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► **Cover picture:** FTS Tourism

59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Date/Location: **4th–9th September 2011, Antalya, Turkey**

President: **Prof. Dr. K. Hüsnü Can Başer**

Dear Colleagues,

It is my great pleasure and honour to hold the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research on September 4–9, 2011 in Antalya, Turkey. This congress series has been organized annually since 1953 and has become the most important and popular congress in Europe in its respected field. It is the first time the congress is organized in Turkey. Turkey is a large peninsula bridging the east and the west at the junction of two continents and has been a passage way between Europe and Asia and even Africa. Due to its geographic location Turkey has been a melting pot of civilizations, cultures and nations, and is full of history and home to diverse traditions. It is a land of many firsts since history starts here. Thanks to its climatically and phytogeographically unique position and its transect ranging from sea level (0 m) to the peak of the Ararat mountain (5137 m) the flora of Turkey is rich and diverse with over 12.000 flowering plant taxa recorded of which 33% are endemic. Anatolia is the land of Galenus of Pergamon and Dioscorides of Anavarza. Pedanius Dioscorides, a physician in the Roman Army had written his famous Materia Medica in the 1st century AD. His birthplace Anavarza is in Kozan, Adana in Southern Turkey not too far from Antalya.
The 59th Congress has attracted global attention and there are participants from all parts of the world. Its scientific level is high thanks to the efforts of the Scientific Committee. High rate of rejects were due to the meticulous work of the reviewers who gave it time and effort to keep the scientific level as high as possible.
Main topics of the Congress are as follows:

- New Trends in Pharmacognosy
- Traditional and Natural Medicines
- Leead Finding from Nature
- Antimicrobials – What's next?
- Endophytes – Importance in Pharmacognosy
- Natural Immune Enhancers
- Nutraceuticals, Cosmeceuticals, Functional Foods – Prevention of Metabolic Diseases
- Essential Oils – Analysis, Bioactivities, Uses, Therapeutical Potential
- Biotechnology and Nanobiotechnology
- Advances in the Analysis of Natural Products

Ten plenary and two keynote lectures will be presented by distinguished scientists. 73 short lectures will be presented in three paralel sessions. Numerous researchers will be able to report their research findings in 900 poster presentations. In addition, young researchers will be able to present their papers at two paralel Young Researchers Workshops. There will also be three more Permanent Committee Workshops of the GA on regulatory affairs, pharmacology, agriculture and quality of natural products. An additional workshop will be held on Traditional Chinese Medicine (TCM). 31 lectures will be presented in the workshops. All in all over 1100 scientific presentation will be made at the congress.

I would like to thank the Executive and the Advisory Board members of the GA for their help and encouragement during the preparatory stages of the Congress. I wish to extend my grateful thanks to Georg Thieme Verlag KG for processing such a huge number of abstracts in a short time. My special thanks go to the members of the Organizing Committee and to the Congress Organizing Company FTS who have done their utmost to offer you a successful, satisfying and enjoyable congress.

I wish you all a fruitful congress which I hope will strengten old friendships and develop new ones in a friendly, scientific and cultural atmosphere. I hope everybody enjoys their stay in sunny Antalya, gets the opportunity to discover hidden beauties of the region and Turkey, and takes home new scientific knowledge and unforgettable memories.

Prof. Dr. K. Hüsnü Can Başer
President of the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Abstracts

Lectures

L1 Natural products in modern life science

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With a realistic threat against biodiversity in rain forests and in the sea, a sustainable use of natural products is becoming more and more important. Basic research directed against different organisms in Nature could reveal unexpected insights into fundamental biological mechanisms but also new pharmaceutical or biotechnological possibilities of more immediate use. Many different strategies have been used prospecting the biodiversity of Earth in the search for novel structure-activity relationships, which has resulted in important discoveries in drug development. However, we believe that the development of multidisciplinary incentives will be necessary for a future successful exploration of Nature. With this aim, one way would be a modernization and renewal of a venerable proven interdisciplinary science, Pharmacognosy, which represents an integrated way of studying biological systems. This has been demonstrated based on an explanatory model where the different parts of the model are explained by our ongoing research. Anti-inflammatory natural products have been discovered based on ethnopharmacological observations, marine sponges in cold water have resulted in substances with ecological impact, combinatory strategy of ecology and chemistry has revealed new insights into the biodiversity of fungi, in depth studies of cyclic peptides (cyclotides) has created new possibilities for engineering of bioactive peptides, development of new strategies using phylogeny and chemography has resulted in new possibilities for navigating chemical and biological space, and using bioinformatic tools for understanding of lateral gene transfer could provide potential drug targets. A multidisciplinary subject like Pharmacognosy, one of several scientific disciplines bridging biology and chemistry with medicine, has a strategic position for studies of complex scientific questions based on observations in Nature. Furthermore, natural product research based on intriguing scientific questions in Nature can be of value to increase the attraction for young students in modern life science. **References:** Bohlin L, Göransson U, and Backlund A (2007) Pure and Appl Chem 79: 763 – 774. Larsson S, Backlund A, and Bohlin L (2008) Phytochem Lett 1: 131 – 134. Bohlin L, Göransson U, Alsmark C, Wedén C, and Backlund A (2010) Phytochem Rev 9: 279 – 301.

L2 Combination of ethnopharmacological knowhow with modern in silico tools

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Computational methods are valuable tools in current drug discovery and development processes. They aim at analyzing, understanding, and predicting the bioactivity of a compound with respect to a specific biological target, and have been applied successfully in medicinal chemistry. Their application in natural product research is however affected with some challenges, such as limited availability of high quality natural product databases, often restricted or laborious access to individual compounds for testing, and lack of chemoinformatics experience with secondary metabolites. This asks for a sensible application of data mining tools in this prospering field of lead finding from nature. The hyphenation of in silico strategies with knowledge from ethnopharmacology offers a unique opportunity to benefit from a combined theoretical and empirical approach. Herbal remedies, which are used since centuries, represent a particularly promising resource for drug leads. These often undefined multicomponent mixtures play a dominant role in healthcare worldwide with increasing information on traditional and biomedical uses and with links between multi-target molecular pharmacology and clinical medicine. This presentation describes strategies how to integrate computational strategies in pharmacognostic workflows to disclose promising bioactive compounds or hidden information about affected pharmacological targets. I will focus specifically on the expectations, possibilities and limits when using computational tools in phytochemistry, and present some recent examples of these highly complementary, but synergistic approaches, viz. in silico – in traditio. **Keywords:** computational methods, in silico, ethnopharmacology, drug discovery

L3 Biological Activities of Essential Oils

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Essential Oils (natural mixtures of single fragrance compounds) do possess biological properties. Besides psychological influences, these rather small and lipophilic molecules exhibit distinct physiological activities upon inhalation and/or topical administration which can be shown in animal experiments as well as in human studies. After a short introduction about the correct definition of the term "Essential Oils" new research results on the application of fragrance compounds in therapy are presented. Examples of the biological properties of these natural compounds will be discussed and a great bow drawn from the effects on the human autonomic and central nervous system to "other effects". These cover e.g. anti-inflammatory activities, anti-oxidative ones and penetration enhancing properties, anti-microbial and insect repellent activities and the possibility to use these small molecules in cancer prevention or therapy and against Alzheimer's disease. Some studies on the biochemical mechanism of such effects are also presented as well as methods to investigate the above activities of essential oils.

L4 Infectious diseases and natural products. What is next?

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Infectious diseases caused by bacteria, viruses, parasites and fungi are results of complex interactions between the pathogen, host and the environment. The early discovery of quinine, followed by the antibiotics and more recently artemisinin has brought a new, realistic hope in the control of infections that once ravaged the humankind. However, the widespread use of these drugs and globalization has led to the development of multidrug-resistant pathogens worldwide. On the other hand, the pharmaceutical industry that previously relied extensively on natural products (NPs) as source of small molecules for anti-infective drug discovery and development has undergone a significant de-emphasis in NP research. The main research activity currently falls on to academia and requires novel approaches for tackling infectious diseases. This lecture will emphasize the opportunities and the challenges in this area and highlight new application forms and areas for natural products. Specific examples from our own research will be presented in order to point out the potential of vast variety of natural products derived from plants, marine organisms and other sources in both prophylaxis and the chemotherapy of infectious diseases caused by parasites (e.g. malaria, trypanosomiasis, leishmaniasis, schistosomiasis and river blindness) and (myco)bacteria (e.g. tuberculosis). Target determination, molecular properties (lipophilicity, permeability, drug-likeliness) and pharmacokinetic properties of some natural and natural product-derived synthetic leads will also be included. **Keywords:** Infectious diseases, natural products, drug discovery, prophylaxis, chemotherapy

L5 Natural products derived from traditional chinese medicine as novel ınhibitors of the epidermal growth factor receptor in cancer cells

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The success of established anticancer drugs is frequently hampered nonsufficient tumor specificity leading to side effects and drug resistance. Oncogene products and tumor suppressors are exquisite targets developing more specific drugs with improved features. The epidermal growth factor receptor (EGFR) became an important target for drug development. However, clinical application of EGFR tyrosine kinase inhibitors resulted in resistance to EGFR-targeting drugs due to the selection of EGFR-mutated variants. This phenomenon forced the search for novel inhibitors of EGFR and downstream signaling cascades. We investigated the prognostic role of the EGFR in human tumor biopsies (lung cancer, glioblastoma, head and neck cancer) by comparative genomic hybridization and immunohistochemistry) [1 – 4,6] and identified phytochemicals (dicentrine, camptothecin derivatives, artesunate etc.) affecting EGFR signaling [5,7 – 9]. Here, we report on recent achievements

in natural products derived from medicinal plants as novel inhibitors of mutated EGFR and EGFR signal transduction pathways [10]. **Keywords:** Cancer, EGFR, Targeted chemotherapy, Traditional Chinese medicine **References:** 1. Volm et al. (2002) Br J Cancer 87: 251 – 7. 2. Volm et al. (2002) Clin Cancer Res 8: 1843 – 8. 3. Volm et al. (2004) Cancer Genomics Proteomics1: 157 – 66. 4. Halatsch et al. (2004)J Neurosurg 100: 523 – 33. 5. Efferth et al.(2004) Biochem Pharmacol 1689 – 1700. 6. Konkimalla et al. (2007) Expert Rev Anticancer Ther 7: 319 – 29. 7. Konkimalla et al. (2009) Curr Cancer Drug Targets 9: 72 – 80. 8. Konkimalla et al. (2010) Biochem Pharmacol 79: 1092 – 1099. 9. Konkimalla et al. (2010) Biochem Pharmacol 80:39 – 49 10. Sertel et al. (2010) Combinat Chem High Throughput Screen 13:849 – 54.

L6 The role of pharmacokinetics in natural products research

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In recent years the number of studies investigating the pharmacodynamic effects of botanicals has increased exponentially, often reporting pharmacological effects of botanical extracts with insignificant bioactivities obtained in irrelevant in vitro bioassays. The data interpretation from these in vitro assays for their efficacy in animals and humans is based on the assumption that a sufficient concentration of active constituents can reach the target sites of action in the body. This interpretation can be misleading since the pharmacokinetic properties of a compound are completely ignored. Although important, there is still limited information available regarding herbal pharmacokinetics. This might be due to the following reasons: (i) the active constituents are not known; (ii) the study of herbal pharmacokinetics is extraordinarily complex because extracts are multicomponent mixtures which contain several chemical constituents. Therefore concentrations of single compounds in the final product are in the lower mg range per dose. (iii) The resulting plasma concentrations are often in the µg to pg per liter range. As a consequence analytical methods determining bioavailability and pharmacokinetics of natural compounds have to be sufficiently sensitive. Advanced techniques such as GC-MS/MS or HPLC-MS/MS can be used nowadays to accomplish these goals. A better understanding of the pharmacokinetics and bioavailability of natural compounds can help in designing rational dosage regimen; and it can further help to link data from pharmacological assays with clinical effects. In this presentation, pharmacokinetic studies will be discussed that have been conducted for some of the top-selling botanicals worldwide, including artichoke, echinacea, mangosteen and valerian.

L7 Overview of Dietary Supplements in USA

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Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question. In the case of botanicals, misidentification of the collected plant, adulteration with other species or contamination with extraneous ingredients are possibilities in which reproducibility may be effected unknown to the manufacturer. Many studies refer to the use of standardized material, but in reality they are referring to chemical standardization. While chemical standardization is important, its utility is limited when the starting material is not well characterized botanically. Although the resulting studies are sound with respect to the actual product tested, adequate authentication of the product cannot be compared to other products on the market. Also, a comparison of one study to another cannot be made due to inconsistencies in the identity of the botanical matrix. The tools needed for authentication of the field plant material also depend on the plant and process involved. This could be as straightforward as botanical/morphological identification or as elaborate as genetic or chemical profiling. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements. **Keywords:** Herbal products, botanical supplements, authentication

L8 Discovery and applications of naturally occurring cyclic peptides

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Over recent years more than 200 examples of ribosomally synthesized head-to-tail cyclised proteins have been discovered in bacteria, plants and animals [1]. The cyclotides [2] are the largest family of these circular proteins and have applications in drug design [3] and agriculture [4]. They occur in plants from the Violaceae (violet), Rubiaceae (coffee) and Cucurbitaceae (cucurbit) families and have a diverse range of biological activities, including uterotonic, anti-HIV, and insecticidal activities, the latter suggesting that their natural function is in plant defence. Individual plants express suites of 10 – 100 cyclotides. Cyclotides typically comprise ~30 amino acids, and incorporate three disulfide bonds arranged in a cystine knot topology. The combination of this knotted structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. This presentation will describe the discovery of cyclotides in plants, their structural characterization, evolutionary relationships and their applications in drug design. Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. **Keywords:** Cyclic peptides; cyclotides; drug design; NMR; protein structure **References:** [1] Craik D J (2006) Science 311: 1561 [2] Gruber C W et al (2008) The Plant Cell 20: 2471 – 2483. [3] Henriques S T, Craik D J (2010) Drug Discovery Today 15: 57 – 64. [4] Barbeta B L et al (2008) PNAS 105: 1221 – 1225

L9 Natural immunomodulators – A Drug Discovery Perspective

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The immune system is an adaptive complex sytem which poses a challenge to therapeutic intervention. Inflammation is known to be involved in numerous disease processes like autoimmune attack and carcinogenesis and can be targeted by immunopharmacological agents. However, it is far less clear how the immune system may be stimulated [1]. In this talk a new view on how the immune system recognizes and senses plant food shall be provided. During the evolution of the immune system different endogenous systems have evolved that modulate inflammatory processes, such as the pattern recognition receptors (TLRs) and the arachidonic acid lipidome. Studying the molecular interactions in these biochemical units by small organic molecules provides insight into how inflammation may be regulated and ultimately manipulated. The endocannabinoid system is a stress signal-integrating lipid signaling network and provides great opportunities to treat inflammatory diseases and bone degeneration with the potential to link nutrition and inflammation [2 – 4]. **Keywords:** Inflammation, immune system, immunomodulation, drug discovery, endocannabinoid system **References:** [1] Gertsch J, Viveros-Paredes JM, Taylor P (2010) Plant immunostimulants-Scientific paradigm or myth? J Ethnopharmacol PMID: 20620205 [2] Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, Altmann KH, Karsak M, Zimmer A (2008) Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 105(26):9099 – 104 [3] Gertsch J (2008) Anti-inflammatory cannabinoids in diet: Towards a better understanding of CB(2) receptor action? Commun Integr Biol 1(1):26 – 8. [4] Schuhly W., Viveros Paredes J M, Kleyer J, Huefner A, Anavi-Goffer S, Raduner S, Altmann KH, Gertsch, J. (2011) Mechanisms of Osteoclastogenesis Inhibition by a Novel Class of Biphenyl-type Cannabinoid CB2 Receptor Inverse Agonists. Chem Biol in press

L10 Pharmacognosy in Turkey

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This presentation will give an overview on the wide range of studies of Pharmacognosy in Turkey, since its establishment as a discipline during the Ottoman Empire, up until contemporary times. Studies of Pharmacognosy have begun in 1839 together with the official education of

pharmacy. Dr. Charles Ambroise Bernard (1808 – 1844), Dr. Mehmed Akif Aykut (1887 – 1939), Prof. Dr. Alfred Heilbronn (1885 – 1961) and Prof. Dr. Sarım Hüsnü Çelebioğlu (1897 – 1982) were the first scholars who had initially developed the education of Pharmacognosy. Prof. Dr. S.H. Çelebioğlu obtained the PhD degree under the supervision of Prof. Dr. E. Gilg at Berlin Friedrich-Wilhems University in 1932. In his dissertation, Çelebioğlu investigated the occurence of the opium alkaloids in the root ends of *Papaver somniferum*, and, its transfer to the whole plant by latex vessels. Çelebioğlu is the founder of the Institute of Pharmacognosy in Istanbul University (1945) and the first author of a Pharmacognosy textbook in Turkey (1949). Three PhD studies performed by Dr. Turhan Baytop (1920 – 2002), Dr. Mekin Tanker and Dr. Nevin Tanker on *Ephedra campylopoda* C.A.May, *Marsdenia erecta* R.Br. and *Juniperus nana* Wild. had been completed under his supervision, respectively. Dr. Baytop, a distinguished scientist focusing mostly on "Medicinal Plants and the Flora of Turkey" and the "History of Pharmacy in Turkey", initiated the National Pharmacognosy Meetings in 1976. Since then, these meetings have been biennially organized by the collaborative efforts of Departments of Pharmacognosy and Pharmaceutical Botany of the different faculties of whose number has risen to twenties. Moreover, the members of these departments have been the founders and pioneers of the Turkish Society of Pharmacognosy & Pharmaceutical Botany. Materials for research carried out by these scientists are provided from the flora of Turkey which represents more than 11000 taxa, including over 3000 endemic species. The richness and diversity of the flora and the knowledge on medicinal plants of this geographical region, continuing the tradition of Hippocrates, Dioscorides, Galenos, Avicenna and Ibn el-Beithar, are still offering an invaluable opportunity to Turkish plant scientists. The scientific studies conducted can be mainly classified as floristic, ethnobotanical, ethnopharmacological, chemical structures and/or activity guided studies. During the last two decades pharmacognostic studies have become interdisciplinary, encouraging collaborative works of multiple disciplines, resulting in most fruitful results.

L11 Agrochemical Applications for Medicinal and Aromatic Plants

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The vast majority of pharmacognosy research is focused on drug discovery for human ailments. However, the discovery of new plant and animal protectants that have low mammalian and environmental toxicity are needed Worldwide. The availability biopesticides to protect humans and animals against insects and insect vectored diseases has come to the forefront following natural disasters such as tsunamis, tropical cyclones, earthquakes, and other natural disasters. Research emphasis at the US Department of Agriculture's Natural Product Utilization Research Group has been on the discovery and development of alternative approaches to utilizing natural plant products for pest management. Discovery and evaluation of natural product based biopesticides is largely dependent upon the availability of suitable miniaturized antifungal and insect bioassays and is a primary facilitator in coordinating the testing of limited source materials. Through research collaborations within the Agricultural Research Service we evaluate natural compounds and their analogs as fungicides against a wide variety of fungal plant pathogens, insecticides for fruits and ornamentals, as adult mosquito deterrents and toxicants, mosquito larvalcides, tick deterrents, and new baits to control imported fire ants. This presentation will focus on a number of chemical compounds and essential oils derived from aromatic and medicinal plants that have application as novel plant and animal protectants. Through a 10 year long ongoing collaboration with Turkish scientists we have collectively published more than 17 peer reviewed journal articles on medicinal and aromatic plants as unique botanical sources for new agrochemical applications.

L12 Metabolomics and systems biology approaches for the investigation of endophytes – plant-interaction – a vision for their importance in biotechnology and natural product research

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Plants have shaped human life forms since their rising. Plants have ever served as food and feed and have a role as one of the richest resources for natural products and lead structures in drug research. Nowadays personalized medicinal and health approaches try to adapt diets to life style. Thus, intimate knowledge of the composition of our plant-derived food is one of the next cornerstones in nutritional physiology. Together with emerging themes, such a renewable energy resources to cope with global climate changes and limited energy resources the relevance of plant biology and biotechnology becomes dramatically important in the next decades. Consequently, it can be anticipated that plant biology and applications will have even more indispensable future roles in all socio-economic aspects of our life [1]. In parallel the last 10 years have recognized a revolution in biology basically as a result of three main developments: (i) shotgun and next-generation genome sequencing, gene reconstruction and annotation, (ii) genome-scale molecular analysis using omics-technologies and (iii) computer-assisted analysis, modelling and interpretation of biological data [1, 2]. Metabolomics – the measurement of the complete small molecule fraction in a biological system – is a relatively young technology, however, has already reached an importance comparable with proteomics and transcriptomics platforms [3]. Metabolomics and proteomics emerged in parallel with the development of novel mass spectrometric techniques. Natural product research is intimately bound to these analytical procedures. Accordingly, metabolomic technology is especially suited for the analysis of a wide range of chemical diversity in biological systems. Many of these biological systems providing the widest range of chemical diversity of natural products comprise symbiotic life forms between plants, fungi and bacteria. Although known and investigated for hundreds of years, this interaction undergoes a revival of deep interest because novel tools as described above are available, especially whole-genome sequencing and genome-scale molecular profiling. Whole plant-endophyte-ecosystems can be observed in nature giving ample opportunities for the search of new natural compounds. Examples for the investigation of plant-endophyte-interactions are given and a vision is presented how the combination with novel technologies such as genome sequencing, metabolomics, proteomics and transcriptomics will increase our understanding of the mechanism of plant-endophyte-interaction and at the same time will amplify our existing portfolio of chemical diversity of natural products.

References: [1] Weckwerth W (2011) Green Systems Biology – from single genomes, proteomes and metabolomes to ecosystems research and biotechnology. Journal of Proteomics in press. [2] Weckwerth W (2011) Anal Bioanal Chem 400: 1967 – 1978. [3] Weckwerth W (2003) Annu Rev Plant Biol 54: 669 – 689

Workshops

WORKSHOP I: Young Researchers Workshop
Rapid Strategies for (phyto) Chemical Characterization of Natural Products
Chairs: J. L. Wolfender, J. Rollinger, A. R. Bilia

WSI IL Impulse Lecture: Metabolic characterization of plants using NMR-based metabolomics

Choi Y, Kim H, Verpoorte R
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Pharmacognosy, particularly using plant-originated natural product has played a central role in natural science. Even in these days, the importance of pharmacognosy is not reduced at all. However, due to innate diversity and dynamic changes of metabolites levels, natural product research had a problem with profiling all metabolites, analysis usually limited to a certain group of metabolites. One of the approaches to solve the problem might be metabolomics, a comprehensive profiling all the metabolites in organisms. It takes a part to provide holistic information of whole metabolome network [1 – 3]. As a part of systems biology, capturing holistic information of the complex metabolic system in unbiased manner has been a dream of natural products researchers for a long time. Recent advances in analytical chemistry, combined with mul-

tivariate data analysis, brought us closer to the final goal of metabolomics, comprehensive evaluation of all metabolites in living organisms including plants. Of many analytical platforms NMR has been thought as one of the most promising techniques to cover all the metabolites in short time despite its' inherent low sensitivity compared with MS-based technology. In addition to the unambiguous advantages of NMR such as broad coverage of metabolite detection, the easiness of data handling for further statistic treatment and signal robustness have been attracting many metabolomists. In this presentation, diverse applications of NMR-based metabolomics for chemical characterization of plants, plant physiology, and screening method of bioactive metabolites will be shown as well as a possible protocol developed by our groups [4]. **Keywords:** Metabolomics, NMR, Chemicals, Characterisation **References:** 1. Kim H K et al. (2011) Trend Biotechnol In press. 2. Verpoorte R et al. (2008) Phytochem Rev 7: 525. 3. Verpoorte R et al. (2007) Phytochem Rev 6: 3. 4. Kim HK et al. (2010) Nat Protoc 3: 536.

WSI 1 On-line coupling of Centrifugal Partition Chromatography (CPC) to High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS)

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Phytochemical analyses of food and medicinal plant extracts require rapid screening and detection strategies to identify bioactive natural products. Centrifugal Partition Chromatography (CPC), a free solid support liquid-liquid chromatography, is a well established method for the isolation of natural products and fractionation of complex samples at the preparative scale [1,2]. Nevertheless, even if the separation is monitored by detectors the composition of the different collected fractions must be evaluated by further High Performance Liquid Chromatography (HPLC) which is time consuming and give delayed information on the fraction composition. We present here the development of a versatile tool for fast screening and rapid detection of bioactive natural products from plant extracts: the on-line coupling of CPC-UV to HPLC-UV-MS/MS. The coupling of CPC and HPLC systems, via a six position switching valve, reduces the time of complete fractionation procedure by direct on-line analyses of collected fractions and permits a guided fractionation step [3]. HPLC columns suitable for fast analysis, monolith and fused core columns, were evaluated to allow rapid analyse of compounds separated by CPC. Furthermore, the use of MS in tandem mode allows to get a direct structural identification of separated molecules. This strategy was applied to the fractionation and purification of bioactive compounds from berries and roots of sea buckthorn (*Hippophaë rhamnoides* L., Elaeagnaceae), a Eurasian medicinal thorny bush, known to have various pharmacological effects [4]. **Keywords:** On-line CPC-HPLC-MS/MS, fractionation, purification, *Hippophaë rhamnoides* **References:** 1. Marston A, Hostettmann K (2006) J Chromatogr A 1112: 181 – 194 2. Ingkaninan K et al. (2000) J Liq Chromatogr Relat Technol 23: 2195 – 2208 3. Michel T et al. J Chromatogr A Available online 1 February 2011 4. Guliyev VB et al. (2004) J Chromatogr B 812: 291 – 307

WSI 2 A rapid LC- MS method for the simultaneous quantification of Oleuropein and its main metabolite, Hydroxytyrosol, in clinical samples after oral administration of commercial herb medicinal products

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Oleuropein (OE) is a secoiridoid glycoside, which occurs mostly in the Oleaceae family presenting several pharmacological properties, including antioxidant, cardio-protective, anti-ischemic, anti-atherogenic effects etc [1]. Based on these findings OE is commercially available in many countries, as food supplement or Herb Medicinal Product (HMP). In the context of investigating the effects of OE on the human blood redox status, a commercial OE enriched HMP has been administered *per-os* to a selected population. A novel LC-MS methodology was developed and validated for the simultaneous quantification of OE and its main metabolite, Hydroxytyrosol (HT), for the aforementioned HMP prior to the clinical study. Modification of the above LC-MS method was performed in order to measure OE and HT in clinical serum samples of the study using a simple pretreatment protocol. Thus, separation of OE, HT and internal standard was achieved on a C 18 Fused Core column with 3.1 min overall run time employing the SIM method for the analytical signal acquisition. The methods were validated according to ICH requirements and evaluated by measuring the selectivity, precision, accuracy, robustness, lower limit of quantification (LLOQ) and found to be linear ($r^2 > 0.99$) over a wide concentration range of 0.1 – 15 µg/mL (n = 12) for both analytes of interest with an LLOQ value of 0.1 µg/mL. The methodological approaches presented in this study allowed the standardization of the administered dose, whereas it was used for the high-throughput analysis of a list of HMP's and clinical samples. In addition PCA-based similarity measurements were performed to the HMP's. **Keywords:** LC-MS, Oleuropein, Hydroxytyrosol, herb medicinal products, serum **References:** 1. Andreadou I, Iliodromitis E, Mikros E, Skaltsounis AL, Kremastinos D (2010) The Use of Oleuropein on Myocardium, Olives and Olive Oil in Health and Disease Prevention. Academic Press. USA

WSI 3 Improved peptidomics screening protocol for the identification of cyclotide-containing plants

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Cyclotides are disulfide-rich plant peptides with unique structural features of a cyclic backbone and three conserved disulfide bonds in a knotted arrangement, known as cyclic cystine knot. So far their presence has only been reported for species of the families of Rubiaceae, Violaceae, Cucurbitaceae and recently Fabaceae [1], but it is very likely that cyclotides are more widely distributed since their predicted number in Rubiaceae alone is ~50.000 [2]. Their sequence diversity and range of bioactivities make them interesting templates for drug development [3]. In this study we investigated > 120 plants in ~20 different families to get novel insights about the distribution of cyclotides within the plant kingdom. Further we improved the identification workflow for new cyclotide-containing species from crude plant extracts optimizing MALDI-TOF/TOF and LC-MS/MS experiments. The presence of 6 cysteines and their cyclic structure gives distinct mass shifts of +348 Da for fully reduced and alkylated peptides and +366 Da for single-site protease cleavages. Novel cyclotide sequences were confirmed by manual and automated peptide sequence assignment using the ERA-tool [4]. We have identified many novel cyclotide-containing species and obtained several sequences. Amongst sequences from Psychotria spp. we identified *Rauwolfia tetraphylla* L. belonging to the Apocynaceae family, to be a 'cyclotide-plant' and for the first time we report sequences in this family confirming previous investigations [2]. This underpins earlier suggestions that cyclotides are one of the largest peptide classes within plants, offering access to a large natural peptide library for multiple biological and pharmaceutical applications. **Keywords:** cyclotides, plant screening, peptide sequencing **Acknowledgement:** *This work is funded by the Austrian Science Fund-FWF P22889.* **References:** 1. Poth et al. (2011) ACS Chem Biol, *in press*. 2. Gruber et al. (2008) Plant Cell 20: 2471 – 2483. 3. Henriques and Craik (2010) Drug Discov Today 15: 57 – 64. 4. Colgrave et al. (2010) Biopolymers 94: 592 – 601

WSI 4 Phytochemical profiling of *Opopanax persicus* Boiss.

Rajabi A[1], Ebrahimi S[2], Neuburger M[3], Wagner T[4], Zimmermann S[5], Quitschau M[2], Amin G[6], Salehi Sourmaghi M[6], Hamburger M[2]
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Opopanax W. D. Koch is a genus of the Apiaceae family, with 11 species distributed throughout the Mediterranean and Iranica region (Iran, Afghanistan, Western Pakistan, Northern Iraq, Azerbaijan, Turkmenistan) [1]. With the exception of some phytochemical studies on O. chironium (L.) Koch growing in the Mediterranean and Balkan regions [2], the genus Opopanax has not been investigated from a chemical nor pharmacological viewpoint. We carried out a phytochemical profiling of O. persicus Boiss., an endemic species growing in parts of Turkey, Iran, Iraq and Transcaucasia [1]. From the dichloromethane extract, a total of 20 compounds were isolated by medium pressure liquid chromatography (MPLC), vacuum liquid chromatography (VLC), and preparative and semi-preparative HPLC. Structure elucidation was carried out by on-line ESI-MS and PDA data, HR-ESI-TOF-MS and off-line 1D- and 2D-NMR spectra recorded in a 1-mm TXI microprobe. Compounds were identified as coumarins with predominantly linear and angular dihydropyranocoumarin scaffold which 10 are reported for the first time as new derivatives. The absolute stereochemistry of isolated compounds was determined by X-ray crystallography and CD spectroscopy. Some of the compounds showed moderate activity against Plasmodium falciparum K1 strain and Trypanosoma brucei rhodesiense (IC50 s 3.6 to 6.9 μg/ml, and selectivity indices (SI) in L-6 cells of 5.7 to 25, respectively). **Keywords:** *Opopanax persicus* Boiss., phytochemical analysis, dihydrofuranocoumarin, absolute stereochemistry, X-ray crystallography, CD spectroscopy **References:** 1. Rechinger KH (1987) Flora Iranica, No. 162: 438 – 439. 2. Appendino G et al. (2004) J Nat Prod 67(4): 532 – 536.

WSI 5 Fungal co-culture as a new source of antifungal metabolites

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Microorganisms are a very rich source of secondary metabolites with antimicrobial potential [1]. In order to produce original metabolites from this source, strategies have to be developed to induce synthetic pathways triggered by genes that are often silent [2]. Nutritional or environmental stress can be used to activate these orphan pathways and lead to novel metabolites. Recently new approaches were developed using genomic approaches or interspecies crosstalk to induce the production of new compounds. In relation with this latter aspect our recent work on co-cultivation of microorganisms shows that new metabolites can be found with this approach [3]. In the course of a screen based on fungal confrontation, we identified intriguing morphological co-culture patterns such as a *Trichophyton rubrum* strain able to inhibit the growth of several other fungi at a distance on agar plates (Figure 1). In order to identify fungal metabolites responsible for this long distance repulsion, confrontation zone and pure fungal strains were compared by UHPLC-TOF-MS fingerprinting using differential metabolomics. Data mining results in an efficient selection of stress induced molecules which were purified using preparative RP-HPLC-MS and subsequently identified by microflow NMR. The antifungal activity of biomarker was assessed in order to verify that the fungal long distance repulsion was related to these mycoalexins. This innovative strategy can be used to search for original antifungal metabolites to eradicate resistant fungi such as *Fusarium*.

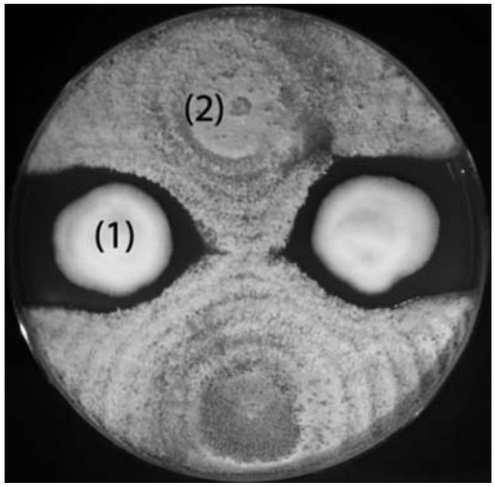

Figure 1: fungal co-culture on agar plates of Trichophyton rubrum (1) and Bionectria ochroleuca (2) showing long distance repulsion

Keywords: fungal metabolites, co-culture, confrontation, anti-fungals
Acknowledgement: *This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W. and K. G.)* **References:** 1. Berdy J (2005) J Antibiot 58: 1 – 26 2. Scherlach K et al. (2009) Org Biomol Chem 7: 1753 – 1760 3. Glauser G et al. (2009) J Agric Food Chem 57: 1127 – 1134

WSI 6 *Achillea collina* response to biotic and abiotic stresses: a comparative evaluation of volatile emissions pathways

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Plants have evolved wide range of mechanisms to cope with biotic and abiotic stresses. It's suggested that hormone signaling pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene, can be involved in plant responses to stress [1]. Our attention was focused on volatile compounds emissions (VOCs) from *Achillea collina* Becker, a medicinal plant, exposed to biotic and abiotic stressing conditions. Headspace Solid-Phase-Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC/MS) "*in vivo*" method [2] was used to evaluate *A. collina* VOCs. Biotic stress was obtained by the infestation of *A. collina* plants with *Myzus persicae* or *Macrosiphoniella millefolii*, the generalist and specialist aphid species respectively. Mechanical stresses were obtained by applying a pressure to the plants using a specially designed equipment or by pricking the leaves with a needle. VOCs emissions are reported in figure1. As shown, some compounds were induced by both the biotic and abiotic stresses (*eg.* 1-Hexanol, Pinocarvone, α-Fenchene) while some other VOCs were specific to the type of stress applied. As an example Spathulenol was only induced by *M. millefolii* and β-Linalool was induced only by the mechanical damage. Aromadendrene, Terpineol-cis-β, Tetradecanal were only induced by the biotic stresses. *A. collina* shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a possible good model for future investigations at a molecular level.

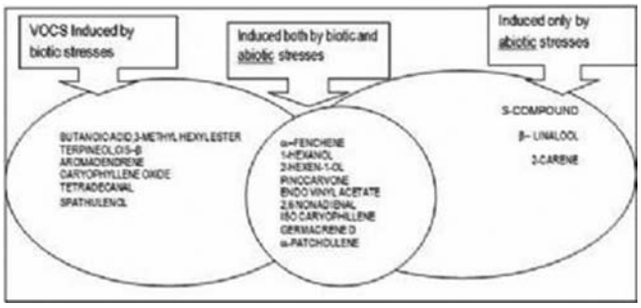

Figure 1: VOCs induced by different stress conditions
Volatile emission pathways of induced VOCs of Achillea collina under biotic and abiotic stresses

Keywords: SPME, VOCs, Aphids, Achillea collina **References:** 1] Fujita M et al. (2006) Current option i n Plant Biology 9: 436 – 442 [2] Giorgi A et al. (2010) Planta Med 76: 1337

WSI 7

A validated HPLC method for standardization of the most active fraction of the antihyperglycemic drug *Cleome droserifolia* using bioactive markers

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The aqueous and ethanolic extracts of the aerial parts of *Cleome droserifolia* (Forssk.) Del. were assessed for their antihyperglycemic effects in male albino rats at the same dose level of the biguanide metformin (150 mg/kg body weight). Diabetes was induced intraperitoneally with a single dose of alloxan (150 mg/kg body weight) [1].The blood glucose level was monitored after 2 and 4 weeks from zero time (Table 1). The four sub-fractions (n-hexane, chloroform, ethyl acetate and n-butanol) of the more active aqueous extract were tested at the same dose level. A validated RP-HPLC method for standardization of the most active ethyl acetate fraction (70% as potent as metformin after 4 weeks of oral administration) was developed. Three flavonoid glycosides; Isorhamnetin-3-O-β-D-glucoside (F1), quercetin-3'-methoxy-3-O-(4''-acetylrhamnoside)-7-O-α-rhamnoside (F2) and kaempferol-4'-methoxy-3,7-dirhamnoside (F3) (were isolated from the ethyl acetate fraction and proved to increase basal glucose uptake, 2-folds as insulin, in C2C12 skeletal muscle cells [2]) were used for the standardization (Fig.1). The parameters of validation of the method (linearity, repeatability, reproducibility, ruggedness, robustness and accuracy) were evaluated. A standard calibration curve, established for the major compound F3 at a concentration range of 44 – 174 µg/ml, showed good linearity with a correlation co-efficient (R2) of 0.998. The recovery of the method was 100.5%. A high degree of repeatability and reproducibility (relative standard deviation values less than 5%) were also achieved.

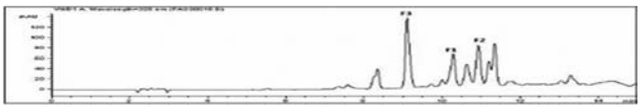

Fig. 1: HPLC chromatogram of the ethyl acetate fraction
HPLC chromatogram of the ethyl acetate fraction showing the flavonoid glycosides F1, F2 and F3. The method involved the use of a Lichrosphere 100 RP-18 column with a guard column. Gradient elution was from 10 to 75% v/v acetonitrile/0.3% orthophosphoric acid in water, in 25 min, at a flow rate of 1.0 ml/min and UV detection at 325 nm.

Table 1. Antihyperglycemic effect of the different extracts of Cleome droserifolia

Time	Zero	2 weeks	2 weeks	4 weeks	4 weeks
Group	M ± S.E.	M ± S.E.	% of change	M ± S.E.	% of change
Diabetic rats (Db) non treated	243.7 ± 8.2	256.8 ± 9.6	-	256.8 ± 9.6	-
Db + metformin	257.3 ± 11.4	129.8 ± 4.3*	49.5	81.9 ± 3.2*	68.2
Db + ethanolic extract	249.2 ± 8.2	216.2 ± 7.6*	13.2	153.2 ± 4.6*	38.5
Db + aqueous extract	256.8 ± 10.1	173.2 ± 6.2*	32.5	141.9 ± 5.5*	44.7
Db + n-hexane fraction	246.9 ± 7.8	214.3 ± 8.6*	13.2	198.6 ± 7.1*	19.5
Db + chloroform fraction	251.9 ± 8.6	186.8 ± 7.4*	25.8	138.9 ± 5.8*	44.8
Db + ethyl acetate fraction	258.4 ± 7.1	187.4 ± 6.3*	27.5	135.3 ± 4.1*	47.6
Db + n-butanol fraction	258.3 ± 10.2	224.9 ± 8.4	13.3	203.7 ± 6.5*	21.1

*Extracts, fractions and the standard metformin were given at a dose of 150 mg/kg body weight. * Statistically significant difference from zero time at P< 0.01. M, mean; S.E., standard error (n = 6).* **Keywords:** validation, bioactive markers, RP-HPLC, *Cleome droserifolia*, hypoglycemia **References:** 1. Eliasson SG, Samet JM (1969). Life Sci 8:493 – 498. 2. Abdel Motaal A, Ezzat SM, Haddad PS (2011) Determination of bioactive markers in Cleome droserifolia using cell-based bioassays for antidiabetic activity and isolation of two novel active compounds. PHYMED-D-11 – 00029R1 (under publication).

WORKSHOP II: Young Researchers Workshop
Rapid Strategies to Assess Bioactivity of Natural Products
Chairs: D. Tasdemir, T. Efferth, A. Hensel

WSII IL

Discovery of neuroactive natural products using behavioral assays in zebrafish

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Despite the advances of modern drug discovery, natural products-not only from plants, but also from fungi and microorganisms-remain the largest and most attractive source of chemical diversity for the development of novel therapeutics across multiple indication areas. Important technological developments within the past decade are now addressing one of the primary bottlenecks of natural product discovery-namely, the bioassay-guided isolation of pure compounds from complex natural matrices. These developments include microgram-scale analytical techniques such as UHPLC-TOF-MS and microflow NMR[1]. Ultimately, the utility of these new methods for natural product discovery will also depend on the biomedical relevance of the bioassays with which they are used. Towards this end, our laboratory is developing *in vivo*, microgram-scale, high-throughput assays based on zebrafish embryos and larvae for the systematic identification of bioactive natural products[2,3]. Zebrafish offer the ability to rapidly evaluate-at a very early stage in the drug discovery process-both the therapeutic potential of natural products, as well as possible hepato-, cardio-, and neurotoxicities. Due to the requirement for only microgram quantities of compounds to be tested, *in vivo* assays based on zebrafish are useful not only for bioassay-guided isolation, but also for the subsequence derivatization of bioactive natural products prioritized for further development as drug discovery leads. This lecture will highlight our laboratory's recent work in the isolation of neuroactive natural products using behavioral assays in zebrafish larvae, particularly in the area of epilepsy. **Keywords:** zebrafish, bioassay-guided fractionation, UHPLC-TOF-MS, microflow NMR, epilepsy **References:** 1. Glauser G et al. (2009) J Agric Food Chem 57:1127. 2. Crawford AD et al. (2008) Planta Med 6:624. 3. Crawford AD et al. (2011) PLoS ONE 6:e14694.

WSII 1 Risk assessment of hERG channel inhibition by natural products – screening and activity directed analysis of spices, food and medicinal plants

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The most prominent determinant of acquired long QT syndrome is inhibition of the hERG potassium channel. Drug-induced QT prolongation can cause undesirable cardiac side effects and has led to several drug withdrawals in the past. In contrast to synthetic drugs, little is known about hERG channel inhibitors of natural origin. For assessing the risk of natural products on hERG channel inhibition, extracts obtained from frequently consumed spices, food, and medicinal plants were submitted to a broad in vitro screening. We established an HPLC-based activity profiling approach for this target by combining HPLC-microfractionation with on-line and off-line spectroscopy, and an automated two-microelectrode voltage clamp assay with transfected Xenopus laevis oocytes. Among the extracts tested, the methanolic extract of the TCM herbal drug Evodiae Fructus (Evodia rutaecarpa [Juss.] Benth., Rutaceae) reduced the peak tail hERG current by 60.9 ± 6.9% at 100 µg/mL. HPLC-based activity profiling of the crude extract led to the identification of dehydroevodiamine and hortiamine, two indoloquinazoline alkaloids, as the active principles. First information on structure activity relationships revealed that both the methyl group at position 14 and the double bond between C(13b) and N(14) are essential for the inhibitory effect of this compound class. We developed a method for removal of both inhibitory alkaloids by filtration over a cation exchange resin (Lewatit® MonoPlus SP 112). The resulting extract was devoid of hERG channel blocking activity. Moreover, we determined the dehydroevodiamine content in different commercial batches of Evodiae Fructus and in various processed TCM products. **Keywords:** hERG channel inhibition, herbal extracts, Evodia rutaecarpa, Rutaceae, HPLC-based activity profiling, indoloquinazoline alkaloids

WSII 2 New cerebrosides and hydroxylated fatty acids from TCM drugs

Rozema E[1], Popescu R[1], Sonderegger H[2], Uhlschmied C[2], Fakhrudin N[1], Reznicek G[1], Atanasov AG[1], Heiss EH[1], Bonn GK[2], Schuster D[3], Urban E[4], Huck CW[2], Dirsch VM[1], Kopp B[1]
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In recent years, increasing attention has been drawn towards the role of lipids in cell signalling pathways and regulation.(1) Plants are a rich source of bioactive lipids. We, therefore, focused on (complex) lipids from plants used as traditional Chinese medicinal (TCM) drugs and their influence on cellular processes. Four new cerebrosides, which belong to the compound class of sphingolipids, were isolated and characterized from *Arisaema amurense* and *Pinellia ternata* (Araceae).(2) Structure elucidation was performed by NMR and mass spectrometry. The compounds were screened for cytotoxicity against cells of 6 different cancer and non-cancerous cell-lines. While the cerebrosides lacked cytotoxic activity, the breakdown product 4,8-sphingadienine showed cytotoxic effect in two types of leukemic cell lines with IC 50 > 20 µM. The effect, however, was similar also in non-cancerous cells (IC 50 > 20 µM). Common fatty acids were found in *A. amurense*, *P. ternata* and *Albizia julibrissin* (Fabaceae). The fatty acids showed high PPARα and -γ activation in a PPAR-luciferase reporter gene assay. Beside these known PPAR ligands, hydroxylated fatty acids, derived from linoleic acid via the lipoxygenase pathway, were isolated from *A. julibrissin*.(2) The influence of the hydroxyl-group(s) on binding to the ligand binding domain (LBD) of PPARγ was studied *in silico* using the program LigandScout.(3) A pharmacophore model was generated based on available complexes of hydroxylated fatty acids with the PPARγ LBD. Thereby, part of the isolated trihydroxylated fatty acids and hydroxy-epoxy fatty acids were predicted to bind to the PPARγ LBD. **Keywords:** cerebrosides, hydroxylated fatty acids, TCM **Acknowledgement:** *This project was supported by the Sino-Austria Project [FF55 – 2004] from the Austrian Federal Ministry and in part by the Austrian Science Fund [NFN S10704-B037 and NFN S10702-B03].* **References:** 1. Evans JF, Hutchinson JH (2010) Nat Chem Biol 6: 476 – 9. 2. Bensky D et al (2004) Chinese Herbal Medicine, Materia Medica. Eastland Press. Seattle. 3. Wolber G, Langer T (2005) J Chem Inf Model 45:160 – 9.

WSII 3 Potential inhibitors of chikungunya and dengue viruses isolated from Malagasy plants

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Chikungunya virus (CHIKV) and dengue virus (DENV) are two emerging arboviruses. CHIKV has recently re-emerged, causing massive epidemics that have moved from Africa throughout the Indian Ocean to India and Southeast Asia. In humans, it is responsible for an acute disease, characterized by a triad of fever, arthralgia and maculopapular rash. [1] Regarding the dengue fever, it affects more than 50 million people annually. [2] Increasing mortality and geographical expansion are the drastic changes noted in the recent epidemiology of the disease. No specific antiviral therapy is currently available, on the market, for these two diseases. A screening on a cellular assay was performed on 400 plants from the Indian Ocean Islands. This screening has led to the selection of *Flacourtia ramontchi* L'Hér. (Salicaceae), a tree distributed in the south of Asia and in Madagascar. Fruits and seeds are used in folk medicine for the treatment of rheumatic arthralgia, cholera and dysentery. [3] Eight new phenolic glycosides and one caffeic acid derivative, together with three known phenolic glycosides and one betulinic acid derivative were obtained by a bioassay-guided isolation from the stem bark. The phenolic glycosides have a salicin core structure; this core may be esterified with benzoic acid and/or 1-hydroxy-6-oxocyclohex-2-en-1-carboxylic acid, on the glucose moiety in 2', 3' and 4' positions and on the primary alcohol function of the salicyl alcohol moiety. Promising results were obtained on the dengue RNA polymerase inhibition assay and preliminary structure-activity relationships were deduced. CHIKV assays are in progress. **Keywords:** *Flacourtia ramontchi*, Salicaceae, phenolic glycosides, chikungunya, dengue, emerging viruses **Acknowledgement:** *This work was financially supported by Museum National d'Histoire Naturelle.* * PHYTOCHIK project was supported financially by CRVOI (Centre de Recherche et de Veille sur les maladies émergentes dans l'Océan Indien) **References:** 1. Solignat et al. (2009) Virology 393: 183 – 197 2. Massé et al. (2010) Antiviral Res 86: 296 – 305 3. Chai X-Y et al. (2009) Planta Med 75: 1246 – 1252

WSII 4 Toxicological risk assessment of *Aristolochia* species

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Aristolochia species are known to contain aristolochic acids, nitrophenantrene derivatives responsible for their nephrotoxic and genotoxic effects [1]. There are numerous aristolochic acid analogues, including aristolactams, a group of compounds with even higher cytotoxic potency than aristolochic acid I [2]. Previous research mainly focused on Aristolochia species used in traditional Chinese medicine, but ethnopharmacological studies indicate that other members of the genus are frequently used medicinally [3]. The aim of our research is to assess the

toxicological risk associated with the use of different *Aristolochia* species as herbal medicines. Metabolomic analysis allows us to take into account all compounds that might be responsible for the nephrotoxic effect. LC-DAD-MS analysis was carried out on *A. manshuriensis* Kom., *A. kankauensis* Sasaki, *A. clematitis* L., *A. elegans* Mast., *A. baetica* L., *A. debilis* Siebold & Zucc. and related species and AA I, AA II, AL I and AA C were quantified. *A. kankauensis* contained the highest levels of AA I and AA II, whereas *A. manshuriensis* contained the largest variety of different AA analogues (AA I, AA II, AA C, AA D, AA C-β-D-glucoside and AA D-β-D-glucoside). The results show that the content of aristolochic acid analogues varies greatly between different parts of the plant, with highest amounts found in the flowers. Extraction of the plant material with aqueous ethanol results in high yields of AA I and AL I, whereas extraction with hot water only yields in small amounts of AA I and AL I, and can therefore be associated with lower toxicological risk. **Keywords:** *Aristolochia*, aristolactam, aristolochic acid, metabolomics **References:** 1. Nortier JL et al. (2000) N Engl J Med 342:1686 – 1692 2. Li J et al. (2010) Toxicol In Vitro 24:1092 – 1097 3. Heinrich M et al. (2009) J Ethnopharmacol 125:108 – 144

WSII 5 Synthesis, detection and quantification of the highly active AhR ligands tryptanthrin, indirubin and indolo[3,2-b]carbazol in Malassezia yeasts

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Malassezia yeasts are part of human skin microbiota and can become pathogenic under currently unclarified conditions. HPLC/UV combined with LC-MS/MS analysis of the extracts of several Malassezia species, revealed the production of compounds like indolo[3,2-b]carbazol (ICZ) [1], indirubin [2] and tryptanthrin[3], which are among the most active Aryl-hydrocarbon Receptor (AhR) inducers known and, interestingly, are preferentially biosynthesized by Malassezia furfur isolates from diseased skin. A previously reported by our group biomimetic synthesis, from indole-3-carboxaldehyde (I3A), the main metabolic product of tryptophan found in all Malassezia studied species, to indirubin and trypranthrin simultaneously [3], showed a common biosynthetic path for these two metabolites. This reaction is a one-step oxidation, using hydrogen peroxide and diphenyldiselenide as a catalyst. The synthesis of the above indole alkaloids allowed us to proceed not only to their quantification by HPLC analysis, but also to the further examination of this biomimetic reaction. Surprisingly, the formation of the same metabolites was achieved even when simple indole was used as starting material. This gave us the opportunity to synthesize a series of symmetric indirubin and tryptanthrin analogues, beginning from the appropriately substituted indoles. Although AhR is an orphan receptor, there are increasing data about its relation with skin homeostasis and skin nosology. Based on our previous work on the activation of AhR in HaCaT cells by Malassezia extracts [3] we could propose that the presence on the human skin of microorganisms able to constantly synthesize potent AhR ligands may play a crucial role in the development of skin diseases. **References:** 1. Gaitanis G et al. (2008) J Invest Dermatol 128:1620 – 1625. 2. Giakoumaki D et al. (2008) Planta Med 74: 1081. 3 Magiatis P et al. (2010) Planta Med 76:1293

WSII 6 A rapid method for detection of alliinase activity in *Allium*, especially in the subgenus *Melanocrommyum*

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In the genus *Allium* about 800 species are currently known belonging to several subgenera [1]. Wild *Allium* species can be found on the northern hemisphere with a main habitat in Central Asia. The enzyme alliinase occurs in *Allium* plants catalyzing the cleavage of cysteine sulphoxides leading to typical odourous compounds [2]. Whereas the alliinases of common *Allium* species like garlic and onion [3] are well analyzed, the properties of other alliinases occurring in further *Allium* species have not been examined yet. Especially the species of subgenus *Melanocrommyum* do not express an alliinase like garlic, as can be shown on SDS-PAGE. The separation of alliinase from other proteins is sometimes difficult because of similar protein properties due to their size and probably also due to glycosilation. Therefore, a new method for direct alliinase activity detection has been developed. A crude alliinase preparation can be separated on a basic-native polyacrylamide gel. This method results in functional enzymes, separated into different spots. These protein spots can be cut out of the gel and screened for their alliinase activity. The L-(+)-S-(3-pyrrolyl)cysteine sulphoxide used as indicator turns into a red dye after the enzymatic cleavage by alliinase (Figure 1) [4]. Although the alliinase is still incorporated inside the gel matrix, a positive reaction can be detected after a few minutes (Figure 2). This new test allows an easy and quick detection of alliinase-like enzymes. Furthermore, the amount of sample needed is very small, allowing tests out of a single bulb.

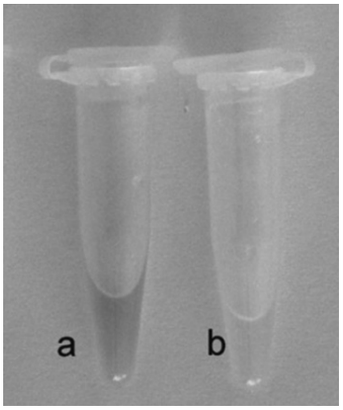

Figure 1: positive reaction b) negative reaction

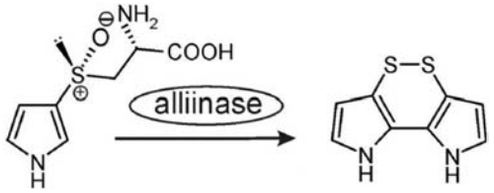

Figure 2: indicator reaction

Keywords: *Allium*, *Melanocrommyum*, alliinase, basic-native polyacrylamide gel **Acknowledgement:** *Institute of Pharmaceutical Chemistry, Regina Gerlach-Riehl* **References:** 1 Fritsch R M. et al. (2010) Phyton (Horn, Austria) 49(2):145 – 220 2 Stoll A, Seebeck E (1949) Helvet Chim Acta 32(1):197 – 205 3 Nock L P, Mazelis M (1987) Plant Physiol 85(4):1079 – 1083 4 Jedelská J et al. (2008) J Agric Food Chem 56(4):1465 – 1470

WSII 7 Electrophoretic mobility in agarose gel as an isolating technique and correlation with synergestic cytotoxicity enhancement

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In previously published work, we reported that Saponinum album dramatically improves the inhibition of tumor growth by targeted toxins in mice in a synergistic way. Herein, we report a simplified slab gel based electrophoretic isolation technique to determine the highly effective fraction of Saponinum album with a relative electrophoretic mobility (Rf) of 0.44 from the mixture. In total, four different fractions were separated at a preparative scale, and evaluated by ESI-MS, HPLC and thin layer chromatographic analysis. Electrophoretic mobility and electrochemical properties of the different fractions of saponins from Saponinum album were set into relation to their ability to enhance the cytotoxicity of epidermal growth factor (EGF)-based targeted toxins. We here treated HER-14 cells, which are NIH-3T3 Swiss mouse embryo cells transfected with the human EGF receptor. Untransfected NIH-3T3 cells

served as control. The major bulk of Saponinum album (72.3%) (Rf 0.62) migrated to the farthest and was found to be significantly ineffective ($p < 0.05$) in enhancing the cytotoxicity of the targeted toxin, while the second fraction (Rf 0.45) showed an enhancement of 9800-fold. The third (EM 0.30 μ) had an enhancement factor of 3200, the fourth (Rf 0.08) was again significantly ineffective ($p < 0.05$) in exhibiting any enhancement of cytotoxicity. This is the first report for the use of slab gel electrophoresis as a convenient isolation technique for saponins. **Acknowledgement:** *Authors would like to acknowledge Alexander von Humboldt foundation for postdoctoral fellowship.* **References:** [1] Heisler, I., Sutherland, M., Bachran, C., Hebestreit, P., Schnitger, A., Melzig, M. F., Fuchs, H., (2005) J Control Release 106: 123 – 137. [2] Bachran, D., Schneider, S., Bachran, C., Urban, R., Weng, A., Melzig, M. F., Hoffmann, C., Kaufmann, A. M., Fuchs, H., (2010) Int J Cancer 127: 1453 – 1461.

WORKSHOP III: Permanent Committee on Regulatory Affairs of Herbal Medicinal Products
Chairs: A. Vlietinck, S. Alban

WSIII 1 Quality of herbal medicinal products and food supplements – EU regulation and practical experience

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Herbs and herbal preparations have been playing an important role for both dietary and medicinal use from paleolithic hunter-gatherers to 21st century urban culture. People have always been aware that the variability of the properties of herbal materials depending, e.g., on the exact plant part, vegetal state, weather conditions, harvesting time and – essentially – the mode of preparation by, e.g., drying, peeling, cooking (or not), fermentation, treatment with inorganic substances, extraction etc., are not less important for the tolerability, digestibility or – in case of medicinal use – success of treatment than the choice of the plant itself. Over the past decades scientific findings have provided a rationale for what our ancestors had established on an empiric basis. We understand why, based on the absence/presence and dose of certain secondary compounds some plants/-preparations are beneficial or harmful. Since both beneficial and potentially harmful properties of herbal ingredients in food supplements are being increasingly assigned to specific secondary compounds it is evident that certain standards are necessary in order to provide for adequate safety and effectiveness of such products. While legal limits have been established in the EU for a large spectrum of possible contaminants in food and thus food supplements including, e.g., pesticides, heavy metals or mycotoxins, no common standards exist for the overall quality assessment of herbal raw materials/preparations for food supplements. While rules established for the quality control of herbal medicinal products and their respective herbal raw materials/preparations are not transferable for various reasons, they provide orientation regarding practicable technical standards and methodology. A comparison will be given of the EU regulatory quality standards in both areas in the light of practical experience.

WSIII 2 Regulatory Options for (Traditional) Herbal Medicinal Products

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All over the world plants have been selected by people because of their healing properties. In Europe, first manuscripts describing medicinal plants were written more than two thousand years ago. Starting from this traditional use, which was often based on ready-to-use receipts, there was a development towards usage of finished herbal medicinal products in central Europe during the 20th century. In parallel with an increasing scientific knowledge on medicinal plants – which included also the identification of highly active purified compounds of plant origin – a common regulatory environment for all medicinal products has been established in Europe. The basic regulatory approach is to control the access to the market and to assess quality, safety and efficacy of medicinal products in order to improve public health and to facilitate supply with medicinal products. There is a clear European regulatory framework for medicinal products (directive 2001/83/EC as amended) providing basic definitions and offering two options to bring products into the market: i) registration of traditional herbal medicinal products and ii) marketing authorisation of herbal medicinal products. Beside applications at the national level there are also options for European procedures. A harmonised scientific evaluation in this field is resulting from the work of the Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA). The major tasks are development of Community Monographs and List Entries on herbal substances as well as relevant guidance. These activities will facilitate handling of procedures and contribute to the harmonisation of the market.

WSIII 3 Traditional Herbal Drugs and Botanical Dietary Supplements: The Italian Experience

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Multicomponent phytotherapic medicines are widely used in the European clinical practice and their registration is nowadays regulated by two distinct registration schemes, based on "well established use" and "traditional use for herbal medicines", now both codified in Dir. 2001/83/EC. In this scenario, also in Italy it is today crucial that the preparation of botanical "Active Pharmaceutical Ingredients" (API) has to fulfil both GAP and GMP guidelines for what harvesting and cultivation of plants and industrial production is respectively concerned. The combination of these two guidelines is essential in order to guarantee quality and reproducibility of the API and also it allows to avoid the following risks: use of inappropriate classified material, pesticides or other harmful agent contamination, API degradation. Botanical derivatives (mostly multicomponent products such as extracts) are widely used in Europe also as ingredients for non-pharmaceutical products which are sold as food supplements in relation to the different national legislations. In Italy, the Ministry of Health has recently (March, 2011) initiated a procedure to transpose into law (as Annex 1 of a draft decree which regulates the use of dietary supplements different from vitamins and minerals) a list of admitted plants which can be utilized for the preparation of food supplements. The quality criteria for the preparation of these products are those requested by the European regulations on food derivatives. Nevertheless some Italian companies, such as Indena are strictly committed to keep the challenge for quality criteria of botanicals used for food supplements as close as possible to "phytotherapic medicines".

WSIII 4 Botanical Food Supplements – Regulatory Situation in the EU

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Botanicals are used as components of both food supplements and medicinal products. The European legal framework explicitly allows both products to exist in parallel. Botanical food supplements need to be in conformity with the full food legislation, including safety provisions, manufacturing requirements and labeling. In addition, Member States have national rules to assure their safe use. Relating to the borderline, the European Court of Justice has establish rules to judge if a product should be considered as medicinal or not. It has ruled that such a decision must consider individual products, taking into consideration all of the product's characteristics. Furthermore, the definition of medicinal product by function should be interpreted restrictively and not cover substances that do not strictly modify the way in which it functions, i.e. that do not have a therapeutic effect. In recent years the EC has put into place two new laws that may help the EU to progress towards harmonisation. It concerns the legislation on health claims under which a claim relating to the effect of a botanical on the body needs premarketing approval and the legislation on addition of nutrients to foods, under which a procedure has been created to deal with emerging safety issues. The claims legislation does not accept traditional use as a valid parameter for the validation of a health claim, while traditional herbal medicinal law product does. This has now created a new situation that the European Commission is reflecting upon.

WORKSHOP IV: Traditional Chinese Medicine Workshop
Chairs: D. Guo, R. Bauer, G. Franz

WSIV 1 Chinese herbal medicine in Europe: Regulatory situation, problems and perspectives

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Herbal medicinal products are regulated in Europe under the drug law. If only health claims are stated, some may be considered as dietary supplements. For drug approval, efficacy, safety and quality have to be demonstrated. A special European regulation exists for traditional herbal medicinal products (Directive 2004/24/EC), which does not require clinical studies, but only 30 years of traditional use, out of them 15 years in Europe [1]. A committee for Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) currently prepares a list of traditional and well-established herbal drugs/preparations/combinations which can be used for drug approval in the European Union. Also Community monographs on herbal drugs or herbal drug preparations that may be used for full marketing authorisations of well-established herbal medicinal products or simplified registrations are elaborated by this committee. However, Chinese herbs have not been considered so far. For all types of products, whether new, well established or traditional, quality needs to be demonstrated individually. Detailed instructions for the documentation of quality are specified in the Guideline on Good Agricultural and Collection Practice (GACP) for starting materials of herbal origin (EMEA/HMPC/246816/2005; 20.2.2006), the Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2819/00 Rev. 1; 30.3.2006), the Guideline on Specifications: Test procedures and Acceptance Criteria for Herbal Drugs, Herbal Drug Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2820/00 Rev. 1; 30.3.2006), and the Guideline on Stability Testing of Existing Active Substances and Related Finished Products' (CPMP/QWP/122/02). Identity, purity, and content are obligatory to be tested. Therefore corresponding methods need to be developed, and monographs are elaborated for the European Pharmacopoeia [2]. Fingerprint analysis is the most appropriate approach to consider the complex composition of herbal products. Therefore, chromatographic techniques, like TLC, HPLC and GC are the methods of choice for quality control [3]. **Keywords:** Chinese herbal medicine, regulation, Europe, quality, efficacy, safety **References:** [1] Silano M et al. (2004) Fitoterapia 75: 107 – 116 [2] Bauer R, Franz G (2010) Planta Med.76(17):2004 – 11 [3] Wagner H, Bauer R, Xiao PG, Chen JM (Eds.) (1996 – 2010).Chinese Drug Monographs and Analysis. Verlag für Ganzheitliche Medizin Dr. Erich Wühr GmbH, Kötzting

WSIV 2 Globalisation of TCM Herbal Drugs: Quality Monographs for the European Pharmacopoeia

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TCM Herbal Drugs are increasingly used in many countries of the EU. Consequently, there is a great need for herbal drugs with consistent quality as a basis for the required safety of the user. All aspects relevant for the quality parameters have to be achieved in an adequate manner, requiring a broad range of analytical methods to be applied for new herbal drug monographs in the European Pharmacopoeia (EP). The actual concern about safety and efficacy of the large number of TCM-Herbal Drugs is based upon reports about problems such as botanical identity, purity and current falsifications. In order to respond europewide to these problems, new monographs in the framework of the EP have to be established, based upon existing data and monographs of the actual Chinese Pharmacopoeia 2005 Engl. Edition and further the new and updated version of the ChP 2010. The appropriate selection of TCM-herbal drugs to be elaborated should at least in part reflect the actual situation and impact of TCM in Europe. The inclusion criteria of TCM Monographs were based upon a priority setting of the EP reflecting the volume of use, problems of toxicity, known problems of quality and risk of substitution. Priority for the elaboration of these new monographs was given for precise definition, identification, test methods and assay procedures, in order to guarantee a reproducible quality. The actual status of this working programme and the current problems related to different issues of the new TCM monographs for the EP will be reported and discussed.

WSIV 3 Current status and future perspective of traditional Chinese medicine in China

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Traditional Chinese medicine (TCM) has over 3000 years of history to treat diseases in China and has played an essential role in the Chinese healthy system. More and more western scientists are now interested in TCM research and hope to learn about the basics of TCM in China. Hence, the following aspects related to TCM will be briefly overviewed in the lecture, including TCM theory, TCM resources, related research institutions and education systems, Chinese Pharmacopoeia on TCM volume, TCM new drug registration regulations by SFDA, TCM-based drug discovery, government funding sectors, TCM hospitals, recent research progress made on TCM research, which may help the audience to understand the basic current situation of TCM in China. In addition, the current research on the quality standard construction of traditional Chinese medicines will also be introduced with some concrete examples. The future directions on TCM modernization will also be insighted as a personal viewpoint. **Acknowledgements:** *Author like to thank Ms. Gangqiang SU (Director General of Department of Scientific Research, State Administration of TCM) for her providing some basic data for the lecture.*

WSIV 4 Fishing and Knockout (FAK) Strategy for Quality Control of Traditional Chinese Medicines (TCMs)

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Herbs and preparations have been the basis of traditional Chinese medicines (TCMs) for thousands of years, and continue to be considered valuable composition in health care system. One of the biggest challenges of herbal research is the complexity of its chemical constituents and their analysis. Thus, a wide range of analytical methods need to be used to fully characterize these 'magical components' and used for quality control (QC) purpose. The effects of TCMs are, of course, induced by their chemical constituents. For effective quality control and efficacy evaluation, we should know: What exist in TCMs? Which are biologically active? And how the components induce integrative functions? During the last decade, our research team used multidisciplinary theory and technology to develop novel methods for multi-component and multi-target evaluation of TCMs. First, a diagnostic ion filtering strategy was proposed for rapid screening and identification of non-target compounds in complex TCM samples by UPLC-Q-TOF/MS. Next, a novel chemical markers' fishing and knockout (FAK) strategy was proposed for screening bioactive compounds and studying component-component interactions. A two-dimensional turbulent flow chromatography on-line coupled with LC-MS strategy was introduced to screen out the bioactive compounds from TCMs binding target proteins. Then, to prove whether the obtained/assumed constituents are active compounds for the extract, a chemical markers' knockout strategy was recommended to prepare an extract in which the assumed compound(s) is removed, called a "knockout" extract. After bioactivity comparison of samples with various components-knockouts or target compounds collections, the fingerprinting-efficacy relationship of TCMs, component-component interactions could be predicted by statistic analysis. With collaborative expertise in chemistry, chemometrics, biology, and bioinformatics, we believe that there are good prospects for obtaining new insights into drug discovery and clinical utility by the continued study of TCMs. **Acknowledgements:** *This work was supported in part by the National Key Technologies R&D Program of China (No. 2008BAI51B01), Program for Changjiang Scholars and Innovative Research Teams in Universities (No. IRT0868).*

WSIV 5 Chemical Mechanism Research during Chinese Medicine Processing

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Chinese medicine processing is a traditional Chinese pharmaceutical technology, and it is also the main characteristic property that distinguish traditional Chinese medicine and natural medicine. There are complex chemical changes during the process of Chinese medicine processing, and these chemical constituents maybe the basis of clinical efficacy changes. To clarify the changes of the chemical constituents in Chinese medicine is the main purpose of the mechanism research of Chinese medicine processing. In recent years, many research institutions at home and abroad have done deeply research in chemical mechanism during the process of Chinese medicine processing, and initially clarify the chemaical reactions and chemical mechanisms during the process of Chinese medicine processing. The main chemical reactions occurred in the Chinese medicine processing are hydrolysis reaction, oxidation reaction, replacement reaction, isomerization reaction, decomposition reaction and so on. This paper reviewed the main achievements in the chemical mechanism research during the process of Chinese medicine processing, and prospected the research directions and future in the research of Chinese medicine processing. **Key words:** Chinese medicine processing; process reaction; chemical constituents; mechanism

WORKSHOP V: Biological and Pharmacological Activities of Natural Products
Chair: V. Butterweck

WSV 1 Identification of PPARγ agonists from natural sources

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The peroxisome proliferator-activated receptors (PPARs) have been in focus for more than a decade for the development of drugs to treat and/or prevent diseases associated with the metabolic syndrome (MS). The PPARs (α, δ, γ) are nuclear receptors (NRs) highly involved in lipid and energy metabolism and hence, targets for treating MS-related disorders. In particular PPARγ that is a key regulator of insulin sensitivity is the target of many conventional drugs, although this may result in severe side effects. However, such side effects could be avoided using selective modulators or partial agonists instead [1,2]. Natural products have proven to be a valuable source of PPAR activators [3] of which some have demonstrated interesting partial agonist activities *in vivo* [4]. In our studies on identification of PPAR modulators from natural sources we use different approaches such as bioassay-guided fractionations, structure-activity relationships, and pharmacophore-modeling [5,6,7,8]. This has led to the identification of new potential PPARγ agonists from purple coneflower (*Echinacea purpurea*) (L.) Moench [5], a plant not traditionally used to treat MS. For traditional anti-diabetic remedies such as sage (*Salvia officinalis* L.) and elderflowers (*Sambucus nigra* L.) PPAR activating properties have been identified suggesting a mechanism of action involving these NRs [6,7]. This was also the case when a pharmacophore-driven approach led to the identification of novel PPARγ partial agonists from mastic gum (*Pistacia lentiscus* L.). Hence, there is a large potential for finding modulators of these versatile NRs amongst natural products and hence important information about their mechanisms of action and their use as drug candidates. **Keywords:** Metabolic syndrome, PPAR, natural products, medicinal plants, bioassay-guided fractionation **References:** 1. Auwerx J et al. (2003) Nuclear Receptor Signalling 1: e006. 2. Berger JP et al. (2005) Trends Pharmacol Sci 26: 244 – 251. 3. Huang TH et al. (2009) Pharmacol Res 60: 195 – 206. 4. Christensen KB et al. (2009) Phytother Res 23: 1316 – 1325. 5. Christensen KB et al. (2009) J Nat Prod 72: 933 – 937. 6. Christensen KB et al. (2009) Phytother Res 24:S129-S132. 7. Christensen KB.et al. (2010) J Ethnopharmacol 132:127 – 133. 8. Petersen RK.et al. (2011) J Comput Aided Mol Des 25: 107 – 116.

WSV 2 Metabolic syndrome, natural compounds and nuclear receptors – are there new targets?

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Roughly 10% of genes of the human genome, among them all 48 human nuclear receptors (NR), are druggable, meaning that the function of their gene products can be modulated by small molecules. The classes NR1I/NR1H comprise molecules which initially have been described as orphan NR. They are now recognized as lipid and drug sensing molecules. Unlike PPARs belonging to the NR1C subfamily which are activated by triglyceride derivatives Constitutive Androstane Receptor Receptor (CAR), Pregnane-X-Receptor (PXR), Farnesoid Receptor (FXR) and Liver-X-Receptor are activated by cholesterol derivatives, bile acids, steroids and bilirubin. A common functional feature of these NRs is induction of a wide range of detoxifying enzymes. NRs are intimately involved in regulation of lipid metabolism and/or they contribute to liver protection. LXRs activated by oxysterols play the most prominent role in regulation of metabolic disease. LXRs have been described as regulators of lipogenesis, of cholesterol and glucose homeostasis and of anti-inflammatory processes, all representing specific features of the metabolic syndrome. FXR upon activation by chenodesoxycholic acid participates in regulation of lipid, lipoprotein and glucose metabolism as well as in hepatic regeneration and hepatoprotection. PXR is activated by pregnane derivatives and together with CAR and FXR guides cholesterol and bile acid homeostasis. Because of the functional link of these NRs to disease they represent valuable drug targets, inlcuding for plant derived natural compounds. The paper presented here will summarize data on plant secondary metabolites modulating the function of these lipid sensos in relation to specific aspects of the metabolic syndrome. **Keywords:** metabolic syndrome, lipid sensing, nuclear receptor, FXR, LXR, PXR, CAR

WSV 3 Plant derived therapeutics for the treatment of Metabolic Syndrome

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Type 2 diabetes is a progressive disorder whose pathophysiology consists of pancreatic dysfunction, hepatic glucose overproduction and insulin resistance in peripheral tissues such as adipose tissue and muscle. Currently, clinical strategies to achieve improved metabolic control endorse the use of a combination of agents from multiple pharmacologic classes and which target specific pathophysiologic defects. Given the role of insulin resistance in the pathophysiology, the improvement of insulin sensitivity for treatment of the metabolic syndrome or type 2 diabetes remains as a primary clinical strategy. Recent data, however, have questioned the safety of the current pharmacologic agents used to enhance insulin sensitivity. As such, alternative strategies, e.g., nutritional supplementation with over-the-counter agents, are extensively practiced by a large number of patients and are frequently done so without the knowledge of the provider. Based on historical human use, there has been great interest in plant extracts (botanicals) as a source for nutritional supplements intended as adjunctive therapies for human diseases. Specifically, isolated compounds identified from plant sources, i.e. phytochemicals or bioactives, have served as a source for therapeutic agents for many diseases, including malignancy, infectious diseases and diabetes. Interestingly, the development of one of the most commonly used anti-hyperglycemic agents in the world today, i.e. metformin, can be traced back to a botanical source. However, the concern with most nutritional supplements, including those considered "natural" (e.g. botanicals) by the consumer, is the paucity of data in humans in regard to efficacy to improve metabolic abnormalities. Thus, there remains considerable controversy regarding the use of botanical supplements for human health. Despite the stated concerns for botanical supplements, there are a number of botanicals that have shown considerable promise for human use but they have to be carefully validated in controlled clinical trials.

WSV 4 Genetic & Nutrient Determinants of the Metabolic Syndrome (Nutrigenomics)

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With the toolbox of the "omics", nutritional science has become a new adventure in understanding the processes that make up mammalian metabolism in health and disease. Essentially unlimited when taken into studies in human cells in culture or in vivo studies in animal models, omics applications in human trials are limited by the availability of biosamples restricted to body fluids, blood cells or biopsy materials. Nutrigenomics research in the last years has delivered some important insights into mechanisms by which dietary constituents affect metabolism and health risks. I shall be presenting findings from human studies in which transcript-, proteome- and metabolite-profiling techniques have been applied – mainly in peripheral mononuclear cells and plasma/urine as biosamples. It also will include some studies on the effects of plant secondary components. Despite enormous efforts, genome-wide association studies as a top-down approach have so far only shown very weak effects of genetic heterogeneity in individual genes and their association with disease initiation or progression. Amongst the reasons that science crossly failed in identifying causal molecular links between diet and disease is that usually only a "snapshot" is taken when applying any of the profiling techniques. But, it is an intrinsic feature of metabolism that it is highly dynamic in time (acute and chronic adaptation) and space (within cell compartments or i. e. the interorgan metabolism). In the future, we therefore should first define the variability in the phenotypic response of mammals as a function of time and space and redefine the homeostatic control as a transient equilibrium. To obtain robust phenotypic alterations it therefore may be advised to challenge the biological system to drive it in a critical state. Such a "critical state" may in terms of nutrition be defined as a severe state of starvation or diets providing extreme nutrient compositions. I shall demonstrate findings from a metabolomics application in humans with such defined challenges. Taken together, nutrigenomics has extended our knowledge on how diets or individual ingredients affect human metabolism on the background of a given genetic make-up, but it is far from delivering predictive parameters for personalized health and/or disease prevention.
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WORKSHOP VI: Quality/Agriculture joint Workshop
Chairs: C. Franz, C. Erdelmeier

WSVI 1 Environmental Contaminants – Heavy Metals Origin – Analytical Methods – Points to Consider

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Environmental relevant heavy metals are lead, cadmium, mercury, arsenic, copper, nickel, zinc and iron. Since Tschernobyl and actually Fukushima caesium 137 and even plutonium 239 might be relevant parameters for some proveniences. The input of these elements into herbal materials is diverse. Some of them like nickel, arsenic or lead are from direct geogenic sources and therefore not avoidable at all. Other inputs are made via air by traffic, industry and combustors also. Even the agricultural industry itself takes part in that scenario by using heavy metal loaded pesticides like some dithiocarbamates (zinc in zineb and others) or anorganic mineral fertilizers (accumulation of cadmium in the soil). Some plants are so called hyperaccumulators for heavy metals at all. Even some of the herbal drugs used for phytopharmacy are found in that group (*e.g. Salix and Populus*). In that group results for cadmium found often exceed the specified limits. Methods and limits are described in the European Pharmacopoeia in the chapters 2.4.27, 2.4.31 and in the monograph "Herbal Drugs" (monograph number 1433). The official testing methods for heavy metals are atomic absorption spectrometry (AAS) or inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS). All methods need to be validated on the herbal materials at least on distinct plant organs like roots or fruits etc. Points to consider in the management of GACP to avoid OOS results for heavy metals are control of the soil, specified pest management, specified selection of plants and varieties. **Keywords:** Heavy Metals, absorption spectrometry (AAS), or inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS)

WSVI 2 The Application of Pesticides in the Production of Medicinal Plants in China

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Traditional Chinese Medicine (TCM) is getting more and more attention all over the world, due to its systematic approach and clinical effectiveness [1]. The quality of TCM determines the safety and efficacy of Chinese-made TCM products. However, the improper use of pesticides in the medicinal plant production has seriously affected the quality of TCM. In the early 1980 s, Chinese scientists had already recognized this problem and put in an effort to study pesticide residue in TCM. During that time, the main focus was mainly involved in the half life of organochlorine and those pesticides that easily caused cumulative toxicity (e. g., hexachlorocyclohexane, DDT, quintozene, drinox) in Chinese herbal materials, herbal preparations, and Chinese patent products. Accordingly, "Pharmacopoeia of People's Republic of China" (CP, the edition of 2000, the first section) set up the assay methods and residual limit of the organochlorine pesticide [2]. During the "9th Five-Year project", the government organized Chinese medicine technology research key project "Standardization of the Quality of Chinese Materia Medica ", which including a systematic way of Research and determination of 71 kinds of Chinese herbal medicines. This project not only improved the research level on the pesticides resides in TCM, but also made us have basic understanding the polluted situations of organic pesticides in the commonly used TCM, providing a substantial base for Good Agriculture Practice (GAP) on Chinese herbal materials in China. During the "10th Five-Year project ", the project, "Researches on the detection and limited standards of 50 kinds of Chinese medicine ", was classified as a major task by the Ministry of National Science and Technology of China. On the basis of this project, we have completed these items, such as: "Detection methods of the pesticide residue of pyrethroid in TCM "; "Detection methods of the pesticide residues of organic phosphorus in TCM". And those above have been officially recorded in 2005 version of Chinese Pharmacopoeia. In recent years, the detection technology of pesticide residues in traditional Chinese herbal medicine has developed rapidly. Application of technologies such as GC, LC, GC-MS, LC-MS, CE-MS, and multiple-stage mass spectrometry techniques, greatly increase the qualitative capability, the detection sensitivity, detection limit and detection coverage. At present, some new analysis methods and techniques are making great progress, especially in the analysis of multi-pesticide residues for many types of pesticides, the analysis of multi-pesticide residues for the same type of pesticides, the analysis in the trial sample for new single pesticides, and rapid analysis and other aspects. According to GAP for TCM herb requirements, pesticide residues should comply with the Green Standards of Medicinal Plants and Preparations for Foreign Trade and Economy of Ministry of Commerce of the P. R. China [3]. At present, China Pharmacopoeia (Edition 2010) supplemented the respective analytical methods of 9 organochlorines (OCPs), 3 pyrethroids and 12 organophosphor- ous pesticides (OPPs), which aim to standardize the cultivation of TCM herbs, improve their quality and bring TCM in line with the international practice. Now the Pharmacopoeia stipulated the MRLs (maximum residue level) of 9 OCPs including BHC, DDT and pentachloronitrobenxene (PCNB) for medicinal materials, among which only two were involved, Radix et Rhizoma Glycyrrhizae and Radix Astragali, but no MRLs for pyrethroids and OPPs have been established for all Chinese medicinal materials [1, 4]. Therefore, implementation of GAP for medicinal plant is an important measure to ensure the quality of TCM. **Keywords:** Traditional Chinese Medicine (TCM); Pesticide; Medicinal Plant Production; China
References: 1. Zhang BG, Peng Y, Zhang Z, Liu HT, Qi YD, Liu S, Xiao PG. (2010) Planta Med; 76:1948 – 1955 2. Yang MH, Wang LN. (2008) World Sci Technol; 10:107 – 112 3. The Ministry of Commerce of the People's Republic of China. (2004) Green standards of medicinal plants and preparations for foreign trade and economy of Ministry of Commerce of the P. R. China. WM/T 2 – 2004. 4. Guo Q, Lv X, Tan L, Yu BY. (2009) Chin J Nat Med; 7:210 – 216

WSVI 3 Legal requirements for the control of contaminants in herbal medicinal products

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Herbal medicinal products require a pre-marketing approval like all other medicinal products and have to prove their quality, safety and efficacy in order to guarantee optimum consumer and patient protection according to the European legislation. Taking into a consideration the natural origin and potential environmental influences, special emphasis is put on tests for contaminants such as heavy metals, pesticide residues, mycotoxins and microorganisms. For heavy metals, the European Pharmacopeia has set limits for lead, cadmium and mercury within the general monograph on herbal drugs. Similar rules are being developed for extracts. In this respect, a German industry's working group has collected a large amount of data on heavy metals occurring in herbal drugs. The respective data base has been evaluated and published [1]. For pesticide residues in plant material, the respective European Pharmacopoeia monograph sets limits for 70 substances. For further substances reference is made to Regulation (EC) 396/2005 on pesticide residues in food. For aflatoxins, specific limits exist for medicinal products which are comparable to the food area. With regard to microbiological purity of herbal medicinal products, the European Pharmacopoeia describes requirements for three different categories of these products as well as determination methods. Raw materials of herbal origin which are intended for use in food supplements have to fulfill the respective European legal requirements for the food area. Besides the above mentioned Regulation (EC) 396/2005 on pesticide residues, limits for further contaminants are described in Regulation (EC) 1881/2006, e.g. for mycotoxins or heavy metals. The Unites States Pharmacopoeia (USP) is currently developing specific rules on heavy metals in dietary supplements of botanical origin. **References:** [1]Gasser U, Klier B, Kühn AV, Steinhoff B (2009) Current findings on the heavy metal content in herbal drugs. Pharmeuropa Scientific Notes 2009 – 1:37 – 49.

WSVI 4 Pesticide testing according to the European Pharmacopoeia (Ph.Eur.) – legal requirements and practical approach

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In 1996 the monograph "Pesticide residues (2.8.13.)" in herbal drugs has been implemented to the European Pharmacopoeia (Ph.Eur.). There were "definition", "limits", "sampling", "qualitative and quantitative analysis of pesticide residues" and a "Test for pesticides" described in the monograph. Refering to the publication in PHARMEUROPA Vol.17 No.1, January 2005 (Pesticide Residues in medicinal Drugs and Preparations) the Ph.Eur. Pesticide Expert Group has been mandated to update the monograph Ph.Eur 2.8.13. The revised monograph (Ph.Eur. 6.2) has been published in 2008. The revision includes following changes: The new harmonised European Pesticide Regulation (EU 396/2005) replaced old European Directives (EC 76/895 and EC 90/642); the list of pesticides has been extended from 34 to 115 substances frequently observed in herbal drugs; limits has been set in view of toxicology and according to a 90 per cent percentile approach; the formula for the calculation of pesticide limits in extracts and other herbal drug preparations has been modified; more details for method validation procedure has been given (cross reference to document SANCO/10232/2006); the method for determination of pesticides ("Test for pesticides") has been deleted. With the updated monograph a framework for quality control of pesticide analysis in herbal drugs has been given. For the frequently found 115 substances pesticides in herbal drugs and herbal drug preparation limits could be found or calculated easily. The allocation from product to limit of all other (not listed) pesticides according to the new harmonised European Pesticide Regulation (EU 396/2005) remains difficult. The scope of testing depends on the methods of analyses used. Using additionally new analytical technics based on LC-MS/MS detection more than 500 substances could be detected analysing pesticide residues in herbal drugs.

Short Lectures

SL1 Phenylethanoid glycosides: Naturally occurring apoptosis inducers

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Natural products have long been regarded as excellent sources for drug discovery given their structure diversity and wide variety of biological activities. Phenylethanoid glycosides are naturally occurring compounds of plant origin and are structurally characterized with a hydroxyphenylethyl moiety to which a glucopyranose is linked through a glycosidic bound and esterified by a cinnamic acid moiety. There can be one to four sugars in their composition and cinnamic acid esterification generally occurs on a glucose directly bound to phenylethanol moiety [1,2]. To date several hundred compounds of this type have been isolated from medicinal plants and further pharmacological studies *in vitro* or *in vivo* have shown that these compounds possess a broad array of biological activities including antibacterial, antioxidant, antitumor, antiviral, anti-inflammatory, neuro-protective, hepatoprotective, immunomodulatory, and enzyme inhibitory actions[1,2]. In this study, we have investigated in vitro anticancer (cytotoxic) activity and structure-activity relationships of 10 different phenylethanoid glycosides against human and murine cancer cell lines, Hep-2 (human epidermoid carcinoma), RD (human rhabdomyosarcoma), and L-20B (transgenic murine L-cells), using MTT method [3,4]. Acteoside, forsythoside B, samioside and teucrioside were exhibited significant cytotoxic activity against tested cancer cell lines in the concentration range of 8 – 50 µg/mL. To determine the selectivity of cytotoxicity, VERO (African green monkey kidney) cell line was used for the comparison and no cytotoxicity was determined. In addition, apoptotic cell death was observed in the histological analysis of tested cancer cell lines. **Keywords:** Phenylethanoids, cancer cells, cytotoxicity, structure activity relationship **Acknowledgement:** *Activity studies were supported by Hacettepe University Research Foundation (Project No: 0302301010).* **References:** 1. Funes L, et al. (2010) Chemistry and Physics of Lipids 163: 190 – 199. 2. Korkina LG (2007) Cellular and Molecular Biology 53(1): 15 – 25. 3.Saracoglu I, et al. (1995) Biological and Pharmaceutical Bulletin 18(10): 1396 – 1400. 4.Saracoglu I, et al. (1997) Fitoterapia 68(5): 434 – 438.

SL2 With a different approach it is possible to develop commercially useful antimicrobial products from plants

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Many compounds from plants play a major role as pharmaceutical products in several therapeutic applications in human and animal health. Despite thousands of publications in the field there has been a remarkable absence of therapeutic products from plants to combat microbial infections. Data will be presented to show that the following factors play a role in this absence: the extractant used, the bioassay used, the unexpectedly low activity of isolated antimicrobial compounds, the presence of synergism in crude extracts and focussing on plants traditionally used. The antibacterial activity of acetone leaf extracts of more than 700 South African tree species on four important nosocomial pathogens *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* were determined. A high proportion of these extracts had antibacterial activities with MICs lower than 0.08 mg/ml. Unfortunately in many cases the extracts are toxic to mammalian cells. Many of these species have a good potential to be used as crude extracts to treat topical infections in humans. Examples will be demonstrated where these extracts have been as effective as commercial products in controlling microbial infections in animals and plants. The limitations associated with commercializing plant extracts as antimicrobials such as quality control, availability of material and potentising the extracts to yield patentable products will also be discussed. It appears that if the focus is on using extracts rather than isolated pure compounds there is a considerable opportunity to use the compounds present in plants to combat microbial infections. **Keywords:** antibacterial activity, synergism, extractant, MIC, plant extract, nosocomial bacteria, commercial product **Acknowledgement:** *The National Research Foundation provided financial support*

SL3 A one-tube assay for four *Hypericum* species – PlantID

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The benefits of DNA-based identification methods for medicinal plant products have been shown – negligible amounts of starting material, high resolving power, increased taxonomic specificity, and fast results (1). However, the simultaneous detection of multiple species in one sample has not until now been possible. We report the design of PlantID for St John's Wort (SJW) (*Hypericum perforatum* L.); a technique capable of detecting both the target species (SJW) and a number of likely adulterants in one sample using a multiplex PCR approach coupled to high resolution DNA fragment analysis. The method is based on the creation of fluorescently labelled amplicons of different lengths which can be resolved via capillary electrophoresis. Each amplicon confirms the presence one of four *Hypericum* species; *H. androsaemum* L., *H. athoum*-Boiss. & Orph., *H. ascyron* Siebold ex Blume and *H. perforatum* L. These amplicons are produced in a multiplex PCR, with all four reactions occurring simultaneously. The target species for design used in this study represent a worst case scenario, with only a few base differences between the ITS regions for each target. It is likely that a selection of target medicinal plants would not be as closely related, and would therefore have significantly more sequence differences. This would dramatically increase the number of species which could be detected in one assay. This technique has the power to both confirm the presence of expected plant material and detect adulterant material in one reaction. The method of design could be replicated for any other medicinal plant, and its problem adulterants. **Keywords:** Molecular Identification, *Hypericum perforatum*, PlantID **References:** 1 Howard C et al. (2009) Planta Med 75: 864 – 869.

SL4 Design and synthesis of natural product-based ligands with high affinity to the kappa-opioid receptor

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Psychoactive natural products play an important role in the discovery and development of new drugs for the treatment of central nervous system (CNS) disorders. Our studies are focusing on identification of plant metabolites responsible for CNS activity and designing new ligands with high affinity to CNS receptors. Salvinorin A, the most potent naturally occurring hallucinogen isolated from the plant *Salvia divinorum* Epling & Játiva (Lamiaceae), has received great attention since the kappa-opioid receptor (KOR) was identified as its principal molecular target. Previously, extensive efforts were made to understand how salvinorin A binds to and activates KOR [1 – 4]. Our goal was to design a series of ligands with high affinity to KOR to further explore the ligand-receptor interactions at the molecular level. Following the synthesis of 22-thiocyanatosalvinorin A [5], the first irreversible KOR ligand, we now report the synthesis and biological evaluation in vitro of new salvinorin A derivatives with Michael acceptor-type functional groups.

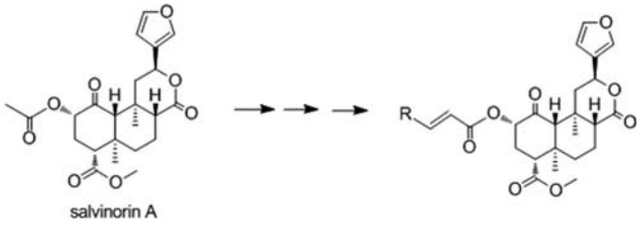

Figure 1: Synthesis of salvinorin A derivatives with Michael acceptor-type functional groups

Keywords: Salvia divinorum, salvinorin A, kappa-opioid receptor, synthesis of new ligands, affinity, ligand-receptor interactions **Acknowledgement:** *This work was financially supported by National Institutes of Health grant R01DA017204* **References:** 1: Roth BL et al. (2002) Proc Natl Acad Sci 99:11934 – 11939. 2. Yan F et al. (2005) Biochemistry 44:8643 – 8651. 3. Vortherms TA et al. (2007) J Biol Chem 282: 3146 – 3156. 4. Yan F et al. (2008) Biochemistry 47:1567 – 1578. 5. Yan F et al. (2009) Biochemistry 48:6898 – 6908.

SL5 Sesquiterpene Lactones and Pentamethoxylated Flavone from *Artemisia kulbadica* Boiss. & Buhse

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Artemisia is a genus of small herbs or shrubs found in Northern temperate regions. It belongs to the important family Compositae (Asteraceae), one of the most bulky vegetal groupings, which comprises about 1000 genera and over 20000 species. Within this family, *Artemisia* is included into the tribe Anthemideae and comprises itself over 500 species. The 500 species of *Artemisia* are mainly found in Asia, Europe and North America. They are mostly perennial herbs and dominating the vast steppe communities of Asia. Asia seems to show the greatest concentration of species with 150 accessions for China, 174 in the ex U.S.S.R, about 50 reported to occur in Japan and 35 species of the genus are found in Iran, of which two are endemic: *A. melanolepis* Boiss. and *A. kermanensis* Pold. *Artemisia* species, widespread in nature, are frequently utilized for the treatment of disease such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria and viruses and this prompted us to conduct a phytochemical investigation of *Artemisia kulbadica*. The aerial parts of *Artemisia kulbadica* afforded a germacranolide and guaianolide type sesquiterpene lactones together with a pentamethoxylated flavone. The structures were elucidated by spectropic methods, including 1D and 2D NMR analysis. **Keywords:** Artemisia kulbadica, Compositae, Sesquiterpene lactones, Guaianolide, Germacranolide, Flavone

SL6 *In silico* approaches to identify FXR-inducing constituents from *Ganoderma lucidum* - the Chinese mushroom of immortality

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The ligand-dependent transcription factor farnesoid X receptor (FXR), belonging to the nuclear receptor superfamily, plays a regulative role in glucose and lipid metabolism. Due to its revealed structural information FXR represents an attractive target for computer-aided drug design. Pharmacophore models based on publicly accessible FXR crystal structures were generated as *in silico* tools to identify novel bioactive compounds with the ability to control endogenous pathways in close relation to inflammatory diseases, like metabolic syndrome, dyslipidemia, atherosclerosis, and type 2 diabetes [1, 2]. Lanostane-type triterpenes, as typically found in the fruit body of the famous TCM fungus *Ganoderma lucidum* (Curtis) P. Karst (Ganodermataceae) were identified as putative FXR ligands by virtual screening of our in-house Chinese Herbal Medicine database [3]. To verify the in silico predictions, 25 constituents isolated from *G. lucidum* were tested on their FXR-inducing potential in a reporter gene assay at 10 μM. A dose-dependent activity could be confirmed for five lanostane triterpenes, i. e. ergosterol peroxide, lucidumol A, ganoderic acid TR, ganodermanontriol, and ganoderiol F, with EC_{50} values between 1 μM and 30 μM [4]. In depth structural insights were gained by molecular docking studies allowing a first structure

activity relationship. Furthermore the five active compounds were tested for general anti-inflammatory effects. At a test concentration of 5 μM they significantly inhibited the TNF or LPS induced expression of IL-8 and E-selectin in human endothelial cells [4]. The observed FXR induction indicates a possible involvement of this nuclear receptor in the mechanism of the inflammatory regulation by these compounds. **Keywords:** farnesoid X receptor, *Ganoderma lucidum*, lanostane triterpenes, molecular modeling, virtual screening, natural products **Acknowledgement:** *This work was supported by the National Research Network (NFN) – project "Drugs from Nature Targeting Inflammation" S 10703/S 10711/S 10713 granted by the Austrian Science Fund (FWF).* **References:** 1. Schuster D et al. (2011) submitted. 2. Lin H R et al. (2006) Bioorg Med Chem Lett 16: 4178. 3. Fakhrudin N et al. (2010) Mol Pharmacol 77: 559. 4. Grienke U et al. (2011) submitted.

SL7 Biological Activities of Andrographolide and Some Semi-synthesis Nonbitter Andrographolides

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Andrographis paniculata Nees, Acanthaceae, is known as the king of the bitter due to its main constituent, andrographolide. The activities of *A. paniculata* and andrographolide were antiviral, analgesics, antipyretics, antibacterials and anti-inflammatory [1]. Andrographolide is an *ent*-labdane containing an α-alkylidene-γ-butyrolactone moiety; two double bonds Δ8(17), Δ12(13); and three hydroxyls at C-3 (a secondary), C-19 (a primary), and C-14 (an allylic). The functional group/s responsible for bitterness of the compound is not yet confirmed, however, esterification of the free hydroxyls of the compound with short chain and long chain fatty acids resulted in diminishing of the bitterness as well as improving those claimed activities of *A. paniculata.* In our studies 14-acetylation increases the antibacterial against some Gram positive bacteria which resulted in the cell division of *B. subtilis* [2]. The 3,19-isopropylidenylandrographolide and 14-deoxy,3 – 19-dipalmitoylandrographolide gave the highest analgesic, antipyretic and antiinflammatory activities [3]. Whereas, only 3,19-isopropylidenylandrographolide gave absolute anti-replication of HSV-1 [4] and interfering 2 glycoproteins synthesis of the virus. **Keywords:** andrographolide, 14-acetylandrographolide, 14-deoxy-3, 19-dipalmitoylandrographolide, 3, 19-isopropylidenylandrographolide, antibacterial, anti-HSV-1, HSV-1 glycoproteins **Acknowledgement:** *We thanks the Thailand Research Fund for the grant of "Nonbitter Andrographolide".* **References:** 1. Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian Medicinal Plants. Council for Scientific and Industrial Research, New Delhi 2. Aromdee C et al. (2011) Arch Pharm Res 34: 71 – 77. 3. Suebsasana S et al. (2009) Arch Pharm Res 32: 1191 – 1200. 4. Aromdee C et al. (2010). Planta Med 76:1 – 7.

SL8 Different effects of the herbal combination STW 5 in small and large intestine of rats

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Herbal medicinal products belong to the most successful treatment options in irritable bowel syndrome (IBS), a functional disorder of the gastrointestinal tract which is still not completely understood in its pathogenesis. The aetiology includes disturbed mechanical transport, caused by hyper- or hypomotility of intestinal smooth muscles. For the herbal combination STW 5 (Iberogast®), the influence on spontaneous and induced contractions of ring preparations from murine jejunum, ileum and colon was determined in an organ bath device. The results show that small intestine shows very regular spontaneous contractions under *in vitro* conditions (amplitude 1.8 ± 0.4 mN, frequency 19.8 ± 3.5 per min) which are inhibited in dose dependent manner by STW 5 and its constituent extracts. (e.g. 50% inhibition by STW 5 in a dilution of 10 μL/mL physiological solution, 90% inhibition by peppermint extract). The spontaneous activity of large intestine is less pronounced and reveals stronger contractions but a very low frequency. With these specimens the inhibitory effect of STW 5 is less pronounced and corresponds to 10 – 25% inhibition at the same given concentration. When contractions were stimulated by acetylcholine (10 μM) or by KCl-induced depolarisation (90 mM KCl) the inhibitory effects of STW 5 were similar in both types of intestine. It can be concluded, that STW 5 has stronger effects in the more proximal parts of the intestine rather than in distal ones under *in vitro* conditions. However, spasmolysis after stimulated contraction can be achieved in small and large intestine. **Keywords:** Irritable bowel syndrome, functional dyspepsia, Iberis amara, Peppermint, Mentha piperita, intestine

SL9 STW 5 is effective in treating experimental ulcerative colitis using the DSS model

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STW 5 (Iberogast®) is an herbal preparation that is effective clinically in functional dyspepsia (1) and irritable bowel syndrome (2). Since STW 5 also has marked anti-inflammatory activity (3), it was of interest to explore its potential effectiveness against inflammatory bowel disease (IBD). An experimental model reflecting ulcerative colitis in man was adopted, whereby colitis is induced in rats by feeding them 5% Dextran Sulfate Sodium (DSS) in drinking water for one week. STW 5 (0.5 ml/kg to 5 ml/kg) and sulfasalazine (300 mg/kg) as a reference standard were administered orally daily for one week before initiation of colitis induction and continued during DSS feeding. The animals were then sacrificed, the colons examined, and tissue samples taken for measurement of relevant parameters. DSS induced a sharp decrease in body weight which was more effectively normalized by STW 5 than by sulfasalazine. It also led to shortening of colon length and an increase in colon mass index, effects that were reversed by treatment with either drug. Changes in myeloperoxidase, reduced glutathione, glutathione peroxidase, superoxide dismutase, TNFα and MIP-2 induced by DSS were also reversed by STW 5 and by sulfasalazine almost to the same extent. The biochemical findings could be substantiated histopathologically. Furthermore, the sensitivity of colon segments towards motor agonists such as carbachol and potassium chloride was assessed ex-vivo. STW 5 normalized the depressed responsiveness of the colon induced by DSS. The findings point to a potential usefulness of STW 5 in the clinical setting of ulcerative colitis. **References:** 1. Schmulson MJ (2008) Nature clinical practice gastroenterology & hepatology 5: 136 – 137 2. Liu JP et al (2006). Cochrane Review, Issue 1. 3. Khayyal MT et al. (2009) Planta Med 75: 1034

SL10 Differentiation of *Allium* subgenera by cysteine sulphoxides and further amino acids

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The genus *Allium* (> 800 species) has been divided into several subgenera, sections, species and subspecies [1]. Cysteine sulphoxides are most prominent amino acid derivatives of this genus. These substances are susceptible for the enzyme alliinase. Alllinase reaction and further non-enzymatic steps do result in volatile sulphur compounds, which exhibit various bioactivities, e.g., antibiotic activities. The pattern of cysteine sulphoxides is rather characteristic for some subgenera. For instance, the subgenus *Melanocrommyum* is characterized by different heteroaromatic cysteine sulphoxides, whereas the subgenus *Cepa* typically shows rather high amounts of isoalliin. In the now presented study, amino acids as well as cysteine sulphoxides have been analyzed as their corresponding o-phthaldialdehyde (OPA) derivatives. Besides the above mentioned differences in the pattern of cysteine sulphoxides, also further amino acids show some relations between their amounts and subgenera. Most prominent are differences in the content of L-arginine in

different subgenera of the genus *Allium*. L-arginine is a valuable nitrogen storage compound in many angiosperms [2]. But this amino acid has been also found in some *Allium* species in high concentrations. As an example, the amount of L-arginine in *Allium cepa* L. was increased during the maturation process [3]. Further on, the amount of L-arginine in samples belonging to the subgenera *Allium* and *Cepa* was significant higher ($p < 0.05$) as the amount of L-arginine found in the subgenus *Melanocrommyum* suggesting different mechanisms of nitrogen storage. These differences should allow chemo-taxonomical classification of subgenera. **Keywords:** *Allium*, cysteine sulphoxides, o-phthaldialdehyde, amino acids **References:** 1. Fritsch RM et al. (2010) Phyton 49: 145 – 220 2. Van Etten CH et al. (1963) J Agr Food Chem 11: 399 – 409 3.Schupan W, Schwerdtfeger E (1972) Qual Plant Mater Veg 21: 141 – 157

SL11 What can phylogeny tell us about chemical diversity?

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Plant secondary metabolites are produced and selected by evolution for their biological activity. Such natural products have always played a major role in traditional medicine and as leads for modern medicine. Only a small fraction of the Worlds biodiversity has been explored for chemical and biological activity. A correlation between phylogeny and biosynthetic pathways is often assumed and could offer a predictive approach enabling more efficient selection of plants for traditional medicine lead discovery. However, formal tests of correlations between phylogeny and chemistry are rare, and the potential predictive power is consequently unknown. As a case in point, we are exploring the Amaryllidaceae subfamily Amaryllidoideae sensu APG, which is known for subfamily specific alkaloids with activity in the central nervous system (CNS). Galanthamine registered for the treatment of Alzheimer's disease was first isolated from the Caucasian snowdrop *Galanthus woronowii* Losinsk. We present a phylogenetic hypothesis of the Amaryllidaceae subfamily Amaryllidoideae based on nuclear, plastid and mitochondrial DNA sequences of over 100 of the circa 850 species, representing all tribes and geographical regions. All major lineages are now well supported and the extended sampling uncovered several genera as non-monophyletic, emphasizing the importance of using phylogenetic rather than classical classification for interpretation of character distribution. Alkaloid profiles and CNS-related bioactivity profiles are significantly correlated with phylogeny using formal tests. Relationships between phylogenetic and chemical diversity are further explored. The predictive power can be used to select candidate taxa for lead discovery and to make recommendations for traditional use. **Keywords:** Amaryllidaceae, phylogeny, chemical diversity, lead discovery **Acknowledgement:** *This research was supported by a Steno grant (N°272 – 07 – 0281) to NR from the Danish Council for Independent Research – Natural Sciences.*

SL12 Determination of bioactive coumarins in Radix angelicae pubescentis by HPLC

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Radix angelicae pubescentis (Duhuo in Chinese) was collected in Chinese Pharmacopoeia 2010 and widely used to treat thrombosis, arthritic disease, and anti-inflammatory. Pharmacological studies and clinical practice have demonstrated that *Radix angelicae pubescentis* possesses bioactive coumarins, including osthole, O-acetyl-columbianetin, and columbianadin. The HPLC method for determination of O-acetyl-columbianetin, osthol and columbianadin in *radix angelicae pubescentis* has been developed in this paper. HPLC separations were carried out with a Diamonsil C_{18} column (250 mm x 4.6 mm, 5 μm). The mobile phase was consisted of water and acetonitrile. The gradient elution was as follows: 0 to15 min, isocratic (52:48, v/v); 15 to 45 min linear gradient (52:48 to 42:58, v/v). The column temperature operated at 40 °C. The detection wavelength was 322 nm. The system suitability tests, including the linearity, limit of detection, limit of quantification, precision, repeatability and accuracy have been made. The developed method provides high selectivity and sensitivity with good accuracy and reproducibility. The established method was applied to simultaneous determination of the three analytes in seven samples of *radix angelicae pubescentis* obtained from different companies and batches. The content ranges of three bioactive coumarins are 3.60 to 1.36 mg/g for O-acetyl-columbianetin, 6.03 to 4.44 mg/g for osthol, and 4.04 to 0.94 mg/g for columbianadin. The results demonstrated the influence of the treatment method, and the storage time on the contents of O-acetyl-columbianetin, osthole, and columbianadin in *Radix angelicae pubescentis*. **Acknowledgement:** *This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients.*

SL13 Novel heparanase activity assay based on a fluorescence sensor technology

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Since tumors and other diseases are characterized by increased heparanase (HEP) levels, HEP is considered a diagnostic marker and a target for antitumor therapy. Therefore, methods are needed to measure HEP and to examine inhibitors. Based on previous findings that HEP degrades not only heparan-sulfate, but also the also the sulfated pentasaccharide fondaparinux (FPX) [1] and that this can be quantified by its effect on the fluorescence intensity (FI) of the sensor molecule Polymer-H [2], we aimed to develop a fluorimetric HEP activity assay. Since the FPX degradation products proved to have no effect on the FI, the remaining FPX can be measured without separation. Optimization of various assay parameters led to the following two-step procedure: (1) Incubation of HEP containing solution with FPX (10 μg/mL). (2) Sample dilution and FPX detection by adding Polymer-H (7.5 μg/mL) and measuring the FI (λ_{em}330 nm, λ_{ex}510 nm). The FXP degradation showed to increase with the concentration of HEP. After 30 min at 37 °C, 1.5mIU/mL HEP led to complete FXP-degradation. By varying incubation time and FPX concentration, the LOD of 0.2mIU/mL HEP can be considerably decreased. Various HEP inhibitors demonstrated the suitability of the assay for inhibitor screening. In conclusion, a rapid, simple and robust microplate HEP activity assay was developed. A major advantage is the use of FPX as substrate. In contrast to heparan-sulfate, it is chemically defined, well available and has not to be labeled with radioactive or other markers for detection. Moreover, its fluorimetric detection is much more convenient than by its anti-FXa-activity or HPLC-MS. **Keywords:** pharmacology, heparanase, assay development, fluoescence sensor technology, heparanase inhibitors **References:** 1. Alban S et al (2009) J Thromb Haemost 7, Suppl.2: PP-WE-506. 2. Lühn S, Schrader T, Sun W, Alban S (2010) J Pharm Biomed Anal 52: 1 – 8.

SL14 Determination of curcumin in turmeric using magnetic iron oxide nanoparticles as solid phase extractor and HPLC

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Curcumin, a derivative of *Curcuma longa* L., is used extensively in the food industry and researches have shown the health benefits of this compound [1]. In this work, a novel, simple and rapid method for extraction and determination of curcumin in turmeric was performed using magnetic iron oxide nanopaticles (MIONs) as solid phase extractor and HPLC. The unique properties of nanoscale materials offer excellent prospects for designing new methods and instrumentation for chemical analysis [2]. The MIONs were synthesized according to the method proposed by Laurent et al. [3]. The average size of nanoparticles was in the range of 90 nm which was determined by using atomic force microscopy (AFM) (Fig. 1). Extraction of curcumin is based on adsorptionof Fe (3+)-curcumin complex on MIONs. Desorption of analyte was performed by NaOH solution containing methanol in order to dissolve the desorbed analyte. Various parameters affecting the extraction recovery such as: pH, volume and concentration of NaOH as desorbing reagent, and con-

centration of Fe (+3) and percentage of methanol were investigated and optimized. These optimized parameters were: pH = 2.0, 1.5 mL of 0.2 M NaOH containing 30% methanol and 0.1 M of Fe (3+), respectively. The intra-day precision (R.S.D.) was 4.0% and inter-day R.S.D. was less than 7.0%. The preconcentration factor of 100 was achieved in this method. The proposed procedure has been successfully applied to the determination of curcumin in turmeric.

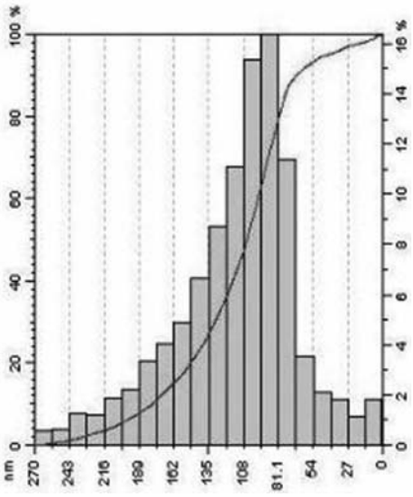

Figure 1: size distribution of MIONs determined by AFM

Keywords: Curcumin, turmeric, Magnetic iron oxide nanoparticles, Solid phase extraction, Determination, HPLC **References:** [1] Srivastava RM, Singh S, Dubey SK, Misra K, Khar A (2011) Inter Immunopharmacol, 11: 331 – 341 [2] Trojanwicz M. (2006) Trends Anal Chem 25: 480 – 489. [3] Laurent S, Forge D, Port M, Roch A, Robic C, Elst LV, Muller RN (2008) Chem Rev 108: 2064 – 2110

SL15 Southern Africa flora: a source of potential antimalarial compounds

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Southern Africa has a diverse botanical flora with numerous plants being used traditionally as phytomedicines. Only a minuscule number of these plants have been scientifically validated for their therapeutic potential as antimalarials and even fewer have had the active chemical compound/s isolated from them. In an attempt to evaluate our plant flora, numerous plants have been assessed for their *in vitro* antimalarial activity. As such plants were collected from diverse areas of southern Africa and extracts and isolated compounds prepared. The antimalarial properties of the extracts/compounds were screened against *Plasmodium falciparum* using the tritiated hypoxanthine incorporation assay. The compounds were evaluated for their haemolytic and cytotoxic properties, and ability to chelate iron and inhibit haemozoin formation. Collation of several years of research reveals that *Crinum bulbispermum* (Burm.) Milne-Redh. & Schweick., *Rosmarinus officinalis* L., *Leonotis leonorus* (L.) R. Br., *Syzygium cordatum* Hochst. ex C.Krauss, *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood, *Agathosma* and Menispermaceae species are highly active against *P. falciparum*, with minimal haemolysis. *A. parva* and *A. purgens* possessed the best safety index against the erythroleukaemia and kidney epithelial cells; while *A. ovata* was the most effective in chelating iron and inhibiting haemozoin formation. Numerous interactions have been investigated, including the synergistic interaction between *Hermannia muricata* Eckl. & Zeyh. and *Hermannia trifurca* L.; indicating a decreased risk of therapeutic failure when clinically combined to eradicate an infection. Plant-derived antimalarial agents have lead the way in the therapy of this disease and the next antimalarial agent or template may be within our flora awaiting discovery to successfully treat drug-resistant strains of *P. falciparum* malaria. **Keywords:** malaria, phytomedicines, *Agathosma*, pharmacology, haemozoin, iron **Acknowledgement:** *The South African NRF Thuthuka Women in Research grant for financial support, all collaborators and postgraduate students who have contributed to the collated research.*

SL16 Evaluation of the *in vitro* antimalarial and malaria prophylactic activity of eight *Ficus* species

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Malaria is a serious health problem in Africa, where approximately 250 million new cases and one million deaths are recorded per year [1]. The absence of long-term prophylaxis and the emerging resistance to the current therapies highlights the necessity of new drugs for its eradication and control. Liver stage malaria parasites show an absolute requirement for type-II fatty acid biosynthesis (FAS-II) [2], rendering this pathway a good target for causal prophylaxis. In Zambia, the genus *Ficus* is traditionally used against malaria [3]. Hence, we collected the leaves, barks and roots of eight Zambian *Ficus* species and screened their crude methanol (CR-Me) extracts for *in vitro* activity against the multi-drug-resistant *Plasmodium falciparum* strain K1 using a modified [3 H]-hypoxanthine incorporation assay. Malaria prophylactic potential of the extracts was also evaluated against three recombinant FAS-II elongation enzymes, FabI, FabG and FabZ, by spectrophotometric assays. All CR-Me extracts were active against *P. falciparum*, with *F. ovata* Vahl bark exhibiting the best activity (IC_{50} 4.76 μg/ml). Most of the root extracts, particularly *F. sycomorus* L. subsp. *gnaphalocarpa* (Miq.) C.C. Berg potently inhibited FabZ (96.6% inhibition at 1 μg/ml). The CR-Me extracts were then subjected to solvent partitioning to yield n-hexane, $CHCl_3$ and aq. MeOH subextracts. Remarkable antiplasmodial potency was observed in $CHCl_3$ subextracts of four species (IC_{50} < 2 μg/ml). The leaf and root aq-MeOH subextracts were active against FabI and FabZ enzymes with inhibition rates > 90% at 1 μg/ml. This is the first screening of Zambian *Ficus* species for antimalarial potential and our results justify their traditional use. **Acknowledgement:** *UK Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding.* **References:** 1. World Health Organization (WHO) (2009) Fact sheet on Malaria WHO, Geneva, Switzerland. 2. Vaughan AM et al. (2008) Cell Microbiol 11: 506 – 520. 3. Fowler DG (2007) Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens. Kew, UK.

SL17 Polyphenolic compounds from Clusiaceae plants modulating angiogenesis and vascular endothelium

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Polyphenolic compounds have created an increasing interest for their potency about cardiovascular diseases for several years[1 – 2]. Nevertheless, most of this research had been focused on polyphenolic compound such as flavanols (*e.g.* catechine from tea), anthocyanin (*e.g.* delphinidin from blueberry) and stilbenoides (*e.g.* resveratrol from grape). The present study was designed to screen the potent effect of polyphenolic compounds isolated from plants belonging to Clusiaceae family on endothelium. A huge number of polyphenols such as xanthones and coumarines have been identified from those species and some of them exhibiting various biological activities such as anti-inflammatory and antioxidant properties[3 – 4]. Their effect on endothelium, more particularly on angiogenesis, is not yet well-known. Firstly, we assessed the capacity of six molecules to induce endothelium-dependent relaxation in mice aortic rings involving nitric oxide production. Isocalolongic acid (A1) and 2-deprenylrheediaxanthone (A2) are able to increase NO production on endothelial cells and to induce endothelium-dependant relaxation. Then, we investigated the effects of these compounds on *in vitro* and *ex vivo* angiogenesis. We showed that A1 treatment promoted the formation of capillary-like network contrary to A2. Endothelial cell adhesion, migration and proliferation were decreased in presence of A2 whereas endothelial migration and proliferation were improved with A1 treatment. We could explain these results with the capacity of A1 to increase VEGF expression and for A2, to decrease ICAM-1 expression. Thus, the strategy used for the screening allows the detection of active molecules from Clusiaceae family that might be of therapeutic benefit in cardiovascular diseases[5]. **Keywords:** Clusiaceae, angiogenesis, endothelium, xanthone, chromanone **Acknowledgement:** *We thank Angers*

Loire Métropole for the PhD grant to the first author and for their financial support. **References:** 1. Tunstall-Pedoe H, Kuulasmaa K, Mähönen M, Tolonen H, and Ruokokoski E (1999) The Lancet 353: 1547 – 1557. 2.Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, El Bedoui J, Chataigneau M, et al. (2004 European Journal of Pharmacology 500: 299 – 313. 3.Hay AE, Aumond MC, Mallet S, Dumontet V, Litaudon M, Rondeau D, Richomme P (2004). J Nat Prod 67: 707 – 709. 4.Hay AE, Guilet D, Morel C, Larcher G, Macherel D, Le Ray AM, et al. (2003) Planta Medica 69: 1130 – 1135. 5.Ferrara N and Kerbel RS (2005) Nature 438: 967 – 974.

SL18 New cytotoxic pregnane glycosides from *Caralluma sinaica* growing in Saudi Arabia

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Certain species of genus *Caralluma* (Asclepiadaceae) are edible and form part of the traditional medicine system of many countries. *Caralluma sinaica* A.Berger, growing wild in the western region of Saudi Arabia, is used by locals as a remedy to treat diabetes [1]. Pregnane glycosides, the key phytochemical ingredients in *Caralluma*, are drawing much attention in recent years because of their antitumor and anticancer activities [2]. Thirteen pregnane glycosides, including six new (Fig.1), were isolated from the cytotoxic chloroform extract of the titled plant using repeated normal and reversed phase chromatographic techniques. The structures of the isolated compounds were characterized using extensive spectroscopic techniques including 1D and 2D microflowNMR methods for compounds available in restricted amount. A detailed profiling of the constituents was obtained by UHPLC-ESI-TOF/MS data [3]. Some isolated compounds were evaluated for their in vitro cytotoxic activity, and quinone reductase induction was also assessed. Abbreviations: Benzoyl: Bz, Tigloyl: Tig, Acetate: Ac, Thevetose: Thev, Cymarose: Cym, Glucose: Glc, Digitalose: Dig.

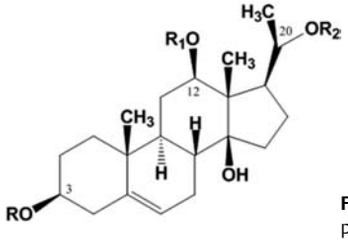

Figure 1: Main structure of pregnane glycoside

List of new compounds

	R	R1	R2
1	Thev-(1→4)-cym-(1→4)-cym	Bz	Ac
2	Glc-(1→4)-cym	Tig	Ac
3	Glc-(1→4)- dig-(1→4)-cym-(1→4)-cym	Bz	Ac
4	Glc-(1→4)- thev-(1→4)-cym-(1→4)- cym	Bz	Ac
5	Glc-(1→4)-cym	Bz	Tig
6	Glc-(1→4)-cym-(1→4)-cym	Bz	Bz

Keywords: *Caralluma sinaica*, Asclepiadaceae, Pregnane glycosides, Cytotoxic **Acknowledgement:** *Acknowledgments: the authors thank Philippe Eugster and Dr. Laurence Marcourt for their help in the recording of the LC-MS profiles and NMR spectra respectively.* **References:** 1. Habibuddin M et al. (2008) J Ethnopharmacol 117: 215 – 220. 2.Abdel-Sattar A et al. (2009) Phytomedicine 16: 659 – 664. 3.Eugster P et al. (2011) J AOAC Int 94: 51 – 70.

SL19 Isolation of Novel Cytotoxic Compounds from a Bangladeshi Medicinal Plant *Acrostichum aureum*

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Natural products and related drugs are used to treat 87% of all categorised human diseases including cancer and immunological disorders [1]. This study reports on the isolation and characterisation of novel cytotoxic compounds from Bangladeshi medicinal plants. Following LC-MS metabolic profiling and cytotoxic screening of 16 Bangladeshi medicinal plants against normal mouse fibroblast (NIH3T3) and three human cancer cell lines (AGS, HT-29 and MDA-MB-435S), *Acrostichum aureum* L. was selected for further phytochemical and pharmacological investigations. A total of 13 compounds were isolated from this plant using SPE and reversed-phase HPLC. The structures of compounds were elucidated by NMR, MS and other spectroscopic methods. Three compounds (1, 2 and 5) were identified as novel natural products. Eight known compounds were isolated for the first time from *A. aureum*; di-(2-methylheptyl) phthalate (3), (2S, 3S)-pterosin C (4), (2R)-pterosin P (7), tetracosane (6), quercetin-3-O-β-D-glucosyl-(6→1)-α-L-rhamnoside (9), quercetin-3-O-α-L-rhamnoside (10) and quercetin-3-O-α-L-rhamnosyl-7-O-β-D-glucoside (11), and patriscabratine (13). Two known flavonoids, quercetin-3-O-β-D-glucoside (8) and kaempferol (12) have previously been isolated from *A. aureum*. The cytotoxic activity of all compounds was assessed against the cell lines mentioned above using the MTT assay. Four compounds (3, 5, 6 and 13) showed moderate to potent cytotoxic activity. Compound 5 displayed the most potent cytotoxicity against gastric and colon adenocarcinoma cell lines (IC_{50} 7.5 – 21.6 $\mu g \cdot mL^{-1}$). The mode of action was revealed as induction of apoptosis. This research has demonstrated that Bangladeshi plants are an exciting sources of bioactive novel compounds and as such a rich basis for ongoing drug discovery research. **References:** 1. Newman DJ and Cragg GM (2007) J Nat Prod 70(3): 461 – 77.

SL20 Combinatory chemo-analytical and physiological concept for the evaluation of immediate physiological effects during inhalation of odorous compounds from plants

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The odorous fractions of plants have been intensely studied regarding their molecular composition and the "key players" eliciting the respective smells. Methodological progress has been especially fast over the last decades, with improved analytical tools for the analysis of odorants even in low traces. Gas chromatographic olfactometric techniques together with mass spectrometric detection in complex matrices, in the headspace or even in vivo during or after inhalation have been developed [1,2,3]. Techniques are now at hand that allow precise determination of the odorant structures and their quantitative distribution in a material and its headspace, as well as monitoring their path along the nasal passage to the olfactory epithelium [4]. Originally starting from food flavour research, the goal of understanding the processes of in vivo odorant transfer in relation to the respective human sensory and immediate physiological response has gained growing momentum. We will present a novel concept of combining in-depth analytical characterisation of the gas phase concentrations of defined plant-constituent odorants when being presented to humans for smelling, enabling us to even follow concentration gradients during inhalation. In relation to the chemo-analytical characterisation physiological responses are recorded both with regard to smell perception and rating, but also biofeedback and behavioural responses of the subjects (sensory evaluation, EEG, skin conductance, heart rate, breathing patterns, mimic analysis). Methods applied in this concept are, amongst others, gas chromatography-olfactometry/mass spectrometry (GC-MS/O) for characterisation of the odour constituents, conventional aroma analytics for quantification in the gas phase, and proton-transfer-reaction mass spectrometry (PTR-MS) for dynamic monitoring.

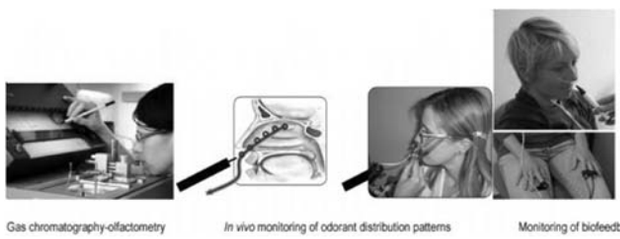

Figure 1: Biofeedback

References: 1. Grosch W (2001) Chem 26: 533 – 545. 2. Buettner A, Beauchamp J (2010) Food Qual Pref 21: 915 – 924. 3. Taylor A, Linforth R. (2010) Food Flavour Technology, 2nd edition, John Wiley & Sons, Oxford, UK. 4. Beauchamp J, Scheibe M, Hummel T and Buettner A. (2010) Conference Human Chemosensation.

SL21 Steering Clear of the Drug Discovery Black-Hole

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There is not one single scientific innovation that you can point to and say, "this has revolutionized drug discovery. " The billions of dollars that have been invested in proteomics, genomics, metabalomics., siRNA, etc. has not created the proportionate output to the dollar input. Mergers and acquisitions have done nothing to invigorate the discovery process. In fact, financial data has indicated that this aggressive strategy to grow the business of a large pharmaceutical company has resulted in $1 trillion reduction of valuation over the past decade. Is this the path towards innovation as an industry? As natural product chemists, the question that we need to ask is, "what about chemical diversity? Why has chemical diversity not been exploited?" Sequoia Sciences identifies novel chemistry from its library of structurally diverse small molecules isolated from plants. The proprietary design of this library allows for the biological screening of these compounds at optimal HTS concentrations without non-drug-like interferences. Sequoia built this analytical process such that rapid isolation and structure elucidation of active compounds could be accomplished. Using the extremely sensitive Bruker TCI 1.7 mm MicroCryoProbe, structure elucidation of active compounds is completed on samples of limited mass at a rate 4 – 10 times faster. The scientific strategy that Sequoia employs in order to rapidly uncover the chemical diversity contained in plant natural products will be outlined. This presentation will outline Sequoia's unique process that is used to create a library of compounds. The MicroCryoProbe has now extended the high-throughput process to include NMR data acquisition. Sequoia's inclusion of the MicroCryoProbe compliments its current platform technologies for high-throughput natural products research for drug discovery allowing it to uncover the chemical diversity contained in natural products.

SL22 The Application of HPLC with Coulometric Electrochemical Array Detection to the Study of Natural Products and Botanicals: From Targeted Analyses to Metabolomics

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Many potentially important bioactive phytochemicals are electrochemically active and can be measured using HPLC with electrochemical detection (HPLC-ECD). Such compounds, come from diverse chemical classes and include phenols, polyphenols, aromatic amines, quinones, isoquinolines, indoles, thiols and conjugated polyenes. Although HPLC-ECD with a single amperometric working electrode offers the analyst excellent sensitivity and some degree of selectivity, this approach is limited as it cannot be used routinely with gradient chromatography, relatively few analytes are measured concurrently, and qualitative information is lacking. The coulometric flow-through graphite working electrode is both sensitive (~100% of an analyte will react) and maintenance free. When used in series, chromatographically co-eluting analytes can be resolved voltammetrically. Such behavior can be used to identify and characterize an analyte in an analogous fashion to the use of spectral data from a diode array detector. Furthermore, the CoulArray® detector, with its array of up to sixteen serially placed coulometric sensors, is fully gradient compatible and can be used for either targeted or metabolomics studies. During this presentation I will illustrate the capability of coulometric array detection for the measurement of specific analytes in plant, animals and human tissues. I will also discuss the use of metabolite profiles/metabolomics with pattern recognition and how this can be used to study product and botanical adulteration, contamination and composition. **Keywords:** HPLC, electrochemical, botanicals, natural products, plasma, urine, adulteration, characterization

SL23 Adaptogens (ADAPT-232) stimulate neuropeptide Y expression in neuroglia cells

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Neuropeptide Y (NPY) is a stress hormone widely distributed in the central and peripheral nervous system. Human studies have revealed a role for NPY in "buffering" the harmful effects of stress (adaptation to stress) [1,2].There is a plethora of pre-clinical and clinical evidence suggesting a mood and cognitive performance improving action for NPY [3,4]. Higher levels of NPY have been observed in soldiers who either present reduced psychological distress or belong to special forces [2]. In contrast, decreased levels of NPY were observed in depression and in brain tissues of suicide victims [1]. Our study for the first time provides evidence that adaptogens, specifically ADAPT-232 – a fixed combination of *Eleutherococcus senticosus* Maxim. root extract SHE-2, *Schisandra chinensis* K.Koch berry extract, *Rhodiola rosea* L. root extract SHR-5 stimulate the expression of NPY, heat shock factor-1 (HSF-1) and release of the heat shock protein (Hsp72) in isolated neurolgia cells. Pre-treatment of human neuroglia cells with NPY-siRNA or HSF1-siRNA (which silences the expression of intracellular NPY and HSF-1 respectively), before treatment with ADAPT-232 resulted in a significant suppression of Hsp72 release. **References:** 1. Morales-Medina JC et al.(2009) Brain Res 1314:194 – 205.. 2. Morgan III CA et al. (2002) Biol Psychiatry 52: 136 – 142; Morgan III CA et al. (2000) Biol Psychiatry 47: 902 – 909. 3. Redrobe JP et al., (2002) Life Sci 71:2921 – 37. 4. Fletcher MA et al. (2010) Behav Brain Funct 6:76 – 85

SL24 Arabinogalactan-proteins from *Echinacea purpurea*: Characterization, localization and immunomodulating properties

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Arabinogalactan-proteins (AGPs) are macromolecular glycoproteins belonging to the putative active compounds of Echinacea preparations [1]. (β-D-Glc)-Yariv phenylglycoside specifically binds to most plant AGPs and has been used to isolate AGPs from pressed juice of the aerial parts and from suspension cultures of *Echinacea purpurea* (L.) Moench (Asteraceae). These AGPs have been structurally characterized and compared concerning their protein- and polysaccharide moiety. The main components of the carbohydrate moiety of AGP from herbal material are 1,6-Galp, 1,3-Galp, 1,3,6-Galp and in the side chains 1,5-Araf, terminal Araf and terminal GlcAp. Side chains of AGP from cell cultures are structurally different with only traces of 1,5-Araf. The protein part of both AGPs mainly consists of Hyp, Asx, Glx, Ser, Thr and Ala. Interestingly, AGP from herbal material showed an amino acid sequence rather untypical for AGPs with predominantly contiguous arrangement of three to four Hyp residues in blocks [2]. For microscopic localization of AGPs in fresh plant tissue, a new method has been developed. Antibodies against Yariv's reagent have been generated in rabbits and used for immunofluorescent labeling of plant tissue. Xylem tracheary elements showed very strong labeling of the cell wall, especially at the inner side of the wall and in the area of pit canals. Preparations of pressed juice from *Echinacea purpurea* are used as herbal medicinal products with immunomodulating properties. In vitro, AGP from the pressed juice of herbal material showed complement stimulating activities [3] as well as binding to human leucocytes [4]. **Keywords:** *Echinacea purpurea*, arabinogalactan-protein, structure elucidation, immunofluorescence, natural immune enhancer **Acknowledgement:** *The authors thank Rottapharm/Madaus GmbH, Köln, Germany, for financial support of this work.* **References:** 1. Classen B et al. (2006) Phytomedicine 13: 688 – 694. 2. Classen B et al. (2005) Planta Med 71: 59 – 66. 3. Alban S et al. (2002) Planta Med 68: 1118 – 1124. 4. Thude S et al. (2006) Phytomedicine 13: 425 – 427.

SL25 Metabolomic profiling of saw palmetto products using proton-NMR spectroscopy and multi-variate analysis

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Saw palmetto products with an often poorly known and variable chemical composition are used in the treatment of Benign Prostatic Hyperplasia.(1, 2) Here we present a method for the metabolomic analysis of saw palmetto products using NMR spectroscopy and multi-variate analysis in order to determine if there are significant differences in metabolytes of the given products and if marker compounds can be identified. Spectra were obtained on a Bruker 500 MHz spectrophotometer. TOPSPIN was used for spectra acquisition and processing. Deuterated methanol and chloroform were selected as solvents. NMR spectra were transferred to AMIX. The spectra were divided into 251 regions and the signal intensity was integrated. Unscrambler was used for PCA analysis. The analysis showed that saw palmetto products can be grouped according to their metabolic profile. Multi-variate analysis showed significant variations between powders, soft extract and tincture products. The largest variation in any product tested was observed for a hexane extract. Oleic acid and caproic acid ethyl ester were identified as potential marker compounds. Additional information regarding TLC analysis and clinical outcomes was supplied by Andy Suter from Bioforce AG, Switzerland. Variations in chemical content were identified using NMR spectroscopy, however, multivariate analysis of the products suggested that there was no significant difference in metabolites between the European extracts tested but differences were observed when compared to non-European products or products that used hexane as the extraction solvent. It is possible to identify marker compounds in saw palmetto using proton-NMR spectroscopy. **Acknowledgement:** *Centre for Pharmacognosy and Phytotherapy, University of London, School of Pharmacy, UK Michael Heinrich, Mire Zloh, Mazlina Said, Andy Suter* **References:** [1] Blumenthal M (2003) The ABC Clinical Guide to Herbs. New York: Thieme: 309 [2] Scaglione F (2008) Pharmacology 82(4): 270 – 275

SL26 Herb-based functional foods: from laboratories to the market

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Consumption of alternative herbal folk medicine has had a tremendous increase in the last decade. A number of medicinal plants contain secondary metabolites which have many biologically active compounds. They are used against hepatic fibrosis and heart ischemia-reperfusion and proved to have antioxidant, antithrombosis, antihypertension, antistress, antivirus, antitumour, antiulcer, antidiabetic, antiaging and anti-inflammatory activities. Non-availability of quality planting materials, low germination, slow plant growth, disease and pest incidence are the major obstacles in conventional medicinal plant cultivation. In Taiwan, many economically important medicinal plants and herbs are produced using various explant materials by tissue culture technique to meet the increasing demand for their medicinal properties. Rapid multiplication through in vitro tissue culture can be advantageous for the continuous supply throughout the year. We have developed and standardized efficient, simple and rapid tissue culture regeneration protocols of many medicinal plants, optimized the conditions in green house and successfully established the regenerated plantlets in the field for the large scale commercial production. Availability of tissue culture protocol is the first step towards the development of the genetic transformation. **Keywords:** Medicinal plant, Tissue culture, Functional foods **Acknowledgement:** *Thanks to the National Science Council of Taiwan for financial support*

SL27 Safety evaluation of licorice consumption from dietary and phytotherapeutic sources

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Licorice (*Glycyrrhiza glabra* L.) contains the triterpenoid glycoside glycyrrhizic acid (GA), which has an intensely sweet taste and is used as a flavouring agent, mostly in confectionary products and tobacco. Licorice also has a long history of medicinal use and is listed in current editions of the British, European and Chinese pharmacopoeias. GA and its aglycone, glycyrrhetinic acid, competitively inhibit 11β-hydroxysteroid dehydrogenase type 2, which converts active glucocorticoids to their inactive metabolites. Mineral corticoid-like effects can result, giving rise to pseudoaldosteronism characterised by hypokalemia, sodium retention, oedema and suppression of the renin-angiotensin-aldosterone system with resulting hypertension.1,2 Licorice-induced pseudoaldosteronism is well documented with most cases involving confectionary licorice. Although it is not possible to accurately identify a NOEL for GA most human studies suggest that a daily intake of more than 100 mg GA over a period of weeks is necessary to produce pseudoaldosteronism. In order to evaluate the potential risk associated with the consumption of Australian made confectionary licorice products and liquid extracts dispensed in phytotherapy, we determined the GA content of ten confectionary products, five liquid extracts and two licorice root samples by reverse-phase HPLC. All but one liquid extract contained GA in concentrations that could readily result in a daily intake in excess of 100 mg if taken in a therapeutic dose. In contrast, confectionary products contained GA in concentrations that varied three orders of magnitude and only a few of them would provide 100 mg/d of GA if consumed in amounts up to 100 g daily. **Keywords:** glycyrrhizic acid; licorice; safety **References:** 1. Isbrucker RA, Burdock GA (2006) Reg Tox Pharm 46:167 – 92. 2. Tanahashi T et al. (2002) J Steroid Biochem Mol Biol 80:441 – 7.

SL28 The molecular cloning of dihydroartemisinic aldehyde reductase and its implication in artemisinin biosynthesis in *Artemisia annua*

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A key point in the biosynthesis of the antimalarial drug artemisinin is the formation of dihydroartemisinic aldehyde which represents the key difference between chemotype specific pathways. This key intermediate is the substrate for several competing enzymes some of which increase the metabolic flux towards artemisinin and some of which – as we show in the present study – may have a negative impact on artemisinin production. In an effort to understand the biosynthetic network of artemisinin biosynthesis, extracts of *A. annua* L. flowers were investigated and found to contain an enzyme activity competing in a negative sense with artemisinin biosynthesis. The enzyme, Red1, is a broad substrate oxidoreductase belonging to the short chain dehydrogenase/reductase family with high selectivity for dihydroartemisinic aldehyde and valuable monoterpenoids. Spatial and temporal analysis of cDNA revealed Red1 to be trichome specific. The relevance of Red1 to artemisinin biosynthesis is discussed.

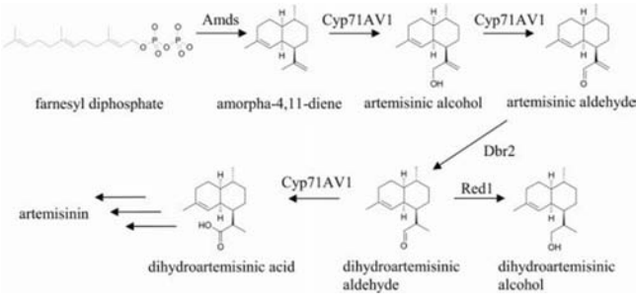

Figure 1: Biosynthetic pathway of artemisinin and the activity of Red1

Keywords: Artemisia annua, red1, dihydroartemisinic aldehyde, dihydroartemisinic alcohol, trichome, reductase, dehydrogenase, trichome, oxidoreductase **References:** Rydén A-M, Ruyter-Spira C, Osada H, Muranaka T, Kayser O, Bouwmeester H (2010) Planta Medica 76:1 – 6

SL29 HPLC-MS and NMR spectroscopy: two integrative analytical tools for the quality control of plant extracts, the case of a commercial blend sold as dietary supplement

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In this study HPLC-DAD-ESI-MS, HPLC-ESI-MS^n and 2D NMR techniques were applied for the quality control of a herbal supplement. Several reports were received by the pharmacovigilance system by patients who fainted after consuming “)Olivis(lrquor)), a dietary supplement for the integrative treatment for hypertension. Declared components of this product are extracts from *Olea europaea* L., *Crataegus oxyacantha* L., *Fumaria officinalis* L., *Capsella bursa-pastoris* (L.) Medik. “)Olivis(lrquor)) sample was subjected, after lyophilization, to direct 2D NMR and HPLC-DAD-ESI-MS analyses to identify the marker constituents of the different Herbal Drugs. Comparison of the NMR and chromatographic profiles of the product with those of the marker compounds of the declared plant species, (such as oleuropein, protopine), showed the lack of the latter. Samples of the declared plant species were taken into consideration as well. Comparison of these samples with the commercial product revealed marked differences in their content. Further, HPLC-DAD and HPLC-ESI-MS analyses showed the presence of *Rauwolfia* sp. type indole alkaloids, while HPLC-ESI-MS^n analyses revealed the presence of reserpine. Parallel phytochemical fractionations led to the isolation of ajmaline. Quantitation studies showed that (i) the content of reserpine in the product was in the therapeutic range and therefore responsible for the collapses of the patients; (ii) ajmaline prevailed against reserpine indicating that a *Rauwolfia* species other than *R. serpentina* was used. The present study shows the importance of extensive controls using combined analytical tools of the botanical products on the market to assure their quality and as a consequence their safety profiles. **Keywords:** HPLC-UV-DAD-MS profile, NMR profile, dietary supplement, adulterant, reserpine, ajmaline

SL30 Isolation and characterization of biosurfactant producing bacteria having antimicrobial activity isolated from oil contaminated sites

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Biosurfactants are surface active amphiphilic compounds that reduce the surface tension of liquids, thereby increase the miscibility of hydrophobic compounds. One of the isolate from oil contaminated site was tested for its ability to produce biosurfactants. The bacterium was identified on the basis of morphological and biochemical characterization and found to be *Pseudomonas putida* SOL-10. The isolate was tested for biosurfactant production under shake flask fermentation and found to be potent biosurfactant producer. The biosurfactant production was analyzed by surface tension and emulsification index (E24%) measurements. In this study the biosurfactant was produced by a newly isolated Pseudomonas putida SOL-10 at optimized conditions. The biosurfactant produced by the isolate reduced the surface tension of culture broth from 43.6 – 29.9 m.N.m^{-1} achieving a maximum biosurfactant concentration of 4.5 g/L after 72 hours of incubation. The biosurfactant also demonstrated a good antimicrobial activity as well as the capability to enhance the antimicrobial effect of some antibiotics. The current study describes the effect of biosurfactant with Ampicillin, Ciprofloxacin and Cefixime, against *Escherichia coli, Bacillus subtilis,* and *Klebsiella pneumonia*. The results demonstrated promising antimicrobial activity enhancing effect of the biosurfactant and suggested a possibility of the biosurfactant to be used with antibiotic formulations in order to increase the effectiveness of antimicrobials against multidrug-resistant pathogenic bacteria. **Keywords:** Pseudomonas putida, biosurfactant, characterization, antimicrobial activity, antibiotics, surface tension

SL31 Egyptian Herbal Drug Industry: Challenges for the Future

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According to the World Health Organization (WHO), the goal of 'Health for All' cannot be achieved without herbal medicines. While the demand for herbal medicines is growing in developing countries including Egypt, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicines. There is, therefore, an increasing consumer demand for herbal medicines in developed countries. Medicinal plants have been used as a source of remedies since ancient times in Egypt. Many plants are still used today in folklore medicine and are sold at herbal venders and shops. Egypt is characterized by abundant production of medicinal and aromatic plants that are exported all over the world and is considered as one of the most important sectors can be relied upon to increase the volume of Egyptian exports due to the growing global demand but several factors pose constraints to their entry into the international market and put them in a disadvantageous position. This lecture explores the situation of the Egyptian herbal drug industry, the economic value, the needs and recommendations for developing this important sector. **Keywords:** herbal medicine, Egypt, drug industry, challenges **References:** References 1. Batanouny K H (1999) Wild Medicinal Plants in Egypt (with contribution: E. Aboutabl, M. Shabana & F. Soliman). 2. Dagmar L (2006) International Trade in Medicinal and Aromatic Plants, Actors, volumes and commodities, Plants.- In Bogers, R.J., Craker, L.E. & Lange, D. (eds.): Medicinal and Aromatic Plants, 155 – 170. 3. Das M, (2005) Medicinal Plants: An Approach towards sustainable development, available at http://www.iamot.org/conference/index.php/ocs/4/paper/viewFile/467/18 4. Saleh NAM (2003) Phytochemistry 63: 239 – 241.

SL32 Bioactivity-guided Isolation of $GABA_A$ Receptor Modulating Constituents from the Rhizome of *Actaea racemosa*

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Black cohosh (*Actaea racemosa* Walter ex Steud.) is a frequently used herbal remedy for the treatment of mild climacteric symptoms. The plants active principle was extensively studied, but its mechanism of action remained unclear [1]. In this study the modulation of GABA-induced chloride currents (I_{GABA}) through $GABA_A$ receptors by black cohosh extracts and isolated compounds was investigated. $GABA_A$-receptors consisting of α_1, β_2 and γ_{2S} subunits were expressed in *Xenopus laevis* oocytes and potentiation of I_{GABA} was measured using the two-microelectrode voltage clamp technique. In a bioactivity-guided isolation procedure the positive modulation of I_{GABA} could be restricted to the terpenoid fractions, resulting in the isolation of 11 cycloartane glycosides, of which 4 compounds significantly enhanced I_{GABA} by more than 150% ($p < 0.05$). The strongest effect was observed for 23-O-acetyl-

shengmanol-3-O-β-D-xylopyranoside (100 µM) enhancing I_{GABA} by 1692 ± 201%, while actein, cimigenol-3-O-β-D-xylopyranoside and 25-O-acetylcimigenol-3-O-α-L-arabinopyranoside were significantly less efficient (range of I_{GABA} enhancement at 100 µM: 256 ± 40% – 378 ± 64%). 23-O-acetylshengmanol-3-O-β-D-xylopyranoside, which exhibited the greatest potentiation of I_{GABA}, was additionally investigated in a mouse model. In these studies a decrease in motoractivity as well as a clear reduction in anxiety behaviour of animals treated with 23-O-acetylshengmanol-3-O-β-D-xylopyranoside was observed, indicating this compound to induce strong sedative effects with concomitant anxiolytic activity. We hypothesise that the established positive allosteric modulation of $GABA_A$ receptors may contribute to beneficial effects of black cohosh extracts in the treatment of climacteric symptoms. **References:** 1. Palacio C, Masri G (2009) Drugs Aging 2623 – 36.

SL33 Pharmacokinetic interactions between botanicals and drugs: *in vitro* investigations

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Herb-drug pharmacokinetic interactions include interferences of plant constituents with drug bioavailability by means of altered absorption, metabolism, distribution and/or elimination [1]. Although *in vitro* pharmacokinetic interactions are not always clinically significant in the *in vivo* situation [2], it may in many cases indicate potential important interactions that can influence the bioavailability of co-administered drugs. This work reports on the effects of extracts from different botanicals such as whole leaf extracts and gels (*Aloe vera, Aloe ferox*) [3], corms (*Hypoxis hemerocallidea*), aerial parts (*Sutherlandia frutescens, Aspalathus linearis*) [4], fruit (*Sclerocarya birrea, Psidium guajava, Dovyalis caffra, Prunus persica, Fragaria ananassa, Prunus domestica*), and vegetables tubers (*Daucus carota, Beta vulgaris*) on *in vitro* drug transport as well as transport of phytoconstituents in crude extracts in comparison to purified compounds (*Hoodia gordonii, Sceletium tortuosum*) [5]. The mechanisms of drug transport alteration was determined for some of the botanicals by means of either measuring transport in two directions (to indicate efflux inhibition/induction) or by measuring the transepithelial electrical resistance (to indicate opening of tight junctions). The results showed that some of the plant extracts increased drug transport in the absorptive direction by decreasing drug efflux transporters, while others induced the efflux transporters. Some plant materials showed the ability to enhance drug transport by opening tight-junctions and thereby allowing paracellular drug transport. These types of pharmacokinetic interactions can lead to potentially significant clinical side-effects, but on the other hand they may be employed in a controlled way to enhance absorption of poorly absorbable drugs. **Keywords:** *In vitro* transport, herb-drug pharmacokinetic interactions, drug absorption enhancement, Caco-2, intestinal mucosa, buccal mucosa **References:** 1. Manzi SF et al. (2005) Clin Pediatr Emerg Med 6: 93 – 102. 2. Farkas D et al. (2008) Expert Opin Drug Metab Toxicol 4: 381 – 393. 3. Chen W et al. (2009) Planta Med 75: 587 – 595. 4. Brown L et al. (2008) J Ethnopharmacol 119: 588 – 592. 5. Vermaak I et al. (2011) Phytomed doi:10.1016/j.phymed.2011.01.017.

SL34 Comparative analysis of gene expression profiles from rats treated with a standardized willow bark preparation, its salicin rich ethanol fraction and imipramine for toxicological endpoints

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We reported earlier that the treatment of CD-rats with a standardized willow bark extract (WB) or its salicin rich ethanol fraction (EtOHFr.) (1) showed antidepressant like effects comparable to the tricyclic antidepressant imipramine in the Porsolt Swimming test (2). Corresponding gene expression profiles were further investigated for potential toxicological endpoints. Gene expression profiles (Agilent Whole Genome Array, n = 4/group) obtained from peripheral blood of male Sprague Dawley rats treated with WB (STW-33 – 1), the EtOHFr. (30 mg/kg bw) or imipramine (20 mg/kg bw) as described (2,3) were comparatively analysed by the Ingenuity Systems Programme, which allows to conduct model calculations of tresholds for theoretical toxic endpoints. The three treatments related to 47 disease clusters. The WB extract reached the treshold for a potential side effect once (cardiac hypertrophy), whereas the EtOHFr exceeded the treshold in 5 disease clusters (cardiac arteriopathy and stenosis, glomerular Injury, pulmonary hypertension). Disease clusters hit by imipramine↑alkaline phosphatase levels treatment (13) matched widely its reported side effects: cardiovascular system: tachykardia, palpitation, myocardial infarction, arrhythmias, heart block, precipitation of congestive heart failure; urinary retention, altered liver functions. Glomerular injury and altered liver functions are part of the side effect profile of aspirin in agreement with the findings for the salicin rich EtOHFr. The applied method appears to be useful for predictions of potential side effects. The phenomena that the WB extract, being effective, reached only once a potential "toxic endpoint" should be further investigated. It questions the commonly assumed principle that substances without side effects will have a poor efficacy. **Keywords:** Willow bark, Imipramine, gene expression, side effects, prediction **Acknowledgement:** *Dr. Anna Koptina is presently holding a scholarship of the DAAD (German academic exchange service). Parts of the work were supported by Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.* **References:** 1. Bonaterra GA et al. (2010) Phytomedicine 17(4): 1106 – 13 2. Ulrich-Merzenich G et al. (2009) Planta MedSL53 3. Ulrich-Merzenich G et al. (2009) Z.f. Phytotherapie 30 (Suppl 1) S 16

SL35 Antimicrobial investigations with impact – what can researchers do to ensure continuity?

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When reflecting on past antimicrobial studies undertaken on medicinal plants, it is clear that those having the most impact have been when a targeted disciplinary approach has been adopted. In order to elaborate on this, past and present studies will be presented with different approaches (pathogen specific, ethnopharmacological correlations, combination studies, structure activity relationships, formulations etc) in order to achieve outcomes that address recommendations made from previous antimicrobial reviews [1,2,3,4]. Medicinal plant use by inhabitants from Maputaland, Zululand (Southern Africa) were undertaken, whereby the *in vitro* antimicrobial investigations against diseases associated with diarrhoea, respiratory and sexually transmitted infections were validated. The outcomes of these studies address the need to investigate specific pathogens, interactive efficacies between different plant species and toxicity profiles. When popular essential oils (*Melaleuca alternifolia* Cheel, *Thymus vulgaris* L., *Mentha piperita* L. and *Rosmarinus officinalis* L.) were combined with conventional antimicrobials (ciprofloxacin and amphotericin B), a predominantly antagonistic interaction was noted, highlighting the need to not only focus on synergistic interactions. Structure activity relationships are important when reporting both the chemistry and antimicrobial activity and pharmacokinetic profiles of *Leptospermum petersonii* F.M.Bailey and the major compounds demonstrate the cidal effect of citral within 1 hr exposure to *Staphylococcus aureus*. Future formulation studies look promising, as demonstrated by the enhanced antimicrobial efficacy of *Melaleuca alternifolia* when encapsulated into polymeric liposomes. With analysis of past shortfalls and future recommendations, researchers should be encouraged to continue producing as much information as possible to allow for a better understanding of plant-based antimicrobial research. **Keywords:** antimicrobial, pathogen specific, ethnopharmacological correlations, combination studies, structure activity relationships, formulations **References:** 1. Rios JL, Recio MC (2005) J Ethnopharmacol 100: 80 – 84. 2. Cos P et al. (2006) J Ethnopharmacol 106: 290 – 302. 3. Van Vuuren SF (2008) J Ethnopharmacol 119: 462 – 472. 4. Van Vuuren SF, Viljoen AM (2011) Plant Med DOI http://dx.doi.org/10.1055/s-0030 – 1250736.

SL36 A structure-oriented approach for the accelerated and focused isolation of possible bioactive natural products based on UHPLC-HRMS/MS methods

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The high impact of Natural Products (NP) in medicine is proven and well sustained [1]. NP are characterized by unique structural diversity and critical drug-like features which rank them as the most promising candidates for possible future drugs [2]. However, several factors constrain their development related mostly to the time-consuming and labor procedures required for their isolation, the high cost and the dereplication problem [3]. In the present work, we propose a structure-oriented approach using UHPLC-HRMS/MS (LTQ-Orbitrap) methodologies trying to release the entire procedure from repeated and unuseful isolation steps and pursuing the targeted determination of the possible bioactive compound. This approach is applied in crude extracts and is based on fast UHPLC methods, high resolution mass spectra and ms/ms accurate mass measurements. As a proof of concept, the Cameroonian tree *Amphimas pterocarpoides* Harms (Leguminosae) was selected and flavonoids – isoflavonoids were chosen as the chemical class of interest. Based on a chromatographic (Rt, polarity) and spectrometric features (UV, accurate m/z, proposed ECs, RDB values and RIAS) as well as ms/ms spectra, the compounds of interest were defined and structurally elucidated. 12 of the 17 traced flavonoids were selectively isolated and characterized using 1 and 2D NMR techniques verifying our concept. Applying this approach, the identification of target-compounds is achieved early in the discovery procedure facilitating the dereplication of known compounds. Consequently much time required for the fractionation, isolation and purification is saved while the possibility of the discovery of novel structures and subsequently novel actives is elevated. **References:** [1] Newman DJ, Cragg GM (2007) J Nat Prod 70: 461 – 477. [2] Yuliana ND, Khatib A, Choi YH and Verpoorte R (2011) Phytother Res 25: 157 – 169. [3] Potterat O and Hamburger M, (2006) Current Organic Chemistry 10: 899 – 920.

SL37 Monodimensional and comprehensive liquid chromatography linked to mass spectrometry for unravelling bioactive components in medicinal plants

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There is considerable evidence nowadays that dietary flavonoids and other phenolic components may exert preventive and/or therapeutic effects in a role of human diseases. Despite the great interest in determining the role of phytonutrients as potential therapeutic agents, and the rising demand of natural sources with nutraceutic benefits, the antioxidant content of many medicinal plants and foodstuffs is unknown, making accurate estimation for human dietary consumption and the correlation to human diseases difficult. High resolution chromatographic techniques and specific and accurate detection may represent a key solution to obtain the identification power required for a complete knowledge on the qualitative composition and contents of these natural sources. This represents mandatory information, for rationale consumption and correlation of beneficial effects to dietary intake. This presentation will show applications of monodimensional and multidimensional comprehensive LC techniques to the study of bioactive compounds in medicinal plants extracts (Mulberry, Mate, Juniperus), by on-line coupling to quadrupole or IT-TOF mass detection. Identification of the separated constituents is carried out on the basis of the complementary information obtained from their migration times, diode array spectra, MS ions, and MS/MS fragments (obtained by CID experiments). Chromatographic resolution will benefit from the employment of partially porous stationary phases (2.7 µm d.p.), while the high mass accuracy and resolution of detection will help in unambiguous structural assignment of unknown molecules **Keywords:** Comprehensive Chromatography, IT-TOF, Bioactive Compounds, Polyphenols **Acknowledgement:** *The authors gratefully ackowledge Shimadzu and Sigma-Aldrich/Supelco for the continuous support*

SL38 Gas chromatography-mass spectrometry combined with mathematical chromatography as a powerful tool in the analysis of citrus fruits essential oils

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Citrus fruits essential oils are valuable natural products that are more popular nowadays in the world due to their effects on health conditions and their role in preventing and curing diseases [1]. Also, they have a broad range of applications in foods, perfumes, cosmetics and human nutrition. Gas chromatography-mass spectrometry (GC-MS) is the most important technique for the analysis of essential oils [2]. However, there are some fundamental problems in their analysis including baseline drift, spectral background, noise, low S/N, changes in the peak shapes and co-elution (overlapped, embedded peaks) [3]. Mathematical chromatography (MC) as a branch of chemometrics [4] attempts to develop new tools to handle these problems. In this work, first, we have extracted the essential oils of the peels of eighteen citrus fruits such as lemon, lime, mandarin, orange and grapefruit using hydrodistillation and then analyzed with GC-MS. Then, their signals were analyzed by MC. Using this strategy, the numbers of identified components were extended and quality of the results was improved significantly. As a positive consequence of using the proposed strategy human time and work are saved. Also, some new components were identified for the first time. In addition, we used our recently developed software, called MCRC Software for performing these techniques [5]. After resolving the volatile components in different samples, principal component analysis (PCA) was used for monitoring the pattern of volatile components in different samples. It is concluded that GC-MS+MC+PCA can open a new window to the comprehensive analysis of essential oils. **Keywords:** Chemometrics, Mathematical chromatography, Essential oil, Gas chromatography-mass spectrometry, Citrus fruits **Acknowledgement:** *Parastar H. would like to acknowledge the Iran National Elite Foundation for their support.* **References:** [1] Fisher K, Phillips C, (2008) Trends Food Sci Technol 19: 156 – 164. [2] Amigo JM, Skov T, Bro R, (2010) Chem Rev 110: 4582 – 4605. [3] Amigo JM, Popielarz MJ, Callejon RM, Morales ML, Troncoso AM, Petersen MA, Toldam-Andersen TB (2010) J Chromatogr A 1217: 4422 – 4429. [4] de Juan A, Tauler R, (2007) J Chromatogr A 1158: 184 – 195. [5] Jalali-Heravi M, Parastar H, Kamalzadeh M, Tauler R, Jaumot J (2010) Chemom Intell Lab Syst 104: 155 – 171.

SL39 Allium species of the subgenus Melanocrommyum are a rich source for cysteine sulphoxides

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The plant genus *Allium* (onions) is highly diverse. About 800 species are currently known belonging to several subgenera [1]. The main centre of distribution is the northern hemisphere, especially the area of Southwest und Central Asia. Inside this area, the subgenus *Melanocrommyum* is most prominent. Methiin **1** seems to be a nearly ubiquitous cysteine sulphoxide for the genus *Allium* (Figure 1). Propiin **2** is a minor compound, which was found in *A. ubipretense* R.M. Fritsch. The L-(+)-S-(3-pyridyl)cysteine sulphoxide **3** is the major cysteine sulphoxide of *A. stipitatum* Regel (0.22%, related to the fresh weight of bulbs) as well as the closely related *A. altissimum* Regel (0.50%) [2]. It seems to be possible that the corresponding N-oxide **3a** is also present in these plants. Most species of the subgenus do also contain the L-(+)-S-(3-pyrrolyl)-cysteine sulphoxide **4** [3]. High amounts of **4** were found in *A. jesdianum* Boiss. & Buhse subsp. *remediorum* R.M. Fritsch (0.52%), *A. macleanii* Baker (0.29%), *A. tschimganicum* (0.23%) and *A. rosenorum* (0.20%). Highest amounts of marasmin **5** were detected in *A. suworowii* (2.25%). Compound **5** is also present in *A. altissimum* and *A. stipitatum*. Especially the latter species is widely used as vegetable, spice and traditional medicine.

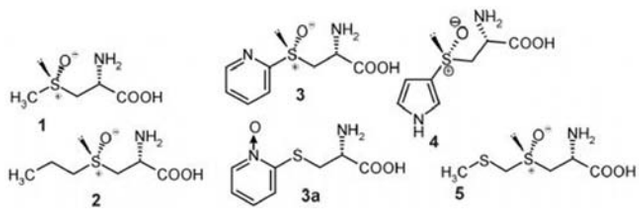

Figure 1: Typical cysteine sulphoxides of the subgenus Melanocrommyum

Keywords: Allium, Melanocrommyum, A. stipitatum, A. altissimum, A. jesdianum, A. rosenorum, A. suworowii, cysteine sulphoxides, marasmin
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SL40 Anticancer, antimicrobial and antiviral potentials of selected medicinal plants from the Island Soqotra

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Soqotra is considered the "jewel" of biodiversity in the Arabian Sea. Surveys have revealed that more than a third of the plant species of Soqotra are found nowhere else [1]. Fifty plants were collected, extracted with methanol and hot water and evaluated for their in vitro anticancer activity against three human cancer cell lines (A-427, 5637 and MCF-7) and for their antimicrobial activity against Gram-positive and Gram-negative bacteria as well as multiresistant *Staphylococcus* strains. Moreover, the antiviral activity of 25 plants has been assayed in two *in vitro* viral systems, influenza virus type A/MDCK cells and herpes simplex virus type 1/Vero cells, at non-cytotoxic concentrations. The methanolic extracts of *Ballochia atro-virgata* Balf.f., *Buxus hildebrandtii* Baill., *Dendrosicyos socotrana* Balfour, *Dracena cinnabari* Balf.f., *Eureiandra balfourii* Cogn., *Hypoestes pubescens* Balf.f., *Jatropha unicostata* Balf.f. and *Punica protopunica* Balf.f. *Withania aduensis* Vierh. and *Withania riebeckii Schweinf. ex Balf.f.* exhibited the highest toxicity on all tumor cell lines with IC_{50} values ranging between 0.29 and 8.2 μg/ml [2,3]. The greatest antimicrobial activity was found by the methanolic extracts of *Boswellia ameero* Balf.f., *Boswellia dioscorides* Thul. & Gifri, *Boswellia elongata* Balf.f., *Boswellia socotrana* Balf.f., Buxus hildebrandtii, *Commiphora ornifolia* (Balf.f.) J.B.Gillett, *Commiphora parvifolia* Engl., *Euclea divinorum* Hiern, *Euphorbia socotrana* Balf.f., *Jatropha unicostata*, *Kalanchoe farinacea* Balf.f., *Leucas samhaensis* Cortés-Burns & A.G.Mill., *Leucas virgata* Balf.f., *Pulicaria stephanocarpa* Balf.f., Punica protopunica, *Rhus thyrsiflora* Balf.f., *Teucrium socotranum* Vierh., *Withania adunensis* and *Withania riebeckii* [3,4]. The methanolic extracts of *Boswellia ameero, Boswellia elongata, Buxus hildebrandtii*, *Cissus hamaderohensis* Radcl.-Sm., *Cleome socotrana* Balf.f., *Exacum affine* Balf.f., *Jatropha unicostata* and *Kalanchoe farinacea* showed anti-influenza virus type A activity with IC_{50}-values from 12.5 to 0.7 μg/ml [5]. In addition, 15 plants of the 25 investigated exhibited anti-HSV-1 activity [5]. **Keywords:** Soqotra, Medicinal plants, Anticancer, Antimicrobial, Antiviral **References:** 1. Miller, G. A., Morris, M. (2004) Ethnoflora of the Soqotra Archipelago. The Royal Botanic Garden Edinburgh, UK: Printed by the Charlesworth Group, Huddersfield, UK. 2. Mothana RAA, Grünert R, Lindequist U, Bednarski PJ (2007) Pharmazie 62: 305 – 307. 3. Mothana RAA, Lindequist U, Grünert R, Bednarski PJ (2009) BMC Complement Altern Med 9: 7. 4. Mothana, RAA, Lindequist U (2005)J Ethnopharmacol 96: 177 – 181. 5. Mothana, RAA, Reiss C, Mentel R, Lindequist U (2006) Phytother Res 20: 298 – 302.

SL41 Toxicological evaluation of DAS-77® – a herbal preparation

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DAS-77® is a herbal preparation that contains the milled bark of *Mangifera indica* L. and root of *Carica papaya* L. (1:1). It is used for the treatment of various ailments in Nigeria hence this toxicity assessment. In the acute toxicity study, DAS-77® (constituted in dH_2O) was administered to mice *p.o.* up to 10 g/kg and *i.p.* at 250 – 3000 mg/kg. Mortality within 24 h was recorded. In the chronic toxicity study, rats were orally treated for 90 days at doses of 80, 400 (therapeutic dose, TD) and 2000 mg/kg. Rats were weighed and observed for feeding and drinking habits. By 90 days, animals were sacrificed and blood samples collected for haematological and biochemical analysis. Organs were harvested for weight determination, antioxidants and histopathological assessments. DAS-77® did not produce any lethality administered *p.o.* up to 10 g/kg but the *i.p.* LD_{50} was 1122 mg/kg. At TD, DAS-77® produced significant changes ($p < 0.05$) only in body weight and food intake (↓), ovary weight (↑), neutrophils (↑), HDL (↑), and K^+ (↓), which were reversible. Histopathological presentations were generally normal. Effects at the other doses were comparable to those at TD except for reversible changes in *in vivo* antioxidants (↑) in the liver, kidney and testes, liver enzymes (↓) and sperm parameters (motility [↓], count [↓] and abnormality [↑]). DAS-77® was found to contain tannins (3.26 ± 0.15%), saponins (2.32 ± 0.04%), phenols (1.31 ± 0.07%), flavonoids (0.54 ± 0.02%) and alkaloids (0.04 ± 0.01%) w/v. Findings in this study revealed that DAS-77® is relatively safe with the potential for enhancing *in vivo* antioxidant activity. However, possibly reversible side-effects include electrolyte imbalance and sterility in males. **Keywords:** DAS-77® herbal preparation, acute toxicity, chronic toxicity

SL42 The design of DNA barcode-specific PCR primers for medicinal plant authentication

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The DNA Barcode of Life initiative aims to obtain designated "barcode" sequences for every known species on Earth. Proponents of plant DNA barcoding have cited medicinal plant authentication as one of the potential applications of barcode information. However, DNA sequencing of barcode regions may not be suitable for routine quality assurance testing of plant materials, which could contain mixtures of plant species and/or degraded DNA. The value of DNA barcoding in this arena may in fact lie in its role as a platform for the design of standardised DNA-based tests. We have studied three groups of plant species (*Hypericum spp. Actaea spp.* and *Rhodiola spp.*), each comprising a target commercial medicinal plant and known or potential adulterant species. The suitability of four "barcode" regions for the design of species-specific PCR primers was determined; the designated plastid *rbcL* and *matK* barcode regions, the candidate plastid *trnH-psbA* spacer barcode region and the nuclear ribosomal ITS region. Problems were encountered with the plastid barcodes (low inter-specific variation of *rbcL*, lack of reliable generic primers for *matK*, repetitive sequences in *trnH-psbA*) and for all three groups of plants the nrITS region proved to be most appropriate for primer design. Targeting the nuclear genome also allows discrimination of hybrids that may not be detected using plastid barcodes. Whilst DNA barcoding may prove to have a role in plant species identification, the current choice of universal plant barcodes may not be ideal for the development of routine authentication tests. **Keywords:** authentication, DNA barcode, PCR primer, ribosomal ITS

SL43 Traditional use as a regulatory category – experiences in Europe

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Directive 2004/24/EC established a regulatory pathway for traditional herbal medicinal products in the European Union that allows the registration and marketing without the standard clinical data packages on safety and efficacy as required for other medicinal products. Five main

criteria have to be fulfilled to guarantee safety in specified conditions: (1) indications suitable for self medication, (2) specified strength and posology, (3) appropriate route of administration, (4) a minimum 30 years period of traditional use and (5) sufficient data to prove that a product is not harmful in specified conditions and effects are plausible based on long-standing use and experience. Thus, the traditional use in the regulatory sense comprises well-defined restrictions that deviate from the concept of traditional use in sensu lato but also standard information generated in ethnopharmacological research. The necessary documentation for compliance with the five criteria are presented using examples of herbal substances from monographs as published by the Committee on Herbal Medicinal Products at the European Medicines Agency. Typical issues of data generation and availability are demonstrated. It is further discussed why not all types of traditionally used medicines or derived preparations fit into the current regulatory framework – even if scientific and historic evidence is available. Conclusions are drawn on the opportunities and limits for use of ethnopharmacological research data for regulatory purposes.

SL44 *Sceletium tortuosum* - an ancient treatment for modern CNS-related disorders

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Members of the genus *Sceletium* (Mesembryanthemaceae) have been used for millennia as masticatories, for the relief of thirst and hunger, to combat fatigue, as medicines, and for social and spiritual purposes by San hunter-gatherers (historically referred to as Bushmen) and Khoi pastoralists [1,2]. Recently, several formulations containing *Sceletium* have been commercialised and marketed to treat anxiety, stress and tension. In 2010 the South African Government granted the country's first ever integrated export and bioprospecting permit to a local pharmaceutical company who will market *Sceletium* in a joint venture with a neutraceutical company in the USA. Based on the interesting ethnobotanical information and the upsurge in commercial interest we embarked on a comprehensive biosystematic and biopharmaceutical study of *S. tortuosum* and its major alkaloids, mesembrenone, mesembrenol, mesembrine and mesembranol. Wild *S. tortuosum* (L.) N.E.Br. plants (n = 150), sampled from different localities (n = 31) in the south western region of South Africa, were examined for the phytochemical variability using GC-MS. The potential effect of chemotypic variation on product formulation and quality assurance protocols will be discussed. *In vitro* permeation studies of the alkaloids and different crude plant extracts (water, methanol and enriched alkaloid acid-base extract) across porcine buccal, sublingual and intestinal mucosa were also conducted to predict the bioavailability of the alkaloids. Absorption across intestinal mucosa was highest, but buccal and sublingual absorption contributed to overall bioavailability. In addition to results generated in our laboratory, a general overview on the botany, chemistry and ethnopharmacology of *S. tortuosum* will be presented. **Keywords:** *Sceletium tortuosum*, alkaloids, bioavailability, quality control **Acknowledgement:** *National Research Foundation, Tshwane University of Technology, R&R abbatoir (Pretoria).* **References:** 1. Smith et al (1996) J Ethnopharmacol 50: 119 – 130 2. Gericke N, Viljoen AM (2008) J Ethnopharmacol 119: 653 – 663

SL45 Antidiabetic Effect of *Juniperus oxycedrus* subsp. *oxycedrus* Berries by Using Bioactivity Guided Fractionation

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Juniperus species are widely used in traditional medicine for many purposes in Anatolia. According to ethnobotanical reports which were carried out in different parts of Turkey, berries of *Juniperus oxycedrus* L. subsp. *oxycedrus*, have been ingested or decoctions of berries have been taken as tea to lower blood glucose levels (1,2). The extracts of berries were given to normal, glucose loaded and streptozotocin induced diabetic rats at 0.5 and 1 g/kg doses. Joso extracts were found to posses antidiabetic effect. Additionally, blood glucose levels of normoglycemic and glucose loaded rats were decreased by administration of ethanol extract. After 10 days administration to diabetic rats, blood glucose and malondialdehyde levels in kidney and liver were decreased significantly. Etanol extract was fractionated by successive solvent-solvent extraction. Sub-extracts were given to the diabetic rats. Results indicated that n-butanol sub-extract (740 mg/kg) were found to possess high antidiabetic activity. n-Butanol sub-extract was fractionated by column chromatography and the active principles were isolated. Shikimic acid, ferulic acid glycoside and oleuropeic acid glycoside were found in the active fraction of the extract. Since shikimic acid was found as major component of the active fraction, it was administrated to diabetic rats at 15 and 30 mg/kg doses. After subacute administration of shikimic acid, some biochemical parameters and tissue antioxidant levels were also evaluated. Whereas insulin level was not elevated blood glucose levels were decreased significantly by shikimic acid (24%). Moreover, malondialdehyde levels in kidney tissues (63 – 64%) and liver enzymes were decreased by subacute administration of shikimic acid. **Keywords:** Juniperus oxycedrus, juniper, antidiabetic, berry, hypoglycaemic, shikimic acid **Acknowledgement:** *We specially thank to Prof. Dr. Erdal Bedir for his help in isolation and identification of the active constituents.* This study was supported by Scientific Research Projects Management of Gazi University (07/2007 – 07). **References:** 1. Tuzlacı E, Erol MK (1993) Fitoterapia 70: 593 – 610. 2. Honda G, Yeşilada E, Tabata M, Sezik E, Fujita T, Takeda Y, et al. (1996) J Ethnopharmacol 53(2): 75 – 87.

SL46 Euphol, a novel cannabinoid agonist, prevents inflammatory and neuropathic persistent pain in rodents

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The cannabinoids have been considered a relevant target for pain management. Persistent pains, related to inflammatory and neuropathic states, are prevalent and debilitating diseases, which remain without safe and adequate treatments. Inflammatory and neuropathic pain was induced by carrageenan, CFA, PSNL, cancer/chemotherapeutic agent, PGE2 and PKCε agonist. Pro-inflammatory mediators were measured by immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) and real time-PCR. Here, we reported that euphol exhibited pronounced and long-lasting oral analgesia in several rodent behaviour models of inflammatory and neuropathic persistent pain. These effects were markedly blocked by CB1 or CB2-selective antagonists and oligonucleotide antisense. Of note, cannabinoid receptor binding experiments showed that euphol directly bound with high affinity to both CB1 (Ki = 71.090 nM) and CB2 (Ki = 0.037 nM), being 1,880-fold more selective for CB2 receptors. Euphol at similar dose inhibited the levels/mRNA expression of pro-inflammatory mediators, as well the myeloperoxidase activity. Our data indicate that euphol activate transcription factors, such as PPAR-γ and inhibiting both NF-κB and CREB activation, associated with the inhibition of COX-2 and PKCε expression, either at the spinal cord or the dorsal root ganglia level. Of relevance, euphol did not display significant central nervous system alterations. Acute toxicological studies carried out in rodents showed that euphol is safe and well tolerate. Therefore, euphol represents a novel orally active and safe natural analgesic for the management of inflammatory and neuropathic pain states. **Keywords:** Euphol, cannabinoid receptors, pain, inflammation **Acknowledgement:** *This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Programa de Apoio aos Núcleos de Excelência (PRONEX), and the Fundação de Apoio a Pesquisa do Estado de Santa Catarina (FAPESC), all of Brazil. R.C.D., K.A.B.S.S., A.F.B., A.F.P. and R.M. are Ph.D. students in pharmacology receiving grants from CNPq.*

SL47 Analogues of a lupane-type triterpene as potential anti-HIV agents

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Infection with human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), continues to be ranked high on the list of the most important health issues facing the world. Although significant progress has been made since the introduction of highly active antiretroviral therapy, it has also led to increased adverse effects and the emergence of multidrug-resistant viral strains. Therefore, there is a need for new classes of drugs involving novel molecular mechanisms [1]. Many classes of natural products and some of their analogues have been tested for their anti-HIV activity [2]. In fact, modification of betulinic acid led to the discovery of bevirimat, a first-in-class drug candidate as a viral maturation inhibitor [3]. The goal of our study was to get new insights into the antiviral potential of lupane-type triterpenes that could inhibit HIV-1 replication and would be useful for the design of new drugs with clinical application [4]. Therefore, we prepared 17 derivatives based on the betulin scaffold, whose structures were determined by spectroscopic studies, and comparison with data previously reported. These derivatives and betulin were tested for their ability to inhibit the HIV replication. Two compounds of this series exhibited a promising activity at 10 μM with replication inhibition percentages of 26 and 31%, respectively. A study of the influence of the substitution pattern on the lupane skeleton revealed that oxidation at C-3, acetylation at C-28 and modification of the isoprenyl moiety play an important role in the activity. **Keywords:** Lupane triterpene; betulin analogues; anti-HIV agents **Acknowledgement:** *We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support. CO thanks the CajaCanarias for the fellowship.* **References:** 1. Mehellou Y, De Clercq (2010) Eur J Med Chem 53: 521 – 538. 2. Cassels B K, Asencio M (2010) Phytochem Rev [accessible online at: http://dx.doi.org/10.1007/s11101 – 010 – 9172 – 2 (25 March 2010)]. 3. Qian K, Kuo R-Y, Chen C-H, Huang L, Morris-Natschke SL, Lee K-H (2010) J Med Chem 53: 3233 – 3141. 4. Lan P et al. (2010) Med Chem Res [accessible online at: http://dx.doi.org/10.1007/s00044 – 010 – 9467 – 2 (21 October 2010)].

SL48 *Hoodia gordonii*: Quality control and biopharmaceutical aspects

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Hoodia gordonii Sweet is a popularly consumed commercially available weight loss product. Therefore, developing rapid quality control methods for raw material and products, and investigating key biopharmaceutical aspects of the perceived active ingredient P57, is of utmost importance. High performance thin layer chromatography (HPTLC) and vibrational spectroscopy coupled with chemometric analysis are attractive alternative quantification methods for P57. The *in vitro* transport of pure P57 and P57 from crude plant extracts across porcine intestinal and buccal tissue was also investigated. The HPTLC system produced good band separation including the P57 band [1] and linear calibration curves with good correlation coefficient (R2) values of 0.9706 – 0.9993 were developed for P57 quantification. For the NIR spectroscopy data, the partial least squares projections to latent structures (PLS) model with 2nd derivative pre-processing predicted P57 content with an R2 value of 0.9629 and a root mean square error of prediction (RMSEP) of 0.03% [2]. Pre-processing of the Raman data with orthogonal signal correction yielded a PLS model with an R2 value of 0.9986 and an RMSEP of 0.004% [3]. Pure P57 was transported across porcine intestinal tissue at a much lower rate and extent than P57 from the crude extract. P57 was transported across the buccal mucosa when applied in the form of a crude plant extract but no transport was detected for pure P57 [4]. The availability of rapid alternative quantification methods may positively influence the quality of distributed raw materials and products. In addition, the current knowledge on biopharmaceutical aspects of P57 was increased. **Keywords:** *Hoodia gordonii*, *in vitro*, P57, quality control, transport **References:** 1. Vermaak I et al. (2010) S Afr J Bot 76: 119 – 124. 2. Vermaak I et al. (2010) Food Chem 120: 940 – 944. 3. Vermaak I et al. (2010) Phytochem Lett 3: 156 – 160. 4. Vermaak I et al. (2011) Phytomedicine in press doi.org/10.1016/j.phymed.2011.01.017].

SL49 Application of liquid chromatography and mass spectrometry methods in pharmacognostic investigations of Traditional Chinese Medicines (TCM)

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TCM has a long history to treat human diseases in China. In our investigations to depict medicinal functionalities of TCM using LC-MS related technologies, a diversity of new chemical structures including novel skeletons were acquired.[1] These molecule-orientated technologies have been integrated effectively into the pharmacognostic investigations of medicinal herbs. In our chemical study of *Stemona* species, a HPLC-MS^n method was developed for the characterization of alkaloids with a pyrido[1,2-a]azepine A, B-ring core from *S. saxorum* Gagnep. based on the ESI-MS^n results of five reference compounds.[2] 41 components were separated, of which 12 compounds (4 new) were identified as *Stemona* alkaloids with such a core. A practical HPLC method was designed to detect the content of shikimic acid, the start material of Tamiflu, in Chinese *Illicium* plants from 21 different species or habitats. The minor toxic anisatin and its analogues were also monitored by an UPLC-MS/MS method. Our results provided scientific evidences for safe usage of fruits of *Illicium* plants. Additionally, new series of sesquiterpenoids and phenylpropanoid flavonoid polymers were identified from these species.[3] *Marsdenia tenacissima* (Roxb.) Moon is rich in C21 steroidal glycosides, of which some were proved to reverse the multi-drug resistance through inhibiting P-gp, ABCG2, and MRP1 transporters. An UPLC/ESI-LTQ method was established to quantitatively monitor the biotransformation of its extract by human intestinal bacteria. The results showed that three major compounds were degraded to their corresponding de-sugar active derivatives within 4 hours. This provided well evidence for TCM as a pro-drug, and a possible new way to verify its efficacy. **Keywords:** HPLC-MS^n, *Stemona saxorum*, *Illicium* plants, *Marsdenia tenacissima*, *Stemona* alkaloids, shikimic acid, anisatin, C21 steroidal glycosides **References:** 1. Lin LG, et al. (2007) Tetrahedron Lett 48: 1559 – 1561. 2. Peng SY, et al. (2009) Rapid Commun Mass Spectrom 23: 3621 – 3631 3. Zhu Q, et al. (2009) J Nat Prod 72: 238 – 142.

SL50 Effect of Catechins Extract of Green Tea on Quality Protection of Black Tea-bag During Storage

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Green tea is one of the richest sources of antioxidants especially catechins, which are studied most because of their health benefits. These valuable compounds may lose their activity and oxidize to thearobigin during fermentation stage or black tea processing. The main objective of this research is applying antioxidant mixtures extracted from green tea on packaging material of black tea and determining the amount of antioxidants released from the tea bags by their immersion into boiling water. The green tea extracts prepared by a two-step extraction process in water at 50 °C and 80 °C in four individual time periods, i.e., 10 min, 10 min; 10 min, 20 min; 20 min, 10 min; 20 min, 20 min (1). Final extract from each process was analyzed by HPLC and DPPH tests and then sprayed on the tea bag packaging material.The activity and concentration of antioxidants (catechins) released from treated tea bags were investigated immediately and after 3 months storage. Sensory properties

of the tea beverage samples were also studied. The results (Tables 1, 2) show that the concentration of antioxidants increases by 66 – 76% as compared to control i. e., untreated black tea. Tea beverage samples were more astringent as compared to untreated black tea. Under different time/temperature conditions the best results were obtained by the sample extracted by a two-step process (20 min at 50 °C) and (10 min at 80 °C) with the highest concentration of antioxidants, lowest astringency and best stability during 3 months storage. **Keywords:** Green tea, Antioxidant activity, Catechins, Black tea bag **References:** 1. Labbe, D, et al. (2008) Food Chem. 111:139 – 143.

Table 1: Effect of extraction time on concentration/activity of the antioxidants released from treated tea bags immediately after preparation

Extraction time (min)	Total antioxidant	
	Concentration (µg/mL)	Activity (%)
10,10	0.52 ± 0.02a	71.70 ± 0.35a
10,20	0.57 ± 0.05a	72.58 ± 0.51a
20,10	0.62 ± 0.07b	72.88 ± 1.26b
20,20	0.64 ± 0.01b	73.34 ± 0.35b

Table 2: Effect of extraction time on concentration/activity of the antioxidants released from treated tea bags after 3 months storage

Extraction time (min)	Total antioxidant	
	Concentration (µg/mL)	Activity (%)
10,10	0.44 ± 0.01a	67.70 ± 0.35a
10,20	0.49 ± 0.01a	66.67 ± 0.42a
20,10	0.56 ± 0.02b	70.54 ± 0.30b
20,20	0.58 ± 0.01b	67.83 ± 0.38b

SL51 Determination of Hypericin in St. John's Wort (*Hypericum perforatum* L.) extracts using HPLC-ED

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The genus *Hypericum*, which comprises about 450 species of herbs, shrubs and trees, belongs to the family Clusiaceae (Guttiferae), formerly Hypericaceae. One of the topmost and commercially renowned species of the genus *Hypericum* is *Hypericum perforatum* L., commonly known as St. John's Wort. *Hypericum perforatum*, is well known for its profound pharmacological activities as antidepressant, anxiolytic, antiviral, antimicrobial and wound healing. In this study, using HPLC-ED system, quantitative analysis of hypericin was carried out in different water-methanolic extracts of St. John's Wort. Hot of water-methanol extracts of St. John's Wort were prepared. The herba (1 g) was powdered and extracted with water-methanol [(1:1) 10 mL]. Afterward 1 mL of that extract was centrifuged, obtaining supernatant which was used for analysis. The standard was hypericin purchased from HWI Analytic GmbH, Germany. Mobile phase: methanol-acetonitrile, water, acetic acid (20+10+70+1), electrochemical detector with range 50 nA, potential + 0.84 V, filter, 0.02 Hz, flow rate, 1 mL/min: and temperature 25 °C. Injection volume: 20 µL. Concentrations of standard were 0.1 µg/20 µL, 0.2 µg/20 µL, and 0.4 µg/20 µL. Retention time of hypericin in the standard solution and herbal extracts were 28.4 minutes. Limit of detection was 100 ng of hypericin. Amount of hypericin in extracts of St. John's Wort was 25 – 26 mg/g dry weight. The very high content of hypericin in Bosnian St. John's Wort give more importance to this plant as traditional drug for its use in official medicine. **Keywords:** hypericin, Hypericum perforatum, HPLC-ED, determination **References:** Robson N, (2003) Hypericum botany. In: Ernst, E. (Ed.), Hypericum: the Genus Hypericum. Taylor and Francis, New York, pp. 196 – 241. Anyżevska M, Kowalczuk A, Łozak A, Jabłczyńska R, Fijałek Z, (2010) Acta Poloniae Pharmaceutica-Drug Research67(6): 587 – 593. Nuevas-Paz L, Molina-Torres J, Prieto-González S (2005) Acta Farm Bonaerense 24(1): 89 – 90.

SL52 Differentiation Effects of Prenylflavanones and Chalcones present in Hops in Neuronal Tissue Cultures

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Hops (*Humulus lupulus* L.) as medicinal plant is abundant in rare polyphenols. Besides so called bitter-acids important in brewing there are chalcones like xanthohumol and flavanones. Particularly 8-prenylnaringenin, a flavanone is the most prominent prenylflavanone since it is known as a very potent phytoestrogen [1]. Besides 6- or 8-prenylnaringenin, hop extracts yield a number of minor compounds related to each other by ring closure reactions, e. g. 8-prenylnaringenin is formed from desmethylxanthohumol, so are many more. The potency of hop flavonoids to induce at least growth arrest in leucemic cell cultures is nearly equipotent and remarkable [2]. The question addressed in this work was if this effect would be observed even in neuronal neoplastic cells types since hop flavonoids may cross the blood brain barrier [3]. A commercial extract is available enriched in rare prenylflavonoids and xanthohumol. It is prepared mostly from a residue of the CO_2 extraction process by solvent extraction. This material was used in a process of various chromatographic techniques to purify pairs of complementary chalcones and flavanones with identical side chains. Xanthohumol and Isoxanthohumol particularly represent such a pair to get some hint of a structure activity relationship. Growth inhibitors frequently induce differentiation in highly proliferating clones. Here some hop flavonoids appeared to be distinghuished since they may have converted proliferation into differentiation. **Acknowledgement:** *Wissenschaftliche Station für Brauerei in München e. V., Dr. Biendl Hallertauer Hopfenveredelungsgesellschaft m.b.H.* **References:** 1. Milligan SR et al. (2000) J Clin Endocrin Metab 85: 4912 – 4915. 2. Diller RA et al., (2007) Planta Med 73: 755 – 761. 3. Butterweck V et al., (2007) J Pharm Pharmacol 59: 549 – 552.

SL53 Evaluation of total phenolics, flavonoids and anti-inflammatory property of ethanolic extracts of *Paulownia tomentosa* var. *tomentosa* bark

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Trees containing polyphenols and flavonoids have been reported to possess strong anti-inflammatory activity [1]. As one chain of our systematically screening the potential bioactivities of *Paulownia tomentosa* (Thunb.) Steud. var. *tomentosa* (Scrophulariaceae), a medicinal hardwood grown native to China and widely used in folk remedies to treat various diseases including inflammatory [2], in the present study, we evaluated the anti-inflammatory property of *P. tomentosa* var. *tomentosa* bark. The anti-inflammatory activity was investigated by evaluating the inhibitory effect of ethanolic extracts of the tree's bark against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in murine macrophages cell line RAW264.7 [3]. Resveratrol, an excellent anti-inflammatory agent, was used as a positive control. The amount of total phenolic compounds (Approx. 180.8 mg/100 g dry sample) and total flavonoids (Approx. 39.6 mg/100 g dry sample) of *P. tomentosa* var. *tomentosa* bark were also determined by Folin Ciocalteu reagent and aluminium chloride method, respectively. Results of the investigation revealed that ethanolic extracts of the *P. tomentosa* var. *tomentosa* bark possessed significant ant-inflammatory activity. Also, the activity was found to be concentration dependent. This work will provide ample opportunities for further investigation to develop high value added anti-inflammatory products from *P. tomentosa* var. *tomentosa*. **Keywords:** total phenolics, total flavonoids, anti-inflammatory property, Paulownia tomentosa var. tomentosa, bark **Acknowledgement:** *This work was financially supported by National Natural Science Foundation of China (NSFC, No. 31000279), Program for New Century Excellent Talents in University (NCET 2010) and Natural Science Foundation of Tianjin City (No. 09JCYBJC 15800).* **References:** 1. Pelzer LE et al. (1998) Farmaco 53: 421 – 424. 2. Si, CL et al. (2009) Holzforschung 63: 440 – 442. 3. Tewtrakul S et al. (2011) J Ethnopharmacol 133: 63 – 66.

SL54 Mite growth regulatory activity of *Blechnum chilense* (Kaulf.) Mett

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The genus *Blechnum* has 13 common and well-distributed species in Chile [1]. *B. chilense* plants have been used for various purposes [1,2]. *Tetranychus urticae* Koch is highly polyphagous species with 1094 host plant species reported worldwide to date [3]. In this research, we report a phytochemical analysis of *B. chilense* and its mite growth regulatory effects. From the n-hexane fraction four phytoecdysones were isolated: ecdysone, ponasterone, shidasterone and 2-deoxycrustecdysone. We conducted a bioassay with *T. urticae* eggs placed on *Phaseolus vulgaris* L. leaf discs previously treated and the development stage was recorded every 24 hours during thirteen days. All treatments showed statistical significant differences regard to Control in emerged and living adults. The EtOAc fraction at 250 ppm and the n-hexane fraction at 250 ppm and 100 ppm caused the greatest mortality of nymphs and almost the total mortality of the low quantity of adults that emerged (Fig. 1). Our results shows that even low concentrations of 10 ppm in both fractions affects the life cycle of *T. urticae* causing a significant decrease in its population. Studies on the identification and physiological role of ecdysteroids in mites are minimal [4], especially in the order Prostigmata. Our results suggest that early exposure of eggs and larvae to phytoecdysones from *B. chilense* may interfere in the natural ecdysteroid metabolism in *T. urticae* leading to the death of nymphs and adults. Our results suggest too that the deaths could be caused by starvation due to the deterrent effects of some phytoecdysones [5,6].

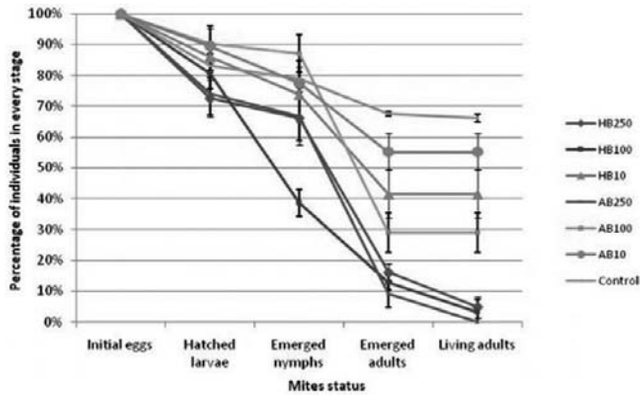

Figure 1: Percentage of T. urticae individuals in every stage treated with different fractions of B. chilense.
HB250: n-hexane fraction at 250 ppm, HB100: n-hexane fraction at 100 ppm, HB10: n-hexane fraction at 10 ppm, AB250: EtOAc fraction at 250 ppm, AB100: EtOAc fraction at 100 ppm, AB10: EtOAc fraction at 10 ppm.

Keywords: Blechnum chilense, phytoecdysones, Tetranychus urticae **Acknowledgement:** *Ministerio de Agricultura y Desarrollo Rural de Colombia, Grupo de Investigaciones Agroindustriales (GRAIN) and Centro de Investigación para el Desarrollo y la Investigación (CIDI) from Universidad Pontificia Bolivariana, Universidad de Antioquia, Ceniflores (Colombia), Dirección de Investigación de la Universidad del Bío-Bío (Grant DIUBB) (Chile) and Prof. David Seigler from Plant Biology Dept. University of Illinois, at Urbana-Champaign (USA).* **References:**
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SL55 New rare atropisomers: structure elucidation, absolute configuration and antimicrobial activity

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Fungi are very known to produce polyketides, which are structurally a very diverse family of natural products with interesting biological activities and pharmacological properties. We report in this study the chemical investigation of two endophytic fungi, *Stemphylium globuliferum* and *Talaromyces wortmanii*. Several rare new atropisomers were isolated and identified, including homo- and heterodimeric bisanthraquinones. The structure of isolated compounds were determined on the basis of one- and two-dimensional NMR spectroscopy and mass spectrometry. The absolute stereochemistry of the new compounds was established by means of TDDFT ECD calculations. Furthermore, the isolated compounds exhibited antibacterial activity against multi drug resistant strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium* and *Enterococcus cloacae*. In addition, the anti-fungal activity of the isolated compounds was measured against drug resistant strains of *Aspergillus fumigatus*, *Aspergillus faecalis*, *Candida albicans* and *Candida krusei*. Interestingly, among the compounds isolated, only the new altersolanol N, tetrahydroaltersolanol B and altersolanol C were active against HV2 and HV8 human viruses. **Keywords:** Endophytes, Atropisomers, Spectroscopy, CD calculation, Antimicrobial activity

SL56 Advances of Infrared Spectroscopic Imaging and Mapping Technologies of Plant Material

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Fourier Transform Infrared (FTIR) spectroscopic imaging and mapping techniques have become essential tools for the detection and characterization of the molecular components of biological tissues [1]. These modern analytical techniques enable high-resolution molecular imaging of complex botanical samples [2]. Imaging and mapping are based on the absorption of IR radiations by vibrational transitions in covalent bonds and their major advantage is the acquisition of local molecular expression profiles, while maintaining the topographic integrity of the tissue by avoiding time-consuming extraction, purification and separation steps. All IR related information is recorded in a so called "hyperspectral cube" from which any ingredient relevant information can be selectively extracted with a resolution of 4 µm and transformed into an image as depicted for e. g. Urtica dioica in Figure 1. These new techniques enable global analysis of biological samples with highest spatial resolution and provide unique chemical-morphological information about the tissue status. With these non-destructive examination methods it is possible to get qualitative and quantitative information of heterogeneous samples. In this presentation recent applications of infrared spectroscopic imaging and mapping technologies of plant material are introduced and discussed. **Keywords:** Phytomics, tissue, instrumentation, data processing, transmission, reflection, hyper spectral imaging

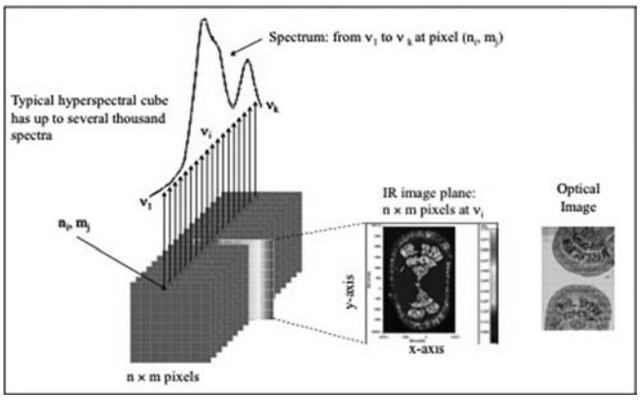

Figure 1: Model of hyperspectral cube

Acknowledgement: *Eurasia-Pacific Uninet (EPU) (Salzburg, Austria), the Ministry for Science and Research and the Ministry for Health, Family and Youth (Vienna, Austria) (Novel Analytical Tools for Quality Control in Traditional Chinese Medicine, Project No. 80855), the Leopold-Franzens University, Innsbruck (Nachwuchsförderung) for financial support.* **References:** 1. Pezzei C, Pallua JD, Schaefer G, Seifarth C, Huck-Pezzei V, Bittner LK, Klocker H, Bartsch G, Bonn GK, Huck CW (2010) Mol Biosyst 6: 2287. 2. Pallua JD, Pezzei C, Huck-Pezzei V, Schönbichler S, Bittner LK, Bonn GK, Saeed A, Majeed S, Farooq A, Najam-ul-Haq M, Abel G, Popp M and Huck CW (2011) Curr Bioactive Comp 7: in press.

SL57 Study on antibacterial and antioxidant activities against dimer, trimer and tetramer resveratrol from Malaysian's *Dipterocarpus verrucosus*

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Dipterocarpus verrucosus Foxw. ex Slooten, known as "keruing merah" by the local is a species of tree in the family Dipterocarpaceae (Symington, 1974). The stem bark was collected from Jengka, Pahang, Malaysia and extracted in acetone and methanol. The phytochemical investigation of this plant led to the isolation of three types of resveratrol compound, laevifonol (dimer), α-viniferin (trimer) and vaticanol B (tetramer). The compound structure was determined by NMR spectra and also in comparison with the previously reported data. Biological activities of the compounds were evaluated against six strains of bacteria; *Pseudomonas aeruginosa*, *Klebsiella pneunomonia*, *Salmonella paratyphi*, *Bacillus subtilis*, *Stapylococcus aureus* and *E.coli* by disc diffusion method (Barry at el., 1994) while antioxidant were evaluated by DPPH (Blois, 1958), TPC (Velioglu et al., 1999), FTC (Osawa and Namiki 1981 and TBA (Kikuzaki and Nakatani 1993). The DPPH radical scavenger test showed that tetramer gave better result (36.6%) as compared to the dimer and trimer. TPC evaluations showed that the tetramer and dimer contain the same amount of phenolics which is 616.15 mg/g of GAEs while trimer displayed lower amount of 340 mg/g of GAEs. FTC and TBA methode revealed that the trimer showed better inhibition among the others with the value of 77.77 and 86.47% each. Antibacterial activity, trimer resveratrol with concentration of 50 mg/ml showed to be the most active with inhibition toward *Stapylococcus aureus* (8.8 mm), *Pseudomonas aeruginosa* (8.5 mm) and *E.coli* (17 mm). These biological data suggested that no regular pattern is observed between molecular size and biological properties. **Keywords:** *Dipterocarpus verrucosus*, Dipterocarpaceae, oligostilbenoid, antibacterial, antioxidant **Acknowledgement:** *We wish to thank to Ministry of Higher Education Malaysia for financial support via FRGS grant 600-RMI/ST/FRGS 5/3/Fst(3/2008) and for Phd study leave as well as UiTM for all the support* **References:** Barry AL, Coyle MB et al. (1979) J Clinical Microbiol 10: 885 – 889. 2-Blois MS (1958) Nature 181: 1199 – 1200. 3-Symington CF (1974) Foresters' Manual of Dipterocarps. University Malaya Publication, Kuala Lumpur, pp. 1 – 356. 4-Velioglu YS, Mazza G, Gao L, Oomah BD, (1998) J Agric Food Chem 46: 4113 – 4117. 5-Osawa T & Namiki M (1981) Agric Biol Chem 45(3): 735 – 739. 6-Kikuzaki H, Nakatani N, (1993) J Food Sci 58: 1407 – 1410.

SL58 Potent inhibitory effects of anthraquinone compounds from *Morinda officinalis* on *in vitro* osteoclastic bone resorption

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The root of *Morinda officinalis* F.C.How has been shown to exert protective effects against bone loss in sciatic neurectomy and ovariectomy rats [1 – 3], and anthraquinones from this plant may contribute to this activity [4]. In the present study, we investigated the effects of 1, 3, 8-trihydroxy-2-methoxy-anthraquinone (1), 2-hydroxy-1-methoxy-anthraquinone (2), and rubiadin (3) from the plant the in vitro bone resorbing activity and mechanism. In the coculture system of osteoblast and bone marrow cells, the compound 1, 2 and 3 decreased the formation of bone resorption pit, the number of multinucleated osteoclasts, tartrate resistant acid phosphates and cathepsin K activity of osteoclasts within the dose range of 0.1~10 μmol/L ($P < 0.01$). Further, the compound 1, 2 and 3 at concentration of 1 μmol/L improved the ratio of mRNA and protein expression of OPG and RANKL in osteoblasts ($P < 0.01$ for compound 1 and 2; $P < 0.001$ for compound 3). In osteoclasts induced from bone marrow cells with MCSF and RANKL, the compound 1, 2 and 3 at concentration of 1 μmol/L enhanced the apoptosis of osteoclast ($P < 0.01$), disturbed the JNK and NF-κB signal pathway ($P < 0.01$), reduced the expression of calcitonin receptor ($P < 0.05$ for compound 1; $P < 0.01$ for compound 2 and 3) and carbonic anhydrase II ($P < 0.05$ for compound 1 and 3; $P < 0.01$ for compound 2). Therefore, the findings of the present study demonstrate that anthraquinones from *Morinda officinalis* is an inhibitor of bone resorption, and potentially explaining some of reported inhibitory effects on bone loss. **Keywords:** Morinda officinalis, anthraquinone, osteoclast, bone resorption, apoptosis **Acknowledgement:** *This study was supported by the National Natural Science Foundation of China (NO. 90709023)* **References:** [1] Wang MY, West BJ, Jensen CJ, Nowichi D, Su C, Palu A, Anderson G. (2002) Acta Pharmacol Sin 23: 1127 – 41. [2] Li N, Qin LP, Han T, Wu YB, Zhang QY, Zhang H (2009) Molecules 14: 2049 – 61. [3] Seo B, Ku SK, Cha EM, Park JH, Kim JD, Choi HY, Lee, HS 2005 Phytother Res 19: 231 – 8. [4] Wu YB, Zheng CJ, Qin LP, Sun LN, Han T, Jiao L, Zhang QY, Wu JZ (2009) Molecules 14: 573 – 83.

SL59 Inhibitory Effects of Indonesian Lemon Pepper Fruit Extract (*Zanthoxylum acanthopodium* DC.) on The Expression of Inflammatory Biomarkers in Lipopolysaccharide-induced Macrophages *In Vitro*

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Gastrointestinal inflammation caused by pathogen infection may lead to the overexpression of pro-inflammatory proteins and cytokines in immune system. Lemon pepper fruits (*Zanthoxylum acanthopodium* DC.; Rutaceae) have been used as a traditional source against stomach ache by Batak people in North Sumatera province, Indonesia. However, its scientific evidence for treatment of inflammatory disorders has not been reported. Here, we investigated the inhibitory effects of lemon pepper fruit extract (LPFE) against inflammatory biomarkers by conducting cell culture experiments *in vitro*. The fruits of lemon pepper were dried and extracted twice in 70% ethanol, followed by evaporation and freeze-drying. The concentrated extract was further tested for its potential inhibition on the protein and gene expression of several inflammatory biomarkers, i.e. tumor necrosis factor (TNF)-α, interleukin (IL)-6, inducible nitric oxyde synthase (iNOS), cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9, in lipopolysaccharide (LPS)-induced macrophages by performing Western blot, gelatin zymography, and reverse transcription-polymerase chain reaction (RT-PCR). LPFE (1 – 10 μg/ml) and LPS (2 μg/ml) had no cytotoxicity effects on macrophages. LPFE dose dependently decreased the expression of TNF-α and COX-2 proteins, and MMP-9 activity in macrophages treated with LPS. At the gene level, LPFE were effectively found to block the mRNA expression of TNF-α, IL-6, iNOS, COX-2, and MMP-9 in the cell system. Our results suggest that LPFE significantly inhibits selected inflammatory biomarkers at the protein and gene levels in LPS-induced macrophages. Further *in vivo* study using animal models is still needed to determine the exact anti-inflammatory potential of LPFE. **Keywords:** lemon pepper fruit, anti-inflammatory activity, *Zanthoxylum acanthopodium*, lipopolysaccharide, macrophages **Acknowledgement:** *This work was funded by the 2010*

DIPA Grant from the Southeast Asian Regional Centre for Tropical Biology (SEAMEO-BIOTROP), Bogor, Indonesia.

SL60 Histomorphometric analysis of bone marrow constituents in ovariectomized *Cimicifuga racemosa* (L.) Nutt. (CR) treated animals

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Following ovariectomy (ovx) rats lose trabecular bone and hematopoietic tissue whereas the amount of fat tissue increases. These fat cells secrete cytotoxic cytokines which cause local inflammation in the bone marrow which augments development of osteoporosis. Whether this can be prevented by estradiol-17ß (E2) or CR BNO 1055 was tested. Rats (3 months old) were ovx and fed with E2 (0.156 mg/animal/day, positive controls) or with CR BNO 1055 (8.22 mg/animal/day) which was shown in previous experiments to prevent osteoporosis. The surface of trabecles and their 3-dimensional structure was determined histomorphometrically. In addition, the amount of fat tissue was quantified. The trabecular surface and the 3-dimensional integrity of the trabecular apparatus in the metaphysis of the tibia was severely disturbed following ovx and this was almost totally prevented by E2 and CR BNO 1055. In addition ovx animals had high fat load in the bone marrow which was largely prevented by E2 and the BNO 1055 extract. These results indicate that the high amount of fat tissue in the bone marrow correlates with decreased trabecular integrity. This can be largely prevented by E2 and by compounds present in CR BNO 1055. Hence, E2 and CR BNO 1055 (which is devoid of estrogenic compounds) protect against the development of osteoporosis in part by decreasing the bone marrow fat load. **Keywords:** Cimicifuga racemosa, osteoarthritis, osteoporosis **Acknowledgement:** *This work was funded by the Bayerische Forschungsstiftung AZ-838 – 08, Germany.*

SL61 Economically Motivated Adulteration of Botanical Raw Materials, Herbal Extracts, and Essential Oils in the Global Marketplace

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The trade of botanical ingredients for the production of herbal drugs and phytomedicines, dietary supplements, and natural cosmetics is global, with supply and quality issues in one geographical region affecting other areas. Chemical complexity of botanicals requires added quality control diligence for raw material suppliers and manufacturers. In recent years there have been numerous cases of accidental misidentification of botanical materials due to nomenclatural confusion, lack of adequate quality control measures, etc. Also, there have been persistent cases of inadvertent contamination with heavy metals, agricultural chemicals, excessive microbial load, excessive solvent levels in extracts, etc. But there is also the disturbing trend of intentional adulteration – economically motivated adulteration (EMA) – as well as the "spiking" of extracts with undisclosed lower-quality and lower-cost ingredients. This includes the spurious and illegal addition of active pharmaceutical ingredients (conventional pharmaceutical drugs), e. g., sildenafil in dietary supplement products for erectile dysfunction and sibutramine in weight-loss products. This presentation reviews many of these quality control challenges and notable cases of safety concerns and economic fraud created by them as is being compiled American Botanical Council and the American Herbal Pharmacopoeia from information supplied by botanical ingredient suppliers, manufacturers, and laboratories in the United States and in other countries.

SL62 The antimicrobial activity of honey in relation to the composition of pollen (Bosnia-Herzegovina, W. Balkan)

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Honey has significant antimicrobial activity (AMA)[1, 2]. As the quality of honey depends on the composition of pollen, and it can expect a different AMA. Honey samples were collected from 12 different locations in the continental part of Bosnia. Honey samples were taken at the end of the season (September – October 2006.). Microscopic preparations were made for the standard method of pollen analysis. For every sample 300 pollen grains were counted. Each pollen grain has been determined. Antimicrobial activity was tested by diffusion method at Muller-Hilton agar. For that clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas* sp. were used. On the edge of prepared pools from strains of microorganisms placed 200 µl prepared honey. All 12 samples of honey caused high inhibition zone for *Staphylococcus aureus* (11 – 21 mm), eight samples of honey caused growth inhibition of *Escherichia coli* (11 – 13 mm) and three samples of honey caused a zone of inhibition at *Pseudomonas* sp. (12 mm). The highest antimicrobial activity has poly-floral mountain honey dominated by pollen of the following species: *Trifolium repens* L. (17%), *Taraxacum officinale* F.H.Wigg. (13%), *Filipendula vulgaris* Hill (13%), *Centaurea jacea* L. (11%), *Trifolium pratense* L. (19%), *Plantago lanceolata* L. (9.5%), *Lotus corniculatus* L. (9%), *Origanum vulgare* L. (5%), *Erica carnea* L. (5%) and others (total 15 plant species). The greatest effect on *Pseudomonas* sp. causes honey dominated of *Lotus corniculatus* (23%). Tested honey shows no effect of the bacterium *Salmonella enteritidis* and *Candida albicans*. Most of these plants are honey and medicinal plants and edible plants [3, 4]. **Keywords:** Honey plants, Biodiversity of plants, pathogenic bacteria, Taraxacum, Trifolium **References:** 1. Ulusoy E et al. (2010) J Food Biochem 34 (Suppl.1): 321 – 335. 2. Bogdanov S et al.(2008) J Am Coll Nutr 27: 677 – 689. 3. Redzic SS (2007) Coll Antropol 31: 869 – 890. 4. Redzic SJ (2006) Ecol Food & Nutr 45(3):189 – 232.

SL63 Immunostimulatory and protective effects of *Aloe vera* components against coccidiosis in broilers

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Present study reports immunostimulatory effect of *Aloe vera* L. extracts in chickens and their protection against coccidiosis. Study was divided into two experiments. Experiment-I was conducted for evaluation of immunostimulatory activity of Aloe vera extracts and experiment-II for protective efficacy against coccidiosis. Results of experiment-I revealed significantly higher ($P < 0.05$) lymphoproliferative responses in chickens administered with ethanolic extract as compared to chickens administered aqueous extract and control group. Microplate haemagglutination assay for humoral response on day 7th and 14th post primary and secondary injections of sheep red blood cells (SRBC) revealed significantly higher ($P < 0.05$) anti SRBC antibody (total Ig, IgG and IgM) titers in experimental groups as compared to control; although both the Aloe vera extracts showed no significant effects on the development of lymphoid organs. Results of experiment-II revealed maximum percent protection (60%) in chickens administered with aqueous extract as compared to ethanolic extract administered chickens (45%). Mean oocysts per gram of droppings in control group was significantly higher ($P < 0.05$) as compared to chickens in both experimental groups. Chickens administered with aqueous extract (40%) showed severe lesions (3.0 – 4.0); whereas 55 and 75 percent severe lesions were recorded in ethanolic extract administered and control chickens, respectively. Daily weight gains from day 3 rd-12th post-challenge were significantly higher ($P < 0.05$) in chickens administered with aqueous extract as compared to those administered with ethanolic extract and control. It was concluded that *Aloe vera* may be potential and valuable candidate to stimulate the immune responses and can be used successfully in immunosup-

pressive diseases like coccidiosis. **Keywords:** Aloe vera, chickens, immunostimulation, coccidiosis

SL64 Effects of nutrient media strength on cardiotonic glycoside accumulation in *Digitalis lamarckii* Ivan, an endemic medicinal species

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The effects of different strengths of Murashige and Skoog (MS) (1) medium on cardiotonic glycoside accumulation in *Digitalis lamarckii* Ivan were investigated. *D. lamarckii*, commonly known as dwarf foxglove (yüksükotu in Turkish), is an Turkish endemic medicinal plant and belongs to the family of Plantaginaceae. The *Digitalis* species are biennial or perennial herbs containing important cardioactive compounds (glycosides) that are used to treat heart problems. *D. lamarckii* has been marked as vulnerable (VU) in the Red Data Book of Turkish Plants (2). Contents of five different cardenolides (namely, digoxigenin, gitoxigenin, lanatoside C, digoxin and digitoxin) in the callus developed from hypocotyl explants, which were cultured for 10 days on different strengths of MS media supplemented with %3 sucrose, 0.5 ppm TDZ and 0.25 ppm IAA, were determined by HPLC. Three different strenghts of MS media were tested; quarter-, half- and full-strength. Of the five cardenolides, only lanatoside C could be detected in the callus cultured on all medium strenghts. The highest concentration of lanatoside C was observed on half-strength MS medium (690 μg/g dry weight, dw), while the callus cultured on full- and quarter-strength media producing 231 and 332 μg/g dw lanatoside C, respectively. In conclusion, the protocol described here is expected to have an important contribution to the future efforts for a large scale production of cardenolides in *Digitalis* species. **Keywords:** Cardenolide accumulation, Digitalis lamarckii, medium strength, Murashige and Skoog basal medium **References:** 1. Murashige T, Skoog F (1962) Physiol Plantarum 15: 473; 2. Ekim T., Koyuncu M., Vural M., Duman H., Aytaç Z., Adıgüzel N (2000) Türkiye Bitkileri Kırmızı Kitabı. University Press, Ankara, Turkey

SL65 A Phase IIA and IIB clinical trial of a quantified extract of *Nauclea pobeguinii* stem bark against uncomplicated *falciparum* malaria

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In a clinical study of type IIA, the quantified 80% ethanol extract from the stem bark of *Nauclea pobeguinii* (Pobég.) E.M.A. Petit containing 5.6% strictosamide appeared to be effective in the treatment of uncomplicated *falciparum* malaria when taken for seven days, at a dose regimen of two 500 mg capsules three times daily for 3 days, followed by one 500 mg capsule three times daily for four days. No serious side effects were noted. The Phase IIA clinical trial was carried out according to the WHO 14 days test (WHO 2003) and the results revealed that all eleven patients were completely cleared of parasitaemia and fever on days 3, 7 and 14, except for one patient. In a Phase IIB study designed as a single blind prospective trial in 65 patients with proven *P. falciparum* malaria, the efficacy and safety of the extract was compared with an artesunate-amodiaquine (AS + AQ) combination as the second arm. Evaluated on the evidence of fever clearance, disappearance of parasitaemia and other symptoms and adequate clinical and parasitological responses (ACPR), according to the WHO criteria (WHO 2003), both treatments were effective in the treatment of uncomplicated malaria. AS + AQ appeared to be slightly more effective while the extract was better tolerated. All these results suggest that the quantified 80% ethanol extract from the stem bark of *Nauclea pobeguinii* should be considered as a candidate for a clinical trial Phase III. **Keywords:** *Nauclea pobeguinii*, quantified extract, clinical trial IIA, clinical trial IIB, malaria

SL66 The *Cimicifuga racemosa* (L.) Nutt. (CR) extract BNO 1055 and thereof purified 2 fractions have cartilage protective effects and prevent accumulation of fat tissue in ovariectomized (ovx) rats

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Estrogens exert beneficial effects in the bone, joint cartilage and prevent obesity. Their use however, bares several risks. Therefore, a search for non-estrogenic alternatives is going on. We showed previously that CR BNO 1055 has bone protective effects. Whether cartilage tissue is also protected and whether this can be attributed to less obesity was not yet studied and was a scope of the present experiments. Rats were treated orally over a period of 4 weeks with CR BNO 1055 and 2 thereof purified fractions (S and R) and estradiol (E2) fed animals served as additional controls. The thickness of the knee joint cartilage layer as well as the size of the Hoffa's fat pad (a fat pad within the knee joint) was determined histomorphometrically. Thickness of the knee joint cartilage was lowest in the ovx, highest in E2 and at intermediate values in the CR and S treated animals. The size of Hoffa'sch fat pad was smallest in E2 and largest in ovx animals and an intermediate size was determined for the CR and S treated animals. It is concluded that E2, CR BNO 1055 and S have chondroprotective effects which correlate with the size of Hoffa's fat pad. The adipocytes in this fat pad are known to secrete cytotoxic cytokines. Therefore, the lower size of the fat pad in E2 and CR treated animals may have resulted in less cytokine production with less chondrotoxic effects. **Keywords:** Cimicifuga racemosa, cartilage, fat tissue **Acknowledgement:** *This work was funded by the Bayerische Forschungsstiftung AZ-838 – 08, Germany.*

SL67 Caribenolide revisited. Reisolation of caribenolide-I together with new congeners

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Caribenolide-I [1] was originally discovered from the cell extract of a free-swimming Caribbean dinoflagellate *Amphidinium gibbosum* by Shimizu and coworkers. The structure was interpreted to be a 26-membered macrolide containing a 6-membered hemiacetal ring, a tetrahydrofuran ring, an epoxide, a ketone carbonyl, four C_1 branches, and five hydroxyl groups. On the other hand, Kobayashi *et al.* reported the isolation of amphidinolide N [2] isolated from a symbiotic dinoflagellate *Amphidinium* species earlier than the report of caribenolide-I. The structure of amphidinolide N was elucidated to be the ring-opening form at the C-21-C-24 tetrahydrofuran ring for caribenolide-I. Caribenolide-I was reported to exhibit strong cytotoxic activity against tumor cell lines and in vivo antitumor activity. Amphidinolide N also showed extremely potent cytotoxic activity. Caribenolide-I as well as amphidinolide N would therefore also appear to be a promising anticancer therapeutic lead. Nevertheless, the scarcity of materials has prevented more detailed studies. Because stereochemistries of caribeolide-I and amphidinolide N have not determined yet, it is difficult to supply the sample by synthesis. In our investigation for anticancer drug leads from the *Amphidinium* dinoflagellates, we have isolated caribenolide-I together a three new caribenolide-I congeners from two benthic *Amphidinium* strains collected off Iriomote Island, Japan. In this symposium, we will discuss the isolation of these four compounds, structural relationship between caribenolide-I and amphidinolide N, and structure elucidation of three new compounds. **References:** 1. Bauer I, Maranda Y, Young K A, Shimizu Y, Fairchild C, Cornell L, MacBeth J, Huang S (1995) J Org Chem 60: 1084 – 1086. 2. Ishibashi M, Yamaguchi N, Sasaki T, Kobayashi J (1994) J Chem Soc, Chem Commun,1445 – 1446.

SL68 *Artemisia annua*: a New Medicinal Plant in the Egyptian Cultivation as a source for Artemisinin

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This study aimed to introduce *Artemisia annua* L. plant to the Egyptian cultivation and to achieve the technological package for its production under Egyptian conditions. The seeds were introduced from Germany and propagated. Several experiments were carried out during two successive seasons in two different locations (clay loamy soil and sandy loam soil). The first experiment aimed to study the effect seasonal variation on growth, yield, essential oil and chemical composition using organic farming system under the Egyptian conditions. The essential oil content and essential oil yield of *A. annua* significantly increased with increasing plant age to reach their maximum values after 180 days from transplanting. The second experiment was carried out in loamy clay soil to study the effect of the mineral fertilization on the growth, yield and the active constituents of *A. annua*. The highest value of artemisinin was obtained from plants treated with 75 kg N/fed + 50 kg K/fed. The third experiment was carried out in loamy clay soil to study the effect of organic fertilizer and/or biofertilizer on the growth and active constituents of *A. annua*. The highest yield of Artemisinin was obtained from plants treated with 30 m^3 compost/fed. without biofertilizer followed by the application of 30 m^3 compost/fed. with biofertilizer. The fourth experiment was carried out to study the effect of soil type on the growth and active constituents of *A. annua*. Plants grown in sandy soil gave a positive increase in the essential oil yield and artemisinin content and yield. Feddan = 4200 m^2

SL69 Production and use of *Artemisia annua* (sweet wormwood) against bacterial diseases in poultry stocks and its effect on food quality

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Necrotic entiritis (NE) in broilers is caused by *Clostridium perfringens* type A (CP) resulting in severe production losses and mortality. Present preventive treatments include the dietary addition of ionophores which may be banned in the EU before long. The plant *Artemisia annua* L. (AA) produces antimicrobial essential oil components (EOCs) [1] that could substitute the use of these antibiotics in poultry production. A first study focused on improving the production of bioactive EOCs in AA by applying physical and chemical stresses during cultivation. Jasmonic acid induced a significant increase in the content of EOCs such as germacrene D and γ-elemene [2]. Extracts of AA aerial parts were tested for antimicrobial activity in overnight cultures of CP strains isolated from diseased broilers. The hexane extract containing EOCs showed the strongest inhibition (MIC = 170 ppm) confirming the potential use of AA EOCs as antimicrobial agents. This extract was incorporated in the diet of broilers applying a NE disease model. The treatment reduced the population of CP and the severity of the associated small intestinal lesions ($p < 0.05$). Furthermore, CP infected broilers fed the diet supplemented with AA hexane extract gained more weight than the control animals ($p < 0.05$). Healthy broilers were fed diets supplemented with dried AA material to ascertain that the palatability of the meat is not affected. Breast filets evaluated by a descriptive sensory analysis did not show any effect of the treatment on meat flavour/taste nor texture or appearance. Hence, AA extracts show promising results as antimicrobial additives in poultry diets. **Keywords:** *Artemisia annua*, necrotic enteritis, *Clostridium perfringens*, essential oil components, sensory analysis **References:** 1. Si W et al. (2009) J App Microb 106: 213 – 220 2. Ivarsen E et al. (2010) Phcog Mag 6(22 Suppl.): 126

SL70 Conventional, stealth and transferrin-conjugated liposomes for artemisinin delivery to cancer cells

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Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua* having an unusual endoperoxyl moiety which is essential for the activity when activated by iron [1]. In addition to its well established antimalarial properties artemisinin has potent anticancer activities in a variety of human cancer cell types [2]. The cytotoxic effect of artemisinin is specific to cancer cells because transferrin-receptors are highly expressed on the surfaces of tumour cells and iron content is higher than in normal cells [3]. The aim of our work was to develop conventional and stealth liposomes for passive targeting and transferrin-conjugated liposomes for active targeting loaded with artemisinin. Multilamellar vesicles were prepared according to the film hydratation method; in order to reduce the dimensions of the vesicles, an high pressure homogenizer Emulsiflex C3® was used. Conventional, stealth and targeted liposomes were fully characterized by particle size, zeta potential, PdI, drug entrapment efficiency and transmission electron microscopy. Coupling of transferrin to the targeted liposomes was obtained by amide bound between Tf and lipid linker and the average amount of transferrin conjugated to the liposome was quantified with bicinchonic acid (BCA). A preliminary study about the cellular uptake of conventional liposomes loaded with fluorescein sodium salt has been performed in K562 cells using flow cytometry analysis and fluorescence microscopy; the highest internalization of fluorescein sodium salt loaded liposomes was after 60 minutes of exposure. **References:** 1. Nakase I et al. (2008) Int J Pharm 354: 28 – 33. 2. Firestone GL et al. (2009) Expert Rev Mol Med 11: e32. 3. Efferth T et al. (2004) Free Radic Biol Med 37: 998 – 1009.

SL71 Biological and chemical study of two Indonesian marine endophytic fungi

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In the search for biologically active natural products from Indonesian marine sources two strains of marine endophytic fungi could be isolated from the marine red alga *Kappaphycus alvarezii*. One strain (KT 30) could be identified as *Xylaria psidii*, the other one (KT 31) remains sterile and could not be identified till now. Ethylacetate extracts from the culture medium displayed considerable cytotoxic activity against a urinary bladder carcinoma cell line with IC_{50} values of 4 and 1.5 µg/ml, respectively. Both strains were also obviously active to inhibit the growth of fish and human pathogenic microorganisms. Most remarkable is the strong antimicrobial activity of ethylacetate extracts against the gram-negative bacteria *Pseudomonas aeruginosa, Escherichia coli, Vibrio anguillarum* and *Aeromonas salmonicida*. A new cyanomethoxy benzoic acid derivative was isolated from *X. psidii* KT30 and a new quinone derivative from the algicolous fungus KT31. Besides, cytochalasin B was detected for the first time in *X. psidii*. **Keywords:** Indonesian marine fungi, algicolous, antifungal, cytotoxic **Acknowledgement:** *We thank PD Dr. Marc Stadler (InterMedDiscovery, Dortmund, Germany) for identification of the Xylaria species.*

SL72 Proanthocyanidins, History, Structure, Occurrence and Biological Activity

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Proanthocyanidins (PCs) are some of the most abundant polyphenolic substances in the plant kingdom. OPCs are an integral part of the human diet, found in high concentrations in fruits such as apples, pear, tea, hawthorn, grapes, and in chocolate. Due to potent antioxidant activity, PCs have been the subject of recent research, demonstrating anticarcinogenic, anti-inflammatory, antimicrobial, and vasodilatory properties, making them a potentially valuable therapeutic tool for the treatment of a variety of conditions. PCs are present in plants as complex mixtures of polymers with an average degree of polymerization between 4 and 11, usually in association with their composing flavan-3-ols. Structural diversity is possible by variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage. The most frequent basic units of proanthocyanidins are derivatives of flavan-3-ols: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin (EGC) and (-)-epigallocatehin gallate (EGCG). PCs, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. Epicatechin, dimeric procyanidin B2 and B5, proanthocyanidin A2 and trimeric procyanidin C 1 of *Crataegus sinaica* Boiss. and/or *Adansonia digitata* L. display potent antioxidant antiviral properties *in vitro*. **References:** 1. De Bruyne T, et al. (1999) Biochem Syst Ecol 27: 445. 2. Shahat FM, et al. (1996) Planta Med 62(1): 10 – 13. 3. Shahat A et al. (2002) Planta Med 68: 539 – 541. 4. Shahat A, Ahmed H, Hassan R, Hussein A (2008) Asian Pacific Journal of Tropical Medicine (Asian Pac J Trop Med) 1(3): 55 – 59

SL73 Structures, absolute configurations and bioogical activities of new unusual metabolites from sponge-associated *Aspergillus* sp

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Over the past years, marine microorganisms have proven to be a prolific source of structurally interesting and biologically active natural products. Marine fungi in particular have attracted considerable interest due to the diversity in chemical structures and biological activities observed for their secondary metabolites. Chemical investigation of the crude extract obtained from the sponge-associated fungus *Aspergillus* sp., isolated from a specimen of the Mediterranean sponge *Tethya aurantium*, yielded new meroterpenoid metabolites of the austalide type, as well as new tryptoquivaline and fumiquinazoline alkaloids, in addition to several known compounds. The structures of the new compounds were unambiguously elucidated on the basis of extensive one- and two-dimensional NMR (^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, and ROESY spectra) and mass spectral analysis. The absolute configurations of te new compounds were established by means of TDDFT ECD calculations. All compounds were evaluated for their cytotoxic activity by the MTT method against the murine cancer cell line L5178Y, as well as the human cancer cell lines K562, A2780, and A2780 cisR, where some of the isolated compounds exhibited moderate to pronounced cytotoxicity. **Keywords:** Aspergillus, marine fungi, Structure elucidation, absolute configuration, cytotoxicity

Poster

Topic A: Analytical Methods

PA1 The Corona Charged Aerosol Detector – A "Universal" Detector for the Measurement of Non-volatile Components in Food, Natural Products and Supplements

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The Corona® Charged Aerosol Detector® is a "universal" mass-sensitive detector capable of measuring any non-volatile and many semi-volatile analytes. Its response is independent of chemical structure and does not require the analyte to possess a chromophore (a prerequisite for UV and fluorescence detection) or to be ionized (essential for MS detection). The Corona CAD is compatible with gradient elution, has a wide dynamic range (4+ orders of magnitude), high sensitivity (sub-nanogram), good reproducibility, and a predictable response independent of chemical structure. As discussed in this presentation, charged aerosol detection overcomes many of the limitations seen with other "universal" detectors including evaporative light scattering, condensation nucleation light scattering, refractive index, and UV. Charged aerosol detection has great applicability to the measurement of foods, supplements, botanicals and natural products. Some of the topics I will discuss include the measurement of carbohydrates, profiling lipids, measurement of fat-soluble vitamins, analysis of antibiotics, examination of potential bioactives in foods and supplements, and the evaluation of the composition of supplements and botanicals.

PA2 Simple and Direct Analysis of Phytosterols in Red Palm Oil by Reverse Phase HPLC and Charged Aerosol Detection

Acworth IN, Bailey B, Plante M, Gamache P
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Phytosterols (PSs) are a group of naturally occurring steroid alcohols found in plants. There is considerable interest in PSs as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. However, recent research suggests that PS supplementation may aggravate atherosclerosis and lead to aortic valve stenosis. PSs are typically measured by gas chromatography (GC), but this approach is time-consuming since it requires saponification of the sample, several extractions, and derivatization. We developed a simplified method using reversed-phase, HPLC and charged aerosol detection (CAD). CAD is sensitive, has a dynamic range of > 4 orders of magnitude, can measure any non-volatile species, and analytes shows similar response independent of their chemical structure. Samples were prepared by simple dilution prior to analysis. Five PSs, campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmastanol, were resolved in < 35 min. Calibration curves showed linear correlation coefficients > 0.997. The LOD was <= 5 ng (on column). Analysis of red palm oil is used as an example. The method is simple to use, has good linearity and sensitivity, and is capable of measuring numerous PSs in plant extracts. This approach can be used to examine product purity, supplement content, and adulteration.

PA3 Simple and Direct Analysis of Falcarinol and other polyacetylenic Oxylipins in Carrots by Reverse Phase HPLC and Charged Aerosol Detection

Acworth IN, Plante M, Bailey B, Crafts C, Waraska J
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Food plants in the Apiaceae (formerly Umbelliferae) family (e.g., carrots, parsley and celery) contain a group of bioactive C 17-polyacetylene compounds, sometimes referred to as the polyacetylenic oxylipins. These compounds have been shown to be highly toxic towards bacteria and fungi and to exhibit a diverse range of biological activities in mammals, both beneficial (e.g., their cytotoxicity is proposed to reduce the risk of developing cancer) and detrimental (*e.g.,* occupational allergic contact dermatitis). Three such compounds, falcarinol, falcarindiol and falcarindiol-3-acetate, natural pesticides produced by carrots in response to fungal diseases, have recently garnered a lot of media attention. Although falcarinols have a distinctive UV spectrum, the consequence of conjugated triple bonds, sensitivity tends to be poor due to the actual number of unsaturated bonds present in their structure. Measurement at 205 nm offers the best sensitivity; however, sample chromatograms tend to be very complicated due to the presence of many other compounds absorbing at this wavelength. Charged aerosol detection (CAD) is a "universal", mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarinol, falcarindiol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne's Lace (root, leaf, and flower) are presented.

PA4 Sensitive Analysis of Commonly Used Artificial and Natural Sweeteners Including Stevia and Their Impurities and Degradation Products

Acworth IN, Crafts C, Plante M, Gamache P
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Many of the recently commercialized sweeteners have increased potency, and therefore the amount of the active ingredient added to beverages and other food products is reduced. This results in costsavings. But, this has contributed to a need for sensitive analytical methods to quantify the active product and detect low levels of breakdown products and impurities. Such product characterization is required for quality and safety issues. Traditional HPLC-UV approaches are inappropriate as these compounds typically do not possess any chromophore. This work describes a number of HPLC-CAD methods that can be used to study common natural sugars (fructose, glucose, turanose, saccharose, trehalose, maltose, melezitose, and raffinose); artificial sweeteners (sucralose, aspartame, saccharin, and acesulfame K); and newly introduced products containing Stevia extracts (rebaudioside A and stevioside). These methods provide sensitivity at low (ng) levels with good reproducibility and accuracy, and correlation to the component concentrations. Stevia products were analyzed by Charged Aerosol Detection and UV; the CAD showed a greater than fivefold improvement in sensitivity over UV for all major components. Finally, the UHPLC methods developed showed a decreased run-time and an increased sensitivity for glucose, lactose, and sucrose. Typical limits of detection were found to be < 500 pg (on column) for glucose and other mono- and disaccharides. HPLC-CAD is a very flexible approach to measuring sweeteners and overcomes many of the limitations of UV, RI, LC-MS, ELSD, and HPLC-pulsed amperometric approaches.

PA5 A Versatile Detector for the Sensitive and Selective Measurement of Numerous Fat Soluble Vitamins and Antioxidants in Human Plasma and Plant Extracts

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Fat-soluble vitamins (FSVs) play essential roles in a wide spectrum of physiological processes. One FSV, Vitamin E (tocopherol), along with a suite of other fat-soluble antioxidants (FSAs) (e.g., carotenoids, CoQ10) mitigate the potentially disastrous effects of oxidative stress linked to numerous diseases. These compounds are thought to exert their beneficial effects by acting as chain-breaking antioxidants, inhibiting lipid peroxidation of polyunsaturated fatty acids contained within biological membranes, thereby preventing the formation of potentially cytotoxic and highly reactive aldehydes (malondialdehyde and 4-hydroxynonenal). Although a number of FSVs and FSAs have been measured by HPLC-UV, this approach typically lacks the sensitivity and selectivity required to measure these compounds in biological samples. Electrochemical detection, however, is both sensitive and selective and makes use of the inherent redox activity of these compounds. The CoulArray® Coulometric Array Detector-the only HPLC electrochemical detector that is fully gradient compatible-uses an array of flow-through, highly efficient electrochemical sensors to generate qualitative, voltammetric data to help identify analytes and resolve co-eluting compounds. The versatility

of this detector is illustrated using a variety of examples including: a global gradient method for determination of FSVs and FSAs in plasma; a gradient method for the analysis of carotenoid isomers in carrots; an isocratic method for the measurement of reactive nitrogen species damage to biomembranes measuring 5-nitro-gamma-tocopherol in rat astrocytes and human plasma; a gradient method for the measurement of tocopherol and tocotrienol isomers in palm oil; and an isocratic method for the determination reduced and oxidized CoQ9 and CoQ10 in human plasma.

PA6 Iridoid and flavonoid patterns of the genus *Veronica* sect. Alsinebe subsect. *Agrestis* (Benth.) Stroh (Lamiales) and their systematic significance

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In most taxonomic schemes *Agrestis* is considered as a subsection of the genus *Veronica* (1,2). The distribution of two Iridoid and six flavonoid compounds in four *Veronica* L. Sect. Alsinebe subsect. *Agrestis* species (23 samples) from Iranian Natural populations was investigated. *Veronica francispetae* M. A. Fisch. and *V. siaretensis* E. Lehm. were studied for these compounds for the first time. The Iridoid and flavonoid patterns showed a good correlation with morphological and chemical features of these taxa. The studied species are closest together according to the flavonoid patterns: species containing quercetin derivatives (*V. persica* Poir., *V. polita* Fr.) and species containing quercetin (*V. francispetae, V. siaretensis*). *V. persica* and *V. polita* are generally related in their morphology, however, *V. persica* can be distinguished from *V. polita* due to the occurrence of 6-O-isovanilloylcatalpol. *V. persica* is an aggressive tetraploid species. Different opinions exist regarding its origin as an autopolyploid from *V. polita* (3). **Acknowledgement:** *This research was supported by the project of the Guilan University.* **References:** 1. Fischer MA, Peev D (1995) Genus Pseudolysimachion Opiz. In: Kozhuharov St., Kozmanov B. (eds.) Flora of the republic of Bulgaria, vol. X. Prof. M. Drinov, Sofia, pp. 190 – 202. 2. Albach DC, Martinez-Ortega MM, Fischer MA, Chase MW (2004) Taxon 53 (2): 429 – 452. 3. Peev D (1978) Taxonomy and microevolution of the wild-growing representatives of the genus Veronica L. in Bulgaria. In: Kozhuharov St, Kozmenov B (eds) Evolution of the flowering plants and florogenesis, vol I. Izd BAN., Sofia, pp. 72 – 106

PA7 Seed and mucilage yield of isabgol (*Plantago ovata* Forsk.) under salinity stress

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Salinity may adversely reduce the overall productivity of plants by inducing numerous abnormal morphological, physiological and biochemical changes. An experiment was conducted in 2010 in the Greenhouse of the University of Tabriz, to investigate isabgol (*Plantago ovata* Forsk.) performance under non-saline (control) and three saline conditions (4, 8, 12 $dS.m^{-1}$ NaCl). The experiment was arranged as completely randomized block design with three replications. Ten seeds were sown 1 cm deep in each pot filled with 800 g perlite. Salinity treatments were applied immediately after sowing. Tap water and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC. After emergence, seedlings were thinned to keep four plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution. At maturity, plants from each pot were harvested and seed yield per plant was determined. Means of seed and mucilage yields per plant decreased with increasing salinity. However, seed yield per plant under 0 and 4 dS/m salinity were statistically similar. Mucilage percentage was not significantly affected by salinity stress. Thus, reduction in mucilage yield was attributed to deductions in seed yield per plant under high salinity treatments.

PA8 Composition of polysaccharides from aqueous extracts of some wound healing plants

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Plant polysaccharides represent ideal candidates for therapeutics with immunomodulatory and wound healing actions. Polysaccharides from several medicinal plants have been shown to exhibit immunomodulatory activities [1 – 2] and stimulate proliferation of keratinocytes and dermal fibroblasts [3 – 6]. In most cases, hot water extracts containing the water-soluble polysaccharides are used for the treatment of wounds [7] and also the isolated pure polysaccharides have been shown to exhibit immunomodulatory and wound healing activities [8]. AQim has been to determine the monosaccharide composition of polysaccharides from aqueous extracts of selected medicinal plants traditionally used in Western Africa as wound healing agents [7] and to identify compounds which possess skin cell-promoting activities under *in vitro* conditions. Raw polysaccharides (RPS) were isolated from aqueous extracts of the selected plants by EtOH precuipitation and dialysis (MWCO 3.5 kDa). TFA 2N hydrolyzed samples were analyzed concerning monosaccharide composition by TLC and HPAEC-PAD (Carbo PacTM PA1 stationary phase). 3 of 11 plants contained substantial amounts (> 3%) of cold-water soluble mucilages. RPS from 3 plants were characterized by high fucose contents, a monosaccharide normally not forming a big part of polysaccharides from higher plants. The high amount of galactose (32%) and arabinose (30%) in the hydrolyzed RPS of *Parquetina nigrescens* (Afzel) Bullock probably gives an indication of presence of arabinogalactans. Glucan structure is reasonable for polysaccharides from *Alstonia boonei* De Wild. Further *in vitro* studies on influence of polysaccharides on skin cell physiology have to be initiated to establish exact structure-activity relationship. **Acknowledgement:** *The authors thank Deutscher Akademischer Austausch Dienst (DAAD) for the fellowship awarded to C. Agyare.* **References:** 1. Diallo et al. (2003) J Ethnopharmacol 84: 279 – 287. 2. Inngjerdingen et al. (2006) Biomacromolecules 7: 48 – 53. 3. Deters et al. (2010) J Ethnopharmacol 127: 62 – 69. 4. Deters et al. (2008) J Pharm Pharmacol 60(2): 197 – 204. 5. Deters et al. (2005) J Ethnopharmacol 102(3): 391 – 399. 6. Deters et al. (2005) J Cell Physiol 202(3): 717 – 722. 7. Agyare et al. (2009) J Ethnopharmacol 125(3): 393 – 403.

PA9 Seasonal variation of kaurane-type diterpenes and cinnamic acid derivatives in leaves of *Mikania laevigata* and *Mikania glomerata* cultivated under different shading conditions

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Mikania glomerata Spreng. and *Mikania laevigata* Sch.Bip. ex Baker are medicinal plants popularly named 'guaco', whose leaves are used to treat respiratory diseases, with coumarin (1) and kaurane-type diterpenes regarded as the bioactive constituents. The goal of the study was to undertake seasonal studies on the contents of chemical markers in leaves under different shading conditions. Species were cultivated under different levels of solar radiation and full sunlight. The leaves were collected in the middle of each season year.The contents of 1, o-coumaric (2), benzoylgrandifloric (3), cinnamoylgrandifloric (4) and kaurenoic (5) acids were quantified in dried leaves of both species by RP-HPLC [1].Significant differences were found in the contents of cinnamic acid derivatives (1 and 2) and kaurane-type diterpenes (3, 4 and 5) for the evaluated harvesting periods and cultivation environments. o-Coumaric acid was solely detected in *M. laevigata* in concentrations bellow the limit of quantification (< 0,045%), in plants under 80% shading, collected in the autumn. Both 1 and 2 were not detected in the analyzed samples of *M. glomerata*.The average concentration of coumarin reached its maximum (0.94%) in the summer, in plants growing under 80% shading.In general, both species presented higher amounts of the kaurane-type diterpenes in plants cultivated under sunlight, except for 3 in *M. glomerata*. Altogether, the obtained results point out that the highest content of coumarin is reached in *M. laevigata* cultivated under 80% shading, preferentially harvested in the summer, but with reduced levels

of kaurane-type diterpenes.On the other hand, *M. glomerata* shall be cultivated under full sunlight for maximum contents of the kaurane-type diterpenes. **Acknowledgement:** *Acknowledgments: FAPEMIG, CAPES and CNPq, for the financial support.* **References:** 1. Bertolucci SKV et al. (2009) Planta Med 75: 280 – 285.

PA10 HPLC-DAD analysis of chemical markers in leaves of *Mikania laevigata* and *Mikania glomerata* submitted to long-term storage

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Mikania laevigata Sch. Bip. ex Baker and *Mikania glomerata* Spreng., known in Brazil as guaco, are medicinal species widely used to treat respiratory affections. Stability analyses of vegetal drugs are crucial to assure the quality of derived products. The present study aimed at undertaking qualitative and quantitative analysis of cheminal markers [coumarin (CO), o-coumaric (OC), kaurenoic (KA), benzoylgrandifloric (BA) and cinnamoylgrandifloric (CA) acids] in dried leaves of *M. laevigata* and *M. glomerata* submitted to long-term storage. The plant materials were stored in a dark room with controlled temperature and humidity, and had their fingerprints analyzed three-monthly up to 18 months. Changes in chemical markers were evaluated by UV spectral purity of the peaks and by quantitative analysis of their contents (% w/w in dried leaves), employing an HPLC method previously reported by us [1]. The concentrations of the chemical markers did not vary significantly within the evaluated storage period ($p > 0.05$) for both species. In contrast, changes in BA, CA and KA peaks were detected for three-months stored samples of both species and CO peak, found only in M. laevigata, was detected after six months of storage, suggesting compound degradation. The CO contents in *M. laevigata* samples ranged from 0.10 ± 0.03% to 0.12 ± 0.03% and therefore fulfill the Brazilian pharmacopoeical requirement established for the species (minimum of 0.1% w/w), except for the 12-month sample (0.09 ± 0.03%) [2].Therefore, the quality control of *Mikania* species should be based both on the quantification of the selected compounds and fingerprint analysis. **Acknowledgement:** *FAPEMIG, CAPES and CNPq, for the financial support.* **References:** 1. Bertolucci SKV et al. (2009) Planta Med75:280 – 285. 2. Farmacopéia Brasileira IV (2005) Sexto fascículo:292.

PA11 Bioavailability and pharmacokinetic of the Algerian propolis constituent naringenin in rats after oral administration

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Till now, a limited number of pharmacokinetics and bioavailability of propolis compounds studies have been performed. So, the absorption of orally administered Naringenin, an active component of Algerian propolis (14.2%), in rats has been studied to evaluate its pharmacokinetics and bioavailability *in vivo* in comparison with those of a standard solution of naringenin. Rats were given 100 mg/kg of body weight of aqueous propolis extract or 14.2 mg/kg of naringenin. Blood was collected from the retro-orbital sinus. Naringenin was quantified by coulometric detection using HPLC-UV system. *In vivo* pharmacokinetic study of propolis extract shows a good and rapid absorption from the gastrointestinal tract and reveal a high bioavailability. The serum concentration of naringenin from propolis was 17.45 nmol/ml, T_{max}= 60 min, the total clearance 9.35 ml/mn, the area under the curve (AUC 0 – 360) 32821 nmol.mn/ml, and the volume of distribution (Vd) was 1949.15 ml. Compared to the standard naringenin, Algerian propolis constituent naringenin shows a better bioavailability and diffusion that may explain the antioxidant effects flavonoids extracted from propolis. **References:** 1. Harmon AW et al. (2004) Breast cancer Res Treat 85:103 – 110. 2. Hou YC et al. (2001) Planta Med 67:538 – 541. 3. Hsiu SL et al. (2002) Life Sci 70:1481 – 1489.

PA12 Development of a rapid isocratic reverse phase -ultra fast liquid chromatographic method for determination of phenolic acids in fruits

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Some low molecular weight phenolic acids namely gallic, chlorogenic, protocatechuic, p-coumaric, vanilic and ferulic, are well-known in their health-promoting properties. Isocratic Ultra Fast Liquid Chromatographic methods (UFLC-DAD) for detecting these compounds are advantageous, due to their simplicity and economy of time and solvent usage. This paper aimed at the development of a rapid and comprehensive isocratic UFLC-DAD method for analysis of phenolic acids in Brazilian fruits mangaba (*Hancornia speciosa* Gomes) and umbu (*Spondias tuberosa* Arruda). Mobile phase compositions (different solvents A – Dihydrogen potassium phosphate, trichloroacetic acid and trifluoroacetic acid and different percentage of solvent B – 8; 10 e 12% of acetonitrile) were combined with flow rates (0.4; 0.5 and 0.6 mL/min) in a statistical factorial design. Among the combinations tried, the trichloracetic acid was found to be the best solvent "A" and 8 – 10% of acetonitrile as the best solvent B, and flow rate of about 0.6 mL/min as the best range of flow. Method presented limits on detection ranging from 0.014 to 0.094 μg and higher recovery percentages were observed to extraction with methanol-acetone (69.51 to 72.59 for protocatechuic acid and 69.58 to 126.31 for the chlorogenic acid). Chlorogenic acid concentrations in mangaba samples (62.93 μg/g) were higher than in umbu samples (8.49 μg/g). Linearity of detector responses (represented by the linear regression coefficient of plots), was higher than 0.999 for all phenolic acids. These results permitted to develop a rapid and practical method for phenolic acids determination in the tropical fruits of umbu and mangaba. **Acknowledgement:** *We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil for the financial support received while the first and last co-authors thank CAPES for fellowships*

PA13 Development of an enzyme-linked immunosorbent assay using monoclonal antibody against asiaticoside

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The monoclonal antibody (MAb) against asiaticoside (AS), the bioactive consituent of *Centella asiatica* (L.) Urban was produced and characterized [1]. As immunogen, AS was conjugated to the carrier protein bovine serum albumin (BSA). In order to confirm its immunogenicity, the ratio of hapten in the AS-BSA conjugate was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). After immunization, hybridomas secreting MAbs against AS were produced by fusing splenocytes with the mouse myeloma cell line, SP2/0-Ag14[2]. After the screening, anti-AS MAb 2B4 was obtained. No detectable cross reactivity with other related triterpenoid glycosides was found except madecassoside which gave a 7.08% cross reaction value. Subsequently, a quantitative ELISA system for AS using the MAb was established and evaluated comparing with HPLC method. The assay was suitable for quantitating AS in the range of 0.78 to 50 mg mL^{-1}. The validation study showed that the method was precise, accurate and sensitive. The ELISA method described should prove useful as an analytical tool for quality control and standardization of medicinal plants and pharmaceutical products containing AS. **Acknowledgement:** *The JSPS-NRCT Core University Program, Dr. Mayuree Tantisira from Chulalongkorn University.* **References:** 1. Juengwatanatrakul T (2011) Analyst 136:1013. 2. Galfre G (1981) Methods Enzymol 73: 3.

PA14 Isothermal Titration Calorimetry as a tool for selection of new natural astringents for cosmetic applications

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The use of astringents in the cosmetic industry is widespread as tonic lotions, cleansers, deodorants and antiperspirants. Aluminium chlorohydrate, among others aluminium salts, is highly used as an astringent. Recently, the use of aluminum in cosmetics raised the concern about its safety to humans. Although the regulatory agencies worldwide assure the safety of this raw material, this issue has led the search for substitutes of aluminium salts to serve consumer needs. In the deveolpment of new raw materials it is wise to evaluate a priori the *in vitro* efficacy to address the extremely complex functional systems of living organisms. Colorimetric titration methods for evaluating tannins based on precipitation of hide powder (1) are very laborious and not specific to tannins (2). The objective of this study was to apply the isothermal titration calorimetry (ITC) method, as described elsewhere by others (3,4,5), to evaluate the interaction of bovine gelatin with 8 commercially available natural and semi-synthetic tannin containing extracts. The bovine gelatin is an especially good source of proline binding sites found in human skin. The tannins belong to the hydrolysable and condensed types present in 5 different plant species. ITC is a reliable and fast technique to evaluate important parameters like enthalpy, entropy, stoichiometry and association binding constant in an unique experiment for further decision support. All raw materials had the efficacy compared to aluminium chlorohydrate. The developed methodology has provided an useful tool for astringency evaluation of tannins and will illuminate the road toward better cosmetics. **References:** 1. Folin O, Ciocalteau V J (1927) Biol Chem 73: 627. 2. Verza S G, Kreinecker MT, Reis V, Henriques A T, Ortega GG (2007) Quimica Nova 30(4): 815 – 820. 3. Frazier R A, Papadopoulou A, Mueller-Harvey I, Kissoon D (2003), J Agric Food Chem 51(18): 5189 – 5195. 4. Frazier R A, Papadopoulou A, Green RJ (2006) Pharm Biomed Anal 41(5): 1602 – 1605 5. Frazier R A, Deaville ER, Green RJ (2010) Pharm Biomed Anal 51(2): 490 – 495,.

PA15 Utilization of High Performance Ion Chromatography (HPIC) method for the determination of total sulphur content in different biological samples

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Sulphur (S) in the plants is assimilated from the soil as a sulphate ion and further being reduced to the organosulphide species. This work has been aimed to assess total S content in the natural and artificial fertilizers and plant material by using a High Performance Ion Chromatographic (HPIC) method. Samples from natural and artificial fertilizers and plant material were prepared by digestion and oxidation with a mixture of perchloric and nitric acid. HPIC method was performed with a Shimadzu Ion Chromatograph equipped with conductivity detector CDD-10A. Deggased and diluted samples were analysed on Shodex IC SI-90G strong exchange column, using a mobile phase: carbonate buffer $NaHCO_3$ (0,0017 M) and Na_2CO_3 (0,0018 M), during the 20 minutes at 40 oC and 1.0 $mLmin^{-1}$ flow rate. The total S content in the analysed fertilizers varies from 15,8 mg/g to 5.0 mg/g (for arificial fertilizers), 2,5 mg/g to 0,8 mg/g (for natural fertilizers), and for different plant material varies from 1,85 mg/g to 0,29 mg/g. Used HPIC method is simple, sensitive and accurate and can be applied for the determination of total S content in the natural and artificial fertilizers and plant material. This experiment illustrates the utility of HPIC as a reliable analytical tool. Results of this analysis could recommend optimal soil fertilization for the cultivation of the plants with a highest S metabolic needs.

PA16 Chemical composition and lipid fraction properties of Iranian pomegranate (*Punica granatum* L.) seeds

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The pomegranate seeds of four commercial varieties (Abanmahi (AB), Malas (MS), Pust Sefid (PS) and Shahvar (SH)) cultivated in Iran were evaluated in terms of quality properties including protein, oil, dietary fiber, mineral contents and fatty acid composition. physicochemical properties and antioxidant activity of pomegranate (*Punica granatum* L.) seed oils (PSOs) also was determined. The oil antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity. Results showed that PS had the highest oil (16.9%) and crude fiber (42.4%), and nutritional value (460.7Kcal/100 g) among selected varieties. PS had the highest level of phosphorus (2766.3 mg/kg) and magnesium (2052.0 mg/kg), while the highest calcium (675.3 mg/kg) and potassium (3724.6 mg/kg) were related to SH. The main fatty acid identified by gas chromatography was punicic acid ranged from 72.07% for SH to 73.31% for MS ($p < 0.05$). The ratios of polyunsaturated/saturated and unsaturated/saturated fatty acids of PSOs were found to be between 9.174 and 9.450, and 10.325 and 10.861, respectively ($p < 0.05$). PSOs obtained presented acid (3.78 – 8.36% punicic acid), peroxide (0.39 – 0.48meq O_2/kg), iodine (216.9 – 220.3 g I2/100 g) and saponification (179.3 – 182.5 mg KOH/g) values. Also, refractive index at 25 °C, viscosity and density of PSOs varied from 1.461 – 1.527, 0.036 – 0.063 Pa.s and 0.9202 – 0.9311 g/cm^3, respectively. The oil obtained from MS showed the lowest level of ortho-diphenols (ODC) and DPPH radical scavenging capability. The relationship between percentage of remaining DPPH and ODC of PSOs also illustrated high correlation among all varieties ($R2 = 0.98$, $p < 0.01$).

PA17 Stereoisomeric composition of two bioflavonoids from *Larix sibirica*

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Nowadays the bioflavonoid complex of *Larix* is well-known and well-studied [1]. Current work is devoted to detailed studies of structure and stereoisomeric composition of two major bioflavonoids from *Larix sibirica* Ledeb.: dihydroquercetin (taxifolin, CAS Nº 480 – 18 – 2, 2,3-dihydro-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H-1-benzopiran-4-on) and dihydrokaempferol (aromadendrin, CAS Nº 480 – 20 – 6, 2,3-dihydro-2-(4-hydroxyphenyl)-3, 5, 7-trihydroxy-4 H-1-benzopiran-4-on). Using preparative RP-HPLC both compounds were isolated from the extract of *Larix* wood and purified to the > 99% of purity. Detailed analysis by RP- and chiral HPLC, UV, LC-MS and high resolution NMR spectroscopy allowed us to characterize these compounds as mainly 2*R*3*R*-isomers with small (up to 4%) content of 2S3S-enantiomers. So we can establish that *Larix sibirica* produces "high quality" optical pure flavonoids. **References:** 1. Kolesnik Yu et al. (2007) Abstr 55th Int Congr Ann Meet Soc Med Plant Res, Graz: 938.

PA18 Absence of Penicillin- derivatives in preparations from *Penicillium* species used in homeopathic medicinal products

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Homeopathic medicinal products produced from preparations of *Penicillium chrysogenum* (Notakehl), *P. glabrum* (Quentakehl), *P. roquefortii* (Fortakehl) or *P. brevicompactum* (Stolonikehl) have been used for over 30 years without reports of any serious or major adverse events. Safety studies and other available data gave no indications for immunotoxic or sensitizing effects. While some species of the genus *Penicillium* do produce antibiotic substances, they are unwanted by-products in the homeopathic use of active substances derived from the *Penicillium* species. The aim of this study was to check for the presence of antibiotic substances in these active substances. For the production of the *Penicillium* species active substance, the biomass yielded by fermentation is purified and then mechanically opened by a cell mill. The liquid phase is separated and the insoluble components are filtered. The filtrate then

undergoes multiple filtration and washing steps prior to sterile filtration and i freeze drying. The resulting starting material is named "e volumine cellulae (lyophil., steril.)" [1] and is raised to homeopathic potencies (D3, D4, D5). HPLC-ESI-MS data is presented to prove the absence of Penicillin- derivatives and precursors (Penicillin G, m/e 335 [M+H]+ and 6-Aminopenicillanic acid, m/e 217 [M+H]+) in used active substances. Whilst being found in the fermented culture broth of e.g. Penicillium chrysogenum, Penicillium G and 6-Aminopenicillanic acid are no longer present in the active substance after processing. During the manufacture of the active substances, unwanted antibiotic compounds are eliminated whilst preserving the products high quality. **Acknowledgement:** *HPLC-ESI-MS analysis was taken out at Phytos GmbH & Co. KG in Neu-Ulm* **References:** [1] Bader G, Akkoyun A, Wiethoff K (2010) Planta Med 76: 1262

PA19 Recovery of alkaloids from leaves and seeds of *Argemone mexicana*

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Research work carried out in Sudan on the weed *Argemone mexicana* L. pointed to the potential of the plant as a source of larvicides against mosquito. Mosquito, *Anopheline arabiensis*, is on focus in Sudan because of its role as a vector of several tropical diseases. The objectives of this study was evaluation or selection of the best solvent used for extraction of the alkaloids and Identification of the alkaloids using spectroscopy as well as chromatographic methods. The result showed that ethanol was the best for extraction of Argemone leaf alkaloids both qualitatively and quantitatively; hexane the poorest while acetone and chloroform were in between. Ethanol was selected as the best organic solvent for extraction and different water dilutions were used (10%, 25%, 50%, 75%, and 100%). The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition pure ethanol extract was comparable to distilled water. The three alkaloids detected in different methanol water dilutions (100%, 75%, 50%, and 0%) and 75%& 50 extract the higher concentration of alkaloids. Pure methanol extract was comparable to the distilled water extract. Distilled water extraction recovery was comparable to acidic water and hot water at [60C°]. Fractionation of the extract from the aerial part of *Argemone mexicana* led to the isolation of 3 major alkaloids which were identified using preparative TLC, IR spectroscopy, HPLC, GC- MS and literature reports as berberine, protopine and benzophenathridine. The seed isolated compound contains Dihydrosanguinarine as a major alkaloid. **Keywords:** Argemone mexicana, extraction, identification

PA20 Effect of exogenous silicon and salt stress on germination and seedling establishment in Borago officinalis

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Borage (*Borago officinalis* L.) is a valuable medicinal plant with a high content of gamma linolenic acid. Seed germination and early seedling growth are critical stage for plant establishment and production.One of the most problems in the field of plant cultivation and production of this plant, is low and uniform seed germination. In addition salt stress in the soil or water is the major factor especially in arid and semi-arid regions which greatly influence plant growth and yield. Thus, in this experiment the influences of exogenous silicon (si) concentration and salinity tolerance during germination and early seedling growth was evaluated. Treatments were 5 levels of silicon (0, 0.2,0.5,0.7,1 mM Na_2SiO_3) and 5 levels of NaCl (0, 20, 40, 80, 100 mM NaCl) in 3 replicates. The results showed that different treatments of salinity and si had considerable effect on the germination rate (GR), germination index (GI) and seedling growth of borage. In all of the si level, seeds possessed more germination rate and germination index than control. The highest germination percentage were obtained at 1 mM si. Significant differences in seed germination were also detected among the NaCl concentrations used. The total dry and fresh weight of seedling were increase in si treatment but reduced by increasing NaCl concenteration. Result of this experiment is consistent with the hypothesis that salinity and si have a priming effect and can prepare a suitable metabolic reaction in seeds and improve seed germination performance and seedling establishment.

PA21 Cichoric acid content and antioxidant activity of commercially available *Echinacea* herbal medicinal products

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Echinacea spp. are very well known medicinal plants with immunomodulatory activity. The purpose of this study was to investigate the phenolic content (cichoric acid and total phenol) and the antioxidant activity of seven medicinal products with *Echinacea purpurea* (L.) Moench commercially available in Croatia. The content of total phenolics was determined spectrophotometrically with Folin Ciocalteu reagent, while the content of cichoric acid was established by using isocratic RP-HPLC. Antioxidant activity was established using the following techniques: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power and β-carotene-linoleic acid assay. The cichoric acid content varied greatly in products with a maximum of 1.6% (w/V) as well as the content of total phenols (0.03 – 16 mg/mL). In all the in vitro analyses the extracts demonstrated marked antioxidant activities. The activity in the reducing power assay correlated very well with the content of total phenols ($r2 = 0{,}995$, $P < 0.0001$), while the activity in the other assays did not correlate neither with the amount of cichoric acid nor with that of total phenolics. It seems that some other substances might be responsible for the activity of the investigated preparations in those assays. The results suggest that cichoric acid, as unstable phenolic compound characteristic for *E. purpurea* products vary as expected. On the other hand, the total phenolic and antioxidant activity of investigated products are greatly influenced by other ingredients of products.

PA22 Deglycosylation of individual flavonoids and flavonoid containing plant extracts by purified human intestinal lactase-phlorizin hydrolase (LPH)

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Lactase-phlorizin hydrolase (LPH, EC 3.2.123/62) is an apically-sorted glycoprotein of the small intestine. One of the two catalytic sites of LPH is responsible for hydrolyzing lactose, the main carbohydrate in milk. The second active site exhibits a broad specificity against substrates like phlorizin and glycosyl-N-acylsphingosines [1]. Phlorizin, a dihydrochalcone, belongs to the group of flavonoids often present as β-glycosides in food and herbal medicinal plant products. Bioavailability of flavonoids may depend on intestinal hydrolysis before absorption and delivery to systemic circulation. The ability of purified LPH to hydrolyze various flavonoid glycosides was investigated. Isolation of human LPH from stably transfected CHO-cells by immunoprecipitation with monoclonal anti-LPH antibodies allows specific activity measurements with known substrates, individual flavonoids and plant extracts. Deglycosylation of approximately 20 putative substrates was tested by determination of the released products by a photometric method (glucose) and by HPLC-DAD (flavonoid aglyca). Flavonoid glycosides with terminal rhamnose (e.g. rutin, naringin and quercitrin) and isoflavone glycosides (e.g. sophoricoside and genistin) were not hydrolyzed by human LPH. A cleavage of flavonoid-mono-/di-β-glycosides with glucose residues in different positions (3, 4', 3', 7) of the aglycone could be observed (e.g. kaempferol-3-O-glucoside, quercetin-4'-O-glucoside, apigenin-7-O-glucoside and luteolin-3'-7-O-diglucoside). Hydrolysis of specific flavonoids could also be shown for plant extracts of onion and curly kale. As an example about 40% of quercetin-4'-O-glucoside, the main component of an onion extract, was deglycosylated by LPH demonstrating its importance in the metabolisation of biologically active compounds. **Acknowledgement:** *We thank the Federal Ministry of Education and Research for financial support, Project-No. 315371E.* **References:** 1. Behrendt M et al. (2009) Gastroenterology 136: 2295 – 2303

PA23 Effect of static magnetic field on seed germination early growth and activities of some enzymes in *Melissa officinalis* seeds

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The objective of the present study was to investigate the effect of static magnetic field (o, 25, 50 and 75 μT) and exposure time (15, 30 and 60 minutes) on *Melissa officinalis* L. seed germination. Treatment of *Melissa officinalis* seeds in these magnetic fields increased the germination rate (GR), germination index (GI), germination rate coefficient (GRC), seedling length and seedling dry and fresh weight under laboratory germination tests. In germinating seeds, enzyme activities of α-amylase, dehydrogenase and protease were significantly higher in treated seeds in contrast to controls. The higher enzyme activity in magnetic-field-treated *Melissa officinalis* seeds could be triggering the fast germination and early vigor of seedlings. **Keywords:** Melissa officinalis, seed germination test, α-amylase, dehydrogenase, protease and magnetic field **References:** 1) Balouchi HR and Sanavy SAM (2009) International Agrophysics 23: 111 – 115. 2) Gholami A and Sharafi S (2010) World Academy of Science, Engineering and Technology 62: 279 – 282. 3) Kavi PS (1977) Sci Cult 43: 405 – 406. 4) Podlesny J, Lenartowicz W and Sowinski M (2003) Zecz Probl Post Nauk Roln 495: 399 – 406. 5) Vashisth A and Nagarajan S (2010) Journal of Plant Physiology 167: 149 – 156

PA24 HPLC-MS/MS Quantitative Determination of Gallic acid and Cyanidin-3-Glucoside Content of Bilberry Fruit Extract from Turkey

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Genus *Vaccinium* is a widespread genus with over 200 species of evergreen and deciduous woody plants varying in size from dwarf shrubs to trees. There is a great interest worldwide in the fruits of bilberry because of their high anthocyanin content. Anthocyanins are flavonoids comprising flavonol glycosides, flavan-3-ols and proanthocyanidins, whereas hydroxycinnamic acids are classified as phenolic acids. Anthocyanins are valued as pigments but are also widely used in natural health products due to their suggested positive effects on night vision, even though firm evidence from clinical trials is still lacking. [1]. In this study, quality control of *Vaccinium myrtillus* L. fruits have been carried out according to the European pharmacopoeia (2). Furthermore, gallic acid and cyanidin-3-glucoside contents of *Vaccinium myrtillus* water and EtOH (70%) extracts were investigated using with HPLC ESI/MSMS MRM method. The assay was performed with different concentrations of gallic acid and cyanidin-3-glucoside chloride as standart solutions. The diagnostic fragmentations of gallic acid 168.7/125 – 79 and fragmentations of cyanidine-3-glucoside 448.7/287 – 150 were used for MRM quantitative determination. Total anthocyanin content of the fruits were shown to be not less than 0.30 per cent and other features were found to conform Pharm Eur requirements. Furthermore, the content of cyanidin-3-glucoside was shown to be 0.0538 g ± 0.001 per cent in the EtOH (70%) extract and 0.045 ± 0.002 per cent in the water extract, respectively. The gallic acid contents measured in the ethanolic and water extracts were 0.001 per cent and 0.136 ± 0,001 per cent, resp. **References:** 1. Riihinen K et al.(2008) Food Chem 110(1): 156 – 160. 2. European Pharmacopoeia 7.1 (2010) Bilbery Fruit Fresh, Myrtilli fructus recens, p 1070.

PA25 Investigation of the existence of five major flavonoids in *Satureja sahendica* Bornm. and optimization of their extraction conditions using experimental design, solid phase extraction and HPLC

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Satureja sahendica Bornm. (SSB) (Lamiaceae) is an endemic species of Iran [1] and in traditional medicine is used as a rapid antidote for food poisoning [2]. In this work, chemometrics, solid phase extraction (SPE) and HPLC methods were used to investigate the existence of five major flavonoids including; myricetin, quercetin, luteolin, apigenin and kaempferol in (SSB) and to optimize the extraction conditions of detected flavonoids. The effects of five experimental factors including; percentage of ethanol, volume of extraction solvent, concentration of HCl, extraction time and temperature on the extraction recovery were investigated using a rotatable, orthogonal central composite design (CCD). Grid search method was used to find the optimum extraction conditions. The SPE was used to preconcentrate the presumably available flavonoids. The SPE parameters including; pH of loading solution, type and volume of elution solvent and break-through volumes were optimized. The preconcentrated extracts were analyzed by HPLC using a C 18 column and methanol:0.5% phosphoric acid (60:40 v/v) with flow rate of 1.0 $mLmin^{-1}$ as mobile phase. Among the investigated flavonoids only quercetin, luteolin and apigenin were found in this species which showed two different patterns for extraction. Quercetin and luteolin were extracted using 20 mL of 68% aqueous ethanol containing 2.0 M HCl, refluxed for 30 minutes at 90 °C while apigenin was extracted sing 20 mL of 68% aqueous ethanol containing 1 M HCl, refluxed for 1 hour at 45 ° C. Concentrations of quercetin, luteolin and apigenin were 10.20, 19.21, and 48.50 $mgKg^{-1}$, respectively. **References:** [1] Rechinger K (1982) Flora Iranica. Akademische Druck-und Verlagsanstalt. p 495 – 504. [2] Taherpour A, Maroofi H, Nasri F (2005) Inter J Appl Chem 1(1):57 – 61.

PA26 Phytochemical analysis of *Anthyllis hermanniae* – Leguminosae, and development of a sensitive UHPLC-HRMS/MS method for the rapid analysis of the phenolic content

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Anthyllis genus includes several species, very few of which are investigated from a phytochemical point of view. Previous works about the phytochemical analysis of the aerial parts of some species describe the isolation and structure elucidation of several glycosides of kaempferol, quercetin and other flavonoid aglycons [1 – 4]. In the present study, a detailed phytochemical analysis of the methanolic extract of the aerial parts of *Anthyllis hermanniae* L. is described, a species for which there are no previous data concerning its metabolic content. Applying several chromatographic techniques (VLC, LC, prep-TLC, HPLC, CPC), twenty-two secondary metabolites, belonging to categories of cinnamic and benzoic acid derivatives, sterols, coumarins, isoflavons and flavonols were isolated and their structures were fully elucidated by means of UV-Vis, MS, HR-MS and NMR (1&2D spectra). Moreover, triglycosides of quercetin and kaempferol, which are new natural products, were isolated and unambiguously elucidated [5]. After the structure elucidation of the isolated metabolites, the twenty-two compounds were used as references for the development of a fast and sensitive method for the simultaneous characterization of the phenolic content of *A. hermanniae*. The analyses were performed on a UHPLC system hypnenated with a hybrid-LTQ-Orbitrap mass spectrometer using ESI & APCI ionization probes, in both positive and negative modes. The study of the full scan spectra together with the accurate MS/MS data enabled the identification of additional phenolics, with high confidence. This newly developed analytical method could be applied for the rapid identification of phenolics in other *Anthyllis* species, as well as in other Leguminosae plants. **References:** [1] Pistelli L et al. (2007) Nat Prod Res 21: 418 – 425. [2] Marco J et al. (1989) Phytochem 28: 1513 – 1516. [3] Adell J et al. (1988) Phytochem 27: 2967 – 2970. [4] Barbera O et al. (1986) Phytochem 25: 2361 – 2365. [5] Halabalaki, M et al. (2011) J Nat Prod *In press*.

PA27 Accelerated Solvent Extraction: development of a representative extraction method from medicinal plants for cosmetic applications

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The first crucial step to discover new cosmetic plant active ingredients is the extraction before the analysis of plant materials. Accelerated Solvent Extraction (ASE) is a fast and automatic sample preparation technique which offers the ability to carry out sequentially multiple extracts (up to 24 samples).Then, cosmetic activities (enzymatic targets and skin cells) can be evaluated. For preliminary phytochemical investigations, an efficient and exhaustive method is traditionally used to extract polar plant

metabolites. The aim of our study was to define ASE conditions to obtain the most complete and representative extraction. In contrary, most of publications deal with the optimization of operational parameters to enhance the selectivity of extraction of only compounds of interest. The transposition of our conventional solvent-based extraction method (hydroethanolic reflux extraction, 1 h, ratio plant/solvent 10%) to ASE technology was performed with respect of amount of extracted material (dry matter, yield) and chemical composition. Among herbs selected, ASE extracts of *Lespedeza capitata* Michx. and *Scutellaria baicalensis* Georgi extracts were obtained and analyzed by Thin-Layer Chromatography. The flavonoids content of the extract was identified by HPTLC coupled with UV densitometric detection. The quantification of orientin/homorientin and baicalin/baicalein was achieved to select the best ASE parameters (50 °C, 10 min, ratio plant/solvent 20%). In addition, the antiradical activity of extracts was evaluated. The results showed that optimized ASE extracts were equivalent to conventional ones concerning phytochemical composition and antiradical activity. To conclude, such standardization of ASE extraction method is a powerful tool for rapid screening of new cosmetic plant active ingredients.

PA28 Determination of vitamin e (α-tocopherol) in canola oils by high performance liquid chromatography

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Tocopherols (Vitamin E) are natural phenolic antioxidants present in vegetable oils and are responsible for many of the healthful properties of these foods. They are effective radical scavengers and defend the body against free radical attack by protecting polyunsaturated fatty acids [1]. Vitamin E plays an important role at the intracellular level since its deficiency increases membrane fragility and promotes the damage of membranes by oxygen-reactive species, ozone, or other free radicals [2]. The tocopherols belong to a group of structurally related compounds called tocols. Foods such as nuts, seeds, some grains, and vegetable oils are good sources of natural tocopherol antioxidants. The various tocopherols may exist in a free or a esterified form. In seed oils, they are mainly present in the free state [3], and the level of antioxidant materials is of great importance in the stability of vegetable oil products. It is known that Vitamin E activity decreases [3] while the antioxidant activity increases in the order α-, β-, γ-, and δ-tocopherols. Canola is one of the most important oil seeds growing in many parts of the world. It is very important to grow canola with high oil levels for agronomical benefits. [4]. There is a growing interest for the use of oil seeds for nutritional, industrial and pharmaceutical usages [5]. The world production of canola oil is higher than soybean and sunflower [6].In this study an effective high performance liquid chromatography (HPLC) method for measuring α-tocopherol in canola gathered from different regions. **References:** [1]. Doelman CJ, in: Emerit I, Anclair C (Eds.) (1989) Antioxidant Therapy and Preventive Medicine, Plenum Press, p. 9. [2] Nesaretnam K, Guthirie N, Chambers AF and Carroll KK (1995) Lipids 30: 1139. [3] Kochar SP, Rossell JB, in: Hudson BJF (Ed.) (1990) Food Antioxidants, Elsevier, London, pp. 19 – 64. [4] Fanaei HR, Akbari Moghaddam H, Keykha GH, Naroueyrad MR and Modares Najafabadi SS (2007) Seed Plant 23: 59 – 74. [5] Carvalho IS, Miranda I and Pereira H (2006) Ind Crop Prod 24: 75 – 78. [6] Flagella Z, Rotunno T, Di Caterina R, de Simone G and De Caro A (2000) Proceedings of XV International Sunflower Conference, Toulouse I pp. C 139-C 144.

PA29 Study of electrooxidation mechanisms and antioxidant properties of flavonols and flavonolignans

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Flavonoids are a large group of naturally occurring polyphenolic compounds that are distributed in vascular plants. A wide range of the biological activities are attributed to flavonoids' antioxidant abilities. Flavonoids are benzo-γ-pyrone derivatives that can be divided into several groups according to their structural differences. Here we focused on quercetin, silybin and their derivatives which belong to the group of flavonols and flavonolignans, respectively. We described basic aspects of electrooxidation of flavonols and flavonolignans at a pyrolytic graphite electrode using cyclic and square wave voltammetry. Flavonols (quercetin, rutin and isoquercitrin) and flavonolignans (silybin, 2,3-dehydrosilybin, 7-O-methylsilybin, 20-O-methylsilybin and isosilybin), were studied in an adsorbed state using ex situ (adsorptive transfer – AdT) voltammetric methods. Under the given conditions, flavonols and flavonolignans are subject to a multistep oxidation. The potential of the oxidation of hydroxy groups and other substituents corresponds with antioxidant properties of studied polyphenols. Using ex situ voltammetry, the following order in antioxidant capacities was proposed: flavonols >> 2,3-dehydrosilybin > silybin and its derivatives. The results provide a solid basis for further study of both the antioxidant and prooxidant parameters of polyphenolic compounds using voltammetric methods and extend our knowledge of their electrooxidation mechanisms. **Acknowledgement:** *This work was supported by the Czech Science Foundation (Grant Projects No. P503/11/P312 and P301/11/0767), by the Ministry of Education, Youth and Sports (MSM 6198959216).*

PA30 A validated HPLC-DAD and HPLC-ESI-MS method for the analysis and quality control of *Viola odorata* aqueous preparations

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A method based on HPLC-UV-DAD coupled to an ESI-MS interface was developed for the determination of the constituents in the aqueous preparations of *Viola odorata* L. flowering tops. The assay was fast, simple and effective and permitted the quality control of the preparations. Aim of this work was to assess the qualitative and quantitative profile of the investigated preparations, with wide applications in food and cosmetic industry, and to propose a validated method for their quality control. HPLC-DAD-ESI-MS analyses supported by extensive preparative chromatographic investigations and NMR analyses revealed the predominance of complex flavonol glycosides. Eleven constituents were unambiguously identified. Main secondary metabolites were flavonol glycosides, principally derivatives of kaemferpol. Flavonol tri- and tetraglycosides are reported for the first time in the genus *Viola*. Their structure was confirmed by NMR analyses. The analytical method provided a good separation of the constituents. Good linearity of the calibration curves was achieved between $0.84\ 10^{-3}$ µg to 0.63 µg ($r^2 > 0.9998$). The assay was validated for LOD, LOQ, intra- and inter-day precision and accuracy. All validation criteria were fulfilled. The proposed qualitative method could be used as a starting method for the evaluation of *Viola* sp. preparations with further optimization case by case, according to the geographical variability. It is noteworthy that at the same time flavonoids baring different aglycones and number of sugar moieties are well separated. This is the first report of detailed analysis of the chemical composition of *Viola odorata* flowers.

PA31 HPLC-DAD, HPLC-ESI-MS and HPLC-MS/MS analyses of aqueous preparations of Tiliae flos (*Tilia platyphyllos*)

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Lime flower is used worldwide for its sedative and antispasmodic properties. Traditionally it is used for migraine, hysteria, feverish colds, and for raised arterial pressure associated with arteriosclerosis and nervous tension [1]. Besides flavonols, little is known for the rest of the phenol content. In the present study extensive HPLC-DAD, HPLC-ESI-MS and HPLC-MS/MS analyses were undertaken in the aqueous preparations of *Tilia platyphyllos* Scop. influorescences. An HPLC-DAD-ESI-MS method was developed and optimised for the quantitative determination of the constituents. Analyses of the ethanol extracts confirmed the predominance of flavonol glycosides and protocatechuic acid. In contrast, both decoction and infusion, which are nevertheless the traditional herbal preparations, were more complex, containing polar simple phenolics and low molecular weight procyanidins. The use of different HPLC col-

umns permitted a good separation of the constituents and enabled their quantitation. The method showed good linearity, (r^2 0.9999 for tiliroside and catechin and 0.9986 for quercetin-3-O-glucoside), intra/inter-day variability (%RSD < 1.56 and 1.33) and real sample repeatability (%RSD < 4.0). Preparative chromatographic investigations (Sephadex LH-20) and NMR analyses revealed the presence of procyanidin B4, while HPLC-MS/MS analyses enabled the identification of procyanidin trimers and tetramers. Overall, 20 constituents were detected and identified, belonging mainly to three classes of compounds: phenolic acid derivatives, condensed tannins and flavonol glycosides. Aqueous extracts contain a higher amount of procyanidins (strong chelating properties) than flavonols and caution should be taken upon frequent use of the drug. This is the first report of detailed analysis of the chemical composition of Tiliae flos. **References:** 1. Barnes J, Anderson LA, Phillipson JD. (2007) Herbal Medicines, Pharmaceutical Press.

PA32 HPLC-DAD/ESI-MS identification and quantification of polar constituents in six *Stachys* taxa from Balkan peninsula

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The genus *Stachys* L. comprises over 450 species [1] and is one of the largest genera of the Lamiaceae. Aerial parts of *Stachys* spp. are used traditionally to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough and ulcers [2]. In previous study an HPLC-DAD-MS method was developed for the quality control of *S. recta* [3]. Aim of the present work was to develop an HPLC-DAD/ESI-MS method which may serve as a tool for future chemotaxonomic and quality control studies of *Stachys* spp. Six mostly endemic *Stachys* taxa from Balkan peninsula were studied: *S. anisochila* Vis. & Pančic, *S. alpina* L. subsp. *dinarica* Murb., *S. atherocalyx* K. Koch, *S. beckeana* Dörfl. & Hayek, *S. nitens* Janka and *S. plumosa* Griseb. A RP-C 18 solvent saving column was chosen and the developed assay permitted a good separation among different constituents of the extracts. Overall, 42 constituents were detected and identified, belonging to three classes of secondary metabolites: flavone glycosides (isoscutellarein and hypolaetin derivatives), phenylethanol glycosides and caffeic acid derivatives. *S. plumosa* instead, contained chrysoeriol/apigenin derivatives. Peak purity analysis and peak assignment were accomplished by means of MS and reference compounds. The analytical method provided a good separation of the constituents, concerning both marker constituents (flavonoids) and the phenylethanol glycosides. Quantification results showed that these plants are a good source of flavonoids. All validation criteria (linearity, LOD, LOQ, intra- and inter-day precision, time precision, accuracy) were fulfilled. This method is a reliable and accurate tool for the detailed analysis of *Stachys* taxa. **References:** 1. Mabberley DJ The Plant-Book, Cambridge University Press, Cambridge, New York, Melbourne 2008. 2. Hartwell JL Plants used against cancer. A survey. Massachusetts: Quarterman Publications Inc. 1982. 3. Karioti A et al. (2010) J Pharm Biomed Anal 53: 15 – 23.

PA33 Validated HPTLC method for quantification of rosmarinic acid in seven *Salvia* species

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The genus *Salvia* (Lamiaceae) encompasses more than 900 species throughout the world. It is represented by approximately 90 species in Turkish flora and half of which are endemic [1]. The genus has attracted a great interest so that it has long been the subject of numerous phytochemical and pharmacological activity studies. Several species of *Salvia* have been utilized in folk medicines for various purposes (i.e.; abdominal pain, stomachache, would healing, carminative) [2,3]. Undoubtedly, rosmarinic acid is one of most significant phenolic compound in Lamiaceae family which is restricted to the subfamily Nepetoideae, including *Salvia* [4]. Mostly the antioxidant activity of many medicinal plants are associated with their rosmarinic acid content and therefore the rosmarinic acid composition of *Salvia* genus has also gained a great interest. In this study, we report a sensitive HPTLC method in order to determine and compare the rosmarinic acid contents of seven *Salvia* species; *S. candidissima* Guss., *S. dichroantha* Stapf, *S. heldreichiana* Boiss. ex DC., *S. sclarea* L., *S. tomentosa* Mill., *S. triloba* L.f. as well as the official sage *S. officinalis* L.. The methanolic extracts of the aerial parts of the plants were migrated on silica gel 60 F_{254} HPTLC plates with toluene: ethyl acetate: formic acid (5:4:1) as mobile phase and densitometric detection of rosmarinic acid was carried out at 330 nm. By this study we calculated the rosmarinic acid contents of seven *Salvia* species as w/w % by using HPTLC densitometric method which is validated in terms of accuracy, precision, repeatability, reproducibility, linearity, limit of detection, limit of quantification, sensitivity and specificity. **References:** 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegan Islands. Edinburgh University Press. Edinburgh. 2. Sezik E et al. (2001) J Ethnopharmacol 75: 95 – 115. 3. Baytop T (1999) Türkiye' de Bitkiler İle Tedavi. Nobel Tıp Kitapevleri. Istanbul. 4. Litvinenko VI et al. (1975) Planta Med 27: 372 – 380.

PA34 Insight into carotenoid structure in a single microalgae cell

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Astaxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in some orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* [1,2], yeast, salmon, trout, krill, shrimp etc. The changes of AXT structure upon thermal stress were investigated for unicellular microalgae *Haematococcus pluvialis* by means of *in situ* Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the ν C = C band at ca. 1520 cm^{-1} along with the change of 1190:1160 cm^{-1} ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

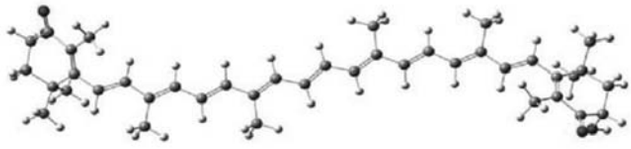

Figure 1: The molecular structure of astaxanthin

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PA35 Quantitative Analysis of Cyanidin-3-glycoside in different elderberry extracts by HPLC

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Anthocyanins widely distributed in fruits and vegetables are water soluble pigments. Anthocyanins and anthocyanin-rich fruits have various different biological activities such as antioxidant, antiviral, anti-aging, antidiabetic and antiproliferative effects (1,2). *Sambucus nigra* L. (Caprifoliaceae) is a shrub or small tree which is known as Mürver and distributed in the Black Sea Region of Turkey (3). It is widely used in Europe. Both fruits and inflorescenses are used as herbal tea for cough, colds and flu. The fruits are also used as a natural dye in fruit juices, wines and jams. In this present study, the fruits were macerated using different solvents and the fruit tea was prepared according to folkloric

use. All extracts were subjected to chromatographic and spectrophotometric analyses for their chemical compositions. In conclusion, the 70% ethanol extract was found to be rich in anthocyanins and cyanidin-3-glycoside when compared with other extracts. **References:** 1. Motohashi N and Sakagami H (2009) Top Heterocycl Chem 16: 1 – 40. 2. Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003) Phytochem 64: 923 – 933. 3. Davis PH (1972) Flora of Turkey and The East Aegean Islands, Vol 4, Edinburgh, University Press, pp. 541 – 543

PA36 Ultra-performance liquid chromatographic (UPLC) determination of the rutin and chlorogenic acid in the *Ribes anatolica* and its antioxidant activity

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A new ultra performance liquid chromatographic (UPLC) method was developed for the determination of rutin and chlorogenic acid in *Ribes anatolica* Behçet (Grossulariaceae) which is an endemic species in Turkey [1]. Good chromatographic separation and determination were performed on an Acquiting UPLCTM BEH phenyl column (100 mm x 1.0 mm, *i.d.*, 1.7 μm) system by using a mobile phase containing acetonitrile and formic acid buffer solution (pH = 3.77) with 3% triethylamine (15:85) (v/v) (See Figure 1). Chromatographic separation is by an isocratic elution with the flow rate of 0.3 mL/min. Calibration graphs for both compounds in the linear concentration range of 5 – 40 μg/mL were obtained by using the relationship between the concentration and the peak area based on the detection at 340 nm. The validity of the UPLC method was done by analyzing the plant samples. The developed UPLC method was applied to the quantitative analysis of *R. anatolica* consisting of rutin and chlorogenic acid and a good agreement was reported. The estimation of the antioxidant activities of water and methanol extracts of the leaf is based on thiobarbituric acid (TBA) assay in order to detect their liposome lipid peroxidation. In this investigation, the significant activities were obtained from the water (IC_{50}= 24.85 ± 6,33) and MeOH (IC_{50}= 27.03 ± 6,64) extracts of *R. anatolica* in the TBA test. Propyl gallate (IC_{50}= 0.04 ± 0,18) was used as a positive control. **References:** 1. Behçet L (2001) Turk J Botany 25: 103 – 105.

PA37 Rapid and efficient isolation of polymethoxylated flavonoids and artemisinin from *Artemisia annua* L. acetone extract

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Artemisia annua L., sweet or annual wormwood (in Chinese qïnghao: green herb) (Asteraceae) is an annual herb that is native to temperate Asia. It has been used in China for more than 2000 years for treating many disorders including malaria [1]. Polymethoxyflavonoids and artemisinin, one of the main compounds presents in *Artemisia annua* L., are among the most promising natural products for antimalarial and anticancer purposes [2]. In this work, three different chromatographic methods, including a rapid and selective isolation method of the main polymethoxyflavonoids (PMFs) – namely eupatin, the isomers casticin and chrysosplenetin, artemetin and 5-OH-3,4',6,7-tetramethoxyflavone – and artemisinin from *A. annua* acetone dried extract, have been compared. Briefly, the method consists of a pretreatment of the original extract between organic and aqueous layers and further purification of the richest extract in PMFs and artemisinin with Sephadex LH-20, silica gel normal phase column chromatografies and flash chromatography equipped with a prepacked normal phase silica column were performed. Quali-quantitative analyses of the main PMFs found in the extract were also reported. The best results in terms of efficiency of isolation were obtained by flash chromatography and to the best of our knowledge this is the first report on the separation of the pair of isomers casticin and chrysosplenetin from *A. annua* by flash chromatography. **Acknowledgement:** *Acnowledgments: Hanze University Groningen, Institute for Life Sciences and Technology, Groningen (NE) in combination with Erasmus European Comission Education and Training for the fellowship to C. Wessels.* **References:** 1. Bilia et al. (2006) Phytomedicine13: 487 – 493 2. Ferreira et al. (2010) Molecules 15: 3135 – 3170

PA38 Finding the most appropriate IR technique for plant species identification

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Quality control of herbal medicinal products is of extreme importance. Procedures for identification of plant species, such as biochemical analysis or macroscopic and microscopic examination of morphological and anatomical properties are time consuming and expensive[1]. A good alternative is infrared spectroscopy since it is rapid, easy to use, non-destructive and low-cost. Identification of species from dried whole leaf samples of pharmaceutically important *Epilobium* and *Hypericum* genera were investigated. To determine which infrared spectroscopy mode gives most informative spectra for plant species identification different modes of infrared spectroscopy were applied. These were diffuse reflectance, attenuated total reflectance (ATR), direct transmission of whole leaves, and KBr tablet transmission with comminuted leaves. First the informative wavenumbers were chosen by one-way analysis of variance. Afterwards the colinearity was reduced with principal component analysis. At last the species identification was determined with discriminant analysis. Best results were obtained with ATR and KBr tablet transmission. Still there were important differences between genera. ATR proved to be appropriate for discrimination among *Epilobium* species (accuracy of plant species identification was 98%), *Epilobium* species differ in distribution and morphology of trichomes on the surface of the leaves[2]. While for *Hypericum* species KBr tablet transmission proved to give best results (accuracy of plant species identification was 97%). *Hypericum* species differ in secondary metabolites that are acumulated in the interior of the leaves[3,4]. Results show that morphological properties of plant material should be taken into consideration when developing an infrared spectroscopy based method for identification of plant species. **References:** 1. European Pharmacopoeia 6th Edition, 2007. EDQM (European Directorate for the Quality of Medicines & Health Care), Council of Europe, Strasbourg Cedex, France 2. Strgulc Krajšek S, Dermastia M, Jogan N (2006). Bot Helv 116: 169 – 178. 3. Umek A, Kreft S, Kartnig T, Heydel B (1999). Planta Med 65: 388 – 390. 4. Maggi F, Ferretti G, Pocceschi N, Menghini L, Ricciutelli M (2004) Fitoterapia 75: 702 – 177.

PA39 Applicability of ultra- and nanofiltration for the concentration of medicinal plant extracts

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The present investigation revealed the potential benefits of ultra- and nanofiltration application in herbal extracts processing. *Helleborus purpurascens* Waldst. & Kit., Geranium robertianum L. and *Salvia officinalis* L. were widely used in traditional medicine. The aqueous extracts of G. *robertianum, H. purpurascens* and *S. officinalis* were concentrated by a ultra- and nanofiltration. The experimental results are reported, regarding polyphenols concentration and to evaluate the extracts antioxidant activity. The total polyphenolic content of the extracts was determined using the Folin-Ciocalteu method [1] and the antioxidant capacities were evaluated using DPPH scavenging methods [2]. Three membranes have been used: Millipore (cut-off 10,000 Da) for ultrafiltration, Millipore (cut-off 1,000 Da) and a new type of composite organic-inorganic membrane preparation in laboratory for nanofitration. Concentrations of active compounds up to 6 – 8 times higher have been obtained in the nanofiltration retentates with composite membrane and 2 – 5 times higher have been obtained in the nanofiltration retentates with Millipore membrane. The concentrated extracts by nanofiltration have a high antioxidant activity (94.8 – 97.2% DPPH inhibition for *Geranium robertianum* concentrated extract, 64.9 – 69.7% DPPH inhibition for *Helleborus purpurascens* concentrated extract and 89.2 – 91.7% DPPH inhibition for *Salvia officinalis* concentrated extract), thus it can be considered a good source for further medicinal applications. **Acknowledgement:** *This work was financially supported by the Romanian National Center for Program Management – PN62076/2008.* **References:** 1. Waterhouse AL (2002) Current Protocols in Food Analytical Chemistry. John Wiley&-Sons. New York. 2. Litescu S, Radu GL (2000), European Food Res. and Technol. Part A p. 211.

PA40 Profiling of African Mimosaceae for the rapid identification of new triterpenoid electrophiles

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Avicins are complex triterpenoid saponins isolated from an Australian Mimosaceae (Leguminosae), *Acacia victoriae* Benth. They are based on an acacic acid core, substituted by two glycosidic units and by a specific side chain at C-21 containing two monoterpene carboxylic acids and a quinovose moiety, conferring thereof particular electrophilic properties [1]. Avicins exhibit potent proapoptotic and anti-inflammatory activities, selectively inhibit the growth of tumor cells and thus appear as a new potential class of anticancer natural substances [2, 3]. In order to discover new avicins analogues and to identify the pharmacophore responsible for the activity, different African Mimosaceae species, including *Acacia, Albizia,* and *Entada* genera, were selected to screen their saponins content. Chemical profiling of saponin-enriched fractions, based on a LC-UV-MS/MS dereplication method and NMR experiments, has pointed out the presence of avicins analogues in the different studied species. Characteristic structural features of avicins consisting of an acacic acid aglycone, a tri- or tetra-saccharide moiety at C-28, a sugar residue at C-3, and the acylation of C-21 were highlighted by the developed method. These findings confirmed that some Mimosaceae species represent an alternative source for the discovery of new avicin analogues [4]. The dereplication approach enabled to quickly identify the compounds of interest, and so to focus on their specific isolation and saving a considerable amount of time. Structures of the isolated triterpenoids will be unambiguously elucidated on the basis of extensive analysis of NMR experiments and mass spectrometry, and further submitted to bioassays to study in depth their mechanism of action. **References:** 1. Jayatilake GS et al. (2003) J Nat Prod 66: 779 – 83. 2. Haridas V et al. (2001) Proc Natl Acad Sci USA 98: 11557 – 11562. 3. Gaikwad A et al. (2005) Clin Cancer Res 11: 1953 – 1962 4. Noté O et al. (2011) Phytochem Rev, *in press*

PA41 Exploitation of HPTLC for methodology development: quantification, fingerprinting and partition coefficient determination

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High Performance Thin Layer Chromatography (HPTLC) is a simple, modern analytical densitometric analysis technique [1 – 5]. In the present study, HPTLC methods were developed, validated and compared to analytical techniques routinely used in the laboratory. The quantification of oleuropein in differently processed extracts of olive leaves was carried out. A calibration curve was created with oleuropein as standard and their content was determined with HPTLC. Quantification was achieved by UV detection at λ 240 nm and excellent linear behaviors over the investigated concentrations were observed. The results were statistically compared with the ones obtained from two well established techniques, namely HPLC and NMR, and proved to be precise and accurate. Furthermore, HPTLC was used for the documentation of the fingerprinting of Genista halacsyi Heldr. in order to detect and quantify the major compounds. The chromatograms allowed the identification of seven main constituents. Moreover, the possibility of elaborating HPTLC for the determination of the partition coefficients used in counter-current chromatography was examined. The obtained results were successfully applied for the purification of the target compounds of the aforementioned plant, indicating that the partition coefficients could effectively be determined with HPTLC analysis and not necessarily with HPLC. HPTLC provided reliable results in all the methods which were developed. It was shown to be sensitive, selective, repeatable, easy to handle, requiring low analysis time and less cost per analysis. Overall, HPTLC could be efficiently employed instead of expensive and time-consuming techniques. **References:** 1. Vanhaelen-Fastre RJ et al. (2000) J. Chromatogr A 868: 269 – 276. 2. Yadav D et al. (2011) J Sep Sci 34: 286 – 291. 3. Rashmi et al. (2011) Phcog J 3: 41 – 44. 4. Dhalwal K et al. (2010) J Med Plants Res 4: 1289 – 1296. 5. Plocharz P et al. (2010) J Chromatogr A 1217: 4868 – 4872.

PA42 Phytochemical study of plants and plant cell cultures of three *Salvia* Species

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The genus *Salvia* L. is widely distributed cultivated in various regions all over the world because of his numerous biological and pharmacological properties. These positive effects have its source in the high diversity of their secondary metabolites and enable its application on pharmaceutical, cosmetics and food industries. The main activities namely adstringent, antibacterial, anti-inflammatory and antioxidative are effected for instance by the essential oils as well as phenolic acids, sterols and higher terpenoids. Beside cultivation parameters, the composition of these secondary metabolites is mainly affected by the species. According to their interesting spectrum of secondary metabolites, *S. officinalis* L., *S. triloba* L.f. and *S. virgata* Jacq. were selected for the induction of *in vitro* cultures. Since the traditional production of selected secondary metabolites by plant is influenced by various parameters like climate, geological conditions and infestation by parasites, the application of plant cell and tissue cultures *in vitro* reveal a potential alternative. In this case cultivation can be conducted under defined and optimized conditions in a bioreactor without the need of herbizides. Different phytochemical methods including extraction, isolation and chromatography techniques were applied on both the plants and their *in vitro* cultures in order to compare their secondary metabolite production. Therefore GC/MS analysis was performed for the identification and structure determination of present secondary metabolites. The observed results of these experiments will be presented. **Acknowledgement:** *This work has been supported by European Social Funds and the Free State of Saxony, project number 080938406.*

PA43 Raman spectroscopy analysis of tobacco alkaloids

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Tobacco plants and products contain alkaloids, mainly nicotine, beside e.g., nornicotine, cotinine, and anabasine. The latter are also metabolites of nicotine produced in the liver by cytochrome P450. The aim of our research was in situ investigation of tobacco alkaloids directly in the plant as well as in some pharmaceutical products. Two-dimensional Raman maps of nicotine distribution were obtained with 1064 nm excitation wavelength, the spatial resolution of 50 – 200 μm and analyzed with the aid of quantum-chemistry calculations (B3LYP/6 – 311++G(d,p) and B3LYP/aug-cc-pVDZ). Additionally, calculations were performed for salts and protonated forms of nicotine. Distribution of nicotine was obtained by integration of characteristic bands of the compound over the measured surface. **Acknowledgement:** *This research was supported by the Polish Ministry of Science and High Education (grant no. N204013635). Computational center "Cyfronet" (Krakow, Poland) is acknowledged for CPU time.*

PA44 Quantification of scopoletin in *Artemisia annua* L. using HPLC-ED

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Artemisia annua L. (Asteraceae) contains various phytochemical compounds such as monoterpenoids, sesquiterpenoids, flavonoids, coumarins, sterols etc. Scopoletin (7-hydroxy-6-methoxycoumarin) is coumarin which can be found in plants of family Asteraceae. In this study, using HPLC-ED system, quantitave analysis of scopoletin was carried out in water extracts of *A. annua*. The shade dried plant material (10 g) was powdered and subjected to extraction with petroleum ether in Soxhlet apparatus. The defatted marc was then extracted with ethanol till complete extraction. 100 mg of ethanolic extract was weighed accurately and dissolved in 10 mL of water with vigorous shaking. It was then filtered and the volume made up to 100 mL with water (stock solution). HPLC conditions were following: Mobile phase: methanol-acetonitrile-water-acetic acid (20+10+70+1); electrochemical detector with range 50 nA, potential +0.840 V, filter 0.02 Hz; flow rate 1 mL/min; temperature 25 °C, column: ODS hypersil. Injection volume was 20 µl. Concentrations of standard were: 10 ng/20 µL, 20 ng/20 µL and 40 ng/20 µL. Retention time of scopoletin in standard solution and extracts of *A.annua* was 11.4 minutes. Content of scopoletin in extracts of two samples of *A. annua* were 109 µg/g and 534 µg/g dry plant. The result showed that very low concentration of scopoletin in plant extracts can be indentified and quantified. **References:** 1. Bakuni R S et al. (2001) Current Science 80: 35 – 48 2. Brown G D (2010) Molecules 15: 7603 – 7698 3. Fernandez Izquierdo ME et al. (2000) Food Chemistry 70: 251 – 258 4. Tzeng TC et al. (2007) Separation and Purification Technology 56: 18 – 24 5. Xia Y et al. (2007) J Chromatography B 857: 332 – 336

PA45 Comparison of total phenolics of ethanolic extracts from Sideritis species, Distributed in the natural flora of Antalya

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The genus *Sideritis* L. (Lamiaceae) includes more than 150 species growing mainly in the Mediterranean areas (1) this genus has 44 species (55 taxa) in Turkey (2). In this study; aerial parts of the flowering 14 perennial *Sideritis* plants (Table1) were collected during July 2010. In order to analyse of the total phenolic in *Sideritis*, samples containing 0.25 g of powder material were processed. The ethanol extraction was performed with 25 mL of 70% ethanol at room temperature for 24 h with a shaker (3). Ethanol extractions were filtered and the total phenolic content was estimated by the Folin-Ciocalteu method (4). Nine hundred microlitres of water were added to 100 µl extracts. 5 ml of 1:10 diluted Folin-Ciocalteu reagent and 4 ml of sodium carbonate (75 g/l) added to extracts. After 2 h of incubation in the dark at room temperature, the absorbance at 765 nm was measured. The amounts of total phenolic substance in 14 species varied in the range of 27.5 and 68.9 mgGAE/g. While the taxa of *Sideritis stricta* Boiss. & Heldr., sampled from Akdeniz University Campus has the highest value (68.9 mgGAE/g), the lowest value (27.5 mgGAE/g) was detected in taxa of *Sideritis albiflora* Hub.-Mor., sampled from Kemer location (Figure1). Because of the close relationship between the total amount of phenolic compounds and antioxidant effect, it has been concluded, that it is able to profit from the *Sideritis* species, containing high amounts of phenolic as a source of natural antioxidants. **References:** 1. Obon De Castro C and Rivera Nunez D (1994) A Taxonomic Revision of the Section Sideritis (Genus Sideritis) (Labiatae), Cramer J, ed., Vol.21, Phanerogamarum Monographiae, Berlin-Stuttgart 2. Duman H, Kırımer N, Ünal F, Güvenç A ve Şahin P (2005) Türkiye *Sideritis* L. Türlerinin Revizyonu, TÜBİTAK Projesi Sonuç Raporu, Proje No: TBAG-1853 (199T090). 3. Petreska J, Stefova M, Ferreres F, Moreno DA, Tomas-Barberan FA, Stefkov G, Kulevanova S, Gil-Izquierdo A, (2011) Food Chem125: 13 – 20 4. Spanos G and Wrolstad R.E (1990) J Agric Food Chem 38: 1565 – 1571.

PA46 Total Phenolics of *Origanum* Species from Natural Flora of Antalya

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The genus *Origanum* L. is represented in Turkey by 26 taxa (23 species, 3 subspecies), 13 being endemic to Turkey. 50% of all 51 Origanum taxa, known in the World are distributed in Anatolia (1,2,3). This high rate suggests that the gene centre of Origanum is Turkey (4) 9 perennial *Origanum* plants from 23 location were collected from natural flora of Antalya. In order to analyse the total phenolics in *Origanum* (leaf and flowers), samples 0.25 g of powder material of each species were processed. The ethanol extraction was performed with 25 mL of 70% ethanol at room temperature for 24 h with a shaker. The total phenolic content was estimated by the Folin-Ciocalteu method. Ethanol extractions were centrifuged and 100 µl extracts were taken, nine hundred microlitres of water were added. 5 ml of 1:10 diluted Folin-Ciocalteu reagent and 4 ml of sodium carbonate (75 g/l) added to extracts. After 2 h of incubation in the dark at room temperature, the absorbance at 765 nm was measured. The total amount of phenolic compounds varied in the range of 56.1 – 109.1 mgGAE/g at the level of location and 61.6 – 98.6 mgGAE/g at the level of species. The highest value at the level of location was obtained from *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw., in Kemer location. However, *Origanum solymicum* P.H.Davis, local endemic species had high values in all three locations and the highest average at the level of species. *Origanum hypericifolium* O.Schwarz & P.H.Davis had the lowest average at the level of species. **References:** 1. Ietswaart J H (1982) Origanum L. In: Davis, P.H. (Editor), Flora of Turkey and the East Aegean Islands, Vol. 7: 297 – 313, Edinburgh University Press, Edinburgh. 2. Davis PH, Mill R R and Tan K (1988) Flora of Turkey and the East Aegean Islands (Suppl. I), Vol 10, Edinburgh University Press, Edinburgh. 3. Güner A, Özhatay N, Ekim T and Başer K H C (2000) Flora of Turkey and the East Aegean Islands (Suppl. II), Vol 11, Edinburgh University Press, Edinburgh. 4. Baser K H C (2002) The Turkish Origanum species. In: Kintzios S E (Editor), Oregano: the genera Origanum and Lippia. Taylor & Francis, pp.109 – 126, London.

PA47 Total Phenolics of *Thymus* species from Natural Flora of Antalya

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The genus *Thymus* (Lamiaceae) is represented by about 250 taxa (214 species, 36 subspecies) worldwide (1). There are represented by 37 species and 55 taxa (species, subspecies, variety) in Turkey, 27 taxa of which are endemic (2,3,4). In this study; aerial parts of the flowering 9 perennial *Thymus* plants from 17 locations were collected from natural flora of Antalya. In order to analyse of the total phenolics in *Thymus* (leaf and flower) samples, 0.25 g of powder material were processed. The ethanol extraction was performed with 25 mL of 70% ethanol at room temperature for 24 h using a shaker (5). The total phenolics content was estimated by the Folin-Ciocalteu method (6). Ethanol extractions were santrifuged and 100 µl extracts taken, nine hundred microlitres of water were added. 5 ml of 1:10 diluted Folin-Ciocalteu reagent and 4 ml of sodium carbonate (75 g/l) added to the extracts. After 2 h of incubation in the dark at room temperature, the absorbance at 765 nm was measured. The total amount of phenolic compounds varied in the range of 45.1 – 76.4 mgGAE/g at the level of location and 45.1 – 66.8 mgGAE/g at the level of species. The highest value at the level of location was obtained from *Thymus zygioides* Griseb., in Saklıkent location. *T. sipyleus* Boiss. subsp. *sipyleus* had the highest average at the level of species. *T. leucotrichus* Halácsy has the lowest average at the level of species. **References:** 1. Morales R (2002) The history, botany and taxonomy of the genus Thymus. In: Stahl-Biskup E, Saez F (Eds) Thyme-the Genus Thymus, Taylor-Francis pp.1 – 43, London. 2. Jalas J (1982) Thymus L. In: Davis, P.H. (Editor), Flora of Turkey and the East Aegean Islands, Vol. 7: 349 – 382, Edinburgh University Press, Edinburgh. 3. Davis PH, Mill R R and Tan K 1988. Flora of Turkey and the East Aegean Islands (Suppl. I), Vol 10, Edinburgh University Press, Edinburgh. 4.Güner A, Özhatay N, Ekim T and Başer K H C (2000) Flora of Turkey and the East Aegean Islands (Suppl. II), Vol 11, Edinburgh University Press, Edinburgh. 5.

Petreska J, Stefova M, Ferreres F, Moreno DA, Tomas-Barberan FA, Stefkov G, Kulevanova S, Gil-Izquierdo A (2011) Food Chem 125: 13 – 20 6. Spanos G and Wrolstad RE (1990) J Agric Food Chem 38: 1565 – 1571.

PA48 Natural product Marie-Lise database development for high-throughput phytochemical profiling of plant extracts

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Modern drug discovery is greatly based on the identification and structural characterization of new lead compounds, stemming from the huge diversity of natural plant chemicals. The process is tedious facing the complexity of plant metabolome, and the time-consuming steps of purification. In order to reduce the number of de novo purification and elucidations of chemical entities, an interesting strategy is to create a natural-product database. Using about two hundred natural compounds including alkaloids, steroids, terpenes and phenolic compounds from commerce and our in-house chemical library (UMR 7200), we developed a database named "Marie-Lise" through a combination of HPLC-DAD and LC-QqTOF-MS/MS analysis using Galaxie and MassLynx softwares. High detection sensitivity and selectivity of these methods permits us to characterize: retention time, UV spectra, mass spectra, MS/MS datas and metabolite specific calibration curve of the two hundred validated pure substances. This data matrix allowed us to build programs that permit to achieve high-throughput screening (HTS) of chemical structures in order to accelerate the discovery of new compounds or to identify and quantified known compounds of specific biological interest or toxicity from complex mixtures such as plant extracts or preparations.

PA49 Combination of LC retention, high resolution TOF-MS information and web database search as dereplication tools in a chemotaxonomic study of *Lippia* spp

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Dereplication of natural products (NPs) in crude plant extracts represents a key process to rationalize bioactivity guided isolation procedures [1]. This process is efficient using LC-MS, notably with instrument specific MS/MS databases, but libraries of spectra have to be built or rebuilt from large collection of pure standards. In order to evaluate how far NP annotations can be made from a single LC-MS profiling using high resolution (HR)MS, online molecular formula assignment and LC-retention-based methods were evaluated in the frame of a chemotaxonomic study on various *Lippia* species from Brazil. In this respect, metabolite profilings were recorded on all crude extracts by HR-UHPLC-TOF-MS using generic long gradient and MS conditions. The high mass accuracy of the TOF-MS routinely provides exact mass determination below 10 ppm, corresponding to 5 – 50 putative molecular formulae per mass detected. To reduce this large number of formulae, heuristic filters were applied, which took into account statistics on characteristic compositional features of organic compounds and database information of existing NPs [2]. This approach mainly reduced the possible hits to one single molecular formula. Search in web databases with chemotaxonomic information provided a few possible NPs for each formula. These were discriminated by retention information, based on the well-known log P – retention relationship [3], comparing the calculated log P of known compounds of the extract with the calculated log P of putative structures. This procedure has been used in a chemotaxonomic study of Brazilian *Lippia* species and more than 40 compounds were annotated in this way. **References:** 1. Wolfender JL (2009) Planta Med 75: 719. 2. Kind T, Fiehn O (2007) BMC Bioinformatics 8: 20. 3. Martel S et al. (2008) Chromatographic Approaches for Measuring log P, in Molecular Drug Properties – Measurement and Prediction. Ed. R. Mannhold, Wiley-VCH, Weinheim, Germany.

PA50 Isolation and characterization of caffeic acid derivates from *Galtonia viridiflora*

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Galtonia is a South African genus of Asparagaceae (former Hyacinthaceae) comprising several bulbous geophytes. So far, little is known about natural products of *Galtonia* species except *G. candicans* Decne. Recent studies showed it to be rich in umpteen cholestane glycosides [1]. This work deals with information about natural products isolated und purified from extracts of *Galtonia viridiflora* I.Verd. *G. viridiflora*, also known as Cape Hyacinth, is a pale green flowering species, which naturally distribution areas are the Orange Free State to Lesotho an the Northeast Cape. Ethanolic and methanolic extracts of bulbous and herbal parts were separated by medium pressure column chromatography on silica gel and polyamide followed by purification *via* preparative HPLC. Several isolated compounds were identified by comparison with authentic substances using FT-IR, UV-Vis and 1 H-NMR. Quantification of the main compounds was carried out by HPLC analysis with UV detection. Beside glycosidic saponins several caffeic acid derivates were found. Above ground and bulbous extracts contain different conjugates of caffeic acid with monoglycosides, mainly rhamnose and glucose. Furthermore, chlorogenic acid and isochlorogenic acids were found in all parts of *G. viridiflora*. Cafteric acid (1) and chicoric acid (1, CA) were mainly detected in extracts of herbal parts. A content of up to 1% CA in the flower's extract is noteworthy, because this substance was found to be a pharmacologically active compound. Several *in vitro* investigations described immune modulating effects and a potential for inhibiting viral integrase [2].

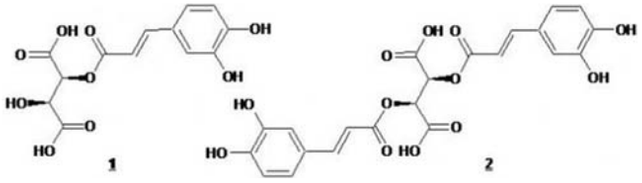

Figure 1

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PA51 Determination of parthenin in Parthenium hysterophorus L. by means of HPLC-UV: Method development and validation

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Parthenium hysterophorus L. or Santa Maria feverfew is a plant used in Cuba since antiquity for the treatment of several diseases. Nowadays, it is still used as an antipyretic and antiparasitic agent (1,2). Parthenin, a sesquiterpene lactone, is the active secondary metabolite and the major component of the plant (3,4). In this study the development and validation of a HPLC method for the determination of parthenin in the powdered plant material are presented, making it possible to perform quality control on preparations containing P. hysterophorus. Firstly, parthenin was isolated from the plant material in order to use this as reference material. During the method development, the extraction procedure, sample preparation, and HPLC conditions were evaluated and optimized. The final method was fully validated in terms of calibration model, precision, accuracy, and specificity. Based on these results, it was concluded that the developed HPLC method is suitable for the determination of parthenin using a single- point calibration. The calibration model was

linear in the concentration range from 0.05 to 0.25 mg/ml. Analysis on different days showed that the method was precise with an average concentration of 4.73% and RSD of 1.39%. A recovery experiment was performed resulting in a 95% confidence interval between 97.5% and 100.4%, meaning that the method is also accurate. Specificity was confirmed by the investigation of the peak purity. Using this newly validated method the quality of the plant material of P. hysterophorus, used as an active principle in pharmaceutical preparations, can be guaranteed. **Acknowledgement:** *Project (Belgium)"Strengthening under graduate and graduate education in PharmaceuticalSciences"* **References:** 1. Belz RG et al. (2007) Planta Med18: 275 – 277. 2. Dominguez XA, Sierra A (1970). Planta Med18: 275 – 277. 3. Khare CP (2007) Indian Medicinal Plants: An Illustrated Dictionary, Springer, New Delhi. 4. Picman AK et al. (1980). Chromatogr 189: 187 – 198.

PA52 Liquid chromatography techniques for separation of flavonoids from Droseraceae

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Flavonoids which are presented in insectivorous plants of the Droseraceae family have wide range of advantageous properties i.e. an antioxidant, anti-inflammatory and antimicrobial activities, antitumor activity was reported as well. The purpose of the research was to develop the most favourable conditions for liquid chromatographic separation and identification of myricetin and quercetin in extracts of insectivorous plants. In the research a methanol and chloroform extracts of the *Drosera binata* Labill., *Drosera capensis* L., *Drosera aliciae* Raym.-Hamet and *Dionea muscipula* Ellis cultivated *in vitro* were used. In the first stage of the research an optimal composition of the eluent for selective separation of the components included in the extracts was selected. The thin layer chromatography (TLC) in the reversed phase and hydrophilic interaction chromatography (HILIC) conditions was used for selection of optimal chromatographic systems. In the second stage of the research a high performance liquid chromatography (HPLC) in RP and HILIC optimal conditions was used for detailed characteristic of analysed mixtures. The results show that thin layer chromatography is helpful technique for pre selection components of the eluent to separation of the flavonoids from complex herbal matrix. In the paper there is reported that application gradient elution is more preferable than isocratic elution in HPLC techniques. **Acknowledgement:** *State Commitee for Scientific Research, Grant No N N405 3757 37. This research work was supported by the European Union in the framework of the European Social Fund. The system project of the Pomorskie Voivodeship "InnoDoktorant – Scholarships for PhD students, II edition".*

PA53 Optimal conditions of naphthoquinones separation from carnivorous plants extracts using thin-layer chromatography and high performance liquid chromatography

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Droseraceae plants are owned by the family of carnivorous plants, which in an unusual way adapt to environments poor in nutrients. Droseraceae obtain essential nutrients catching insects and other small invertebrantes. Plumbagin, chloroplumbagin, ramentaceon and droseron are naphthoquinones, which could be found in leaves and shoots of plants from Droseraceae family. In the literature there are a lot of information about the pharmacological properities of these secondary metabolites. The purpose of this research was to develop optimal conditions for separation of naphthoquinones contained in extracts of insectivorous plants of the species *Dionaea muscipula* Ellis, *Drosera aliciae* Raym.-Hamet, *Drosera capensis* L. and *Drosera binata* Labill. The experimental studies have been divided into two stages, the first included study using thin layer chromatography (TLC) and the second using high performance liquid chromatography (HPLC) technique. Both the studies by thin layer chromatography and high performance liquid chromatography was performed in normal and reversed phase system. Optimal conditions of naphthoquinones separation using TLC and HPLC in normal and reversed phase system will be presented. **Acknowledgement:** *State Commitee for Scientific Research, Grant No N N405 3757 37. This research work was supported by the European Union in the framework of the European Social Fund. The system project of the Pomorskie Voivodeship "InnoDoktorant – Scholarships for PhD students, II edition".*

PA54 Application of HPTLC-MS for the *on-line* identification of oxypregnan glycosides in *Hoodia gordonii*

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Hoodia gordonii (Mass.) Sweet is a succulent plant from South Africa and Namibia which has been used by the indigenous people to suppress appetite. Oxypregnan glycosides (hoodigosides) are considered as active principles [1]. HPLTC methods have been previously developed for the fingerprint analysis and identification of extracts from *Hoodia gordonii* [2,3]. Ion-trap tandem mass spectrometry and liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry have been used for the identification of isolated steroidal glycosides in *Hoodia gordonii* [4]. Recently, an HPTLC-MS Interface became available, which semi-automatically can extract zones of interest directly from a TLC/HPTLC plate and can direct them into a LC-MS system so that mass spectra can be obtained [5,6]. Previously we have tested the HPTLC-MS Interface for analysis of flavonoid containing herbal drugs [7]. We now have investigated the HPTLC-MS Interface for the identification of hoodigosides in extracts of *Hoodia gordonii*. Extracts have been applied as bands onto HPTLC plates using an automatic TLC sampler. Separated zones were eluted from the plate by the HPTLC-MS interface using methanol as solvent delivered by an HPLC pump at 100 µl/min. The interface was hyphenated to a Finnigan LCQ Deca XP Plus ion trap mass spectrometer equipped with an electro spray ionization (ESI) source. Hoodigosides M, L, U, O, E, F, J, N, and C could be identified on the basis of the mass spectra obtained by HPTLC-MS. Therefore, the HPTLC-MS interface is a quick and powerful tool for the on-line identification of hoodigosides in TLC separations and can complement the classical TLC detection tools. **References:** 1. Vermaak I et al. (2011) Planta Med [Epub ahead of print]. 2. Widmer V et al. (2008)J Planar Chromatogr (2008) 21(1): 21 – 26. 3. Rumalla Ch et al. (2008)J Sep Sci 31: 3959 – 3964. 4. Avula B et al. (2008) Rapid Comm Mass Spectrom 22(16): 2587 – 2596. 5. Luftmann H et al. (2007) Rapid Commun Mass Spectrom 21: 3772 – 3776. 6. Reich E, Widmer V (2009) Planta Med 75(7):711 – 718. 7. Bauer R et al. (2010) Planta Med 76: 1334.

PA55 Application of near-infrared spectroscopy (NIRS) as a tool for quality control in Traditional Chinese Medicine (TCM)

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Traditional Chinese Medicine is becoming more and more popular all over the world. Novel analytical tools for quality control are highly demanded enabling analysis starting at breeding and ending at biological fluids including urine or serum. Compared to analytical separation methods (chromatography, electrophoresis) near-infrared spectroscopy (NIRS) allows analyzing matter of interest non-invasively, fast and physical/chemical parameters simultaneously. It can be used for the quantitative control of certain ingredients. In many cases identification can only be achieved by pattern recognition. Therefore, NIRS combined with cluster analysis offers huge potential to identify e.g. species, geographic origin, special medicinal formula etc (Figure 1). In the present contribution the fundamentals, possibilities of NIR applied in quality control of TCM are pointed out and its advantages and disadvantages are discussed in detail by several practical examples [1,2]. A Büchi FT-NIR spectrometer was used for recording. Cluster analyes and PLS calibration models were generated with NirCal 4.21 and/or The Unscrambler. A Perkin Elmer 400 spectrometer in combination with a microscope with a nitrogen cooled MCT detector-array was used to acquire the hyperspectral images. NIR imaging is highly useful to judge the botany and morphology of the sample and allows visualizing the distribution of active plant

ingredients. Stable PLS calibration models can be applied for quantitative determination of API's, judgment of raw materials, during the production and the preparation of medicinal formulations. Cluster analyes are highly suitable for identifying falsification, species and georgraphic regions. Both methods in combination are applied to monitor the quality of patented formulations.

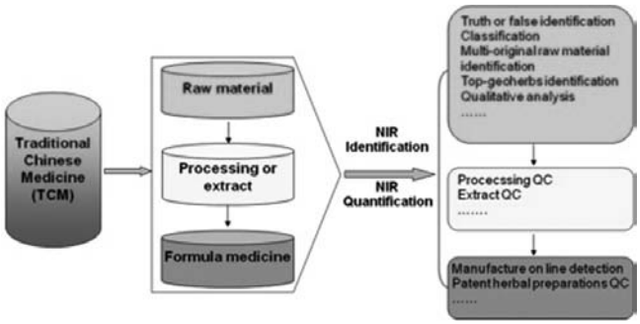

Figure 1: Flow diagram of NIRS application fields in TCM

Acknowledgement: *Eurasia-Pacific Uninet (EPU) (Salzburg, Austria), the Ministry for Science and Research and the Ministry for Health, Family and Youth (Vienna, Austria) (Novel Analytical Tools for Quality Control in Traditional Chinese Medicine, Project No. 80855), the Leopold-Franzens University, Innsbruck (Nachwuchsförderung) for financial support* **References:** 1. Pezzei C, Pallua JD, Schaefer G, Seifarth C, Huck-Pezzei V, Bittner LK, Klocker H, Bartsch G, Bonn GK, Huck CW (2010). Mol Biosyst 6: 2287. 2. Pallua JD, Pezzei C, Huck-Pezzei V, Schönbichler S, Bittner LK, Bonn GK, Saeed A, Majeed S, Farooq A, Najam-ul-Haq M, Abel G, Popp M and Huck CW (2011) Curr Bioactive Comp 7: *in press.*

PA56 Study and comparison of the *Pistacia atlantica* Desf. oleoresins from Iran

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Pistacia atlantica Desf. is one of the species which is valued because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases [1, 2]. Furthermore, the extracted oleoresin from *P. atlantica* is used in the chewing gum industries. One of the difficulties in chewing gum industries is the influence of the preliminary processing of the raw materials on the quality of the final products. It may caused by the variation of the different geographical area where the raw materials were collected or the process of preliminary preparation. These factors were monitored according to the chemical and physical examinations. The preparation process was monitored over the final physical properties of samples using thermal gravimetric method, (TG) and differenzial scanning calorimetry, (DSC). Moreover, In order to evaluate the geographical influence, the fragrance and the structural compounds of the raw samples collected from different areas were studied. The essential oil from the oleoresin, collected from six different locations in Iran, was obtained and its chemical composition was determined with GC and GC-MS. The yield range of essential oil was 17 – 22% (w/w), and the major compound is α-pinene, (92%). Because of the versatile usage in food industries and high concentration of α-pinene, it subjected for the antimicrobial activity against variety of respiratory and gastrointestinal microorganisms. In addition, because the oleoresin has a carbohydrate structure [3], the monosaccharides constituted of extracted polysaccharides were studied. The results demonstrate that the major constitutions were arabinose and galactose. **References:** 1. Meickle RD (1977) Flora of Cyprus The Bentham-Moxon Trust, Royal Botanic Gardens. Kew. 2. Bellakhder J (1997) La pharmacopée marocaine traditionnelle; Médicine arabe ancienne et savoir populaire. Ibis Press. Saint Etienne. 3. Kottakis F et al. (2008) Amino Acids 34: 413 – 420.

PA57 ^{1}H NMR Metabolomic analysis of coffee and tea samples for the quantative determination of the main constituents

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In metabolomics the aim is to detect and quantify all metabolites present in a certain organism, tissue or cell at a specific moment. One of the major problems in the analysis is the incomplete extraction [1]. In NMR based metabolomics generally aqueous solutions are used. In studies with humans or animals, where the samples are generally derived from body fluids this seems logic, but in plants a much larger variety of metabolites is encountered and the metabolites are obtained through the extraction of tissues. For the analysis of samples of tea and coffee the extraction procedure was investigated. Direct extraction with commonly used NMR solvents, did show that no single solvent provided a complete profile, but the best results were obtained with an extraction with a two-phase system consisting of water and chloroform. Water was found to be essential to obtain a good extraction of the apolar constituents with chloroform. The direct use of chloroform on dried plant material yielded incomplete extractions. The application of the developed protocol on the coffee and tea samples permitted an accurate quantification of the caffeine content and the detection of specific metabolites in different types of coffee and tea. **Acknowledgement:** *CNPq, FAPERJ, CAPES.* **References:** 1. Schripsema J (2010) Phytochem Anal 21: 14 – 21

PA58 Quantitative and qualitative analyses of rosmarinic acid in South African *Salvia* species using two chromatographic techniques

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The genus *Salvia* (Lamiaceae) encompasses about 900 species worldwide of which 26 are indigenous to southern Africa. The South African species are used in traditional medicines [1], as spice or tea due to reported good anti-oxidant properties [2]. The anti-oxidant capacity of these plants has been ascribed to the presence of phenolic compounds such as rosmarinic acid (RA), caffeic acid, carnosic acid and carnosol [2]. HPTLC-densitometric and HPLC-UV chromatographic techniques were used for qualitative and quantitative analyses of RA in 18 methanol:-chloroform extracts from sixteen *Salvia* species. Polynomial and linear regression analyses were used to estimate the amount of RA in solvent extracts by HPTLC-densitometric and HPLC-UV techniques, respectively. RA was identified in all the samples investigated and ranged from 13.1 μg/mg (*S. stenophylla* Burch. ex Benth.) to 113.0 μg/mg (*S. muirii* L.Bolus). The paired sample t-test showed no statistical significant difference in the estimation of the amount of RA in the solvent extracts using the two chromatographic techniques. A strong correlation (*r2*= 0.93) was found between the estimation using the HPTLC-densitometric and the HPLC-UV calibrations. **Acknowledgement:** *National research Foundation (South Africa); Tshwane University of Technology, South Africa National Biodiversity Institute (Pretoria).* **References:** 1. Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. E and S. Livingstone, Edinburgh. 2. Kamatou GPP et al. (2010) Food Chem 119: 684 – 688.

Topic B: Biotechnology

PB1 Expression of amorpha-4,11-diene synthase (ADS) gene in Iranian *Artemisia annua* L. genotypes

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Malaria disease is caused by unicell of *Plasmodium falsiparum*. Nowadays, malaria has been reported in more than 100 countries. The artemisinin is a sesquiterpene that is produced by two pathways of isoprenoid and mevalonate in *Artemisia annua* L. Artemisinin as new and effective drug is widelyused. *Artemisia annua* is annual plant and native to Asia and most probably China. In this study, six different genotypes *Artemisia annua* was collected from the province of Golestan. Amorpha-4, 11-diene synthase (ADS) promoter was analyzed by using different

cis-elements database of PLATcare, TRANSFAC and PLACE. The result showed that there are different cis-elements responding to plant hormones and abiotic stress in ADS promoter. We also identified two new putative transcription factors in EST library of *Artemisia annua* then studied the expression of ADS gene and three transcription factors by using real time PCR technique. The result showed that WRKY transcription factor had more important role than other transcription factors.

PB2 Development of NaCl-tolerant line in *Tanacetum cinerariaefolium* through shoot organogenesis of selected callus line

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Plants were regenerated successfully through shoot organogenesis of a NaCl-selected callus line of *Tanacetum cinerariaefolium* (Trevir.) Schultz-Bip developed through stepwise increase in NaCl concentration in MS medium. Increasing NaCl level concentration (0, 5, 10, 15, 20, 25, 30, 35, 40, 45mM) from low level to high level was found to be a better way to isolate NaCl-tolerant callus line, since direct transfer of callus to high saline medium was detrimental to callus survival and growth. Among different media and growth regulator treatments, MS media containing 1 mgl^{-1} BA and 1 mg l^{-1} NAA or 1 mgl^{-1} BA, 2 mgl^{-1} NAA and 0.5 mgl^{-1} GA3 for shoot organogenesis in selected callus line and B5 medium supplemented with 2 mgl^{-1} NAA showed best response for root regeneration. As increasing NaCl concentrations (From 0 to 45 mM) the ability of shoot and root regeneration were decreased. The selected callus line showed significance increase in proline content and decrease in pyretrine content. Based on growth performance and proline content (20 mM in callus line and 35 mM in shoot culture) could be considered as NaCl-tolerant line showing all positive adaptive features towards the salinity stress. Further studies about agronomic performance of obtained plants under saline soil condition are necessary for understanding to check the genetic stability of the induced salt-tolerance plants.

PB3 Stable Over expression of codeinone reductase gene in transgenic *Papaver somniferum* plant

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Papaver somniferum today is the commercial source of the narcotic analgesics morphine and codeine. By conversion of codeinone and morphinone to morphine, codeinone reductase is a key gene in metabolic engineering of isoquinoline alkaloids pathway. In this project, at first we optimised expression of gus gene in *P. somniferum via Agrobacterium tumefaciens* containing pBI121 plasmid. Encoding gene of COR enzyme was isolated using primers which designed on the base of gene sequence available on data banks (NCBI) for *P. somniferum*. This gene then cloned in expression vectors under controlled of CaMV35 promoter and transferred to plants by agro transformation. The result of evaluation showed the qualitative and quantitatives changes in metabolite production of transgenic and control plants.

PB4 Accumulation of phytoalexins in potato tuber treated with plant extract

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One of the best and longest-studied defense responses of plants to infection is the induced accumulation of antimicrobial, low-molecular-weight secondary metabolites known as phytoalexins. A role for these compounds in defense has been revealed through several experimental approaches. Samples of *Olea europeae* L. leaves were collected from Béjaia in January 2009. The whole samples were dried in shade and crushed to fine powder. 20 g of dried powder of olive leaves were submitted to extractions which were carried out twice for 24 h with 400 mL of ethanol according to Ranalli et al (2006). The total phenolic contents of the samples were determined with the Folin Ciocalteu reagent. The half tuber cultivars Desiree and Spunta, was treated by depositing 100 µl of one of the previously prepared phenolic extracts in the hole drilled with a cork as described by val et al. (2006). They were then inoculated with an inoculum of *Pectobacterium atrosepticum* (108 cfu/ml). The tubers were assessed after five days for the development of diseases symptoms, and were used to evaluate production of phytoalexin The rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site, of potato tubers treated with plant extract was studied. The results of the half-tuber inoculation treated by various extracts showed a remarkable reduction in the amount of rotted tissue. Evidence in support of phytoalexins in resistance as well some recent advances in phytoalexin biosynthesis are reviewed. Criteria for evaluating a role for phytoalexins in disease resistance are also discussed. **Keywords:** Phytoalexins, potato tubers, protection, plant extracts **References:** Ranalli. et al. (2006.) J Agric Food Chem 54: 434 – 440. Val F et al D (2006) Phytoma, la défense des végétaux 596: 33 – 36

PB5 Effect of Plant Growth Promoting *Rhizobacteria* (PGPR) on agronomic characteristic and root colonization in fennel

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In order to study the effect of Plant Growth Promoting Rhizobacteria (PGPR) on agronomic characteristic and root colonization in fennel (*Foeniculum vulgare* Mill.), an experiment was conducted in western of Iran in 2008 and 2009 growing seasons. The factors were Plant Growth Promoting *Rhizobacteria* (Azotobacter inoculation with and non-inoculated) and nitrogen application (0, 40, 80 Kg/ha-1). The treatments were arranged as factorial in a randomized complete blocks design with three replications. Results showed that the highest grain yield, umbrella per plant, biological yield and root colonization percent were obtained with Azotobacter treatment. Nitrogen application was significant affected on studies traits. The highest grain yield, biological yield and root colonization percent obtain by 80 Kg/ha-1. Interaction effect PGPR x nitrogen application was affected on grain yield and colonization percent. The highest grain yield and root colonization percent obtained Azotobacter x 80 Kg/ha-1 nitrogen.

PB6 Effect strains of *Mycorrhiza* on root characteristic and concentration of phosphate, iron and zinc in cumin (*Cuminum cyminum* L.)

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Symbiosis between plants and mycorrhizal fungus are very important for agriculture system and natural resource. In soil whit, less fertility mineral matter absorbed by *Mycorrhiza* can lead to improvement in growth that results increase in mycorhizal plant resistance in comparison whit non-mycorrhizal plants in stress condition. In order to test the influence of the strains of *Mycorrhiza* on root characteristic and nutriments on *Cuminum cyminum* L. an experiment was conducted based on completed design with three replications in western of Iran in 2009 – 2010 growing season. Strains of *mycorrhiza* including of *Glomus fasciculatum, Glomus etanicatum, Glomus mosseae* and *Glomus intraradices*. In these research traits as: root length, root length, root length/root dry weight ratio, total dry weight of colonization and concentration of phosphate, iron and zinc. Results of variance of analysis showed that strains of mycorrhiza had significant effected on studies characteristic, as *Glomus fasciculatum* had the highest root colonization, root dry weight, root length/root dry weight ratio. Strains of *Mycorrhiza* had significant affect on absorption of nutriments. *Glomus fasciculatum* had a more ability in absorption of phosphate, iron and zinc other then strains of mycorrhiza.

PB7 Bioactive metabolites isolated from the endophytic fungus *Penicillium chrysogenum* isolated from Red Sea algae

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Endophytic fungi constitute one of the most interesting sources of bioactive natural products. They are synergistic to their respective host and at least some of them are thought to play an important role in the host's defence by producing secondary metabolites that protect the host from being attacked by pathogenic fungi [1,2]. Ten known compounds alatinone, emodin, w-hydroxyemodin, 2-Acetylquinazolin-4(3 H)-one, chrysophanol, cyclo-L-Alo-L-Leu, cis-cyclo (pro,val), 2',3'-Dihydrosorbicillin, meleagrine, and uracil were identified from the EtOAc-extract of a Czapk's-peptone culture of the endophytic fungus *Penicillium chrysogenum* isolated from red algae (*Liagora viscid* (Forsskål) C. Agardh) collected from the Egyptian Red Sea. The structures of the compounds were elucidated on the basis of comprehensive NMR spectral analysis (1 H- and 13C- NMR, HHCOSY, HSQC, HMBC) as well as mass spectrometry. The crude organic extract and some of the pure compounds showed moderate to strong antimicrobial activity. **References:** [1] Stierle A et al. (1993) Science 260: 214 – 216. [2] Strobel G et al. (1997) Aust J Bot 45: 1037 – 1082

PB8 Microbial Transformation of β-Phellandrene

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Derivatives of natural and aromatic compounds obtained by biotransformation constitute an important resource for natural pharmaceutical, fragrance and aroma ingredients or active compounds. Monoterpenes and their new metabolites are very important resources in such applications and uses. At the present study the monoterpene β-phellandrene was biotransformed by fungal whole cell cultures for the first time to best of our knowledge. The substrate β-phellandrene was primarily isolated and purified from its natural source *Angelica archangelica* seed essential oil (the main compound of essential oil 78%) and then subjected to biotransformations by *Corynespora cassiicola*, *Fusarium heterosporum*, *Aspergillus alliaceus*, *Yarrowia lipolytica*, *Alternaria alternata* fungal cultures. As a result cis-*p*-Menth-2-en-7-ol and 4-isopropylcyclohexene-2-on (syn. cryptone) were determined as metabolites by using chromato-spectral methods. Key words: β-phellandrene, biotransformation, monoterpenes

PB9 Transformation of h6 h gene from *Atropa belladonna* to *Hyoscyamus kurdicus* in order to enhance scopolamine production

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Hyoscyamine and scopolamine are medicinally important tropane alkaloids, which possess anticholinergic and central nervous system activities. They have well stablished therapeutic uses (in ophthalmology, cardiology, gastroenterology, etc). For medicinal purpose, scopolamine is much more useful and valuable because of its higher physiological activity and fewer side effects. These natural substances are exclusively extracted from plants [1,4]. Several species from the family Solanaceae like the genus *Atropa, Datura, Duboisia, Hyoscyamus, Scopolium* produce these alkaloids. *Atropa belladonna* L. is a perennial herbaceous plant and most importantly commercial source of pharmaceutical tropane alkaloids in the family Solanaceae. *Hyoscyamus* have 18 species in Iran; one of them is *Hyoscyamus kurdicus* Bornm. from Kurdestan province [2]. Hyoscyamine 6B hydroxylase (h6 h) is a bifunctional enzyme which catalyzes the last two oxidative reactions of tropane alkaloid biosynthetic pathway, converting hyoscyamine to scopolamine [3]. We expressed the h6 h gene from *A. belladonna* in *H. kurdicus*, which caused over accumulation of scopolamine. For this purpose RNA was extracted from leaf disk of *A. belladonna* plants. cDNA was synthesized and amplified by specific h6 h primers. PCR products were sequenced and constructed to pBI121 shuttle vector. *H. kurdicus* seeds were collected from its natural habitats in Kurdestan-Iran and cultured. **Keywords:** Hyoscyamus kurdicus, Atropa belladonna, scopolamine, h6 h gene transformation **Acknowledgement:** *This research was supported by the National Institute of Genetic Engineering and Biotechnology* **References:** 1. Liu X, et al. (2010)J MedPlant Res 4(17):1708 – 1713. 2. Bahmanzadegana A, Sefidkona Fand Sonboli A (2009) Iranian J Pharmaceutical Res 8(1): 65 – 70 3. Hashimoto T, Yun D-J and Yamada Y (1993) Phytochemistry 42:713 – 718 4. Yang Ch et al. (2011) Plant Omics Journal 4(1):29 – 33

PB10 Effects of different nitrogen sources on production of polysaccharides by *Agaricus blazei*

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Mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. A variety of polysaccharides from a number of mushroom varieties have been demonstrated to enhance the immune system. Yield and functionally of polysaccharides by fermentation are highly dependent on their culture conditions, such as different culture compositions and environmental parameter (1). In this study the effects of different Nitrogen sources including; yeast extract, Mycological peptone, poly peptone, ammonium nitrate, ammonium sulfate and ammonium oxalate in two different media (Complex and synthetic liquid culture) were investigated. For the determination of polysaccharides produced by *Agaricus blazei* Murrill the total polysaccharides which were precipitated by absolute alcohol were weighed. The experiments showed that the highest growth and polysaccharide production were obtained when yeast extract used as nitrogen source. The concentration of polysaccharide in both complex and synthetic media when yeast extract was used were similar. The lowest growth and productivity were also seen in medium containing ammonium sulfate. **References:** 1) Shu CH, Lin K-J, and Wen B-J (2004)J Chem Technol Biotechnol 79: 998 – 1002.

PB11 Morphological and molecular analyses for the characterization of Iranian olives (*Olea europaea* L.) in Kermanshah province

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In the present study, we evaluated genetic diversity between seventy one samples of olive (*Olea europaea* L.) germplasms (40 accessions, 5 Iranian cultivars and 26 foreign cultivars) growing in Kermanshah province by Morphological, RAPD and ISSR markers. Morphological characters were compared to the molecular data obtained using RAPD and ISSR markers. Thirty-four RAPD primers and 8 ISSR primers amplified 412 and 118 polymorphic fragments, respectively. The dendrograms based on UPGMA cluster analysis using Jaccard's similarity index for RAPD, ISSR markers and combined both markers include 7, 5 and 7 groups, respectively. The results of mantel's test indicated significant correlation between grouping obtained by RAPD and ISSR markers (r = 0.493) and also between morphological and molecular markers. The morphological and molecular data led to similar representations of the cultivar relationships. The results indicated not a relationship between genetic diversity and different geographical regions in Kermanshah Province. This suggests that cultivar selection has occurred in different genetic pools and in different areas. The results of these analyses showed the existence of a genetic divergence between accessions and this diversity can be used in olive breeding programs. This study allowed us to analyze genetic diversity for further prospecting, to provide additional genetic information on the agronomic and quality characteristics of the olive varieties, and for introducing new olive accessions. **Keywords:** Genetic diversity, *Olea europaea* L., RAPD, ISSR, Kermanshah province

PB12 Production of phytohormone auxin by rhizospheric cyanobacterium *Leptolyngbya* sp. MMG-1

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The genus *Leptolyngbya* is one of the most flourishing filamentous cyanobacterium in rice fields. *Leptolyngbya* sp. MMG-1 was isolated from the rhizosphere of rice plants. The strain was characterized morphologically by light microscopy and confocal laser scanning microscopy and later identified by 16S rDNA sequence analysis. The ability of this strain to synthesize the auxin like bioactive compound was demonstrated under various culturing conditions. Auxin was extracted from the culture of *Leptolyngbya* strain MMG-1 and its identity was confirmed as IAA (indole-3-acetic acid) by thin layer chromatography (TLC) as well as by high performance liquid chromatography (HPLC). The IAA precursor L-tryptophan was required for IAA biosynthesis. Highly significant correlation was recorded between the IAA secreted by the strain and the initial concentration of L-tryptophan in the medium as well as the incubation time. *Leptolyngbya* strain MMG-1 tends to accumulate more IAA than it released it into the medium. The bioactivity of the secreted IAA was established by its effect on the formation of roots by *Pisum sativum*. There was a significant positive effect of the supernatant of cultures of *Leptolyngbya* MMG-1 on the number of lateral roots of *P. sativum* L. while a negative effect on root length was observed. This study highlighted the ability of rhizospheric *Leptolyngbya* to produce auxin by which they thrive there and effect plants.

PB13 *In vitro* regeneration and analysis of total phenolics in *Ocimum basilicum* L. (sweet basil)

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An efficient *in vitro* regeneration system via direct and indirect shoot organogenesis was developed from cotyledonary leaf and hypocotyl explants of *Ocimum basilicum* L., commonly known as a sweet basil, belonging to the family Lamiaceae. Sweet basil is used in traditional medicine as a culinary herb and a well known source of flavouring properties. Various types and concentrations (ranging from 0.1 to 3.0 mg/l) of plant growth regulators in different combinations (TDZ+IAA, BAP+IAA, KIN+IAA, TDZ+NAA) were tested using Murashige and Skoog medium. The highest number of shoots (1.3 shoots per explant) was obtained from hypocotyl explants on medium supplemented with the growth regulators 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA). For rooting, regenerated shoots were transferred to auxin-containing media. After the rooting stage, healthy regenerants were transferred to pots for acclimitization process, through which almost all of them grew vigorously, attained maturity and produced fertile seeds. We also analysed variations in patterns of total phenolics in the *in vitro* cultured callus, regenerated plantlets from callus as well as in leaves of *ex vitro* plants by UV-spectrophotometer. The phenolic contents of regenerated plantlets and leaves of *ex vitro* plants were found very similar but considerably higher than the callus. However, the greatest difference of phenolics content between callus and regenerated plantlets was observed when they were cultured on media containing combinations of BAP and IAA; callus produced 492,75 mg/g dry weight (dw) while regenerated plantlets yielded 1258,81 mg/g dw.

PB14 Enhanced glycyrrhizin production in Glycyrrhiza inflata hairy roots cultures using elicitation

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Glycyrrhiza inflata Batal (Leguminosae) has been used as a source of licorice. Licorice has had a high market demand due to its high medicinal value, whereas the licorice resources in the world regions are limited [1]. *In vitro* culture of plant is an alternative source for the production of valuable secondary metabolites [2]. In the present study, we report the condition for optimizing cell growth and glycyrrhizin production and the influence of elicitors on glycyrrhizin accumulation in hairy roots cultures of *G. inflata*. Hairy roots were induced by infecting stems and leaves of *G. inflata* with *Agrobacterium rhizogenes* ATCC 15834. Transformed roots were grown in ½MS liquid medium without hormones. The optimization of growth and glycyrrhizin accumulation of *G. inflata* hairy root was studied. The maximum biomass of the hairy roots culture occurred on nine weeks of culture 0.29 ± 0.05 g/flask dry wt. Glycyrrhizin production reached the maximum level in the fourth week of culture (34.79 ± 4.11 μg/g dry wt). Sucrose (6%, w/v) was optimum for growth and glycyrrhizin accumulation in *G. inflata* hairy roots (76.07 ± 6.82 μg/g dry wt). Effects of elicitors like chitosan, methyl jasmonate, and yeast extract on glycyrrhizin production in hairy root of *G. inflata* were studied. Methyl jasmonate (100 μM) was the most efficient for enhancing the glycyrrhizin production up to 108.9 ± 1.15 μg/g dry wt on day 5 of elicitation. The results from this investigation indicate that application of elicitors can enhance the capacity of *G. inflata* hairy roots to produce glycyrrhizin. **Acknowledgement:** *Khon Kaen University, The Japan Society for the Promotion of Science (JSPS)* **References:** 1. Rauchensteiner F et al. (2005) J Pharmaceut. Biomed 38: 594 – 600. 2. Wongwich, W et al. (2008) Z. Naturforsch 63C: 413 – 417.

PB15 SPOTLight: Sustainable Production of Thapsigargin using Light – *turning moss into a terpenoid producer*

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Terpenoids is the biggest group of secondary metabolites among plants. Their accumulation in species belonging to the Apiaceae and Asteraceae family is the reason why several of these plants possess biological activities that are used in the treatment of various diseases [1,2]. Thapsigargin's ability to induce apoptosis by inhibiting the endo/sarcoplasmatic calcium ATPase (SERCA) makes it a promising agent for the therapy of cancer. The development of a pro-drug targeted to prostate cancer cells allows its selective use [3]. Comparison with related sesquiterpenes for which biosynthetic enzymes have been identified has enabled us to propose an enzymatic pathway by which thapsigargin could be generated from farnesyl diphosphate via several intermediates [1]. Large scale High Throughput Sequencing of expressed mRNAs from *Thapsia* species was undertaken to provide contig database of gene fragments, to date we have identified and cloned several genes of interest, which are undergoing characterization. Our first targets are two sesquiterpene synthases. Secondly, we have cloned 12 P450's in the CYP71 clade, which is believed to be involved in secondary metabolism. These are currently undergiong characterization in yeast. To optimize *Physcomitrella* as a production host for thapsigargin we aim at constitutively upregulate the expression of enzymes involved in the upstream part of the isopentenyl diphosphate (IPP) biosynthesis [4]. These metabolic modifications will increase the pool of the terpenoid precursor IPP that is available for sesquiterpene biosynthesis. We aim at establishing the moss *Physcomitrella* patents as the system of choice for the production of all kinds of terpenoids [4]. **Acknowledgement:** *We would like to thank The Danish Council for Strategic Research for their financial support.* **References:** 1. Drew DP et al. (2009) Phytochem Rev 8: 581 – 599 2. Drew DP et al. (2011) Phytochem Anal *In press* 3. Søhoel H et al. (2006) Bioorg Med Chem 14: 2810 – 2815 4. Simonsen HT et al. (2009) Perspec Med Chem 3: 1 – 6

PB16 The challenges of *Podophyllum* tissue culture

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Podophyllotoxin is obtained from the rhizomes and roots of wild populations of *Podophyllum hexandrum* Royle. This is a low-growing plant with a long juvenile phase, making the availability of this natural pro-

duct limited (1). Demand for podophyllotoxin was created with the introduction of its semi-synthetic derivatives in cancer chemotherapy (2). The species is endangered in the Himalayan region (3) through over collecting and lack of organized cultivation (4). Efforts remain to facilitate the *in vitro* propagation (5, 6) of *Podophyllum*. In the present investigation on the tissue culture of *P. hexandrum*, seeds germinated within 35 to 40 days in moist, dark conditions, with *in vitro* grown seedlings being obtained either on 0.2 normal strength semi-solid B5 medium (7) or full-strength MS medium both lacking growth regulators. Although callus induction from root explants cultured on 0.5 normal strength B5 medium containing 1.0 mgl^{-1} 2,4-D, 1.0 mgl^{-1} BAP and 1.0 mgl^{-1} GA_3 was slow, tissue became embryogenic after sucessive subcultures. Embryogenic cell suspensions were established in the dark from root-derived callus, cultured in liquid MS medium containing 2,4-D and kinetin, at 2.0 mgl^{-1} and 0.25 mgl^{-1}, respectively. Differentiation of somatic embryos and subsequent shoot formation occurred on either full-strength or half-strength MS medium with 0.45 mgl^{-1} BAP. Rooting of somatic embryo-derived plants was stimulated by the inclusion of 10^{-5} M lipo-oligosaccharide in the culture medium. A robust explant-to-plant micropropagation system for *Podophyllum* will reduce the pressure on wild resources and may offer an alternative source of podophyllotoxin production. **Acknowledgement:** *To RHAE/CNPq and Funed for their financial support to CGS, which is greatly appreciated.* **References:** 1. Choudhary DK et al. (1998) J Med Aromat Plant Sci 20: 1071 – 1073. 2. Farkya S et al. (2004) Appl Microbiol Biotechnol 65: 504 – 519. 3. Airi S et al. (1997) Plant Genet Resour Newsl 110: 20 – 34. 4. Nadeem M et al. (2000) Biol Conserv 92: 121 – 129. 5. Chakraborty A et al. (2010) Indian J Biotechnol 9: 217 – 220. 6. Silva CG (2000) Ph. D. Thesis. University of Nottingham. Nottingham, UK. 7. Heyenga AG et al. (1990) Plant Cell Rep 9: 382 – 385.

PB17 Another CAPS DNA marker for sex identification in jojoba seedlings

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Jojoba [*Simmondsia chinensis* (Link) Schneider] plant has immense industrial value. Jojoba is grown for its seed oil, which is a unique liquid wax consisting of esters formed from acids and alcohols with chain lengths of 20 or 22 carbon atoms. Higher seed yield is obtained when well balanced male and female plants are established in jojoba orchard. However, the sex of jojoba plants cannot be determined with morphological characters until the plants reach reproductive maturity. DNA markers are commonly used for plant genetic studies [1,2,3,4], Up-to-date several male and female specific DNA markers have been developed for sex identification in jojoba seedlings [5,6]. This study reports another cleavage-amplified polymorphic sequence (CAPS) assay, which easily identify male individuals. Genomic DNAs of 16 male and 16 female jojoba plants growing in an orchard were extracted using a DNA extraction protocol [4]. A touch-down PCR approach was used to amplify genomic DNAs of jojoba using J818F 5'-AGGGGATAAATGAGCCGAAT-3' and J818R 5'-GACCCAGAGGATGAGGAATG-3' primer pair [6]. Amplified products were digested using Hind III restriction enzyme. Electrophoresis separation of the digested products as shown in Figure 1 indicated that male and female jojoba plant can be easily identified. CAPS marker reported in this study could be used in breeding studies and in the sex allocation of seedlings in seed orchard establishment.

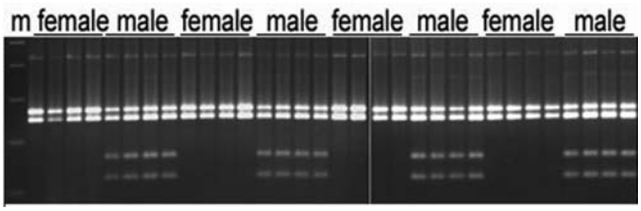

Figure 1: Male specific CAPS marker
M: DNA size marker

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PB18 Comparison of multiple DNA alignment algorithms for Labiatae molecular phylogeny inferences

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Multiple alignment of sets of nucleotide or amino acid sequences are usually required prior to phylogenetic studies for phylogeny inferences. There are several widely used programs for carrying out automatic multiple alignment of nucleotide sequences [1,2,3]. Since there exist different type of multiple alignment programs, the selection of proper alignment program is important for true phylogeny inferences. In order to compare different alignment algorithms implemented in five different multiple alignment programs (ClustalW, T-Coffee, MAFFT, Kalign and MUSCLE, all implemented in the Ugene software) were used to align internal transcribed spacer sequences of 24 taxa of Labiatae family. MetaPIGA 2.0 software was used to obtain phylogenetic trees. Analysis parameters of MetaPIGA 2.0 were set to heuristic search using stochastic consensus pruning (metaGA), GTR model of likelihood rate test and other parameters were kept at default values of the program. Evaluations of the multiple alignment algorithms were based on bootstrap values and groupings at the genus level of consensus trees. Analyses indicated that phylogeny inferences were affected with the multiple alignment algorithms used. Among the tested multiple alignment programs, sequences aligned with MAFFT produced better phylogenetic tree. Further studies will be useful to reveal whether the use of different phylogeny programs and different gene sequences could overcome the effects of multiple alignments on phylogeny inferences. **Acknowledgement:** *This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University.* **References:** 1. Ince AG et al. (2005) Akdeniz Univ Ziraat Fak Derg 18: 157 – 162. 2. Helaers R, Milinkovitch MC (2010) BMC Bioinformatics 11: 379. 3. Ince AG et al. (2010) Genet Resour Crop Ev 57: 773 – 779.

PB19 Molecular authentication of Thai medicinal plant, *Vitex glabrata*, by PCR-RFLP

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Several species in *Vitex*, a genus in the family Labiatae, are medicinal plants used in folk remedy in many countries with different effects. *Vitex glabrata* R. Br. is used as anthelmintic, wound healing and sexual enhancer, which are different from the uses of other *Vitex* species. These effects are related to the presence of ecdysteroids in the plant (1) since it has high amount of 20-hydroxyecdysone (2). Crude drugs of many *Vitex* species have similar appearance, which lead to confusion and misuse. Therefore, an accurate authentication of *V. glabrata* is essential for medicinal purpose. In this study, PCR-restriction fragment length polymorphism (PCR-RFLP) based on the chloroplast maturase K (matK) gene analysis was applied to identify *V. glabrata* from other *Vitex* species commonly found in Thailand. Among five *Vitex* species, a distinctive site recognize by a restriction enzyme HindIII in *V. glabrata* was found. A pair of new primers, Vt1110matKF and Vt1516matKR, was designed based on the sequence of V. glabrata to amplify a smaller fragment of 407 bp in length from genomic DNAs of the leaves of these five species. Only the PCR product of *V. glabrata* could be digested with HindIII into two fragments of 324 bp and 83 bp while the other species remained undigested. This result suggests that PCR-RFLP analysis is an effective and accurate method for authentication of *V. glabrata*. **Acknowledgement:** *the Faculty of Pharmaceutical Sciences Research Funds, Faculty of Sciences, Chulalongkorn University, Thailand* **References:** 1. Báthori M, Pongrácz Z (2005) Curr Med 12:153 – 172. 2. Werawattanametin K (1986) J Nat Prod 9(2):365 – 366

PB20 Phenolic compounds and antioxidant capacity of chickpea seed

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Phenolic compounds have free radical scavenging abilities, antimutagenic and anticancerogenic activities and there is increasing interest for phenolics compounds in food, today [1,2]. In our work the content

and composition of phenolics compounds as well as their antioxidant activity of chickpea (*Cicer arietinum* L.) were examined. The chickpea seed were milled and the plant extracts were prepared by using 80% (v/v) ethanol. The total phenolics compounds content was determined based on a standard curve with chlorogenic acid concentrations covering the range from 50 to 1000 µmol and the antioxidant activity was determined by using DPPH radical scavenging capacity [3].The composition of phenolics compounds was determined by HPLC analyses on an Agilent 1100 Series HPLC system, Agilent Eclipse XDB-C 18 column and spectrophotometric detection in the UV region at 350 nm was used [4]. The phenolics compounds content was 8.37 µmol of chlorogenic acid per g of dried extract residue i.e. 0.33 µmol of chlorogenic acid per g of milled chickpea seed. Results of antioxidant capacity of investigated phenolic extracts showed the maximum DPPH radical scavenging capacity was 39% at extract's concentration of 10.9 mg/ml and the extract's concentration sufficient to obtain 50% of maximum scavenging capacity was 2.9 mg/ml. By using the HPLC analysis, chlorogenic acid (4.26%), hydroxycinnamic acid "C1" (10.86%), 5-O-caffeoylshikimic acid (5.21%), kaempferol 3-O-7-O-diglucoside (7.78%), kaempferol 3-O-rhamnoside (33.52%), kaempferol-7-O-rhamnoside (7.66%) and genkwanin 4,-O-glucoside (4.59%) of phenolics compounds were found. In total sum, the content of kaempferol glucosides was the biggest (48.96%). **Acknowledgement:** *This work was supported under the projects No.OI 172047 by the Ministry of Science of the Republic of Serbia.* **References:** 1. Bravo L (1998) Nutr Rev 56: 317 – 333. 2. Nakamura Y et al.. (2003) J Agric Food Chem 51: 3309 – 3312. 3. Choi CW et al. (2002) Plant Sci 163: 1161 – 1168. 4. Viet M et al. (1995) Phytochem 38(4): 881 – 891.

PB21 Effects of cAMP modulators on flavonoid accumulation in cell cultures of *Hypericum androsaemum* L.

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Hypericum androsaemum L. has been used in traditional medicine for its diuretic and hepatoprotective properties [1], which are attributed to the diverse flavonoids and phenolic acids found in this species. Cell suspension cultures established from hypocotyl-derived callus of *H. androsaemum* were reported [2] to accumulate low amounts of flavonoids, with maximum levels occurring on the 14th day of the growth cycle. More recently [3], it was shown that treatment of 11-day-old cultures for 72 h with 15 mM $CaCl_2$ or 5 µM calcium ionophore A23187 increased considerably the accumulation of flavonoids and the activity of phenylalanine ammonia-lyase (PAL, a key regulatory enzyme of phenylpropanoid metabolism). Since adenylyl cyclases can be regulated by Ca^{2+}, similar experiments were carried out in this study using three different modulators of intracellular cAMP: dibutyryl-cAMP (100 µM, a membrane permeable cAMP analogue), IBMX (100 µM, a cNMP phosphodiesterase inhibitor) and forskolin (20 µM, an adenylyl cyclase activator). The first two treatments induced a marked increase in both PAL activity and flavonoid content of cells, as compared to control cultures. Increased levels of flavonoids were also found in forskolin-treated cells, but in this case accompanied by an insignificant rise in PAL activity. Considered together, these findings are in agreement with the involvement of cAMP signaling in flavonoid metabolism of *H. androsaemum* cell cultures. **Acknowledgement:** *FCT and POCTI/FEDER for financial support.* **References:** 1. Novais M et al. (2004) J Ethnopharmacol 93: 183 – 195. 2. Paranhos A (2006) Planta Med 72: 1060 – 1061. 3. Paranhos A (2007) Planta Med 73: 1017.

PB22 Towards the sustainable and continuous *in-vitro* production of active pharmaceutical ingredients from medicinal plants

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Medicinal plants have been used for the past millennium to treat various conditions, especially in the oncology market. Despite an increasing interest in the translation of traditional knowledge of medicinal plants into clinical drugs, progress has been quite slow since the discovery of Paclitaxel and Camptothecin in the 1970 s. One aspect which could explain the limited number of complex molecules entering clinical trials and reaching the patient is the restricted supply chain of the plant raw material, thus limiting the availability of the Active Pharmaceutical Ingredients (API). Still today, most of the raw materials needed for the extraction of the active ingredients are harvested from cultivated or wild plant populations, posing a threat to the bioavailability of certain medicinal plants and strong variability in the yield of API. We have developed a new in-vitro propagation method based on the use of temporary immersion bioreactors that allows for the rapid and abundant generation of a leafy-biomass from transgenic plants (Michoux et al., 2010). This technology is now being applied to medicinal plants and the results will be discussed. This technology provides a unique opportunity for the sustainable production of complex APIs which require plant cell differentiation. **References:** Michoux F, Ahmad N, McCarthy J and Nixon PJ (2010) Plant Biotechnology Journal Online Nov 24.

PB23 Hairy roots induction in *Nepeta crispa* (Lamiaceae) from Iran by using *Agrobacreium rhizogenes*

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Nepeta crispa Willd. (Lamiaceae) is an aromatic endemic plant of Iran. This plant with the common local name Mofarrah has been of great interest to Iranian traditional medicine. Infusion and beverage obtained from the aerial parts of *N. crispa* were used traditionally as sedative, relaxant, carminative, restorative tonic for nervous and respiratory disorders. *N. crispa* has antimicrobial activity against bacteria and fungi. The main constituents were 1,8-cineole (47.9%), 4-aα,7α,7aβ-nepetalactone (20.3%), α-pinene (5%) and β-terpineol (4.1%). *Agrobacterium rhizogenes* causes hairy root disease in plants. The neoplastic (cancerous) roots produced by *A. rhizogenes* infection are characterized by high growth rate, genetic stability and growth in hormone free media. Hairy root cultures offer promise for high production and productivity of valuable secondary metabolites (used as pharmaceuticals, pigments and flavors) in many plants. Hairy roots were induced from cotyledon explants excised from seven day old aseptically grown seedlings of *N.crispa* using *Agrobacterium rhizogenes* 15834. The cefotaxime concentration of 250 mgL^{-1} was found to be most suitable for hairy root induction in *N.crispa*.To confirm transformation, PCR analysis was performed by using of specific primers for rol B gene amplification. Results of PCR analysis showed the presence of diagnostic 780 bp rol B product amplification and thus confirmed the transformation of hairy roots. This is the first report on the induction of hairy roots in *N.crispa*. **References:** 1. Sonboli A, Salehi P, Yousefzadi M (2004) Z Naturforsch 59: 653 – 656 2. Jamzad Z, Grayer R-J, Kite G-C, Simmonds M-S-J., Ingrouille M, and Jalili A (2003) Biochem Syst Ecol 31: 587 – 600. 3. Mojab F, Nickavara B, Tehrania H H (2009) IJPS 5(1): 43 – 46

PB24 Hairy roots induction in purslane (*Portulaca oleracea* Linn.) using *Agrobacterium rhizogenes*

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Portulaca oleracea Linn. Is a medicinal plant found in Europe and Asia. *P. oleracea* has a variety of pharmacological activity, including analgesic, anti-inflammatory, anti-fungal, wound healing, and hypoglycemic. This plant contains a variety of bioconstituents, including catecholamine, 1-noradrenalin, dopamine, l-dopa, α-amyrin, β-amyrin, and portuloside A. The development of genetically transformed by *Agrobacterium rhizogenes* (hairy roots), is a key step in the use of *in vitro* culture for the production of secondary metabolites. In our research hairy roots system were induced from cotyledon explants exited in seven day old aseptically grown seedling of *P. oleracea* using *Agrobacterium rhizogenes* 15834 strain. The cotyledon segment were soak in the bacterial suspension for infection. After 2 day of co-cultivation at 24 °C in the dark, the explants were transferred onto 1/2Ms medium containing 300 mgl cefotaxim to remove *Agrobacterium rhizogenes*. Hairy roots for further proliferation were transferred to liquid 1/2 Ms medium and maintained in 16 h light/8 h dark photo period at 24 ° on orbital shaker (110 rpm). To confirm transformation, PCR analysis was performed by using of specific primers for rol B gene amplification. Results of PCR analysis showed the presence

of diagnostic 780 bp rol B product amplification and thus confirmed the transformation of hairy roots. This is the first report on the induction of hairy roots in *P. oleracea*. **Acknowledgement:** *A.Eskandary, B. Mehrabani and J.Salary of the Garden of Medicinal Plants* **References:** 1. Chen J, Shi YP, and Liu JY (2003) J Chromatogr A 1003:127 – 132. 2. Chan Ket al. (2000) J Ethnopharmacol 73:445 – 451. 3. Feng PC, Haynes LJ and Magnus KE (1961) Nature 191: 1108. 4. Awad NE (1994) Bull Fac Pharm 32(1):137 – 142.

PB25 Optimization of biomass production with enhanced bioactive compound content by the medicinal mushroom *Ganoderma australe* under submerged culture

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Mushrooms have become attractive as functional foods while they represent an untapped source for the development of drugs and nutraceuticals. Comparing with the established field cultivation, their submerged cultivation has significant industrial potential for the effective production of biomass and valuable metabolites [1]. In this study, *Ganoderma australe* (Fr.) Pat. (strain ATHUM 4345, collected from Greece), which is a species of pharmaceutical interest [2] was investigated for maximum biomass production with enhanced dietary fiber and bioactive β-glucan content in a batch-stirred tank bioreactor. Specifically, when the optimized culture medium was tested in a 20-L stirred tank bioreactor, high biomass yields (10.1 ± 0.4 g/L) and productivity of 0.09 g L-1 h-1 were obtained. The yield coefficients for total glucan and dietary fibers on biomass formed were 94.9 ± 6 and 341.2 ± 12.3 mg/g mycelium dry weight, respectively [3]. Additionally, the biomass produced by the process described above, was lyophilized and finally extracted with ethyl acetate:cyclohexane. Subsequently, the fractionation of the resulting extract by chromatographic techniques led to the isolation and identification of linoleic acid, 19-octacosenoic acid, 9-palmitoleic acid, ergosta-5,7,22-trien-3β-ol and a derivative of the triterpene austrolactone. All compounds isolated, were identified by means of spectral data (1 H-NMR and 2D NMR), HRMS and direct comparison with the respective literature data. The findings of this study are valuable, as the established fermentation process, led to the efficient production of biomass containing bioactive compounds. Those results demonstrate the potential of producing high added value bioactive compounds from *G. australe* strain under submerged culture conditions on an industrial scale. **References:** 1. Tang YZ et al. (2007) Food Technol Biotechnol 45: 221 – 229. 2. Paterson RMM (2006) Phytochemistry 67: 1985 – 2001. 3. Papaspyridi L-M et al. (2011) Eng Life Sci11: 65 – 74.

PB26 Changes of taxane production and gene expression during the development of *in vitro Taxus* plant cultures

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The production and accumulation of secondary metabolites in plants is always regulated by the expression of genes involved in their biosynthesis. There are very few reports about the regulation of the biosynthesis of the anticancer agent taxol and other related taxanes and the rate-limiting steps involved, especially during the development of *Taxus* plants. Using *Taxus baccata* L. plantlets grown *in vitro* for 1 year, our group has studied the relationship between the profile and production of taxanes and the expression of genes codifying for enzymes that participate in early and late steps of taxane biosynthesis (*TXS*, *DBAT*, *BAPT* and *DBTNBT*). A far higher taxane content was observed in the aerial part of the plantlets than in the roots, 10-deacetylbaccatin III being the most abundant taxane, with very low conversion to baccatin III and taxol. The mRNA accumulation of the studied genes was also higher in the aerial part than in the corresponding roots. Our results indicate that the low taxane levels in the roots could reflect the low transcript accumulation of the aforementioned genes in this part of the plant, although an active metabolism or translocation of taxanes to the aerial part could also be responsible. The high content of 10-deacetylbaccatin III and very low levels of baccatin III, together with the low mRNA accumulation of *DBAT* in the aerial part, suggest that this gene could control a limiting step in the taxane biosynthetic pathway in *T. baccata* plantlets grown for 1 year in *in vitro* conditions.

PB27 *In vitro* propagation and total alkaloid evaluation of *Catharanthus roseus* L

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The Madagascar periwinkle (*Catharanthus roseus* L.) is an important medicinal plant from the family Apocynaceae produces over ninety terpenoid indole alkaloids. Among its alkaloids, ajmalicine and serpentin are used in the treatment of hypertension and vincristine and vinblastine applied in cancer chemotherapy. The aim of this study was to evaluate the effect of various hormone treatments on callus growth and regeneration during tissue culture and to study the total alkaloid content of callus. The explants were sterilized and cultured in the MS media consisting of different concentrations and combinations of hormones. Traits such as fresh callus weight, color, vitrification and the quality of tissue callus were studied. Analysis of variance of data showed that comparisons were significantly different at 1% probability level. The comparison of callus weights in various hormone levels indicated that the 14.42 mg/l concentration of 6-Benzyladenine (BAP) combined with 3 mg/l concentration of 1-Naphthalene Acetic Acid (NAA) resulted highest callus weight. The combination of 1 mg/l of NAA and 1 ml/l of BAP in foliar callus and NAA (2 mg/l) with BAP (8 mg/l) in single lateral buds, gave the highest number of plantlets regeneration. The highest amount of alkaloids in foliar callus was obtained when 1.5 mg/l of BAP and 0.25 mg/l of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) were employed. The highest amount of alkaloids at plantlets was produced using NAA (3 mg/l) and BAP (14.42 mg/l). The results of the present study emphasized the potential of production of periwinkle active compounds through *in vitro* cultivation.

PB28 Characterization and *in vitro* evaluation of a new chitosan-based propolis tooth varnish

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Propolis has been recently studied due to its cariostatic activity. Although its activity has been exhaustively demonstrated, there is no formulation commercially available using this agent in tooth care. Chitosan has been extensively studied due its film-forming properties. Recent studies had also shown that chitosan can adhere on tooth surface and has an effective inhibition effect on the initial adherence of oral bacteria onto human tooth surface. Chitosan-based propolis varnish was successfully developed and characterized by ATR-FTIR spectroscopy SEM, hydration potential, casting time and mucoadhesive properties. The formulation presented good tooth surface adherence and ability to form films very fast on tooth surface when compressed air was used as casting agent. Also, the varnish presented interesting hydration potential (Figure 2), which suggests that the formulation will not be easily removed from tooth surface by saliva. Propolis varnish has, also, shown antimicrobial activity against *Streptococcus mutans* (MD 8.67 ± 0,52) e *Streptococcus sanguinis* (MD 11.70 ± 2.11). Its cytotoxicity was made by direct contact with osteoblasts and evaluated by the MTT method. After 24 hours, the varnish reduced 20% of the cells, showing low toxicity (ISO 10993 – 5). The results were analyzed statistically by ANOVA in a significance of $p < 0,05$. **Acknowledgement:** *The authors thank for the financial support to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNpQ), Coordenação de Aperfeiçoamento de Pessoal de Nivel*

Superior (CAPES), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and Silvana Maria de Souza for the techinical laboratory support. **References:** References 1- Hayacibara MF et al. (2005) J Ethnopharmacol 101: 110 – 115. 2- Libério SA et al. (2009) J Ethnopharmacol 125: 1 – 9. 3- Liu H, Chen B, Mao Z, Gao C (2007) J Appl Polymer Sci 106: 4248 – 4256. 4- Ashraf H, Taherian A, Kerda AN (2010) Aust Endod J 36: 24 – 28.

PB29 Biotransformation of *cis*-jasmone by fungal strains

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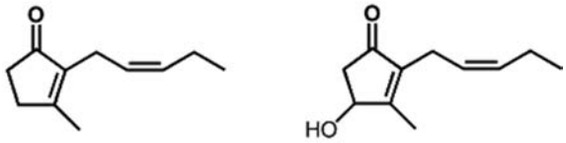
Cis-jasmone, well known as a component of plant volatiles, is produced also by damaged plant vegetative tissues [1]. This natural ketone is considered to be the final product in the jasmonic acid biosynthetic pathway from linolenic acid [2]. This pale yellow, viscous liquid compound posses strong jasmine fragrance and interesting biological activities. It is an activator of chemical defence in plants, causing the release of volatile semiochemicals e.g. bean plants, *Vicia faba*, treated with *cis*-jasmone showed a significant increase in the production of (*E*)-ocimene [3]. *Cis*-jasmone is also responsible for plant-insect interactions. The population of grain aphid *Sitobion avenae* (Fabricius) is reduced by *cis*-jasmone which plays a role of repellent [4, 5] while members of two families of insect parasitoids (*Braconidae* and *Sarcophagidae*) in hop (*Humulus lupulus* L.) cultivation are attracted by *cis*-jasmone [6]. In our study we focused on the biotransformations of *cis*-jasmone by fungal cultures: *Penicillium, Absidia, Syncephalastrum, Botrytis, Aspergillus, Cunninghamella, Chaetomium, Didymosphaeria*. Screening procedure led to the selection of fifteen microorganisms that have ability to biotransformation of *cis*-jasmone. Microbial transformations were used as a tool to obtain new biologically active oxyderivatives. Major product of biotransformations was 4-hydroxyjasmone which was formed in regio- and stereoselective process of hydroxylation.



cis-jasmone 4-hydroxyjasmone

Fig. 1

Acknowledgement: *This Project was financed by European Union from the European Regional Development Found Grant No. POIG.01.03.01 – 00 – 158/09 – 03.* **References:** 1. Loughrin JH, Manukian A, Heath RR, Tumlinson JH (1995) J Chem Ecol 21: 1217 – 1227. 2. Koch T, Bandemer K, Boland W (1997) Helv Chim Acta 80: 838 – 850. 3. Birkett MA et al. (2000) Proceedings of the National Academy of Sciences USA 97: 9329 – 9334. 4. Bruce TJA, Martin LJ, Pickett JA, Pye BJ, Smart LE, Wadhams LJ (2003) Pest Manag Sci 59: 1031 – 1036. 5. Bruce TJ, Pickett JA, Smart LE (2003) Pesticide Outlook 14: 96 – 98. 6. James G (2005) J Chem Ecol 31: 481 – 495.

PB30 Synthesis and cytotoxic activity of new betulin and betulinic acid esters with conjugated linoleic acid (CLA)

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Novel ester derivatives of betulin (**3a-c**) and betulinic acid (**4**) with conjugated linoleic acid (mixture of isomers: 9c, 11 t – 43.4%, 10 t, 12c – 49.5%, other isomers – 7.1%) were obtained. Betulin (**1**) and betulinic acid (**2**) are natural lupane-type triterpenoids with numerous biological activities: anti-inflammatory, antifungal, antibacterial, antimalarial, antiviral, anticancer [1]. Conjugated linoleic acid (CLA) is group of isomers of linoleic acid, found in milk of ruminants and bovine meat. CLA is known for their antioxidant, antitumor, antiatherogenic properties [2,3]. One can expect that the ester derivatives containing both molecules can also posses the valuable biological activity and they can find an application as medicine for many diseases. Esterifications of CLA with betulin and betulinic acid were carried out with using of *N,N'*-dicyclohexylcarbodiimide (DCC) as coupling agent, in presence of 4-dimethylaminopyridine (DMAP) in dichloromethane for betulin derivatives or in pyridine for betulinic acid derivative respectively. The cytotoxic activity of betulin (**1**), betulinic acid (**2**), mixture of CLA and their derivatives (**3a-c, 4**) *in vitro* was determined by performing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cytotoxicity assay. Five different cell lines were used: P388 (mouse leukemia), CCRF (human leukemic lymphoblasts), CEM/C2 (camptothecin resistant derivative of the human T cell leukemia cell), Hl-60 (human promyelocytic leukemia) and HT-29 (human colon). The preliminary tests indicated that betulin (**1**), betulinic acid (**2**) and CLA are the most active agents against cancer cell lines studied. However the betulinic acid ester (**4**) showed comparable activity as CLA.

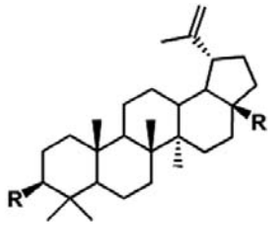

Fig. 1

	R^1	R
1	OH	CH_2OH
2	OH	COOH
3a	$OC(O)C_{17}H_{31}$	$CH_2OC(O)C_{17}H_{31}$
3b	$OC(O)C_{17}H_{31}$	CH_2OH
3c	OH	$CH_2OC(O)C_{17}H_{31}$
4	$OC(O)C_{17}H_{31}$	COOH

Fig. 2

Acknowledgement: *This work was co-financed by the European Union within the European Regional Development Fund (Project no. POIG.01.03.01 – 00 – 133/08)* **References:** 1. Alakurtti S et al. (2006) Eur J Pharm Sci 29: 1 – 13. 2. Pariza MW et al. (2001) PLipRes 40: 283 – 298. 3. Hur SJ et al. (2007) Livestock Science 110: 221 – 229.

PB31 Callus culture studies on *Jasminum malabaricum* - An endemic medicinal plant

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Jasminum malabaricum Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber, with white flowers and fragrance and known for its ethno medicinal importance like antibacterial, antioxidant, blood purifier, anti-tumor properties. The extensive exploitation of this species has led to reduction of its natural population. Owing to its attributes callus culture studies was carried out using Murashige and Skoog medium with different combinations and concentrations of BAP, NAA and 2,4D. The leaves and stem segments were used as explants for callus growth and leaves responded significantly to produce callus. The total phenolics present in the callus culture were estimated. **References:** 1. Bhattacharya S, Bhattacharyya S (1997) Journal of Plant Cell, Tissue and Organ Culture 51(1): 57 – 60. 2. Murashige T, Skoog F (1962) Physiol plant 473 – 9. 3. Mann H H (2008) Journal of the Linnean Society of London, Botany, 45(302): 155 – 8.

PB32 Development of low-Gly m Bd 30K(P34) allergen breeding lines using molecular marker in soybean

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Soybean (*Glycine max* (L.) Merr.) is an important source of vegetable oil and high protein. Use of soybean meal by the food industry is increasing, but severely limiting dietary choices and the quality of life of food-allergic individuals. Gly m Bd 30K (P34) is known as the main seed allergens in soybean-sensitive patients. The objective of this work was to determine the molecular basis of the low mutation of soybean P34 and to design molecular marker for the selection of the causative mutations for wild homozygous, heterozygous and mutant homozygous. We developed a co-dominant marker based on the sequence of Glyma08g12270 containing a four-base pair insertion at the P34 start codon. Also, we made a polyclonal antibody for investigation of P34 protein levels. Using a co-dominant marker and a polyclonal antibody, polymorphism and amount of protein for Glyma08g12270 were analyzed in F2 and F3 generation crossing PI 567476 and Hwanggumkong, Korean cultivar. To investigate the association of the P34 genotype with the P34 protein phenotype, segregating populations in F2 were developed from crossed Hwanggum and PI567476. For the 258 samples analyzed, the ratio of homozygous wild-type: heterozygous: homozygous mutant P34 genotypes was 34:94:30 = 1:2:1(test for goodness of fit by X2 analysis). As results, the polymorphism analysis was accustomed to a difference of protein level of wild homozygous, heterozygous and mutant homozygous. **References:** Bilyeu K et al. (2009) The plant Genome 2: 141 – 148 Joseph LM et al. (2006) Crop Sci 46: 1755 – 1763 Herman E M et al. (2003) Plant Physiology 132: 36 – 43

PB33 Effect of taxine B feeding on taxol production and cell viability in *Taxus* suspension cultures

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After several years, still the affair of paclitaxel (Taxol) and its analogs is one of the most stimulating subjects in anticancer remedy production studies [1]. Many reports have described that the yield of taxol is very low or sometimes not detectable in dedifferentiated cells such as callus tissues or suspension cultured cells. In order to obtain products in concentrations high enough for commercial manufacturing, many efforts have been made to stimulate or restore biosynthetic activities of cultured cells using various methods. Addition to the culture media of appropriate precursors or related compounds sometimes stimulates secondary metabolite production. This approach is advantageous if the precursors are inexpensive [2, 3]. To achieve a better understanding of the effect of taxoids addition to Taxus cell culture, an Iranian yew cell line growing 14 days in a selected growth medium was treated with concentration of Taxine B (a basic Taxoids with a C-4(20) Double Bond) in the range of 1.25 – 10 mgL^{-1}. The results showed that different concentration of taxine B can lead to various influence on taxoids production such as taxol (a taxoid with an oxetane Ring). Thus, taxine B caused to change the metabolomics profile of the biosynthesis of taxol preparation. Compared with an untreated control, all the taxol concentrations affected cell viability. **References:** 1. A. Ghassempour A et al. (2010) Chromatographia 72: 833 – 839. 2. Ketchum REB et al. (2007) Phytochemistry 68: 335 – 341. 3. Expo'sito O et al. (2009) New Biotechnol 25: 252 – 259.

PB34 Tissue culture studies on *Semecarpus kathalekanensis* an endangered medicinal plant

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Semecarpus kathalekanensis Dasappa & M.H.Swaminath is a evergreen tree with very large simple leaves, attains a height of about 30 m, belonging to the family Anacardiaceae is a critically endangered swamp tree and consists of major chemical compounds like phenols, biflavanoids and traditionally having high medicinal importance being used as an antimicrobial, antioxidant and anticancer. Hence considering its population, it is an endangered species and endemic to western ghats region of shimoga district in India. The tissue culture of different plant parts was carried out on MS medium using different concentration of plant growth regulators in the culture tubes and the explants were incubated at 25 ± 20 C under 48 hrs photoperiod. Due its high phenolic content callus initiation was not occurred further isolation and identification of endophytic fungi from Semecarpus kathalekanensis plant was performed. **References:** 1. Dasappa & Swaminath MH (2000) Indian For 126: 78 – 82 2.Vasudeva R, Raghu HB, Dasappa, Uma Shaanker R & Ganeshaiah KN (2001) Population structure, reproductive biology and conservation of Semecarpus kathalekanensis: A critically endangered freshwater swamp tree species of the Western Ghats. In Forest Genetic Resources: Status, Threats and conservation Strategies (Eds Uma Shaanker, R., Ganeshaiah, K.N., and Bawa, K.S.) 211 – 223 (Oxford & IBH, New Delhi,). 3.Murashige T, Skoog F (1962) Physiol plant 15:473 – 9.

PB35 Metabolic engineering: an effective approach for optimal production of secondary metabolite compounds

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Plants have a limited capacity to produce secondary metabolites in natural environmental conditions; however, recent developments in genetic engineering and recombinant DNA technology have had a great impact on their production. Metabolic engineering is considered as an efficient tool towards achieving higher level of the secondary metabolites. Globally, metabolite engineering is widely used for increasing the content of secondary metabolites or even producing novel medicinal compounds. Iran has a high level of genetic and phytochemical variability for different types of medicinal plants. Knowing the biochemical pathways and manipulating these native plants improve their commercial production. Consequently, our research group has started large-scale experiments on native medicinal plants such as different species of genus Artemisia, Cannabis, Papaver, and Salvia. We aim to increase and/or decrease and change metabolites by using anti-sense and RNAi technologies, isolation and transformation of related genes, promoter analysis and changes in regulatory gene expression. Such studies would definitely provide a great chance to improve the production of secondary metabolites in these plants and to better understand the novel genes involve in bio-synthetic pathways.

PB36 Biotransformation of trans-farnesol by *Ceriporiopsis subvermispora*

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Terpenoids which are included in various plants are important for the food, animal feed, cosmetics and pharmaceuticals industries greatly. However they are expensive due to low concentration and difficult isolation. Therefore biotransformation of terpenoids could be an alternative way to produce them. In this study, whole cell of *Ceriporiopsis subvermispora* was used in biotransformation as biocatalyst. *C. subvermispora* is a naturally occurring fungus able to remove phenolic compounds than other compounds. Farnesol is a sesquiterpene which is an acyclic ses-

quiterpene alcohol derivative of farnesol Pyrophosphate and the building block of most acyclic sesquiterpenoids and is an important starting compound for organic synthesis. Therefore it has been chosen as starting material for biotransformation. The object of this study was to investigate the microbial biotransformation of trans-farnesol by *C. subvermispora* at different culture condition in order to obtain the more valuable sesqui-terpenoid stuructures. Biotransformation was started after inoculation by added 0.005 g M-1 of homogenized strains into the 100 ml SSC medium culture flasks. After 48 hours, substrate 200ul was added in the 250 ml flask with 0.1 % Tween 80. Per 24 h, one flask were stopped for cultivation and extracted with solvents at 250 rpm, 3 times using the shaker. The final solutions were subjected to gas chromatography FID spectrometry (GC-FID) and GC-MS. After 10 days, when used the *C. subvermispora* as the biocatalyst, novel compounds produced in SSC medium. These products were nerolidol, farnesol, β-farnesol, bisabolene, eudesm-7(11)-en-4α-ol. **References:** 1. De Carvalho C and da Fonseca M (2006) Biotechnology Advances 24(2): 134 – 142. 2. Asther A and Lesage-Meessen L (1999) Trends in Biotechnology 17(7): 186 – 194 3. Bauer K, Garbe D and Surburg H (1990) Common Fragrance and Flavor Materials. VCH Weinheim. 4. Limberger R et al. (2007) Electronic Journal of Biotechnology 10: 500 – 507.

PB37 In vitro regeneration studies on Hydnocarpus pentandra

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Hydnocarpus pentandra Buch-Ham (Flacourtiaceae) an endangered species from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 ± 2°C under photoperiod of 16 hrs.

PB38 Study of endophytic fungi isolated from leaves of *Hydnocarpus pentandra*

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Hydnocarpus pentandra Buch-Ham (Flacourtiaceae) an endangered species from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. Ten endophytic fungi were isolated from the plant which are are a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity. They represent a relatively unexplored ecological source and their secondary metabolism is particularly active because of their metabolic interactions with their hosts.The APEF-12,13 *(Dematicious sp., Fusarium sp.)* showed maximum activity against bacteria *B. subtilis* and *E.coli* and APEF-06,15 (*Cladosporium sp*., non sporulating hyaline form) were most active against fungi *C.albicans.* **References:** 1. Uche C (2004) Technology in Society 26: 4537 – 550. 2.Farago J, Psennkova I, Faragova N (2009) Nova Biotechnologica 9: 279 – 293. 3.Hsin-Shengtsay (2007) Chaoyang University of Technology, Food & Fertilizer Technology Center, Taiwan.

PB39 Effects of aqueous extracts of *Urtica dioica* L. leaves on lifespan of *Caenorhabditis elegans*

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Urtica dioica L. (Urticaceae) is widely distributed throughout the temperate regions of the world. Leaves of the plant recommended not only for the complaints associated with rheumatoid arthritis [1], cardiovascular diseases and diabetes [2] but also has antiviral, antioxidant, anti-inflammatory activities. It is also used as traditional medicine as panacea in Turkey [3]. Active constituents of nettle leaves are phenolic compounds [4]. It's suggested that the combination of antioxidant/anti-inflammatory polyphenol compounds found in plants may show efficacy in reversing aging[5]. We therefore investigated the effects of aqueous extract of nettle leaves on lifespan and aging of the nematode, *Caenorhabditis elegans*. Aqueous extracts of *U. dioica* leaves extended mean lifespan of the *C. elegans* in concentration of 250 μg/ml and increased the maximum lifespan for1 – 3 days. Further investigations are necessary to identify active compunds of *Urtica dioica* and its mechanism of action. **References:** 1. Riehemann K et al. (1999) Febs Letters 442: 89 – 94 2.Ziyyat A et al. (1997)J Ethnopharmacol 58: 45 – 54 3.Sezik E et al. (1992) Int J Pharmacogn 30: 233 – 39. 4. Pinelli P et al. (2008)J Agric Food Chem 56(19): 9127 – 32 5. Joseph J et al. (2005) Am J Clin Nutr 81(Suppl.): 313 – 316

PB40 Screening of sage suspension cultures for triterpenic acids and other metabolites

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Two pharmacological interesting substances present especially in sage species are oleanolic acid (OA) and ursolic acid (UA). These triterpenic acids possess several biological activities like hepatoprotective, anti-inflammatory, antimicrobial as well as anticancer properties [1]. A biotechnological way to produce valuable biological-active substances is the cultivation of cell suspension cultures in controlled bioreactor systems. Before up scaling a suspension culture to bioreactor level an important step is to establish stable suspension cultures and screen them for their potential. Interesting features of suspension cultures are the size of the cell aggregates, the growth and the production rates of target metabolites. The present study focuses on the screening of sage suspension cultures on growth and metabolic activity. Different suspension cultures from *S. officinalis* L., *S. triloba* L.f. and *S. virgata* Jacq. were cultivated in the modern Respiratory Activity Monitoring System (RAMOS, Hitec Zang, Germany). It allows the measurement of the oxygen transfer rate (OTR) at shake flask scale and in parallel. Using the OTR the growth properties of the cultures have been detected. Samples were taken at the starting point, in the exponential growth phase and the stationary phase. Ethanolic extracts were analysed with HPLC to quantify the production rates of the target substances OA and UA. Using GC/MS the metabolite spectra were investigated to search for other high valuable substances and receive information about the metabolic activity of the cultures. All these data are important for the subsequent culture optimisation. **Acknowledgement:** *This work has been supported by a PhD fellowship from the German Academic Exchange Service and a grant from the Max Buchner Research Foundation.* **References:** [1] Dzubak P et al. (2006) Nat Prod Rep 23: 394 – 411.

PB41 Biotransformation of Cycloartane-Type Sapogenols, Cycloastragenol and Cyclocanthogenol, by *Cunninghamella blakesleeana* NRRL 1369

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Cycloastragenol is a cycloartane-type sapogenol found in *Astragalus* species. It is a minor metabolite mainly present in the roots of the plant and possesses very interesting pharmacological activities [1, 2]. The bio-

transformation of cycloastragenol by the fungus *Cunninghamella blakesleeana* was investigated previously by our group [3]. Inspired by the diversity of the transformed products, further studies were carried out on cycloastragenol (CG) and another secondary metabolite, named cyclocanthogenol (SKG). The biotransformation process was conducted in two scales; analytical scale and preparative scale. One-stage fermentation protocol was followed where the saponins were fed to the biotransformation media 72 hours after the inoculation. In the analytical scale, both submerged (30 °C, 200 rpm) and surface (30 °C) culture conditions were tested, taking 2 mL samples for 3 weeks for the evaluation of the chemical profiles, followed by preparative scale studies by using 500 mg of SKG and 1000 mg of CG. Incubation period was continued with centrifugation, extraction with ethyl acetate and n-butanol, and evaporation under vacuum. The isolation and purification studies performed on the extracts yielded total of 13 metabolites, 10 from CG and 3 from SKG. Structures of the isolated metabolites were elucidated by 1D- and 2D NMR techniques, and LC-MS analyses. The major products obtained from each sapogenol Cb_CG_MF_01 and Cb_SKG_MF_01 have the same tetracyclic steroidal framework with a primary alcohol substitution at C-11 position, encountered for the first time in microbial transformation studies [3]. **Acknowledgement:** *ARS Culture Collection, TUBITAK (108T654 – 109S345).* **References:** 1. Bedir E et al. (2000) Biol Pharm Bull 23: 834 – 837. 2. Valenzuela HF et al. (2009) J Immunol 182: 90.30. 3. Kuban M et al. (2010) Org Lett 12: 4252 – 4255.

PB42 Induction of changes in secondary metabolites and essential oils of *Calendula officinalis* L. by methyl jasmonate

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In this study, effect of Methyl jasmonate as a chemical elicitor on the secondary metabolites and essential oils of *Calendula officinalis* L. shoot were evaluated. The plants were grown in hydroponic conditions in Hogland nutrient solution and were treated with 50 and 100 μM methyl jasmonate. The results showed that lignin content were increased significantly in treated plants. In comparison, the content of total wall associated phenolic compounds and anthocyanins and flavonoid of shoots of treated plants decreased. However, there was no significant difference in membrane lipid peroxidation rate of treated plants and control ones. In the essential oils of *C. officinalis* shoots, α- Cadinol was the major constituent. A sesquiterpene compound, α-Muurolene, with anti fungal properties was induced in those plants treated with methyl jasmonate, therefore, this chemical elicitor can be suggested for inducing changes in isoprenoid biosynthesis pathway and special phytoalexin production. **References:** 1. YukimuneY, Tabata H, Higashi Y, Hara Y (1996) Nat Biotechnol 14: 1129 – 1132. 2. Kim DG, KimYJ, Lee SH, Lee (2005) Plant Biol 48(3): 298 – 303. 3. Gazim ZC, Rezende CM, Fraga SR, Svidzinski TIE., Cortez DAG (2008) Braz J Microbiol 39(1):61 – 63. 4. Pourcel L, Routaboul JM, Cheynier V, Lepiniec L, Debeaujon I (2006) Trends in plant Science 12:1 5. Perez AG, Sanz C, Olias R, Olias JM (1997) J Agric Food Chem 45: 3733 – 3737.

PB43 Polyphenols and their antioxidant activity in callus-cultured *Malva neglecta* cells under UV-B and UV-C irradiation

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Malva neglecta Wallr is a perennial plant with high mucilage content, expectorant and cough-suppressing actions. The leaves and flowers of *M. neglecta* and some *Malva* species are used in traditional phytotherapy. Ultraviolet radiation in sunlight has diverse effects on humans, animals, plants and microorganisms. UV can cause damage to membrane by excitation of UV-B receptors, resulting in generation of reactive oxygen species and ultimately oxidative burst. Consequently organisms need to protect against and repair UV damage to survive in sunlight. Antioxidants are an important group of medicinal preventive compounds as well as being food additives inhibiting detrimental changes of easily oxidable nutrients. Polyphenols are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects, including antioxidant activity. In the present research callus cultures from leaf explants of *M. neglecta* were initiated in vitro, and their capacity to produce UV absorbing compounds was analyzed, after 90 minutes exposure to UV. The results showed that the levels of apigenin and delphinidin decreased after illumination with UV-B and UV-C, while Malvidin increased in UV-B and UV-C exposed Malva cells. The results demonstrate that polyphenols play important role in UV protection of *Malva* cells **References:** Deters A et al. (2010) J Ethnopharmacol127: 62 – 69. Hosseini Sarghein, Carapetian J and Khara J (2008) International Journal of Botany 4: 486 – 490. Jansen Marcel AK, Hectors K, O'Brien N M, Guisez Y, Potters G (2008) Plant Science 175: 449 – 458. Li J, Ou-Lee TM, Raba R, Amundson RG, and Last RL (1993) The Plant Cell 5: 171 – 179. Zacchini M, de Agazio M (2004) Plant Physiology and Biochemistry 42: 445 – 450.

PB44 Antioxidant capacity of phenolic phytochemicals from peel of apples, pears, plums, red and white grapes

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Polyphenolic phytochemical extractions from peel of apples, pears, plums, red and white grapes were performed using 70% ethanol and 0.9% NaCl (7:3) with ultrasound assistance and extracts were analyzed for total phenolics, flavonoids, and antioxidant capacity. In the tested fruit the highest content of flavonoids was found in the red grapes (4.337 ± 0.218 mg/g), followed by the pear and the plum (3.333 ± 0.035 and 3.108 ± 0.157 mg/g), which have almost identical content, and the white grapes and the apples which again have an approximately equal content of flavonoids (2.126 ± 0.039 and 2.072 ± 0.096 mg/g). The antioxidant capacities of analysed fruit extracts were assayed for antioxidant activity by DPPH radical scavenging and total reducing power. The percentage of neutralize DPPH radicals can be reported by EC50 value or by the concentration of extract required to neutralize 50% of DPPH radicals. The lowest EC50 value, and therefore the highest antioxidant activity was in the extracts of red grape, followed by the extracts of pear, apple, white grape, and plum.The highest content of phenolic compounds were in extract of the red grape (12,884 ± 0,444 mg/g), than followed by the pear (9,590 ± 1,031 mg/g), the plums (9,296 ± 0,268 mg/g), the apples (3,676 ± 0,135 mg/g), and the lowest content was in the extract of the white grapes (3,009 ± 0,161 mg/g). **Acknowledgement:** *This work was supported under the projects No.OI 45001 by the Ministry of Science of the Republic of Serbia.* **References:** 1. Halliwell B (1997) Nutr Rev 55: S44-S52. 2. Aquino R et al. (2002) J Ethnopharmacol 79: 183 – 191. 3. Xu BJ, Chang SKC (2007) J Food Sci 72: S159-S166.

PB45 Secondary Metabolites from *Phomopsis amygdali*, an Endophytic Fungus Isolated from Hazelnut (*Corylus avellana*)

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Endophytes are microbial entities that live within living tissues of plants. In most cases their relationship with the host plant is symbiotic and probably mutualistic. Many are capable of synthesizing bioactive compounds that have been proven useful for novel drug discovery. The early literature reports that species of *Phomopsis* isolated from plants produce different bioactive metabolites. The main aim of the study was to isolate endophytes from different parts of hazelnut, to extract bioactive secondary metabolites and then to elucidate their structures. Different plant materials including the roots, branches and leaves were collected from BlackSea region of Turkey and surface sterilized with 3% sodium hypochlorite (NaOCl). The outer layers removed with a sharp, sterilized blade and cut into pieces. Small pieces of the inner tissue were placed on the surface of potato/dextrose/agar (PDA) medium and incubated at 28 °C. Subsequently 7 fungal species was isolated and grown in 1 L flask containing 250 ml of Malt Extract Broth medium and cultured at 150 rpm at 28 °C for 21 days in a rotary shaker. Then the fermentation broths were extracted with chloroform. The chloroform extracts were screened for their cytotoxic activities by MTT method. Based on the activity results, the isolate L1, identified as *Phomopsis amygdali*, was

selected for further studies. After large scale fermentation and purification studies, a new metabolite (L1F3) together with a known compound, named (-)pestalotin (L1F2) were obtained. The structure of the new metabolite was elucidated as (R)-4-butoxy-6-((S)-1-hydroxybutyl)-5,6-dihydro-2 H-pyran-2-one by the extensive use of 1D- and 2D NMR.

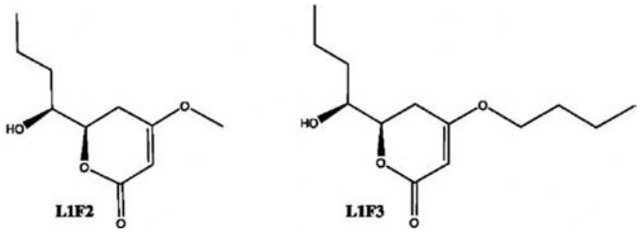

Figure 1

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PB46 Biotechnological process for obtaining bioaromas from leaves of cashew apple trees

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The cashew tree (*Anacardium occidentale* L.) is native to mainland Central and South America belonging to the family Anacardiaceae. The cashew apple fruit and nut are widely used and appreciated for its flavor quality. The aim of this work was to obtain bioaromas produced from the fermentation of the leaves of the cashew tree. Fermentations were performed at room temperature for 48 hours in three conditions: natural (leaves only), leaves in sterile distilled water and leaves in a solution containing 10% glucose. Every 24 hours of processing, sensorial (by a panel of five trained judges), microbiological (total mesophilic bacteria, molds and yeasts) and chromatographic (extraction by dynamic headspace method) analysis was performed on fermented products. Sensorial analysis revealed the presence of sweet fruity aromas characterizing citrus and green odor notes in all samples. The citric note of aroma was more prominent in all three types of fermentation without significant difference between them. However, the aroma was less intense in the fermentations realized with green leaf alone and it was significantly different ($p < 0.05$) between the other fermentations. Furthermore the production of bioaromas generated after 24 hours led to an increase of total bacteria and fungi to the order of 102 CFU/mL. A large number of volatile compounds belonging to esters, terpenes, ketones and aldehyde classes were identified in the fermented products. **Acknowledgement:** *We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil for the financial support received while the first, second and third co-authors thanks CNPq for fellowships while the last co-author thanks CAPES for fellowships*

PB47 Assessment of somaclonal variation in *Ducrosia anethifolia* plants regenerated from long-term callus cultures using AFLP

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Somaclonal variation may be defined as tissue-cultured-induced variation that has relevance in the micropropagation of endangered germplasms. It has been studied in some plant species, but only a few studies have reported on the assessment somaclonal variation in medicinal plants using molecular markers. *Ducrosia anethifolia* Boiss. is an endangered medicinal herb belonging to the Apiaceae family. In this study somaclonal variation in plants regenerated from long-term cultured calluses of *Ducrosia anethifolia* was characterized using amplified length polymorphism profile. Genomic DNA was double-digested with two Bgl II and MseI and the digested fragments were ligated to double stranded adaptors appropriate with the Bgl II and MseI restriction sequences. Results revealed that banding patterns were different between various explants in different subcultures. A total of 112 polymorphic fragments were scored, with an average of 22.4 fragments per primer combination. Results also showed that this method is reliable and effective way of assessing somaclonal variation in tissue culture-derived plants. **Acknowledgement:** *The research was supported by funds received from the Institute of Medicinal Plants, Karaj, Iran* **References:** 1. Xu M, Li Xand Korban S S (2000) Plant Molecular Biology Reporter 18: 361 – 368 2. Lei CP, Jiun KS, Choo CS and Singh R (2006) Asia Pacific Journal of Molecular Biology and Biotechnology 14(2): 47 – 55

PB48 Responses of zygotic embryos of galbanum in *in vitro* conditions

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Galbanum (*Ferula gummosa* Boiss.) is a valuable medicinal-industrial plant native to Iran which is at risk of extinction due to irregular and overharvesting from natural habitats. The objective of the study was to investigate the growth response of zygotic embryos of galbanum, originated from center of Iran, in *in vitro* conditions. The results however, showed that zygotic embryos of galbanum had not a suitable germination in *in vitro* conditions. However, the best treatment for *in vitro* germination of embryos was ¼ MS (MS with ¼ macro elements) medium supplemented with 0.3 mg l^{-1} GA_3. The results also showed the embryos had good callus production in ¼ MS medium (MS with ¼ macro elements) supplemented with 2 mg l^{-1} BA and 10 mg l^{-1} NAA. In this study, the results showed that although applied treatments did not lead to normal germination of zygotic embryos of galbanum in *in vitro* conditions, but these treatments were able to force zygotic embryos into the callus production phase with good quality and quantity. The pH of primary media for zygotic embryo germination which was adjusted before adding plant growth regulators, can be effective on good callus production. Nevertheless, more experiments are needed to reveal the effect of pH on callogenesis in galbanum plant. **References:** 1. Bernard F et al. (2007) Pakistan Journal of Biological Sciences 10: 1977 – 1983 2. Irvani N et al. (2009) Plant Cell, Tissue and Organ Culture 100: 293 – 299 3. Tafreshi RS et al. (2008) Iranian Journal of Medicinal Plants 27: 71 – 81

PB49 Conservation and multiplication of an endangered medicinal plant – *Caralluma arabica* – using tissue culture

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Caralluma arabica N.E.Br. (Asclepiadaceae) is a succulent, perennial herb that grows in arid regions, in West Asia and in the Middle East, including Oman and the United Arab Emirates. This plant is highly valued for its medicinal properties, and is commonly used in the preparation of traditional medicine for the treatment of diabetes, liver ailments, and painful and inflammatory conditions (1). Pharmacological studies revealed that *C. arabica* extract has anti-nociceptive, anti-gastric ulcer, cytoprotective, and anti-inflammatory properties (1, 2). Unfortunately, this plant is facing considerable pressures which threaten its survival. Therefore, the development of a protocol for the propagation of *C. arabica in vitro* is of high importance for the conservation of this species and its commercial cultivation. Plant regeneration via organogenesis was initiated for *C. arabica* using stem segments excised from young shoots and used as explants for i*n vitro* culture. Stem explants were cultured on Murashige and Skoog (MS) (3) medium containing different concentrations of kinetin and indol-acetic acid (IAA). Preliminary results showed that differentiation of adventitious shoots was initiated within 5 weeks of culture on a medium containing 1 mM Kinetin and 3 mM IAA. Root induction was obtained on half-strength MS medium containing Indol-3-butyric acid. Further investigation is underway to establish optimal culture conditions for the regeneration of this important medicinal plant. **References:** 1. Zakaria et al. (2001)J. Ethnopharmacol 76(2): 155 – 158 2. Zakaria et al. (2002) Pharmaceutical Biology 40(3): 225 – 230 3. Murashige, Skoog (1962) Physiol Plant 15: 473 – 497

PB50 *In vitro* study of callus induction and regeneration in Iranian *Cichorium intybus*

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Cichorium intybus L. belongs to the *Asteraceae* family and is one of the medicinally important plant that contains some useful secondary metabolites such as bitter sesquiterpene lactones, coumarins and flavonoids. This plant is conventionally propagated through seeds. In this research, *in vitro* culture of Iranian *Cichorium intybus* was studied, thus an experiment was laid out as a completely randomized design (CRD) in a factorial arrangement with there replications that investigated factors were different concentration of plant growth regulators (NAA and BAP) and explants (hypocotyls and cotyledon). The results indicated that significant and non-significant differences among levels of explants and other factors for callus induction and plant regeneration, as we observed the highest percentage of callus formation from MS medium supplemented with 1.5 mg/l NAA and 2 mg/l BAP. Mean comparisons shown that the best explant was cotyledon versus hypocotyls for the traits and maxium of regeneration recorded for MS medium with 0.4 mg/l NAA and 5 mg/l BAP.

Topic C: Clinical Studies

PC1 Clinical Evaluation of *Cissus quadrangularis*, *Moringa oleifera* and combination of two as Osteogenic agents in Mandibular fractures

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Fractures of the jaw bones renders not only physical trauma but also makes the person miss out on work productivity and other social obligations for a period ranging from 4 – 8 week on an average. Ayurveda the ancient science system of the medicine describes various herbs preparation that achieves the hastening of bone healing. Hadjor (*Cissus quadrangularis* L.) and Moringa (*Moringa oleifera* Lam.) showed clinical efficacy in treatment of fractures. Our study also showed reduction in time of inter maxillary fixation time from 6 weeks to 3 – 4 weeks with Ostoeseal (combination of *Cissus quadrangularis*, *Moringa oleifera*) lesser with Hadjor, least with Moringa and in the case of Placebo there was no reduction in time of inter maxillary fixation. Serum Ca level both ionic and total and serum phosphorous level was significantly increased in other three groups but decreased in placebo group.

PC2 Gemmotherapy – adjuvant treatment in juvenile spondyloarthropathy

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The spondyloarthropathies (SpA) are a group of rheumatic diseases that predominantly affect the axial skeleton's joints accompanied by enthesitis. SpA are strongly associated with HLA-B27 histocompatibility antigen. Gemmotherapy is a form of herbal medicine that uses remedies made from the embryonic tissue of various trees and shrubs, the reproductive parts and from newly-grown tissues. Monitoring the response to gemmotherapy of a selected pediatric cohort of spondyloarthropaty. The assessment of the 21 children included complete clinical and functional evaluation (disease activity score, visual analogue scale, disability index), lab tests, x-ray and genetic study. Reevaluation was performed at 3 and 6 months. According to the European Spondyloarthropathy Study Group criteria, we diagnosed 4 children with juvenile ankylosing spondylitis (AS), 7 cases of undifferentiated spondyloarthropathy (USp), 4 cases with inflammatory bowel disease (IBD) associated spondylarthropathy, 6 patients with reactive arthritis (RA). Treatment of AS and of arthropaties with IBD included nonsteroidal antiinflammatory drugs (NSAID), Sulfalazine, biologics and glucocorticoids. In USp and RA, NSAID and gemmotherapy was used. Cases with mild or no inflammation in lab test was selected for exclusive herbal therapy. Administration of extracts of *Ribes nigrum* L., *Abies pectinata* Poir., *Pinus montana* Schur, *Vaccinium vitis-idaea* L., *Ampelopsis veitchii* Hort. was daily, during 6 months, in adapted dose and was accompanied by physical therapy. Reevaluation (3 and 6 months) denoted an amelioration of clinical signs and functional scores in all 13 cases and remission in 8 cases. Gemmotherapy associated with exercises minimized symptoms, ameliorated joint mobility and allowed a well tolerated, natural therapy. **References:** Pitera F (2000) Compendio di gemmoterapia clinica (Meristemoterapia) con indice clinico, Genova, Cassidy JT, Petty R E, Laxer RM, Lindsley CB (2006) Textbook of Pediatric Rheumatology, fifth edition, Elsevier Saunders

PC3 The effects of a combination of silymarin and selenium on prostate health

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Complementary and alternative medicine increasingly being used by men who wish to decrease their susceptibility to prostate cancer. The aim of this 6 months intervention study was to assess the effects and safety of a research preparation based on a combination of silymarin and selenium on healthy men with a prostate specific antigen (PSA) level lower than 2.0 μg/L. In this double-blind, placebo-controlled pilot study, a total of 55 participants were randomized to either treatment with 570 mg silymarin, and 240 μg selenium as selenomethionine per day (n = 26) or placebo (n = 29). Baseline clinical and demographic characteristics were comparable. Outcome measures were changes in the International Prostate Symptom Score (IPSS), quality of life score, safety clinical chemistry and hematology parameters, serum selenium, PSA and testosterone levels, antioxidant status, transrectal ultrasound prostate volume, urinary flow rate, ultrasound estimated postvoid residual urine volume at baseline and 180 day. The results showed statistically significant differences between treatment and control groups for the following parameters: decreased PSAtot value, improved selenium level, IPSS, quality of life score, urination parameters including voiding parameters-rate of urine flow (Qmax), average flow (Qave), total volume V and postvoid residual urine volume (RV). There was no effect on blood testosterone level. Overall the treatment was well-tolerated with no adverse effects. In conclusion, the chosen combination of silymarin and selenium proved effective and may be beneficial for the maintenance of prostate health in men. **Acknowledgement:** *Financial support from the Czech Ministry of Education, Youth and Sport (Grant No. MSM 6198959216) is gratefully acknowledged.* **References:** 1. Vidlar A et al. (2010) Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154(3): 239 – 244

PC4 Molluscicidal Activity of Some *Solanum* Species Extracts against the Snail *Biomphalaria alexandrina*

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Snails' species are associated with transmission parasitic disease as intermediate host. Biological control stands to be a better alternative to the chemical controls aimed against snails. The search of herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative molluscicides control. Solvent extracts of fresh mature leaves of *Solanum nigrum* L., *S. villosum* Mill., and *S. sinaicum* Boiss. were tested against *Biomphalaria alexandrina*, a common intermediate host of *Schistosoma mansoni*. A phytochemical analysis of chloroform: ethanol extract was performed to search for active toxic ingredient. The lethal concentration was determined. Extracts isolated from mature leaves of *Solanum* species were found to be having molluscicidal properties. S. nigrum extract was recorded as

the highest mortality rate. When the mortality of different solvent extracts was compared, the maximum (P< .05) mortality was recorded at a concentration of 90 ppm of ethanol extract of *S. nigrum*. Extract of mature leaves of *S. nigrum* exhibited molluscicidal activity followed by S. sinaicum and the less one was *S. villosum*. The study provides considerable scope in exploiting local indigenous resources for snails' molluscicidal agents. **Acknowledgement:** *The authors thank all the participants who shared their time for working on this study.* **References:** Ahmed AH, Kamal IH, and Ramzy RM (2001) Journal of the Egyptian Society of Parasitology, 31(3): 843 – 852. Massoud AM and Habib FS (2003) Journal of the Egyptian Society of Parasitology, 33(2): 585 – 596.

PC5 Efficacy of Punica granatum extract on in vitro and in vivo control of Trichomonas vaginalis

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Trichomoniasis vaginalis is now an important worldwide health problem. Metronidazole has so far been used in treatment, but the metronidazole resistant strains and unpleasant adverse effects have been developed. Treatment of patients with metronidazole refractory vaginal trichomoniasis constitutes a major therapeutic challenge and treatment options are extremely limited. The last 7 years have seen over seven times as many publication indexed by Medline dealing with pomegranate (*Punica granatum* L.) than in all the years preceding them, because of this, and the virtual explosion of interest in pomegranate (Roman) was *in vitro* investigated for its efficacy against *T. vaginalis* on Diamond media. Besides, infected women (18/20) who accepted to be treated with *P. granatum* juice were completely cured and followed- up for two months. The anti-trichomoniasis vaginalis activity of *P. granatum* extract (*in vitro* and *in vivo*) gave very promising results. **Acknowledgement:** *The authors thank all the participants who shared their time for working on this study.* **References:** 1. Abdel Hady NM, El- Sherbini GT, Morst TA (2008) J Egypt Soc Parasitol 38(3): 1024 – 5 2. Adams LS, Zhang Y (2010) Cancer Prev Res (Phila Pa) 3(1):108

PC6 Plasma oligoelements levels in pediatric cohort of spondyloarthropathy

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The importance of trace elements in chronic inflammatory diseases is related to their cofactor role in immune system functions and in different metabolic processes in articular tissues. Spondyloarthropathies (SpA) are a group of rheumatic diseases linked by common pathology, including inflammatory back pain and peripheral enthesitis. To investigate the status of plasmatic trace elements in a pediatric cohort of spondylorthropathy, to establish the relationship between these trace metals and the main biological and clinical parameters of the disease. We studied plasma concentrations of zinc (Zn), copper (Cu), iron (Fe), cadmium (Cd), nickel (Ni), plumb (Pb), manganese (Mn), calcium (Ca), magnesium (Mg) in 11 patients with juvenile spondyloarthropathy and compared them with 14 sex- and age-matched healthy subjects. Disease activity was measured by lab tests. Oligoelements concentrations were determined by atomic absorption spectrophotometry. There were no significant differences in plasma concentrations of Cd, Ni, Pb, Mn among the two groups ($p > 0.05$). Plasma zinc was significantly lower in cases with SpA than control group ($p < 0.05$) and was correlated with numerous of the biohumoral as well as clinical markers of SpA. Plasma zinc was found to be lower in SpA patients taking anti-inflammatory drugs. Cu concentrations were higher, but not significantly, in patients with SpA than those of healthy subjects. Ca and Fe plasma levels was significantly lower in children with SpA ($p < 0.05$). Administration of supplements with the proper quantity of oligoelements could balance the plasma concentrations of these trace elements in juvenile SpA.

PC7 Evaluation of the protective effect of *Urtica dioica* leaf extract on Beta cell islet langerhance of diabetic rats

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Herbal medicine is a complementary way to improve the health. The traditional Iranian Medicine introduces many plants for treatment. This study investigates the anti-diabetic effects of *Urtica dioica* L. leaves that were introduced as anti-diabetic plant in the traditional Iranian medicine. The animal was made diabetic with intra tail vein injection of 50 mg/kg STZ (streptosotocine). Animals with fasting blood sugar > 250 mg/kg were considered as diabetic. One group of diabetics were treated with *Urtica dioica* leaf extract (1 ml/kg/day intra peritoneal). After one month animals were decapitated to take the blood sample and pancreas tissue. Tissue was observed by histologist. The tissue parameters were studied in both diabetic and experimental group. Blood glucose in treated group decreased from 400 ± 54.2 mg/kg to 87.9 ± 11.9 mg/kg whereas no change was observed in diabetic group. In diabetic group, necrosis and infiltration of mononuclear cells were produced in plenty, Capillaries, islet cells, Beta cells and secretary vacuoles were damaged while in treated group the necrotic tissues was repaired and infiltration of mononuclear cells were a bit. Beta cells increased and secretary vacuoles were appeared. The number of capillaries and undifferentiated cells also increased. *Urtica dioica* repairs pancreas tissue and improves its function. This may lead to increase insulin secretion and Urtica Dioica direct influence in decreasing blood sugar. **References:** 1. Kavalali G et al. (2003) J Ethnopharmacol 84(2 – 3): 241 – 245 2. Farzami B et al. (2003).J Ethnopharmacol 89: 47 – 53 3. Kumar V et al. (2003) Basic Pathology, the Pancreas 7 th ed, Saunders; pp: 635 – 657

PC8 Antibacterial activity of some medicinal plants against antibiotics

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In this study, the antibacterial activity of 6 species of Lamiaceae family: *Mentha spicata* L., *Mentha aquatica* L., *Stachys byzantina* K.Koch, *Marrubium vulgare* L., Rosmarinus officinalis L. and *Melissa officinalis* L. were investigated. The methanolic extracts of aerial parts of plants were evaluated in different concentrations according to the disk diffusion method by using *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Streptococcus fecalis* and *Klebsiella pneumoniae* and all the extracts were compared with standard antibiotic discs like vancomycin, ampicillin and chloramphenicol. The methanolic extracts of *Stachys byzantina* and *Rosmarinus officinalis* were shown to inhibit, to different degrees, the growth of microorganisms (15 – 20 mm). Furthermore, *Stachys byzantina* showed significant antimicrobial activity against *Staphylococcus aureus* resistant to vancomycin. The other plant extracts had shown lower antibacterial activity in comparison with standard antibiotics. This study showed that some medicinal plants that were used in folk medicine significantly *Stachys byzantina* could be comparable with antibiotics and potential sources of new antimicrobial agents.

PC9 How does long term exposure to base stations and mobile phones affect human hormones profile?

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This study is concerned with assessing the role of long-term exposure to high frequency non-ionizing electromagnetic radiation (RFR) emitted either from mobile phones or from base stations. One thousand volunteers from different areas in Egypt exposed to radio frequency non-io-

nizing electromagnetic radiation emitted from mobile phones or from base stations for period extended to six years suffered tangible effects on the pituitary – adrenal axis. Volunteers were divided into three subgroups according to non-ionizing radiation exposure; according to the time of exposure to RFR per day. In addition to negative control subjects of compatible age ranges, sex and socioeconomic status. Volunteers' plasma ACTH and serum cortisol levels were measured. Also thyroid hormones were detected for all individuals. In addition, each of their serum prolactin, progesterone and testosterone level were measured to detect the different relations between all these seromarkers in all volunteers who expose to non-ionizing electromagnetic radiation emitted either from mobile phones or from base stations. Authors have previously found no association between self-reported illness symptoms and the exposures to microwave radiation emitted by mobile phones or electromagnetic field induced by other major sources [1]. The thyroid gland is one of the most exposed vital organs and may be a target for electromagnetic radiation. It has been established that even a small change in circulating thyroid hormone levels is sufficient to alter the brain functions [2]. **Keywords:** base stations, mobile phones, long-term exposure, electromagnetic radiation, ACTH, cortisol, thyroid, prolactin, progesterone, testosterone

Topic D: Cultivation and Breeding

PD1 Response of seed priming on seed germination and seedling growth in basil

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In order to evaluate the effect of priming on seed germination and seedling growth in basil (*Ocimum basilicum* L.), an experiment was conducted based factorial in randomized complete block design with three replications in western of Iran. Priming factor including: witness, KCL 2% and priming time including: 0, 3, 5, 7 and 9 hours. Results showed that Priming was affected on radicle length, radicle dry weight, stem let dry weight, germination percentage and speed germination. KCL 2% had better results due to negative osmotic adjustment. Seed priming had significant affected on radicle length, radicle dry weight, stem let length and stem let dry weight. Seed priming in 7 hours had positive affected on all studied traits. The highest radicle length, radicle dry weight and stem let length obtained from 7 hours. According to the results Priming and priming time role played in plant germination. Among treatments KCL 2% and 7 hours had an important role in germination. **Keywords:** Seed priming, Basil, Germination, Ocimum basilicum

PD2 Effect of plant growth promoting rhizobacteria (PGPR) on the healthy and productivity of soy bean plant

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A pot and field experiment were conducted to evaluation some rhizobacteria namely *Pseudomonas fluorescens*, and *Bacillus subtilis*. The pot experiment was executed to evaluate probable suppressive effect of rhizobacteria as bioagents against *Macrophomina phaseolina, Fusarium solani* and *Sclerotium rolfsii* under artifically infested soil. Results showed that co-inoculation of soy bean with rhizobacteria led to a significant decrease in pre- and post-emergence damping-off caused by all pathogens under investigation. In addition to enhance the nodulation status, growth, N-content and pod yield plant under uninfested or infested soil. Field experiment were carried out in El-Sharqia governorate to evaluate the promotive and suppressive disease effects of rhizobacteria on nodulation, plant growth and yield of soy bean. Results showed that the inoculation with rhizobacteria led to a significant increase in the nodulation status, shoot dry matter and N-content after 15, 45, 75 days of planting. Moreover, the co-inoculation of *Bacillus subtillis* with *Rhizobium* sp. Salient superiority in suppressive disease. The obtained results explained that the synergy between rhizobacteria (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Rhizobium* sp.) considered the efficient manner to save the protection against the phytopathogenes and promote the nodulation and symbiotic nitrogen fixation leading to a high quality yield of soy bean.

PD3 Effect of plant density and application rates of vermicompost on essential oil content and composition of Balm (*Melissa officinalis* L.)

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In order to investigate plant density and application rates of vermicompost on essential oil content and composition of Balm, the experiment was conducted during 6 months in Dineh phytomedic company in 2010. This experiment was carried out in complete randomized block design with 3 replications at three plant densities (6, 8 and 10 plant/m^2) and four application rates of vermicompost (0, 5, 10 and 15 ton/ha). In floral imitation, plants harvested and essential oil were extracted by water distillation. The essential oils were analyzed by GC and GC/MS. The results showed a significant difference (%1) among plant densities and application rates of vermicompost on essential oil yield. maximum amount of essential oil obtained from 10 plant/m^2 and 10 ton/ha vermicompost consumption. Identification of essential oil components showed that plant density had no effect on essential oil composition but some compounds of the oil decreased with more application of vermicompost, whereas some other compounds increased with most application of vermicompost.

PD4 Study the effect of different levels of phosphobiofertilizer's inoculation on some traites of *Anethum graveolens* L. in Rudhen

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It is well known that In nature, a considerable number of microorganisms (*e.g.* bacterial species), mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield.To inspect the influence of phosphobiofertilizer, we used 6 shapes of phosphobiofertilizers inoculation, included: (B seeds inoculated, B top dressing, E seeds inoculated, E top dressing, E-B & control) of Iranian (B) and non-Iranian (E) microorganisms, on hight of plant, yield of seed and essential oil production of the plant named experimental Dill (*Anethum graveolens* L.) in the form of factorial on the basis of Randomized Complete Block design in three replication was conducted in Rudhen university (Kolyak state). Results showed that in all traits, There was a significant difference between utilization of both types phosphobiofertilizers and other treatments. **Acknowledgement:** *The authors are grateful to Dr. Baghi and Dr. Seyed Hadi for their scientific support to the present project.*

PD5 Effect of different nitrogen and phosphorus application on qualitative a quantities characteristic of beebalm

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In order to test the effect of nitrogen and phosphorus fertilizers on qualitative and quantitative characteristic of beebalm, an experimental was carried out using factorial with randomized complete block design with three replication in llam, Iran in 2009 – 2010 growing season. Experimental factor including different of nitrogen fertilizer (70, 100 and 130 kg/ha) and phosphorus fertilizer (50, 70 and 90 kg/ha). Results showed that nitrogen fertilizer was affected on essential oil content, essential oil yield, plant height, number of tillering, stem diameter, root length, root weight and shoot ratio. The highest essential oil yield, plant height, number of tillering, stem diameter, root length was obtained 130 kg/ha nitrogen application. Phosphorus fertilizer affected essential

oil content, essential oil yield, plant height and root weight. The highest essential oil yield, plant height, number of tillering, stem diameter, essential oil yield and root weight was obtained 70 kg/ha phosphorus application. Interaction effect nitrogen x phosphorus application was affect on essential oil yield and plant height. The highest and the lowest essential oil yields were obtained 130 nitrogen and 70 kg/ha phosphorus application, respectively.

PD6 Effect of salinity on essential oil content and composition of *Agastache foeniculum* Kuntze

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Water and soil salinity on the environmental agents limit plant growth and its productivity in Iran. Anise Hyssop (*Agastache foeniculum* Kuntze) is a perennial aromatic plant, belonging to the family Lamiaceae. The essential oil of Anise Hyssop is used in food industries, pharmacy, perfumery and making soda. This experiment was conducted in a randomized complete blocks design with four salt treatments including 0 (control), 50, 100, 100 and 150 mM NaCl and four replications in green house. Some parameters such as content and composition of essential oil were modulated. The results showed that salt stress had significant effects on estimated parameters. Salinity decreased the fresh and dry weight of leaves and shoots, herbage yield and the amount of essential oil. In the composition of essential oil β-pinene, myrcene, anisaldehyde and β-bourbonene increased and the content of linalool and methyl chavicol decreased. High salinity (100 and 150mM) destroyed the plants.

PD7 Allelopathic effect of cumin (*Cuminum cyminum* L.) on seed germination of three weeds

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The allelopathic effects of cumin (*Cuminum cyminum* L.) were evaluated on seed germination of velvet flower (*Amaranthus retroflexus* L.), flixweed (*Descurainia sophia* (L.) Webb ex Prantl) and wild oat (*Avena fatua* L.) in laboratory using the aqueous extracts of dried powdered of cumin leaves. The treatments were 1, 2, 5, 10 and 15% extract of cumin and distilled water control. According to the results, extracts significantly inhibited seed germination of weed species and the degree of inhibition increased with increasing concentration of extracts. Germination of *Amaranthus retroflexus* seeds was inhibited at concentrations greater than 5%. (In addition, radicle and plumule lengths of *Amaranthus retroflexus* were significantly reduced at 1% compared to the distilled water. Results indicated germination percentage, germination rate and radicle and plumule lengths of *Avena fatua* were significantly reduced by the extracts compared to the distilled water. Results confirm germination of *Descurainia sophia* seeds was inhibited at concentrations greater than 2%. Accordingly germination rate and radicle lengths of *Descurainia sophia* were significantly reduced by the extracts compared to the distilled water. Therefore, extract of cumin might be useful as natural herbicides and might also contain numerous growth inhibitors that could be used for the development of biological herbicides.

PD8 Growth, yield and essential oil content of *Marrubium vulgare* as affected by three levels of nitrogen fertilizer

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Marrubium vulgare L. (Lamiaceae) is a perennial herb commonly known as "White horehound" and grows wild in the Egyptian desert and commonly distributed in Europe, North and South America, the Mediterranean district and Western Asia. The plant is used in the folk medicine of several countries for the treatment of a variety of diseases, including inflammatory, gastroenterical and respiratory disorders. For the first time in Egypt, the plant was cultivated under systematic agriculture regime to estimate the nitrogen doses for the best plant growth. Three nitrogen doses were applied (N1 (33.5), N2 (50.25) or N3 (67) kg N/fedden = 4200m2). Nitrogen fertilization had significant effects on most of agronomic parameters studied. Plant height (cm), number of branches per plant, plant fresh and dry weight (g) increased with the increase in nitrogen fertilization. Among various levels of nitrogen (N2) exhibited the best growth attributes, although the differences were not significant in most harvests (cuttings) between the rates of N2 and N3. On the other hand, oil content was not influenced by nitrogen fertilization in all harvests. **References:** 1. Boulos L (2002) Flora of Egypt. Al Hadara Publishing. Egypt. 2- Stulzer HK, Tagliari MP, Zampirolo JA, Cechinel-Filho V, Schlemper V (2006) J Ethnopharmacol 108: 379 – 384. 3- Sahpaz S, Garbacki N, Tits M, Bailleul F (2002) J Ethnopharmacol 79: 389 – 392.

PD9 Changes in essential oil content and composition of *Agastache foeniculum* under water stress at second harvest

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To study the effect of different levels of water stress on essential oil content and composition of *Agastache foeniculum* Kuntze at second harvest, a field experiment in randomized complete block design with three replications was conducted. Water stress treatments were: 100% of field capacity (FC), 85% of FC, 70% of FC, 55% of FC, 100 – 85% of FC (100% at vegetative and 85% at reproductive phases), 100 – 70% of FC (100% at vegetative and 70% at reproductive phases), 85 – 100% of FC (85% at vegetative and 100% at reproductive phases). The highest of essential oil content was observed at 70% of FC (1.69) and 100 – 85% of FC (1.67). Also the treatment 85% of FC (1.33) caused the lowest of essential oil content. There was not significant different between other treatments. The main essential oil constituent was methyl chavicol which showed a increasing with progress water stress till 70% of FC and then decreased at 55% of FC treatment. Limonene, another main component was decreased with increasing water stress levels from 100 of FC to 70% of FC. The highest amount of methyl chavicol (98.3%) and limonene (2.4%) were found at 70% of FC and 85 – 100% of FC, respectively.

PD10 Influence of biofertilizers on the essential oil content and constituents of *Dracocephalum moldavica* L.

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In this study the effect of three levels of vermicompost (0, 15 and 30% V/Pot), two levels of biophosphate (application and not application) and two levels of azotobacter (application and not application) on content and constituents of essential oil of *Dracocephalum moldavica* L. was investigated. The results showed that the essential oil content of dragonhead (*Dracocephalum moldavica*) and its constituents were significantly affected by biofertilizer treatments. The highest essential oil content (0.74%) was obtained when 30% pot volume was vermicompost. Fifteen components were identified from the oil of plants which were fertilization by biofertilizers. The highest geranyl acetate content (61.1%) of essential oil were obtained when 30% pot volume was vermicompost, while the highest geraniol content in essential oil (24.2%) was obtained when 15% pot volume was vermicompost with application of biophosphate. and highest geranial content of essenitial oil (18.2%) was obtained with 15% pot volume was vermicompost with application of azotobacter. Vermicompost had a promoting influence on most of vegetative growth parameters and it has been led to make accumulation of essential oil, chemical constituents including total carbohydrate and photosynthetic pigments content.

PD11 Effect of sowing date and seeding levels on quantitative and qualitative yield of chamomile (*Matricaria recutita*)

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In order to study the effect of sowing date and seeding levels on quantitative and qualitative yield of chamomile (*Matricaria recutita*) L., an experiment was conducted. The experimental design was split-plot in the basic of randomized complete blocked design with three replications. Main plots consisted of three sowing dates (6 Nov, 5 Mar, and 4 Apr) and sub-plots included three seeding levels (0.2, 0.4 and 0.8 g/m^2). On the basis of the results, highest plant (47.4 cm), the most number of plants in plot (135.4 plants), the most yield of fresh and dry flower yield (749.1 and 175.1 g/m2) was obtained from the plants were sown on 6 of Nov but the most percentage of essential oil and chamazulene (0.59 and 5.62 percent respectively) and essential oil yield (0.79 g/m^2) was obtained from the plants were sown on 5 of Mar. On the basis of the results of their interaction, highest plant (49.7 cm), the most yield of fresh and dry flower yield (810 and 198.2 g/m^2) was obtained from the plots were sown on 6 of Nov with 0.8 g/m^2 but the most essential oil and chamazulene content (0.63 and 5,9 w/w percent respectively) and essential oil yield (0.97 g/m^2) was obtained from the plots were sown on 5 of Mar with 0.4 g/m^2. According to the results, the most suitable sowing date and seeding level in Mashhad condition is 5 of Mar with 0.4 g/m^2 seeds. **References:** 1. Letchamo W, Marquard R (1993) Acta Hort 331: 357 – 361. 2. Zalecki R (1972) Herba Polonica 910: 70 – 88.

PD12 Effect of GA3 and KNO_3 treatments on improving *Nepeta crispa* seeds germination

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Nepeta crispa Willd. is an aromatic endemic plant of Iran. This plant with the common local name Mofarrah (because of its sweet odor) has been of great interest to Iranian traditional medicine. Infusion obtained from the aerial parts of *N. crispa* was used traditionally as sedative, relaxant, carminative, restorative tonic for nervous and respiratory disorders. Therefore, study of different characteristics of this plant, including propagation and increasing is essential. The effect of GA3 (gibberellic acid) and KNO_3 (potassium nitrate) was tested for seed germination of *N. crispa* by measuring the germination percentages and rate also dry and fresh weight under pre- soaking seeds with concentrations 50, 100, 200, 300 and 500 ppm of GA3 at 48 h and 0/1%, 0/2% and 0/4% of KNO_3 at 72 h, along with control. The results showed that the highest germination percentage and rate were obtained with seeds which were presoaked 300 ppm GA3. Also the highest dry and fresh weight in seeds which were obtained with seeds was pre- soaked 300ppm GA3. But the lowest germination percentage and rate were provided by pre- soaking seeds in 500 ppm of GA3. **Acknowledgement:** *A.Eskandary, Y.Ahmadi Moghadam, M.Avizegosh*

PD13 Evaluation the effects of harvesting management and drying methods on chemical indices of barberry

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Seedless Barberry (*Berberis vulgaris* L.) is a medicinal shrub that all parts of the plant used for many diseases treatment [5]. Since the managemental procedure are critical for quality of medicinal products, the aim of this research was determininig the best harvesting and drying methods for seedless barberry. This research was conducted as factorial experiment based on Complete Randomized Block Design with three replications in southern Khorassan province, Iran, in 2010. Exprimental factors included picking off method (branch and berry picking) and drying method (sun drying and shade drying). The chractestics such as pH, soluble solids (Brix) and acidity were determined in barberry sampels, also the amount of anthocyanin was defined by Timberlak and Bridle method [6]. Maturity index (MI) defined as brix to acidity ratio. Results showed that all chractestics were superior in sun drying and berry picking off methods tretments, except pH (Table 1). It has been reported that the amounts of anthocyanin was reduced at low light level [2], whereas the others reported that increasing light reduced it [1]. This differents related to plant, variety, location, season and growth phase [3]. Anthocyanines are more stable at high pH [4], Since in sun drying and berry picking treatmetns, amounts of acidity and anthocyanin were increased and pH was decreased, it seems that this pigments in seedless barberry could maintain with applying these treatments. Our results indicated that sun drying and berry picking off methods were more effective to improve qualitative proprrties of seedless barberry. **References:** 1- Bergqvist J et al. (2001) J Enology and Viticulture 52: 1 – 7. 2- Dokoozlian N K, Kliewer WM (1996) Soc.Hort Sci 121: 869 – 874. 3- Jyothi A N et al. (2005) Int J Food Prop 8: 221 – 232. 4- Inami O et al. (1996) J Agric Food Chem 44: 3090 – 3096. 5- Shamsa F et al. (1999) J Ethnopharmacol 64: 161 – 166. 6- Timberlake CF, Bridle P (1982) Distribution of anthocyanins in food plant. Anthocyanins as food colors. London: Academic Press.

PD14 Selecting superior variety of *Atractylodes lancea* through photosynthetic characters and chlorophyll fluorescence parameters

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Atractylodes lancea (Thunb.) DC., which had been highly appreciated in medical literature of past dynasties, is a kind of authentic herb, and has a long history of medicinal in China. The selection of superior variety is an important measure to cultivate the *Atractylodes lancea* Wild *Atractylodes lancea* grown in Maoshan mountainous area, Jiangsu province, China, was divided into four types according to leaf shapes: incised leaf-type, ovate leaf-type, long lanceolate leaf-type, and short lanceolate leaf-type. The photosynthetic activity and chlorophyll fluorescence parameter of four types of *Atractylodes lancea* were measured. There were significant differences in photosynthetic activity among the four types of *Atractylodes lancea*. The net photosynthetic rate of incised leaf-type was much higher than that of other types. Substantial differences in the overall performance of the photosynthetic apparatus also existed among the four types of *Atractylodes lancea*. The capacity to regenerate the photosynthetic apparatus, photochemical quenching capacity and PS II electron transport activity of the incised leaf-type *Atractylodes lancea* were greater than those of other types, and the capture efficiency of light energy of the short lanceolate leaf-type was the lowest among the four types of *Atractylodes lancea*. This suggests that the growth rate of the incised leaf-type *Atractylodes lancea* was greater than that of other types in growing environment. It was consistent with the measurers to the growth of stem and leaves in the field [1]. The incised leaf-type *Atractylodes lancea* can be selected as the superior variety. **Acknowledgement:** *The authors gratefully acknowledge the financial support from the High-tech Agriculture Research Program of Jiangsu Province, China (No. BG2006322).* **References:** Sang X (2008) J Anhui Agri Sci 36: 7726 – 7727.

PD15 Effect of plant growth regulators on the growth of *Orychophragmus violaceus* plantlets *in vitro*

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Orychophragmus violaceus (L.) O.E.Schulz, which belongs to *Orychophragmus* Bunge (Cruciferae), is an annual or biennial wild plant. Much attention has been paid to it from researchers for its adaptability to karst and great economic worth and medical value including anticancer role (contaning anticancer substance glucoraphanin in seeds) [1,2]. High efficient propagation is necessary for mass production of *Orychophragmus violaceus*. The successive information of plantlets *in vitro* was obtained via using image analysis technique. The effect of plant growth regulators on the growth of *Orychophragmus violaceus* plantlet *in vitro* was studied. Nine treatments with different additions of 6-benzyl aminopurine (6-BA) and naphthalene acetic (NAA) supplemented to MS medium were carried out. The biomass of plantlets *in vitro* in sterilized condition was acquired *via* image analysis technique. There is a significant correlation

between the biomass obtained from the image analysis and those from the manual measurement ($Y = 0.9497X + 0.0018$, $R^2 = 0.9861$, $n = 29$, $P < 0.01$). The difference of effect on the growth rates by various growth regulators assembly is significant. The high level of 6-BA and NAA is disadvantageous for the growth of *Orychophragmus violaceus* plantlet *in vitro*. MS medium supplemented with 0.02 mg l^{-1} NAA and 2.00 6-BA was the optimal for the growth of *Orychophragmus violaceus* plantlets *in vitro*. **Acknowledgement:** *The authors gratefully acknowledge the financial support from National Basic Research Program of China (973 Program) (No. 2006CB403206).* **References:** 1. Wu YY (1997) Comprehensive studies on plant of adaptability to karst-Orychophragmus violaceus. Guizhou Sci. Publ. House. Guiyang. 2.Wu YY et al. (2004) The studies on Orychophragmus violaceus' adaptability to karst. Guizhou Sci. Publ. House. Guiyang.

PD16 Assessment diversity and cultivation potential of *Coridothymus capitatus* (L.) Reichenb. fil growing wild in Jordan

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Coridothymus capitatus (L.) Reichenb. fil. is a medicinal and aromatic plant growing wild in Jordan and locally known as Za'tar Farisi. The phenotypic diversity and potential cultivation study comprised fifteen wild populations of *Coridothymus capitatus*, one wild population of *Thymbra spicata* and two *Thymbra. spicata* landraces. The investigated wild populations of *Coridothymus capitatus* showed various degrees of phenotypic variation based on the characters under investigation. Significant variations were obtained for quantitative characters, the coefficient of variation percentage (C.V %) ranged from 12.60%. to 39.20%. The average estimate of Shannon's diversity index (H') was 0.58. The genetic distance among pairs of populations was low. *Coridothymus capitatus* populations introduced for cultivation showed a good stand and potential toward producing dry herbage yield (3046 kg/ha). Cultivated populations showed phenotypic variation in the investigated traits. The results of this study indicate that a broad range of genetic variation exist among populations of *Coridothymus capitatus* collected from wild habitats in Jordan, and among *Thymbra spicata* populations. Seeds of *Coridothymus capitatus* and *Thymbra spicata* were conserved (*ex situ*) in seed bank and in the field bank. The results obtained pave the road for a potential commercial and large-scale cultivation and oil production from *Coridothymus capitatus* species. **References:** Faleiro L et al. (2005) J Agric Food Chem 19: 8162 – 8168. Goren A et al.. (2003) J Biosci58: 687 – 690. Haddad N and Turk M (2002) Medicinal and herbal plants cultivation. Ministry of Agriculture, National Center for Agricultural Research and Technology Transfer (NCARTT). Conservation of medicinal and herbal project preparation grant, Global Environment Facility (GEF), Amman. Jordan. Morales R (1996) Lamiales newsletter 4: 6 – 8. Morales R (1989) Biocosme Mésogéen 6:205 – 211. Morales R (1986) Ruizia 3: 1 – 324.

PD17 Safranal variations in saffron (*Crocus sativus* L.) under irrigation regimes in Iran

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Saffron is a spice derived from the flower of the saffron crocus (*Crocus sativus* L.). Together with the styles stalks that connect the stigmas to their host plant the dried stigmas are used in cooking as a seasoning and colouring agent is native to Southwest Asia. Saffron also contains a carotenoid dye, crocin, which imparts a rich golden yellow hue to dishes and textiles. In order to the safranal variations in saffron under irrigation regimes, an experiment was carried out using a randomized complete blocks design with three replications at Iran in 2010. The factors including irrigation regimes (control, irrigation interrupted from stem elongation stage, irrigation interrupted from flowering stage) were studied. The flower yield in saffron increased under control irrigation into interrupted irrigation but safranal variations was increased under interrupted irrigation into control irrigation. The findings may give applicable advice to medicinal and aromatic plants researchers for management and concern on water strategy and estimate of irrigation carefully for increase of quantity and quality yields in medicinal and aromatic plants farming.

PD18 18 The study on the effect of irrigation levels and mulch application on growth indices and essential oil content of peppermint (*Mentha piperita* L.)

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In order to study the effects of different irrigation regime and mulch types on growth indices and, essential oil content of peppermint (*Mentha piperita* L.) in 2010 in research field of Agricultural college of Ferdowsi University of Mashhad as factorial randomized complete block design was performed in four replicates. Irrigation treatments included three levels (100, 80 and 60 percent of water requirements calculated by evaporation pan class A and two types of mulch (black plastic and wood chips) and the uncoated control. Traits include inter-node distance, number of flowers/plant, branch number/plant, plant height, fresh weight, dry weight, chlorophyll content, leaf relative water content, leaf area, essential oil percentage and yield were evaluated during full flowering. The results showed that the effect of irrigation on fresh weight, dry weight, leaf relative water content and leaf area was significant (at 0.05 level). The effect of mulch on fresh weight, dry weight, leaf area, inter-node distance, number of branch and leaf relative water content was significant (at 0.05 level). Interaction between irrigation and mulch on inter-node distance, number of branches, fresh weight, dry weight, relative water content of leaves and leaf area and height were significant (at a 0.05 level). The effect of irrigation, mulch and interaction effects on traits such as flower number, chlorophyll content, percentage essential oil (at 0.05 level) was not significant.

Topic E: Essential oils

PE1 The effect of harvesting time on total phenolic content and antioxidant activity of five plants of the family Labiatae

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Regarding the effects of the harvesting stage on the amount of phenolic content and antioxidant activity of medicinal herbs *Satureja hortensis* L., *Origanum majorana* L., *Salvia virgata* Ait., *Melissa officinalis* L. and *Hyssopus officinalis* L. an experiment has been tested three S.D in form of randomized complete block design.The results of this survey in herbal medicine showed the effects of the harvesting stage on the amount of phenolic content and antioxidant activity in the pre-flowering had the least and flowering time had the most phenolic content and antioxidant activity. The most amount of phenolic content and antioxidant activity in *Satureja hortensis* herbal medicine are (25.15mgGAE/gdw) in flowering and (8.38 μg/ml) in pre-flowering stage in sequence. The most amount of phenolic content and antioxidant activity in *Origanum majorana* herbal medicine are (46.73 mg GAE/gdw) in pre- flowering and (6.53 μg/ml) in flowering stage in sequence. The most amount of phenolic content and antioxidant activity in *Salvia virgata* herbal medicine are (47.27mgGAE/gdw) in flowering and (7.77 μg/ml) in pre- flowering stage in sequence. The most amount of phenolic content and antioxidant activity in *Melissa officinalis* herbal medicine are (42.60 mg GAE/gdw) and (7.32 μg/ml) in flowering stage in sequence. The most amount of phenolic content and antioxidant activity in *Hyssopus officinalis* herbal medicine are (21.88 mg GAE/gdw) and (9.53 μg/ml) in flowering stage in sequence. **Keywords:** Phenolics content, Antioxidant activity, Harvesting time, Satureja hortensis, Origanum majorana, Salvia virgata, Melissa officinalis, Hyssopus officinalis

PE2 Antinociceptive mechanisms of *Bunium persicum* essential oil in the mouse writhing test

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Antinociceptive profiles of *Bunium persicum* B.Fedtsch. were examined in NMRI mice. Essential oil of *Bunium persicum* administered intraperitoneally (0.001, 0.01, 0.05, 0.1, 0.5 and 1%; 10 ml/kg) in Tween-80(0.5%)

showed an antinociceptive effect in a dose-dependent manner as measured by writhing test as a model of visceral pain. Furthermore to reveal the antinociceptive mechanisms of *Bunium persicum*, we examined the effect of opioidergic, serotonergic, and histamine receptor antagonists on *Bunium persicum* -induced antinociception. Intraperitoneal (*i.p.*) pretreatment with naloxone, chlorpheniramine and cimetidine attenuated inhibition of the pain response induced by *Bunium persicum*. However, cyproheptadine did not affect inhibition of the pain response induced by *Bunium persicum*. Our results suggest that *Bunium persicum* shows an antinociceptive property in writhing test. Furthermore, antinociception of *Bunium persicum* may be mediated by opioidergic and histamine H1 and H2 receptors.

PE3 Essential oils composition and antioxidant properties of three *Thymus* species

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The essential oils of three wild-growing *Thymus* species (*Thymus kotschyanus* Boiss. & Hohen., *Thymus eriocalyx* (Ronniger) Jalas. and *Thymus daenensis* Celak subsp *lancifolius* (Celak) Jalas. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum extraction and analysis conditions, 44, 38 and 38 constituents (mainly monoterpenes) were identified in *T. kotschyanus, T. eriocalyx* and *T. daenensis* subsp. *lancifolius* which represented 89.9%, 99.7% and 95.8% of the oils, respectively. The main constituents were thymol (16.4 – 42.6%), carvacrol (7.6 – 52.3%) and γ-terpinene (3.0 – 11.4%). Antioxidant activity was employed by two complementary test systems namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid systems. Antioxidant activity of polar sub-fraction of *T. daenensis* subsp *lancifolius* was found to be higher than those of the others in DPPH assay while non-polar sub-fraction of *T. eriocalyx* has most antioxidant activity in β-carotene/linoleic acid test (19.1 ± 0.1 μg/ml and 96.1 ± 0.8% inhibition rate, respectively).

PE4 Effect of heating on *Zataria multiflora* and *Cinnamon zeylanicum* essential oils for the evaluation of their antiradical activities by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH)

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Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration. Since application of natural antioxidants may be one of the technically simplest ways of reducing fat oxidation, The present study was designed to survey the effect of heating on antiradical property of *Cinnamon zeylanicum* Breyne and *Zataria multiflora* Boiss.(avishan-e-shirazi) essential oils. The essential oils were heated in three tempratures (100, 140, 180 °C) for 1, 2, 3 hours and the antiradical property was compared with samples before heating. The antiradical property was evaluated by using DPPH° assay. All the data were analysed by SPSS software (version11.5). In the ambient conditions, EC_{50} of *Zataria multiflora*. and *Cinnamon zeylanicum* essential oils were 4026.67 ± 2.2 and 2605.01 ± 3.7 ppm, respectively. According to the results, different behavior of essential oils, based on different heat conditions, were observed. In conclusion, the essential oils under study exhibited good antiradical properties and might be efficiently used to control lipid oxidation during food processing. **Keywords:** essential oil, *Cinnamomum zeylanicum*, *Zataria multiflora*, DPPH assay **References:** 1. El-Baroty GS et al. (2010) African Journal of Biochemistry Research 4: 167 – 174. 2. Jayaprakasha G K, Negi P S, Jena B S and Roa L (2007) J Food Composition and Analysis 20: 330 – 336. 3. Kulisic T, Radonic A and Katalinic V (2004) Food Chem 85: 633 – 640. 4. Marinova E M and Yanishlieva N V (1996) Food Chem 58: 245 – 248. 5. Marongiu B et al. (2007) J Agric Food Chem24: 10022 – 10027.

PE5 Effect of Salt Stress on Growth and Essential oil of *Matricaria chamomilla* L.

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A pot experiment based on complete block design was carried out to investigate the effect of salinity on growth traits and essential oil content of chamomile (*Matricaria chamomilla* L.). Four levels of salinity including control (0 mM), 50, 150 and 250 mM Nacl and CaCl2 in 5:1 molar ratio were used. Result indicated that increased salinity caused reduction in plant height, number of branches per plant, number of flowers per plant. Increased salinity also significantly decreased plant dry weight, flower dry weight and essential oil content. The highest values of growth traits such as number of flower per plant, flower dry weight and essential oil content were observed under control condition (non-salinity stress). The effect of salinity on flower dry weight is greater than other traits. Flower dry weight of plants at low (50mM) level of salinity was decreased 12.2% compared to control (non-stressed plant) while essential oil content increased 18.2% at the same salt concentration. At the highest level of salt stress (250mM) flower dry weight and essential oil content was decreased by 79.8 and 45.5% compared to non stressed plants, respectively. Number of flower per plant was decreased by 16.1 and 69.2% at lowest (50 mM) and highest (250 mM) salinity concentration respectively. Salinity affects flowering time of plants. Flowering time of non-stressed plants started 50 days after plant transplanting while flowering time of plants treated by 250 mM salinity started 64 days after seedlings transplanting to pots. **Acknowledgement:** *I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support. The author is grateful to Mr Hamid Eskandari BSc student of medicinal plant production for his excellent assistance.*

PE6 Effect of Salinity on Germination and Seedling Growth of Four Medicinal Plants

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This experiment was conducted in germinator in order to study the effects of water potential on seed germination, rate of germination and seedlings growth of four medicinal plants (*Coriandrum sativum* L., *Plantago psyllium* L., *Descurainia sophia* (L.) Webb ex Prantl and *Portulaca oleracea* L. Four water potential including distilled water as control (0), -0.37, -0.59 and -0.81 Mpa which made by different salts (NaCl, $CaCl_2$ and NaCl+$CaCl_2$ in 5 to 1 molar ratio). The experiment was carried out based on completly randomized design with six replications. Results showed that the effects of water potential, type of salt on germination percentage, rate of germination, root and shoot length were significant. With decreasing water potential, germination percentage and rate of germination declined but the response of plant were differ. Germination of *Portulaca oleracea* was not affected by decreasing water potential whereas others significantly decreased. The effect of salt composition was significant on rate and percentage germination. The percentage of germination at lower water potential (-0.37 MPa) which made by NaCl + $CaCl_2$ significantly was higher than the same water potential made by only NaCl and $CaCl_2$. Although, percentage and rate germination of *Portulaca oleracea* were not affected by different water potential, seedling growth of *Portulaca oleracea* significantly decreased. **Acknowledgement:** *I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.*

PE7 Effects of Different Level of Nitrogen and Phosphorous Fertilizers on Yield Quantity and Quality of *Matricaria recutita*

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In order to investigate the effects of different levels of nitrogen and phosphorous fertilizers on qualitative yield and some quality components of German chamomile (*Matricaria recutita* L.), a factorial experiment based on a randomized complete block design with four replications was carried out in the Medicinal Plant Research Farm of Shirvan College of Agriculture in 2007. Nitrogen had three levels (0, 100, 200 kg/ha as source of urea) and phosphorous also had three levels including 0, 30, 60 kg/ha triple super phosphate.The results showed that application

of nitrogen and phosphorous fertilizers to the soil imposed a significant effects ($p < 0.01$) on plant height, number of branches per plant, number of flower per plant and flower yield. The main effects of nitrogen fertilizer appeared in improvement of vegetative growth of plants. Plants treated with 200 nitrogen per hectare and 60 kg phosphorous per hectare significantly produced the highest flower yield per square meter. Fertilizer did not affect the percentage of chamazulene. However, plant treated with 200 kg nitrogen and 60 kg phosphorous produced highest essential oil and chamazulene per square meter due to higher flower yield. **Acknowledgement:** *I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.* **References:** Balak R, Misra PN, Sharma NL, Nagari AA (1999) J Med Aroma Plant Sci 21: 969 – 971. Franz Ch (1983) Acta Hort 132: 203 – 216. Franz Ch and Kirsch C (1974) Hort Sci21: 11 – 19.

PE8 Enantiomeric composition of α-pinene in essential oils of leaves and unripe cones of *Juniperus communis* L

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The leaves and unripe cones of common juniper (*Juniperus communis* L.) were sampled from 11 habitats across Lithuania. The essential oils of leaves and cones from 110 samples (= trees) each were isolated by hydrodistillation in a European Pharmacopoeia apparatus during two hours. The enantiomers of α-pinene were separated by the chiral-phase capillary GC and identified by matching their retention times to the optically pure analytical standards. The average content, the min-max values and the variation coefficient of (1R)-(+)-α-pinene were 74 ± 1%, 7 – 92% and 16.4% in leaves and 69 ± 2%, 0 – 96% and 24.9% in unripe cones, respectively; the average content, the min-max values and the variation coefficient of (1S)-(-)-α-pinene were 26 ± 1%, 8 – 93% and 45.8% in leaves and 31 ± 2%, 4 – 100% and 55.7% in unripe cones, respectively. It was established that the most of samples of leaves and unripe cones of the studied *J. communis* individuals were rich in (1R)-(+)-α-pinene, while (1S)-(-)-α-pinene dominated in 2.7% samples of leaves and 10.9% those of unripe cones only. However, the (1S)-(-)-α-pinene absolutely predominated in leaves of two *J. communis* individuals. The absolute predomination of the (1R)-(+)-α-pinene was not detected neither in leaves nor in unripe cones. **Acknowledgement:** *This research was funded by a grant (No. MIP-56/2010) from the Research Council of Lithuania.*

PE9 Antifungal activity and chemical composition of *Mentha cervina* L. essential oils

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Mentha cervina L. is a very aromatic plant with a characteristic flavour, which can be found on some regions from central eastern and south of Portugal. In the present paper, we analysed the chemical composition of essential oils from fresh and dried leaves of *M. cervina* and its antifungal activity against strains of *Aspergillus niger, Penicillium* sp. and *Fusarium oxysporum* isolated from soils. M. cervina was collected in Almaceda – Vila Velha de Ródão, Central Eastern of Portugal during the flowering period. Yellowish essential oils were obtained in a yield 1.1% and 1.8% (v/w) to fresh and dry plant, respectively. Major component of the oils was identified as pulegone (78.0 and 80.4%). Both essential oils of *M. cervina* at the doses of 10 µL, inhibited totally the growth of the tested fungi. Doses of 5 µL of each essential oil also showed activity against the fungi strains used in this work, in particular against *Penicillium* sp. Taking into account the high level of pulegone observed in both essential oils and the antimicrobial activity of this compound reported by Duru [1], these results may suggest that this compound could be the main responsible component for the antifungal activity observed. **References:** 1. Duru M E, Ozturk M, Ugar A, Ceylan Ö (2004) J Ethnopharmacol 94: 43 – 48.

PE10 The effect of drying temperature, storage and distillation times on the essential oil content and composition of anis hyssop (*Agastache foeniculum*)

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This paper deals with the effect of different drying temperatures, storage and distillation times on the essential oil content and composition in anis hyssop (*Agastache foeniculum* Kuntze). The treatments were two levels of temperature (ambient temperature and 40 °C), two levels of material storage time (immediately after drying and two month after drying) and two levels of distillation time (2 and 4 h). The treatments were arranged in factorial design in base of Complete Randomized Design (CRD) with three replications. The findings show that essential oil content was declined with increasing temperature degree and storage time. Distillation time did not have significant effect on essential oil content. The highest essential oil content was observed in samples air dried and immediately after drying. According to the GC and GC-MS analyses, twelve components were recognized. Methyl chavicol (97.21 – 98.07%) and limonene (0.76 – 1.44%) were main composition of essential oil. The temperature degree, storage time and distillation time did not have a significant effect on the composition of the essential oil extracted from anis hyssop.

PE11 Chemical constituents of the essential oil of *Ferulago carduchorum* Boiss. et Hausskn. and *Levisticum officinale* Koch

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The volatiles obtained by hydrodistillation and methanol extraction of the aerial parts of *Ferulago carduchorum* Boiss. et Hausskn. and *Levisticum officinale* Koch., two Umbelliferae species of Iran, were analyzed by GC and GC/MS. The oil and the extract obtained by hydrostillation and extraction of *F. carduchorum* were characterized a high amount of monoterpene hydrocarbons (93.8% and70%, respectively). The main components of the oil and extract were (Z)-β-ocimene (12.7% and 20.0%), terpinolene (13.1% and 6.0%), α-Phellandrene (12.7% and 8.3%) and β-Phellandrene (10.9% and 8.8%), respectively. The water distilled oil and methanol extract of the air-dried *Levisticum officinale*, were also both rich in monoterpene (85.8% and 52.9%, respectively). In the oil, α-terpinyl acetate (40.5%) and β-Phellandrene (16.7%) were the main constituents, whereas in the extract, β-Phellandrene (23.0%), naphthalene (20.6%) and γ-terpinene (12.1%) were the major components. **References:** 1- Baser KHC, Koyuncu M and Vural M (1998) J Essent Oil Res10: 665 – 666. 2- Masoudi S, Rustaiyan A and Ameri N (2004) J Essent Oil Res 15: 143 – 144. 3- Sedaghat S, Khossravi M, Masoudi S, Larijani K and Rustaiyan A (2002) J Essent Oil Res14: 447 – 448.

PE12 Major volatile compounds of 50 Thymus taxa naturally grown in Antalya region of Turkey

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A large number of medicinal and aromatic plant species naturally grown in the Mediterranean Basin of Turkey contain secondary metabolites that are used in the food, pharmaceutical, cosmetic, and pesticide industries [1 – 5]. This study used 50 taxa consisting of 9 species or subspecies of the genus *Thymus* grown wild in Antalya area in the Mediterranean part of Turkey to determine their volatile compounds. The major constituents of the volatile constituents noted in six taxa of *Thymus longicaulis* C.Presl subsp. *chaubardii* (Boiss. & Heldr. ex Rchb.f.) Jalas var. *chaubardii* were borneol, nerol, geraniol, thymol, γ-terpinene, ρ-cymene, camphene, 1,8-cineole and linalool. The main components of three taxa of *T. sipyleus* Boiss. subsp. *sipyleus* var. *rosulans* (Borbas) Jalas

were α-pinene, 1,8-cineole, β-caryophyllene, α-terpineol and intermedeol. Three taxa of *T. sipyleus* Boiss. subsp. *sipyleus* var. *davisianus* Ronniger contained 1,8-cineole, ρ-cymene, β-caryophyllene, neral, α-terpineol and geranial as major constituents. Eight taxa of *T. sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus* mainly contained myrcene, 1,8-cineole, linalool, (E)-nerolidol, ρ-cymene, α-terpineol, carvacrol and hexadecanoic acid. Nine taxa of *T. zygoides* Griseb. var. *lycaonicus* (Celak) Ronninger contained γ-terpinene, ρ-cymene, borneol, thymol and carvacrol. Three taxa of *T. leucotrichus* Hal. var. *austroanatolicus* Jalas contained α-pinene, camphene, myrcene, camphor, linalool, bornyl acetate, thymol and carvacrol. Three taxa of *T. cherlerioides* Vis. var. *isauricus* Jalas contained 1,8-cineole, borneol, γ-terpinene and ρ-cymene. Six taxa of *T. revolutus* Celak contained borneol, 1,8-cineole, γ-terpinene and ρ-cymene. Nine taxa of *T. cilicicus* Boiss. & Balansa mainly contained 1,8-cineole, linalool, α-terpineol, geraniol, caryophyllene oxide, α-pinene, camphene, camphor and borneol. The occurrence of volatile compounds among clearly indicated that Thymus taxa show significant inter- and infra-specific variations. **Acknowledgement:** *This work was supported by the Scientific and Technological Research Council of Turkey.* **References:** 1. Elmasulu SY et al. (2009) Planta Med 75: 932. 2. Ince AG et al. (2009) Planta Med 75: 932. 3. Karaca M et al. (2008) J Sci Food Agric 88: 2508 – 2516. 4. Ince AG et al. (2009) Genet Resour Crop Ev 56: 211 – 221. 5. Ince AG, Karaca M (2009) J Sci Food Agric 89: 168 – 176.

PE13 Classification of 63 Origanum taxa based on microsatellite markers and essential oil composition

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A large number of aromatic plant species naturally grown in the Mediterranean basin of Turkey contain and produce essential oil [1]. In this study 63 taxa of eight *Origanum* species grown in the Mediterranean region of Antalya, Turkey were DNA typed using microsatellite markers, and oil compositions of these taxa were determined using method described in [2,3,4,5]. All the 8 *Origanum* species were separated from one another according to classical taxonomic groups using DNA markers. Individuals of two *O. vulgare* L. subsp. *hirtum* (Link) Ietsw., two *O. majorana* L., two *O. solymicum* P.H.Davis and two *O. saccatum* P.H.Davis taxa could not be differentiated in the DNA typing studies. There were high level of similarities between a dendrogram obtained from DNA markers and oil composition types among the taxa studied. *O. bilgeri* P.H.Davis consisted of two chemotypes (caryophyllene oxide and alpha-thujene) and they were clearly separated by DNA analyses. *O. husnucanbaseri* H.Duman, Aytaç et A.Duran was also separated from other species and it was the only species containing trans-sabinene hydrate. Taxa collected from Elmali location of *O. onites* L. were linalool types and they were distinctly separated from other individuals within the species. *O. majorana* consisted of two chemotypes (carvacrol and linalool). In conclusion, present study indicated that chemotypes could be identified using DNA markers. Thus, DNA markers developed in this study could be used in the identification of species in herbal mixtures, selecting the individual plant for desired oil compositions and the most importantly these DNA markers are valuable in *Origanum* improvement programs. **Acknowledgement:** *This work was supported in part by the Scientific and Technological Research Council and The Scientific Research Projects Coordination Unit of Akdeniz University.* **References:** 1. Ince AG, Karaca M (2009) J Sci Food Agric 89: 168 – 176. 2. Karaca M et al. (2005) Anal Biochem 343: 353 – 355. 3. Karaca M et al. (2008) J Sci Food Agric 88: 2508 – 2516. 4. Ince AG et al. (2010) Biochem Genet 48: 83 – 95. 5. Coskun S et al. (2008) Parasitol Res 103: 259 – 261.

PE14 Chemical composition of the essential oil from leaf, root and fruit of *Diplotaenia damavandica*, an endemic species of Iran

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Diplotenia damavandica Mozaff., Hedge & Lamond (Apiaceae), which is locally named "Kozal" is an endemic species of Iran and grows in Damavand area in Tehran province (1). Fresh aerial parts of kozal are used as flavoring agent for local dairy products. Our previous studies showed that this plant is a rich source of furanocoumarins like xanthotoxin and Angelisin (2). In this study the essential oil of the leaves, roots and fruits of the plant obtained by hydrodistillation and analyzed by GC and GC/MS. Average yields of essential oil were 1.5%, 2.5%, 0.3%, for leaves, roots and fruits respectively. The most abundant components of the leaves were trans-ocimene (22.5%), α-phellandrene (19.0%), linalool (7.3%) and cis-ocimene (6.8%). The root essential oil was characterized by high amounts of α-phellandrene (20%), α-pinene and α-terpinolene (12%). The main components of the fruits were α-phellandrene (17.1%), γ-terpinene (16.9%), limonene (13.2%) and α-terpinolene (11.2%). **References:** 1) Mozaffarian V (2007) Flora of Iran. Research Institute of Forests and Rangelands. Tehran. 2) Aynehchi Y et al. (1999) Pharm Biol 37: 161 – 162

PE15 Microvawe-Assisted Distillation Kinetics and Chemical Composition of Ginger Oil

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Rhizomes of ginger (*Zingiber officinalis* L.) are widely used ingredients in food technology, pharmaceutical and cosmetics industries. The characteristic aroma and taste of ginger as well as medicinal effect is attributed to essential oils and oleorezins. In this work, the microwave distillation kinetics, yield and composition of the essential oil obtained at different dry plant material-to-water ratios (1:10, 1:15 and 1:20) and microwave powers (350W, 450W and 600W) were examined. The oil yield strongly depend on the dry plant material-to-water ratio and the applied microwave power. Oil yield is increasing with increasing applied microwave power and lowering dry plant material-to-water ratio. The highest oil yield (1.23 mL/kg) was achieved at dry plant material-to-water ratio of 1:20 and microwave power of 600W. The essential oil composition was identified using GC-MS techniques. There are no significant differences in the chemical composition of the oils obtained at different microwave distillation conditions. **Acknowledgement:** *This work was supported under the projects OI 172047 by the Ministry of Science and Technological Development Republic of Serbia.*

PE16 Essential oil variability and trichomes morphology from *Lavandula pedunculata* (Mill.) Cav. grown at *Mata Experimental do Escaroupim* (Portugal)

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The Experimental Forest of Escaroupim [*Mata Experimental do Escaroupim* (MEE), Salvaterra de Magos, Portugal], is a protected forest area with over 175 years and under the Total Forestry Regime since 1901. *Lavandula pedunculata* (Mill.) Cav. is an aromatic shrub common in the Iberian Peninsula [1], and frequent in the understory of the MEE *Pinus*, *Quercus*, *Ulmus* and *Eucalyptus* spp. forests. In the present work, the essential oils and trichomes morphology from flowering aerial parts of two populations of *L. pedunculata* collected in two years were evaluated. The essential oils were isolated by hydrodistillation, and analyzed by GC and GC-MS [2]. The indumentum of *L. pedunculata* field grown plants was studied by LM and SEM, according to [3]. *L. pedunculata* essential oils were obtained in an average yield of 2% (v/w). Thirty six components were identified, representing 97 – 99% of the total essential oils, which were dominated by fenchone (62 – 70%) and 1,8-cineole (6 – 28%). cis-Verbenol (traces-5%), camphor (1 – 5%) and limonene (traces-4%) were also relatively abundant. Previous studies also showed essential oils fenchone-, 1,8-cineole- and camphor-rich [2,4,5]. *L. pedunculata* showed a morphologically complex indumentum of i) non-glandular uni- and bicellular unbranched trichomes and multi-cellular branched trichomes of the stellate type; ii) peltate and capitate trichomes, the last with three different morphological types; iii) multi-cellular branched stellate type with only glandular arms and iv) multi-cellular branched stellate type with both glandular and non-glandular arms. These results are in agreement with a previous study on the indumentum of *L. pedunculata* field- and *in vitro*-grown plants [5]. **Acknowledgement:** *Telmo Nunes, Paula Paes, A. Sofia Borges, Prof. Ana Monteiro.* **References:** 1. Morales R (2010) Flora Iberica, Lavandula. Vol. XII. Real Jardín Botánico. CSIC. Madrid 2. Matos F et al. (2009) J Essent Oil Res 21: 327 – 336. 3. Antunes T, Sevinate-Pinto I (1991) Flora 185: 65 – 70. 4. Zuzarte M et al. (2009) Chem Biodivers 6: 1283 – 1292. 5. Zuzarte, M et al (2010) Ind Crops Prod 32: 580 – 587.

PE17 Comparative assessment on efficiency and compounds of *Ferula gummosa* Boiss. essential oils at two different harvesting areas of Alborz mountains in Iran

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Ferula gummosa Boiss. from Umbelliferae family (Baridje in Persian and Galbanum in English) is one of the most important medicinal plants of Iran mountains area which has industrial applications, too. The northern steeps with 2000 – 4000 altitude and soils of well drained and deep, rich of humus and different quantity of lime are the best growth region for Galbanum, as ecological investigation have been shown. Galbanum exudes sap-like exudate which contains 10 – 26% essential oils, 60 – 75% resins and 5 – 30% carbohydrates, roughly[1]. It must be noticed that no alkaloids and phenol compounds have been found; except some trace of saponin and tannin. Non -carbohydrate portion of Galbanum can be extracted with ethanol. Incineration is used to determine the quality of exudates, results in ash content that must not exceed 10%. In this study, the percentage and essential oil components of Galbanum harvested from Tehran and Semnan provinces were investigated. The oleo-gum-resin was submitted to steam distillation. The essential oil, with yellowish appearance and specific gravity of 0.865 – 0.89, was analysed using GC/MS. α-pinene, β-pinene, myrcene, δ-3 carene, limonene, fenchyl acetate and guaiol identified, among which limonene and fenchyl acetate ranked highest and lowest percentage, respectively[2]. The identified compounds have significant differences at the two locations, that means at northern slope of Alborz in Tehran, essential oils yields 2.16 – 2.44%, whereas 1.23% was assigned to southern slope in Semnan. **Acknowledgement:** *We are grateful to the International Desert Researches Center, University of Tehran for financial support of this work.* **References:** [1] Zargari A (1986) Medical Plant, Vol 2, University of Tehran Press. Tehran. p 598 (in Persian). [2] Mozaffarian V (1987) Umbelliferae Family Plants in Iran. Researches Institute of Forests and Rangeland press. Tehran (in Persian).

PE18 Essential oil composition of *Artemisia spicigera* C. Koch

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he genus *Artemisia*, which comprises about 400 species, belongs to the Asteracae family and is the largest of flowering plants [2]. Most representatives are aromatic herbs or shrub. *Artemisia spicigera* C. Koch is a member of this family (Asteraceae) and from fodder and medical point of view is an important plant [4]. In this research the aerial parts of plant were collected from Azerbaijan province (Iran). The dry material of plant was subjected to steam distillation in Clevenger apparatus and a light yellow residue obtained. The essential oil was submitted to column chromatography. Gas chromatography was done and careful analysis by GC and GC-Ms of essential oil from *Artemisia spicigera* allowed us to identify most components (99.01%). The chromatogram showed the presence of approximately 17 components. The results of analysis revealed the presence of camphor (24.6%), 1,8-cineole (23.3%), β-thujone (20.7%) and α-thujone (17.1%) as major components in this plant. The essential oil, having a very strong odor and acrid taste, is described as neurotoxic due to the high thujone content [3]. The composition of the volatile oil in *Artemisia spicigera* varies widely according to geographical location, climate, day length, soil type and cultivar [1]. **References:** 1. Aleskerova AN et al. (1986) Khim Prir Soedin (1):116 – 117. 2. Bremer K and Humphries C J (1993) Bull Br Mus (Nat Hist) Bot 23: 71 – 171. 3. Miller Y et al. (1981) Clinical Toxicology 18: 25 – 32. 4. Naseri HR et al. (2009) Cytologia 74(1): 56 – 64.

PE19 Chemical Composition and Antioxidant Activity of *Salvia virgata* Jacq. and *S. verticillata* L. Volatile Oils from Iran

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Many species of Salvia are aromatic and rich in essential oils and are called Maryam goli in Persian (1). Some species are used in cosmetics, perfume, pharmacy and aromatherapy and as species (2). *Salvia virgata* Jacq. and *S. verticillata* L. (Lamiaceae) were collected from Chalus (Gachsar), in Mazandaran Province, in north of Iran. Hydrodistilled essential oils from the leaves and flowers of these plants were analyzed by GC and GC/MS. The oils yield for leaves and flowers of *S. virgata* were 0.15% and 0.19% (v/w), 19 and 30 compounds of the essential oils were identified respectively. The main constituents of the leaves oil were phytol (29.1%), β-caryophyllene (19.2%), caryophyllene oxide (17.0%) and hexadecanoic acid (8.2%). The Major components of flowers oil were β-caryophyllene (21.1%), germacrene-D (13.2%), bicyclogermacrene (7.0%), α-humulene (6.7%) and β-pinene (6.7%). The oils yield for leaves and flowers of *S. verticillata* were 0.1% and 0.12% (v/w); which 54 and 36 compounds were identified respectively. β- Caryophyllene (13.6%), β-phellandrene (12.9%), germacrene-D (11.5%), β-pinene (7.5%) and α-humulene (5.6%),

were dominant substances of leaves oil. Spathulenol (23.6%), β-caryophyllene (17.2%), caryophyllene oxide (16.4%) and sabinene (8.4%) were the main compounds of flowers oil. Antioxidant capacities of the oils were measured using DPPH (2, 2-diphenyl 1-picrylhydrazyl). Results showed that the antioxidant properties of 50 µl of *S. virgata* flowers oil was equal with 200 µg/mL of BHA ($P > 0.05$), but flowers oil of *S. verticillata* and leaves oils of *S. virgata* and *S. verticillata* were not as potent as BHA ($P < 0.03$). **References:** 1. Mozaffarian V (1996) A Dictionary of Iranian Plant Names. Farhang Moaser Publisher, Tehran. p. 207. 2. Ozcan M et al. (2003) Flav Fragr J 18: 325 – 327.

PE20 Yield and Quality Parameters of *Thymus x citriodorus* (Pers.) Schreb. (synonym *T. fragrantissimus, T. serpyllum citratus* and *T. serpyllum citriodorum*) Cultivated under Ankara Ecological Conditions

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Lemon thyme (*Thymus x citriodorus* (Pers.) Schreb) is widely grown as ornamental aims in Turkey and has no natural distrubition. It's a hybrid of *Thymus vulgaris* L. and *Thymus pulegioides* L. Compared with other Thyme species, *Thymus citriodorus* has a herbaceous oblique structure and rhizome formation. By means of this research, *T. citriodorus* was firstly cultivated in a field experiment and its yield and quality parameters recorded in semiarid conditions. Field trials were conducted in the Central Research Institute for Field Crops, Ankara, at complete randomized block design with four replications during the years of 2009 and 2010. Total yield of green herb, drug herb and drug leaf were 3769 – 4707 kg/da, 1193 – 1475 kg/da and 742.3 – 844.2 kg/da, respectively. The average content and total yield of essential oil were detected 1.430 – 1.308% and 16.93 – 19.42 L/da. In comparison to other lemon odor sources such as *Melissa officinalis* L. (0.01 – 0.03%) and *Cymbopogon citratus* [DC] Staph. (0.2 – 0.5%), its essential oil content (1.3 – 1.4%) was found rather satisfactory. Though most Thymus species are thymol containing, the main component of *Thymus citriodorus* essential oil was determined as 'geraniol' with lesser amount of lemon scented citral (geranial + neral) (from twenty-one constituents). **Keywords:** Thymus x citriodorus, essential oil, geraniol, citral

PE21 Phytochemical analysis of essential oil from *Rosmarinus officinalis* L. of Iran

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Rosemary (*Rosmarinus officinalis* L.) belongs to the family Lamiaceae (Labiatae) and has been a very important medicinal and aromatic plant since earliest times. It is a small evergreen which grows in most Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas wildly.The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa. Rosemary is a food flavoring and medicinal herb for its powerful antibacterial, antimutagenic properties, and as a chemopreventive agent. Owing to its antioxidant properties of leaves, *R. officinalis* has been widely accepted as one of the spices with the highest antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent [1, 2, 3]. The aerial parts of *R. officinalis* grown at Hamadan in the west of Iran were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 2.08% (w/w) of pale yellow oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4 °C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Twenty-nine compounds were characterized in the essential oil of Rosmarinus officinalis, representing 98.5% of the oil, of which α- pinene (22.4%), 1,8-cineole (10.8%), verbenone (10.2%), camphor (9.9), camphene (8.4%) were found to be the major components. **Acknowledgement:** *The authors acknowledge the financial contribution from the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for supporting this research.* **References:** 1. Oluwatuyi M, Kaatz G W & Gibbons S (2004) Phytochemistry 65: 3249 – 3254. 2. Peng Y, Yuan J, Liu F, & Ye J (2005) J Pharm Biomed Anal 39: 431 – 437. 3. Ozcan M M, Chalchat J (2008) Int J Food Sci Nutr 59: 691 – 698 4. Adams R P (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL.

PE22 Analysis of the essential oil of *Thymus kotschyanus* from Iran

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The genus *Thymus* L. (Labiatae) consists of about 215 species of herbaceous perennials and subshrubs. This genus is represented in Iranian flora by 14 species, four of which are endemic. Studies indicating *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities [1, 2,3] Among them, *Thymus vulgaris* L. and *Thymus kotschyanus* Boiss. et Hohen known as two members of thyme species is used as traditional medicine among people. We report on the essential oil composition of *Thymus kotschyanus* cultivated in Hamadan. Aerial parts of dry plant were steam distilled for three hours using a Clevenger-type system. Essential oils were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis. The essential oil was isolated in yield of 0.6% (w/w) the yellowish oil. The sample was analyzed by GC and GC-MS. The components of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [3]. Thirty nine components were identified in the oil of *Thymus kotschyanus* of which linalool (24.8%), carvacrol (24.5%) and trans-caryophyllene (8.6%) were reported as the main constituents. **Acknowledgement:** *The authors are grateful to the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for its financial support.* **References:** 1. Rechinger K H (1982) Flora Iranica. Akademische Druck and Verlagsanstalt. Graz, Austria 152. 2. Mozaffarian V (1996) A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran, Iran, 547 – 548. 3. Marandi R J (2010) J Med Plants Res 4: 2424 – 2430. 4. Adams R P (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL

PE23 Determination of the chemical main composition and anti bacterial effectiveness of the volatile oils in two Syrian wide spread plants *Micromeria rubestris* L., *Mentha viridis* L. (Lamiaceae)

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Micromeria rubestris L., *Mentha viridis* L. (Lamiaceae), two widespread plants in Syria are commonly used in herbal medicine. The aim of this research was to determine the chemical composition and antibacterial activities of the volatile oils in dried leaves of these plants. Using water distillation method, the volatile oils were obtained and their main chemical composition was determined using GC/MS. To determine the effectiveness of the volatile oils against bacteria, disk diffusion methods, MIC and MBC, were used (2). The results showed that the yield of volatile oil was 1.2% in *Micromeria rupestris* (main components β-caryophyllene 49% and piperitone oxide 29%; the best antibacterial effect was against *Staphylococcus aureus*) and 1.5% in *Mentha viridis* (main components carvone 53% and limonene 21%, the best antibacterial effect against *Micrococcus luteus*).

PE24 The effects of different drying methods (natural method, oven and microwave) on drying time, essential oil content and composition of Savory (*Satureja hortensis*)

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To determine the effect of different drying methods on drying time, essential oil content and composition of Savory (*Satureja hortensis* L.), this experiment was carried out during 2008. The experimental design was completely randomized block design with three replications and treatments were: two temperatures: 50 and 70 °C, six microwave powers: 100, 180, 300, 450, 600 and 900 w and drying in shaded and sunny area. The drying process was continued until the mass of the sample reduced to a moisture content of about 0.10 on a dry basis or 10% on a wet basis. The results indicated that different treatments of drying had a significant effect on drying time and essential oil content. Minimum and maximum drying times (4.5 minutes and 96 hours respectively) obtained at 900 w microwave power and drying in shaded area. The maximum essential oil content (3%) obtained at drying by 70 °C and drying in shaded area and minimum content (0/9%) obtained at drying in sunny area. 100 and 300 w microwave powers had average content of essential oil (2/3%). Maximum carvacrol content (63.9%) obtained at 300 w microwave drying. Maximum γ-terpinene content (28.2%) obtained at drying by 70 °C that it had little difference with 50 °C, 100 and 300 w. According to the results, because of reduction of drying time and suitable essential oil content and composition in drying by low microwave powers, these methods counseled. **References:** 1. Szumny A et al. (2009) J Food Engin 97(2): 253 – 260. 2. Soysal Y (2004) J Food Engin 89(2): 167 – 173.

PE25 Chemical composition variation in essential oils of *Calamintha hispidula* (Boissier and Reuter) Maire, endemic in North-eastern Algeria

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The essential oils from three samples at the full blossom stage of wild *Calamintha hispidula* Boiss. and Reut. M. native of the mountain of Texanna, (Algeria), (one sample taken at the altitude of 625 m south facing and the other two at 526 m and 620 m north facing), have been extracted by hydrodistillation and analysed by GC-MS. The oil yileds were 1,06%, 0,59% and 1,49%, respectively. The main essential oil constituents were isomenthone (68,7%, 68,2% and 57,9%), pulegone (18,1%, 15,1% and 22,2%) and piperitone oxide (3,6%, 6,6% and 22,2%), respectively. The variation in the yields of essential oils was considerable between the two altitudes 526 m, 620 m north facing. Isomenthone was found to be the major constituent in all cases. **Acknowledgement:** *The authors are grateful to Mr Gérard de Bélair Lecturer at the University of Annaba for species identification and writing assistance, and Mr Desdous Abderrachid for the GC/MS analysis.*

PE26 Relationships among Yield and Yield Components and Essence in Cumin (*Cuminum cyminum* L.) Under Different Climatic Conditions

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An experiment was conducted to study effects of nitrogen fertilizer and plant density on quantitative relationships between yield and yield components and essential oil in cumin (*Cuminum cyminum* L.) under three different climate testing sites (cold, tropical and moderate climate locations) in western of Iran. Density (80, 120, 160 plant m^{-2}) and Nitrogen fertilizer (0, 25, 50, 100 kg ha^{-1}) were main and subsidiary factors respectively. Maximum yield (1275 kg ha^{-1}) and essential oil (2.78%) were obtained at 100 kg nitrogen ha^{-1} and 160 plant m^{-2} in moderate location. Analysis of variance for testing contrasts of density and nitrogen fertilizer showed significant linear relationship between nitrogen fertilizer and yield components. A quadratic relation was found to be significant between nitrogen fertilizer and yield, yield components as well as between nitrogen fertilizer and essential oil percent. Significantly positive correlations were also observed between yield and number of seed per plant, number of umbel per plant, number of seed per umbel, thousand seed weight and biologic yield at tropical location. To gain maximum seed and essential oil of cumin, moderate location is the recommended site. Our research was a first investigation to evaluate the variation of path analysis and correlation in cumin at different locations. Complicated relationships between seed yield and essential oil of cumin have been detected. **Keywords:** Cumin, Cuminum cyminum, path analysis, essential oil, yield components, Climate condition **References:** 1. Abdollah M (2009) Acta Horticulture 826: 301 – 308. 2. Azizi K, Kahrizi D (2008) Asian Journal of Plant Sciences 7(8): 710 – 716. 3. Hashemi P, Yarahmadi A, Azizi K, Sabouri B (2007) Chromatographia 67: 253 – 257. 4. Li XM, Tian SL, Pang Z., Shi JY, Feng ZS, Zhang YM (2009) Food Chemistry 115(3): 1114 – 1119.

PE27 The combination effects of the essential oils from *Perilla frutescens* var. *acuta* with antibiotics against antibiotic-resistant and susceptible *Vibrio* and *Salmonella* species

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Salmonella and *Vibrio* species comprise many of the common pathogens causing food-borne diseases. There has been an increased emergence of antibiotic resistant strains in recent. This is thought to have largely resulted from the consumption of processed food and agricultural products that have been in contact with antibiotics. *P. frutescens* Britton var. *acuta* Kudo is an annual herb cultivated in Korea, mainly in the southern district. It has been used in traditional medicine to disperse cold causing fever, chills, headache, nasal congestion, or cough. It is also has effects to treat nausea, vomiting and other gastrointestinal symptoms. In this study, the essential oil fraction obtained from the fresh and dried leaves of *P. frutescence* var. *acuta* by steam distillation and analyzed by GC-MS. Its main components were isolated by column chromatography. The inhibiting activities of the essential oil fraction and isolated compounds were evaluated against antibiotic-susceptible and -resistant strains of *Vibrio* and *Salmonella* species. In addition the potential of synergistic effects were accessed by combing the oils with ampicillin or trimethoprim-sulfamethoxazole (T/S). The essential oil fraction of *P. frutescence* var. *acuta* and its main component, apiol showed significant inhibitory activity against most of the tested strains of antibiotic-susceptible and -resistant bacteria. The FICs and FICI of its main component combined with ampicillin or T/S depicted on the basis of results of checkerboard microtiter test indicated synergism or additive effects. **Acknowledgement:** *This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (NRF-531 – 2008 – 1-E00106).* **References:** Shin S (2005) Arch Pharm Res 28: 765 – 769.

PE28 Investigation on Development of Zein Antimicrobial Edible Film and Essential oil of Zataria multiflora Boiss. on Salmonella enteritidis, Listeria monocytogenes, Escherichia coli and Staphylococcus aureus

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Active packaging is a type of packaging that can control or react to things arranged inside (2). An antimicrobial Active Packaging is made by incorporating antimicrobial agents in food packages. *Zataria multiflora* Boiss. is a plant that belongs to the Lamiaceae family and grows

only in Iran, Pakistan and Afghanistan (1). The antimicrobial activities of the plant are also well established against a wide variety of bacteria (3). In this study zein is based on the films that contain essential oil of *Zataria multiflora* (0, %1, %2, %3 and %4 w/v) and glycerol (%30 v/v). The objective of this study was to determine the effectiveness of the edible film in inhibiting *Salmonella enteritidis*, *Listeria monocytogenes, Escherichia coli* 0157: H7 and *staphylococcus aureus*. The bacterial strains were transferred onto the surface of nutrient agar plates using sterile swabs, and then discs of zein films containing different percent of the essential oil of the *zataria multiflira* put onto the surface of the plates. After that incubated overnight at 37 °C. Finally after 24 hours the inhibitory zones was measured with calliper. Overall, there was a decline in the test organisms that was generally increased in the presence of the Zataria multiflora Boiss' essential oil. The mentioned bacteria showed significant reductions in bacterial survival in the higher extract concentration. To conclude zein antimicrobial edible film and *Zataria multiflora* essential oil might help to decrease the risk of food borne illness related to these microorganisms. **References:** 1. Ali MS et al (2000) Phytochemistry 55: 933 – 936 2. Del Nobile MA et al. (2009) Journal of Dairy Research 10: 1 – 6 3. Palmer A S et al (2001) Food Microbiology 18: 463 – 470

PE29 Effect of different solvents for seed oil extraction of *Melia azedarach* L. (Meliaceae) on insecticidal activity of this plant extract

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Developing of new pesticides from botanical pesticides has been attempted in the past [1]. Botanical pesticides are safe to use in different propose and IPM program [2]. *Melia azedarach* L. (Meliaceae) is characterized by containing allelochemicals compounds, with a high level of bioactivity against insect. In this study *M. azedarach* seeds were collected from Kerman, Iran. The seeds were milled and powdered mechanically using commercial electrical stainless steel blender to extract their oil. The experiments were directed to determine the effect of different solvents on insecticidal activity of seed oil of *M. azedarach* on 3 – 4-day-old individuals of *Aphis fabae* Scopoli. The oil was extracted with different solvents. The solvents were included acetic acid, acetone and n-Hexane. All experiments were conducted by topical test bioassay in laboratory, at 25 ± 1 °C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. Water and DMSO (Dimethyl sulfoxide) were used as control treatments. The results indicated that in the concentration 80 µl/ml, the mortality of *A. fabae* treated with n-Hexanic seed extract of *M. azedarach* after 24 hours, was more than 72%, while it was 40.75% and 60% in *A. fabae* treated with acetic acid and acetonic seed extract of *M. azedarach* respectively. The mortality of *A. fabae* treated with n-Hexanic seed extract of *M. azedarach* was significantly higher than acetic acid seed extract of *M. azedarach*. It could be concluded that some plant extracts may be applicable as a safe insecticide to aphid's control. **References:** 1. Isman M (2005) Biopesticides of Plant Origin. Lavoisier Tech & Doc. Paris. 2. Raja N et al. (2001) J Stored Prod Res 37: 127 – 132.

PE30 Chemical composition, Antioxidant and Mosquito larvicidal activities of essential oils from *Tagetes filifolia*, *Tagetes minuta* and *Tagetes elliptica* from Perú

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The chemical compositions of the essential oils from the aerial parts of *Tagetes filifolia* Lag. ("Anís serrano"), *Tagetes elliptica* Sm. ("Chincho") and *Tagetes minuta* L. ("Huacatay") from Perú were determined by gas chromatography-mass spectrometry. The main constituents of the essential oils were: *trans*-anethole (88.2%) and methyl chavicol (10.9%) for *T. filifolia*; *trans*-ocimenone (51.7%), *cis*-tagetone (17.7%) and *cis*-ocimenone (7.7%) for *T. minuta*; and 6,7-epoxy myrcene (31.9%), dihydrotagetone (22.0%), *trans*-ocimenone (12.0%) and *cis*-tagetone (8.0%) for *T. elliptica*. Antioxidant activities of the essential oils were determined by the DPPH test. The essential oil of *T. minuta* exhibited the highest antioxidant *in vitro* (EC_{50}= 0.8 mg/ml), compared to *T. elliptica* (EC_{50}= 3.4 mg/ml) and *T. filifolia* (EC_{50}= 20.2 mg/ml). Mosquito larvicidal tests were runned against third instar larvae of *Aedes aegypti*, considered an important vector of dengue and yellow fever. Essential oil from *T. filifolia* showed the strongest larvicidal activity (LC_{50}= 47.7 ppm), compared to *T. minuta* (LC_{50}= 52.3 ppm), and *T. elliptica* (LC_{50}= 111.0 ppm). The essential oil of *Tagetes filifolia* might be considered as a natural alternative to chemical larvicides for the control of *Aedes aegypti*. **Acknowledgement:** *Fondo Concursable para Estímulo a la Investigación de los Estudiantes de la UPCH, 2010* **References:** 1. Adams RP (2009) Identification of Essential Oils by Gas Chromatography Mass Spectrometry. Allured Bussiness Media. Illinois, USA. 2. Li C et al. (2009) Food Chem 115: 801 – 805. 3. Dharmagadda V et al. (2005) Biores Technol 96: 1235 – 1240.

PE31 Chemical composition of the oil of *Cicuta virosa* L. from Kazakhstan

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The present work is a part of our ongoing research into the volatiles of Apiaceae species. A well-known toxic plant, water hemlock, *Cicuta virosa* L. is widely distributed in the temperate regions. Herbal parts of *C. virosa* were collected in Karkaraly Mountain (Kazakhstan) during blossoming in 2009. The essential oil (yield 0.1%) was obtained by hydrodistillation using a Clevenger type apparatus. Chemical composition of the oil was analyzed by GC-FID and GC/MS methods. The oil was found to be rich in sesquiterpenic compounds with (*Z*)-β-farnesene (22.7%), α-humulene (5.4%), humulene epoxide II (5.9%), caryophyllene oxide (3.4%), germacrene D (3.2%) and (*Z,E*)-α-farnesene (3.6%) as major constituents. Among the monoterpenes myrcene (7.8%) was detected in high percentage. Fatty acids were represented mostly by hexadecanoic acid (8.4%). To the best of our knowledge, the essential oil of *C. virosa* from Kazakhstan was not investigated previously.

PE32 Sulphur containing volatiles of *Barbarea vulgaris* W.T. Aiton from Kazakhstan

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Aim of the present study was the investigation of the chemical composition of *Barbarea vulgaris* W.T.Aiton volatiles. The plant material was collected in Akmolinskaya and Karagandinskaya provinces of Kazakhstan in 2009. Different plant parts (flower, leaf and herb) were subjected to microdistillation to obtain the volatiles. The volatiles were then analyzed by GC-FID and GC/MS methods. The most of samples were rich in sulfur containing compounds. Methyl (methylthio) methyl disulfide (14.5%), dimethyl trisulfide (11.2%), dimethyl sulfide (3.4%) were detected in the herb of *B. vulgaris* from Akmolinskaya province. Chemical composition of the flower and leaf volatiles was found to show some differences. Isopropyl isothiocyanate (36.7%) was the main constituent in flower, while phytol (25.7%), hexadecanoic acid (9.3%), hexahydrofarnesyl acetone (7.4%), dodecanoic acid (5.5%) and isopropyl isothiocyanate (5.3%) were detected in the leaf. It should be noted that another sample of *B. vulgaris* from Akmolinskaya province was found to be very different in volatile composition. Namely, borneol (20.3%), camphene (13.5%), bornyl acetate (8.0%) and germacrene D (5.4%) were the major constituents. In the flower and leaf of *B. vulgaris* from Karagandinskaya province, methallyl cyanide (50.5% and 12.9%), 3-butenyl isothiocyanate (15.6% and 43.8%) and isohexyl cyanide (4.2% and 0.9%) were the major

volatile compounds. To the best of our knowledge, the volatiles of *B. vulgaris* from Kazakhstan were not investigated previously.

PE33 Essential oil composition of different populations of *Thymus caramanicus* Jalas growing wild in Iran

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Thymus species are well known as medicinal plants because of their biological and pharmacological properties. *Thymus caramanicus* Jalas is an endemic species of Iran. Essential oils of the air-dried aerial parts of seven populations of *Thymus caramanicus* collected from different locations of Iran were obtained by hydrodistillation with a yield of 0.41 – 2.9% (w/w). The essential oils were analyzed by combination of GC and GC-MS techniques. Oxygenated monoterpenes were the main group of constituents in all samples. carvacrol (24.9 – 97.6%), Thymol (25.6 – 36.9%), p-cymene (2.9 – 16.1%), and γ-terpinene (1.3 – 8.1%), represented as the major compounds.

PE34 Antioxidant activity of the essential oils of five species of the family Lamiaceae

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Species in the family *Lamiaceae* are praised medicinal and aromatic plants. They are used against various inflammations, stomach problems, as expectorant, as well as spices [1]. The aim of the present study was to evaluate the antioxidant activity of essential oils from wild growing herbs in South and South-East in Serbia: *Hyssopus officinalis* L., *Origanum vulgare* L., *Satureja kitaibelii* Wierzb. ap. Heuff., *Nepeta nuda* L., and *Thymus serpyllum* L. The essential oils were isolated by hydrodistillation in a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate, filtered and stored at +4 °C in a well-filled, airtight container, protected from light, until the analysis. Two antioxidant assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] and FRAP (Ferric Reducing Antioxidant Power) assay [3] were used to evaluate vitamin C antioxidant activities, were used as standard. Essential oil from *Thymus serpyllum* L. exhibited the highest antioxidant activity (EC_{50}= 0,69 ± 0,14 μl/ml), while the lowest activity was determined for *Hyssopus officinalis L.* oil (EC_{50}= 47,50 ± 11,62 μl/ml). Compared with vitamin C (EC_{50}= 0,04 ± 0,05 μg/ml) all essential oils were of lower DPPH antioxidant activity. In the FRAP assay, the reducing power decreased in the following order: *Thymus serpyllum* L.>L-ascorbic acid>*Origanum vulgare* L.>*Satureja kitaibelii* L.>*Nepeta nuda* L.>*Hyssopus officinalis* L. Our results confirm that the traditional use of medicinal and aromatic plants in mitigating oxidative stress is an initiator of many diseases. **Acknowledgement:** *This work was supported by the Ministry of Science and Technological Development, Republic of Serbia projects OI 172047.* **References:** 1. Anon. (2004) PDR for Herbal Medicines, 3 rd Ed., Thomson PDR at Montvale, USA. 2. Choi CW et al. (2002) Plant Sci 163 (6): 1161 – 1168. 3. Benzie IFF et al. (1996) Anal Biochem 239: 70 – 76.

PE35 Essential oil composition of *Ducrosia assadii* aerial parts and fruits from Iran

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In Iran the *Ducrosia* genus (Apiaceae) is represented by three species: *D. assadii* Alava. *D. flabellifolia* Boiss. and *D. anethifolia* (DC.) Boiss. of which the first two are endemic plants (1,2). There are few reports on analysis of the essential oils from *Ducrosia* species. The fresh aerial parts and fruits of *Ducrosia assadii* were collected in May and September 2007 from Dehbakry of Kerman Province respectively and Voucher Specimens were deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI) in Tehran, Iran. Hydrodistilled essential oils from the aerial parts and fruits of this plant were analyzed by GC and GC/MS, yielded 0.2% and 1.1% (v/w) respectively. Twenty four 82.2% components of the aerial parts and Thirty five 97.9% compounds of the fruits oils were identified. The main constituents of the oil of aerial parts on HP5-MS column were α-pinene (14.5%), decanal (10.1%), decanoic acid (10.4%), β-myrcene (6.1%), benzyl acetate (4.5%), dodecanal (4.1%), E,E-α-farnesene (3.5%) and limonene (2.57%). The major components of fruits oil on DB1 column were verbenol + myrtenal (71.0%), allo-ocimene + delta -3-carene (4.0%), crysanthenyl acetate (3.3%), β-caryophyllene (3.0%), trans-pinocarvyl acetate (2.8%) and β-pinene (2.2%). The amounts of monoterpens of aerial parts and fruits were 29.9% and 87.7% respectively. Fruits oil was rich of oxygenated monoterpenes (78.6%). Sesquiterpenes were minor compounds in the oils of this plant. The oil of the aerial parts comprised 48.3% of non-terpenoids constituting dominant portion of the oil. **References:** 1-Rechinger KH (1987) Ducrosia, In: Flora Iranica, Umbelliferae. No.162. Edits., K.H. Rechinger and I.C. Hedge, Akademische Druck and Verlagsanstalt, Graz, Austria, pp. 471. 2-Mozaffariyan V (1996) A Dictionary of Plant Names. Farhang Moaser Publishers. Tehran.

PE36 Whitening effect and antioxidant activity of essential oils from *Cryptomeria japonica*

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The aim of this study was to investigate whitening effect and anti-oxidation effect by determining the tyrosinase inhibition activity, DPPH radical scavenging activity and superoxide dismutase (SOD) like activity of essential oil from *Cryptomeria japonica* D.Don. Essential oil of *C. japonica* was extracted by steam distillation of Clevenger-type. Essential oil of *C. japonica* was divided into crude oil and 6 fractions by column chromatography and thin layer chromatography. Crude oil and 6 fractions of *C. japonica* essential oil inhibited the oxidation of L-tyrosine and L-dihydroxyphenylalanine (L-DOPA) by mushroom tyrosinase. In tyrosinase inhibitory activity of essential oils of *C. japonica*, the activity was effective at the fraction A (L-tyrosine: 86.76%, L-DOPA: 88.45%) and B (L-tyrosine: 87.53%, L-DOPA: 85.03%). In examination of anti-oxidation activity, the *C. japonica* essential oils were determined using the free radical and the stable reductant, 1,1-diphenyl-2-picrydrazyl (DPPH) and pyrogallol. Fraction B (44.11%), C (86.91%) and D (44. 40%) were highly effective of DPPH radical scavenging, and in examination of SOD like activity, fraction B (96.19%) was appeared as extremely high effective. Fraction B of *C. japonica* essential oil, effective fraction of whitening activity and anti-oxidation activity, was mainly consisted of bornyl acetate and nezukol, which are terpenoids having hydroxyl group. These compounds were inhibition of acting on tyrosinase in melanin biosynthesis and block up DPPH radical scavenging and auti-oxidation by supplying hydrogen. Thus, they can apply for cosmeceutical material as functional raw material containing highly active compounds for whitening and anti-oxidation. **References:** 1. Blois MS (1958) Nature 181: 1199 – 1200. 2. Brand-Williams et al. (1995) LWT-Food Sci Technol 28(1): 25 – 30. 3. Kulisic T et al. (2004) Food Chem 85(4): 633 – 640. 4. Marklund S et al. (1974) European J Biochem 47(3): 469 – 474. 5. Yagi A et al. (1986) Planta Med 52: 517 – 519.

PE37 Compositions of the Essential Oils of *Salvia fruticosa* Mill. Populations in the Flora of Marmara Region

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Salvia is the largest genus of the family Lamiaceae with *ca.* 900 species distributed around the world. Its centre of origin is considered to be south west and central Asia (1). *Salvia* is represented in Turkey by 97 species including 4 subspecies and 8 varieties. The rate of endemism in Turkey is 52.5% with 51 species (2). *Salvia fruticosa* Mill. has a wide distribution in Turkey and its dried leaves are sold in local markets for consumption as herbal tea and dried leaves are exported especially to European Countries. We have distilled essential oils from samples collected from 20 localities by water distillation and analyzed them by GC and GC/MS techniques. Oil yields in the samples varied between 2.0% to 3.0% and the main components were characterized as 1,8-cineole (20.7% to 46.9), β-caryophyllene (6.0% to 16.9) β-pinene (5.3% to 11.3), and camphor (2.8% to 17.5). **References:** 1. Hedge I C (1992) A Global Survey of the Biogeography of the Labiatae. In R. M. Harley and T. Reynolds (eds.), Advances in Labiatae Science, Royal Botanic Gardens, Kew, pp. 7 – 17. 2. Ipek A, Gurbuz B (2010) Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 19 (1 – 2): 30 – 35.

PE38 Variation in the essential oil composition of the fruits of *Vitex agnus-castus*

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Vitex agnus-castus L. (Lamiaceae) is widely distributed in Turkey, mainly in coastal areas of the West and the South West. The fruits are used in folk medicine to treat illnesses and the essential oil obtained from the fruits is known is used as a substitute for kekik oil in Turkey (1). Essential oil compositions of the fruits of *Vitex agnus-castus* collected from 5 different regions, Balıkesir (Altınoluk) 1, Muğla (Bodrum) 2, Antalya (Manavgat) 3, Edirne (Enez) 4 and Zonguldak (Devrek) 5 have been analyzed by GC and GC-MS to document the variability in their composition. The results indicate chemovariability in oils of the fruits sourced from different sites. There are also marked differences in contents of the major constituents. Sample 2 and 3 contains 1,8-cineole [17% (1), 13.2% (2)], sabinene [12.8% (2), 12.1% (3)], β-caryophyllene [12.7% (2), 11.4% (3)] and bicyclogermacrene [11.0% (2), 12.1% (3)] as major constituents respectively. Sabinene (15.4%), 1,8-cineole (17.3%) and bicyclogermacrene (12.1%) are the major constituents together with (Z)-β-farnesene (13.5%) in the sample 1. The highest content of bicyclogermacrene (22.1%) is shown in sample 4. Sample 5 is the only one which contains α-pinene (10%) as the main constituent. **References:** Reference: 1) Baytop T (1999) Türkiye'de Bitkiler ile tedavi (Therapy with plants in Turkey), 2nd ed. Nobel Tıp Kitabevleri Ltd. İstanbul

PE39 Composition of the essential oils of *Centaurea aphrodisea, C. polyclada, C. athoa, C. hyalolepis and C. iberica*

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The genus *Centaurea* L. (Asteraceae) is represented in Turkey with 192 taxa, 114 of which are endemic (1 – 4). The present study aims at investigating the volatile compounds of two endemic *Centaurea* species (*C. aphrodisea* Boiss. and *C. polyclada* DC.) and three widespread (*C. athoa* DC., *C. hyalolepis* Boiss. and *C. iberica* Trev. ex Sprengel) *Centaurea* taxa from Turkey by GC and GC/MS. The analyses revealed 109 constituents, accounting between 87% and 93.4% of the oils. Major constituents of the oils of *Centaurea* were as follows; in *C. aphrodisea*: spathulenol (8.1%) and hexahydrofarnesyl acetone (7.8%); in *C. athoa*: caryophyllene oxide (17.1%) and heptacosane (8.1%); in *C. iberica*; hexadecanoic acid (27.9%) and cyclosativene (13%); in *Centaurea hyalolepis*: hexadecanoic acid (8.2%) and phytol (5.6%) and in *C. polyclada*: hexadecanoic acid (8.1%) and hexahydrofarnesyl acetone (7.1%). **References:** 1. Wagenitz G (1975) Centaurea L. (Asteraceae). In: Flora of Turkey and the East Aegean Islands, vol. 5. Edit., P.H. Davis, Edinburgh University Press, Edinburgh, UK. 2. Güner A, et al. (2000) Flora of Turkey and the East Aegean Islands, vol.11, Edinburgh University Press, Edinburgh, UK 3. Duran A, Duman H (2002) Ann Bot Fennici 39: 43 – 48 4. Turkoglu I et al. (2003) Bot J Linn Soc, 143:207 – 212

PE40 *Phoenix dactylifera* L. essential oil: Chemical composition, antimicrobial and insecticidal activities

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Date palm, *Phoenix dactylifera* L. (Arecaceae), is very common in the Arabian Peninsula. The essential oil of *P. dactylifera* from the spathe was obtained by hydrodistillation. The oil was subsequently analysed by GC and GC-MS, simultaneously. Overall, 16 components were characterized representing 99% of the oil. 3,4-Dimethoxytoluene (73.5%) and 2,4-dimethoxytoluene (9.5%) were found as the main components. First the antimicrobial activity of the essential oil was determined using the broth microdilution method against various human pathogens, where a low inhibitory range was observed (MIC 1000 µg/ml). The oil was evaluated further for antifungal activity against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography assay. As a result the essential oil showed no antifungal activity at 80 and 160 µg/spot concentrations compared to standard antifungal agents. In addition, the oil was subsequently investigated for its insecticidal properties against *Aedes aegypti.* The oil showed repellent activity against the yellow fever mosquito *A. aegypti* using the "cloth patch assay" with 0.051 mg/cm2, however, the oil exhibited weak activity against the mosquito's first instar larvae in a high throughput bioassay and adult topical assay. As a conclusion, the *Phoenix* essential oil and other fractions may have potential against parasites for further evaluation.

PE41 Structural and developmental studies on secretory epidermis in *Rosa damascena* Mill. petals

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Rosa damascena Mill. is one of the most important *Rosa* species, for its wide application in perfumery and cosmetics, and as a valuable natural drug possessing diverse effects (1). Therapeutic activities of *R.damascena* oil and water have been reported both in traditional Iranian medicine and modern pharmacological studies (2). The essential oil is secreted by epidermal cells in petals of *Rosa* species, but no histological data is available on the mode of secretion in this genus. The present research has focused on the secretory structure in *R.damascena* petals in relation to flower development. Flowers were collected at four successive ontogenic stages from Shiraz, south western Iran. Petals were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in resin. Semithin sections were stained with toluidine blue. The two petal surfaces were distinguishable from the stage with the emerging intensely colored petals. They differed by the shape and content of the epidermal cells. The upper epidermal cells revealed a very dense cytoplasm, and numerous small vacuoles in half open flowers. Their polyphenolic content diminished during flower development. At full bloom, the vacuolar polyphenolic compounds had totally disappeared and only a few cells had a dense cytoplasm. Structural features of the epidermal cells suggest the petals of the half open flowers to be in the most active secretory phase. Further analytical studies will correlate these histological data with the essential oil secretion process during *R.damascena* flower development. **References:** 1. Libster (2002) Delmar's Integrative Herb Guide for Nurses. Delmar's Thamson Learning, Albany. 2. Rakhshandeh et al. (2007) Iranian Journal of Pharmaceutical Research 6: 193 – 197.

PE42 Quality profile of Chios mastic (mastiha) essential oil

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Mastic oil is the essential oil of mastic (mastiha), the resin of *Pistacia lentiscus* L. var. *chia* Duham (Anacardiaceae), uniquely produced in the Greek island of Chios. It is a valuable product with a small annual yield (~300 kg), as well as mastic itself. Its characteristic odor and its established beneficial activities [1,2,3,4] have created an increased demand in cosmetics industry, leading to a high purchasing price (>€ 2,500/kg) and an adulteration danger. Therefore, the determination of mastic oil quality profile, considering the factors that potentially contribute to it (resin origin, storage time), could be used to afford an unambiguously labeled product. For this purpose, several mastic oil samples of different local origin, as well as different resin and oil storage time were analyzed with chiral GC-MS in order to set content limits for its compounds. Locality has little influence in the proportions, while mastic storage time before distillation clearly affects them, as the differences in the major compounds' contents show (α-pinene increases from 69.7% for samples distilled 2 months after harvest to 78.9% after 9 months, and myrcene decreases from 19.5% to 11.1%, respectively). The effect of mastic oil storage time was smaller (differences of < 3% within 1 year), but the analysis of a 40-year-old sample revealed a dramatic drop in myrcene content, appointing it as the most characteristic criterion of mastic oil aging. Content limits for all mastic oil compounds were determined, within which a mastic oil sample should lay in order to be characterized as authentic and non-aged. **References:** 1. Magiatis P et al. (1999) Planta Med 65: 749 – 751. 2. Paraschos S et al. (2007) Antimicrob Agents Chemother 51: 551 – 559. 3. Heo C et al. (2006) Korean J Med 71: 354 – 361. 4. Loutrari H et al. (2006) Nutr Cancer 55: 86 – 93.

PE43 Structural changes in secretory canals associated with fruit development in Ajowan (*Trachyspermum ammi* Sprague)

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Ajowan (*Trachyspermum ammi* Sprague) fruits (*Apiaceae*) accumulate essential oil in compartments referred to as canals (vittae). *T.ammi* is one of the most important herbs used in Ayurveda (1). Iranian traditional herbal medicine recommends the use of Ajowan fruits also as digestive stimulant. A high proportion of thymol has been selectively demonstrated by Magnetic Resonance Imaging in the essential oil of mature Ajowan fruits (2). It has also been reported that different harvest stages affect the number of oil components in Ajowan fruits (3). The present research has focused on the relationship between the secretion process and reproductive development in *T.ammi*. Flowers and fruits were collected from wild growing plants at 6 ontogenic stages. Samples were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in resin. Semithin sections were stained with toluidine blue. Six canals were distinguishable in the ovary wall of the partly open flowers. A single layer of orderly arranged cells reperesented the schizogenous cavity. The cytoplasm of these cells increased in density, and reached the highest level in flowers at full bloom. The immature fruit exhibited the features of the most active secretory phase, i.e., higher amount of the secreted material lining the cavity and maximum width of the canal. The cells underwent post-secretory phase throughout the fruit maturation. They were compressed, with a degenerative protoplasm in dry mature pericarp. Results of the present histological study confirm previous analytical data (3) and recommend the use of immature stage for the best efficiency of Ajowan. **References:** 1. Pathak et al. (2010) Journal of pharmacy Research 3(4): 895 – 899. 2. Gersbach & Reddy (2002) Annals of Botany 90: 253 – 257. 3. Saharkhiz et al. (2005) Journal of Essential Oil Bearing Plants 3:300 – 303.

PE44 Trichomes in *Echium amoenum* Fisch & Mey petals: A micromorphological survey

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Echium amoenum Fisch & Mey (Boraginaceae) grows widely in the northern highlands of Iran. Dried petals of *E. amoenum* have long been used for their anxiolytic, sedative, anti-inflammatory and analgesic effects in Iranian folk medicine (1). Phytochemical studies revealed a variety of substances of which rosmarinic acid and flavonoids showed antioxidant activity in humans (2). Inhibition of humoral antibody synthesis has also been reported (3). All the published studies on *E. amoenum* have concentrated on the therapeutic uses and/or the phytochemical analyses. No botanical data is available and the secretory structure has not been reported so far. This study was carried out to provide elements on the morphology and localization of the secretory structure, with regard to floral development. *E. amoenum* flowers were collected from Ghazvin, at four developmental stages. Petals were double fixed in glutaraldehyde and osmium tetroxide, dehydrated in acetone, air dried in hexamethyldisilazan, coated with gold and viewed under the scanning electron microscope. The youngest floral buds were densely covered with protective non glandular trichomes of different length. The same trichomes covered the outer epidermis of the young petals emerging from the sepals. Short stalked capitate trichomes, with one globular secretory head were observed between the protective hairs. During development, the number of non glandular trichomes decreased, but capitate trichomes increased in number, reaching their maximum in 3.5 cm long petals at full bloom. Further histochemical studies will elucidate the variable nature of the secreted material, as well as the phases of the secretion process. **References:** 1. Zargari A (1996) Medicinal Plants, vol. 3. Tehran University Publications. 2. Ranjbar A et al. (2006) eCAM 3(4): 469 – 473. 3. Amirghofran Z et al. (2000) Iranian Journal of Medical Sciences 2(3 & 4): 119 – 124.

PE45 Composition and antioxidant activities of the essential oil of *Murraya paniculata* leaves growing wild in Cubans mountain

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Murraya paniculata (Linn.) Jack, syn. *M. exotica* Linn, known as orange jessamine, belongs to the family Rutaceae and is commonly grown in gardens is commonly grown in gardens for its glossy green foliage and large clusters of fragrant flowers (1). This plant has been used in ethnomedicine. Infusion of the leaves and flowers of *M. exotica* is tonic and stomachic. It is said to be aromatic, refrigerant, digestive, and beneficial in rheumatic fever, coughs, giddiness, hysteria, thirst, and burning of the skin (2,3). The essential oil was obtained by hydrodistillation, were analyzed by gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was evaluated using several *in vitro* studies. The results showed that the essential oils tested differed in their chemical compositions although there is coincidence in the most abundant constituents. The analysis of *Murraya paniculata* volatile oil showed the presence of eighteen compounds identified, accounting for 95.1% of the total amount. The major component of both oils was found the Caryophyllene (30%), with the other components in lesser amounts. The antioxidant activity has shown good activity for the inhibition of primary and secondary oxidation products in crude *Cucurbita* oil added at the concentration of 0.02% which were evaluated using peroxide, thiobarbituric acid, p-anisidine values. Moreover, it was further supported by complementary antioxidant assay in linoleic acid system, comparable with synthetic and natural origin antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), carvacrol and timol). **References:** 1. Roig JT (1974) Plantas medicinales, aromáticas o venenosas de Cuba. Ed. Científico-Técnica. La Habana. 2. Jiangsu New Medical College (1977) Dictionary of Chinese Herbal Medicine; Shanghai Science & Technology Press: Shanghai, China. 3. Pery LM (1980).Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; MIT Press: Cambridge

PE46 Chemical composition of essential oil from *Ocimum selloi* Benth. (Lamiaceae) collected in Paty do Alferes (Brazil)

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Brazil presents the highest floristic genetic diversity of the world. Among the native plants of Brazil is *Ocimum selloi* Benth., an herbaceous annual plant of Lamiaceae family. This medicinal specie has been used as anti-diarrheic, antispasmodic and anti-inflammatory and these properties have been observed in pre-clinical tests. Leaves of *Ocimum selloi* were collected in a private property in Paty do Alferes district (Rio de Janeiro State) in 11/2010, and dried at room temperature (28 °C +- 2) at shade conditions. Essential oil was obtained by hydrodistillation (Clevenger-type apparatus) for 4 h and analyzed by GC-MS (Shimadzu, QP 5050-DB-5 capillary column – 30 m x 0.25 mm x 0.25 μm). Carrier gas was Helium (1.7mL/min); split ratio: 1:30. Temperature program: 50 °C, rising to 180 °C at 5 °C/min, 180 °C, rising to 280 °C at 10 °C/min. Injector temperature: 240 °C and detector temperature: 230 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known compounds reported in the literature. In the essential oil were found 16 chemical compounds. The major compound characterized was methyl-chavicol (85.3%), followed by trans-caryophyllene (1.8%), germacrene-D (2.9%), bicyclogermacrene (3.3%), germacrene B (0.5%) and spathulenol (0.6%). Anethole was not observed in this essential oil. This results showed that this quemotype is similar to the one observed by Martins [1], founded in Viçosa- Minas Gerais State (Brazil) **References:** [1] Martins ER (1998) in Ming et al. Plantas Medicinais, Aromáticas e condimentares: avanços na pesquisa agronômica. UNESP. Botucatu. p. 97 – 126

PE47 Antimicrobial activity of the essential oils and non-polar extracts from leaves and flowers of *Tithonia diversifolia* against cariogenic bacteria

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Bacteria of the *Streptococcus* and *Lactobacillus* genera, such as *S. salivarius, S. mutans, S. mitis, S. sobrinus, S. sanguinis, S. salivarus* and *L. casei* are the main microorganisms responsible for human dental caries. As part of our ongoing project on the prospection of natural products with anticariogenic potential, we have investigated in this work the antibacterial activity of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), which is used in the Brazilian medicine folk as anti-inflammatory [1]. The minimum inhibition concentration (MIC) values of the essential oils of flowers (EOf) and leaves (EOl), and their respective n-hexane extracts were determined by using the broth microdilution method. The n-hexane extracts of leaves and flowers, and the EOl were inactive against the panel of selected bacteria, having displayed MIC values between 1000 to 2000 μg/mL, whereas the EOf exhibited moderate activity (MIC = 250 μg/mL) against *S. mitis, S. sanguinis, S. sobrinus* and *L. casei*. The lowest MIC value was obtained for the EOl against S. mutans (MIC = 125 μg/mL). β-caryophyllene (11.04%) was found to be the major constituent in the EOl, however it has been reported to exhibit weak activity (MIC = 500 μg/mL). Further studies to verify the occurrence of possible synergistic effects between β-caryophyllene and other minor constituents in the EOl are in progress. **Acknowledgement:** *FAPESP (Proc. 2009/09491 – 1), CAPES, CNPq.* **References:** [1] Owoyele VB et al. (2004) J Ethnopharmacol 90: 317 – 321.

PE48 Antibacterial effects of the essential oil from of *Rosmarinus officinalis* against some pathogenic antibiotic-resistant *Staphylococcus aureus* strains

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Rosmarinus officinalis L. (Labiatae) is an evergreen shrub native in Mediterranean region which has been used as condiments mainly with meat dishes. It has been cultivated in many herbal gardens also in Korea and used for herbal remedies and etc. To develop the new natural antibiotics from the plant resources against antibiotic-resistant *Staphylococcus aureus*, the antibacterial activities of the essential oil from *R. officinalis* and its isolated main components were accessed by microbroth dilution method. In addition, synergistic effects of the essential oil with antibiotics were investigated for enhancing the relatively weak activity of the oil compounds compared to current antimicrobial drugs. As the results of the experiments, the *Rosmarinus* oil showed significant inhibiting activities against most of the tested strains with minimum inhibiting concentrations (MICs) between 0.5 – 16.0 mg/ml. Potential synergism was identified when antibiotics were combined with the oil. The activity of erythromycin, norfloxacin, or oxacillin, against antibiotic-susceptible and also against -resistant strains of *S. aureus* was enhanced significantly by combination with *Rosmarinus* oil and its main component, 1,8-cineole with fractional inhibition concentration indices (FICIs) between 0.26 and 1.00. In conclusion, the combination of *R. officinalis* essential oil or 1,8-cineole with antibiotics could be used to reduce the effective dose of antibiotic and to modulate the resistance of *S. aureus* strains. **Acknowledgement:** *This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (2010 – 07 – 1-M020 – 0006).* **References:** 1) Shin S (2005) Kor J Pharmacog 36: 252 – 256 2) Shin S (2010) Yakhak Hoeji 54: 122 – 125

PE49 Chemical composition and antibacterial activity of the essential oil of *Achillea filipendulina* (Asteraceae)

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There are 19 *Achillea* species available in the Iranian flora and 7 of them are endemic. Various part of different species of the genus *Achillea* are widely used in folk medicine due to numerous pharmacological properties, such as antimicrobial, antiinflammatory, antiallergic and antioxidant activities. The antimicrobial activities of the essential oils and various extracts from several *Achillea* species have been reported before. As far as our literature survey confirms, the antimicrobial activities of leaves and flowers essential oil of *Achillea filipendulina* Lam. (Asteraceae) have not been studied before. Although there was a report concerning the chemical analysis of flowers essential oil, the chemical composition of leaves oil has not been investigated previously. The chemical composition and antibacterial activity of the essential oils of the leaves and flowers of *Achillea filipendulia* were investigated. Essential oils were isolated by hydrodistillation and analyzed by GC and GC -MS. Overall, 95.3% and 92% of the constituents were characterized for the leaf and flower oils. The main components of the leaves and flowers were: 1, 8-cineole (17.2 – 19.0%) and chrysanthenyl acetate (18.5 – 19.3%), respectively. The antibacterial activity of the essential oils against seven gram positive and gram negative bacteria were investigated and high antibacterial activity was observed. **References:** 1. Simon JE, Chadwick AF and Craker LE (1984) Herbs: An Indexed Bibliography 1971 – 1980. Elsevier, Amsterdam. 2. Konemann (1999) Botanica. Gordon Cheers Publication, Hong Kong, p. 1020. 3. Esmaeili A et al. (2006) Flavour Fragr J 21: 253 – 256. 4. Mishurova SS, Abbasov RM, Malinovskaya, TA and Mamedalieva FM (1985) Rastit Resur.21: 69 – 73.

PE50 Antimicrobial Activities in Cultivated *Origanum vulgare* subsp. *hirtum* Populations of Different Origin

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Origanum L. genus belongs to Lamiaceae (Labiatae) family. It has antimicrobial activities on high rates. Especially *Origanum vulgare* L. has very efficient antimicrobial activities because of the high essential oil with its main components; thymol and carvacrol. *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, main components of the essential oil are carvacrol, thymol, γ-terpinene and p-cymene. This study was conducted in order to determine antimicrobial activities in cultivated *Origanum vulgare* subsp. *hirtum* populations of different origin, and to correlate harvest times with antimicrobial activity. Species of bacteria tested were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 29212, *Bacillus cereus* CCM 99, *Salmonella typhimurium* CCM 5445, *Pseudomonas aeruginosa* ATCC 27853. Species of fungi tested were *Candida albicans* ATCC 10231, *C. tropicalis* RSSK 665, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 2219, *C. dubliniensis* CD 36, *Aspergillus fumigatus* NRRL 2999. CLSI (The Clinical Laboratory Standards Institute') broth microdilution method was used for the determination of MIC (Minimum inhibition concentration). MIC was determined according to the CLSI M27-A2 for *Candida* species, CLSI M38-A microdilution for *Aspergillus fumigatus* and CLSI M2-A7 microdilution for bacteria.

PE51 Effect of potassium nutrition on essential oil of *Calendula officinalis* L. flowers

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Effects of potassium (K) by rates of 0.00 (control), 115.5, 173.2, 231.00, 288.7 and 346.4 kg ha $^{-1}$ on essential oil (EO) extracted from marigold (*Calendula officinalis* L.) flower heads were investigated by GC and GC/MS. The highest accumulation of EO (0.29% &0.095 g plant^{-1}) was recorded at the treatment of 173.2 kg ha^{-1} compared with control treatment (0.13% &0.015 g plant-1). 28 constituents were identified in the EO. The main constituents (α-cadinol, Δ-and γ -cadinene) increased with K level increased. The highest amount of main constituents [α-cadinol (33.11%), Δ-cadinene (18.41%), and γ -cadinene (9.99%)] produced from the 346.4 kg ha^{-1} treatment compared with other and control treatments. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of Calendula EO. α-Cadinol represents the highest concentration among the alcohols. This indicates that Calendula EO grown under K belongs to the α-cadinol chemotype.

PE52 Composition of *Artemisia abrotanum* and *A. pontica* Essential Oils and Their Repellent Activity against *Aedes aegypti*

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Mosquito-borne diseases such as malaria, encephalitis, and Yellow, Dengue, and Rift Valley fevers are diseases that result in significant morbidity and mortality in humans and livestock globally. Currently, the development of natural product-based insecticides and repellents are under exploration to increase and improve our ability to protect humans from mosquito bites, and ultimately to reduce the incidence of mosquito-borne illnesses [1]. We have undertaken a collaborative research project to discover new natural compounds for personal protection and control of mosquitoes. *Artemisia abrotanum* L. leaves have reportedly been used as a moth and insect repellent. Therefore, we evaluated *Artemisia abrotanum* and *A. pontica* L. essential oils for mosquito repellent activity against *Aedes aegypti* L. *Artemisia* oils obtained by hydrodistillation of aerial parts were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main *Artemisia* oil constituents were as follows: *A. abrotanum*: 32.6% 1,8-cineole, 13.5% borneol, 10.2% presilphiperfolan-9α-ol and 8.0% *p*-cymene; A. pontica: 35.6% artemisia ketone, 30.1% α-thujone, 22.3% 1,8-cineole and 3.7% β-thujone. *Artemisia abrotanum* oil showed repellent activity down to a minimum effective dosage of 0.219 mg/cm^2 (± 0.143) using cloth patch assay. Whereas *A. pontica* oil exhibited no repellent activity at the highest concentration tested, 0.375 mg/cm^2. Our research into exploring the repellency of specific compounds in the *A. abrotanum* oil will continue and be expanded to include other mosquito vectors and pesticide resistant mosquito strains. **Acknowledgement:** *This study was supported by a grant from the Deployed War-Fighter Protection (DWFP) Research Program and the U.S. Department of Defense through the Armed Forces Pest Management Board (AFPMB), and by a grant from the Mississippi Agricultural and Forestry Experiment Station.* **References:** 1. Hoel D et al. (2010) Wingbeats 21(1): 19 – 34.

PE53 The composition of Taif rose oil

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Fresh flowers of *Rosa damascena* Miller var. *trigintipetala* Dieck cultivated in Taif, Saudi Arabia are the source of Taif Rose Oil. The oils were sourced from two dealers in Riyadh, Saudi Arabia in 2011. They were analysed by GC and GC/MS. Both oils gave a similar profile with quantitative differences. The main components characterized were citronellol (23 – 28%), geraniol (14 – 20%), nonadecane (11 – 16%), nerol (6 – 11%), linalool (8%) and heneicosane (7%), resp.

PE54 Application of vibrational spectroscopy in the quality assessment of Buchu oil obtained from two commercially important *Agathosma* species (Rutaceae)

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Agathosma species (Rutaceae) are medicinal shrubs used traditionally to treat renal and chest ailments1. In addition, 'Buchu oil' from two South African species; *Agathosma betulina* (P.J. Bergius) Pillans and *Agathosma crenulata* (L.) Pillans is an important ingredient in flavour and fragrance formulations2,3. The use of vibrational spectroscopy as possible alternatives to conventional chromatographic techniques for the rapid and inexpensive assessment of 'buchu oil' was investigated. Samples of *A. betulina* (55) and *A. crenulata* (16) were collected from natural populations and cultivation sites in South Africa. The essential oil was scanned on NIR, MIR and Raman spectrometers. The spectral data was processed using orthogonal partial least squares discriminant analysis (OPLS-DA). Using GC-MS data, calibration models were developed for the MIR, NIR and Raman spectral data to predict the major compounds in 'buchu oil'. The results showed that OPLS-DA technique is a useful tool in the differentiation of *Agathosma* species using a non-targeted approach. Identification of wave regions that contain peaks separating the two species was possible. The PLS calibration model developed using MIR data was the best with $R^2X = 0.96$; $R^2Y = 0.88$ and $Q^2Ycum = 0.85$ for the quantification of six oil constituents. The model showed high predictive power for pseudo-diosphenol ($R^2 = 0.97$), isomenthone ($R^2 = 0.97$), menthone ($R^2 = 0.90$), limonene ($R^2 = 0.91$), pulegone ($R^2 = 0.96$) and diosphenol ($R^2 = 0.85$). These results illustrate the potential of MIR spectroscopy as a rapid and inexpensive alternative to predict the major compounds in commercially important buchu oil. **Acknowledgement:** *The financial support of National Research Foundation (SA), Tshwane University of Technology and Jagiellonian University is gratefully acknowledged. S. Chicken Naturals (Cape Town) are thanked for logistic arrangements to source plant material.* **References:** 1. Van Wyk B-E, Wink M (2004) Medicinal plants of the world, Briza publications, Pretoria. 2. Simpson D (1998) Scott med J 43:189 – 191 3. Turpie JK, Heydenrych BJ, Lamberth SJ (2003) Biol Cons 112: 233 – 251.

PE55 Antibacterial effects of some essential oils on the growth of *Ralstonia solanacearum*

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Biological control has been considered as one of the most interesting approaches to reduce plant disease during the last decades while the essential oils are among the most important antibacterial agents. In this study, the efficacy of the essential oils from 5 plant species was evaluated *in vitro* against *Ralstonia solanacearum* (race 3, biotype 2) causing potato wilting incidence. Essential oils of *Thymus vulgaris* L. (leaf), *Rosmarinus officinalis* L. (leaf), *Coriandrum sativum* L. (seed), *Cuminum cyminum* L. (seed) and *Eucalyptus globulus* Labill. (leaf) were extracted by hydrodistillation and tested against the bacterium species by the paper disc diffusion method at the concentrations of 0, % 0.01, % 0.05, % 0.1, % 0.5, % 1, % 5, % 10, % 25, % 50, % 75, % 100 (v/v). The minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBC) was determined by twofold broth dilution method. The most inhibition zone (34.8 mm) and MIC (1 µl/ml) were shown by *Thymus vulgaris*, followed by *R. officinalis* > *C. cyminum* > *C. sativum*. The efficacy of essential oil from *E. globulus* (inhibition zone 6.5 mm) was insignificant. MIC and MBC values of essential oils were 1 – 250 µl/ml. Results indicate that thyme essential oil has a potential to be used as an antibacterial against bacterial wilting disease in potato. Thyme in the greenhouse experiments, prevented 65% from potato wilting disease.

PE56 Antimicrobial activity of essential oils onto pathogenic microorganisms of interest to dentistry

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Essential oils have been used in folk medicine as active ingredients for medicines and cosmetics for the treatment of oral microbial diseases. This work studied the sensitivity of pathogenic microorganisms *Streptococcus mutans*, *Staphylococcus aureus*, *Aggregatibacter actinomycetemcomitans*, and *Candida albicans* to 12 different essential oil components: Myrcene (Mir), ocimene (western), limonene (Lim), bisabolene (Bis), linalool (Lin), citral (Cit), caryophyllene (Car), citronellol (Cin) acetate, eugenol (Eug), and geraniol (GER). The cultivation and preparation of inoculum, as well as antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) by the agar diffusion and successively dilution, were made in accordance CLSI (2007). 20 µL of each oil were soaked in sterile blanc discs and placed on the agar surface previously inoculated with 1.5 x 108CFU/mL specific organism and incubated at 37 °C for 48 h. The inhibition zones were measured and the mean and standard deviations (M ± SD) were tabulated. Discs containing Amoxicillin and Nystatin served as positive control of inhibition. The results showed that, except for geraniol and myrcene, all aromachemicals inhibited the microorganisms growth. The lowest MIC concentration was observed for bisabolene (0.88 mg) while the highest concentration was observed for ocimene (4.25 mg). These results suggest that essential oils tested could be used as active ingredients for oral use. However, further studies *in vitro* and *in vivo* should be made to confirm the potential antibiotic and low toxicity of the products in the oral environment. **Acknowledgement:** *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal Ensino Superior (CAPES), Fundação de Auxilio a Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação dos Cursos de Pós-Graduação da Faculdade de Odontologia da UFMG (CCPG/FOUFMG).* **References:** 1 Ennajar – M et al. (2010) J Sci Food Agric 90: 962 – 970 2- Ahonkhail Iet al. (2009) Pak J Pharm Sci 22: 405 – 409. 3- PereiraEM et al. (2011) Planta Med 77: 401 – 404.

PE57 Investigation on the improvement of essential oil distillation efficiency by changing the osmotic potential of distillation system

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Just as different environmental factors influence the amount and quality of essential oil, changes in osmotic potential of the extraction system can also be effective on the amount and quality of essential oils extracted. The purpose of this experience was possible increasing the efficiency of essential oil extraction by changing the osmotic potential of extraction system using NaCl. The research was conducted on the four important medicinal plants including, Ammi, Peppermint, Thyme and Eucalyptus. The research was set as completely randomized block design with three replications. The results showed that the osmotic potential of the extraction system affect oil extraction efficiency significantly. The highest essential oils (in all samples) obtained using NaCl salt solution at 3%w/v that adjusts the extraction temperature of the system equal to 98.5 °C. The eessential oil content of Eucalyptus, Thyme, Ammi and Peppermint were 2.717, 3.233, 3.25 and 2.167 v/v respectively. According to the results of this study osmotic potential of the extraction system affect extraction efficiency of essential oils. **Keywords:** extraction efficiency,

osmotic potential, NaCl, Carum copticum, Mentha piperita, Zataria multiflora, Eucalyptus globulus **References:** 1. Babu KGD, Singh B, Joshi VP and Singh V (2002) Flavour Fragr J 17: 136 – 140. 2. Clifford AA, Basile A, Jimenez-Carmona MM, Al-Saidi SHR (1999) High Pressure Chemical Engineering 6271: 189 – 192.

Topic F: Ethnopharmacology/Traditional and natural medicines

PF1 Phytochemical properties of *Aspilia africana* leaf

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Aspilia africana (Pers.) C.D.Adams is used in herbal medicine for the perceived presence of some bioactive components in the leaves. Scientific reports suggest that different crude extracts of the plant contain specific bioactive constituents that could have varied effects on its biological activities. This study evaluated the phytochemical activities in the meal; aqueous, chloroform and ethanolic extracts of *A. africana* leaves. The anti-microbial activity of the four samples of *A. africana* were tested on nine micro-organisms of six bacteria and three fungal strains using the agar well diffussion technique. Results of the phytochemical screening and subsequent quantification revealed the presence of high amount of some bioactive compounds; saponins, tannins, alkaloids, flavonoids, terpenoids and phenols, but the absence of steroids (leaf meal and all extracts), phylobatannin (chloroform and ethanolic extracts) and cardiac glycoside (ethanolic extract) in the *A. africana* leaf products. Though the chloroform leaf extract had higher concentrations ($P < 0.05$) of these phytochemicals, significant ($P < 0.05$) improvements were observed in the chemical composition of the aqueous and ethanolic extracts. The anti-microbial activities observed indicated that biological activities were dependent on the types of extractants and the concentrations of principles present such as alkaloids and tannins. These activities were comparable to those obtained for ampicillin and gentamycin. The susceptibility of the microorganisms to the different *A. africana* leaf samples demonstrated its anti-bacterial and anti-fungal potentials and validated its use as an anti-microbial agent in ethnoveterinary medicine. Further investigations are required on their efficacies as phytobiotics in animal production.

PF2 A comparative analysis of two medicinal plants used to treat common skin conditions in South Africa

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Infectious dermatological diseases are a common occurrence in southern Africa. Plants showing dermatological properties are highly sought after due to their ability to stop bleeding, speed up wound healing and to soothe skin exposed to burns. An attempt was made to validate the use of *Haworthia limifolia* Marloth and *Aloe excelsa* A.Berger against microbial properties from extracts of leaves against five gram positive, four gram negative bacteria and six species of fungi. All gram positive bacteria were inhibited by both the ethyl acetate and acetone extracts for leaves of *H. limifolia*. However, only one gram negative bacteria was inhibited by the same extracts. Ethyl acetate extract of *A. excelsa* was only effective against three gram positive bacteria whilst acetone extract was effective against all bacteria except for *Shigella sonnei* and *Enterobacter aerogenes*. Both ethanol and aqueous extracts of *H. limifolia* and *A. excelsa* showed antifungal activity. *H. limifolia* extracts showed greater antibacterial activity than *A. excelsa* whilst *A. excelsa* showed greater antifungal activity than *H. limifolia*. Use of either species as traditional medicine will therefore depend on the type of infection or condition presented by the patient.

PF3 An ethnobotanical survey of medicinal plants used by traditional healers in Durban, South Africa

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Medicinal plants have been extensively used for the treatment of infectious diseases by majority of the world's population. Many of the rural communities in KwaZulu-Natal have no access to western medical practioners and rely on traditional medicines for their cures. It has been noted that approximately 20% of the plants found in the world have some pharmacological properties. An ethnobotanical survey of medicinal plants used for various treatments including stomach ailments, skin diseases, blood purifiers, rashes, burns and other infections used in KwaZulu- Natal, South Africa was conducted through the use of structured questionnaires. Respondents included traditional healers, herbalists and herb sellers. The information collected revealed that 25 plant species belonging to various families are currently being exploited for their curing properties. The most frequently used parts are the leaves followed by root, rhizome or bulb. Stems, flowers and fruits are seldom used. The survey has indicated that Traditional healers administer their medications via extracts that are obtained by boiling, either as a tea or concoction.

PF4 The use of wild medicinal plants in the traditional therapy of respiratory diseases in high mountain region of W. Balkan

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Due to various disturbances in the environment is increasing the proportion of patients with respiratory diseases. A very high proportion are obstructive lung disease in relate to climate changes [1]. Especially children suffer from asthma and bronchitis. In chronic respiratory disease than the classic drugs, often used in various herbal medicines. Many of them used since ancient times in traditional phytotherapy, particularly in high altitudes [2, 3]. In order to find effective herbal means in the prevention and treatment of respiratory diseases are carried out ethnobotanical research in high mountain region of Bosnia and Herzegovina. It has been use of classical ethno-botanical interview with 52 adults informants in various locations (SE Herzegovina; mountains Maglic, Zelengora and Volujak in SE Bosnia and mountains in surrounding of Sarajevo). It has been determined 35 plant species used in traditional treatment of respiratory disorders. Most commonly cited species are: *Cetraria islandica* (L.) Ach., *Primula intricata* Gren. & Gordr and *P. veris* L., *Plantago reniformis* G. Beck, *Pinus mugo* Turra, *Picea abies* (L.) H.Karst., *Abies alba* Mill., *Allium ursinum* L., *Telekia speciosa* (Schreb.) Baumg., *Thymus balcanus* Borb., *Malva moschata* L., *Orchis* sp. (several species), *Dactylorhiza* sp. (several species) and others. Most used were aerial part of plants in the flower, then leaves, root, bulb and rhizome. Those plants use to make infusions, decoctions, wraps, and "cigarettes." Many of the plants should be identified and examined through various laboratory pharmacological tests in order to put some of them in the use as an official phytotherapeutics. **References:** 1. Frumkin H et al. (2008) Am J Public Health 98: 435 – 445. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S (2006) Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141

PF5 Evaluation of Some Medicinal Plant Extracts against Neuroinflammation Characterizing Alzheimer's Disease in Experimental Rat Model

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The main purpose of the present study is to evaluate the role of *Salvia triloba* L.f. and *Ruta graveolens* L. extracts in management of neuroinflammatory insults characteristic for Alzheimer's disease in rat model. Male Sprague Dawley rats were classified into five groups: (1), control group; (2), AD group which was orally administered with aluminum chloride in a dose of 17 mg/kg b. wt. daily for one month; (3), AD group which was treated with rivastigmine in a dose of 0.3 mg/kg b. wt. daily for three months; (4), AD group which was treated with total extract of the aerial part of *Salvia triloba* daily for three months and (5), AD group which was treated with total extract of the aerial part of *Ruta graveolens* daily for three months. Serum Ach and brain AchE activity, Bcl2, NF-κB, and CRP were estimated. Histological investigations of brain sections of all studied groups were also carried out. The results showed that administration of AlCl3 resulted in significant elevation in AchE, NF-κB and CRP levels accompanied with significant depletion in Ach and Bcl2 le-

vels. Histological investigations of the brain of rats administered $AlCl_3$ showed the appearance of amyloid plaques characterizing AD. While, treatment of rats with the extracts caused marked improvement in the measured biochemical parameters as well as in the histological features of the brain. In conclusion, *Salvia triloba* and *Ruta graveolens* have a potent anti-inflammatory effect against neuroinflammation characterizing Alzheimer's disease. **Keywords:** Alzheimer's disease, Salvia triloba, Ruta graveolens, anti-inflammatory, Rat

PF6 Bioactivity Guided Evaluation of Antinociceptive and Anti-inflammatory Properties of *Cnestis ferruginea* Vahl ex DC (Connaraceae)

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Cnestis ferruginea Vahl ex DC (Connaraceae) (CF) is a shrub widely used in traditional African Medicine (TAM) for the treatment of various painful and inflammatory conditions. This study sought to isolate, identify and investigate the anti-inflammatory and antinociceptive activity of the active constituents of CF through bioassay guided fractionation. The crude methanolic root extract of CF was sequentially fractionated into four sub extracts (chloroform, ethylacetate, n-butanol and the remaining aqueous fraction). The aqueous – butanol fractions having showed significant inhibition of inflammation and pain was subjected to fractionation through successive column chromatography on silica gel 60 – 120 mesh, eluted with a gradient of $CHCl_3$- MeOH. Sixty five fractions were collected; fractions with similar TLC profile were grouped into seven major fractions (1 – 7). Fraction 4 being most active in bioassay was rechromatographed to yield CF-2 and CF-5. The effect on inflammatory mediators was studied in rat astrocytoma cells (C6), nitrite release in culture supernatant, ROS in cells and TNF-α in cell lysate were estimated. The methanolic extract, aqueous and n-butanol fraction showed significant ($p < 0.05$, $p < 0.01$, $p < 0.001$) inhibition of acute inflammation, peripherally and centrally mediated forms of pain in the bioassay procedures. The aqueous/n-butanol fraction afforded two active major compounds, identified as amentoflavone (CF-2) and an amino acid compound (CF-5) following various spectroscopic experiments. CF-2 and CF-5 significantly attenuated nociception, inflammation, nitrite release, ROS generation and down regulated TNF-α. This study confirmed the anti-inflammatory and antinociceptive activity of *Cnestis ferruginea* and its active constituents based on folkloric uses.

PF7 W9, a medicinal plant from the pharmacopeia of the Eastern James Bay Cree, exhibits anti-diabetic activities in two mouse model of diabetes

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Aboriginal populations are particularly at risk for developing type 2 diabetes mellitus and its complications. In Canada, diabetes prevalence for these populations is at least three times higher than that of the general population. W9 has been identified among species used by the Cree of Eeyou Istchii of northern Quebec to treat symptoms of diabetes. In a previous study, the ethanol extract of W9 enhanced glucose uptake in C2C12 muscle cells via stimulation of AMP-activated protein kinase (AMPK) pathway. In this study, we investigated the in vivo effect of this plant in two mouse models of type 2 diabetes. In the first one, KKAy mice received W9 extract in drinking water (1%) for 10 days. In the second model, C57BL/6 mice were fed a high fat diet (HFD; ~35% lipids) for 8 weeks until they became obese and insulin resistant (diet-induced-obesity; DIO). Treatment then began by adding W9 extract to HFD at 3 different concentration (125, 250 and 500 mg/Kg) for another 8 weeks. In both models, W9 significantly decreased glycemia, strongly tended to decrease insulin levels, and this was accompanied with reduced fluid intake in the KKAy model. This correlated with either a tendency or a frank increase in GLUT4 content and activation of the AMPK and/or Akt pathways in skeletal muscle. W9 treatment also improved hepatic steatosis by decreasing hepatic triglyceride levels and significantly activating the AMPK and Akt pathways. The results of the present study confirm that W9 represents a culturally relevant treatment option for Cree diabetics. **Acknowledgement:** *This work was supported by a Team Grant from the Canadian Institutes of Health Research (CIHR Team in Aboriginal Antidiabetic Medicines) to P.S.H. and J.T.A. and was conducted with the consent and support of the Cree Nation of Mistissini,*

PF8 Evaluation of the Hepatoprotective and Antioxidant Activities of an Indigenous Triherbal Formulation from South Eastern Nigeria using Wistar Albino Rats

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A triherbal formulation prepared from a mixture of the leaves of *Gongronema latifolium* Benth., *Ocimum gratissimum* L. and *Vernonia amygdalina* Delile (GOV) was evaluated for hepato-nephro protective and antioxidant properties against D-galactosamine-induced hepatic and renal toxicity in Wistar albino rats. Normal Wistar albino rats were divided into seven groups of seven animals each. Two control experiments were setup which included normal rats treated with D-galactosamine and normal rats that received only distilled water. Three groups were treated with different doses of GOV extract (2000, 4000 and 8000 mg kg-1 b. wt) and some standard hepatoprotective drugs such as Liv 52 and silymarin for 13 days prior to intoxication with D-galactosamine. The activities of serum liver enzymes, concentrations of some biochemical analytes, effect on heamatologic parameters and antioxidant status were monitored. The results showed that rats treated with GOV dose dependently exhibited significant ($p < 0.05$) decrease in levels of ALP, ALT, AST, GGT, LDH, cholesterol, creatinine, triglycerides, urea and MDA and subsequently significantly ($p < 0.05$) increased the albumin, total protein, catalase, GPx, GSH, GST and SOD levels when compared to the toxin control rats. The data from this study suggest that the triherbal formulation possess hepato- and nephro-protective potential against D-galactosamine induced hepatotoxicity in rats, thus providing scientific rationale for its use in traditional medicine for the treatment of liver diseases.

PF9 The most useful herbs of Traditional Iranian Medicine prescribed for Insomnia

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Insomnia is a highly prevalent condition, and due to ongoing demand from patients suffering with this condition, new pharmacological treatments are actively being sought.[1]Insomnia is a well-known disorder in Iranian Traditional Medicine (ITM). Scholars of ITM described insomnia in their manuscripts precisely. According to ITM references, Insomnia is an excess and abnormal form of awakening. Various causes such as bilious temperament, pain, Indigestion, melanotic swelling around the brain and fever will result in insomnia.[2] Herbal therapy was the major treatment prescribed by Iranian physicians. Six Iranian ancient medical texts *i.e.* Canon of Medicine (Avicenna 980 – 1037 AD), Alhavi (Razes 865 – 925 AD) Tohfat ul-Mo,menin (Mo,men tonekaboni 17th century), Makhzan ul-Advia (Aghili 18thcentury), Ekhtiarat Badi,i (Ansari 1329 – 1404 AD), and Al-abnia An-Haghyegh el-advia (Heravi 11thcentury) were studied for the treatment of insomnia. Subsequent to our study, the herbal medicines were listed and scored based on the frequency of their prescriptibility. Moreover, the effort has been taken to provide the best scientific name for each plant. This study showed that *Papaver somniferum* L., *Crocus sativus* L. and *Lactuca sativa* L. were the most frequent herbs mentioned in ITM prescriptions and in conclusion they can be introduced as new anti insomnia herbal medicines for clinical researches. **Keywords:** Insomnia, Herbal, Traditional Iranian Medicine
References: 1. Richey SM, Krystal AD (2011) Pharmacological Advances

in the Treatment of Insomnia. Curr Pharm Des. (in print). 2. Avicenna, Canon of Medicine, (1987) Translated by Sharafkandy A, Soroush pub., Tehran p. 110 – 111.

PF10 Molluscicidal Potential of the Fruit Pericarp of *Blighia unijugata* Baker Against *Biomphalaria pfeifferi*

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Blighia unijugata Baker (Sapindaceae) is a small to medium-sized tree up to 30 m tall widespread in tropical Africa. The leaves are eaten as vegetable and various part of the tree are considered to have sedative and analgesic properties and are used in traditional medicine for the treatment of rheumatism, kidney pain and muscular stiffness. The macerated twigs, leaves, flowers and fruit as a fish poison by the coastal people in Nigeria and there is a high correlation between plants employed as fish poison or soap substances and molluscicidal activity. Powdered pericarp was macerated with 50% ethanol, filtered and the filtrate concentrated to dryness under vacuum to yield 10.30 g of the dried extract and out of this 9.80 g was dissolved in water and partitioned between ethyl acetate, butanol and water to give 2.77 g of ethyl acetate, 2.81 of Butanol and 3.35 of water fractions respectively Snails for the experiment were collected from streams that have not been subjected to either synthetic or plant molluscicides. They were allowed to acclimatize in the laboratory for two weeks before use. The methods of Al-Zanbagi et al. (1) and Truiti et al. (2) were modified and used. The crude ethanolic extract has LC_{50} of 15ppm and for the fractions ethyl acetate was the most active with LC_{50} of 7.6ppm while the butanol fraction had a LC_{50} of 15 ppm and water fraction was the least active with LC_{50} of 25ppm. Efforts are being made to isolate the active compounds from each fraction. **Keywords:** Blighia unijugata, fruit pericarp, crude extract, fractions, Biomphalaria pfeifferi **References:** 1. Al-Zanbagii NA et al. (2000) J Ethnopharmacol 70:119 – 125 2. Truiti MCT et al. (2005) Braz J Med Biol Res 38: 1873 – 1878.

PF11 Antibacterial and anticancer activity of kaurenoic acid from root bark extract of *Annona senegalensis*

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The antibacterial [1] and anticancer [2] activities of extract of *Annona senegalensis* Pers. (Annonaceae) have been reported. Bioactive-guided fractionation of the methanol-methylene chloride root bark extract of *A. senegalensis* afforded a potent antibacterial ethyl acetate fraction (EF) which on further fractionation, gave two active sub-fractions F1 and F2. F1 yielded a lipophilic liquid component while F2 on purification, precipitated a white crystalline compound, ASI, that was characterized by proton NMR and X-ray crystallography as kaur-16-en-19-oic acid. F1 was analyzed using GC-MS to obtain 6 major constituents: 1- dodecanol, kaur-16-en-18-oic acid, 1-Naphthalenemethanol, 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-ethanol, 3,3-dimethyl-2-(3-methylbuta-1,3-dienyl) cyclohexan-1-methanol and 3-hydroxyandrostan-17-carboxylic acid. Agar well diffusion method, using a 0.5 McFarland standard, was employed to obtain the MIC's for F1 and ASI. The MIC's against clinical isolates of *Bacillus subtilis* in μg/ml of the EF, F1 and ASI were 180, 60, and 30 respectively. However, ASI exhibited appreciable activity against *Staphylococcus aureus* with an MIC value of 150 μg/ml while F1 was active against *Pseudomonas aeruginosa* with an MIC value of 40 μg/ml. The standard agent ciprofloxacin exhibited MIC values of 0.28, 1.18 and 3.6 μg/ml against *B.subtilis*, *Staph aureus* and *Ps. aeruginosa* respectively. Additionally, ASI was screened for cytotoxicity on 3 Cancer cell lines, human embryonic kidney cells expressing SV40 Large T-antigen (293 T), Pancreatic tumour (PANC-1) cell lines and Henrietta Lacks' cervical cancer cell line (HeLa), using the standard MTT assay method. 50% cellular toxicity (TC50) of ASI was calculated to be 125.89, 211.35, 266.07 μg/ml for 293 T, Hela and PANC-1 cells respectively.

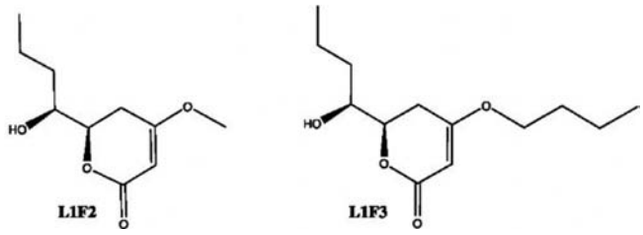

Fig. 1: Kaur- 16-en- 19-oic acid

Acknowledgement: *The authors are grateful to IOT World Bank assisted award grant of Step-B project of Federal Government of Nigeria.* **References:** 1. Apak L, Olila D (2006) Afr Health Sci 6 (1): 31 – 35. 2. Abubakar MS, Musa AM, Ahmed A, Hussaini IM (2007) J Ethnopharmacol.111: 625 – 629.

PF12 Microscopic and histochemical characterization of leaves of the medicinal plant *Ocimum obovatum*

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Ocimum obovatum E. Mey. subsp. *obovatum* var. *obovatum* has been valued for its hair restorative properties for decades on the African continent. The member of the Lamiaceae is also traditionally prescribed as a remedy for infantile abdominal cramps and a hot water extract of the leaves is used to treat epigastric conditions in children. Commonly known as 'cat's whiskers', the aromatic plant can be seen growing along the KwaZulu-Natal coastline and the Western Cape of southern Africa. *Ocimum obovatum* is also common in Zimbabwe and Swaziland as well as northern and west Africa [1]. The medicinal properties of the plant are attributed to the essential oils supposedly produced and secreted by appendages on the foliar surfaces referred to as trichomes [2]. Traditional light and electron microscopy studies revealed the presence of two types of glandular trichomes and one type of non-glandular trichome across all stages of leaf development. The glandular trichomes were classified as large, four-celled peltate trichomes and smaller capitate trichomes. The latter were further classified into two subtypes; Type I capitate trichomes with a single basal cell and two head cells and Type II capitate trichomes with a single basal and stalk cell and an ovoid head cell. Histochemical and phytochemical studies showed that essential oils of a terpenoid nature were present in the head cells of glandular trichomes. Flavonoids, triterpenoids, tannins, saponins, fixed oils and fats, phenolics and cardiac glycosides were also detected in a crude ethanolic extract of the leaves using phytochemical test methods. **Acknowledgement:** *The National Research Foundation (South Africa) is gratefully acknowledged for the funding of this research.* **References:** 1. Hutchings et al. (1996) Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg. 2. Werker et al. (1993) Annals of Botany 71: 43.

PF13 Effect of drying methods on the antioxidant activity of *Anacardium occidentale* L. (Cashew)

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Studies on the drying characteristics of Cashew leaves are scarce in the literature; particularly the traditional sun drying properties as well as oven drying properties of plants are not adequately investigated. The aim of the work was to determine the sun, oven, shade dried and fresh leaves drying characteristics of Cashew and to compare the effect of the same on the antioxidant property of the extracts. The extracts of leaves exposed to various drying conditions were prepared using various solvents. The phytochemical tests were carried out for various extracts prepared in order to ascertain the presence/absence of various phytoconstituents. The effect of drying conditions on the antioxidant activity of the extracts, the extracts were evaluated for their antioxidant effect by DPPH. Assay, Greiss assay and determination of total phenol content.

The Phytochemical investigations of the extracts revealed the presence of tannins and phenolics, saponins, flavonoids, alkaloids, steroids and sugars. From the results obtained, it was observed that shade dried leaves contained the maximum content of polyphenols. And the order of content to polyphenol was found to be Sun Dried leaves> Fresh Leaves > Oven Dried leaves > Shade dried leaves. **Acknowledgement:** *The authors thank ICMR, New Delhi, India for funding the research project.* **References:** 1. Chang SS, Ostric-Manjasevic B, Hsieh OL, Huang CL (1977) J Food Sci 42: 1102 – 6. 2. Yağcıoğlu A, Değirmencioğlu A, Cağatay F (1999) Drying characteristics of laurel leaves under different drying conditions. In: Bascetincelik A, editor. Proceeding of the 7th international congress on agricultural mechanization and energy in agriculture, 26 – 27 May, Adana, Turkey. Faculty of Agriculture, Cukurova University.

PF14 Antihyperglycaemic and antihyperlidaemic effects of *Raphia hookeri* root extract on alloxan induced diabetic rats

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The antihyperglycaemic and antihyperlidaemic effects of *Raphia hookeri* G.Mann & H.Wendl. (RH) root extract used for diabetic treatment were evaluated against alloxan diabetic rats. Adult rats weighing 150 ± 10 g were fasted for 18 hrs and induced with diabetes for three days using alloxan monohydrate (150 mg/kg body weight). Animals with blood glucose level >= 250 mg/dl were used. Diabetic rats were divided into four groups and treated as follows: groups 1 – 3 received graded doses of RH (50, 100 and 200 mg/kg) by gavages; group 4- glibenclamide (10 mg/kg); groups 5 and 6 as normal and diabetic controls. Each comprised of 5 rats. Blood was collected at days, 0, 3, 5, 7, 9, 11, 13 and 15 and analyzed for glucose by oxidase method; Lipid profile, by modified enzymatic procedure1; Insulin assay, using Diagnostic Automation Kit 2 and glycated haemoglobin by standard protocol 3. Results showed that the extract exhibited significant ($P < 0.05$) dose dependent decrease in glycaemia from day 3 to 15 with highest dose exerting 87% decrease at day 15. RH dose at 500 mg/kg on normal rat caused hypoglycaemia after 4hrs with maximum decrease (54.7%) observed after 8 hrs. Total cholesterol, triglycerides and low density lipoprotein cholesterol levels decreased with dose while high density lipoprotein cholesterol showed dose increase. RH stimulated insulin secretion and equally exerted significant increase in glycated haemoglobin (HbA1c). Photomicrograph of RH treated showed significant beta cell survivors. RH exhibited antihyperglycaemic and insulin stimulatory effects with beneficial effect on lipid homeostasis. **Acknowledgement:** *Prince Musibau Sikiru a herbalist at Sagamu, Ogun State, Nigeria, for assisting with the plant material.*

PF15 Ethnobotanical survey and antifungal activity of plants identified for the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa

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In a study to document plants used to treat opportunistic fungal infections (OFIs) seen in HIV/AIDS patients in the Eastern Cape, South Africa, ethnobotanical information was obtained through questionnaires and conversations with 22 traditional healers and 101 HIV/AIDS patients. Thirty two plant species, belonging to 26 families, were identified as being used for this purpose. Two frequently used plants, *Arctotis arctotoides* K.Lewin and *Gasteria bicolor* Haw., were examined for validation by recording antifungal effects and minimum inhibitory concentrations (MICs) of their hexane, acetone and water extracts against 10 opportunistic fungi, using agar well-diffusion and broth micro-dilution methods. Among the 6 plant extracts, all the hexane and acetone extracts were active against at least one of the fungi with zones of inhibition varying from 8 to 32 mm (control: 14 – 27 mm). For both plants the lowest MICs were obtained with the hexane extracts (*A. arctotoides*: 0.005 mg/ml against *Trichophyton mucoides* and *G. bicolor*: 0.04 mg/ml against *Aspergillus fumigatus*). The inhibitory activity of the active extracts, based on the mean inhibition diameters, was in the order: *A. arctotoides* (hexane) > *A. arctotoides* (acetone) > *G. bicolor* (hexane) > *G. bicolor* (acetone). The most susceptible fungi were *Candida glabrata*, *C. krusei* and *Microsporum canis*, while *Cyptococcus neoformans*, *Trycophyton tonsurans* and *M. gypseum* were not susceptible to any of the extracts even at 5 mg/ml which was the highest concentration used. This study not only documents thirty two plants used, but validates the use of two of these plants in traditional medicine for the management of OFIs in HIV/AIDS patients. **Acknowledgement:** *The Govan Mbeki Research and Development Centre of the University of Fort Hare is acknowledged for financial support for the research and conference attendance.*

PF16 An Investigation of Antimicrobial and Wound-Healing Potentials of *Boesenbergia rotunda*

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The antimicrobial activity accelerates the wound-healing process by preventing the wound against the microorganism infections as previously reported[1]. The purposes of this study are to evaluate antimicrobial potentials of *Boesenbergia rotunda* (L.) Mansf.] from rhizome extracts against the common microorganisms causing skin infections, and to validate the wound healing activity in rats. The antimicrobial activity of aqueous and ethanolic extracts of *Boesenbergia rotunda* rhizomes has been tested against selected six bacterial and two fungal strains using a broth microdilution method to determine the minimal inhibition concentrations (MIC) and the minimal microbicidal concentrations (MMC). Both extracts possessed potential antimicrobial and antifungal activities by inhibiting gram positive bacteria *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* and *Bacillus subtilis* ATCC 6633 and fungi *Candida albicans* ATCC 10231 and *Sacharomyces cerevisiae*. MIC and MMC varied from 0.039 to 25 mg/ml and 0.156 to 25 mg/ml, respectively and all the microorganisms were more sensitive to ethanolic extracts than aqueous extracts. In the wound-healing studies, the topical application of 20% ethanolic extract has indicated a significantly increased percentage of wound contraction (83.49%) on day 14 compared to the control groups (71.9%). The results of histological evaluation have confirmed a remarkable wound healing activity of *Boesenbergia rotunda*. **Acknowledgement:** *This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.* **References:** 1. Srinivas Reddy B et al. (2008) J Ethnopharmacol 115:249 – 256

PF17 The study of hypoglycemic effects of the *Morus alba* L. leaf extract and histology of the pancreatic islet cells in diabetic and normal rats

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The objectives of this study were to evaluate the hypoglycemic effects and histological changes of pancreatic islets after receiving water extract of *Morus alba* L. leaf in normal and diabetic rats. Diabetes was induced by injection of streptozotocin (STZ, 45 mg/kg) intraperitoneally. All diabetic rats were divided into 5 groups, each of which was orally received one of the following; vehicle, glibenclamide and leaf extract (150, 300 and 600 mg/kg) for 12 days. The results showed that the leaf extract at the doses of 300 and 600 mg/kg significantly ($p < 0.05$) reduced blood glucose levels in diabetic rats. Moreover, the study of *Morus alba* leaf extract in decreasing of acute hyperglycemic effect was undertaken by oral glucose tolerance test (OGTT) which revealed that the leaf extract could not reduce blood glucose levels in acute hyperglycemia in both diabetic and normal rats. The histological examination of pancreas

showed that all doses of leaf extract could recover the damaged islet cells in diabetic rats in a dose dependent manner. The pancreatic islets of diabetic rats receiving the extract were larger and the cells within the islets were rounder and less congestive when compared to diabetic control rats. Conclusion, the results of this study suggested that *Morus alba* leaf extract could reduce blood glucose levels and improve the histological features of pancreatic islets in diabetic rats. **Acknowledgement:** *This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.*

PF18 Hepatoprotective effect of the ethanolic extract of *Anethum graveolens* L. on paracetamol-induced hepatic damage in rats

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The present study was conducted to evaluate the hepatoprotective effect of the *Anethum graveolens* L. extract on paracetamol-induced hepatic damage in rats. Thirty rats were divided into 5 groups of 6 rats. Group I served as control that received 5% tween 80. Group II and III was pre-treated with 5% tween 80, for 7 day before 3 g/kg BW of paracetamol was administered on day 8 whereas group III also received N-Acetyl Cysteine (NAC) at 2 hours after paracetamol administered. Group IV and V were pre-treated with 1 g/kg and 500 mg/kg of the plant extract respectively for 7 days following by 3 g/kg BW of paracetamol on day 8. The extract was examined for antioxidant effects by DPPH radical scavenging activity while the biochemical parameters such as serum Aspartate transaminase (AST), Alanine transaminase (ALT) and histopathological examination were determinated at the end of the experimental period. The result showed that the ethanolic extract has the antioxidant activity with the EC 50 value by DPPH radical scavenging activity is 97.66+5.65 μg/ml. Moreover, the ethanolic extract has a hepatoprotective action by decreasing the AST and ALT levels in Group IV and V. Moreover, hepatotoxicity was observed in rats treated with paracetamol alone as shown by hepatic steatosis and hepatic necrosis while these were reduced in the pre-treatment with ethanolic extract and NAC treatment rats. In conclusion, the ethanolic extract of *Anethum graveolens* L. has an antioxidant activity and exhibits hepatoprotective effect against paracetamol-induced hepatic damage in rats. **Acknowledgement:** *This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.*

PF19 The effects of ethanol extract of *Raphia hookeri* seed on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats

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The activity of *Raphia hookeri* G.Mann & H.Wendl. (RH) seed extract used locally in the treatment of benign prostatic hyperplasia (BPH) was investigated on exogenous induced prostatic enlargement. Adult male rats weighing 200 ± 10 g/kg were induced with BPH by exogenous administration of testosterone and estradiol in staggered doses (three times a week) for three weeks (1). The induced animals were divided into five groups. Groups 1 and 2 received the extract at 50 and 100 mg/kg body weight by gavages for forty five days; group 3- fenasteride (0.1 mg/kg); group 4- was left untreated for forty five days; group 5- (negative control) was sacrificed immediately after induction. Group 6- received the extract (100 mg/kg) and the steroid hormones simultaneously while group -7 was normal control. Prostate specific antigen (PSA) and testosterone levels were determined from blood serum. The oxidative activity; Catalase (CAT), Superoxide dismutase (SOD), Lipid peroxidation and glutathione (GSH) were assayed as described by Rukkumani et al,(2). The result showed significant decrease ($P < 0.05$) in PSA level in RH treated compared to the negative control. There was also decrease in testosterone level in RH treated. The levels of CAT and SOD (Table 1) in RH treated were comparable to normal. However, GSH showed comparably higher level to normal while the extract peroxidative activities decrease slight. Prostatic tissue morphology of the extract treated (Fig. 1) showed extensive shrinkage while hypertrophy of prostate gland occurred in the untreated (Fig. 2). RH effectively reduced enlarged prostate mass, lowered PSA and testosterone levels and also exhibited anti-oxidative activity. **Acknowledgement:** *Prince Musibau Sikiru, herbalist, Ogun State, Nigeria assisted with the plant material.* **References:** (1) Bernoulli J (2008) An experimental model of prostatic inflammation for drug discovery. Medica – Odontologica. (2) Rukkumani R et al. (2004 J Pharm Sci 7(2): 274 – 283.

PF20 Insecticidal properties of extracts and phytochemicals isolated from three Egyptian plants against *Culex pipiens*

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The chemistry of three Egyptian plants, namely *Ambrosia maritima* L., *Achillea santolina* L. and *Adhatoda vasica* L. was studied. Integration use of chromatographic methods led to isolate fifteen compounds of sesquiterpenes, flavonoids and alkaloids from these plants. The chemical structures of the isolated compounds were determined by using spectral data of ^{1}H NMR, ^{13}C NMR, MS and UV. In the preliminary test, the extracts and compounds were evaluated at concentration of 500 mg/l against the fourth instar larvae of *Culex pipiens*. Among the tested extracts *A. vasica* alkaloidal fraction was the most effective with complete mortality of *C. pipiens* larvae followed by *A. santolina* extract which caused 43.3% mortality. Two sesquiterpenes (neoambrosin and damsin, and two flavonoids (santoflavone and eupatorin) and the alkaloid vasicine caused complete mortality of the larvae at this concentration. The extracts and compounds that display mortality higher than 50% were further evaluated at a series of concentration to calculate the lethal concentration values (LC_{50}). Eupatorin revealed the strongest toxicity with LC_{50} with of 5.61 mg/l. The alkaloid vasicine showed good insecticidal activity since LC_{50} value was 88.88 mg/l. Among the tested sesquiterpenes, damsin had the highest activity, followed by neoambrosin while hymenin was the less effective one. The obtained results suggest that eupatorin, neoambrosin, damsin and ambrosin may serve as new natural compounds for control *C. pipiens*.

PF21 Antinociceptive activity of the fractionated extracts of *Muntingia calabura*

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Non-steroidal Anti-inflammatory Drugs (NSAIDs) have been shown to be effective in inflammatory and pain management. However, this group of drugs can also cause various undesirable side effects such as dyspepsia, upper gastrointestinal and renal effects. Thus, it is necessary to continue searching for new sources of anti-inflammatory and pain-relieving agents from natural sources, which exert lesser or, possibly, no side effects. *Muntingia calabura* L. is known locally as *Kerukup siam* [1]. This plant has been claimed by the Peruvian folklore to possess medicinal values which include soothing gastric ulcers, relieving headache and cold, reducing swelling of the prostate gland and antiseptic [1]. This study focuses on understanding the antinociceptive effects of the extracts using Sprague Dawley (S.D.) rats. In the present study, activity-guided studies of the methanolic extract of *Muntingia calabura* collected in Shah Alam, Selangor, Malaysia were conducted for their antinociceptive property using formalin test [2]. Seven fractions of petroleum ether extract labelled A-G were separately administered (orally) with distilled water or 10% DMSO as negative controls and morphine and aspirin as a positive control. Fraction D showed the most significant antinociceptive activity when compare to other fractions (Table 1). At the dose of 300 mg/kg, fraction D exhibited 66.2% and 81.4% antinociception in first (analgesic) and second phase (inflammation) respectively. Fraction D showed no significant different ($p < 0.5$) when compared to aspirin (100 mg/kg) as a positive control. In further experiment, active com-

pounds from fraction D will be identified and their antinociceptive and anti-inflammation properties will be investigated.

Table 1: Antinociceptive activity for petroleum ether fraction of Mutingia calabura

Fraction (mg/kg)	First phase	Percentage %	Second phase	Percentage %
A (300)	79.667 ± 1.585	2.6	145.667 ± 2.246	0.45
B (300)	74.833 ± 1.922	8.6	137.833 ± 2.056	5.8
C (300)	57.167 ± 2.056	30.1	86.167 ± 1.851	41.1
D (300)	27.667 ± 2.418	66.2	27.167 ± 1.778	81.4
E (300)	42.167 ± 3.381	48.5	77.833 ± 1.537	46.8
F (300)	81.82 ± 3.26	0.01	86.667 ± 1.819	40.8
G (300)	74.333 ± 1.429	9.2	143.833 ± 2.428	1.7

Acknowledgement: *This study was supported by the National Science Fellowship (NSF) scholarship from Ministry of Science Technology and Innovation Malaysia (MOSTI).* **References:** [1]. Morton JF (1987) Jamaica cherry in fruit of warm climates. Miami. pp: 65 – 69. [2]. Rosland JH, Tjolsen A, Maehle B & Hole K (1990). Pain 42: 235 – 242.

PF22 Protective effect of *Polygonum odoratum* L. on acetaminophen-induced liver injury in rats

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This study aims to investigate the protective effect of the *Polygonum odoratum* L. on acetaminophen-induced liver injury in rats. 30 male Spraque Dawley rats were divided into 5 groups. Group I, II and III were given 5%Tween 80 whereas group IV and V were given 500 and 1000 mg/kg body weight/day of the plant extract, respectively for 7 days. On day 8th, animal in group II-V were received acetaminophen 3 g/kg body weight; group II also received N-Acetyl Cystein (NAC) 400 mg/kg body weight at 2 hours after acetaminophen administrated. All animal were sacrificed on day 9 th and blood samples were collected for the determination of Aspartate transaminase (AST), Alanine transaminase (ALT), Malondialdehyde (MDA) and nitrite formation. AST, ALT, MDA and nitrite levels were significantly higher in rats treated with acetaminophen alone compared with normal control ($p < 0.05$). Pretreatment of the animal with the extract and administration of NAC significantly reduced oxidative stress and liver injury induced by acetaminophen as shown by reduction of MDA, nitrite levels, AST and ALT levels compared to the rats treated with acetaminophen alone ($p < 0.05$). In conclusion, the result of this study demonstrated that pre-treatment with the ethanolic extract of *Polygonum odoratum* can ameliorate oxidative stress and liver injury induced by acetaminophen. **Acknowledgement:** *This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.*

PF23 Ethnobotanical study of medicinal plants used by local Bedwins in the Badia region of Jordan

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An ethnobotanical study was conducted as a part of the local knowledge study which was carried out in 2010. The target participants were the Livestock owners in the arid Jordanian Badia region. The objective of the study is to document the traditional knowledge in using wild plants in treating health problems in order to conserve this valuable knowledge from loss; to identify the key plant species used and to calculate the Informant Consensus Factor (ICF) for the health disorder categories. The data was collected from interviewing 70 participants, 21% of them were women, the women interviews were very important as they are considered experts in the field of the local medicinal plants. The participants were interviewed face to face and few focus groups were conducted. A questionnaire that helps in the data gathering was prepared, video recording was taken to show the procedures that the local communities were using in their process. A total of 48 plant species are used by local Bedwins for medicinal purposes, the majority of these are native to the study area, e.g. *Artemisia judaica* Lour., *Citrullus colocynthis* (L.) Schrad., *Ducrosia anethifolia* Boiss., *Ecballium elaterium* (L.) A.Rich., *Paronychia* spp and *Rheum palaestinum* Feinbrun. The study showed that the plant species with the highest use value is *Artemisia herba-alba*. Moreover, the highest value of Informant Consensus Factor (ICF) was scored for Jaundice disease (0.87) followed by Gastrointestinal disorders (0.86) and dental disorders (0.81). This may indicate the high incidental occurrences of these diseases and the lack of dental care services in the rural areas.

PF24 Current status, role and challenges of traditional medical practitioners involved in management of diabetes mellitus in Nigeria

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Patients suffering from Diabetes mellitus in Nigeria have resulted to consulting Traditional Medical Practitioners (TMPs) to manage their health conditions. There are no available data on the role and status of traditional medicine practice in the management of the disease in the country. A study was iniated in 2009, to document this in six geographical zones of the country. Data was collected through oral interviews in the local languages of over 90 practitioners and responses documented in a specially designed questionnaire along with prior informed consent form and entered into a database. This paper intends to highlight the results obtained which include collection of over 80 recipes in various forms, (mostly of plant based), preliminary screening which yielded 4 most active recipes along with pharmacognostic standards, adequate referral system of the practise, low percentage of women involved in the practise, inadequate educational background of practitioners, good understanding of disease diagnosis, inadeqiate record keeping and improved shelf life of products. Challenges include need for training on standardization methods of thier products and practise, establisgment of botanical gardens due to derorestation, establishment of clinics and more opportunities for product resgistartion. These results are required to aid the promotion, standardization and integration of the practice into National Health Care System. **Acknowledgement:** *The authors wish to acknowledge the STEPB Project of the World Bank for award of Research Grant for this study as part of a larger study.*

PF25 Ethnobotanical Evaluation of Some Medicinal Plants in Eskişehir, Turkey

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An ethnobotanical survey was made to collect information by means of oral and written questionnaire about the use of medicinal plants in Eskişehir with the assistance of herbal markets. A list of medicinal plants and their reported folkloric uses was compiled during the survey. Information regarding latin name, local name, part(s) used, medicinal uses, recipe preparations of plants. According to the survey 49 plant taxa were members of Aquifoliaceae (1 taxon), Apiaceae (1 taxa), Asteraceae (4 taxa), Caprifoliaceae (1 taxon), Compositae (2 taxa), Brassicaceae (1 taxon), Equisetaceae (1 taxon), Ericaceae (1 taxon), Fabaceae (1 taxon), Ginkgoaceae (1 taxon), Poaceae (2 taxa), Juglandaceae (1 taxon), Labiatae (5 taxa), Lauraceae (1 taxon), Loranthaceae (1 taxon), Lycopodiaceae (1 taxon), Malvaceae (5 taxa), Oleaceae (1 taxon), Onagraceae (1 taxon), Plantaginaceae (1 taxon), Primulaceae (1 taxon), Ranunculaceae (1 taxon), Rosaceae (6 taxa), Rubiaceae (2 taxa), Theaceae (1 taxon), Tiliceae (2 taxa), Urticaceae (1 taxon), Zingiberaceae (1 taxon), Zygophyllaceae (1 taxon). Most of the remedies were prepared from single species. The majority of the plants were obtained from Turkey (about 85.7%).The highest number of taxa were used for cardiovascular-cholesterol (22 plants) disorders, diuretic (20 plants), gastrointestinal (15 plants), respiratory (12 plants) and diabetic (14 plants) illnesses. **Keywords:** Ethnobotany, survey, medicinal plants, Eskişehir

PF26 In vitro and in vivo antioxidant activities of the leaves of *Chrysophyllum albidum*

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Chrysophyllum albidum G. Don. (Sapotaceae) which is distributed in Nigeria is used for the treatment of yellow fever, malaria, diarrhea, vaginal disorders, etc [1]. The study was aimed at investigating the antioxidant

properties using *in vitro* and *in vivo* models. The effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) antiradical activity on ethanol, petroleum ether, ethylacetate, butanol, and water fractions of *C. albidum* was determined. The ethylacetate fraction was purified in column chromatography which led to the isolation and characterization of a myricetin rhamnoside [2]. The structure was elucidated by NMR and mass spectroscopic techniques. Furthermore, ethanol extract was administered to five groups of eight rats per group.The positive control animals were administered with vehicle on the first four days, and with the vehicle and CCl_4 on the fifth, sixth and seventh day [3]. The animals in the treatment category were respectively administered (by gastric intubation) with 500, 1000 and 1500 mg/kg bw of extract & distilled water for the first four days, and with distilled water, extract and CCl_4 on the last three days. Animals were anaesthetized and blood samples were collected for some antioxidant assays. Petroleum ether fraction showed the least antiradical activity (4057.5 ± 809.6 g/kg) while ethyl ether fraction exhibited the highest activity (414.4 ± 92.0 g/kg). Myricetin rhamnoside also exhibited an excellent radical scavenging activity (314.1 ± 60.2). *C. albidum* exhibited significant ($p < 0.05$) differences on the activity of malondialdehyde, catalase, and reduced gluthathione. The plant therefore possesses antioxidant activities and could be employed as natural antioxidant boosters.

PF27 Possible mediators underlying Linalool effect on HepG-2 but not primary hepatocytes: Comparative study

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Linalool is the major component of coriander-sativum seeds. We have recently reported (1) a 100%-decrease in the viability of HepG2 treated with 2 μM linalool. No effect was observed with other cell lines. Linalool resulted in a decrease in the ATP and GSH levels; increase in ROS; and inhibition of ETC-complexes I and II. ROS are known to affect level of UCP2 and ANT. Recent report showed Leukemias cells treated with linalool induced apoptosis mediated by P-53 up-regulation (2). We investigate the effect of linalool on 10ry -hepatocytes, variation in UCP2, ANT and P53 expression in HepG2 and 10ry-hepatocytes. Viability of 1ory-hepatocytes (3), treated with varying concentration of linalool was determined using MTT assay. Expression of P53, ANT, & UCP2 in 10ry-hepatocytes was compared to those of HepG2 cells, using western blotting and was expressed relative to GAPDH. We report that: a) 10ry-hepatocytes were not sensitive to linalool treatment; Comparing 1ory-hepatocytes to HepG2 cells, a 250 fold of linalool concentration was needed to demonstrate a 100% b) Increase in P53 expression was obtained in HepG-2 cells whereas P53 was not detected in 10ry- hepatocytes; c) Down regulated of the expression of ANT and UCP-2 in HepG-2 cells. Linalool effect is specific to HepG2 cells but had no significant effect on 10ry- hepatocytes. There is a role of P53, and the mitochondrial proteins ANT and UCP2 in rendering HepG2 cells more sensitive. Biotransformation into toxic metabolites of linalool by HepG2 cells, but not 10ry -hepatocytes, may not be disregarded. **Acknowledgement:** *Medical Practice Plan and University research Board at the American University of Beirut* **References:** 1. Usta J et al. (2009) Chem- Biol Interact 180: 39 – 46 2. Gu Y et al. (2010) Toxicology 268:19 – 24 3. Schaffner I et al. (2005) Assay Drug Dev Technol 3(1)27 – 38

PF28 The aqueous root extract of *Aristolochia ringens* (Vahl.) prevents chemically induced inflammation

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Aristolochia ringens Vahl Aristolochiaceae belongs to a family with many medicinal uses, but also reported toxic (1). Based on its use in traditional medicine, the antiinflammatory activity of the aqueous root extract of *Aristolochia ringens* (AR) (10 – 100 mg/kg p.o.) was evaluated using the carrageenaan and egg albumin induced rat paw oedema (2), formaldehyde induced arthritic inflammation (3) and xylene induced mouse ear oedema methods (2). AR (10 – 50 mg/kg) produced a dose-dependent decrease in rat paw oedema in the carrageenan and egg albumin induced inflammations at the time intervals studied. The maximum inhibitory effects of AR (50 mg/kg), 57.1% and 65.6% in both experiments were comparable to the 57.9% and 63.9% of standard drugs, indomethacin and diclofenac (10 mg/kg p.o.) respectively. AR (10 – 50 mg/kg) also dose dependently inhibited the arthritic paw oedema induced by formaldehyde over the 10 day period of study. The maximum inhibition by AR (50 mg/kg) (50%) was greater than the 40.8% inhibition by diclofenac (10 mg/kg i.p.). AR (10 – 50 mg/kg) also produced a significant ($p < 0.05$) dose dependent inhibition of mouse ear oedema, with a peak effect at 50 mg/kg of 84.78%, which was greater than the 65.21% inhibition by dexamethasone (1 mg/kg). No mortality was observed in 24 hours, with AR (up to 10 g/kg p.o.), but an LD_{50} of 453 mg/kg was obtained with the intraperitoneal route of administration in mice. Results suggest that the aqueous root extract of *Aristolochia ringens* possesses antiinflammatory activity, inhibiting chemically induced inflammation. **Acknowledgement:** *Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria, Chijioke M.C.* **References:** 1. Pacheco AG et al. (2009) Molecules 14: 1245 – 1262. 2. Adeyemi OO et al. (2002) Fitoterapia 73: 375 – 380. 3. Hosseinzadeh & Younesi (2001) BMC Pharmacol 2: 7 – 14.

PF29 Medicinal plants and their traditional uses in Kabylia (Algeria): an ethnobotanical survey

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This study aims to assess ethnobotanical knowledge in Kabylia, focusing on the use of traditional medicinal plants, at eight rural municipalities in the department of Tizi Ouzou. This region has remained relatively isolated and agro-industrial development is not led to a significant decline in traditional practices, including the use of plants in traditional medicine. Ethnobotanical information was gathered using a questionnaire among herbalists, traditional healers and local populations in the study area. Overall, 98 vascular plants were identified and recorded, a large majority of them live in a wild habitats (forests and wetlands, especially), with the exception of 6 crops. They belong to 48 families, the most represented are the Lamiaceae (13 species) and Asteraceae (12 species). The many diseases listed in the survey are grouped into 10 major disease groups. The most pathologies treated are those of the digestive system (40 plants), skin diseases (29), circulatory system (24) and respiratory system (21). In contrast, the visual system, too precious, is treated with a single plant (*Ocimum basilicum* L.). The toxicity of some herbs used with caution is well known (*e.g. Nerium oleander* L.). Medicinal plants are often multipurpose plants (food, flavor, feed, veterinary, crafts, etc.). Moreover, 31 of these wild plants yet still have an interest in food for rural populations. Finally, a large majority of medicinal plants used in Kabylia are also known for their therapeutic properties in the Mediterranean basin. For example, 73.5% of the plants of this study are cited by the project Rubia. **References:** Gonzalez-Tejero MR et al. (2008) J Ethnopharmacol 116: 341 – 357.

PF30 Evaluation of anti-fertility of *Lawsonia inermis* L. (Lythraceae) roots found in Kaduna State, Nigeria

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Traditional contraceptive practices and use of medicinal plants is a common occurrence in Kaduna State, Nigeria [1]. The use of *Lawsonia inermis* L. (Lythraceae) roots was evaluated for antifertility activity. Extraction of the plant root and phytochemical studies of the extract were carried out according to methods described Evans [2]. Apparently healthy female and male Wistar rats were used. Pre-implantation and mating ratio using methods of Ambali et al. [3] were carried out. Determination of implantation sites was by method of Cavieres et al. [4], determination of Corpora Lutea was the method described by Armanda-Dias et al. [5] and the effects of extract on weight of the rats were also determined. It was observed that the extract effect on contractility of isolated rat uterus was less than oxytocin. There were loss of implantation sites and decrease in body weight. The number of implantation sites showed dose-response relationship significantly ($P <= 0.05$) among

the dose of extract and to standard drug (ethinyl estradiol). There was also significant ($p <= 0.05$) difference observed in the number of corpora lutea in all experimental and control groups. Similarly, there was significant ($p <= 0.05$) difference observed in all the experimental and control groups on percentage pre-implantation loss. Since extracts gave positive tests for steroids, and sex hormones being steroidal compounds, the plants' sterols (phytosterols) may be suspected to be responsible for the anti-fertility effects of the extract. The finding may explain the traditional use of the plant as antifertility agent **Acknowledgement:** *The authors appreciate the support of Dr. S. F. Ambali of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria.* **References:** 1. Samagoro C (2010) MSc thesis, A.B.U., Zaria, Nigeria 2. Evans C (2002) Trease and Evans Pharmacognosy. Saunders Elservier Ltd. London. 3. Ambali S F et al. (2010) Agric Biol J N Amer 1(2): 152 – 155 4. Cavieres F M et al. (2002) Environ Health Perspect 110(11): 1081 – 1085 5. Armanda-Dias L et al. (2001) Braz J Med Biol Resour 34(9): 1209 – 1215

PF31 Assessment of wound-healing, anti-inflammatory and antioxidant activities of *Helichrysum graveolens* (Bieb.) Sweet

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Helichrysum graveolens (Bieb.) Sweet is used for the treatment of jaundice, as a wound-healing and diuretic agent in Turkish folk medicine. In order to prove the claimed utilization of the plant, effects of the extracts and the fractions were investigated by using the *in vivo* linear incision and circular excision wound models. Antioxidant and anti-inflammatory activities, which are correlated to wound healing activity, were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and the acetic acid-induced increased vascular permeability model, respectively. The methanolic extract, which demonstrated potent effect on above-mentioned models, was then subjected to successive solvent extraction with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Each solvent extract was also applied on the same experimental models. The results of the histopathological examination also supported the outcome of both incision and excision wound models. Bioassay-guided fractionation, and thorough phytochemical analysis led to the determination of active principle/s.

PF32 Chilean medicinal plants traditionally used for wound healing therapy studied for activity against resistant *Staphylococcus aureus* strains

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Plants traditionally used for wound healing therapy by the Huilliche people in Chile were investigated for their activity against a selection of *Staphylococcus aureus* strains. *S. aureus* is a frequently encountered pathogen in skin infections and ethnopharmacological knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 30 plant samples of 24 species were collected in the Valdivian rainforest west of Osorno in Chile. Material was extracted with three different organic solvents and antibacterial activity against susceptible and resistant *S. aureus* was evaluated. An agar-overlay diffusion assay and a MIC-determination were utilized for comparative purposes. Total phenolics and tannins were determined and antibacterial contribution of the tannins evaluated. Extracts of 19 species were active against susceptible *S. aureus* at 100 µg extract. At the same concentration 16 species showed activity against resistant *S. aureus*. Extracts without tannins rendered only six samples active. The MIC-determination showed antibacterial activity of 20 extracts on all eight strains, and the highest effect was 64 µg/ml. Species *Aristotelia chilensis* (Mol) Stuntz, *Baccharis magellanica* (Lam.) Pers., *Baccharis sphaerocephala* Hook et Arn., *Berberis buxifolia* Lam. and *Crinodendron hookerianum* Gay being among the most active. Activity against multidrug resistant *Vanthida* strain was remarkable with 36 active extracts. The results support Huilliche traditional knowledge, and the hypothesis that their wound healing plants are potential sources of anti-staphylococcal agents. These results will form the basis for a selection of plant species for further investigation of new antibacterials in the fight against resistant pathogens. **Acknowledgement:** *Robert Leo Skov, SSI, the National Reference Center in Denmark, for strains and helpful advice. Arife Önder and Betül Asar for technical support and Sara Nilean for valuable knowledge during plant collection.*

PF33 *In vitro* antiplasmodial activities and cytotoxicity of water extracts of *Piper rostratum* Roxb., *Sida rhombiforia* Linn. and *Tiliacora triandra* (Colebr.) Diels

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This study aims to evaluate the *in vitro* antiplasmodial activity and cytotoxicity of *Piper rostratum* Roxb., *Sida rhombiforia* Linn. and *Tiliacora triandra* (Colebr.) Diels., herbs traditional used to treat malaria in Thailand. The water extracts of these Thai medicinal plants were tested for their antiplasmodial activity by assessing their ability to inhibit the uptake of [^{3}H] hypoxanthine into the multidrug-resistant strain *Plasmodium falciparum* K1. The antiplasmodial activity was expressed by the concentration that inhibited 50% of parasite growth (IC_{50}). Cytotoxicity of the extracts was determined on Vero cells, and the Cytotoxicity Index ($CI = IC_{50}$ on Vero cells/IC_{50} on *Plasmodium falciparum*) was calculated to evaluate the safety of tested extracts. *Tiliacora triandra* (Colebr.) Diels. was the only one of three plants that showed the *in vitro* antiplasmodial activity ($IC_{50} = 43.43 \pm 0.90$ µg/ml) with good Cytotoxicity Index ($CI = 5.92$) whereas *Piper rostratum* Roxb. and *Sida rhombifora* Linn. did not show this activity. Further study is needed to evaluate an *in vivo* antiplasmodial activity of *Tiliacora triandra* (Colebr.) Diels. extract. **Acknowledgement:** *This study was supported by The Annual Government Statement of Expenditure for Thammasat University.*

PF34 Total phenolics content of the ethyl acetate extract of *Salvia tomentosa*

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Salvia generally including flavonoids is one of the largest genera in Lamiaceae family and this genus has 900 species. It is composed of 88 species in Turkey and mostly benefited in the treatment of skin infections, colds, stomach ache, headache and tuberculosis. In the present study, aerial part of *Salvia tomentosa* Mill. was used and extraction procedure was applied to its ethyl acetate extract. Yield was obtained as percentage (%). In addition, it was investigated for its total phenol content (TPC) and performed by Singleton and Rossi method with a few modifications. Gallic acid was used as standard and results were expressed as micrograms of total phenolics including of extract as gallic acid equivalents (GAE). In conclusion, its TPC was found as 103.75 ± 4.32 GAE µg/mg of extracts. Each datum was calculated as a average of duplicate measurements acquired from at least three separate experiment sets. According to this result, when its total phenol content was compared with other *Salvia* species, *S. tomentosa* showed higher TPC than that of other *Salvia* species. **References:** 1. Kelen M, Tepe B (2008) Bioresource Technology 99: 4096 – 4104. 2. Kivrak I, Dur ME, Öztürk M, Mercan N, Harmandar M, Topçu G (2009) Food Chemistry116: 470 – 479. 3. Singleton V L, & Rossi J A (1965) American Journal of Enology Viticulture 16:144 – 158.

PF35 Proteomics study in *Pueraria mirifica*

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Proteomics are commonly used in direct protein study of tissues, cells and living organisms for functional component analysis. This technique is widely applied in biological science because it provides more information on living systems than the genomics approach. Proteomics are applied by researchers for medical proteomics, pharmaceutical proteomics and plant proteomics. The principles of proteomics comprise of 4 main steps; protein separation, protein identification, protein quantitative and protein analysis. Application of this technique in soybean (*Glycine max*) has established reference map in nodule cytosol in which 69 glycolysis enzymes has been found [1]. In soybean leaf, a total of 71 unique proteins are identified [2]. High levels of flavonoids in soybean leaf are confirmed to be sensitive to UV-B at the proteomics level [3]. Since very few proteomics study is established in herbal plants, we thus will focus on the proteomics study of *Pueraria mirifica* Airy Shaw & Suvatab. or "White Kwao-Krua", an indigenous Thai medicinal plant is traditionally consumed for the treatment of menopausal symptoms belong to legume family the same as soy bean. The plant phytoestrogens and their estrogenic activity have long been investigated. This will enable an investigation into the key proteins related with metabolite production in the Thai herbal plant tissues. **Acknowledgement:** *Thailand Research Fund DBG5180025, Department of Biology, Department of Chemistry, Department of Biotechnology, Laboratory of Biochemistry, Chulabhorn Research Institute* **References:** 1. Oehrle NW, Sarma AD, Waters JK, Emerich DW (2008) Phytochemistry 69: 2426 – 2438. 2. Xu C, Garrett WM, Sullivan J, Caperna T., Natarajan S (2006) Phytochemistry 67: 2431 – 2440. 3. Xu C, Sullivan J, Garrett WM, Caperna TJ, Natarajan S (2008) Phytochemistry 69: 38 – 48.

PF36 Phytoconstituent of Petroleum Ether Extract of *Atriplex lindleyi* Moq. aerial Part and Its Hepato -Renal protection

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The work aimed the detailed description of the lipoidal profile and hepato-renal protective effect of *Atriplex lindleyi* Moq. aerial part against bromobenzene (BB) intoxication in rats. Column chromatography of petroleum ether (60 – 80) extract and GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters were qualitatively and quantitatively investigated. Oxygenated and non-oxygenated hydrocarbons, alcoholic, phenolic, steroidal and triterpenoidal compounds were identified in the petroleum ether extract. GC/MS analysis of fatty acid methyl ester led to identification of 20 compounds. *In vivo* examination of the petroleum ether extract against bromobenzene (BB) intoxication using hepaticum as a reference drug was included.five groups of albino rats were selected in this study. Group1: normal control group, group 2: *i.p* injected with BB (460 mg/kg b.wt) two times/week for three weeks, group 3: received oral doses of plant extract (150 mg/kg b.wt.) at the same time and duration of BB injection. Group 4: served as group 3 and treated with hepaticum® (Medical Union pharmaceutics company, Egypt) (100 mg/kg b.wt.) as a reference drug. Group 5: received plant extract only. The Drastic changes observed after BB intoxication in liver function enzymes (AST, ALT and ALP), hepatic cell organelles marker enzymes (SD, LDH, G-6-Pase, AP and 5'- nucleotidase), kidney disorder parameters (creatinine and urea) and certain antioxidants; glutathione, lipid peroxide and superoxide dismutase. Treatment with petroleum ether extract improved all biochemical parameters under investigation as well as the histopathological chromatogram of liver and kidney. The petroleum ether of *A. lindleyi* contains bioactive compounds exhibiting hepato-renal protective effect. **References:** 1. El-Sharaky AS, Newairy AA, Kamel MA, Eweda SM (2009) Food Chem Toxicol 47(7): 1584 – 90. 2. Shaker E, Mahmoud H, Mnaa S (2010) Food Chem Toxicol 48(3): 803 – 6. 3. Said O, Fulder S, Khalil K, Azaizeh H, Kassis E, Saad B (2008) Evid Based Complement Alternat Med 5(4): 421 – 428.

PF37 In vitro effect of purified plumbagin of *Plumbago indica* against motility of *Paramphistomum cervi*

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The crude extract of *Plumbago indica* L. has been used as Thai traditional medicine for treating digestive tract disorders [1]. Reportedly, the effect of purified plumbagin of *P.indica* inhibited the motility of *Caenorhabditis elegans* and infective cercariae stage of *Schitosoma mansoni* [2]. However, the anthelmintic activity of plumbagin on *P.cervi* has not been studied. Therefore, this work aimed to investigate the effect of purified plumbagin of *P.indica* root on adult *P.cervi* was evaluated after incubating parasite in M-199 medium containing plumbagin in the serial concentrations of 10 fold dilution of 100 μg/ml, for 3, 6, 12 and 24 h, relative motility assay and histopathological changes. It was found that the complete inhibition of worm motility and subsequent mortality was observed at 100 μg/ml, in early 3 h observation. The motility of *P.cervi* was progressively decreased since 3 to 12 h exposure, and few activity of *P.cervi* was observed at 24 h exposure at the concentraton 0.1,1.0 and 10.0 μg/ml, respectively. Observation under the stereo-microscope, adult *P.cervi* appeared brown to black color, numerous blebs and peeling of tegument after 12 h exposure. Light microscopic observation showed the numerous blebs, erosion and desquamation of tegument of P.cervi. They also revealed the cecal epithelial cell lining detachment. Although, the previously report that crude extract of *Artocarpus lakoocha* Roxb.affected on *P.cervi* [3], but its dosage is higher than plumbagin. These results suggest that plumbagin of *P. indica* could be against the motility of adult stage of *P.cervi* better than *A.lakoocha*. **Acknowledgement:** *This work was supported by the Thailand Research Fund (Senior Research Scholar Fellowship to Prof. Prasert Sobhon), Mahidol University and research grants from Suranaree University of Technology.* **References:** [1] Wuttidhammaved W (1997) The Encyclopedia of Medicinal Plants of Thailand: The Thai Traditional Medicine, The O-dien Store Publishing Co., Ltd, Bangkok, Thailand (Published in Thai), 681. [2] Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ (2009) J Ethnopharmacol 123(3):475 – 82. [3] Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V, Sobhon P (2009) Exp Parasitol 122(4):289 – 98.

PF38 *Diospyros lotus* L. fruit extract protects G6PD-deficient erythrocytes from hemolytic injury *in vitro* and *in vivo*: prevention of favism disorder

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Favism is a life-threatening hemolytic crisis that can result from the ingestion of fava beans by susceptible individuals who have G6PD enzyme deficiency (1). The aim of this study was to evaluate the protective effect of *Diospyros lotus* L. fruits extract against the hemolytic damage induced by fava beans extract in both G6PD enzyme-deficient human and rat erythrocyte in vitro and in vivo models. in vitro model, venous blood samples were obtained from five subjects with known G6PD deficiency, which was also confirmed with standard techniques. Erythro-

cyte hemolysis was induced by fava beans extract in the presence and absence of *Diospyros lotus* fruits extract. The hemoglobin release in the supernatant, as well as the value of the hematocrit was determined by recording optical density at 540 nm in a spectrophotometer and microhematocrit, respectively.in vivo model, G6PD enzyme deficiency was induced in rats by intraperitoneal (*i.p.*) injection of DHEA (Dehydroepiandrosterone) (100 mg/kg), a specific G6PD enzyme inhibitor, for 35 consecutive days (2). Then the animals were pre-treated with different doses of *Diospyros lotus* (500, 750, 1000, and 1500 mg/kg) by oral administration for seven consecutive days after induction of G6PD deficiency. Rats were administered orally on the seventh day with Vicia faba beans extract (40 mg/kg b.w.), the blood was removed for evaluation of its value of erythrocyte hemoglobin and hematocrit after one hour. Our results have shown that *Diospyros lotus* fruits extract with an antioxidant activity has protective effect against hemolytic damage induced by fava beans extract in both G6PD-deficient human and rat erythrocytes. **Acknowledgement:** *The in vitro modeling of this investigation was the subject of Pharm. D thesis of Emran Habibi as a student of the Mazandaran University of Medical Sciences. This research was supported by a grant from the Research Council of Mazandaran University of Medical Sciences.* **References:** 1. Neto EC et al. (1993) Hum Genet 91: 293 – 294. 2. McIntosh MK (1993) J Nutrition 123: 216 – 224.

PF39 Ethnopharmacological evaluation of male contraceptive efficacy of *Dendrophthoe falcata* in albino rats

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Search for male-antifertility agent in plants remains a potential area of investigations. Though, antispermatogenic activity has been reported in some plants, only few are reported.. Therefore, present study was undertaken to evaluate the contraceptive-efficacy of *Dendrophthoe falcata* (L.f.) Ettingsh. in male albino rats as reported in folk remedies[1]. Shade-dried stems of *D. falcata* were extracted in methanol and then fractionated with different solvents. A part of isolated-fractions were also processed for various phytochemical-techniques to identify active constituents. Spectral studies revealed that it has one compound in major quantity i.e. kaempferol, a natural flavonol[2,3]. Male rats were gavaged with kaempferol rich fraction at 50 mg/rat/day for 60days. On day 61st animals were autopsied and testes, epididymides, seminal-vesicle and ventral-prostate were dissected out and weighed. Sperm motility and density were assessed. Biochemical and histological analysis were also performed. A marked reduction in weight of testes, epididymides, seminal vesicle and ventral prostate was observed. The sperm motility and density were significantly reduced. The histoarchitecture of testes revealed degenerative changes in the seminiferous-tubules and arrest of spermatogenesis at the stage of round-spermatids. A marked alteration in Leydig cell differenzial counts was also noticed. Serum-testosterone levels were decreased significantly. Protein, glycogen, sialic acid, acid and alkaline phosphatase content of testes, protein and sialic in cauda and seminal vesicular fructose was decreased, whereas testicular-cholesterol increased significantly. Results of present study showed a significant effect of *D. falcata* on testicular and epididymal spermatozoa. Findings of present study supports the use of this plant in folk remedies as an antifertility agent. **Acknowledgement:** *Author Dr. Jai Bahadur Singh Kachhawa, acknowledges the financial assistance from UGC, New Delhi for Dr. D.S. Kothari Postdoc Research Fellowship.* **References:** 1. Jain SK, Defillips R. (1991) Medicinal plants of India. Vol. 1. Reference Publication.Inc Michigan, pp. 392. 2. Kumar, Dixi and Khanna P (1989) Plantes Medicinales et Phytotherapie 23: 193 – 201. 3. Wang H et al. (2008) IUBMB Life 60(8): 549 – 554.

PF40 Popular medicinal plants in Iran for the treatment of GI disorders

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This paper is a collection of plant samples and their derivative products (gum and extracts) that have been used traditionally for Centuries to treat GI disorders as it has been mentioned in Iranian old reference books Considering today's applications of these medicinal plants, reveals the great value & effect of traditional medicine in Iran. Of course, GI disorders, according to ancient texts in Iran, include: different types of abdominal pains due to flatulence and gastritis types, Constipation, diarrhea, stomach cramps, which can be classified according to the traditional treatments and their comparison with the current one. These plants can include: Labiatae (*Mentha spicata* L., *Zataria multiflora* Boiss.), Compositae (*Matricaria chamomilla* L.), Umbelliferae (*Carum carvi* L., *Pimpinella anisum*L.), Zingiberaceae (*Zingiber officinale* Roscoe, *Curcuma longa* L.), Anacardiaceae (*Pistacia lentiscus* L., *Pistacia atlantica* Desf.), etc. We selected 2 – 3 sample, from each family. Most important parts of these plants are their leaves with 35%, then their fruits about 30%, roots & Rhizomes with the lowest standing 10%, and the whole plant is about 20%. Of course, gums and other parts of plant such as flowers, with the very low percentage, are also used in the treatment of GI disorders. In this case, the leaves are consumed as boiling in the first stage and taking into distillates (aqueous and alcoholic extract) in the second stage Most of these plants are used as carminative and antispasmodic and sometimes antidiarrheal ones, and in comparison with chemical drugs (Pantoprazole, Dicyclomine, Sucralfate, Magnesium Hydroxide. they have the same or sometimes better effects. **Acknowledgement:** *Farsam H. Amin GH of School of Pharmacy, Tehran University of Medical Sciences*

PF41 Antioxidant potential of *Arnica montana* and *Urtica dioica* hydroalcoholic extracts on mouse fibroblasts *in vitro*

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The use of medicinal plant derivatives is an alternative to conventional medicine to treat diseases associated with oxidative stress [1 – 3]. In this study, we have determined the total phenolic and flavonoid contents of the hydroalcoholic extracts obtained from two Romanian traditional medicinal plants: *Arnica montana* L. and *Urtica dioica* L. and we assayed their antioxidant capacity using TEAC and ORAC methods. The effect of the two plant extracts on mouse fibroblasts (NCTC clone 929 cell line) growth, as well as their antioxidant protective effect against hydrogen peroxide-induced cell damage were also investigated. The degree of fibroblast growth and protection against hydrogen peroxide damage was quantified by Neutral Red and LDH assays. The results showed that the studied extracts presented high phenolic and flavonoid content values and possessed a good ability to scavenge free radicals. Both plant extracts had a significant dose-dependent effect on the growth of mouse fibroblasts up to a concentration of 100 µg/ml for *Arnica montana* and 500 µg/ml for *Urtica dioica*; higher concentrations of extracts were toxic to the cells. The extracts have also protected fibroblasts against oxidative damage. Treating the cells with *Arnica montana* and *Urtica dioica* for 24 h prior to 0.05 mM hydrogen peroxide increased the cell viability from 52% in hydrogen peroxide treated cells to more than 80%. The results obtained in cell culture correlated well with the antioxidant potential of the plant extracts. Our data indicate that the studied plants may be useful as agents for skin diseases caused by oxidative stress. **Acknowledgement:** *This study was supported by Romanian Project PN II 62059.* **References:** 1. Lee HS et al. (2005) Biol Pharm Bull 28: 1639 – 1644 2. Svobodova A et al. (2006) Burns 32: 973 – 979 3. Annan K et al. (2008) J Ethnopharmacol 119: 141 – 144

PF42 *In vitro* effect of Myrrh extracts on the viability of *Schistosoma mansoni* larvae

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Schistosomiasis, a parasitic disease caused by trematode flatworms of the genus *Schistosoma*, represents a growing concern in the Sub-Saharan Africa, where up to 80% of the population is infected. Mirazid®, a commercial drug obtained by combination of two solvent extracts of Myrrh, the oleo-gum-resin from the stem of *Commiphora molmol* Engl. ex Tschirch (Burseraceae), is marketed in Egypt since 2001 as an alternative treatment for schistosomiasis [1]. However, recent independent studies question its efficacy. All experiments conducted with Myrrh so far are either *in vivo* tests or clinical trials, but no *in vitro* data is available. In order to shed light into controversy around Myrrh, two commercial Myrrh samples (from S. Africa and M. East) were extracted and/or combined as described a) for Mirazid®; first with petroleum ether (A), subsequently with MeOH (B) b) hydrodistillation to yield volatile oil c) for myrrh (8.5 parts of resin + 3.5 parts of volatile oil) [2 – 4]. They were also extracted with MeOH and then partitioned between hexane, $CHCl_3$ and aqueous MeOH. As the combination ratios of the extracts A+B in Mirazid® are unclear, extracts A/B were combined in simple ratios. In the medium throughput visual *S. mansoni* larval assay, all lipophilic extracts, the combinations, and the essential oils showed moderate, but differenzial activity (IC_{50}s 7.18 – 32.69 µg/ml). The extracts and the essential oils were also different phytochemically (by TLC, ^{1}H NMR, GC-MS). This study shows that Myrrh has antischistosomal potential, but the origin of the plant material and extraction method is of importance. **References:** 1. Badria F et al. (2001) Pharm Bio 39: 127 – 131. 2. Massoud A et al. (1998) Parasitol Int 47: 105 – 131. 3. Sheir Z et al. (2001) Am J Trop Med Hyg 65: 700 – 704. 4. Abdel-Hay MH et al. (2002) Spectroscopy Lett 35: 183 – 197.

PF43 Evaluation of four traditional Romanian medicinal plants as wound healing agents

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Achillea millefolium L., *Hyssopus officinalis* L., *Equisetum arvense* L. and *Echinacea purpurea* (L.) Moench, are four medicinal plants traditionally used in Romania for the treatment of skin disorders and wound healing. In order to support their use for new plant derived products the aim of this study was to evaluate their potential to stimulate the wound healing process. We investigated the four herbal extracts by HPLC-MS for the presence of polyphenolic compounds, we also assessed the antioxidant activity by the DPPH photometric method, BHT was used as positive control (1) and by Sircol assay we evaluated the rate of soluble collagen produced in cell culture medium by fibroblasts (cell line L-929) treated with various concentrations from 35 µg/ml to 140 µg/ml of herbal extract. The HPLC-MS analysis revealed the presence of antioxidant compounds, phenolic acids and flavonoids such as: rosmarinic acid, chlorogenic acid, caffeic acid, luteolin -7-O- D glucoside, apigenol 7-O- glucoside, rutin and apigenol. High antiradical capacity was detected in ethanolic extracts and flavonoids and phenolic acids may be reasonable. Collagen excretion was increased in culture medium of fibroblasts treated with herbal extracts when compared to the culture medium of untreated cells. At the highest concentration of herbal extract, the highest collagen synthesis was observed which was almost 2 times higher as compared to the synthesis of untreated cells. The current study explains the medicinal utility of these plants due to their antioxidant activity and to their ability to stimulate the collagen synthesis, activities that would accelerate the wound healing process. **Acknowledgement:** *This study was supported by Project PN II 62071* **References:** 1. Huang HJ, Cheng HJ (2005) Bot Bull Acad Sin 46: 99.

PF44 Study of apoptosis induction effects of traditional remedies and quality control strategies

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Traditional medicine is an important part of health care system in Vietnam [1]. Nevertheless, lack of scientific and therapeutic evidences as well as quality control system limit its development. Many anticancer products used in cancer therapy act by inducing apoptosis in cancer cells [2]. In addition to chromatographic analysis with standard compounds, biological response fingerprinting are suggested for quality control of traditional formulations [3]. We investigated five traditional remedies reported in traditional pharmacopoeia as having anticancer effects. We determined apoptosis inducing capacity and cell cycle arrest of these remedies and their components on HeLa cells by DNA fragmentation assay, fluorescence microscopy, caspase activity assay and flow cytometry-based method. We showed that the modified remedy "Hoang Lien Giai Doc Thang" (HLGDT) caused cell death by inducing apoptosis, independently of caspase-3 activation. Three components, *Coptis sinensis* Franch, *Scutellaria baicalensis* George and *Phellodendron amurense* Rupr have higher cytotoxicity than the whole remedy on HeLa cells. Microarray data analysis performed on HeLa cells treated with HLGDT for 24 and 36 hours showed differenzial, increased or decreased, expression of 408 genes. Some overexpressed genes – *DDIT3, TRIB3, FAM129A, STC2, GDF15, SERPINE2* were reported as involved in ER-stress. Expression level of these genes was confirmed by real-time RT-PCR. Real-time RT-PCR amplification of these genes are furtherused to set up biological fingerprints. Baicalin and berberin were used as chemical fingerprints through chromatographic analysis. These fingerprints could be considered for quality control purposes of the remedy. **Acknowledgement:** *These work was supported by grants from the Department of Science and Technology – Ho Chi Minh City. We are grateful to Prof. Sangho Lee and the Microarray platform from Sungkyunkwan University for microarray analysis* **References:** 1. WHO (2002). WHO traditional medicine strategy 2002 – 2005. 2. Fulda S (2010) Planta Med 76(11): 1075 – 9 3. Chavan P, Joshi K, & Patwardhan B (2006) eCAM: 1 – 11

PF45 Evaluation of the effects of *Parinari curatellifolia* seed and *Anthocleista vogelli* root extracts individually and in combination on postprandial and alloxan-induced diabetes in animals

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Parinari curatellifolia Planch. ex Benth. seed and *Anthocleista vogelii* Planch.roots extracts mixture (1:1) have been used locally for the treatment of diabetes. The postprandial effects were evaluated on albino rats (20) randomly distributed into four groups. Each received orally 500 mg/kg of the extract mixture, *P. curatellifolia* and *A. vogelli* respectively and the control 0.5 ml (2% w/v) acacia solution. Blood glucose levels were monitored at 30, 60, and 120 min. intervals as described by Ogbonnia et al (1). Twenty five diabetic albino rats with plasma glucose>= 200 mg/dl were randomly divided equally into five groups and treated orally for 30 days as follows: Groups I, II and III received orally 500 mg/kg body weight of the mixture of *P. curatellifolia* and *A.vogelii* respectively while group IV received glibenclaimide 600 µg/kg body weight (2), while V diabetic control received 0.5 ml acacia solution. Results showed a significant reduction ($p <= 0.05$) in postprandial plasma sugar level after 30 min in all treatments. Also significant reductions ($p <= 0.05$) in the plasma glucose, LDL-cholesterol, AST and ALT levels, and increase in HDL-cholesterol were observed in the treated diabetic groups. The pancreas tissue of diabetic animals treated with the extract mixture showed marked necrotic changes while pancreatic tissue of diabetic untreated animals showed more severe necrosis of beta cells which formed mass of amorphous oesinophilia. The glibenclamide treated animals showed

spots of necrotic changes; otherwise they had predominantly viable beta cells. The results showed that the extracts and mixture had both good hypoglycaemic activity and beneficial effects on cardiovascular risk factors. **Acknowledgement:** *I would like to acknowledge traditional medical practitioners T.U Agu and GO Ozougwu for supplying me the plant materials and information on their ethnobotanical uses* **References:** (1) Ogbonnia SO, Odimegwu JI and Enwuru VN (2008) Afr J Biotech 7(15): 2935 – 2939 (2) MahdiA A et al. (2003) Indian J Clinical Biochem 18 (2): 8 – 15

PF46 *In vitro* screening of selected medicinal plants against *Schistosoma mansoni* larvae

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Schistosomiasis, caused by various members of the trematode flatworms (*Schistosoma* species) is the second most important parasitic disease next to malaria. Resistance and low susceptibility towards praziquantel, the only available schistosomicidal drug, urge the search for new drugs. This study aimed at assessing the in vitro schistosomicidal effects of several medicinal plants traditionally used for the treatment of schistosomiasis or other helminths [1]. The crude MeOH extracts of selected plants *Artemisia absinthium* L. and *A. abrotanum* L. (aerial parts) *Phytolacca dodecandra* L. (roots), *Curcuma longa* L. (roots), *Zingiber officinale* Roscoe (roots), *Punica granatum* L. (peels, fruits and the aqueous extract of the fruits) and *Citrus reticulata* Blanco (peels) were tested against juvenile worms (schistosomulae) of *S. mansoni* by using the standard visual larval assay. All crude extracts, except *P. granatum* and *C. reticulata* exhibited significant antischistosomal effect. The highest activity was displayed by *C. longa* (IC_{50} 4.06 μg/ml), followed by *A. abrotanum* and *Z. officinalis* extracts with IC_{50} values of 11.1 μg/ml and 11.73 μg/ml, respectively. In the next step, the crude MeOH extracts were subjected to a liquid-liquid partitioning scheme between aqueous MeOH, hexane and $CHCl_3$ and the subextracts were retested in the same assay. Generally, the lipohilic subextracts retained the initial schistosomicidal potential, whereas the aq. MeOH subextracts were mostly inactive at 100 μg/ml concentration. The current study highlights the potential of plants against *Schistosoma* infections and confirms their use in traditional medicine. It also warrants phytochemical studies on the active plants to identify their active principles. **References:** 1. Sanaa AA (2011) Res J Med Plant 5: 1 – 20.

PF47 Changing of some elements during phonological stages in *Nitraria Schoberi* L

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Nitraria Schoberi L. from Nitrariaceae as pharmaceutical, industrial and fodder plants can tolerate harsh environmental conditions especially sandy and saline soil. This plant is natural sources of some important chemical components like schoberine nitraraine, dihydronitraraine, nitraramine, nitraroxine and etc. More than 25 Mha of salt affected land exists in Iran and this plant can offer an economic and practical alternative towards achieving saline land and water resources in order to produce natural chemical components for pharmaceutical industries. Changing in some elements (Nitrogen, Phosphor, Sodium, Potassium, and Calcium) during flowering and forming seed stages (2009) were investigated. Samples of the aerial part of *Nitraria Schoberi* collected in the Kashan region were dried, grained and analyzed in Laboratory. The results showed that elements level indices values including P, K, Ca and N were significantly differed among phenological stages but Na did not show any difference between two stages. Decreasing in P, K and N showed chemical component of this plant could change by sequence of phenological stages and finally these changes affect on quality of fodder and medicinal properties.

PF48 Phytochemical characterization of antimycobacterial crude extracts from medicinal plants traditionally used in Mozambique

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A number of medicinal plants have long been used by traditional healers to treat tuberculosis and related diseases in Mozambique [1,2]. The present study was aimed to evaluate selected medicinal plants for their in vitro antimycobacterial activity, and reveal the main classes of compounds which might account for the observed activity. Four organic solvents (n-hexane, dichloromethane, ethylacetate, and 70% ethanol) were used for the sequential extraction. Decoction of each plant material was prepared according to traditional use. Different species of mycobacteria, namely, *M. smegmatis*, *M. bovis* BCG, *M. avium,* and *M. tuberculosis* were employed to screen extracts by broth microdilution method. The cytotoxicity against human macrophages from the monocytic THP-1 cells was also evaluated. Overall, n-hexane extracts of *Maerua edulis* Gilg & Gilg-Ben. and *Securidaca longepedunculata* Fresen, ethyl acetate extract of *Tabernaemontana elegans* Stapf and dichloromethane extract of *Zanthoxylum capense* (Thunb.) Harv. were found to possess considerable activity against *M. bovis* BCG and *M. tuberculosis* H37Ra with MIC = 15.6 – 62.5 μg/mL. *Tabernaemontana elegans* ethyl acetate extract displayed strong activity against *M. tuberculosis* H37Rv (MIC 15.6 μg/mL) as well as potent cytotoxic effects in THP-1 cells (IC 50 < 4 μg/mL). Based on 1 H NMR spectroscopic analysis, major components in both *Maerua edulis* and *Securidaca longepedunculata* n-hexane extracts were linear chain unsaturated fatty acids. *Zanthoxylum capense* dichloromethane extract contained more complex constituents (mostly phenolic compounds), and the prominent compounds in ethyl acetate extract of *Tabernaemontana elegans* were identified as indole alkaloids. **Keywords:** Antimycobacterial activity, medicinal plants, Mozambique, tuberculosis
Acknowledgement: *This study was supported by FCT, Portugal (SFRH/BPD/37179/2007; PTDC/SAU-MII/098024/2008).* **References:** 1. Bandeira SO et al. (2001) Pharm Biology 39:70 – 3. 2. Janse, PCM, Mendes O (1982, 1983, 1990, 1991) Plantas Medicinais-Seu Uso Tradicional em Mocambique. Gabinete de Estudo da Medicina Tradicional. Maputo.

PF49 Genome wide expression analysis of the effect of wwcsw, a traditional Korean herbal formula, on rat intracerebral hemorrhage

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Woo-whang-chong-shim-won (WWCSW) is a traditional Korean herbal formula which is commonly used for treating patients with hypertension, arteriosclerosis, coma and stroke in China and Korea. WWCSW is composed of various kinds of chemical components, it would be difficult to isolate major components having pharmaceutical effect. Therefore, high throughput screening systems such as microarray analysis is essential process to elucidate the molecular effects of herbal extract on animal disease model. In this experiment, we measured the effect of WWCSW on ICH in rat using microarray technology. ICH was induced by injection of collagenase type IV, and total RNA was isolated. Hierarchical clustering was implemented using CLUSTER and TREEVIEW program, and for Ontology analysis, GOSTAT program was applied in which p-value was calculated by Chi square or Fisher's exact test based on the total array element. WWCSW-treatment restored the gene expression

altered by ICH-induction in brain to the levels of 76.0% and 70.1% for up- and down-regulated gene, respectively. Co-regulated genes by ICH model of rat could be used as molecular targets for therapeutic effect of drug including WWCSW. **References:** 1. Choi EW et al. (2000) Journal of Applied Pharmacology 8: 255 – 61. 2. Kim BY et al. (2009) Korean J Orient Int Med 30(3): 594 – 60. 3. Andrew IS et al. (1979) Progress in Cardiovascular Diseases 22(1): 31 – 52.

PF50 Isoflavonoid biosynthesis in *Pueraria mirifica* leaves

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Pueraria mirifica Airy Shaw & Suvatab. is a Thai indigenous herb with long-term consumption among Thai menopausal women for menopausal treatment. Researches in the tubers of this plant are mostly focused on their estrogenic potency and application to human health. The tubers are sources of active ingredients including the potent estrogenic miroestrol and deoxymiroestrol, and also isoflavonoids, however, the plant tubers show limited growth rate. Isoflavonoids are the abundance secondary metabolites in *P. mirifica* [1], [2] which play important roles in estrogenic effects [3] in animal assays, especially daidzein and genistein are potent anti-cancer, including breast cancer [4]. This group of chemicals is also needed for dietary supplement and cosmetic products. The plants produce a lot of leaves during their growth and development. In this study, the leaves were collected for 12 consecutive months and tubers were collected for every 4 month. The leaves were dried and extracted for isoflavonoids in the absence of chlorophyll for HPLC analysis together with the dried tubers. The analysis revealed that plant leaves contain significant amount of isoflavonoids. Thus it would initiate impact not only on isoflavonoid extraction industry but also commercialized products derived from these chemicals. In addition, proteomics approach is introduced in our study which resulted in finding some interesting appearance proteins in the plant tubers. **Acknowledgement:** *Thailand Research Fund DBG5180025.* **References:** 1. Cherdshewasart W, Subtang S, Dahlan W (2007)J Pharm Biomed Anal 43: 428 – 434. 2. Cherdshewasart W, Sriwatcharakul S (2007) Biosci Biotechnol Biochem 71: 2527 – 2533. 3. Cherdshewasart W, Sriwatcharakul S, Malaivijitnond S (2008) Maturitas 61: 350 – 357. 4. Cherdshewasart W, Kitsamai Y, Malaivijitnond S (2007)J Reprod Dev 53: 385 – 93.

PF51 Larvicidal and antimalarial activity of some Zulu medicinal plants

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Gardenia thunbergia T.A Sprague, *Siphonochilus aethiopicus* (Schweif.) B.L Burt, *Schotia brachypetala* Sond., *Acorus calamus* L., *Withania somnifera* (L) Dunal in DC., *Elaeodendron transvalense* (Burtt Davy) R.H. Archer, *Hypoxis hemerocallidea* Fisch., C.A. Mey. & Ave-Lall, *Vernonia adoensis* Sch. Bip. Ex Walp.and *Acanthosperum australe* (Loefl.) Kuntze are medicinal plants commonly used by traditional healers in South Africa to treat malaria. Aqueous, dichloromethane and methanol extracts of these plants were screened for larvicidal, antioxidant, *in vivo* antipyretic, and *in vitro* antiplasmodial activities. The plant extracts either killed or reduced spontaneous movement in *Culex quinquefascitus* larvae after 24 hours following treatment. Methanol extracts exhibited antioxidant (DPPH, ABTS scavenging, Fe^{2+} chelating) activity, albeit to varying degree of efficiency. The dichloromethane and methanol extracts significantly ($p <= 0.05$) reduced pyrexia with activity increasing in a concentration dependent manner. The antiplasmodial activity against chloroquine sensitive strain of *Plasmodium falciparum* (D10) showed that the methanol extracts of *G. thunbergia*, *V. adoensis* and the dichloromethane extracts of *E. transvalense*, *A. australe* and *W. somnifera* were active (IC_{50} of 1.04 – 5.07 μg/ml). The results suggest that these plants contained constituents that could be developed as potent antimalarial drugs (mosquito larvicide, anti-fever and anti-plasmodial). Possibly, the compounds target metabolic pathways common to the *C. quinquefascitus* larvae and *P. falciparum*. **Acknowledgement:** *University of Zululand Research Committee Medical Research Council, South Africa*

PF52 Ethnobotanical studies in Astor valley, Nanga Parbat, Pakistan

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Astor valley adjoin the eastern part of Nanga Parbat (Pakistan) consists of more than 100 villages. Local communities are dependent on surrounding plant resources for fruits, vegetables, fodder, shelter and healthcare. Data was collected through questionnaire and interview with 30 respondents from three villages Khumadas, Gorikot and Harcho. In total 33 plant species were recorded along with their local names, part used, purpose and diseases treated. The most commonly used medicinal plants are *Mentha longifolia* Huds. (Phhileel), *Thymus linearis* Benth. (Tumoro), *Saussurea petiolata* Komarov ex Lipsch. (Mumiran), *Berberis lyceum* Royle (Ishkeen), *Cichorium intybus* L. (Chitay iskanaji), *Swertia petiolata* Royle (Mumiran), *Viola pilosa* Blume (Lillio), *Ferula narthex* Boiss. (Sup), *Bergenia ciliata* Stein (Sanspur). The medicinal plants are used for stomach problem, cough, asthma, acidity, dysentery, pneumonia, cold, fever, blood pressure, eye diseases, dental diseases, swelling, anti-allergic, burning, skin problems, typhoid *etc*. The area depleted, due to over-exploitation of herbs and trees should be protected completely and attempt should be made to cultivate these plants at different localities and elevations. There is need to assess and identify the factors affecting biodiversity and indigenous knowledge system for mountain natural resource utilization and conservation. **Acknowledgement:** *We are thankful to the people who participated in the study.* **References:** Khan W S and Khatoon S (2007) Pakistan Journal of Botany 39(3): 699 – 710

PF53 The determination of total phenolics and flavonoid contents, and antioxidant activity of some sage populations of *Salvia fruticosa* Mill., *Salvia pomifera* Mill. and *Salvia tomentosa* Mill. in the Marmara region of Turkey

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Methanolic extracts of 40 different population of three species of Salvia (*Salvia fruticosa* Mill. 20 samples, *Salvia pomifera* Mill. 5 samples and *Salvia tomentosa* Mill. 15 samples) were analyzed for their antioxidant properties. Samples were collected from different natural ecological areas in Marmara Region in Turkey. The antioxidant capacity (TAC) was investigated with the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and Flavonoid contents in the extracts were determined by a colorimetric method. The TAC values of the spices ranged from 288. 57 to 3608.32 μmol (TE)/100 g dw. The total phenolic and flavonoid content ranged from 488.07 to 3277.97 mg of gallic acid equivalents (GAE)/100 g DW and 664.03 to 4046.77 mg of catechin equivalents (CE)/100 g DW, respectively.

PF54 An investigation of the contents of phenolics, flavonoid compounds and antioxidant activity of some wild mushrooms

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The antioxidant activity and properties of 12 wild mushrooms (*Lactarius piperatus* (L.) Pers., *Tricholoma calignatum* (Viv.) Ricken, *Amanita caesarea* (Scop.) Pers., *Lactarius delicious* (L.) Gray, *Lactarius salmonicolor* R. Heim & Leclair, *Cantharellus cibarius* Fr., *Hydnum repandum* L., *Picoa lefebvrei* (Pat.) Maire, *Ramaria aurea* (Schaeff.) Quel., *Lactarius semisanguifluus* R. Heim & Leclair, *Craterellus cornucopioides* (L.) Pers., *Laccaria laccata* (Scop.) Fr.) collected from Turkey were evaluated. Their methanolic extracts were used to determine antioxidant capacity (TAC), total phenolics and flavonoids. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were measured to evaluate antioxidant capacity of the extracts and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and Flavonoid contents in the extracts were determined by a colorimetric method. Wild mushrooms were found to be high in antioxidant phytochemicals, such as phenolics (575.10 – 2156.40 mg GAE/100 g DW), flavonoids (103.01 – 346.53 mg CE/100 g DW). The TAC values of the spices ranged from 525.32 to 1693.85 μmol (TE)/100 g DW. and the antioxidant activity was found to vary in the order: *Hydnum repandum* L.> *Ramaria aurea* (Schaeff.) Quel.> *Lactarius salmonicolor* R. Heim & Leclair > *Craterellus cornucopioides* (L.) Pers. > *Lactarius delicious* (L.) Gray > *Lactarius piperatus* (L.) Pers. > *Picoa lefebvrei* (Pat.) Maire > *Tricholoma calignatum* (Viv.) Ricken > *Amanita caesarea* (Scop.) Pers. > *Cantharellus cibarius* Fr.> *Laccaria laccata* (Scop.) Fr. > *Lactarius semisanguifluuss* R. Heim & Leclair.

PF55 Identification of proteins in preparations of *Candida* species used in homeopathic medicinal products

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Preparations with *Candida albicans* and *C. parapsilosis* have been safely used as active substances in isopathic remedies for over 30 years. Isopathy is a special kind of homeopathy [1]. Water-soluble fractions after cell mill treatment and different purification steps are obtained from both yeast and named e volumine cellulae (lyophil., steril.) [2]. D3 to D5 potencies of these starting materials are used *e.g.* for the treatment of eczema or mycotic skin disorders (marketing authorizations for different dosage forms of Albicansan and Pefrakehl in Switzerland). The aim of the study was to characterize the protein fraction of the active substances. The proteins in the extracts were quantified by colorimetric assays according to standard protocols [3]. This revealed an amount of 11% (C. parapsilosis) and 35% (C. albicans) of protein of the whole dry mass. The proteins were then identified by LC-MALDI mass spectrometry in a bottom-up approach. The crude protein extract was directly digested using trypsin followed by separation of the peptides by nano-rpHPLC coupled to mass spectrometry. 99 proteins from *C. albicans* and 131 proteins from *C. parapsilosis* could be identified. According to the gene ontology data, approximately 80% of the proteins belong to cellular metabolic processes. **References:** [1] Wiethoff K, Kracke A (2010) Report Naturheilkunde 14: 56 – 60 [2] Bader G, Akkoyun A, Wiethoff K (2010) Planta Med 76: 1262 [3] Lowry OH et al. (1951) J Biol Chem 193: 265 – 275

PF56 *In vitro* activity and chemical characterization of an apolar fraction of *Morus alba* leaf hot water extract

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White mulberry (*Morus alba* L.) leaf is a well known traditional medicine of type II. diabetes, a progressive disease with a broad spectrum of complications which has increasing incidency worldwide. Most typically it is taken as tea, often in combination with other phytotherapeutics. Many constituents were found to contribute to the antidiabetic activity of mulberry leaf, including iminosugars, flavonoids and related compounds, glycoproteins and ecdysteroids. Moreover, the role of phenylpropanes and megastigmane glycosides was also hypothesized by our group [1,2]. Here we report the investigation of a fraction of mulberry leaf hot water extract obtained by solvent-solvent partition between water and dichloromethane. A significant increase in the 24 h glucose consumption of fully differentiated adipocytes was found when treated with 50 μg/mL of fraction with 0.32 μM insulin or rather 200 μg/mL without it, as compared to the corresponding controls. In the latter case, the activity was similar to that of 50 μg/mL rosiglitazone that was used as positive control. Chemical composition of the pleasant odour, oily fraction was investigated by using HPLC-DAD, GC-MS, GC-FID and LC-MS/MS. The main constituent (GC-FID: 56.2%) is suggested to be a chain-saturated cinnamaldehyde derivative, and benzyl alcohol, ethyl benzoate, t-cinnamic acid, p-hydroxyacetophenone, t-coniferyl alcohol and sinapyl alcohol were also identified as minor constituents. Attempting to perform *in vitro* activity guided isolation, further fractionation was done by using rotational planar chromatography. All fractions obtained were found inactive, which may suggest synergy between certain constituents. *In vivo* investigation of antidiabetic activity is currently in process. **Acknowledgement:** *This project was supported by the Hungarian National Research Fund (OTKA; PD75383), the New Hungary Development Plan (TÁMOP-4.2.2 – 08/1 – 2008 – 0013 and TÁMOP-4.2.1/B-09/1/KONV-2010 – 0005) and by the grant from the National Science Council of Taiwan (NSC 982314B037011MY3).* **References:** 1. Hunyadi A et al. (2007) Planta Med 73: 941. 2. Hunyadi A et al. (2008) Planta Med 74: 1117.

PF57 Ethnobotany and ethnopharmacology of the genus *Veratrum* L. in veterinary medicine (Bosnia and Herzegovina, W. Balkan)

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Genus *Veratrum* L. (Liliaceae) in flora of Bosnia and Herzegovina (BiH) is represented with three taxa – white hellebore *Veratrum album* L., black hellebore *Veratrum nigrum* L. and green hellebore *Veratrum lobelianum* Bernh. White and green hellebore grows on mountain meadows, and black hellebore grows in strap of termofille oak-hornbeam forests. During ethno-botanical researches on area of BiH [1], determined was that white hellebore in some areas has been used in human traditional medicine. However, it is still actively used in traditional veterinarian medicine in mountain areas of BiH in places rich in cattle breeds (sheep, goats, cows and horses). Using method of ethno-botanical interview of over 86 informants, 20 of them confirmed use of underground parts of this plant (Veratri rhizoma) in treatment of skin diseases caused with parasites, as well as for removal of parasites. Rhizome is dug during vegetation periods, cook and use in preparation of a special form of mild decoction. Decoction is used cold to wash sheep and goats, occasionally cows. This preparation is used only externally, with special attention to keep it away from eyes. In same manner is used rhizome of black hellebore Veratri nigrae rhizoma in warmer areas (sub-Mediterranean and Mediterranean). In the past, this was the only way to treat skin parasites with domestic animals. Rhizome and leaves of *Veratrum album* contain wide spectrum of steroid alkaloids [2], that effectively affects maggots of house flies [3]. In some cases it has been used in human therapy, as well. **References:** 1. Redzic SS (2007) Coll Antropol 31 (3): 869 – 890. 2. Rahman A et al. (1996) Phytochemistry 43 (4): 907 – 911. 3. Bergmann ED,

Zwi H, Mechoulam L, Mechoulan R (1958) Journal of Insect Physiology 2 (3): 162 – 177.

PF58 Phytochemical Study from *Sonchus arvensis* L. Leaves for Standardizing Traditional Medicine Extract

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Sonchus arvensis L. leaves is empirically used as a traditional medicine for asthma, cough, anti-inflammation and diuretic [1,2]. To ensure quality through identification and standardization of its extract, fingerprint/phytochemical study is needed. In this research, the phytochemical study was carried out by TLC (Thin Layer Chromatography) scanner and HPLC (High Performance Liquid Chromatography). From the results, n-hexane extract showed a better separation with toluen: ethyl acetate (93:7 v/v) and had specific retention factor 0.80; 1.30; 2.18 (254 nm) and 0.88; 1.29; 2.14 (366 nm). Chloroform extract showed specific retention factor 1.13; 1.30; 2.14 (254 nm) and 0.88; 1.29; 2.14 (366 nm). Otherwise, clear separation of ethyl acetate extract was shown in chloroform: toluen: ethanol (4:4:1 v/v/v) with specific retention factor 0.98; 1.44; 1.9; 2.34; 2.49 (254 nm) and 0.98; 1.84; 1.95; 2.34; 2.50 (366 nm). From HPLC chromatogram at 254 nm, using acetonitrile-phosporic acid mixture showed specific retention time at 2.46; 4.09; 4.83; 7.69; 10.02; 11.06; 11.73 minute for hexane extract and 3.66; 5.84; 6.98 minute for ethyl acetate extract. In conclusion, the specific retention time from both extracts can be used as fingerprint for standardization of traditional medicine extract of *Sonchus arvensis* leaves. **References:** 1. Foster S. & Duke J.A (1995) A Field Guide to Medicinal Plants. Eastern and Central N. America. Houghton Mifflin Co., ISBN 0395467225. 2. Xu et al. (2008) Food Chemistry 111: 92 – 97.

PF59 HM-61, a Korean native plant extract, inhibits high glucose-induced ocular vessel alteration in zebrafish and prevents the development of diabetic ocular complications in diabetic db/db mouse

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Diabetes alters the structure and function of most cell types in the eye. The injury of ocular cell and retinal vasodilation are the hallmark of diabetic ocular changes. HM-61 is an 80% ethanolic extract of *Litsea japonica* (Thunb.) Jussieu, Korean native medicinal plant, with beneficial effects on diabetes. In this study, we investigated the preventive effects of HM-61 on diabetic ocular complications in zebrafish and diabetic db/db mouse. Tg(flk-1:EGFP) zebrafish larvae which specifically express EGFP in all blood vessels were immersed in high glucose medium with or without HM-61 for 5 days post fertilization (dpf). HM-61 (100 and 250 mg/kg body weight) was also treated once a day orally for 12 weeks in db/db mouse. In zebrafish model of diabetes, HM-61 effectively inhibited high glucose-induced ocular vasodilation ($p < 0.01$ vs. control). In diabetic db/db mouse, the treatment of HM-61 prevented the breakage of retinal-blood barrier and the injury of retinal vascular cells. In addition, diabetes-induced microvascular and neuronal cell apoptosis was significantly reduced in HM-61-treated db/db mouse ($p < 0.01$ vs. vehicle). Similarly, the administration of HM-61 also inhibited the development of diabetic cataract through the inhibition of sorbitol accumulation in lens fibers. These results indicate that HM-61 could provide a valuable therapeutic approach against diabetic ocular complications. **Acknowledgement:** *This research was supported by a grant [K10040] from the Korea Institute of Oriental Medicine (KIOM).*

PF60 Effects of Angelicae Gigantis Radix (AGR) on Polycystic Ovary induced by Estradiol Valerate in rats

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Angelicae Gigantis Radix (AGR) is the most frequently used medicinal plats for patients with gynecological problems, especially pregnancy (1,2). This study was designed to investigate the effects of (AGR) on Polycystic Ovary (PCO) induced by Estradiol valerate (EV) in female rats. We investigated the effects of AGR on Changes in body weights and food and water uptake for 5 weeks. In addition, we examined the effects on ovary weights. Finally, we also observed histopathological changes in PCO rats. In our results, AGR administration group restored ovary/body weight ratio to normal levels, which were lowered by induction of PCO (3). In histopathological observation, formation of cysts was suppressed in AGR group compared with non-treated PCO group). In conclusion, these results suggest that AGR can be used for patients with PCO to prevent formation of cystic follicles and malfunction of ovary. **References:** 1. Yoo DL (2001) J Oriental Gynecology 14(1): 453 – 460. 2. Shin YW et al. (2003) J Oriental Gynecology 16(4):180 – 188. 3. Farookhi R, Hemmins R, Brawer J R (1985) Biology of Reproduction 32: 530 – 540.

PF61 Traditional Chinese medicines and their effective components for the treatment of rheumatoid arthritis: a systematic review

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Rheumatoid arthritis (RA) is a systemic, autoimmune, and one of refractory disease, which belongs to “arthromyodynia” in Chinese medicine. Traditional Chinese medicines (TCM) were utilized for treatment of RA in clinic for a long history. In recent years, a large number of experiments were carried out for anti-RA effects of TCM A and their effective components in China and many other countries. In this review, more than 20 Chinese medicines which are commonly used such as *Tripterygium giumwilfordii* Hook, *Salvia miltiorrhiza* Bunge, *Paeonia sterniana* Fletcher, etc. and their effective components such as triptolide, sinomenine, total glucosides of paeony for the treatment of RA are summarized. Furthermore, sinomenine injection, tripterygium glycosides tablet and total glucosides of paeony capsules as well as other new drugs prepared from TCM have been developed for therapy of RA and used popularly in clinic. Further investigations are required to elucidate the possible action mechanism of these medicines and components and determine their potential for clinical use needs to be demonstrated in clinical trials. **Acknowledgement:** *This review was supported by the Science and Technology Commission Research Projects of Shanghai Municipality (10DZ1972000).*

PF62 Evaluation of antimicrobial effects of three traditional medicinal plants from Iran

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There is growing interest in use of plants as natural antimicrobial agents because they do not induce antibiotic resistance which is usually happened with the synthetic antibiotics. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infection diseases from various sources such as medicinal plants (1). The antimicrobial effects of different fractions of seed extract of *Securigera securidaca* L., fresh petal of *Rosa damascena* Mill. and aerial parts of *Tripleurospermum disciforme* Sch.Bip. extract were examined against four gram positive and four gram negative bacteria and 2 fungi, which were obtained

from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. The seed of *Securigera securidaca,* petals of *Rosa damascena* and top flowered of *Tripleurospermum disciforme* were collected in September, May and July 2009 around the Fars, Gilan and Tehran Provinces of Iran, respectively. The antibacterial and antifungal activity were studied by cup plate diffusion method as described by Warnock DW (2) and Soybean Casein Digest Agar and Saburouad Dextrose Agar were used as medium for the growth of bacterial and fungal strains, respectively. The petroleum ether and chloroform fractions of S. securidaca showed antibacterial activities against *Staphyloccus aureus* and *Pseudomonas aeruginosa*, while methanol fraction had no antibacterial effects. *R. damascena* extract had antibacterial activities against *Bacillus cereus*, *Staphyloccus aureus, Staphyloccus epidermidis* and *Pseudomonas aeruginosa*. *T. disciforme* extract demonstrated antibacterial effects against *Staphyloccus aureus* and *Staphyloccus epidermidis*. All the fractions of plants had no antifungal activities. **References:** 1-Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA (2007) J Ethnopharmacol 112: 426 – 429 2-Warnock DW (1991) Methods with antifungal drugs In: Evans EG and Richardson MD. (Eds.) Medical Mycology: A Practical Approach. IRL Press, Oxford University Press. 179 – 200.

PF63 One of the Korean mistletoe species, *Loranthus yadoriki* Sieb. exhibited potent inhibitory activities against monoamine oxidases

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It is well known that *Viscum album* L. var. *coloratum* Ohwi has anticancer, hypotensive, antimicrobial and antivirus activities. The European mistletoe (*V. album* var. *album*) white fruit has been studied for its anticancer activity as it is widely used for the treatment of various malignant tumors and their supplement. Lectin and viscotoxin are the active anticancer ingredients. Homoflavoyadorinin-B was reported as an antioxidant component of the mistletoe, and oleanolic acid was also reported as an anticancer component. There are five kinds of Mistletoe species in Korea. Most of them have above constituents evenly besides only one species. One of the Korean mistletoes, *Loranthus yadoriki* was very different constituent pattern. There were no main indicational bioactive ingredients, such as lectin, homoflavoyadorinin-B, and oleanolic acid was also smaller amount other than species. To investigate the bioactivities of *Loranthus yadoriki* several bioassays were applied. *Loranthus yadoriki* showed potent inhibitory activity against both types of monoamine oxidases and dopamine beta hydroxylase. But *Loranthus yadoriki* did not inhibit NO production in the cell. We are isolating the bioactive compounds from this plant with MAO inhibitory activity as a guide assay. **Acknowledgement:** *This work was supported by grants from Scientific research (KNA1 – 2-11,10 – 2) of Korea National Arboretum.* **References:** 1. Hajto T, Berki T, Boldizsar F, and Nemeth P (2003) Immunol Lett 86(1): 23 – 27 2. Siegle I, Friz P, McClellan M, Gutzeit S, and Murdter T E (2001) Anticancer Res 21(4A): 2687 – 2691 3. Kim YK, Kim YS, Choi SU, and Ryu SY (2004) Arch Pharm Res 27(1): 44 – 47 4. Karagoz A, Onay E, Arda N and Kuru A (2003) Phytother Res 17(5): 560 – 562 (). 5. Fernandez T et al. (2003) J Ethnopharmacol 85(1): 81 – 92

PF64 Contents of ephedrine-like alkaloid synephrine in traditional Chinese decoctions

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The FDA's ban on ephedra has led to an increase in the use of the ephedrine-like alkaloid synephrine, in dietary supplements for the purpose of body loss. Synephrine naturally occurs in bitter-orange (*Citrus aurantium* L.) and other *Citrus* species. Concerns have been raised about the safety of products containing synephrine. Tangerine peel (*Citrus reticulata* Blanco; Chenpi) is a herbal drug used in traditional Chinese medicine (TCM) and also contains small amounts of synephrine [1]. Traditional decoctions [2] of this drug are evaluated: *i.e.* the extraction yields for synephrine in dependence to extraction time. Thereof, an assumed daily intake is calculated for synephrine. Results showed a content of synephrine of 3.0 mg/g in the herbal drug (batch 1). Traditional decoction resulted in extraction yields of 69% synephrine, referred to dried drug. An extraction profile over time showed similar yields (about 67%) also after 3 hours of decoction. Maceration in cold water was about the same effective, yielding up to 71% of synephrine after 3 h. The analysis of a second herbal drug batch showed a synephrine content of 1.7 mg/g. Traditional decoction of it resulted in an extraction yield of 75% synephrine. A longer decoction lasting 2 hours lead to extraction yields up to 93%. It can be concluded that, assuming a daily dose of Chenpi of 3 – 9 g, there could result a daily intake of up to 19 mg synephrine. Such doses are below the levels exhibiting pharmacological effects, which are reported to be of 100 to 150 mg [3]. **Acknowledgement:** *We thank Lian Chinaherb, Switzerland, for the supply of herbal drug material.* **References:** 1. EDQM (2010) Mandarin epicarp and mesocarp (draft monograph), In: Pharmeuropa 22.4 2. EDQM (2010) Preparation of drugs for traditional Chinese medicines, In: Pharmeuropa 22.2 3. Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (2009) Illegale Schlankheitsmittel, In: LGL Jahresbericht 2009

PF65 Antioxidant activity of *Rhodomyrtus tomentosa* (Ait.) Hassk. of Terengganu coastal area

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Rhodomyrtus tomentosa (Ait.) Hassk. has shown interesting capability to scavenge free radicals and hydrogen peroxide as well as play effective role inhibition of lipid peroxidation. Results of DPPH scavenging assay shows the IC_{50} of 30 μg/mL and the 80% maximum inhibition at the concentration of 100 μg/mL. The 50% inhibition against hydrogen peroxide is at the concentration of 0.17 μg/mL and the maximum inhibition of 98% at the concentration of 0.25 μg/mL. FTC and TBA assay shows the 77.11% and 95.88% inhibition, respectively. **Acknowledgement:** *The authors are thankful to the Faculty of Agricultural and Biotechnology, University of Sultan Zainal Abidin for the funding and research facilities*

PF66 Antinociceptive Activity of *Eryngium kotschyi* Boiss. Root Extracts

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Eryngium species, belonging to *Apiaceae* family are well known plants in ethnobotanical culture in the world and also in Turkey. They are used as antitussive, diuretic as well as for analgesic and antiinflammatory purposes in traditional medicine. Although they have a wide usage in traditional medicine, there are only a few number of studies concerning biological activity of *Eryngium* species to confirm their usage. In our previous studies, it was reported that the roots of *Eryngium kotschyi* Boiss. have significant antinociceptive and antiinflammatory activity. Based on these results, we tried to find compounds responsible for the antinociceptive activity of this plant by a bioguided fractionation of methanolic extracts using different nociception models (acetic acid induced writhing test and hot plate test). This procedures allow us to isolate a new triterpene saponin (Compound 1), with a moderate antinociceptive activity (control: 8.33 ± 0.67 s, Compound 1: 14,33 ± 0,33 s, $P < 0.05$ with hot plate test), which is characterized as 3-O-β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucurunopyranosyl-22-O-angeloyl-A1-barrigenol. The isolation was done by using several chromatographic steps (medium pressure liquid chromatography, flash chromatography, on normal and reversed phase silica gel), and the structure elucidation was achieved by 1D and 2D NMR spectroscopy (COSY, TOCSY, HSQC, HMBC) and FABMS.

PF67 Anti-inflammatory effects of novel gel-formulations with traditional used plants in Benin

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In African and Asian countries with low incomes up to 80% of the populations depend on traditional medicine for primary health care (WHO, 2008 World Health Report). In West-African countries and especially in the northern region of the Republic of Benin patients with pain-associated diseases used traditionally some plants like *Entada africana* Guill. & Perr., *Ficus thonningii* Blume, *Combretum collinum* Fresen., *Fadogia agrestis* Schweinf. ex Hiern, *Piliostigma thonningii* (Schumach.) Milne-Redh. and *Chasmanthera* sp. Primary goal of this project was to test a potential analgesic effect of novel gel formulations containing a defined combination of ethanolic or aqueous extracts of these plants on pain associated with irritation provoked by arthrosis or musculoskeletal trauma. Second objective was to compare effectiveness of the novel gel-formulations with diclofenac gel. The analgesic effect was assessed using criteria of evaluation within 10 days. 81.8% of the patients (n = 11) responded with reduced pain score after topical application twice daily of the gel formulation containing the ethanolic extracts, whereas the water-based formulation was less effective (57.1%; n = 7). Diclofenac gel (25 mg) reduced the pain by 58.3% (n = 12) of the patients within two weeks. The results demonstrated that the ethanolic extract was more effective than the aqueous extract and the well established diclofenac gel. Our study involving pain-associated patients explained the importance of an adequate formulation for extracts used in the traditional medicine and pointed out that a combination of plant extracts could an effective alternative to topically applied synthetic analgesics. Further studies are necessary to examine the mechanisms contributing to the analgesic effect of the plant extracts.

PF68 Antiflammatory activity from *Limonium brasiliense* (Boiss.) Kuntze

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Limonium brasiliense Kuntze (Plumbaginaceae) is a medicinal plant, known as "Guaycuru" from southern Argentina. Infusion from the roots is popularly used in the treatment of hemorrhage, menstrual disorders, rheumatism and it is believed to have cardioprotective properties [1] The aim of this work was to evaluate the antiflammatory activity of methanolic extract from roots of *L. brasiliense* and its major constituent, myricetin 3-O-rhamnoside, *in vitro*. [2], [3] This extract was partitioned with different solvents of increasing polarity to obtain sub-extracts that were fractionated by silica gel column chromatography, for isolation and purification of the active compounds. The fractions and isolated compounds have been tested in cultures cell lines. These were not cytotoxic against RAW 264.7 and HL60 cells lines. Thus, these fractions and the isolated compound have been tested on inhibition of Nitric Oxide (NO) overproduction on LPS-stimulated RAW 264.7 cells. The best anti-inflammatory potency (40 µg/ml = 63% inhibition) was provided by a fraction coming from the ethyl acetate sub-extract. This fraction contains myricetin 3-O-rhamnoside. (IC50 = 13.29 µM/ml) Also, we investigated the antioxidant effects of these fractions and the isolated compound on inhibition of intracellular and extracellular production of reactive oxygen species (ROS). These have inhibited both ROS production. The results presented demonstrate that myricetin 3-O-rhamnoside displayed a typical antioxidant activity; it markedly inhibited extracellular and intracellular ROS production. These results also support the claims of traditional medicine about the use of *L. brasiliense* roots in the treatment of inflammatory diseases. Therefore antioxidative research should also be extended to *in vivo* models. **Acknowledgement:** *This work was supported financially by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP N°6056), Universidad Nacional del Sur (UNS, PGI 24/Q009), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT, PICT N°25775), Erasmus Mundus External Cooperation Window (EMECW, 2009 – 1796/001 – 001-ECW), Comisión Interministerial de Ciencia y Tecnología (CICYT) and Xunta de Galicia, Spain. Murray A.P.is a research member of CONICET* **References:** [1] Murray AP et al. (2004) Z Naturforsch 59c: 477 – 480 [2] Leiro J et al. (2004) J Leukoc Biol 75: 1156 – 1165 [3] Orallo F et al. (2002) Br J Pharmacol 121: 1627 – 1636

PF69 Anti-inflammatory Evaluation of *Ageratum conyzoides* L. Leaves

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Alcohol extract (70%) as well as successive extracts of the air dried powdered *Ageratum conyzoides* leaves have been evaluated for their anti-inflammatory and chemical constitution. All extracts showed high significant anti-inflammatory activities against carragenan induce edema. Moreover, crude extract and ethyl acetate exhibited good anti-inflammatory activity against cytokine interlukin (1L)-6 than the other extracts. Quantitative and qualitative estimation of the total flavonoid, steroidal, triterpenoidal, protein and carbohydrate contents of 70% alcohol extract were determined. The mucilage of crude extract was isolated, identified using HPLC and re-evaluated for their anti-inflammatory activity. The free and glycosidal flavonoids of ethyl acetate extract were isolated. Kaempferol, p- hydroxyl benzoic acid, quercetin-3-O-rhamnopyranoside and quercetin-3,7- diglucopyranoside were isolated from ethyl acetate extract. The isolated flavonoidal fractions and compounds are evaluated for anti-inflammatory activity. The glycosidal flavonoid fractions proved greater anti-inflammatory effect than the isolated compounds.

PF70 Folk medicines of Çamlıdere (Ankara)

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In this study, folk medicines which are being currently used in Çamlıdere district (Ankara) were determined. For this purpose, scientific trips were organized to the Çamlıdere. During these trips, interviews were conducted with those who have knowledge on folk medicines. When the plants that collected during the trips and constituted the majority of folk medicines were evaluated systematically, it was determined that 65 species belonging to 61 genera of 29 families are being used in the treatment of various diseases. Besides, 19 medicines of animal origin and 34 mixtures were recorded to be used in the district. The plants of the Asteraceae family were determined to be used mostly, Lamiaceae and Rosaceae were the other frequently used families. The folk medicines were mainly used for respiratory tract diseases, dermatologic problems, gastrointestinal system diseases and rheumatic complaints in study area. Best of our knowledge, eight plant taxa were determined at the first time to be used as folk medicine in Turkey. Additionally, different usages of 41 predefined folk medicines were designated in this study. Finally, striking erosion observed in ethnobotanical heritage and the urgent need of systematic ethnobotanical researches to record this precious knowledge before it is completely lost were also emphasized.

PF71 Evaluation of acute and sub chronic hepatotoxicity of hydroalcoholic extract of *Teucrium polium* L. in non-diabetic rats

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While it is well-known and widely used for its hypoglycemic and anti spasmodic properties in traditional medicine of many countries (1, 2), probable side effects of *Teucrium polium* L. (T.p), especially hepatotoxicity in diabetics, needs more investigation. The purpose of this study is to determine the acute and subchronic hepatotoxicity of T.p hydro alcoholic extract in non-diabetic rats. In acute phase, rats were given doses from 50 – 7000 mg/kg of the solution by gastric gavages. However, our paraclinic and histopathologic studies were focused on the dose of 3000 mg/kg. In sub chronic phase, 1000 mg/kg of the solution was given through drinking water once daily. On the day 45, liver damage was again evaluated through blood samples and biopsy. (3) There was no mortality seen. AST and ALT rose, more in females, but not to a significant level in either sex. Histopathologic examination revealed signs compatible with non specific reversible hepatic inflammation. The results were the same in both phases. Our study suggests that hydro alcoholic extract of *Teucrium polium* 1:1 is non-toxic *in vivo* and does not induce hepatotoxicity. However, the same result may not be seen in diabetic rats and that entails more investigation. Additionally, the growth place of T.p may have some effects on the results. **References:** 1. Hasani-Ranjbar S, Nayebi N, Larijani Band Abdollahi M (2010) International Journal of Pharmacology 6(4): 315 – 325 2.Ljubuncic P, Azaizeh H, Portnaya I, Cogan U, Said O, Saleh KA, Bomzon A (2005) J Ethnopharmacol 99(1): 43 – 7 3. Ecobichon DJ (1997) The basis of toxicity testing. CRC press ll c (Second edition), Boca Raton, New York

PF72 Antioxidant and hepatoprotective activity of *Tragopogon porrifolius* methanolic extract

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Tragopogon porrifolius L. (Asteracea), commonly known as purple salsify, is cultivated for its edible root and shoot. The present study investigates the in vitro and in vivo antioxidant activity of the methanolic extract of the aerial part of *T. porrifolius* as well as its protection against CCl_4-induced hepatotoxicity in rats. Total phenolic and flavonoid contents, were assessed using the Folin-Ciocalteu and the aluminum chloride colorimetric methods and found to be 37.1 mg/g GAE and 16.6 mg/g QE respectively per gram dry weight of the extract. The FRAP assay showed an antioxidant activity of 744 μmol Fe^{2+}/g. In vivo, the extract (50, 100 and 250 mg/kg body weight) exhibited a dose dependent increase in the activity of liver antioxidant enzymes. The highest dose used increased the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) by 222, 149 and 68% respectively. *T. porrifolius* extract also showed substantial hepatoprotective capacity against CCl4-induced hepatic injury by restoring the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) to normal levels at 250 mg/kg body weight dose. These findings suggest that *T. porrifolius* methanolic extract possesses antioxidant and hepatoprotective activity and can be used to prevent liver disorders. **Acknowledgement:** *Mr. Jean Karam.* **References:** 1. Tawaha K et al. (2007) Food Chem 104: 1372 – 1378. 2. Chandra T et al. (1987) Fitoterapia 58: 23 – 31.

PF73 *In vitro* schistosomicidal activity of triterpenoids from the African plant *Momordica balsamina*

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Schistosomiasis, also known as bilharzia, is a chronic liver and intestinal parasitic disease caused by trematode worms of the genus Schistosoma. Praziquantel is the only available drug against all forms of schistosomiasis. The development of praziquantel resistance is a great concern and new drugs are urgently needed [1]. *Momordica balsamina* L. (Cucurbitaceae), commonly known as African pumpkin, is a vegetable widespread in tropical and subtropical regions that has been used as food, mainly in sub-Saharan Africa. It has also been widely used in traditional medicine in Africa to treat various disease symptoms, mostly diabetes and malaria. In previous work, bioassay-guided fractionation of the methanol extract of the aerial parts of *M. balsamina* led to the isolation of several cucurbitane-type triterpenoids. Most of the isolated compounds as well their acylated derivatives displayed antimalarial activity [2, 3]. Continuing our search for antiparasitic compounds, the aim of this work was to evaluate the in vitro schistosomicidal activity of several triterpenoids isolated from *M. balsamina* against *Schistosoma mansoni* adult worms [4, 5]. Praziquantel was used as positive control. A remarkable schistosomicidal activity was observed for two of the ten compounds tested (at 50 and 100 μM), which caused the death of all *S. mansoni* adult worms after 24 h of incubation. Both compounds, at 10 – 50 μM, induced significant reductions in the motor activity of the worms and significantly decreased the egg production. Furthermore, they were able (at 10 – 100 μM) to separate the adult worm pairs into male and female after 24 h. **Acknowledgement:** *This study was supported by FCT, Portugal (SFRH/BD/22321/2005) as well as Fapesp (2006/60132 – 4) and CNPq, Brazil* **References:** 1. WHO (2010). Fact sheet n° 115. 3. Ramalhete C et al. (2010) Bioorg Med Chem 18: 5254 – 60. 4. Ramalhete C et al. (2011) Bioorg Med Chem 19: 330 – 8. 3. Magalhães LG et al. (2010) Parasitol Res 106: 395 – 401. 4. Magalhães LG et al. (2009) Parasitol Res 104: 1197 – 120

PF74 Phytochemical screening and the effects of aqueous extracts of *Phyllanthus amarus* leaves on the lipid profile and cardiac muscle cyclic guanosine monophosphate of male Guinea pigs

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Extracts of the leaves of *Phyllanthus amarus* Schumach. (Euphorbiaceae) are used as folk medicine for the treatment of jaundice and other diseases in Nigeria and other countries. Recently the extract is becoming popular for increasing and or improving libido and reproductive functions in men. The effects of the aqueous extracts of the leaves of *Phyllanthus amarus* on lipid profile and the cardiac muscle cyclic guanosine monophosphate (cGMP) in male Guinea pigs was investigated and compared to the effects of sildenafil citrate on the same parameters. The phytochemical screening was also carried out. The results showed that the administration of aqueous extract of the *Phyllanthus amarus* leaves to the animals (100, 200 and 400 mg/kg body weight) caused a statistically non significant ($p > 0.05$) increase in cholesterol, triacylglycerol, low density lipoprotein and high density lipoprotein level while the administration of 100 mg/kg body weight of sildenafil citrate caused a non significant ($p > 0.05$) decrease on lipid profile levels but a non significant increase in the level of triacylglycerol. However the administration of aqueous extract of *Phyllanthus amarus* (100 and 200 mg/kg body weight caused a non significant *($p > 0.05$)* decrease in the level of cardiac cGMP, while the administration of 100 mg/kg body weight of sildenafil citrate and 400 mg/kg body of the aqueous extract cause a non significant increase $p > 0.05$ in the levels of cGMP. Furthermore, the phyto-

chemical screening of the leaves of *Phyllanthus amarus* revealed the presence of flavonoids, tannins, alkaloids, terpenoids, steroids, saponins and cardiac glycosides.

PF75 The effects of *Cucurbita pepo* seeds on testosterone induced benign prostatic hyperplasia

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The *Cucurbita pepo* L. (pumpkin) seeds are considered a snack food in most social gatherings in Lebanon and the Middle East. Many herbal combinations containing pumpkin seeds are used to treat symptoms of benign prostatic hyperplasia (BPH). Because the seeds are the most commonly consumed and not the seed oil, this study was carried out to examine the effects of pumpkin seeds on testosterone (3.57 mg/kg body weight) induced BPH in rats (1). After achieving hyperplasia (30 days), treatment with pumpkin seeds (10, 20, 30 and 60% w/w of chow) or finasteride (5 mg/kg body weight) was initiated for 12, 24, and 36 days. Results showed that pumpkin seeds exerted maximum inhibition (86.7, 98.0 and 98.4%) of hyperplasia at 30% w/w dose after 12, 24 and 36 days respectively. They were comparable to finasteride (78.1, 89.5 and 96.4%). There was no significant effect on weight gain in rats treated with testosterone and pumpkin seeds. Additionally, no significant effects were observed on levels of sGOT-AST enzyme and ALP, while slight increase was observed on sGPT-AST. The findings on prostatic hyperplasia were confirmed by histopathological studies where tissue showed abundant stoma between glandular cells and lack papillary projections into the lumen of the glands. In conclusion, pumpkin seeds inhibit prostate hyperplasia induced by testosterone, and improve the histology of the prostate **Acknowledgement:** *Mr. Jean Karam.* **References:** 1. Gonzales G (2007) Asian J Androl 9(2): 245 – 251.

PF76 The most common medicinal plants of Sistan (Sistan & Balouchestan province, Iran) and some ethnobotanical aspects

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The uses of herbs to cure diseases have been common in folk medicine of Iran since the ancient times. In the present work, the most common medicinally important species used by local inhabitants of Sistan region in (south-east of Iran) were collected from various localities and identified by using related flora and comparison with herbaria specimens. This area is severely affected by arid and semi-arid climates with relatively poor vegetation. For each species botanical name, vernacular name, used part (s), popular medicinal usage, forms of preparation and application were also provided on the bases of ethnobotanical and folk beliefs in local culture. The essential oil content of some species is also determined by hydrodistillation method. Thirty species belonging to 22 families and 29 genera were recognized as the most common medicinal plants in the study area from which 2 species are cultivated (*Eucalyptus camaldulensis* Dehnh. and *Aloe vera* L.), while the others grow as wild species. Also, only 2 species are monocotyledons and the others are dicotyledons. Some of the most important medicinal plants such as *Eucalyptus camaldulensis* (4%), *Cuminum cyminum* L. (3.5%), *Trachyspermum copticum* Link (3%), *Foeniculum vulgare* L. (2%), *Nigella sativa* L. (1%), *Mentha longifolia* Huds. (1%) and *Citrullus colocynthis* (L.) Schrad. (0.75%) showed high quantity of essential oils (Table 1), while *Peganum harmala* L. had the least amount (0.015%). The life forms of these plants were also determined. These medicinal plants are used in traditional medicine as diuretic, stomach improver, wound healing agent, antipyretic, expectorant, etc.

PF77 Prevention of carcinogen-induced mouse skin papilloma by *Daucus carota* (wild carrot) aqueous extract

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Daucus carota L. ssp. *carota* is a part of the folk medicine in Lebanon where it is used to enhance immunity and protect against many ailments among which are inflammation, gastric ulcer, and diabetes. Earlier we reported anti-inflammatory and anti-ulcer activities of the aqueous and methanolic extracts of the wild carrot umbels.[1] Additionally, the aqueous extract of *D.carota* was found to possess anticancer activity against human promyelocytic leukemia HL-60 cells[2], while the oil extract exhibited chemopreventive effect against chemically induced skin cancer.[3] The present study investigates the anticancer effects of the aqueous extract of *D. carota* umbels on DMBA-induced skin cancer in mice. The extract was administered to animals via either gavage or intraperitoneal routes for 20 weeks. Significant antitumor effects were observed with the intraperitoneal (250 mg/kg body weight) mode of treatment, where the percentage of papilloma incidence, yield and volume were reduced by 28, 23 and 86.4% respectively. Gavage treatment failed to inhibit tumor incidence, yield and volume. Intraperitoneal treatment decreased hyperplasia and dermal infiltration with an increase in the level of hyperkeratosis. These findings demonstrate that *Daucus carota* aqueous extract possesses anti-tumor activity against DMBA-induced skin cancer. **Acknowledgement:** *Mr. Jean Karam.* **References:** 1. Diab-Assaf M et al. (2007) ACCR International Conference on Molecular Diagnostics in Cancer Therapeutic Development, Atalanta, USA. 2. Wehbe K et al. (2009) Journal of Complementary and Integrative Medicine6(1): Article 7. 3. Abou Zeinab R et al, Pharm Biol *in press*

PF78 Evaluation of cytostatic potential of *Helleborus purpurascens* extracts concentrated by membrane techniques

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Helleborus sp. is used in the adjuvant treatment of different tumors. Indications include various types of brain tumors in children, as well as prostate cancer, leukemia and lymphoma [1]. Our aim was to evaluation of cytostatic potential of *Helleborus purpurascens* Waldst. & Kit. concentrated extracts. Particular attention was devoted to investigate the possibility to purify and concentrate *Helleborus purpurascens* extracts by microfiltration, ultrafiltration and nanofiltration process, allowing the preservation of thermolabile compounds from the extracts and their antineoplastic properties examination. The bioactive compounds characterization has been effected by UV-VIS spectroscopy and HPLC. The concentrated Hellebore extracts "*in vitro*" testing, on HeLa neoplastic cell cultures, has highlighted the cell proteinsynthesis alteration; protein dynamics modification; decrease of total cell number; cell viability diminution; inhibitory impact upon the cell cultures development. Concentrated Hellebore extracts induces an inhibitory effect upon cell cultures development of 90.7% at 72 h, thus it can be considered a good source for further medicinal applications. **Acknowledgement:** *This research was supported by the Romanian National Center for Program Management – PN62076/2008 projects.* **References:** 1. Jesse P et al. (2009) Pediatr Blood Cancer 52(4): 464 – 469

PF79 NMR based Metabolomic Investigation of the Brazilian Medicinal Plant Carqueja: *Baccharis trimera* (Less.) DC

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In 2010 the ANVISA (the Brazilian Agency equivalent to the FDA in the USA) recognized 66 species of plants as medicinal plants and regulated its use [1]. Of most of these species only limited phytochemical information is available and doubts exist about the active components. On several species we iniciated research with the objective to obtain the phy-

tochemical fingerprints and relate them to the pharmacological activities. From *Baccharis trimera* (Less.) DC., a plant widely used in popular medicine, and known in Brazil under the name Carqueja, a series of samples was obtained from local markets and pharmacies. Using a protocol developed in our laboratory, the samples were directly extracted with deuterated solvents to provide two types of extracts: A polar aqueous extract and a apolar chloroformic extract. These were subsequently analysed by NMR spectroscopy. In this comparison large differences were encounterred in the chemical composition: *e.g.* the quantity of the flavonoid Eupatorin ranged between 0 and 10 mg/g of plant material. The differences in the phytochemical profiles generate large doubts about the efficient use of this medicinal plant. But, considering that the active components have not been identified, it also offers a possibility to find novel drug leads, by metabolomics, in which activity profiles of the individual samples are directly compared with the chemical profiles. With sufficient samples and efficient biological tests the active components will be revealed. **Acknowledgement:** *CNPq, FAPERJ, CAPES.* **References:** 1. Diário Oficial da União, Resolução RDC no. 10, de 9 de março de 2010.

PF80 Effect of *Garcinia kola* Heckel seeds on bioavailability of two commonly used drugs in nigeria (sulphamethazine and paracetamol)

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Garcinia kola Heckel (Guttiferae), is a large forest tree found throughout Western and Central Africa. Widely known in commerce as 'bitter kola', the seeds are used extensively in African traditional medicine as a social masticatory agent and for the treatment of various diseases, especially cough, mouth and respiratory tract infections, and are also claimed to reduce the effectiveness of drugs and toxic substances in general. They are thus used locally as antidote to poisons and in cases of drug overdose. The effects of concurrent administration of *G. kola* whole seed suspension were investigated on the bioavailability of two commonly used drugs in Nigeria, namely, sulphamethazine, a sulphonamide antibiotic, and paracetamol, an analgesic and antipyretic, in albino rabbits. Two groups of rabbits (n = 4 each) were treated by gavage with a concentration of 0.5 g/kg body weight of seed suspension given concurrently with 150 mg/kg body weight of sulphamethazine and paracetamol respectively. Control groups were given equivalent doses of either drug alone. Blood was withdrawn from the left ear at one hour intervals for five hours. Results showed that *G. kola* seeds decreased significantly ($p < 0.05$) the bioavailability of the two drugs. Relative bioavailability was calculated to be 77.56% for sulphamethazine and 75.39% for paracetamol. The time of peak and peak concentrations were also reduced, while the concentration at one hour was only significantly different for paracetamol at $p < 0.05$. These results suggest that *G. kola* seeds may reduce bioavailability by interfering with drug absorption across the gastrointestinal mucosa. **Acknowledgement:** *The Management and Staff of Emmanuel Research Laboratory, Enugu Nigeria for allowing the use of their facilities* **References:** 1. Esimone C O et al. (2002) American Journal of Therapeutics 9(4): 275 – 280 2. Braide VB (1990) Phytother Res 4: 39 – 41. 3. Akintonwa A and Essien A R (1990) J Ethnopharmacol 29(2): 207 – 211.

PF81 Comparison of mangiferin in mango leaf and honeybush infusions

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Mangiferin reportedly exhibits antioxidant, anticancer, antimicrobial, antischlerotic, anti-allergenic, anti-inflammatory, analgesic and immunodilatory action in humans (1). Xanthone has demonstrated a potent arresting effect on the proliferation of tumours and malformed cells. Mango (*Mangifera indica* L.) has high concentrations of mangiferin content in the pulp, peel, stone and leaves. Honeybush (*Cyclopia genistoides* Vent.), an indigenous South African herbal tea, used for its antioxidant property and other health benefits, also contains high levels of mangiferin (2). The aim of this study was to determine the levels of mangiferin in the leaves of three mango cultivars to investigate the possibility of using mango leaves as a health beverage. Mangiferin was extracted by infusing the dried leaves in boiling water and the extract was subsequently quantified using UV-Vis (3), HPLC and HPTLC (4). Spectroscopic methods were used to analyse the powdered leaf materials. Chemometric analysis (O-PLS) was used to develop a predictive model for mangiferin. Extracts of the mango leaves were added to fruit juices and the stability of mangiferin determined regarding time, light and pH. The levels of mangiferin in mango were compared to those found in honeybush teas. Mangiferin levels found in a leaf infusion of mango indicated that mango leaves may have more health benefits than honeybush tea. **References:** 1 Mashibo M, He Q (2008) Compr Rev Food Sci and Food Safety 7: 309 – 319 2 McKay DL, Bloomberg JB (2007) Phytother Res 21:1 – 16 3 Joubert E et al. (2008) Phytochem Anal 19:169 – 178 4 Rastogi S et al. (2007) J Planar Chromat 20: 317 – 320

PF82 Purification of verbascoside from plant extracts using column and countercurrent chromatography

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Verbascoside, a phenylethanoid glycoside, displays diverse biological activities. The antifungal activities of polar extracts of Lippia species against *Penicillium digitatum*, a common pathogen of citrus, was attributed to the presence of verbascoside1. Partial purification of such plant extracts to increase the verbascoside content could provide natural mycobiocides for postharvest control of pathogens on fruit. Verbascoside was extracted from dried *Lippia wilmsii* H.Pearson plant material using aqueous methanol. The extracts were purified using silica gel column chromatography and the verbascoside concentrations were compared to that obtained by countercurrent chromatography (CCC). The crude extract contained 4.8 g/L verbascoside, corresponding to 12.5% of the extract. After column chromatography, the verbascoside content of the purified extract was substantially increased, but was lower than that obtained by CCC (40.8%). High performance liquid chromatography was used for the analyses of the target compound. The widespread invader weed *Lantana camara* L., was investigated as an alternative source of the compound. Five specimens of *L. camara* from 13 populations in South Africa were analysed by HPLC. Both intra- and interpopulation variability was observed in the verbascoside content. Combinations of flowers and leaves from *L. camara* could serve as a good source of verbascoside. Several stability tests were conducted to evaluate the stability of the compound under different conditions. The shelf life stability study proved that the compound is stable in a dry form when stored in the dark, but decomposes rapidly when exposed to light. Verbascoside also proved to be reasonably stable under steam distillation conditions **References:** 1 Shikanga et al. (2009) Fruits 64:3 – 12.

PF83 Inhibitory Effect of Crude Aqueous *Brucea amarissima* Extract on the Growth Profile of Oral *Candida*

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The prevalence of oral *Candida* infections has increased, due to immunosuppressive effect of antifungal agents on resistant hosts [1,2]. Growth rate is a key attribute of virulence among infectious microorganisms including *Candida* species. The aim of this study was to evaluate the growth inhibitory effect of *Brucea amarissima* (Lour.) Merr. leaves extract based on changes in the pattern of growth profile of *Candida* sp. *Candida albicans*, *Candida tropicalis* and *Candida dubliniensis*were used in this study. Crude extract of *B. amarissima* was prepared and the minimal inhibitory concentrations (MICs) towards *Candida* sp. were determined. The growth responses were recorded based on changes in the doubling time (g-values) and specific growth rates (μ-values). The values in the presence of extract were computed as percentage in the optical density (OD) relative to the total cells suspension in the absence of extract. 0.12% w/v chlorhexidine (CHX)-containing mouth rinse and sterile distilled water were used as controls. *C. tropicalis* was found to have the highest growth rates indicating high bioactivities and reproducibilities. *C. dubliniensis* and *C. tropicalis* showed the highest reduction of μ-values at a minimal concentration with 87.04% and 57.28%, respectively. At higher concentration (6 mg/mL), the extract exhibited significant reduction towards the growth ($p < 0.05$). Also, was able to reduce the μ-values of all *Candida* strains with more than 90% reduction. The extract

exhibited fungistatic (< 6 mg/mL) and fungicidal (> 6 mg/mL) activities towards oral *Candida* sp. hence, may be considered as a promising candidate for the development of antifungal agent of natural products. **Acknowledgement:** *University of Malaya Research Grant (UMRG) (RG095/09HTM), Postgraduate Research Fund (PS160/2010B).* **References:** 1. Sánchez-Vargas LO et al.. (2005) Rev Iberoam Micol 22: 83 – 92. 2. Bastert J, Schaller M, Korting HC, and Evans EG (2001) Int J Antimicrob Agents 17:81 – 91.

PF84 Plants known as "Node-to-dog" (*Heteropterys aphrodisiaca* O. Mach. (Malpighiaceae) and its medicinal use in southwestern Mato Grosso, Brazil

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There are several species of medicinal plants known as "Node-to-dog". The most used is the *Heteropterys aphrodisiaca* O. Mach. (Malpighiaceae). We conducted field and laboratory studies and also review on popular usage, occurrence and bioactivity of *H. aphrodisiaca* in southwestern Mato Grosso (MT-sw), Brazil from 2003 – 2009. The plant occurs in savannah and field areas. It is widely used as an aphrodisiac by local communities. Its beautiful flowers are ornamental, yellow, often visited by wild bees. The plant has long, expanded and nodulated roots, hence the name of "node-to-dog". It spreads easily by seeds, adapts well in adverse environments and resists fires, regenerating after. Experiments on more appropriate systems of cultivation, developed in Cáceres (MT-sw), revealed that the species adapts equally well in mono and polyculture. The root of the adult plant is used in folk medicine to treat nervous disorders, sexual problems, high cholesterol, and is physical invigorating. The root is macerated in wine for consumption as an appetizer. There are substances in the *H. aphrodisiaca* promising to treat fatigue, memory loss and Alzheimer's disease. Phytochemical analysis revealed the presence, in root extracts, of polyphenols, tannins, alkaloids; cardiotonic, aromatic and flavonoid glycosides; and of saponins. In MT-sw it is a commonly used medicinal plant and the roots are sold in boxes. **Acknowledgement:** *Fapemat – financial support, and for UNEMAT – institutional support; to the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)*

PF85 Plant known as "Sangra d'água" (*Croton urucurana* Baill. (Euphorbiaceae) and its medicinal use in southwestern of Mato Grosso state, Brazil

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"Sangra d'água" is a medicinal tree plant (genus *Croton*). There are several species, including: *C. urucurana* Baill., *C. salutaris* Casar., *C. lechleri* Müll.Arg., *C. planostigma* Klotzch. [*Croton urucurana* (Euphorbiaceae) is the best known. We conducted "filed, lab studies" and a literature review on popular usage, occurrence and bioactivity of *C. urucurana* in southwestern Mato Grosso (MT-sw), Brazil, 2003 – 2007. In MT-sw it grows spontaneously and quickly in watercourses margins. The additional light plant exposure accelerated its reproduction. The inflorescence releases dust that causes allergies-skin irritation. In MT-sw it is used to treat female (discharge, sores, inflammation, cyst) and male genital disorders (prostate); cancer, gastritis, intestinal- stomach ulcers, bruises, infections, hemorrhoids, pain-in-legs, blood-purifying. In other regions of Brazil, it is also used as anti-hemorrhagic, anti-inflammatory, antiseptic, anti-viral, healing, and haemostatic. Obtaining bark and tree sap should be done in the morning and opposite to the rising sun. The water from boiling the bark is used for bathing. Other research on *C. urucurana* latex discloses that the healing action is attributed to alkaloid taspine; in mice it develops peripheral analgesic activity; in rats, the ingestion was highly toxic, why should it have external use only. The users of these plants should make sure the proper procedures have been taken, consulting qualified professionals to target efficiency and avoiding side effects. In Cáceres (MT-sw), bark and sap are sold by healers, whose expertise is primarily inherited from their ancestors. From this plant the honeybees extract resin to produce propolis. It also has other uses within the conception of sustainable development. **Acknowledgement:** *For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)*

PF86 Plant known as "Leather hat" (*Echinodorus* spp. (Alismataceae) and its medicinal use in southwestern Mato Grosso, Brazil

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Several plant species (> 10) known as "Leather hat" (*Echinodorus* spp. – Alismataceae) used in traditional medicine. We made field and laboratory studies and review on popular usage, occurrence and bioactivity of "Leather hat" in Mato Grosso (MT), Brazil, from 2003 – 2007. The following species have been known to occur in MT: *E. grandiflorus* Mitch., *E. lanceolatus* Rataj, *E. macrophyllus* Kunth and *E. teretoscapus* Haynes. They prefer humid tropical conditions. The species *E. grandiflorus* and *E. macrophyllus* are also used as tea. Studies of ethnic knowledge in southwestern MT revealed that leaves of "Leather hat" are used as antiophidic, antiseptic, diuretic, laxative, astringent, for gargling, washing sores, treat kidney, liver, arthritis, rheumatism, high blood pressure; roots – to treat atherosclerosis, hernia, boils; leaves and flowers – to treat syphilis, purgative; roots, leaves and flowers – for skin disorders. The healers collect the plant in the dry season, and indicate that *E. grandiflorus* and *E. macrophyllus* are used as tea, poultice, and tincture. Some scientific studies show significant results with "Leather hat": leaf infusion confirmed analgesic, anti-inflammatory and diuretic effect in experimental animals; tea showed diuretic activity; aqueous extract of dried leaves of the plant showed abortifacient activity in rats; prolonged use can lower blood pressure; and tincture, in excessive dose, can cause diarrhea. "Leather hat" has multi-use potential: medical, industrial, ornamental, ecological. **Acknowledgement:** *Fapemat team – for financial support; UNEMAT team – by institutional support; Colleagues of the research group FLOBIO- (Plants carrier of bioactive substances) who collaborated in this study.*

PF87 Antidiabetic effects of *Allium ascalonicum* methanolic extract in experimental diabetes

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In the present study we have investigated the antidiabetic effects of bulbs of Persian shallot *Allium ascalonicum* L., (Alliaceae) methanolic extract (AAE) at doses of 500 and 250 mg/kg bw, on Alloxan-induced diabetic male Wistar rats in comparision with Acarbose (as a reference drug), by measuring postprandial blood glucose (PBG), oral glucose tolerance test (OGTT), inhibition of rat intestinal α-glucosidases activities, changes in the levels of plasma lipids profiles antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), pancreatic Insulin and cardiac Glut-4 mRNAs expression. In diabetic Wistar rats, in short term period, effects of AAE on PBG showed significant reduction after 24 h of oral administration. After 3 weeks of treatment, significant chronic decrease in the PBG was observed. For OGTT, the increase in PBG levels reduced mildly in AAE treated diabetic rats, at 1hour. Intestinal sucrase and maltase activities were inhibited by AAE, 17.41% and 14.62% respectively. In diabetic rats, AAE also increased the activities of SOD by 65%, GPx by 43% and CAT by 55%, showing strong antioxidant effects. AAE demonstrated antihyperlipidemic properties by reducing serum TG, LDL, VLDL and TC. In addition, we have observed increased expression of Ins and Glut-4 genes in diabetic rats treated with methanolic extract of *Allium ascalonicum*, compared to control group. **Acknowledgement:** *The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support.* **References:** Vincent AM, Russell JW, Low P, Feldman EL (2004) Endocr Rev 25: 612 – 28. Marcus AO (2001) Postgrad Med110: 113 – 23. Day C (1998) Br J Nutr 80: 5 – 6. Augusti KT & Sheela CG (1996) Experientia 52: 115 – 120. Feshani AM, Kouhsari SM, Mohammadi S (2010) J Ethnopharmacol 133: 67 – 74

PF88 Molecular and biochemical effects of the methanolic extracts of the leaves of Salvia officinalis on diabetic male wistar rats

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Common sage (*Salvia officinalis* L.) is among the plants that are claimed to be beneficial to diabetic patients and previous studies have suggested that this plant has hypoglycaemic effects in normal and diabetic rats. In the current study, we have investigated the effects of methanolic extract of *S.officinalis* leaves on blood glucose, plasma biochemical parameters, pancreatic Insulin and cardiac Glut-4 mRNAs expression, inhibition of rat intestinal α-glucosidases activities and erythrocyte antioxidant enzymes. Treatment of Alloxan monohydrate -induced diabetic rats with oral administration of sage leaves methanolic extract for 3 weeks, resulted in a significant reduction in blood glucose (Glucose oxidase assay).Total cholesterol (TC), triglycerides (TG), LDL/HDL ratio and VLDL were mildly decreased after treatment of diabetic rats by plant extract. We have also observed significant enhancement in activity of blood antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). we have observed increased expression of Ins and Glut-4 genes in diabetic rats treated with methanolic extract of *S. officinalis*, compared to control group. The extract showed strong inhibition effect on intestinal α-glucosidases enzymes activities. **Acknowledgement:** *The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support.* **References:** Lin YF, Tsai HL, Lee YC, Chang SJ (2005) Journal of Nutrition 135: 2457 – 61. Lu YR and Foo LY (2001) Tetrahedron Lett 42: 8223 – 8225. Wang M et al. (1998) J Agric Food Chem 46: 4869 – 4873.

PF89 New Approaches in Characterisation of Herbal Preparations

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New techniques have recently been established in instrumental analytics and molecular biology, which are also applicable to medicinal plants. We could demonstrate the suitability of such methods for identification of herbal preparations by PCR-based methods and for characterisation of herbal preparations by NMR-fingerprinting in combination with principal component analysis [1, 2]. The next step is characterisation of biological activities and correlation with fingerprint profiling. Extracts with solvents of different polarity (Ethanol, Ethanol/Water, Water, Dichloromethane) were obtained from herbal substance of *Chelidonium majus* L. and characterised by means of HPLC and NMR-fingerprinting. Extracts were applied to human liver cells (HepG2) and cell proliferation was monitored in a real time cellular monitoring system, xCELLigence (Roche). Growth of HepG2 cells was drastically inhibited compared to control by ethanolic extracts and partially by ethanolic/aequeous extracts. For aqueous extracts and dichloromethane extracts only minor growth inhibition was observed. Toxic effects of the extracts will be further investigated with expression profiling using DNA microarray technology to check if there is a correlation with known toxic effects which have been reported to herbal preparations containing *Chelidonium majus* **References:** 1. Daniel C et al. (2008) Zeitschrift für Phytotherapie 29: 270 – 274 2. Kersten T et al. (2008) Zeitschrift für Phytotherapie 29: 122 – 128

PF90 An investigation of the *in vitro* transport of *Sceletium tortuosum* alkaloids across porcine buccal, sublingual and intestinal membranes

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Sceletium tortuosum (L.) N.E.Br. is indigenous to South Africa and has traditionally been used for its mood enhancing properties. Recently, products containing *S. tortuosum* have become increasingly popular and are commonly administered as tablets, capsules, teas, decoctions or tinctures, while the dried plant material has traditionally been masticated and the resulting extracts swallowed. In this study, the in vitro transport of four major *S. tortuosum* alkaloids across porcine intestinal, sublingual and buccal mucosa in their pure form as well as in the form of three different crude extracts; water, methanol and an acid-base alkaloid-enriched extract, was evaluated. The permeability of mesembrine across intestinal tissue was higher than that of the highly permeable reference compound, caffeine, in its pure form as well as in the form of crude extracts. The intestinal permeability of mesembranol was similar to that of caffeine, while that of mesembrenol and mesembrenone was lower than that of caffeine but much higher than that of the poorly permeable reference compound, atenolol. In general, the permeability of the alkaloids was lower across the sublingual and the buccal tissues than across the intestinal tissue. However, comparing the transport of the alkaloids with that of the reference compounds there are indications that absorption from the oral cavity may contribute considerably to the overall bioavailability of the alkaloids when the plant material is chewed. The results from this study predict relatively good bioavailability of the alkaloids of *S. tortuosum* in purified or crude extract form when administered orally.

PF91 Antimicrobial activity of the various plant parts of *Warburgia salutaris*

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Warburgia salutaris (G. Bertol.) Chiov. is an aromatic plant found in forests and ravines of northern KwaZulu-Natal, Swaziland, Mpumalanga and the Northern Province of southern Africa. The vernacular names for this tree is Ishibhaha (Zulu), Shibaha (Tsonga), pepper bark tree and Peperbasboom (Afrikaans). Although the bark has a variety of uses, it is mainly used as an expectorant and for the treatment of upper respiratory tract infections. The aim of this study was to determine or confirm the antimicrobial activity of the bark, roots and leaves when exposed to a host of pathogens. Water and methanol/chloroform extracts were prepared and tested against the selected pathogens using minimum inhibitory concentrations (MIC) and then determining possible antibiofilm activity using the crystal violet assay. The majority of the extracts were effective against *Mycobacterium smegmatis* with the solvent root extract exhibiting the noteworthy antimicrobial activity (MIC of 0.25 mg/ml). The bark (water extract) had antimicrobial activity within the MIC range of 1.00 – 2.00 mg/ml) against most of the pathogens while the MIC's of the methanol/chloroform extract of the root ranged between 0.25 to 4.00 mg/ml. None of the extracts showed more than fifty percent inhibition of a 24 hour biofilm.

PF92 Assessing the in vitro gastric stability and intestinal transport of selected natural molecules

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Natural antioxidants in foods and plants play a major role in helping the body's defense system to fight the destruction caused by reactive oxygen species. They may act by decreasing oxygen concentration and as metal inactivators, hydroperoxide decomposers, oxygen scavengers and synergists (Shahidi, 1997). Several studies have indicated the possible beneficial effects of natural antioxidants in the human body, without considering the influence the gastrointestinal tract may have on the composition, activity and absorption of these compounds (Cao et al., 1998; Serrano et al., 2007). This study aimed at assessing the in vitro gastrointestinal stability and intestinal transport of orally ingested antioxidants in food and plants. Curcumin, epicatechin, ferulic acid, gallic acid, quercetin, resveratrol, rosmarinic acid, and rutin were selected. Compounds were incubated in simulated gastric fluid (SGF, pH 1.2) for 1 hour and in simulated intestinal fluid (SIF, pH6.8) for 3 hours. Concentrations were detected before and after incubation. The results indicated that all the compounds were stable in SGF, only epicatechin and rutin were unstable in SIF (with 6.27%, 5.16% degradation, respectively). The in vitro transport experiment was conducted across porcine intestinal tissue in the apical-to-basolateral direction in a Sweetana-Grass

diffusion apparatus. Apparent permeability coefficient (Papp) was calculated. Ferulic acid gallic acid and rutin exhibited low transport with Papp value 8.57 x 10 – 7, 4.89 x 10 – 7 and 1.78 x 10 – 7, respectively. Epicatechin, rosmarinic acid, curcumin. quercetin and resveratrol showed poor transport. **Acknowledgement:** *We appreciate the financial support from Tshwane University of Technology* **References:** 1. Cao G, Booth SL, Sadowski JA, Prior LR (1998) AJCN 68: 1081 – 1087. 2. Serrano J, Goni I, Saura-Caltixo F (2007) Food Research International 40: 15 – 21. 3. Shahidi F (1997) Natural Antioxidants. The America Oil Chemistry Society.

PF93 Antihyperlipidermic and antidiabetic effects of *Pinus koraiensis* leaf oil

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Metabolic disease is a complex syndrome to develop cardiometabolic risk factors, including central obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension. In the present study, anti-diabetic and hyperlipidermic activities of essential oil from leaves of *Pinus koraiensis* Siebold & Zucc. (EOPK) were evaluated. EOPK significantly reduced the blood glucose concentration in streptozotocin (STZ)-induced diabetic mice without weight loss, while significant weight loss was observed in STZ treated mice. Furthermore, EOPK significantly suppressed the production of α-amylase, an enzyme that catalyzes the breakdown of carbohydrate into glucose, in a dose-dependent manner and also prevented the STZ-induced cytotoxicity and nitric oxide (NO) production in HIT-T15 pancreatic β-cells. In addition, EOPK significantly inhibited the level of human cholesterol acyltransferase (hCAT)-1 and -2 and reduced low-density lipoprotein (LDL) oxidation in a dose-dependent manner with an IC_{50} value of 40 μg/ml. Also, Gas chromatography-mass spectrometry (GC-MS) revealed that EOPK contains alpha-pinene (21.3%), alpha-terpineol (11.0%), δ-3-carene (10.2%), terpinolene (7.2%), camphene (6.2%) and limonene (5.2%). Taken together, our findings suggest EOPK can be a potent pharmaceutical agent for prevention and treatment of metabolic syndrome including diabetes and hyperlipidermia. **Acknowledgement:** *This work was supported by a grant from the Next-Generation BioGreen 21 Program (PJ007998), Rural Development Administration, Republic of Korea.* **References:** 1. Yang X et al. (2008) Fitoterapia 79(3):179 – 81. 2. Lee JH et al.(2008)J Microbiol Biotechnol 18(3):497 – 502. 3. Nyenwe EA et al. (2011) Minerva Endocrinol 36(2):129 – 45.

PF94 An ethnobotanical survey of medicinal plants in Valopei district of Savadkouh (Mazandaran-Iran)

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This study was aimed to identify wild plants collected for medical purposes by the local people of Valopei County, located in the Northern of Iran, and to establish the uses and local names of these plants. Field study was carried out over a period of two years (2009 – 2011). Valopei district with over thousand years oldness, 52 villages and high rich knowledge about useful plants is situated in central Elburz region of Iran. A total of 66 plant species belonging to 61 genera and 39 families have been reported from the study area [1]. The most encountered medicinal plant family was Lamiaceae with seven species. The plants are used as medicinal, food and vegetable, provender, treatment of animal diseases, hunting, building construction, manure, weaving and dyeing, respectively based on significance. The use of several species such as *Ligularia persica* Boiss., *Albizia julibrissin* Durazz., *Ruscus hyrcanus* Woronow, *Gleditschia caspica* Desf., *Tamus communis* L., *Hyoscyamus niger* L., *Quercus castaneifolia* C.A.Mey., *Punica granatum* L., *Calystegia sepium* (L.) R.Br. and *Potentilla reptans* L. are reported for the first time. Mode of preparation and administration are discussed along with the family and local names of plants and plant parts used [2]. **References:** 1. Rechinger KH (1963 – 1999) Flora Iranica, Vol. 1 – 174. Akademische Druck und Verlagsanstalt Graz. Austria. 2. Martin GJ (1995) Ethnobotany. A methods manual. London: Chapman & Hall.

PF95 Antioxidant activity, total phenolic and flavonoid content of *Ficus deltoidea* Jack varieties

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Six varieties of *Ficus deltoidea* Jack namely *F. deltoidea* Jack var. *deltoidea*, *F. deltoidea* Jack var. *angustifolia* (Miq.) Corner, *F. deltoidea* Jack var. *intermedia* Corner, *F. deltoidea* Jack var *bilobata* Corner, *F. deltoidea* Jack var. *trengganuensis* Corner and *F. deltoidea* Jack var. *kunstleri* (King) Corner were collected from various locations in Peninsular Malaysia. The total phenolic content in *Ficus deltoidea* var. *intermedia* was remarkably high, followed by varieties *kunstleri, trengganuensis, deltoidea* and *bilobata*. The lowest flavonoid content was observed in the *angustifolia* variety. Antioxidant activity of aqueous extracts was determined by measuring DPPH and H_2O_2 scavenging activities. Very strong antioxidant activity was observed for the extract of *intermedia* variety with IC_{50} of 40 μg/mL, whereas moderate activities were recorded in the extracts of *bilobata* and *kunstleri* varieties with IC_{50} of 150 and 200 μg/mL, respectively. Low DDPH scavenging activities were observed in the extracts of *trengganuensis, deltoidea* and *angustifolia* varieties; with IC_{50} of 325, 380 and more than 500 μg/mL, respectively. Higher H_2O_2 scavenging activity was observed in all varieties studied, when measured at 500 μg/mL as compared to vitamin C.

PF96 Ethnobotanical and ethnopharmacological study in the practice of midwifery in pastoral Iran

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In this study the ethnobotanical and ethnopharmacological features of twenty medicinal plants native to Iran were investigated. Midwives in pastoral communities across the world are very important as main health care providers, but few researches have recognized the therapeutic plants engaged in this age-old practice. Semi-structured interviews were carried out with 5 midwives in 12 pastoral communities near Kermanshah, Iran, concerning the plants they utilize during child delivery as well as pregnancy. Twenty different plant species used to treat 5 conditions happening during the pregnancy, birth and postpartum stages were recorded. Most plants and uses were reported by only one or two midwives. It is interesting to mention that most midwives in this area had emigrated from different parts of the country. Approximately all the midwives used or knew of plant remedies for the treatment of miscarriages, postpartum abdominal pain and hemorrhages, retained placenta, and for speeding up contractions during labor. The most commonly cited plants as well as those for which there was greatest consensus tended to be widespread cultivated or wild species. Although use of medicinal plants by midwives has been reduced as a result of retraining programs by government health centers, midwives' knowledge of medicinal plants may provide an important resource for improving maternal-infant health in Iran and elsewhere. **References:** 1. Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B (2007)J Ethnopharmacol 110: 105 – 117. 2. WHO (2000), www.who.int/diabetes/facts/world-figures (World Health Organization, Switzerland).

Topic G: Isolation and structure elucidation

PG1 Myrosinase hydrolysates of *Brassica oleraceae* L. var. *italica* Plenck

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Phytochemicals, especially the secondary metabolites synthesized by plants, play key roles in human nutrition, health, wellness and disease prevention. Preliminary studies using LC-ESI, provides a simple, rapid technique for the analysis, that is suitable for routine screening of plant materials. Two glucosinolates were identified in the aqueous extract of *Brassica oleraceae* L. var. *italica* Plenck, glucoiberin and 3-hydroxy, 4(α-L-rhamnopyranosyloxy) benzyl glucosinolate, they were identified by liquid chromatography- negative ion electrospray mass spectrometry (LC-ESI) [1]. Two compounds were isolated after enzymatic hydrolysis of the aqueous extract by myrosinase, one of them was identified as 4-vinyl-3-pyrazolidinone. The second compound (sulphoraphane) 1-isothiocyanate-4-methyl-sulphinyl butane, converted to the most stable form of thiourea, (sulphoraphane thiourea) [2]. The crude extract (80% alcohol extract) of broccoli florets was examined for cytotoxic activity against different cell lines [3], it showed good inhibition to colon tumor (IC50 3.88 μg). But when the same test was repeated on each successive extract no significant cytotoxic activity produced with any of them. When myrosinase hydrolysate was tested for cytotoxic activity on colon tumor cell line, it showed very high activity 95% lethality up to 0.78 μg/mL..
Acknowledgement: *Hamed A. for performing LC-ESI. Institute of natural medicine, Toyama university, Toyama, Japan.*
References: 1. William CK et al. (1998) J Agric Food Chem 46: 1018 – 1012. 2. Hashem FA, Wahba HE (2000) Phytother Res 14: 284 – 287. 3. Zhang Y, Talalay P (1994) Cancer Res 54 (Suppl.): 1976 s-1981 s.

PG2 Phytochemical Investigations on the Leaves of *Bafodeya benna* using LC-SPE-NMR

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Bafodeya benna (Scott-Elliot) Prance ex F.White or *Parinari benna* Scott-Elliot (Chrysobalanaceae) is a tree that only grows in the savanna of Mali, Guinea-Conakry and Sierra Leone. Since it is used in traditional medicine against infectious diseases such as malaria, phytochemical investigations were started in order to identify potentially active constituents using LC-SPE-NMR and LC-MS. A crude extract was prepared from the leaves using 80% methanol, and 4 subfractions of different polarity (petroleum ether, 90% methanol, chloroform and water). The tannins were removed from the aqueous fraction, and this fraction was submitted to LC-SPE-NMR analysis. Five major constituents were observed in the HPLC chromatogram and they were identified as neoastilbin (1), astilbin (2), neoisoastilbin (3), isoastilbin (4), and quercetin-3-O-α-L-rhamnoside. Moderate antiplasmodial activity has already been reported for taxifolin, however not for astilbin ((2R,3R)-taxifolin-3-O-alpha-L-rhamnoside). It is possible that the glycosides are hydrolysed first in the gastro-intestinal tract. It was concluded that these flavonoid glycosides contribute, at least in part, to the traditional use against malaria.

PG3 Phytochemical and Biological Studies on *Aster novi-belgii*

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Aster is a large genus of the family Asteraceae (Compositae) comprising more than 200 species distributed around the world (1). Some of *Aster* species have been used in traditional medicine for the treatment of fever, cold, tonsillitis, snake bite and bee sting (2). Members of this genus are rich in triterpenoid saponins, two oleanan type saponins and three flavonoid compounds were isolated from Aster novi (3,4). Phytochemical investigation of the methanolic extract of *Aster novi-belgii* L. (Asteraceae) aerial part has led to the isolation and identification by spectroscopic means of four triterpenoid saponins, oleanolic acid 3-O-β-D-galactopyranosyl (1→3)-α-L-arabinopyranoside; 3-O-β-D- glucopyranosyl- 3β, 16 α- dihydroxyolean- 12- en- 28-oic acid-28-O-α-L- arabinopyranoside; {3-O-β-D- glucouronopyranosyl- 3β,16 α- dihydroxyolean- 12-en -23-oic acid 28-O-β-D- xylopyranosyl- (1→3)-β-D- xylopyranosyl- (1→4)- α-L-rhamnopyranosyl-(l→2)-α-L-arabinopyranoside} ester and {3-O-β-D-glucouronopyranosyl-3β,16 α- dihydroxyolean- 12-en-23-oic acid 28-O-β-D- xylopyranosyl- (1→3)- β-D-xylopyranosyl (1→4)-[α-L- arabinopyranosyl- (1→3)] -α-L- rhamnopyranosyl- (l→2)- α-L-arabinopyranoside} ester, and four flavonoids; kaempferol, quercetin, kaempferol 3-O-β-D-glucopyranoside and quercetin 3-O-β-D-glucopyranoside. Moreover, the extract showed a molluscicidal activity against *Biomphalaria alexandrina* snails.
References: 1. Shao Y et al. (1997) J Nat Prod 60: 743 – 746. 2. Shao Y et al.(1995) Planta Med 61: 246 – 249 3. Abdel Khalik SM (2006) Bull Fac Pharm Cairo Univ 44: 85 – 89 4. Abdel Khalik SM (2006) Bull Fac Pharm Cairo Univ 44: 253 – 256

PG4 Investigation of Flavonoidal Constituents and Hepatoprotective Activity of *Myoporum laetum*

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Myoporum laetum G.Forst. (Myoporaceae) is an evergreen ornamental shrub and it flowers from May to June [1].Fractionation and isolation of the butanol extract of *Myoporum laetum* yielded five major flavonoids, luteolin 4'-O-α-L rhamnoside, 5-methoxy-luteolin 7-O-β-D-arabinoside, 5'-hydroxy-luteolin 7-O-β-D-glucoside (tricetin 7-O-β-D-glucoside), luteolin and apigenin. Their structures were determined by chromatographic and spectroscopic methods. The hepatoprotective and antioxidant activities of the butanol extract against liver injury induced by repeated doses of the profenofos as hepatotoxicant were investigated. Oral supplementation of butanol extract to profenofos treated animals successfully modulated the hepatotoxicant induces deviation in the liver function markers (liver oxidative and antioxidant markers) indicating its potential hepatoprotective and antioxidant abilities.
References: 1. Blackburne et al. (1972) Aust J Chem 25: 1787 – 96.

PG5 Biologically active saponins from *Apodytes dimidiata*

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In previous studies *Apodytes dimidiata* E.Mey. ex Arn. (Icacinaceae) displayed activity against leishmaniasis [1]. Therefore, a bioassay guided isolation was performed in order to isolate the active constituents. Six saponins, never isolated from nature before, were identified by LC-MS/MS, GC-MS and 1D and 2D NMR: apodytine A (compound **1**), apodytine B (compound **2**), apodytine C (compound **3**), apodytine D (compound **4**), apodytine E (compound **5**), apodytine F (compound **6**). Compounds **1**, **2** and **3**, having an acetyl group at the same position in the aglycon part of the molecule, were more active against *Leishmania infantum* (IC_{50} values < 1 μM) and cytotoxic against MRC-5 cells (CC_{50} values < 10 μM) than compounds **4**, **5** and **6** (IC_{50} < 10 and CC_{50} < 20 μM, respectively), which contain a hydroxyl functionality at the same position. These compounds, responsible at least in part for the antileishmanial activity of the plant, also showed a haemolytic activity, and antiangiogenic activity in the rat aorta ring assay. The latter might be due to a non-selective toxicity. Since saponins are known for their molluscicidal activity, the presence of these compounds might explain the use of *Apodytes dimidiata* against the snails *Bulinus africanus* and *Biomphalaria pfeifferi*, intermediate host snails of *Schistosoma* spp. [2].

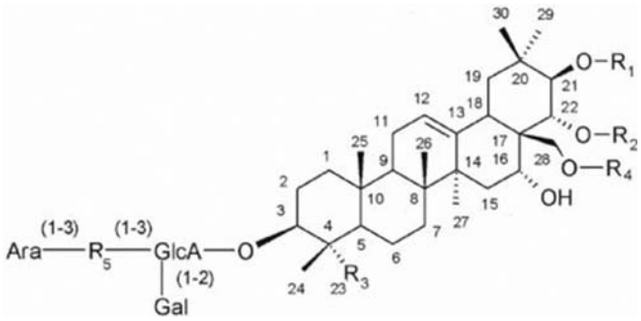

Figure 1: Compound 1 – 6 (Apodytine A – F)

Compound	R_1	R_2	R_3	R_4	R_5
1	isopentanoyl	acetyl	CHO	H	galactosyl
2	isopentanoyl	acetyl	CHO	H	arabinosyl
3	isopentanoyl	acetyl	CH_3	H	galactosyl
4	isopentanoyl	H	CHO	acetyl	galactosyl
5	isopentanoyl	H	CHO	acetyl	galactosyl
6	isopentanoyl	H	CH_3	acetyl	galactosyl

References: 1. Maes L. (2003) PhD Thesis: Lead identification and development of a new anti-leishmanial saponin PX-6518, isolated from the Vietnamses plant Maesa balansae, University of Antwerp, Antwerp. 2. Pretorius SJ et al. (1991) J Trop Med Hyg 94: 159 – 165.

PG6 Rapid identification of the main constituents of an antimalarial plant extract using LC-SPE-NMR

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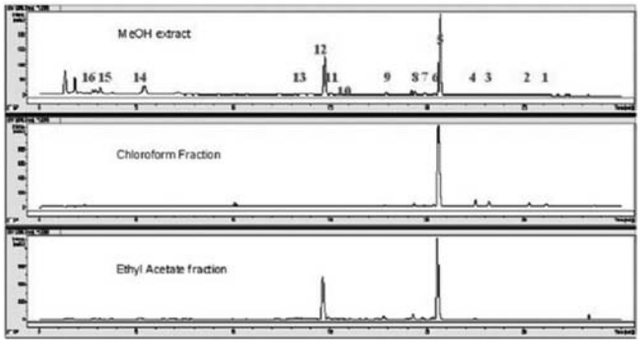
Previous phytochemical investigation of *Ormocarpum kirkii* S.Moore (Papilionaceae) has led to the isolation and identification of (I-3,II-3)-biflavonoids, some of them with antiplasmodial activity (Dhooghe et al., 2010). The major compound of the total extract (80% MeOH) was (+)-chamaejasmin. However, a series of minor constituents remained unidentified. The aim of this work was to further characterise the 80% MeOH ectract from *O. kirkii* and to identify the minor constituents using an integrated platform of LC-MS and LC-SPE-NMR. The crude 80% MeOH extract of *O. kirkii* was partitioned into four fractions by means of liquid-liquid extraction: *n*-hexane, chloroform, ethyl acetate and water. The chloroform and ethyl acetate fractions were used for the LC-SPE-NMR study. By using the multiple trapping technique several components were enriched on the SPE cartridges. After drying and eluting with deuterated methanol into 3 mm NMR tubes, high-resolution NMR spectra were recorded. The structure elucidation of these compounds was based on 1D and 2D NMR, and MS data. A total of sixteen compounds were identified and assigned in the HPLC chromatogram: sikokianin B (**1**), sikokianin C (**2**), glabroisoflavanone A (**3**), diphysolone (**4**), (+)-chamaejasmin (**5**), isochamaejasmin (**6**), apigeninyl-(I-3,II-3)-naringenin (**7**), liquiritigeninyl-(I-3,II-3)-naringenin (**8**), (I-3,II-3)-biliquiritigenin (**9**), 5,5"-dimethoxydiphysin (**10**), 4"-hydroxydiphysolone (**11**), 7-O-glucosylchamaejasmin (**12**), diphysin (**13**), ormocarpin (**14**), 7-O-glucosyldiphysin (**15**), and isovitexin (**16**). In addition to the twelve constituents (**5-16**) that had been isolated before using semi-preparative HPLC, four more compounds were obtained (**1-4**).



References: Dhooghe L et al. (2010) Phytochemistry 71: 785 – 791

PG7 Flavonoids from the flowers of *Lantana camara* L. with *in vitro* antioxidant activity

Abou El Kassem LT[1], Mohammed RS[1], Salah El Din S[2], El Ansari A[1], Hawas UW[3], Mahmoud K[1]
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Lantana camara L., Family Verbenaceae commonly known as wild or red sage, is the most widespread species of this genus, growing luxuriantly in tropical, sub-tropical and temperate regions [1]. *L. camara* is used in folk medicine as vulnerary, diaphoretic, carminative, antiseptic, antispasmodic and tonic and [2 – 3]. A phytochemical investigation of the aqueous-methanolic extract of its flowers had led to isolation of a new flavonoidal compound, apigenin 7-*O*-β-D-galacturonopyranosyl-(2→1)-*O*-β-D-galacturonopyranoside with the eleven flavonoids, luteolin 7-*O*-β-D-glucuronopyranosyl-(2→1)-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-β-D-glucuronopyranosyl-(2→1)-*O*-β-D-glucuronopyranoside, vitexin, isovitexin, apigenin 7-*O*-β-D-galacturonopyranoside, luteolin 7-*O*-β-D-glucopyranoside, luteolin 7-*O*-β-D-galactopyranoside, luteolin 4'-*O*-β-D-glucopyranoside, apigenin 7-*O*-β-D-glucopyranoside, luteolin and apigenin. The aqueous methanolic of *L. camara* flowers and some compounds proved a significant antioxidant effect. **References:** [1] Sharma OP, Makar HPS, Dawra RK (1988) Toxicon26: 975. [2] Kirtikar KR, Basu BD (1935) Indian medicinal plants, vol. III. Dehra Dun: Bishen Singh and Mahendra Pal Singh,:1914. [3] Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian medicinal plants. New Delhi: CSIR,149.

PG8 Phytochemical constituents of the root of *Combretum micranthum* G. Don (family: Combretaceae)

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The root of *Combretum micranthum* was evaluated phytochemically. β-sitosterol was isolated and characterized from the hexane extract of *C. micranthum* root. Epicatechin and catechin as penta-acetates; epigallocatechin, gallocatechin and bartogenic acid – 28 – β – D – glucoside as hexa-acetates were isolated and characterized from the acetylated methanol extract of the root. The structures of the compounds were established by comparing their spectroscopic data (IR, proton, carbon-13, DEPT 90/135) with data published in the literature [1, 2, 3, 4]. **Acknowledgement:** *[1] Prof. F. O. Shode, School of Chemistry, University of KwaZulu Natal (UKZN), Durban in the Republic of South Africa for providing space in his laboratory to HD Umar to carry out the phytochemical analysis. [2] STEP-B project for IOT grant to HD Umar.* **References:** [1] Kolak U et al. (2005) Turk J Chem 29: 177 – 186. [2] Balde AM et al. (1991) Phytochem 30 (1): 337 – 342. [3] Weinges K and Schick H (1995) Phytochem 38 (2): 505 – 507. [4] Arramon G et al (2002) Phytochem Anal 13: 305 – 310.

PG9 Oligostilbenoids from the stem bark of *Dryobalanops aromatica*

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Dryobalanops is one of the Dipterocarpaceae families which contain oligostilbenoid that show various biological activities [1]. The oligostilbenoid in *Dryobalanops* genera is very unique, as some compounds such as cis- and trans-diptoindonesin B and malaysianol A have difference oxidative pattern compared to other oligostilbenoids in Dipterocarpaceae [2, 3]. The aim of this study is to isolate the oligostilbenoid constituents in Malaysian *D. aromatica* C.F.Gaertn. and to determine their cytotoxic activity. The dried powder of the stem bark of *D. aromatica* was macerated with acetone and evaporated under reduced pressure. The crude of acetone extract was subjected to vacuum liquid chromatography (VLC) to give 10 major fractions. Purification of the sixth fraction with radial chromatography gave laevifonol (**1**) (93 mg) and ampelopsin E (**2**) (397 mg) while the fifth fraction afforded α-viniferin (**3**) (91 mg) and ε-viniferin (**4**) (20 mg) and the tenth fraction yielded diptoindonesin A (**5**) (30 mg). The effect of the isolated compounds (**1 – 3**) against HL-60, MCF-7, HepG2, A-549 and WRL-68 cell lines were evaluated by using

MTT assay [4]. Compound **3** was found to inhibit very strongly the growth of HL-60 cell lines (IC_{50} 2.7 μg/ml) and display moderate activity against MCF-7 cell line (15.7 μg/ml). Besides that, **2** was found to moderately inhibit MCF-7 cell line (14.3 μg/ml). **Acknowledgement:** *The authors would like to thank The Ministry of Higher Education, Malaysia for the financial support under the Fundamental Research Grant Scheme; 600-RMI/ST/FRGS/5/3/Fst (12/2010).* **References:** [1] Sotheeswaran S, Pasupathy V (1993) Phytochemistry 32: 1083 – 1092. [2] Syah YM et al. (2003) Phytochemistry 63: 913 – 917. [3] Wibowo A et al. (2011) Fitoterapia doi:10.1016/j.fitote.2011.02.006. [4] Mosmann T (1983) J Immunol Methods 65: 55 – 63.

PG10 A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigerian mistletoe with potent immunostimulatory activity on C57BL6 splenocytes

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In our continued efforts to identify the immunoactive constituents of a local mistletoe species in Eastern Nigeria, a novel sesquiterpenoidal acid, 2, 3-dimethoxy-benzo [a, b] cyclopentenyl-3', 3', 5'-trimethyl pyran-4-carboxylic acid (1), and a known alkaloid, lupinine (2) [1] were isolated from a bioactive chloroform fraction of the leave extract of the mistletoes parasitic on kola tree, *Cola acuminata* (P.Beauv.) Schott & Endl.. These compounds were screened for immunostimulatory activities on isolated C57BL/6 mice splenocytes at concentrations of 10, 25 and 100 μg/ml. Their effects on the expression of CD69, an early immune cells activation marker [2], were determined using flow cytometry techniques and compared to Lipopolysaccharide (LPS; 10 μg/ml) and Concanavalin A (ConA; 2 μg/ml) as standards. The compounds (1 and 2) at a concentration of 25 μg/ml showed statistically significantly ($p < 0.05$) stimulatory activity on the isolated splenocytes compared to the non-stimulated control cells with values of 56.34 ± 0.26% and 69.84 ± 0.19% respectively compared to 7.58 ± 0.42% recorded for the control. Similarly, the CD69 expression assay at the above dose showed that the compounds were stimulatory with statistically significant values ($p < 0.05$) of 2.31 ± 0.07% and 2.71 ± 0.03% respectively compared to 1.69 ± 0.05% recorded for the non-stimulated control. The compounds were characterized using a combination of UV/visible, IR, NMR (13C-NMR and 1 H-NMR) and DEPT, MS and 2-dimensional correlation (H-H COSY, HSQC, HMBC, NOE, and NOESY) studies. These compounds may be responsible in part, for the immunostimulatory activities already established for the Eastern Nigerian mistletoes. **Acknowledgement:** *The author wish to thank Mr Alfred Ozioko of BDCP Nsukka for identifying the plant material and Associate Professor Akira Kawamura of CUNY for the detailed spectral studies.* **References:** 1. Michael JP (1998) Nat Prod Rep 16: 675 – 696. (2) Borrego F et al. (1999) Immunol 97: 159 – 165

PG11 Flavonoids from *Centaurea glomerata* and antioxidant activity of its extract

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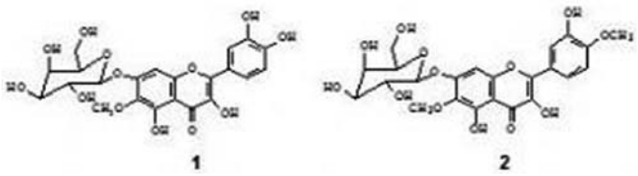
In the early nineties the presence of flavonoids in herbal began to attract the attention of a number of researchers, as a result of their biological and physiological importance [1]. The present study deals with the isolation and identification of flavonoids from *Centaurea glomerata* Vahl. and evaluation of antioxidant activity of the extract. The aqueous alcoholic extract (MeOH:H_2O 7:3) of *Centaurea glomerata* aerial parts was subjected to extensive repeated column chromatography on polyamide, and Sephadex LH-20 resulted in two new flavonoids named quercetin 6-methoxy -7-O-galactoside (1) and quercetin 6,4- dimethoxy -7-O-galactoside (2) as well as apigenin 8-C-glucoside, apigenin 6-C-glucoside, quercetin 6-methoxy, quercetin and apigenin. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D $^1H/^{13}C$ NMR spectroscopy. The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The effective dose 50 (ED_{50}) of the extract was compared with that of standard antioxidants as vitamin C.



1 **2**

References: 1. Havsteen B (2002) Pharmacology & Therapeutics 96: 67 – 202

PG12 Amaryllidaceae alkaloids from *Rauhia multiflora* (Kunth) Ravenna

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The taxonomy of the American Amaryllidaceae subfamily Amaryllidoideae sensu APG (Amaryllidaceae s.s.) is currently undergoing revision based on molecular phylogenetic analyses [1]. The genus *Rauhia* Traub comprises three to five species endemic to Northern Peru [1,2]. *Rauhia* resolves as sister to *Phaedranassa* Herb. in the primarily Andean tribe Stenomesseae Traub based on a combined nrITS and gap matrix [2]. Family specific alkaloids [3] with CNS activity such as galanthamine are phylogenetically constrained and have chemotaxonomic value [3,4]. However, the chemistry of the American Amaryllidaceae is sparsely investigated and the chemistry of the genus *Rauhia* has to our knowledge not previously been investigated. In this first investigation of alkaloids from the genus *Rauhia*, galanthamine and sanguinine were isolated from *Rauhia multiflora* (Kunth) Ravenna. Dried bulbs of *Rauhia multiflora* (23.15 g) were extracted following standard methods [4]. The alkaloids were identified using ^1H NMR and ^{13}C NMR spectroscopy and optical rotation. Galanthamine has been reported from several species of primarily Eurasian Amaryllidaceae, but also for example from South American *Eucharis amazonica* Linden [3]. Sanguinine was first isolated from *Lycoris sanguinea* Maxim. and subsequently from other Eurasian Amaryllidaceae, as well as South American *Phaedranassa dubia* (Kunth.) J.F.Macbr. [3]. The finding of sanguinine in *Rauhia multiflora* may support the proposed sister relationship of *Rauhia* and *Phaedranassa*, but the taxonomic value of both galanthamine and sanguinine within American Amaryllidaceae in general is unknown. **Acknowledgement:** *We thank the Botanical Gardens of the University of Copenhagen for material of Rauhia multiflora (Voucher: Rønsted 374, Herbarium C). This research was supported by a Steno grant (N°272 – 07 – 0281) to NR from the Danish Council for Independent Research – Natural Sciences.* **References:** 1. Meerow AW (2010) Diversity, phylogeny and evolution in the monocotyledons, Aarhus University Press, Denmark, pp. 145 – 157. 2. Hannon DP (2009) Herbertia 63: 131 – 150. 3. Jin Z (2009) Nat Prod Rep 26: 363 – 381. 4. Jensen BS et al (2011) Biochem Syst Ecol 39: 153 – 155.

PG13 Trimer and Tetramer Resveratrols from *Shorea macroptera*

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Four oligomer resveratrols have been isolated from the stembark of *Shorea macroptera* Dyer by using various chromatographic techniques. The dried and ground bark of *Shorea macroptera* (6 kg) was soaked with acetone for 72 hours. The acetone extract was concentrated *in vacuo* to yield a crude extract (300 g) that was partitioned further using diethyl ether. The diethyl extract was concentrated (115 g) and subjected to vacuum liquid chromatography (VLC) using gradient system of Hexane: Ethylacetate (Ea) and seven fractions were afforded. Fraction 4 was further purified by second VLC (CHCl3-acetone system), and repeated

radial chromatography ($CHCl_3$-MeOH system) to yield compound **1**. Fraction 5 was subjected to VLC (Hex: Ea: Ac system) and purified by radial chromatography repeatedly ($CHCl_3$:Ea:MeOH system) to give compound **2**, **3** and **4**.The structures of compounds **1 – 4** were determined based on spectroscopic data including UV, IR, 1D an 2D NMR and comparison with those previously reported. The similarity to these published data from Tanaka *et al*, 2000 [1], Ito *et al*, 1997 [2] and Tanaka *et al*, 2001 [3] suggested compound **1** as davidiol A, a trimer resveratrol and compound **2, 3** and **4** as tetramer resveratrols namely hopeaphenol, isohopeaphenol and hemsleyanol D, respectively. **Acknowledgement:** *Scholarship of one of the authors was financed by the National Science Fellowship (NSF) from Ministry of Science, Technology and Innovation Malaysia (MOSTI).* **References:** [1] Tanaka T et al. (2000) Phytochemistry 53: 1009 – 1014. [2] Tukiran AS et al. (2005) Biochemical Systematics & Ecology 33: 631 – 634. [3] Tanaka T et al. (2001) Heterocycles 55:729 – 740.

PG14 Isolation of Trimer stilbenoids from the bark of *Shorea maxwelliana*

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The stem bark of *Shorea maxwelliana* King (2.85 kg) was collected from reserved forest Jengka, Pahang, Malaysia. Three major trimer stilbenoids have been isolated from acetone extract of the stem bark of *Shorea maxwelliana*. Sample of *S. maxwelliana* was macerated in acetone for five times, and the solvent evaporated using rotary evaporator to produce crude acetone extract. The acetone extract (178.90 g) was fractionated by a series of solvent partitions into an EtOH soluble phenolic fraction (25.78 g). This fraction was repeatedly chromatographed on silica gel columns with Hex-EtOH (1:1) and EtOH, and then with a $CHCl_3$-MeOH gradient of increasing MeOH to provide four fractions (A-D). Fraction A was the n-hexane soluble fraction (12.6 g). Fraction B (2.26 g) was subjected to silica gel chromatography eluted with $CHCl_3$-MeOH (95:10 to 80:20) to give compound **1** (500 mg). Fraction C (9.8 g) was subjected to silica gel chromatography eluted with $CHCl_3$-EtOH-MeOH (85:1:0.5) to afford compound **2** (250 mg). Compound **3** (185 mg) was obtained from fraction D (1.12 g) by silica gel chromatography eluted with $CHCl_3$-MeOH (8:2) [2]. The molecular structures of **1, 2** and **3** were determined based on spectroscopic data, including UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and comparison with that reported in the literature. Spectral data of compounds **1, 2** and **3** showed very close similarity to α-viniferin [1], vaticanol A [1] and copalliferol A [2]. **Acknowledgement:** *The authors would like to thank the Ministry of Science, Technology and Innovation, Malaysia (MOSTI) for funding the research grant, Fundamental Research Grant Scheme (FRGS), 600-RMI/FRGS 5/3/Fst (19/2009) and National Science Fellowships (NSF) for financing the study of one of the authors.* **References:** [1]. Sahidin EHH et al. (2007) Journal Matematika dan Sains. 12(3): 113 – 118. [2]. Sotheeswaran S, Sultanbawa M U S and Surendrakumar S (1983) Journal of Chemical Society Perkin Transactions (1): 699.

PG15 A study on exudates and micromorphology of *Primula*

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The genus *Primula* L. comprises more than 400 species, grouped in 6 subgenera and 37 sections [1]. Especially the production of oily or farinose exudates on aerial surfaces of leaves, stems, calyces and flowers is a conspicuous character of this genus. These exudates consist primarily of un-substituted flavones and other flavones with unusual substitution patterns, which are probably derived from a still unidentified biogenetic pathway [2]. Exudate profiles were monitored by HPLC and TLC, and known structures were identified by comparison of their UV-spectra and retention times with those of reference compounds of our spectral library. New structures were elucidated additionally by NMR spectroscopy. The auto-fluorescent property of some of these flavonoids was used for studying their accumulation in glandular hairs in vivo by using an epifluorescence microscope. Different colors of fluorescence were observed within a single leaf of *P. vulgaris* Huds., while leaves of *P. vialii* Delavay ex Franch. showed uniform fluorescence. The significance of our findings in relation to chemodiversity, morphology, and micromorphological character differentiation will be discussed. **Acknowledgement:** *Hochschuljubiläumsstiftung der Stadt Wien, Gesellschaft zur Förderung der Pflanzenwissenschaften.* **References:** 1. Richards J (2002) Primula. B.T. Batsford Ltd. London. 2. Valant-Vetschera KM et al. (2009) NPC 4: 365 – 370.

PG16 New Lanostane Triterpenoids from *Antrodia camphorata*

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Many polypores are used for medicinal purposes in traditional Chinese medicine. *Antrodia camphorata* (M. Zang et C.H. Su) Sheng H. Wu, Rywarden et T.T. Chang, known as "niu-chang-chih", is restricted to the endemic tree, *Cinnamomum kanehirae* Hayata. Traditionally the fungus has been used for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer. The components of this fungi have shown activities such as anti-inflammation, immune-modulation, anti-Helicobacter pylori, and neuroprotection from Aβ damage. Here, we present our chemical studies on the mixture of fruiting body and mycelia of solid cultures of *A. camphorata*. As a result, eight new lanostane triterpenoids, 3,7,11-trioxo- 5α-lanosta-8,24-(E)-dien-26-oic acid (1), methyl 11α-hydroxy-3,7-dioxo-5α-lanosta-8,24-(E)-dien-26-oate (2), methyl 3,7,11,12,15,23-hexaoxo-5α-27ζ-lanost-8-en-26-oate (3), ethyl 3,7,11,12,15,23-hexaoxo-5α-27ζ-lanost-8-en-26-oate (4), ethyl lucidenate A (5), ethyl lincidenate F (6), acetyl ganolucidic acid A (7), 3,11,15,23-tetraoxo- 5α-27ζ-lanosta-8,16-dienoic acid (8), were isolated and elucidated. These compounds were evaluated for their cytotoxicity against several human tumor cell lines.

PG17 New type of polyacetylene sesquiterpenoid conjugates from *Notopterygium incisum*

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Qiang Huo, the dried rhizome and root of both *Notopterygium incisum* Ting ex H. T. Chang and *N. forbesii* Boiss. (Umbelliferae) are used widely in China for treating cold, and inflammatory diseases. Peroxisome Proliferator Activated Receptor gamma (PPAR-gamma) is involved in inflammatory processes, and has become an important pharmacological target.[1] From the dichloromethane extracts of the underground parts of *Notopterygium incisum*, we have obtained through bio-guided isolation, a series of falcarindiol derivatives with significant PPAR-gamma agonistic activity.[2] In addition, we have isolated eight polyacetylene derivatives (**1-8**), which were identified by NMR as unique polyacetylenes fused with sesquiterpenoids. They are a second type of polyacetylene adducts connected through an ether bond, besides previously reported coumarin adducts.[3,4] The sesquiterpenoid moiety of **5** and **6** is reported for the first time.

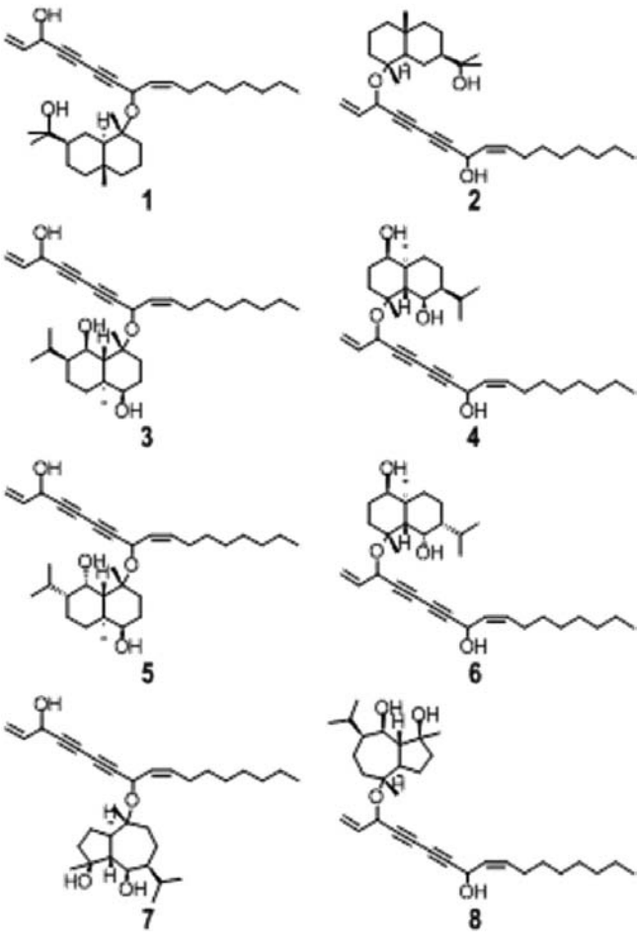

Figure 1. structures of polyacetylene sesquiterpenoid conjugates from N. incisum.

Acknowledgement: *We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF) within project NFN S 10705-B13.* **References:** 1. Schmidt MV et al. (2010) Scientific World Journal 10: 2181 – 97 2. Blunder M. et al. (2011) Planta Medin preparation 3. Furumi K et al. (1998) Bioorg Med Chem Lett 8: 93 – 6. 4. Fujioka T et al. (1999) Chem Pharm Bull 47: 96 – 100.

PG18 New Pyrones from the Mangrove Endophytic Fungus *Pestalotiopsis* sp

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Pestalotiopsis is generally found as endophytes of tropical plants and as prolific producers of structurally unusal natural products [1]. Our previous chemical investigations on fungus species *Pestalotiopsis* JCM2A4 isolated from the Chinese Mangrove plant *Rhizophora mucronata* Lam. have led to the isolation of a series of natural products and yielded over twenty different compounds with seventeen of them being new natural products including chromones, cytosporones and coumarins [2 – 3]. Following cultivation of the fungus now yielded eight new pyrone derivatives (1) as well as a known compound. The structures of all compounds were unambiguously established from their spectroscopic data, that included HR-ESIMS and 1- and 2-dimensional NMR spectroscopy, and by comparison with the literature [4]. Our findings proved endophyte genus *Pestalotiopsis* to be particularly productive.

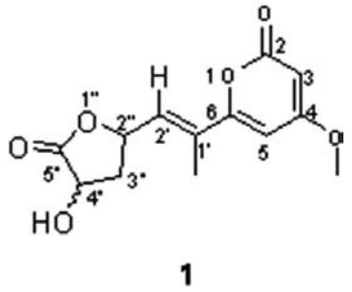

Figure 1: Structure of pyrone

Acknowledgement: *Financial support by grants of Hainan University Youth Foundation (qnjj1005) and Dr. Research Starting Found (kyqd1066) are gratefully acknowledged.* **References:** 1. Xu J et al. (2010) Fungal Divers 44:15 – 31. 2. Xu J et al. (2009) J Nat Prod 72: 662 – 665. 3. Xu J et al. (2009) Bioorg Med Chem 17: 7362 – 7367. 4. Xu J et al. (2011) Tetrahedron Lett 52: 21 – 25.

PG19 Alkaloids constituents from the stem bark of *Polyalthia cauliflora* var. *cauliflora* (Annonaceae)

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Polyalthia cauliflora Hook. f. & Thomson var. *cauliflora* from the Annonaceae family is known as '*Semukau*' among 'Kelabit' community in Bario Sarawak, Malaysia. It is a small tree with 14 m tall and 13 cm diameter and has purplish fruits at the trunk of the plant and is widely distributed in Peninsular Malaysia, Borneo, Sumatra and Thailand [1]. This plant has been used traditionally for birth control [2]. Alkaloidal crude extract (4.94 g) was subjected to vacuum liquid chromatography (VLC) on silica gel using Hex/DCM/MeOH mixtures of increasing polarity to obtained 52 fractions. The same patterned of spots were pooled together into 10 fractions. Fraction 3 was subjected to radial chromatography to yield compounds **1** (1.3 mg) and **2** (1.2 mg). Fraction 5 and 6 were subjected to preparative thin layer chromatography to afford compounds **4** (4.1 mg) and **3** (3.6 mg). The spectral data analysis (1D and 2D NMR spectroscopy, MS) of compounds **1 – 4** revealed that **1** and **2** are oxoaporphine, **3** is isoquinoline and **4** is aporphine alkaloids. Comparison with literature determined compounds **1 – 4** as liriodenine [3], lanuginosine [4], 6-hydroxy-7-methoxy-2-methyl-3,4-dihydroisoquinolinone [5] and dehydrodomesticine [6], respectively. The synthesis of **3** and **4** have been reported previously but this is their first occurence naturally. **Acknowledgement:** *This study was supported by the grant from FRGS (No.: 600-RMI/ST/FRGS/5/3/Fst (32/2009)) and scholarship of one of the authors was financed by National Science Fellowship (NSF) from Ministry of Science, Technology and Innovation, Malaysia (MOSTI).* **References:** 1. (http://www.nationaalherbarium.nl/sungaiwain/Annonaceae/Polyalthia_cauliflora.html) 2. Fasihuddin BA, Ipor I, Din L (1995) Medicinal Plants Used by the Kelabit Community in Bario Sarawak. Chemical Prospecting in the Malaysian Forest. Pelanduk Publication (M) Sdn. Bhd. Selangor. 3. Lavault M, Cabalion P, Bruneton J (1981) Planta Med 42: 50. 4. Leboeuf M, Cave A, Bhaumik PK, Mukherjee B, Mukherjee R (1982) Phytochemistry 21: 2783 – 2813. 5. Irie H, Shiina A, Fushimi T, Katakawa J, Fujii N, Yajima H (1980) Chemistry Letters 9(7): 875 – 878. 6. Guinaudeau H, Leboeuf M, Cave A (1983) Journal of Natural Products 46(6): 761 – 835.

PG20 Isolation of three oligostilbenes from the bark of *Shorea bracteolate*

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Shorea species is the largest subfamily of Dipterocarpaceae and are the source of resveratrol oligomers (oligostilbene), sesquiterpenes and triterpenes (Aminah et al., 2002). *Shorea bracteolata* Dyer – also called white meranti is locally known as "Meranti Pa'ang" and is widely distributed in Sumatera, Peninsular Malaysia, Indonesia and Singapura. The

tree is up to 50 m in height and the timber is light hardwood. The study was undertaken to extract and isolate the chemical constituents from the stembark of *Shorea* species namely *Shorea bracteolata* and to elucidate the structures of the chemical constituents isolated by using modern spectroscopic methods. The stembark of *S. bracteolata* (4 kg) was dried and cut into small pieces and ground to powdery about 1 mm mesh size using grinder. The sample was extracted with acetone, filtered and evaporated in vacuo at 400ºC to yield crude extract (320 g). Diethyl ether was added to crude extract to remove the tannin. The tannin free crude extract was fractionated using vacuum liquid chromatography (VLC) to give six fractions. Fraction 2 was subjected to radial chromatography 1 [Hex:EtOAc (5:5)] and 2 [$CHCl_3$:MeOH (9.5:0.5] to yield ε-viniferin (1) (Muhtadi et al., 2005) and α-viniferin (2) (Sutopo, 2009). Double purification of fraction 5 using radial chromatography [EtOAc:-MeOH (9:1)] and [$CHCl_3$: Acetone:MeOH (7:2:1)] afforded hopeaphenol (3) (Guebailia et al., 2006). The structure of the isolated compounds was determined based on analysis of spectroscopic data, including NMR, UV, IR and comparison with previous reported studies.

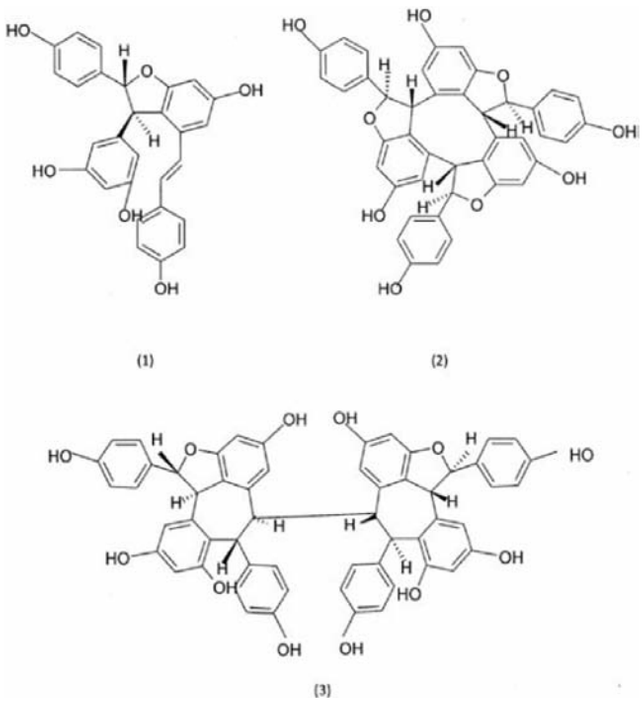

Figure 1: Structure of compound 1 – 3

Acknowledgement: *The authors would like to thank Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia for financing this research project. The scholarship of one of the authors was financed by UiTM Fellowship.* **References:** Aminah NS et al. (2002) Fitoterapia 73(6): 501 – 507 Guebailia HA et al. (2005) J Agric Food Chem 54: 9559 – 9564. Muhtadi HEH et al. (2005) J. Matematika dan Sains, 10(4): 137 – 143. Sutopo H (2009) Modern Applied Science 3(4): 45 – 51.

PG21 Synthesis and Evaluation of Gamgogic Acid Analogs as Cytotoxic Agents

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Gambogic acid (1, GA) was isolated from the resin of *Garcinia hanburyi* Hook. f. (Clusiaceae), a tree growing in Southeast Asia. The resin is used in folk medicine and as coloring agent in China (1,2). The chemical structure of GA shows a unique 4-oxatricyclo[4.3.1.0]decan-2-one ring system attached to a xanthone backbone.3 In order to analyze structure-activity relationships (SAR) of GA, we converted it into xanthonic derivatives (Figure 1) and performed the synthesis of some structurally related prenylated derivatives, using as building blocks 1,3,6-trihydroxyxanthone (2) and tested them for cytotoxic activity. All newly synthesized compounds were assayed for *in vitro* cytotoxicity against 4 human cancer cell lines: KB (nasopharyngeal), KBvin (multidrug-resistant nasopharyngeal over-expressing P-gp), A549 (lung) and DU-145 (prostate). Among them, compounds 9 and 10 showed remarkable IC_{50} values of 0.91 and 0.82 μg/mL, respectively, against KBvin cells.

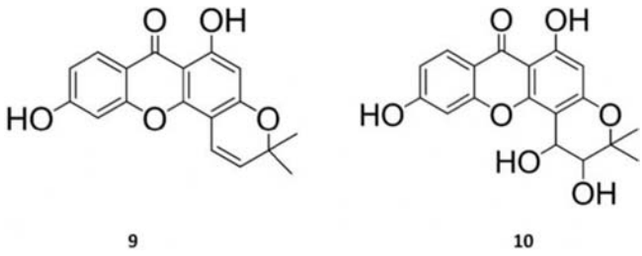

Figure 1: The structures of compounds 9 and 10

References: 1. Ollis WD, Redman BT, Sutherland IO, Jewers K (1969) J Chem Soc Chem Commu. 15: 879 – 880. 2. Kumar P, Baslas RK (1980) Herba Hung 19: 81 – 91. 3. Li NG et al. (2007) Chin Chem Lett 18: 659 – 662.

PG22 Linalool Effect on HepG2 cells: Structure Function Relation

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Linalool is a monoterpene that is widely used in fragrance industry and cosmetics. The effect of linalool on cultured cells (HepG2, MCF7, Hek293, Caco2 and NIH3T3) was investigated. We have recently reported a significant decrease of 50% and 100% in the viability of HepG2 by 0.4 μM and 2 μM linalool, respectively (1). Other studies reported the cytotoxicity of linalool on hematopoietic malignancies but not on normal human cells (2). These findings identify linalool as a potential anticancerogenic molecule. The aim of this study was to investigate the importance of the structural features of linalool in exerting the effect on HepG2 cells. Eleven chemicals with were tested. HepG2 cells were treated with various chemicals at 2 – 500 μM for 24 hours and the viability was estimated using MTT. None of the screened compound had the same potent effect of linalool (2 μM). No effect was demonstrated at concentrations lower than 50 μM. We obtained cell death in HepG2 74% with myrcene & nerolidol (100 μM); 55% with trans-2-nonenal, Decanal (100 μM); 20% with Nonyl aldehyde, citronellal, citronellal, citral, Perillaldehyde, trans-2-octen1-ol, and 1-octen-3-ol at (500 μM). Our findings suggest that the effect of linalool is specific to 1-ene 3-ol moiety. The hydroxyl group needs be tertiary. Hydration of myrcene and L-perillaldehyde did not have any significant effect on HepG2 viability. This may be attributed to: favorability of the hydration reaction and to possible steric effect. Alternative metabolism of linalool into other products may not be ruled out. **Acknowledgement:** *Medical Practice plan and University Research Board of the American University of Beirut* **References:** 1. Usta J et al. (2009) Chem-Biol Interact 180: 39 – 46 2. Gu Y et al. (2010) Toxicology 268: 19 – 24

PG23 New Alkaloids, Sessilifoliamides K and L from the Roots of *Stemona sessilifolia*

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Plants belonging to the genus *Stemona* (family Stemonaceae) are noted for producing a series of alkaloids with unique structures, most of which are characterized by incorporating a pyrrolo[1,2-a]azepine core. Of the genus *Stemona* plants, *Stemona japonica* (Blume) Miq., *S. tuberosa* Lour.,

and *S. sessilifolia* Franch. & Sav. have been used in China and Japan as an insecticide and also as a remedy for cough, and their biological activities are considered to be related to their alkaloid components. In our studies on the chemical constituents of *S. sessilifolia,* we isolated eleven new alkaloids, sessilifoliamides A – J and sessilifoliamine A, with novel alkaloid skeletons. In this meeting, the isolation and structure determination of further new Stemona alkaloids, sessiliamides K and L are represented.

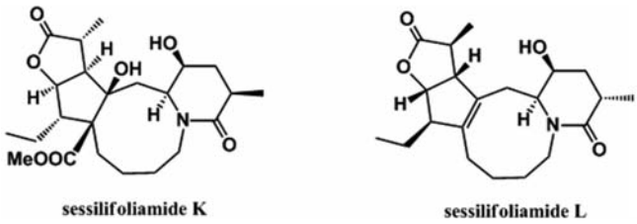

Figure 1

PG24 Triterpenoids and Flavonoids from *Pavetta corymbosa*

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The genus Pavetta have long being used in ethnomedicine as anti-malaria, remedy for tuberculosis and for relieve of stomach pain (1).The literature does not report any phytochemical studies on *Pavetta corymbosa* (DC.) F.N. Williams.The dichloromethane and the ethyl acetate extracts were investigated for phytochemical constituents. Fractionation of the dichloromethane extract by Flash column chromatography, sephadex LH-20 and Preparative TLC afforded the trieterpenes: α-amyrin, lupenol, ursolic acid and a mixture (1:1) of β-sitosterol and stigmasterol, while the ethylacetate extract was fractionated over sephadex LH-20 to give the known flavonoids; quercetin, quercetin 7-O-rhamnoside and kaempferol. The structures were elucidated by NMR spectroscopy and compared with literature (2, 3, 4) and are reported here for the first time. **References:** 1 Dalziel J.M and Hutchinson J (1955) Useful plants of West Africa. Crown Agents for Oversea Publication, London 2. Reynold WF et al. (1999) J Braz Chem Soc 10(3): 237 – 240 3. Ahmad SH and Nordin HL (1998) ARBEC II:1 – 6 4. Mabry T.J et al. (1970) The Systematic Identification of Flavonoids Springer-Verlag Publication, New York.

PG25 Chemodiversity of *Pentadesma grandifolia* (Clusiaceae) from Cameroon

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Within the family Clusiaceae, the genus *Pentadesma* is represented by three species only, which are distributed in the tropical regions of Africa and America. *Pentadesma grandifolia* Baker f. is used in African folk medicine, and the roots and stem bark are applied to treating fever and malaria in the western part of Cameroon [1]. Xanthones, biflavanoids and triterpenoids are the major secondary metabolites of this genus as reported recently [1]. A new glycosidic biflavonoid and a further xanthone derivative were identified now from the same accession originating from Cameroon [1]. Profiling of different plant organs was done both by TLC and HPLC, and isolated structures were identified on basis of ^{13}C NMR, ^{1}H NMR, HRMS and ESI-MS. Structure elucidation and the distribution of isolated compounds in different parts of the plant will be presented. Tests for antifungal activity were performed by bio-autography on TLC against spore suspensions of *Cladosporium sphaerospermum*. The significance of bioactivity results and reported bioactivities of *Pentadesma* compounds are shortly discussed. **Acknowledgement:** *The financial support of OEAD for G. L. Djoufack is gratefully acknowledged.* **References:** 1. Djoufack Nwabouloun GL et al. (2010) Nat Prod Commun 5: 1055 – 1060

PG26 Seeds from South African plants as a source of bioactive metabolites

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South Africa has a rich diversity of plant species which contain various classes of bioactive compounds [1]. Seeds of these plants have been little studied from a chemical viewpoint and a survey of their constituents is of high interest. The source plants of these seeds may grow in areas with extreme climatic conditions, thus increasing the chances of finding original metabolites. Seeds from plants (mainly trees) growing in different areas of South Africa were extracted with methanol and screened for radical scavenging activity in a TLC assay with the 2,2-diphenyl-1-picrylhydrazyl radical. Inhibition of acetylcholinesterase by TLC bioautography [2] was also performed, as compounds which inhibit this enzyme may have some application in the management of Alzheimer's disease. The extracts were also screened for antimicrobial and antimalarial activities. Two species were selected for further study: *Schotia brachyphylla* Sond. (Fabaceae) and *Colophospermum mopane* (J. Kirk ex Benth.) J. Kirk ex J. Leonard (Fabaceae). The bioactive constituents of the seeds, including two new 4-coumaroyl derivatives of flavonoids (**1**, **2**), were isolated by a combination of high-speed countercurrent chromatography and classical chromatographic techniques. **References:** 1. Mulholland DA and Drewes SE (2004) Phytochemistry 65: 769 – 782. 2. Marston A, Kissling J and Hostettmann K (2002) Phytochemical Analysis 31: 51 – 54.

PG27 Chemical constituents of *Artocarpus xanthocarpus* and their inhibitory effects on melanin biosynthesis

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Artocarpus xanthocarpus Merr. is an evergreen monoecious tree with milky juice, distributed in Philippines, Borneo and Taiwan (only on Lanyu) [1]. Various phenolic compounds, including isoprenylated flavonoids, stilbenoids, and 2-arylbenzofurans are widely distributed in plants of the genus *Artocarpus*. Many of these compounds exhibit cytotoxic, anti-inflammatory, anti-platelet, antibacterial, antimalarial, antitubercular, antiviral and antioxidant activities [2, 3]. In continuation of our research on natural whitening agents, the present study aims to characterize the chemical constituents of *A. xanthocarpus* and demonstrate their potential whitening potency. Three new compounds, artoxanthocarpuones A-C (**1-3**), and twelve known compounds (**4-15**) have been isolated from the root of *A. xanthocarpus*. Their structures were determined on the basis of spectroscopic evidence. These compounds were evaluated for their antioxidative property, tyrosinase inhibitory potential and cytocoxicity. In B16F10 melanoma cells, compound **1**, norartocarpetin (**4**), oxyreserveratrol (**5**), albanin A (**8**) and steppogenin (**9**) reduced tyrosinase activity and also inhibited the α-MSH induced melanin production. We may conclude that isolates of *A. xanthocarpus* with antioxidant and tyrosinase reducing activities may be considered as depigmenting agents. **Acknowledgement:** *The authors are thank to the financial support from the National Science Council of Taiwan (NSC-99 – 2320-B037 – 021).* **References:** 1. Liao JC (1996) Moraceae in Flora of Taiwan, 2nd edition. Vol. 2: 136 – 137. Editorial Committee of the Flora of Taiwan. Taipei. 2. Hakim EH et al. (2006) J Nat Med 60: 161 – 184. 3. Jagtap UB, Bapat VA (2010) J Ethnopharmacol 129: 142 – 166.

PG28 Evaluation of effects of climate condition on the quality of sugar beet production in Iran

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This research has been carried out during the 9 years (1998 – 2008) and in some provinces (Chehar Mahal Bakhtiari, Isfehan, Qazvin, Zanjan, Azarbayejan Gharbi) in 5 sugar factories. Our aim was the evaluation of qualitative specifications of sugar beet produced in different climates and their effects on the amounts of sugar produced. Conclusions of this research were based on the analysis of 13811 samples of sugar beet consignments received during 9 years. The results have shown that amounts of sugar changed from min. 15.38% to max. 62%. K^+ contents varied between min. 5.06 and max. 8.1; while Na^+ varied from min. 1.8 meq to max 5.49 meq., and harmful nitrogen ranged between 1.96 to 4.37. These results showed the annual variations in the quality of sugar produced in Iran with respect to geographical variations.

PG29 Structural analysis of arabinogalactan-proteins from suspension cultures of *Pelargonium sidoides* DC.

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Pelargonium sidoides DC. is a traditional medicinal plant from South Africa. An aqueous-ethanolic formulation of the roots is approved for the treatment of acute bronchitis. The main effects could be related to antibacterial activities and the stimulation of the non-specific immune system by the main components of *Pelargonium sidoides*: coumarins, phenols and tannins [1]. Due to wild harvesting, *Pelargonium sidoides* is an endangered species. Therefore the propagation of the plant material by cell cultures and the extraction of ingredients are interesting tasks. From suspension cultures of *Pelargonium sidoides* high amounts of pure Arabinogalactan-proteins (AGPs) could be isolated by precipitation with β-glucosyl Yariv reagent. These AGPs have been investigated with regard to their structure. Quantification of neutral sugars by acetylation pointed out arabinose (Ara) and galactose (Gal) as dominating monosaccharide residues in a ratio of 1:2. Colourimetric determination of uronic acids revealed an amount of 6 – 8%. Linkage type analysis in combination with the reduction of the uronic acids showed that the main components are 1,3,6-Gal(p), 1,3-Gal(p) and 1-Ara(f) as well as minor amounts of 1,6-Gal(p), 1-GlcA(p), 1,4-Gal(p), 1-Gal(p), 1,5-Ara(f) and 1,2-Ara(f). Molecular weight of AGPs has been determined by size exclusion chromatography with laser light scattering detection and found to range between 80 and 85 kDa. The characterisation of the AGP-protein moiety pointed out an untypical low protein content for AGPs with 1%. According to the amino acid analysis the protein moiety consists of high amounts of Hyp (42.8 – 51.1%) as well as Pro, Gly, Glx, Asx, Ser, Ala, Leu and Thr. **References:** 1. Kolodziej H (2008) Planta Med 74: 661 – 666

PG30 Thymol, benzofuranoid, and phenylpropanoid derivatives: anti-inflammatory constituents from *Eupatorium cannabinum* subsp. *asiaticum*

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Eupatorium cannabinum L. subsp. *asiaticum* Kitam. (Compositae) [1] is a perennial herb distributed in Himalaya mountain range, China, and Taiwan. *E. cannabinum*, locally called 'Taiwan ze-lan' or 'liu-yue-xue', has been used as a folk medicine to treat hepatitis, headache, diarrhea, hypertension, and Diabetes mellitus in Taiwan. Sesquiterpene lactones, diterpenoids, flavonoids, pyrrolizidine alkaloids, thymols, benzofurans, and their derivatives are widely distributed in plants of the genus *Eupatorium*. Many of these compounds were found to exhibit cytotoxic, antimicrobial, and anti-inflammatory activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* inhibitory activity on neutrophil pro-inflammatory responses, and *E. cannabinum* has been found to be an active species. Five new compounds, 9-*O*-angeloyl-8,10-dehydrothymol (**1**), 9-(3-methylbutanoyl)-8,10-dehydrothymol (**2**), eupatobenzofuran (**3**), 2-hydroxy-2,6-dimethylbenzofuran-3(2*H*)-one (**4**), and 1-(2-hydroxy-4-methylphenyl)propan-1,2-dione (**5**) and 16 known compounds have been isolated and identified from the aerial part of *E. cannabinum* subsp. *asiaticum*. Compounds 6 – 8, 11, 13, and 15 exhibited inhibition (IC_{50} values < 18.4 μM) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds 2, 3, 10, 13, and 15 inhibited fMLP/CB-induced elastase release with IC_{50} values < 18.3 μM.

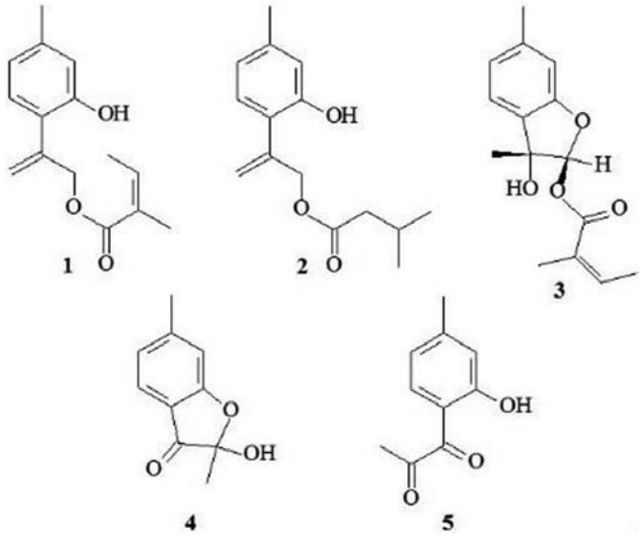

Figure 1: Structures of new compounds 1 – 5

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to J.-J. C. We also thank the National Center for High-Performance Computing (NCHC, Taiwan) for providing computer resources and chemical database services.*
References: 1. Peng C.I. et al. (1998) 'Compositae' in 'Flora of Taiwan', 2nd ed., Editorial Committee of the Flora of Taiwan: Taipei, Taiwan, 1998; Vol. 4, pp 804 – 1101.

PG31 Value-added products from *Pinus banksiana* wood

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Pinus banksiana Lambert (Pinaceae) is a boreal conifer species, with a sparse, variable crown and spreading branches at maturity. The species is widely available in Canada, and an important timber species for pulp and lumber [1]. In this work, the chemical composition, such as ash, lignin, cellulose and hemicellulose contents, of the P. banksiana wood chips from Eastern Canada, was determined. In addition to timber and pulp, *P. banksiana* wood may be a rich source of unexploited potentially novel bioactive compounds. A previous study showed that *P. banksiana* wood extracts possessed strong antioxidant and anti-tumor activities [2]. In this work, the chemical constituents of the extractives from *P. banksiana* wood were further investigated. The GC-MS results of essential oils from *P. banksiana* wood showed that there were 76 volatile compounds presented, including phenolic acids, phenylpropanoids, alkaloids and terpenoids. Among those determined, 1-Naphthalenecarboxylic acid was the most abundant (29.06%). Based on the successive Sephadex LH-20 column chromatographic separation of *P. banksiana* wood aided by Thin Layer Chromatography, 5 yellowish low-molecular-weight natural compounds, including 2 flavan-3-ols [(+)-Catechin (I) and (-)-Epicatechin (II)], a phenolic acid [Caffeic acid (III)], a phenylpropanoid [Isoconiferin (IV)] as well as a lignan [Cedrusin (V)], were isolated. Structure elucidation of the isolates was based on their physiochemical and spectroscopic data. To the best of our knowledge, this was

the first time of isolation low-molecular-weight natural compounds from *P. banksiana* wood. The results in the study might lead to the further development of high value-added products from this pine species. **Acknowledgement:** *This work was financially supported by National Natural Science Foundation of China (NSFC, No. 31000279), Program for New Century Excellent Talents in University (NCET 2010) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800).* **References:** 1. Poncsak S et al. (2009) J Wood Chem Technol 29: 251 – 264. 2. Phelan M et al. (2009) J Med Food 12: 1245 – 1251.

PG32 Activity-guided supercritical CO_2 isolation of antioxidative constituents from *Eucommia ulmoides*

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Increasing evidence exhibited that antioxidants played very important role in protecting against various diseases like cancer, atherosclerosis, diabetes, cataracts, neurodegenerative disorders [1]. *Eucommia ulmoides* Oliv., the sole species in *Eucommia* genus and Eucommiaceae family, is a large medicinal hardwood native to China and widely cultured in Eastern Asia and has widely been used as a tonic to strengthen the kidney and liver, against diabetics, strong bones, ache knees, treat lower back pain, prevent fatigue and miscarriage [2, 3]. In present study, crude extracts of *E. ulmoides* wood were initially obtained by supercritical CO_2 isolation. And DPPH free radical scavenging assay, a standard in vitro model, was employed for the activity-guided purification to identify the antioxidative constituents of E. ulmoides. The crude extracts were suspended in water, and then successively partitioned to a serious of polar solvents to yield fractions soluble in n-hexane, dichloromethane, ethyl acetate, n-buthanol and water. Ethyl acetate fraction exhibited most significant capacity to scavenge DPPH radical, and thus was further subjected to repeated sephadex LH-20 open column to separate the individual antioxidative compounds. Guided by DPPH assay, the fraction having superior activity was identified as containing five major yellow amorphous compounds, including gallic acid, ellagic acid, caffeic acid, (+)-catechin and kaempferol and were obtained. The structures of the isolated antioxidants were mainly elucidated and established by spectroscopic analysis, such as NMR and MS, as well as cellulose TLC. To the best of our knowledge, this was the first time of phytochemical investigation of *E. ulmoides* wood. **Acknowledgement:** *This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010), Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616), National Natural Science Foundation of China (NSFC, No. 31000279) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800).* **References:** 1. Si CL et al. (2009) Planta Med 75: 1165 – 1167. 2. Chang H., Yan SZ (1979) Flora Republicae Popularis Sinicae. Science Press. Beijing. 3. Takamura C et al. (2007) J Nat Pro. 70: 1312 – 1316.

PG33 Phytochemical investigation of *Galanthus transcaucasicus* Fomin, as a source of isoquinoline alkaloids

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Galanthus transcaucasicus Fomin (Amaryllidaceae) is an endemic species of the Caucasus region and the Alborz mountains in Iran and is used in folk medicine to recover paralysis and nerve pain (1, 2). All species of *Galanthus* are famous for their bioactive alkaloids such as galanthamine, an acetylcholinesterase inhibitor, which is used for the treatment of Alzheimer's disease (2). This study is designed to identify major constituents of the alkaloid fraction of *G. transcaucasicus* Fomin. The plant was collected from Alborz mountain area (Rostam abad) in February 2008. The bulbs of the plant were percolated with 96% ethanol and alkaloid fraction of the plant was prepared. Major constituents of alkaloid fraction were purified using different chromatographic methods. Finally, five isoquinoline type alkaloids involving galanthamine, narwedine, lycorine, caranine and tazettine were identified with spectroscopic methods. The results showed that this species can be considered as a source of isoquinoline type alkaloids especially galanthamine which is a long acting, competitive and reversible acetylcholinesterase inhibitor (2). **References:** 1. Bastida J et al. (2000) The alkaloids. Elsevier Scientific Publishing, Amsterdam 63: 87 – 179. 2. Heinrich M et al. (2004) J Ethnopharmacol 92: 147 – 162.

PG34 New skeleton polyacetylene ferulic acid conjugates from *Notopterygium incisum*

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In our search for natural products activating PPAR-gamma we have isolated three polyacetylene ferulic acid conjugates (**1**-**3**) from the underground parts of *Notopterygium incisum* Ting ex H. T. Chang (Qiang Huo), in addition to previously isolated polyene-yne derived compounds.[1,2] Their structures were elucidated by NMR and MS. Compound **1** is formed by an ester bond, while compound **2** and **3** represent two new skeletons. Pharmacological evaluation of the isolated compounds is in progress.

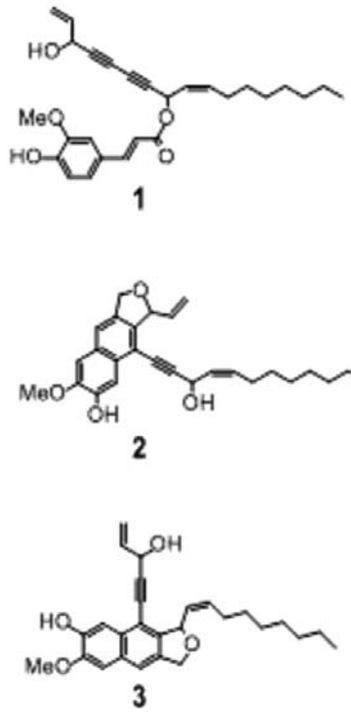

Figure 1: structures of polyacetylene ferulic acid conjugates from N. incisum.

Acknowledgement: *We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF) within project NFN S 10705-B13.* **References:** 1. Liu X et al. (2011) Planta Med in preparation 2. Blunder M et al. (2011) Planta Med in preparation

PG35 Isolation of antocyanins with identified qualitative-quantitative properties

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Technology of natural substances isolation is substantial element affecting on the quality of natural preparations. The isolation of the plant substances from a raw material is carried out by distillation and extractions with different solvents. The methods used for anthocyanin separation due to high temperatures, etc. destroy their structures and decrease their therapeutic effects. The present study is aimed to establish optimal procedures for anthocyanins after their extraction to secure the stability of substances by lyophilisation. The freeze drying technology seems to be an appropriate method to keep the structure and qualitative-quantitative properties of these natural components. The focus of lyophilisation technology is vacuum sublimation of ice crystals, i.e. phase transition from solid to gas state, which is created during freezing the water solution. The process is carried out in three phases: freezing, primary drying and secondary drying. LC/MS/IT/TOF equipment is used for the determination of anthocyanin qualitative-quantitative characteristics before and after lyophilisation. The biological and antimicrobial properties of these natural components are tested as well. The freeze drying technology used is established by Mediproduct Company in Lipany, the East Slovakia. **Keywords:** Hydrolyzation, lyophilisation, plant material, secondary metabolites **Acknowledgement:** *The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).*

PG36 Secondary metabolites from the Root of *Rhaphiolepis indica* var. *tashiroi* and Their Anti-inflammatory Activity

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Rhaphiolepis indica (L.) Lindl. ex Ker var. *tashiroi* Hay. ex Matsum (Rosaceae) is an evergreen shrub or small tree which distributes from India to southern China, the Ryukyus, Japan, Korea, and Taiwan at low altitudes. The methanolic extract of the root of this species showed anti-inflammatory activity using *N*-formylmethionylleucylphenylalanine (fMLP)-induced production of superoxide anion, an inflammatory mediator produced by neutrophils *in vitro*. Previously, we reported seven compounds including four new dibenzofuran derivatives, rhaphiofurans A – D (**1-4**), two new biphenyl derivatives, rhaphiobiphenyls A – B (**5-6**) along with 3-hydroxy-5-methoxybiphenyl (**7**) from the active EtOAc-soluble layer of this plant's root. Continuing investigation of the active EtOAc-soluble layer of this plant's root led to the isolation of three new triterpenoids, namely 2α,3β-dihydroxy-olean-11,13(18)-dien-28,19-olide (**8**), 2α, 3β-dihydroxy-olean-13(18)-en-28-oic acid (**9**) and 3β,5α-dihydroxyglutinanol (**10**). The structures of these isolates were elucidated by spectral analyses. Among the isolates, **3**, **6**, and **7** exhibited potent inhibition against fMLP-induced superoxide production with IC_{50} values less than 8.36 μM. **Acknowledgement:** *National Science Council of the Republic of China (NSC 97 – 2320-B-037 – 010-MY3)*

PG37 Secondary Metabolites from the Root of *Neolitsea daibuensis* and Their Anti-inflammatory Activity

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Neolitsea daibuensis Kamikoti (Lauraceae) is a small semideciduous trees, endemic to Taiwan, confined to broad-leaved forests from 800 to 1000 m in the southern part. Recently, approximately 40 species of Formosan Lauraceous plants have been screened for anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and the methanolic extract of the root of this species has been shown with potent inhibition of NO production without any cytotoxicity on RAW 264.7 cells. Besides, the chemical constituents of its root have not extensively been studied yet. Bioassay-guided fractionation of the ethyl acetate soluble layer of the root of this species led to the isolation of three new alkaloids: daibucarbolines A-C (**1-3**), three new sesquiterpenoids: daibulactones A-B (**4-5**), and daibuoxide (**6**), together with twenty known compounds. The structures of these compounds were determined by spectroscopic analysis. Among the isolates, daibucarboline A (**1**), hiiranlactone B, isolinderalactone, 7-*O*-methylnaringenin, and prunetin showed iNOS inhibitory activity with IC_{50} values as 18.41 ± 0.47, 29.30 ± 0.92, 0.30 ± 0.01, 19.55 ± 0.89, and 10.50 ± 0.33 μM, respectively. **Acknowledgement:** *National Science Council of the Republic of China (NSC 99 – 2300-B-037 – 009)*

PG38 Phytochemical investigation of *Himatanthus sucuuba* bark leading to the identification of novel and anti-inflammatory compounds

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Himatanthus sucuuba (Spruce ex Müll. Arg.) Woodson (Apocynaceae) is used in the Amazonian region for the treatment of inflammatory diseases [1]. The bark of this medicinal plant was phytochemically investigated guided by an LPS/TNF stimulated assay measuring E-selectin and IL-8. Out of bioactive fractions 11 constituents were isolated and identified by MS and 1D and 2D NMR experiments as iridoids (plumericin, plumieridin, allamandicin, and the new natural product (2'R,3R,4R,4aS,7aR)-methyl 3-hydroxy-4'-((S)-1-hydroxyethyl)-5'-oxo-3,4,4a,7a-tetrahydro-1 H,5'H-spiro[cyclopenta[c]pyran-7,2'-furan]-4-carboxylate), flavonoids (biochanin A, dihydrobiochanin A, dalbergioidin, naringenin, ferreirin, and dihydrocajanin), and the lignan pinoresinol. Except for plumericin and pinoresinol this is the first time these compounds are reported to be isolated from *Himatanthus sucuuba*. The structure of the new iridoid was determined using X-ray crystallography. Interestingly, NMR experiments showed the presence of two compounds indicating stereochemical conversion. The isolated constituents were analyzed for their anti-inflammatory activity. They showed only moderate or no effects with the exception of plumericin which exhibited dose-dependent inhibitory activity on LPS or TNF induced expression of IL-8 and E-selectin in the low μM range. In conclusion, several known components were isolated from *Himatanthus sucuuba* for the first time together with one new natural product. Plumericin revealed as the most active compound in this general anti-inflammatory assay. The elucidation of the molecular mechanism of action is currently under evaluation. **Acknowledgement:** *This work was granted by the Austrian Science Fund (S 10703).* **References:** 1. Amaral ACF et al. (2007) Pharmacogn Rev 1: 305 – 313.

PG39 New isoflavones and bioactive constituents from the fruits of *Psoralea corylifolia*

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Psoralea corylifolia (Chinese name Buguzhi), dry fruits of leguminous plant *P. corylifolia* L., is one of the most popular traditional Chinese medicines. This crude drug has used for the treatment of pollakiuria, enuresis, osteoporosis, depression, and various kidney problems. It is reported to contain coumarins, flavonoids, alkaloids, essential oil, and terpenoids. Many of these compounds were found to exhibit anti-allergic, antioxidant, antitumor, insecticidal, and antimicrobial activities. Investigation on n-hexane- and EtOAc-soluble fractions of the fruits of *P. corylifolia* has led to the isolation of two new isoflavones, 7-*O*-methylcorylifol A (**1**) and 7-*O*-isoprenylcorylifol A (**2**), together with eight

known compounds, including angelicin (**3**), psoralen (**4**), bavachalcone (**5**), bakuchiol (**6**), 12,13-dihydro-12,13-epoxybakuchiol (**7**), p-hydroxybenzaldehyde (**8**), b-sitosterol (**9**), and stigmasterol (**10**). The structure of new compounds **1** and **2** was determined through spectroscopic and MS analyses. Among the isolated compounds, psoralen (**4**) exhibited inhibition (IC_{50} value = 1.10 ± 0.60 μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB).

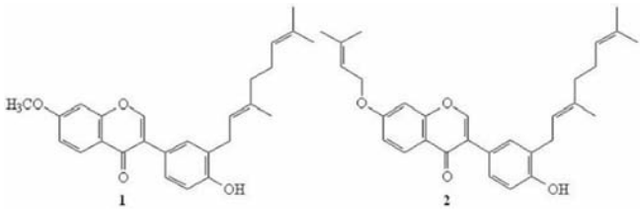

Figure 1: Structures of new compounds 1 and 2

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to J.-J. C.* **References:** 1. Huang T C, Ohashi H (1993) 'Leguminosae' in 'Flora of Taiwan', 2nd ed., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. 3, pp. 160 – 396.

PG40 A new ferulic acid ester, a new ellagic acid derivative, and other constituents from *Pachycentria formosana*: Effects on neutrophil pro-inflammatory responses

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Pachycentria formosana Hayata (Melastomataceae) is an endemic creeping shrub distributed in 300 – 2100 m forests throughout Taiwan [1]. Various tannins, flavonoids, and triterpenoids are widely distributed in plants of the family Melastomataceae. Many of these compounds exhibit antioxidant, anti-inflammatory, cytotoxic, antifungal, antiprotozoal, and antimicrobial activities. In our studies of Formosan plants for *in vitro* anti-inflammatory activity, *P. formosana* was found to be an active species. Investigation on the CH_2Cl_2-soluble fraction of the leaves and twigs of *P. formosana* led to the isolation of a new ferulic acid ester, 1,24-tetracosandiol di-(*Z*)-ferulate (**1**) and a new ellagic acid derivative, 3,4,3',4'-dimethylenedioxyellagic acid (**2**), along with eight known compounds. Among the isolated compounds, oleanolic acid (**6**), ursolic acid acetate (**7**), and 3-*epi*-betulinic acid (**9**) exhibited potent inhibition (IC_{50} values < 21.8 μM) of $O_2^{\cdot-}$ generation by human nutrophils in response to *N*-formyl-L- methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). In addition, oleanolic acid (**6**), 3-*O*-(*E*)-feruloylursolic acid (**8**), 3-*epi*-betulinic acid (**9**), and lawsonic acid (**10**) also inhibited fMLP/CB-induced elastase release with IC_{50} values < 18.6 μM.

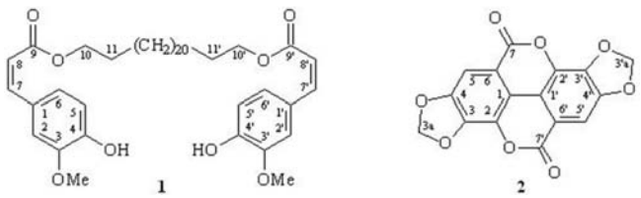

Figure 1: Structures of new compounds 1 and 2

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001- MY3), awarded to Prof. J.-J. Chen.* **References:** 1. Huang SF, Huang TC (1993) 'Melastomataceae', in 'Flora of Taiwan', 2nd edn., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. 3, p. 905.

PG41 A new benzoylphloroglucinol derivative with an adamantyl skeleton and other constituents from *Garcinia multiflora*: Effects on neutrophil pro-inflammatory responses

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Garcinia multiflora Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan [1]. The genus *Garcinia* (Guttiferae) comprises about 400 species with pantropical distribution. In Taiwan, the genus *Garcinia* is represented by three species, viz., *G. linii*, *G. multiflora*, and *G. subelliptica*. Plants of this genus contain a variety of secondary metabolites including xanthones, benzophenones, phloroglucinols, terpenoids, biflavonoids, and their derivatives. Many of these compounds exhibit antioxidant, trypanocidal, cytotoxic, antitubercular, anti-inflammatory, and anti-HIV activities. As part of our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses. In the course of this screening, an AcOEt-suluble fraction of the fruit of *G. multiflora* exhibited inhibitory activities with IC_{50} values of 7.21 ± 1.07 and 6.01 ± 0.37 μg/ml, respectively, against fMLP/CB-induced superoxide anion generation and elastase release. Investigation of the active fraction afforded a novel benzoylphloroglucinol derivative, garcimultiflorone D (**1**), with an unusual adamantly caged skeleton and four known compounds. The structure of **1** was determined through extensive 1D/2D NMR and mass-spectrometric analyses. Garcimultiflorone D (**1**) exhibited inhibitory activities with IC_{50} values of 7.21 ± 1.07 and 6.01 ± 0.37 μg/ml, respectively, against fMLP/CB-induced superoxide anion generation and elastase release.

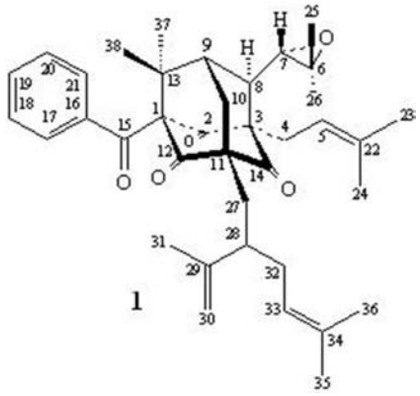

Figure 1: Structure of new compound 1

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.* **References:** 1. Robson NKB. (1996) 'Guttiferae', in 'Flora of Taiwan', 2nd edn., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. 2, p. 694.

PG42 New biphenyl derivatives and anti-inflammatory constituents from the stem bark of *Magnolia officinalis*

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The stem bark of *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae) has been used as a traditional medicine for the treatment of gastrointestinal disorders, bronchitis, and emphysema, in China, Taiwan, Japan, and Korea [1]. Chemical studies have revealed a variety of neo-lignans and

alkaloids as constituents of this plant. Many of these compounds exhibit central depressant effect, muscle relaxation, and antigastric ulcer, antibacterial, antiallergic, vasorelaxant, and neurotrophic activities. Investigation on EtOAc-soluble fraction of the stem bark of *M. officinalis* has led to the isolation of three new biphenyls, 5-allyl-5'-(1-hydroxyallyloxy)biphenyl-2,2-diol (**1**), 5,5'-diallyl-2'-(allyloxy)biphenyl-2-ol (**2**), and 5,5'-diallyl-2'-(3-methylbut-2-enyloxy)biphenyl-2-ol (**3**), together with 12 known compounds, including four neolignans, magnolol (**4**), honokiol (**5**), (-)-monoterpenylmagnolol (**6**), and randainal (**7**), two norlignans, magnaldehyde D (**8**) and randaiol (**9**), and six steroids, β-sitostenone (**10**), stigmasta-4,22-dien-3-one (**11**), β-sitosterol (**12**), stigmasterol (**13**), 3β-hydroxystigmast-5-en-7-one (**14**), and 3β-hydroxystigmasta-5,22-dien-7-one (**15**). The structure of new compounds (**1-3**) were determined through spectroscopic and MS analyses. Among the isolates, magnolol (**4**) and honokiol (**5**) exhibited potent inhibition against fMLP-induced superoxide production with IC_{50} values of 4.42 ± 0.24 and 0.68 ± 0.20 μg/mL, respectively. In addition, magnolol (**4**) inhibited fMLP/CB-induced elastase release with an IC_{50} values of 1.45 ± 0.20 μg/mL.

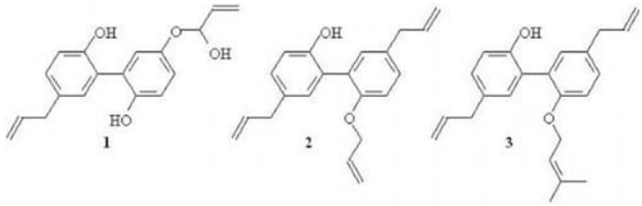

Figure 1: Structures of new compounds 1 – 3

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.*
References: 1. Xia NH et al. (2008) 'Magnoliaceae' in 'Flora of China', Science Press, Beijing. Vol. 7, pp. 48 – 91.

PG43 New Coumarin Derivative and Bioactive Constituents from the fruits of *Cnidium monnieri*

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Cnidium monnieri (L.) Cusson (Apiaceae)1 is a crude drug "Fructus Cnidii" (Chinese name Shechaungzi) used in traditional Chinese to treat impotence, frigidity, and skin-related disease. Coumarins, chromones, and their derivatives were isolated from this plant in previous studies. Many of these compounds were found to exhibit insecticidal, antiallergic, antifungal, and antibacterial activities. Investigation on EtOAc-soluble fraction of the fruits of *C. monnieri* has led to the isolation of a new coumarin, 3'-*O*-methylmurraol (**1**), together with 12 known compounds, including 11 coumarins, meranzin hydrate (**2**), peroxyauraptenol (**3**), auraptenol (**4**), demethylauraptenol (**5**), peroxymurraol (**6**), murraol (**7**), osthol (**8**), bergapten (**9**), isopimpinellin (**10**), xanthotoxin (**11**), and xanthotoxol (**12**), and a chromone, cnidimol A (**13**). The structure of new compound **1** was determined through spectroscopic and MS analyses. Among the isolates, osthol (**8**) completely inhibited ADP-induced platelet aggregation at 100 μg/mL. Xanthotoxin (**11**) showed complete inhibitory activity on platelet aggregation at 100 μg/mL induced by arachidonic acid.

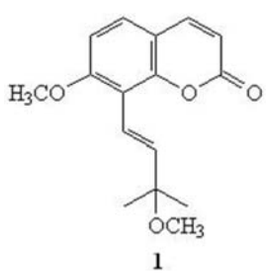

Figure 1: Structure of new compound 1

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.*
References: 1. Sheh ML et al. (2005) 'Apiaceae' in 'Flora of China', Science Press, Beijing, China. Vol. 14, pp. 1 – 205.

PG44 Secondary Metabolites from the Root of *Helicia rengetiensis*

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Helicia rengetiensis Masamune (Proteaceae) is an endemic evergreen trees, growing in thickets at lower elevations in the central and southern parts of Taiwan. Flavonol glycosides, phenolic glycosides, benzenoid glycosides, and their derivatives distributed in plant of genus Helicia. In our studies on the antitubercular constituents of Formosan plants, 1,200 species have been screened for in vitro antitubercular activity, and the H. rengetiensis has been found to be an active species. However, the chemical constituents and biological activities of this plant have never been studied. Bioassay-guided fractionation of the active EtOAc-soluble fraction of the root of this species has led to the isolation of three new compounds, including helicinol A (**1**) helicinol B (**2**) and helicinone (**3**), together with eleven known compounds, including one cyclophane, kermadecin H (**4**), three flavones, 5-hydroxy-3,7,4'-trimethoxyflavone (**5**), 5-hydroxy-3,6,7,4'-tetramethoxyflavone (**6**), and5-hydroxy-3,6,7,8,4'-pentamethoxyflavone (**7**), one fatty acid, stearic acid (**8**), one benzoquinone, α-tocopheryl quinone (**9**), and five steroids, β-sitostenone (**10**), a mixture of β-sitosterol (**11**) and stigmasterol (**12**), and a mixture of 3-*O*-β-D-glucopyranosyl β-sitosterol (**13**) and 3-*O*-β-D-glucopyranosyl stigmasterol (**14**). The structures of these new compounds were determined through spectroscopic analyses including 2D-NMR data. The successive isolation and antitubercular assay are still in progress. **Acknowledgement:** *National Science Council of the Republic of China (NSC 99 – 2320-B-037 – 010)*

PG45 Anti-HIV activity of Δ18-oleane triterpenoids from *Cassine xylocarpa*

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[1]Instituto Universitario de Bio-Orgánica "Antonio González" Universidad de La Laguna, La Laguna, Tenerife, Spain;
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The species of the Celastraceae have a long history in traditional medicine, and they produce an extraordinary variety of bioactive metabolites, including pentaciclic triterpenoids. Thus, a diversity of oleane-type triterpenoids have been isolated from this family, mainly those corresponding to the Δ12-oleane skeleton, however, only a few examples of Δ18-oleane-type have been reported[1]. The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). Although antiretroviral therapy (ART) is able to stop the progression of the disease, drug toxicity and viral resistance are important limitations[2]. Therefore, an unprecedented effort is being performed to find new anti-HIV drugs with acceptable toxicity, good resistance profiles and novel mechanisms of action. Thus, bevirimat, a derivative of betulinic acid represents a unique first-in-class of anti-HIV compounds known as maturation inhibitors (MIs), which has succeeded in phase IIb clinical trials. In addition, moronic acid, a Δ18-oleane triterpene, also exhibits promising anti-HIV activity as a lead compound with an EC_{50} of 0.1 μg/mL and a good therapeutic index (TI > 186) relative to its cytotoxicity[3]. As a part of our research for bioactive compounds from Celastraceae species, herein we report the isolation of eleven new Δ18-oleane triterpenes, along with two known ones, from the stem of *Cassine xylocarpa* Vent.. Their structures were determined by spectroscopic methods, including 1D and 2D NMR techniques. The compounds were tested for their activity as inhibitors of HIV replication. Ten of the compounds showed significant inhibitory effects, ranging from 66 to 99% at a concentration of 10 μM. **Acknowledgement:** *We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support.Osorio AA. thanks to MAEC-AECID for the fellowship.* **References:** 1. Alveranga N, Ferro E (2005) Studies in Natural Products Chemistry (Part K). Elsevier. 30: 635 – 702. Amsterdam. 2. Mehellou Y, De Clercq E (2010) J Med Chem 53: 521 – 538. 3. Qian K et al. (2010) J Med Chem 53: 3133 – 3141.

PG46 Chemical Constituents and Bioactivities from the Root of *Piper taiwanense*

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Piper taiwanense Lin & Lu (Piperaceae) is a woody climber, endemic to Taiwan, distributed in forests at low to medium altitudes throughout the island. Previously, four new compounds and 25 known compounds, along with anti-platelet aggregation activity were reported from the stem of this species. The methanolic extract of the root of this plant showed potent antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and inhibitory activities against platelet aggregation induced by collagen. The aim of this study is the isolation of chemical constituents and their bioactivities of the root of this species The methanolic extract of root was partitioned into EtOAC and H2O-soluble layer. Bioassay-guided fractionation of the active EtOAC-soluble layer led to the isolation of eight new compounds, taiwanensols A, B (**1**, **2**), taiwandimerols A, B (**3**, **4**), neotaiwanensol A, B (**5**, **6**), 1,2-diacetoxy-6- methoxy-4-allylbenzene (**7**), and 1-acetoxy-2-hydroxy-4-allylbenzene (**8**), and the last two were first isolated from nature, along with 16 known compounds. The structures of these isolates were elucidated by spectral analysis. Among the isolates, the mixture of **8** and 2-acetoxy-1-hydroxy-4-allylbenzene (**9**), 1,2-dihydroxy- 4-allylbenzene (**10**), and 1,2-diacetoxy-4-allylbenzene (**11**) showed potent inhibitory activities against platelet aggregation induced by collagen, with IC_{50} values as 1.7, 0.8, and 0.5 μg/mL, respectively. The successive isolation and bioactivity assay of the isolates are under progress.

PG47 Flavonoid triglycosides from *Astragalus armatus*

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Astragalus armatus Willd. is an endemic shrub of the Northern Africa (Algeria, Morocco, Tunisia), is distributed in the pre-Saharian zone and is associated with the desertification in arid areas due to overgrazing [1]. In Tunisia it is used as tonic, stimulant and in cases of anaemia [2]. From the aerial parts of *A. armatus* a new acylated flavonoid triglycoside, isorhamnetin-3-*O*-(4'''-*p*-hydroxybenzoyl)-α-apiofuranosyl-(1→2)[α-rhamnopyranosyl-(1→6)]-β-galactopyranoside (**1**), and one new flavonoid triglycoside, tamarixetin-3-*O*-α-apiofuranosyl-(1→2)[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**2**), have been isolated together with six known flavonoids: isorhamnetin-3-*O*-α-apiofuranosyl-(1→2)[α-rhamnopyranosyl-(1→6)]-β-galactopyranoside, kaempferol-3-*O*-α-rhamnopyranosyl-(1→2) [α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, kaempferol-3-*O*-α-rhamnopyranosyl-(1→2)[α-rhamnopyranosyl-(1→6)]-β-galactopyranoside, isorhamnetin-3-*O*-α-rhamnopyranosyl-(1→2)[α-rhamnopyranosyl-(1→6)]-β-galactopyranoside, nikotiflorin and narcissin. The structures of the isolated compounds were established by means of 2D NMR, HPLC-DAD-MS, HR-MS, and UV spectral analyses. Pivotal role in the structure elucidation and in particular in the determination of the sugar sequence, played ROESY and HSQC-TOCSY experiments.

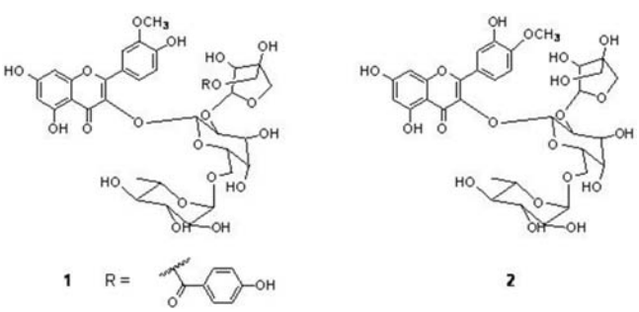

Figure 1: Structures of the new naturally occurring compounds

Acknowledgement: *The authors are grateful to ANDRS and DG-RSDT (MESRS, Algeria) for financial support and to Prof. Gérard De Bélair (Faculty of Sciences, University Badji-Mokhtar-Annaba) for the identification of the plant material and to Dr. Elena Michelucci (Mass Spectrometry Center, University of Florence – CISM) for recording the HRESI mass spectra. The authors would like to thank Stefano Rocchi for technical assistance*
References: 1. Hirche A et al. (2010) Environ Monit Assess DOI 10.1007/s10661 – 010 – 1744 – 5 2. Bouaziz et al. (2009) Afr J Biotechnol 8: 7017 – 7027.

PG48 Bioactivity-guided isolation of leishmanicidal chalcones from *Piper delineatum*

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Leishmaniasis affects approximately 12 million people worldwide, primarily in developing regions [1]. Conventional chemotherapy with pentavalent antimonials is considerably toxic and prone to induce resistance. Second-line drugs, such as amphotericin B and its lipid formulations, are either too toxic or expensive for routine use in developing countries. At the same time, the efficacy of miltefosine against cutaneous leishmaniasis remains to be ascertained [2,3]. Therefore, there is an urgent need to search for novel, effective and safe drugs for the treatment of these diseases [4]. In our ongoing study of potential leishmanicidal agents from *Piper* species [5], we have carried out a bioguided fractionation of the ethanolic extract of the leaves of *Piper delineatum* Trel. This extract was subsequently partitioned between water and organic solvents of increasing polarity, giving CH_2Cl_2 and EtOAc fractions. These extracts and the remaining aqueous layer were tested for their leishmanicidal activity against promastigote forms of two strains of *Leishmania* (*L. amazonensis* and *L. braziliensis*). The bioactive CH_2Cl_2 fraction was subjected to column chromatography, yielding thirteen fractions. The most active fraction (IC_{50} 1.1 and 1.3 μg/mL against *L. amazonensis* and *L. braziliensis*, respectively) was further subjected to repeat chromatography, affording two new bioactive *trans*-chalcones: 2',3,4'-trihydroxy-6'-methoxy-chalcone and 2',3,4'-trihydroxy-5,6'-dimethoxy-chalcone, whose structures were elucidated by means of spectrometric and spectroscopic techniques. These results support the use of the Piper species as a traditional remedy in the treatment of parasitic diseases. **Acknowledgement:** *We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) and RED RAPMA/OPS projects for financial support. TJC is grateful to MAEC-AECID for a fellowship.* **References:** 1. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis (2010). WHO Technical Report Series 949. Switzerland. 2. Griensven J et al. (2010) Lancet Infect Dis.10: 184 – 194. 3. Bethony J et al. (2011) Immunol Rev 239: 237 – 270. 4. Nogueira C et al. (2011) Molecules 16: 2146 – 2190. 5. Parmar V et al. (1997) Phytochemistry 46: 597 – 673.

PG49 Chemical constituents from *Salvia dichroantha*

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The genus *Salvia*, being the largest genus of Lamiaceae family, contains around 900 species worldwide. There are 90 *Salvia* species growing wild in Turkey half of which are endemic including *S. dichroantha* Stapf [1]. Some *Salvia* species are used as carminative, spasmolytic, diuretic, antiseptic as well as against cold and cough in Anatolian folk medicine [2]. The only phytochemical work on *S. dichroantha* which was conducted on the roots revealed the presence abietane-type diterpenes [3]. In the continuation of our work on the secondary metabolites of Lamiaceae, we herein report the isolation and structure elucidation of diverse compounds from the aerial part of title plant. Chromatographic studies on the H_2O and $CHCl_3$ subextracts of the MeOH extract led to the isolation

of two megastigmane glycosides, premnaionoside (**1**), and salvionoside B (**2**), an aliphatic alcohol glycoside, (*3R*)-1-octen-3-ol-3-O-β-D-xylopyranosyl-(1 – 6)-O-β-D-glucopyranoside (**3**), a flavonoid, 5-hydroxy-3,7,4'-trimethoxyflavone, two hydroxycinnamic acid derivatives, rosmarinic acid and 3-O-methyl-rosmarinic acid and sucrose. The structures of the compounds were established by means of 1D- and 2D-NMR experiments and MS. To the best of our knowledge, compound **1** is being reported for the first time from Lamiaceae, while compounds **1** and **3** are new for the genus *Salvia*. This work also constitutes the first phytochemical work on the aerial parts of *S. dichroantha*. **References:** 1. Hedge IC (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegan Islands. Edinburgh University Press. Edinburgh. 2. Baytop T (1999) Therapy with Medicinal Plants in Turkey, Nobel Tıp Kitapevleri, Istanbul. 3. Kawazoe K et al. (1999) Phytochemistry 50: 493 – 497.

PG50 Iridoid, phenylethanoid and flavonoid glycosides from *Sideritis trojana*

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The genus *Sideritis* is represented by 45 species in the flora of Turkey [1]. *S. trojana* Bornm., a perennial herb endemic to Kazdaglari (Ida Mountains), is utilized as an herbal tea for various purposes. Previously, several diterpenes were reported from *S. trojana* [2]. However, there is no report on its iridoid and phenolic constituents. In the continuation of our work on the bioactive secondary metabolites from Lamiaceae family, we now describe the isolation and structure elucidation of the secondary metabolites from the roots of *S. trojana* as well as their antioxidant activity. From the H_2O-soluble portion of the MeOH extract, a new iridoid glycoside, 10-*O*-(*E*)-feruloylmelittoside (**1**) was obtained in addition to four known iridoid glycosides (melittoside,10-*O*-(*E*)-*p*-coumaroylmelittoside, stachysosides E and G). Moreover, five phenylethanoid glycosides (verbascoside, isoacteoside, lamalboside, leonoside A, isolavandulifolioside), three flavone glycosides (isoscutellarein 7-O-[6'''-*O*-acetyl-β-allopyranosyl-(1 – 2)]-β-glucopyranoside, 4'-*O*-methyisoscutellarein 7-*O*-[6'''-*O*-acetyl-β-allopyranosyl-(1 – 2)]-β-glucopyranoside, 3'-hydroxy-4'-*O*-methyisoscutellarein 7-*O*-[6'''-*O*-acetyl-β-allopyranosyl-(1 – 2)]-β-glucopyranoside) and a benzyl alcohol derivative (di-*O*-methylcrenatin) were obtained and identified. Characterization of the isolates was carried out by using NMR experiments and HR-MS. All compounds were tested for their antioxidant activity by *in vitro* TEAC assay and some of them exhibited moderate activity (0.97 – 1.44 mM) if compared with the reference compound (quercetin, 1.86 mM). **References:** 1. Aytac Z and Aksoy N (2000) Flora Mediterranea 10: 181 – 184. 2. Topcu G et al. (2001) Nat Prod Let 16: 33 – 37.

PG51 Isolation an identification of rotenoids in *Pachyrhizus tuberosus* seeds

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Amazonian yam bean (*Pachyrhizus tuberosus* Spreng., Leguminosae) is a perennial twining plant indigenous to South America used for food and as a fodder. Large tuberous roots, which are eaten fresh or roasted, contain high amount of protein and easily digestible carbohydrates. Leaves, stems, roots, ripe pods, and seeds can be toxic to humans due to the presence of insecticidal and piscicidal compounds of isoflavonoid type called rotenoids [1]. Aqueous/methanolic extract obtained from dried seeds (collected in Peruvian Amazon) was pretreated on an immunoaffinity column (IAC) [2] and subsequently analyzed by reverse phase HPLC with diode array detector. Immunosorbents for IAC are characterized by high molecular selectivity so that single group of structurally related compounds can be targeted. Certain levels of compounds with immunoreactivity similar to isoflavonoids were identified. The extract was subsequently separated by flash chromatography into fractions and semipreparative HPLC column into individual compounds. Their structures were identified by ^1H and ^{13}C NMR and confirmed by HR-MS. A study of chemical constituents of the seeds of *P. tuberosus* led to the isolation and identification of one new rotenoid of chemical formula $C_{20}H_{16}O_7$, along with five other known rotenoids (rotenone, pachyrrhizine, neotenone, erosone and 12α-hydroxydolineone) [3]. The new rotenoid was assigned structure of 12α-hydroxyerosone based on the detailed analysis of ^1H and ^{13}C NMR spectra. According to previous literary data describing various biological activities of rotenoids, we suppose that future research on this new rotenoid may lead to new findings in the phytochemistry of these bioactive compounds.

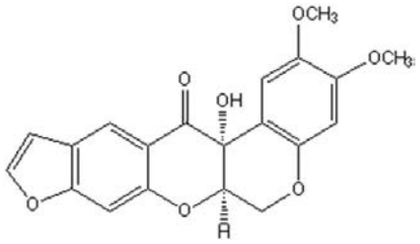

Figure 1: 12α-hydroxyerosone

Acknowledgement: *1. Czech Science Foundation (Project GA 525/09/0994), 2. Ministry of Education, Youth and Sports (Institutional research plan MSM 6046070901)* **References:** 1. Sørensen M (1997) Biodivers Conserv 6: 1581 – 1625, 2. Delaunay N et al. (2000) J Chromatogr B 745: 15 – 37, 3. Krishnamurti M et al. (1970) Tetrahedron 26: 3023 – 3027.

PG52 Searching for iridoids from tropical plants: detection, isolation and antibacterial activity

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Iridoid glycosides, which form an important group of cyclopentane monoterpenoids, are biosynthesized by a large number of plant species belonging to approximately twenty important botanical families. Although they possess a wide range of pharmacological and biological properties, such as anti-allergic, anti-inflammatory, antibacterial, antifungal, antiviral, anti-oxidative, immunomodulatory, neuroprotective, etc. [1,2], no molecule is currently used as a drug. However, iridoids, which possess a highly functionalized aglycon, may be regarded as starting material for the synthesis of a number of new chiral molecules. In this context, we have launched a research project aimed at searching for new chiral scaffold of iridoid-type from higher plants of the tropical biodiversity. This project, part of an ANR program called IRNA-CHIR, focuses on species, which contain high iridoids content. For this study, approximately 500 species were selected from iridoid-containing families, and were subjected to a methanolic extraction followed by dichloromethane recovery. Then a methodology, based on the combination of different analytical and spectroscopic techniques such as TLC, LC/UV/DEDL, LC/MS and NMR, was developed in order to select the plants of interest. Parallel to this study, we conducted an evaluation of the antimicrobial potential of extracts and isolated compounds. Our initial results show that no new iridoid was discovered to date, despite the fact that the species selected have never been studied, and few containing-iridoid extracts exhibit potent antibacterial activity. We will present and discuss the methodology used to detect and isolate iridoids, and the antibacterial activity of the extracts selected and pure compounds. **Acknowledgement:** *This 4-years project is funded by the French National Research Agency. We are gratefull to our foreign partners: Dr K. Awang (University Malaya, Malaysia), Dr. P. Rasoanaivo (IMRA, Malagasy), Dr. V.H. Nguyen (VAST, Vietnam), Dr. B. Kiremire (University of Makerere, Uganda) enabling us, through official agreements between CNRS and var-*

ious public institutions, to have access to a rich biodiversity. **References:** 1. Dinda B et al. (2007) Chem Pharm Bull 55(5): 689 – 728 2. Dinda B et al. (2009) Chem Pharm Bull 57(8): 765 – 796

PG53 NMR and HRMS/MS studies of acetophenone derivatives isolated from *Acronychia pedunculata* (L.) Miq.-Rutaceae

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Phenylated acetophenone derivatives constitute a characteristic chemical group of constituents of *Acronychia pedunculata* (L.) Miq. [1]. In continuation of a previous study, seven acetophenone dimmers were isolated among them five structural isomers [2,3]. Such acetophenones exhibit particular structural characteristics as fully substituted and polyhydroxylated aromatic rings. The presence of inter- and intra-molecular hydrogen bonds and their conformational behavior due to the occurrence of rotamers complicates their structure elucidation. In the present study, NMR spectroscopy was used in order to determine the structures and the different rotamers of all isolated acetophenones. The developed methodology included variation of different solvents (DMSO-d_6, $CDCl_3$, C_6D_6) as well as acquisition of NMR spectra in a broad range of temperatures (0 to 47ºC) where acrovestone was used as a model compound. Two principal rotamers of acrovestone are the most populated in $CDCl_3$ solution at 0ºC, while at 47ºC their representative NMR signals are not resolved due to fast inter-conversion between the rotamers. According to our study, *Acronychia* acetophenone rotamers' determination can be accomplished with NMR spectroscopy, by changing the polarity of solvent used as well as by altering temperature conditions of measurements. In parallel, an LC-APCI(+)-HRMS and MS/MS method was developed for the analysis of acetophenone derivatives using a LTQ-Orbitrap mass analyzer. A characteristic ion corresponding to the major fragment at *m/z* 319 was defined and used as diagnostic peak of the isolated phenylated acetophenone dimers. This novel developed LC-MS/MS method could be applied for the detection and identification of acrovestone-type prenylated acetophenone dimers in other substrates. **References:** 1. Adsersen A et al. (2007) Biochem Syst Ecol 17: 447 – 453. 2. Kouloura E et al. (2008) Planta Med 74: 1051 – 1052. 3. Kouloura E et al. (2009) Planta Med 75: 914.

PG54 Alkaloids from *Meiogyne virgata* (Annonaceae)

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The genus *Meiogyne* (family Annonaceae) consists of about 24 species, widely distributed in Indo-china, Thailand, Peninsular Malaysia, Sumatera, Java, Borneo and the Philippines. Several plants of the genus *Meiogyne* has been used as a folk medicine in Malaysia. Genus *Meiogyne* has been reported to contain various types of alkaloids. In this work, we investigated the chemical constituents of the bark of *Meiogyne virgata* Miq. The phytochemical procedures adopted were acid-base extraction followed by vacuum liquid chromatography, column chromatography and preparative thin layer chromatography. Isolation and purification of alkaloids from the bark of *Meiogyne virgata* afforded nine alkaloids; four oxoaporphines, liriodenine **1**, lanuginosine **2**, asimilobine **3** and lysicamine **4**; four aporphines, anonaine **5**, N-methylanonaine **6**, nornuciferine **7** and norushinsunine **8**; and one azaanthracene alkaloid, cleistopholine **9**. This paper reports presence of alkaloids **2**, **4**, **6** and **7** for the first time from *Meiogyne virgata*. The structural elucidation was accomplished by spectroscopic methods such as ID-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (COSY, HMQC, HMBC), UV, IR and MS and comparison with published data. **Acknowledgement:** *Universiti Teknologi MARA, Universiti of Malaya, Ministry of Higher Education for research grants and Ministry of Science, Technology and Innovation Malaysia for scholarship awarded to Alias, A.* **References:** 1. Tadic D, Cassels BK, Leboeuf M and Cave A (1987) Phytochemistry 26(2): 537 – 541 2. Alias A, Hazni H, Mohd Jaafar F, Awang K and Ismail NH (2010) Molecules 15: 4583 – 4588

PG55 New labdane-type diterpenoids and anti-inflammatory constituents from the rhizome of *Hedychium coronarium*

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Hedychium coronarium J.Koenig (Zingiberaceae) is a terrestrial epiphytic herb, which is distribution in India, Malaysia, Vietnam, southern China and Taiwan [1]. Two new labdane-type diterpenoids, hedychicoronarin (**1**) and peroxycoronarin D (**2**), together with eleven known compounds, including calcaratarin (**3**), (+)-coronarin A (**4**), coronarin D (**5**), coronarin D methyl ether (**6**), (*E*)-labda-8(17),12-diene-15,16-dial (**7**), ergosta-4,6,8(14),22-tetraen-3-one (**8**), β-sitostenone (**9**), stigmasta-4,22-dien-3-one (**10**), oleic acid (**11**), stearic acid (**12**), and palmitic acid (**13**) have been isolated from the rhizomes of *Hedychium coronarium*. The structure of new compounds **1** and **2** was determined through spectral analyses including extensive 2D NMR data. Among the isolates, calcaratarin (**3**), coronarin A (**4**), (*E*)-labda-8(17),12-diene-15,16-dial (**7**), and oleic acid (**11**) exhibited potent inhibition (IC_{50} < 6.17 μg/mL) against fMLP-induced superoxide production and elastase release.

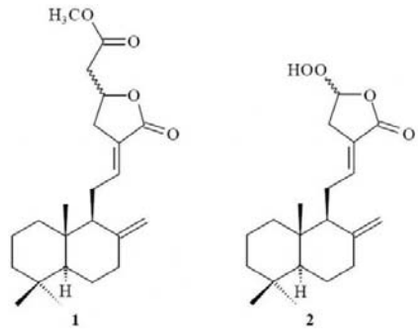

Figure 1: Structure of new compounds 1 and 2

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.* **References:** 1. Wang J C et al. (2000) 'Zingiberaceae' in 'Flora of Taiwan', 2nd edn., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. 5, p. 707 – 724.

PG56 Sesquiterpenoids from the root of *Solanum erianthum*

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Solanum erianthum D. Don (Solanaceae) is an evergreen shrub or small tree which is native of South America, widespread in tropical Asia and Oceania [1]. It is a traditional folk medicine used for the treatment of metrorrhagia, edema, gout, carbuncles, eczema, toothache and dermatitis [2]. In a screening program of Formosan plants, the MeOH extract of the root of this plant showed significant cytotoxic activities and was partitioned into *n*-hexane, EtOAc, *n*-BuOH and H_2O-soluble layers. Investigation of the active EtOAc-soluble layer led to the isolation of a new sesquiterpenoid solanerianone, along with 8 known compounds, including 4 sesquiterpenoids: solavetivone, anhydro-β-rotunol, solafuranone, lycifuranone A; 1 phenylalkanoid: acetovanillone, and 2 steroids: β-sitosterol and stigmasterol. Solavetivone, the major constituent, was reported owing cytotoxicity against OVCAR-3 (IC_{50}: 0.1 mM) [3]. The structure of the new sesquiterpenoid was determined by spectral analyses. **Acknowledgement:** *This work was kindly supported by a grant (NSC 98 – 2320-B-039 – 015-MY3) from the National Science Council of the Republic of China.* **References:** 1. William GD, Peng CI (1998) Solanaceae in Flora of Taiwan, 2nd edition. Editorial Committee of the Flora

of Taiwan, Taipei, Taiwan: Vol. 4: 549 – 581. 2. Tuan NH et al. (2008) Adv Nat Sci 9: 163 – 169. 3. Syu WJ et al. (2001) J Nat Prod 64: 1232 – 1233.

PG57 Pharmacognostic study and safety evaluation on *Illicium* plants

Sheng Y, Ping TC, Qiang KC, Qin Z, Yang Y
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The fruits and seeds (star anise) of *Illicium* plants are used traditionally as spices and folk medicines in Southern China. Those are one of the most important natural resources of shikimic acid (SA, Fig. 1), which is the raw material of the antiviral drug Tamiflu. China is the largest star anise supplier in the world and 80% of raw resources are from Guangxi province. A simple and rapid HPLC method was established to analyze the content of SA in the fruits and leaves of 22 samples from different species and habitats, and SA with high content was found in the fruits of three species (> 8%) and leaves of eight species (> 5%). Thus these materials can be used as the raw resources of SA. Researches found that even the trace amount of anisatin and its analogs could arouse toxic effects. The mechanism studies revealed they are non-competitive antagonists of GABA receptor[1]. Chemical investigations on three *Illicium* plants resulted in 14 sesquiterpenoids including anisatin and it's analogues to be obtained[2]. Based on the summarized MS behaviors of anisatin and its analogues, a qualitative analytical method was developed to detect anisatin in the fruits of the above species, the results turned out that most species contained anisatin and its analogues including the edible species (*I. verum* Hook.f.) from Rongshui County, while three edible species (*I. verum* and *I. majus* Hook.f. & Thomson in Jinxiu County, *I. jadifengpi* B.N. Chang in Lingyun County) did not contain anisatin or its analogues and they are safe to use as spices and folk medicines.

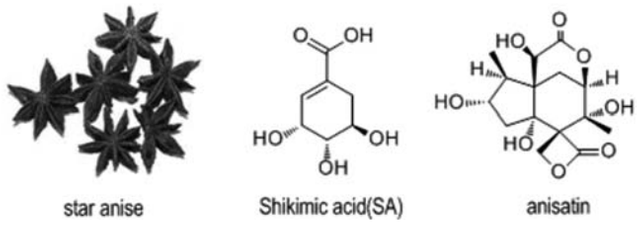

Figure 1: Star anise and the structures of shikimic acid (SA) and anisatin.

Acknowledgement: *Prof. Shen JG of Department of Natural Products Chemistry, Shanghai Institute of Materia Medica, Prof. Song GQ. of Department of Analytical Chemistry, Shanghai Institute of Material Medica* **References:** 1. Matsui T et al. (2007) Planta Med 73: 662 – 665. 2. Zhu Q et al. (2009) J Nat Prod 72: 238 – 242.

PG58 New sesquiterpene derivatives and anti-inflammatory constituents from *Pachira aquatica*

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Pachira aquatica Aublet (Bombacaceae) is an evergreen tree, distributed in tropical America and introduced to Guangdong, Yunnan, and Taiwan as a cultivated plant [1]. Cadinane sesquiterpenoids, sesquiterpene lactones, and triterpenes are widely distributed in plants of the family Bombacaceae, and many of these compounds exhibit antiangiogenic, hypotensive, and antimicrobial activities. Investigation of *n*-hexane-soluble fraction of the stem of *P. aquatica* has led to the isolation of two new sesquiterpene derivatives, 11-hydroxy-2-*O*-methylhibiscolactone A (**1**) and *O*-methylhibiscone D (**2**), together with 18 known compounds, including 5-hydroxyauranetin (**3**), kaempferol-3,7,4'-trimethyl ether (**4**), santi-7-methyl ether (**5**), 3,5,6,7,8,3',4'-heptamethoxyflavone (**6**), calycopterin (**7**), retusin (**8**), 5,4'-dihydroxy-3,7-dimethoxyflavone (**9**), isohemigossylic acid lactone-7-methyl ether (**10**), hibiscolactone A (**11**), hibiscone C (**12**), hibiscone D (**13**), 2-*O*-methylisohemigossypolone (**14**), scopoletin (**15**), benzophenone (**16**), 2α,3β-dihydroxylupene (**17**), lupenone (**18**), 24-methylenecycloartenol (**19**), and (23*E*)-cycloart-23-ene-3β,25-diol (**20**). The structures of new compounds **1** and **2** were determined through spectroscopic and MS analyses. Among the isolates, 5-hydroxyauranetin (**3**) and isohemigossylic acid lactone-7-methyl ether (**10**) exhibited potent inhibition against *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine-induced superoxide production with IC_{50} values of 28.84 ± 2.26 and 12.77 ± 2.48 μM, respectively.

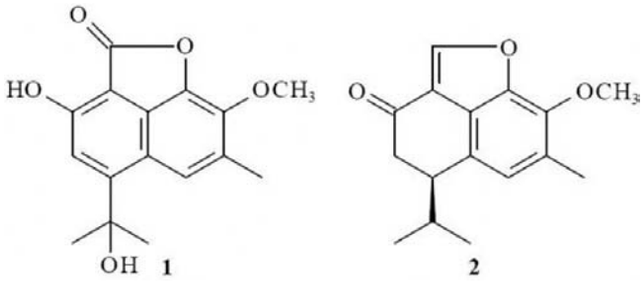

Figure 1: Structure of new compounds 1 and 2

Acknowledgement: *This research was supported by grants from the National Science Council of the Republic of China (NSC 95 – 2320-B-127 – 001-MY3 and NSC 98 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.* **References:** 1. Tang Y et al. (1995) 'Bombacaceae' in 'Flora of China', Science Press, Beijing, China. Vol. 12, pp. 264 – 299

PG59 A new natural Pepstatin from Kitasatospora (Actinomycetales)

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The pepstatins are linear peptides biosynthesized and excreted into culture filtrates by several species of Actinomycetes and are well known inhibitors of aspartic proteinases, such as pepsin and cathepsins D and E. Apart from their role as proteinase inhibitors, their other pharmacological and cellular activities remain unclear. Our lab has isolated Pepstatin A [1] and a new member of this family from an actinomycete strain belonging to the genus *Kitasatospora*. BLAST searches and sequence alignments of partial ribosomal DNA sequences revealed that this strain is closely related to strains of the species *Kitasatospora mediocidica*. The producing organism was isolated from the rhizosphere of a juniper-tree (*Juniperus communis* L.) after plating and incubation of a soil suspension on a selective glycerol arginine agar isolation medium. How was it fermented? Fermentation discovery strategy? A 1L fermentation of the strain was extracted with acetone and, after evaporation of the organic solvent, fractionated on a SPE resin (SP207ss) on reverse phase mode. Acetonitrile/water semi-preparative HPLC gradient of one of these fractions led us to the detection (LC/MS) of two components related to the pepstatin family. Both secondary metabolites were isolated by preparative HPLC under similar conditions. Structural elucidation of both components was based on NMR (1 H, 13C, COSY, HSQC, HMBC) and low and high resolution Mass Spectrometry (LC/MS-ESI) data. The isolated compounds were finally identified as pepstatin A and the new derivative we designate as pepstatin K. Data on the isolation, structural characterization and biological properties of both compounds will be presented.

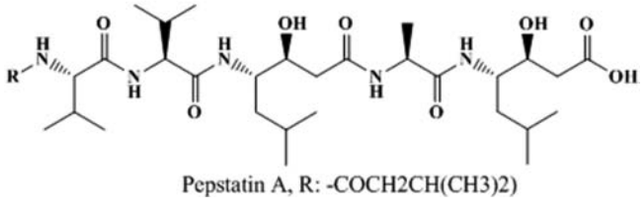

Figure 1: Pepstatin Family

References: [1]. Umezawa H et al. (1970) J Antibiot 23: 259

PG60 Isolation and structural elucidation of coumarins from *Micromelum falcatum* (Rutaceae)

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Micromelum falcatum Tanaka (Rutaceae) is a small tree growing in Southeastern Asia [1] showing protective and therapeutic effects against cold and rheumatoid arthritis according to the traditional medicine of China [2]. The leaves of the plant, collected in Cambodia, after drying and pulverization (20gr) were extracted successively with CH_2Cl_2 (200 ml x 3), MeOH (200 ml x 3) and H_2O (200 ml x 3). In parallel, a protocol using NH_3 and extraction (150gr) with EtOAc and MeOH was followed for focused isolation of alkaloids [3]. All the resulted extracts were evaluated qualitatively using a novel HPLC-DAD method. The analytical profiling revealed the presence of coumarins as the major class of constituents in both CH_2Cl_2 and EtOAc extracts before and after the alkalization. The HPLC-DAD method was transferred to semi-preparative scale and was used for the isolation of the detected coumarins. Nine known and two new coumarins, microfalcrin (1) and microcoumaririn (2) were isolated. Three among them, micromarin A, B and C were isolated for the first time from *Micromelum falcatum*. The identification of the isolated compounds was performed by HRMS and NMR (1 &2D) spectroscopy.

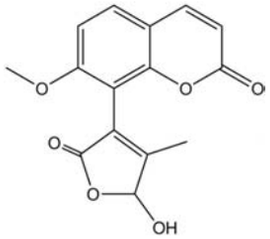

Figure 1: Microcoumaririn

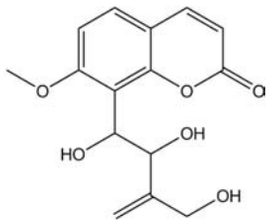

Figure 2: Microfalcrin

References: 1. Perry LM (1980) In Medicinal Plants of East and Southeast Asia. MIT Press. Cambridge. 2. Kham L (2004) Medicinal Plants of Cambodia, Bendigo Scientific Press. 3. Pusset J et al. (1991) Planta Med 57: 153 – 155.

PG61 Anti-oxidative and anti-inflammatory activities of caffeoyl derivatives from the barks of *Ilex rotunda*

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The barks of *Ilex rotunda* Thunb. (IR) have been used for the treatment of common cold, tonsillitis and intestinal ulcer. As part of our continuous search for new anti-oxidative and anti-inflammatory agents from natural sources, we tried to isolate caffeoyl derivatives from the bark of IR. Activity guided isolations of the 80% MeOH extract yielded two new caffeoyl hemiterpenes (**1** and **2**), tentatively named rotundarpene and rotundarpenoside B, together with 6 caffeoyl derivatives which were methyl caffeic acid (**3**), 5-caffeoylquinic acid (**4**), 3,5-dicaffeoylquinic acid (**5**), 1,4-dicaffeoylquinic acid (**6**), 3,4.5-tricaffeoylquinic acid (**7**) and 3,5-dicaffeoyl shikimic acid (**8**), respectively. In order to evaluate anti-oxidative and anti-inflammatory activities of **1-8**, DPPH radical scavenging activity and inhibitory activity on nitric oxide production in LPS-stimulated RAW 246.7 cells were measured *in vitro*. All caffeoyl derivatives (**1-8**) showed potent anti-oxidative and anti-inflammatory activities, and these results suggested that caffeoyl derivatives from IR might be developed as anti-oxidative and anti-inflammatory agents.

Acknowledgement: *This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091121)* **References:** 1. Damtoft S, Jensen SR (1995) Phytochemistry 39: 923 – 924. 2. Jiang JH et al. (2005) J Nat Prod 68: 397 – 399.

PG62 Four new kaempferol glycosides from the leaves of *Brugmansia suaveolens*

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Brugmansia suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & C. Presl (Syn. *Datura suaveolens*; Common name: Angel's trumpet) is a flouring shrub of Solanaceae family and it is native from coastal regions of the rainforest in Southeast Brazil. This plant has been investigated due to its inflammatory and wound healing activities [1] and mainly due to the presence of alkaloids [2]. Nevertheless, only few studies related the characterization of flavonoid glycosides [3]. This prompted us to investigate the ethanolic extract prepared from its leaves. In order to have pure compounds, the plant material was submitted to successive chromatographic separations using open column chromatography and HPLC on RP-18. Up to now, four new flavonol glycosides, namely, kaempferol 3-O-β-D-glucopyranosyl-(1'''→2'')-O-α-L-arabinopyranoside-7-O-β-D-glucopyranoside (1), kaempferol 3-O-β-D-[6'''-O-(3,4-dihydroxy-cinnamoyl)]-glucopyranosyl-(1'''→2'')-O-α-L-arabinopyranoside-7-O-β-D-glucopyranoside (2), kaempferol 3-O-β-D-[2'''-O-(3,4-dihydroxy-cinnamoyl)]-glucopyranosyl-(1'''→2'')-O-α-L-arabinopyranoside-7-O-β-D-glucopyranoside (3), and kaempferol 3-O-β-D-glucopyranosyl-(1'''→2'')-O-α-L-arabinopyranoside (4) were isolated and identified by means of extensive spectroscopic methods including 1D-(1 H and 13C) and 2D NMR experiments (COSY, HSQC and HMBC) as well as ESI-MS.

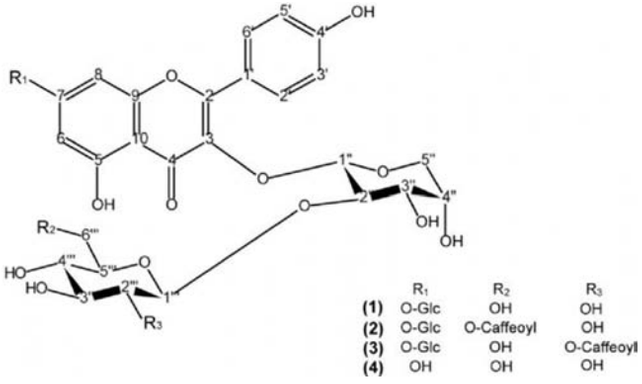

Figure 1: Isolated flavonol glycosides

Acknowledgement: *Financial support from the government Baden-Württemberg (Zukunftsoffensive IV) is gratefully acknowledged.* **References:** 1. Sebold DF (2003) Master thesis, UFRGS/Brazil. 2. Alves MN et al. (2007) J Chem Ecol 33: 297 – 309. 3. Begum S et al. (2006) Nat Prod Res 20: 1231 – 1236.

PG63 Phytochemical investigations of *Alyssum corsicum*

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The genus *Alyssum* belonging to family of Cruciferae is represented by 89 species in Turkey, 52 of them being endemic [1]. Although there are no reports of the medicinal uses of *Alyssum corsicum* Duby, the aerial flowered part, flowered stems and inflorescences of *A. maritimum* (L.) Lam. are employed as renal lithotripter in infusion and decoction in the Iberian Peninsula. It is also claimed to be a hepatic lithotripter, and to have other benefits associated to the hepatic function (hepatoprotective, antiicteric, the last use in veterinary) [2]. In Iran, seeds of *A. minutum* Patrin ex DC. are used as a treatment for fevers and other ailments. Glucosinolates, hydrocarbons, fatty acids and flavonol 7-glucuronides were isolated from the genus *Alyssum* previously[3]. Glucosinolate profiles of the seeds of the various *Alyssum* species were also screened by Ion-Pair LC-MS method [4]. This is the first phytochemical report on *Alyssum corsicum*. In this study three known compounds (Tamarixetin 3,7-diglucoside, Tamarixetin 3-O-β.-D- glucopyranoside-7-O-α-rhamnopyranoside, Tamarixetin 3-O-β-D-glucoside) were isolated from the MeOH extract of of *Alyssum corsicum* by using preparative chromatographic methods. The structure elucidation of the isolated compounds was based on analyses of their spectroscopic data (1D and 2D NMR). **References:** 1. Davis PH (1965) Flora of Turkey and East Aegean Islands. University Press. Edinburgh. 2. Parada et al. (2009)J Ethnopharmacol 124: 609 – 618. 3. Afsharypuor S, Lockwood GB (1986)J Nat Prod 49: 944 – 945. 4. Bennett RN et al. (2004)J Agric Food Chem 52: 428 – 438.

PG64 Constituents of Verbascum reeseanum

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The genus *Verbascum* (Scrophulariaceae) is represented by 228 species, of which 185 are endemic in the flora of Turkey and East Aegean Islands [1]. *Verbascum* species contain biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, and iridoid and monoterpene glycosides [2]. The leaves and flowers of *Verbascum* are reported to have expectorant, mucolytic and demulcent properties, and are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine [3]. In this study, dried and grinded plant material (*Verbascum reeseanum* Hub.-Mor. – whole plant) was extracted with MeOH, and then treated with *n*-hexane. The *n*-butanol fraction from the methanol extract was submitted to several preparative chromatographic methods such as VLC, normal phase silica gel CC and reversed phase RP-18. The structures of the known compounds were determined as ajugol, lawsoniaside B, ajugoside, coniferin and sinuatol, by a combination of one- and two-dimensional NMR techniques, and mass spectrometry. **References:** 1. Davis PH (1978) Flora of Turkey and East Aegean Islands. University Press. Edinburgh 2. Tatli II, Akdemir ZŞ (2004) Fabad J Pharm Sci 29: 93 – 107. 3. Tatli II, Akdemir ZŞ (2006) Fabad J Pharm Sci 31: 85 – 96.

PG65 Efficient isolation of bioactive constituents from Greek Fabaceae species through elaboration of counter-current chromatography (CCC) approaches

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Fabaceae family contains plants that are characterized by significant biological activities. The main edible plants of this family constitute an important part of the Mediterranean diet and contain secondary metabolites with considerable estrogenic, antioxidant and chemopreventing activity1. Recent years CCC has become a method of choice in separation and purification of natural products. The advantage of this method is the ability to separate substances from large volume of crude and complex extracts which is crucial in further analysis as far as identification and biological control activity (2,3). In the present study the application of CCC is demonstrated as a main separation technique in the phytochemical analysis of seven Fabaceae species growing in Greece.The analysis of active extracts of *Vicia faba* L., *Lotus siliquosus* L., *Tetragonolobus purpureus* Moench and *Genista hassertiana* (Bald.) Buchegger took place by of line coupling of CCC technique with sephadex column or preparative HPLC. In the case of *Lotus edulis* L., *Lathyrus laxiflorus* (Desf.) Kuntze, and *Genista halacsyi* Heldr. the direct isolation of active compounds (flavonoids, isoflavones and phenolic acids) was achieved from the complex extracts using dual mode or gradient mode CCC. The purity and identity of isolated compounds was confirmed by NMR spectroscopy. It is worth noting that the phytochemical analysis of *L. siliquosus, T. purpureus* and *G. hassertiana* is presented for the first time. In conclusion, it is clearly indicated that counter-current chromatography is a valuable technique and can be successfully employed for rapid and effective separation of natural compounds from crude active extracts of Fabaceae species. **References:** 1. Spanou C et al (2008)J Agric Food Chem 56: 6967 – 6976. 2. Bertoth A et al. (2009) Pure Appl Chem 81(2): 355 – 387. 3. Sutherland IA et al. (2009)J Chromatogr A 1216: 740 – 753.

PG66 Constituents of *Asperula cypria*

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The family Rubiaceae is represented by about 500 genera and 6000 species, with about 200 known *Asperula* species [1]. *Asperula* species are traditionally used by Lebanese people to reduce blood pressure and inflammation/edema, and for diabetes [2]. Moreover they are rich sources of antimicrobial agents [3]. The chemical composition of genus *Asperula* characterized by the presence of iridoids, flavonoids and anthraquinone glycosides [4,5,6]. During our search on the aerial parts of *Asperula cypria* Ehrend., an endemic plant of Cyprus, a number of secondary metabolites were isolated from the methanol extract of the plant. The isolation was carried out with several chromatographic steps using VLC and open column chromatography over silica-gel, reverse phase silica gel (RP-18) and Sephadex LH-20 to produce a new and seven known compounds, four flavonoids together with two iridoids, and a triterpene. The structure of the known compounds was determined as kaempferol (1), kaempferol 3-O-β-D glucopyranoside (Astragalin) (2), quercetin-3-O-β-D-glucoside (isoquercitrin) (3), quercetin-3-O-β-D-galactopyranoside (hyperin) (4), asperulosidic acid (5), asperuloside (6), and oleanolic acid (7). The new compound was elucidated to be a phe-

nylethane-C-glycoside, encountered for the first time in nature, by the extensive use of 1 H NMR, 13CNMRĐEPT, 1 H-1 H COSY, NOESY, HSQC, HMBC and LC-MS experiments. **References:** 1. Trease GE, Evans WC (1989) Pharmacognosy. English Language Book Society. Balliere Tindall. London. 2. Loizzo MR et al. (2008) J Ethnopharmacol 119: 109 – 116. 3. Kalyoncu F (2009) Iran J Pharm Res 8: 263 – 268. 4. Corrigan D et al. (1978) Phytochemistry 17: 1131 – 1133. 5. Borisov MI et al. (1972) Khim Prir Soedin 3: 281 – 285. 6. Constantinescu E (1974) Farmacia 22: 335 – 344.

PG67 A New Guaianolide from the aerial parts of *Centaurea pannonica* (Heuffel) Simonkai

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Centaurea is a complex genus of about 500 species belonging to the Asteraceae family [1]. Sesquiterpene lactones are the main chemical taxonomic markers of the genus [2,3]. Some members of this genus are used in folk medicine [4]. In the present study, we report the main compounds isolated from *Centaurea pannonica* (Heuffel) Simonkai, a taxon belonging to the section Jacea. The plant was collected in Šumadija region-Serbia, on September 2008. The aerial parts were extracted according to the Bohlmann isolation method, slightly modified [5]. One new naturally occurring sesquiterpene lactone (Fig. 1), six known guaianolides, namely babylin A, chlorohyssopifolin C, chlororepdiolide, repdiolide, janerin, 19-deoxyjanerin and three known lignans arctigenin, matairesinol, arctiin were isolated by repeated CC and RP18-HPLC. The structure of the isolated compounds were elucidated by spectroscopic methods, particularly high-field NMR spectroscopy (1 H-NMR, 1 H-1 H COSY, HSQC, HMBC). So far, the presence of guaianolides is characteristic for the taxa of the section Jacea [6, 7].

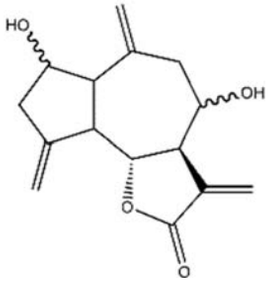

Figure 1

Acknowledgement: *Greek foundation of Scholarships (IKY) and Ministry of Science, Republic of Serbia (project III 43004).* **References:** 1. Mabberley DJ (1997) The Plant Book. Cambridge University Press. Cambridge. 2. Djeddi S et al. (2008) Biochem Syst Ecol 36: 336 – 339. 3. Bruno M et al. (2005) Biochem Syst Ecol 33: 817 – 825. 4. Platéarius (1986) Le livre de simples médecines. Ed. Ozalid et Textes Cardinaux. Paris. 5. Bohlmann F et al. (1984) Phytochemistry 23: 1979 – 88. 6. Gonzalez AG et.al. (1974) Phytochemistry 13: 1193 – 1197. 7. Gonzalez AG et.al. (1978) Can J Chem 56: 491 – 494.

PG68 Discovery of bioactive metabolites from the leaves of *Vitex pinnata* using high-throughput flash chromatography

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In our study we report the chemical investigation and various biological activities of secondary metabolites isolated from the leaves of *Vitex pinnata* L. In Malaysia, leaves of *V. pinnata* were traditionally used to treat cuts and wounds while the bark decoction is used for stomach ache and post-childbirth medicine [1,2]. High-throughput flash chromatography proved to be fast, robust, efficient and reproducible in the isolation and purification of the biologically active natural products. Ten compounds were isolated and chemical investigation revealed that retusin, kaemferol trimetyl ether, pheophytin *a*, β-sitosterol, and a new diterpene are the major compounds found in this leaves. The structures of all isolated compounds were determined by using 1D and 2D-NMR and also LC-MS. Antioxidant and antibacterial activities were tested on isolated compounds and fractions. In the qualitative antioxidant TLC assay, new diterpene and pheophytin *a* bleached the DPPH (0.2% in MeOH) purple colour indicating antioxidant activity. Retusin and β-sitosterol isolated from hexane extract showed to have antibacterial activity against *Microbacterium marinum*. Other bioassays such as antitrypanosomal and cytotoxicity studies are still under investigation. Consequently, this plant is a promising source for various biological activities. **Acknowledgement:** *The Ministry of Higher Education, Malaysia Universiti Sultan Zainal Abidin, Kuala Terengganu Malaysia* **References:** 1. Burkill IH (1968) A Dictionary of the Economic Products of the Malay Peninsula (Vols. I and II), Ministry of Agriculture Publication Unit, Kuala Lumpur. 2. Ong HC, Nordiana M (1999) Fitoterapia 70: 502 – 513

PG69 Enrichment of bioactive phenolic compounds from aqueous solution by foam separation

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Olive mill wastewater (OMWW) is an abundant source of polyphenols [1]. Due to their different bioactivities, OMWW would be a worthwhile source of highly valuable compounds for pharmaceutical and food industry. However, a simple and cost-effective extraction technique has still to be found. The present study aimed to evaluate foam separation for the isolation of phenolic compounds from OMWW. Thereby, surface-active substances can be enriched in an up-rising foam produced by introducing gas in an aqueous solution [2]. Running the process with inert gas at room temperature provides a mild technique for heat- and oxygen-sensitive substances. Aqueous solutions of phenolic acids (vanillic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, protocatechuic acid), all found in OMWW, were used as simple models of OMWW. The added cetyltrimethylammonium bromide (CTAB) acted as a foaming agent and as an anion-collector for the deprotonated reference substances. Enrichment factors (concentration in the collected foam relative to the concentration in the feed solution verified via HPLC analaysis) for the phenolic acids were optimized by varying important process parameters like pH, CTAB concentration, biophenol concentration, and gas flow rate. As a result, enriched extracts were obtained for all tested substances. **Acknowledgement:** *This work was funded by the Bundesministerium für Bildung und Forschung, BioDisc.* **References:** 1. Obied HK et al. (2005) J Agric Food Chem 53: 823 – 937. 2. Lemlich R (1986) Ind Eng Chem Res 60: 16 – 29.

PG70 Microgram-scale, *in vivo* natural product discovery using zebrafish bioassays, UHPLC-TOF-MS and microflow NMR: Identification of anticonvulsants in the Philippine medicinal plant *Solanum torvum*

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The rapid acquisition of structural and bioactivity information on natural products at the sub-milligram scale is key for performing efficient bioactivity-guided isolations. We have recently established zebrafish as an ideal *in vivo* system for natural product discovery[1,2]. Zebrafish offer

the possibility of rapid *in vivo* bioactivity analysis at the microgram scale, an attractive feature when combined with high-resolution fractionation technologies and microgram-scale analytical methods such as UHPLC-TOF-MS and microflow NMR[3]. Using this platform, we have performed high-resolution *in vivo* bioactivity profiling of *Solanum torvum* Schltdl., one of several Solanaceae species used as medicinal plants for the treatment of epilepsy[4–6]. The crude methanolic extract of *S. torvum* stem bark exhibited strong anti-convulsant activity in a microtiter plate-based zebrafish seizure assay. UHPLC-TOF-MS profiling revealed the presence of numerous steroid glycosides. An initial enrichment step localized the bioactivity to the steroid fraction. High resolution microfractionation by semi-preparative LC-MS enabled the resolution of most constituents directly in a single 96-well microtiter plate. The analysis of these wells by microflow NMR in combination with LC-MS profiling allowed dereplication of the active compounds, identifying them as spirostanol glycoside derivatives and estimating their microquantities for quantitative assessment of anticonvulsant activity in zebrafish. These results indicate the potential of zebrafish bioassay-guided microfractionation, in combination with UHPLC-TOF-MS and microflow NMR, to rapidly identify bioactive natural products. **References:** 1. Crawford AD et al. (2008) Planta Med 6: 624. 2. Crawford AD et al. (2011) PLoS ONE 6:e14694. 3. Glauser G et al. (2009) J Agric Food Chem 57: 1127. 4. Quisumbing E (1951) Medicinal Plants of the Philippines, Bureau of Print, Manila. 5. Barber CF (1895) JAMA 25: 1023. 6. Trusch D (1904) Journal de Mèdecine de Paris 8: 74.

PG71 Chemodiversity and biological activity of the genus Alpinia (Zingiberaceae)

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Several species of the large and taxonomically complex genus *Alpinia* are of ethnobotanical importance in (sub)-tropical regions of Asia and Oceania. Different parts of the plants (rhizomes, leaves, fruits) are used mainly for medicinal purposes, as food additives and spices (e.g. A. *officinarum* Hance, *A. galanga* Willd., and *A. oxyphylla* Miq.). Current comparative studies on the chemical profiles of rhizomes of 25 Alpinia species revealed the presence of labdane diterpenes, acetylated phenylpropenes, kavalactones, specific flavonoids, and diarylheptanoids, each being derived from distinct genetically defined pathways. Phytochemical methods applied include various chromatographic techniques for profiling (HPLC-UV/Vis, TLC) and isolation (CC, MPLC, pTLC), and spectroscopic methods (NMR, MS) for structure elucidation of obtained compounds. Methanolic extracts and purified compounds were routinely checked for bioactivity, against the phytopathogenic fungus Cladosporium sphaerospermum and the pest insect Spodoptera littoralis. In addition, some of the pure compounds isolated were studied for their anti-angiogenic properties in a zebrafish model system. The labdane-diterpene zerumin A dose-dependently inhibited vessel formation on both wild type and Tg(fli1a:EGFP)y1 zebrafish embryos through effects on multiple molecular targets related to angiogenesis. The occurrence of different secondary metabolites within rhizomes of *Alpinia* is presented, and bioactivity results are discussed.

PG72 New lathyrane-type diterpenes from *Euphorbia boetica*

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Euphorbia species, commonly named spurge, have been widely used in traditional medicine to treat several diseases, like tumors and warts [1]. From these species, a wide range of structurally unique polyoxygenated macrocyclic diterpenes as jatrophanes, lathyranes and their polycyclic derivatives, have been isolated. These compounds have shown several important biological activities including antiproliferative effects, modulation of multidrug resistance in cancer cells and apoptosis induction [2 – 4]. *Euphorbia boetica*Boiss. is an herb endemic to Europe, commonly found in Southwest of Iberic Peninsula (Algarve and Alentejo). In our continuing investigation for biologically active molecules, the methanol extract of *E. boetica* aerial parts has been studied. The crude methanolic residue was suspended on a methanol-water mixture and extracted with ethyl acetate. Repeated column chromatographic fractionation and further purification by HPLC of the ethyl acetate soluble part afforded six diterpenes with the lathyrane skeleton that have a new acylation pattern. In addition, a cycloartane triterpene was also isolated and identified. The chemical structures of the isolated compounds, including stereochemical features were deduced from their physical and spectroscopic data, which include: Infrared Spectroscopy, low and high resolution Mass Spectrometry (MS), and extensive one- and two-dimensional Nuclear Magnetic Resonance studies (1D and 2D-NMR). **Acknowledgement:** *This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008)* **References:** 1. Hartwell J (1969) Lloydia 32: 153 – 205 2. Lage H et al. (2010) Phytomedicine 17: 441 – 448 3. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 9330 4. Duarte N. et al. (2007) Bioorg Med Chem 15: 546 – 554

PG73 Alkanoyl and aroyl derivatives of a lathyrane-type macrocyclic diterpene

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Multidrug-resistance phenomenon (MDR) to anti-cancer drugs is one of the most serious obstacles in the success of a chemotherapeutic treatment. P-glycoprotein (P-gp) is often implied in the efflux of drugs as anthracyclins, vinca alkaloids, taxanes and other non-related drug, lowering the effective concentration of such drugs in the cytoplasmatic compartment [1]. One of the most promising approaches to overcome MDR is the development of molecules that can effectively modulate the activity of P-gp, thus inhibiting the drug efflux. In the past decades, several natural and synthetic compounds have been reported as MDR modulators, but none is currently available for the clinical practice. In previous works, we have isolated, from *Euphorbia* species (Euphorbiaceae), several macrocyclic jatrophane [2,3] and lathyrane-type [3,4,5] diterpenes with strong P-gp modulation activity. In order to obtain a library of bioactive lathyrane and jatrophane diterpenes, required for QSAR studies and further refinement of an in-house P-gp modulators pharmacophore model, the phytochemical study of *Euphorbia piscatoria* Ait., an endemic species from Madeira island traditionally used in fishing activities, has been carried out. Fractionation by chromatographic methods of the crude methanolic extract of the aerial parts of *Euphorbia piscatoria* yielded a large amount of a lathyrane-type diterpenoid that was acylated, using different alkanoyl and aroyl chlorides/anhydrides. Several new esters were obtained whose structures were assigned based on spectroscopic methods namely 1D NMR (^{1}H, ^{13}C, DEPT) and 2D NMR (COSY, HMBC, HMQC) data. **Acknowledgement:** *This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008 and grant SFRH/BD/72915/2010).* **References:** 1. Teodori E et al. (2002) Il Farmaco 57: 385 – 415 2. Valente C et al. (2004) Planta Med 70: 81 – 84 3. Duarte N et al. (2006) Planta Med 72: 162 – 168 4. Duarte N et al. (2007) Bioorg Med Chem 15: 546 – 554 5. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 9330

PG74 Unusual Cycloartane Saponins with Cytotoxic Activity from *Astragalus stereocalyx* Bornm.

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The genus *Astragalus* L., which is included 10 subgenera and approximately 150 sections, today it contains about 2500 – 3000 species and subspecies according to some sources (1 – 2). In Turkish folk medicine, the aqueous extracts of some *Astragalus* species (declared by the healer) are used to treat leukaemia as well as for wound healing (3). Six new cycloartane-type triterpene glycosides **(1 – 6)** were isolated from *Astragalus stereocalyx* Bornm. along with six known cycloartane-type glycosides (Figure). Their structures were established by the extensive use of

1D and 2D-NMR experiments along with ESIMS and HRMS analysis. Compounds **1-3** are based on a new aglycon characterized by the occurrence of an unusual hydroxyl group at position 20, whereas compounds **4-6** are based on cycloasgenin C as aglycon, so far reported from *Astragalus* spp. All of the compounds tested for their cytotoxic activities against a number of cancer cell lines. Among the compounds, only 10 exhibited activity versus human cervical cancer (Hela) at 10 μM concentration. **References:** 1. Heywood VH (1978) Flowering Plants of the World. Oxford University Press. London. 2. Maassoumi AA (1998) Astragalus in the Old World, Check-List. Islamic Republic of Iran Ministry of Jahad-e-Sazandegi Research Inst of Forests and Rangelands. Iran. 3.Çalış I, Yuruker A, Taşdemir D, Wright AD, Sticher O, Luo YD, Pezzuto JM (1997) Planta Med 63: 183 – 186.

PG75 Antimicrobial constituents from the African medicinal plant *Zanthoxylum capense*

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The genus *Zanthoxylum*, comprising approximately 250 species, is well known for its ethnobotanical uses among Rutaceae family [1]. Previous studies have demonstrated that plants belonging to this genus are rich sources of biologically active compounds, such as alkaloids, aliphatic and aromatic amides, coumarins, as well as lignans [2]. *Zanthoxylum capense* (Thunb.) Harv. is a medicinal plant indigenous to Zimbabwe, South Africa, and Mozambique. Traditional healers use the decoction of its roots for snakebites, and the decoction of its root barks to treat tuberculosis, paralysis, and relief of toothache. However, until date there have been relatively few phytochemical studies on this species [3, 4]. During our search for bioactive compounds from the methanolic extract of *Z. capense* roots, we have isolated a new benzophenanthridine alkaloid and two new 2-arylbenzofuran neolignans. In addition, several known compounds were also isolated, including six alkaloids with the benzophenanthridine scaffold, one furoquinoline-type alkaloid and a lignan. The structures of the compounds were elucidated by means of MS, extensive 1D and 2D-NMR analyses and by comparison of their physical and spectroscopic data with those reported in the literature. All the isolated compounds were evaluated for their *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. Some compounds showed inhibitory activity mainly against *Staphylococcus aureus* ATCC 6538 with MIC values ranging from 12.5 to 50 μg/mL. These compounds might be promising leads for the development of new antimicrobials. **Acknowledgement:** *This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007).* **References:** 1. Sun XW et al. (1996) Acta Pharma Sin 31: 231 – 240. 2. Chen JJ et al. (2008) J Nat Prod 71: 212 – 217. 3. Calaerwood JM et al. (1970) Phytochemistry 9: 675. 4. Fish F et al. (1973) Phytochemistry 12: 2553 – 2554.

PG76 Isolation and Tandem Mass Spectometric Characterization of Selected *Crocus sativus* L. (Saffron) Bioactive Compounds

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Saffron, the dried stigmas of *Crocus sativus* L., is an expensive spice that is used mainly as a medicine or food coloring and flavoring agent in different parts of the world. The major biologically active ingredients of saffron are crocin analogues, including crocins 1 – 4, which are all glycosides of trans-crocetin, a carotenoid derivative. cis-Crocetin and its glycosides are also present, although they make up the minor components in saffron [1, 2]. In addition, saffron also contains the flavonoid derivatives, picrocrocin and its aglycone safranal, in lower quantities. In this study, extraction of saffron was carried out using 50% methanol and was evaluated qualitatively using HPLC-DAD profiling. According to the Rt and UV spectra, crocin analogues (cis and trans), kaempherol glycosides, picrocrocin and safranal were detected. The analytical method that was used for the profiling was transferred to preparative MPLC and HPLC scale and was used for the fractionation and purification of the aforementioned constituents, respectively. The complete identification of the purified compounds was performed using NMR (1 & 2D) and HRMS/MS spectrometric methods. Fingerprinting of crocus extracts was performed by LC-electrospray (ESI) mass spectrometry (MS) on Orbitrap and QqRTOF systems. Moreover, selected saffron compounds (e.g., transcrocin, cis-crocin and picrocrocin) were further characterized by various tandem mass spectrometric techniques such as vacuum MALDI-TOF/RTOF-MS in combination with collision-induced dissociation intermediate pressure MALDI-QqRTOF-MS/MS, ESI-QqRTOF-MS/MS, ESI-LTQ-orbitrap-MS/MS and ESI-IT-MS/MS. Furthermore, high-energy CID on the stable [M+Na]+ adduct ions yielded the highest content of structural information. **References:** 1. Choi HJ et al. (2001) Dyes Pigment 49: 15 – 20. 2. Tarantilis AP et al. (1995) J Chromatogr A 699: 107 – 118.

PG77 Anti-Influenza Viral Flavonoids from *Persicaria filiforme*

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Persicaria filiforme Nakai, which belongs to the family Polygonaceae, is a perennial herb growing in mountains area of Korea [1]. It has been used as a traditional Chinese medicine for the treatment of various bleeding, diarrhea, dysentery, and stomachache [2]. In the course of a search for anti-influenza viral compounds from natural products, we have found that the methanol extract of *P. filiforme* has anti-viral activity against influenza A virus. To date, there are no reports on the phytochemical constituents and biological activities of *P. filiforme*. A new flavonol glycoside and eight known flavonoids were isolated from the EtOAc and *n*-BuOH fractions of *P. filiforme*. The known compounds, quercitrin (**2**), juglanin (**3**), avicularin (**4**), afzelin (**5**), quercetin (**6**), kaempferol (**7**), quercitrin 2''-O-gallate (**8**) and quercitrin 3''-O-gallate (**9**) were identified by comparing their spectral data with literature values. The structure of a new flavonol glycoside (**1**), named persicarioside A, was determined as quercetin 3-O-β-D-apiofuranosyl-(1→2)-α-L-rhamnopyranoside from spectral data and chemical evidence. The anti-influenza viral activity of the isolates **1-9** were evaluated using cytopathic effect (CPE) inhibition assay against influenza A/NWS/33 (H1N1) virus [3, 4]. Compounds **1**, **2**, and **7** showed antiviral activity. The anti-viral compounds were subjected to inhibitory activity against neuraminidase in a virus. The effect of compounds was evaluated using neuraminidase inhibition assay in influenza A/NWS/33 virus [5]. The activity of neuraminidase decreased significantly by tested compounds, however half reduction of enzymatic activity was shown at relatively high concentration. **References:** 1. Lee TB (1999) Illustrated flora of Korea. Hyangmoonsa. Seoul. 2. Zhonghuabencao Compilation Committee (1999) Zhonghuabencao (2). Shanghai Science and Technological Publisher. Shanghai. 3. Player MR et al. (1998) Proc Natl Acad Sci USA 95: 8874 – 8879. 4. Jeong HJ et al. (2009) Bioorganic & Medicinal Chemistry 17: 6816 – 6823. 5. Song J-M et al. (2005) Antiviral Research 68: 66 – 74.

PG78 Isolation, Purification and Structure Elucidation of Diterpenoids from the Roots of *Salvia chorassanica* Bunge

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Salvia contains around 900 species worldwide, mainly in central Asia. The genus has 58 species and one hybrid in Iran, in which 17 of them are

endemic to the country [1]. *Salvia chorassanica* Bunge is one of the Iranian endemic species of *Salvia* that only grows in Iran that belonges to the Lamiaceae family. There is not any reported literature on *S. chorassanica* so the present project set to start search for finding various diterpenoids from this plant. Air- dried and powdered roots of *S. chorassanica* were extracted with EtoAc (3 x 3L), for about 24 h at ambient temperature. After filtration, the combined extracts were consentrated yielding 20 g of total extract. two diterpenoids, Taxodione (1) [2] and Ferruginol (2) [3], were isolated by means of chromatographic methods mainly column chromatography checked by TLC, and purified by preparative RP-HPLC. The structures of compounds 1 – 2 were determined on the basis of spectroscopic data [4,5] using 1 H NMR, 13C NMR, DEPT-135, HMBC, HSQC, COSY and NOESY experiments. In conclusion, based on the result obtained from our study *S. chorassanica* can be considered a rich source of different abeitane diterpenoids. This is the first report of compounds isolated from this plant. **References:** 1. Emami A et al. (2008) de l'Universite d'Iran des Sciences Medicales pp. 362 – 391. 2. Kupchan SM et al. (1968) Journal of the American Chemical Society 90: 5923 – 5924. 3. Son Kh et al. (2005) Bioorganic & Medicinal Chemistry Letters 15: 2019 – 21. 4. Marcos IS et al. (2010) Tetrahedron 66: 7773 – 7780. 5. Tezuka Y et al. (1998) Pharmaceutical Society of Japan 46: 107 – 112.

PG79 A new cycloartane-type glycoside from *Astragalus schottianus* Boiss

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Astragalus L., the largest genus in the family Leguminosae, is represented by 445 species, of which 224 are endemic. They can be attributed to 62 sections in the flora of Turkey [1,2,3]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug [4]. In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [4]. In our continuing search on Turkish *Astragalus* species, we have isolated a new cycloartane-type triterpene glycoside from methanolic extract of *A. schottianus* by combined chromatography on reverse phase C-18 and silica gel. The structure of the new compound was determined as 3-O-β-D-xylopyranosyl-3β,6α,16β,20(S),24(S),25-hexahydroxycycloartane by the extensive use of 1D and 2D-NMR techniques and mass spectrometry. This compound represents the first entry of the series of cycloartane-type compound possessing a 20-OH functional group in *Astragalus* genus.

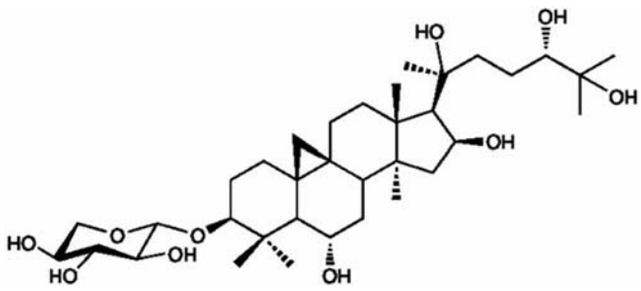

Figure 1

References: 1. Davis PH (1978) Flora of Turkey and East Aegean Islands Vol.4, University Press: Edinburg. 2. Güner A, Ozhatay N, Ekim T, Başer KHC (2000), Flora of Turkey and the East Aegean Islands Vol.11, University Press: Edinburg. 3. Özüdoğru B et al. (2011) Ann Bot Fennici 48: 1797 – 2442. 4. Tang W, Eisenbrand G (1992) Chinese Drugs of plant Origin, Springer-Verlag: Berlin.

PG80 Semi-synthesis of Cytotoxic Molecules From Cycloartane Type Sapogenols

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Semi-synthetic anticancer drug-discovery programs focusing on saponins mainly engaged with commercially available triterpenoids such as oleanolic acid and ursolic acid (1,2,3), not including less common miscellaneous aglycons such as cycloartanes, lanostanes and hopanes. Cycloartanes occupy a special position among low molecular bioregulators because they are produced by photosynthesizing organisms only, and one from the initial representatives of this range, cycloartenol, serves as the key link in the biosynthesis of different phytosterols (4). In general, the plants of *Astragalus* genera proved to be the richest source of this class of compounds. As part of our continuing studies on cycloartane-type sapogenols of Astragalus genus, twenty molecules were synthesized starting from cycloastragenol and its isomer astragenol, and their cyctotoxicities were tested against three different cancer cell lines (HT-29: human column cancer cell line; MDA-MB-231: human breast cancer cell line; PC-3: human prostate cancer cell line) together with a transformed cell line (HEK 293: human embryonic transformed kidney cell line). Some of the semi-synthetic derivatives such as A2 and C5 exhibited more potency compared to the starting molecules. Further studies are in progress to prepare more potent compounds versus cancer lines.

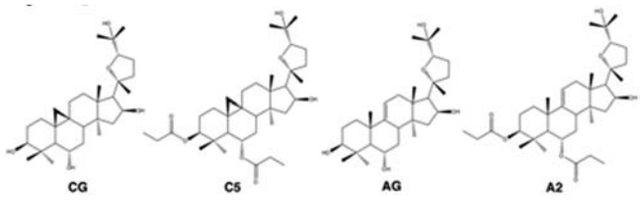

Figure 1

	IC50 (μM)			
	HT-29	MDA-MB-231	PC-3	HEK 293
AG	41.59	88.97	93.99	60.13
A2	19.62	19.26	13.71	12.14
CG	30.49	> 100	> 100	> 100
C5	11.61	63.03	30.25	9.15

Acknowledgement: *TUBITAK (109S345)* **References:** 1. Honda T et al. (2000) J Med Chem 43: 1866 – 1877. 2. Honda T et al. (1999) Bioorg Med Chem Lett 9: 3429 – 3434. 3. Gao X et al. (2007) J Neurooncol 84: 147 – 157. 4. Davis PH (1970) Flora of Turkey and East Aegean Islands Vol 4. University Press. Edinburgh.

PG81 Flavonol glycosides and a saponin from *Chenopodium foliosum* Asch

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Three new flavonol glycosides and a new saponin, namely 6-methoxy-kaempferol-3-O-β-gentiobioside, gomphrenol-3-O-β-gentiobioside, gomphrenol-3-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside and 3-O-β-D-glucopyranosyl-30-normedicagenic acid-28-β-D-glucopyranosyl ester as well as the known flavonol glycosides patuletin-3-O-β-gentiobioside and spinacetin-3-O-β-gentiobioside were isolated from the aerial parts of *Chenopodium foliosum* Asch. The structures of the compounds were established by means of spectroscopic methods (1D and 2D NMR, UV, IR, and HRMS). DPPH free radical scavenging activity and cytotoxicity (MTT-test) of the new compounds were assessed as well. **Acknowledgement:** *This study was supported by Medical Science Council at the Medical University of Sofia (Project 36/2011)*

PG82 Terpenoids from the Root of *Salvia hypoleuca* Benth

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The genus *Salvia* comprises nearly 900 species and is one of the largest members of the lamiaceae family. Fifty eight species of this genus are found in Iran, 17 species of them are endemic. In this study, the roots of *Salvia hypoleuca* Benth., were collected at flowering stage from Tehran province (Iran) and dried at room temperature, in shade. Dried plant materials were cut into small pieces and extracted with ethyl acetate by percolation method. Three sterols, sitosteryl oleate, sitosterol and stigmasterol, two diterpenoids, manool and 7α-acetoxy royleanone and five triterpenoids, ursolic acid, oleanolic acid, 3-epicorosolic acid, 3-epi-maslinic acid and coleonolic acid, were isolated and purified by column chromatography (silicagels normal and reverse phases, Sephadex LH20). The structures of these compounds were identified by spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, HMBC and H-H COSY. These compounds have been reported for the first time from *Salvia hypoleuca* of which coleonolic acid has not been previously reported from the genus *Salvia*. *Keywords: Lagochilus cabulicus, flavonoid, chromatography, spectroscopy* **Acknowledgement:** *This research was supported by the Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences.*

PG83 Phytochemical study of *Lagochilus cabulicus* Benth

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The genus, *Lagochilus*, belongs to Lamiaceae family and consists of 44 species all over the world, 33 of which grow in central Asia. Five species of this genus have been found in Flora Iranica and 4 species exclusively grow in Iran. Chemical studies on some *Lagochilus* species have studied [3 – 12]. One of these species, *Lagochilus cabulicus* Benth., was collected during flowering stage, dried at ambient temperature and shade condition and cut into small pieces. Plant material was successively extracted with ethyl acetate and methanol solvents using percolation method. Main compounds were separated and isolated by column and thin layer chromatography. The isolated compounds were identified by spectroscopic methods, including ^{1}H-NMR and ^{13}C-NMR. In conclusion, four flavonoids, Tricetin 3'-methyl ether (1), Quercetin (2), Quercetin 3-O-β-D-glucopyranoside (3), Quercetin 3-O-α-L-rhamnopyranosyl (1®6) β-D-glucopyranoside (4), two steroids, β-Sitosteryl acetate (5), Stigmasteryl acetate (6) and one triterpenoid, Lupeol (7), have been identified, which not previously reported from this plant species.

PG84 Unusual flavones in *Cytisus* Desf.

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Cytisus Desf. (Fabaceae) is a diversified genus enclosing approximately sixty species, which are particularly found around the Mediterranean Sea. Many plants of this genus exhibit bioactive properties such as diuretic, hypnotic, anxiolytic, antiparasitic, antidiabetic and antioxidant [1] and, in particular the latter, has been closely associated to the high content in flavonoids [2]. The present work aims to contribute to the knowledge of *Cytisus* chemical composition, through the identification of new flavonoids in that genus. The compounds in focus were detected in ethanolic extracts of *Cytisus multiflorus* (Aiton) Sweet flowers by means of HPLC-DAD, ESI-MS and MS^{n} analyses. These included the two isomers 2''-*O*-pentosyl-6-*C*-hexosyl-luteolin and 2''-*O*-pentosyl-8-*C*-hexosyl-luteolin (MW 580 Da), the two isomers 2''-*O*-pentosyl-6-*C*-hexosyl-apigenin and 2''-*O*-pentosyl-8-*C*-hexosyl-apigenin (MW 564 Da), the 6''-*O*-(3-hydroxy-3-methylglutaroyl)-2''-*O*-pentosyl-*C*-hexosyl-luteolin (MW 724 Da) and the 6''-*O*-(3-hydroxy-3-methylglutaroyl)-2''-*O*-pentosyl-*C*-hexosyl-apigenin (MW 708 Da). Attending that half of these compounds were herein described for first time in Fabaceae, overall, the present work is a valuable contribution for the phenolic elucidation of *Cytisus* genus as well as of Fabaceae family.

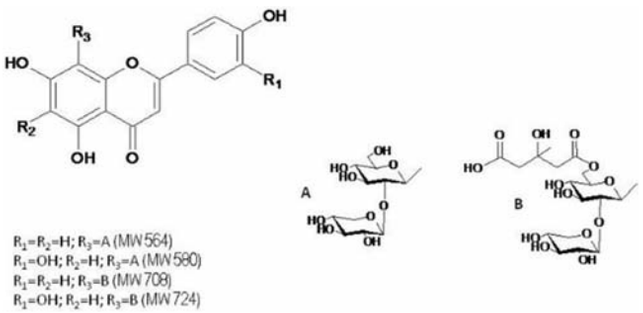

Figure 1: Structures for flavones identified in Cytisus multiflorus

Acknowledgement: *Pereira OR thanks for the PROTEC grant SFRH/PROTEC/49600/2009* **References:** 1. Gião MS et al. (2007) J Sci Food Agr 87: 2638 – 2647 2. Luis A et al. (2009) J Med Plants Res 3: 886 – 893

PG85 Polymeric biophenols in olive mill wastewaters

Cardoso SM[1], Falcão SI[2], Peres AM[3], Pereira OR[4], Domingues MR[5]
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Olive mill wastewater (OMW), the effluents generated in the olive (*Olea europaea* L.) oil extraction industry operating in three-phases mode, are phytotoxic mainly due to its high phenolic content [1]. On the other hand, attending to the potential health-benefits of some of their phenolic compounds, OMW are now regarded as a potent source of biophenols for food and pharmaceutical industries. An important portion of the OMW biophenols include the secoiridoids found in olive pulp and their derivatives formed along the olive oil extraction process [2]. Still, due to the complex composition of OMW, many phenolic compounds remain unknown. Their structural identification can encourage the search of new bioactive compounds in OMW and contribute to further valorize this waste. In the present work, electrospray ionization-mass spectrometry analysis in the negative mode with direct infusion of OMW aqueous acetone purified extracts allowed to identify new major polymeric compounds, detected as [M-H]- ions at *m/z* 909, 1071, 1457, 1075 and 1613. These compounds could be classified into two groups: I- derivatives of a ligstroside glucoside isomer and II- oleuropein oligomeric compounds. Attending that the scavenging ability of a polyphenolic compound is increased by its degree of polymerization [3], bioactivities related to that capacity are expected at least for some of these compounds.

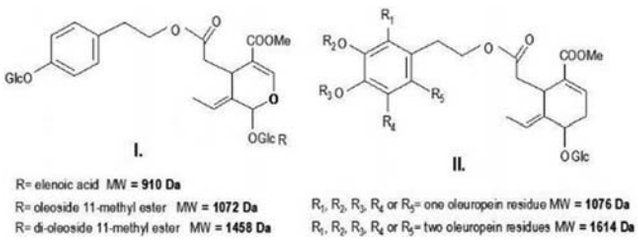

Figure 1: New secoiridoids in olive mill wastewaters

Acknowledgement: *Work supported by Fundação para Ciência e a Tecnologia, project Biotechnological valorization of olive mill wastewaters (OMWalor) – PTDC/AMB/69379/2006* **References:** 1. Casa R et al. (2003) Chemosphere 50: 959 – 966. 2. Obied HK et al. (2007) Anal Chim Acta 603: 176 – 189. 3. Roesler R et al. (2007) Food Chem 104: 1048 – 1054.

PG86 Lavandulifolioside B and Allomonomelittoside; two new glycosides from *Stachys lavandulifolia* Vahl

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The genus Stachys (lamiaceae), consists about 200 – 300 species widespread throughout the world (1). In Iran, 34 species of this genus are present, including *Stachys lavandulifolia* Vahl (2). This species widely distributed in different regions of Iran and is known as the names of "Tuklidjeh" and "Chaaye Koohi". In Iranian folk medicine, decoction of aerial parts of *S. lavandulifolia* is used in painful and inflammatory gastrointestinal disorders (3). anxiolytic and sedative effects of this species also are known in traditional medicine when use as tea (4). in previous phytochemical studies two phenylethanoid glycosides; Acteoside and Lavandulifolioside have been reported from this plant (5). In continuation of our phytochemical studies on medicinal plants from Iran we now report three known phenylethanoid glycosides; Acteoside, Lavandulifolioside, leucosceptoside A and two new compounds; 4, 3',4' trimethoxy lavandulifolioside and one iridoid glycoside, 5-O-β allopyranosyl monomelittoside that were named lavandulifolioside B and allomonomelittoside respectively. These five glycosides were isolated from methanolic extract's Sep-pak fractions by using semi-preparative reversed-phase HPLC and its structures were elucidated by 1D-NMR and 2D-NMR spectroscopic techniques, and also by comparison of experimental data with literature data. Previous reports have indicated that phenylethanoid and iridoids possess anti-inflamatory, antioxidant and analgesic properties, thus it is reasonable to assume that these compounds can be responsible for traditional medicine uses and pharmacological effect of *S. lavandulifolia*. **References:** 1- Rechinger KH, Hedge IC, (1982) Flora Iranica, Vol. 150. Akademiche Druck Verlagsanstalt, Graz, Austria, pp. 360 – 361. 2- Mozaffarian V, (1996) A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran, p. 522. 3- Zargari A (1990) Medicinal Plants. Vol 4. Tehran University Publications, Tehran, Iran, p. 238. 4- Amin G (1991) Popular Medicinal Plants of Iran. Iranian Research Institue of Medicinal Plants, Tehran, Iran, p. 80. 5- Basaran A et al. (1988). Helv Chim Acta 71: 1483 – 1490.

PG87 The Constituents from the Stem of *Clausena lansium* and their Bioactivities

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Two new glycosides, clausenosides A (**1**) and B (**2**) and three new carbazole alkaloids, clausenaline A (**3**), claulamine A (**4**) and claulamine B (**5**), together with fifty known compounds were isolated from the stem of *Clausena lansium* Skeels. Their structures were determined by spectroscopic methods, including CD spectroscopy and 1D and 2D NMR spectra. Compound **4** has an 1-oxygenated carbazole framework with a rare 2,3-lactone ring. Compound **5** represents the first acetal carbazole alkaloid, of which the absolute configuration was determined by Mosher's method. The cytotoxicity of compounds **3-6** against a limited panel of cancer cell lines and the anti-inflammatory activity of **8-27** were evaluated.

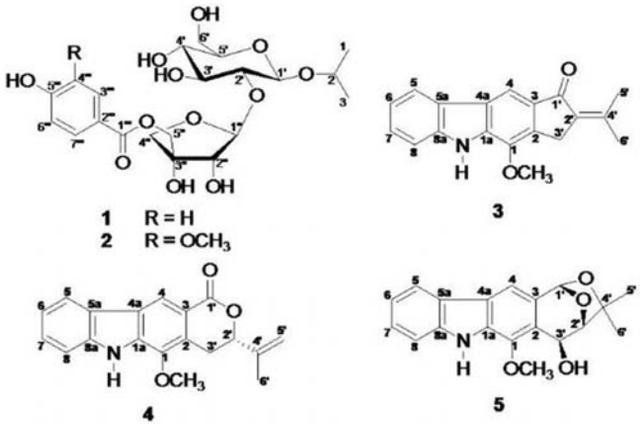

Figure 1: Structures of compounds 1 – 5 from the stem of *Clausena lansium*

Acknowledgement: *The authors are grateful for financial support from the National Science council of Republic of China awarded to T.-S. W. Thanks are also given to Associate Prof. Vu Xuan Phuong (Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology). This study was supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH 100-TD-B-111 – 004).*

PG88 Bioactivity guided isolation of iridoid and flavonoid glycosides from four *Veronica* species

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The genus *Veronica* L. (Plantaginaceae) has been used traditionally for the treatment of a number of diseases. The use of *Veronica* species for influenza, coughs, inflammation, rheumatic pains and cancer was reported in many countries [1,2]. Earlier investigations performed on *Veronica* species resulted in the isolation of mainly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid and flavonoid glycosides [3,4]. It is represented by 79 species in Turkish flora, 26 of which are endemic [5]. In this study, the antioxidative activity of four different Turkish *Veronica* species were investigated and bioactivity guided isolation was carried out to examine the chemical composition of *V. serpyllifolia* L. further. 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide (SO) radical scavenging assays

were performed for guiding of the bioactivity. *V. chamaedrys* L. was found to be the most bioactive species, followed by *V. serpyllifolia. V. fuhsii* Freyn & Sint. was found to be the least active species. Thin layer chromatografies of their water extracts showed *V. chamaedrys* to contain a large proportion of phenylethanoid glycosides, the remaining species showed the presence of a large proportion of flavonoid glycosides. Chromatography of *V. serpyllifolia* water extract gave five pure compounds. Their structures were determined as iridoid glucosides verproside, catalposide, veronicoside and flavonoid glycosides 4'-*O*-methylapigenin-7-*O*-rhamnopyranosyl-acetylglucopyranoside, 3'-*O*-methylluteolin-7-*O*-rhamnopyranosyl-acetylglucopyranoside using different 1D and 2D NMR techniques. Isolation and structure determination studies on bioactive compounds of genus *Veronica* are still continuing. **Acknowledgement:** *This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Project No: 108T518.* **References:**
1. Baytop T (1984) Therapy with Medicinal Plants in Turkey (Past and Present). Publications of Istanbul University. Istanbul. 2. Fujita T et al. (1995) Econ Bot 49: 406 – 422. 3. Harput US et al. (2003) Z Naturforsch 58c: 481 – 484. 4. Saracoglu I et al. (2004) Phytochemistry 65: 2379 – 2385. 5. Davis P. (1978) Flora of Turkey and the East Aegean Islands. Vol. 6. University Press. Edinburgh.

PG89 High-speed countercurrent chromatography of *Harpagophytum procumbens* constituents and their identification by TLC-MS

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Harpagophytum procumbens DC. (Pedaliaceae), known as Devil's claw, is native to the arid regions of Southern Africa including the Kalahari desert. The dried secondary root tubers have been used to reduce pain and inflammation especially in rheumatism and arthritis [1]. Iridoid glycosides are considered to be the main pharmacologically active constituents with other constituents such as phenylethanoid glycosides and flavonoids contributing to the effect [2]. Rapid isolation and identification of the constituents was necessary in order to acquire sufficient quantities of the reference compounds for use in further biological studies as well as to develop quantitative calibration models. To achieve these goals, a methanol extract of the secondary root tubers was rapidly filtered over silica gel to remove sugars and other polar compounds. The resulting fraction, which consisted mainly of iridoid and phenylpropanoid glycosides, was subjected to high-speed countercurrent chromatography (HSCCC). This allowed a one-step separation of the major constituents. The minor constituents were obtained either by a second HSCCC operation or by a final column chromatographic step. In order to distinguish close-running compounds in the absence of reference standards, TLC-MS [3] was performed on the extract and the isolated constituents. This method could be used, for example, to distinguish the close-eluting pair 8-E-p-coumaroylharpagide and 8-E-p-feruloylharpagide [2]. **Acknowledgement:** *The authors thank the National Research Foundation of South Africa for financing this study.* **References:**
1. Qi J et al. (2006) Phytochemistry 67: 1372 – 1377. 2. Karioti A et al. (2011) J Pharm Biomed Anal 55: 479 – 486. 3. Reich E, Widmer V (2009) Planta Med 75: 711 – 718.

PG90 Flavonoid constituents from *Morettia philaena* and their antimicrobial activity

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A successive petroleum ether, diethyl ether, ethyl acetate, butanol and methanol extracts of *Morettia philaena* (Delile) DC. (Cruciferae) flowering aerial parts were tested for their antimicrobial activity. The ethyl acetate and methanol extracts were found to be most effective against most of the tested organisms. The chemical investigation of these extracts afforded nine flavonoids using chromatographic techniques. These are kaempferol, kaempferol 3-O-β-glucoside, kaempferol 3,7 di-O-β-glucoside, kaempferol 3-O-β-sophroside-7-O-β-glucoside, quercetin, quercetin 3-O-β-glucoside, quercetin 3-O-β-gentobioside, orientin and isoorientin. Their structures were established through chemical and spectral analysis. All flavonoids were evaluated to show a broad antimicrobial spectrum of activity on microorganisms including seven bacterial and two fungal species. Among them, the isolated aglycones had stronger bioactivity than their glycosides. **Acknowledgement:** *This work was financially supported by the Phytochemistry and Plant Systematic Department, National Research Centre, Giza, Egypt.*

PG91 New Hexadecan-2-ol and 3-Hydroxymethyloctadecanoate with Hepatoprotective Activity and Cytotoxicity Activity from *Grindelia camporum* Greene (Asteraceae)

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New hexadecan-2-ol (8) and 3-hydroxymethyloctadecanoate (10), together with 14 known compounds (1 – 7, 9, 11 – 16) have been isolated from *Grindelia camporum* Greene var. *camporum*. The new compounds were characterized through spectroscopic studies including 1D (1 H and 13C NMR) and 2D (COSY, HSQC and HMBC) NMR and mass spectroscopy. The known compounds (Compounds 3 and 4 are hitherto unreported from the family Asteraceae) were identified by comparison of their spectral data with those reported in the literature, chemical evidence or authentic samples [1 – 3]. The total methanolic extract and the total aqueous extract exhibited cytotoxic and hepatoprotective activities respectively. Also antimicrobial, toxicological (LD_{50}, anti-inflammatory, antipyretic and analgesic activities of the different fractions were studied [4 – 6]. **References:**
1. Pande A et al. (1995) Phytochemistry 39(3): 709 – 711. 2. Ageta H et al. (1995) Chem Pharm Bull 43(2): 198 – 203. 3-Chiu P et al. (1985) Phytochemistry 24(2): 263 – 266. 4-Ashour ML et al. (2009) Journal of Pharmacy and Pharmacology 61: 1079 – 1087. 5-Bisignano G et al. (1994) Pharm Biol 32: 400 – 405. 6-Achliya GS et al. (2003) Ind J Pharmacol 35: 308 – 311.

PG92 Antiplasmodial and antitrypanosomal triterpenoids from *Salvia hydrangea* with rare carbon skeletons

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Salvia hydrangea DC. ex Bentham, endemic to Iran, has been widely used in traditional Iranian medicine. Fractionation of the hexane extract of the aerial parts of this plant led to the isolation of hydrangdione-A (1) and hydrangdione-B (2), two new triterpenoids with rare carbon skeletons. Their structures were established on the basis of an extensive spectroscopic analysis, including 1D and 2D NMR, and by comparison of their NMR data with those of the related compounds. The IC_{50} of the compound 1 and 2 were determined against two parasites and rat myoblast (L6) cells. Hydrangdione-A (1) and hydrangdione-B (2) exhibited in vitro antiplasmodial activity against P. flaciparum K1 strains with IC_{50} value 1.43 and 0.18 μM with great selectivity index (SI) 86.2 and 69.6. Also these compounds were tested against T. brucei rhodesiense STIB 900, they exhibited significant inhibition of growth with IC_{50} values of 4.33 and 15.92 μM. Triterpenoids with these carbon skeletons are rare in the nature and have been previousely reperted only from two other species; *Salvia bucharica* Popov [1] and *Perovskia abrotanoides* Kar. [2]. It is interesting to note that all three species belong to the flora of Iran and the genus Perovskia is a closely related to the genus *Salvia*. In a suggested proposal for the biosynthesis of hydrangdione A and B they are presumably constructed by the addition of a monoterpenic unit (myrecene for hydrangedione A and trans-β-ocimene for hydrangdione B) to a diterpenic unit (an icetexone precursor) and this coupling may proceeds via Diels-Alder type reaction.

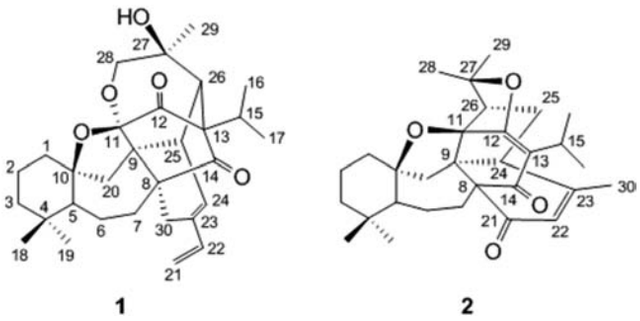

Figure 1: Hydrangdione A (1) and Hydrangdione B (2)

References: [1] Ahmad V U et al. (1999) J Org Chem 64: 8465 – 8467. [2] Parvez A, et al. (1992) J OrgChem 57: 4339 – 4340.

PG93 MAO Inhibitory Activity of Xanthones from Dichloromethane Extract of *Polygala supina*

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Eight xanthones as inhibitors of monoamine oxidase have been isolated from dichloromethane extract of aerial part and roots of *Polygala supina* Schreb. The isolated xanthones were characterized by spectroscopic methods such as 1D, 2D NMR, and MS data analyses as 6,8-Dihydroxy-2,3,4-trimethoxyxanthone (**1**), 2,4,6,8-Tetrahydroxy-3,7-dimethoxyxanthone (**2**), 2,3,7-Trimethoxyxanthone (**3**), 3,7-Dihydroxy-1,2-dimethoxyxanthone (**4**), 1,3,6-Trihydroxy-2,7-dimethoxyxanthone (**5**), 1,3,5-Trihydroxy-2,6,7-trimethoxyxanthone (**6**), 3,6,8-Trihydroxy-1,2-dimethoxyxanthone (**7**), 1,3,7-Trihydroxy-2,6-dimethoxyxanthone (**8**). MAO-A (monoamine oxidase-A) activity of isolated xanthones (**1 – 8**) from dichloromethane extract was assayed according to the method of Holt et al.[1] Compound **5** showed the best activity with IC_{50} value of 0.24 mM, also **1** and **4** showed good activity with IC_{50} values of 2.12 mM and 3.64 mM, respectively. The compounds **2**, **6**, and **7** showed mild activity with IC_{50} values of 12.21, 43.80, and 23.98 mM. However, compounds **3** and **8** were not active. **References:** 1. Holt A et al. (1997) Analytical Biochemistry 244: 384 – 392

PG94 New *N*-alkylamides from *Anacyclus pyrethrum*

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The roots of *Anacyclus pyrethrum* DC (Asteraceae) are frequently used in traditional medicine *e.g.* as aphrodisiac [1]. Depending on the extraction method and solvent, different yields of *N*-alkylamide constituents can be found, possibly resulting in alterations in biological effects and toxicity. Therefore, analytical profiling of the bio-active *N*-alkylamides in these plant preparations is an inevitable quality parameter, with liquid chromatography-electrospray mass spectrometry (HPLC/ESI-MS) as recommended technique for comprehensive analysis of alkylamides in plant extracts [2 – 4]. An *N*-alkylamide profiling from an ethanolic *Anacyclus pyrethrum* root extract was performed using a gradient reversed phase HPLC/ESI-MS method on an embedded polar column. MS^1 and MS^2 fragmentation data were used for identification purposes, while UV was used for quantification. Thirteen *N*-alkylamides (five *N*-isobutylamides, three *N*-methyl isobutylamides, four tyramides and one 2-phenylethylamide) were detected. Five of them are novel compounds, which have never been identified in Nature. **Acknowledgement:** *Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) (no. 091257) and the All India Council for Technical Education, New Delhi, India.* **References:** 1. Sharma V, Thakur M, Chauhan N, Dixit V (2010) Planta Med 76: 1214 – 1214. 2. Sharma V, Boonen J, Chauhan N, Thakur M, De Spiegeleer B, Dixit V (2011) Phytomedicine, In press. 3. Kartal M, Kan, Gulpinar AR (2007) Planta Med 73: 253. 4. Boonen J et al. (2010) J Pharmaceut Biomed 53: 243 – 249.

PG95 Hydrangdione C, a novel triterpenoid with an unprecedented skeleton from *Salvia hydrangea* DC. ex Benth

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The genus Salvia is a rich source of terpenoids with structural diversity. Apart from sesterterpenoids as unusual constituents of *Salvia* species [1,2], it is a source of di- and triterpenoids with unprecedented carbon skeletons [3]. Aiming at identifying structurally interesting and bioactive metabolites from the *Salvia* species, we examined the extract of *Salvia hydrangea* DC. ex Benth. In our search for new bioactive natural products, a novel triterpenoid (hydrangdione C, 1) was isolated from the hexane extract of this plant. The skeletal type displayed by hydrangdione C was noticeable for its unusual carbon ring skeleton with a unique five membered ring D substituted by an acetyl group. This is the first report of a natural triterpenoid with a five-membered ring D. The structure of 1 was established by comprehensive 1D NMR, 2D NMR, and HRMS spectroscopic analysis and subsequently confirmed by a single-crystal X-ray diffraction study.

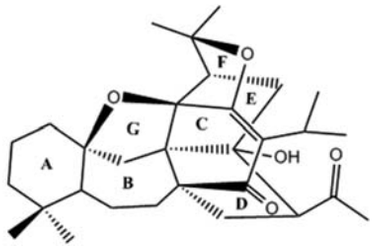

Figure 1: Hydrangdione C

A plausible biosynthetic pathway for 1 was proposed. The formation of 1 could be explained through a pathway including a [3 + 2] cycloaddition type reaction between a monoterpenic unit (trans-β-ocimene) and a diterpenic unit (an icetexone precursor) followed by an oxidation reaction. **References:** (1) Moghaddam FM et al. (2010) J Nat Prod 71: 1601 – 1605. (2) Rustaiyan A, Masoud S, Anaraki MT (2007) Nat Prod Comm 10: 1031 – 1042. (3) Xu G et al. (2007) Org Lett 9: 291 – 293.

PG96 Sesquiterpene lactones and other constituents from *Hedyosmum brasiliense*

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Hedyosmum brasiliense Miq. is an endemic, aromatic arborescent shrub, which is the only representative of Chlorantaceae in Brazil. There have been few studies that seek to determine its chemical composition and/or pharmacological effects. This work describes five new compounds: two guaianolides, two elemanolides and a dimeric lindenanolide, which were tested against *Mycobacterium tuberculosis*, together with some widespread phenolics. All the structures of the isolated compounds were elucidated based on extensive analysis of 1D and 2D NMR and MS data, and also in comparison with the published data. The new compounds found were: 1,2-epoxy-10α-hydroxy-podoandin (3), 1-hydroxy-10,15-methylenepodoandin (4), 15-acetyl-isogermafurenolide (5), 15-hydroxy-isogermafurenolide (6) and brasiliensolide (7) – which is the first dimeric sesquiterpenolide identified in the *Hedyosmum* genus. The phenolic compounds isolated were scopoletin, vanillin, vanillic acid, protocatechuic aldehyde and ethyl caffeate. The isolated sesquiterpene lactones, at concentrations in the range of 1 – 30 μM, did not show in vitro anti-mycobacterial activity, since *M. tuberculosis* cultures exposed

to the *Hedyosmum brasiliense*-derived substances kept growing but were sensitive to isoniazid, an antibacterial agent.

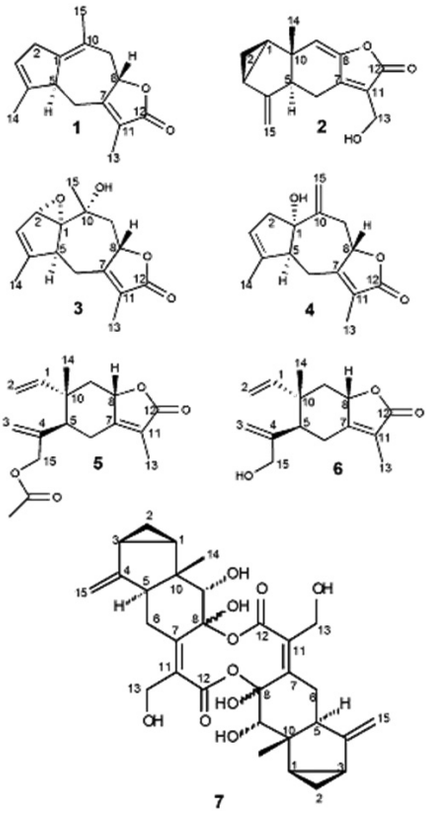

Figure 1: Isolated compounds of Hedyosmum brasiliense

Figure 2: Key NOE correlations observed for compounds 4 and 5

Acknowledgement: *The authors are grateful to CNPq and CAPES for financial support, and Norberto P. Lopes and José C. Tomaz (FCFRP-USP) and Luciano F. Huergo (Departamento de Bioquimica e Biologia Molecular-UFPR) for the MS analysis and André Báfica (Laboratório de Imunofarmacologia e Doenças Infecciosas-UFSC) for the antimicrobial assay.*

PG97 Chemical diversity investigation of *Stemona* species using LC-MS technologies

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Stemona species (Stemonaceae) are plant resources of traditional Chinese medicine 'baibu', which had long been used as antitussive and insecticidal agents. *Stemona* alkaloids, featuring a pyrrolo[1,2-α]azepine or pyrido[1,2-α]azepine nucleus, are believed to be responsible for their medicinal usages [1,2]. Our previous work has let to the isolation of 90-plus alkaloids from different *Stemona* species. In our effort to identify alkaloidal constitutents from the extract of a Vietnamese species, an LC-MS method was established for a rapid and sensitive screening of the specific compounds [3]. On the basis of more than 90 alkaloids, we established a MS database of all these reference compounds by UPLC/ESI-LTQ. By picking the specific peak in the LC chromatogram, extracting its MS, MS^2 and MS^3 spectra, and then comparing with those of the standard samples, we can do a rapid identification of main structures in the extract. Such technology was successfully applied for the chemical diversity investigation of three *Stemona* species – *S. sessilifolia* Franch. & Sav., *S. japonica* Franch. & Sav., and *S. tuberosa* Lour. The results revealed that the alkaloids varied greatly with species and habitats, but not with collecting seasons. *S. tuberosa* is the commonly-used species while having the most complicated metabolites which structural types were influenced extremely by ecological environment. *S. sessilifolia* growing in Tsuchou, Anhui province with the highest amount of the most active stemospironine, was determined to the best species for medicinal usage. All these data, combined with the pharmaceutical experiments, supplied the scientific evidence for guiding the usage of TCM baibu. **Keywords:** *Stemona*, alkaloid, chemical diversity, LC-MS, UPLC/ESI-LTQ **Acknowledgement:** *Department of Analytical Chemistry, Shanghai Institute of Materia Medica, Prof. Yang YM.* **References:** 1. Lin L-G et al. (2006) J Nat Prod 69: 1051 – 1054. 2.Tang C-P et al. (2008) J Nat Prod 71: 112 – 116. 3. Peng S-Y et al. (2009) Rapid Commun Mass Spectrom 23: 3621 – 3631.

PG98 Saskatoon (Amelanchier alnifolia Nutt.) as a source of bioactive phytochemicals

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Bioactive polyphenols are found in high amounts in berries, fruits and in other plant parts. The polyphenols of saskatoon berries grown in Canada has been studied to some extent, while there are no studies concerning Finnish varieties. Although the potential importance for nutraceutical and other food/non-food applications, limited information is available on the phytochemicals in different plant parts of useful crops. Polyphenols in leaves, stems and berries of four saskatoon cultivars grown at agricultural farms in Finland were identified and quantified with HPLC and LC/MSD systems. Proanthocyanidins (condensed tannins) were determined with butanol-HCl test. HPLC-analysis revealed over 30 individual phytochemicals in a saskatoon plant. The polyphenolic composition varied among plant parts and the concentrations of compounds varied among cultivars. The most significant cultivar differences were detected in the accumulation of polyphenols in berries. The main berry components were cyanidin-based anthocyanins, quercetin-derived flavonol glycosides and chlorogenic acid derivatives. The leaves consisted of quercetin and kaempferol derived flavonol glycosides, catechins, neolignans and hydroxycinnamic acids. In stems, the main components were flavanones/flavonols, catechins and benzoic acid derivatives. Saskatoon cultivars were also rich in proanthocyanidins: 3 % of dry berry biomass and 10-14 % of dry biomass of stems and leaves. Cultivars were observed similar in their proanthocyanidin contents. Due to the high concentrations of phytochemicals, cultivation of saskatoon plants may have a great potential in the production of functional raw material to a wide range of food products. **Keywords:** Flavonoids, phenolic acids, proanthocyanidins, saskatoon, cultivars **References:** [1] Bakowska-Barczak, A. M.; Kolodziejczyk, P. (2008). J. Agric. Food Chem., 56: 9933-9940. [2] Hu, C.; Kwok, B. H. L.; Kitts, D. D. (2005). Food Res. Int., 38: 1079-1085. [3] Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. (2004). Am. J. Clin. Nutr., 79: 727-747. [4] Ozga, J. A.; Saeed, A.; Reinecke, D. M. (2006). Can. J. Plant Sci., 86: 193-197. [5] Scalbert, A.; Johnson, I. T.; Saltmarsh, M. (2005). Am. J. Clin. Nutr., 81: 215S-217S.

Topic H: Marine natural product research

PH1 Production of some biologically active secondary metabolites from marine-derived fungus *Penicillium brevicompactum*

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The fungal isolate *Penicillium brevicompactum* which isolated from the associated marine alga *Pterocladia* sp. in autumn season was able to produce 11 clear and active compounds, separated by the best solvent system dichloromethane: methanol (95:5 v/v). Compounds 4 and 9 were considered as antibacterial compounds, active against Gram positive (*B. subtillis*) and Gram negative (*E. coli*) bacteria. Malt extract broth medium with initial pH 4 when incubated at 28 °C in an incubator shaker at 200 rpm for 12 days were the most favorable conditions for compound 4 production (19.87 mg/l). The suitable conditions for compound 9 production (121.13 mg/L) were potato carrot broth medium, initial pH 4, incubation temperature 26 °C at 180 rpm after incubation period for10 days. Structural elucidation of the pure compounds suggested that compound 4 may be [Di(2-ethyl hexayl) phthalate], and compound 9 may be fungisterol or one of its isomers. Pure compounds were evaluated for cytotoxicity towards 6 different types of tumor cell lines performed in Cancer Biology Department, National Cancer Institute, Cairo, Egypt. The results revealed that, the maximum concentration of compound 4(100 μg/mL) kills about 30% of lung cells. The maximum concentration of compound 9 (100 μg/mL) kills approximately 40% of the viable infected liver cells and also kills approximately 50% of the viable infected lung cells at concentration equal to 91.6 μg/mL. It can be concluded that compound 9 can be recommended as an anticancer compound.

PH2 Phycoconstituents of Some *Sargassum* Species and Their Evaluation on Insecticidal and Nucleopolyhedroviral (NPV) Replication *in vitro* and *in vivo*

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Multiple resistance and environmental pollution to chemical pesticides with increasing world population led to the development and the improvement of new trends to pesticides from natural product. The application of natural product as well as the biological control specific agents especially nucleopolyhedro virus (NPV) are considered very important tool to avoid the contradiction between pest control and clean environment. Furthermore, the biological control specific agents have many advantages such as their low mammalian toxicity and no adverse effect on plant growth and seed viability. The brown algae *Sargassum* species (Family: Sargassaceae) were widely distributed in worldwide. They have been used as food, as well as in industry and medicine for various purpose. Pharmacopeial constants, phytochemical screening, determination of minerals and trace elements of *Sargassum asperifolium*, *Sargassum dentifolium* and *Sargassum linifolium* from the Red Sea, Hurghada, Egypt, were investigated. These studies revealed that *Sargassum* species have high ash content and gave positive for sublimenable matter, volatile constituents, carbohydrate content, sterols and/or triterpenes. In addition, they have high Ca, K, Mg & Fe contents. The protein content as well as amino acid composition of three algae were performed using Kjeldal method and amino acid analyzer, respectively. The alcoholic extracts (70%) of *Sargassum* under investigation were subjected to screen their insecticidal activities in *vitro* and in *vivo* on *Spodoptera littoralis* and *Spodoptera fungipende* and their effects on the replication of *Spodoptera littoralis* nucleopolyhedro virus and *Spodoptera fungipende* nucleopolyhedro virus. The evaluation proved that the tested algae have various insecticidal and antiviral activities.

PH3 Effect of dieckol from *Ecklonia cava* on glucose transport in L6 muscle cells

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Ecklonia cava, a brown sea algae that is grown in the coast of Jeju island in Korea, has a variety of biological activities. We investigated the effect of dieckol (DEK) isolated from *Ecklonia cava* on glucose transport and its related signaling pathways in L6 muscle cells. DEK increased the basal glucose transport independent of insulin. Glut4 was also translocated from cytoplasm to plasma membrane in response to DEK. PKB/Akt as well as ERK was phosphorylated by DEK in a dose-dependent manner. DEK also stimulated phosphorylation of AMPK, an insulin-independent stimulator of glucose uptake. DEK-stimulated increase of Glut4 translocation and of glucose transport were sensitive to both inhibition of PI3 kinase (by wortmannin) as well as AMPK (by compound-c). These results suggest that DEK from *Ecklonia cava* can be applied to ameliorate abnormalities in glucose metabolism like as insulin resistance or diabetes mellitus.

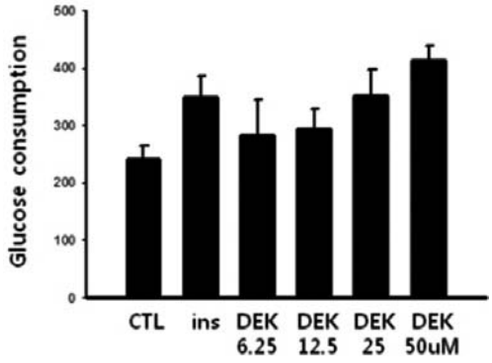

Figure 1: DEK stimulate glucose consumption independent of insulin in L6 muscle cells

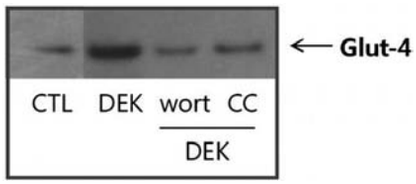

Figure 2: DEK-induced Glut4 translocation to plasma membrane is sensitive to inhibition of PI3-kinase as well as AMPK in L6 muscle cells

Acknowledgement: *This work was supported by a grant from National Research Foundation of Korea (2010 – 0022036).*

PH4 Composition of fatty oil of sea urchin eggs from Barents Sea

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The regular sea urchin *Strongylocentrotus pallidus* is a widespread species in high-Arctic waters. However, little is known about lipid composition of sea urchin eggs from northern Barents Sea. Sea urchin was harvested by divers in Barents Sea close to Murmansk (Russia) in November 2010. The eggs have been (or were) found to contain 20% (dry matter) total lipid fraction. Lipids were extracted from eggs with chloroform/methanol (2/1) by sonification in 30 min. Bound fatty acids were esterified by sodium methoxide, and the methyl esters were analysed by GC using a DANI 3865 GC equipped with SP 2340, 60 m; 0.25 mm i.d. (Supelco, Switzerland) column. The oven temperature was programmed from 70 °C to 130 °C at a rate of 10 °C/min, to 235 °C at a rate of 2.5 °C/min. Programmed temperature vaporizer (PTV) increased from 70 °C to 235 °C, and FID temperature was set at 235 °C. Peak identification was

based on retention order of known fatty acid compositions of marine lipids, pure substances and literature. The study shows that sea urchin eggs are a rich source of omega-3 PUFA, especially EPA (20:5n-3).

PH5 Iriomoteolide-12a, a 12-membered macrolide from dinoflagellate *Amphidinium* species

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Marine dinoflagellates of the genus *Amphidinium* are well-known as a producer of unique cytotoxic metabolites. We have isolated a new 12-membered macrolide, iriomoteolide-12a, from the benthic dinoflagellate *Amphidinium* species collected off Iriomote Island, Japan. In this symposium, we will discuss the isolation and structural elucidation of this new macrolide. The dinoflagellate *Amphidinium* sp. (strain KCA09053) was cultivated in 50 L of 1% Provasoli's enriched seawater (PES) medium, 16 h light and 8 h dark. The algal cells obtained from 50 L of the medium were extracted with MeOH/toluene (3:1). The toluene-soluble materials of the extract were subjected to a SiO_2 column, C 18 column and one of a macrolide containing fractions were separated by C 18 HPLC to afford a new compound, iriomoteolide-12a. Iriomoteolide-12a was obtained as colorless amorphous solid, and the molecular formula of $C_{25}H_{40}O_5$ was revealed by HRESIMS data. ^{13}C NMR data disclosed the presence of a total of 25 carbon atoms due to five quaternary carbons, one of which is three carbonyl carbons, eight methine carbons, five methylene carbons, and seven methyl carbons. The planar structure of iriomoteolide-12a was elucidated on the basis of detailed analyses of 2D NMR data and MS spectra. Iriomoteolide-12a is a new 12-membered macrolide with six one-carbon branches, two ketone carbonyls, and a hydroxyl group. The skeleton is related to that of amphidinolide Q [1,2]. **References:** 1. Kobayashi J, Takahashi M, Ishibashi M (1996) Tetrahedron Lett 37: 1449 – 1450. 2.Takahashi Y, Kubota T, Fukushi E, Kawabata J, Kobayashi J (2008) Org Lett 10: 3709 – 37111.

PH6 Constituents of the Red Alga *Laurencia obtusa*

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The genus *Laurencia* Lamouroux (Rhodomelaceae) includes about 140 species distributed throughout the world except in the Arctic and Antartic zones [1]. The red algae of the genus *Laurencia* (Rhodomelaceae) are known as a rich source of the halogenated sesquiterpenes, diterpenes and acetylenes [2,3]. Although a number studies have been done on *L. obtusa*, investigations are still going on this species due to high biodiversity of its constituents. In our continuing research on secondary metabolites of the alga *L. obtusa* Lamoroux, which has different colors in different regions of Turkey and at different times, we have obtained several halogenated sesquiterpenoids [4,5]. In this study, a sample of *L. obtusa*, collected from North eastern part of Aegean Sea (Bademli- Ayvalık) in Turkey and an extract was obtained by exhausting in chloroform-methanol (1:1) solvent mixture as 14 g. Fractionation of the extract on a Si-gel column carried out by the elution starting petroleum ether, and gradients used were first dichloromethane, and then acetone, finally methanol with increasing amounts. The fraction, obtained by elution with the petroleum ether-dichloromethane mixture (6:4) afforded two halogenated compounds, their 1 H-NMR spectra indicated that they have probably sesquiterpene skeleton. Another compound was obtained during elution with the dichloromethane-acetone solvent mixture (8:2). Structure elucidation studies are still going on using by intensive NMR and mass spectral analyses. There are a number of compounds which have not purified yet. After purification and structure elucidation studies, the pure compounds will be investigated for their potential bioactivity including cytotoxic and anti-cholinesterase activity tests. **References:** [1] Rodriguez MCG et al. (1992) Bot Mar 35: 227 – 237 [2] Faulkner D J (1999) Nat Prod Rep16: 155 – 198 [3] Scheuer PJ (1989) Med Res Rev 9: 535 – 545 [4] Topçu et al. (2003)J Nat Prod66: 1505 – 1508 [5] Öztunç A et al. (2001) Tetrahedron 47: 2273 – 2276

PH7 Antimicrobial active compounds of green alga *Ulva rigida* collected from Ghar El Melh lagoon (North of Tunisia)

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The green alga *Ulva rigida* is wellspread within Tunisian coast mainly in the northern region of Ghar El Melh lagoon with important blooms particularly in warm seasons [1]. The aim of this study is to evaluate its antimicrobial potential against pathogens bacteria and fungi. Thus, polar and non polar organic crude extracts of dried *Ulva rigida* collected from Ghar El Melh lagoon (37 ° 10.8' N, 10 ° 16.8' E), were tested against eighteen pathogenic species of bacteria and the yeast *Candida albicans*. The dichloromethane/methanol extract which exhibited the most significant activity was therefore subjected to column gradient chromatography on silica gel and led to one hundred thirty seven fractions. Eluates were subsequently grouped into twenty nine sub-fractions based on their similarity in Thin Layer Chromatography (TLC). According to TLC visualisation by phosphomolybdic acid and Libermann's spraying, and UV examination, we retained sub-fractions considered of further interest, to isolate antibacterial compounds through bioassay-guided fractionations. Purification processes (column chromatography with Silica gel and Sephadex LH-20 and preparative TLC) of the most active fractions led to purified and semi purified compounds with strong antibacterial activity especially against *Staphylococcus aureus* and *Micrococcus sp*. which are recognized amongst most common human pathogens. Nevertheless, active compounds showed weak value of CMI. 1 H NMR and 13C NMR provided structural information about active compounds. **References:** 1- Shili A et al. (2000) Journal of Costal Conservation 8: 127 – 134.

PH8 Antimicrobial potentialities of *Ulva rigida* epiphytic bacteria

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Marine epiphytic bacteria (72 strains) were isolated from green alga *Ulva rigida* collected in two different biotopes (Cap Zebib, rocky shore: 30 strains and Ghar El Melh lagoon: 34 strains) and alga surrounding water (eight strains). All isolates were identified based on their 16S rDNA sequences and tested for antimicrobial effect against several human and fish pathogens (18 Gram+ and Gram- bacteria and the fungus *Candida albicans*) using *in vitro* drop method. Results obtained revealed high activities of *Ulva rigida* epiphytic bacteria; amongst alga isolates 36% were active with variable antimicrobial spectrum. Within active isolates, 69.5% were from the alga collected in the lagoon. High level activities were observed for six isolates identified as, *Bacteroidetes bacterium*, *Pseudoalteromonas sp*., *Octadecabacter sp*., *Stappia marina*, *Stappia sp*. and *Ruegeria sp*.. Nevertheless, all free living bacteria from surrounding water were inactive. Else, we noted variable sensitivity spectrum of indicators used, *Staphylococcus aureus*, *Micrococcus sp*., *Streptococcus sp* and *Salmonella typhimurium* were mostly inhibited by the isolates tested, while, *Vibrio* species (*V. anguillarum*, *V. tapetis* and *V. alginolyticus*) were resistant. *Candida albicans* was inhibited only by the two *Stappia* species isolated from *Ulva rigida* of Cap Zebib locality. Further investigations continue on the inhibition effect of *Ulva rigida* organic crude extracts against epiphytic bacteria isolated, in order to assess degree of affinity of epibionts to their proper host. **Keywords:** *Ulva rigida*, *Epiphytic bacteria*, *Antimicrobial activities* **Acknowledgement:** *The authors thank Ms. Veronique Confurius-Guns, Department of Marine Microbiology, Netherlands Institute of Ecology, NIOO-KNAW, Yerseke, The Netherlands, for her assistance and help with PCR and DNA sequencing.*

PH9 Optimized isolation and pharmacological activities of sulfated polysaccharides from the red seaweed *Delesseria sanguinea*

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The red seaweed *Delesseria sanguinea* dominantly populates a large artificial reef at Nienhagen in the Baltic Sea. It contains substantial amounts of sulfated polysaccharides (D.s.-SP), which consist of a homogenous fraction of branched sulfated xylogalactans (gal:xyl ~5.4) and exhibit a pharmacological profile indicating anti-inflammatory and anti-skin aging potencies [1 – 3]. Compared with heparin, D.s.-SP revealed stronger inhibitory effects on the enzymes elastase, hyaluronidase, heparanase, collagenase as well as on complement activation, cell adhesion to P-selectin and cytokine release from LPS-activated monocytes, but have only moderate anticoagulant activity. Their hyaluronidase and complement inhibitory activities proved even superior than those of the anti-inflammatory β-1,3-glucan sulfate PS 3. Crucial for an economic use is the availability of adequate amounts of D.s.-SP with reproducible high quality. For evaluation and optimization, 30 D.s. batches were harvested and extracted since 2005 resulting in almost 200 D.s.-SP batches. By a standardized procedure (extraction (EX) with water for 8 h at 85 °C), the D.s.-SP can be isolated in reproducible high quality. However, as found by a second 8 h-EX, the first 8 h-EX is incomplete. Subsequently modified EX-procedures led to following yields: 8.8 %(1 x 8 h-EX), 13.3 %(2 x 4 h-EX), 15.0 %(2 x 2 h-EX) and 17.9 %(4 x 2 h-EX). Consequently, a 2 x 2 h-EX (15.0 %) seems to be a rational compromise. Moreover, the D.s.-SP obtained by shorter EX contained less glucose, which partly represents co-extracted starch: 14.4 %(1 x 8 h-EX), 10.92 %(2 x 4 h-EX), 9.0 %(2 x 2 h-EX) and 11.74 %(4 x 2 h-EX). The glucose content was further reduced by precipitating the extracted D.s.-SP with 70% instead of 90% ethanol. In conclusion, after stepwise optimization of the isolation procedure, the D.s.-SP from Nienhagen are ready for an economic use. **Acknowledgement:** *This project is financed by the EU (FIAF/EFF) and the LFALF Mecklenburg-Vorpommern.* **References:** 1. Groth I, Grünewald N, Alban S (2009) Glycobiology 19: 408 – 417. 2. Grünewald N, Alban S (2009) Biomacromolecules 10(11): 2998 – 3008. 3. Grünewald N, Groth I, Alban S (2009) Biomacromolecules 10(5): 1155 – 1162.

PH10 Effects of Nitrogen Fertilizer on Growth, Seed Yield and Oil Seed Content of Naked Seed Pumpkin (*Cucurbita pepo* subsp. *pepo* var. *styriaca*)

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Naked (Styrian, Green Gold, Medicinal) pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) is one of the valuable medicinal plants in pharmaceutical industries of developed countries. Nitrogen influenced on the growth, development and productivity of the plants. In this study, nitrogen applied at four levels (0, 50, 100 and 150 $Kgha^{-1}$) with three replicates in each treatments. Nitrogen fertilization were applied at three different stages of the plant growth: seed sowing time, fourth leaves and flowering stage. In order to study the effects of increasing nitrogen level on leaf total chlorophylls content, leaf dry matter, fruit and seed yield and oil seed content of the medicinal pumpkin determined. The results indicated that nitrogen fertilization increased the amount of total leaves chlorophyll content in comparison to the check. The highest rate of chlorophylls content of the leaves obtained when nitrogen applied at 150. There were significant different between some treatments on fruit and seed yields, seed dry matter and oil seed content of the plants. The highest oil levels obtained at 100 and 150 $Kgha^{-1}$ of nitrogen fertilization.

PH11 Characterization and purification of the crude laccase enzyme produced by the marine-derived fungus *Trematosphaeria mangrovei*

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The extracellular laccase produced by the marine-derived fungus *Trematosphaeria mangrovei* was purified and characterized. The crude enzyme reached its maximal activity at 35 °C, pH 4.5, enzyme concentration of 5.429 mg protein/reaction mixture and substrate concentration of 40mM ABTS. The enzyme was stable for 60 min at 35 °C and retained about 80 to 90% of its activity after treatment for 60 min at 40 °C up to 50 °C. And the enzyme activity showed the highest stability after 60 min exposure at pH 4.5. Also at pH 4 the enzyme retained about 91.60% of its activity after 60 min exposure. Fractional precipitation of the fungal extracellular *T. mangrovei* laccase enzyme with different methods showed that, the enzyme fraction precipitated at 60% acetone was the most favourable enzyme fraction, exhibited 4.84 purification fold. Purification of laccase on sephadex G-100 column showed that, the fraction number 8 of laccase component was the most active has the highest specific activity (1466.49 U/mg protein) and revealed 6.5 purification fold (purification fold 31.47 compared to the culture filtrate). Native polyacrylamide gel electrophoresis (PAGE) of fraction 8 showed a separated single band similar to that of standard enzyme indicating its purity and homogeneity, and different from that of partial purified enzyme.

Topic I: Molecular Biology

PI1 Molecular characterization of daurichromenic acid synthase from *Rhododendron dauricum*

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Rhododendron dauricum L. (Ericaceae) produces daurichromenic acid (DCA), an anti-HIV component [1]. DCA is a terpenophenol, and would be biosynthesized from grifolic acid via oxidative cyclization of the farnesyl group, the reaction analogous to those reported for cannabinoid biosynthesis [2]. We attempted to amplify cDNA fragments encoding DCA synthase by homology-based RT-PCR with degenerate primers designed from conserved sequences in cannabinoid synthases and related plant oxidases. Then, the 3' and 5'-end regions of cDNA were obtained by rapid amplifications of cDNA ends. Consequently, three cDNA clones, that encode polypeptides named RdOx 1~3, were cloned. RdOx 1~3 consisted of 533, 533 and 534 amino acids containing a FAD binding motif. In addition, these polypeptides had > 50% identities with cannabinoid synthases. The heterologous expression system for RdOxs was established using *Pichia pastoris* as a host. The recombinant RdOx1 and 2 could produce DCA from grifolic acid, whereas RdOx-3 showed no DCA-producing activity, suggesting that RdOx 1 and 2 are active DCA synthase in *R. dauricum*. DCA synthase would be applied for biotechnological production of DCA because the substrate grifolic acid has been isolated from a mushroom *Albatrellus dispansus* in a large amount [3].

Figure 1: The reaction catalyzed by daurichromenic acid synthase

References: 1. Kashiwada Y et al. (2001) Tetrahedron 57:1559 – 1563. 2. Taura F et al. (2007) Chem Biodiv 4:1649 – 1663. 3. Hashimoto T et al. (2005) Heterocycles 65: 2431 – 2439.

PI2 Development of new genomic and genic SSR primer pairs for carrot

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Carrot (*Daucus carota* L.) is one of the most economically important and the most popular vegetables cultivated worldwide among the members

of family Apiaceae. Despite its importance for human nutrition, health, and development of new drugs, genomic resources in carrot relatively underdeveloped and the use of molecular markers in carrot has limited to a few results of several researches [1]. Among the molecular markers microsatellite or simple sequence repeat (SSR) has much superiority in genetic studies since they are co-dominant, highly polymorphic, and reliable PCR procedure [2,3,4]. But, the number of microsatellite primer pairs flanking the microsatellites in ESTs and genomic DNA library limited in carrot. In order to utilize microsatellites in carrot genetic studies, new microsatellite primer pairs are required. To date at the NCBI, 3845 nucleotide sequences and 93 expressed sequence tag (EST) sequences are available for all Daucus species (March 2011). We developed 14 microsatellite primer pairs using ESTs and genomic DNA library data bases in the NCBI databases. Microsatellites were determined using Exact-Tandem Repeat Analysis program and primer pairs flanking these microsatellites were designed using Primer3 software [5,6]. Microsatellite primer pairs developed in the present study (Table 1) will enhance genetic studies in carrot. Besides, transferability of these microsatellite primer pairs from carrot to other members of the Apiaceae family is important for future genetic studies in the Apiaceae family. **Acknowledgement:** *This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University.* **References:** 1. Cavagrona PF et al. (2009) Mol Genet Genomics 281: 273 – 288. 2. Karaca M, Ince AG (2008) J Genet 87: 83 – 863. 3. Ince AG et al. (2010) Mol Breeding 25: 645 – 658. 4. Ince AG et al. (2010) Mol Breeding 25: 491 – 499. 5. Ince AG et al. (2008) Plant Cell Tissue Organ Cul. 94: 281 – 290. 6. Ince AG et al. (2010) Plant Mol Biol Rep 28: 285 – 291.

PI3 Transferability of EST-Microsatellite Markers to some Labiatae Genera

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In recent years, molecular markers have been used in identification and differentiation of chemical compositions in some aromatic species [1,2,3]. Among molecular markers, SSRs or microsatellites are the marker of choice, however, the development of microsatellite markers is often a laborious and costly process since the construction of a DNA library and screening of the library with probes corresponding to the repetitive sequences required. Fortunately after the discovery of microsatellites in ESTs, ESTs became an important source for development of microsatellite markers [4]. Many studies indicated that unlike genomic microsatellites, genic ones could amplify genomic regions of related genera [5,6]. Based on these findings this study developed one hundred microsatellite primer pairs using a total of 13641 ESTs from *Origanum*, *Salvia*, *Stenogyne* and *Thymus* species. A total of 20 primer pairs obtained from *Origanum* ESTs were used to investigate transferability of EST based microsatellites to *Salvia*, *Sideritis*, *Melissa*, *Teucrium*, *Rosmarinus*, *Thymus*, *Pholomis* and *Capsicum*. All 20 primer pairs could amplify genomic DNA of *Origanum*. Three of the 20 microsatellite primer pairs were failed to amplify genomic DNAs of the species tested. Analyses indicated that the level of transferability of *Origanum* EST microsatellite markers were considerable higher in species belonging to Labiatae genera while a few markers could be transferable to *Capsicum*. In many cases amplified products were high in molecular mass, however, the use of CAPS-microsatellite technique described in [6] could be used on these microsatellites for detection of polymorphisms. **Acknowledgement:** *This work was supported by the Scientific and Technological Research Council of Turkey.* **References:** 1. Karaca M et al. (2008) J Sci Food.Agric 88: 2508 – 2516. 2. Karaca M, Ince AG (2008) J Genet 87: 83 – 86. 3. Ince AG et al. (2010) Biochem Genet 48: 83 – 95. 4. Ince AG et al. (2010) Plant Mol Biol Rep 28: 285 – 291. 5. Ince AG et al. (2010) Mol Breed 25: 645 – 658. 6. Ince AG et al. (2010) Mol Breed 25: 491 – 499.

PI4 Enhancing effect of Methyl jasmonate on antioxidative capacity of *Bunium persicum* under Cadmium stress

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Methyljasmonate (MeJA) is a compound that used as plant growth regulation and is currently being used in cancer research. *Bunium persicum* B.Fedtsch. is one of important medicinal plants that cultivated widely and has numerous usage in medicine.The present study emphasizes the important of MeJA (0, 0.01 and 0.1 μM) against oxidative stress in this plant that exposed to cadmium (0, 30 and 60 Mm $CdCl_2$) stress. Our results showed that in plants that only treated with Cd growth parameters, flavonoids, ascorbic acid and total phenol content reduced significantly but protein, MDA and H2O2 content and superoxide dismutase (SOD) activity enhaced significantly. On the other hand, in plants that were pretreated with MeJA and then treated with Cd, MDA and H_2O_2 content significantly decreased but growth parameter and free radical scavenging compounds increased. We concluded that MeJA increased antioxidative capacity in this plant against Cd as heavy metal stress.

PI5 The effect of silicon on membrane integrity and antioxidative pigments on *Echium amoenum* that exposed to cadmium stress

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Some researchers reported that silicon (Si) increase tolerance in some higher plants against biotic and abiotic stress. The beneficial effects of Si are mainly associated with its high deposition in plant tissue and enhancing their strength and rigidity. We investigated the role of Si against cadmium stress in (*Echium amoenum* Fisch. & C.A.Mey.) in greenhouse condition. When the seventh leaf appeared, plants were pretreated with five levels of Si: 0,0.2, 0.5, 0.7and 1.5 mM Si (as sodium trisilicate, Na2(sio2)3) and then the plants were treated with two levels of Cd (30 and 90 mM). The effects of Silicon and Cd were investigated on some physiological and biochemical parameters such as: lipid peroxidation (malondialdehyde (MDA) and other aldehydes), anthocyanin and flavonoid content. Our results showed that Cd significantly increased MDA, other aldehydes, antocyanin and flavonoids content in *Echium* and silicon offset the negative effect and increased tolerance of Echium against Cd stress. From this results we concluded that Si increase membrane integrity and antioxidative ability in this plant against cd stress.

PI6 Effect of the extracts of *Piper cumanense* and *Piper eriopodon* in the behavior of genes involved in oxidation process of the skin

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Nowadays the role of the genes is a tool of study to achieve an accurate form to avoid in this particular case the skin degeneration. The propose of this study was the method design to evaluate the behavior of certain genes as MMP1, MMP7, MMP9, MMP11, MMP12, COL3A1, COL1A2 and ELASTIN, (which are involved in degradation process caused by the oxidation), in in vitro assays with Human Dermal Fibroblast through the use of Real Time RTPCR, as well as, the evaluation of butanolic extracts of *Piper cumanense* Kunth and *Piper eriopodon* C.DC. over these cells to determine the effect to above genes; said extracts were rich in flavonoids. The concentration used for *Piper cumanense* was 18 μg/mL and for *Piper eriopodon* was 6 μ/mL. The Real Time RTPCR study showed an important relative change in the expression of MMP1 (0,00005), MMP2 (0,00020), MMP3 (0,18956), MMP9 (3,64782), MMP12 (5,34601), COL1A2 (0,21653), COL3A1 (0,01603), ELASTIN (0,03221) genes of the cells trated with *Piper cumanense* extract; and for the *Piper eriopodon* extract, the important relative change was, MMP1 (0,00003), MMP3 (04,56702), MMP9 (0,23369), MMP12 (0,54334), ELASTIN (10,69215), These results suggest that the extracts could have an influence over the oxidation process in which these genes are involved, and the use of the Real Time RTPCR methodology allows the elucidation of this influence. **Acknowledgement:** *Dr. Fabio Aristizábal, professor, Department of Pharmacy, National University Colombia and Linamaría Escobar.*

PI7 Identification of methyl jasmonate-inducible cytochrome P450 s and diterpene cyclase involved in cyclic diterpene biosynthesis in *Scoparia dulcis*

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Diterpenes including the phytoalexins and phytohormone gibberellin are one of biologically important pharmaceutical sources from natural origin. *Scoparia dulcis* L. (Scrophulariaceae), a tropical medicinal plant, produce tetracyclic diterpenes such as scopadulcic acid B (SDB) and scopadulciol (SDC). SDB exhibits various pharmacological activities including inhibitory effects on replication of herpes simplex virus type 1 and antitumor. Furthermore, SDB formation in *S. dulcis* leaf tissue is rapidly and transiently stimulated by addition of methyl jasmonate (MJ) as an elicitor. In order to gain insight into the molecular mechanisms underlying diterpene biosynthesis, we have focused on cytochrome P450 (P450) enzymes often appear to form key regulatory steps in plant secondary metabolism. As a first step, we performed differenzial display analysis of P450 genes induced during elicitation of SDB biosynthesis after MJ addition to *S. dulcis* leaf tissues. As a result, nine genes were found to be up-regulated and were highly homologous to the corresponding region of P450 cDNA. We further examined the change in these gene expressions in the course of induction of SDB synthesis by MJ or Yeast extract. In addition, we also isolated a gene encoding *ent*-kaurene synthase (KS) which catalyzes the cyclization of copalyl diphosphate to *ent*-kaurene from *S. dulcis* and analyzed its function. The KS gene has been duplicated in the *S. dulcis* genome and is highly expressed in mature leaves. Here, we discuss the physiological roles of these isolated P450 s and KS in diterpene biosynthesis in *S. dulcis*.

PI8 Effects of black cohosh (*Cimicifuga racemosa*) extract on apoptosis and proliferation rates in hmscs, mcf7, mda-mb-231 and lncap cells

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Recently, herbal therapeutics such as *Cimicifuga racemosa* (L.) Nutt. (CR) has gained interest as an alternative to hormone replacement therapy. New findings, which additionally demonstrate osteoprotective effects of CR in ovariectomized rats, would make CR an optional herbal drug for osteoporosis prevention and treatment. Purified fractions of CR containing saponin (S) and aqueous (A) soluble contents were examined *in vitro* to determine their effects on cell viability and proliferation on a series of tumor cells and osteogenic precursors, respectively. Human mesenchymal stem cells (hMSCs), estrogen receptor-positive (MCF7) and estrogen receptor-negative (MDA-MB-231) human breast adenocarcinoma cell lines as well as androgen-sensitive human prostate adenocarcinoma cells (LNCaP) (n = 3) were stimulated with the whole CR extract, S and A fractions in a range of 0 up to 1000 ng/ml. After 72 h incubation, apoptosis (Caspase-Glo 3/7-Assay, Promega) and proliferation (CellTiterGlo-Luminescent Cell Viability Assay, Promega) rates were determined. The apoptosis rate is decreased down to 33% compared to untreated cells in all investigated cells and cell lines. No noticeable effect regarding the proliferation rate of hMSCs, MCF7 and MDA-MB-231 cells was detected except for a border line stimulating effect on LNCaP cells. The overall toxicity of such extracts appeared to be very low, cell death occurred beyond concentrations of 1 mg/ml. The obtained data show inhibitory effects of CR extracts on apoptosis but no cytotoxicity as measured by proliferation assays in hMSCs, MCF7 and MDA-MB-231 cells. Hence, CR does not possess toxic effects at concentrations of up to 1000 ng/ml extract upon the cells mentioned.

PI9 Genomic characterization of γ-terpinene synthase from *Thymus caespititius*

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Thymus caespititius Brot., commonly known as 'tormentelo' or 'erva-úrsula', is a Lamiaceae aromatic species endemic of the NW Iberian Peninsula, and of the Azores and Madeira archipelagos characterized for showing high essential oil chemical variability [1, 2]. Using eight chemically distinct *Thymus caespititius* accessions, collected at Pico and São Jorge islands (Azores) and in the Mainland Portugal, the genomic characterization of exon and intron numbers, sizes and placement, of a putative gene encoding a monoterpene synthase, γ-terpinene synthase (*TcTPS*2), was performed. *TcTPS*2 is responsible for the first step of the 'cymyl'-pathway, giving rise to phenolic terpene isomers thymol and carvacrol and related compounds, main components in two of the chemotypes from *T. caespititius* essential oils. The putative gene was organized in seven exons and six introns. With almost no variability on the plants analysed, *TcTPS*2 putatively encoded for a protein sequence of 598 amino acids from an open reading frame of 1794 bp, comprising a total of 2291 bp nucleotide sequence content. The deduced amino acid sequence of the putative gene showed a 98% pairwise identity, sharing 93% similarity with closely related *Origanum* species. A BLASTP search on GenBank revealed a high identity (65 – 58%) with other known terpene synthases from different members of other Lamiaceae species. Herewith reported for the first time for the genus *Thymus*, this nucleotide identification approach improved the understanding of the genome organization of these genes. **Acknowledgement:** *This study was partially funded by the Fundação para a Ciência e Tecnologia (FCT) under research contracts PTDC/AGR-AAM/70136/2006 and PTDC/AGR-GPL/101334/2008.* **References:** 1. Figueiredo AC. et al. (2010) Natural Product Communications 5: 1465 – 1476. 2. Figueiredo AC et al. (2008) Cur Pharm Design 14: 3120 – 3140

PI10 PCR-based Assays for the Authentication of Black Cohosh Products

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Black Cohosh (*Actaea racemosa* L.) is one of the highest selling medicinal plants, ranking as the eighth best seller in the US in 2005. However, this popularity has been damaged by links to cases of hepatotoxicity and other significant health implications. The investigation of these reports has not been able to confirm that Black Cohosh plant material is responsible. This has led to the suspicion that some cases of adverse reactions may result from substitution or adulteration with Asian species of *Actaea*, rather than to *A. racemosa* (1). This context demonstrates the requirement for correct identification of *A. racemosa* in Black Cohosh products. We report the development of the PlantID assay for *Actaea* species; a DNA-based assay capable of discriminating *A. racemosa* from potential adulterant species, particularly those associated with hepatotoxicity. A group of cohosh species were chosen on the basis of their widespread growth, commercial availability and/or knowledge of use as an adulterant of Black Cohosh preparations. DNA sequences for each species were aligned to identify hotspots of sequence variation. Species-specific primers were then designed to these regions and optimised for qPCR and multiplex PCR. The product from each reaction was designed to differ in size to enable their resolution by capillary electrophoresis; fluorescent labels attached to each forward primer allow detection of each fragment. The profile of peaks generated is indicative of each species present in the sample. **References:** 1 Jordan S A, Cunningham DG & Marles RJ.(2010) Toxicology and Applied Pharmacology 243: 198 – 216.

PI11 Insights from P-Glycoprotein *in-silico* modelling

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P-glycoprotein (P-gp) is the most representative member of the ABC transmembranar transporter superfamily, often implicated in the multidrug-resistance phenomenon (MDR) [1]. Several models have been proposed for the efflux mechanism, namely the hydrophobic pore, the flipase model and, more recently, the hydrophobic vacuum-cleaner model. Using the bacterial transporters Sav1866, BtuCD or MsbA, several homology models have been constructed. However, the majority proved to be inaccurate due to errors introduced during the homology process, originating misleading results. The recently published crystallographic structure of the murine P-glycoprotein [2] constitutes a more suitable working model. However, a linker known to regulate substract's specificity and to be involved in the conformational changes that accompanies ATP hydrolysis was not mapped [3]. Starting with the murine P-gp crystallographic structure, we built a system comprising a correctly protonated P-gp structure inserted in a lipid bilayer inside a molecular dynamics simulation box with respective counter-ions and waters to solvate all the system. Variations on this system were studied that allowed examining the influence of the linker and lipid type on the P-gp structure stability. The lipid environment and bilayer rigidity was also tested by studying systems with and without cholesterol. Different force field parameterizations were used for quality assessment. The molecular dynamics systems were simulated for tens of nanoseconds using the GROMACS simulation package and the new insights gathered from the simulations namely dynamic and static properties both from P-gp and lipids will be presented and discussed. **Acknowledgement:** *This study was supported by FCT, Portugal (project PTDC/QUI-QUI/099815/2008)* **References:** 1. Juliano R et al. (1976) Biochimica et Biophysica Acta 455: 152 – 162 2. Aller S. et al. (2009) Science 323: 1718 – 1722 3. Sato T et al. (2009) FEBS 276: 3504 – 3516

PI12 DNA-based molecular screening and identification of *Veronica* sp

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Southeastern Europe represents an important center of genetic diversity for many groups of *Veronica*. It was estimated that about 80 species of *Veronica*, representing 10 subgenera, are found in Europe [1]; out of these, about 40 have been reported in literature as being present on the Romanian territory [2]. Data about the chemical composition have been found for ten *Veronica* species from the Romanian flora; these species have a complex and variable biochemical composition, with many secondary metabolites used in pharmacognosy [3]. We aimed to apply molecular techniques to different *Veronica* species present in Romania, in order to obtain reliable means of authentication of the raw plant material and finished herbal medicinal products which contains *V. officinalis* and other species of the genus. Nuclear ribosomal internal transcribed spacer region (ITS) and plastid DNA (cpDNA) intron sequences have been used for PCR amplification. The *rpoB-trnC* spacer region, one of the most variable plant markers of the plastid genome [4] and the *psbA-trnH* spacer, a highly variable cpDNA region [5] were amplified from different veronica species. The length of both DNA fragments taken into evaluation for their putative usefulness as markers for plant authentication were highly variable among the *Veronica* species tested; the length variability in coherent with the molecular data reported from phylogenetic studies [6]. These two spacers could be successfully used as potential DNA barcode marker and as an alternative way to rapidly authenticate the plant species. **Acknowledgement:** *This study was supported by UEFISCDI/project 32151/2008.* **References:** 1. Albach DC et al. (2004) Taxon 53: 429 – 452. 2. Ichim MC et al. (2010) Bulletin UASVM Agriculture 67(2): 482. 3. Crisan G et al. (2009) Rev Med Chir Soc Med Nat 113(2): 81 – 85. 4. Shaw J et al. (2005) Am J Bot 92: 142 – 166. 5. Kress WJ et al. (2005) PNAS 102(23): 8369 – 837. 6. Albach DC, Meudt HM (2010) Mo. Phyl Evol 54: 457 – 471.

PI13 Evaluation of the Effect of Licorice Extract on Proliferation and Differentiation of Human Mesenchymal Stem Cells into Osteoblast cells

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Estrogen deficiency caused osteoporosis during the first decade after Menopause. Estrogen replacement therapy is effective in osteoporosis caused by menopause, but it has some effects, such as carcinogenesis and uterine bleeding. Recent studies have focused on replacement natural compound that contain phytoestrogen. phytoestrogen is natural compound derived from plant, which exibit estrogen-like activities. Licorice is one of the medicinal plant that have phytoestrogen and its extract indicate activity as Estradiol in some parameters. To evaluate the effect of licorice extract on the proliferation and osteogenesis of human, mesenchymal stem cells were determined by MTT method and real-time PCR. Our results show that licorice extract were increased the proliferation and differentiation of hMSC in a dose dependent manner (significant at 10,25,50,100 μg/ml). Real-time pcr analysis shown that licorice extract treatment induced an increase in the expression of BMP-2, Runx-2, Alp, osteocalcin and spp-1 in day 6 and 12, hence ICI 182780, an specific estrogen receptor antagonist inhibit the effect of licorice extract on differentiation, we found that licorice extract stimulates osteoblastogenesis *via* estrogenic activity and can be used as alternative natural medicine for bone disease such as osteoporosis

PI14 Methyl jasmonate-induced biosynthesis of taxol and expression of certain related genes by Hazelnut (*Corylus avellana* L.) cells

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Taxol (Paclitaxel), a diterpene alkaloid against cancer, was originally isolated from *Taxus* sp. and recently was shown to be produced by hazelnut as well. To develop an optimal bioprocess for paclitaxel supply, taxane biosynthetic pathway regulation must be better understood. In the present study, the effects of methyl jasmonate (MJ) on taxol production and phenyl alanine ammonia- lyase (PAL), deoxyxylulose phosphate reductoisomerase (DXR) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) genes expression were investigated in suspension-cultured hazelnut cells. The cultures were treated with MJ (0, 25, 50 and 100 μM) 8 days after subculture. According to the results, cell growth and viability decreased but lipid peroxidation rate, phenolics and taxol production increased by these treatments. In those cells treated with 100 μM of MJ, extracellular and intracellular taxol were respectively 168 μg/L and 20.5 μg/g DW (dry weight) for, 3 and 2.3 times higher than those of the control cells. The expression of the PAL and DXR genes were respectively maximum after 48 h and 72 h of the MJ treatment, but the expression of HMGR gene was suppressed by MJ suggesting that the terpenoid part of taxol is more derived from non-mevalonate route and is originated from plastidic terpenoid pathway rather than cytosolic route of terpenoids production. **References:** Bestoso F et al. (2006) BMC Biotechnol 6: 45. Hoffman A et al. (1998) Spectroscopy 13: 22 – 32. Ottaggio L et al. (2008) J Nat Prod 71: 58 – 60. Rezaei A, Ghanati F, Behmanesh M (2010) 6th International Workshop on Biological Effects of Electromagnetic Fields pp 70 – 71. Wu J, Lin L (2003) Appl Microbiol Biotechnol 62: 151 – 55.

Topic J: Nutraceuticals and Dietary Supplements

PJ1 Quantitation of Underivatized Omega-3 and Omega-6 Fatty Acids in Foods by HPLC and Charged Aerosol Detection

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The omega fatty acids are a group of compounds that include essential n-3 and n-6, and nonessential n-9 analytes. The omega-3 fatty acids,

which also include eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], are required for normal growth and health. Although both omega-3 and -6 fatty acids can give rise to eicosanoid-signaling molecules (prostaglandins, prostacyclins, thromboxanes and leukotrienes), the omega-6 eicosanoids are generally pro-inflammatory and may play a role in disease. It appears that the amounts and balance of omega fatty acids in a person's diet affect their eicosanoid-controlled functions. A proper balance of omega fatty acids in the diet is important. Traditionally, omega fatty acids are measured using gas chromatography (GC). For foods, analytes are extracted from the samples prior to hydrolysis to release the fatty acids from their triglycerides, and then converted to their volatile methyl-esters prior to analysis by GC. Regardless, this approach is tedious, time-consuming, and the high temperatures can affect polyunsaturated fatty acid stability. Charged aerosol detection (CAD), a universal mass-based approach, is sensitive, has a wide dynamic range, and has a major advantage in that all nonvolatile analytes give similar response independent of chemical structure. No derivatization is required, and unlike UV detection, the analyte does not need to contain a chromophore. Presented here is a simple and direct HPLC-CAD method that can be used to measure omega-3, -6, and -9 fatty acids in traditional and commercially produced meat, fish, and oils, as well as over-the-counter supplements.

PJ2 Potential preventive and therapeutic effects of date palm (*Phoenix dactylifera*) pollen grain on cadmium – induced testicular injury in rats

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This study was investigated the possible preventive and therapeutic effects of date palm (*Phoenix dactylifera* L.) pollen grain (DPP) on cadmium (Cd)-induced testicular damage using quantitative, biochemical and histopathological approaches. A total of 25 adult male rats were randomly divided into five groups. control; DPP treated group received a 56 days DPP suspension; Cd treated group received only $CdCl_2$; pretreated group received a 15 days of DPP suspension before $CdCl_2$ injection while post-treated group received a 56-day of DPP suspension following $CdCl_2$ injection. $CdCl_2$ (1.2 mg/kg bwt) was intraperitoneally injected as a single dose and DPP (120 mg/kg bwt) given by gavage suspended in distilled water. Cd treated group showed significantly decrease reproductive organs index weight, sperm count and motility, reduced glutathione, serum testosterone and Johnsen's score. Meanwhile, sperm abnormalities, lipid peroxidation were significantly elevated. Necrotic changes with poor spermatogenesis to complete spermatogenic arrest were the key histopathological finding. Although the mechanism is not clear, improved sperm quality and antioxidative status, elevated testosterone level, restored testicular histology and reclaimed spermatogenesis were noticed in DPP post-treated group as therapeutic intervention. While, DPP pretreatment as preventive intervention failed to attenuate the adverse effects of cadmium.

PJ3 Anti-metalloproteinase-9 Activities of Selected Indonesian Zingiberaceae Rhizome Extracts in Lipopolysaccharide-induced Human Vascular Endothelial Cells *In Vitro*

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Atherosclerosis arises from chronic inflammation triggered by bacterial infection that activates degradation process by matrix metalloproteinases (MMPs). Zingiberaceae, a group of tropical food crops grown in Indonesia and other Southeast Asia regions, has been traditionally used for food coloring, seasoning, culinary, and medicinal purposes. However, its efficacy as natural vascular protection has not been explored. Our previous studies demonstrated that *Kaempferia pandurata* Roxb. possesed MMP-2 and MMP-9 inhibitory effects in human gingival and oral epithelial cells induced by *Porphyromonas gingivalis*, suggesting its potential therapeutic for natural periodontal therapy. Here, we examined the effects of 10 Indonesian Zingiberaceae rhizome extracts on inhibition of MMP-9 expression in human vascular endothelial cells treated with lipopolysaccharide (LPS) *in vitro* by conducting gelatin zymogram, Western blotting, and RT-PCR assays. LPS (2 μg/ml) significantly elevated the expression of MMP-9 secretion, protein, and mRNA in vascular endothelial cells. Selected Zingiberaceae exctracts (1 and 5 μg/ml), i.e. *Curcuma xanthorrhiza, C. aeruginosa, C. mangga, C. longa, Kaempferia galanga, Alpinia galanga,* and *Zingiberaceae officinale*, effectively attenuated the expression of MMP-9 secretion, protein, and mRNA in LPS-induced vascular endothelial cells. Furthermore, MMP-9 expression was specifically blocked by MAPK inhibitors, i.e. PD98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor), and PI3K inhibitor (LY294002), indicating that MAPK and PI3K signaling pathways are involved in regulation of MMP-9 gene expression in LPS-induced vascular endothelial cells. These results suggest that selected Indonesian Zingiberaceae rhizomes with potent MMP-9 inhibitory activity may have potentials on prevention and protection of vascular diseases particularly atherosclerosis.

PJ4 Formation of 5-HMF in making aged garlic (*Allium sativum* L.) under different condition

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5-Hydroxymethylfurfural (HMF) is derived from dehydration of sugars and has been identified in processed foods [1]. The biological function of HMF have revealed as antisickling agent and thyrosinase inhibitor [2,3]. This study was performed to find out the amount of HMF and free sugars from the aged garlic when it is treated by temperature at 60 and 75°C and different incubation period from 7 to 35 days. HMF and free sugars from the hot-water extracts of aged garlics were analyzed with GC/MS, LC/MS, and HPLC. The amount of HMF was high at 75°C and 35 days incubation. Among free sugars, the only fructose except glucose and sucrose was formed and converted to HMF at high temperature and long incubation period. However, fructose formed in low temperature making aged garlic was rarely converted to HMF. This result indicates that formation of HMF can be dependent on the temperature and incubation period for making aged garlic. **References:** 1. Chen S et al. (2009) Food Chem 114: 582 – 588. 2.Abdulmalik O et al. (2005) British J Haematol 128: 552 – 561. 3. Sharma V et al. (2004) Phytotherapy Res 18: 841 – 844.

PJ5 Sesamin and sesamolin contents in various commercial sesame oils

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Sesame (*Sesamum indicum* L.) seed and oil have been categorized as one of the representative health food and widely used for good flavor and taste in China, Japan, and other East Asian countries for a long times. Sesame seed and oil contain abundant lignans [1] such as sesamin, sesamolin, and others. Sesamin and sesamolin are major lignans in sesame seed and oil, and its biological effects have been extensively studied. These components are believed to play an important role in the oxidative stability of sesame oil [2]. It is important to understand the variation in the contents of these physiologically active constituents in sesame oil. This knowledge of their levels and forms in sesame seed, sesame oil, and functional foods contained sesame is beneficial to control the quality of the sesame seed and sesame oil, and to develop the sesame oil manufacturing technique. The aim of this study was to establish the methods of sample pretreatment and simultaneous determining the sesamin and sesamolin in the sample of sesame oils by RP-HPLC. The methods developed in this work were used to determine the sesamin and sesamolin in ten different brands of sesame oils collected in the Chinese markets. The mean contents of total lignans, sesamin and sesamolin were 8.16, 5.14, and 3.02 mg/g respectively. The results shown that can be used to control the quality of sesame oils, and to estimate the dietary intake of sesame lignans. And also it will be beneficial to improve the processing technique in industry. **Acknowledgement:** *This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients.* **References:** [1] Daisuke N et al. (2006) J Pharm Exp Ther 318: 328 – 335 [2] Nakai M et al (2006) Biosci Biotechnol Biochem 70: 1273 – 1276.

PJ6 Studies on the stability of secoisolariciresinol diglucoside in flaxseed powder

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Secoisolariciresinol diglucoside (SDG) (Fig. 1) is an essential component (11.9 to 25.9 g/kg [1]) of lignans in flaxseeds. Recently, SDG has drawn more and more attention because of its health benefits. A number of animal studies have shown that SDG may help fight many diseases in the modern society, including breast cancer [2], cardiovascular malfunction [3], diabetes [4] and prostatic hyperplasia [5]. With a novel perspective, we focused our study on the effect of food processing methods, including steamed, boiled, fried and deep fried, and processing times on the stability of SDG in flaxseed powder. In this paper, the samples of four processing times were prepared for each processing method. The concentrations of SDG in different samples were determined by RP-HPLC [6]. The chromatographic analysis was performed on a Diamonsil C_{18} column (150*4.6 mm, 5 μm). Acetonitrile and 1% aqueous acetic acid was selected as the mobile phase. The detection wavelength was 280 nm. A comprehensive study on the effects of different processing methods was made in Fig. 2. For steaming process, little effect on SDG content was observed. While for the boiled dishes, i.e. the medicated soups, although little impact was exerted on the content of SDG in flaxseed powder remnant, small amount of SDG was released from flaxseed into the filtrate (the soup). As for fried and deep fried dishes, the loss of SDG was inevitable, but shorter processing time lead to less decrease in SDG, flaxseed could also be employed if cooked properly.

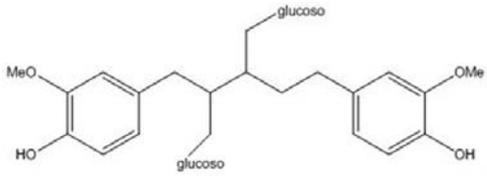

Figure 1: Chemical structure of SDG

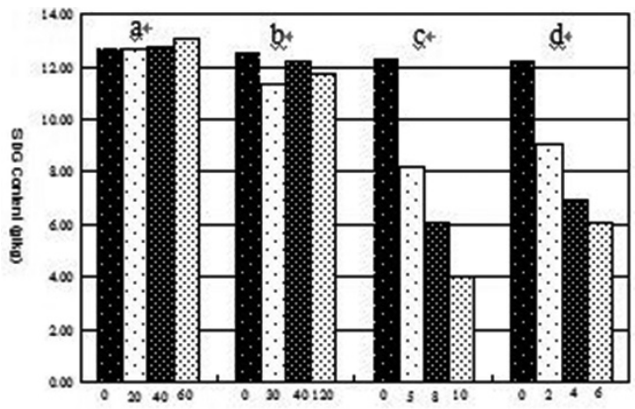

Figure 2: Comprehensive comparison of SDG content in different processing methods. a. Steaming; b. Boiling; c. Frying; d. Deep Frying.

Acknowledgement: *This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients.* **References:** [1] Eliasson C et al. (2003) J Chromatogr A 1012:151 – 159 [2] Saggar J K et al. (2010) Nutr Cancer 62:533 – 542. [3] Prasad K (2008) Atherosclerosis 197: 34 – 42. [4] Prasad K (2001) J Lab Clinic Med 138: 32 – 39. [5] Zhang W et al. (2008) J Med Food 11: 207 – 214. [6] Chen J et al. (2007) J Liq Chromatogr 30: 533 – 544.

PJ7 Quality control of red clover based nutritional supplements by FTIR and chemometric analysis

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Botanical preparations of red clover (*Trifolium pratense* L.) have gained interest as an alternative treatment for menopausal problems such as hot flushes. This shows the need for simple and rapid analysis methods. In the present study, FTIR-ATR spectroscopy has been applied for the characterization and identification of the active compounds of red clover belonging to the class of isoflavones, e.g. formononetin and biochanin A. Information about functional groups and chemical composition could be obtained, making FTIR a powerful tool for a fast and non-destructive quality control. Moreover, using chemometrics calibration models based on the partial least squares (PLS) regression were employed. The results of the multivariate calibration demonstrated the potential of this method to quantify the main isoflavones in red clover.

PJ8 Lectins from *Vigna radiata* - A potential health supplement

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There has been growing interest in use of nutraceuticals of plant origin, because of their impact on the status of human health and disease prevention. Plant lectins, a unique group of proteins and glycoproteins with potent biological activity, occur in commonly consumed foods such as legumes. The lectins can be used as dietary supplements due to their potential benefits of enzyme inhibition. The present research work discusses lectins from *Vigna radiata* (L.) R.Wilczek as an effective and economical source of natural antioxidants and α-amylase inhibitors which can prove to be a potential dietary and health supplement. Lectins from *Vigna radiata* were extracted by macerating the seed meal in 50 mM phosphate buffer saline (pH 5.2) containing D-(+)-Galactose at 40°C. The lectin rich extract was prepared by salt precipitation. The precipitate was dialysed against distilled water for 48 hours. The lectins were purified by Size exclusion Chromatography by using Sephadex G75. The presence of lectins was confirmed by hemmagglutination assay. The fractions showing agglutination were pooled together and lyophilized. The yield of relatively purified lectins was found to be 0.044%w/w. Lectin rich extract was evaluated for anti-oxidant property by TBARS assay. The IC_{50} value of the lectin rich extract was found to be 598.32 ± 3.2 μg/ml. The extract was also subjected to the evaluation of α-amylase inhibitory activity. The IC_{50} value was found to be 552 ± 6.09 μg/ml. This study provides evidence on the potential health benefits of lectins from *Vigna radiata*, thus confirming the traditional claim. **References:** 1. Heller VG (1927) JBC 435 – 442.

PJ9 Protective effects of saffron and trans-crocetin on glutamate mediated excitotoxicity in rat neuroblastoma cells

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Neuronal dysfunctions or even cell death are often accompanied by an exceeding release of glutamate. Excessive glutamate level induces unregulated stimulation of NMDA receptors. In previous studies we found an antagonistic effect of hydro-ethanolic saffron extract (CSE) and trans-crocetin, a carotenoid from saffron, on NMDA receptors (Berger et al., Neuroscience, 180; 238 – 247, 2011). In this study we evaluated theprotective effects of CSE and trans-crocetin on glutamate mediated excitotoxictiy on rat B104 neuroblastoma cells using cell-based cytotoxicity tests and a fluorescent stain with 4',6-diamidino-2-phenylindole (DAPI-staining). Glutamate applied for 24 h decreased concentration dependently (0.1 – 20 mM) cell viability (MTT-test) and increased LDH activity (LDH-test). The number of annexin-V and 7-AAD positive cells was also augmented and DAPI staining showed an enhanced number of pycnotic nuclei. The glutamate effect was partially inhibited by kynurenic acid (10 mM), an antagonist on excitatory amino acid receptors. After preincubation with CSE or trans-crocetin for 2 h followed by 24 h co-incubation with 10 mM glutamate a reduced excitotoxicity could be found. CSE 500 μg/ml significantly increased cell viability from 27 ± 4% to 63 ± 5% and decreased LDH activity from 207 ± 5% to 125 ± 18%). DAPI-staining showed no differences between CSE treated and control cells. Trans-crocetin 50 μM fully abolished the glutamate effects on cell viability, LDH activity, and DAPI-staining. We conclude that the neuroprotective effects of saffron and trans-crocetin which was previously demonstrated on cortical neurons are partially mediated by attenuation of glutamate mediated excitotoxicity

PJ10 Isoflavone profiles in different plant parts of red clover

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Red clover (*Trifolium pratense* L., Fabaceae) is important source of isoflavones, among which the most present are: daidzein, genistein, formononetin and biochanin A [1]. These substances are considered to be beneficial for reduction of menopausal symptoms, prevention of osteoporosis, cancer and cardiovascular diseases. Since red clover extracts are used for production of dietary supplements, it is important to evaluate profile of these active compounds in plant parts. The aim of this study was to determine content of daidzein, genistein, formononetin and biochanin A in different plant parts of red clover, and to investigate which isoflavone is present in highest concentration. Stems, leaves and flowers of five red clover cultivars were grounded and mixed with water 30 minutes on 37 °C. After that, 3 M HCl and 96% ethanol were added and mixture was heated to boiling. Extracts were than purified by solid phase extraction on HLB cartridges. Isoflavones were identified and quantified in samples by high-performance liquid chromatography (HPLC), using corresponding standard compounds [2]. Total isoflavone content was on average the highest in leaves (2,73 mg/g), and the lowest in stems of red clover cultivars (0,47 mg/g). Isoflavone content in flowers varied between 0.53 – 1.05 mg/g. In leaves formononetin was dominant (1,62 mg/g), while the lowest average content had biochanin A in stems (0,04 mg/g). The highest individual concentrations of all investigated isoflavones were found in leaves of different cultivars. On average, in all analyzed samples formononetin was the most present isoflavone. **References:** 1. Sivesind E, Seguin P (2005) J Agric Food Chem 53: 6397 – 6402. 2. Krenn L et al. (2002) J Chromatogr B 777:123 – 128.

PJ11 Fatty acid profile from samples of hemp seeds of dioecious and monoecious hemp varieties approved in Romania

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Cannabis sativa L. hemp seeds can be a complete and balanced source of fatty acids with an optimal omega 6/omega 3 ratio of 3:1 [1], but with limited use in Romania because of the stigma of drug. Whole hemp seeds have oil content of about 25 – 35% [2], but in Russia there is a cultivated variety called "olifera" which contains 40% oil [3]. About 30 – 35% of the weight of hempseed is an edible oil that contains about 80% as essential fatty acids (EFAs), linoleic acid, omega-6 (LA, 55%), alpha-linolenic acid, omega-3 (ALA, 22%), in addition to gamma-linolenic acid, omega-6 (GLA, 1 – 4%) and stearidonic acid, omega-3 (SDA, 0 – 2%). This study aims to investigate the impact of the extraction technology of oil content and fatty acid profile from samples of hemp seeds of dioecious and monoecious varieties hemp obtained in research stations from different areas of the country and approved in Romania. Hemp oil has been extracted from the seeds by cold pressing using a press and by Soxhlet method with a Velp block of mineralization. Investigation of fatty acid profile and oil content was performed by gas chromatography GC-MS whit Shimadzu GC MS QP 2010. Hemp seeds not only contain essential fatty acids, but come with substantial contribution of 20 – 25% protein, essential amino acids, which make hemp seed an ideal food for vegetarians, successfully replacing the lack of animal protein, with a high nutritional value for human consumption in salads, bread and even chocolate. **References:** [1] Callaway JC. (2004) Euphytica 140: 65 – 72 [2] Şandru ID, Paraschivoiu R, Găucă C (1996) Cultura cânepii, Helicon, Timişoara [3] Deferne JL and Pate DW (1996) Journal of the International Hemp Association 3(1): 4 – 7

PJ12 The contents of heavy metal (Pb, Cd and Zn) in plant *Taraxacum officinale* Weber

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The species of *Taraxacum officinale* Weber (Asteraceae) is a very popular medicinal and edible herb. Since time immemorial have been used in traditional phytotherapy in Bosnia and Herzegovina (B-H) [1]. The young shoots and inflorescences are used as health food [2]. Dandelion is widespread. Most often inhabit different anthropogenic habitats that are loaded with different pollutants, including heavy metals [3]. This is a serious limitation for safe and sustainable use of this plant in medicine and dietetics. Investigation the content of heavy metals in roots and aerial part of the dandelion included 30 localities of B-H which are under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in *Taraxacum officinale* varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under severe anthropogenic pressure and a larger site outside the pollution. In many localities have been established concentrations that are not allowed. **References:** 1. Redzic SS (2007) Coll Antropol 31: 869 – 890. 2. Redzic SJ (2006) Ecol Food & Nutr 45(3): 189 – 232. 3. Redzic S et al. (2009) Planta Med 75: 902 – 902.

PJ13 Evaluation of dissolution profiles of alpha lipoic acid soft gelatin capsules and tablets

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Alpha lipoic acid (ALA) has become a common ingredient in many commercially available supplements. The aim of the present work is to compare the dissolution profiles of the certain samples of ALA soft gelatin capsules and tablets (300 mg and 600 mg) commercially available from the local market. Fixed volumes of the dissolution medium were withdrawn at 15, 30, 45 and 60 minutes. Dissolution tests were performed on the USP Apparatus 2 (Dissolution tester ERWEKA DT 800; rotating speed 75 rpm at 37 ± 0.5°C, 900 mL, distilled water). Also, the purpose of this work was to adapt and use the HPLC method proposed by Salem for the determination of the amount of the active ingredient released [1,2]. HPLC was performed with a mobile phase composed of 0.05 M phosphate buffer pH 3.5:acetonitrile = 650:350$_{v/v}$, and peaks were detected at 330 nm. Degassed and diluted samples were analysed on Zorbax Eclipse XDB-C 18 column (250 x 4.6 mm, 5 μm), at 25°C and 1.5 mLmin^{-1} flow rate. The dissolved amounts of ALA in soft capsules and tablets at the end of testing were in the range of 12.9 ± 2.8%- 18.6 ± 1.9% and 85.2 ± 7.6%- 90.6 ± 4.9%, respectively. The results of dissolution studies are summarized in Figure 1. which show the percentage of ALA dissolved as a function of time. The results obtained in this study indicated problems in drug release from the investigated ALA soft gelatin capsules. According to the results obtained, we can presume differences in therapeutic response of the investigated ALA soft gelatin capsules and tablets.

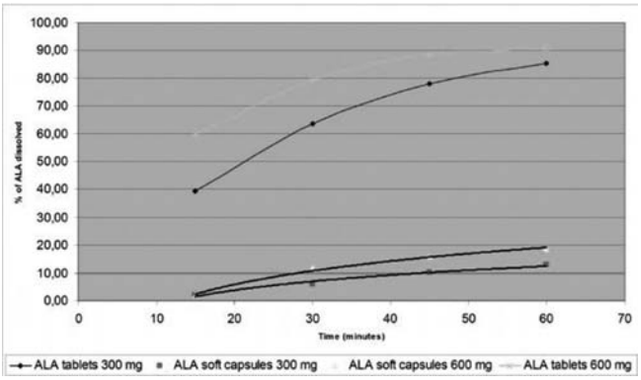

Figure 1: Comparative display of dissolution profile for ALA soft capsules and tablets (300 mg and 600 mg)

References: [1] Salem H (2010) Chromatographia 72: 327 – 330. [2] Kataoka H (1998) J Chromatogr B 717: 247 – 262.

PJ14 Beta-ecdysone prevents the metabolic syndrome in ovariectomized rats: effects on metabolic parameters

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Ovariectomized (ovx) rats develop a metabolic syndrome which includes hypercholesterinemia, hypertriglyceridemia and an impaired oral glucose tolerance test. These impaired metabolic parameters are due to increased cytokine secretion by the increased number of adipocytes. Estradiol (E2) and beta-ecdysone (Ecd) are known to reduce visceral and subcutaneous fat loads and E2 normalizes the deranged metabolic parameters. Whether this can also be achieved by a treatment with Ecd was not studied hitherto. Ovx rats were orally treated with E2 (0.108 mg/animal/day) or Ecd (18.56 mg/animal/day) and following necropsy 4 weeks later serum cholesterol, LDL, HDL and triglycerides were determined. In addition an oral glucose tolerance test (OGTT) was performed. Serum cholesterol, HDL and LDL were reduced by E2 whereas triglycerides were increased. Ecd decreased cholesterol, LDL and also triglycerides but increased HDL. Clearance of glucose following an OGTT lasted longer in the ovx controls than in the E2 and Ecd treated animals. It is concluded that Ecd shares the positive effects of E2 on cholesterol and glucose clearance but prevents the adverse acting stimulation of triglycerides by E2. Hence, Ecd may be a novel non-estrogenic alternative for prevention and treatment of the metabolic syndrome. **Acknowledgement:** *This work was in part funded by VerdeVital GmbH*

PJ15 Beta-ecdysone (Ecd) prevents the metabolic syndrome in ovariectomized (ovx) rats: joint cartilage tissue

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We have recently shown that Ecd prevents osteoporosis and decreases visceral fat mass in ovx rats. This animal model is known to develop a metabolic syndrome and this disease was shown to be associated with increased visceral and bone marrow fat tissue. The adipocytes in bones secrete large amounts of cytotokines which have cytotoxic effects on osteoblast thereby augmenting estrogen deficiency induced osteoporosis. The reduction of bone mass by Ecd is suggestive that fat load in the knee joints – the Hoffa's fat pad – are also reduced thereby exerting preventive effects on osteoarthritis. Ovx rats (n = 10 – 12 per group) were orally treated with Ecd (56 mg/day/animal) and the amount the size of the knee joint fat depot as well as the knee joint height of cartilage tissue were determine histomorphometrically following 4 weeks of treatment. Control rats received soy free food, positive controls received estradiol-17β (E2; 0.108 mg/animal/day) supplemented to the chow. The size of the knee joint cartilage tissue was significantly lower in ovx and higher in E2 and Ecd treated animals while the Hoffa's fat pad was significantly larger in ovx than in E2 and Ecd treated animals. These results suggest that the high fat load in the knee joint is a source of high cytokine production which exerts inhibitory effects on development of cartilage tissue in the knee joint. Both, E2 and Ecd seemed to prevent these lipotoxic effects. **Acknowledgement:** *This work was in part funded by VerdeVital GmbH*

PJ16 Elemental Compositions of Soybean Cultivars Cultivated in Turkey

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Soybean (*Glycine max* (L.) Merr.) is annual plants species and is locally known as "Soya Fasülyesi". Soybean is consumed as a food and vegetable plant and its recorded folkloric usages for the medicinal purposes. In this present study macro (N, P, K, Ca, Mg, Na,) and trace elements (Fe, Mn, Zn, Cu) of soybean cultivars cultivated under the controlled conditions in Turkey have been studied. Macro and trace elements were determined by using various techniques. N was determined by the dry combustion method using elemental analyses, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectrocopy (AAS). All experiments were performed qualitatively and quantitatively with comparison to a certified reference plant material statistically. The results of elemental analyses showed that N ranged 5.41 – 5.82%, P ranged 5250 – 6100 ppm and K ranged 15915 – 19645 ppm. To the best of our knowledge, this is the first report on micro and macro elements of cultivated Turkish soybean cultivars. As a conclusion, the elemental composition and the nutritional value soybean cultivars are worthwhile to investigate with comparison to other *Glycine* sp. used medicinally.

PJ17 Analysis of ascorbic acid content in various fruits and vegetables by spectrofluorimetric methods

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Ascorbic acid (AA) is one of the most important vitamin for human nutrition that is supplied by fruits and vegetables. In this study, a simple and sensitive fluorimetric method determination of ascorbic acid was used in 25 different samples of fruits and vegetables. This method is based on the condensation reaction of AA with o-phenylenediamine (OPDA) in the absence of the oxidant. The fluorescence intensity is measured at excitation and emission wavelengths of 330 nm and 430 nm, respectively. The effect of pH and OPDA concentration on fluorescence intensity of the system was examined. Optimum pH was between 9.4 and 9.5, and optimal concentration of OPDA was 0.05% (w/v). Under optimal conditions, a linear relationship is obtained between the fluorescence intensity and the concentration of AA in the range 1 – 10 micro g/mL. The regression coefficient was 0.9999. The content of AA were found between 3 and 60 mg/100 g of fresh fruits and between 17 and 50 mg/100 g of fresh vegetables. The detection limit (3σ) was found to be 0.20 micro g/mL of AA (σ from 4 determination of 5 micro g/mL). A relative standard deviation of 1.0% was recorded for 4 measurements of 5 micro g/mL standard AA solution. Limit of quantification AA was 0.68 micro g/mL. The results obtained with this method are comparable with the results obtained by other methods [1]. Presented method seems to be useful in the nutritional analytical practice. **References:** 1. Eitenmiller RR, Ye L, Landen WO (2008) Vitamin analysis for the health and food sciences, CRC press

PJ18 Histopathological and immunohistochemical study of the effect of *Punica granatum* extract on Azoxymethane induced colon cancer in Rats

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Chemoprevention has become an important area in cancer research due to the failure of current therapeutic modalities. Polyphenol-rich dietary foodstuffs have attracted attention due to their cancer chemopreventive and chemotherapeutic properties. The modulating effects of aqueous methanol of *Punica granatum* L. peels at doses (200 and 400 mg/kg) on colon carcinogenesis initiated with azoxymethane (AOM), were investigated in male rats by weekly s.c. injections of 15 mg/kg body wt for 12 weeks. Histopathological studies on AOM-treated rats revealed dysplasia of the colonic histoarchitecture, which showed signs of improvement following *P. granatum* administration, was found to significantly and dose dependently decrease the total number of aberrant crypt foci (ACF) per rat. Cell proliferation in the colon, as shown by proliferating cells nuclear antigen (PCNA), was also reduced in those treatments. AOM-treated rats exhibited alterations in cancer tumour markers gamma glutamyl transpeptidase (gamma-GT), carcinoembryonic antigen (CEA), pathophysiological markers (alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)) and oral administration of *P. granatum* restored the levels of these marker enzymes. Also, pro-inflammatory proteins (inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines tumour necrosis factor (TNF)-alpha and interleukin (IL-6) in AOM group exhibited elevated expressions of all these inflammatory proteins. *P. granatum* administration reduced COX-2, iNOS, TNF-alpha and IL-6 as confirmed by immunohistochemical analysis during AOM -induced colon carcinogenesis. Our results suggest that *P. granatum* could exert a significant chemopreventive effect on AOM induced colon carcinogenesis is probably due to combined effect of polyphenolic compounds.

PJ19 Investigation on Compositions of Seed Oil and Yield of Silymarin of Seeds from Silybum marianum (L.) Gaertn. Cultivated in Konya Ecological Conditions

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Milk thistle (*Silybum marianum* (L.) Gaertn.) is an herbal supplement used to treat liver and biliary disorders. The active constituent of milk thistle is silymarin, a mixture of flavonolignans. It is also contains important faty oil acid ansaturated. In this study, researchs have been conducted in Medicinal – Aromatic Plants laboratory and Medicinal and Aromatic Pants Experimental Farm of Field Crops Department, Agriculture Faculty, Selcuk Universty. The aim was to determine the effect on yıeld and quality some characters of organic fertilizers applied at the different doses on milk thistle (*S. marianum*) cultivated under Konya (Turkey) ecolojical conditions. It were applied at the three different doses sheep manure as organic fertilizer. In this study; plant height, plant seed yield, yield of crude oil, composition and yield of oil and flavonoid (silymarin) were examined. According to results of this research; plant height, seed yield, yield of crude and composition and yield of oil and flavonoid (silymarin) varied between 75 – 118 cm, 720 – 1480 kg/ha, 20 – 28 % and % 1.1 – 3.1, respectively. In this research; high silymarine yield, crude oil yield, fatty acid composition and drug of milk thistle (*S. marianum*) cultivated in Konya ecological conditions were obtained from 15000 kg/ha from applied organic fertilizer.

PJ20 Antioxidant properties of wild *Solanum nigrum* ripe fruit

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This work was to examine hepatoprotective activities of *Solanum nigrum*, commonly known as Black Nightshade, a medicinal herb grown in Egypt used traditionally in oriental medicines and believed to have various biological properties. A crude ripe fruit ethanol extract was made, lyophilized to give 2.86 g/100 g. Two sets of experiments were done, *in vitro* antioxidant experiments and *in vivo* biological assays. The results revealed that crude ethanol extracts of Black Nightshade ripe fruit had strong antioxidant activity, for example in a DPPH assay at 500 ppm of the extract, a 68 % reduction of DPPH radicals was detected, with total antioxidant capacity at 8.45 ± 0.031 as ascorbic acid equivalents. *In vivo*, the extract; 100, 200 or 300 mg/Kg; showed valuable activity as a hepatoprotective agent on oral one dose CCl_4-treated experimental rats shown by an increase in total serum soluble protein, albumin, and a remarkable reduction in the serum activity of AST, ALT and ALP as well as bilirubin and uric acid. For example, the serum total albumin level was reduced from 6.29 ± 0.12 g/dl in the healthy normal control animals to 3.35 ± 0.37 g/dl (53 % at normal control) for CCl_4 intoxicated control rats, but recovered to 5.62 ± 0.39 g/dl by 300 mg extract per kg body weight for rats on daily oral post-treatment for 5 days. Collectively, Black Nightshade ripe fruit ethanol extracts were shown to be an effective hepatoprotective agent *in vivo* due to their high content of antioxidant and bioactive plant secondary metabolites. **Keywords:** *Solanum nigrum* L., Black Nightshades, Hepato-protective, Antioxidant, CCl_4 **Acknowledgement:** *The authors thanks Dr. Emam Abdel-Mobdee, Biochemistry department, Faculty of Agriculture, Cairo University for his great support. Thanks to Kamela Alegre, American PhD student at Universitat Autonoma de Barcelona, for Language revision.* **References:** Lin H et al. (2008) Chemico-Biological Interactions 171: 283 – 293 Loganayaki N, Siddhuraju P and Manian S (2010) Food Science and Biotechnology 19(1): 121 – 127

PJ21 Hypocholesterolemic Effects of *Glyphaea brevis* (Spreng.) Monach. in Normal And Streptozotocin-Induced Diabetic Rats

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Hypercholesterolemia, sometimes linked to diabetes, is a public health concern since it paves the way to severe complications such as hypertension and stroke. The search for innovative, natural and safe treatments to reverse the condition remains imperative. This study was aimed at evaluating the hypocholesterolemic potential of *Glyphaea brevis* (Spreng.) Monach. aqueous extract (AE) in both normal and streptozotocin-induced diabetic rats. AE was given on a daily basis to rats by gastric intubation at 500 mg/kg for 30 days in a controlled study. At the end of experiment, administration of AE significantly reduced the levels of fasting blood total cholesterol (normal: -38.54 %, $p < 0.01$; diabetic: -22.08 %, $p < 0.01$), LDL-cholesterol (normal: -72.85 %, $p < 0.01$; diabetic: -38.15 %, $p < 0.01$) while no significant change was observed in triglycerides. Moreover, atherogenicity indices total cholesterol/HDL-cholesterol (TC/HDL-c) and LDL-cholesterol/HDL-cholesterol (LDL-c/HDL-c) were significantly reduced at the end of study (TC/HDL-c: normal: -43.88 %, $p < 0.01$; diabetic: -32.43 %, $p < 0.01$; LDL-c/HDL-c: normal: -76.14 %, $p < 0.01$; diabetic: -44.11 %, $p < 0.01$). These results suggest the hypocholesterolemic effect of G. brevis. Such effect would be accountable of the presence of flavonoids (revealed by phytochemical screening) that may inhibit enzymes such as hydroxymethyl-CoA (HMGCoA) reductase that are involved in cholesterol biosynthesis. The outcome of our study could find applications in the development of alternative means of treatment or prevention of hypercholesterolemia and its associated complications.

PJ22 The pericarp of *Pisum sativum* L.(Fabaceae) as a biologically active waste product

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Food industries generate large amounts of wastes and byproducts which contain biologically active compounds. The recycling of these wastes could be of economic benefits. Pericarp of *Pisum sativum* L. pods is separated from the seeds which are processed as frozen foods. Most of the phytochemical studies on Pisum sativum dealt mainly with the seeds. With the aim of utilization of waste products as biologically ac-

tive natural sources, the pericarp of *Pisum sativum* (Pea), cultivated in Egypt was phytochemically and biologically studied. The phenolic content amounted to 27.5 mg/g. HPLC analysis revealed the presence of eight phenolic acids (cinnamic, chlorogenic, vanillic, coumaric, ferulic, caffeic, gallic and syringic acids) and three isoflavones (daidzein, genistein and formononetin). GC/MS analysis of the unsaponifiable and saponifiable fractions of the lipoidal matter revealed the presence of two major sterols: stigmasterol and β-sitosterol and twelve fatty acids, palmitic acid being the major component (30%). Soluble carbohydrate content determined by phenol-sulfuric acid method amounted to 0.6 g/g of aqueous extract, the identified sugars being glucose, fructose, sucrose and rhamnose. Acute toxicity of the chloroform and 80% aqueous – acetone extracts, antimicrobial screening of the saponifiable fraction and the anti-inflammatory activity of the unsaponifiable matter were studied, together with the antidiabetic activity of the 80% aqueous – acetone extract. Results showed that the two extracts were relatively safe. The saponifiable fraction showed significant antibacterial activity, but no effects against fungi or yeast. The unsaponifiable matter displayed significant anti-inflammatory activity. The 80% aqueous – acetone extract showed potential antihyperglycaemic activity.

PJ23 Developing a nutraceutical from Egyptian stabilized rice bran: a pharmacological approach

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Rice bran is known to contain bioactives which are potentially useful for human health. These include the tocotrienols, policosanol and gamma-oryzanol. Rice bran has been stabilized immediately after collection from the milling stations in order to preserve the integrity of its active constituents. An extract of the stabilized rice bran has been prepared and standardized to contain 2% g-oryzanol. The extract has been subjected to a battery of pharmacological testing to establish its potential therapeutic usefulness. When given orally in doses of 30 and 100 mg/kg for 1 week to rats which had been rendered hypertensive by L-NAME, the extract was shown to protect against the rise in blood pressure without affecting the heart rate of animals. Equally, in the same oral doses, it was shown to be effective as an anti-inflammatory in models of acute (carrageenan paw edema) and chronic inflammation (adjuvant-induced arthritis) in rats. It also reduced the level of inflammatory mediators and cytokines in the blood. The extract also showed good antioxidant activity as measured in various *in vitro* as well as in vivo models and has a beneficial effect on insulin secretion in *in vitro* models and in preliminary animal experiments. Safety studies on the extract in rats showed its lack of toxic side effects over a prolonged period of time. The present results point to the potential usefulness of the extract as a nutraceutical to guard against inflammatory disorders, hypertension, and diabetes. **Keywords:** rice bran, nutraceuticals, diabetes, hypertension, anti-inflammatory, herbal drugs

Topic K: Pharmaceutical Applications

PK1 High-performance liquid chromatography (HPLC) analysis of phenolic compounds in two edible mushrooms extracts and their protective effect against oxidative damage in BHK-21 cell line

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Mushrooms have been used for many years in oriental culture as tea and nutritional food and because of their special flavour and texture [1]. *Auricularia auricula-judae* (Bull.) J.Schröt. and *Pleurotus eryngii* (DC.) Quél. are two edible mushrooms and they have been reported to have many biological activities [2,3,4]. In this study we investigated the phenolic composition, protective and cytotoxic effects of these mushrooms. Analysis of phenolic compounds in these edible mushrooms species has been carried out by high-performance liquid chromatography coupled to photodiode array detector (HPLC-DAD). Twelve of the 14 phenolic compounds were identified and quantified by comparing their chromatographic characteristics and absorption spectra with that of the standard compounds. The analysis showed that *p*-hydroxybenzoic acid, catechin, gallic acid and caffeic acid were the major phenolic components in the extracts. Protective effect of these mushroom on H_2O_2 induced oxidative cell damage was determined by using MTT (3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay. All the extracts exhibited protective effect against H_2O_2 induced oxidative cell damage but the highest activity was observed for *A. auricula-judae* aqueous extract (89.5 ± 1.8% cell viability at 0.1 mg/ml) (Fig. 1.). *A. auricula-judae* extracts (at concentration of 0.025 – 0.100 mg/ml) were not toxic to baby hamster kidney fibroblast cell line (BHK 21) (Fig. 2.). The results of this study indicated that the extracts exhibited interesting protective effect against H_2O_2-induced baby hamster fibroblast cell line and they may be used as natural sources in pharmaceutical industry for the prevention of conditions that occur due to oxidative damage.

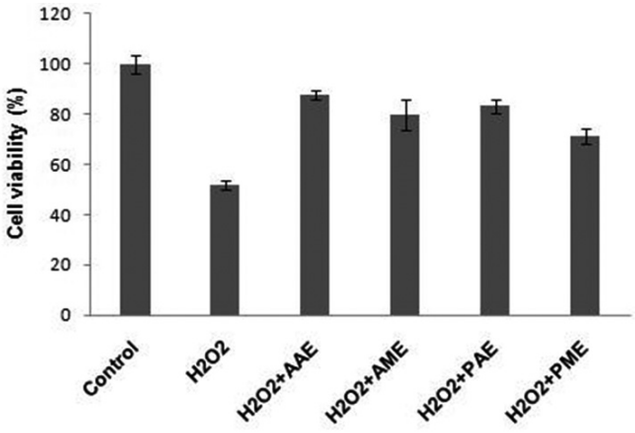

Figure 1: Protective effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.100 mg/ml) for 24 h and exposed to 1 mM H2O2-induced oxidative stress. Each value represents the mean ± SD of five wells.AAE; A.auricula-judae aqueous extract-AME; A. auricula-judae methanol extract; PAE; P. eryngii aqueous extract; PME; P. eryngii methanol extract

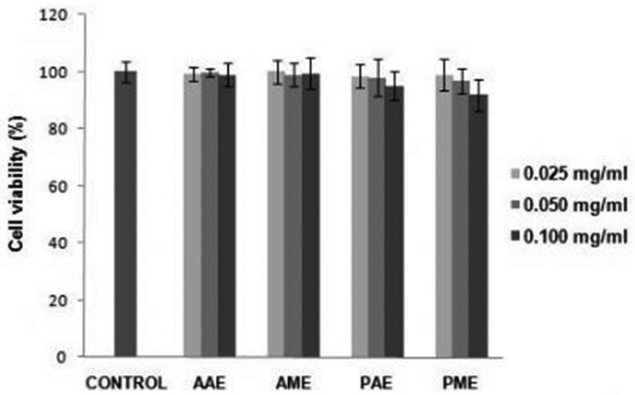

Figure 2: Cytotoxic effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.025, 0.050 and 0.100 mg/ml) for 24 h. Each value represents the mean ± SD of five wells.

Keywords: *Pleurotus eryngii*, *Auricularia auricula-judae*, HPLC, Phenolic composition, Protective effect, Oxidative damage, Cytotoxicity **References:**
1. Manzi P et al. (1999) Food Chem 65: 477 – 482. 2. Acharya K et al. (2004) Indian J Exp Biol 42: 538 – 540. 3. Luo Y et al. (2009) Innov Food Sci Emerg 10: 215 – 221. 4. Wasser SP, Weis AL (1999) Int J of Med Mushrooms 1: 31 – 62.

PK2 Apollon, a new *Artemisia annua* variety with high artemisinin content

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Artemisinin, a sesquiterpene lactone endoperoxide isolated from the herb *Artemisia annua* L. (Asteraceae), is a highly potent antimalarial compound, which is efficient againt multidrug-resistant strains of *Plasmodium falciparum*. The promotion of artemisinin-based combination therapies (ACTs) by the WHO during the past years lead to a strong pressure on the world market of artemisinin. The artemisinin world market is volatile and therefore efforts to improve performance of this culture are often limited. The use of varieties with high artemisinin content is a key factor for the development of such cultures. This should secure the supply of artemisinin, lower its cost of production and improve the competitiveness of this new culture with other commercial crops. After the variety Artemis, Mediplant launches a new variety called Apollon with about 20% yield increase. Performances of this new hybrid, with artemisinin content nearing 1.6%, are being presented.

PK3 Use of paclitaxel on balloon catheters against restenosis

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Besides the established use as chemotherapeutic agent against breast or ovarian cancer, paclitaxel coated on medical devices such as stents and balloon catheters has recently been applied in local prophylaxis and therapy of arterial stenosis/restenosis [1]. Paclitaxel is particularly suitable to inhibit injury-induced excessive intravascular scar formation following balloon angioplasty because of its strong and persistent antiproliferative properties [2]. The aim of this work was to optimize the balloon coating for clinical application. The challenge is to guarantee firm adherence of coated paclitaxel on its way through a hemostatic valve and atherosclerotic arteries to the target, and to allow optimal release at the lesion site. Paclitaxel was coated on the surface of angioplasty balloon catheters by a semiautomatic Hamilton microsyringe. HPLC showed a coating of ca. 3.0 µg/mm2 of paclitaxel. When introducing the catheter into the artery, the loss of paclitaxel was shown to be in the range of 15%. Upon the inflation of a balloon catheter in the stenotic segment of the artery, the surface gets in contact with the vessel wall for a few seconds up to one minute only [3]. After removal of the device from the artery ca. 10% of the drug were retrieved on the balloon, indicating the release of ca. 90%. The proportion absorbed by the vessel was ca. 10% as assessed in a porcine model. The pharmacological effect measured by angiography using the diameter stenosis as a parameter supported the benefits of the processed coating for clinical practice. **References:** 1. Rowinsky EK and Donehower RC (1995) N Engl J Med 332: 1004 – 1014. 2. De Labriolle A et al. (2009) Catheter Cardiovasc Interv 73: 643 – 652. 3. Waksman R and Pakala R (2009) Circ Cardiovasc Interv 2: 352 – 358.

PK4 Preclinical evaluation of red grapes seeds extract from *Vitis vinifera*, Burgund Mare, Recas, Romania as skin photochemoprotective agent

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Several studies have shown that polyphenols from grape seeds possess anti-inflammatory, antioxidant effects and inhibit the oxidative stress – mediated activation of MAPK and NF-kB involved in carcinogenesis pathways [1]. For the development of newer and more effective photochemopreventive agents we assessed the effect of grapes seed extract from Vitis vinifera L, Burgund Mare variety, Romania, with proved antioxidant and anti-inflammatory effects in vitro and on SKH-1 hairless mice [2]. Two groups of volunteers (n = 10) were exposed to one minimal erythema doses (1 MED) of UVB. For each subject we used 3 skin study areas, of the same size, from the posterior thorax: 1. ctrl – irradiated with 1 MED; 2. HA+I – pre-treated with vehicle (40 µl/cm2 hydroalcoholic solution – HA) and irradiated after 30 min with 1 MED; 3. BM+I – pretreated with BM extract 4 mg polyphenols/40 µl/cm2 in HA, irradiated at 30 min with MED. Skin biopsies were sampled 1 hour respectively at 24 hours after UVB irradiation. Samples were examined histopatologically and by immunohistochemistry for DNA damage and apoptosis (using Anti Cyclobutane Pyrimidine Dimers – CPD antibody and Caspase 3 antibody). The BM extract reduced sunburn cells number, acute inflammation and formation of UVB radiation-induced DNA damage as demonstrated by reduced amounts of CPD, ultimately leading to reduced apoptosis. Our results suggest that BM extract might be a potential chemopreventive candidate in reducing UV-induced skin cancer risk. **Acknowledgement:** *Project PN II-42104/2008* **References:** 1. Chakraborti S, Chakraborti T (1998) Cellular Signalling 10: 675 – 683. 2. Muresan A (2010) Acta Physiol Hung 97(2): 240 – 246.

PK5 Improvement of high-fat-diet-induced metabolic syndrome by ethanol extract of *Polygonatum falcatum* (ID 1215B) in mice

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Sirtuin, a NAD+-dependent class III histone deacetylase, is closely related to calorie restriction (CR) – mediated life span expansion in yeast and rodents. Recently, it has been also reported that sirtuins improve various chronic diseases associated with metabolic dysfunction such as diabetes and obesity. In this study, we screened to find Sirt1 (a mammalian homolog of sirtuin) modulating herbal extracts. We identified the ethanol extracts of *Polygonatum falcatum* A.Gray (ID 1215B) increased the expression of Sirt1 protein in HEK293 cells and further investigated the effects of ID 1215B on metabolic syndrome in mice. The metabolic effect was evaluated in male C57Bl/6J mice administered in high fat (HF) diet that were orally given a dose of 250 or 750 mg/kg/day of ID 1215B for 8 weeks. ID 1215B significantly decreased body weight gain, lipid accumulation in adipose tissue, serum triglyceride and free fatty acid levels and improved insulin resistance. In addition, ID 1215B increased the expression of genes related to mitochondrial biogenesis and fatty acid oxidation in the HF-diet mice. Taken together, our results indicate that ID 1215B may be a promising anti-obesity therapeutic agent that could improve the metabolic syndrome including the insulin resistance and hyperlipidemia as well as body weight gain. **References:** 1. Rodgers JT et al. (2005) Nature 434: 113 – 8 2. Lagouge M et al. (2006) Cell 127: 1109 – 22 3. Milne JC et al. (2007) Nature 450: 712 – 6

PK6 Researches regarding skin anti-photoaging effect of *Linum usitatissimum* L. oil by using an in vivo skin imagistic dermatologic evaluation

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Due to its content of unsaturated fatty acids and lignans (acting like phytoestrogens) *Linum usitatissimum* L. oil have been proved several pharmaceutical properties in dermatologic and cosmetic field. The current stage of knowledge concerning the dermatological uses of this oil is: - photoprotection effect, an well-known propriety, already used by a lot of production pharmaceutical laboratories, - seboregulating and noncomedogenic effect of linum seeds lignans. The main lignan in flaxseed is secoisolariciresinol diglucoside (SDG) which plays a major role in the sebum rate decrease, by being a 5 alpha-reductase inhibitor. - unsaturated fatty acids are essentials for maintain the epidermal physiology. LA is the most abundant fatty acid in the epidermis. Importantly, it is the precursor to ceramides, a major component of the extracellular lipid matrix that forms the stratum corneum permeability barrier (SCPB). [2,3] Additionally, omega 3 fatty acids are involved in prevention of skin cancers. Our study is advocated the skin anti-photoaging effect of *Linum usitatissimum* oil. This effect was performed in vivo, on healthy voluntaries, by registering the wrinkles involution with ProDerm Skin Analyzer. We admitted in study 22 females volunteers, ages between 33 an 56 years. The imagistic evaluations were registered after 14 days, 21 days and respectively 28 days of daily application. The oil content of the

studied varietes Lirina 29.9%, Alexin 26.1%, Floriana 22.5%, Florinda 31.3% and Iunia 96 28.9%, determined by Soxhlet method with a Velp block of mineralization. Investigation of fatty acid profile was performed by GC-MS.

PK7 Inclusion studies of falcarinol in β-cyclodextrin

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Falcarinol is a natural C17-polyacteylenic pesticide (phytoalexin) present in Apiaceae vegetables such as carrot (*Daucus carota* L.). Recently has attracted a lot of attention due to its interesting biological activities such as cytotoxic, antibacterial, antimycobacterial [1]. However, falcarinol suffers from photo- and thermal degradation, due to the presence of unstable triple bonds in its structure, which limits its possible applications. In the present work the thermal and photo-stability of falcarinol alone and in complex with β-cyclodextrin was studied. Falcarinol was isolated from the endemic Sardinian plant *Seseli praecox* (Gamisans) Gamisans (Apiaceae) [2]. Falcarinol/β-cyclodextrin complexes were prepared and the inclusion complex was initially characterised by NMR (ROESY) spectroscopy. Accelerated thermostabilty testing proved to be an extrememely agressive method for this type of constituent resulting in the complete degradation of both, the compound and its inclusion complex. On the other hand, photostability studies were carried out successfully as the β-cyclodextrin complex provided protection to the substance which kept its macroscopically properties and protected the substance from degradation. In comparison the photostability assay generated a loss of 15% in uncomplexed falcarinol. Therefore, inclusion in β-cyclodextrin was proved to be a good method for the photoprotection of falcarinol.

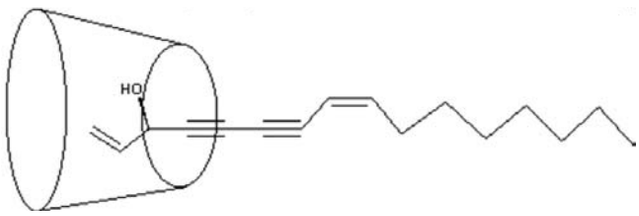

Figure 1: Falcarinol/ß-cyclodextrin inclusion complex

References: 1. Santos, PAG et al. (2005) Plant Science 168: 1089 – 1096. 2. Leonti M et al. (2010) Biochem Pharmacol 79: 1815 – 1826.

PK8 Development and stability of semisolid preparations based on *Gardenia jasminoides* Ellis extract

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The fruit of *Gardenia jasminoides* Ellis has been traditionally used as a Chinese medicine for centuries in China, as well as in other Asian countries [1]. The major and characteristic constituents of Gardenia fruit are iridoid glycosides such as geniposide, gardenoside, genipin-1-β-gentiobioside, geniposidic acid, acetylgeniposide, scandoside methyl ester, shanzhiside and gardoside. Modern clinical and pharmacological research has revealed that Gardenia fruit has anti-inflammatory properties, cytotoxic effects, as well as protective against oxidative damage [1,2]. In the Chinese Pharmacopoeia a topical application of the fruit of gardenia, powdered and mixed with water is reported [8]. Gardenia fruit also represents the principal effective ingredient of a preparation with antiinflammatory and analgesic properties, "Zhongtong Caji", a kind of liniment for external use, formulated with more than ten different herbal drugs [3]. The development of three semisolid preparations (anphyphil cream, sepigel and natrosol gel) based on *Gardenia jasminoides* Ellis extract is reported. Aqueous methanol (50% v/v) was selected in order to exhaustively extract the active constituents, iridoids (20% w/w). Stability of the developed preparations were investigated according to ICH guidelines. In vivo permeation of three selected formulations is investigated using the "skin stripping" test, according to the FDA, in healthy subjects. Analysis of iridoids both in the extract and in the stratum corneum were performed by HPLC-DAD-MS method. The sepigel gel showed the best stability and release profile in the in vivo tests. **References:** 1. Wang Y et al. (2010) Int J Pharm 392: 72 – 77. 2. Park E-H et al. (2003) Phytother Res 17: 961 – 962. 3. Pharmacopoeia of the People's Republic of China (2005) vol.1, 95 – 96.

PK9 Enhanced water solubility and stability of curcumin by microinclusion in natural and semi-synthetic cyclodextrins

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Curcumin is a natural polyphenolic constituent of *Curcuma longa* L. It has been generally associated with a large number of biological activities, including anti-oxidant, anti-inflammatory [1] and anti-cancer [2] properties. Although curcumin is a safe molecule even at high doses, its therapeutic use is limited by its low hydro-solubility in acid or physiological pH [3] and, consequently, by its poor bioavailability. Another drawback for clinical application of curcumin is its rapid hydrolysis under alkaline conditions and its photochemical degradation. The overall aim of this project is to increase solubility and stability of curcumin by microinclusion in cyclodextrins. Solubility studies of curcumin in presence of different concentrations of natural (α, β, γ) and semi-synthetic cyclodextrins (HEβ, DMβ, TMβ, RAMEB, HPβ, HPγ) were carried at different temperatures (25 – 37 °C). Thermodynamic parameters related to complex formation (ΔG, ΔH and ΔS) were also evaluated. Stoichiometry of curcumin inclusion and apparent equilibrium constants (K1, K2) were evaluated by Job's plot method using UV detection. Inclusion of curcumin into selected cyclodextrins was obtained by co-fusion, co-lyophilization, co-evaporation and physical mixture. Complex characterization was achieved by DSC, UV, NMR and HPLC/DAD analysis. Between the cyclodextrins tested the most efficient in order to maximise curcumin solubilisation was DMβ and the most effective complexation technique was the co-lyophilization. This latter was then employed, after curcumin complexation, for the realization of a topical formulation, useful as local anti-inflammatory medicament, and pharmacokinetic was evaluated by *in vitro* test using Franz cells apparatus. **References:** 1. Dong-Oh M (2008) Bioch Bioph Res Comm 375: 275 – 279 2. Preetha A et al. (2008) Cancer Lett 267: 133 – 164 3. Tonnesen HH et al. (2002) Int J Pharm 244: 127 – 135.

PK10 Effects of different irrigation intervals on yield and yield components of black cumin (*Nigella sativa*)

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Black cumin (*Nigella sativa* L.) is a medicinal plant with economic influences, especially in medicine production. A randomized complete block (RCB) experiment with three replications was conducted in 2010, to evaluate yield and yield components of black cumin (*Nigella sativa*) under no irrigation and three different irrigation intervals (7, 14 and 21 days) at research station of Islamic Azad university of Maragheh. Plants were sown in plots 20 cm plant to plant distance and 50 cm apart rows. Three irrigation intervals had significant effects on all studied characteristics. Results showed that increasing irrigation intervals to 14 days, increased number of capsules per plant, number of seeds per capsules and grain yield per plant, but produced smaller seeds. The lowest numbers of capsules and grain yield per plant were obtained in no irrigation treatment. Lowest Number of seeds per capsules and the largest grains produced in 21 days intervals. Increasing yield per plant in 14 irrigation interval was mainly attributed to the highest number of capsules per plant and number of seeds per capsules. **References:** 1. Ghamarinia H, Khosravy H, and Sepehri S (2010) J Medicinal Plants Research 4(16): 1612 – 1616. 2. Mohhebi M and Maleki H (2010) Advances in Environmental Biolog 4(1): 10 – 13. 3. Nourouzpour Gh and Moghadam P (2007) Agronomy and Horticulture 19: 43 – 47

PK11 Pharmacoeconomic evaluation of peppermint tea-bag products using graph theory

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Peppermint (*Mentha x piperita* L.) has very long tradition of medicinal use due to its essential oil content. It is often also used as tea and food flavoring. The content of essential oil of peppermint leaf is crucial for its medicinal, but also flavoring effects. There are plethora of tea products on the market for the consumers to choose from, usually based on the product's price and the external package appearance. The consumers commonly don't have the insight into the tea-bag's content pharmacognostic and chemical quality. We have used pharmacoeconomic framework to make the surveillance of ten peppermint tea-bag products present on Bosnian and Herzegovinian market. Consumers were asked to give grades 1 – 10 for the products external packaging. The unit price was determined for each product. Pharmacists were asked to rate organoleptic appearance of herbal content of tea-bags. Modified methods of GC and GC-MS analysis described by Kowalski and Wawrzykowski where used for the essential oil determination. The graph theory was chosen to sum up all results of different parameters for each product and give a quantitative estimate of the pharmacoeconomic acceptability. Relationship between tested parameters is presented in Figure 1. The obtained results reflect balance between external value on one side (< 1) and pharmacognostic quality (> 1), while value of 1 represents perfect balance of tested opposites. Of ten tested products, in 8 predominated external value (0,19 to 0,70), while in 2 products predominated pharmacognostic quality (1,67 and 1,77). The graph theory was useful in assessment of herbal products.

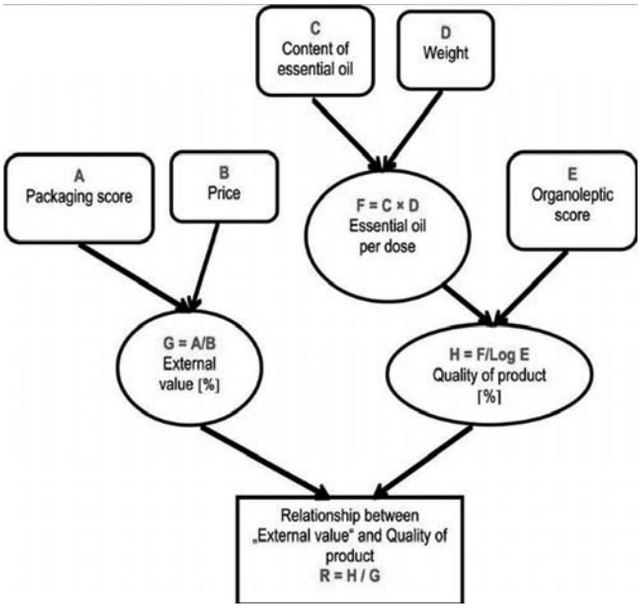

Figure 1: Relationship between tested quality parameters

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PK12 Phytochemical and hypoglycemic effect investigation of methanolic flower extract from *Piper claussenianum*

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Species of the genus *Piper*, the most important genus of Piperaceae Family, are widely used in traditional medicine for the treat many conditions. Chemical investigations of *Piper* species revealed many bioactive metabolites as amides, lignans, alkaloids, terpenes, flavonoids among others. In order to investigate native Piper species, phytochemical analysis of *Piper claussenianum* C. DC. flower extracts were performed. A flavonoid rich methanolic extract from *P. claussenianum* flowers was tested for hypoglycemic effect in rats with type 1 diabetes. Seven days after the induction, rats with glucose levels above 200 mg/dL began to be treated with vehicle or extract. Treatment lasted for 14 days and rats had glucose levels measured on days 0, 5 and 14. Glucose levels of both groups were also measured after 7 days of treatment interruption. Glucose levels of vehicle and extract groups were: day 0 (346.14 ± 41.67 and 275.29 ± 27.13, $p > 0.05$, $n = 7$), day 5 (290.14 ± 32.65 and 122.71 ± 7.19, $p < 0.05$, $n = 7$), day 14 (370.75 ± 77.89 and 137.50 ± 17.70, $p < 0.05$, $n = 4$). Seven days after treatment interruption, glucose levels were 304.14 ± 71.16 (vehicle, $n = 7$) and 255.50 ± 114.86 (extract, $n = 4$). Thus, the results suggest the remarkable presence of 2',6'-Dihydroxy-4'-Methoxychalcone on methanolic flower extract of *Piper claussenianum* has a noteworthy role to reduce blood glucose levels in rats with type 1 diabetes. **Acknowledgement:** *The authors thank to CNPq.*

PK13 Stability and staining property of gel from roselle calyx extract and butterfly pea flower

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Roselle calyx and Butterfly pea flower are among plants which have been used for coloring of food and beverage. The combination of the water extraction of the two plants present tanning color and was found interesting to study the property of an herbal staining gel. The herbs were extracted with boiling water for 2 hours, filtered and lyophilized until dried. The gel base was prepared by using combination of sodium carboxy methyl cellulose gel base and hydroxyethylcellulose 4000 gel base in 1:1 ratio. The herbal extractions were varied amount to add to the gel base. The color number in an expanded color chart were use to evaluate the usable color. The staining gel was tested for cracking, precipitation, changing of color, changing of pH before and after heating cooling cycle. The gel was tested for staining property by applied 0.5 g of gel on the pig skin and left for 1 hr then determine the color by using a color scale.The Mexameter MX 18 was used for determined of color uniformity of skin. The permanent of staining was tested by stirring the pig skin after staining for 24 hrs with 500 millilitres of water for 20 minutes and compare the color change. The gel with 1.35% of roselle extracted and 0.15% of butterfly pea extract was selected to be a staining gel. The color pH and physical property of gel are not changing after freeze and thaw for 3 cycles. The gel present consistency and permanent of staining. **Acknowledgement:** *The faculty of pharmaceutical science Khon Kaen university for supportive of grant.* **References:** 1. Kazuma K et al (2003) Phytochemistry 64: 1133 – 1139. 2.Therkildsen P et al (1998) Skin Res Technol 4: 174 – 179.

PK14 Stem gum of *Moringa oleifera* as pharmaceutical excipient

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The herbal gum exudates from the stem of *Moringa oleifera* Lam. is sparingly soluble in water but swells in contact with water, giving a highly viscous solution. It consists of arabinose, galactose, and glucoronic acid. In the present study the formulation of paracetamol tablets by

using *Moringa oleifera* gum as a binder was performed. The four different tablet formulations loaded drug as paracetamol were prepared by wet granulation method. The binder concentrations used in the formulation were 2, 4, 6 & 8% w/w of *Moringa oleifera* gum. Tablets were subjected for evaluation of hardness, friability, drug content uniformity. The preliminary evaluation for granules was done by measuring the granule size, angle of repose and percentage fines. The percent friability was in the range and tablet showed 98% to 99% of labeled amount of paracetamol indicating uniformity in drug content with 6 to 20 min disintegration time and more than 90% dissolution at 80 to 90 min. Tablets at 6% w/w binder concentration showed more optimum results as tablet binder. Paracetamol tablets were prepared using newly developed herbal gums as binder for the controlled release. The results indicated that tablets were successfully formed and displayed good binding properties to become the new source of binder. The herbal gums obtained from *Moringa oleifera* stem gum could be utilized in the development new pharmaceutical formulations.

PK15 Development and evaluation of conventional and PEGylated curcumin liposomes, absorption and tissue distribution studies in mice

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Curcumin is the main biological active polyphenolic compound present in the rhizomes of turmeric (*Curcuma longa* Linn.), has a wide biological and pharmacological profile. It has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic properties [1 – 3]. Many clinical study reports have revealed that curcumin has many beneficial properties in the treatment of various diseases in man such as pancreatic cancer and inflammatory bowel disease [4,5]. Despites these promising effects a poor oral absorption due to its extremely low aqueous solubility and rapid metabolism result in very low oral systemic bioavailability, thus limiting its clinical use. In order to overcome bioavailability drawbacks, this work proposes inclusion of curcumin into liposomal carriers. Liposomes were prepared by thin layer evaporation technique using phospholipon 90G, cholesterol and PEG-GSPE. Temperature of rehydration of thin film and curcuminamounts were optimized in order to maximise efficiency of entrapment of drug inside the vesicles. Vesicles were characterized by dynamic light scattering and HPLC/DAD. The pharmacokinetic profile was tested in mice after i.p administration using stealth and conventional vesicles and an alcoholic solution of the drug. After two hours administration organs (liver, spleen, intestine, mesentery and lung) were removed and curcumin dosed by HPLC/DAD/MS analysis. Liposomal inclusion increases bioavailability of curcumin in plasma, in particular, stealth formulation. A different accumulation was found among the different tested formulations. A further advantage of PEGylated liposomes is the high EE% of curcumin in the vesicles (70% compared to 47% of conventional liposomes). **References:** [1] Ruby et al. (1995) Cancer Lett 94: 79 – 83. [2] Lukita et al. (2002) Shock 17: 399 – 403. [3] Johnson et al. (2007) Cancer Lett 255: 170 – 181. [4] Dhillon et al. (2006) J Clin Oncol 24: 14151. [5] Holt et al. (2005) Dig Dis Sci 50: 2191 – 2193.

PK16 Supercritical CO_2 Extraction And Optimization of Total Phenols From Strawberry tree (*Arbutus unedo* L.) Fruits: A Comparative Study

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Arbutus unedo L. is an evergreen shrub of *Ericaceae* family growing on rocky slopes or in pine forests at the Mediterranean countries. Fruits of *A. unedo* were reported to possess astringent, diuretic and antiseptic properties and to have high flavonoid content, majorly proanthocyanidins, anthocyanins as glycosides of cyanidin and delphinidin with cyanidin-3 galactoside. Moreover, elagic acid, vitamin C, vitamin E and caretenoids were also quantified. The aim of this study was to optimize total phenol composition and radical scavenging activities of *A. unedo* fruits by supercritical fluid extraction, using response surface methodology and to compare the total phenol contents with that of traditional water and ethanol extractions. The independent variables were temperature (30 – 80 °C), pressure (50 – 300 bar) and co-solvent ratio (0 – 20%). The antioxidant capacities were determined by Folin-Ciocalteu and DPPH radical scavenging methods, whereas β-carotene bleaching method was used to evaluate the oxidative stability of the extracts in the linoleate emulsion model. The results demonstrated that temperature and co-solvent were more effective on yields of the compounds, whereas the effect of pressure was not noticeable. Optimum extraction conditions were elicited as 60 bar, 48 °C and 19.7% yielding 25.72 mg GAE total phenols/g extract and 99.9% radical scavenging capacity which were significantly higher than water (24.89 mg/g; 83.8%) and ethanol extracts (15.12 mg/g; 95.8%), whereas oxidation rate ratio (0.892) was close to that of water extract (0.661) demonstrating challenges as a green separation process for industrial applications.

PK17 Anticarcinogenic effect of *Cyclotrichium origanifolium* on human colorectal cancer cell line and associating with its antioxidative properties

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The aim of the study was investigating anticarcinogenic and antioxidative effects of *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. The anticarcinogenic activity of the methanolic and the water extract from *C. origanifolium* (at 10 – 1000 μg/ml concentrations) in CCL-221 (colorectal cancer line) and Caco-2 (colon cancer cell line) was determined using trypan blue exclusion test. On the other hand, antioxidant activity of the extracts was evaluated by using DPPH radical scavenging, metal chelating, plasma lipid peroxidation and β-carotene beaching assays. In addition, the measurement of total antioxidant compounds in the extracts were carried out. The most effective anticancer activity has been shown at concentrations 500 and 1000 μg/ml of the extracts against CCL-221. The water extract showed anticarcinogenic properties in CCL-221 with maximum inhibition of 81.3 ± 2.2% over the control at 1000 μg/ml. Also, no cytotoxic effect of the extracts was observed against normal cell line (human fibroblast cell line). *C. origanifolium* water extract (36.17 ± 0.35%) showed the higher inhibitory effect against plasma lipid peroxidation than the methanolic extract (20.02 ± 2.17%). *C. origanifolium* water and methanol extract showed effective scavenging activity against DPPH radicals (IC_{50} = 0.049 ± 0.001 mg/ml and IC_{50} = 0.051 ± 0.003 mg/ml, respectively). These results showed that *C. origanifolium* may be used in pharmaceutical applications due to its remarkable antioxidant and anticancer activity against CCL-221. Further studies such as fractionation and purification of the extract must be carried out in order to identify natural compounds with anticancer activities. **Keywords:** *Cyclotrichium origanifolium*, anticancer effect, antioxidant effect, cytotoxicity

PK18 Effect of *Urtica dioica* on proliferation of HCT-116 colon cancer cell line

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Urtica dioica L. (stinging nettle), a member of the Urticaceae family is a plant which has been used as a remedy for diabetes mellitus (1), benign prostatic hyperplasia (2), arthritis (3), allergic rhinitis (4), hypertension and cardiovascular disease (5). In recent studies it was shown that extract of *Urtica dioica* exhibited significant growth reduction in human prostatic epithelial cells (6) and inhibition on adenosine deaminase activity in prostate tissue. In this study we aimed to investigate the effect of *Urtica dioica* on proliferation of HCT-116 colon cancer cell line. Herbal preparation of *Urtica dioica* was made by ethanol extraction which was followed by evaporation. HCT-116 cells were incubated with different doses of *Urtica dioica* ranging from 3,33 mg/mL to 42,8 mg/mL for 24 hours. Cell viability was measured with MTT test. Results showed that Urtica dioica (33,3 mg/mL, 42,8 mg/mL) inhibited HCT-116 colon cancer cell proliferation significantly ($p < 0{,}001$). Further studies are needed to reveal the effectiveness of *Urtica dioica* as an alternative therapy for colon cancer. **References:** 1) Rasal VP, Shetty BB, Sinnathambi A, Yeshmaina S, Ashok P (2006) Int J Pharmacol 4(2): 22. 2) Krzeski Tet al.

(1993) Clin Ther 15: 1011 – 1020. 3) Chrubasik S, Enderlein W, Bauer R, Grabner W (1997) Phytomedicine 4: 105 – 108. 4) Mittman P (1990) Planta Med 56: 44 – 47. 5) Daher CF, Baroody KG, Baroody GM (2006) Fitoterapia 77: 183 – 188. 6) Konrad L, Müller HH, Lenz C, Laubinger H, Aumüller G, Lichius JJ (2000) Planta Med 66(1): 44 – 7.

PK19 Smart Nanoparticles as new Drug Delivery Systems: Bioapplications

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In recent years, the design of multifunctional polymeric materials in the submicrometer size has been considerably improved due to their wide applications in the fields of biomedicine. Particularly, hollow polymeric nanospheres and micelles have attracted a great deal of attention due to their wide range of applications. These structures have potential utility in encapsulation and controlled release of various biomolecules such as drugs, peptides and genes. A variety of multi stimuli-responsive nanoparticles have been synthesized that are capable of conformational and chemical changes on receiving an external signal. These changes are accompanied by variations in the physical properties of the polymer. The signal is derived from changes in the materials' environment, such as a change in temperature or in pH. On the one hand, we have synthesized, characterized and study organic micro- and nano-spheres for magnetic and non magnetic properties. Specifically, pH and thermal responsive hollow microspheres were prepared using the distillation precipitation polymerization method with magnetic nanoparticles encapsulated either in the shell or in the core. These novel hybrid microstructures were characterized with transmission electron microscopy, scanning electron microscopy, dynamic light scattering, vibrating sample magnetometry, X-ray diffraction and FT-IR spectra. On the other hand, polymeric micelles seem to be one of the best carriers for delivering hydrophobic drugs.They are formed by the self-assembly of amphiphilic block copolymer in aqueous solutions and have a spherical shape and a size in nano-range. Anticancer drugs that are incorporated into micelles were shown to improve their stability and efficiency. **Acknowledgement:** *This work was supported by scientific programme "IDEAS", ERC Advanced Grand Nanotherapy. Project Reference: 232959.* **References:** [1] Kataoka K. et al. (2003) Angew Chem Int Ed. 42: 4640 – 4643. [2] Minko S et al. (2010) Nature Materials 9:101 – 113. [3] Piskin E et al. (2007) Prog Polym Sci 32: 534 – 595. [4] Sukhorukov GB et al. (2004) Langmuir 20: 7265.

Topic L: Pharmacognosy/Pharmaceutical Biology and Biodiversity

PL1 Evaluation of Burn Healing Activity of Black Seed Oil in Rats

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This study investigated the burn healing efficiency of Black seeds (*Nigella sativa* L.) oil on the second degree burn wound models in rats. Many of pharmacological activities of *Nigella sativa* seeds such as anti-inflammatory and antioxidant were due to unsaturated fatty acids and essential oil [1,2,3]. In this study the hexanic extract of seeds was topically applied to evaluate the healing activity of seeds oil. Animals were randomly divided into three groups of six for each group. Burn wounds were created on dorsal part of shaved rats by a soldering iron with a flat contact surface (diameter 1.5 cm) on top (100 °C for 10 seconds). Silver sulfadiazine (SSD) was used as an antiseptic standard drug. Wound healing was evaluated by the rate of contraction and histological characteristics in treated and untreated groups. On day 12, the extract-treated animals showed 81.20% decreasing in the wound district and were significantly ($P < 0.05$) more than control group 63.31%. Histological study showed fully grown regenerated epidermis on day 12 in treated animals. The results of this study suggest that burn wound healing potential of seeds may be due to anti-inflammatory, antioxidant and antimicrobial activities of main compounds oil.

PL2 Genetic variation study among *Lepidium sativum* resources in Egypt

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Three local resources (Rajab, Haraz and Khider) of *Lepidium sativum* L. were used to study the genetic variation in Egypt. The study included quantitative characters, fatty acid and DNA fingerprint. Five quantitative characters were studied among three successive seasons. Separated and combined statistical analysis presented significant variation among resources and among interaction between resources and seasons in the plant characters. GLC analysis of fatty acid methyl esters were carried out for each resource. Arachidonic acid was the most abundant acid followed by linoleic in Khider resource, while behenic acid was the most abundant acid followed by arachidonic acid in both Haraz and Rajab resources. By using six primers, DNA fingerprint showed differences in the number of bands among resources. The variations among *Lepidium sativum* resources were confirmed on quantitative characters, fatty acids and DNA fingerprint level.

PL3 Anti-Dengue virus activity of *Polygonum spectabile* (Polygonaceae)

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Dengue is considered a worldwide public health problem and is responsible for thousands of deaths annually. However, the main strategies for prevention and control of dengue are focused on the virus vectors, since there are no specific therapeutic agents or vaccine for dengue viruses. In the quest of new anti-dengue drugs we have screened plant extracts which disclosed the activity of the aerial parts of *Polygonum spectabile* Mart., a plant traditionally used in Brazil for treatment of several infection diseases. However, the compounds isolated have shown no activity against DENV-2 (1) what has motivated a further investigation of this extract. We report here the evaluation of fractions obtained from the crude ethanol extract by partition between immiscible solvents and of the fractions from the ethyl acetate chromatography over a Sephadex LH20 column®. The *in vitro* cytotoxicity (LLCMK2 cells) and antiviral activity were evaluated by the MTT colorimetric method. Three, out of the seven fractions from the Sephadex LH20 column of the ethyl acetate fraction, have inhibited the viral multiplication cycle with EC_{50} values between 41.52 ± 3.7 μg/ml and 5.58 ± 2,9 μg/ml. The determined CC_{50} were between 197,78 ± 19,7 μg/ml and 257,34 ± 24,1 μg/ml. Good SI values (CC_{50}/EC_{50}) have been calculated: 5.88, 11.52 and 46.11. These results show that chemical constituents of these fractions might be promising as anti-dengue drugs. Phytochemical and molecular studies are on progress aiming to determine the active compounds and mechanisms of action. **Keywords:** Dengue virus, antiviral activity, *Polygonum spectabile* **Acknowledgement:** *To VIRIONTECH and CNPq-National Research Council, Brazil* **References:** 1. Brandão GC et al. (2010) Phytomed 17: 926 – 929.

PL4 Inter-population variation in phenolic content of *Teucrium chamaedrys* L. from the localities in the Balkan Peninsula

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Total phenolic content and flavonoid concentrations in methanolic extracts obtained from *Teucrium chamaedrys* L. in five natural populations of the Balkan Peninsula and a garden population were investigated and compared. The above-ground parts of plants were collected during the flowering phase and the methanolic extracts were prepared. The total phenolic content of the extracts was determined using Folin-Ciocalteu reagent and expressed as gallic acid equivalent. The obtained values varied between 142.04 mg GA/g and 265.91 mg GA/g. The concentration of flavonoids was determined using $AlCl_3$ and expressed as rutin equivalent. The obtained values ranged between 55.66 mg Ru/g and 90.48 mg Ru/g. The highest phenolic content was found in the plants collected from the mountain areas (Bulgaria, Serbia, Bosnia and Herzegovina) and somewhat lower content was found in plants from Mediterranean localities (Montenegro, Croatia). The lowest level was found in the extract obtained from the cultivated plant (Greece). The highest concentration of flavonoids was found in the plants from Mediterranean localities (Croatia, Montenegro), while the levels were lower in the other samples and ranged between 50 and 70 mg Ru/ml. On the basis of comparative analysis, the plants collected at higher altitude localities were found to be richer in total phenolics, while higher concentration of flavonoids was found in T. chamaedrys from Mediterranean localities. A cultivar of *T. chamaedrys* had lower concentration of phenolics in comparison with natural populations. The results obtained in the analysis point out that the concentration of phenolics depend on the ecological properties of the plant habitats. **Acknowledgement:** *Ministry of Science and Education, Republic of Serbia (III41010)*

PL5 Phytochemical and pharmacological studies of *Ficus auriculata* Lour. (Family Moraceae) cultivated in Egypt

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This study scientifically examined the phytochemistry, antibacterial and anti-inflammatory potencies of two extracts of *Ficus auriculata* Lour. Eight known compounds, including: betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, β-sitosterol-3-O-β-D-glucopyranoside, myricetin and quercetin-3-O-β-D-glucopyranoside were isolated from the petroleum ether, chloroform and ethyl acetate fractions of alcoholic extracts of the leaves and fruits of *Ficus auriculata*. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from *Ficus auriculata* (Moraceae). Concerning the biological studies, the results revealed that both extracts were effective against gram + ve bacteria (*Staphylococcus aureus*) and gram – ve bacteria (*Esherichia coli*) by agar well diffusion method. However, ethanolic extract of leaves exhibited greater antibacterial activity than the ethanolic extract of fruits. Meanwhile, the ethanolic extract of leaves at dose of 500 mg/kg exhibited significant anti-inflammatory effect using carrageenin-induced rat hind paw oedema model. **Keywords:** *Ficus auriculata*, Moraceae, antibacterial activity, anti-inflammatory

PL6 *Moringa oleifera*-treated dry season-turbid Well-water in Enugu Metropolis, Nigeria: A comparative evaluation

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Water and sanitation services provide a cost-effective solution for alleviating the impact of water-borne diseases. Polluted water is gateway to infectious pathogens leading to a both acute and chronic-diseases worldwide. With the ultimate objective of contributing to the improvement of the quality control of drinking water, we report here, the main application of *Moringa oleifera* Lam. seed extract in the treatment of 25 natural underground well-water samples randomly collected from the three most populous cities in Enugu Metropolis, in southeastern Nigeria. The assessed parameters were salinity, pH, conductivity, total dissolved solid (TDS), total solids (TS), total suspended solids (TSS), turbidity and microbial load before and post-treatment with both alum (as a standard agent) and *M. oleifera* aqueous and ethanolic extracts at equal concentrations of 60 mg/L. The result of the finding showed the ability of *M. oleifera* seed extract to remove organic matter (natural humic substances and micropollutants) thereby avoiding water degradation (mainly bad odours and taste, formation of disinfection by-products such as trihalomethanes) and in addition to having a potent antimicrobial activity which alum naturally lacked. The ethanolic extract of *M. oleifera* had broader spectrum of antibacterial activity than aqueous extract. The alum-treated water samples showed increased salinity and pH in addition to other by-products. From the foregoing, the use of *M. oleifera* aqueous and ethanolic seed extracts as alternative biocompatible flocculants in water treatment in Enugu Metropolis could be recommended. **Acknowledgement:** *This work is a product of research for a Fellowship award of Nigerian Institute of Science Laboratory Technology (NISLT).*

PL7 The effect of *Salvia virgata* on GSH-Px Activities of HepG2 cells.

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Turkey is an important country for *Salvia* species. The flora of Turkey includes 88 species of the genus *Salvia*. *Salvia virgata* Jacq. which has shown to be extremely rich with the phenolic compounds that allows this species to be an important member of antioxidant plants. This study was performed to investigate the effect of different *Salvia* extracts on GSH-Px activities of HepG2 hepatocarcinoma cells. The 70% methanol and water extracts were prepared from the aerial parts of *S. virgata* collected from Bursa, Turkey. Gallic acid and rosmarinic acid were used as positive controls. The cells at a number of 2×10^5 cells per well were incubated for 24 h with the extracts and the positive controls under %5 CO_2 at 37 °C. The GSH-Px activities of the cells were than analysed spectrophotometrically via a multifunctional microplate reader. Phenolics rich extract of aq. methanol has enhanced the GSH-Px activity more than water extract where their effect was just in between the rosmarinic acid and gallic acid positive controls. These results reveal that both extracts mostly the phenolics rich extract of aq. methanol supports the antioxidant activity in the hepatocarcinoma cell line and these results confirm that it can further effect the glutathione reserves of these cells. This peliminary results needs to be further investigated over the GSSG, GSH and total gluathione and selenium levels. **Keywords:** *Salvia Virgata*, HepG2, GSH-Px, antioxidant **References:** 1. Kosar M, Goger F, Baser KHC (2008) J Agric Food Chem 56(7):2369 – 74 2. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E (2009) Biol Res 42(2):175 – 81 3. Tepe B (2008) Bioresour Technol 99(6):1584 – 8

PL8 Anti-inflammatory effect and lipoidal content of *Acrocarpus fraxinifolius* Wight & Arn leaves

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Acrocarpus fraxinifolius Wight et Arn commonly known as mundane and shingle tree. it is a stately deciduous tree, attaining heights of 30 – 60 m; stem cylindrical, free of branches for up to 75% of its total height. It can achieve a diameter of over 200 cm. The dried powdered leaves of *Acrocarpus fraxinifolius* was successively extracted with solvents of increasing polarities (petroleum ether, chloroform, ethyl acetate and methanol) as well as the total ethanol extract of the powder was prepared. The petroleum ether extract was fractionated into unsaponifiable matter (USM) and fatty acids (FA). The FA fraction was methylated to give FAME fraction. GC/MS analysis of the USM revealed the identification of twenty five compounds represented 74.2% of the total USM, with phytol (43.73%) as the major compound followed by butylated hydroxy toluene (9.96%), n-hentriacontane (6.02%) and squalene (3.56%). Oxygenated compounds represented 59.8% of the total USM. GC/MS analysis of FAME fraction revealed the identification of twenty two compounds represented 85.49% of the total fraction, with methyl 9,12,15- octadecatrienoate (21.05%) as the major compound followed by methyl hexadecanoate (20.09%), methyl 9,12- octadecadienoate (19.98%) and methyl octadecanoate (8.96%). The unsaturated FA represented 41.61% of the total fraction. The acute anti-inflammatory effect of the total ethanol and successive extracts was evaluated by measuring the percentage of reduction of rat hind paw oedema induced by carrageenan [1] which revealed good effects exhibited by all extracts. Ethyl acetate extract was found to be the most potent (93.11% potency) in comparison with indomethacin (100% potency). **References:** 1. Winter GA et al. (1962) Proc Soc Exp Biol Med III: 1544 – 1547

PL9 The first antibacterial activity report of three selected Malaysian rainforest medicinal plants

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Despite increasing resistance among clinically important gram-negative and gram-positive pathogens to many common antibacterial agents, many large pharmaceutical companies are showing decreased interest in this product area that is so critical to public health. This downturn in antibacterial discovery and development, in turn, is leaving us vulnerable to emerging resistance, particularly to recently arrived vancomycin-resistant *Staphylococcus aureus* and multiply resistant, gram-negative bacilli for which we do not have adequate antimicrobial therapy in the future. Rainforest plants possess therapeutic potential, including antimicrobial activity [1,2]. Therefore, this study is a screening program of several extracts from three endemic medicinal plants in Malaysian rainforest which have not been fully discovered and investigated for their antimicrobial properties against several Gram-positive and Gram-negative bacteria strains. These plants included families namely Annonaceae, Ebenaceae and Burseraceae. The antibacterial activity of hexane, chloroform and ethanol fractions of some parts of *Uvaria grandiflora* Roxb. (Annonaceae), *Diospyros wallichii* King & Gamble (Ebenaceae) and *Canarium patentinervium* Miq. (Burseraceae) was determined against Gram-positive bacteria *Bacillus cereus* ATCC 10876, Staphylococcus aureus ATCC 11632, Methicilin resistant Staphylococcus aureus ATCC 43300 and Gram- negative bacteria *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 10536 using the disk diffusion method. Results showed that the bark ethanol fraction of *U. grandiflora*, the fruit hexane fraction of *D. wallichii* and the leaf ethanol fraction of *C.patentinervium* are active (Table 1). The results indicate that these medicinal herbs can be used as active and potent ingredients in the formulation of natural antibacterial products. **References:** 1- Wiart C (2006) Medicinal Plants of the Asia-Pacific: Drugs from the Future, World Scientific, Singapore. 2. Harvey AL (1999) Trends Pharmacol Sci 20: 196 – 198.

PL10 Bioactive Constituents from *Gleditsia triacanthos* L. leaves

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Gleditsia triacanthos L. is a deciduous tree belonging to family Fabaceae. It flowers in July, and the seeds ripen from October to November, Different extracts of *Gleditsia* posses important pharmacological activities in treating rheumatoid arthritis [1], as anti-mutagenic [2], anticancer [3] and have significant cytotoxic activity against different cell lines [4].The dried powdered leaves of *G.Triacanthos* was successively extracted with solvents of increasing polarities (petroleum ether, chloroform, ethyl acetate and methanol) as well as the total ethanol extract of the powder was prepared. The petroleum ether extract was fractionated into unsaponifiable matter (USM) and fatty acids (FA).The FA fraction was methylated to give FAME fraction. GC/MS analysis of the USM revealed the identification of twenty four compounds represented 76.86% of the total USM, with squalene (22.68%) as the major compound followed by nonacosane (21.03%) and isophytol (14.70%). The oxygenated compounds represented 23.28% of the total fraction. GC/MS analysis of FAME fraction revealed the identification of twenty one compounds represented 85.87% of the total fraction, with methyl 9,12,15- octadecatrienoate (31.37%) as the major compound followed by methyl hexadecanoate (19.35%), methyl 9,12- octadecadienoate (13.52%) and methyl octadecanoate (8.52%). The unsaturated FA represented 45.64% of the total fraction. The acute anti-inflammatory effect of the total ethanol and successive extracts was evaluated by the carrageenan induced rat hind paw oedema test Winter, et al. [5], which revealed a moderate effect of all extracts. The most potent effect was exhibited by 100 mg/kg b.wt. of the total ethanol extract (74.60% potency) in comparison with indomethacin (100% potency). **References:** 1. DaiY, Ye W, Fu L (2002) Patent Appl. 2,002,160,095,31 2.Lim JC et al. (2005) Chem Pharm Bulletin 53(5: 561 – 564 3. Klyosove A., Platt D (2001) Patent Appl. Woo 276, 474 4. Zhong L et al. (2004) Planta Med 70(9): 797 – 802 5. Winter GA et al. (1962) Proc Soc Exp Biol Med III: 1544 – 1547

PL11 Statistical software R and Wolfram Mathematica7 in studying variability of *Satureja montana* in Albania

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Albania is one of the most important exporters of medicinal and aromatic plants in Europe. *Origanum vulgare* L., *Thymus capitatus* (L.) Hoffmanns and *Satureja montana* L., are are endangered species and are included in Albanian National Red Data Book. All of these three plants produce essential oils which are rich in phenolic compounds (Carvacrol or thymol are the dominant phenols in their essential oils) and are used as oregano spices. Using R and Mathematica7 to study the variability of these medicinal plants, provide a very interesting example for further essential oils research on less known MAP resources of the European flora and protect biodiversity. The combinations of modern statistical analyses provide the clear method to analysis the variability of essential oils in MAPs. This will lead to better statistical understanding of the data and higher economic gains.

PL12 Effect of biostress on accumulation of secondary metabolites in two *Hypericum* species

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In the presence of a pathogen attack, plants developed a vast array of metabolic defense responses sequentially activating the production of bioactive secondary metabolites [1]. The present study aimed to detect how fungal and bacterial biostress alter the phytochemical profile of *Hypericum perforatum* L. and *H. triquetrifolium* Turra known for their pharmacological activities.The greenhouse grown plants were inocu-

lated with four different doses of the fungal pathogen *Seimatosporium hypericinum* and the soil bacterium *Pseudomonas putida*. Secondary metabolites were analysed by HPLC-DAD. An analysis of covariance was used to measure the overall effect of different inoculation doses of microorganisms on concentrations of metabolites. According to the results, inoculation of *H. perforatum* with both microorganisms had a significant effect on positive changes of hypericins, hyperforin, rutin, hyperoside, isoquercitine and total phenolics. In *H. triquetrifolium* the amounts of hypericins and phenolic compounds did not vary significantly under the higher doses of inoculums with exception of a positive accumulation of hyperforin. The increased accumulation of hyperforin can be described to be very important in plant defense mechanism triggered by some of the components excreted by the microorganisms. The increased accumulation of hyperforin can be described as a most important compound in plant defense mechanism triggered by some of the components excluded by the microorganisms. The comparison of the microorganisms' effect on the biosynthesis of secondary metabolites showed that pathogenic fungi seem to have more influence than bacteria. The two species of *Hypericum* showed differences in the accumulation of secondary metabolites induced by biostress. Biological stimuli of microorganisms may allow a specific modulation of the biosynthesis of some desirable metabolites in plants. **Acknowledgement:** *The research was supported by Research Council of Lithuania; project number MIP-57/2010.* **References:** 1. Conceição et al. (2006) Floriculture, Ornamental and Plant Biotechnology Vol. 3:483 – 487. Global Science Books. UK.

PL13 The content of fagopyrin and polyphenols in common buckwheat (*Fagopyrum esculentum* Moench) sprouts depends on growing conditions and the phase of development

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Dried buckwheat herb (*Fagopyrum esculentum* Moench) is used in medicinal products and the fresh green plant parts, especially sprouts, are consumed as a vegetable [1,2]. The herb contains fagopyrins, which cause sensitivity to light after the ingestion of large amounts of the green parts of buckwheat [3]. The aim of this study was to investigate the impact of different growing conditions and development phase on the content of fagopyrin and phenolic compounds in buckwheat sprouts. Total flavonoid and total phenol contents, fagopyrin content and antioxidant activity were determined spectrophotometrically. Fagopyrin and flavonoids were located almost exclusively in cotyledons. It was found that the content of fagopyrin in 14-days-old buckwheat sprouts grown in a sprouter was nearly the same as reported for mature plants, but the content of polyphenols was only at approximately 20 to 30%. The safe intake of buckwheat sprouts was then estimated to be at least 40 g per day. **References:** 1. Hinneburg I, Neubert Reinhard HH (2005) J Agric Food Chem 53: 3 – 7. 2. Kreft I et al. (2006) Food Chem 98: 508 – 51 3. Chick H, Ellinger P (1941) J Physiol 100: 212 – 230.

PL14 Comparative phytochemical study on *Veronica officinalis* L. and *Veronica chamaedrys* L

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Criteria to avoid the substitution of *Veronica officinalis* L. (common speedwell) with other species of the genus *Veronica* (Plantaginaceae sensu APG 2003, formely Scrophulariaceae) are urgently needed [1], especially *Veronica chamaedrys* L. (germander speedwell), widely spread and without therapeutic action. We have studied the differenzial phytochemical characters, for the two species regarding the iridoids and polyfenolic compounds content. In these species we have determined the aucubin and catalpol content by using a HPLC analysis with mass spectrometry detection. The content of aucubin is 107.4 μg% for *V. officinalis* and 328.6 μg% for *V. chamaedrys* The content of catalpol is 232.2 μg% for *V. officinalis* and 144.4 μg% for *V. chamaedrys* [2]. The polyphenolic compounds were determined in the two species before and after acid hydrolysis. The identification of these compounds was achieved through a HPLC analysis with mass spectrometric detection, by comparison with 18 polyphenol standards. The quantitative analysis of the polyphenols, based on UV detection, was performed using an external standard method [3]. The most significant difference between the two species is in the qualitative and quantitative content of polyfenolic compounds and it can be a criteria to phytochemical differentiation of *V. officinalis* and *V. chamaedrys* **Acknowledgement:** *This work was supported by the grant PN II 32151/2008 financed by MECI Romania* **References:** [1] APG (Angiosperm Phylogeny Group). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II (2003) Bot J Linn Soc, 141: 399 – 436 [2] Crişan Get al. (2010) Farmacia 58(2): 237 – 242 [3] Crisan G, Vlase L, Balica G, Crisan O (2009)., Rev Med Chir Soc Med Nat Iaşi 113(2): Supliment nr. 4, 81 – 85

PL15 Pharmacognostic studies and establishment of quality parameters of *Albizia altissima* (Hook.f) Hutch et Dandy

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Albizia altissima (Hook.f) Hutch et Dandy is a deciduous tree that grows up to 15 m in height and 25 cm in diameter and is found in various parts of Africa from Sierra Leone to West Cameroon, Sudan and up to Angola. It is used as a fish poison and in traditional medicine for the treatment of mental illness, snake bites, stomachache and toothache. The use in ethonomedicine for the treatment of mental disorders has been scientifically investigated and and the results validated the ethno medicinal use Pharmacognostic studies of the leaves were carried out following the World Health Organization guidelines on the establishment of quality standards for medicinal plants. Other physiochemical parameters were also determined. Pharmacognostic investigations include macro and microscopic studies on fresh and powdered leaves, physiochemical constants like total ash, extractable (water and alcohol) material and chromatographic fingerprint analysis. The results showed the presence of paracytic stomata in the leaves exculsively on the lower surface, free prisms of calcium oxalate and clusters of calcum oxalate crystals embedded in the epidermal cells. The amount of water extractable matter was 130 mg/g, that of alcohol extractable matter 150 mg/g and the ash value was 30 mg/g. The results of this study are helpful for the preparation of a monograph for the *Albizia altissima*. **Acknowledgement:** *The authors acknowledge Ajibesin K K and Raji R* **References:** World Health Organization (1998) Quality control methods for medicinal plants, Geneva

PL16 Development of a novel botanical drug (DA-9701), as a new prokinetic agent

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Functional dyspepsia (FD) is a highly prevalent chronical gastrointestinal disorder that causes a considerable burden to both the patient and society. In the past ten years, several herbal extracts were reported from natural sources in our laboratory. This research was also carried out as a continuous work on bioactive extracts and for the development of prokinetic drugs from natural sources. Based on our prokinetic prescreening data, Corydalis tuber (*Corydalis ternata* Nakai) and Pharbitis seed (*Pharbitis nil* Choisy) were selected for this research. A prokinetic agent, DA-9701 has newly formulated with Corydalis tuber and Pharbitis seed. We evaluated the gastroprokinetic effects of DA-9701 to develop a therapeutic for FD. Corydalis tuber have been used as traditional Chinese medicine (TCM) in the treatment of gastric and duodenal ulcer. Pharbitis seeds are the seeds of Pharbitis nil Choisy of the Convolvulaceae family, has been used as a folk medicine for analgesic effects on the abdomen in the TCM. Oral administration with DA-9701 significantly accelerated gastric emptying and gastrointestinal transit. Furthermore, DA-9701 increased the gastric accommodation in Beagle dogs. These results indicate that DA-9701 has potential as a safe and effective prokinetic agent capable of lessening gastrointestinal symptoms and increasing quality of life in FD patients with abnormalities in GI motor function. At the present time, product development is in progress for complement of phase

III clinical study in 2010, NDA and release of product in 2011. **Acknowledgement:** *This work was supported by grants from the Plant Diverse Research Center of 21C Frontier R&D programs from the Ministry of Science & Technology in Korea.*

PL17 A novel botanical drug (DA-9801) for the treatment of diabetic neuropathy

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Diabetic neuropathy is one of the most common causes of chronic neuropathic pain. In our search for bioactive constituents from plant sources, we found a diabetic neuropathy agent, DA-9801. Ethanol extract of two herbal mixture (the rhizome of *Dioscorea japonica* Thunberg and *Dioscorea nipponica* Makino. DA-9801 induces increases in endogenous Nerve growth factor (NGF) levels, and thereby has a protective effect against diabetic neuropathy. NGF plays an important role in the survival and maintenance of neurons in the nervous system and in nerve injury repair. The rhizome of *D. japonica* has been used in traditional medicine and as food in East Asia to strengthen stomach functions and to dilute sputum in TCM. The rhizome of *D. nipponica* has been used in traditional medicine in East Asia for treatment of rheumatoid arthritis and diabetes. After phytochemical investigation we found 2 new furostanol saponins besides 13 known compounds in DA-9801. We evaluated the anti-diabetic neuropathic effect of DA-9801 in a streptozotocin (STZ)-induced animal model. After treatment with DA-9801, NGF levels increased significantly in STZ-induced diabetic rats. Results from a nociceptive test (thermal & mechanical hyperalgesia) showed an increased latency time in groups treated with DA-9801 when compared with control and reference drug groups. The results suggest that DA-9801 may improve the damage produced by diabetic neuropathy via increasing the level of NGF in target tissue, shows improvement on nerve conduction velocity (NCV) and recovery from nerve degeneration. Therefore DA-9801 might have a potential therapeutic effect in patients with diabetic neuropathy. **Acknowledgement:** *This study was supported by grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (2011-A111082)*

PL18 A polyphenol enriched *Theobroma cacao* bean extract for cosmetic application

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It was found that long-term ingestion of cocoa flavonols was able to prevent a variety of dermal disorders associated to UV exposure, like decrease of skin thickness and skin density (1), possibly by means of production of glycosaminoglycans and collagen (2). The antioxidant activity seems to be the underlying mechanism (3). These findings led to the development of a polyphenol enriched cocoa bean (*Theobroma cacao* L.) extract for further use as a cosmetic ingredient. The proposed method of extraction of polyphenols was able to to avoid the formation of insoluble tannins that normaly occurs in the production of chocolate (4). The extract was evaluated by three different and complementary DPPH, Lipoperoxidation and Plasmidial DNA Protection Assay. In The DDPH assay, the extract showed an IC_{50} of 7×10^{-3} mg/ml, the same protection achived by the control BHT at 0,01%. In the lipoperoxidation assay the lowest concentration tested, 25 μg/ml, showed a reduction of oxidation of 33,8% of the liposomes. The maximum level of protection was achieved by 50ug/ml and was not surpassed by greater concentrations. In the Plasmidial DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4,7 J/cm^2) and riboflavin (phototoxic under UVA radiation) to form the damnified open circle (OC) DNA. The greater concentration tested *i.e* 183,4 μg/ml exhibited the same level of protection of quercetin at 1mM. The results showed support indirect evidence for the use of the present extract as an anti-aging ingredient. **References:** 1. Heinrich U et al. (2006) J Nut. 46: 1565 – 1569 2. Gasser P et al. (2008) International Journal of Cosmetic Science 30: 339 – 345 3. Han B & Nimni ME (2005) Connective Tissue Research 46: 251 – 257 4. Wollgast J, Ankla, E (2000) Food Research International 33: 423 – 447

PL19 Phytochemical and pharmacological studies of *Ficus auriculata* Lour. (Moraceae) cultivated in Egypt

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This study scientifically examined the phytochemistry, antibacterial and anti-inflammatory potencies of two extracts of *Ficus auriculata* Lour. (Moraceae). Eight known compounds, including: betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, β-sitosterol-3-O-β-D-glucopyranoside, myricetin and quercetin-3-O-β-D-glucopyranoside were isolated from the petroleum ether, chloroform and ethyl acetate fractions of alcoholic extracts of the leaves and fruits of *Ficus auriculata*. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from *Ficus auriculata*. Concerning the biological studies, the results revealed that both extracts were effective against gram + ve bacteria (*Staphylococcus aureus*) and gram – ve bacteria (*Escherichia coli*) by agar well diffusion method. However, ethanolic extract of leaves exhibited greater antibacterial activity than the ethanolic extract of fruits. Meanwhile, the ethanolic extract of leaves at dose of 500 mg/kg exhibited significant anti-inflammatory effect using carrageenin-induced rat hind paw oedema model. **Keywords:** Ficus auriculata, Moraceae, antibacterial activity, anti-inflammatory

PL20 *Lavandula luisieri* pharmacognostic studies and antimicrobial activity against *Mycobacterium smegmatis*

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Lavandula luisieri (Rozeira) Rivas-Martinez is a Lamiaceae endemic in Iberian Peninsula (1). The morphology and histochemistry of vegetative and reproductive structures of specimens collected in SW Portugal, during 2007 – 2010, were investigated by LM and SEM. Non-glandular multi-cellular branched stellate hairs and peltate and capitate I and II, glandular hairs were identified on those structures. Glandular hairs exhibit different secretory modes and almost all showed mixed secretions, hydrophilic and lipophilic in their nature, except peltate hairs, where lipophilic secretions prevail. A preliminary phytochemical screening through TLC on silica gel plates on ascending polarity plant extracts confirmed the results of the histochemical tests: phenolics, flavonoids and terpenes were present and alkaloids were absent. The antibacterial activity of *L. luisieri* extracts was determined against different bacteria responsible for infectious diseases in human: Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 51299); Gram-negative bacteria (*Salmonella typhimurium* ATCC 13311, *Klebsiella pneumoniae* ATCC 9997, *Pseudomonas aeruginosa* ATCC 9027) and the alcohol-acid resistant bacillus *Mycobacterium smegmatis* ATCC 19016. Appropriate antibiotics were used as positive controls. All extracts exhibited low activity against Gram-negative bacteria (MICs > 125 μg/mL) and were active against *Staphylococcus aureus*. The n-hexane extract inhibited *S. typhimurium* at a concentration of 62 μg/mL; while the dichloromethane and methanol extracts showed the same MIC value of 62 μg/mL for Gram-positive bacteria. Dichloromethane, methanol and water extracts inhibited the *Mycobacterium smegmatis* bacillus growth with MICs of 32, 15 and 32 μg/mL, respectively. These results are strongly promising and are worthy of further studies. **Acknowledgement:** *Telmo Nunes, Paula Paes* **References:** 1. Morales R (2000) Portugaliae Acta Biol 19: 31 – 48

PL21 *Sanguisorba hybrida*: pharmacognostic and antimicrobial activity evaluation

Moreira I[1], Madureira AM[1], Duarte A[1], Feijão MD[2], Correia AI[3], Teixeira G[4]
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Sanguisorba L. is a Rosaceae distributed throughout the northern hemisphere. Some species are known to show hypoglycemic and hemostatic properties (1), antimicrobial (2) and antiviral activities (3). *Sanguisorba hybrida* (L.) Nordborg is endemic in Portugal (4) and was selected for pharmacognostic studies including a preliminary phytochemical survey and an evaluation of its potential against human pathogens. Samples were collected in SW Portugal (38 ° 8' N – 8 ° 33' W) during 2009 – 10 and identified at LISU. Under microscopy techniques non glandular and glandular multicellular trichomes were seen on both leaf surfaces. With histochemical tests the terpenoids and phenols were the most relevant compounds detected. Powdered plant material was extracted with n-hexane, dichloromethane, ethyl acetate, methanol and water. Their phytochemical survey, through TLC on silica gel plates and the proper reagents, was performed and the previous tests were confirmed. All extracts were tested against reference and multiresistant bacterial strains: Gram-positive (*Enteroccocus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Mycobacterium smegmatis*); Gram-negative (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*) and the yeast *Candida albicans*. The minimum inhibitory concentrations (MIC) were determined by the serial broth microdilution method. Appropriated antibiotics were used as controls. The methanol and water extracts showed better antimicrobial activity than n-hexane, dichloromethane, ethyl acetate extracts. Gram-positive bacteria were the most sensitive and the MIC values of 3.50 – 1.75 μg/mL were obtained using those polar extracts against *Staphylococcus aureus*, including strains resistant to meticillin (MRSA- Meticillin Resistance *Staphylococcus aureus*). **Acknowledgement:** *Telmo Nunes* **References:** 1. Reher G et al. (1991) Planta Med 57: A 57 – 58. 2. Kokoska L et al. (2002) J Etnopharmacol 82: 51 – 53. 3. Kim T et al. (2001) Phytother Res 15: 718 – 720. 4. Navarro C, Garmendia F (1998) Flora Iberica, Sanguisorba. Vol. VI. Real Jardín Botánico, CSIC. Madrid.

PL22 New results on saponins and saponin-rich plant-extracts enabling synergistic cytotoxicity with type-I-RIPs/lectins

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The synergistic cytotoxicity between saponins and lectins, especially the naturally very low cytotoxic activity showing type-I-RIPs (ribosome-inactivating protein type I)/lectins is known for years by now [1]. It has become a promising strategy in anti-cancer research [2]. While the preappliance of certain saponins can drastically amplify the cytotoxicity of the type-I-RIPs [3, 4], it may also minimize the required effective dose of these very expensive (especially when linked to human antibodies) and time-consuming to purify/to create substances [5]. In our work we searched for new saponins and saponin-rich plant-extracts capable of increasing the cytotoxicity of the naturally very low cytotoxic activity showing lectin saporin, considered as a standard type-I-RIP. The spotlight of our research was put on the plant-family of Caryophyllaceae, but saponins and saponin-rich plant-extracts from other plant-families were also tested when fulfilling certain structural conditions. All tests were performed in a cell culture model using ECV-304 cells. The cytotoxicity was measured by MTT assay and DNA quantification. **References:** 1. Hebestreit P, Melzig MF (2003) Planta Med 69: 921 – 925. 2. Bachran C et al. (2009) J Immunother 32: 713 – 725. 3. Hebestreit P et al. (2006) Toxicon 47: 330 – 335. 4. Weng A et al. (2008) Chem Bio. Int 176: 204 – 211. 5. Bachran C et al. (2010) Brit J Pharmacol 159: 345 – 352.

PL23 In vitro antiviral activity and cytotoxicity of the extracts of *Salvia wiedemannii* Boiss.

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Herpes simplex type 1 (HSV-1) and *Parainfluenza-3* (PI-3) viruses are important pathogens for humans. Although antiviral drugs are available, resistance to these antiviral medications have been increasing (1,2). *Salvia* L. (Lamiaceae) is widely distributed in Turkey by 94 taxa belonging to 89 species, with a 50% ratio of endemism (4). Some parts of *Salvia* species have been used in Turkish folk medicine for the treatment of various disorders and symptoms, including catarrh, cold, wounds, stomachache, flatulence, constipation, rheumatic pain, wards, sunstroke, and hemorrhage. In addition, there are also some reports on the antiviral effects of *Salvia* species. In this study, we evaluated the antiviral efficacy of *S. wiedemannii* Boiss. extracts on HSV-1 and PI-3 viruses by using Vero cell lines. Antiviral efficacy of these extracts, obtained from aerial parts of *S. wiedemannii*, was compared to that of acyclovir and oseltamivir. The H_2O, $CHCl_3$, and EtOH extracts of *S. wiedemannii* (16 – 0.0625 μg mL^{-1}) showed a significant antiviral activity on HSV-1 with the MNTC of 16 μg mL^{-1}. Only the BuOH extract of *S. wiedemannii* demonstrated important antiviral activity on PI-3 with a range of 64 – 16 μg mL^{-1} of inhibitory concentration for CPE, which was close to the anti PI-3 activity of oseltamivir. This study has showed that *S. wiedemannii* extracts have important antiviral activities and can be used as a source for drug development. **References:** 1. Rebecca CB et al. (2004) Antiviral Res 61: 73 – 81. 2. Hall CB (2001) N Engl J Med 344: 1917 – 28. 3. Davis PH. (1982) Flora of Turkey and the Aegean Islands. Edinburgh.

PL24 Evaluation the bioactivities of some extracts of *Cistus laurifolius*

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Some *Cistus* species (Cistaceae) can be found in Turkey; five of them have been identified (1). The leaves of *Cistus* species have been used against high fever, rheumatic pain, peptic ulcer, stomachache and urinary inflammations in Turkish folk medicine (2,3). For *Cistus* some biological effects are reported, including antimicrobial, antibacterial, antiviral, anti-inflammatory, anti-Helicobacter pylori, antiulcer, analgesic, antioxidant, antihepatotoxic, antiaggregant, and anticoagulant activity. In the present study, ethanol, hexane, chloroform, butanol, and water extracts of *C. laurifolius* L. were screened for their in vitro antibacterial, antifungal and antiviral activity. Antibacterial and antifungal activities were tested by the microdilution method against both, standard and isolated strains, of gram negative (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii*) and gram positive (*Staphylococcus aureus, Enterococcus faecalis*) bacteria, as well as fungi (*Candida albicans, C. parapsilosis*). Antiviral activities of these extracts were tested against Herpes simplex virus Type-1 (HSV-1) and Parainfluenza-3 virus (PI-3) by using Madin-Darby Bovine Kidney and Vero cell lines. All extracts (32 – 64 μg mL-1) exerted strong antimicrobial activity against isolated gram-negative strains of *E. coli* which are close to effects with the control antibiotic ampicilline (MIC; 64 μg mL^{-1}). The hexane extract (CPE of 32 – 8 μg mL^{-1}) had remarkable antiviral activity against PI-3. **References:** 1. Davis PH (1998) Flora of Turkey and the East Aegean Islands. Edinburgh. 2. Yeşilada E et al. (1995) J Ethnopharmacol 46: 133 – 52. 3. Honda G et al. (1996) J Ethnopharmacol 53: 75 – 87.

PL25 Biologically active flavonoid glycosides from *Horwoodia dicksoniae* Turrill

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Three flavonoid glycosides and one aglycone have been isolated from the ethanolic extract of *Horwoodia dicksoniae* Turrill for the first time, and their structures were assigned from ^{1}H- and ^{13}C-NMR spectra (DEPT) and from negative ESI-MS, as luteolin-7-O-β-D-glucopyranoside (1), apigenin-6-C-β-D-galactopyranoside (2), luteolin-6-C- β-D-galactopyranoside (3) and luteolin (4). The SRB cytotoxicity assay was used to investigate the antitumor activities of the ethanolic extract, compounds 1, 3 and 4. Compound 1 showed the highest cytotoxic activity against the three human cell lines; HEPG2, HCT 116 and MCF 7 (IC_{50}= 10.7, 9.3 and 9.9 μg/ml, respectively), compared with the standard antitumor drug doxorubicin. Compound 4 showed selective antitumor activity against the colon cell line (IC_{50}= 9.5 μg/ml). The present investigation also demonstrates the protective effect of compounds 1, 3 and 4 with antioxidant potential, in glycerol-induced myoglobinuric acute renal failure in rats. Treatment with each of these compounds attenuated renal dysfunction, and restored the oxidant balance by decreasing renal MDA levels, increasing the activity of the depleted renal antioxidant enzymes, and the non enzymatic antioxidant GSH. They also, decreased the elevated serum inflammatory marker (TNF- α), and ameliorated apoptotic kidney damage by reduction in caspase-3 activity. Compound 1 showed the highest biological activity.

PL26 Biodiversity assessment of *Veronica* sp. in Romania for their characterization, preservation and sustainable use in pharmacognosy

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Veronica is the most species-rich genus of Plantaginaceae family with about 500 species. The majority of the species are herbaceous annuals or perennials and they are very diverse from an ecological point of view [1]. Based on morphological and molecular analyses it was estimated that about 80 species of *Veronica* are found in Europe; about 40 species found in nine different subgenera are endemic to Europe and about 40 species have been reported in literature as being present in Romania [1, 2]. The genetic diversity and the chemical composition, useful for pharmacognosy [3] (*V. officinalis*, especially), of the *Veronica* species have encouraged different types of research to be carried out. Our aim is to asses the biodiversity of the species from *Veronica* genus on the Romanian territory in order to contribute to the protection of their biodiversity. These studies will subsequently result in offering new *Veronica* species as alternatives to pharmacognosy and to long-term biodiversity reconstruction and sustainable use of these sources of pharmacologically active compounds. Different *Veronica* sp. have been identified have been identified in different types of habitats, including some NATURA 2000 sites, and distributed all across Romania: West (Timisoara, Arad and Hunedoara counties), South (Gorj and Valcea counties), Center (Mures, Cluj and Harghita counties) and North-East (Iasi, Neamt and Suceava counties). Have been identified 21 taxons, out of which 20 species and one sub-species. The most abundant *Veronica* species proved to be: *V. chamaedrys* L., *V. officinalis* L., *V. beccabunga* L., *V. persica* Poiret, *V. spicata* L. and *V. spicata* L. ssp. *orchidea* (Crantz) Hayek. **Acknowledgement:** *This study was supported by UEFISCDI/project 32151/2008.* **References:** 1. Albach DC et al. (2006) Mol Ecol 15: 3269 – 3286. 2. Ichim MC et al. (2010) Bulletin UASVM Agriculture 67(2): 482. 3. Crisan G et al. (2010) Farmacia 58(2): 237 – 242.

PL27 *Centipeda cunninghamii*, an australian traditional medicinal plant

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Centipeda cunninghamii (DC.) A.Braun & Asch. is an endemic Australian Asteraceae with a long history of traditional use as a medicinal plant for treating wounds, infections and inflammation. Whilst its essential oil composition, principally chrysanthenyl and sabinyl acetates, has been known for some time, there was little scientific information regarding its phytochemistry and biological activity. Investigations on aqueous ethanolic extracts confirmed its anti-inflammatory and antioxidant (ORAC) activity. Detailed investigations suggest the extract acts against a range of inflammatory markers including COX-1, COX-2, NO and TNF-α, but not through the lipoxygenase pathway. Seventeen compounds were isolated and subsequent bioassays indicated that the anti-inflammatory activity was linked to flavonoids, whilst the antioxidant activity was attributed to both flavonoids and a group of novel heptenedioic acid cinnamoyl esters. The latter compounds are ring-opened quinic acid derivatives and appear to be unique to this species. Optimisation of growing, post-harvest and extraction conditions based on quality markers have been developed for future production and product development.

PL28 Biodiversity of carrot genetic resources – variation in secondary metabolites

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Daucus genetic resources comprises a few thousand accessions collected in gene banks mainly in USA, Europe and Russia. In 2009 a sub collection of 94 accessions representing edible and wild carrots was established that should represent available biodiversity. The choice of the accession was done mainly based on their passport data supplemented with data obtained during European programme on carrot characterization during which morphological characters were assessed. The aim of the presented work was to assess variation of the chosen accessions with regard to their composition of secondary metabolites. The analytical investigation was focused on carotenoids, including alpha-, beta-carotene, lutein, lycopene and their precursor phytoene, reducing and non-reducing sugars, phenolics, including anthocyanins, flavonols and phenylopropanoids and tocopherol. The results obtained revealed considerable variation of secondary metabolites content depending on genetic background. Edible carrots possessed higher carotenoid content, while phenolics dominated in wild relatives. Several accessions with high level of these compounds with importance for human health were identified. These materials may be prioritized in genetic and breeding programs for the development of high nutritional carrot cultivars. **Acknowledgement:** *Research was supported by Polish Ministry of Agriculture and Rural Development (grant No. HOR hn-078 dec-1/10).*

PL29 Quantitative Determination of Lycorine in *Galanthus* x*valentinei* nothosubsp. *subplicatus*

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Galanthus x*valentinei* (J. Allen) Beck nothosubsp. *subplicatus* (N. Zeybek) A. P. Davis (Amaryllidaceae) is a hybrid between *G. nivalis* L. and *G. plicatus* M. Bieb. subsp. *byzantinus* (Baker) D. A. Webb. This *Galanthus* L. hybrid is endemic and it occurs naturally in north-western Turkey [1,2]. Lycorine, a common alkaloid found in Amaryllidaceae plants, has been shown to possess important biological activities including antiviral [3], cytotoxic [4] and antimalarial activities [5]. In the present study, a reversed-phase high-performance liquid chromatographic method has

been used for the quantitative determination of lycorine in the aerial parts and bulbs of *G. xvalentinei* nothosubsp. *subplicatus* [6]. A simple method for the extraction of lycorine in low-mass plant samples was employed utilizing pre-packed columns with diatomaceous earth (Extrelut®) [7]. The chromatographic separation was carried out using an isocratic system with a mobile phase of trifluoroacetic acid-water-acetonitrile (0.01: 95: 5) applied at a flow rate of 1mL min^{-1} using diode array dedector. The linearity of the method was studied by injecting five known concentrations of lycorine in the range of 0.5 – 8 µg mL^{-1}. The calibration curve for lycorine was determined as Y = 14.9668622x + 0.7717199. The content of lycorine in the bulbs of *G. xvalentinei* nothosubsp. *subplicatus* was found to be 0.0028%. Lycorine was not detected in the aerial parts of this hybrid. **Acknowledgement:** *This study was financially supported by Ege University Research Fund (09/ECZ/037) and partially supported by TUBITAK (TBAG-104T272) and EBILTEM (2007-BIL-007).* **References:** 1. Davis AP et al. (2001) Kew Bull 56: 639 – 647. 2. Davis A (2006) The Genus Galanthus-Snowdrops in the Wild, in Bishop M., Davis A.P., Grimshaw J. (Eds.), Snowdrops, A Monograph of Cultivated Galanthus. Griffin Press Publishing Ltd. Cheltenham. 3. Szlàvik L et al. (2004) Planta Med 70: 871 – 873. 4. Weniger B et al. (1995) Planta Med 61: 77 – 79. 5. Sener B et al. (2003) Phytotherapy Res 17: 1220 – 1223. 6. Mustafa NR et al. (2003) J Liq Chromatogr R T 26:3217 – 3233. 7. Berkov S et al. (2008) Phytochem Anal 19: 285 – 293.

PL30 Medicinal plants of the Royal Botanic Garden site at Tell Ar-Rumman in Jordan

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The present study describes the floristic features and some of the documented pharmaceutical values of the medicinal plants that are present at the Royal Botanic Garden site in Tell Ar-Rumman in Jordan. A total of 574 plant species are recorded through a plant survey that was carried out during the period of 2006 to 2010. Among the recorded plant species, fifty nine species (10%) are considered medicinal e.g. *Varthemia iphionoides* Boiss. & C.I.Blanche, of these 6% are medicinal poisonous e.g. *Mandragora autumnalis* Bertol. The recorded medicinal plants are distributed among 51 genera and 24 families while Compositae is the dominating family with 11 species followed by 8 species for Labiatae. The majority of them belong fully or partially to the Mediterranean chorotype. The life form spectrum showed that the annuals comprised (24%) followed by (Chamaephytes) dwarf shrubs (20%) and hemicryptophytes (15%). The recorded plants are reported in the literature to have important medicinal values, e.g. *Ballota undulata* Benth. (hypolipidaemic, antimicrobial); *Capparis spinosa* L. (arthritis and gout, liver dysfunction); *Anchusa strigosa* Labill. (anti ulcer); *Hyoscyamus aureus* L. (Psychoactive) and *Pistacia atlantica* DC. (antioxidant). Furthermore, Chemical analysis was also made by Jordanian researchers for some of the plants e.g. *Anchusa strigosa*, *Alhagi maurorum* Medik. and *Urginea maritima* Baker. This work is considered a base for further investigations about the pharmaceutical values and documentations of traditional knowledge of the plant uses.

PL31 From structural studies of natural products to the discovery of a selective antiplasmodial derivative: a serendipity story

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In our continuing effort directed to the search for new antiplasmodial natural compounds from plants of the tropical biodiversity, the phytochemical study of *Canthium majus* Drake, a Madagascan plant belonging to the family Rubiaceae, was carried out. Bioguided fractionation of the ethyl acetate leaf extract led to the isolation of eight new diarylheptanoid glucosides together with the known β-sitosterol, which showed a weak antiplasmodial activity due to a possible stomatocytogenic[1] effect. The structures of the diarylheptanoid glucosides were similar to those isolated from the rhizomes of *Tacca chantrieri* André by Yokosuka and co-workers[2]. After chemical modifications of the natural glucosides, including hydrolytic cleavage, methylation and esterification, the determination of their absolute configuration using the CD exciton chirality method [3] applied to acyclic 1,3 dibenzoates [4] systems has been successfully achieved. Naturally-occuring diarylheptanoid glucosides and their derivatives were evaluated, in vitro, for their antiparasitic activity against *Plasmodium falciparum* (FcB1), *Leishmania donovani* (amastigote and promastigote forms) and *Trypanosoma brucei* as well as for their cytotoxic activity against HL-60, KB and MRC5 cell lines. Among 17 compounds investigated, one diarylheptanoid exhibited a selective antiplasmodial activity and no cytotoxicity. **Acknowledgement:** *This works was supported by an ICSN-CNRS grant to one of us (M.A B.). We express our thanks to G. Aubert for the cytotoxicity assay, Pr. P. Loiseau and Pr. C. Bories for the antileishmanial and antitrypanosomal assay.* **References:** 1. Ziegler H L et al. (2002) Antimicrob Agents Chemother 46: 1441. 2. Yokosuka A et al. (2002) J Nat Prod 65: 283. 3. Harada N et al. (1972) Acc Chem Res 5: 257. 4. Harada N et al. (1991) J Am Chem Soc 113: 3842.

PL32 Antiprotozoal and cytotoxic activities of some mushrooms from Turkey

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Protozoal infections still constitute a major health problem worldwide. Due to emerging resistance to common antiprotozoal agents, new drugs are urgently needed. Mushrooms are simple, non-photosynthetic organisms widespread in the world flora. Some are inedible or toxic, but many of them are used medicinally or in cooking. Some biological effects, e.g. antioxidant, immunomodulatory, antitumor, antimicrobial and antiprotozoal of mushrooms have been shown [1 – 4]. In this study, we evaluated the in vitro antiparasitic and cytotoxic potential of ethanolic extracts of some mushrooms growing in Turkey, namely *Polyporus gilvus, P. sulphureus, P. annosus, P. rheades, P. pinicola, P. volvatus, P. badius, Cantharellus cibarius, Ganoderma applanatum, Fomes fomentarius, Clavulina cinerea, Cortinarius orellanus* and *Trametes versicolor*. The test organisms used were *Trypanosoma brucei rhodesiense, T. cruzi, Leishmania donovani* and *Plasmodium falciparum*. Cytotoxic effects of the extracts were also assessed towards primary mammalian L6 cells. All mushroom extracts were active against *T. brucei rhodesiense* with *P. rheades* being the most potent (IC_{50} 0.59 µg/ml). The most potent extracts against *L. donovani* and *P. falciparum* were those of *P. gilvus, P. sulphureus, P. annosus, P. pinicola, F. fomentarius, C. cinerea* and *T. versicolor* (IC_{50} values 1.39 – 2.73 µg/ml). The extracts dispayed low or no cytotoxicity versus L6 cells. To our knowledge, this is the first biological activity and antiprotozoal screening study carried on Turkish mushrooms. The activity-guided isolation of the most active extracts is in progress. **References:** 1. Ribeiro B et al. (2008) Food Chem 110: 47 – 56. 2. Guerra DCM et al. (2007) Int Immunopharm 7: 1160 – 1169. 3. Kaneno R et al. (2004) Food Chem Toxicol 42: 909 – 916. 4. Samchai S et al. (2009) J Biol Sci 9: 778 – 783.

PL33 Climate change impact on conservation status of wild *Melissa officinalis* L. (Lamiaceae) populations in Armenia

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Climate change and temperature may lead to long-term irregularities in inter-specific interaction and may alter plant populations' dynamics, its structure and ecosystem functioning in the region [1,3]. Studies on possible effects of climate change on medicinal plants biodiversity and conservation status are particularly significant due to their value within traditional systems of medicine and as economically useful plants. Currently, only limited information on conservation status under the impact of global climate change of these species is available in Armenia [2]. Anthropogenic threats to biodiversity (overpopulation, deforestation and urbanization) have simultaneously hindered research and increased the need for it. From 2006 – 2009, field studies were conducted to find

out changes the growth, phenological and habitat characteristics of *Melissa officinalis* L., population size and location (GPS mapping). In 2010, we have implicated these research data to carry out future assessment of the risk analyze and impact of global climate change on its population distribution and conservation status. Neural network and genetic algorithms have been identified as stochastic self-learning methods to investigate hidden regularities between different data. Certain factors, such as biological characteristic of plants, habitat of the populations, anthropogenic threats and climate change have been identified as the key elements. In fact, vulnerability of plant population, particularly will increase central and northern part of the country, as they identified to be comparatively stressful environment under global climate change and anthropogenic threats, which included: poor land management, increasing population pressure, and excessive collection of plants. **References:** 1. Hughes L (2000) Trends Ecol Evol 15: 56 – 61 2. IUCN-WHO-WWF (1993) Guidelines on the Conservation of Medicinal Plants, IUCN, Gland, Switzerland, 50 p. 3. Bishop JG, Schemske DW (1998) Ecol 79: 534 – 546

PL34 NMR and MS profiling of chemosystematic markers in triploid *Populus tomentosa*

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Chemosystematics, or chemotaxonomy, is the attempt to classify and identify plants, according to demonstrable differences and similarities in their secondary metabolites. Thus, chemotaxonomic markers are powerful tools for the identification of a wide variety of plants [1]. Chinese forest scientists have been making significant efforts to develop fast-growing trees due to the extreme shortage of wood resources. Triploid *Populus tomentosa* Carr. (Salicaceae), the cloned hardware poplar species from *Populus tomentosa*, has been receiving the most attention [2]. However, secondary metabolites of triploid P. tomentosa have never been studied to date, though poplars have been widely used in folk medicines for the treatment of various diseases [3]. This work was carried out to investigate the secondary metabolites and the chemosystematic markers from triploid *P. tomentosa*. Column chromatographic purification of triploid P. tomentosa extracts resulted in the isolation of twelve phenolics: grandidentatin, isograndidentatin A, isograndidentatin B, caffeic acid, populoside A, salireposide, luteolin, salicortin, apigenin, populoside, ρ-coumaric acid, and 7-O-caffeoylsalirepin. The structures of the isolated secondary metabolites were extensively elucidated and characterized by spectroscopic method, including 1D and 2D NMR, and EI, FAB and MALDI-TOF MS. This was the first investigation of the secondary metabolites of triploid *P. tomentosa* wood. The isolation of isograndidentatin A, isograndidentatin B, grandidentatin, here in triploid *P. tomentosa* was interesting and glucosides of 1,2-dihydroxycyclohexane acylated by ρ-coumaric acid (or ρ-coumaric acid derivatives) could be considered as useful chemosystematic marks within the Salicaceae family, which was also well in accord with the our previous conclusion [4]. **Acknowledgement:** *This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010), Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616), National Natural Science Foundation of China (NSFC, No. 31000279) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800).* **References:** 1. Bohm BA (1987) The Bot Rev 53: 197 – 279. 2. Si CL et al. (2011) Bioresources 6: 232 – 242. 3. Si CL et al. (2009) Chem Nat Compd 45: 634 – 636. 4. Si CL et al. (2009) Biochem Syst Ecol 37: 221 – 224.

PL35 Effects of autumn and spring sowing on yield, oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) cultivars in Shirvan region

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Yield, oil content and fatty acid sythesis of crop are influenced by a lot of factors such as genotype, ecology, morphology and management (planting date, plant density, fertilization etc.). The aim of this study was to determine effect of Autumn and spring sowing on yield, oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) cultivars under Shirvan conditions. This research was conducted at Research Farm of the Faculty of Shirvan Agriculture, Ferdowsi University of Mashhad, Iran in 2009 – 2010. Five safflower cultivars (Cinaa, CW-440, Sahuripa-88, Ghochan local and Isfahan local) were used in this study. The research was randomized complete block, split plot design with three replications. Different sowing time significantly affected yield, oil content and fatty acid compositin of the genotypes used in this study. There was interaction between genotypes and sowing times. According to the results of this research; Cina had highest yield in autumn and spring sowing (2989 and 2120 kg/ha respectively). Also Sahuaripa-88 has showed the highest oil content in autumn and spring sowing (32 and 28.9% respectively). Yield and oil content in autumn sowing was highest (847 kg/ha and 2.1%). The palmitic, stearic and oleic acid increased but linoleic, and linolenic acid decreased in autumn sowing. According to the results of the study it was found that autumn sowning was suitable than spring sown and Cina genotype have desirable potential for planting in Shirvan region.

PL36 Discovery of new indoleamine-2,3-dioxygenase inhibitors from *Carthamus tinctorius*

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Indoleamin-2,3-dioxygenase (IDO) is the rate limiting step of tryptophan catabolism. As its expression is induced by Type II interferone (INF-γ), it is involved in inflammatory diseases [1]. In neurological disorders, degradation of tryptophan can reduce serotonin synthesis, which is related to major depression [2]. Furthermore, quinolinic acid originating from tryptophan catabolism has neurotoxic effects. In cancer cells, the expression of IDO leads to a local suppression of T-cell responses and promotes immune tolerance. Therefore, IDO is an interesting target for therapeutic intervention in these conditions [1]. As *Carthamus tinctorius* L. was used in ethnopharmacology against inflammatory diseases and also against cancer [3] and depression [4], this plant was investigated for IDO inhibitors. Three lignans isolated from *Carthamus tinctorius* seed oil cake were tested for inhibition of IDO in peripheral blood mononuclear cells (PBMCs), namely arctigenin, trachelogenin and matairesinol. Arctigenin and trachelogenin inhibited IDO with IC_{50} of 26.49 and 57.35 μM whereas matairesinol showed only slight activity. **Acknowledgement:** *This work was supported by the TWF ("Tiroler Zukunftsstiftung") and the Austrian Science Fund (FWF; S10703).* **References:** 1. King NJ and Thomas SR (2007) Int J Biochem Cell Biol 39: 2167 – 2172 2. Miura H et al. (2008) Stress 11(3): 198 – 209 3. Blaschek W et al (eds) (2007) Hagers Enzyklopädie der Arzneistoffe und Drogen (4) 6 ed. Wissenschaftliche Verlagsgesellschaft mbH Stuttgart73 – 5 4. Zhao G et al. (2009) Eur Neuropsychopharmacol 19: 749 – 758

PL37 Effects of chemical and organic fertilizers on number of corm and stigma yield of saffron (*Crocus sativus*)

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Saffron (*Crocus sativus* L.) is the world's most expensive spice and 95% of the production is coming from Iran [1]. The aim of this study was to better understanding the effects of different organic and chemical fertilizers on number of corm and stigma yield of saffron. This expriment was conducted in Organic Farm of Ferdowsi University of Mashhad, Iran, based on CRBD with three replications. The experimental treatments were four different fertilizers including chemical (50 – 250; 100 – 250 and 300 – 250 kg/ha N-P_2O_5), cow manure (20, 40 and 60 t/ha), sheep manure (20, 30 and 40 t/ha) and hen manure (5, 10 and 15 t/ha). The results which is reporting here, came from fifth year of the experiment. Results showed that the highest fresh flower and dry stigma yield were

obtained from chemical fertilizer (300 – 250 kg/ha N-P2O5) and then from cow manure (20 t/ha) treatments. Fresh flower and dry stigma yield were increased by increasing the nitrogen level in chemical fertilizer and incresing sheep manure levels. The same results has been reported by Behnia et al [1]. Behzad et al [2] showed that application of 200 kg ammonium phosphate plus 30 tons of cow manure produced the highest stigma yield. Rezvani moghddam et al. [4] reported that cow and chemical fertilizers produced more flower and stigma yield than hen manure. Sheep manure at 40 t/ha produced the highest mother corm and replacement corm per clump. Saffron is a low nutrient demand plant and requires a modest amount of nutrients [3]. **References:** 1- Behnia MR et al. (1999) J Agron Crop Sci 182: 9 – 15. 2- Behzad S et al. (1992) Acta Hort 306: 337 – 339. 3- Housini M (1998) Iranian Scientific and Indusrial Research Organization, Press -Khorasan Center. 4- Rezvani Moghaddam P et al. (2006) 2nd International Symposium on Saffron Biology and Technology. Iran.

PL38 Proteolytic activity in latices of Asteraceae and Campanulaceae

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In the order Asterales only two species are known to have proteolytic activity in their latices, first Taraxalisin from dandelion *Taraxacum officinale* Webb s.l., and second Parthenain from Guayule *Parthenium argentatum* L.. Both are characterized as serine endopeptidases [1]. Proteolytic enzymes isolated from plant latex have received special attention in the pharmaceutical industry and biotechnology due to their property of being active over wide range of temperature and pH. Nearly half of the commercially available enzymes are proteases, frequently used in food processing, tenderization of meat, brewing, cheese elaboration, bread manufacturing, leather and textile industries [1]. In this investigation the latex of 40 species of the Asteraceae family and 8 species of the Campanulaceae, which are not biochemical characterized before, were collected in the Botanical Garden Berlin. To determine proteolytic activity we used the fluorogenic substrate BODIPY FL- casein (Molecular Probes, Inc., USA) [2]. To investigate the type of endopeptidases, the latex samples were pre-incubated with specific inhibitors for serine proteases (AEBSF (4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride), cysteine proteases (E64 (4-(2-Aminoethyl) benzenesulfonyl-fluoride hydrochloride)), aspartatic proteases (Pepstatin A) and metalloprotease (EDTA) and the remaining activity was determined. In both families highly active serine proteases were found. **References:** 1. Domsalla A, Melzig MF (2008) Planta Med 74: 1 – 13 2. Menges DA et al. (1997) Anal Biochem 251: 144 – 147

PL39 Antioxidant Properties and Phenolic Composition of *Viburnum opulus* from Turkey

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Viburnum opulus L. (Caprifoliaceae) growing in Kayseri and surroundings is named as gilaburu. The fruit juice of gilaburu is consumed as a traditional drink. In many parts of the world, gilaburu is used for antispasmodic, anti-inflammatory, anti-allergic, sedative and diuretic purposes. Research on antioxidant effects of gilaburu is limited. In this study the antioxidant effects of different extracts of gilaburu are investigated. Water and methanol (70%) extracts were prepared. The antioxidant activity of the extracts was determined using 1,1-diphenylpicrylhydrazin (DPPH°), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+°) radicals and lipid peroxidation activity by the β-carotene bleaching test. The content of total phenols, flavonoids, flavonols and anthocyanins, and the reductive activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, and gallic acid were used as positive controls. Methanol extracts of dry fruits rich in phenolics showed more scavenging activity on DPPH° (IC_{50}: 0.104 mg/ml) than other extracts, whereas methanol extracts of fresh fruits rich in anthocyanins (0.47 ± 0.05 mg cyanidin-3-glycoside/g extract) more scavenged the ABTS+° radical (TEAC: 0.92 mM). The aq. methanol extract better reduced ferric(III) to ferro(II) than the water extract. All extracts inhibited linoleic acid peroxidation in the β-carotene bleaching test to almost the same degree. **References:** 1. Özer E (2000) MSc Thesis, Selçuk University Institute of Sciences, Konya. 2. Özer E, Kalyoncu IH (2007) Selçuk Journal of Agriculture and Food Sciences 21: 46 – 52. 3. Çam M, Hışıl Y (2005) Erciyes University Project, Project No: EUBAP-FBT- 03 – 51. 4. Aksoy A, Güvensan A, Akçiçek E, Öztürk M (2004) International Symposium on Medicinal Plants: Linkages Beyond National Boundaries, Islamabad, pp 65 – 70.

PL40 Phytochemical and Antimicrobial Investigation of *Gontscharovia popovii*

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Aerial parts of *Gontscharovia popovii* B. Fedtsch. et Gontsch. from Lamiaceae family (1) was collected from Hadjiabad in South of Iran (Hormozgan Province) on May 2008. It is used for the treatment of cold and infectious disease in folk medicine of South Iran. There is no report on secondary metabolites of this plant. Aerial parts of *G. popovii* were finely powdered and 531 g of sample was extracted with 80% methanol in a percolator. After evaporation of solvent, the gummy reminder was fractionated with petroleum ether (5.49 g), chloroform (1.29 g), ethyl acetate (2.87 g) and the residue named methanol extract (56.42 g). Methanol extract tested for detection of secondary metabolites. It was rich of luteolin glycosides which were isolated with different chromatographic methods and identified with spectroscopic methods. Luteolin, luteolin-7-O-glucoside, luteolin-7-O-rhamnoglucoside and luteolin-7-O-diglucoside were identified from this fraction. Antimicrobial activities of all fractions were tested against four G+ (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus* and *Bacillus subtilis*), two G- (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and two fungi (*Aspergillus niger* and *Candida albicans*) with micro-broth dilution methods (2). The results showed that methanol extract had antimicrobial activity only on *Bacillus subtilis*. Petroleum ether fraction showed considerable properties on most microorganisms but had no effect on *P. aeruginosa*. Chloroform fraction indicated antimicrobial activities on bacteria and fungus except on *P. aeruginosa*. Ethyl acetate fraction was effective on all strains. **References:** 1. Rechinger KH (1982) Gontsharovia Popovii (Labiatae). In: Flora Iranica, Rechinger KH, ed Akademische Druck-u. verlagsanstalt, Craz Austria.150: pp. 504 – 505. 2. NCCLS. (2000) Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standards 5th ed. NCCLS document M7-A%. NCCLS: Wayne, PA, USA.

PL41 Quantitative Determination of Galanthamine and Lycorine in an endemic *Galanthus* species: *G. cilicicus*

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Galanthus cilicicus Baker, an endemic species of the genus *Galanthus* L. (Amaryllidaceae), is distributed in southern Turkey mainly in the province of Içel [1]. Galanthamine, the most important alkaloid found in Amaryllidaceae species, is used for the treatment of Alzheimer's disease [2]. Lycorine, another important and also a widespread alkaloid found in Amaryllidaceae plants has been proven to have several biological activities [3,4]. A reversed-phase high-performance liquid chromatographic method has been used and validated for the determination of lycorine and galanthamine in *G. cilicicus*. The extraction of both alkaloids in low-mass plant samples, were carried out by a simple and a rapid method utilizing pre-packed columns with diatomaceous earth (Extrelut®) [5]. The chromatographic separation was performed using an isocratic system with a mobil phase of trifluoroacetic acid-water-acetonitrile (0.01: 92.5: 7.5) and diode array dedector [6]. The linearity of the method was studied by injecting five known concentrations of lycorine in the range of 1 – 10 μg mL^{-1} and five known concentrations of galanthamine in the range of 2.5 – 20 μg mL^{-1} The calibration curves for lycorine and galanthamine were determined as Y = 13.2828995x + 0.4488635 and Y = 10.1354031x +0.5465348, respectively. Validation procedures showed that the method was specific, accurate and precise. The above-mentioned method was applied to the aerial parts and bulbs of *G.cilicicus*. The contents of galanthamine and lycorine in the bulbs of *G.cilicicus*

were found to be 0.016% and 0.0035%, respectively. In the aerial parts, lycorine was not detected, however the content of galanthamine was found as 0.015%. **Acknowledgement:** *This study was financially supported by Ege University Research Fund (09/ECZ/037) and partially supported by TUBITAK (TBAG-104T272) and EBILTEM (2007-BIL-007).* **References:** 1. Davis AP (2006) The Genus Galanthus-Snowdrops in the Wild, in Bishop M., Davis A.P., Grimshaw J. (Eds.), Snowdrops, A Monograph of Cultivated Galanthus. Griffin Press Publishing Ltd. Cheltenham. 2. Heinrich M, Teoh HL (2004) J Ethnopharmacol 92:147 – 162. 3. Szlàvik L, et al. (2004) Planta Med 70: 871 – 873. 4. Sener B et al. (2003) Phytotherapy Res 17: 1220 – 1223. 5. Berkov S et al. (2008) Phytochem Anal 19: 285 – 293. 6. Mustafa NR et al. (2003) J Liq Chromatogr R T 26: 3217 – 3233.

PL42 Screening of Zambian *Ficus* species for antibacterial and antimycobacterial activity

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Members of the genus *Ficus* (Moraceae) are traditionally used in Zambia against many infectious diseases, including bacterial (oral, chest and diarrhoeal), mycobacterial and fungal (ringworms) infections [1,2]. Based on this information, we collected different plant parts (leaves, stem and root barks) of eight Zambian *Ficus* species; *F. ovata* Vahl, *F. wakefieldii* Hutch, *F. natalensis* Hochst, *F. sansibarica* Warb. subsp. *macrosperma*, *F. lutea* Vahl, *F. ingens* (Miq.) Miq., *F. sycomorus* L. subsp. *gnaphalocarpa* (Miq.) and *F. sycomorus* L. subsp. *sycomorus*. The dried plant materials were extracted with methanol (CR-Me) and further partitioned to obtain n-hexane (K-Hex), chloroform (K-$CHCl_3$) and aqueous methanol (K-MeOH) subextracts. We recently investigated the antifungal effect of CR-Me extracts against *Trichophyton* species, the causative agents of ringworm infections [3]. Herein we screened the CR-Me extracts and the subextracts for antibacterial and antimycobacterial activity using agar disc diffusion and MTT assays, respectively. Test organisms were Gram-positive [*Staphylococcus aureus* NCTC 12695, methicillin-resistant *Staphylococcus aureus* MRSA 1199B, *Enterococcus faecalis* 13379] and Gram-negative [*Escherichia coli* NCTC 10418] bacteria, plus *Mycobacterium tuberculosis* (H37Rv strain). The CR-Me extracts of the stem barks were active against all Gram-positive microorganisms. Of the subextracts, K-MeOH-solubles exhibited the best activity with inhibition zones of 11 mm at 100 µg/disc concentration against all three Gram-positive bacteria. Moderate antitubercular activity was observed in some K-Hex and K-$CHCl_3$ subextracts, with K-$CHCl_3$-solubles of *F. ovata* stem bark exhibiting the highest activity (MIC 128 µg/ml). These results provide a scientific basis supporting the use of *Ficus* species in traditional herbal preparations in Zambia. **Acknowledgement:** *UK Commonwealth Scholarship Commission and the Rick-Cannell Travel Fund of the School of Pharmacy are acknowledged for funding.* **References:** 1. Kuete V et al. (2008) J Ethnopharmacol 124: 556 – 561. 2. Fowler DG (2007) Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens. Kew, UK. 3. Bwalya AG et al. (2010) Planta Med 72: 1301.

PL43 Impact of nitrogen nutrition on growth and plant quality of *Centella asiatica*

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Due to its bioactive triterpene saponins, *Centella asiatica* (L.) Urb. has been used as a medicinal herb since ancient times and its economical importance is still rising [1,2,3]. Up to now plants are collected spontaneously which implicates a large variation in plant and product quality depending on the origin, genotype and time of harvest of the plants [1 – 4]. To assure high plant quality, it will be necessary to encourage cultivation of *Centella*. To our knowledge there is scarce information on cultivation techniques, especially on mineral nutrition of *Centella* plants. The aim of this study was to investigate the effects of nitrogen on growth and saponin biosynthesis of *Centella asiatica* and to find appropriate parameters to evaluate quality of plant material by non-destructive measurements in situ. Plants were grown for eight weeks in rock wool cubes in a greenhouse and fed with five nutrient solutions differing in their nitrogen concentrations. Number of leaves and stolons, length of stolons, assimilation rate and leaf green intensity were monitored weekly. Non-destructive measurements were conducted with a portable optical sensor (Multiplex® Research, Force-A, France) recording the fluorescence signature which is associated with plant constituents. Fresh and dry weight of leaves and stalks, leaf area and specific leaf weight were ascertained biweekly, while examination of leaf chlorophyll and leaf nutrient content was carried out once at the end of the study. Leaf samples for determination of triterpenoid content were harvested four times. Analyses of asiaticoside and asiatic acid by HPLC are in progress and will be discussed. **Acknowledgement:** *Regionale 2010, State of North Rhine-Westphalia (Germany); Institute of Systematic Botany, The New York Botanical Garden (USA), A.N. Nicolas; Institut Malgache de Recherches Appliquées (Madagascar), D. Randriamampionona; National Center for Natural Products Research, University of Mississippi (USA), B. Avula; Unité d'Analyse Chimique et Physico-Chimique des Médicaments et Pharmacognosie, Université Catholique de Louvain (Belgium), M.H. Rafamantanana; Institute of Nutrition and Food Sciences, University of Bonn (Germany), B.F. Zimmermann.* **References:** 1. Thomas MT et al. (2010) Ind Crop Prod 32: 545 – 550. 2. Devkota A et al. (2010) Biochem Syst Ecol 38: 12 – 22. 3. Randriamampionona D et al. (2007) Fitoterapia 78: 482 – 489. 4. Sritongkul J et al. (2008) Acta Hort 804: 367 – 372.

PL44 Antioxidant Properties and Phenolic Composition of *Salvia virgata* from Turkey

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Several biochemical reactions generate reactive oxygen species and these are capable of damaging crucial bio-molecules (1). Free radicals are very important in food products, because oxidative degradation of lipids is one of the main factors limiting their shelf-life (2). In recent years, natural antioxidants have been focused on because of the harmful effects of synthetic antioxidants (3). *S. officinalis* L. (Lamiaceae), is an important source of antioxidants used and have wider implications for the dietary intake of natural antioxidants (3). Turkey is an important country for *Salvia* species. The flora of Turkey includes 88 species of the genus *Salvia*. The 70% methanol and water extracts were prepared from the aerial parts of *S. virgata* Jacq. collected from Bursa, Turkey. All the extracts were analyzed by HPLC and in in vitro antioxidant assays. The 1,1-diphenylpicrylhydrazin (DPPH°), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+\circ}$) radical scavenging activity and β-carotene bleaching methods were used. Total phenolic compounds and reductive activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, gallic acid and rosmarinic acid were used as positive controls. Phenolics rich extract of aq. methanol showed more scavenging activity on DPPH° than water extract whereas water extract more scavenged the $ABTS^{+\circ}$ radical. The aq. methanol extract more reduced the ferric(III) to ferro(II) in a certain proportion than water extract. All extracts were inhibited linoleic acid peroxidation in β-carotene bleaching test and shown more activity than rosmarinic acid. Rosmarinic acid was found as the main component and caffeic acid, ferulic acid and luteolin-7-*O*-glycoside were identified in the extracts. **References:** 1. Kumaran A, Joel Karunakaran R (2006) Food Chem 97: 109 – 114. 2. Pizzalle L et al. (2002) J Sci Food Agric 82: 1645 – 1651. 3. Kintzios SE (2000) Sage The Genus Salvia. Harwood Academic Publishers, 27 – 53 and 185 – 192.

PL45 Antioxidant Properties and Phenolic Composition of *Alchemilla mollis* from Turkey

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Alchemilla mollis (Buser) Rothm. (Rosaceae) is also known as Lady's Mantle and native to southern Europe and grown throughout the world as an ornamental garden plant (Evenor et al., 2001). In folk medicine, lady's mantle was also used to soothe infections of the mucous membranes of mouth and throat. The leaf tea and dewdrops from the leaves of the living plant are most commonly employed to help female conditions such as menorrhagia, menopause and painful periods. Lady's mantle was also used traditionally for treating blood sugar control diseases, although no evidence exists to support its usefulness (Kisilova et al., 2006; Shrivastava et al., 2007). Air-dried *A. mollis* herb material (100 g)

was powdered and sequentially extracted with hexane, ethyl acetate, methanol, and n-butanol using a Soxhlet apparatus for 8 h for each. Thereafter, the extract was filtered and evaporated to dryness in vacuo at 40 °C. All the extracts were analyzed in in vitro antioxidant assays. The free radical scavenging activity of the extracts were investigated using 1,1-diphenylpicrylhydrazin (DPPH°) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+\circ}$) radicals. Total phenols, flavonoids and flavonols, and reductive activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, and gallic acid were used as positive controls. Polar fractions were found to be more active as antiradical assays. These active fractions were contained more tannins, especially galloyl tannins. Chemical composition and antiradical activity results of the A. mollis are the first report in the same research. **References:** 1. Evenor D et al. (2001) Plant Cell Tiss Org 65: 169 – 172. 2. Shrivastava R et al. (2007) Phytother Res 21: 369 – 373. 3. Kiselova Yet al. (2006) Phytother Res 20: 961 – 965.

PL46 Justicidin B – a potent cytotoxic arylnaphtalene lignan from in vitro cultures of *Linum leonii*

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The plants are one of the attractive sources of novel antitumor compounds. Isolation of pharmaceuticals from plants is difficult due to their extremely low concentrations. To extend the research to human clinical studies, we needed to find a reliable supply of plant material, produced target compounds. As part of our ongoing program on the investigation of *Linum* species, cell cultures of *L. leonii* F.W.Schultz, were examined. We have established several callus and suspension cultures and checked for the occurrence of lignans. The main component in cell cultures of *L. leonii* was isolated and analyzed by means of GC-MS and NMR. The EI-MS of the isolated compound showed an ion at m/z 364 and mass fragmentation, which is consistent with the data for an arylnaphthalene lignan. the 1 H NMR spectrum showed that the isolated compound is justicidin B. Justicidin B produced by in vitro cultures of *Linum leonii* was tested for cytotoxic activity and induction of apoptosis in MDA-MB-231 and MCF-7 breast cancer derived cell lines. The tested lignan evoked strong, concentration-dependent cytotoxicity in both cell lines, whereby MCF-7 proved to be more sensitive. The 24 h treatment of both cell lines increased the level of apoptotic DNA fragmentation; however the proapoptotic activity is completely inhibited if the cells are co-incubated with the non-selective pan-caspase inhibitor Boc-Asp(OMe)-fluoromethyl ketone (PCI), which implies that justicidin B, activates PCD via caspase-dependent mechanisms. Exposure of MDA-MB-231 cells with justicidin B leads to concentration-dependent decrease in the NFkB expression; strong NFkB expression is observed in MCF-7 cells. **Acknowledgement:** *Financial support from Ministry of Education and Science, Sofia, Bulgaria (grant D 002 – 128/08 I. Ionkova) is acknowledged.*

PL47 Investigation on the antimicrobial activity of *Acroptilon Repens* (L.) DC

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Acroptilon repens (L.) DC. (Russian knapweed)) is a perennial herbaceous plant belonging to the family Asteraceae. This plant is native to Mongolia, western Turkestan, Iran, Turkish Armenia and Asia Minor [1]. The objective of this study was to evaluate antimicrobial activity of aerial parts extract of *A. repens* against 4 pathogenic bacteria. The samples were collected from Takestan, Ghazvin province in june 2007. The CH_2Cl_2, EtOAc and MeOH extracts of the aerial parts of the plant obtained by percolation method were investigated for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Bacillus subtilis*. The present study showed that all the extracts of *A. repens* had significant antibacterial activity against gram positive bacteria at dilutions of 1/10, whereas no effect was observed against gram negative bacteria. In comparison, the MeOH extract showed the most potent effect on *B. subtilis*. A. Repens, with potential activity on *S. aureus* and *B. subtilis* can be considered as a prominent source for obtaining new natural antibiotics. So the isolation of the active compounds and understanding the mechanism of inhibition would be of interest. **Acknowledgement:** *This research has been supported financially by Student's Scientific Research Center, Tehran University of Medical Sciences grant number: 7132 – 61 – 02 – 87.* **References:** 1. Maddox DM, Mayfield A, Poritz NH (1985) Weed Sci 33: 315 – 327.

PL48 Curcumin As Anti-Oxidative Stress In Iron Toxicity

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Iron is an important element for normal cellular physiology, but an excess might induce the formation of oxygen free radicals (1). Thus, in the present study the oxidative stress induced by chronic iron intoxication ws investigated by analysis of some enzymatic and non-enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT), ceruloplasmin (Cp), glutathione (GSH) and ascorbic acid (AsA) in liver and spleen homogenates of treated and control rats. The average values of TBARS, NO and OH radicals were significantly ($p < 0.001$) elevated in iron-overloaded rat groups compared with the corresponding control group. The average amount of iron was significantly ($p < 0.001$) elevated whereas that of copper was significantly ($p < 0.001$) reduced in iron-overloaded rat groups. Inversely, the administration of curcumin as iron-chelating agent with SOD- and CAT-like activities before setting the iron overload improved the above biochemical parameters (2). The administration of curcumin as iron chelating therapy ameliorated the oxidative stress of excess iron either by decreasing iron level or by scavenging reactive oxygen intermediates, which could be clinically useful. **References:** 1. Lucesoli F, Caligiuri M, Roberti MF, Perazzo JC, Fraga CG (1999) Arch Biochem Biophys 372(1): 37 – 43 2. Abou-Seif M, Badria F, and Houssein W ((2004) Arab J Lab Med 30(2): 193 – 206).

PL49 Screening methods to determine potential bioactivity of endophytic fungi from *Vitis vinifera*

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Fungal endophytes are widespread in plants and colonize living internal tissue of their hosts symptomlessly [1]. They are well known for their beneficial effects for their hosts, providing increased tolerance against abiotic and biotic stresses, enhancing inter alia resistance to insect pests and fungal or microbial infection. This is largely due to their production of bioactive secondary metabolites [2]. Recently, Zingiberaceae species were studied and several bioassays successfully developed to test for antifungal activities [3]. These tests were now applied to the analysis of *Vitis* plants and their endophytic fungi, including routinely: a) competition tests against *Cladosporium sphaerospermum*, to investigate the dominance of the endophyte compared to its competitor *in vitro* [4]; b) thin layer bio-autography with the crude endophytic extract and subsequent determination of inhibition halos, caused by separated compounds after spraying with conidiospores of *C. sphaerospermum*; c) establishing species-specificity by cultivation of the endophytic fungus on media containing the crude extract of the respective plant; d) performing a modified ELISA-test with the fungal crude extract to determine the median effective concentration (EC_{50}) for inhibition of the growth of *C. sphaeospermum*. Several fungal endophytes have so far been isolated from *Vitis vinifera* L. cultivars, and respective results will be presented. The methods described provide tools not only for testing for antifungal activities, but also for subsequent isolation of bioactive compounds, and eventually for their practical applications in pest control. **Acknowledgement:** *The financial support of "Society for the Advancement of Plant Sciences" is gratefully acknowledged.* **References:** 1. Petrini O (1991) Microbial Ecology of Leaves, Springer Verlag, New York. 2. Gao F et al. (2010) Afr J Microbiol Res 4(13): 1346 – 1351. 3. Zahradnik C (2010) Pilzendophyten aus Alpinia malaccensis und Curcuma sp.: Kultur, Sekundärstoffprofil und Bioaktivität. Diploma thesis, Univ. of Vienna. 4. Yuen TK (1999) Microb Ecol 37: 257 – 262.

PL50 Quantitative Determination of Gallic acid and Cyanidin-3-O-Glucoside within Sumac Extracts by HPLC-MS/MS

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Rhus coriaria L., commonly known as sumac (also spelled sumach), grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and southeast Anatolian region of Turkey [1]. The fruits are red colored and contain one seed. It's dried and ground leaves have been used as a tanning agent due to their high tannin content. Previous phytochemical studies of this plant reported that it contained flavones, tannins, anthocyanins, and organic acids [2]. In this study gallic acid and cyanidin-3-O-glucoside contents of Water and MeOH %70 extracts of sumac were investigated using with HPLC ESI/MSMS MRM method. The assay performed in different concentrations of gallic acid and cyanidin-3-O-glucoside chloride as standard solutions. The diagnostic fragmentations of gallic acid and cyanidin-3-O-glucoside were used 168.7/125 – 79 and 448.7/287 – 150 respectively for MRM quantitative determination. Cyanidin-3-O-glucoside contents of 100 g each of aq. Methanol and water extracts of sumac were found as 0.007 ± 0.001 g and 0.015 ± 0.005 g, response. Gallic acid contents varied between 0.923 ± 0.010 g and 0.566 ± 0.005 g in 100 g each of aq. Methanol and aqueous extracts, resp. **References:** 1. Dogan M and Akgul A (2005) Chem Nat Comp. 41(6): 724 – 725. 2. Kosar M et al. (2007) Food Chem. 103(3): 952 – 959.

PL51 Free Radical Scavenging Activities of Flavonoids from *Cistus salviifolius* L

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The genus *Cistus* (Cistaceae) is represented by 21 species on worldwide and 5 species in Turkish flora. The *Cistus* genus has widespread utilization in Turkish folk medicine such as against rheumatism, for hemorrhoids, to cure sterility, kidney and urinary inflammations, as a hemostatic, antipyretic, expectorant, sedative, and for peptic ulcer, as well as diabetes mellitus. Anti-inflammatory, anti-helicobacter pylori, antihypertensive, anti-microbial activities and cytotoxic effects of *Cistus* species were also reported. In this study, pharmacognostical investigations have been carried out on *Cistus salviifolius* L. The aerial parts of the plant were extracted with *n*-hexane and MeOH, respectively. The methanolic extract was partitioned with petroleum ether and *n*-BuOH. Isolation of secondary metabolites from *n*-BuOH extract were performed with various chromatographic methods. The *n*-BuOH extract yielded three flavonoid aglycones; CS-1: Kaempferol, CS-2: Quercetin, CS-3: Myricetin, four flavonoid glycosides; CS-4: Kaempferol 3-*O*-β-(6''-*O*-*trans*-*p*-coumaroyl)-galactopyranoside, CS-5: Quercetin 3-*O*-α-arabinopyranoside, CS-6: Quercetin 3-*O*-β-galactopyranoside and CS-7: Myricetin 3-*O*-β-galactopyranoside. The structures of these compounds were elucidated using spectroscopic methods (UV, IR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR and MS). The free radical scavenging activity of these compounds was measured by DPPH method and found as Quercetin (IC_{50}: 4,23) > Kaempferol (IC_{50}: 6,24) > Quercetin 3-*O*-β-galactopyranoside (IC_{50}: 8,26) > Myricetin (IC_{50}: 9,76) > Myricetin 3-*O*-β-galactopyranoside (IC_{50}: 10,05), respectively. According to these results quercetin is more active than ascorbic acid and has similar activity with the caffeic acid used as standards.

PL52 Screening of *Dracocephalum kotschyi* accession for surface flavonoids and rosmarinic acid

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Dracocephalum kotschyi Boiss, of the Labiatae family, is an endemic and native herbaceous plant of Iran, where it is known as Badrandjboie-Dennaie and Zarrin-Giah[1]. It has been traditionally used as a folk medicine and an additive to improve the taste and scent of tea and yogurt. *D. kotschyi* is an important source of essential oils [2] and flavonoids such as xanthomicrol[3], calycopterin and cirsimaritin with anticancer properties. In this study we collected plant samples in 13 natural habitats to locate valuable accessions for domestication and breeding. Methanolic (80%) extract of leaves was used to quantify xanthomicrol, cirsimaritin, calycopterin, apigenin and rosmarinic acid, which were also identified and quantified by ESI-MS and HPLC-DAD methods. Plants collected from the central regions of Iran showed the highest levels of methylated flavonoids whereas the plants from the north of the country contained the most rosmarinic acid. Taken as a whole, our results show that flavonoid contents in *D. kotschyi* depend on environmental conditions; a semi-arid climate and high exposure of the plants to UV radiation leads to an accumulation of methoxy derivatives, while wet and cold conditions increase rosmarinic acid accumulation. **References:** 1. Mozaffarian V (1998) A Dictionary of Iranian Plant Names. Farhang Moaser Pablication. Tehran. 2. Fattahi M et al. (2010) Planta Med 76: 1271. 3. Jahaniani F et al. (2005) Phytochemistry 66: 1581 – 1592.

PL53 Anti-obesity effects of *Geranium thunbergii* extract via improvement of lipid metabolism in high-fat diet-induced obese mice

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Geranium thunbergii Siebold & Zucc. (Geraniaceae) is a traditional herb with anti-diarrhetic, anti-inflammatory, and anti-oxidative effects. This study investigated the anti-obesity properties of an extract of *Geranium thunbergii* (GTE) in high-fat diet-induced obese mice. GTE treatment significantly reduced body weight, adipose tissue mass, adipocyte size, and serum triglyceride, total cholesterol, and low density lipoprotein-cholesterol levels in obese mice relative to the high-fat diet-fed mice. It also decreased serum leptin levels and increased adiponectin levels. The serum levels of aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine were not significantly changed in GTE-treated mice compared to their levels in normal diet and high-fat diet-fed mice. Furthermore, GTE suppressed the mRNA levels of sterol regulatory element-binding protein 1c, peroxisome proliferator-activated receptor γ, adipocyte fatty acid-binding protein, and fatty acid synthase in the adipose tissues of obese mice. These results suggest that GTE ameliorated high-fat diet-induced obesity by altering adipokine levels, and downregulating the expression of transcription factors and lipogenic enzymes involved in lipid metabolism.

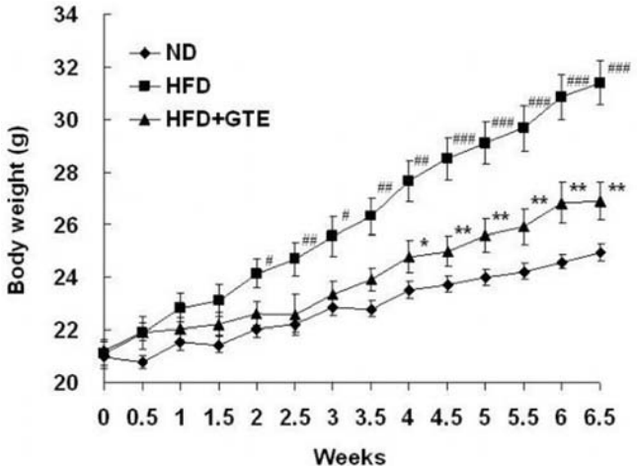

Figure 1: Effect of GTE on body weight in high-fat diet-induced obese mice

Acknowledgement: *Jinan Red Ginseng Oriental Medicine Cluster Corporation, Ministry for Food, Agriculture, Forestry and Fisheries of Korea.*
References: 1. Pokharel YR et al. (2007) Biol Pharm Bull 30: 1097 – 1101. 2. Hiramatsu N et al. (2004) Biofactors 22: 123 – 125. 3. Ushio Y et al. (1991) Int Arch Allergy Appl Immunol 96: 224 – 230.

PL54 Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophaë rhamnoides* L.) organs

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Hippophaë rhamnoides L. (Elaeagnaceae), commonly known as sea buckthorn, is a thorny bush with orange berries naturally distributed in Asia and Europe. Fruits of *H. rhamnoides* have been used by Chinese, Mongolian and Tibetan medicines for decades, and possessed considerable medicinal and nutritional values like antimicrobial, antitumoral, antioxidant and dermatological effects [1,2]. However the therapeutic potential and phytochemical diversity of the other *H. rhamnoides* parts remain unexplored. In this work we present the phytochemical and bio-activities screening of seed, leaf, stem and root of H. rhamnoides. The crude extracts were obtained by Pressurised Liquid Extraction (PLE) using ethanol. Each extract was then partitioned by liquid-liquid extraction using three solvents of different polarities: aqueous, ethyl acetate and hexane. The antimicrobial effect, the total phenolic content, the reducing power (FRAP), and the free radical scavenging activity (DPPH) of crude extracts and their fractions were evaluated. *H. rhamnoides* organs have all antibacterial values and that the most antioxidant potential was found in root and seed extracts. Furthermore, the antimicrobial and the antioxidant activities were found in the aqueous fraction. High Performance Thin Layer Chromatography (HPTLC) analyses of aqueous fractions showed that they were mainly constituted of sugar and polyphenolic compounds. The bio-activities were consequently attributed to the polyphenolic compounds present in active fractions of seed, leaf, stem and root of *H. rhamnoides*. **References:** 1. Guliyev VB et al. (2004) J Chromatogr B 812: 291 – 307 2. Zeb A (2004) J Bio Sci 4: 687 – 693

PL55 New vobasinyl-iboga bisindole alkaloids with antiparasitic activities from *Muntafara sessilifolia*

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Muntafara sessilifolia (Baker) Pichon or *Tabernaemontana sessilifolia* is an endemic plant of Madagascar which belongs to the Apocynaceae family. The stem-bark is traditionally used for the treatment of fevers and as tonic. Screening based on inhibitory activity against the chloroquine-resistant strain FcB1 of *Plasmodium falciparum* allowed the selection of this plant for a phytochemical investigation. Selective acid-base extraction with gradient of pH performed on methanol extract from the powdered stem-bark, yielded a crude alkaloid and EtOAc extracts. Both extracts were active *in vitro* on *P. falciparum* with an IC_{50} value of 1.6 and 6.5 µg/ml, respectively. The bioassay-guided fractionation of EtOAc extract by combined chromatographic methods (preparative TLC, CC (SiO_2, Al_2O_3), Sephadex ®LH 20 gel, MPLC, preparative HPLC) led to isolation of indole along with vobasinyl-iboga bisindole alkaloids as active constituents. Their structures were elucidated by spectrometric techniques (IR, UV, ESI-MS, 1D and 2D NMR (COSY, HSQC, HMBC, NOESY)). Six compounds are new among the thirteen isolated[1], [2]. NMR spectra at low temperature allowed the characterization of bisindole alkaloids whose ^{1}H NMR spectra were not resolved at room temperature. A hypothesis of biogenesis is proposed.The antiparasitic on *Plasmodium falciparum*, *Trypanosoma brucei brucei* and *Leishmania donovani* and cytotoxic activities were evaluated on all obtained molecules. The selectivity towards parasites was determined. The attempt for the access to the target of active molecules on *Plasmodium falciparum* is discussed.

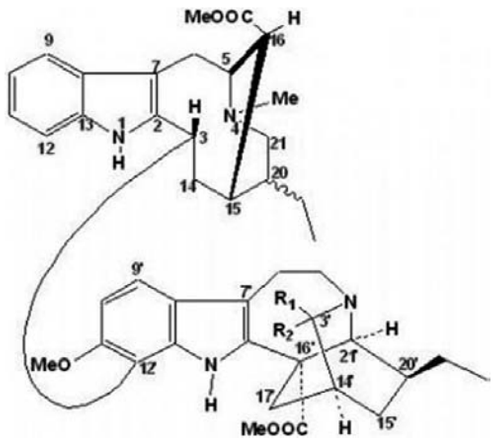

Figure 1: bisindole alkaloids from Muntafara sessilifolia

Acknowledgement: *the MENESR* **References:** 1 Garnier et al. (1984) J Nat Prod 47(6): 1055 – 1056 2 Le Men et al. (1975) Phytochemistry 14: 1120 – 1122

PL56 Bioactive compounds from *Phyllanthus atropurpureus* Bojer cultivated in Egypt

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From the ethyl acetate soluble fraction of the *Phyllanthus atropurpureus* Bojer, six compounds were isolated and identified based on spectral data (IR, UV, Mass (FAB, EI), ^{1}H-NMR and ^{13}C-NMR). Four compounds are isolated for the first time from the plant, the structures were established as di [3, 4, 5- trihydroxy- phenyl] ether, 5, 6, 8, 4'-tetrahydroxy isoflavone, Robustaside A, 6'- (4"- hydroxy cinnamoyl) arbutin, and 6'- (3", 4"- dihydroxy cinnamoyl) arbutin. The other two isolated compounds are Demethoxysudachitin, and quercetin-7- O-glucoside. Concerning the biological studies, the results revealed that the total extracts can be considered an efficient antimicrobial, when ethyl acetate fraction of leaves shows significant gram -ve antibacterial activity (E. coli) than the other tested materials as well as Ampicillin. All tested materials show significant gram +ve antibacterial activity (*S. aureus*) compared to the effect of Gentamycin. Also robustaside A was found to produce a specific strong anti-tumor activity against hepatocellular carcinoma.

PL57 Anticholinesterase and free-radical scavenging activity of *Corydalis cava* alkaloids

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The tubers of *Corydalis cava* Schweigg. & Kort. were extracted with ethanol and the summary alkaloid extract was fractionated in silica gel chromatography column using step gradient elution with hexane, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of fifteen isoquinoline alkaloids. The chemical structures of isolated compounds were determined on the basis of spectroscopic techniques and by comparison with literature data. Isolated alkaloids were tested on ability to inhibit human erythrocyte acetylcholinesterase, serum butyrylcholinesterase (IC_{50}) and for its free-radical scavenging activities (EC_{50}). Cholinesterase inhibitory activities were determined *in vitro* by modified spectrophotometric Ellman's method [1]. (+)-canadaline inhibited acetylcholinesterase as well as butyrylcholinesterase in a dose-dependent manner with IC_{50} values 20.1 ± 1.1 μM and 85.2 ± 3.2 μM, respectively. (+)-canadine with an IC_{50} value 12.4 ± 0.9 μM was the most potent inhibitor of acetylcholinesterase, whilst (±)-corycavidine and (+)-bulbocapnine were effective inhibitors of butyrylcholinesterase with IC_{50} values 46.2 ± 2.4 μM and 67.0 ± 2.1 μM. Other isolated alkaloids were considered inactive (IC_{50} > 100 μM). Free-radical scavenging activities of isolated alkaloids were tested *in vitro* by means of the DPPH test [2]. The highest activities exhibited (-)-scoulerine, (-)-sinoacutine and (+)-bulbocapnine with EC_{50} values 102 ± 6.2 μM, 209 ± 8.1 μM and 279 ± 16.7 μM, respectively. Other isolated alkaloids were considered inactive (EC_{50} > 1000 μM). **Acknowledgement:** *The study was supported by grants of GA UK No. 122309 and SVV-2010 – 263 – 002.* **References:** 1. Ellman L, Courtney D, Andreas V, Featherstone R (1961) Biochem Pharm 7: 88 – 95. 2. Polášek M, Skála P, Opletal L, Jahodár L.(2004) Anal Bioanal Chem 379: 754 – 758.

PL58 Biodiversity of high mountain flora as a source of new medicines – Dinaric Alps (W. Balkan)

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The biodiversity of high mountain flora is very rich [1]. It still pharmacologically poorly investigated. This is especially true in areas that are rich in endemic species like this Dinarides (Western Balkans). This is an important resource in getting new drugs [2]. The aim is to make identification of potentially endemic medicinal plants and their biochemical background. In order to achieve of objectives, the following methodology was applied: field research on different profiles, including ethnobotanical interviews, followed at the end by comparative taxonomic-biochemical method. In the mountainous zone of the western Balkans was found 2500 species [3]. Very small number used in the official pharmacy and medicine. As potentially are 1500 species of medicinal plants. On the basis of their taxonomic similarity is expected and biochemical similarity, the pharmacological activity, as well. As a real or potential sources of alkaloids are the species of the genera: Onosma, Moltkaea, Colchicum, Senecio, Cynanchum, Astragalus, Oxytropis, Vicia, Papaver, Euphorbia, Edraianthus, Campanula; heterosides are species of genera: Arctous, Ferulago, Atamantha, Pancicia, Bupleurum, Seseli, Genista, Gentianella, Gentiana, Frangula, Rhamnus; saponosides are: Verbascum, Scrophularia, Primula, Soldanella, Dianthus, Silene, Arenaria, Minuartia, Knautia, Scabiosa, Viola, etc.; tannins are: Geum, Potentilla, Sibirea, Crataegus, Dryas, Saxifraga, Geranium, Asplenium, etc.; terpenoides are species of genera: Centaurea, Hieracium, Hypochoeris, Amphoricarpos, Petasites, Homogyne, Stachys, Satureja, Micromeria, Scutellaria, Euphrasia, Pedicularis, Veronica, Iris, Pinus, etc.; carbohydrates are: Orchis, Gymnadenia, Dactylorhiza, etc. and lipids are species of genus Linum. It opens new possibilities in modern phytotherapy [4]. **References:** [1] Redzic S (2007) Planta Med 73: 1013 – 1013. [2] Redzic S (2008) Planta Med 74: 1143 – 1144. [3] Redzic S (2007) Planta Med 73: 1013 – 1013. [4]. Redzic SS (2007) Coll Antropol 31: 869 – 890.

PL59 Chemical characterization and antimicrobial activity of the needle essential oil of *Pinus mugo* (Pinaceae) from Macedonian flora

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Pinus mugo Turra (Pinaceae) or Mountain pine is low and shrubby conifer which can be found in Republic of Macedonia in very huge population, only in central part of the country, on Karadzica Mountain. Withal, this location is the southernmost extensive point for this plant. The needle essential oil was obtained by hydrodestilation in Clevenger apparatus after removing the needles from the branches and was yielded from 0.15 to 0.65%. The chemical composition of the essential oil was analyzed by gas chromatography/mass spectrometry and the most abundant components were monoterpenes alpha-pinene (6.2 – 12.9%), beta-pinene (1.3 – 3.3%), delta-3-carene (10.1 – 18.7%), limonene + beta-phellandrene (3.1 – 5.7%), alfa-terpeinolene (2.1 – 3.0%) and bornyl acetate (2.0 – 3.7%) and sesquiterpenes trans-caryophyllene (5.7 – 6.4%), germacrene D (2.4 – 11.8%), bycyclogermacrene (3.0 – 6.8%), delta-cadinene (4.0 – 6.6%), tau-muurolol + tau-cadinol (2.5 – 4.4%) and alpha-cadinol (3.4 – 5.0%). The essential oil was screened for antimicrobial activities against 13 bacterial isolates representing both Gram positive and Gram negative bacteria and one strain of *Candida albicans* using hole-plate method. The broth dilution method was used for testing the minimal inhibitory concentration (MIC) of the essential oil. The needle essential oil was confirmed to have significant antimicrobial activity, especially against Gram positive bacteria such as *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* with minimal inhibitory concentrations 15.26 μl/ml, 7.5 μl/ml and 31.25 μl/ml, respectively.

PL60 Basic pharmacognostic research of *Gentiana cruciata* L. species from Bosnia and Herzegovina (W. Balkan)

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Gentiana cruciata L. (Gentianaceae) is a widely distributed species in the area of Dinarides. [1]. It is used in traditional medicine in some mountainous areas of Bosnia [2]. As related species of *Gentiana lutea* L. is endangered, [3] similar uses of the other species of *Gentiana* in modern phytotherapy are investigated. *Gentiana cruciata* has such capabilities. As a precondition for its use, we planned to conduct basic botanical and pharmacognostic research. The material was sampled 2007 at the mountains of Sarajevo (800 to 1500 m). All studies were carried out in accordance with the European Pharmacopoeia IV monograph. The leaves have dorsi-ventral histological structure. Stomata indexes are 26.22. Type of stoma is anomocytic. Root has the primary and later secondary structure. Use of spectrophotometric analysis and paper chromatography fortified the ratio between chlorophyll a and b 2:1. Chlorophyll a is 0.6 mg/g; chlorophyll b is 0.383 mg/g and carotenoids 0.285 mg/g. The proportion of plant pigments indicate the potential antioxydative activity of this plant. The roots of this species has been prepared for chemical analyses. For the separation of metabolites was used thin layer chromatography method. Standard analysis showed the presence of sucrose and amarogentin. The method of micro-sublimation proved the presence of gentisin. Preliminary and basic results suggest that the roots and aerial parts of *Gentiana cruciata*could be a useful replacement for the very popular and highly endangered species *Gentiana lutea* L. subsp. *symphyandra* (Murb.) Hayek. **References:** 1. Redzic S (2006) Proc. 1st IFOAM Intern. Conf. Organic Wild Production 117 – 141. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S et al. (2009) Planta Med 75: 902 – 902.

PL61 Elemental compositions of *Echinacea purpurea*, *E. pallida* radix and herba cultivated in Turkey

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Echinacea sp. (Asteraceae) are one of the most popular medicinal plants used in phytotherapy. In this present study, *E. purpurea* (L.) Moench and *E. pallida* (Nutt.) Nutt. were successfully cultivated under controlled conditions in experimental fields in Konya. Dried areal parts and the roots of 36 cultivation samples were investigated for their macro (N, P, K) and micro (Ca, Mg, Na, Fe, Mn, Zn, Cu) trace elemental compositions using various techniques. N was determined by the dry combustion method using elemental analyses, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with statistical data comparison to a certified reference plant material, respectively. The total dry matter content, including those of the areal parts of the crops ranged 25 – 30%. The results of elemental analyses showed that N ranged 0.54 – 1.69%, P ranged 1100 – 2600 ppm and K ranged 9990 – 29585 ppm. To the best of our knowledge, this is the first report on micro and macro elements of cultivated Turkish *Echinacea* sp. As a conclusion, the elemental composition and the nutritional value *E. purpurea* and *E. pallida* are worthwhile to investigate with comparison to other Echinacea sp. used medicinally.

PL62 Bioassay-guided fractionation and cytotoxic activity of flavonoids from *Echinochloa crus-galli* L. (Barnyard Grass)

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Echinochloa crus-galli L. (Family *Poacae*) is a problematic summer weed found in rice fields and moisten soil worldwide and known as Barnyard Grass [1]. Bioassay-guided fractionation of the seeds of *Echinochloa crus-galli* L. lead to isolation of two cytotoxic flavonoids after screening against four human cancer cell lines: MCF-7 (breast cells), HCT-116 (colon cells), HELA (cervical cells) and HEPG-2 (liver cells) using the sulforhodamine B (SRB) colorimetric assay [2]. Different extracts showed a dose dependent inhibition in a range of 5 – 50 µg/ml. The ethanolic extract (95%) proved to be the most active extract against HELA cell line (IC 50 = 12 µg/ml). On the other hand, the hexane and chloroform fractions exhibited moderate activities against HEPG-2 (IC_{50}= 15,5 µg/ml) and HCT-116 (IC_{50}= 17.1 µg/ml) cell lines, respectively. Two flavonoids were isolated from the chloroform fraction and identified as 5,7-dihydroxy-3',4',5'-trimethoxy flavone (1) and quercetin (2) [3]. Compound **1** exhibited potent cytotoxic activities against HELA cell line (IC_{50}= 4.5 µg/ml) and HEPG-2 cell line (IC_{50}= 4.5 µg/ml), which were comparable to doxorubicin (IC_{50}= 4.3 µg/ml). Compound **2** showed moderate cytotoxic effects against MCF-7, HCT-116, HELA and HEPG-2 cell lines with IC_{50} values of 12.7, 20.4, 13.9 and 11.3 µg/ml, respectively. **Acknowledgement:** *The authors are grateful to Prof. Dr Osama El Kopacy, Professor of Botany, Faculty of Agriculture, Cairo University for authentication of the plant and Prof. Dr. Samia Shouman, Professor of Clinical Biochemistry, National Cancer Institute, Cairo, for her help in carrying out the cytotoxic assays.* **References:** 1. Apfelbaum S, Sams C (1987) Natural Journal 7: 68 – 74. 2. Skehan P, Stroreng R (1990) G Natl Cancer Inst 82: 1107 – 1112. 3. Mabry TJ, Markham KR, Thomas MB (1996) The Systematic Identification of Flavonoids, 2nd ed., Springer- Verlag, Berlin.

PL63 Plant pigments in some medical plants of family Lamiaceae (Bosnia and Herzegovina, W. Balkans)

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Plant pigments chlorophyll and carotenoids are very important group of primary and secondary metabolites, resp. Besides their role in process of photosynthesis and plant protection from extensive radiation, they have huge appliance in pharmaceutical industry, cosmetology and dietetic. Plant pigments are also given significant role in anti-oxidant activity [1, 2]. Goal of these studies has been qualitative – quantitative analysis of main and side pigments in selected medicinal species of wild flora in BiH, including endemic species. Plant materials were gathered during different seasons. They were transported fresh to the laboratory where qualitative (paper and thin layer chromatography) and quantitative (spectrophotometric) analyses took place. Results (Table 1) showed significant presence of chlorophyll a, chlorophyll b and carotenoides. Ratio between chlorophyll a and chlorophyll b was rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medicinal and gives them higher potential for antioxidant capacity [3].

Tab. 1: Contents of plant pigments in selected plants of Lamiaceae family

Plant species	Locality (100 – 2,000 m altitude)	Chlorophyll a (mg in g fresh of leaves)	Chlorophyll b (mg in g fresh of leaves)	Carotenoides (mg in g fresh of leaves)
Origanum vulgare L.	Igman Mt.	4,592	4,114	1,619
Origanum heracleoticum L.	Mostar	5,210	4,252	1,731
Satureja subspicata Bartl. ex Vis.	Velez	4,750	3,870	1,670
Satureja montana L.	BH coast	6,452	4.445	1.756
Micromeria thymifolia (Scop.) Fritsch	Sarajevo	5.780	2,100	0,890
Thymus aureopunctatus (Beck) K.Maly	Konjic	5.723	1,124	0.679
Thymus balcanus Borbas	Bjelasnica Mt.	4.678	1,670	1,620
Thymus bracteosus Vis. ex Benth	Trebinje	5,120	2,020	1,230
Acinos orontius (K. Maly) Silic	Konjic	4,345	1,970	0.890
Nepeta pannonica L.	Visoko	5,670	2,230	1,120
Salvia officinalis L.	Ljubuski	5,100	1,800	1,234

References: 1. Redzic S, Hodzic N, Tuka (2005) Bosn J Basic Med Sci 5(2): 53 – 8. 2. Redzic S, Tuka M, Pajevic A (2006) Bosn J Basic Med Sci 6(2): 25 – 31. 3. Davies K ed. (2004) Plant pigments and their manipulation. Annual Plant Reviews, 14, 368 p., Blackwell Publishing, Oxford.

PL64 Protective effects of *Sedum caespitosum* and its polyphenolic compounds against to H_2O_2 induced cytotoxicity

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There are 33 *Sedum* species growing in Turkey and some *Sedum* species have been employed in folk medicine for their antidiarrheic, wound healing, and diuretic properties as well as treatment of chronic viral hepatitis.The crude MeOH extract prepared from the aerial parts of *Sedum caespitosum* (Cav.) DC. and its EtOAc, n-BuOH, H_2O subextracts were screened for their protective effect against to H_2O_2 induced cytotoxicity at different concentrations in human red blood cell. The EtOAc subextract were found to be the most protective one and its chemical composition was further analysed. Five polyphenolic secondary metabolites including gallic acid (1), kaempferol 3- α-rhamnopyranoside (2), quercetin 3-O-β-glucopyranoside (3), quercetin 3-O-α-rhamnopyranoside (4), myrcetin 3-O-α-rhamnopyranoside (5) were isolated from the EtOAc extract by successive chromatographic methods. The structures of the isolated compounds were elucidated by 1D- and 2D-NMR techniques. This is the first phytochemical work on *S. caespitosum*. The protective effect of the isolates against to H_2O_2 induced cytotoxicity in human red blood cells also evaluated at 5 and 10 µg/ml and kaempferol 3-α-rhamnopyranoside was shown most protective effect. **Acknowledge-**

ment: *Authors are grateful to Prof. Dr. Hayri Duman, Gazi University, Ankara for identification of plant material.*

PL65 Fagopyrin and its derivatives in buckwheat (*Fagopyrum* sp.)

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Fagopyrin and its derivatives are dimerized anthraquinone polyphenolic substances from buckwheat. They act as photosensitizers upon excitation with visible light (540 – 610 nm), which causes phototoxic effect after ingestion of large amounts of buckwheat called fagopyrism (1). According to known structures and transformations of hypericin derivatives from St. John's wort and their similarity to fagopyrin, several forms of fagopyrin derivatives have been postulated as pseudofagopyrin and protofagopyrin (2). It was shown that pre-forms of fagopyrin transform to fagopyrin in plant extract under daylight exposure (3). We optimized the extraction method of fagopyrins from plant material. We developed a HPLC method coupled with fluorescence detector (excitation wavelength 330 nm, emission 590 nm) for separation and detection of those compounds. The HPLC method yielded several chromatogram peaks with close retention times presenting different forms of fagopyrin. Various buckwheat products available on the market were analyzed using this method. The highest amount of fagopyrins was found in buckwheat herb samples and less in fruit samples, which contain most of the compounds in peels. Buckwheat herb was used for further observation of the nature of fagopyrins in plant extract. As described previously (4), we observed that the content of different forms of fagopyrin varied due to different extraction conditions (time, temperature, solvent). Since the differences were observed even if the conditions changed after the end of the extraction (removal of the herbal substance), we assume that this changes were due to transformations of fagopyrins and not only due to extraction efficacy. The transformations were at least partly reversible. **References:** 1. Chick H, Ellinger P (1941) J Physiol 212 – 230. 2. Brockmann H, Lackner H (1979) Tetrahedron Letters 18: 1575 – 1578. 3. Habermann B (2000) Arch Farm, Farm Med Chem 333, Suppl. 2. 4. Hinneburg, Neubert Reinhard HH (2005) J Agric Food Chem 53: 3 – 7.

PL66 Seasonal variation of lipophilic constituents in roots of *Echinacea purpurea* and *E. pallida*

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Echinacea purpurea (L.) Moench and *E. pallida* (Nutt.) Nutt. are widely used for the unspecific enhancement of the immune system. The plants origin from North America and are grown all over the world as garden flowers or as medicinal plants. Lipophilic constituents such as alkamides and ketoalkenes/ketoalkynes are believed to be among the active metabolites in *E. purpurea* and *E. pallida*, respectively, with the highest concentrations being found in the roots. Most investigations on roots have been conducted on plants younger than one or two seasons and few have investigated the season variation of lipophilic constituents in roots of *Echinacea* species. From early winter 2009 to fall 2010 five 4 – 5 year old *E. pallida* and seven 3 – 4 year old *E. purpurea* roots from the same population of plants were collected throughout one year (*i.e.* before and after soil freeze in winter, mid spring, at high soil temperature in the summer and mid fall). Lipophilic constituents were extracted from milled freeze dried roots with EtOH-H_2O (70:30) and analyzed by HPLC-PDA and LC-MS/MS. The highest concentration of alkamides in *E. purpurea* roots was found when soil temperature was just above 0 °C after winter and during summer, when the soil temperature was high. In the first case dodeca-2E,4Z-diene-8,10-diynoic acid isobutylamide was the major alkamide and in the latter case dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides were the major constituents. For *E. pallida* roots the highest concentration of 2-ketoalkenes and -alkynes were found when the soil temperature was just above 0 °C after winter and here the major constituent was pentadeca-8Z,13Z-dien-11-yn-2-one.

PL67 Review of Natural and synthetic tyrosinase inhibitors

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Tyrosinase is a multifunctional copper-containing enzyme found in a broad spectrum of animal and plant species [1]. It catalyses the initial steps in the formation of the pigment melanin from tyrosine. In addition tyrosinase is responsible of browning of certain fruits and vegetables during their handling, processing and storage after harvest [2] and It is one of the most important enzymes in the insect molting process[3]. It also is linked to Parkinson disease and other neurodegenerative diseases[2]. Recently, safe and effective tyrosinase inhibitors have become important for their potential application in improving food quality [4]and insect control agents[3] and preventing or treat pigmentation disorders (melasma, freckles, senile lentigines[5] and other melanin-related health problems in human beings. These causes have encouraged researchers to seek new potent tyrosinase inhibitors for use in foods, cosmetics and pharmaceuticals This article overviews tyrosinase inhibitors from natural and synthetic sources including all herb species, active ingredients, semi synthetic and synthetic components from 2000 – 2011 discussing on natural skeletons, semisynthetic and synthetic compounds, SAR of components and the richest families, genuses,.. **References:** 1. Hearing VJ et.al. (1987) Int J Biochem 19(12): 1141 – 7. 2.Fais A et al., (2009) Molecules 14(7): 2514 – 20. 3.Arung ET et al.,(2005) Journal of Wood Science 51: 520 – 525. 4.Hsu CK et al. (2007) Food Chemistry 105(3): 1099 – 1105. 5.Zhang X et al. (2009) Biological and Pharmaceutical Bulletin 32(1): 86 – 90.

PL68 Study of content and composition of anthocyanins in selected plants species

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Anthocyanins are heteroglycosides composed of aglycone – anthocyanidine and sugar moiety. They are the final product of flavonoid production in secondary metabolism of plant cells. They are characteristic with antioxidant effects, through which they have positive effect on human organism. There is a large number of anthocyanidins, out of which only six are of the greatest importance, those with hydroxylic group at C-3 location. They are cyanidine, pelargonidine, peonidine, delfinidine, petunidine, malvidine. These are present in large amounts in plant species *Vitis vinifera* L., *Vaccinum corymbosum* L. and *Sambucus nigra* L. In the berries of *Vitis vinifera* they are accumulated in hypodermal cell layer of peel, or in the pulp of some cultivars. Except for pelargonidine, it contains all important anthocyanidines, with predomination of malvidine. Total content of anthocyanins in fresh berries ranged from 0,50 to 4,99 $g.kg^{-1}$ and in peels from 20,7 to 66,6 $mg.g^{-1}$ of peels dry matter. In the anthocyanins of *Vaccinum corymbosum* there were identified cyanidine, delphinidine, malvidine and peonidine. Their total content varies significantly depending on variety. Total determined content of anthocyanins ranged from 290,16 to 1343,08 $mg.dm^{-3}$. *Sambucus nigra* contains five important anthocyanidines: cyanidine 3-sambubiosid-5-glucoside, cyanidine 3,5 diglucoside, cyanidine 3-sambubioside, cyanidine 3-glucoside and cyanidine 3-rutinoside. The content of identified anthocyanins in fruits of this species ranges from 602,9 to 1265,3 $mg.100\,g^{-1}$. The amount of accumulated anthocyanins pigments depends on variety, ecological conditions standard of agricultural technology, and particularly on the temperature and solar radiation. **Acknowledgement:** *The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/ 2010 – 11).*

PL69 Genetic and Environmental Variations in Baicalin, Baicalein and Wogonin Contents in *Scutellaria baicalensis*

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Scutellaria baicalensis has long been used to treat fever, cough, diarrhea and infection in Chinese medicine, and flavonoids such as baicalin (BA), baicalein (BE), and wogonin (WO) have been identified. To understand genetic and environment-dependent variations, 15 *Scutellaria baicalensis* landraces were collected from Korea and China, and cultivated under different nitrogen fertilizer application and planting density conditions, and resultant changes in BA, BE, and WO were evaluated. Tested 15 landraces exhibited BA, BE, and WO contents ranging 4.56 to 13.59%, 0.28 to 5.54%, and 0.50 to 1.63% with an average of 9.66%, 2.09%, and 0.52%, respectively. Among tested 4 levels (0 to 500 kg/ha) of nitrogen fertilizer application 300 kg/ha resulted in highest BA (10.3%) and BE (1.3%), as well as WO (0.4%) contents, corresponding to about 10% increment compared to 0 kg/ha. Six different levels of planting density (2 and 3 Rows x 1, 5, 10 cm distance), however, showed no difference in flavonoid contents. Similarly no difference in flavonoid contents could be observed between *Scutellaria baicalensis* harvested from early June 6th to Aug. 3 rd. When flavonoid contents in different plant parts were compared, leaf and stem of *Scutellaria baicalensis* showed no BE and WO under our experimental conditions, while relatively low BA (0.67% in leaf and 1.56% in stem) could be found in leaf. Higher composition of BA could be observed in top part of the root, while higher WO could be observed in lower root part close to root hair. **References:** 1. Cole et al. (2008) Planta Med 74(4): 474 – 481. 2. Rhee J, Park H (1997) Analytical Science & Technology 10: 91 – 104.

PL70 Antioxidant activity of *Jasminum malabaricum* – A medicinal plant from Western Ghats

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Jasminum malabaricum Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber with white flowers and fragrance, which is known for its ethnomedicinal importance as blood purifier and anti-tumor properties. The extensive exploitation of this species has led to reduction of its natural population. In the present study the leaves and stems were subjected for continuous shaking extraction (CSE) and microwave assisted extraction (MAE) using methanol. The antioxidant studies were performed with various concentrations of methanolic extract by DPPH and FRAP radical scavenging assay. The leaf extract showed significant activity (i.e. 92.86 ± 00.08% for CSE and 80.21 ± 00.14% for MAE) for DDPH method and for FRAP method the results showed significant activity (i.e. 4241.40 ± 212.07 for CSE and 5547.40 ± 277.37 for MAE) at concentration 0.2% which was compared with the standard ascorbic acid. **References:** 1. Mann HH (2008) J Linnean Soc (London) Botany 45(302): 155 – 8. 2. Thangavelu NR, Thomas S (2010) Int J Biol Med Res 1(4): 188 – 192

PL71 Antioxidant potential of Brazilian plants

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Oxidative stress, the imbalance between free-radical formation and elimination, is part of the pathophysiology of many diseases[1]. Exogenous antioxidants, such as those from plants, can help to restore the normal redox state of tissues[2]. The aim of this study was to evaluate the antioxidant potential of plant species found in the Brazilian *cerrado*. The ethanolic extracts of different anatomical parts of 22 plant species (13 botanic families) were evaluated *in vitro* using two distinct approaches: the DPPH free-radical scavenging method (determination of EC_{50}) and the β-carotene bleaching test (125; 62,5 e 31,25 μg/mL), both performed in microplates and with pyrogallol and quercetin as antioxidant standards. In this study, the sample was considered active when showed EC_{50} < 30 μg/mL in the DPPH method and inhibition of 50% of β-carotene oxidation at 31.25 μg/mL. All the 46 extracts evaluated showed concentration-dependent responses and 19 of them were considered active. Two species showed the best antioxidant profile: *Qualea grandiflora* Mart. (Volchysiaceae), with EC_{50} of 4.62 ± 0.69 μg/mL (leaves) and 4.94 ± 0.34 μg/mL (barks) on the DPPH method and 71.6 ± 6.1% (leaves) and 74.7 ± 2.38% (barks) inhibition of β-carotene oxidation at 31.25 μg/mL; and *Lafoensia pacari* A.St.-Hil. (Lythraceae) with DPPH EC_{50} of 4.68 ± 0.37 μg/mL (leaves) and 4.62 ± 0.63 μg/mL (barks) and 62.4 ± 2.9% (leaves) and 64.5 ± 3.8% (barks) inhibition of β-carotene oxidation. These species will be further studied as a source of products for use in the prevention and treatment of diseases in which oxidants or free radicals are implicated. **Acknowledgement:** *Fapemig, for the financial support (FAPEMIG DEG-AUC-43/10); Prof. Braga, F.C and Prof. Castilho, R.O. for gently allowing the use of the laboratory facilities.* **References:** 1. Valko A et al. (2007) Int J Biochem Cell B 39: 44 – 84. 2. Halliwell B. (2001) Free Radical Bio Med 46: 531 – 542.

PL72 Phytochemical investigation of the Neotropical plant *Strychnos aff. darienensis*

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Strychnos darienensis Seem. of the family Loganiaceae, initially identified by Seemann [1], is widely distributed in the region of Central and South America [2] and it was used in the preparation of curarizing arrow poison from the South American Indian hunters. Generally, *Strychnos* species are rich in alkaloids, whereas the content of other secondary metabolites is in many cases neglected. In continuation of our investigation of plants from the Amazonia [3], *S. aff. darienensis* that has not been previously investigated was collected from Peru. The evaluation of the best extraction procedure in order to recover a vast range of metabolites was of great importance. Profiling of the extracts with TLC revealed that the best approach was the maceration of the stem bark with EtOAc-EtOH-NH3 (96:3:1) and percolation with EtOAc and then with MeOH [4]. We report herein the investigation of the EtOAc and MeOH extracts, which has lead to the isolation of flavonoids and alkaloids. The isolation procedure was performed using Medium Pressure Liquid Chromatography (MPLC), molecular exclusion chromatography on Sephadex LH-20, semi preparative HPLC and preparative TLC. All compounds isolated, were identified by means of spectral data (1D and 2D NMR, HRMS). Two monomeric flavonoids and one bisflavonoid were identified, namely luteolin (**1**), 3-methoxyquercetin (**2**) strychnobiflavone (**3**) and the monoterpene alkaloid venoterpin. Compound **2** is for the first time reported in the genus and strychnobiflavone (**3**) is isolated for the second time from a plant extract [5].

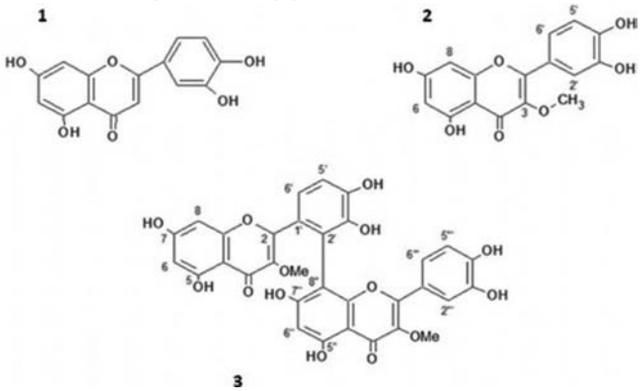

Figure 1: Isolated flavonoids of Strychnos aff. darienensis

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PL73 Phenolic acids and free radical scavenging activity of Bulgarian endemic - *Alchemilla jumrukczalica* Pawl

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In the phytotherapy *Alchemilla vulgaris* L. complex is widely used as astringent, diuretic, anti-inflammatory agents, characterized by the presence of phenolic acids, flavonoids, tannins, triterpenes, etc. [1]. *Alchemilla jumrukczalica* Pawl. is rare plant, unstudied for chemical constituents and biological activity until now. The present study aims to establish the antiradical potential and phenol content of *A. jumrukczalica* (cultivated materials) and *A. vulgaris* complex. The antioxidant activity of the methanol extracts was evaluated by the scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH.) radicals. The extracts showed significant antiradical activity with IC_{50} values of 12,09 and 19,62 μg/ml respectively for *A. jumrukczalica* and *A. vulgaris* complex. Commercial antioxidant butylated hydroxytoluene (BHT) and syringic acid were used as positive controls and their IC_{50} values are respectively 12.65 and 4.40 μg/ml. The methanol extracts of the studied samples were examined before and after acid hydrolysis for free and bounded phenolic acids. Ten free and seventeen bounded phenolic acids were identified and quantified by performed of gas chromatography mass-spectrometry (GC-MS). The extracts of the both samples contain phenolic acids in comparable amounts. Among the identified free phenolic acids gentisic, protocatechuic, salicylic and caffeic acids are represented in the greatest quantity. Salicylic, protocatechuic, caffeic, trans-cinnamic, gentisic and vanilic acids were the major bounded phenolic acids in the studied extracts. The present study revealed the extract of *A. jumrukczalica* as potential source of antioxidant activity. **Acknowledgement:** *The authors are grateful for the financial support provided by the Bulgarian National Science Fund, Ministry of Education, Youth and Science (Project DTK-02/38)* **References:** 1. Nikolov S (ed.) (2007) Specialized Encyclopedia of the Medicinal Plants in Bulgaria. Publishing House Trud, Sofia

PL74 High-mountain medicinal plants in Bulgaria – protection, phytotherapy, reproductive capacity and sustainable use

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The object of this study are rare and endemic species of the Bulgarian flora: *Alchemilla mollis* (Buser) Rothm; *A.jumrukczalica* Pawł. (Bulgarian endemic); *A.achtarowii* Pawł. (Bulgarian endemic); *Sideritis scardica* Griseb. (Balkan endemic); *Gentiana lutea* L. ssp. *symphyandra* (*Murb.)* Hayek and *Arnica montana* L. The species are spreaded in the subalpine and alpine zones of the Bulgarian mountains. They are included in the List of protected species of the Biological Diversity Act and Red Book of R Bulgaria Vol. 1 – Plants, excepting *A. montana*, reported to grow in the Rila Mt. [1], but so far its distribution has not been confirmed. In this study were used seeds of *A. montana*, originating from Ukraine and Germany. The harvesting of plant material from the natural populations of the species is prohibited. At the same time they are widely applied in the medicine and phytotherapy. The plant material for the pharmaceutical industry and phytotherapy can be supply only by cultivation through conventional and biotechnological methods. These practices will ensure the sustainable use and conservation of the species. The aim of the study is to reveal the reproductive capacity and opportunities for cultivation of the plants. With regard to this, we have carried out studies on the biological features of *A. mollis* [2], *A. montana* [3], reproductive biology and *in vitro* propagation of *G. lutea* [4] and *A.montana*. The results obtained demonstrate the real opportunities for growing the species in field conditions and provide the needed plant material for practical use. **Acknowledgement:** *The authors are grateful to the National Science Fund for the financial support of the study under the Contract DTK 02/38.* **References:** 1. Assyov B & Petrova A (eds) (2006) Conspectus of the Bulgarian vascular flora. Distribution Maps and Floristic Elements. Ed.3. Bulgarian Biodiverity Fundation, Sofia. 2. Vitkova A, Gavrilova A & Tashev A (2011) Phytol Balcan 17(1): 83 – 88. 3. Balabanova V & Vitkova A (2010) Compt Rend Acad Bulg Sci 63(2): 1301 – 1306. 4. Yankova E, Baldziev G, Petrova M, Zayova E & Yurukova P (2010) Biotechnol & Biotechnol EQ, Special edition/On line, 24(2): 45 – 48.

PL75 The *in vivo* angiogenic evaluation of *Achillea biebersteinii* Afan. and *Achillea teretifolia* Willd. essential oils

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In the present study, *Achillea biebersteinii* Afan. and *Achillea teretifolia* Willd. collected from Turkey were investigated for their *in vivo* angiogenic or antiangiogenic properties to correlate the folk medicine uses [1]. The essential oils were obtained from aerial parts by hydrodistillation, which were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), simultaneously. The main constituents of the oils were identified as 1,8-cineole (36, 34%) and camphor (22, 11%), respectively. Using the *in vivo* chick Chorio Allantoic Membrane (CAM) assay the oils and their main constituents were tested at various concentrations (5 – 50 μg/pellet). As a result, *A. teretifolia* essential oil showed strong antiangiogenic effect with no irritation whereas, *A. biebersteinii* oil showed no antiangiogenic effect with slight irritation (> 10%) at 50 μg/pellet when compared with cortisone, suramin, thalidomide and sodium dodecyl sulphate. 1,8-Cineole and camphor showed weak to strong antiangiogenic effect with no irritation at the same concentration in a scoring system [2]. Furthermore, none of the tested samples showed embryotoxicity, confirming its safe use. **Acknowledgement:** *The authors would like to thank TUBITAK – SBAG-107S262 (3756) for financial support of the project.* **References:** 1. Baytop T (1999) Therapy with Medicinal Plants in Turkey 2nd Edition. Nobel Tıp Kitapevleri, İstanbul. 2. Krenn L, Paper DH (2009) Inhibition of angiogenesis and inflammation by an extract of red clover (*Trifolium pratense* L.). Phytomedicine 16(12): 1083 – 1088.

PL76 NMR- and UHPLC-MS-based metabolomics for the discrimination of different resistant *Vitis vinifera* L. cultivar woods

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Vitis cultivars exhibit different susceptibility to pathogens such as botrytis or downy mildew and the selection of resistant species is important for a sustainable wine production without use of harmful pesticides. In order to highlight biomarkers that can be related to *Vitis* resistance to common diseases, woods of resistant *vitis* cultivars were profiled by NMR and UHPLC-TOF-MS [1] and analysed based on differenzial metabolomics [2]. Three different samples of *Vitis* wood, one resistant to botrytis, one resistant to downy mildew and one susceptible to both phytopathogenic microorganisms were used in this study. The wood samples of specific specimens were divided in 18 groups (6 per cultivar) and extracted separately with EtOAc to offer statistical confidence. Two different sample preparation protocols were developed and applied in parallel for the NMR (600 MHz) and UHPLC-TOF-MS analysis of the extracts, respectively. Multivariate data analysis using both supervised (PLS-DA) and unsupervised (PCA) methods and different scaling methods revealed a clear distinction between the three groups as well as in the discrimination between the two different resistant species. A high convergence regarding the discrimination patterns between NMR and UHPLC-TOF-MS data was obtained. The NMR and MS variables derived from the loading plots were attributed to specific biomarkers. This statistical model could be efficiently applied for the determination of resistant cultivars of *Vitis* as well as for the identification of novel biomar-

kers involved in resistance phenomena. **References:** 1. Eugster P et al. (2011) JAOAC 94: 51 – 70. 2. Wolfender JL et al. (2010) Curr Org Chem 14: 1808 – 1832.

PL77 Assessment of antiradical activity of high-mountain medicinal plants in Bulgaria – *Alchemilla achtarowii*, *A. mollis*, *Gentiana lutea* ssp. *symphandra*, *Arnica montana*

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The objects of present study are protected, endemic and rare high-mountain medicinal plant in Bulgaria – *Alchemilla achtarowii* Pawl., *A. mollis* (Buser) Rothm., *Gentiana lutea* L. ssp. *symphandra* (Murb.) Hayer, *Arnica montana* L. The species are widely used in modern phytotherapy, they are demand raw materials on national and international markets. This requires their cultivation by conventional and biotechnological methods. The purpose of present study is to evaluate antiradical capacity and total phenols of plants which were grown *in situ* (*A. achtarowii*, *G. lutea*), *ex situ* (*A. mollis*) and *ex vitro* (*A. montana*). The methanol extracts of examined species were estimated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu assays. The extracts of aerial parts of *A. mollis* and *A. achtarowii* showed significant antiradical activity with IC_{50} values below 50 µg/ml. The lowest activity was found of extract of *G. lutea* > 200 µg/ml. The extracts of folia and flowers of *ex vitro* plants of *A. montana* revealed high radical scavenging activity too, their IC_{50} values are 64,01 and 85,73 µg/ml respectively. Commercial antioxidant butylated hydroxytoluene (BHT) was used as positive control and its IC_{50} value is 12,65 µg/m. The antiradical properties of the studied extracts positively corresponded with their total phenol content. The results obtained showed high antiradical qualities of the examined species. It is especially important that the *ex situ* and *ex vitro* grown plants kept its valuable properties. These results will be basis for a future comparative analysis of antioxidant capacity and the content of active components of these species. **Acknowledgement:** *The authors are grateful for the financial support provided by the Bulgarian National Science Fund, Ministry of Education, Youth and Science (Project DTK-02/38)*

PL78 Effects of different drying methods on caffeic acid derivatives content of *Echinacea purpurea* cultivated in Turkey

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Echinacea sp. are promoted primarily in oral dosage forms as an immune stimulant that helps increase resistance to colds, influenza and other infections; topical products for wounds and inflammatory skin conditions are also avaible (1,2). *E. purpurea* (L.) Moench aerial parts contain at least 4 major groups of compounds generally considered to be a medicinal interest: alkamides, caffeic acid derivatives (phenylpropanoids), polyacetylenes, and polysaccharides (3). In this work, *E. purpurea* aerial parts and radix were dried under different drying conditions; sun-drying, shade-drying and oven-drying. Aerial parts and radix were dried under the sun for 48 h at average 28 °C, under the shade for 96 h at average 26 °C, in the oven for 72 h at 40 °C. When the amount of moisture in the plant reduced below 14%, drying process was ended. Agilent 1100 Series HPLC system was used for analysis of caffeic acid derivatives (caftaric acid, cichoric acid), and Zorbax ODS (250 x 4,6 mm, 5um) column was used with gradient elution. According to HPLC results; oven drying was found to be the best method compared with the others. The highest levels of caftaric and cichoric acids in *E. purpurea* aerial parts were 0,5019 ± 0,0088% and 1,3742 ± 0,0354%; respectively. And the content of caftaric and cichoric acids in the radix were found as 0,1872 ± 0,0285% and 1,4768 ± 0,1567%; respectively. As a result, oven drying is the best appropriate method for *E. purpurea* drying process. **References:** 1. Ebadi M (2002) Pharmacodynamic Basis of Herbal Medicine. CRC Press. USA. 2. Stuart DL, Wills RBH (2003) J Agric Food Chem 51: 1608 – 1610. 3. Upton et al. (2007) American Herbal Pharmacopoeia and Therapeutic Compendium. American Herbal Pharmacopoeia. USA.

PL79 Determination of lycorine of *Sternbergia candida* by HPLC

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Amaryllidaceae is one of the most widely used medicinal plant family [1]. *Sternbergia candida* Mathew & T. Baytop (Sc) is an endemic member of the family from Mugla, Turkey [2]. Lycorine is the major alkaloid of Sternbergia species. HPLC analysis of the bulbs of the species has been done previously [3]. In this study, it is aimed to determine the lycorine in chloroform and methanolic extracts of both bulbs (SCBC: SCBM) and leaves (SCLM) of Sc by HPLC. The analysis was performed with a shorter column (3 µm, C 18 150 mm x 3 mm) in a gradient solvent system without acetonitrile (A: 97.5% 10 mM ammonium bicarbonate with 2.5% methanol; B: 2.5% 10 mM ammonium bicarbonate with 97.5% methanol pH: 7.8) with a flow rate of 0.3 ml/min. The results are given in Table 1. Lycorine was found in the leaves of the plant however it was higher in the methanolic extract of the bulbs.

HPLC analysis of lycorine of Sternbergia candida

Sample	RT	RSD% RT	Area	%RSD Area	mg/g
Scbc	26.46	0.03	11.81	2.25	0.59
Scbm	26.46	0.03	23.80	1.45	0.63
Sclm	26.47	0.01	2.87	2.91	0.30

(Scbc: Sc bulb chloroform extract; Scbm: Sc bulb methanolic extract; Scbm: Sc leaves methanolic extract) (n = 3). **Acknowledgement:** *This work was supported by grants of Ernst Mach Grants; authors are thankful to Ernst Mach Grants and OEAD for the supports given.* **References:** 1. Calderon AI et al. (2010) Pharm Biol 48(9): 988 – 993. 2. Davis PH (1984) Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, Vol. 8, 363. 3. Citoglu GS et al. (2008) Chem Nat Comp 44: 6.

PL80 Investigation of stability of *Hypericum perforatum* l. total extract due to temperature and humidity

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Hypericum perforatum L. has been used to treat a variety of medical illnesses for centuries [1]. Stability represents a crucial part of the testing program for drug substances [2]. Meanwhile the stability problems of the constituents of the species is known [2 – 6]. However the stability of the total extract considering the heat and humidity under ICH test conditions has not been published. In this study total methanolic extract of *Hypericum perforatum* is investigated for stability including ten active molecules (hypericin, pseudohypericin, hyperforin, chlorogenic acid, rutin, hiperoside, isoquercitrin, quercitrin, quercetin, amentoflavone) due to the temperature and humidity for six months. As a result, degradation percentage of each molecule due to stability testing conditions were determined. Flavonoids were less effected in storing conditions. While chlorogenic acid amount was rising in forth month degragation over 32% was determined in the end. Hypericin, pseudohypericin, hyperforin degradated more than 50% in 40 °C, 75% Humidity. **Acknowledgement:** *Authors are thankful to Research and Application Center of Drug Development and Pharmocokinetics (ARGEFAR) and Prof. Dr. Ulvi Zeybek for facilities.* **References:** 1. Shelton RC (2009) J Clin Psychiatry 70(5): 23 – 7. 2. Bilia AR (2001) Int J Pharm 213(1 – 2): 199 – 208. 3. Isacchi B (2007) J Pharm Biomed Anal 45(5): 756 – 61. 4. Ang CY (2004) J Agric Food Chem 52(20): 6156 – 64. 5. Li W (2001) J Chromatogr B Biomed Sci Appl 765(1): 99 – 105. 6. Bilia AR (2002) Drug Dev Ind Pharm 28(5): 609 – 19.

PL81 Hepatoprotective action of the Egyptian variety of *Phyllanthus atropurpureus*

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Genus *Phyllanthus* (Family Euphorbiaceae) is considered as one of the important medicinal and ornamental plants. A phytochemical investigation of the extracts was performed to search for the active ingredient. Results from the investigation of the hepatoprotective activity of *Phyl-*

lanthus atropurpureus Bojer, revealed that its extracts is quite similar to silymarin. Both of them improve the parameters of CCl_4-induced liver injury including serum AST and ALT. Among the extracts tested, root extract showed maximum activity as compared with aerial part extract relative to silymarin.

PL82 Response of germination and seedling growth of, hyssop (*Hyssopus officinalis*) and Marguerite (*Chrysanthemum x superbum*) as medicinal plants to water stress

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In order to study the effects of five levels of water stress (0, -2, -4, -6 and -8 bar) on germination characteristics and seedling growth of two medicinal plants (*Hyssopus officinalis* L. and *Chrysanthemum x superbum* Bergmans ex J.Ingram), two experiments were conducted at physiology laboratory of Faculty of Agriculture Ferdowsi University of Mashhad as a Completely Randomized Design with four replications. The results showed that the effects of different levels of water stress were significant in all of the studied characteristics of two plants. Germination percentage was decreased and mean germination time were increased by increasing in water stress levels and, germination percentage was zero in levels of -6 bar in two types of plants. It is suggested that decrease in seed germination and depression in seedling growth under drought conditions related to limited hydrolysis of food reserves from storage tissues as well as due to impaired translocation of food reserves from storage tissue to developing embryo axis [3]. Root length of Hyssop and Marguerite respectively, were increased and decreased by increasing in water stress levels. Plumule length had a decreasing trend in two studied plants, but amounts of this trend was less in hyssop and the root length/plumule length were increased in each of plants. Many researchs were shown that an increased root/shoot ratio resulting in more efficient water and nutrient uptake [1,2]. Also, dry weight root had increased trend and dry weight plumule had decreasing trend but root dry weight/plumule dry weight was increased in two types of plants. **References:** 1- Fallahi J et al. (2008) Iranian J Environ Str Agric Sci 1(1): 57 – 67. 2- Gorham J et al. (1999) Plant Soil 89: 15 – 40. 3- Misra N, Dwivedi UN (2004) Plant Sci 166: 1135 – 1142.

PL83 *Omididun* (corn liquor): an economic solution to xerostomia

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Xerostomia is dry mouth resulting from reduced or absent saliva flow associated with dehydration, use of drugs, various syndromes (Plummer-Vinson syndrome) and side effects of radiotherapy and chemotherapy in cancer treatment. It can affect nutrition and dental as well as psychological health. *Omididun* is the liquor obtained from fermented ground and sieved maize or sorghum. While the ground wet flour obtained is boiled into a semisolid cereal (*Ogi*) for breakfast in Nigeria. Lactoperoxidase (LPO) presence was confirmed in *omididun* obtained from four varieties of fermented corn [*Zea mays* Linn. (Poaceae) white and yellow varieties and *Sorghum bicolor* Linn. (Poaceae) white and red varieties] using the principle of LPO decomposition of hydrogen peroxide and the oxidation of colorless 1, 4-phenylenediamine into the purple indophenol. LPO was estimated with a reaction mixture of hydrogen peroxide and potassium iodide solutions, incubated at room temperature to achieve equilibrium and absorbance read at 350 nm in a UV spectrophotometer against a blank without omididun and procedure repeated for commercially available dry mouth wash and toothpaste. The colour intensity was proportional to the LPO's concentration in the order of yellow corn> white corn>red sorghum>white sorghum. LPO content increases from 3.528 ± 0.451 % in white sorghum to 34.713 ± 0.068 % in yellow corn. It is proposed that *omididun* could be used as a mouth rinse or incorporated in tooth paste because of the natural LPO content to treat xerostomia and as well reduce oral bacteria and consequently the acid produced by those bacteria.

PL84 Fungal transformation of pimaradienoic acid and its schistosomicidal activity against *Schistosoma mansoni*

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In the present work, the microbial transformation of pimaradienoic acid (PA, 1) (Figure 1) was performed using submerged shaken liquid culture of *Aspergillus ochraceus* (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PA was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 3 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPA. Chemical and NMR studies of AoPA allowed us to isolate and to identify two PA derivatives (Figure 1: Compounds 2 and 3). The *in vitro* schistosomicidal activity of these metabolites was performed against male and female *S. mansoni* adult worms [2], and the results denote that PA is very effective with respect to the separation of coupled pairs, mortality, decrease in the motor activity and tegumental alterations. In addition, PA is able to reduce the percentage of eggs number and eggs development. In this context, the schistosomicidal effects of PA indicate that *ent*-pimarane diterpenes could be considered a promising source for discovery of new agents to treat human schistosomiasis.

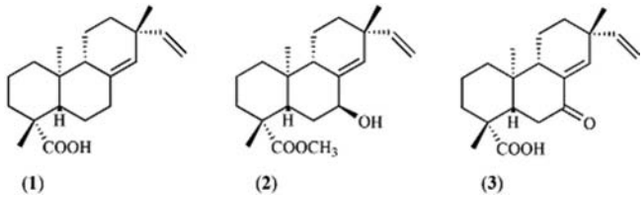

Figure 1: Chemical structures of PA (1) and its derivatives obtained through fermentation for 3 days with *A. ochraceus*.

Acknowledgement: *FAPESP (Proc. 2007/54762 – 8)* **References:** 1. Bastos DZL et al. (2007) Phytochemistry 68: 834 – 839. 2. Magalhães LG et al. (2010) Parasitol Res 106: 395 – 401.

PL85 Biotransformation of *ent*-8(14),15-pimaradiene and antimicrobial activity of the obtained derivatives against multi-resistant Gram-positive bacteria

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In the present work, the microbial transformation of *ent*-8(14),15-pimaradiene (Figure 1; 1; PI) was performed using submerged shaken liquid culture of *Aspergillus ochraceus* (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PI was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 7 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPI. Chemical and NMR studies of AoPI allowed us to isolate and to identify four PA derivatives (Figure 1: Compounds 2, 3, 4 and 5). The antimicrobial activity of these metabolites was evaluated against a panel of 14 multi-resistant Gram-positive bacteria. For this purpose, the broth microdilution method was applied and the minimal inhibitory concentration (MIC) values were determined [2]. Diterpene 2 displayed significant inhibitory effect on the growth of these pathogens, showing MIC values very promising [3].

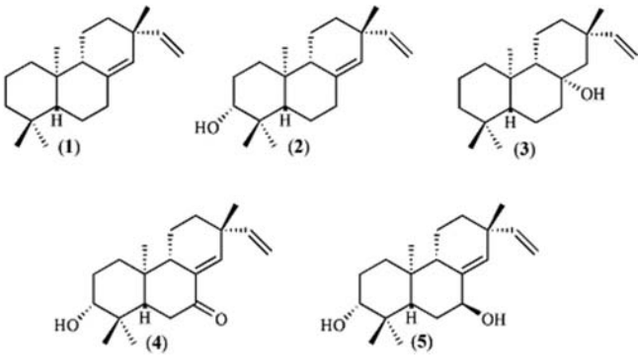

Figure 1: Chemical structures of PI (1) and its derivatives obtained through fermentation for 7 days with *A. ochraceus*.

Acknowledgement: *FAPESP (Proc. 2007/54762 – 8)* **References:** 1. Bastos DZL et al. (2007) Phytochemistry 68: 834 – 839. 2. Porto TS et al. (2009) Molecules 14: 191 – 199. 3. Gibbons S (2008) Planta Med 74: 594 – 599.

PL86 Antioxidant capacity of *Matricaria chamomilla* L. extract and its effect on neural tube structure in diabetic rat offspring

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Increased oxidant stress has been suggested to play a role in the pathogenesis of disturbed embryogenesis in diabetic pregnancies also cause several types of histopathologic changes in the placenta (1) The present study was conducted to determine whether *Matricaria chamomilla* L. extract, a well known medicinal herb with appropriate antioxidant activity, would reduce the incidence of diabetic embryopathy in the streptozotocin-induced diabetic rat model. Antioxidant capacity of extract was measured using DPPH method. Diabetic and control rats were administered 100,300,500, mg/kg chamomile extract. Mating condition was prepared by putting male rats and diabetic female rats together. vaginal plaque mentioned as a positive sign of pregnancy and treatment started with extract or vehicle from 1th to 17th day of gestation by gastric gavages. Blood glucose was measured during 17 days. results shows level of blood glucose was reduced about 1.62 fold ($p < 0.00$) in treated diabetic rats. At 17th day, rats were scarified. The fetuses were released from the yolk sacs and surrounding deciduas and were neural tube of fetuses was examined by Light microscopy and electro microscope.Neural tube defect was significantly reduced and only some changes in increasing Lumen size of spinal cord was observed in treated group. The obtained results of antioxidant capacity by DPPH method showed IC 50 equal to 0.73 mg/ml. Our findings showed that *Matricaria chamomilla* L. extract may have a protective effect against diabetes-related embryopathy and it may be due to its antioxidant activity. **Acknowledgement:** *This study is a part of a joint project between Ahvaz Jundishapur university of medical sciences and Pharmaceutical research network.* **References:** Wiznitzer A et al. (1999) Am J Obstet Gynecol 180: 188 – 93

PL87 Antioxidant activity of crude foliar extracts and alkaloids of *Psychotria* spp. (Rubiaceae)

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Current studies on the chemodiversity of the paraphyletic and pantropical genus *Psychotria* revealed the presence of various types of alkaloids [1]. Several species are used in traditional medicine of indigenous people for treatment of different diseases, as health tonics and as depurative [2]. In order to asses eventual antioxidative activities, crude methanolic extracts from more than 20 species of *Psychotria* s.l. were subjected to the diphenylpicrylhydrazyl (DPPH) free radical-scavenging assay and EC_{50} values were determined. In addition, total phenolic compounds were assessed using Folin-Ciocalteus reagent. Within different taxonomic groups of *Psychotria*, strongest observed activity was restricted to members of subg. *Psychotria*. At comparable concentration, alkaloids isolated from different *Psychotria* spp. [1] showed lower activity than crude extracts. Thus, it may be assumed that total antioxidant activity of crude extracts is not only caused by the presence of alkaloids but also due to the accumulation of phenolic compounds. Results of the present analysis indicate that members of subg. *Psychotria* are promising sources of natural antioxidants due to the observed radical scavenging properties of their extracts. The antioxidant activity may be causal for the use of these species in folk medicine. From the chemotaxonomic point of view, differentiation in the degree of antioxidant activity within *Psychotria* correlates well with current taxonomic views [3], confirming that species of subg. *Heteropsychotria*, devoid of strong activity, should be placed in a separate genus. **References:** 1. Berger A et al. (2010) In: Program and Abstracts, 19th International Symposium "Biodiversity and Evolutionary Biology", German Botanical Society (DBG), Univ. Vienna, Austria, p. 76. 2. Sanz-Biset J, Campos-de-la-Cruz J, Epiquién-Rivera MA, Cañigueral S (2009) J Ethnopharmacol 122: 333 – 362. 3. Nepkroeff M, Bremer B, Systma KJ (1999) Syst Bot 24: 5 – 27.

PL88 Quantitative analysis of rosmarinic acid in *Rosmarinus officinalis* growing in Turkey by LC-MS/MS

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Rosmarinic acid is naturally occuring polyphenolic compound mostly found in Lamiaceae family herbs such as rosemary, perilla, oregano, sweet basil. It exhibits various biological activity like antioxidant, antiviral, antibacterial and antiinflammatory (1,2). LC-MS/MS has an important role in the studies of identification and characterization of natural compounds, drug metabolism, discovery of new drug candidates because of its both sensivity and specificity. In this present study, we aimed to quantify of rosmarinic acid in methanol extract of *Rosmarinus officinalis* leaves collected from İzmir by using a rapid LC-MS/MS method. Therefore we used turbo spray ionization for LC-ESI-MS method in negative mode. Rosmarinic acid was characterized by its MS/MS spectrum and LC retention time. The assay performed in different concentrations of rosmarinic acid as standart solutions. The diagnostic fragmentations 358.9/160.9 and 358.9/197.0 of rosmarinic acid were used for the quantification. As a result, 0.31 g rosmarinic acid measured in 100 g methanol extract of rosemary leaves. **References:** 1. Petersen M, Simmonds MSJ (2003) Phytochem 62:121 – 125. 2. Park SU, Uddin MR, Xu H, Kim YK, Le, S (2008) African J Biotechn 7(25): 4959 – 4965.

PL89 Chronic anti-inflammatory potential of aqueous extract of *Capraria biflora* L

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Capraria biflora L. is a plant with a long history in the traditional medicine, commonly used in chronic pathologies associated to inflammatory processes (1,2). Extracts of this plant were active in models of acute inflammation in studies developed previously (3) but information of its action in models of chronic inflammation does not exist. The present study was designed to investigate anti-inflammatory activity of aqueous extract of *Capraria biflora* using cotton pellet-induced granuloma method (4). Male Sprague-Dawley rats weighing 180 – 220 g were used. Six groups of six animals each one were used. Group I served as control (water distilled) and group II as standard (Indomethacin 5 mg/kg/). Group III, IV and V received extract at the doses of 200, 400 and 800 mg/kg respectively. The results were analyzed for to SPSS, version 11.5 for Windows, using the tests of Kruskal-Wallis and Mann-Witney for independent samples. Oral administration of the aqueous extract decreased significantly ($p < 0.01$) the weight of the granuloma forma-

tion, indicating its effectiveness in the proliferative phase of inflammation. The results suggest that this extract can be used in the treatment of chronic inflammatory illnesses and they justify the uses of this plant in the traditional medicine **Acknowledgement:** *The author wishes to express his grateful thanks to VLIR (Vlaamse Interuniversitaire Raad) in Belgium.* **References:** 1-Roig JT (1988) Editorial CientíficoTécnico La Habana 510 – 11. 2. Lans C (2007) Journal of Ethnobiology and Ethnomedicine 3: 13; 3-Acosta SL et al. (2003) Acta Farm Bonaerense 4 – Mizushima Y et al (1972)J Pharm Pharmacol 24: 781 – 85.

PL90 Medicinal and aromatic plants in generating new values for the 21st century

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The population is growing exponentially. The needs for food and medicine is increasing. Regional, and global poverty is increasing. A way to reduce the galloping growth of poverty is sustainable use of medicinal and aromatic plants (MAP). Especially in countries in transition [1,2]. The biggest global market of MAPs is China, Germany, France, Italy, Japan, Spain, United Kingdom and the United States. The International Council for MAPs has announced that global growth during 2001. and 2002 was 8 – 10% per year. The world market was estimated at 60 billion U.S. $ in 2003. Europe is a major world trader of MAPs. Today at the market are at least 2000 species of MAPs, of which 1200 to 1300 species are associated only to the European continent [3,4]. In current situation needs for herbal products at the international market is increasing. It is high opportunity for generating of sustainable benefit using of natural resources. There are more chances for regional and global economy to improve. Particularly it is great opportunity for global poor (especially in the Third and Fourth World). For the sustainable use of MAPs, it is necessary to develop programs of organic certification [5]. In addition, it is necessary to apply international law, particularly the Convention on Biological Diversity and the CITES Convention. Sustainable use of MAP is a prerequisite in generating ecologically sustainable benefit. MAPs are a great opportunity for new medicines and bio-materials [6] in both, developed and developing countries. **References:** 1. Redzic S (2006) Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S (2010)J Med Plant Res 4(11): 1003 – 1027. 4. Redzic S (2008) Planta Med 74: 1143 – 1144. 5. Redzic S et al. (2009) Planta Med 75: 902 – 902. 6. Barudanovic S et al.(2009) Planta Med 75: 938 – 938.

PL91 Comparison of chemical composition of *Artemisia annua* volatile oil from Romania

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The aim of our work was to obtain and compare, quantitatively and qualitatively, the composition of volatile oil from the *Artemisia annua* L. (sweet wormwood). The fresh plant material, harvested from a Romanian natural population and Anamed (A3) cultivar, was distilled with the classical Clevenger (HD) and the microwave assisted (MWHD) laboratory hydrodistillation [1]. The MWHD apparatus, with original design, was equipped with a microwave oven (750 W), a rotating head and a Clevenger extension. The volatile oil samples were analyzed by thin layer chromatography (TLC) and gas chromatography (GC). The amount of volatile oil obtained was 0.57 respectively 0.58% v/w (HD) and 0.69% v/w (MWHD). The operation time for MWHD was 20 minutes and 180 minutes for HD. With TLC 11 spots were visualised; in case of MWHD volatile oil, the intensity and area of several spots were greater. The GC analysis has shown significant differences in the chemical composition of volatile oil between Romanian population and Anamed (A3) cv., which is consistent with other researches performed on natural populations [2]. About 100 peaks were obtained and 17 constituents were identified by GC. For Anamed (A3) cv. the main components, for both HD and MWHD, were artemisia ketone (38.0/41.2%), 1.8-cineole (11.9/13.0%), borneol (8.7/8.8%) and camphor (8.4/9.1%). MWHD method was faster then HD and the quantity of volatile oil was higher by 19%. The main components ratio was also slightly higher in this case. Hence, the MWHD methods seem to be more efficient for essential oil hydrodistillation at laboratory scale. **References:** [1] Toth ET et al. (2010) Acta Medica Marisiensis 56(2): 61. [2] Hethelyi EB et al. (1995)J Essent Oil Res 7: 45 – 48.

PL92 Systemic Studies on Arctii Fructus

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Arctii fructus is the dry seeds of *Arctium lappa* L. and generally used as an herbal medicine in traditional Chinese medicine. The pharmacognosy and anticancer constituents of Arctii fructus as well as the ecological suitability of Arctium lappa and its suitable cultivation regions in China were studied. Our research established a method for distinguishing Arctii fructus from its adulterations. In Chinese patent medicine, the processed Arctii fructus was mostly used. The optimal procedure for the processing of Arctii fructus were studied and the processing principle of Arctii Fructus was determined to be: 1) Protect arctiin from degradation by hydrolytic enzyme in fructus arctii; 2) Extract the active constituents from arctii fructus easier by making the pericarp texture of arctii fructus crispy; 3) Abate the nature of arctii fructus by attenuating the purgative action by a decreasing content of lipids and arctiin. Dao-Di-Yao-Cai means the best and highest quality of Chinese medicine materials. It is a unique index for evaluation of Chinese medicine in traditional Chinese medicine and the result of long-time clinical experience of practitioners. The suitable cultivation regions for Arctii fructus in China were determined on the basis of its ecological suitablility.

PL93 Determination of alkannin/shikonin derivatives in endemic Greek *Alkanna* species

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Alkannin and Shikonin (A/S) derivatives are optical antipodes of plant origin with a verified wide spectrum of antimicrobial, wound healing, anti-inflammatory and antioxidant activity. Although the aforementioned antipodes were originally introduced as wound healing agents, recent studies on cancer chemotherapy revealed that A/S also exhibit antitumor activity. A/S have been found in roots of several Boraginaceous species[1 – 3]. Determination of A/S, their esters and the total A/S content in Boraginaceous roots of different origin was reported in several papers[4 – 7]. *Alkanna* species grown wild in Greece have been analysed for their A/S esters by LC-PDA-MS previously [5], whereas the total A/S content (A/S and their derivatives) has not been reported. In the present study endemic *Alkanna* species (*tinctoria* (L.) Tausch, *pindicola* Hausskn., *orientalis* (L.) Boiss., *methanea* Hausskn., *calliensis* Boiss., *graeca* Boiss. & Spruner, *primuliflora* Griseb., *stribrnyi* Velen., *sieberi* DC., *noneiformis* Griseb.) grown in various Greek regions were collected and analyzed for their total A/S content for the first time. A comparison was additionally performed among species and different regions. Quantitative analysis revealed that specific root samples of *A. tinctoria, A. pindicola* and *A. sieberi* showed the highest amount of A/S and derivatives (1.41, 1.38, 1.00 mg/100 mg root respectively), but the A/S content varied from one region to another even within the same species. Yet, a significant difference in A/S content was observed among species. With this study it can be concluded that some of the examined *Alkanna* species of the Greek flora could serve as alternative sources for medicinally valuable A/S derivatives. **Keywords:** Alkannin, shikonin, *Alkanna*, Boraginaceae, naphthoquinones, wound healing **References:** 1. Papageorgiou VP, Assimopoulou AN et al. (1999) Angewandte Chemie, Int. Edition 38(3): 270 – 301. 2. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(16): 2123 – 2142. 3. Papageorgiou VP et al. (2008) Current Medicinal Chemistry 15(30): 3248 – 3267. 4. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(5): 583 – 622. 5. Assimopoulou AN et al. (2006) Biomedical Chromatography 20: 1359 – 1374. 6. Pekin G. et al. (2007) Planta Med 73: 267 – 272. 7. Akgun IA et al. (2009) Chromatographia 70: 963 – 967.

PL94 Fast Centrifugal Partitioning Chromatography (FCPC) towards the recovery of secondary metabolites coming from roots of Argemone mexicana

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Mexican poppy (*Argemone mexicana* L.) is an annual thorny herb which belongs to Papaveraceae family. It is commonly spread in Mexico and in south-western North America where it is growing in the wasteland. *Argemone mexicana* contains high variety of isoquinoline alkaloids which influence its activity (*e.g.* cholagogue, hipotensive, antifebrile, antimalarial, spasmolytic and depressive towards the CNS). In the course of current study main active constituents coming from methanolic extract of Mexican poppy's roots were separated using Fast Centrifugal Partition Chromatography. Several solvent systems were elaborated according to their affinity to Mexican poppy's alkaloids. Petroleum ether, ethyl acetate, methanol and water (15:30:21:20 v/v/v/v) was responsible for the most successful separation of its secondary metabolites. The obtained alkaloidal fractions were analyzed by means of ESI-octopole-orthogonal acceleration time-of-flight (oa TOF)-mass spectrometry (MS) with high mass accuracy. Among well known and described alkaloids in this species (sanguinarine, chelerithrine, argemonine, protopine, berberine or coptisine), magnoflorine, palmatine and galanthamine were confirmed in Argemone mexicana for the first time. Selective and sensitive TLC-bioautography screening test for natural acetylcholinesterase (AChE) inhibitors was performed for isolated alkaloids [1]. **References:**
[1] Mroczek T (2009) J Chromatography A 1216(12): 2519 – 2528.

PL95 Antioxidant capacity and total phenolic content of *Stachys aucheri* endemic plant to Persia

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Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [1]. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases. The phenolic compounds in herbs act as anti-oxidants due to their redox properties. According to the studiese about antioxidant capacity of others species of Stachys, *Stachys aucheri* Benth. that has not yet been studied was choosed. Total extract of aerial parts was tested by FRAP (ferric reducing antioxidant power) and DPPH[2] (1,1-diphenyl -2-picrylhydrazyl) methods. Quantitation of total phenolic content, Folin Ciocalteu method was used. DPPH IC_{50} (an antioxidant concentration which is capable to inhibit 50% of generated DPPH free radicals) in DPPH method and, EC 1 (antioxidant concentration which can change the absorption equal to one mM of Fe^{2+}) in FRAP method and, Total phenolic (equal mg gallic acid) per 100 g herb were used for expression of the results. IC_{50} of hydroalcoholic extract was1.20 mg/ml and EC 1, was 14.21 mmol Fe^{2+} equal per 100 g of herb powder. Results show that this plant is good antioxidant candidate to be used in Food industry and as neutriceutical. **References:** 1. Srinivasa K e al. (2010) Food and Chemical Toxicology 48: 729 – 732 2. Dejian H et al. (2005) J Agric Food Chem 53: 1841 – 1856

PL96 Quantitative and Qualitative Studies on Five Endemic *Hypericum* Species of Turkey

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In this study quantitative determination of hypericin and qualitative analysis of the phenolic compounds of the flowering aerial parts of five endemic *Hypericum* species of Turkey, namely, *Hypericum kotschyanum* Boiss., *H. salsugineum*Robson et Hub.-Mor., *H. scabroides* Robson et Poulter, *H. thymopsis* Boiss. and *H. uniglandulosum* Hausskn. ex Bornm. were performed by HPLC (1). The results were also compared to each other. It was observed that the *H. salsugineum* had the highest hypericin content among the others. In the phenolic compounds side of view, quercetin and isoquercitrin were determined in all species. The rutin was observed in the *H. kotschyanum*, different than the other species. **References:**
1. European Pharmacopoeia (2008). Herbal Monographs: St John's Wort (Hyperici herba), EDQM, Strasbourg, pp: 3839 – 3842.

PL97 New synthetic flavones of natural origin as antimalarial agents

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Recent reports of increased tolerance to artemisinin derivatives, the most recently adopted class of antimalarials, have prompted a need for new treatments. In this context, we tested the antimalarial activity of lanaroflavone, a biflanonoid isolated from the methanol extract of the aerial part of *Campnosperma panamense* Standl. (Anacardiaceae), an endemic tree species of Colombia (1). Lanaroflavone showed good *in vitro* antimalarial activity but was inactive in a rodent model. Here, 10 new simplified synthetic analogs of lanaroflavone were tested for the first time against blood stage culture of different chloroquine sensitive and resistant strains of *P. falciparum* (3D 7, Africa and 7G8, Brazil). We used immunoenzymatic technique based on the *Plasmodium* Lactate Deshydrogenase production to evaluate the inhibition of parasitic growth and to determine the target stage throughout the erythrocytic cycle of *Plasmodium*. Haemotoxicity and cytotoxicity were also evaluated. Out of the 10 compounds tested, MR27770 strongly killed the early blood stages of *P. falciparum* at nanomolar concentrations. We noted no haemolytic effect in vitro on red blood cells or cytotoxicity on several cultured cell lines (hepatic mouse cells Hepa 1 – 6 and Normal Human Dermal Fibroblasts), with selectivity indexes values above 1000. Evaluation of MR27770 in a rodent malaria model will also be presented. **References:**
1. Weniger et al. (2006) Phytomedicine 13: 176 – 180

PL98 Findings on antifungal-activity and phytochemistry of Brazilian Amazon plant extracts against *Candida albicans*

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Candida albicans represents one of the most common opportunistic microorganisms influencing the quality of life of patients committed by severe diseases as diabetes, hypothyroidism, hyperparathyroidism, hypoadrenocortical activity, malnutrition, trauma, Sjögren Syndrome, long-term systemic infection, infancy, advanced age or immunosuppressed patients, among others. The introduction of new chemotherapeutics to fight opportunistic mycosis provoked by *C. albicans* is necessary to expand possibilities of diminishing suffering and improve quality of life for those patients. Nature is a known source of pharmacological-active compounds. For that reason, more than 2,000 plant extracts were tested against *C. albicans* using the disk diffusion method (DDM), in Sabouraud dextrose agar medium. Extracts showing any degree of inhibition were considered active in the initial screening. The active extracts were retested in triplicate in order to confirm antifungal activity in DDM, and for the six extracts that confirmed activity, diameter of inhibition-growth zones were measured both horizontally and vertically, and standard drugs amphotericin B, nystatin and chlorhexidine digluconate were used. Means and standard deviations were obtained and compared. CE 849, obtained from *Diospyros guianensis* Gürke, and CE 689, obtained from *Abarema auriculata* (Benth.) Barneby & J.W.Grimes, were the most active extracts and both has shown a significant activity when compared to the standard drugs. The Brazilian Amazon rain forest can be considered an extremely important source of new chemical lead compounds to be introduced as pharmacological weapons to improve the quality of life of patients suffering from extreme health

conditions. **Acknowledgement:** *FAPESP-grant#2008/58706 – 8, UNIP* **References:** 1. Suffredini IB et al. (2006) Anti-Cancer Agents in Med Che. 6: 367 – 75

PL99 Comparison of the antioxidant activity and total phenolic contents in some species of Lamiaceae family

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Antioxidant compounds in food play an important role as a health protecting factor. In this study, The methanolic extracts of the aerial parts of nine Lamiaceae species: *Mentha spicata* L., *Mentha aquatica* L., *Mentha piperita* L., *Stachys byzantina* K.Koch, *Marrubium vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L. and *Melissa officinalis* L. were investigated for their antioxidant activity and total phenolic and flavonoid content using DPPH and Folin-Ciocalteu and potassium chloride assays respectively. The IC_{50} of the methanolic extracts ranged between 42.67 – 489.97 µg/ml, total phenolic content were between 38.27 – 59.14 $mgGAEg^{-1}$dw. R. officinalis and M. vulgare showed the most content of antioxidant activity. There was a direct correlation between total phenol and antioxidant activity which indicates that polyphenols are the main antioxidants.

PL100 Comparative analysis of polyphenols and flavonoids in natural populations of *Crataegus monogyna* from Eastern Carpathians

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Our aim was to obtain and compare, quantitatively and qualitatively, the polyphenols and flavonoids from six natural populations of *Crataegus monogyna* Jacq. harvested from the spontaneous flora of Eastern Carpathians. The Crataegi fructus and Crataegi folium cum flore samples were collected from Neamt County -Cernegura Hill (no. 1), Batca Doamnei (no. 2), Cheile Bicazului (no. 6) and other three from Harghita County – Praid area (no. 3, 4, 5). The qualitative analysis of polyphenols and flavonoids was performed by TLC and HPLC. The quantitative analysis was performed by UV/VIS spectrophotometry [1] (rutoside for flovonoids and chlorogenic acid for polyphenols). TLC analysis of Crataegi fructus samples has revealed four polyphenols (chlorogenic and caffeic acids and two caffeic compounds) and two flavonoids (hyperoside and vitexine). TLC analysis of Crataegi folium cum flore samples has revealed two polyphenols (chlorogenic and caffeic acids) and three flavonoids (rutin, hyperoside and a 6-O-glycoside of luteolin). Sample no. 6 seems to contain also vitexine. The HPLC analysis of an additional Crataegi folium cum flore harvested from Piatra Neamt has revealed UV spectra of the main seven compounds (the chlorogenic acid was identified). The spectrophotometric quatification of flavonoids from Crataegi folium cum flore samples has detected a higher content (1.0175% – 1.9175%) then the one detected in fruit samples (0.1218% – 0.2801%). The quantification of polyphenols from Crataegi fructus has identified high amounts (2.11% – 2.70%). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations were samples no. 3 and 4. **References:** [1] Farmacopeea Romana (1998) Xth Edition, Editura Medicala, Bucharest, 325 – 327.

PL101 Antitubercular activity of pimarane and kaurane diterpenes against *Mycobacterium tuberculosis*

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Tuberculosis (TB) is still a public health problem and causes millions of deaths every year [1]. According to the World Health Organization, 8.8 million new TB cases occurred in 2007 [2]. Since the increase of bacterial resistance and the emergence of new infections are common problems [3], the search for new antibacterial or antimycobacterial agents is a urgent matter. In the course of our investigation about diterpenes and their biological activities, we have performed some antimycobacterial assays against *Mycobacterium tuberculosis* H37Rv, ATCC 27294 with five diterpenes. All of them showed at least moderate activity a MIC of $<= 31.25\ \mu g.mL^{-1}$, that can be classified as promising, as according to Cantrell et al. [4] a MIC value below $64\ \mu g.mL^{-1}$ for isolated compounds can be considered to be of interest. In the present work, compound 1 was the less active with a MIC of $250\ \mu g.mL^{-1}$. Compounds 2 and 4 are of moderate activity each with a MIC of $125\ \mu g.mL^{-1}$. Most active compounds were 3 and 5 with a MIC of $<= 31.25\ \mu g.mL^{-1}$. Further assays will determine detailed final MIC values for compounds 3 and 5 and other diterpenes of both classes will be investigated in the test system. **Acknowledgement:** *FAPESP (Proc. 2009/09491 – 1), CAPES, CNPq* **References:** [1] Higuchi CT et al. (2008) Quim Nova 31: 1719 – 1721. [2]www.whqlibdoc.who.int/hq/2007/WHO_HTM _TB_2007.378_eng.pdf (accessed april/04/2011) [3] Porto TS et al. (2009) Fitoterapia 80: 432 – 436. [4] Cantrell et al. (2001) Planta Med 67: 1 – 8.

PL102 Biological effects and phenolic content of felty germander (*Teucrium polium* L. subsp. *polium*)

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Felty germander – Lamiaceae is popular species of *Teucrium* genus in the folk medicine and used for treatment of appetite loss and gastrointestinal ailments [1]. In the present study, antioxidative and anti-acetylcholinesterase activity, total phenolic content as well as flavonoid concentration of methanolic, acetone and ethyl acetate extracts were investigated. Ferric reducing/antioxidant power (FRAP) [2] was assayed and values were between 235 and 846 µmol Fe^{2+} equ/l. The antioxidant capacity have been evaluated using the Briggs-Rauscher oscillating reaction method [3], expressed as a time requierd for regeneration of osscillations in minutes and obtained values were: 43.5 for methanolic, 2.0 for acetone, while ethyl acetate extract did not show activity. The ability to scavenge DPPH radicals [4] was determined and expressed as IC_{50} values that ranged from 59.37 to 622.96 µg/ml. Acetylcholinesterase inhibition was measured using slightly modified Ellman's method [5] and results indicate a weak inhibitory activity of extracts. Total phenolic content was determined using Folin-Ciocalteu reagent and the values ranged from 41.37 up to 124.62 mg of GA/g of extract. The content of flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. Methanolic extract was most active in comparison with other extracts for all measurments. That indicates that the methanol, as a polar solvent, is the very effective for phenolic compounds extraction from *T. polium* L. subsp. *polium*. Based on the obtained results, *T. polium* subsp. *polium* extracts are rich sources of phenolic compounds and promising candidates for further development as natural antioxidant agents. **Acknowledgement:** *Ministry of Science and Education, Republic of Serbia (III41010).* **References:** 1. Sharififar F et al. (2008) Food Chem 112: 885 – 888. 2. Benzie IFF & Strain JJ (1996) Analytical Biochem 239: 70 – 76. 3. Briggs TS & Rauscher WCJ (1973) Chem Educ 50: 496. 4. Stankovic SM et al. (2010) J Med Plant Res 5. Ellman GL et al. (1961) Biochem Pharm 7: 88 – 95.

PL103 Comprehensive Analysis of Artemisiae scopariae herba from different growing areas

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Artemisiae scopariae herba (Yinchen) is used in Traditional Chinese Medicine (TCM) for treatment of hepatic diseases like jaundice. Since TCM is getting popular in Europe, monographs are developed for the German and European pharmacopoeias [1,2]. Moreover the Bavarian State Research Center for Agriculture (Lfl) successfully cultivates Chinese medicinal plants to ensure authenticity of the plant material and quality of the preparations thereof. Plant material provided from suppliers of Chinese origin and the Lfl showed after morphological analysis a significant different habitus between German and Chinese plants. Furthermore, phytochemical analyses by TLC and GC also revealed a different spectrum of polyphenols and volatile compounds between the two geographical sources. To examine the variation of the essential oil in more detail the water-distilled essential oil from the aerial parts of *Artemisia scoparia* Waldst et Kit was analyzed with GC- FID and GC-MS for both provenances revealing capillene as one characteristic differentiating compound. PCR-Analysis of the Internal Transcribed Spacer (ITS) authenticated from the genetical point of view that despite all morphological and phytochemical differences both sources of Artemisiae scopariae herba belong to the same source plant named *Artemisia scoparia*.
Acknowledgement: *The BfArM (Kurt-Georg-Kiesinger-Allee3, 53175 Bonn, Germany) is gratefully acknowledged for financial support. The Bavarian State Research Center for Agriculture (Am Gereuth 8, 85354 Freising, Germany) is gratefully acknowledged for providing samples and support.*
References: 1. German Pharmacopoeia 2010 (DAB 2010) 2. European Pharmacopoeia 2011 (Ph. Eur. 2011)

PL104 Contribution of two agronomic characteristics to yield and oil content of safflower germplasm in eastern Algeria

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Safflower (*Carthamus tinctorius* L.) is one of humanity's oldest crops, but generally it has been grown on small plots for the grower's personal use and it remains a minor crop. Since safflower is a drought tolerant crop, the objective of this research was the investigation of the seed yield and oil content under semi-arid conditions in eastern Algeria. The results showed that plant height (PH) and plant dry matter weight (PDMW) showed 63,37 – 107,63 cm and 65,55 – 123,04 g of variation respectively. Syprus variety gave the highest PDMW (123,04 g) and yield of seeds (YS) (420.53 g/m2). While Finch variety gave the highest PH (107,63 cm). Considering the yield of the fixed oil (YO) (% of seeds), Gila variety produced the highest percentage (38.47%). The research revealed that the most suitable safflower variety, under semi-arid conditions of eastern Algeria was Syprus variety which was provided by ICARDA (International Center for Agricultural Research in Dry Areas, Syria). Analyses of variance showed highly significant differences among the varieties for yield agronomic traits and oil content. Correlation coefficients between variables (traits) are calculated, and the cluster analysis of observations (varieties) is also used to clarify the clustering pattern of genotypes tested.

PL105 Pharmacognostic study of two medicinal species of *Rytigynia* (Rubiaceae): *Rytigynia nigerica* (S. Moore) Robyns and *Rytigynia umbellulata* (Hierns) Robyns from Nigeria

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Micromorphological and phytochemical studies were carried out on the leaves of *Rytigynia nigerica* (S. Moore) Robyns and *Rytigynia umbellulata* (Hierns) Robyns. The epidermal cells of both the adaxial and the abaxial surfaces have irregular shape and the anticlinal cell wall patterns are either curved or undulate. Remarkable diagnostic features of the two plants which in a way justify their grouping in the same genus are paracytic stomatal type, hypostomatic leaf and centrally located vascular bundles in the midrib and spatial deposition of crystals of calcium oxalate in the perivascular tissue. But the distinctive features of each species include higher epidermal cell number in *R. nigerica* than *R. umbellulata*. Thin cell wall of 1.0 (1.6 ± 0.2) 3.0 µm on the abaxial surface of *R. nigerica*. Higher stomatal size of 6.0 (12.4 ± 1.2) 20 µm x 5.0 (13.4 ± 1.3) 20 µm in *R. umbellulata* and long and tip bent trichomes reported on the abaxial surface of *R. nigerica* and multicellular glandular type on the adaxial layer of *R. umbellulata*. Phytochemical screening showed that in both *R. nigerica* and *R. umbellulata*, bioactive compounds such as alkaloids, tannins, saponins, reducing sugar, glycosides, flavonoids and terpenes were present; whereas anthraquinones, cardiac glycosides, cyanogenetic glycosides and phlobatannins were absent. However, only the extracts of *R. nigerica* were positive for steroids. These bioactive compounds found in the leaves of these plants play a major role in their medicinal potentials. The two species are well known plants used in folkloric medicine in Nigeria.

PL106 Bioassay-guided fractionation of a hepatoprotective and antioxidant extract of pea by-product

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Fruits and vegetables waste products offer a cheap and practical source of potent antioxidants that could be used as functional ingredients. The hydroalcoholic extract (PE) of pea (*Pisum sativum* L.) waste (husks) was evaluated for hepatoprotective and antioxidant activities, using CCl_4-induced oxidative stress and hepatic damage in rats. PE significantly inhibited CCl_4-induced elevation of serum ALT and AST by 45.3, 17.8%, respectively and normalized the levels of serum total protein and albumin in hepatotoxic rats. It afforded 31.2% protection against hepatic lipid peroxidation, recovered hepatic glutathione and protein thiols levels (by 161.3, 55.9%, respectively), restored the glutathione-peroxidase activity (by 42.7%) and significantly increased the glutathione-S-transferase activity (by 10%). PE also inhibited CCl_4-induced elevation of hepatic NO levels by 34.2%. The active PE extract was fractionated using different solvents of increasing polarity and its fractions were tested for their hepatoprotective and antioxidant activities, using the same model. Chromatographic fractionation of the active n-butanol fraction led to the isolation of four flavonoid glycosides viz., quercetin-3-sophorotrioside (F1), quercetin-3-O-(6-O-feruloyl-β-D-glucopyranosyl (1→2)- β-D-glucopyranosyl (1→2)- β-D-glucopyranoside (F2), quercetin-3-O-(6-O-sinapoyl-β-D-glucopyranosyl (1→2)- β-D-glucopyranosyl (1→2)- β-D-glucopyranoside (F3) and quercetin-3-O-rutinoside (F4). The isolated compounds were quantified in PE, using a validated HPLC method. F2 (0.1312%) was the major compound, followed by F1 (0.0753%, calculated as rutin) and F3 (0.0273%, calculated as F2). Besides, low amounts of F4 (0.0049%) were also detected. According to our findings, pea by-product contained biologically active constituents which can be utilized for upgrading of this by-product to obtain high value added products for nutraceutical use.
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PL107 Biological Study and Phytochemical Screening of Several *Aloe* Species Cultivated in Egypt

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Aloe, has long been used in traditional medicine for the treatment of digestive system diseases, skin troubles, wounds and burns. Recently it was proved to have antitumor activity. About 19 species were cultivated in Egypt whereas *Aloe arborescens* Mill., *Aloe ciliaris* Haw., *Aloe eru* Berger and *Aloe grandidentata* Salm-Dyck were found to be most abundant. They were subjected to biological and phytochemical investigations. The biological study includes toxicological (LD_{50}) and pharmacological investigation for ethanolic extracts. Acute anti- inflammatory activity was done using paw edema method in rats with standard indomethacine. Chronic anti-hyperglycemic activity was carried out using standard metformine. Antitumor activity was investigated using available human cell lines, U251 (brain), MCF7 (breast), H460 (lung), HELA (cervix), HCT116 (colon) and HEPG2 (liver). Antimicrobial activity was investigated against Gram +ve, Gram -ve bacteria and fungi. Phytochemical screening was performed according to the usual published methods. The ethanolic extracts of *A. ciliaris* and *A. grandidentata* were found to produce significant dose-dependant anti-inflammatory effects equivalent to 85.7% and 92%, respectively comparing to the standard. While *A. grandidentata* showed potent anti-hyperglycemic effect equivalent to 95% and 87% after 4 weeks and 8 weeks respectively comparing to the standard. *A. ciliaris* showed significant antitumor effect on brain and liver cell lines. The ethanolic extracts of *A. arborescens*, *A. ciliaris* and *A. grandidentata* possessed significant antimicrobial and antifungal activity against the tested microorganisms. Phytochemical screening for biologically active extracts indicated that carbohydrates and/or glycosides, sterols and/or triterpines, combined and free anthraquinones are the main constituents present.

PL108 Biosynthesis of Bioactive Secondary Metabolites in Herbs

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Plant secondary metabolites are the major source of bioactive compounds of Herb. Metabolic engineering has opened a new promising perspective for the improved production of these valuable secondary metabolites in plant cell factory. Apparently, the key to metabolic engineering is the detailed knowledge of pathways of interest. We have developed RACE (rapid amplication of cDNA ends) method for the isolation of genes involved in certain biosynthesis pathway or crucial regulation process [1], which prompted the possibility of a key gene-based metabolic engineering for the synthesis of active compounds. In addition, we have successfully developed several plant cell culture systems such as hairy root, suspension cell as well as *Saccharomyces cerevisiae* cultures [2], which not only facilitated gene manipulation such as transformation and knockout, but also feasible for the industrial production of desired compounds in the near future. In our study, several metabolic engineering strategies have been successfully used to channel metabolites into pathways leading to desired products, including overexpression of rate-limiting enzyme genes, suppression or knockout of competitive enzyme genes, regulation of signal molecular pathway, and transformation of important transcription factors or transporters, etc [3]. Furthermore, for the unidentified secondary metabolite pathways, we are now using isotope tracing and 2-dimensional electrophoresis technology to explore them [4]. The identification and isolation of the enzymes involved will certainly help us to elucidate the whole biosynthesis pathway(s), and ultimately enable the possibility of metabolic engineering for the production of specific bioactive secondary metabolites in herbs. **Acknowledgement:** *This research was financially supported by National Natural Science Foundation of China (20572130, 30900786) and Modernization of traditional Chinese medicine foundation (08DZ1971502), Shanghai Science and Technology Committee.* **References:** 1. Xiao Y et al. (2009) Mol Biol Rep 36: 2019 – 2029. 2. Huang BB et al. (2011) Metabolomics 7: 134 – 146. 3. Xiao Y et al. (2009) Physiol Plantarum 137: 1 – 9. 4. Xiao Y et al. (2010) Biosci Rep 30: 33 – 40.

PL109 Effect of Ailanthus altissima (Mill.) Swingle and Ailanthus excelsa Roxb. stem bark extracts on Streptozotocin Induced Diabetes

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The inhibitory effects of methanol (70%) extracts of *Ailanthus altissima* (Mill.) Swingle and *Ailanthus excelsa* Roxb. stem bark on streptozotocin (ST) – induced diabetes mellitus were studied using ST – treated diabetic model. When the effects of the extracts on ST-induced ATP/ADP ratio of islets were assayed, the extracts were effective in restoring of ATP/ADP ratio and when the islets (200/condition) were treated with ST (5 mM for 30 min.) and then the extracts were added to the ST-treated cells, the extracts concentration (200 μg/ml) showed increased insulin production in pancreatic islet cells. **Keywords:** Ailanthus excelsa, Ailanthus altissima, stem bark, Antidiabetic activity

Topic M: Pharmacology/Biological Activitiy

PM1 Antibacterial potential of essential oil of medicinal plant *Satureja bachtiarica* Bunge against human pathogenic bacteria

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we investigated, antibacterial activity of essential oil of different parts (stem and leaves) of *Satureja bachtiarica* Bunge by well diffusion method. The essential oil tested antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella dysenteriae* and *Proteus mirabilis*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested against the bacteria. Phytochemical analysis of the essential oil was done. The essential oil showed antibacterial activity against tested bacteria. The (MIC) for *P. aeruginosa* were found to be 400 μg/ml while for the *K. pneumoniae* a lower MIC value (200 μg/ml) was obtained. The minimum bacterial concentration for the essential oil against all the pathogens tested was 400 – 500 μg/ml. The number of compounds detected the essential oil extracted from the stem and leaves parts by GC/MS technique, was 20 and the main compounds were: Carvacrol (48.2%), cis-jasmone (13.1%) and geranyl acetate (4.0%). **Keywords:** S. bachtiarica, essential oil, antibacterial

PM2 Composition of volatile oil and antioxidant activity of the oil and methanolic extracts of *Ferula microcolea* Boiss

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The essential oils of *Ferula microcolea* Boiss. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum distillation and analysis conditions, 22 constituents (mainly monoterpenes) were identified in *Ferula microcolea* which represented 93.6% of the oil. The main constituents were α-pinene (27.3%), β-pinene (16.4%), nonanal (8.7%), β-caryophyllene (8.5%) and thymol (6.7%). The samples were also subjected to

screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In the first case, the free radical scavenging activity of polar sub-fraction of methanol extract was superior to all other extracts (IC_{50}= 34.3 ± 0.3 μg/ml). non polar sub-fraction of methanol extract exhibited stronger activity than the essential oil. In the case of the linoleic acid system, oxidation of the linoleic acid was effectively inhibited by the polar sub-fraction of methanol extract, while the oil and non polar sub-fraction of methanol extract were less effective. **Keywords:** Ferula microcolea, Antioxidant activity, Essential oil

PM3 Targeted modification of trilobolide and search for related sesquiterpenes with immunobiological properties

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Trilobolide and its analogues belong to guaianolide type of sesquiterpene lactones, widely distributed within families Asteraceae and Apiaceae [1]. Trilobolide (1), structurally related to thapsigargin (4), is quite specific in its structure and biological activities [2]. Certain guaianolides evoked attention for their promising anti-inflammatory, anticancer, anti-infectious and SERCA inhibitory activities. However, due to their alkylation capabilities, they are generally toxic. Search for compounds with significant immunobiological properties, but with minor cytotoxicity is a challenge for immunopharmacological research also in our case [3]. (Fig. 1) We extended investigation of the immune interventions of trilobolide [2, 3] also on related guaianolides (2 – 7) isolated either from Laser trilobum (L.) Borkh., or from related Laserpitium siler (L.). For the structure-activity relationship study, we included also a series of structurally related exomethylene lactones (e.g. well recognised helenalin) [1]. For better relationship evaluations, additional series of transformed deacyl derivatives were prepared, either by alkaline hydrolysis or by hydrogenolysis of trilobolide. Implication of the specific vicinal diol (glycol), located on the lactone moiety, combined with the presence of various esters or other structure functionalities, is particularly assessed and evaluated. **Keywords:** sesquiterpenes, guaianolides, trilobolide, structure-activity relationship, immunomodulation, Laser trilobum

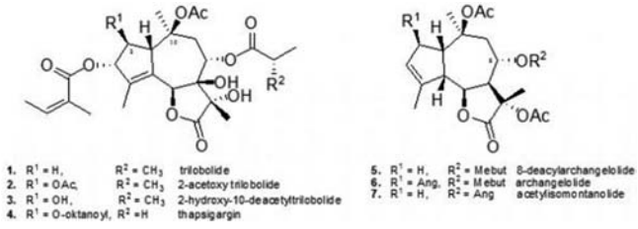

Figure 1

Acknowledgement: *Supported by GACR grant No 305/07/0061* **References:** 1. Holub M et al. (1986) Phytochemistry 25: 2015 – 2026. 2. Kmoníčková E et al. (2008) Eur J Pharm 588: 85 – 92. 3. Kmoníčková E et al. (2010) Fitoterapia 81: 1213 – 1219.

PM4 Evaluation of inhibitory effects of some Iranian plants against *Phytophthora drechsleri*

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Crude aqueous and methanolic extracts of 121 plant species belonging to 41 families collected from the west of Iran were screened for antifungal activity against mycelial growth of *Phytophthora drechsleri*. Bioassay was performed based on paper disc diffusion method with four replicates. Thirty eight of 121 (about 31%) plant species showed inhibitory activity against this phytopathogenic fungus, among which 23 species measurably inhibited the growth of *Phytophthora drechsleri*. Results indicated that methanolic extract of *Xanthium strumarium* L. showed the maximum activity (17.79 ± 1.35 mm) against *P.drechsleri* followed by *Glycyrrhiza glabra* L., *Hypericum perforatum* L., *Centaurea depressa* M.Bieb., *Lamium amplexicaule* L., *Haplophyllum perforatum* (M.B.) Vved. The investigation on the effect of plant parts on mycelial inhibition of tested fungus using paper disc method indicated that inflorescence and fruits of cocklebur (*Xanthium strumarium*) has significantly more inhibitory effect against *P. drechsleri*. The study on the antifungal activity of two common species of cocklebur grown around city of Kermanshah, *X. strumarium* and *X. spinosum* L., showed that both of them have inhibition on mycelial growth of tested fungus, but the *X. strumarium* showed significantly more inhibitory effect against *P. dreschleri* than *X. spinosa*. **Keywords:** Phytophthora activity, Xanthium strumarium, Iranian plants, Paper disc **References:** Bahraminejad S et al. (2008)J Phytopathol 156: 1 – 7. Kim DK et al. (2002) Plant Pathol J 18(5): 288 – 292. Koko WS (2007) Nat Prod 15: 1 – 10. Qi LY et al. (2008) Agr Sci Tech-Hunan 9(4): 144 – 148.

PM5 Structure-Activity Relationship of 9,10-Anthraquinone Analogues from *Rennellia elliptica* and Their Antiplasmodial Activity

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Anthraquinones isolated from the roots of *Rennellia elliptica* Korth. demonstrated interesting antiplasmodial activity. The activity however, varies depending on substitution pattern of the anthraquinone skeleton which warrants further investigation. This paper reports preliminary structure-activity relationship of a series of 9,10-anthraquinones and their antiplasmodial activity. The natural anthraquinones were isolated from roots extract of *R. elliptica*. The analogues of bioactive anthraquinones were synthesized through Friedel-Craft reaction between phthalic anhydride and various benzene derivatives in the presence of eutectic mixture of aluminium chloride and sodium chloride. The antiplasmodial activity was determined based on inhibition of the compounds against *Plasmodium falciparum* (3D7) growth *in vitro*. Combination of methyl and hydroxyl substituents at different positions on the anthraquinone skeleton caused strong antiplasmodial activity. The ortho-arranged substituents at 2,3 positions exhibited strongest activity with IC50 value of 0.08 μg/ml followed by the compound with substituents at 1,2 positions. The para-arranged (1,4) and meta-arranged (1,3) substituted anthraquinones showed less potent activity. On the other hand, analogues of dihydroxyanthraquinones displayed a reverse order of activity with the strongest inhibition shown by 1,3-dihydroxyanthraquinone. The hydroxy-methyl anthraquinones and dihydroxyanthraquinones substituted with additional methyl group at C-6/C-7 on ring A showed similar pattern of activity but much weaker than those substituted only on ring C. Protection of hydroxyl group via methylation reaction caused significant variation in antiplasmodial activity. Anthraquinones substituted at C-2 and C-3 and anthraquinones substituted at C-1, C-2 and C-6/C-7 promotes antiplasmodial activity. Structural differences due to different substitution pattern affects antiplasmodial activity of 9,10-anthraquinones. **Keywords:** Anthraquinones, Antiplasmodial, Structure-Activity Relationship, Rennellia elliptica

PM6 Assessment of Anti-angiogenic and Anti-tumoral Potentials of *Origanum onites* L. Essential Oil

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Medicinal plants and culinary herbs with anti-angiogenic and little toxicity properties have gained importance in the last decade. Non-toxic anti-angiogenic phytochemicals are useful in combating cancer by preventing the formation of new blood vessels to support the tumor growth. We have investigated the essential oil of *Origanum onites* L., which is commonly used as a condiment, with reported antibacterial, antifungal, antioxidant, insecticidal and anti-carsinogenic activities for a possible anti-angiogenic activity. Essential oil of *Origanum onites* L. was

analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The antiproliferative activities (by MTT assay, 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2 H-tetrazolium bromide), the anti-angiogenic activities (by matrigel tube formation assay), cell migration inhibiting capability (migration assay) and apoptotic potential (DAPI staining) of the *Origanum onites* essential oil (OOEO) were evaluated on rat adipose tissue endothelial cells (RATECs) and 5RP7 (c-H-ras transformed rat embryonic fibroblasts) cells. Our experimental results revealed that OOEO could markedly inhibit cell viability and induced apoptosis of 5RP7 cells and also could block *in vitro* tube formation and migration of RATEC. These results imply that OOEO having anti-angiogenic activity might be useful in preventing angiogenesis-related diseases and combating cancer. **Keywords:** Essential oil, *Origanum onites* L, antiangiogenesis, cytotoxicity, apoptosis, cancer

PM7 Neuropharmacological Activity of *Pimenta Pseudocaryophyllus* (Gomes) L.R. Landrum

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Pimenta pseudocaryophyllus (Gomes) L.R. Landrum (Pp) is popularly used as a tranquilizer in the treatment of emotional tension in the city of Campos do Jordão, São Paulo, Brazil. The aim of this study was to evaluate behavioral changes induced by ethanol extract of the Pp leaves (PpEE), seeking to identify the most active fraction. PpEE was obtained by soaking the dried leaf powder in ethanol (95%;1:5). The hexane (HF), dichloromethane (DF), ethyl acetate (ACF) and aqueous (WF) fractions were prepared through PpEE Fractionation with solvents of different polarities. Swiss male mice (25 – 35 g) were treated orally with PpEE 1 g/kg; HF160 mg/kg; DF260 mg/kg; ACF420 mg/kg, or WF 640 mg/Kg in proportion to their respective yield. After 1hr of treatment, anxiolytic effect was evaluated in sleep induced by sodium pentobarbital (50 mg/kg, i.p.), open field (OF) and elevated plus maze (EPM) models. Group treated with Diazepam (1 or 5 mg/kg, i.p.) and vehicle 10 mL/Kg were used as a positive and negative control respectively. The PpEE increased sleep duration by 32% and reduced sleep latency by 20%. DF prolonged sleep time by 62%; increased the number of squares crossed and time spent at the center by 10,8% and 39% respectively in the OF. Number of entry and time spent on the open arm of EPM were also increased by 31% and 42% respectively. These results suggest the presence of compounds with anxiolytic activity in the DF. **Keywords:** Pimenta pseudocaryophyllus, Anxiolytic effect, Medicinal plant, Elevated plus maze

PM8 Anti-*Salmonella* and anti-inflammatory activities of *Z*-ligustilide from *Ligusticum chuanxiong*

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Ligusticum chuanxiong Hort. (Umbelliferae), a perennial herb cultivated mainly in Korea and China, is one of the main plant sources of Cnidii Rhizoma which were shown to exhibit cardiovascular, antiplatelet, anti-inflammatory, and also antimicrobial and insecticidal effects. To develop an effective agent against antibiotic-resistant *Salmonella* infections the essential oil was extracted from the rhizomes of *L. chuanxiong* and analyzed by GC-MS to identify its composition. The antibacterial activity of the oil fraction and its main components against *Salmonella* species was estimated using the broth dilution method. Moreover, to determine the combined effect of essential oil components and antibiotics, checkerboard microtiter tests were performed. the fractional inhibiting concentrations (FICs) were calculated. In addition, the anti-inflammatory properties of *L. chuanxiong* oil and its components evaluated using RAW 264.7. Cell viability was determined by an MTT assay after treatment with various dilutions of the compounds. Inhibition of nitric oxide production in cells treated with lipopolysaccharide (LPS) was evaluated by reaction with Griess reagent. The m-RNA expression levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were investigated by PCR with corresponding primers and agar gel electrohoresis. As the results, the minimum inhibiting concentrations (MICs) ranged in value from 1~> 4 mg/ml indicating differences between the tested *Salmonella* species and strains. The *L. chuanxiong* oil and its components combined with antibiotics showed additive or synergistic activities with FICs between 0.28 and 0.63. The increases of m-RNA expression levels of iNOS and COX-2 were indentified by image analysis of the bands using Gel-doc system. **Keywords:** *Ligusticum chuanxiong*, essential oil, *Salmonella*, antibiotic-resistant, anti-inflammatory **Acknowledgement:** *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011 – 0011249)* **References:** 1) Shin S (2010) Nat Prod Sci 16: 259 – 264 2) Shin S (2008) Arch Pharm Res 31: 497 – 502

PM9 Reduction toxicity by doxorubicin entrapped in liposomes nanocapsules

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Doxorubicin induced an irreversible congestive heart failure, renal and hematological toxicity that are often fatal. The molecular mechanisms involved are only partially known and are complex and different from the anticancer mechanism involving oxidative stress. Encapsulation of doxorubicine in liposomes was elaborated in order to prevent the toxicity observed with the free form. The study was conducted in vivo by treatment of Wistar rats with doxorubicin encapsulated in liposomes or naked at different doses (10, 20 and 30 mg/kg) and in vitro on H9c2 cells. In addition to the oxidative status, different mitochondrial parameters (CR, Swelling, bioenergy...) were evaluated. The study is complemented by MTT and LDH tests. In vivo doxorubicin causes oxidative stress more pronounced than liposomal doxorubicin. A activity inhibition cytochrome c oxidase, depletion of tissue glutathione concomitant with increased production of ROS, swelling of mitochondria are observed. The mitochondrial dysfunction at origin of the cardioticity is confirmed by the MTT assay and LDH test. We observed also renal dysfunction and aplasia in blood, spleen and bone marrow more serious with naked doxorubicin than with the encapsulated one. In conclusion, doxorubicin would be responsible for cytotoxicity by apoptosis involving the mitochondria. Theses disorders may be prevented by its encapsulation in liposomes. **Keywords:** Cardiotoxicity, Nephrotoxicity, Haematotoxicity, Doxorubicin, Liposomes, Oxidative stress **References:** 1. Plassat V et al. (2007) Int J Pharm 118 – 127. 2. Leite A et al. (2007) Life Sciences 80: 1327 – 34. 3. Lahouel M et al. (1987) Drugs Exptl Clin Res 10: 593 – 599.

PM10 Total phenolic content, flavonoids and Superoxide radical scavenging activity of some *Citrus* peels

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The Genus *Citrus* is a shrub, from Rutaceae family. The main constituents of *Citrus* are phenolic compounds, acids, volatile oil, pectin, carotenoids, flavonoids and vitamin C. Polyphenols of citrus have antioxidative effect to prevent lipoperoxidation, increase serum antioxidant capacity and decrease oxidative stress in geriatrics. In this study three different extracts (methanolic extraction, chloroform and flavonoids fraction) from peel of 8 species (orange, mandarin, sour orange, citron, grape fruit, lemon, sweet lime, lime) of citrus were prepared. The total phenolic content, inhibition of superoxide radical capacity and antioxidant activity of citrus were evaluated using the Folin-Ciucalteu method, NBT, DPPH and FRAP assays. In NBT assay Citron flavonoids fraction (IC_{50}= 0.035 mg/ml) has the the highest capacity of inhibition and were comparable to Vitamin C (IC_{50}= 0.058 µg/ml). in DPPH assay lime methanolic extraction (IC_{50}= 9.40 mg/ml) and in FRAP assay Lime methanolic extraction have the highest capacity of inhibition. in DPPH assay Vit C has (IC_{50}= 0.072 µg/ml). The maximum amount of phenolic compound was observed in grapefruit flavonoids fraction (142.47 mg acid tannic/

1 g extraction). The maximum amount of flavonoids compounds was observed in Lime methanolic extraction (12.91 mg Rutin/1 g extraction). Methanolic extract in DPPH and NBT method have the same results for all species, but in Folin-Ciucalteu method all species except grapefruit and lemon have the results of DPPH and NBT methods. Methanolic extract of lime have maximum effects in DPPH, FRAP and NBT methods and almost the highest amount of polyphenol compounds. The results showed that lime peel have maximum antioxidant effects in *Citrus* sp. **Keywords:** Citrus, NBT, DPPH, Folin-Ciucalteu, FRAP, Antioxidant, Flavonoids **Acknowledgement:** *This research has been supported by Ahwaz University of Medical Sciences.*

PM11 Antibacterial Activity of Different Extracts of *Clidemia hirta* (L.) D. Don leaves

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Clidemia hirta (L.) D. Don (Melastomataceae), locally known as "senduduk bulu" by a local tribe in Malaysia, has been used traditionally to stop bleeding [1] and in the treatment of venom fever [2]. The use of this species as traditional medicine for several bacterial infections has also been recorded in several references [3, 4]. Thus, this study was conducted to investigate the antibacterial properties of this species and determine its MIC towards several bacteria, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*. Several extracts has chosen by extracting (cold extraction) the leaves with different polarity of solvents such as hexane, ethyl acetate and methanol, respectively. The initial antibacterial property identification was done by using disk diffusion method. The active extract was further investigated for its mechanism and MIC's value by dilution technique. Only the hexane extract was not showing any antibacterial property against selected bacteria. Meanwhile, none of these extracts showed activity against *E. coli*. Interestingly, bactericide activity was exerted against *P. aeruginosa* by ethyl acetate extract (MIC 0.625%), followed by its bacteriostatic activity against *E. faecalis* (MIC 0.625%). Conversely, the methanol extract showed bacteriostatic activity against *P. aeruginosa* (MIC 1.25%) and bactericide activity against *E. faecalis* (MIC 1.25%). **Keywords:** *Clidemia hirta*, Melastomataceae, antibacterial **References:** 1. Musa N (2007) The Forgotten Jungle Medicine of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang. 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. Pusat Pengurusan Penyelidikan UKM. Bangi. 3. Franca F Lago EL, Marsden PD (1996) Rev Soc Bras Med Trop 29: 229 – 232. 4. McClatchey W (1996) J Ethnopharmacology 50: 147 – 156.

PM12 Preventive Role of Cactus (*Opuntia ficus-indica*) Cladodes on methotrexate-induced Biochemical, Hematological and oxidative damage in rat liver

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Methotrexate is widely used in the therapy of various types of malignancy as well as in the treatment of various inflammatory diseases. Its efficacy is limited by severe side effects, it may affect liver, kidney, and blood. The ability of Extract of Cactus to restore MTX – liver and Hematological Damage was tested. Cactus cladode Extract (*Opuntia ficus-indica* Mill.) was injected intraperitoneally alone or simultaneously with an intraperitoneal Methotrexate (MTX) administration to wistar male rats for 10 days. The rats were randomly divided into four groups as follows; I- Control group; II- cactus group (0.4 g/kg); III-MTX group (20 mg/kg); IV-MTX+cactus (0.4 g/kg of cactus+20 mg/kg MTX). On the tenth day rats were scarified by decapitation, blood samples were colleted for hematological and serum biochemical parameters measurements. Our results clearly showed that MTX treatment significantly decreased hematocrit, hemoglobin, white blood cells, and increased the most of biochemical serum parameters. While the mixture of the extract of cactus cladodes with the MTX induced a reestablishment of hematological parameters, and levels of serum biochemical enzyme activities. In conclusion, it appears that cactus cladodes extract protects against methotrexate-induced oxidant organ injury and it may become a promising treatment in the prevention of undesired side effect of MTX. **Keywords:** Opuntia ficus indica, Methotrexate, Biochemical and Hematological Parameters, Prevention, Cladodes of Cactus **Acknowledgement:** *This research was funded by the Tunisian Ministry of Scientific Research and Technology through the Research Unit of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa*

PM13 Antihyperglycemic effect of *Derris reticulata* Craib extract in alloxan-induced diabetic rats

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The global prevalence of diabetes mellitus (DM), a metabolic disorder characterized by chronic hyperglycemia, has been estimated to be increasing worldwide [1]. It is known that the present synthetic drugs available for treatment of DM can cause several undesirable side effects. As recommended by WHO, the quest for effective and safer antidiabetic plant drugs is an important topic [2]. In Thailand, *Derris reticulata* Craib (DC) which belongs to Leguminosae family has been traditionally used for diabetic treatment. However, limited scientific data are available. The aim of this work was to investigate the antihyperglycemic potential of the aqueous extract from DC stem in alloxan-induced diabetic rats. The result showed that administration of DC extract at the daily dose of 250 mg/kg for 15 consecutive days significantly decreased fasting blood glucose levels compared with diabetic control group. More groß pathological lesions were found on the pancreas of diabetic control rats than that of DC-treated rats. An association between antioxidant property and antihyperglycemic activity of plant extract has been reported [3]. Total phenolic content and the IC50 of antioxidant potential against DPPH radical of the extract were 78.84 ± 0.01 mg gallic acid equivalent/g and 239.85 ± 0.13 μg/ml respectively. In conclusion, the DC extract exerts antidiabetogenic effect which may be associated with its antioxidant-mediated pancreatic protection. **Keywords:** diabetes mellitus, *Derris reticulata*, antioxidant **References:** 1. Wild S et al. (2004) Diabetes Care 27:1047 – 1053. 2. Gupta S et al. (2009) J Ethnopharmacol 123: 499 – 503. 3. Alarcon-Aguilar FJ et al. (2010) J Ethnopharmacol 132:400 – 407.

PM14 Echinocystic acid inhibits acute-lung injury by inhibiting TLR4/LPS complex formation

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Orally administered lancemaside A isolated from *Codonopsis lanceolata* Trautv. (Campanulaceae) showed anti-inflammatory effects in vivo and produced 3 metabolites by the incubation with human intestinal microflora in vitro [1, 2]. Among lancemaside A and its 3 metabolites, echinocystic acid most potently suppressed the production of the pro-inflammatory cytokines, TNF-α and IL-1β, as well as of the activation of their transcription factor NF-κB in LPS-stimulated alveola macrophages. Echinocystic acid also down-regulated the production of inflammatory markers, including inducible nitric oxide synthase and cyclooxygenase-2, as well as the inflammatory mediators, nitric oxide and prostaglandin E2 in LPS-stimulated macrophages. Echinocystic acid also inhibited the activation of IL-1 receptor-associated kinase, the phosphorylation of IKK-β and IκB-α, the nuclear translocation of NF-κB. Furthermore, echinocystic acid potently inhibited the interaction between LPS and TLR4. Echinocystic acid suppressed LPS-induced acute-lung injury in mice, as well as the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, and the activation of their transcription factor, NF-κB. When lancemaside A was orally administered for mice, its metabolite echinocystic acid alone was detected in the blood. Based on these findings, echinocystic acid may express anti-inflammatory effects by inhibiting the binding of LPS to TLR4 on alveola macrophages *in vitro* and *in vivo*. **Acknowledgement:** *This study was supported by a grant from World Class University Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R33 – 2008 – 000 – 10018 – 0).* **References:** 1. Joh EH et al. (2010) Int J Colorectal Dis 25: 545 – 551. 2. Joh EH et al. (2010) J Chromatogr B Analyt Technol Biomed Life Sci 878: 1875 – 1880.

PM15 Antioxidant and antibacterial activities of the extract of *Aquilaria crassna* leaves

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Aquilaria crassna Pierre ex Lecomte (Thymelaeaceae) or Krisana agarwood has long been used for the production of high valued incense, cosmetic and pharmaceutical products in Asia [1]. In Thailand, leaves of young *Aquilaria crassna* are used to produce commercial herbal teas. In addition to its aroma, it is believed that Krisana leaves possess many interesting medicinal properties, such as antidiarrheal, antidiabetic and antibacterial activities. However, scientific study on its pharmacological activity is very limited. The aims of this study were to investigate the safety and antibacterial activity of the aqueous extract of *Aquilaria crassna* leaves. Acute toxicity test showed that even at high dose (15,000 mg/kg) the extract did not cause death or overt signs of toxicity when observed for 14 consecutive days in mice. It was found that the extract exhibited antibacterial activities against *Staphylococcus aureus* and *Staphylococcus epidermidis* with MIC of 12.0 and 4.0 mg/ml, respectively. Since the correlation between antioxidant and antibacterial activities of plants has been reported [2], the total phenolic compound (TPC) and antioxidant property were also examined. The TPC of the extract was 162.4 ± 0.3 mg gallic acid equivalent/g. The extract showed strong antioxidant activity against DPPH radical with IC_{50} of 6.04 ± 0.18 μg/ml. It is concluded that the aqueous extract of *Aquilaria crassna* leaves may be beneficial for treatment of diarrhea caused by *Staphylococcus aureus* and skin infection associated with *Staphylococcus epidermidis*. **Keywords:** *Aquilaria crassna*, Acute toxicity, Antibacterial, Antioxidant **Acknowledgement:** *This study is funded by the Office of the Higher Education Commission, Thailand.* **References:** 1. Eurlings MCM et al. (2010) Forensic Sci Int 197: 30 – 34. 2. Bajpai VK et al. (2009) Food Chem Toxicol 47: 1876 – 1883.

PM16 Potential antibiotic and anti-infective effects of rhodomyrtone from *Rhodomyrtus tomentosa* (Aiton) Hassk. on *Streptococcus pyogenes* as revealed by proteomics

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Rhodomyrtone from *Rhodomyrtus tomentosa* (Aiton) Hassk. leaf extract has a strong antibacterial activity against the bacterial pathogen *Streptococcus pyogenes*. Our previous studies indicated that the bactericidal activity of rhodomyrtone might involve intracellular targets. In the present studies we followed a proteomics approach to investigate the mode of action of rhodomyrtone on S. pyogenes. For this purpose, S. pyogenes was cultivated in the presence of 0.39 μg/ml rhodomyrtone, which corresponds to 50% of the minimal inhibitory concentration. The results show that the amounts of various enzymes associated with important metabolic pathways were strongly affected, which is consistent with the growth-inhibiting effect of rhodomyrtone. Additionally, cells of *S. pyogenes* grown in the presence of rhodomyrtone produced reduced amounts of known virulence factors, such as the glyceraldehyde-3-phosphate dehydrogenase, the cAMP factor, and the streptococcal pyrogenic exotoxin C. Taken together, these findings indicate that rhodomyrtone has both antimicrobial and anti-infective activities, which make it an interesting candidate drug. **Keywords:** glycolysis, proteomics, rhodomyrtone, Rhodomyrtus tomentosa, Streptococcus pyogenes, two-dimensional gel electrophoresis **Acknowledgement:** *We thank Jan Arends, and members of the Department of Medical Microbiology and Department of Molecular Genetics for strains and technical support. We thank Assoc. Prof. Dr. Wilawan Mahabusarakam and Mr. Asadhawut Hiranrat for rhodomyrtone isolation. The work was funded by the Thailand Research Fund through the Royal Golden Jubilee, Ph.D. Program (PHD/0029/2548). Funding was furthermore provided by the National Research University Project of Thailand's Office of the Higher Education Commission, the Van Leersumfonds, The Netherlands (VLF/DA/3689), and the CEU projects LSHM-CT-2006 – 019064 and LSHG-CT-2006 – 037469.* **References:** [1] Limsuwan S, Hesseling-Meinders A, Voravuthikunchai SP, van Dij JMl, Kayser O (2011) Phytomedicine, in press [2] Limsuwan S et al. (2009) Phytomedicine 16: 645

PM17 *In vivo* evaluation of an herbal remedy for antimalarial activity

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The antimalarial activity of an herbal remedy (HR) formulated, based on ethnomedical claims followed by observational experiences, was investigated in *Plasmodium berghei* NK 65- infected mice. The decoction of the HR was prepared, concentrated *in vacuo* and freeze-dried. Evaluation of the antimalarial activity involved the use of early malarial (4 -day test) and established infection models [1, 2]. The HR was tested at 15 – 240 mg/kg while the positive control, amodiaquine (AQ) was tested at 1.25 – 10 mg/kg. For the established infection test, the HR was tested at 60 – 240 mg/kg with AQ (10 mg/kg) as positive control. Distilled water was used as negative control in both test. The HR and AQ gave ED_{50} of 40 and 3.8 mg/kg respectively, while for the established infection test, the highest dose of 240 mg/kg gave 54.45% clearance on day 5. The HR showed higher suppressive than curative activity. **Keywords:** Herbal remedy, *Plasmodium berghei*, Amodiaquine **Acknowledgement:** *Prof. G. A. Ademowo, Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria for access to the Plasmodium berghei NK 65 parasite* **References:** 1. Peters W (1965) Exp Parasitol 17: 80 – 87 2. Ryley J, Peters W (1970) Am J Trop Med Parasitol 84: 209 – 211

PM18 Procyanidins of *Nelia meyeri* SCHWANT. elicit endothelium-dependent relaxation in porcine coronary arteries by activation of the PI3/Akt signalling pathway

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Nelia meyeri Schwant. (Mesembryanthemacea) is a South African succulent known to contain procyanidins [1]. The aim of this study was to examine the molecular mechanism by which the extract from leaves of this plant elicits blood vessel relaxation. For this, a highly purified fraction comprised of bi- to tetrameric flavan-3-ols was applied to porcine coronary arterial rings suspended in organ chambers containing Krebs-Henseleit solution maintained at 37 °C. In endothelium-intact rings precontracted with the thromboxane A2 mimetic U46619, the sample produced a concentration-dependent relaxation that was abolished by mechanical removal of the endothelium. Concentration-response curves to the defined procyanidin fraction were shifted to the right in the presence of L-NAME (NG-nitro-L-arginine methyl ester), an inhibitor of eNOS. The observed relaxation was also abolished by wortmannin, an inhibitor of PI3K (phosphoinositide 3-kinase). However, the relaxant response to the *Nelia* extractives remained unaffected in the presence of ICI 182,780, an estrogen receptor antagonist, and pertussis toxin, an inhibitor of Gi proteins. These observations confirm the essential role of EDRF in the relaxant response to *Nelia* procyanidins. In addition, relaxation to the *Nelia* fraction was abolished by MnTMPyP, a cell permeable mimetic of superoxide dismutase but not by tiron, a superoxide anion scavenger. The relaxation was insensitive to charybdotoxin plus apamin (Ca2+-activated K+ channel blockers) but was abolished by the combination of charybdotoxin plus apamin plus L-NAME. Taking together, these findings suggest that the endothelium-dependent relaxation induced by *Nelia* procyanidins is mediated by EDHF and EDRF following activation of PI3/Akt. **Keywords:** *Nelia meyeri*, procyanidins, endothelium relaxation, EDHF, EDRF **References:** 1. Kolodziej H (1984) Phytochemistry 23: 1745 – 1752

PM19 *In vitro* antileishmanial activity of resveratrol appears associated with cell cytotoxicity rather than antiparasitic properties

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Recently, we reported the antileishmanial activity of resveratrol against *Leishmania major* GFP in infected BMMθ. In parallel we observed host cell cytotoxicity in a concentration-dependent manner, contrasting with claimed cell tolerability [1]. This apparent discrepancy prompted the present study using the reported resveratrol-tolerable J774-G8 cell line. When *L. major* GFP-infected J774-G8 cells were exposed to resveratrol (5 – 75 μg/mL), the resulting GFP signal was similarly reduced from 95% to 5% as a reflexion of antileishmanial activity. However, host cell cytotoxicity invariably increased with sample concentrations as assessed by FACS analysis following staining with propidium iodide and apparent changes in cell morphology (Diff-Quick-Staining). The MTT-assay provided an IC_{50} of 96 μM (22 μg/mL) and 83 μM (20 μg/mL) of resveratrol for non-infected J774-G8 and BMMθ, respectively. This finding provides evidence for similar cytotoxicity of the test compound in both cell lines. Its antileishmanial activity appears to be associated with cytotoxic effects on host cells at concentrations of 25 – 40 μg/mL rather than selective antiparasitic properties. Having in mind that J774-G8 is a murine macrophage-like cancer cell line, the observed pronounced cytotoxic effects are in line with reports on anticancer/chemopreventive properties of resveratrol [2]. Current studies include staining techniques to discriminate between apoptotic and necrotic effects. Preliminary results suggested the induction of apoptosis, consistent with reports on apoptosis-associated proteins [3]. **Keywords:** resveratrol, antileishmanial, cytotoxicity, BMMθ, J774-G8 cells **References:** 1. Kedzierski L et al. (2007) Parasitol Res 102: 91 – 97 2. Bhat et al. (2001) Antioxidants & Redox Signaling 3: 1041 – 1064 3. Li, G. et al. (2011) Phytomedicine doi.10.1016/j.phymed.2010.11.015

PM20 Targeting intestinal digestive enzymes by natural products: synergistic effect of flavonoids

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Diabetes is a common metabolic disorder that is caused by either inherited and/or acquired deficiency in insulin secretion or due to decreased responsiveness to insulin. One of the common approaches in the treatment of diabetes is decreasing postprandial hyperglycemia by inhibiting key enzymes for the hydrolysis of carbohydrates in the small intestine. In our laboratories, the effects of various natural products on key digestive enzymes, α-glucosidase and α -amylase, are routinely assessed [1]. It was found that some flavonoids including kaempferol-3-O-rutinoside (KR, Fig. 1) are potent inhibitors of these enzymes. A synergistic enzyme inhibitory effect; e.g, between KR and flavonoid aglycones, was observed for some flavonoids. The structure activity relationship established from the study and potential therapeutic implications are discussed.

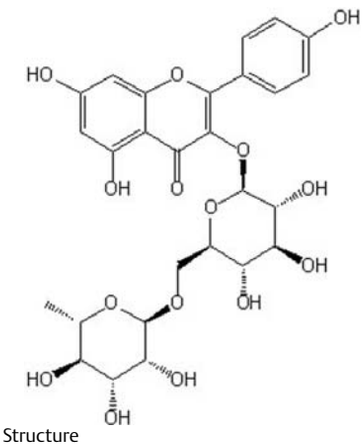

Figure 1: KR Structure
Structure of exemplary flavonoid glycoside with potent a-glucosidase inhibitory activity.

Keywords: Diabetes, α-glucosidase, α -amylase, flavonoids, kaempferol-3-O-rutinoside **References:** 1. Habtemariam S (2011) Nat Prod Commun 6:201 – 203.

PM21 The effect of Boswellia serrata on Giardia duodenalis

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Giardia duodenalis is a parasite that colonizes the small intestine of various mammalian hosts especially in humans. The common treatment for giardiasis includes metronidazole, furazolidone and benzimidazole-based drugs which cause many side effects besides an increasing resistance problem [1,2]. Having in mind that *Boswellia serrata* Roxb. is used for the treatment of chronic inflammatory disorders and that this parasite is known to facilitate these conditions [3], the gum resin of this plant source was tested for antigiardial effects. A crude extract standardized to 85% boswellic acids reduced the viability of the parasite by ca. 65% at a concentration of 20 μg/mL. At the highest concentration tested (80 μg/mL), the antigiardial effect was ca. 80% based on the metabolic conversion of resazurin [4]. Metronidazole (50 μg/mL) served as a positive control. In search for the active principle, the extract was subjected to HPLC separation showing two major peaks at Rt 12.3 and 14.7 min, respectively. The former, comprising a complex mixture of boswellic acids, exhibited pronounced antigiardial activity at 20 μg/mL, as evident from a ca. 80% reduction in parasite viability. HPLC analysis showed also the presence of oleanolic acid. Preliminary analyses proved this triterpenoid only moderately active (parasite viability ca. 65% at 45 μM corresponding to 20 μg/mL). This finding suggested boswellic acids as the active principle. Owing to the complexity of the fractions, the isolation of distinct boswellic acid members for antigiardial activity studies is still in progress. This is the first report on antigiardial effects of boswellic acids. **Keywords:** *Boswellia serrata*, antigiardial, boswellic acids, oleanolic acid **References:** 1. Gardner B and Hill D (2001) Clin Microbiol Rev 14: 114 – 128 2. Upcroft P and Upcroft J (2001) Clin Microbiol Rev 14: 150 – 164 3. Layton MA et al. (1998) Brit J Rheumatol 37: 581 – 583 4. Bénéré E et al. (2007) J Microbiol Methods 71: 101 – 106

PM22 Anti-ulcerogenic Activity of the Standardized Water Extract of *Phyllanthus emblica* Linn

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Phyllanthus emblica Linn. (synonym: Emblica officinalis Gaertn.), Family Euphorbiaceae is native to the tropics of South and Southeast Asia. It is also called Emblic, Emblic myrobalan, Indian Gooseberry, Malacca tree and Myrobalan. In Thailand, it is known as Ma-kham-pom. *P. emblica* is an herbal plant commonly used in Asian traditional medicine systems for treatment of many disorders including anorexia, indigestion, and anemia (1, 2). The fresh or dry fruit is used in traditional medicine for the treatment of diarrhea, jaundice and inflammatory disorder (2, 3). The *P. emblica* water extract was prepared according to the Thai Herbal Pharmacopoeia and standardized. The phytochemical study, the *P. emblica* water extract contained tannins about 42.51%. The HPLC analysis of *P. emblica* water extract showed the presence of 20.48% gallic acid. Preliminary study, *P. emblica* water extract elicited the inhibitory effect on both COX-1 and COX-2 enzymes. Thus, the gastric ulcer may be one of the potential side effect of *P. emblica* water extract caused by its inhibitory effect on COX-1 enzyme. The oral administration of the *P. emblica* water extract at the dose of 600 mg/kg did not produce gastric lesions. On the contrary, the extract at the doses of 150, 300 and 600 mg/kg reduced ulcer formation in all tested acute gastric ulcer models i.e. EtOH/HCl-, indomethacin-, and stress-induced gastric lesions. These results indicate that *P. emblica* water extract possess anti-ulcerogenic effect. **Keywords:** Anti-ulcerogenic, Phyllanthus emblica Linn **Acknowledgement:** *Royal Golden Jubilee Ph.D. Program and the National Research Council of Thailand.* **References:** 1. Santisuk T et al. (2005) Floral of Thai-

land. Vol. 8 Part 1 (Euphorbiaceae). Prachachon. Bangkok. 2. Khan KH (2009) Bot Res Intl 2(4): 218 – 28 3. Deokar AB (1998) Medicinal plant grown at Rajegaon, 1st ed. DS Manav Vikas Foundation. Pune.

PM23 Inhibition of angiogenic factors by laserolide, a sesquiterpene lactone from *Laser trilobum* Borkh. ex Gaertn

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Sesquiterpene lactones (SLs) are plant secondary metabolites, widely distributed within the families of Asteraceae and Apiaceae. SLs have received ever increasing attention for their beneficial effects in pathologies etiologically associated with angiogenesis, such as chronic inflammation and cancer. We have investigated the interference of SLs isolated from the non-pharmacopoeia C./E. European plant *Laser trilobum* Borkh. ex Gaertn., i.e. laserolide, isolaserolide, eudeslaserolide, archangelolide and 2-deangeloyl-archangelolide with production of angiogenic factors such as nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines vascular endothelial growth factor (VEGF), interleukins IL-1β and IL-6. The recognized SL costunolide was included for comparative purposes. The cytocidal effects of the compounds were evaluated as well. The immunobiological experiments were done under in vitro conditions using rat resident peritoneal cells. They were cultured at a density of 2×10^6/mL in RPMI-1640 medium for 24 h. The production of NO, PGE2 and cytokines were triggered by lipopolysaccharide (1 μg/mL). Formation of NO was assayed using Griess reagent. Concentrations of PGE2 and cytokines were determined by ELISA. In contrast to costunolide, the SLs from *L. trilobum*. are devoid of cytocidal effects up to a concentration of 50 μM. Laserolide may be considered as a promising candidate for further preclinical investigations because its immunosuppressive effectiveness is very close to that exhibited by costunolide. It inhibits the cytokine (including the major angiogenic factor VEGF), PGE2 and NO production at IC 50 s of approximately 5 – 10 μM. **Keywords:** *Laser trilobum*; laserolide; nitric oxide; prostaglandins; cytokines **Acknowledgement:** *The work was supported by the grant 305/07/0061 from GACR.*

PM24 Participation of citral in the relaxation of isolated rat tracheal smooth muscle induced by ginger oil

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Ginger (*Zingiber officinale*) Roscoe is a common food plant that has been used as alternative medicine for a number of ailments. The rhizome of this plant is well known for the treatment of gastrointestinal tract disorders, such as dyspepsia, nausea and vomiting, as well as respiratory illnesses [1]. Its hydroethanolic extract has been shown to inhibit airway hyperreactivity and remodelling, and lung inflammation [2 – 3]. The inhibitory effects of aqueous and methanolic crude extracts of ginger on tracheal smooth muscle were described [4], however, the effects of ginger oil and its active compound on airway smooth muscle have never been reported. The aim of this study is to investigate the effect of ginger oil and its constituents on tracheal smooth muscle *in vitro*. Chemical compositions of ginger oil were determined by GC-MS. Citral and camphene appeared to be its major components. For the relaxation study, two-cartilage segments of rat trachea were prepared and mounted vertically in an organ bath. The contraction of tracheal smooth muscle was induced by acetylcholine (Ach) before ginger oil, citral or camphene were added. Ginger oil and citral, but not camphene, were found to reverse the Ach-induced airway contraction in concentration-dependent manner. This result indicates that citral is, at least partly, responsible for the myorelaxant effect of ginger oil on rat trachea. **Keywords:** Ginger oil, Citral, Tracheal smooth muscle, Relaxation **Acknowledgement:** *This study is supported by the National Research Council of Thailand.* **References:** 1. Ghayur MN et al. (2008) Can J Physio Pharmacol 86: 264 – 271. 2. Kuo PL et al. (2011) J Agric Food Chem (in press). 3. Aimbire F et al. (2007) Prostaglandins Leukot Essent Fatty Acids 77:129 – 38 4. Ghayur MN, Gilani AH (2007) Eur Food Res Technol 224:477 – 481.

PM25 Screening on cytotoxicity, antioxidant and antimicrobial of stem bark from Malaysian *Vatica odorata* and *Vatica bella* (Dipterocarpaceae)

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Dipterocarpaceae have proven to be rich sources of variety biological activities (Zain et al.2010). In our continuing investigation on this family we wish to report the screening on three biological activities: cytotoxicity, antioxidant and antimicrobial of methanol and acetone extract from *Vatica odorata* (Griff.) Symington and *Vatica bella* Slooten. The antioxidant activity were evaluated by 1,1-diphenyl-2-picrylhydrazil (DPPH), Total Phenolic Content (TPC), Ferric thiocyanate method (FTC) and Thiobarbituric acid (TBA) method. The cytotoxicity activities were screened against Chang and HepG2 cells line (Mackeen et al. 1997). Meanwhile antimicrobial activity was conducted against six types of bacteria *(Escherichia Coli, Stapylococcus aureus, Bacillus subtilis, Salmonella typhi, Pseumodonas aerugonisa* and *Klebsiella pneumonia*) and four dermatophyte fungal species (*Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum gypseum* and *Candida glabrata*) by disc diffusion method (Barry et al.1979). The results indicated that acetone extract of *Vatica bella* displayed moderate activity against Chang's liver cell and HepG2 cell with IC_{50} values of 14 ± 0.50 μg/mL and 20.5 ± 2.68 μg/mL respectively. Both the *Vatica* extract displayed total phenolic content with range of 331.54 – 482.31 mg/g GAE and are weak DPPH scavenger as compared to standard with the range between 35.60 – 66.2%. In FTC and TBA test, the *Vatica* extracts exhibited antioxidant potential with percent inhibition between the ranges 26.60 – 88.36%. The antimicrobial screening showed that both the crude extract inhibited moderately *S.aureus* except for acetone extract of *Vatica bella*. Meanwhile methanol extract of *Vatica bella* gave the active result where it inhibited moderately against *Trichophyton mentagrophyte, Trichophyton tonsurans* and *Microsporum gypseum*. **Keywords:** *Vatica bella*, *Vatica odorata*, Dipterocarpaceae, oligostilbenoid, cytotoxicity, antimicrobial, antioxidant **Acknowledgement:** *We wish to thank to Ministry of Higher Education Malaysia for financial support via FRGS grant (011000070006) and UiTM for all the support* **References:** 1-Zain WZWM, Ahmat N, Nawi L.& Jusoh, K (2010) World Applied Science Journal 8(9):1050 – 1055. 2-Mackeen MM et al. (1997) Int J Pharmacognosy 35: 174 – 178 3- Barry AL et al. (1979). Journal of Clinical Microbiology 10: 885 – 889.

PM26 Isolation, cytotoxic and antiplasmodial activities of 6-farnesyl-3',4',5,7-tetrahydroxyflavanone from the flower of *Macaranga triloba*

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The genus *Macaranga* is one of the largest genera of the Euphorbiaceae, with approximately 300 species [1]. *Macaranga triloba* Müll.Arg. locally known as "Mahang merah" is a tree endemic to Southeast Asia at forest margins and its water extract is used as pain relief for stomach trouble in Java [2]. The flower of *Macaranga triloba* was collected from Pasir Raja, Hulu Terengganu (Malaysia), macerated successively with hexane, dichloromethane and methanol for 72 hours. The dichloromethane (DCM) extract (44.41 g) was dissolved in MeOH and subjected to vacuum liquid chromatography (VLC). The sub-fractions was further chromatographed on a reverse phase column chromatography (RPCC) that led to the isolation of MT1, 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (58.1 mg) [3]. The biological activity of MT1 was evaluated for the first time. The cytotoxicity of MT1 was evaluated by using MTT assay. 6-Farnesyl-3',4',5,7-tetrahydroxyflavanone exhibited strongly, moderately and very strongly the growth of HL60, MCF-7 and HeLa cell lines with IC_{50} value of 6.71, 11.38 and 2.64 μM respectively. The DCM extract was also subjected to antiplasmodial screening which displayed an IC_{50} = 2.01 μg/mL indicating the good potential as anti-plasmodial agents. The antiplasmodial property of MT1 was evaluated using *Plasmodium falciparum* with concentration of 10, 1, 0.1, 0.01 and 0.001 μg/mL. 6-Farnesyl-3'.4',5,7-tetrahydroxyflavanone (MT1) was found to exhibit a strong antiplasmodial activity with an IC 50 value of 0.06 μM. This study

indicates the potential of MT1 as anti-cancer and anti-plasmodium agents. **Keywords:** *Macaranga triloba*, 6-farnesyl-3', 4', 5, 7-tetrahydroxyflavanone, farnesyl, cytotoxic, antiplasmodial

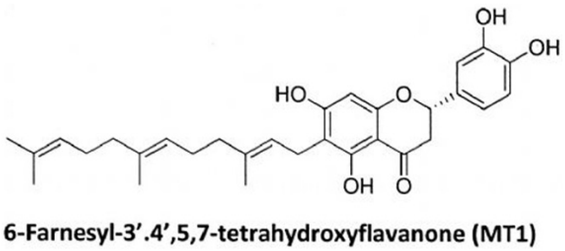

Figure 1: MT1

Table 1: Cytotoxic and antiplasmodial activities of MT1

cytotoxicity		Antiplasmodium	
HL 60 (μM)	MCF-7 (μM)	HeLa (μM)	Plasmodium falciparum (μM)
6.71	11.38	2.64	0.06

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PM27 Antifungal activity of the extract of *Alpinia officinarum* Hance rhizomes on *Candida albicans*

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Alpinia officinarum Hance, known as lesser galangal, is a pungent and aromatic plant which is used as spice for flavoring food throughout Asian countries [1]. This plant has also been used as traditional medicine for several purposes such as relieving stomachache and pain, treating colds, invigorating the circulatory system, and reducing inflammation and swelling [2]. *Candida albicans* is a major causative microbe associated with fungal infection, especially in patients with endocrine disorders, immunosuppression, malignant disorders and AIDS [3]. Nowadays, choices of antifungal against candidiasis are quite limited due to drug toxicity and resistance. The crude extract of the rhizomes of *Alpinia officinarum* has been shown to possess antibacterial activity [4], however, antifungal activity of this plant has not been reported. In the present study, we found that lesser galangal exhibited antifungal activity against *Candida albicans*. The minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) value were 1.2 mg/ml and 2.0 mg/ml, respectively. The assessment of cell damage produced by the crude extract of *Alpinia officinarum* rhizomes was conducted through scanning electron microscope (SEM) observation. SEM analysis showed that the extract induced deformation of *Candida albicans*. The treated cells had coarse surface and changed from oval to rounder shape. The result suggested that the extract damaged cell wall, causing *Candida albicans* to form spheroplast. This postulated mechanism may contribute to the antifungal activity of the crude extract of the *Alpinia officinarum* rhizomes against *Candida albicans*. **Keywords:** *Alpinia officinarum* Hance, *Candida albicans*, minimum inhibitory concentration, minimal fungicidal concentration, scanning electron microscope **References:** 1. Ly TN et al. (2003) J Agric Food Chem 51: 4924 – 4929. 2. Lee J et al. (2009) J Ethnopharmacol 126: 258 – 264. 3. Kumar R et al. (2010) Fungal Biol 114: 189 – 197. 4. Zhang BB et al. (2010) Fitoterapia 81: 948 – 952.

PM28 The role of rice bran extract on acetyl CoA carboxylase in liver of rats fed a high-fat diet

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Rice bran water extract (RBE) was shown to reduce fat mass in rats fed a high-fat diet [1]. The involvement of RBE in metabolic alteration were investigated in 7 groups of Sprague Dawley rats, 8 rats each. High-fat fed group 3 to 7 were either co-treated daily with RBE (220.5, 2205, 4410 mg/kg) or metformin (19.10, 38.20 mg/kg). Oral glucose tolerance was tested at the end of forth week. Rats were killed and specimens were collected. The results showed that the mean ± SEM of abdominal fat weight, epididymal fat cell size and triglyceride level in blood and liver were increased whereas cholesterol in high-density lipoprotein was decreased in rats fed a high-fat diet alone as compared to rats fed with chow (7.65 ± 0.29 vs. 4.73 ± 0.39 g.; 4919.76 ± 453.59 vs. 2835.23 ± 249.15 μm2; 44.67 ± 2.72 vs. 35.00 ± 2.02 mg/dL; 2.50 ± 0.26 vs. 1.48 ± 0.08 mg/mL; 67.14 ± 1.62 vs. 77.20 ± 2.82 mg/dL, respectively). At least 2205 mg RBE/kg or 19.10 mg metformin/kg were able to significantly reduce abdominal fat weight and triglyceride levels in liver. ACC activity was increased in high-fat feeding group but the activities retuned to normal when they were also received RBE or metformin, though there was no statistic significance. In conclusion, fatty acid flux might induce fat synthesis in liver as an initial step in increasing fat accumulation [2]. Both RBE and metformin were able to reduce the alteration. **Keywords:** Rice bran, acetyl CoA carboxylase, high-fat diet **Acknowledgement:** *Research Unit, Faculty of Medicine, Thammasat University, the National Research Council of Thailand.* **References:** 1 Kandee N et al. (2009) Thamm Med J 9:140 – 7. 2 Samuel VT, Peterson KF, Shulman GI (2010) Lancet 375:2267 – 77.

PM29 Wound healing potentials of a herbal homeopathic remedy on NIH 3T3 fibroblasts *in vitro*

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This study was aimed to investigate the effect of the commercial homeopathic herbal remedy Similasan® Arnica plus Spray (Similasan AG, Jonen, Switzerland), which is an ethanolic (22% m/m) preparation of *Arnica montana* L. D4, *Calendula officinalis* L. D4, *Hypericum perforatum* L. D4 and *Symphytum officinale* L. D6 (0712 – 2), on wound healing in cultured NIH 3T3 fibroblasts. Wound healing requires the coordination of complex cellular and molecular interactions. Therefore we investigated the cell proliferation, migration and wound closure promoting effect of the preparation and its potentized hydroalcoholic solvent (0712 – 1) using BrdU uptake, transwell chamber assay and wound healing scratch assay, respectively. All assays were performed in a controlled, blinded manner at least in three independent experiments. The preparation (0712 – 2) exerted a stimulating effect on cell migration 31.7% vs. 15% solvent (0712 – 1) at 1:100 dilutions ($p < 0.05$, $n = 3$). Mean wound closure reached 59.5% by preparation in comparison to 22.1% by solvent ($p < 0.05$, $n = 3$) at the same concentration. Positive control (5% FCS) caused 63.0% closure. There was no effect of cell proliferation. In conclusion, the Similasan® Arnica plus homeopathic remedy showed wound healing activity in the NIH 3T3 fibroblasts scratch assay, which may be partly a result of fibroblasts migration and does not seem to be related to mitotic activity.

Figure 1: Wound closure of 3T3 fibroblasts by Similasan Arnica plus preparation
Representative microphotographs of wound healing effect of proparation (1:100) on 3T3 fibroblasts after 24 h treatment. Indicated percentages of wound closure are normalized to the untreated control (medium).

Keywords: wound healing, 3T3 fibroblasts, homeopathic preparation

PM30 Antioxidant capacity of *Pistacia lentiscus* and *Fraxinus angustifolia* extracts and their fractions

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Oxidative stress is thought to be the main cause of several pathologies; that is why research is focussing on the characterization of bioactive natural substances with antioxidant activity to replace synthetic molecules. The antioxidant activity of extracts of *Fraxinus angustifolia* Reut. ex Nyman and *Pistacia lentiscus* L., two medicinal plants used to treat inflammatory-related disorders was determined. The results indicated that aqueous chloroform extract of *Pistacia lentiscus* exhibited a great reducing power of 657.86 ± 35.25 mg Ascorbic Acid Equivalent/g of the extract, compared to that of the aqueous extract from *Fraxinus angustifolia* (260.73 ± 22.38 mg Ascorbic Acid Eq/g of extract). Extracts issued from both plants showed an outstanding capacity in scavenging the (DPPH) 2, 2-diphenyl-1-picrylhydrazyl (90%) and (ABTS) 2,2-azobis-ethylbenzothiazoline-6-sulphonic acid (85%) radicals at a concentration of 100 µg/ml, even higher than that of the standards: (BHA) butylhydroxyanisol and quercetin. Moreover, *Pistacia lentiscus* extracts showed relatively high scavenging activity (64.86% at 100 µg/m) against hydrogen peroxide, better than that of ascorbic acid (40.09%) and caffeic acid (45.59%) at the same concentration, while *Fraxinus angustifolia* extracts showed significant activity only at high doses (400 µg/ml). Column chromatography associated with thin layer chromatography analysis of plant extracts allowed the recovery of fractions responsible for high antiradical activity. Determination of total phenols and tannins plead for a major role of these compounds in the observed high antioxidant activity and may well explain the use of these plants in traditional medicine. **Keywords:** *Fraxinus angustifolia*, *Pistacia lentiscus*, Phenolic compounds, antioxidant, active fractions **Acknowledgement:** *We wish to thank the Ministry of Education and Scientific Research of Algeria for sponsoring this work Grant number: F00620070022* **References:** 1- Oyaizu et al. (1986) Japanese Journal of Nutrition 44: 307 – 315. 2- Pitchaon et al. (2007) Food Chemistry 10 (4): 1409 – 1418. 3- Atmani et al. (2009) Food Chemistry 112 (2): 303 – 309.

PM31 Antioxidant activity of crude extract from Algerian chemlal olive leaves and application in stored meat

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In this study an aqueous crude extract and oleuropein have been obtained from algerian olive leaves (variety chemlal). The antioxidant effect of these compounds was determined in stored meat by TBA-RS methods. These compounds were added to chicken meat at 500 mg/kg. All meat samples have been stored in the aerobic conditions at 4 ± 2 °C for one week. The results showed that compounds of olive leave have a remarkable antioxidant activity throughout the storage phase. However, crude extract showed higher activity on lipid oxidation. The sensory analysis showed that meat "off-odour" added to these compounds remains acceptable during storage. The results of the bioassays support the possibility of using compounds of olive leaves as potent natural preservatives to contribute in the oxidative stability in stored chicken meat. **Keywords:** olive leaves, crude extract, oleuropein, Antioxidant activity, stored chicken meat **Acknowledgement:** *The authors are grateful to Ministerio de Asuntos Exteriores y Cooperación of Spain (AECID) and Ministère de l'Enseignement Supérieur et de la Recherche Scientifique of Algeria for financial assistances to this work within the Programa de Cooperación Interuniversitaria e Investigación Científica PCI/MED Algeria-Spain (grant ALI A/011170/07; A/019342/08; A/023365/09; A/033506/10) and CNEPRU (F00520090025), respectively.*

PM32 Antioxidant potential, cytotoxic activity and phenolic content of *Clematis flammula* leaf extracts

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Five fractions of *Clematis flammula* L., a plant widely used in the Mediterranean traditional medicine, were isolated from the leaves using a selective extraction procedure and their total antioxidant capacity was measured by both the ABTS and ORAC tests. Furthermore, their capacities to inhibit microsomal lipid peroxidation and to scavenge the hydroxyl radical were assessed. The cytotoxic potential of the crude ethanolic extract and the aqueous fraction obtained from chloroform was also evaluated on three human hepatoma cell lines CHL, PLC and HuH7. The results showed a stronger antioxidant capacity for the two aqueous phases obtained from ethyl acetate and chloroform concerning ABTS (7.9 and 10.5 mmoles Trolox eq/g of plant extract, respectively), ORAC (487 and 387 mmoles Trolox eq/g of plant extract, respectively) and hydroxyl radical scavenging activity (IC_{50}= 56.5 and 48.4 µg/mL, respectively), compared to their organic counterparts which, however, inhibited microsomal lipid peroxidation more efficiently (IC_{50}= 390.7 and 523.5 µg/mL, respectively). The ethanol crude extract exhibited a fairly good cytotoxic potential on the two cell lines CHL and PLC (IC_{50}= 58.5 and 47.3 µg/mL, respectively), in contrast to the aqueous phase obtained from chloroform (IC_{50}= 457.7 and 304.9 µg/mL, respectively). A positive correlation was also found between the phenol content and the different activities. These results provide experimental support for the therapeutic virtues of *Clematis flammula* leaf extracts. **Keywords:** *Clematis flammula*, anti-cancer, antioxidant, phenolic compounds **Acknowledgement:** *We wish to thank the Ministry of Education and Scientific Research of Algeria for sponsoring this work, Grant number: F00620070022* **References:** 1- Re et al. (1999) Free Rad Biol Med 26: 1231 – 1237 2- Huang et al. (2002) Food Chem 50: 4437 – 4444. 3- Fee and Teitelbaum (1972) J Agri Biochem Biophys Res Comm 49: 150 – 158. 4- Mosnann (1983) J Immunol Methods 65: 55 – 63.

PM33 Involvement of serotonergic system in anxiolytic effect of dichloromethane fraction of *Pimenta pseudocaryophyllus* (Gomes) Landrum

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The purpose of this study was to verify the putative anxiolytic-like activity of dichloromethane fraction (DF) prepared from the leaves of *Pimenta pseudocaryophyllus* (Gomes) Landrum (Pp) and the mechanism of action involved using the elevated plus maze (EPM) and light dark box (LDB) tests. Male Swiss mice (25 – 35 g) were treated orally with the vehicle (10mL/Kg), DF (125,250 and 500 mg/kg p.o.) or positive controls diazepam (1 mg/Kg) and buspirone (10 mg/Kg) 1 h before behavioral evaluation in the EPM and LDB. A treatment of DF significantly increased the percentage time spent and the number of entries into the open arms of the EPM as well as latency, number of transitions and time spent at the light part of the LDB in a dose dependent manner. The effects of DF in 250 mg/kg were antagonized by the 5-HT1A receptor antagonist NAN-190 (0.5 mg/kg i.p.). However, the effects could not be blocked by the benzodiazepine antagonist flumazenil (2 mg/kg i.p.). These results indicate an effective anxiolytic activity of Pp mainly mediated via the Serotonergic system without compromising motor function of the mice. Although there is need for the isolation of the less polar constituent responsible for this effect, further clinical investigations are necessary for its possible application as an alternative for the treatment of anxiety disorders to other medications currently in use. **Keywords:** Anxiolytic effect; Benzodiazepines, Elevated plus maze, light-Dark box, Pimenta pseudocaryophyllus, GABA receptor, 5-HT1A receptor **Acknowledgement:** *CNPq, CAPES, FAPEG, FUNAPE/UFG*

PM34 Hypoglycemic properties of banana pseudo-stems

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Water extract of banana (*Musa sapientum* L.) pseudo-stems has been claimed by Lebanese herbalists to be efficient in the treatment of diabetes mellitus. This work aimed at verifying the alleged effect and at elucidating its possible mode of action. Administration of the extract in replacement of drinking water to streptozotocin-induced diabetic rats, did reduce significantly blood glucose levels. The mechanism of action of the extract was studied by investigating its involvement in intestinal glucose absorption and its effect on the Na+/K+ ATPase and glucose transporters SGLT1 and GLUT2 in the rat jejunum. Rat jejuna were perfused *in situ* with Krebs Ringer buffer containing [^{14}C] 3-O-methyl-D-glucose, and the activity of the Na+/K+ ATPase in jejunal homogenates was assayed *in vitro*, by measuring the amount of inorganic phosphate released in presence and absence of inhibitors of the ATPase. The extract induced a significant reduction in glucose absorption and Na+/K+ ATPase activity, but did not affect the protein expression of SGLT1 and GLUT2 glucose transporters. It was concluded that the extract acts by reducing the Na+/K+ ATPase activity and consequently the sodium gradient required for sugar transport by SGLT1. Reduced activity of SGLT1 leads to a decrease in intracellular glucose and in the number of apical GLUT2 [1], which contributes to the observed hypoglycemic effect. The current on going work focuses on identifying the active ingredient(s) in the extract. **Keywords:** Banana, Glucose absorption, Na+/K+ ATPase, SGLT1, GLUT2 **Acknowledgement:** *This work was supported by a grant from the University Research Board.* **References:** 1. Kellet GL et al (2000) Biochem J 350:155 – 162

PM35 Radical scavenging activity and phenolic components in different plant parts of *Saraca asoca*

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The therapeutic properties of herbal drugs depend on certain chemical constituents (secondary metabolites) which varies according to age and maturity of the plant. Phenolic compounds have multiple biological properties and also act as antioxidants. They protect the human body against damage by reactive oxygen species. Medicinal plants have been focused for antioxidant compounds because of safety concerns associated with synthetic antioxidants. *Saraca asoca* (Roxb.) Wilde (Fabaceae) an important medicinal tree has been well known for its effectiveness in menorrhagia and dysmenorrhea. Its bark has stimulating effect on the endometrium and ovarian tissue and has been used traditionally for gynecological disorders. Different plant parts: bark, leaves and twigs of various aged group trees of *Saraca asoca* were evaluated for their total phenols (TP), total flavonoids (TF), tannins (T), phenolic acids contents and radical scavenging activity. TP varied from 5.27 – 8.65%, TF from 0.16 – 0.28%, T from 20.88 – 51.17%. This is the first study in which different phenolic acids were estimated in *Saraca asoca*; vanillic acid varied from 2.34 – 5.07%, caffeic acid from 1.37 – 7.15%, chlorogenic acid from 8.51 – 25.59%, gallic acid from 0.17 – 0.46% and catechin from 4.78 – 7.95 mg/100 g. Radical scavenging activity showed significant variation among different girth classes and IC_{50} values ranged between 2.29 – 4.82 mg/ml. Bark was found to contain maximum concentration of active ingredients. The results revealed that the optimum girth class to obtain quality produce is 61 – 90 cm as it contains maximum concentration of active ingredients and possess high radical scavenging activity. Thus it can be used for making various formulations containing natural antioxidants. **Keywords:** Antioxidants, phenolic acids, harvesting age, Saraca asoca **Acknowledgement:** *The authors are thankfull to the Director, Tropical Forest Research Institute for providing neccessary facilities to carry out the research work. The work was supported by a grant from the National Medicinal Plant Board (NMPB), Govt. of India, New Delhi.*

PM36 Decrement of body fat and hypolipidemic effect of 3,4,5-trihydroxybenzaldehyde isolated from *Geum japonicum* in high fat diet-induced obese rats

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3,4,5-trihydroxybenzaldehyde(THBA) is isolated from the aerial part of *G. japonicum* Thunb., a Korean herb belonging to the family of Rosaceae [1]. In a recent study we have shown that the ethyl acetate fraction of *G. japonicum* inhibited NO production by LPS-activated RAW 264.7 cells and the natural THBA showed a remarkable scavenging activity on the DPPH radical[2]. Being on these findings, we determined the preventive effect of 3,4,5-trihydoxybenzaldehyde(THBA) for adiposity and dyslipidemia using high fat diet-induced obese rats. As a result of the investigation for lipid and leptin metabolism in obese rats, body weight, adipocyte cell size and visceral fat mass was significantly reduced by feeding with THBA. The concentration of triglyceride and leptin in serum was also significantly reduced whereas HDL-cholesterol level in serum was significantly increased. These results suggest that THBA isolated from *G. japonicum* is beneficial for the suppression of diet-induced obesity and hyperlipidemia. **Keywords:** Geum japonicum, 3, 4, 5-trihydroxybenzaldehyde, hyperlipidemia, antioxidant **References:** 1. Kim J et al. (2006)J Food Drug Analy14(2): 190 – 193. 2. Kang S et al. (2006) Nutritional Science 9(2): 117 – 123.

PM37 Cytotoxicity activities of *Dicranopteris linearis* extracts and fractions

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Cancer is currently a second leading cause of death in the world [1]. Therefore the newer anticancer drugs from natural product are needed to replace the synthetic drug from the chemical compound. Based on the traditional medicinal value, *Dicranopteris linearis* (Burm.f.) Underw. from the Gleicheniaceae family was shown to possess pharmacological potential such as cytotoxic activity. For initial investigations, the dried leaves of *D. linearis* were extracted with aqueous, chloroform and methanol. The extracts were tested against HL 60 (acute promyelocytic leukemia cell lines) and WRL 68 (normal liver cell line) using MTT assays. The methanol extract (Table 1) showed the promising cytotoxic activity against HL 60 (IC_{50} = 7.9 µg/ml). The methanol extract was further partitioned in sequence with n-hexane, chloroform and methanol to determine which extract contain the most active constituents. The result showed the methanol fraction to be significantly active against HL 60 with the value IC_{50}= 12.88 µg/ml and non toxic to normal cell. The methanol fraction was then subjected to the bioassay-guided fractionation. The fractionation by vacuum liquid chromatography (VLC) gave eleven fractions labeled as F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 and F11. The fraction F7 (Table 2) demonstrated cytotoxic activity with the best value (IC_{50} = 25.12 µg/ml). In addition, it was also found to be non-toxic against normal cell. Further work involving the isolation of active compounds in this potent ferns would be necessary to elucidate the actual source of the observed bioactivities. **Keywords:** *Dicranopteris linearis*, Gleicheniaceae, MTT assays, acute promyelocytic leukemia cell lines, normal liver cell line **Acknowledgement:** *This study was supported by the research grant (02 – 01 – 01-SF0182) from the Ministry of Science, Technology and Innovation, Malaysia.* **References:** [1]. Pratt WB, Ruddon RW, Ensminger WD, Maybaum J (1994) The Anticancer Drugs. 2 Eds. Oxford University Press: New York, 3 – 16.

PM38 Antinociceptive activity of *Muntingia calabura* leaves

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The aims of the present study were to develop the *in vivo* antinociceptive profile of methanol crude extracts of *Muntingia calabura* L. leaves and it's fractions. The antinociceptive activity of orally-administered test solutions was assayed using the formalin test in rats. Based on the data obtained, the methanol extract of *M. calabura* (MEMC) exhibited the most effective ($P < 0.05$) antinociception in the 1st and 2nd phases of the assay in a dose-dependent manner followed by the aqueous and chloroform extracts. The MEMC was then partitioned with petroleum ether (PEP) followed by ethyl acetate (EAP) and the remaining residue was dissolved in distilled water (AQP). Following subjection to the formalin test, the PEP (100, 500 and 1000 mg/kg) exerted the most effective antinociception ($P < 0.05$) in both phases of the assay in a dose-dependent manner followed by the EAP and AQP. The PEP was subjected to the fractionation processes and yielded 7 types of fractions labelled as FA, FB, FC, FD, FE, FF and FG. All fractions, in the dose of 300 mg/kg, were subjected to the assay and only fractions FC, FD, and FF demonstrated significant ($P < 0.05$) antinocicpetion at least in the 2nd phase of the formalin test. In conclusion, the antinociceptive activity of *M. calabura* involved modulation of central and peripheral pain mechanisms and attributed to the presence of flavonoids. **Keywords:** Muntingia calabura; Elaeocarpaceae; Antinociceptive activity; Formalin test; Flavonoids **Acknowledgement:** *The authors would like to thank Universiti Putra Malaysia for providing the Research University Grant Scheme (RUGS; Reference no: 04 – 02 – 10 – 0925RU) and Ministry of Science, Technology and Innovation (MOSTI) for granting the eScience Fund* **References:** 1. Zakaria ZA et al. (2006) Fundamental and Clinical Pharmacology 20(4): 365 – 372. 2. Zakaria ZA et al. (2007) Journal of Natural Medicines 61(4): 443 – 448. 3. Zakaria ZA et al. (2011) American Journal of Chinese Medicine 39(1): 183 – 200.

PM39 Neural mechanisms and sedative effect of different fractions of *Holarrhena floribunda* (Apocynaceae) stem bark in mice

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Holarrhena floribunda G. Don (Apocynaceae) stem bark is used locally in the treatment of mental illness in Nigeria.This work examined the neural mechanism and the sedative properties of the crude extract and its fractions. Acute toxicity studies were carried out on the crude extract and fractions by oral and intraperitoneal administration using Lorkes (1983) method. The effect of the crude extract of *Holarrhena floribunda* and the fractions; hexane, chloroform, ethylacetate, butanol and aqueous was examined on novelty-induced rearing and grooming behaviors and on pentobarbital-induced sleeping time in mice, The results shows that the crude extract and the fractions were not toxic with the exception of butanol fraction. The crude extract and fractions reduced novelty-induced rearing and grooming behavior in mice. The inhibitory effect of the crude extract and the fractions were not reversed by atropine, cyproheptadine, yohimbine and naloxone; however the crude extract and the fractions blocked the facilitating effect of flumazenil. This suggests that the crude extract and the fractions appear to facilitate GABA-ergic transmission. The crude extract, aqueous, butanol and chloroform fractions prolonged pentobarbital-induced sleeping time in mice which were blocked by flumazenil a GABAA antagonist, indicating that the crude extracts, aqueous, butanol and chloroform fractions contain GABAA agonist. The result suggests that the stem bark of *Holarrhena floribunda* possess sedative effects which may be mediated through GABA-ergic neurotransmission. **Keywords:** Holarrhena floribunda, Rearing, Grooming, Sedative, GABA **References:** Lorke D (1983) Arch Toxicol 54: 275 – 287.

PM40 Studies of analgesic activity of the different fractions of *Holarrhena floribunda* stem bark in mice

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The antinociceptive effect of crude extract of *Holarrhena floribunda* T.Durand & Schinz together with its fractions prepared with hexane, chloroform, ethylacetate, butanol and aqueous were investigated using hot plate, tail immersion and acetic acid-induced abdominal constriction tests in mice. Acute toxicity studies were carried out on the crude extract and fractions by oral and intraperitoneal administration using Lorkes (1983) method. The crude extract was prepared by soaking the stem bark in 70% ethanol for 72 hours. It was filtered and evaporated using rota vapour. The dry crude extract was dissolved in distilled water followed by butanol, ethylacetate, hexane and chloroform in a separating funnel to obtained the fractions. The result shows that the crude extract and the fractions were not toxic with the exception of butanol fraction. The crude extract and the fractions shows dose independent significant increase in latency period in the hot plate and tail immersion tests. They also reduced the abdominal constriction induced by acetic acid in mice. The analgesic effect of the extract and fractions were reversed by naloxone an opioid receptor antagonist in the hot plate and tail immersion test; while naloxone did not reverse the inhibitory effect of the extract and fractions on the acetic acid-induced abdominal constriction test. The results show that the central analgesic effect of the crude extract and fraction is mediated through opioid receptor in the brain. The peripheral analgesic effect of the extract and fractions is not mediated through opioid receptors. **Keywords:** Analgesia, Holarrhena floribunda, hot plate, tail immersion, writhing, opioid **References:** Lorke D (1983) Arch Toxicol 54: 275 – 287.

PM41 Hepatoprotective effects of *Artemisia monosperma* and silymarin on carbon tetrachloride-induced hepatic damage in rat

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The hepatoprotective effect of aqueous ethanol extract of *Artemisia monosperma* Delile aerial parts was investigated against carbon tetrachloride-induced acute hepatotoxicity in rat. The hepatoprotective activity of *A. monosperma* was evaluated by determination of liver enzymes marker in the serum (aspartat amino transferase AST; serum alanine transaminase ALT and alkaline phosphatase ALP). The histopathological studies were also carried out to support the above parameters. Oral administration of *A. monosperma* (100 and 200 mg/kg) markedly reduced the elevated values of marker enzymes caused by CCl_4 – treatment. Glutathione (GSH) significantly decreases in the group treated with CCl_4. The two doses of *A. monosperma* and silymarin (25 mg/kg) significantly increased GSH values when given in combination with CCl_4. However, silymarin normalized liver enzymes and increased GSH levels than *A. monosperma* (two doses) when compared with the control group. A comparative histopathological study of liver of rat treated with *A. monosperma* exhibited almost normal architecture, compared to CCl_4-treated group. Image analysis of liver revealed a marked reduction damage area after treatment with *A. monosperma* (100 or 200 mg/kg) and silymarin compared with CCl_4-treated group. Phytochemical study of *A. monosprema* resulted in the isolation of a quercetin 3-O-β-glucopyranoside; quercetin 5-O-β-glucopyranoside; isorhamnetin 3-O-β-glucopyranoside; 5, 4' – dihydroxy 6, 7-dimethoxy flavone; 5, 3' – dihydroxy 6, 7, 4'- trimethoxy flavone; 5, 7, 3' – trihydroxy 3, 6, 4' – trimethoxy flavone; quercetin and isorhamnetin. Hepatoprotective effect of *A. monosperma* is probably due to combined effect of flavonoids. **Keywords:** Artemisia

monosperma, Carbon tetrachloride, Hepatoprotective activity, Flavonoids

PM42 Kaerophyllin Suppresses Hepatic Stellate Cell Activation by Apoptotic Bodies and Ameliorates Hepatic Fibrosis in Rats

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Hepatocyte apoptosis is a central feature of many liver diseases, leading to liver inflammation and fibrosis. In this study, we screened potential drugs inhibiting hepatic stellate cell (HSC) migration induced by hepatocyte apoptotic bodies (ABs) and evaluated the in vivo therapeutic effects in a rat model of hepatic fibrosis induced by thioacetamide (TAA). Rat HSCs were exposed to UV-irradiated hepatocyte ABs or TNF-α to investigate the anti-fibrotic effects of kaerophyllin. Liver fibrosis was induced by TAA injection into rats twice weekly for 6 weeks. Kaerophyllin (10 or 30 mg/kg) or curcumin (150 mg/kg, as a positive control) was given by gavage twice daily for 4 weeks starting 2 weeks after TAA injection. Kaerophyllin (α-(trans-3,4-dimethoxybenzylidene)-β-(3,4-methylenedioxy-benzyl)-γ-butyrolactone, a lignan isolated from a Chinese herb Bupleurum scorzonerifolium by bioactivity-guided fractionation) attenuated ABs- and TNF-α-induced HSC migration, protein levels of collagen I and α-SMA, and the mRNA levels of ICAM-1, MCP-1 and IL-1β genes, but elevated PPAR-γ luciferase activity. Furthermore, kaerophyllin reduced TNF-α- and ABs-induced NF-κB luciferase activity with decreased IκB phosphorylation and p65 nuclear translocation. In TAA rats, kaerophyllin and curcumin treatment significantly protected liver from injury by reducing serum AST and ALT levels, and improved the histological architecture and fibrosis score. In addition, kaerophyllin treatment suppressed α-SMA protein expression, and mRNA levels of collagen I, TIMP-1, TNF-α, IL-1β and MCP-1 genes in TAA rats. Our results demonstrated that kaerophyllin protected the rat liver from TAA-caused injury and fibrogenesis by suppressing hepatic inflammation and inhibiting HSC activation. **Keywords:** hepatic stellate cells, liver fibrosis, kaerophyllin, inflammation, Bupleurum scorzonerifolium **Acknowledgement:** *This work was supported by the National Science Council and the National Research Institute of Chinese Medicine in Taiwan.* **References:** [1] Friedman SL (2008) Gastroenterology 134: 1655 – 1669. [2] Marra F (2002) Front Biosci 7: d1899 – 1914. [3] Canbay A et al. (2003) Hepatology 38: 1188 – 1198.

PM43 Bioassay-guided Isolation of Cytotoxic Fractions from *Muntingia calabura* Leaf

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M. calabura L. or locally known as "Kerukup Siam", belongs to the family Elaeocarpacea [1]. This plant is native to American continent and is widely cultivated in warm areas of Asian region, including Malaysia [2]. The leaf is used to provide relief from gastric ulcers and to reduce swelling of the prostate gland as reported in Peru folklore medicinal uses. The aim of the present study is to determine the in vitro cytotoxic activity of *Muntingia calabura* leaf against cancer (HL60 and MCF-7) and normal (WRL68) cell lines using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Mosmann [3] but with slight modifications. The crude methanolic extract of *M. calabura* (MCME) was suspended in distilled water to afford an aqueous MeOH solution and then partitioned with petroleum ether and EtOAc to give petroleum ether, EtOAc and aqueous extracts. The EtOAc extract showed significant cytotoxic activity when tested against HL60 (IC_{50}= 27.48 ± 3.60 μg/ml) and was further fractionated using vacuum liquid chromatography with gradient mixture of hexane-EtOAc (from 9:1 to 1:9) as solvent systems. Seven fractions were obtained (F1-F7), and subjected to cytotoxic activity against HL60, MCF7 and WRL68 cell lines. The IC_{50} values of the *M. calabura* extracts and fractions are shown in Table 1. Fraction 5 tested against HL60 showed strong inhibition (IC_{50}= 3.98 ± 0.09 μg/ml) as compared to the other cell lines as well as other fractions. Fraction 5 will be isolated further and the bioactive compounds responsible for the activity will be determined in the future study.

Table 1: IC_{50} values of the *M. calabura* extracts and fractions

	HL60 – IC_{50} (μg/ml)	MCF7 -IC_{50} (μg/ml)	WRL68 -IC_{50} (μg/ml)
MCME	30.90 ± 4.73	36.56 ± 5.40	> 100
Partitions:			
Petroleum ether	29.46 ± 3.95	43.07 ± 3.25	68.51 ± 8.71
Ethyl acetate	27.48 ± 3.60	40.72 ± 6.18	76.57 ± 5.32
Aqueous	> 100	> 100	> 100
Fractions			
F1	32.14 ± 1.68	> 100	> 100
F2	35.89 ± 3.69	35.65 ± 4.41	43.93 ± 6.06
F3	84.49 ± 2.26	> 100	37.07 ± 4.66
F4	34.22 ± 5.83	30.80 ± 3.60	33.15 ± 2.04
F5	3.98 ± 0.09	35.47 ± 5.44	32.29 ± 4.23
F6	5.99 ± 0.95	35.27 ± 2.51	40.75 ± 6.16
F7	28.13 ± 3.59	42.16 ± 4.34	36.60 ± 1.29

Keywords: *Muntingia calabura*, Elaeocarpacea, cytotoxic, MTT assay **Acknowledgement:** *The authors wish to thank Faculty of Pharmacy, UiTM Malaysia for financial assistance.* **References:** [1]. Morton, J.F. (1987) Jamaica Cherry. In Fruits of Warm Climates. Miami: J.F. Morton; p. 65 [2]. Chin WY (1989) A guide to the wayside trees of Singapore. BP Singapore Science Centre, pp: 145. [3]. Mosmann T (1983) Journal of Immunol Methods 65: 55 – 63.

PM44 Pharmacokinetics of linalool and linalyl acetate, the two main constituents of silexan, an essential oil from *Lavandula angustifolia* flowers, in rats

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Silexan is the active ingredient in Lasea®, which has recently been approved for the treatment of restlessness and mild anxiety in Germany. The naturally occurring enantiomers R-(-)linalool L and R-(-)linalyl acetate (LA) are the main constituents of silexan representing 70 – 80% of the total oil. We investigated the bioavailability and organ distribution of L and LA in rats by headspace GC-MS after administration of silexan or the single constituents. The peak concentrations of L after 100 mg/kg silexan was 77 ng/ml in plasma, 2287 ng/g in liver, 670 ng/g in kidney, 2085 ng/g in fat and 164 ng/g in brain tissue. LA was only measured in the brain (31 ng/g). Administration of 28,9 mg/kg L which corresponds to the amount contained in 100 mg silexan, resulted in peak concentrations of 33 ng/ml in plasma, 218 ng/g in liver, 541 ng/g in kidney, 1140 ng/g in fat and 43 ng/g in brain tissue. The gavage of 36.8 mg/kg of LA, the equimolar amount to 28.9 mg L resulted in peak L concentrations of 10 ng/ml in plasma, 274 ng/g in liver, 255 ng/g in kidney, 244 ng/g in fat and 0 ng/g in brain tissue. LA itself was only found in the brain and in fat tissue. The results indicate that the bioavailability of L is generally higher when applied as total oil in comparison to the application of the single constituents. Interestingly, LA is very rapidly metabolized into L and can only be detected in the brain and in fat tissue. **Keywords:** Pharmakokinetics, Lavandula, Silexan, Bioavailability

PM45 Antiviral Activity of *Aloe hijazensis* against Some Haemagglutinating Viruses Infection and its Phytoconstituents

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On our ongoing for investigating the bioactive compounds of *A. hijazensis* Lavranos & Collen. (Abd-Alla et al., 2009), one of 24 species of *Aloe* in Saudi Arabia (Collenette, 1999); the flowers and flower-peduncles were selected for the present study. Thirteen compounds were isolated from both flowers and flower-peduncles. The isolated compounds were classified into; five hydroxyquinones; ziganein, Ziganein 5-methyl ether, aloesaponarin I, chrysophanol, aloe-emodin, one dihydroisocoumarin; feralolide, four flavonoids; homoplantaginin, isoorientin, luteolin 7-glu-

curonopyranoside, isovitexin, one phenolic acid; p-coumaric acid, the anthrone; aloin together with aloenin. Eleven compounds were attributed to the flowers and seven to the flower-peduncles. Homoplantaginin and luteolin 7- glucuronopyranoside are reported here for the first time from *Aloe* spp. Evaluation of the antiviral activities of flowers, flower-peduncles, leaves, and roots of *A. hijazensis* against haemagglutinating viruses of avian paramyxovirus type-1, influenza virus type A, Newcastle disease virus, and group III adenovirus; egg-drop syndrome virus in specific pathogen free chicken embryos were carried out. In general, the flowers and leaves showed the highest antiviral effect. This is the first report on the isolation of phytoconstituents from this plant parts and also the first time for its biological evaluation. **Keywords:** *Aloe hijazensis*, Phytochemical Constituents, Haemagglutinating Viruses **Acknowledgement:** *Department of Botany, King Abdul-Aziz University, Jeddah, Saudi Arabia, Dr. Farag Abd-Allah Elghamdi.* **References:** 1. Abd-Alla HI et al. (2009) Nat Prod Res 23: 1035 – 1049. 2. Collenette S (1999) Wild Flowers of Saudi Arabia. National Commission for Wild Life Conservation and Development. Riyadh.

PM46 The use of a new phytodrug Suttigen in gynaecology for treatment of inflammatory processes

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The aim of the research is an assessment according to the microbiological parameters of 3% suttigen ointment on the basis of Suttigen substance from a grass of *Euphorbia soongarica* Boiss., as vaginal swabs. In 185 female patients with inflammatory processes of the pelvic organs suttigen ointment was applied. The levomekol ointment (55 patients) and 10% the metiluratsil ointment (43 patients) was applied in the control groups. Along with a local therapy there was a general treatment with antibiotics, desensibilization and detoxification drugs. During the treatment, a quantitative microbiological examination of smears from the cervical canal, urethra and vagina was carried out in women with inflammatory conditions of the pelvic organs. The intake of material was within the time of patients admission and continued in the dynamics for 2 – 3 days. Before the treatment, semination wounds of *Staphylococcus aureus* were 105 · 106 microbial bodies in genital habitats. On the 5th and the 6th days of the therapy with the use of this ointment was a completed resulting in a complete clearance of the genital habitat of *Staphylococcus aureus*. The content of *Staphylococcus aureus* in genital swabs in the control groups on the first day there were 105 · 106 microbial bodies, while on the third day 105 and on the fifth day – 103. In comparison with the ointments of levomekol and 10% metiluratsil, 3% suttigen ointment reduced the duration of a genital cleansing of biotopes from microflora to more than 1.3 – 1.5 times and improved therapy.

PM47 Mathematical model for Glucose-Insulin interactions after administration of the *Arctium lappa* extract in diabetic rats

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Diabetes is a widespread chronic disease which is increasing at an alarming rate in the world. It can lead to a variety of vascular, neurological or metabolic complications. Maintaining blood glucose levels within the normal range by exogenous insulin administration or oral administration of plant extracts which increase plasma insulin levels can decrease these effects. Mathematical models have provided one means of understanding diabetes dynamics. In this study we used one of these models "minimal model" (1) based on our experimental data for estimation of plasma insulin from plasma glucose. We used a modified Michelis Menten equations (2) in our model. Diabetes was induced by intraperitoneal injection of sterptozotocin (80 mg/kg). After 24 hours of food deprivation, blood samples were collected from the orbital sinus before, and at 1, 2, 3 hours after oral administration of *Arctium lappa* L. extract. Blood glucose and insulin level were determined by glucose oxidase and standard radioimmunoassay methods, respectively. In diabetic rats, plant extract increased blood insulin levels ($p < 0.05$) and decreased blood glucose levels ($p < 0.01$). Results showed the above model can predict plasma insulin level from plasma glucose value. **Keywords:** diabetes, Arctium lappa, insulin-glucose **References:** (1) Bergman RN, Cobelli C (1980) Federation Proc 39: 110 – 115. (2) Lin J (2007) Robust modeling of the glucose-insulin system for tight glycemic control of critical care patients, Ph.D. Thesis, Department of Mechanical Engineering, University of Canterbury, New Zealand,

PM48 Evaluation of acute toxicity of betulin

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Betulin is a pentacyclic triterpene alcohol belonging to the lupane series of compounds. It is extracted from the outer birch bark. The birch triterpenes have known antiallergic, antiviral, antimicrobial, antitumor and hepatoprotective effects [1 – 3]. Although to our knowledge no reports about acute toxicity of betulin were published. Acute toxicity of betulin was studied on rats and mice. Betulin (98%) was provided by VTT. Male and female outbreed rats and mice were administered with betulin in single dose of 1000 – 16000 mg/kg intragastric and rats in single dose of 250 – 4000 mg/kg intraperitoneally. No significant effect of betulin administration on body weight of animals and no lethal effect were observed during 14 days after administration in rats and mice in all doses tested. LD 50 was not reached in all experiments. Skin irritation, edema or infiltration at the injection site after intraperitoneal injection was not observed. Irritation of the gastrointestinal tract of rats and mice, and peritoneum in rats at the site of drugs injection has been not fixed. The form and location of all internal organs were without pathology booth in rats and mice and there were no significant changes in mass coefficients of organs. In result of single intragastric administration or intraperitoneal injection of betulin in rats and mice no toxic effects were observed. Results of 14 days of observation of animals and data of necropsy evidenced about safety of this substance. Betulin is non-toxic and may be classified as substance of VI class toxicity [4]. **Keywords:** betulin, intragastric administration, intraperitoneal injection, mice, rats **Acknowledgement:** *the study was done in frame of FORESTSPECS project, grant agreement 227239* **References:** 1. Alakurtti S et al. (2006) Eur J Pharm Sci 29: 1 – 13 2. Krasutsky PA (2006) Nat Prod Rep 23: 919 – 942 3. Shikov AN et al. (2011) Phytomedicine doi:10.1016/j.phymed.2011.01.021; 4. Gosselin RE et al. (1976) Clinical Toxicology of Commercial Products. Acute Poisoning, 4th edition. Baltimore: Williams and Wilkins.

PM49 *Trigona laeviceps* propolis: Chemical compositions and antiproliferative activity on cancer cell lines

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Cancer is a leading cause of death worldwide and continue rising. Many cancer patients resisting to recent chemotherapeutic agents, so it is very important to search for new compounds with antitumor activity and develop to be anticancer drugs. Propolis of stingless bee (*Trigona laeviceps*) is focused in this research. It was extracted by 95% ethanol and partitioned through their polarities with 40% MeOH, CH_2Cl_2 and hexane. All parts were tested for antitumor activities against five tumor cell lines (Chago, KATO-III, SW620, BT474 and Hep-G2) by MTT assay. In addition, the cytotoxicity against two normal cell lines (Fibroblast and CH-liver) was performed by the same assay. Due to IC_{50} value, the hexane part had the highest antiproliferative activity and the lowest cytotoxicity on normal cell lines. The hexane part was therefore purified next by quick column chromatography. Eight fractions (100HEX, 10DCM, 30DCM, 50DCM, 70DCM, 100DCM, 5MET and 10MET) were obtained. After MTT assay, the most effective fractions were 30DCM and 100DCM. Both were separately purified by size exclusion chromatography, yielding totally 8 fractions (30DCM-F1, 30DCM-F2, 30DCM-F3, 30DCM-F4, 100DCM-F1,

100DCM-F2, 100DCM-F3 and 100DCM-F4). After MTT assay, 30DCM-F3 fraction was the most potential one. An attempt to identify active compounds, using spectroscopic techniques is under investigation. In conclusion, it presents that *T. laeviceps* propolis prone to have active compounds which are not only effective in antiproliferation on cancer cell lines, but also nontoxic to normal cell lines. In the future, the expected active compound would be a template for anticancer-drug developing program. **Keywords:** *Trigona laeviceps* propolis, Bioassay-guided isolation, MTT assay, Chromatography, Spectroscopy **Acknowledgement:** *We thank the National Research Council of Thailand, the Asahi Glass Foundation, the Thai Government Stimulus Package 2 (TKK2555), under the Project for Establishment of Comprehensive Center for Innovative Food, Health Products and Agriculture, the Ratchadapisek Somphot Endowment Fund (AG001B), and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission for financial supports.*

PM50 Ascorbic acid content, phenolic compounds and antioxidant capacity of Brazilian exotic fruits açai (*Euterpe oleraceae* Mart.) and cupuaçu (*Theobroma grandiflorum* Schum.)

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It is well-known that diets high in fruits and vegetables may decrease the risk of chronic diseases, due to their low fat content and high levels of fiber and antioxidant substances, such as ascorbic acid and polyphenols. Current work describes the characterization of two Brazilian exotic fruits namely açai (*Euterpe oleraceae* Mart.) and cupuaçu (*Theobroma grandiflorum* Schum.), for their: antioxidant capacity; ascorbic acid content; and total polyphenolic compounds. Antioxidant capacity was determined in pulps by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Ascorbic acid was quantified by ultra-fast liquid chromatography using a Shimadzu™, UFLC-20A chromatograph with a reversed-phase octadecylsilane column XR-ODS™, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Polyphenolic compounds were determined by the Folin-Ciocalteau method. Antioxidant capacity, expressed in terms of grams of pulp per 100 g of DPPH, was 1666.76 for the açai fruit, and 4366.71 for the cupuaçu fruit. Ascorbic acid was not detected in açai pulp, and its content was 7.04 mg per 100 g of pulp in cupuaçu pulp. Total phenolic compounds content, was 108.5 expressed in terms of galic acid equivalent per 100 grams of pulp for the açai and 91.85 for the cupuaçu pulp. Results pointed out the nutritional and therapeutic potentialities of these exotic fruits, for their antioxidants properties. **Keywords:** food phenolics, data base, liquid chromatography, nutritional properties **Acknowledgement:** *We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the fourth and fifth co-authors also thank CAPES for fellowships*

PM51 Chemical Constituents and pharmacological activities of *Zilla spinosa*

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Zilla spinosa T.Durand & Schinz is very wildely distributed in the Egyptian deserts, it is used by the natives for the treatment of the kidney stones. The present study deals with the isolation and identification of chemical constituents of the aerial parts of *Zilla spinosa* and evaluation of pharmacological activities of its extract. Chemical investigation of the n-hexane and methanolic extract of the aerial parts of *Zilla spinosa* lead to the isolation of campesterol, spinasterol, β-Sitosterol, α-amyrine, β-amyrine, squalene as well as quercetin 3-O-α-rhamnopyranoside (6→1) β- glucopyranoside, kaempferol 3-O-α-rhamnopyranoside(6→1) β-glucopyranoside, quercetin 3-O- β- glucopyranosid, kaempferol 3-O- β-glucopyranoside, quercetin, kaempferol. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D 1 H/ 13C NMR spectroscopy.The methanolic extract showed potent analgesic, anti-inflammatory and hepatoprotective activities. **Keywords:** Zilla spinosa, Flavonoids, hepatoprotective, analgesic, anti-inflammatory

PM52 Phenolic metabolites from *Acacia nilotica* flowers and evaluation of its free radical scavenging activity

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Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to phenolic compounds, such as simple phenolic acids, flavonoids, isoflavonoids, hydrolyzable tannins and condensed tannins. The present study deals with the isolation and identification of the phenolic metabolites from *Acacia nilotica* (L.) Delile (Leguminosae) flowers and evaluation of its free radical scavenging activity. The aqueous alcoholic extract (MeOH: H_2O, 8: 2) of *Acacia nilotica* flowers was subjected to extensive repeated column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in catechin, catechin 7-O- gallate, gallic acid, naringenin 7-O-β- glucopyranoside, quercetin 3-O- β- glucoside (2→1) glucopyranoside, quercetin 3-O- β-glucopyranoside, chalconaringenin 4'-O-β-glucopyranoside, naringenin and quercetin. The structure of the isolated compounds was elucidated on the basis of spectral analysis (UV, HRESI, 1/2D NMR). The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The total polyphenols showed excellent antioxidant potency when tested by radical scavenging methods. **Keywords:** Acacia nilotica, Phenolic compounds, antioxidant activity, DPPH

PM53 *In vivo* analgesic activity of *Salvia wiedemannii* Boiss. used in Turkish Folk Medicine

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Salvia species belongs to the Lamiaceae are widely distributed in Turkey, 50% of the 89 *Salvia* species is endemic (1). Various parts of some *Salvia* species have been reported to have traditional uses (2,3). The common indications include GIT symptoms/disorders (colic, diarrhea, indigestion, and abdominal pain), respiratory tract symptoms/disorders (colds, sore throat, and cough), infections (tuberculosis, bacterial infections, influenza, and parasitic infections), pain (headache and arthralgia), and miscellaneous disorders (diabetes mellitus, liver diseases, barrenness, urticaria, and hemorrhage). In this study the analgesic activity of ethanol, butanol, chloroform and water extracts of *S. wiedemannii* Boiss. has been evaluated by using tail flick and acetic acid- induced writhing tests. The chloroform extract (500 mg/kg, *i.p.*) obtained from *S. wiedemannii* showed significant analgesic activity on tail flick assay, its efficacy was very close to morphine. The water, ethanol and butanol extracts showed analgesic activities similar to that observed with aspirin. Chloroform extract (500 mg/kg, *i.p.*) also inhibited number of writhings induced by acetic acid. Chloroform extract provided analgesic effects similar to morphine. Its effect was quick and durable. This *in vivo* study demonstrates that *S. wiedemannii* has strong analgesic effect in accordance with the public belief. **Keywords:** *Salvia wiedemannii*, analgesic activity, folk medicine **References:** 1. Davis PH (1982) Flora of Turkey and the East Aegean Islands. Vol. 4, Edinburgh. 2. Honda G et al. (1996) J Ethnopharmacol 53: 75 – 87. 3. Sezik E et al. (2001) J Ethnopharmacol 75: 95 – 115.

PM54 Antihyperglycemic and antioxidative potential of *Acacia nilotica* pods in streptozotocin-induced diabetic nephropathy

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Although a wide array of medicinal plants plays a role in the prevention and treatment of diabetes, there are few reports of the application of herbal medicines in amelioration of renal damage. The present study examined the effect of methanolic extract (25 and 50 mg/kg body weight) of *Acacia nilotica* (L.) Delile (Leguminosae) pods in streptozotocin-induced diabetic rats for 30 days, and its biochemical, histopathological and histochemical study in the kidney tissues. Diabetic rats exhibited loss of body weight, hyperglycemia, elevated of serum urea and creatinine. Significant increase in lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) was observed in diabetic kidney. Histopathological examination revealed infiltration of the lymphocytes in the interstitial spaces, glomerular hypertrophy, basement membrane thickening and tubular necrosis with loss of their brush border in some of the proximal convoluted tubules in diabetic rats. Daily oral administration of *Acacia nilotica* extract reversed the adverse effect of diabetes in rats. *Acacia nilotica* extract lowered blood glucose levels, restored serum urea and creatinine and body weight loss. In addition, *Acacia nilotica* extract attenuated the adverse effect of diabetes on LPO, SOD and GSH activity. Treatment with *Acacia nilotica* was found to almost restore the normal histopathological architecture of kidney of streptozotocin-induced diabetic rats and ameliorate mitochondrial succinic deydrogenase. However, glomerular size and damaged area showed ameliorative effect after treatment with the extract. In conclusion, the antioxidant and antihyperglycemic properties of *Acacia nilotica* extract may offer a potential therapeutic source for the treatment of diabetes. **Keywords:** Acacia nilotica, streptozotocin, biochemical, histopathological, antioxidant activity

PM55 Antibacterial activity of *Salvia hydrangea* extract against oral bacteria and comparison with *vancomycin* antibiotic *in vitro*

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The three oral bacteria (members of the normal flora in the mouth) including *Streptococcus mutans*, *Lactobacillus* and *Streptococcus sanguis* play a major role in the production of dental caries so that the first two bacteria accelerate dental caries but the third reduce this process. The purpose of this study was to determine the antibacterial activity of hydro and methanolic extract from *Salvia hydrangea* DC. ex Benth. against the three oral bacteria and comparison with *vancomycin* antibiotic *in vitro.* At First, a sample of hydro and methanolic extract of the *Salvia hydrangea* was prepared and then its antibacterial activity against *S. mutans* (PTCC 1601), *Lactobacillus* (PTCC 1608) and *S. sanguis* (PTCC 1449) was evaluated by well diffusion (with concentration of 100 mg/ml) and agar serial dilution methods for determining of MIC (minimum inhibitory concentration) with dilution of 0.195 to 100 mg/ml. Also, we studied the antibacterial activity of *vancomycin* antibiotic on them by disk diffusion method. The results from the antibacterial tests *Salvia hydrangea* hydro and methanolic extract had not been affected against any of the bacteria. While *S. mutans* was sensitive to *vancomycin* and *Lactobacillus* and *strep. Sanguis* were resistant to it. This study demonstrated that hydro and methanolic extract of *Salvia hydrangea* may not be an effective antibacterial medication in the prevention of dental caries. **Keywords:** *Salvia hydrangea*, hydro and methanolic extract, antibacterial activity, *vancomycin*, oral bacterial

PM56 Anti-proliferative Effect of *Brucea javanica* Fruit Extract Against Human Hepatocarcinoma Cell Lines and Its Mechanism

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The fruits of *Brucea javanica* (Linn.) Merr (Simaroubaceae) are used as herbal remedies for treatment of human amebiasis, as well as for cancer in Chinese folklore. Here, we studied the anti-proliferative activity of the extract from fruit of *B. javanica* (BJE) and the fraction (BJEF5) against Hep G2 human hepatocarcinoma cells, and explored their mechanisms. From these studies, BJE showed growth inhibitory activity effect by the MTT assay in a dose-dependent manner with an IC_{50} value of 1.56 ± 1.02 μg/mL, and by HP20 resin chromatography, the fraction (BJEF5) of elute washing by 50% EtOH showed more potent effect in a dose-dependent manner with an IC_{50} value of 0.44 ± 0.02 μg/mL. However, cell cytotoxic activity was not observed in the peripheral blood mononuclear cell (PBMC) treated with BJE or BJEF5 less than 30 μg/mL. Results from flow cytometry analysis also showed, BJE and BJEF5 induced cell cycle arrest in G1 phase as compared with the control groups, and the β-catenin transcription activity was inhibited in Hep G2 cells when treated with BJE and BJEF5, respectively. Furthermore, western blot analysis indicated that BJE and BJEF5 significantly reduced c-Myc, cyclin D1 protein levels leading to cell cycle arrest, and survivin protein level leading to apoptosis in a dose-dependent manner, respectively. Therefore, the BJE or BJEF5 deserves further exploration for it's use as a potential agent in the therapy for hepatocellular carcinoma (HCC). **Keywords:** antiproliferation, *Brucea javanica*, hepatocarcinoma **Acknowledgement:** *The authors would like to thank the Ministry of Economic Affairs for the financial support of this research under contract No. MOEA 99-EC-17-A-02 – 04 – 0317.*

PM57 Antibacterial effects of two Iranian plant extracts and their synergistic effects on *Staphylococcus aureus* in laboratory medium

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The aim of this work was to evaluate the antimicrobial effects of wild garlic (*Allium hirtifolium* Boiss.) and peppermint (*Mentha piperita* L.) extracts and their combination on *Staphylococcus aureus*. In the present work the antimicrobial effects of the mentioned plant extracts were evaluated using disk diffusion method as a preliminary step and microdilution method. The mentioned extracts were introduced into TSB Broth at ten concentrations from 50% to 0.09%(v/v) in order to determine minimum inhibitory concentration (MIC) for *Staphylococcus aureus* using Bioscreen C device, which is based on optical density measurements. Results indicated that wild garlic and peppermint extracts showed MIC of 3.17%%(v/v) and 12.5%(v/v) respectively for *Staphylococcus aureus*.The antimicrobial activity was enhanced in response to extract mixture than individual extracts of each species, as no growth was observed at the concentrations from 50 to 0.09%(v/v). In conclusion, edible plants can be a potential source for inhibitory substances for some pathogens. Both extracts studied in this research were effective on *Staphylococcus aureus* and the combination of them showed synergistic effect on the inhibition of the growth, so the potential of plant extracts when combined with eachother can be used as a more effective barrier for preservation. **Keywords:** antimicrobial, extract, Staphylococcus aureus, Mint, Wild Garlic

PM58 Simultaneous determination of some phenolic compounds and antioxidant activity of *Inula viscosa* (L.) Aiton

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Inula species are widespread in the world and used traditionally for ages by different cultures due to their various biological activities. The members of this genus contain terpenic compounds, especially sesquiterpene lactones, flavonoids, glycolipids and anthranilic acid derivatives (1,3). *I. viscosa*, suffrutescent and rank-smelling herb up to 1 – 2 m, is widespread in Mediterranean area (4). In this study, antioxidant activity of freeze dried water, methanol and ethyl acetate extracts of flower, leaf and radix of *I. viscosa* were evaluated via DPPH and ABTS methods. All the extracts showed antioxidant activity in different concentrations. Water extract of *I. viscosa* flower expressed strong antioxidant activity with lower IC_{50} values with both methods compared with the other extracts. Ethyl acetate extracts of the investigated parts of the plant showed less antioxidant activity compared with the water and methanol extracts. It's obvious that phenolics are responsible for the antioxidant potential of the plants. For this reason, phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempherol were analyzed qualitatively and quantitatively in the flower, leaf and radix methanol extracts of *I. viscosa* by RP-HPLC. Chlorogenic and caffeic acids were found in all the investigated parts of the plant. Only myricetin was absent in the flower extract and chlorogenic acid was found in significant amount in radix extract. While myricetin was not determined in the plant, kaempherol was found only in the flower extract. Therefore, most of the investigated phenolics could be responsible for the potent antioxidant activity of *I. viscosa* **Keywords:** *Inula*, Antioxidant activity, DPPH, ABTS, RP-HPLC **References:** 1. Zhao Y-M et al. (2006) Chem Biodivers 3: 371 – 384. 2. Danino O et al. (2009) Food Res Int 42: 1273 – 1280. 3. Shan J-J et al. (2006) Biol Pharm Bull 29(3): 455 – 459. 4. Davis PH (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh.

PM59 Metal chelating, radical-scavenging and anti-lipid peroxidative activities of various extracts from two endemic species belonging to the genus *Prangos* Lindl. (Umbelliferae)

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Medicinal applications have been reported for some *Prangos* species as emollient, carminative [1], antifungal [2], antioxidant [3], antibacterial, cytokine release inhibitor [4], and anti-HIV [5]. This study was undertaken to determine metal chelating, radical scavenging, and anti-lipid peroxidative properties of leaf extracts from two endemic species; *Prangos denticulata* Fisch. & Mey. and *Prangos platychloena* Boiss. ex Tchihat. subsp *engizekensis* H. Duman et M.F. Watson. In addition, the amounts of total phenol compounds were determined.The methanol and the hot water extracts were more effective in all assays than the acetone extracts. *P. denticulata* hot water extract showed the highest radical scavenging ability (IC_{50}= 0.048 ± 0.001 mg/ml). The strongest chelating effect was obtained from the *P. platychloena* subsp. *engizekensis* water extract (IC_{50}= 0.76 ± 0.02 mg/ml). A significant relationship between the antioxidative effects and total phenolic contents were found ($p< 0.05$). Moreover, the hot water extracts showed a notable capacity to suppress lipid peroxidation. This study suggests that two endemic *Prangos* species can potentially be used as a readily accessible source of natural antioxidants. They can be exploited for its use against a number of disorders including cardiovascular diseases and cancer. **Keywords:** *Prangos denticulata*, *Prangos platychloena* subsp. *engizekensis*, endemic plant, radical scavenging, lipid peroxidation, metal chelating, antioxidant, total phenol **References:** 1. Zargari A (1988) Medicinal Plants. Tehran University Publications, Tehran. 2. Ozcan M (1999) Acta Alimentaria 28: 355. 3. Mavi A et al. (2004) Biol & Pharm Bull 27: 702. 4. Tada Y et al. (2002) Phytochemistry 59: 649. 5. Shikishima Y et al. (2001) Chem & Pharm Bull 49: 877.

PM60 Antimicrobial activity and characterization of some phenolic compounds of *Inula peacockiana* (Aitch. & Hemsl.) Krovin

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The genus *Inula* (Asteraceae) has more than one hundred species and is found in Europe, Asia, Africa and mainly in the Mediterranean region. Some of the members of this genus are used as traditional herbal remedies throughout the world due to their antitussive, expectorant, diaphoretic, bactericidal, antiinflammatory, antihepatic, antioxidant and antitumoral properties (1,2). *Inula peacockiana* (Aitch. & Hemsl.) Krovin is a perennial herb up to 2 m and naturally growing in Iranian-Turkey region (3). There are still many *Inula* species which were not studied or received a little attention, and one of these species appears as *I. peacockiana*. In this study, antimicrobial activity of the methanol extracts of flowers, leaves and radix of *I. peacockiana* were determined by agar dilution method against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis*. All parts of the plant exhibited antimicrobial activity against all the investigated bacteria and yeasts. Flower extract was seemed to be more active against Gram positive bacteria and yeasts with lower MIC values. Some phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempherol were investigated qualitatively and quantitatively by RP-HPLC in the methanolic extracts of the plant parts. While chlorogenic and caffeic acids were found in all the investigated parts of *I. peacockiana*, quercetin was found in significant amount in the flower extract. All the investigated compounds were determined in the flower extract in various amounts. Consequently, some of the antimicrobial potential of the plant could be due to the presence of the investigated phenolics. **Keywords:** *Inula*, Antimicrobial activity, Agar dilution method, RP-HPLC **References:** 1. Zhao Y-M et al. (2006) Chem Biodivers 3: 371 – 384. 2. Bai N et al. (2005) J Food Lip 12: 141 – 149. 3. Davis PH (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh.

PM61 Anti-inflammatory effect of peat distillates in animal models

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Until now, the medical uses of peat derivatives have been very limited. One medicinal product made from peat is Torfot, a Soviet Union product used as a stimulator of regeneration processes, as well as non-specific immunomodulator. The aim of present study was to investigate anti-inflammatory effect of peat distillates. Two samples of peat (upper -PD1 and deep -PD2) were collected in October 2010 by Dr. N. Demidova (Northern Research Institute of Forestry, Arkhangelsk, Russia). The peat was distilled with steam. Female rats were injected intravenous with peat distillates in single dose of 0.3, 0.6 and 0.9 ml. Indomethacin was used as positive control. Edema was induced 1 h later by injection of 0.5% carrageenin solution in the planter aponeurosis of the right hind paw. The edema volume was determined using the oncometric method at 3 h after the injection of carrageenin, and inhibition of edema rate was calculated [1]. Analgesic studies was carried out using the hot plate test [2]. Inhibition of edema rate after injection of PD1 in dose 0.6 ml was 55.2% and PD2 in dose 0.3 ml was 50.5%, while after administration of indomethacin it was 17.5%. Analgesic properties of distillates were observed in the hot-plate test at a dose of 0.9 ml. The latency time was increased in 2.4, 1.9 and 1.6 times comparing to control group for indomethacin, PD1 and PD2 respectively. This is the first evaluation of anti-inflammatory effect of distillates of peat which exhibited significant anti-inflammatory and analgesic activity in rats. **Keywords:** upper peat, deep peat, indomethacin, edema, analgesic **Acknowledgement:** *the study was done in frame of FORESTSPECS project, grant agreement 227239.* **References:** 1. Shikov AN et al. (2010) Phytomedicine 17: 463 – 468 2. Shikov AN et al. (2008) J Nat Med 62: 436 – 440.

PM62 Diuretic activity of Olive (*Olea europaea* L.)

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Diuresis is important in the treatment of many diseases ranging from acute cases as renal failure to chronic cases as hypertension. Olive, *Olea europaea* L., is a species of a small evergreen tree in the family Oleaceae, native to the coastal areas of the Mediterranean region. Olive leaves are used as anti-rheumatic, anti-inflammatory, antinociceptive, antipyretic, vasodilatory, hypotensive, diuretic and hypoglycemic agents in traditional medicine. Recently, it has been shown that olive leaf extract (OLE) has calcium channel blocker property. The mechanism of the diuretic activity was studied through determination of saluretic, natriuretic and carbonic anhydrase inhibition indices, in addition to assessing glomerular and tubular functions in lithium clearance experiments. Results showed that the petroleum ether extract and aqueous methanol extract of olive leaves possess efficient diuretic activity. Significant increase in creatinine urinary excretion and saluretic index was noticed on administration of aqueous methanol extract. Phytochemical study of aqueous methanol extract led to isolation of three flavonoid compounds. There structures were determined through spectroscopic assay as luteolin, apigenin, apigenin 7-O-β-D-neohesperopyranoside. **Keywords:** Olea europaea, Diuretic activity, Oleaceae, Flavonoids

PM63 Antiradical Activity and Total Phenolic Contents of Wild and Cultivated Myrtle (*Myrtus communis* L.) Fruits

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Myrtle is a typical Mediterranean ecosystem plant. White fruited types are more common compared to black fruited ones in Turkey. Myrtle fruits and leaves are considered commercial commodity and are sold in different regions of the country for using as antiseptic, disinfectant and hypoglycemic agent. Myrtle leaves are mainly used for myrtle oil production in Turkey (1). Fruits of wild and cultivated plants with both black and white in color were used as plant materials. Total phenolic contents which were expressed as gallic acid equivalents in milligrams per 100 gram of fresh fruit weight (mg GAE/100 g FW) were measured with Folin Ciocalteu method (4) while antioxidant activity (EC50) was assessed by DPPH method (3). Antiradical activity was calculated by formulae of 1/EC50 (2). Total phenolic content and antioxidant activity were higher in wild plants when compared to cultivated ones. Black colored fruits have higher antioxidant activity than white colored ones. Cultivated plants have greater fruits than wild ecotypes. Total phenolic content (GAE/100 g FW) ranged from 506,8 to 527,1 for black colored and from 380,6 to 4l8,1 for white colored myrtle fruits of cultivated and wild plants, respectively. Antiradical activity was highly correlated with total phenolic content of fruits. Antiradical activity (1/EC50) of black colored fruits were higher than white colored ones ranging from 3.17 and 3.09 to 2.99 and 2.67 for wild and cultivated plants, respectively. Black colored myrtle fruits with high antiradical activity and total phenolic content can be a promising fruit for fresh and dried fruit consumption in Turkey. **Keywords:** Myrtle, Fruit, Antiradical activity, Phenol, Myrtus communis **References:** 1. Baytop T (1999) Nobel Tıp Kitapevi, Istanbul 2. Guendez R, et al. (2005) Food Chem 89: 1 – 9. 3. Lafka I et al. (2007) Food Chem 104: 1206 – 1214. 4. Spanos G and Wrolstad RE (1990) J Agric Food Chem 38: 1565 – 1571.

PM64 Anti-inflammatory evidence of a standardized *Passiflora alata* dry extract

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It is well established that aging of human skin is enhanced by continuous inflammatory process triggered by intrinsic and extrinsic factors, such as ultraviolet radiation, pollutants and reactive oxygen species (ROS) (1). Interleukins play a pivotal role in skin inflammation (2). IL-6 contributes to production of matrix metalloproteinases 2, that degrades collagen type IV (3). IL-8 induces the expression of urokinase-type plasminogen activator (uPA) that enhances extracelluar matrix degradation (4). After these statements, the search for cosmetic ingredients showing anti-inflammatory activity is of great value to prevent or reduce the process of skin aging. The objective of this study was to evaluate the *in vitro* anti-inflammatory activity of a *Passiflora alata* Curtis extract, a *Passifloracea* species endemic to Brazil. The extract was standardized in vitexin-2-O"-rhamnoside, the major component responsible for the biological activity. The content of vitexin-2-O"-rhamnoside in the dried extract is of 12%. The anti-inflammatory activity was evaluated by ELISA analysis for IL-6 and IL-8 in an *in vitro* model of human fibroblasts. The inflammatory *stimuli* were either UVB radiation or lipopolysaccharide. The results indicate the use of the presented extract as an anti-aging cosmetic ingredient. **Keywords:** inflammation, *Passiflora alata*, interleukins, IL-6, IL-8, aging, cosmetics **References:** 1. Giacomoni PU and Rein G (2004) Micron 35(3): 179 – 184. 2. Chung HY et al. (2002) Microscopy Research and Technique 59:264 – 272 3. Luckett LR & Gallucci RM (2007) British Journal of Dermatology 156: 1163 – 1171. 4. Han YP, Hughes MW, Nien YD., Garner WL J (2002) Surg Res 106(2):328 – 34.

PM65 Activity of (-) mammea A/BB from the Leaves of *Calophyllum brasiliense* on *Mycobacterium tuberculosis*

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Despite the development of a number of effective treatments over the past half century, tuberculosis remains one of the most destructive bacterial infections in humans. The emergence of multidrug-resistant *Mycobacterium tuberculosis* leads to research of new classes of antimycobacterial agents (1). *Calophyllum brasiliense* Cambess (Clusiaceae) is a tree popularly known as "guanandi" being a rich source of bioactive substances, including coumarins (2). Previous studies of (-) mammea A/BB reported leishmanicidal (3) and anti-HIV (4) activities. This compound is a coumarin-type mammea was purified from a dichloromethane crude extract of *Calophyllum brasiliense* leaves by chromatographic methods. In the current study, we evaluated the citotoxicity and in vitro antimycobacterial activity of the (-) mammea A/BB. The compound was identified by spectroscopic methods and comparison with literature data (5). Antimycobacterial activity determination was carried out using resazurin microtiter assay plate (REMA) to determine the minimum inhibitory concentration (MIC) (6) of (-) Mammea A/BB against M. tuberculosis H37Rv (ATCC 27294). The cytotoxicity assay was carried out by Sulforrhodamine B colorimetric method (7). The cytotoxicity for J774G8 macrophages was compared using the selectivity index (SI). The coumarin (-) mammea A/BB showed significant activity against M. tuberculosis with MIC value of 31,2 µg/mL. The cytotoxicity against J774G8 macrophages showed SI of 0.823. These results provide new perspectives on the development of novel drugs obtained from natural products with anti *M. tuberculosis* activities. **Keywords:** Calophyllum brasiliense, Mycobacterium tuberculosis, antimycobacterial **Acknowledgement:** *The authors are grateful to CNPq for providing a research grant and fellowships* **References:** 1. Luciani F et al. (2009) PNAS 106: 14711 – 14715. 2. Ito C (2002) J Nat Prod 65: 267 – 272. 3. Brenzan MA et al. (2008) Pharmaceutical Biology 46: 380 – 386. 4. Reyes-Chilpa R (2004) Life Sci 75: 1635 – 1647. 5. Brenzan MA et al. (2008) Biomedicine & Pharmacotherapy 62: 651 – 658. 6. Palomino J C et al. (2002) Antimicrob Agents Chemother 46: 2720 – 2722. 7. Papazisis KT (1997) J Immunol Methods 208: 151 – 158.

PM66 Anti-biofilm Activity of Pimarane Diterpenoids Against Anaerobes

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One of the greatest challenges in endodontic treatment is the presence of bacteria as a biofilm, which confers stronger bacterial resistance to antimicrobial compounds [1]. In the present work, the *in vitro* anti-biofilm activity of two natural pimarane-type diterpenes and one semi-synthetic derivative were investigated against nine bacteria responsible for dental root canal infections. The following anaerobic bacteria were evaluated in the present study: *Porphyromonas gingivalis* (ATCC and clinical isolate), *Prevotella nigrescens* (ATCC), *Prevotella intermedia* (clinical isolate), *Prevotella buccae* (clinical isolate), *Bacteroides fragilis* (ATCC), *Actinomyces naeslundii* (ATCC), *Peptostreptococcus micros* (clinical isolate), and *Aggregatibacter actinomycetemcomitans* (ATCC). The diterpenes *ent*-pimara-8(14),15-dien-19-oic acid (**1**), its sodium salt (**2**), and *ent*-8(14),15-pimaradien-3β-ol (**3**) (Figure 1) were used for determination of the minimum biofilm inhibition concentration ($MBIC_{50}$) [2]. $MBIC_{50}$ results varied between 6.25 and 25.0 μg/mL for the studied compounds. All the examined compounds displayed 50% or higher inhibition activity concerning biofilm formation. A maximum value of approximately 20-fold the MIC was attained for *P. gingivalis* (ATCC) [3] in the case of compound **1**, and a minimum value of approximately onefold the MIC was achieved for most of the tested bacteria. The present results suggest that pimarane-type diterpenes are able to inhibit biofilm formation in vitro, and that their structure influence this antimicrobial activity [3]. So this class of diterpenes should be considered in the search for new irrigating substances in the area of endodontic infections treatment. Studies of antibacterial activity linked to biofilm formation versus cytotoxicity of these compounds are being undertaken by our research group.

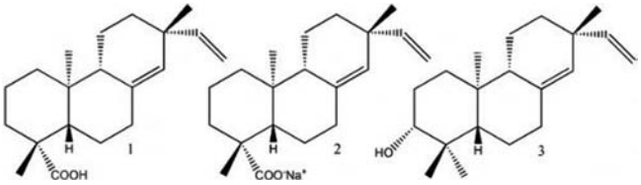

Figure 1: Chemical structure of diterpene type-pimarane

Keywords: Diterpene, Biofilm, Antibacterial activity, Anaerobe **Acknowledgement:** *FAPESP (Proc. 2009/18278 – 0)* **References:** 1. Stewart & Costerton (2001) Lancet 358: 135 – 138. 2. Wei et al (2006) J Antimicrob Chemother 57: 1100 – 1109. 3. Carvalho et al (2011) Molecules 16: 543 – 551.

PM67 Chemical analysis and biological activities of methanol extracts from *Astragalus gombiformis* Pomel (Fabaceae)

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In the present study, wild *Astragalus gombiformis* Pomel extracts were tested for their biological activities and phenolic amounts. Antibacterial activity of this species against various bacteria was tested by the paper disk agar diffusion method and determination of the minimal inhibitor concentration. DPPH and ABTS assays were used to evaluate the antioxidant activity of methanol extracts. These extracts were also chemically investigated by spectrophotometric and HPLC analysis. For DPPH test, inhibitor concentrations 50% were 473.33 ± 64.29 and 626.66 ± 64.29 μg/ml, respectively, for aerial part and roots methanol extracts. Ascorbic acid, used as positive control, showed an inhibitor concentration 50% of 7.36 ± 0.70 μg/ml. ABTS test showed that roots and aerial part extracts contain respectively, 47.13 ± 0.05 and 79.81 ± 1.31 μmoles of Trolox equivalents per g of dry plant material weight. Chemical investigation showed that total polyphenols and flavonoids were three folds higher in aerial part methanolic extracts. The antioxidant potential seems to be correlated to the phenolic contents. Five among the tested extracts exhibited diameter of inhibition zone equal or above 12 mm and with a minimal inhibitor concentration ranging between 233 and 1250 μg/ml. However, no insecticidal effect of aerial part extracts was shown against *Culex pipiens*. It appears that both roots and aerial part of *A. gombiformis* extracts possess antioxidant and antibacterial effects and should be more studied for identification of active compounds. **Keywords:** Astragalus gombiformis, Antibacterial, insecticidal, Antioxidant, phenolic amounts

PM68 Phenolic amounts, antioxidant and antimicrobial potential of *Crithmum maritimum* cultivated in Tunisian arid zones

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Polyphenols are bioactive molecules exhibiting a lot of scientific attention due to their multiple biological activities. The present study aimed at assessing the phenolic content, antimicrobial and antioxidant activities of different organs (leaves, flowers, seeds and stems) of *Crithmum maritimum* L. cultivated in the south east of Tunisia (Medenine). The analyzed organs exhibited different total polyphenol amounts (8,60 ± 2,64 to 24,89 ± 2,44 mg of gallic acid equivalents per g of dry weight), flavonoids (2,04 ± 0,64 to 16,58 ± 0,64 mg of quercetin equivalents per g of dry weight) and tannins (0,532 ± 0,488 to 1,06 ± 0,77 mg of catechin equivalents per gram of dry weight). Evaluation of antioxidant potential showed that seeds extracts displayed the highest DPPH· scavenging ability with the lowest IC_{50} value (406 ± 11 μg/ml). The antimicrobial activity of *C. maritimum* was evaluated by method disc diffusion against different pathogens and expressed as diameter of zone inhibition. Results showed that the bacteria tested responded differently according to each organ. Among tested bacteria, *E. coli* and *P. aeruginosa* are the most sensitive strains to *C. maritimum* extracts. Tested extracts showed also antifungal effect against *Candida albicans*. We can conclude that *C. maritimum* has an antioxidant activity, which is correlated to their phenolic amounts. This species exhibited also antimicrobial effects against several human pathogens. Thus, the identification of responsible bioactive compounds is under progress in our laboratory to reinforce the notion of a potent valorization of such plant extracts. **Keywords:** *Crithmum maritimum*, Phenolic content, Antioxidant, antimicrobial

PM69 Seaweeds: new source of MAO-A inhibiting compounds

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The intense phytochemical study of terrestrial ecosystems, in the past decades, resulted in the identification and characterization of several new bioactive compounds. However, along the years, the scientific community became motivated to change its route of investigation into aquatic systems, which are known to possess a rich and unexplored biodiversity. Therefore, seaweeds are now being exploited by the pharmaceutical industry. Since they have to overcome highly competitive habitats, seaweeds developed some morphological and chemical survival strategies, and these last include the biosynthesis of a wide variety of primary and secondary metabolites that shall be studied in order to assess their bioactivities. In the present study the antidepressant capacity of 11 seaweed belonging to Rhodophyta, Phaeophyta and Chlorophyta phyla was evaluated in vitro, by the inhibition of MAO-A enzyme. Their aqueous

extracts were tested, revealing five very active brown seaweeds: *Cystoseira usneoides* (EC_{50}= 0.084 mg/mL), *C. tamarascifolia* (EC_{50}= 0.073 mg/mL), *C. nodicaulis* (EC_{50}= 0.041 mg/mL), *Stypocaulon scoparium* (EC_{50}= 0.265 mg/mL), and *Fucus spiralis* (EC_{50}= 0.110 mg/mL). After liquid-liquid partitioning, activity was found in the ethyl acetate fractions. These fractions were further analysed by HPLC-DAD, which showed compounds absorbing at 254, 280 and 350 nm. An attempt to identify these compounds by HPLC-NMR is currently in progress. **Keywords:** seaweeds, MAO-A inhibition, HPLC-NMR **Acknowledgement:** *Clara Grosso thanks the Fundação Para a Ciência e a Tecnologia for the Post-Doc fellowship (SFRH/BPD/63922/2009)*

PM70 Diterpenes from *Copaifera langsdorfii* oleoresin against anaerobic oral pathogens

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Anaerobic bacterial infections are the major cause of pulp and periradicular diseases [1] and *Phorphyromonas gingivalis* can be considered as the beginner of these pathological processes. Our research group has demonstrated that some diterpenes isolated from *Copaifera langsdorfii* Desf. oleoresin are able to inhibit the growth of various aerobic cariogenic bacteria with very promising MIC values [2]. In view of these significant results against oral bacteria, this works aimed the investigation of the effect of these compounds on a panel of representative microorganisms responsible for root canal infections using the microdilution broth method [3]. The results indicate that (-)-copalic acid was the most active compound, displaying very promising MIC values against the main pathogen associated with these disease (*P. gingivalis*). The other compounds also displayed some activity against the tested microorganisms. **Keywords:** *Porphyromonas gingivalis*, diterpenes, *Copaifera langsdorfii* **Acknowledgement:** *FAPESP (Proc. 2009/09438 – 3 and Proc. 2009/12796 – 9).* **References:** 1. Gomes BPFA et al. (2004) Oral Microbiol Immun 19: 71 – 76. 2. Souza AB et al. (2010) Phytother Res 25: 215 – 220. 3. NCCLS (2007) Methods for antimicrobial susceptibility testing of anaerobic bacteria, approved standard.

PM71 Observations with rapid micro-colony assay to screen antifungal activity of *Origanum vulgare* L., *Zostera marina* L. and *Centaurea ensiformis* P.H. Davis extracts

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Currently, synthetic antifungal drugs are the main option to treat fungal pathogens e. g. *Candida albicans* C.P. Robin in humans. In turn, fungal pathogens generate resistance upon clinical treatments. Moreoever, human pathogen *Aspergillus fumigatus* Fresenius isolates were suggested to generate resistance to azole antifungal drugs due to the exposure to control plant fungal diseases in the field conditions. Thus, antifungal agents derived from natural products have vital importance for sustainable control of fungal infections. Plants accumulate plethora of antimicrobial compounds e. g. alkaloids, iridoids, flavonoids and lignans which could target the different sites *e.g.* cell wall formation and protein biosynthesis in fungi. Alternative antifungal agent screening methods should be assayed for faster and sound detection. Previously, the micro-colony method i. e. the measurement of the early fungal development using microscopy was developed to screen dose response in the filamentous fungal species *Fusicladium effusum* Winter. The micro-colony assay was tested to detect dose response in *Candida albicans* (ATCC 10231), *A. fumigatus*, *Fusarium oxysporum* Schlecht. emend. Snyder&-Hansen on *Origanum vulgare* L. (Lamiaceae), *Zostera marina* L. (Zosteraceae) and *Centaurea ensiformis* P.H. Davis (Asteraceae) extracts and reference fungicide flucanozole. The *O. vulgare* essential oil was toxic in all testing concentrations, but *Z. marina* methanol extracts were ineffective. *C. ensiformis* methanol extract showed slight growth inhibition on *C. albicans* and *A. fumigatus*. This method provides a dose response in 24 hours. Additionally, method could be used to evaluate topical treatments, pigmentation and filament morphology in fungi. **Keywords:** Antifungal drugs, natural products, *Candida albicans*, *Aspergillus fumigatus*, *Fusarium oxysporum*, dose response in fungi, rapid detection, *Centaurea spp.*, *Centaurea ensiformis*, *Zostera marina* **Acknowledgement:** *Authors thank Assoc. Prof. Dr. Sibel Konyalıoğlu for the utilization of the microscopy facility.*

PM72 Can it be possible to use *Caulerpa* species for the treatment of some diseases?

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Many algae species have important secretions which are used for defensive purposes. These secretions generally have significant potential in terms of pharmaceutical industry. Among these secretions caulerpenyne (CYN) which is the main metabolite of *Caulerpa* species may have an important position in medical investigations as a result of its determined properties such as cytotoxic, antiviral, antiproliferative and apoptotic effects (Lemée et al., 1993; Fischel et al., 1995; Galgani et al., 1996; Nicoletti et al., 1999; Barbier et al., 2001; Cavas et al., 2006). In the present study, the inhibitory effects of CYN isolated from *Caulerpa prolifera* (Forsskål) J.V. Lamouroux (Caulerpaceae) on monoacylglycerol lipase and lipoxygenase were investigated. The results of the study presented that purified caulerpenyne inhibited soybean lipoxygenase with an IC_{50} of 5.1 μM. The results of the investigation conducted with monoacylglycerol lipase revealed that the inhibition of this enzyme were well in line with CYN concentration. The IC_{50} value of monoacylglycerol lipase inhibition with CYN was also determined as 98.4 μM. In conclusion, *Caulerpa* species can be a promising material for the treatment of monoacylglycerol lipase and lipoxygenase related diseases. **Keywords:** *Caulerpa* species, caulerpenyne, lipoxygenase, monoacylglycerol lipase, inhibition **Acknowledgement:** *We thank Prof.Dr. Georg Pohnert, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University of Jena for sharing his valuable knowledge about CYN purification with us. The authors are grateful to the Research Foundation of Dokuz Eylül University (Project No: 2008. KB. FEN. 019) for financial support. Sevilay Cengiz thanks to The Scientific and Technological Research Council of Turkey (TÜBİTAK) for the scholarship. TÜBİTAK Project (109T512) is also acknowledged for financial support.* **References:** 1. Barbier P et al. (2001) Life Sci 70: 415 – 429. 2. Cavas L et al. (2006) J Exp Mar Biol Ecol 339: 111 – 119. 3. Fischel JL et al. (1995) Anticancer Res 15: 2155 – 2160. 4. Galgani I et al. (1996) J Biochem Toxicol11: 243 – 250. 5. Lemée R et al. (1993) J Appl Phycol 5: 485 – 493. 6. Nicoletti E et al. (1999) Phytotherapy Res 13: 245 – 247.

PM73 Screening of Indian medicinal plants for their antimicrobial property

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Medicinal plants play a key role in human health care. Over the side effects of allopathic drugs, the medical world move towards to the plant kingdom for the treatment of various ailments[1]. Many people are infected by venereal diseases, where most of them are causing by infectious microbial agents. Indigenous people tend to use several medicinal plants to treat these infectious diseases rather than western medicines. Therefore, the present study was carried out to screened the different herbs to evalutes their antimicobial properties. The dried plant materials *i.e.* flowers, stem, fruit, seeds and leaves of ten medicinal plants (*Aloe vera* (L.) Burm.f., *Alstonia scholaris* (L.) R.Br., *Annona squamosa* L., *Cressa cretica* L., *Mangifera indica* L., *Momordica dioica* Wall., *Pterocarpus marsupium* Roxb., *Rosa centifolia* L., *Thevetia peruviana* K.Schum. and *Zamia furfuracea* L.f.) were extracted with 100% methanol (MeOH). The extracts were evaluated for their antimicrobial properties against Gram-positive (*Bacillus coagulans*), Gram-negative (*Escherichia coli*) bacteria, using Well diffusion method[2]. The presented results offer supporting evidence for effective use of selected plant extracts. It also supports the wealth of nature and shows that most of the plant materials possess antimicrobial property. They have different inhibition zones but gives a basic idea of the uses of these plants as antimicrobial agents. However, more *in vitro* confirmatory tests using other assays and/or *in vivo* tests are required.

Keywords: Medicinal plants, antimicrobial activity, *Bacillus coagulans*, *Escherichia coli* **References:** 1. Brindha D, Saroja S and Jeyanthi GP (2010) J Basic Clin Physiol Pharmacol 21(4): 401 – 413. 2. Baur AW, Kirby WMM, Sherris JC and Truck M (1966) Am J Clin Pathol 45: 493 – 496

PM74 Antibacterial capacity of *Juglans sigillata* green husks

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Juglans sigillata Dode., a fast growing deciduous tree species in the family Juglandaceae, is indigenous to mountain slopes and valleys of Tibet, Yunnan, Sichuan, and Guizhou provinces of southwest China [1, 2]. The green husk, cortex, kernel, nutshell, root and leaf of *J. sigillata* have a long history of being used in folk medicines to treat oxidative, inflammatory, rheumatic and nociceptive diseases, as well as to relief eczema, cancer, kidneys and stomach disorders. In the present study, antibactierial properties of J. sigillata green husk extractives were studied by hole-plate diffusion assay method described by Rios *et al.* [3] against Gram-negative bacteria, including *Salmonella enterica* and *Escherichia coli* and Gram-positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*. Aqueous EtOH (95%, v/v) extracts from green husk were successively partitioned with a serious of polar solvents to get fractions soluble in n-hexane, CH_2Cl_2, EtOAc, n-BuOH and H_2O. Results, expressed by diameter of inhibition zone, revealed that the 95% aqueous EtOH extracts and all resulting soluble fractions from *J. sigillata* green husk revealed moderate or significant antibacterial effects against the four Gram bacteria, which indicated that *J. sigillata* green husk extractives have potential to destroy bacteria or suppress their growth or their ability to reproduce and could be used as excellent antibacterial agents. **Keywords:** antibacterial capacity, green husks, Juglans sigillata, Juglandaceae, hole-plate diffusion assay **Acknowledgement:** *This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010), Foundation for the Development of Science and Technology in Tianjin Universities (No. 20080616), National Natural Science Foundation of China (NSFC, No. 31000279) and Natural Science Foundation of Tianjin City (No. 09JCYBJC 15800).* **References:** 1. Wu ZY, Raven PH (1999) Flora of China, Vol. 4. Science Press. Beijing. 2.Si CL et al. (2009) Planta Med 75: 922 – 922. 3. Rios JL et al. (1998) J Ethnopharmacol 23: 27 – 149.

PM75 Gastroprotective and anti Helicobacter pylori activities of propolis

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Helicobacter pylori (HP) determines the most common human infection overall. Important gastric diseases are associated with HP with percentage of correlation up to 90% (duodenal ulcer and gastric lymphoma). The complexity of the pathogenetic factors of HP has prompted research to identify "phytocomplexes" able to act on different mechanisms of HP pathogenesis [1]. Our group investigated propolis regarding its antibacterial activity and focusing the protection from oxidative stress and massive inflammatory response, key elements of the progression of HP gastric diseases related, scarcely considered in classic antibiotical therapies [2]. We studied propolis marketed in Italy: classic propolis dry extract, hydrodispersible propolis, water-soluble propolis and a new formulation of propolis (75% italian propolis dry extract, 15% green tea catechins, 10% grape seed procyanidins), 50% total polyphenols UV method standardized. The different chemical composition of samples determines different biological activity as evidenced by *in vitro* and in human cells assays [3,4,5]. Propolis water solutions at different concentrations were tested. Results indicated that propolis has high radical scavenging capacity (IC_{50} in DPPH test ranging from 8.2 to 65.4 ?g/mL) and protect cell membranes from oxidative stress. Antioxidant activity was directly proportional to the effectiveness against HP: the new propolis formulation MBC and MIC against $HPcag^+$ and $HPcag^-$ is 0.250 mg/mL. Tests on human PBMC stimulated with LPS showed that all samples (conc. 200 μg/mL) exhibited anti-inflammatory activity respect to LPS group. This research clearly demonstrated that antioxidant properties of propolis preparations and effectively promote gastric protection acting in different steps of HP pathogenesis. **Keywords:** Helicobacter pylori, propolis, anti-inflammatory, antioxidant, gastroprotective **References:** [1] Molnar B, Galamb O, Sipos F, Leiszter K, Tulassay (2010) Z Dig Dis 28(4-5):604-608. [2] Calvino-Fernández M, Parra-Cid T (2010) Rev Esp Enferm Dig 102: 41-50. [3] Biagi M, Miraldi E, Figura N, Giachetti D (2009) Nat Prod Commun 4(2):255-260. [4] Rapta P, Misík V, Stasko A, Vrábel I (1995) Free Radic Biol Med 18 (5):901-908. [5] Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, Mitov I, Krastev Z (2005) J Med Microbiol 54:481-483.

PM76 Role of polyphenolic compounds on biological activity of collagenous materials

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Collagen, a unique connective tissue protein, is extensively used as biocompatible biomaterial in wound healing [1], cosmetics [2] and tissue engineering [3]. Due to its high sensitivity to enzymatic degradation, cross-linking of collagenous materials must be a compulsory step in their fabrication. Chemical cross-linking agents are able to introduce new covalent bonds in collagen structure, but are cytotoxic. The ability of natural plant polyphenolic compounds to stabilize collagen structure while preserving its cytocompatibility it is now established [4]. This study aimed to investigate the interaction of small molecules from plants with collagen and their effect on its biological properties. Three mixtures of collagen with a polyphenolic extract of *Urtica dioica* L., a plant-derived flavonoid (quercetin) and a flavin from milk (riboflavin), respectively, were conditioned as porous materials by freeze-drying technique. A collagen-glutaraldehyde mixture was used as control. Free amino groups present in the mixtures were spectrophotometrically assayed. An *in vitro* experimental model using bacterial collagenase was used to mimic the enzymatic attack on the collagenous materials implanted *in vivo*. The swelling capacity and *in vitro* cytocompatibility tested according to ISO 10993 – 5 on fibroblasts from NCTC cell line were evaluated. Results showed a good correlation between the free amino groups and the biodegradability of each mixture. The values of swelling capacity were at least 70-fold higher than the initial weight. Fibroblast viability and morphology showed a high cytocompatibility of the polyphenol-collagen mixtures. In conclusion, all these tests indicated an improved applicability of these mixtures for tissue wound healing. **Keywords:** polyphenols, collagen, cross-linking, degradation, cytocompatibility, Urtica dioica **Acknowledgement:** *This study was supported by Project PN II 62059.* **References:** 1. Powell HM et al. (2006) Biomaterials 27: 5821 – 5827 2. Helfrich YR et al. (2008) Dermatol Nurs 20: 177 – 183 3. Craciunescu O et al. (2011) Cent Eur J Biol 6: doi: 10.2478/s11535 – 011 – 0012 – 1 4. Chuang TH et al. (2009) Tissue Eng Part A 15: 2837 – 2851

PM77 Effect of Abnormal Balgham Munziq Aqueous Extracts and Ethanol Extracts on Proliferation, Tyrosinase Activity and Melanogenesis of B16 Murine Melanoma

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Abnormal Balgham Munziq (ABMq) is a traditional Uighur medicie herbal preparation commonly used to preventing and treating the vitiligo. To study the mechanism of this preparation its effect on the activity of mushroom tyrosinase, the melanogenesis and proliferation of B16 murine melanoma cell were estimated using non-cell vitro system as well as in culture. The results showed that at concentration of 0.5 – 200 μg, aqueous extracts and ethanol extracts of ABMq significantly activate the mushroom tyrosinase, melanogenesis in the non cells system in vitro. The ethanol extracts strongly activate the tyrosinase activity and increase melanogenesis than aqueous extracts, also show dosage depend manner. The aqueous extracts and ethanol Isolation from ABMq also has

a strong potential to promote the proliferation and melanogenesis of B16 cell, also can promote cell tyrosinase activity. The results of the present study suggest that the putative mechanisms of ASMq in treatment vitiligo may at least involve enhance the proliferation of melanocyte, melanogenesis, the tyrosinase activity of melanocyte. This activity may play an important role in its treatment of vitiligo. **Keywords:** Abnormal Balgham Munziq, mushroom tyrosinase, melanogenesis, B16 murine melanoma cell **References:** Abulimiti A, Yusup A, Upur H (2000) Pharmacology and Clinics of Chiniese Materia Medica16: 34 – 36. Upur H, Yusup A (2003) Xinjiang Science and Technology publishing house, Urumqi, pp. 42 – 53. Xu SY, Bian RL, Chen X (2002) Experimental Methodology of Pharmacology (third version). Peoples' Medical Publishing House, Beijing, pp. 826 – 828. Cheng DQ, Wei D, Wang Q (2000) Chinese Journal of Dermatology 33: 173 – 174.

PM78 Antioxidant Compounds and Antioxidant Activities of the Methanolic Extracts from Cockscome (Celosia cristata L.) Flowers

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Cockscome (*Celosia cristata* L.) is a traditional medicine herb used for treatment of fatigue, atherosclerosis, leucorrhoea and osteoporosis (1). The chemical constituents of this plant include mainly flavonoids (2). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts and solvent fractions from cockscome flowers. To determine the antioxidant compounds in the methanolic extract and solvent fractions, the content of total polyphenol, flavonoid and tannin were measured by spectrophotometric methods. These were evaluated for antioxidative activities by DPPH and ABTS radical scavenging activities. The yield of methanolic extracts, hexane, chloroform, ethyl acetate, butanol, and water fractions of cockscome flowers were 23.33, 10.27, 20.00, 13.63, 17.55 and 38.54%, respectively. The total polyphenol, flavonoids and tannin contents of methanolic extracts on the cockscome flowers were 6.80, 2.34 and 6.23 mg/g extract residue, respectively. The DPPH and ABTS radical scavenging activities of the methanolic extracts on the cockscome flowers were 52.43 and 107.01 mg Trolox equivalent antioxidant capacity per g extract residue, respectively. The results of this study show that notable antioxidant activities in cockscome flowers are considered to have significant health benefits. **Keywords:** Cockscome (*Celosia cristata* L.), polyphenol, flavonoid, antioxidant activity **References:** 1. Wang Y et al. (2010) Fitoterapia 81: 1246. 2. Weng DB et al. (1995) Acta Nutrimenta Sinica 17: 59.

PM79 Antioxidant Compounds and Antioxidant Activities of the Methanolic Extracts from *Lespedeza cuneata* G. Don

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Lespedeza cuneata G. Don is a member of the Pea or Fabaceae family. This invasive plant has spread throughout the eastern Asia. *L. cuneata* is used as a medicinal herb to treat ailments such as skin ulcerations, dysentery, enteritis, and hernias (1). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts and solvent fractions from *L. cuneata*. To determine the antioxidant compounds in the methanolic extract and solvent fractions, the content of total polyphenol, flavonoid and tannin were measured by spectrophotometric methods. These were evaluated for antioxidative activities by DPPH and ABTS radical scavenging activities. The yield of methanolic extracts, hexane, chloroform, ethyl acetate, butanol, and water fractions of *L. cuneata* were 19.52, 3.85, 21.00, 6.73, 7.31 and 61.11%, respectively. The total polyphenol, flavonoids and tannin contents of methanolic extracts on the *L. cuneata* were 12.44, 2.94 and 8.75 mg/g extract residue, respectively. The DPPH and ABTS radical scavenging activities of the methanolic extracts on the *L. cuneata* were 206.15 and 338.64 mg Trolox equivalent antioxidant capacity per g extract residue, respectively. The results of this study show that notable antioxidant activities in *L. cuneata* G. Don are considered to have significant health benefits. **Keywords:** *Lespedeza cuneata* G. Don, polyphenol, flavonoid, antioxidant activity **References:** 1. Altom JV et al. (1992) Weed Technology Journal of the Weed Science Society of America 6: 573.

PM80 Change in Chemical Components and Antioxidant Activity of Adzuki Beans during Germination

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In East Asian countries such as Korea, China, and Japan, adzuki beans are consumed as 'an' or 'ann' (adzuki paste) or boiled and sweetened whole beans, and used in desserts, snacks, and confectionery items (1). Germination is a processing method by which the quality of this cereal can be improved for both digestibility and physiological function (2). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts from adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) sprouts. To determine the antioxidant compounds in the methanolic extract, the content of total polyphenol, flavonoids, and anthocyanins were measured by spectrophotometric methods. These were evaluated for antioxidative activities by ABTS and DPPH radical scavenging activities. The adzuki bean sprouts extracted by separation with buds and grains. The methanolic extracts from adzuki beans sprouts showed generally higher antioxidant compounds and antioxidant activities than the extracts from ungerminated adzuki beans. The total polyphenol, flavonoids, and anthocyanins contents of the ungerminated adzuki beans were 5.90 – 27.45, 8.17 – 27.23 and 2.70 – 7.32 mg/g, respectively. The total polyphenol contents on buds and grains of the germinated adzuki bean were 25.20 – 32.35 and 14.69 – 16.13 mg/g sample, respectively. The ABTS and DPPH radical scavenging activities of the ungerminated adzuki beans were 0.70 – 1.09 and 0.34 – 0.85 mg Trolox equivalent antioxidant capacity (TEAC) per g sample, respectively. The ABTS and DPPH radical scavenging activities on buds and grains of the germinated adzuki bean were 45.43 – 55.96 and 31.74 – 40.30, and 6.07 – 8.81 and 5.21 – 9.39 mg TEAC per g sample, respectively. **Keywords:** Adzuki Beans, germination, polyphenol, antioxidant activity, Vigna angularis **References:** 1. Yousif AM et al. (2003) Lebensmittel-Wissenschaft und-Technologie 36: 601. 2. Yang F et al. (2001) Journal of Food Science and Nutrition 52: 319.

PM81 Anti-inflammatory properties of polyphenols from *Cymbopogon citratus* by inhibition of NF-κB pathway

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Inflammation is associated with several diseases and still exits an urgent need for the development of new and safer anti-inflammatory drugs. Since plant polyphenols are described to possess anti-inflammatory properties through modulation of pro-inflammatory signaling pathways, they could be used as source of a new anti-inflammatory drug. In a previous study using *Cymbopogon citratus* Stapf (Cy), an herb used in traditional medicine, we have demonstrated that polyphenolic compounds (PFs) from Cy have anti-inflammatory properties by inhibiting

lipopolysaccharide (LPS)-triggered nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression [1]. To further understand the underlying molecular mechanisms of PFs activity, the effect of Cy polyphenolic fractions was evaluated on the LPS-induced nuclear factor (NF)-κB pathway in the mouse macrophage cell line Raw 264.7. In Western blot assays, we observed that PFs inhibited the degradation and phosphorylation of inhibitory protein-κB (IκB). Next, the NF-κB transcriptional activity was assessed using cells transiently transfected with a NF-κB-dependent luciferase reporter plasmid. In addition to the interference with LPS-induced NF-κB activation observed in western blot, PFs inhibited the LPS-induced NF-κB transcriptional activity. In summary, these results demonstrate that PFs from *Cymbopogon citratus* inhibited the NF-κB pathway and therefore could be used as a natural anti-inflammatory drug. **Keywords:** Anti-inflammatory, *Cymbopogon citratus*, polyphenols, NF-kB **Acknowledgement:** *Research supported by FCT PhD fellowship SFRH/BD/46281/2008, FCT project PTDC/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096).* **References:** 1. Francisco V et al. (2011) J Ethnopharmacol 133: 818 – 827

PM82 Evaluation of antinitrosative activities of selected plant polyphenols

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The involvement of free radicals as reactive oxygen (ROS) and reactive nitrogen species (RNS), specially their increased production, appears to be a common feature to most human diseases, including cardiovascular disease, neurodegeneration and cancer [1, 2]. The treatment with antioxidant substances and other strategies leading to the reduction of oxidative and nitrosative stress may represent a therapeutic intervention that could reduce the progression of the pathological process. As such, plant polyphenolics have been suggested to play particularly important role to fight against these diseases, by affording protection towards free radical damage in cellular DNA, lipids and proteins[3 – 7]. Our goal was herein to investigate the scavenging capacity of some plant polyphenolic derivatives using different antinitrosative assays at different concentrations (from 0 to 300 μM). In addition, the anti-proliferative activity against different human tumor cell lines was estimated using the MTT and LDH assayes. The ability of eight plant polyphenolic derivatives to react with the biologically relevant reactive nitrogen species, nitric oxide, peroxynitrite and nitrous acid were investigated indirectly by measurement of their ability to inhibit RNS-induced tyrosine nitration *in vitro*. All the investigated plant polyphenolic derivatives were found to be potent reactive nitrogen species scavengers and resulted in a significant inhibition of 3-nitrotyrosine (3-NT) formation in a dose-dependent manner. All the IC_{50}s were being found at the μM level. These results indicate that these compounds may be utilized as promising sources of therapeutics. **Keywords:** reactive nitrogen species (RNS), nitric oxide, peroxynitrite and nitrous acid, inhibition, 3-nitrotyrosine **Acknowledgement:** *This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 260.* **References:** 1. Roberts RA et al. (2010) Toxicology 276: 85 – 94. 2. Lamas S et al. (1998) Trends in Pharmacological Sciences 19: 436 – 438. 3. Stephanie YH et al. (2006) Free Radical Biology & Medicine 40: 323 – 334. 4. Pollard SE et al. (2006) Biochemical and Biophysical Research Communications 350: 960 – 968. 5. Choi JS et al. (2002) Phytotherapy Research16 (3): 232 – 235. 6. Oldreive C, Rice-Evance C (2001) Free Radical Research 35: 215 – 231. 7. Bartsch H et al. (1993) Basic Life Science 61: 27 – 44.

PM83 Adaptogens stimulate molecular chaperon Hsp70 expression in neuroglia cells

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The seventy-kilo Dalton heat shock protein (Hsp70) plays an important role in the deterrence of protein damage during aging and their expression is required for longevity [1]. Recently, we demonstrated that ADAPT-232, a fixed combination of the extracts of three adaptogenic plants – *Rhodiola rosea* L., *Schisandra chinensis* K.Koch and *Eleutherococcus senticosus* Maxim., significantly increases the levels of circulating Hsp70 in the blood of rats [2]. Further, the long term treatment of aged rats with ADAPT-232 diminished or prevented a range of age-related disorders including malfunction of the central nervous system, loss of memory and loss of learning ability [3]. Similarly, ADAPT-232 improves cognitive function and mental performance in humans [4]. In this study, for the first time we demonstrate that ADAPT-232 stimulates the release of the heat shock protein (Hsp72) in isolated neuralgia cells via the upregulation of heat shock factor-1 (HSF-1). Taken together, our data suggests that the stimulation of HSP expression by adptogens is associated with their anti-aging activity. **Keywords:** Adaptogens, Heat Shock Proteins, Neuroglia Cells, ADAPT-232, HSF-1, Hsp70 **Acknowledgement:** *This work was supported in part by the Swedish Herbal Institute; Scott & White Memorial Hospital and Clinic, the Texas A&M Health Science Center College of Medicine, the Central Texas Veterans Health Administration and an Endowment from the Cain Foundation.* **References:** 1. Calderwood et al. (2009) Gerontology 55: 550 – 558. 2. Panossian et al. (2009) Phytomedicine 16: 617 – 622. 3. Makarov et al. (2007) Abstract of International Congress Stress, Budapest, p. 242. 4. Aslanyan et al. (2010) Phytomedicine 17:494 – 499.

PM84 Antifungal activity of supercritical fluid extract obtained from *Calophyllum brasiliense* Cambess

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Calophyllum brasiliense Cambess (Clusiaceae/Guttiferae) is a native Brazilian medicinal plant that is traditionally used in folk medicine for the treatment of several diseases, including infectious pathologies1. Leaves of *C. brasiliense* were extracted with supercritical fluid using CO_2 as solvent (SFE) at 40 and 60 °C and pressures of 109.2 and 244.1 bar. The extracts were tested against clinical isolates from patients' mouths, containing one of the following microorganisms: *Candida tropicalis, Candida albicans* or *Candida glabrata*, by determination of the minimal inhibitory concentration (MIC). The results indicated that both extracts exhibited antifungal activity against *C. glabrata*. The extract obtained by supercritical fluid at 60 °C and 244.1 bar showed better antifungal activity against *C. glabrata*, with MIC = 31.25 μg/ml for nine samples. This extract contained 30% of a mixture of mammea- -type coumarins and the majority compound was identified as (-) mammea A/BB by spectroscopy analyses. We conclude that SFE is an efficient method for obtaining bioactive compounds from plants, and that this method preserved the properties associated with antifungal activity. **Keywords:** Calophyllum brasiliense, coumarins, supercritical fluid, antifungal activity **Acknowledgement:** *The authors are grateful to CNPq for providing a research grant and fellowships* **References:** 1. Reyes-Chilpa R et al. (1997) Chem Ecol 23: 1901.

PM85 Phytochemical and biological characterization of *Agrimonia eupatoria* L.: an approach to structure-activity

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Plant polyphenols are well-known antioxidants, and recent studies have reported that they play an important role in prevention and treatment of

oxidative-related disorders, such as inflammatory diseases and cancer. In this work, a phenol-rich fraction from *Agrimonia eupatoria* dry aerial parts (AePRF) was studied. Results revealed p-coumaric and ellagic acid derivatives, flavonol and flavone glycosides, and monomers and oligomers of flavan-3-ols (proanthocyanidins). Some key features in molecular structure of flavonoids seem to be crucial to anti-inflammatory mechanisms: 4-oxo functional group and C2-C3 double bond at C-ring, 5- and 7-OH on A-ring and also OH functions on B-ring[1]. Polymerization degree of proanthocyanidins plays a significant role in bioactivity, since dimers and higher oligomers are more effective than monomers, in inhibiting NO production. On the other hand, catechol moiety increases antioxidant activity, leading to presume that catechin-type proanthocyanidins are very active[2]. Anti-inflammatory effect was evaluated in LPS-stimulated Raw 264.7 macrophage cell line by measuring the nitric oxide (NO) production through the Griess assay and AePRF cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Moreover, antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Total phenols and total flavonoids were also evaluated, and phenolic compounds were identified by high performance liquid chromatography, coupled to photodiode-array and electrospray ionization mass spectrometry detectors. Anti-inflammatory and antioxidant activities verified, as well as the phenolic profile established corroborate the traditional use of AePRF in inflammatory-related pathologies, since generous amount of the cited compounds were found in the phenol-rich fraction studied. **Keywords:** anti-inflammatory, antioxidant, phenolic, agrimonia, eupatoria **Acknowledgement:** *This work was supported by FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096) and FCT, by the project PTDC/SAU-FCF/105429/2008 and the PhD fellowship SFRH/BD/46281/2008. A special thank to LEM/UC integrated in RNEM of Portugal for the HPLC/MS analyses.* **References:** 1. Takano-Ishikawa et al. (2006) Phytomedicine 13: 310 – 7 2. Terra et al.(2007)J Agric Food Chem 55: 4357 – 65

PM86 Chilean medicinal plants as a source of norA efflux pump inhibitors against resistant *Staphylococcus aureus* strains

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Staphylococcus aureus is a highly encountered pathogen in skin infections and multidrug resistance in this strain caused by native norA effluxpump is a growing clinical problem. A path to solve this problem is the synergistic combination of an antibiotic with an effluxpump inhibitor (EPI) providing an effective drug. Ethnopharmacological knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 24 plants traditionally used by the Huilliche people in southern Chile for wound healing therapy were used. Plant extracts were tested for norA effluxpump inhibitory activity in an assay based on fluorometric measurement of ethidium bromide transport by norA. Synergy studies was performed using the microtiter checkerboard method and MIC to rule out intrinsic activity A total of 24 plant species were collected. Seven crude extracts was active (> 50% inhibition) at 100 µg/ml, compared to reference drug reserpine at 20 µg/ml. None of the seven plants revealed antimicrobial action in the concentration range tested. The two most potent efflux inhibitors were tested for dose-response activity and showed similar profile as reserpine, but had higher IC_{50} -values of 11 and 14 µg/ml compared to 6 µg/ml of reserpine. Synergy studies of the two extracts G and M showed a 4-fold reduction in Moxifloxacin MIC at an extract concentration of 100 µg/ml. Extracts of Huilliche medicinal plants is likely to inhibit *S. aureus* norA facilitated EtBr efflux. Combination of extracts G and M with Moxifloxacin enhanced antibiotic action 4 fold. **Acknowledgement:** *Glenn W. Kaatz of John D. Dingell VA Medical Center, Detroit for encouraging help on setting up the assay. Luca Guardabassi at LIFE-faculty University of Copenhagen for hosting our experiments.*

PM87 Contribution of the components of STW 5 to its mode of action on inflamed rat small intestinal preparations

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STW 5 (Iberogast®) is successfully used in therapies of functional dyspepsia and irritable bowel syndrome. Given that clinical data suggest an inflammatory etiology of IBS, STW 5 and its components were examined on the production of the pro-inflammatory cytokine TNF-α and inflammation-induced cell death. The inflammation was induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 mM, 30 min). The gene expression of TNF-α was determined in rat ileum preparation by realtime-RT-PCR. The release of TNF-α was measured in LPS-stimulated human monocytes using a commercially available ELISA. The cell death of THP-1 cells was determined using a commercially available LDH (lactate dehydrogenase)-assay. The TNBS-induced inflammation in ileal preparations was accompanied by increased TNF-α gene expression. STW 5 (500.5 µg/ml) inhibited the increase in gene expression and reduced significantly the release of TNF-α by 87% in LPS (100 ng/ml)-stimulated human monocytes, while having no effect in untreated cells. In equivalent concentrations to STW 5, caraway, milk thistle, lemon balm and greater celandine had no effect on the LPS-induced increase in TNF-α release. Bitter candytuft, peppermint, chamomile, liquorice and angelica reduced the TNF-α release, though less pronounced as compared to STW 5. STW 5 (500.5 µg/ml) reduced TNBS (100 µM)-induced cell death significantly by 51.2%. Apart from caraway all other components revealed a significantly decreased cell death in differentiated THP-1 cells after co-incubation with TNBS. Lemon balm had the strongest effect and caused a reduction to 2.64%. The results indicate that the herbal components contribute differently to the effects of STW 5. **Keywords:** Iberogast, Rat ileum, Inflammation, TNF alpha, THP-1 cells, LDH-test

PM88 Effects of STW 5 and STW 6 on rat ileal and colonic preparations: A comparative study

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STW 5 (Iberogast®) is a fixed combination of nine plant extract with *Iberis amara* L. (STW 6) as its main component. It is successfully used for treatment of functional dyspepsia or irritable bowel syndrome (IBS). Because clinical data suggest an inflammatory etiology of IBS the influence of STW 5 and STW 6 on tone and acetylcholine (ACh)-induced contractions of intact and inflamed intestinal preparations was examined. We used 1 – 1.5 cm long ileum and colon preparations of male Wistar rats to analyze region specific differences. Inflammation was induced by intraluminal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 10mM, 30 min). STW 5 (64 – 512 µg/ml) concentration dependently reduced the tone and decreased ACh-induced contractions of intact ileal and colonic preparations. STW 6 in equivalent concentrations (3 – 24.1 µg/ml) neither affected tone nor contractility. TNBS-induced inflammation was accompanied by a significantly reduced ACh contractions and morphological disturbances. Co-incubation of TNBS with STW 5 (512 µg/ml) or STW 6 (24.1 µg/ml) partially normalized the TNBS-induced attenuation of ACh-induced contractions andmorphological damage in ileum preparations, whereas in inflamed colon segments only the co-incubation of TNBS with STW 6 in a high concentration (24.1 µg/ml) revealed similar effects. In conclusion, STW 5 influenced ACh contractions and tone in intact ileal and colonic preparations, whereas STW 6 does not contribute to these effects. In TNBS-inflamed ileum preparations STW 5 as well as STW 6 normalized morphological and contractile disturbances, while in colon preparations STW 6 but not STW 5 was effective. Our study confirms region specific effects of STW 5 and its main component STW 6. **Keywords:** Inflammation, Ileum, Colon, Gastrointestinal motility, Iberogast, Iberis amara

PM89 Agar-overlay assay; a useful and cost benefit method for detection of antibacterial peptides in plant seeds

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Multi-drug resistant bacteria are considered as a worldwide problem. Plant material have been an attractive candidate for overcoming human pathogens and amongst them plant defensins has been noticeably identified as a new class of promising antimicrobial substances. In this study, antimicrobial activity of some plant seeds components were assessed in two series of experiments. 8 different plant seeds were chosen according to data obtained from screening experiments which had been planned for accession of antimicrobial potential of different plant seeds methanol extract. Then an agar-overlay method, using fully separated proteins on SDS-PAGE gels was used for initial determination of active putative proteins in total water soluble proteins of seeds. 4 different gram positive and gram negative bacteria were subjected to the assay. For 2 of 8 selected plant seeds, there were clear and remarkable zones of inhibition in a region correspond to low molecular weight proteins in agar-overlay assays for all of tested gram positive bacteria but a smaller inhibition zone with several colonies for the gram negative bacterium. Clear and noticeable inhibitory zone in the case of our gram positive strains would be promising results and characterization of the effective peptides is now progressing in our laboratory. Even more important this approach makes it possible to estimate the amount of antimicrobial activities of the peptides in a semi-quantitative manner which was not possible in routine screening test by using organic solvent extracted materials which are very complex matrix with unknown antagonistic or synergistic effects on their components. **Keywords:** Plant defensins, Multi-drug resistant bacteria, Bioassay **Acknowledgement:** *We acknowledge the financial support of Medicinal Plants and Drug Research Institute of Shahid Beheshti University.* **References:** 1. Wisplinghoff H, Bischoff T, et al. (2004) Clin Infect Dis 39: 309 – 317. 2. Thevissen Ket al. (2007) Drug discovery Today 12(21/22): 966 – 972. 3. Ko S-K, Ahn C (2000) Food Sci Biotechnol 9(4): 263 – 296. 4. Sarker SD, Nahar L, Kumarasamy Y (2007) Methods (San Diego, Calif.) 42(4): 321 – 324.

PM90 New pentacyclic diterpene polyesters isolated from *Euphorbia falcata* L. as resistance modulators in cancer cells

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Resistance is a major cause of failure of chemotherapy and efflux is one of its major mechanisms that render the cancer cell resistant to more than one anti-cancer agent. The use of adjuvants that restore the activity of already existing chemotherapeutics is a promising way to overcome resistance. Four new premyrsinane- (1 – 4) and three cyclomyrsinane-type (5 – 7) diterpene polyesters isolated from *E. falcata* L. [1] were tested for their activity as efflux modulators. The compounds were identified as di- (1), tetra- (2), penta- (3), hexa- (4,5) and heptaester (6,7) derivatives of a polyfunctional diterpene alcohol, acylated with acetic, propanoic, isobutanoic, 2-methyl-butanoic, n-hexanoic and benzoic acids Compounds 2, 3, 4, 6 and 7 increased the retention of rhodamine123 in L5178 mouse T-cell lymphoma cells transfected with pHa *MDR*1/A retrovirus[2] yielding fluorescence activity ratios between 12.63 and 46.15 at 0.002 mM. The compounds when used in combination with doxorubicin synergistically decreased resistance of the cells yielding combination index values at ED_{50} between 0.13 and 0.34 and at ED_{90} between 0.03 and 0.3. Among premyrsinanes the presence of 7-OBz and 15-Ac groups seem to be important for the activity of the compounds. The acetyl group at position C-17 slightly decreases activity. In case of cyclomyrsinanes, compounds with benzoyl group have lower activity while lack of substitution at C-17 increases activity. The results of this work contribute to the understanding of important structural elements of diterpene polyesters for the design of new effective compounds that may be used as adjuvants for therapy of multidrug resistant cancer. **Keywords:** Diterpene polyesters, Resistance modulator, Cancer therapy, ABCB1, efflux pump **Acknowledgement:** *This work was supported by the Hungarian Research Fund (OTKA K72771), the New Hungary Development Plan (TÁMOP-4.2.2.-08/1 – 2008 – 0013 ad TÁMOP-4.2.1/B-09/1/KOV-2010 – 005) and the Szeged Foundation of Cancer Research. A. Vasas acknowledges to the János Bolyai Scholarship from the Hungarian Academy of Sciences.* **References:** 1. Sulyok E et al. (2010) Planta Med 76: 1257 2. Kars MD et al. (2006) Anticancer Research 26: 4559 – 4568.

PM91 Effect of methanolic extract of Harmal (*Peganum harmala* L.) on greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae)

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The greenhouse whitefly, *Trialeurodes vaporariorum* is one of most serious pests of vegetables in the world [1]. The current insecticides mainly were caused resistance to insects and outbreaks of whiteflies [2]. The plant kingdom is by far the most efficient producer of chemical compounds, synthesizing many products that are used in defense against herbivores. Plants provide for pest control alternative to pesticides as a rich source of bioactive chemicals [3]. This experiment was studied to determine the effect of methanolic extract of harmal (*Peganum harmala* L.) on hatching rate and hatching time of the whitefly. The leaves of bean plants with eggs of the whitefly were placed in the round plastic Petri dishes (5 cm diameter) that filled with agar gel. The number of eggs on the leaf discs was counted and the eggs were treated with two concentrations of the harmal extract (40 & 80 mg/ml). In the concentration of 80 mg/ml, eggs were treated immediately after laying, while in 40 mg/ml, they were treated seven days after the expired incubation period. In control treatments distilled water were applied. In concentration of 40 mg/ml, hatching rate and hatching time were 82.4% and 9.3 days, respectively. While the results of hatching rate and hatching time in the other concentration (80 mg/ml) were 72.3% and 10.1 days, respectively. Hatching rate and hatching time in the both of concentrations were significantly different than control treatment. So, this extract could be affected on hatching rate and hatching time of this insect. **Keywords:** Trialeurodes vaporariorum, Peganum harmala, Hatching rate, hatching time **References:** 1. Lindquist R K (1972)J Econ Entomol 65: 1406 – 1408. 2. Andover D G (1990) Whiteflies; their bionomics, pest status and management. Intercept Ltd. UK. 3. Kim HG et al. (2005) Food Sci Biotechnol 14 (5): 685 – 688.

PM92 Potent anti-inflammatory compounds identified in *Zingiber officinale* Roscoe var. *rubrum Theilade*: Mechanisms of action in psoriasis

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Psoriasis is an autoimmune inflammatory skin disease associated with aberrant activation of T and B-lymphocytes. Increasing evidence indicates that T-helper 1 (Th1) and Th17 lymphocyte subsets play key roles in the immunopathogenesis of the disease. In such a setting, activated Th1/Th17 cells interact with keratinocytes leading to their proliferation and hyperplasia. Our studies are focused on developing new approaches for targeted therapy for psoriasis. Recent studies from our laboratories have identified therapeutic effects for compounds extracted from the ginger species *Zingiber officinale* Roscoe var. *rubrum Theilade*, on pathogenic mechanisms in psoriasis. Initially, the therapeutic effects of chloroform extract (HB02) and selected fractions were assessed for their ability to suppress the production of pro-inflammatory mediators produced by macrophage. Four fractions, F5, F6, F7 and F10 with dual sup-

pressive effects on NO and PGE_2 production were identified. The fractions had higher potency than L-NAME, a specific inhibitor of iNOS, and exhibited comparable effects to indomethacin in inhibiting of PGE_2. F6 had particularly potent inhibitory effects on inhibiting NO (IC_{50}= 6.7 ± 2.7 µg/ml) and suppressing *iNOS* gene transcription by 82.3 ± 3.73 % at 20 µg/ml. Two compounds, 6-shogaol and a ferulate derivative were isolated from F6. Interestingly, the 2 compounds had additive effects in down-regulating *iNOS* and *il23* gene transcription. These compounds may, therefore, be key components responsible for the anti-inflammatory effects of HB02. Current experiments are focused on mechanisms of action and therapeutic efficacy of these compounds on suppressing psoriasis involving chemokine and cytokine production and keratinocytes proliferation using an *in vitro* human psoriatic skin model. **Keywords:** Psoriasis, *Zingiber officinale* Roscoe var. *rubrum Theilade*, 6-shogaol, ferulate derivative **Acknowledgement:** *1. Standards and Industrial Research Institute of Malaysia (SIRIM Berhad), Malaysia 2. Ministry of Science, Technology & Innovation (MOSTI), Malaysia*

PM93 Effect of some medical plants on nymphal development and mortality of *Brevicoryne brassicae* (L.) (Homoptera: Aphididae)

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The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food. Therefore, several efforts have been created to reduce the use of synthetic pesticides particularly the use of synthetic insecticides. One of the efforts is the development of botanical insecticides as a novel and safer alternative strategy. Botanical insecticides, which contain plant extracts as active components, are safer as well as environmentally friendlier than synthetic insecticides [1]. Botanical pesticides are easily biodegradable [2] and their use in crop protection is a practically sustainable alternative. Ethanolic solution of three plant species, clove flower buds (*Syzygium aromaticum* L.), harmal seeds (*Peganum harmala* L.), Persian lilac fruits (*Melia azedarach* L.) were tested for their activities on duration of development and mortality of cabbage aphid [*Brevicoryne brassicae* (L.)] in the laboratory. The first instars (one day old) were sprayed with ethanolic solution (30 mg/ml) of different plant extracts, and nymphal duration as well as mortality were estimated. In the control treatment, the insects were sprayed with ethanol (95 %). In harmal treatment, the duration of nymphal development was considerably longer than control with a mean of 8.0 days. Total mortality (%) during the development of the aphid from N1 to adult emergence were 50.0 %, 36.6 %, 30.0 % and 21.4 % in clove, harmal, Persian lilac and control treatment, respectively. **Keywords:** *Brevicoryne brassicae*, plant extract, nymphal development, mortality **References:** 1. Dadang ED et al. (2009) J ISSAAS 15(1): 42 – 51. 2. Devlin JF, Zettel T (1999) Ecoagriculture: Initiatives in Eastern and Southern Africa. Weaver Press. Harare.

PM94 An approach to studying the mechanism of action of STW 5 in functional dyspepsia using the restraint stress model in rats

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While the clinical efficacy of medicinal plants as therapeutic options in treating functional gastrointestinal diseases is widely accepted, the understanding of their mechanisms of action still remains uncertain. Two models for stress-induced functional dyspepsia were performed in order to choose the more adequate one for testing sensitivity changes of the fundus to various mediators. In one model, maternal separation (1) was performed on weanling rats starting from postnatal day 2 for 3 h each day for 3 weeks. Rats were then allowed to mature to an adult age. The other model was that of restraint stress (2,3). Adult animals were restrained for 90 min/day for 1 week. The animals were eventually sacrificed, the stomach fundus was isolated and its sensitivity in vitro to carbachol, potassium chloride, serotonin and adrenaline was tested. The sensitivity of the fundus strips from restrained rats towards these agents was more depressed than those from maternally separated ones. That model was therefore chosen to test the efficacy of STW 5 in restoring sensitivity to the agents mentioned. A group of animals received STW 5 orally once daily for 2 weeks before subjecting them to restraint stress. Treatment with STW 5 was effective in normalizing the depressed responses exhibited by animals subjected to restraint stress. Samples of blood were taken to assess levels of CRF and ghrelin. The findings throw further light on the mechanisms underlying the therapeutic usefulness of STW 5 in functional dyspepsia, especially when triggered by psychological stress. **Keywords:** Functional dyspepsia, STW 5, Stomach fundus, restraint-stress **References:** 1. Cheung CK et al. (2010) Gastroenterology138: S-766 2. Zhang H et al. (2008) Phytomedicine 15: 602 – 611. 3. Zheng J et al. (2009) Am J Physiol Regul Integr Comp Physiol 296: R1358- R1365.

PM95 Reproduction and longevity of the cabbage aphid [*Brevicoryne brassicae* (L.)] after exposure to ethanolic extract of clove (*Syzygium aromaticum* L.)

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Botanical pesticides are an important group of naturally occurring, often slow-acting crop protection that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Therefore the use of plant pesticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risks [1, 2]. Especially botanical insecticides have long been a subject of research in an effort to develop alternatives to conventional insecticides. Therefore, this research was conducted to assess the effectiveness of ethanolic solution (30 mg/ml) of clove flower buds extract (*Syzygium aromaticum* L.) on reproduction and longevity of the cabbage aphid [*Brevicoryne brassicae* (L.)] in the laboratory. In this experiment, 50 newly 1st nymphal instars of the cabbage aphid were placed together into the round plastic Petri dishes on rape leaves and sprayed with the ethanolic solution. In control treatments only ethanol (95 %) were applied. After 24 hours, the nymphs were transferred into the new Petri dishes with fresh leaves and reared until the adulthood. Afterwards, the adults were confined singly in other Petri dishes and reared until death. During the longevity experiments, reproduction of the adults was estimated during one week. The ethanolic plant extract caused a significant reduction in longevity of the adults (8.7 days) when compared with the control treatments. Moreover, the plant extract had a significant deleterious effect on the mean total number of laid nymphs during the seven days with the mean of 5.8 nymphs. **Keywords:** *Brevicoryne brassicae*, plant extract, *Syzygium aromaticum*, Reproduction, longevity **References:** 1. Isman MB (2006) Ann Rev Entomol 51: 45 – 66. 2. Pavela R (2007) Pest Technol 1: 47 – 52.

PM96 Toxic effect of three medicinal plant extracts on *Myzus persicae* (Sulzer) (Hem.: Aphididae)

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The peach potato aphid *Myzus persicae* (Sulzer) (Hem.: Aphididae) is one of the most noxious species [1]. It can infest plants of over 40 different families including many economically important ones world wide, and it is able to transmit over 100 plant viruses [2]. Therefore, in the present study the efficacy of acetonic leaf extracts from three medicinal plants

were evaluated against 3 – 4-day-old individuals of the *M. persicae*. The plants were included *Eucalyptus globulus* Labill. (Myrtaceae), *Teucrium polium* L. (lamiaceae) and *Otostegia persica* Boiss. (Labiatae). In order to obtain the crude extracts, the dried leaves were powdered and extracted with acetone. Experiments were carried out at 25 ± 1 °C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. In control treatments only distilled water and DMSO (dimethyl sulfoxide) were applied. Topical treated aphids with three acetonic extract emulsion (in distilled water with DMSO) were placed on the broad bean leaf discs (4.5 cm diameter) in the round plastic Petri dishes (5.5 cm diameter), filled with a 0.5-cm-thick agar gel layer. The highest percentage of mortality (55.6%) was observed in the acetonic leaf extract of *O. persica* in the concentration of 80 µl/ml after 48 hours. While, it was less than 10% in the acetonic leaf extract of *E. globulus*. The acetonic leaf extract of *T. polium* caused 14.5% mortality of *M. persicae*. It is concluded that *O. persica* is the most promising for future development and use as botanical pesticide. **Keywords:** *Myzus persicae*, medicinal plant, Toxic effect, Topical test **References:** 1. Blackman RL, Eastop VF (1984) Aphids on the World's Crops. John Wiley and Sons. New York. 2. Clements KM et al. (2000) RevToxicol 3: 1 – 23.

PM97 Evaluation of antibacterial and antioxidant activities of *Ziziphus vulgaris* (Rhamnaceae)

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Zizyphus vulgaris Lam. is a deciduous shrub, native of the Mediterranean region and used to treat sore throats, alleviate stress and helps in the common colds. In present work 10 g of the dried plant material was soaked in 100 ml methanol and shaken for 24 h and clear filtrate was obtained. The fresh methanolic crude extracts were qualitatively screened for secondary metabolites. Results showed that: flavonoids, hydrolysable tannins, alkaloids, terpenes and saponins had reasonably high contents but anthraquinones and coumarines were low. In vitro antimicrobial assay and MIC determination growth inhibition activities of methanolic leaves extract of *Z. vulgaris* against gram-positive and gram-negative bacterial species using the conventional paper disc assay showed good inhibitory effects only against gram-positive with no antagonistic effects against gram-negative bacterial species testedThe MIC values of the crude methanolic *Z. vulgaris* extract on gram-positive was in range to 12.5 – 25.0 µg/ml, whereas extract exhibited very weak antimicrobial activity against gram-negative with very high values (1000 µg/ml) of MICs. Total phenolics assay by the Folin-Ciocalteu method in plant extract also showed reasonably high contents of polyphenolics (300 mg/ml extract). Results collectively suggest that *Z. vulgaris* is not only reliable natural sources of antimicrobials but also potential sources of phenolic antioxidants and hence could be nominated for future intensive studies. **Keywords:** Ziziphus vulgaris, antimicrobials, phenolic antioxidants

PM98 Antioxidant activity and phenolic content of different extracts of *Gentiana cruciata* L

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Gentiana cruciata L. is a perennial plant belonging to the genus *Gentiana* (fam. Gentianaceae) [1]. *Gentiana* species are widely used throughout the world as potential stomachic and hepatoprotective agents [2]. *G. cruciata* is used in the traditional medicine for loss of appetite, as a stomachic as well as component in preparations showing beneficial effects in gall and liver diseases [3]. The aim of this study was to evaluate the antioxidant and radical-scavenging activities of methanol extract, chloroform, ethyl acetate and *n*-butanol fractions obtained from the methanol extract of aerial part of *G. cruciata*. Amounts of total phenols and flavonoids were also determined. The total phenolics contents in the fractions and extract were determined as gallic acid equivalent (GA) using Folin-Ciocalteu's reagent, while the spectrophotometric method with aluminium chloride was used for the determination of total flavonoids. The total amount of flavonoids was calculated as the rutin equivalent (RU).The extracts were investigated for antioxidant capacity using two different assays: DPPH assay and inhibitory activity toward lipid peroxidation. The highest content of total phenols (109.8 mg GA/g) and flavonoids (110.9 mg RU/g) was determined in the *n*-butanol fraction. The most effective DPPH radical scavenger was *n*-butanol fraction (IC_{50}= 114.7 µg/mL), while the methanol extract showed the highest inhibitory activity toward lipid peroxidation (IC_{50}= 69.9 µg/mL). The results show a significant antioxidant activity of the investigated extracts compared to referent antioxidant compounds, such as butylated hydroxytoluene (BHT), ascorbic acid (AA), gallic acid (GA) and α-tocopherol. **Keywords:** *Gentiana cruciata*, antioxidant activity, phenolic content **Acknowledgement:** *This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia (project No. III 43004).* **References:** 1. Struwe L, Albert V (2002) Gentianaceae-systematics and natural history, Cambridge University Press, Cambridge 2. Jiang R et al. (2005) Phytochemistry 66: 2674 – 2680. 3. Menkovic N et al. (2011) J Ethnopharmacol 133: 97 – 107.

PM99 *Melissa officinalis*: an important dietary source of phenolic compounds with high antioxidant capacity

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Balm, *Melissa officinalis* L. a perennial herb native to southern climates of Europe and North America and is cultivated in Mediterranean and central Asian areas [1]. Oil of balm has been shown to have antiviral, antibacterial and antispasmodic activity [2,3]. In this research, total phenolic content and related total antioxidant capacity of plant infusions was analyzed. Infusions were prepared in common way in which teas are prepared for human consumption. The total phenolics were measured by Folin-Ciocalteau assay. The total antioxidant capacity was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay. Also, the phenol antioxidant coefficient (PAC) was calculated for plant infusion. The obtained results for Melissa infusions showed: high phenolic concentration, very high FRAP (> 20 mM/L) and PAC > 3. The ffect of infusion time and temperature on the phenolic content, FRAP, and free radical scavenging ability was tested. Preparation of Balm infusions with hot (98 °C) and cold (20 °C) revealed that although antioxidants were liberated from leaves into the water at both of the temperatures studied, infusions prepared at higher temperature had more than 2-fold higher antioxidant capacity determined as FRAP. DPPH radical scavenging ability of Balm phenolics was similar to (+)-catechin but not as good as for quercetin. Compared to Trolox and vitamin C, Melissa phenolics were more efficient free ABTS radical scavengers. The results indicate that *Melissa officinalis* infusions could be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea. **Keywords:** Phenolic compound, antioxidant capacity; Infusions; Melissa officinalis; FRAP; DPPH; ABTS **References:** 1- Kennedy D, Little OW, Haskell CF, Scholey AB (2006) Phytother Res 20: 96 – 102 2-Weiman Z, Alkrinawi S, Golfarb D, Bitran C (1993) J Pediatr122:650 – 652 3-Wake G, Court J, Pictering A, Lewis R, Wilkins R, Perrey E (1999) J Ethnopharmacol 69: 105 – 114

PM100 *In vitro* and *In vivo* Antitumor Effects of Deoxyelephantopin on Human Breast Cancer Cells

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Breast cancer is one of the most common cancers in women, and a leading cause of death worldwide. It is often highly resistant to chemotherapy, and there is often no effective cure for patients in the advanced stages of the disease. In this study, we evaluated the effect of deoxyelephantopin (DET), a phytocompound extracted from *Elephantopus scaber* L. (Asteraceae) for possible anti-tumor activities in the human breast cancer cell-line MDA-MB-231. Cell-apoptosis assay showed that DET treatment was able to effectively suppress the growth of test tumor cells in vitro. In addition, DET treatment significantly decreased expression level of transforming growth factor-beta (TGF-β), effectively inhibited cell growth by inducing G2-M phase cell cycle arrest and apoptosis, and reduced the clonogenicity in a concentration-dependent manner in MDA-MB-231 cells. DET also significantly inhibited the invasion and migration of test breast tumor cells. The effect of DET on suppression of NF-κB, via activation by TNF-α, was examined using electrophoretic mobility shift analysis (EMSA). Decreased levels of expression of phospho-NF-κB and the downstream molecules of the NF-κB signaling path-

way, including survivin, Bcl-2, MMP-9 and VEGF, were observed in DET-treated MDA-MB-231 cells. *In vivo*, DET significantly inhibited tumor growth and the myeloid derived suppressor cell (MDSC) population in nude mice. Taken together, our findings suggest that DET may warrant a further systematic investigation for potential applications in the chemoprevention or control of breast cancers. **Keywords:** Deoxyelephantopin (DET), Elephantopus scaber, Asteraceae, anticancer

PM101 The anticancer potential of *Artemisia afra*

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Artemisia afra Jacq. is one of the oldest, most well known and widely used traditional medicinal plants in South Africa. It is used to treat many different medical conditions, particularly respiratory and inflammatory ailments (Liu et al., 2009). There is no reported evidence of its use for the treatment of cancer but due to its reported cytotoxicity (Fouche et al., 2008; Mativandlela et al., 2008), we investigated the effect of *A. afra* extracts on 2 cancer cell lines. IC_{50} values of 18.21 µg/mL and 31.88 µg/mL of ethanol extracts were determined against U937 and HeLa cancer cells, respectively. An IC_{50} value of the aqueous extract was greater than 250 µg/mL. Dose response assays were also performed using confluent HeLa cells, yielding an IC_{50} value greater than 250 µg/mL. The effect of the cytotoxic ethanolic *A. afra* extract (20 µg/mL) on U937 cells and their progression through the cell cycle, apoptosis and mitochondrial membrane potential was investigated. Melphalan was used as a positive control. After 24 hours of treatment with melphalan using U937 cancer cells, an increase in sub G1 phase was evident. Treatment of cells with *A. afra* showed a delay in G2/M phase of the cell cycle. Apoptosis was confirmed using the TUNEL assay for DNA fragmentation, which was evident with the positive control and *A. afra* treatment at 24 and 48 hours. JC-1 staining showed a decrease in mitochondrial membrane potential at 24 hours. The results obtained suggest that *A. afra* potentially has medicinal anticancer properties. **Keywords:** *Artemisia afra*, apoptosis, cytotoxicity **References:** 1. Liu et al. (2009) S Afr J Bot 75: 185 – 195. 2. Fouche et al. (2008) J Ethnopharmacol 119: 455 – 461. 3. Mativandlela et al. (2008) Phytother Res 22: 841 – 845.

PM102 *In vitro* inhibition of 5-lipoxygenase by natural quinone compounds

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Dual inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways is promising approach in treatment of inflammatory diseases. Drugs able to block production of prostanoids together with leukotrienes should provide better anti-inflammatory properties and fewer side effects than non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors [1,2]. Our previous studies revealed that some natural quinone compounds such as primin, alkannin, or shikonin are potent in vitro COX inhibitors [3]. In the current study, we tested 19 quinone compounds for 5-lipoxygenase (5-LOX) inhibition using in vitro assay according to [4] where neutrophile granulocytes with 5-LOX activity isolated from the human blood are incubated with arachidonic acid (substrate) and tested samples. After the incubation, the amount of leukotriene B_4 (LTB_4) is determined by commercial LTB_4 EIA kit (Assay Designs). The most active quinone 5-LOX inhibitors were benzoquinone primin and naphthoquinone shikonin which decreased LTB_4 production by 93 and 87% at 50 µM concentration, respectively. Reference inhibitor zileuton reduced LTB_4 production by 91% at the same concentration. Based on these preliminary results obtained in 5-LOX assay together with data from previous studies targeted on COX inhibition the plant quinones such as primin and shikonin should be considered for further studies aimed on their potential of dual COX/LOX inhibition. **Keywords:** natural quinones, 5-lipoxygenase, enzyme inhibition, leucotriene B_4, arachidonic acid pathway **Acknowledgement:** *This study was supported by Czech Science Foundation project 525/09/P528.* **References:** 1. Celotti F et al. (2001) Pharmacol Res 43: 429 – 436. 2. Leone S et al. (2007) Curr Top Med Chem 7: 265 – 275. 3. Landa P (2009) Planta Med 75: 1059. 4. Adams M et al. (2004) Planta Med 70: 904 – 908.

PM103 Comparative assessment of antioxidant profile of *Daucus carota* L. ssp. *sativus* Hoffm. var. *atrorubens* Alef. and a fermented local beverage, "Şalgam"

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Şalgam is a well-known traditional local beverage which is prepared by the lactic acid fermentation of black carrot [1]. It has been widely consumed in Turkish daily meal. This study aims to compare the antioxidant potentials of Şalgam and its main ingredient, black carrot. Both şalgam and black carrot juice were freeze-dried prior to analysis. *In vitro* colorimetric systems were used for this purpose. DPPH test was performed for free-radical scavenging activity. IC_{50} value of black carrot were found to be 646 ± 90.11, whereas 919 ± 13.43 for şalgam. Total antioxidant capacities equivalent to ascorbic acid of şalgam and black carrot juice showed a good activity at 125 mg/L concentration with values 91.76 ± 1.24 and 100.8 ± 1.72 mg AA/g dry extract, respectively. Ferric-reducing antioxidant power assay gave a result of 1012 ± 13.27 µM $FeSO_4$/g dry extract for black carrot and 964 ± 7.21 µM $FeSO_4$/g dry extract for şalgam. These results indicate that although antioxidant potentials are close to each other, black carrot juice shows higher *in vitro* antioxidant activity when compared to that of şalgam. Further *in vivo* studies are in progress. **References:** [1] Erten et al. (2008) Food Reviews International 24:352 – 359

PM104 Bioactivity of *in vitro* glycoalkaloids from *Solanum nigrum*

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Glycoalkaloids were produced from callus and regenerated plants of *Solanum nigrum* L. (Solanaceae) using different concentrations of auxins and cytokinins.The glycoalkaloids were separated by acid base precipitation, and determined by high performance liquid chromatography. The produced glycoalkaloids found to have antioxidant activity against the free radical DPPH. Also, they exhibited cytotoxic activity against the selected carcinoma cell lines including liver, breast and lymphoplastic leukemia cell lines. Examination of the antiviral activity showed that glycoalkaloids had virucidal effect against the tested virus strains. In addition, callus glycoalkaloids were found to have antischistomiasis and antifasciolos activities. **Keywords:** *In vitro* glycoalkaloids, *Solanum nigrum*, cytotoxicity, antiviral, antiparasitic

PM105 Determination of *in vitro* antioxidant potential of *Cornus mas* L. and its polyphenol content

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Cornus mas L. (Cornaceae) is one of the two species of the genus *Cornus* L. represented in the Turkish flora [1]. Its leaves have been widely used in Anatolian folk medicine against diarrhea and diabetes [2]. This study was designed to investigate the antioxidant potential of the 80% methanolic extract prepared from the leaves. The antioxidant properties were examined by using different *in vitro* systems. Ascorbic acid and BHT were used as reference substances. In the DPPH test IC50 value was

found to be 418 ± 2.26 μg/mL. The extract showed a strong superoxide scavenging activity (IC 50 = 114.75 ± 3.12 μg/mL). Moreover, the result of total antioxidant capacity as equivalent to ascorbic acid was 228 ± 5.08 mg AA/g dry extract. Ferric-reducing antioxidant power and metal-chelating activities of 1 mg/mLextract were 1487 ± 7.46 μmol $FeSO_4$/g dry extract and 32.7%, respectively. Total phenolic content 248 ± 0.41 μg gallic acid/mg extract, the flavonoid content was 75 ± 0.24 μg quercetin/mg extract. These results suggest that *Cornus mas* L. leaf extract exert significant *in vitro* antioxidant potential and further *in vivo* studies are in progress. **References:** [1] Chamberlain DF (2001) Flora of Turkey and The East Aegan Islands, Davis P.H (ed.), Edinburgh, Vol 4, pp. 539 – 540 [2] Yeşilada et al. (1999) Journal of Ethnopharmacology 64: 195 – 210

PM106 Mechanism of action of *Fragaria vesca* leaf extract on LPS treated macrophages

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Fragaria vesca L., commonly known as Strawberry, has been used over the years by traditional medicine for the treatment of several diseases. However, scientific reports of its molecular action mechanism are lacking. Thus, this work aims to investigate the anti-inflammatory effects, of a *Fragaria vesca* leaves extract obtained by successive extractions with ethanol and 50% aqueous ethanol, on the macrophage cell line, Raw 264.7, stimulated with lipopolysaccharide (LPS). For this purpose nitric oxide (NO) production, scavenging activity and cytotoxicity of the extract were assessed. Furthermore, was evaluated the expression of proteins that are potential targets to prevent or treat chronic inflammation, namely iNOS, COX-2, phospho-IκB-α and IκB-α. The results demonstrated that *Fragaria vesca* leaves extract was not cytotoxic and inhibit the production of NO triggered by LPS. Using (S)-Nitroso-N-acetylpenicillamine as NO donor, the extract promoted a significant decrease of NO in the culture medium. Western Blot analysis showed that LPS triggered a significant increase on iNOS and COX-2, though no significant differences were observed between cells treated with LPS or co-treated with the extract. Furthermore, in cells stimulated with LPS we observed a strong decrease on the content of IκB-α, while phosphorylated IκB-α strongly increased. However, an increase on the phosphorylation of IκB-α occurred in cells co-treated with the plant extract and LPS, suggesting a potential reduction of proteasome degradation, since phospho-IκB-α is a target for the ubiquitin-proteasome pathway. In conclusion, our data show that *Fragaria vesca* decreased the level of nitrites, mainly through direct NO scavenging activity of the extract. **Keywords:** Fragaria vesca, strawberry, anti-inflammatory properties, scavenger activity, proteasome inhibition **Acknowledgement:** *Research supported by FCT PhD fellowship SFRH/BD/72918/2010 and FCT project PTDC/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096).*

PM107 *In vitro* antiprotozoal activity of organic and aqueous extracts of several Turkish Lamiaceae species

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The *in vitro* antiprotozoal activities of methanolic extracts and subextracts prepared from the aerial parts of five Lamiaceae plants (*Salvia tomentosa* Miller, *S. sclarea* L., *S. dichroantha* Stapf., *Nepeta nuda* L. subsp. nuda and *Marrubium astracanicum* Jacq. subsp. *macrodon* (Bornm.) P.H. Davis were evaluated against four parasitic protozoa, *Trypanosoma brucei rhodesiense, T. cruzi, Leishmania donovani* and *Plasmodium falciparum*. The cytotoxic potentials of the extracts on L6 cells were also evaluated. Melarsoprol, benznidazole, miltefosine, chloroquine and podophyllotoxin were used as reference drugs. MeOH extracts showed antiprotozoal potential against three or four parasites. Hence, they were dispersed in water and partitioned against n-hexane and chloroform, respectively, to yield three subextracts that were screened in the same test systems. The n-hexane extract of *N. nuda* was the most active against *T. brucei rhodesiense* with IC 50 value of 0.62 μg/mL, whilst the $CHCl_3$ extracts of *S. tomentosa* and *S. dichroantha* showed significant activity against *L. donovani* (IC_{50} 1.81 and 2.31 μg/mL, respectively). All organic extracts displayed moderate trypanocidal potential against *T. cruzi* with hexane extract of *S. sclarea* being the most active one (IC 50 18.17 μg/mL). Again all organic extracts exhibited remarkable antimalarial activities and with IC 50 values in the range of 2.54 – 3.78 μg/ml, the chloroform subextracts appeared to be slightly more potent than the hexane subextracts (IC_{50} values 3.37 – 4.64 μg/mL). The extracts displayed low or no cytotoxicity towards mammalian L6 cells. This is the first study reporting the antimalarial, leishmanicidal and trypanocidal effects of the genera *Salvia, Nepeta* and *Marrubium* that are native to Turkey. **Keywords:** Lamiaceae, Nepeta, Salvia, Marrubium, Antiprotozoal activity

PM108 Antinociceptive effect of chronic administration of green tea epigallocatechin gallate in a model of diabetic hyperalgesia in rat

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Considering antidiabetic and antiinflammatory potential of geen tea epigallocatechin-gallate (EGCG), this study was designed to investigate the analgesic effect of two-month EGCG using formalin and tail-immersion tests in diabetic rats. Male rats were divided into control, EGCG -treated control, diabetic, EGCG -treated diabetic, and sodium salicylate-treated control and diabetics. EGCG was administered *p.o.* at doses of 20 and 40 mg/kg for seven weeks one week after diabetes induction. At the end of the study, pain threshold and nociception were evaluated using hot water tail immersion and formalin tests respectively. Diabetic rats exhibited a higher score of pain at both phases of the formalin test and EGCG-treated diabetic rats dose-dependently exhibited a lower nociceptive score at both phases of the test ($p < 0.05$). Regarding pain threshold, diabetes significantly reduced tail immersion latency ($p < 0.05$) and EGCG treatment did not produce a significant change in this respect. Although chronic treatment with EGCG does not affect pain threshold but significantly reduces nociception in an experimental model of hyperalgesia and this may be considered as an auxiliary treatment for diabetic hyperalgesia. **Keywords:** Epigallocatechin-3-gallate, Pain, Hyperalgesia, Diabetic rat

PM109 Antinociceptive effect of *Allium schoenoprasum* L. oral feeding in diabetic rats

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Hyperalgesia is considered as one of the marked signs of subchronic diabetes mellitus that could affect the life style of the patients. This study was designed to investigate the antinociceptive effect of chronic feeding of *Allium schoenoprasum* L. (AS) leaf in streptozotocin-diabetic rats using formalin and hot tail immersion tests. Rats were divided into control, AS leaf-treated control, diabetic, sodium salicylate (SS)-treated diabetic, and AS leaf-treated diabetic groups. The treatment groups received oral administration of AS leaf-mixed pelleted food (3%) for 8 weeks. Finally hyperalgesia were assessed using standard formalin and hot tail immersion tests. AS leaf treatment of diabetic rats reduced pain score in chronic phase of formalin test from 2.41 ± 0.14 to 2.01 ± 0.12 ($p < 0.05$). Regarding hot tail immersion test, diabetic rats showed a significant reduction (5.9 s) in tail flick latency as compared to control ones ($p < 0.05$) and AS leaf treatment of diabetic rats did not significantly increase this latency relative to untreated diabetics. Taken together, 8-week administration of AS leaf could attenuate nociceptive score in chronic phase of formalin test in streptozotocin-induced experimental model of diabetes mellitus and has no effect on thermal pain and anti-inflammatory property of the plant is perhaps responsible for its analgesic effect. **Keywords:** Allium schoenoprasum, Diabetic hyperalgesia, Antinociceptive

PM110 The anti-inflammatory pharmacological profile of herbal extracts used in Tonsipret®

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Tonsipret® is a commercially available medicinal product for the treatment of sore throat and tonsillitis. The herbal extracts used in this preparation are hydro-alcoholic tinctures of dried, ripe fruits from *Capsicum annuum* L., of resin from *Guaiacum officinale* L., and of freshly collected roots from *Phytolacca americana* L.. The anti-inflammatory activity of the single extracts was evaluated *in-vitro* concerning inhibition of prostaglandin biosynthesis by cyclooxygenases (COX-1 and -2) [1], inhibition of leukotriene biosynthesis in human neutrophile granulocytes [1], inhibition of nitric oxide (NO) production in RAW 264.7 macrophages [2], and inhibition of Nuclear Factor kappa B1 (NF-κB1) and COX-2 expression in THP-1 cells [3]. Each herbal extract appeared to have an anti-inflammatory tendency *in-vitro*. The extract of *Guaiacum officinale* showed the strongest inhibitory effects on the assessed parameters. Its active constituents interfered with NF-κB1 expression, as well as with COX-2 expression, the enzymatic activity of inducible nitric oxide synthase (iNOS), and 5-lipoxygenase – genes containing an NF-κB binding site in their promoter regions. The other examined extracts also revealed a certain anti-inflammatory activity. The extract of *Capsicum annuum* exhibited a good inhibition on the enzymatic activity of COX-2, whereas the extract of *Phytolacca americana* showed a higher inhibitory activity on COX-2 expression. **References:** 1. Blunder M et al. (2010) Bioorg Med Chem 18: 2809 – 2815 2. Konkimalla VB et al. (2010) Biochem Pharmacol 79:1573 – 1580 3. Gusenleitner S, Bauer R (2007) Planta Med 73: 844

PM111 Histological analysis of rat cutaneous wounds treated with a semi-solid formulation of linseed (*Linum usitatissimum* L.) oil

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The oil linseed of *Linum usitatissimum* L. (Linaceae) is popularly known as linen. Its chemical composition shows the presence polyunsaturated fatty acids, linolenic (56.6%) and linoleic acid (13.2%), and the monounsaturated fatty acid oleic (17.8%) which are important for the maintenance of normal dermal structure [1, 2]. The purpose of this study was to investigate the effects of a a semi-solid formulation of linseed oil – SSFLO (1%, 5% or 10%) on re-epithelialization of excision wound model. Surgically standardized circular (± 78.5 mm^2) wounds were made on the dorsum of Wistar rat. The animals were divided into five groups ($n = 6$) and treated for 14 days with SSFLO (1%, 5% or 10%), petrolatum jelly (negative control) or commercial emulsion of sunflower oil (positive control). At 14th days the animals were euthanized and the scar tissue was collected for histological and histomorphometric analysis to evaluate re-epithelization, quantification of inflammatory cells, fibroblast cells, blood vessels and collagen density. All animals treated with SSFLO (1% or 5%) or commercial emulsion of sunflower oil (positive control) showed complete re-epithelialisation, against only 33.33% showed by negative control group. In the morphometric evaluation a significant increase ($p < 0.05$) in the number of inflammatory cells in the group treated with 10% SSFLO was observed compared to the ESO control group. Among the remaining variables, there were no significant differences observed. The results clearly demonstrate that locally administered 1% and 5% SSFLO promote a significant re-epithelization during the healing process, and might represent a novel therapeutic approach in cutaneous wounds. **Keywords:** Linum usitatissimum, re-epithelialization, cutaneous wounds **References:** [1] Joshi K et al. (2006) PLEFA 74:17 – 21. [2] Otranto M et al. (2010) WRR 18: 629 – 636.

PM112 Tyrosinase inhibitory activity of selected medicinal plants

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Melanin is a pigment that is distributed widely in bacteria, fungi, plants and animals (1). Melanogenesis is initiated with oxidation of L-tyrosine by tyrosinase that is rate-limiting step in this process. Next reactions can proceed spontaneously (2). Tyrosinase that is a key enzyme in formation of melanin pigments, widely exists in animals and plants. Tyrosinase have a monophenolase activity as well as diphenolase activity that oxidizes o-diphenols to o-quinones (3). This study evaluated inhibitory effect of four plants including *Physalis alkekengi* L., *Alcea rosea* L., *Bunium persicum* B.Fedtsch. and *Marrubium vulgare* L. on mushroom tyrosinase. In this study L-Dopa (Dihydroxyphenilalanin) is used as substrate, so diphenolase activity of mushroom tyrosinase was evaluated. kojic acid was used as possitive control. Extracts of *physalis alkekengi, Alcea rosea, Bunium persicum* (Total), *Bunium persicum* (defatted) and *Marrubium vulgare* showed IC_{50} values of 0.09, 0.38, 0.37, 0.38, 2.76 mg/ml respectively. IC_{50} values were defined as concentration of inhibitor that inhibited 50% of tyrosinase activity (4). Etract of *Physalis alkekengi* showed greatest inhibitory effect on mushroom tyrosinase activity with IC_{50} value of 0.09 mg/ml. Kinetic and Inhibition parameters (Km, Vm, Ki) were calculated. In this study kinetic parameters (Km, Vm) are evaluated and Ki evaluated for *P. alkekengi, A. rosea* and *B. persicum*. Data has revealed that these three plants display a mixed- type inhibition. However *Marrubium vulgare* shows an uncompetitive inhibition (Table1). *P. alkekengi* that had the greatest tyrosinase inhibitor showed Ki value of 0.52 mg/ml comparing another study on total extract of *Lavandula stoechas* L. showed Ki value of 0.183 mg/ml (4). Finally calcu-

lation methods, types of inhibition (herbs & pure compounds) will be discussed.

Table 1: effect of Extracts and kojic acid on the kinetic parameters of mushroom tyrosinase.

	N.I(a)	Kojic acid (b)	Physalis alkekengi	Alcea rosea	N.I	Bunium persicum	Bunium persicum (defatted)	N.I	Marrubium vulgare
Km(mM)	0.18	0.45	0.22	0.3	0.11	0.17	0.13	0.14	0.12
Vm(U)	4.5	2	1.5	2.2	4.1	1.6	1.5	4.1	2.1

Keywords: *physalis alkekengi, Alcea rosea, Bunium persicum, marrubium vulgare*, Tyrosinase, Inhibitor, Kinetic **References:** 1. Kima Y-J et al. (2005) CMLS Cell Mol Life Sci 62: 1707 – 1723 2. Chang T-S (2009) International J Molecular Sci 10(6): 2440 – 2475 3. Wanga Q et al. (2007) Bioorganic & Medicinal Chemistry 15(3):1568 – 1571 4. Hsu C-K et al.(2007) Food Chemistry 105(3): 1099 – 1105

PM113 Antioxidant capacity of *Bifora radians* Bieb

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Thegenus *Bifora* Hoffm. (Apiaceae = Umbelliferare) is represented by two species in Turkey, namely *Bifora testiculata* (L.) Sprengel and *B. radians* Bieb. [1] *B. radians* is an annual herb with typical odor in the fieldsides especially chalky of Central Anatolia and known locally as "yabani kişnişotu, küçük kişnişotu or aşuti" [1,2]. This species is used in traditional medicine as a stomachic and carminative in Turkey. Furthermore, the aerial parts of this plant has been used as an aromatic in foods especially soup in Doğubeyazıt-Van [2]. *B. radians* is rich in alkanals and alkenals [3]. The phenolic contents of the samples were determined using Folin-Ciocalteu's phenol reagent.The antioxidant activity of diethyl ether and methanol extracts were studied by two different techniques: qualitative DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and the TBA assays. Both extracts showed a slightly antioxidant activity with the qualitative $DPPH^{\cdot}$ test. Methanol extract of the plant was found to possess moderate inhibitory activity (IC_{50}= 89.43 ± 3,09) on the lipid peroxydation whereas the nonpolar fraction (IC_{50}= 261.32 ± 2,72) showed a slightly antioxidant activity. Propyl gallate (IC_{50}= 0.09 ± 0,18) is used as a positive control. **Keywords:** Bifora radians, antioxidant activity, Apiaceae **References:** 1. Hedge IC, Lamond JM (1972) Bifora Hoffm. in Flora of Turkey and the East Aegean Islands, Ed. Davis, P.H., University Press, Edinburgh, Vol. 4, pp. 332 – 333. 2. Baytop T (1999) "Türkiye'de Bitkiler ile Tedavi. Geçmişte ve Bugün". 2nd ed. Nobel Tıp Kitabevleri, İstanbul. pp. 151 – 152. 3. Baser HC, Demircakmak B, Ermin N, Demirci F, Boydag I (1998)J Essent Oil Res 10(4): 451 – 452.

PM114 Evaluation of the hypoglycemic activity of extracts from *Boldoa purpurascens* Cav

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Boldoa purpuracens Cav. (1), a plant belonging to Nyctaginaceae family, is traditionally used for its diuretic effect comparable to furosemide (2). In the species described the presence of several flavonoid compounds widely used for its diversity of actions among which is the hypoglycemic. The phytochemical analysis by 1 H and 13C NMR spectroscopy allowed the presence of D-pinitol in the ethanol extract from the leaves of the plant (3). The aimed of the investigation was checking the hypoglycemic effect of aqueous and alcoholic extracts obtained from *Boldoa purpurascens* at doses of 50, 100 and 200 mg/kg using insulin 5UI/kg as a positive control and NaCl 0.9% as negative control. Another experiment was performed similar but dried aqueous extract was used at doses of 50, 100 and 200 mg/kg; using metformin at dose of 50 mg/kg as a positive control and keeping the 0.9% NaCl as a negative control. The statistical analysis was carried out by the test of Kruskal Wallis with an interval of trust of 99%. The study concluded that the species possesses hypoglycemic activity at all doses tested in both extracts, being the reduction greater for the ethanol extract (40%), comparable to insulin. **Keywords:** Boldoa purpurascens, antidiabetic **References:** 1. Roig J T (1988) Dictionary of Cuban Common names, pp 225226. 2. Gonzalez D (2006) Doctoral Theses. 3. Bates S, Jones R, Baylei C (2000) Brit J Pharmacol 130:1944 – 1948.

PM115 Influence of a semi-solid formulation of Persea americana oil fruit on the healing of cutaneous wounds in rats

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Persea americana Mill. (Avocado) oil fruit presents polyunsaturated (oleic (ω-9) and linoleic (ω-6)) and monounsaturated fatty acids (linolenic (ω-3) [1,2]. Several studies have shown a role for ω-9, ω-6 and ω-3 in the process of tissue repair [3,4,5]. The purpose of this study was to investigate the effects of a semi-solid formulation of avocado oil (SSFAO) on cutaneous wound healing of rats. Wistar rats (200 – 250 g) were anesthetized with intraperitoneal injection of ketamine (75 mg/kg) plus xylazine (15 mg/kg) followed by shaving of the skin at wounding site and an circular area (78.5mm2) of skin was surgically removed from dorsal region of the animals. After surgery, the animals were divided in groups (n = 6) and treated with topical application of SSFAO (1%, 5%, 10% or 50%), avocado oil (AO), CuratecAge® (positive control) and petroleum jelly (negative control) once daily for 14 days. Concerning to wound area (mm2) evolution, in the second day of treatment a statistically significant difference was observed between the AO group (118.88 ± 14.37) compared to positive control (86.56 ± 16.23), and in the fifth day the difference was observed between SSFAO 1% (59.52 ± 9.74) compared to negative control (92.09 ± 14.91). No difference was observed on the qualitative aspects (crust color and presence of fibrin, exudates, granulation and re-epithelialization) between SSFAO groups (1%, 5%, 10%, 50%) or AO when compared to controls. In conclusion, the topic use of SSFAO (1%, 5%, 10%, 50%) or the AO appears to have no influence over the wound healing of rats. **Keywords:** Persea americana, wound healing **References:** [1] Salgado JM et al. (2008) CTA 28: 20 – 26. [2] Tango JS et al. (2004) RBF 26: 17 – 23. [3] Manhezi AC et al. (2008) RBE 61: 620 – 629. [4] Hatanaka E Curi R (2007) RBF 88: 53 – 58. [5] Cardoso RB et al. (2004) WRR 12: 235 – 243.

PM116 The new source of biologically active substances – *Barbarea vulgaris* W. T. Aiton

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The aboveground parts of *Barbarea vulgaris* W.T.Aiton (Brassicaceae) werecollected in the Akmola region of the Republic of Kazakhstan in 2009. By means of spectrophotometric method the quantitative content of biologically active substances in the leaves, flowers and stems were determined: flavonoids (0,70%, 2,25%, and 0%) [5], carbohydrates (3.60%, 1.01%, and 0.36%) [3] and tannins (2.03%, 2.06%, and 0.08%); by method of titrimetry- organic acids (2.59%, 3.16%, and 0.58%) [2,4]. Determination of the mineral composition of ash from above-ground parts of plant was determined by mass spectrometry with inductively coupled plasma. There was found 31 elements in samples under analysis. As a result it was found that the plant is prone to the accumulation of such elements as iron (24,25*10 – 4%), silicium (8,99*10 – 4%), calcium (0,07%), potassium (0,14%), strontium (7,7*10 – 4%), magnesium (0,015%), sodium (0,014%), aluminum (9,94*10 – 4%) – the given elements contained in the most concentration in the plant. A separation scheme was developed for the study of flowers. Water-alcohol extract was concentrated to a complete removal of ethanol. The resulting aqueous extract was separated from the sediment and then the liquor was exhaustively extracted with ethyl acetate. The separated sediment was processed with petroleum ether, benzene, ethanol, aqueous alcohol, water coherently [1]. Quercetin was identified in the ethyl acetate extract while rutin was found in the alcohol extract. **References:** 1. Fedoseeva L et al. (2005) Chem Plant Substances 3: 45 – 50. 2. State Pharmacopeia USSR (1990) 11:296 – 297. 3. Zaprometov M et al. (2003) BiochemCcarbohydrates 1:324 – 326. 4. Grinkevich N et al. (1983) Chem Anal Med Plants 1: 87 – 118. 5. Khaled A et al. (2004) Quantitative Content of Flavonoids 1: 356 – 358.

PM117 Total phenolic contents and antioxidant activities of *Michauxia* L'Hérit (Campanulaceae) species growing in Turkey

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The genus *Michauxia* L'Hérit (Campanulaceae) is represented by five species in Turkey, namely *Michauxia campanuloides* L'Hérit ex Aiton, *M. laevigata* Vent., *M. tchihatchewii* Fisch. et Mey. (E); *M. thyrsoidea* Boiss. & Heldr. (E), and *M. nuda* A.DC. The fresh stem and roots of *M. campanuloides* and *M. tchihatchewii* have been used as a vegetable.In the present study, the phenolic contents and antioxidant activities of the water, methanol (MeOH), dichlormethane (DCM), Ethyl acetate (EtOAc) and butanol (BuOH) extracts obtained from the root and herb of 5 species of Michauxia collected in different parts of Turkey were compared. The phenolic contents of the samples were determined using Folin-Ciocalteu's phenol reagent. Antioxidant activities of the extracts were studied by qualitative and quantitative DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) assay to detect the free radical scavenging activity and by thiobarbituric acid (TBA) assay to detect their liposome lipid peroxidation. The total phenolic contents of extracts in the five different polarity were found the EtOAc extracts ranged from 108.1 to 439.1 mg/g in dry weight expressed as gallic acid equivalents (GAE). All extracts showed a moderately antioxidant activity with the qualitative DPPH· test. In the quantitative DPPH· method the highest activity were determinated in EtOAc extracts of five plants. High activity was observed in the herb EtOAc extracts of *M. tchihatchewii* (IC_{50}= 4.94 ± 6,46) and the MeOH extracts of *M. campanuloides* herb (IC_{50}= 97.58 ± 6,03) when compared to the other extracts in the TBA test. Propyl gallate (IC_{50}= 0.09 ± 0,18) is used as a positive control. **Keywords:** Michauxia campanuloides; M. laevigata; M. tchihatchewii; M. thyrsoidea; M. nuda; Antioxidant activity

PM118 Digitonin reverses doxorubicin resistance in cancer cells

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A decrease of the intracellular concentration of doxorubicin by activation of ABC-transporters, mainly P-glycoprotein, leads to a reduction of its chemotherapeutical efficacy. To overcome multidrug resistance, digitonin, steroidal saponin, was selected to enhance cell permeability, increase intracellular accumulation, and anticancer effect of doxorubicin. We investigated the cytotoxicity and P-glycoprotein modulatory effect of digitonin in combination with doxorubicin in resistant leukemia and colon cells. MTT assay was applied to evaluate the cell viability and reversal effect of this combination. Rhodamine123 and calcein efflux assays were used for investigate P-glycoprotein function by flow cytometry. At the molecular level, RT-PCR confirmed the data obtained. Digitonin exhibits a significant effect on viability of Caco-2 and CEM/ADR5000 cells with IC_{50} values 15.17 μM and 16.02 μM, respectively. The co-incubation of doxorubicin with non-toxic concentration of digitonin (5 μM) resulted in an enhance doxorubicin cytotoxicity in Caco-2 and CEM/ADR5000 cells by 1.9- and 1.2-fold, respectively. Digitonin increase Rho123 and calcein accumulation in Caco-2 and CEM/ADR5000 cells in dose dependent manner. Moreover, 5 μM digitonin increases the accumulation of Rho123 and calcein 1.3- and 1.1-fold of verapamil activity in Caco-2 cells. RT-PCR data indicate that 5 μM digitonin down-regulated P-gp/MDR1 mRNA to 80% of the control level. In conclusion, digitonin enhances the antitumor effect of doxorubicin and exhibits P-glycoprotein modulatory effect, so it considered as an efficient additive to the chemotherapeutic principle. **Keywords:** digitonin, anticancer, doxorubicin resistance

PM119 Cytogenetic analysis of genotoxicity of *Cynoglossum officinale* L. (Boraginaceae) extract from Bosnia and Herzegovina (W. Balkan)

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The *Cynoglossum officinale* L. have always been used in traditional medicine in Western Balkans [1]. The young shoots in some regions are used in nutrition [2]. A large number of medicinal plants of the family Boraginaceae contains pyrolizidine alkaloids and show genotoxic effects [3]. To expect a similar action of *Cynoglossum officinale*. For the analysis were taken samples of *Cynoglossum officinale* from different localities. The extracts were made of fresh aerial parts at concentrations of 0.5% and 1%. The genotoxicity was done by Allium-test. For each concentration were taken in 10 specimens. The effect of treatment, the extract was observed after 24 and 48 hours. After that, taken root and a fixed. It was examined in 10 preparations for each concentration and five for control. then determined the mitotic index, the frequency of certain phases of mitosis, the frequency of chromosome aberrations and disturbances in the meiotic spindle. The analysis examined 10 x 1000 cells. It was found that extract of *Cynoglossum* affect on mitotic activity and on other investigated parameters. Index mitosis after 48 hours is 3.5 (conc. 0.5%) and 2.7 (conc. 1%); control (4.8). The most cells were in prophase, at least in anaphase. The both of concentration of the extract cause of chromosome aberrations. There have been C-mitosis, anaphase abnormalities, irregular metaphase and a few cells with two nucleus. More intensive effects express of 1% concentration. On the basis of research can be talking about cytostatic and genotoxic activity of the extracts of *Cynoglossum officinale*. **Keywords:** genotoxicology, cytogenetics, alkaloids, Allium test, medicinal plant, human therapy, Cynoglossum officinale **References:** 1. Redzic SS (2007) Coll Antropol 31: 869 – 890. 2. Redzic SJ (2006) Ecol Food & Nutr 45(3):189 – 232. 3. Redzic A et al. (2009) Planta Med 75: 987 – 987.

PM120 Modulation of P-glycoprotein, cytochrome P450, and glutathione-S-transferase by resveratrol in human cancer cells

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Resistance of cancer cells to chemotherapy is controlled by a decrease of intracellular drug accumulation, increase of detoxification, and diminished propensity of cancer cells to undergo apoptosis. ABC-membrane transporters together with intracellular metabolic enzymes contribute to the complex and unresolved phenomenon of multidrug resistance (MDR). Resveratrol, a polyphenol of *Fallopia japonica* (Houtt.) Ronse Decr., has antiinflammatory and antioxidant properties [1]. However, it is also interesting in the field of cancer therapy [2]. The mechanisms by which resveratrol might produce anticancer effects are not well understood. In this study, resveratrol was shown to increase Rho123 and calcein accumulation in a concentration dependent manner (1 – 500 μM) in Caco-2 cells by 3 – 167% and 5 – 361% of verapamil. Morever, the treatment of CEM/ADR5000 with 10 – 100 μM resveratrol significantly inhibited the Rho123 and calcein efflux by 107 – 407%, and 164 – 460% as compared with verapamil (100%), respectively. The cytotoxicity of doxorubicin was enhanced by using 20 μM resveratrol; IC_{50} values were decreased from 4.15 to 1.23 μM, and from 33.67 to 1.81 μM, respectively. Furthermore, resveratrol significantly inhibited GST and cytochrome P450 enzyme activity in a dose dependent manner with IC 50 values 33.30 μM and 11.49 μM, respectively. RT-PCR reveals a significantly down-regulation of ABC-transporters and of smetabolic enzymes mRNA levels in Caco-2 cell lines in response to resveratrol treatment. In conclusion, the inhibition of both ABC-transporters and of metabolic enzymes could explain the advantages of resveratrol in cancer therapy. **References:** 1. Harmsen S et al. (2007) Cancer Treat Rev 33: 369 – 380. 2. Szakacs G et al. (2006) Nat Rev Drug Discov 5: 219 – 234.

PM121 Variability of phenolic contents, antioxidant and antimicrobial activities of *Inula crithmoides* from Tunisia

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Inula crithmoides L. is a spontaneous halophyte thriving on waterlogged zones. This species is harnessing edible, medicinal, aromatic and economic potentialities. In fact, this plant is known for its richness on bioactive compounds, mainly on essential oils. In this study, we tried to carry out the richness of this species on phenolic compounds and to evaluate their biological activities. Different parts of the plant were collected from Kairouan (center of Tunisia) air dried, grounded to a fine powder then subjected to a selective extraction with petroleum ether, acetone 60% then ethyl acetate in order to have a phenolics enriched fraction. Dried extracts were dissolved in methanol to be used in the colorimetric quantification of phenolics and to estimate their antioxidant activities (DPPH, total antioxidant activity, reducing power and inhibition of the β-carotene bleaching tests) and antibactrial activity against four human pathogenic bacteria. Results revealed that I. crithmoides extracts contain interesting amounts of these phytochemicals, significantly variable within the different plant parts, with highest amounts recorded in flower extracts. Besides, the entire investigated antioxidant test showed that *I. crithmoides* extracts exhibited high antioxidant activities, especially flower extracts. The effect of *I. crithmoides* extracts on the degree of inactivation of selected food borne pathogenic bacteria was variable and depended on the strains in question and on the part of the plant. These finding suggest that *I. crithmoides* is an interesting source of phenolics having antioxidant and antibacterial potentialities allowing them to be used as preservative ingredients in the food, pharmaceutical, and cosmetic industry. **Keywords:** Inula crithmoides, phenolic compounds, biological activities

PM122 Inhibitory activities of selected medicinal plants on mushroom tyrosinase

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Tyrosinase is a key enzyme in melanin synthesis from tyrosine. Using tyrosinase inhibitors has become increasingly important in medicinal and cosmetic products to prevent or treat pigmentation disorders (1).To evaluate inhibitory effects the extracts of *Urginea maritima* (L.) Baker [1], *Zhumeria majdae* Rech.f. & Wendelbo [2] and *Physalis divaricata* D.Don [3] on mushroom tyrosinase this study was designed. L-Dopa as used as substrate. Ethanolic extracts of *U. maritima*[bulb], *Z. majdae* [leaves] and *P. divaricata* [air organs] were used for their inhibitory effect in vitro on diphenolase activity of tyrosinase, using a specterometric method. The extracts showed anti tyosinase activity weaker than positive control (Kojic acid). The inhibitory activity of tested plants *Urginea maritima*, *Zhumeria majdae* and *Physalis divaricata* against mushroom tyrosinase is expressed using IC_{50} (concentration of inhibitor that inhibited 50% of tyrosinase activity) values of 2.79, 2.37, 3.34 mg/ml respectively.The kinetic study indicated that all extracts were uncompetitive inhibitors for tyrosinase. **Keywords:** *Urginea maritima*, *Zhumeria majdae*, *physalis divaricata*, mushroom tyrosinase, Inhibitor **Acknowledgement:** *This research is a part of a project granted by Ahvaz Jundishapur University of Medical Sciences.* **References:** Zhang X Hu et al. (2009) Biological and Pharmaceutical Bulletin 32(1): 86 – 90.

PM123 Anti-inflammatory activity of ointments with dry extracts of rhizome and herb of *Aremonia agrimonoides*, (L.) DC (Rosaceae)

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Aremonia agrimonoides,(L.) DC (Rosaceae), also known as Bastard Agrimony, is a native plant species in the central Europe. The aqueous extracts of rhizome and herb were prepared by method of percolation with water. The liquid extracts were then evaporated in stream of nitrogen. The content of phenolics was determined by the method with Prussian Blue (1). The ointments with 1% dry extract of rhizome and 1% dry extract of herb were prepared in paraffin ointment. The anti-inflammatory effect of ointments was tested through the model of mouse ear model. Inflammation of both ears of albino mice (both sexes) was induced by applying 10 µl of 3% solution of acetone-based croton oil (2). As control we used 1% hydrocortisone ointment. Ointments were applied once a day in the period of three days on the left ear, two hours after inducing inflammation. The right ear was not further treated. The appearance of the ears observed during three days was expressed in scores on 0 – 14 scale. The mean values recorded on the third day after the causing of ear inflammation were: for ears treated with ointment with extract of rhizome 6 ± 1, for ears treated with the extract of aerial part of plant 7 ± 1, for untreated ears 12 ± 2, and for ointment with 1% hydrocortisone 6 ± 1. Content of phenolic compounds in the extracts was 12,09% for the rhizome and 12,76% for the herb. The pharmacological response to both ointments was similar with the ointment with 1% hydrocortisone. **Keywords:** antiinflammatory, aremonia, mouse ear **References:** 1. Price ML, Butler LG (1977)J Agric Food Chem 25:1268 – 1273. 2. Williamso EM, Okpako DT, Evans FJ.(1996) Selection, Preparation and Pharmacological Evaluation of Plant Material in: Pharmacological Methods in Phytotherapy Research; John Wiley Sons p. 131 – 153.

PM124 Change of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn

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This study was carried out to evaluate changes of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn. Black waxy corns have pericarps colored black and Black pericarps contain anthocyanins. Anthocyanins relate to antioxidant activities. Thirty black waxy corn inbred lines were planted at upland crop fields of National Institute of Crop Science in Korea, 2009. They were evaluated total anthocyanins content, respectively. Base on these results, they were classified by 3 groups. Three groups were mutually crossed. Their F1 seeds were planted in upland crop fields of National Institute of Crop Science in Korea, 2010. They were classified 8 crossing groups by crossing combinations. These crossing groups were harvested at 19 days, 21 days, 23 days and 25 days after silking date, respectively. And their products were evaluated by total anthocyanins content and lightness. As increasing of harvest days, total anthocyanins content were increased, but lightness was not. The total anthocyanins content and lightness were analyzed correlation by SAS Enterprise Guide 4.2. They have negative correlation and coefficient of determinant (R2) was 0.7151. **Keywords:** black, waxy, corn, anthocyanin, days after silking date **References:** 1. Lopez Martinez et al. (2009) Food Science and Technology 42: 1187 – 1192

PM125 Quercetagetin, a component of premature *Citrus unshiu* (Swingle) Marcow., suppress the chemokines related with atopic dermatitis by regulating STAT1 signal

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Atopic dermatitis (AD) is an ithchy and relapsing inflammatory skin disease. It was known that a predominant systemic Th2 dysbalance with

increased IgE levels and eosinophilia is widely accepted in the pathogenesis of AD [1]. Thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22) are related with AD and are elevated in serum and lesional skin of AD patients [2, 3]. Citrus unshiu (CU) contains various flavonoids that have various bioactive effects [4]. In present study, we investigated the effect of a component of premature CU, quercetagetin, on the production of TARC and MDC in HaCaT human keratinocytes. As results, quercetagetin showed inhibitory activity on the protein production and mRNA expression of TARC and MDC in IFN-γ and TNF-α-stimulated HaCaT human keratinocytes. Also, quercetagetin inhibited the phosphorylation of STAT1, key transcription factor initiating IFN-γ signaling pathway, in a time- and dose-dependent manner. These results suggest that quercetagetin, a component of premature CU, may have an anti-atopic activity by inhibiting the inflammatory chemokines (TARC and MDC) via the STAT1 pathway. **Keywords:** quercetagetin, Citrus unshiu, Atopic dermatitis, TARC/CCL17, MDC/CCL22, Jak-STAT pathway **References:** 1. Bieber T (2010) Ann Dermatol 22 (2): 125 – 137. 2. Hijnen D et al. (2004) J Allergy Clin Immunol 113 (2): 334 – 340. 3. Leung TF et al. (2003) Pediatr Allergy Immunol 14 (4): 296 – 301. 4. Kim YD et al. (2009) Korean J Nutr 42(3): 278 – 290.

PM126 Solvent extracts of *Carpinus tschonoskii* suppress the expression of atopic inflammatory cytokines and chemokines in RAW264.7 macrophages and HaCaT keratinocytes

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Atopic dermatitis (AD) is a common, chronic relapsing, inflammatory skin disease characterized by pruritus and inflammation and accompanied by cutaneous physiological dysfunction through chemokine-mediated infiltration of numerous mononuclear cells in lesional skin [1]. TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22) that bind to the chemokine receptor CCR4 which is highly expressed on T-helper 2 cells lead to preferential influx of Th2-type lymphocytes to the lesianal skin in AD [2]. Furthermore, cytokines are another triggers of AD and the expressions of inflammatory cytokines (TNF-α, IL-1β and IL-6) increase in lesional skin macrophages of AD patients [3]. In present study, we investigated the anti-inflammatory effects of *Carpinus tschonoskii* Maxim. in RAW264.7 murine macrophage and HaCaT human keratinocytes. As results, the CHCl3 sub-fractions (C-4, -5, and -6 fr.) dose-dependently inhibited the production of TNF-α, IL-1β and IL-6 in the LPS-stimulated RAW264.7 murine macrophage. Also, they inhibited the mRNA expression and protein level of TARC and MDC *via* suppressing the phosphorylation of STAT1 protein in IFN-γ-stimulated HaCaT human keratinocytes. These results suggest that *C. tschonoskii* may be an effective source for improving atopic dermatitis by inhibiting the inflammatory cytokines and chemokines. **Keywords:** Carpinus tschonoskii, Atopic dermatitis, TARC/CCL17, MDC/CCL22, RAW264.7 macrophages, HaCaT keratinocytes **References:** 1. Bieber T (2008) N Engl J Med 358:1483 – 94. 2. Sekiya T et al. (2000) J Immunol 165:2205 – 2213. 3. Grossman RM et al. (1989) Proc Natl Acad Sci USA 86: 6367 – 71.

PM127 Effect of *Moringa oleifera* extract on experimental reflux oesophagitis in rats

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Moringa oleifera Lam. (Family: Moringaceae) is commonly known as drumstick tree or horseradish tree. The leaves are highly nutritious, being a significant source of beta carotene, Vitamin C, protein and antioxidants [1]. In the present study, protective effect of hydroalcholic extract of the leaves of *M. oleifera* on experimental reflux oesophagitis in rats was investigated. Rats received *M. oleifera* extract (200, 400 mg/kg), omeprazole (30 mg/kg) given at 1 h prior to surgery [2]. *M. oleifera* extract at doses 200, 400 mg/kg significantly inhibited the oesophagitis index ($P < 0.001$) as compare to control. Further, acid and pepsin out put of gastric contents were significantly decreased in treated groups. *M. oleifera* extract (400 mg/kg) significantly inhibited the lipid peroxidation (from 0.58 ± 0.03 to 0.38 ± 0.02 nmol of malonyldialdehyde (MDA)/mg protein) ($P < 0.001$) and increased in levels of catalase to 25.4 ± 2.8 units of catalase activity/mg protein and superoxide dismutase (SOD) to 71.2 ± 5.8 units/mg protein ($P < 0.001$). *M. oleifera* extract (200 mg/kg) and omperazole also showed significant inhibition in lipid peroxidation ($P < 0.05$) and enhanced the activities of catalase ($P < 0.01$) and SOD activity. Further, it altered the elevated levels of sialic acid and hexose contents in oesophageal tissue. Indeed, *M. oleifera* significantly decreased the elevated plasma histamine content ($P < 0.05$). The results suggested that antioxidants potential of *M. oleifera* could attenuate the severity of reflux oesophagitis and prevent the oesophageal mucosal damage. **Keywords:** *Moringa oleifera*, Reflux Oesophagitis, Antioxidant **References:** 1. Verma A R (2009) Food Chem Toxicol 47: 2196 – 2201 2. Rao ChV, Vijayakumar M (2008) Eur J Pharmacol 589: 233 – 238

PM128 *In vitro* anticariogenic effects of polyphenolics from *Potentilla recta*

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Most pharmacological studies have confirmed the traditional use of *Potentilla* species in many diseases. These pharmacological effects of *Potentilla* species can be explained by the high amount of polyphenolic compounds present in all plant parts [1,2]. *Potentilla recta* L. (sulfur cinquefoil) is a long-lived invasive perennial plant from Eurasia that has become one of the most serious invaders of natural area grasslands in North America and Canada. It has been reported to contain 2-pyrone-4,6-dicarboxylic acid, which seems to be a chemotaxonomic marker for the *Potentilla* species. Ranges of seasonal changes in the content of chlorophyll, carotenoids, free organic, ascorbic and triterpenic acids, neutral triterpenoids and fat oils in some organs and overground phytomass of *P. recta* have also been determined. More recently, from the aerial parts of *P. recta*, ten compounds including a neolignan glycoside and flavonol derivatives have been isolated [3,4]. The purpose of the current study was to investigate *in vitro* the antibacterial activity (by determination of the MICs) of selected polyphenolic compounds: methyl brevifolincarboxylate (I), tiliroside (kaempferol 3-O-β-D-(6"-E-p-coumaroyl)-glucopyranoside) (II), ellagic acid 3, 3'-di-O-methyl ether 4-O-β-D-xylopyranoside (III), apigenin 7-O-β-D-glucopyranoside (III) as well as luteolin 7-O-β-D-glucopyranoside (IV) against cariogenic *Streptococcus* spp. strains (*S. mutans* CAPM 6067, *S. sobrinus* CAPM 6070, *S. sobrinus* GCM 20381 and *S. sobrinus/downei* CCUG 21020). MIC values the tested substances were above 400 μg/mL (MIC> 400 μg/mL). Chlorhexidine was used as positive control. In addition their inhibitory effects on insoluble glucan (mutan) and artificial dental plaque formation were also determined [3]. **Keywords:** *Potentilla recta*, polyphenolic compounds, anticariogenic activity **Acknowledgement:** *This study is financially supported by the Polish Ministry of Science and Higher Education (Grant No. N N405 621638)* **References:** 1. Tomczyk M, Latté KP (2009) J Ethnopharmacol 122: 184 – 204. 2. Tomczyk M, Pleszczyńska M, Wiater A (2010) Molecules 15: 4639 – 4651. 3. Tomczyk M, Wiater A, Pleszczyńska M (2011) Phytother Res 25: 343 – 350. 4. Şöhretoğlu D, Kırmızıbekmez H (2011) Biochem Syst Ecol 39: 132 – 134.

PM129 Phytoestrogenic activity of *Sophora flavescens* extract and its constituents

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Sophora flavescens Ait. is monographed in the Chinese Pharmacopoeia as Radix Sophorae flavescentis (Kushen) [1]. The crude drug is traditionally used for the treatment of diarrhoea, gastro-intestinal haemorrhage and eczema. *S.flavescens* roots contain quinolizidin alkaloids, non-glycosidic flavones, and triterpene glycosides. Although some of the flavones found in this medicinal plant hint to potential estrogenic properties, surprisingly, still up to now, very few reports on the estrogenic activity of extracts from this drug have been published [2]. Due to the presence of the alkaloids, *S. flavescens* roots have some toxic potential. Thus, we have engaged in the development of a special extract from this drug

which is characterized by the absence of alkaloids [3]. This extract was tested for estrogenic activity in a panel of suitable test models. Besides a significant competitive binding to estrogen receptors alpha (ER alpha) and ER beta, induction of alkaline phosphatase in Ishikawa endometrial adenocarcinoma cell was observed. Unfortunately, the extract did not display any estrogen receptor selectivity and promoted uterine growth in ovariectomized rats. Hence, it was considered inappropriate for the treatment of climacteric complaints and precluded from further product development. **Keywords:** Sophora flavescens, antiestrogenic activity **References:** 1. Kuang L, Zhang K (2005) Pharmacopoeia of the Peoples Republic of China, Vol.I, People's Medical Publishing House, Beijing. 2. Hillerns PI, Wink M (2005) Planta Med 71: 1065. 3. Dr. Willmar Schwabe GmbH & Co., European Patent EP 1294388 B1 (granted 2004)

PM130 Antibacterial activity of plant extracts highly depends on extraction solvent

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As an alternative to antibiotic growth promoters in animal nutrition, that have been banned in the EU in 2006, the demand for plant derived substances (phytogenics) is emerging to counteract bacterial infections in swine and poultry. In contrast to antibiotics, phytogenics are expected to refrain from causing transmissible bacterial resistances and leaving critical residues in animal tissue. Looking for potential phytogenics, five different plant raw materials (*Berberis aristata* DC. root, *Sophora flavescens* Aiton root, *Holarrhena antidysenterica* (L.) Wall. bark, *Bridelia ferruginea* Benth. bark and leaves) were selected. Dry extracts were produced of each material using different extraction solvents (ethanol abs., water and 50/50 (v/v) ethanol/water). The antibacterial activity of the extracts on two pathogenic bacteria, *Salmonella typhimurium* and *Clostridium perfringens* Type C, was examined with a turbidimetric microdilution method. The bacterial cultures with defined microbial count were incubated together with different concentrations of the extracts. The change in optical density of the bacterial culture led to a quantitative result, indicated as the MIC_{50} value. The lowest MIC_{50} values were reached by the ethanol extracts of *B. aristata* (78 mg/l) and *S. flavescens* (156 mg/l) against *C. perfringens*. The ethanol and ethanol/water extract of *H. antidysenterica* showed higher activity against *S. typhimurium*. In fact, the ethanol extracts of all plant materials were most effective, except for the extracts of *B. ferruginea* bark, whereof the water extract was most effective against *C. perfringens* (MIC_{50} value 156 – 625 mg/l). Based on these findings about extraction solvent-dependent activity, further investigations towards active substance identification will be accomplished.

PM131 Phytochemistry and biological activities of the ethanolic extract of *Onosma aucherianum*

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This study was aimed at evaluating the antioxidant activity and efficacy of the ethanolic extract of the endemic plant species *Onosma aucherianum* DC. in inhibiting the development of selected fungi and bacteria. The highest susceptibility to the ethanolic extract of *O. aucherianum* among the bacteria tested was exhibited by B. subtilis and S. aureus (MIC = 15.62 µg/ml). Among the fungi, *A. niger* (MIC = 15.62 µg/ml) showed the highest susceptibility. Total phenolic, flavonoid, condensed tannin and gallotannin contents were 90.26 ± 0.69 mg GA/g, 35.24 ± 0.55 mg RU/g, 74.65 ± 0.75 mg GA/g and 31.74 ± 1.05 mg GA/g, respectively. Total antioxidant capacity was 78.45 ± 0.98 µg AA/g. IC_{50} values were determined for each measurement: 21.45 ± 1.55 µg/ml for DPPH free radical scavenging activity, 36.46 ± 1.68 µg/ml for inhibitory activity against lipid peroxidation, 99.11 ± 0.23 µg/ml for hydroxyl radical scavenging activity and 45.91 ± 0.88 µg/ml for chelating ability. The rosmarinic acid was found to be the dominant phenolic compound of the extract. **Keywords:** antimicrobial activity, antioxidant activity, Onosma aucherianum, HPLC analysis, phenolic compounds **Acknowledgement:** *Serbian Ministry of Agriculture, Forestry and Water Management, STAR Project No. 401 – 001972/2010 – 03.*

PM132 Topical anti-inflammatory activity of *Plantago lanceolata* L. leaves: the relevance of triterpenic acids

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The leaves of *Plantago lanceolata* L. (Plantaginaceae) are used in traditional medicine for the topical treatment of skin inflammatory affections [1]. Although *P. lanceolata* leaf extracts and some of their constituents have been shown to inhibit *in vitro* enzymes involved in inflammation [1, 2], the *in vivo* topical anti-inflammatory properties of the leaves have not been investigated. Therefore, *P. lanceolata* leaves have been studied for their topical anti-inflammatory activity by the Croton oil-induced ear dermatitis assay in mice [3]. *P. lanceolata* leaves were sequentially extracted with *n*-hexane, chloroform and methanol and the relevant extracts were evaluated for their ability to inhibit the mouse ear edema induced by Croton oil. Each extract (300 µg/cm^2) provoked a significant edema reduction, the chloroform one being the most active. Its potency was only two fold lower than that of the reference non steroidal anti-inflammatory drug indomethacin: their ID_{50} (dose inducing 50% edema inhibition) values were 186 and 97 µg/cm^2, respectively. By column chromatography, the chloroform extract was separated in five fractions (A-E), concentrating its activity into fraction C, which was constituted mainly by ursolic acid (44%) and oleanolic acid (27%). These compounds induced a dose-dependent edema inhibition, and ursolic acid (ID_{50}= 56 µg/cm^2) was more active than oleanolic acid (ID_{50}= 132 µg/cm^2) and indomethacin. The two triterpenes, which give a significant contribution to the anti-inflammatory activity of the parent extract, can be proposed as parameters in the quality control of *P. lanceolata* leaf preparations for the topical use against skin inflammations. **References:** 1. Beara IN et al. (2010) J Pharm Biomed Anal 52: 701 – 706. 2. Vigo E et al. (2005) J Pharm Pharmacol 57: 383 – 391. 3. Tubaro A et al. (1985) Agents Actions 17: 347 – 349.

PM133 *Cyathula prostrata* inhibits *in vitro* cancer cell growth via multiple targets

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The *in vitro* anticancer activity of an 80% ethanol extract of *Cyathula prostrata* (L.) Blume, an annual branching shrub used by traditional healers in Nigeria to treat cancer was investigated. IC_{50} values were 100.8 µg/ml and 64.4 µg/ml for HeLa (cervical cancer) and U937 (myelo-monocytic) cell lines, respectively. Further experiments were performed using 125 µg/ml *C. prostrata* extract and 50 µM cisplatin as positive control. More than 80% of the cells were arrested in the G1 phase after 48 hours of *C. prostrata* treatment. The annexin V-FITC/PI assay revealed an increase in percentage apoptotic cells from 4.9% to 53.1% at 24 h. Cell cycle arrest was not accompanied by increased levels of the cyclin-CDK inhibitor p21. Increase in caspase-8 activation was observed in response to treatment with the extract with no cyt-c release from the mitochondria. The lack of cyt-c release was due to no change in mitochondrial membrane potential, which was investigated with the aid of fluorescent mitochondrial dyes and flow cytometric techniques. The results therefore show that *C. prostrata* extract induces apoptosis via the extrinsic pathway and this activation is independent of the mitochondria. Levels of hTERT, the catalytic subunit of telomerase, were also shown to decrease upon *C. prostrata* treatment. The findings from this study suggest that the extract acts through multiple targets, by inducing: cell cycle arrest in the G1 phase through an unknown mechanism; apoptosis through an extrinsic death receptor pathway and replicative senescence through inhibition of telomerase. **Keywords:** *Cyathula prostrata* apoptosis, caspase 8, telomerase, cell cycle arrest **Acknowledge-**

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PM134 Total Phenols, Antioxidant potential and Antimicrobial Activity of the Methanolic Extracts of *Ephedra sarcocarpa*

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The antioxidant activities of the methanolic extracts of *Ephedra sarcocarpa* Aitch. & Hemsl. growing in Iran was evaluated using ferric reducing antioxidant power (FRAP) [1] and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] free radical scavenging assays.FRAP values 2.1 mmol eq quercetin/g extracts, and IC_{50} values in the DPPH assay 4.6 mg/mL. this plant showed the high antioxidant activities.this plant showed the high antioxidant activities. FRAP and DPPH assay results showed good correlations with the total phenolic contents [3] of the plants, measured by the Folin-Ciocalteau assay.(r^2= 0.920and 0.893, respectively, $p < 0.0001$). The antimicrobial capacity [4] was screened against Gram-positive and Gram-negative bacteria and fungi. the extract inhibited the growth of Gram-negative bacteria, being *pseudomonas aeroginosa* the most susceptible one with MIC of 16 μg/mL for the extract.The results obtained indicate that *E.sarcocarpa* may become important in the obtainment of a noticeable source of compounds with health protective potential and antimicrobial activity. **Keywords:** Antioxidant(s), FRAP, DPPH, Total phenol, Antimicrobial, Ephedra sarcocarpa **References:** 1. Benzie I F, Strain JJ (1996) Journal of Analytical Biochemistry 239: 70 – 76. 2. Hwang BY et al. (2001) Journal of Natural Products 64: 82 – 4. 3. Singleton VL, Rossi JA (1965) American Journal of Enology and Viticulture 16: 144 – 158. 4. Bauer AW, Kirby WMM, Sherries JC, Turck M (1966) American Journal of Clinical Pathology 45: 493 – 496.

PM135 Is the inhibition of STAT3 phosphorylation in vascular smooth muscle cells by indirubin-3'-monoxime redox-dependent?

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Indirubin is a natural product found in the traditional Chinese antileukemic recipe, Danggui Longhui Wang.[1] Its reported anti-proliferative activity makes it a promising candidate in the treatment of cardiovascular diseases (CVDs). We showed recently that the derivative indirubin-3'-monoxime (I3MO) inhibits the proliferation of vascular smooth muscle cells (VSMC) by inhibition of STAT3 phosphorylation.[2] The importance of reactive oxygen species (ROS) in STAT3 activation has been reported[3] and oxidative stress has been implicated in many CVDs.[4] Here, we examine the role of ROS as a putative target of I3MO acting upstream of STAT3. Employing the fluorescence probes 2',7'-dichlorodihydrofluorescein and Amplex Red™, I3MO was shown to inhibit PDGF-induced intracellular ROS production and H_2O_2 release. Interestingly, the compound did not exhibit radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Western blot analyses revealed that STAT3 phosphorylation triggered by exposure of VSMC to exogenous H_2O_2 is also blunted by I3MO. Furthermore, diphenyliodonium (3 μM), an inhibitor of NAD(P)H oxidases (Nox) and other flavoproteins, mimicked the selective effect of I3MO on PDGF-induced STAT3 phosphorylation. Selective downregulation of Nox1 and Nox4 isozymes, using the inhibitory peptide, gp91tat and the siRNA approach, respectively, did not prevent PDGF-induced STAT3 phosphorylation. This study shows for the first time that I3MO reduces PDGF-induced oxidative stress. Additionally, I3MO-mediated STAT3 inhibition, which was linked to its anti-proliferative effect on VSMCs, was shown to be redox-dependent. Despite being considered the major ROS-source in VSMCs, Nox isozymes, Nox1 and Nox4, are not targets of I3MO responsible for the effect on STAT3 phosphorylation. **Keywords:** indirubin, vascular smooth muscle cells, proliferation, atherosclerosis, STAT3, PDGF, reactive oxygen species, NAD(P)H oxidase **Acknowledgement:** *This work was supported by the Austrian Science Foundation (FWF) (P23317 to E.H.H., NFN S107-BO3 to V.M.D. and P 18982 to V.M.D.)* **References:** 1. Meijer L et al. (1999) Nature Cell Biol 1: 60 – 7. 2. Schwaiberger AV et al. (2010) ATVB 30: 2475 – 81. 3. McCormick J et al. (2006) FASEB J 20: 2115 – 17. 4. Bronas, UG, Dengel DR (2010) Am J Lifestyle Med 4: 521 – 534.

PM136 Antioxidant, antimicrobial, anti-inflammatory and anticancer activities of *Carthamus tinctorius* flowers

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Carthamus tinctorius L. (Asteraceae) is an aromatic and folkloric medicinal plant thanks to its multiple virtues. However, few scientific studies investigated its biological activities. For that, this study aimed to investigate antioxidant, antibacterial, anti-inflammatory and anticancer activities of methanolic flower extracts of *Carthamus tinctorius* in order to validate some of its ethnopharmacological claims. Antioxidant activity was assessed via the ABTS radical scavenging and β-carotene inhibition tests, the antibacterial capacity were tested against human pathogen strains. Whereas, anti-inflammatory activity were estimated using inhibition NO release in LPS-stimulated Raw 264.7 macrophages, in comparison with N(G)-nitro-L-arginine methyl ester (L-NAME) which used as a positive control. In addition, anticancer activity was evaluated against Human lung carcinoma (A-549) and Human colorectal adenocarcinoma (DLD-1) cell lines. Main results showed that the flowers exhibit interesting biological activities. Indeed, flower extract displayed an inhibition percentage against ABTS equal to 30% and over 40% for the β-carotene inhibition assay. Antimicrobial activities were important especially against *M. luteus* strains (100% inhibition). Concerning anti-inflammatory activity, methanolic extract was able to inhibit NO release by 80% at 160 μg/ml. Furthermore, *C. tinctorius* extract showed an anticancer activity against tumor cell lines DLD-1 with an IC_{50} value of 72 ± 9 μg/ml. These findings demonstrate the interesting potentiality of *Carthamus tinctorius* flowers as valuable source of antioxidant compounds which exhibit novel biological activities as antibacterial, anti-inflammatory and anticancer capacities. **Keywords:** Carthamus tinctorius, antioxidant capacity, antibacterial activity, anti-inflammatory activity, anticancer ability

PM137 Evaluation of Hydroalcoholic extract of *Astragalus fasciculifolius* Boiss. on Immunological factors IFN-γ, IL-4 in early sensitized mice induced by Ovalbumin

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The genus *Astragalus* is a very large group of more than 2,000 species and about 800 species in Iran. Currently, much of the pharmacological research on *Astragalus* is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immune deficiency conditions. *Astragalus* has demonstrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. *Astragalus* can modulate the balance of Th 1 /Th 2 cytokines; it decreases IL-4 and increases IFN-γ. Since, allergy conversely disturbs the balance of Th 1 /Th 2, increases IL-4 and decreases IFN-γ; we decided to use *Astragalus fasciculifolius* Boiss. to improve the balance. Hydroalcoholic extract of *Astragalus fasciculifolius* assessed by phytochemical tests to recognize the main active constituents. Mice were sensitized with subcutaneous injection of 100 μg of ovalbumin, 1 mg aluminum hydroxide, days 1 and 7. efficiency of sensitization was assessed by blood IgE levels. then 14

days 250 mg/kg & 500 mg/kg extract was interaperitoneally injected.on day 14, mice were challenged with intraperitoneal injection of 10 μg of ovalbumin. IL-4 and IFN-γ levels in bronchoalveolar lavage (BAL), was assessed by ELISA kits., The results showed significant differences ($P < 0.05$). Phytochemical tests showed, terpenoids and flavonoids. Intraperitoneal injection of *Astragalus fasciculifolius* was able to decrease IL-4 and increase IFN-γ, consequently Herbal species has the potential to modulate the balance of Th1/Th2 cytokines in allergy. **Keywords:** *Astragalus fasciculifolius* , Immunologic factors, IFN-γ, IL-4 **Acknowledgement:** *Ahvaz Jundishapur University of Medical Sciences*

PM138 Betulinic acid enhances glucose uptake in 3T3L1 adipocytes after long term treatment

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The metabolic syndrome including hyperglycaemia and insulin resistance is on the rise worldwide and consequently also cardiovascular diseases and Diabetes Mellitus Type 2. Currently used drugs for these indications are effective, but possess side effects when used chronically. Nature could provide a variety of compounds with undiscovered potential to treat and prevent these disorders. In this study, we tested betulinic acid (BA), a naturally occurring pentacyclic triterpenoid, in two diabetes-related assays, namely inhibition of the Protein Tyrosine Phosphatase 1B (PTP1B) in vitro and 2-deoxy-D-glucose (3 H-DOG) uptake in 3T3L1 adipocytes. We found no inhibition of PTP1B activity by BA despite its close structural similarity to ursolic acid, a known natural PTP1B inhibitor. However, in differentiated 3T3L1 adipocytes, BA (10 μM) elicited a 1.8-fold increase of the basal glucose (3 H-DOG) uptake rate after 48 hours of treatment. The observed increase in glucose uptake was further enhanced by insulin stimulation and not accompanied by a decrease in cell viability as evident by unaltered cell morphology under the microscope and lack of procaspase 3 cleavage shown by immunoblots. Interestingly, incubation of RAW264.7 macrophages and immortalized human umbilical vein endothelial cells (HUVECs) with 10 μM BA also increased their basal glucose uptake rate approximately 1.4-fold and 1.7-fold, respectively. Given the vast number of so far reported anti-fungal, anti-viral, anti-bacterial and anti-cancer properties of BA [1], our data indicate that BA may be successfully repurposed also for metabolic disorders (hyperglycemia), and warrant further investigations concerning the underlying mode of action. **Keywords:** adipocytes, hyperglycemia, triterpenoids, metabolic syndrome **References:** [1] Mullauer FB et al. (2010) Anticancer Drugs 21(3):215 – 227

PM139 Evaluation of antidiabetic and anti-inflammatory properties of Malaysian Rubiaceae and correlation to their antioxidant potential

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We have previously reported the antioxidant activities of methanolic extracts of 22 species of Rubiodeae plants (family Rubiaceae) [1]. In this paper, we now report the antihyperglycemic and anti-inflammatory properties of the 22 species. The assays employed were α-glucosidase inhibition assay for antidiabetic potential and Griess assay for the measurement of nitric oxide (NO) inhibition in lipopolysaccharide (LPS) and interferon-γ (IFN-γ)-treated RAW 264.7 cells. In the α-glucosidase inhibitory assay, extracts of *Hydnophytum formicarum* Jack, *Psychotria griffithii* Hook.f. and *Urophyllum griffithianum* Hook.f. were shown to be effective inhibitors against α-glucosidase. The results indicated that *H. formicarum* and *P. griffithii* showed high percent inhibition in the α-glucosidase inhibitory assay with percent inhibition of 89.8% and 87.7%, respectively. *U. griffithianum* showed moderate activity with percent inhibition of 68.4% while other species showed no activity. In the anti-inflammatory assay, *Hedyotis philippinensis* (Wild. ex Spreng.) Merr. ex C.B. Rob. (leaves and stems), *Spermacoce exilis* (L.O.Williams) C.D.Adams and *Spermacoce latifolia* Aubl. showed potent inhibitory activity on NO production in LPS and interferon-γ (IFN-γ)-induced RAW 264.7 cells. We have also found a good correlation ($R^2 = 0.684$) between DPPH radical-scavenging activity and α-glucosidase inhibition. Our results support the findings that antioxidants (specifically, radical-scavengers) play an important role in the control and management of diabetes [2]. **Keywords:** Rubiaceae, Rubioideae, antidiabetic, anti-inflammatory, α-glucosidase, NO inhibition **Acknowledgement:** *Research and Management Institute (RMI), Universiti Teknologi MARA and Ministry of Higher Education (MOHE) for research grant 600-RMI/ST/FRGS 5/3/FST(35/2009) and Dr Shamsul Khamis from Universiti Putra Malaysia for identification of plants.* **References:** 1. Ahmad R et al. (2010) African Journal of Biotechnology 9: 7948 – 7954. 2. Rahimi R, Nikfar S, Larijani B & Abdollahi M (2005) Biomedicine and Pharmacotherapy 59: 365 – 373.

PM140 Anti-sickling studies of Nigerian plants

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Sickle cell disorder is a public health problem in many countries particularly in Africa. It is one of the most prevalent heamatologic genetic disorders which results from a single point mutation of βGlu6 in Hb to βVal6 in Hbs (1). No drug could effectively cure the disorder but a potentially useful drug if available should effectively provide relief by alleviation or prevention of its symptoms. Nonetheless, there are few anti-sickling agents to date available for clinical use (2). In view of its genetic origin, advocacy remains the only option for the prevention of the disorder. However, with over 1 million individuals worldwide with sickle cell disorder, the search for ideal anti-sickling drugs is a major priority. The present screening study reports the anti-sickling properties of eight Nigerian plant species with inhibitory and reversal properties. Extracts of the *Cola* species tested, in particular, showed the same order of activity as p-hydroxybenzoic acid, the positive control. **Keywords:** Sickle cell disorder, plants, *Cola* species, anti-sickling properties **References:** 1. Quattara B et al. (2009) Phytomedicine 16: 125 – 129 2. Martin KS et al (2004) J Med Chem 47: 4665 – 4676

PM141 *In vitro* COX-1, COX-2 and 5-LOX inhibitory activity of plant family Ranunculaceae

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The arachidonic acid metabolism is the main target for non-steroidal anti-inflammatory drugs (NSAIDs). Two cyclooxygenases (constitutive COX-1 and inducible COX-2) and lipoxygenase (5-LOX) enzymes are responsible for transformation of arachidonic acid into the potent biologically active lipid mediators that are intimately involved in inflammation [1]. The newly developed COX-2 selective inhibitors seem to possess lower risk of unwanted side-effects than traditional NSAIDs. In our previous study, we identified potential COX-2 inhibiting plant material in Ranunculaceae family [2]. It is now being perceived, that dual blocking of both COXs and 5-LOX is promising approach to treatment of inflammatory diseases [3]. Thus we decided to evaluate the *in vitro* inhibitory activity against both COXs and 5-LOX of ethanolic extracts prepared mainly from roots of more than 30 plant species belonging to plant family Ranunculaceae using method previously described by Reininger and Bauer [4] and Adams et al. [5], respectively. The amounts of prostaglandin E_2 (for COX) and leukotriene B_4 (for 5-LOX) were determined by commercial EIA kits (Assay Designs). The highest prevention against production of COXs and 5-LOX derived eicosanoids possessed extract form roots of *Helleborus purpurascens* Waldst. & Kit., where COX-1/COX-2/5-LOX scavenging rate 1,5/1/1,2 was recorded. The consequent bioactivity guided fractionation showed, that isomers of linoleic acid seem to be responsible for blocking of even COXs or 5-LOX. *Cimicifuga racemosa* (L.) Nutt. and *Trollius altissimus* Crantz had been determined as other promising plant materials, suggesting these species potential for further research for new anti-inflammatory substances. **Keywords:** cyclooxygenases, lipoxygenase, anti-inflammatory, Ranunculaceae, *in vitro* **Acknowledgement:** *This research was supported by Czech Science Foundation (Project No. 525/08/1179).* **References:** 1. Claria J,

Romano M (2005) Curr Pharmacol Des 11: 3431 – 3447. 2. Malik J et al. (2009) Planta Med 75: 1059 – 1059. 3. Charlier C, Michaux C (2003) Eur J Med Chem 38: 645 – 659. 4. Reininger EA, Bauer R (2006) Phytomedicine 13: 164 – 169. 5. Adams M et al. (2004) Planta Med 70: 904 – 908

PM142 *Sutherlandia frutescens* targets adipose tissue mitochondrial metabolism

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Sutherlandia frutescens (L.) R.Br. ex W.T.Aiton is an indigenous South African medicinal plant traditionally used to treat a number of ailments including diabetes. While previous *in vivo* studies have confirmed its anti-diabetic properties, the precise molecular mechanism of action has not been elucidated. In the present study we have established that *S. frutescens* treatment specifically attenuates a number of adipose tissue related paramaters, including circulatory and adipose tissue free fatty acid and triglyceride levels. The lack of any significant changes in adipose tissue nitrotyrosine and plasma MCP-1, both classical markers for adipose inflammation, indicates that these effects are not attributable to anti-inflammatory properties. In 3T3-L1 preadipocytes, treatment led to a significant increase in the rate of glucose consumption despite the complete absence of triglyceride accumulation. This increased glucose consumption is reflected by a corresponding dose dependent increase in lactate production, suggesting an increased glycolytic flux in treated cells. Taken together our *in vivo* and *in vitro* findings are consistent with a hypothesis in which *S. frutescens* induces mitochondrial uncoupling in adipose tissue, resulting in a reduced efficacy of oxidative phosporylation and a consequent up-regulation of glycolysis. In this manner the carbon flux is redirected away from lipid synthesis resulting in both decreased free fatty acid production and triglyceride accumulation in adipose tissue. Increased markers for mitochondrial function, elevated levels of phosporylated AKT and the effects on PI3K regulated glucose uptake in 3T3-L1 treated cells provide further support that *S. frutescens* counteracts adipocyte dysfunction associated with the development of diabetes. **Keywords:** *Sutherlandia frutescens*, adipose tissue, lipid metabolism, mitochondria

PM143 Wound healing and anti-inflammatory activities of the *Michauxia* L'Hérit (Campanulaceae) species native to Turkey

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Michauxia L'Hérit (Campanulaceae) species are used as vegetable and medicinal plants in Turkey, and five species of this genus grow naturally in Turkey. The leaf of *M. campanuloides* L'Hérit ex Aiton is used in traditional medicine the treatment of wounds in Kahramanmaraş (Turkey). The present study was designed to investigate the wound healing and anti-inflammatory activities of the water and methanol (MeOH) extracts obtained from the root and herb of five *Michauxia* species. The incision by using tensiometer and excision models were used in order to assess the effect of the plant extracts on wound healing in mice and rats. Results were also evaluated histopathologically. In vivo inhibitory effect of the extracts on acetic acid-induced increase in capillary permeability was studied for the assessment of anti-inflammatory activity. The wound healing effect was comparatively evaluated with a reference ointment Madecassol®. Noteworthy wound healing activity was observed for the ointment formulation prepared with 1% *M. nuda* A.DC. (root MeOH) and *M. tchihatchewii* Fisch. et Mey. (herba MeOH) extracts. The results of histopathological evaluation supported the outcome of both incision and excision wound models. Moreover, the root extract of the *M. nuda* exerted remarkable anti-inflammatory activity also demonstrated. The experimental study revealed that Mictauxia displays remarkable wound healing and anti-inflammatory activities. **Keywords:** Michauxia, wound healing

PM144 Antimicrobial evaluation of hydroalcoholic extract and Volatile components of aerial part of *Kelussia odoratissima* Mozaff.

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Kelussia odoratissima Mozaff. is a sweet-smelling, wild plant which is traditionally consumed in Iran as a garnish. Only Little, is known about its potential antioxidant activity. In this study.The antibacterial activities. against *Staphylococus aureus*, *Escherichia coli, Bacillus cereus* and *Pseudomonas aeruginosa* were investigated by paper Disc and immersion bioautography Methods. Separately Ethanolic extract was taken by maceration method and Volatile components was taken by cold solvent extraction method using petroleum ether. Inhibitory zones of blank discs, impregnated with defined amounts of extract were observed. and MIC were measured using E-Test. method. In bioautographic Method, extract and volatile components were separated on silica gel TLC plates to the best resolution then were placed on agar plate inoculated with bacterial suspension. After incubation for 16 – 18 hr at 37 °C, visualization of inhibition zones was done by spraying the surface of culture media with MTT solution. Clear zone in dark purple background was the positive answer. Another TLC sheet developed with the same solvent, in the same condition was sprayed with known spraying reagents commonly used for detecting known phytochemical groups. Hydroalcoholic extract of *Kelussia odoratissima* were effective against *E. coli, B. cereus, P. aeruginosa and S. aureus* with MIC (0.64, 0.25, 0.43, 0.43 mg/disc) and volatile components on S. aureus. B. cereus with MIC (0.43, 0.18 mg/disc). **Keywords:** *Kelussia odoratissima*, Antimicrobial, Bioautography **References:** 1- Ahmadi F et al. (2007) Food Chemistry 105(1): 57 – 64 2- Moshefi et al. (2004)11(2):109 – 118 3 -Wagner H et al. (1984) Plant Drug Analysis. 3th ed. Berlin, pp.299 – 304.

PM145 Effects of the extracts from Turkish medicinal plants on NF-κB activation on LPS-induced RAW 264.7 macrophages

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NF-κB is a transcription factor mediating the expression of several genes involved in inflammation and its inhibition might be a valuable strategy to develop effective anti-inflammatory agents [1]. *Sambucus ebulus* L., *S. nigra* L. (Caprifoliaceae) and *Cistus laurifolius* L. (Cistaceae) which are used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity [2,3]. Each plant was extracted with ethanol or methanol and then fractionated by successive solvent extractions to obtain subextracts; i.e. hexane, chloroform, ethyl acetate, *n*-butanol and remaining water. Effects of the extracts on the viability of RAW 264.7 macrophages were determined by using WST-1 cell viability assay. Effects on NF-κB activation in lipopolysaccharide (LPS) induced RAW 264.7 macrophages were studied by using Electromobility Shift Assay (EMSA). RAW 264.7 cells were preincubated for two hours with indicated non-toxic concentrations of extracts and then stimulated with LPS (1 μg/mL). Cells were harvested, nuclear proteins were extracted and assayed for NF-κB-DNA binding affinity by EMSA. Results were quantified by densitometric analysis using Image J program. *S. ebulus* hexane subextract (50 μg/mL) exhibited the highest activity which led to 51.3% decrease of NF-κB activation followed by ethyl acetate (100 μg/mL) and chloroform (100 μg/mL) subextracts which showed 32.6%, 28.1% inhibition respectively, while the remaining water subextract (100 μg/mL) showed the lowest activity (9.3%). Only hexane extract of *S. nigra* exhibited an inhibition (25.3%) of NF-κB activation at 50 μg/mL. The hexane, chloroform and remaining water subextracts of *C. laurifolius* showed 10.7%, 11.9% and 18.8% inhibition at 100 μg/mL, respectively. **Keywords:** NF-κB, RAW 264.7, *Sambucus*, *Cistus*, anti-inflammatory **Acknowledgement:** *This study is supported by Turkish Scientific and Technological*

Research Council of Turkey (Project no: SBAG-110S197) **References:** 1. Lee J et al. (2006)J Pharmacol Exp Ther 316: 271 – 8. 2.Yesilada E et al. (1993)J Ethnopharmacol 39: 31 – 38. 3.Yesilada E et al. (1999)J Ethnopharmacol 64: 195 – 210.

PM146 Wound Healing Effects of New Cream Formulations

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Some of the many potentially beneficial ingredients that are traditionally used in wound healing, are obtained from some plants but their effectiveness has not been scientifically evaluated yet (1,2). In this study, new cream formulations (Levant storax and Complex creams) with the same cream base were developed. The composition of the cream base was as follows; (1) *Butyrospermum parkii* Kotschy (2) squalane, (3) cetearyl olivate and sorbitan olivate, (4) cetyl stearyl alcohol, (5) caprylic/capric triglyceride, (6) petrolatum, (7) glycerine, (8) tetrasodium EDTA, (9) methylparaben, ethylparaben, propylparaben, butylparaben and isobutylparaben and (10) deionized water. In addition to the ingredients used in the cream base, Levant storax cream also contained balsam of oriental sweet gum, while complex cream contained (1) calendula oil, (2) St. John's wort extract, (3) escin (4) freeze dried powder of *Aloe vera* (L.) Burm. f. leaf juice and (5) allantoin. The aim of the study is to comparatively assess the wound healing potential of the two formulations against a reference cream, Madecassol® and the cream base by using *in-vivo* excisional wound model on rats. All wounds were photographed in the presence of a standard ruler by DLite Analog Microscope on the first and last day (tenth day) of the study. The wound areas were computed by using the Image J software and the wound contraction rates were calculated as a percentage of the reduction in wounded area and analyzed for statistical significance. According to the results, Levant storax cream was the best formula with the highest contraction rates, the Complex cream was as effective as the reference cream and better than the cream base. **Keywords:** Wound, Excision, *Liquidambar orientalis*, Wound healing **References:** 1. Bruneton J (1999) Pharmacognosy, Phytochemistry, Medicinal Plants. 2nd edition, Lavoisier Tec&Doc, Paris, 520 2. Hafizoglu H, Reunanen M, Istek A (1996) Holzforschung 50: 116 – 117

PM147 Hair-growth promoting effect of bimatoprost

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Importance of prostaglandin pathway in hair growth has been reported and, in particular, PGF2α was reported to promote hair growth. We thus examined the efficacy of several PGF2α analogues such as latanoprost, bimatoprost, unoprostone and travoprost, on the proliferation of dermal papilla cells (DPC), regulator of hair cycle and length of hair follicles, uging immortalized DPC from rat vibrissa follicles. Among these compounds, bimatoprost showed outstanding effectiveness on the proliferation of DPC. When rat vibrissa follicles were treated with bimatoprost, hair-fiber length of vibrissa follicles significantly increased. When we examined the effect of bimatoprost on the regulation of cell cycle, bimatoprost was found to decrease the Sub-G1 population and to increase the expression of cell cycle regulated proteins such as CDK2 and Cyclin E in DPC. Bimatoprost also increased the expression of β-catenin as well as the expression of Cox-2, target gene of β-catenin. Taken together, our results suggest that bimatoprost increased the hair growth by progression of cell cycle through upregulation of CDK2, Cyclin E and β-catenin. **Keywords:** bimatoprost, hair growth, dermal papilla cells, cell cycle, vibrissa follicle

PM148 Anticancer effect of a cembrenolide diterpene LS-1 in colon cancer cells through activation of oxidative stress

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We observed that (1*S*,2*S*,3*E*,7*E*,11*E*)-3,7,11,15-Cembratetraen-17,2-olide (LS-1), marine cembrenolide diterpene, inhibited growth and induced apoptosis in colon cancer cells via a ROS dependent mechanism. Treatment of HT-29 cells with LS-1 resulted in ROS generation, which was accompanied by disruption of mitochondrial membrane potential, cytosolic release of cytochrome *c*, sub-G1 peak accumulation, activation of Bid, caspase-3, -8, and -9, and cleavage of PARP along with the suppressive expression of Bcl-2. All these effects were significantly blocked on pretreatment with the ROS inhibitor *N*-acetylcystein (NAC), indicating the involvement of increased ROS in the proapoptotic activity of LS-1. Moreover, we showed that LS-1 induced the phosphorylation of JNK and dephosphorylation of p38, ERK, Akt, Src and STAT3, which were effectively attenuated by NAC. In addition, the expression of antioxidant catalase was abrogated by treatment using LS-1 with or without NAC. These findings reveal the novel anticancer efficacy of LS-1 mediated by the induction of apoptosis via ROS generation in human colon cancer cells. **Keywords:** LS-1; cembrenolide diterpene; HT-29; Apoptosis; ROS **Acknowledgement:** *This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2009 – 351 – 2-E00072) and Jeju National University Hospital Research fund (2010).*

PM149 Cytotoxic properties of five *Centaurea* L. species from Anatolia

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The genus *Centaurea* L. (Asteraceae) comprises about 192 taxons in the flora of Turkey distributed througout the Anatolian peninsula, with 61% being endemic (1 – 3). Many species of the genus have long been used traditionally to treat various ailments e.g. cough, hemoroid, peptic ulser and abscess (4). Pharmacological studies on some Centaurea species have reported antiinflammatory, antimicrobial, antipyretic, cytotoxic and immunological activities (5). In this study, methanolic extracts of five *Centaurea* L. species (*C. aphrodisea* Boiss., *C. athoa* DC, *C. hyalolepis* Boiss., *C. iberica* Trev. and *C. polyclada* DC) were investigated for their cytotoxic activities against three human cancer cell lines; MCF7 (human breast cancer), A549 (human lung cancer), U20S (human osteocarcoma) and one non-cancer cell line, 293HEK (human embriyonic kidney) by cell proliferation assay using WST-1 reagent. *C. polyclada* extract was the most active one against MCF7 (IC50:61 µg/ml), U2OS (IC_{50}: 63 µg/ml) and 293HEK (IC_{50}:72 µg/ml) cell lines. *C. aphrodisea* also showed significant effect on 293HEK (IC_{50}:85 µg/ml) and MCF7 (IC_{50}:90 µg/ml). This is the first cytotoxic activity report for the *Centaurea* species mentioned above. **Keywords:** Centaurea, Asteraceae, cytotoxicity **References:** 1. Bona B et al. (2008) IUFS J Biology 67(1):55 – 63 2. Wagenitz G (1975) In: Flora of Turkey and the East Aegean Islands, Davis P.H. (Ed.) Edinburgh, Edinburgh University Press, Vol. 5, p: 465 – 585 3. Uysal T (2008) Ann Bot Fennici 45: 135 – 137 4. Baytop T (1999) Türkiye'de Bitkilerle Tedavi (Geçmişte ve Bugün), Nobel Tıp Kitabevleri, İstanbul, 2.baskı, s:316 5. Arif R, Küpeli E, Ergun F (2004) GUJ Science 17(4): 149 – 164

PM150 Investigation of in vivo anti-inflammatory and analgesic effects of rose hip powder (*Rosa canina* L.)

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The standardized rose hip powder LitoMove® (*Rosa canina* L.) is a widely used herbal remedy. Consumption of LitoMove® has been shown to reduce pain in patients with osteoarthritis (1). The dichloromethane extract of LitoMove® possess *in vitro* immunomodulating effects which have been correlated to the presence of triterpene acids (2). To establish if the clinical effect of LitoMove® is caused by anti-inflammatory or analgesic effects, the dichloromethane extract was tested in the paw edema model of inflammation and the 1:1 methanol: dichloromethane extract in the hot plate test of acute pain. In both models, extracts were administered orally once daily in the indicated period. Treating rats with 100 mg dry extract/kg for three weeks did not result in a significant reduction of the paw edema compared to the control group. In the hot plate test, mice were treated with 500 mg dry extract/kg for five days. No significant difference in pain threshold between the treatment and the control group could be observed. It thus appears that the tested extracts neither possess anti-inflammatory nor analgesic effects in the chosen animal models. However, the paw edema model and the hot plate test are general models of inflammation and pain and thus, may not represent the inflammation and pain in arthritis. Therefore, further studies in specific animal models of arthritis inflammation and pain are needed before the clinical effects of LitoMove® can be understood. **Keywords:** *Rosa canina*, arthritis, inflammation **Acknowledgement:** *HybenVital and the Danish Rheumatism Association are thanked for financial assistance.* **References:** 1. Christensen R et al. (2008) Osteoarthritis Cartilage 16: 965 – 972, 2. Saaby L et al. (2011) Phytother Res 195 – 201.

PM151 Anti microbial activities of leaves and stems of *Ulmus minor* Miller subsp. *minor*

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Ulmus L. (Ulmaceae) is represented by three species in Turkey. *Ulmus minor* Mill. subsp. *minor* is a tree to 30 m but often much smaller, suckering. Twigs glabrous or sparsely pubescent [1]. Although there are no reports of the medicinal uses of *Ulmus minor* Miller subsp. *minor* in Turkey, the bark of the root and stem of *Ulmus davidiana* var. *japonica* has been used as a traditional Korean medicine to treat inflammatory disorders. This plant reportedly exhibits antioxidant, anticancer, and anti-inflammatory effects [2]. *Ulmus* species contain biologically active compounds, such as sesquiterpenoids, triterpenes and flavonoids [3,4]. In this study, n-hexane, dichloromethane, ethyl acetate, methanol, and methanol:water (20:80) extracts of leaves and stems of *Ulmus minor* subsp. *minor* were tested separately against selected Gram-positive, Gram negative bacteria and *Candida albicans*, an unicellular yeast, using a broth microdulition broth susceptibility assay. All of the extracts exhibited antimicrobial activity against *Enterococcus faecalis* and *Salmonella thyphimirium* resulting MIC values 0,81 and 25 mg/ml. Ethyl acetate extract of the stems of the plant was found to be active against all tested microorganisms with a range of MIC values extended from the concentration of 6, 25 – 0, 2 mg/ml. **Keywords:** *Ulmus minor*, Ulmaceae, antimicrobial activity **References:** 1. Davis PH (1982) Flora of Turkey and East Aegean Islands, University Press, Edinburgh. 2. Choi SY et al. (2010) J Med Food 13: 1019 – 1023. 3. Zheng MS et al. (2010) Biomol Ther 18: 321 – 328. 4. Lee GY et al. (2008) Planta Med 74: 1800 – 1802.

PM152 Antimicrobial studies on Semecarpus kathalekanensis

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Semecarpus kathalekanensis Dassapa & Swaminath, an evergreen tree with very large simple leaves, which attains a height of about 30 m belonging to the family Anacardiaceae which is critically endangered swamp tree and consists major chemical compounds like phenols, bioflavonoids and traditionally having high medicinal importance also used as an antimicrobial, antioxidant, and as an anticancer. The endophytic fungi were isolated from plant species and subjected for antimicrobial studies which showed significant results against gram positive and gram negative bacteria. **Keywords:** Endemic, Endophytic and *Semecarpus kathalekanensis*

PM153 The influence of extracts from *Potentilla* species on normal human colon cells

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The biological activity of extracts obtained from aerial parts of *Potentilla* species: *P. erecta* (L.) Rauschel, *P. anserina* L., *P. argentea* L., *P. rupestris* L., *P. grandiflora* L. was analyzed. Extracts were tested using MTT, NR and DPPH tests on two human normal cell lines: CCD 841 CoTr and CCD-18Co. Fluorescence staining of the cellular cytoskeleton after rhodamine-phalloidyne addition and IL-6, IL-10 (ELISA) in culture supernatants after 24 h of incubation with *Potentilla* extracts and nitric oxide (NO) analysis with a Griess method were performed. Extracts were tested at the range of 25 – 225 μg/mL concentrations while to the ELISA two non-toxic doses (15 and 30 μg/mL) were chosen. We found that all extracts stimulated metabolism of epithelial cells while myofibroblasts' mitochondrial dehydrogenase activity was stimulated at concentrations higher than 125 μg/mL. The exception was *P. grandiflora* which activated succinyl dehydrogenase just at low extract dose (25 μg/mL). Extracts from *P. erecta* and *P. argentea* had no toxic effect on colon epithelial cells while other extracts significantly decreased viability of cells even when added at 25 μg/mL concentration. Only *P. grandiflora* and *P. rupestris* significantly decreased viability of myofibroblasts. All extracts showed free radical scavenging effect in a concentration dependent manner. *Potentilla* extracts inhibited IL-6 and IL-10 production by myofibroblasts while in epithelial cells slightly induced or had no effect on the cytokine level. *Potentilla* extracts influenced F-actin filament composition and changed the cellular cytoskeleton and morphology of cells. Modulation of NO production after plant extracts addition has also been observed. **Keywords:** Potentilla, cytotoxicity, normal human colon cells

PM154 Antimicrobial and cytotoxic activities of roots of *Centaurea cadmea* Boiss.

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Centaurea cadmea Boiss. is an endemic taxon for Anatolia, growing wild in N, W & SW of Turkey (1). Phytochemical studies revealed the presence of a sesquiterpene lactone, ivalin, which is known cytotoxic com-

pound on several tumor cell lines (2), together with eupatorin, 5-hydroxy-3',4',6,7- tetramethoxyflavone and β-sitosterols from the aerial parts of *C. cadmea* (3). *In vitro* anti-inflammatory, antioxidant, antiprotozoal and antimicrobial activities of the aerial parts of *C. cadmea* extracts have been reported before (4, 5), but no bioactivity study has been performed on roots of the plant, yet. The present study aims at investigating the antimicrobial and cytotoxic activities of roots of *C. cadmea*. The antimicrobial activities of extracts of the plant were investigated by MIC method. The antibacterial activities of the extracts were tested against four gram negative (*Escherichia coli* ATCC 23999, *Pseudomonas aeroginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445 and *Klebsiella pneumonie* CCM 2318) and four gram positive (*Staphylococcus au*reus ATCC 6538/P, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* ATCC 7064) bacteria strains. While the chloroform extract of the plant has no activity against the tested microorganisms, the methanol extract has weak anti-bacterial activity (MIC = 64 – 256 μg/ml). The cytotoxic activity were analyzed by cell proliferation assay using WST-1 reagent against three human cancer cell lines; MCF7 (human breast cancer), A549 (human lung cancer), U20S (human osteocarcoma) and one non-cancer cell line; 293HEK (human embriyonic kidney). *C. cadmea* extract was found active against U2OS (IC_{50}: 138 μg/ml). **Keywords:** *Centaurea cadmea*, Asteraceae, cytotoxicity, antimicrobial **Acknowledgement:** *Authors are appreciated to U. Karabay-Yavasoğlu, Ph.D. and P. Ballar, Ph.D. for their scientific contribution.* **References:** 1. Wagenitz G (1975) Centaurea L. (Asteraceae). In: Flora of Turkey and the East Aegean Islands, vol. 5. Ed. P.H. Davis, Edinburgh University Press, Edinburgh, UK. 2. Lee J et al. (2002) Planta Med 68:745 – 747 3. Karamenderes C et al. (2007) Chem Nat Comp 43: 694 – 695 4. Karamenderes C et al. (2007) Phytother Res 21: 488 – 491 5. Karamenderes C et al. (2006) Pharm Biol 44: 534 – 539

PM155 Antibacterial activity of the essential oil and main components of two *Dracocephalum* species from Iran

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Antibacterial activity of *Dracocephalum polychaetum* Bornm. and *D. surmandinum* Rech.f. essential oils and two main components were investigated. Essential oils of the plants were analyzed by GC and GC-MS[1]. Twenty-three components were characterized in the essential oil of *D. polychaetum*. The oil was rich in oxygenated compounds (73.1%) and hydrocarbons (25.0%). Monoterpenes including perilla aldehyde (63.4%) and limonene (22.1%) were the major constituents. Among the 25 identified compounds (97.8%) in the oil of *D. surmandinum* perilla aldehyde (54.3%) and limonene (30.1%) were the main constituents. The bioassays exhibited that all of the Gram-positive and Gram-negative bacteria tested were highly inhibited in the presence of the oils and main components investigated. The most sensitive microorganism to the oils was found to be *Staphylococcus epidermidis* with the lowest MIC value of 0.3 mg/ml. The resistant Gram-negative *Pseudomonas aeruginosa* was highly inhibited by the oil of *D. polychaetum* with MIC value of 2.4 mg/ml. **Keywords:** *Dracocephalum*, antibacterial activity, essential oil, perilla aldehyde, limonene **References:** [1] Adams RP (2007) Identification of essential oils components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing. Carol Stream, IL.

PM156 Screening of Anti-inflammatory Activity of Natural Products through A Panel of Target Based Assays

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Inflammation is considered as a risk factor for several types of cancers, obesity and metabolic disorders. Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, metastasis and found to mediate a wide variety of diseases, including cardiovascular diseases, cancer, diabetes, arthritis, Alzheimer's disease, pulmonary diseases, and autoimmune diseases [1 – 3]. Phytochemicals such as curcumin, genistein, resveratrol, 6-gingerol and saponins are believed to suppress the inflammatory process and are considered as protective agents against cancer and other chronic diseases [4]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammatory diseases. Activation of the NAG-1 gene is involved in the action of NSAIDS and has been associated with the apoptotic elimination of cancer cells [5]. NF-kB and iNOS are also considered as important targets for inflammation [1,5]. A collection of standard compounds known for anti-inflammatory activity (curcumin, genistein, resveratrol, berberine, parthenolide, quercetin, aspirin, diclorofenac, ibuprofen, indomethacin, ciglitazone, rosiglitazone) were screened through a panel of assays that can determine the activity of these targets in various cell lines. Genistein, parthenolide, rosiglitazone, berberine, curcumin and quercetin inhibited iNOS activity in mouse macrophages whereas ciglitazone, parthenolide, resveratrol, curcumin and quercetin inhibited NF-kB mediated transcriptional activity in human chondrosarcoma cells. Genistein, ibuprofen, diclorofenac and resveratrol induced NAG-1 expression in human colon cancer cells. These assays were utilized to screen the anti-inflammatory activity of some selected medicinal plant extracts. The results will be presented. **Keywords:** Anti-inflammatory Activity, Natural Products, Target Based Assays **Acknowledgement:** *The Council of Higher Education (YOK) from Turkey and USDA-ARS specific cooperative agreement no 58 – 6408 – 2-0009 are acknowledged for support of this work.* **References:** 1. Aggarwal (2009) Curr Opin Pharmacol 9:347 – 350 2. Coussens LM, Werb Z (2002) Nature 420: 860 – 867. 3. Philip M et al. (2004) Semin Cancer Biol 14:433 – 439. 4. Aggarwal BB, ShishodiaS (2006) Biochem Pharmacol 71:1397 – 1421. 5. Baek SJ et al. (2005) Mol Pharmacol 67: 356 – 364.

PM157 Antioxidant and anti-inflammatory properties of *Rumex patientia* L

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Rumex patientia L., a member of Polygonaceae family, is a perennial plant widely distributed and cultivated in Eastern Europe. The roots of *R. patientia* have been used extensively in traditional medicine worldwide for treatment of different disorders due to their laxative, diuretic, antipyretic, wound cure and anti-inflammatory properties. It has been reported that *R. patientia* contains anthraquinones, tannins, flavonoids and phenolic acids. Some of these compounds have shown anti-inflammatorry and antioxidant effects. Therefore the present study was undertaken to evaluate the chemical composition, antioxidant and anti-inflammatory properties of ethanolic extracts of aerial plants and roots of *R. patientia*. Phytochemical profile was determined by measuring total phenolic and total flavonoid content and by quantitative LC-MS/MS analysis of the extracts. The antioxidant activity was evaluated by measuring ferric reducing ability (FRAP) of the extracts and their radical scavenging capacity towards DPPH, OH, NO and superoxide radicals [1]. The anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was investigated [2]. Experimental data, obtained by the study of *R. patientia* specimens collected at three different locations in Serbia, showed that ethanolic extracts of aerial parts have higher total phenolic and total flavonoid contents, reducing capacity and OH scavenging ability in comparison to root extracts. On the other hand, root extracts exhibited higher DPPH and superoxide scavenging activity. Both root and herb extracts showed dose-dependent inhibition of 12-HETE, 12-HHT, PGE2 and TXB2 production. Herb extract exhibited higher COX/LOX pathway inhibitory activity than root extract. **Keywords:** *Rumex patientia* L., antioxidant, anti-inflammatory, LC-MS/MS **Acknowledgement:** *Ministry of science and technological development, Republic of Serbia, grant No. OI 172058* **References:** [1] Beara IN et al. (2009) J Agric Food Chem 57: 9268 – 9273 [2] Beara IN et al. (2010) J Pharm Biomed Anal 52:701 – 706.

PM158 Long-term effects of the rhapontic rhubarb extract ERr 731® on estrogen-regulated targets in the uterus and on the bone in ovariectomized rats

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The efficacy of the commercially available extract ERr 731® from *Rheum rhaponticum* L. regarding attenuation of menopausal complaints like hot flushes, depression, anxiety and vaginal dryness has been proven in a two-year clinical study. Further, no undesired side effects like uterotrophy or proliferation of the endometrium became apparent while testing ERr 731® in a 3-day uterotrophic assay. The present study aimed at further substantiating the safety of application of ERr 731® regarding endometrial hyperplasia and at the same time test for potential bone sparing effects in the preclinical ovariectomized (ovx) rat model. For this purpose we performed a 90 d dietary feeding study in ovx rats. The impact of exposure on uterine proliferation was investigated by assessing the mRNA levels of proliferation marker genes (Mki67, Pcna) in comparison to the expression of the mRNAs of the estrogen receptors ESR1 and ESR2 and the estrogen response gene C3. To test for potential effects on the bone, we additionally performed densitometry analysis of the proximal tibia metaphysis using peripheral computed tomography and quantified bone homeostasis marker in the serum. With this study design, neither an uterotrophic response nor a modulation of mRNA levels of proliferation markers was detected after 90 d of dietary exposure with the rhapontic extract. Furthermore, no effect of the two administered doses of ERr 731® on E2 deprivation induced bone loss became apparent. In conclusion, the observations from previous trials regarding the endometrial safety of ERr 731® application were substantiated, but no effect on the Bone Mineral Density could be observed. **Keywords:** *Rheum rhaponticum*, ovariectomized rat, bone loss, endometrium, proliferation

PM159 Phenolic profile and biopotential of *Plantago schwarzenbergiana* Schur

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Ancient use of plantains (genus *Plantago* L., Plantaginaceae) as herbal remedies is a consequence of their astringent, anti-toxic, antimicrobial, expectorant and diuretic properties. *Plantago schwarzenbergiana* Schur. is distributed in the Balkan Peninsula, but there is no data about biological activity of this species. In order to valorize medicinal use of *P. schwarzenbergiana*, some tests on antioxidative and anti-inflammatory activities of methanolic extract of this plantain, collected from area of Vojvodina (Serbia) have been undertaken. The extract has been characterized regarding phenolics composition by LC-MS/MS, where the contents of apigenin (5.08 ± 0.1 mg/g of d. e.), p-hydroxybenzoic (0.63 ± 0.03 mg/g of d. e.) and caffeic (0.59 ± 0.08 mg/g of d. e.) acid were the highest. The radical scavenger capacity (RSC) was evaluated towards several radicals using spectrophotometry [1] and following IC_{50} were found: diphenylpicrylhydrazyl (10.6 ± 0.6 μg/ml), hydroxyl (181.6 ± 6.0 μg/ml), superoxide anion (54.6 ± 0.8 μg/ml) and nitric oxide radical (2.0 ± 0.3 mg/ml), inhibition of lipid peroxidation (34.0 ± 1.2 μg/ml), indicating comparable or higher extract activity than activity of synthetic antioxidants as BHT or BHA (butylated hydroxytoluene/hydroxyanisol). Anti-inflammatory activity was examined by means of cyclooxygenase-1 (COX-1) and 12-lipooxygenase (12-LOX) inhibition, quantifying the COX-1 product 12-HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) and 12-LOX product 12-HETE (12-hydroxy-5,8,10,14-eicosatetraenoate) by RP-HPLC-MS/MS [2]. Extract inhibited both COX-1 and 12-LOX (IC_{50}= 5.8 ± 0.4 and 4.0 ± 0.2 mg/mL, respectively). In this study, we report for the first time about phenolic profile, antioxidant and anti-inflammatory activity of *P. schwarzenbergiana*, and accordingly consider this species as a promising source of natural antioxidant and anti-inflammatory agents. **Keywords:** *Plantago schwarzenbergiana*, Antioxidant activity, Anti-inflammatory activity, Phenolic profile, LC-MS/MS **Acknowledgement:** *Autonomous Province of Vojvodina – Provincial Secretariat for Science and Technological Development, Grant No. 114 – 451 – 1991/2011 – 01.* **References:** 1. Beara I et al. (2009) J Agric Food Chem 57: 9268 – 9273. 2. Beara I et al. (2010) J Pharm Biomed Anal 52: 701 – 706.

PM160 Anti-infammatory property of *Juniperus communis* L. var. *communis* needles and cones extracts and essential oils

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All over the world plants from the *Juniperus* genus have always been regarded as a well-known traditional remedy and spice. These plants are extensively used in the folk medicine for healing various disorders: common cold, urinary and kidney infections, dermatological disorders, bronchitis, pneumonia, dysentery, hemorrhage, rheumatic arthritis, stomachache, diarrhea and for regulation of the menstruation and in relieving menstrual pains [1]. However, there are only few literature data about their pharmaceutical activity and chemical composition. In this study anti-inflammatory properties of methanol extracts and essential oils of leaves and cones of the *Juniperus communis* L. var. *communis*, were determined using assays which measure inhibitory potency toward COX-1 and 12-LOX enzymes in human platelets, by novel optimized method which was based on method previously described [2]. Extended assay is using LC-MS/MS technique for the quantification of three products (12-HHT, TXB_2 and PGE_2) of COX-1 and one product (12-HETE) of 12-LOX metabolism. Both essential oils showed markedly anti-inflammatory activity, with needless and cones essential oils having higher potency concerning inhibition of production of all four inflammation mediators, than extracts. Regarding 12-LOX inhibition, most potent was essential oil from cones (IC_{50}= 260.79 nL/mL), while most potent regarding lowering production of COX-1 metabolites was essential oil from needless (12-HHT/IC_{50}= 234.31 nL/mL, TXB_2/IC_{50}= 259.46 nL/mL, PGE_2/IC_{50}= 223.28 nL/mL). According to obtained results examined *Juniperus communis* L. var. *communis* species could be regarded as a promising source of bioactive natural compounds, which can be used both as a food supplement and as a remedy. **Keywords:** *Juniperus communis*, Anti-inflammatory activity, extracts, essential oils, LC-MS/MS **Acknowledgement:** *The Ministry of Sciences and Environmental Protection, Republic of Serbia (Grant No. OI 172058) supported this research work. We thank Goran Anackov, PhD for the plant specimen determination.* **References:** 1. Lesjak M et al. (2011) Food Chem 124: 850 – 856. 2. Beara I et al. (2010) J Pharm Biomed Anal 52: 701 – 706.

PM161 Regulatory effect of 4-O-methylhonokiol on TGF-β1-induced cell cycle arrest in human keratinocyte cell line (HaCaT)

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Transforming growth factor-β (TGF-β) signal pathway has a pivotal role in the progression of catagen phase in hair growth cycle. 4-O-Methylhonokiol, a neolignan compound from *Magnolia Officinalis*, has various biological activites such as anti-inflammatory, neurite outgrowth activity and anti-acetylcholinesterase activity. Recently we have reported the hair-growth promoting effect of 4-O-methylhonokiol. However, the hair-growing mechanisms of 4-O-methylhonokiol on the TGF-β signal pathway have not yet been elucidated. We thus examined whether 4-O-methylhonokiol has an inhibitory effect on TGF-β signal pathway in HaCaT cells. When HaCaT cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Sp1 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory

action on TGF-β-induced cell cycle arrest and ROS production. **Keywords:** 4-O-methylhonokiol; *Magnolia. offici*nalis; TGF-β; HaCaT cells; cell cycle arrest, NOX4

PM162 Chemical composition, antioxidant and antimicrobial activities of the lichen *Toninia candida* (Weber) Th. Fr (Catillariaceae)

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In the present investigation, methanol, chloroform and petroleum ether extracts of the lichen *Toninia candida* (Weber) Th.Fr. were assayed for their antioxidant and antimicrobial activities. The phenolic composition of the extracts was determined by HPLC-UV analysis. The predominant phenolic compound in all the extracts was the depsidone norstictic acid. Apart from the norstictic acid, the tested extracts of T. candida contain different amounts and ratios of atranorin and stictic, protocetraric and usnic acids. The lichen extracts showed comparable and strong antioxidant activity, exhibited higher DPPH and hydroxyl radical scavenging capacity, chelating activity and inhibitory activity towards lipid peroxidation. The lichen extracts demonstrated major antimicrobial activity against 8 strains with MIC values ranging from 16.62 to 62.50 μg/ml. This is the first report of the chemical composition, antioxidant and antimicrobial activities of the lichen Toninia candida. **Keywords:** Toninia candida, HPLC-UV, chemical composition, antioxidant activity, antimicrobial activity **Acknowledgement:** *This work was supported by the Serbian Ministry of Science and the Environment, Project No. 172015*

PM163 An *in vitro* approach to neuroprotective activity of *Rosa damascena* Mill., a medieval age traditional medicine used for memory enhancement

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Rosa damascena Mill. was recorded to be used traditionally for memory enhancement in the medieval age. Therefore, neuroprotective effect of the essential oil and aromatic waters of *R. damascena* was investigated by *in vitro* and *in silico* methods. The essential oil and its components (citronellol, geraniol, nerol, and phenylethyl alcohol), and two samples of the aromatic water (Eau de rose) of *R. damascena* were tested for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) at 100, 200, 500, and 1000 μg/ml. Since oxidative damage is associated with neurodegeneration, antioxidant activity of the samples was determined by DPPH radical scavenging, metal-chelation, and ferric-reducing antioxidant power (FRAP) assays. Chemical composition of the samples was elucidated by GC-MS. The rose essential oil showed a noteworthy inhibition against AChE (60.86 ± 1.99%) and BChE (51.08 ± 1.7%) at 1000 μg ml-1, whereas the aromatic waters did not have any inhibition. The essential oil exhibited moderate activity in antioxidant assays. Phenylethyl alcohol exerted higher cholinesterase inhibition than other components. None of the double and triple combinations of citronellol, geraniol, nerol, and phenylethyl alcohol could reach at inhibition level of phenylethyl alcohol. Phenylethyl alcohol was theoretically studied utilizing molecular docking simulations into the active site gorge of AChE and BChE and the data revealed that this compound is more selective towards BChE than AChE. Our findings confirmed traditional use of *R. damascena* for memory enhancement, which is suggested to come into view through mainly cholinesterase inhibition, and antagonistic interaction presumably exists between phenylethyl alcohol and other components. **Keywords:** Rosa damascena, rose water, memory enhancement, enzyme inhibitory activity

PM164 Studies on anticholinesterase and DPPH radical scavenging effects of 41 species of *Fritillaria* L. genus of Turkish origin

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The genus *Fritillaria* L. (Liliaceae) is a member of geophytes with attractive flowers, which are cultivated as ornamental plants. Many of the European *Fritillaria* species are found in the Alps, the Pyrenees, the Balkans, and northern Turkey. There are 41 *Fritillaria* species growing in Turkey, 26 of which are endemic. The research carried out on *Fritillaria* species are focused on the alkaloid content of the plant. In the present study, the dichloromethane and methanol extracts prepared from the bulbs of 59 samples belonging to 41 *Fritillaria* cultivated in Turkey have been investigated for their cholinesterase inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the enzymes linked to Alzheimer's disease, at 50, 100, and 200 mg mL-1 using ELISA microplate reader. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of the extracts was also tested 12.5, 25, and 50 mg mL-1 final concentrations. According to our results; the highest inhibition against AChE was caused by the dichloromethane and methanol extracts of *F. persica* L. (21.03 ± 0.34% and 27.39 ± 2.26%, respectively). The most active extracts against BChE were found as the dichloromethane extract of *F. pinardii* Boiss. (49.72 ± 2.56%) and two samples of *F. persica* (48.27 ± 1.98% and 47.29.± 1.72%). Among the methanol extract, the best BChE inhibitions were found to be caused in *F. minima* Rix (54.69 ± 3.40%), *F. persica* (51.85 ± 4.68%), and *F. caucasica* J.F. Adam (46.14 ± 2.96%). On the other hand, all of the extracts displayed low profile of DPPH scavenging effect below 25%. The present results indicate that especially *F. persica* could be a potential source of natural compounds with anticholinesterase effect. **Keywords:** Fritillaria, anticholinesterase activity, free radical scavenging effect

PM165 Methanolic *Alnus glutinosa* bark extract affect ROS and TNF-α production

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Alnus glutinosa (L.) Gaertn., commonly known as 'European alder', presents several types of secondary plant metabolites, including anthraquinones, phenolic glycosides, flavonol glycosides, terpenoids or xanthones, that had been previously reported in barks, buds, leaves and pollens. *Alnus glutinosa* stem bark (AGSB) is traditionally used as alterative, astringent, cathartic, febrifuge, emetic (fresh), haemostatic and tonic. In addition, a decoction of AGSB is used to treat swelling, inflammation and rheumatism. These traditional uses suggest that AGSB may contain active metabolites related to the inflammation process. Inflammation is associated with the progression of numerous diseases and is accompained by the chronic release of cytokines and reactive oxygen species (ROS), which may be involved in tissue injury increment. Moreover, ROS, as well, contribute to the expression of a variety of different inflammatory cytokines such as TNF-α, which is considered to be a primary mediator of the inflammatory response. In this sense, the present study was designed to evaluate the capacity of the AGBS extract to reduce ROS generation in H2O2-induced oxidative stress in HeLa cells. In addition, the extract effect on TNF-α production using HL60 cell line, was also tested. Results show that the AGSB extract is able to protect cells from induced oxidative stress and may be able to decrease TNF-α production. These biological properties are linked to a successful reduction in inflammatory processes and may support, in part, its ethnopharmacological use. **Keywords:** *Alnus glutinosa*. TNF-α. ROS

PM166 Anti-amnesic activity screening of the seed ethanol extracts of Turkish *Paeonia* taxa by *in vitro* methods

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The genus *Paeonia* L. (Paeoniaceae), known as "şakayik, ayi gülü, bocur, etc." in Turkey, was recorded to be used in Chinese traditional medicine against amnesia. *Paeonia* species were recorded in Turkey, which is the most important gene center worldwide for this genus. Consequently, the ethanol extracts of the defatted seeds of 7 *Paeonia* taxa; (*P. arietina* Anders., *P. daurica* Andrews, *P. mascula* Miller subsp. *bodurii* N. Özhatay, *P. cf. mascula* L. (Mill.) subsp. *mascula*, *P. peregrina* Miller, *P. tenuifolia* L., and *P.xkayae* N. Özhatay) were screened against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), linked to Alzheimer's disease and tyrosinase (TYRO), connected to Parkinson's disease using ELISA microplate reader. As amnesia is a neurodegenerative situation associated with oxidative damage, antioxidant activity of the extracts was also measured by radical scavenging activity tests against 2,2-diphenyl-1-picrylhydrazyl (DPPH), *N,N*-dimethyl-*p*-phenylendiamine (DMPD), and nitric oxide (NO) as well as metal-chelation capacity and ferric-reducing antioxidant power (FRAP) tests. Total phenol and flavonoid contents were determined spectrophotometrically. All of the extracts strongly inhibited AChE (85.69 ± 0.58% – 96.68 ± 0.44%), BChE (73.34 ± 1.92% – 98.87 ± 1.08%), and TYRO (60.50 ± 1.68% – 76.16 ± 0.30%) at 200 µg mL^{-1}. The extracts displayed scavenging activity below 40% against DPPH and DMPD radicals, whereas they were not able to quench NO. They exhibited moderate FRAP values and very low metal-chelation capacity. As conclusion, our findings reveal that *Paeonia* species possess potent anti-amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The present study confirms the claimed utilization of the plant against amnesia in traditional medicine.

PM167 Spasmodic response and neurogenic mechanism of water extract of *Vernonia cinerea* (L.) Less. on rat duodenum

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The pharmacological study was conducted on adult female albino rats (175 – 225 g). Rats were anaesthetized with pentobarbitone sodium (40 mg/kg, IP) and sacrificed by cervical dislocation and exaguination. A small piece of duodenum 4 cm apart stomach was removed, cleaned and mounted on an organ bath containing Tyrode solution.The bathing fluid was continuously bubbled with air and maintained at constant temperature (37 ± 0.5 °C).The tissue was allowed to equilibrate for a period of 30 min under a resting tension of 0.5 gm. During this period the tissue was washed every 15 min. After equilibration, drugs were administered in bath fluid to record their effect on physiograph. Every time responses of 2 successive doses of acetylcholine (ACh), at an interval of 10 min were recorded to confirm optimum responsiveness of the tissues. Serial solutions of the water extract of *Vernonia cinerea* (L.) Less. whole plant (VWE) were prepared directly in Tyrode solution and mixed properly with the help of an Ultra-Sonicator. Effect of cumulative doses (0.25 to 25 mg/ml) of VWE on rat duodenal smooth muscle was recorded. This response indicates that the plant contains an active ingredient, which has spasmogenic response on rat duodenal smooth muscle. The responses of VWE were fully reversible on wash. This finding suggests that VWE's responses were mediated through an agonistic action without blockade of any receptor or enzyme. Extract possibly stimulates presynaptic cholinergic nerve endings to produce spasmodic response in rat duodenal smooth muscle. **Keywords:** Vernonia cinerea, Rat duodenum, Aqueous extract, spasmodic response **Acknowledgement:** *Thanks are due to authorities of G.B. Pant University of Agriculture & Technology, Pantnagar, India for providing necessary research facilities.*

PM168 Estrogenic activity of the methanolic extract of *Ebenus cretica* L

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Plant derived compounds when friendly to the human organism tend to become very important ingredient of our nutrition. Members of the Leguminosae family are known to exhibit a mild estrogenicity due to their rich phenolic profile. In the present study, methanolic extracts of *Ebenus cretica* L. (ECME), *Ebenus sibthorpii* DC., *Medicago marina* L. (MMME) and *Medicago falcata* L. have been investigated for their estrogenic activity on ERb. All extracts were subjected to ERβ-binding screening using stably transfected HeLa cells. Luciferase assay was used as a tool to monitor the ERβ-binding. At the concentration of 400 µg/ml ECME and MMME exhibit 2.7-fold and 3.2-fold respectively stronger binding with the ERβ compared to its natural agonist, estradiol. Followingly, in order to isolate bioactive compounds from *E. cretica*, ECME was submitted to fast centrifugal partition chromatography and sephadex analysis and was proved rich in flavonoids such as rutin and isoflavones such as 4', 8-dimethylether-7-O-β-D-glucopyranosylisoflavone. An aurone, maesopsin glucoside, was also isolated. Additionally, an in vivo experiment was performed in order to evaluate ECME's osteoprotective role. Female Wistar rats were treated with the ECME dissolved in drinking water. Rats were submitted to ovariectomy prior to the treatment. Natural products from plants traditionally provide the pharmaceutical and food industry with one of the most important sources of "lead" compounds. Findings of our study offer valuable information on the beneficial effects of *E.cretica*, which could be used as the basis of food supplement, functional food or even drug. **Keywords:** Ebenus, Medicago, estrogenic activity **Acknowledgement:** *The present study has been funded by the project IRAKLEITOS II*

PM169 Larvicidal activities of selected Nigerian plants

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The methanol extracts of *Bryophyllum pinnatum* (Lam.) Kurz leaves, *Dysoxylum lenticellare* Gillespie leaves, *Newboldia laevis* Seem stem bark, *Markhamia tomentosa* K.Schum. ex Engl. stem bark, ethylacetate extract of *Jatropha multifida* L. leaves and *Jatropha gossipifolia* L. stem bark were investigated for larvicidal activity against the larvae of *Aedes aegyptii*, the vector of dengue and yellow fevers. After 48hours, *D. lenticellare* extract with LC_{50} and LC_{90} values of 1.54 ± 0.31 mg/ml and 3.32 ± 0.15 mg/ml respectively and *J. gossipifolia* extract with LC_{50} and LC_{90} values of 1.96 ± 0.26 mg/ml and 3.50 ± 0.25 mg/ml respectively were the most active. Work is in progress to purify these most active extracts and isolate their active constituents. **Keywords:** plant extracts, *Aedes aegyptii*, larvicidal activity

PM170 Appraisal of *in vitro* neuroprotective effects of Turkish *Pinus* L. species and pycnogenol and essential oil analyses

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Pycnogenol, the French maritime pine bark extract, has been popular recently for its various health effects including memory enhancement. Therefore, we aimed to determine neuroprotective effect of the acetone, ethyl acetate, and ethanol extracts and essential oils of the shoots and needles of *P. brutia* Ten., *P. halepensis* M.Bieb., *P. nigra* Link, *P. pinea* L., and *P. sylvestris* L., which are the *Pinus* species growing in Turkey, and

pycnogenol by *in vitro* experiments using enzyme inhibition and antioxidant assays. Inhibitory activity of the extracts, essential oils, and pycnogenol was assessed against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), connected to Alzheimer's disease. Since neurodegeneration is associated with oxidative damage caused by free radicals and metal accumulation, antioxidant activity of the extracts, essential oils, and pycnogenol was measured using the methods; 2,2-diphenyl-1-picrylhydrazyl (DPPH) and N,N-dimethyl-p-phenylendiamine (DMPD) radical scavenging activity as well as ferric ion-chelation capacity and ferric-reducing antioxidant power (FRAP) tests. Chemical compositions of the essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Accordingly, the best AChE inhibition was caused by the shoot essential oil of *P. halepensis* (83.91 ± 3.95%), while the needle ethanol extract displayed a high profile of BChE inhibition (82.47 ± 5.57%) at 200 µg/mL. AChE and BChE inhibitions by pycnogenol were 63.33 ± 0.22% and 83.67 ± 0.22%, respectively. The extracts and essential oils exerted moderate activity in antioxidant tests. However, many of them displayed similar or greater activity ferric ion-chelation capacity (26.49 ± 4.47% – 67.77 ± 3.33%) than that of pycnogenol (29.14 ± 2.00%). Our findings revealed that the Turkish pine species and pycnogenol possess neuroprotective effects by the *in vitro* methods applied herein. **Keywords:** Antioxidant activity, *Pinus* sp., essential oil

PM171 Black Cohosh and the protective effects on bone metabolism as measured by computer tomography (qCT) in ovariectomized (ovx) rats

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Osteoporosis is a major disease in postmenopausal women. There is compelling evidence that the special extract from *Cimicifuga racemosa* (L.) Nutt. (CR) BNO 1055 influences positively bone metabolism and prevents induced osteoporosis by ovariectomy (ovx). Aim of the study was the investigation of CR BNO 1055 extracts and their protective effects on bone metabolism. *Via* liquid-liquid extraction, extracts were separated into two groups, *i.e.* a lipophilic rich in saponins and a hydrophilic rich in sugars and phenylpropanoids. Preparative fractions were characterized by TLC and by HPLC-UV-ELSD and HPLC-MS. Analytical data clearly prove the presence of triterpene glycosides in the lipophilic fraction and the presence of carbohydrates and phenylpropanic acid derivatives in the hydrophilic fraction. To investigate the activity on bone metabolism of CR, ovx rats received either nutrition containing CR, 17ß-estradiol or food without any active component. All types of food were soy-free food. QCTs were performed at the level of the metaphysis of the tibia and the trabecular density measured prior to the ovariectomy and at the end of the four weeks lasting application of the extracts. Within these four weeks the bones of the ovx rodents lost nearly half of their cancellous density. This effect did not appear by rats fed with nutrition including 17ß-estradiol and CR. The lipophilic fraction shows a significant high activity on bone metabolism while the hydrophilic fraction was inactive. The qCt results demonstrate that the prevention effect of CR BNO 1055 on bone is up to the lipophilic compounds of the extract. **Keywords:** Black Cohosh, Cimicifuga racemosa, bone metabolism **Acknowledgement:** *This work was funded by the Bayerische Forschungsstiftung AZ-838 – 08, Germany*

PM172 Biologically active lupane triterpenoids from Anatolian *Salvia* species

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Salvia species are used in perfumery, food and drug industry, belonging to Lamiaceae family which contain annual or perennial plants with nice fragrance. Totally 900 *Salvia* species grown wildly all over the world, and Turkey has over 90 *Salvia* species, half of them being endemic [1]. Since ancient times, *Salvia* species have been traditionally used as antiseptic, digestive, carminative, and sedative as well as in the treatment of bronchitis, tuberculosis, menstrual and neurological disorders. *Salvia* species are rich in terpenoids [2,3], steroids, flavonoids and other phenolics. In our continuing studies on *Salvia* species, we have isolated many oleanane and ursane type triterpenoids, namely oleanolic and ursolic acids in addition to diterpenoids, flavonoids and phenolics. Besides oleanane and ursane triterpenoids, we have also isolated lupane triterpenoids from *Salvia* species, but with poor biodiversity. In this presentation, a number of lupane triterpenoids, obtained from the extracts of several *Salvia* species (*S. montbretii* Benth., *S. cedronella* Boiss., S. *macrochlamys* Boiss. & Kotschy ex Boiss., *S.trichoclada* Benth., *S. hypargeia* Fisch. & Mey.) [4,5] will be presented with their promising activity results. Structures of the pure triterpenoids were identified by spectroscopic analysis using extensive NMR (1D and 2D), UV, IR, and mass spectroscopic techniques. Antioxidant activity of the triterpenoids was established by β-caroten bleaching and radical scavenging methods. The most promising activity was found for glochidone and monogynol A against AChE and BChE enzymes by Ellman Method [6]. Monogynol A and its three natural derivatives, isolated from *S. macrochlamys* were also found to be highly active in a metal chelating test system on ferrous ion [5]. **Keywords:** *Salvia* species, *S. montbretti, S. cedronella, S. macrochlamys, S.trichoclada, S.hypargeia*, antioxidant activity, Ellman Method, lupane **References:** [1] Davis PH (1982) Flora of Turkey and East Aegean Islands, V.7, pp.401 – 463, University Press, Edingburgh. [2] Topçu G (2006) J Nat Prod 69: 482 – 487. [3] Kommera H et al. (2011) Investigational New Drugs 29: 266 – 272. [4] Ulubelen A et al. (1994) Phytochemistry 36: 413 – 415. [5] Topçu G et al. (2007) Arkivoc 7: 195 – 208. [6] Ellman et al. (1961) Biochemistry and Pharmacol 7: 88 – 95.

PM173 Improved pharmacophore description of P-glycoprotein modulators

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Cancer is one of the diseases with increased prevalence in the 21st century. Since the 1970 s that a class of transmembranar proteins called ABC transporters are known, being P-Glycoprotein (Pgp) the most representative member. They are often involved in the efflux of drugs preventing their accumulation in the cytoplasm and thus decreasing the therapeutic effect. This key factor strongly contributes to the phenomena of resistance to anticancer drugs, preventing the success of chemotherapy regimens [1]. Despite the recent publication of the murine P-gp crystallographic structure, the characterization of the drug-binding site is still limited and, therefore, the theories for the protein's functioning cannot be validated and more suitable modulators for the effective inhibition of the multidrug-resistance phenomenon cannot be developed. In this case, pharmacophores can give important input on the subject matter. Several pharmacophores were already published that identified hydrophobic and acceptor/donor groups as essential characteristics for the recognition by the transporter [2,3]. However, the majority of the already published pharmacophores only cover a small variety of compounds, frequently derived from a primary scaffold, not being able to detect different structures or to select from a database only the active ones. In addition, the literature-derived pharmacophores fail to detect our in-house macrocyclic diterpenes [4]. Inspired on the published literature and based on the lathyrane-type scaffold, we developed a new pharmacophore (Figure 1) capable of detecting not only the literature (84.2%) but also all in-house compounds, with lower detection of inactive molecules, in a database comprising 272 compounds.

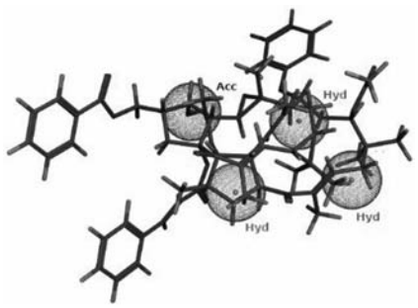

Figure 1: In-house pharmacophore

Keywords: P-glycoprotein; pharmacophore; modulators; lathyrane; macrocyclic diterpenes **Acknowledgement:** *This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008)* **References:** 1. Tsuruo T et al. (1981) Cancer Research 41: 1967 – 1972 2. Pearce HL et al. (1989) Proc Natl Acad Sci USA 86: 5128 – 5132 3. Pajeva IK.et al. (2002) J Med Chem 45: 5671 – 5686 4. Duarte N et al. (2006) Planta Med 72(2): 162 – 168

PM174 MHC-II loading enhancement (MLE) – a new immunological activity of natural essential oils and their constituents

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Enhancement of MHC-II peptide loading by low-molecular weight chemicals is of twofold interest in immunological research. Compounds that elicit an increased loading of MHC-II molecules with immunogenic peptides (MLEs) may be involved in the pathophysiology of autoimmune diseases. On the other hand, such compounds might be of potential use to enhance the activity of vaccines and of antitumour immunotherapies [1]. We have now discovered that some natural essential oils and their constituents are able to increase the loading of MHC-II allele HLA DR1 to a very significant extent. In a screening based on Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA) [2]), we found that a variety of essential oils as well as isolated constituents could increase the spontaneous loading of soluble HLA DR1 [3] with an influenza A haemagglutinin peptide (HA 306 – 318, [4]). Quite interestingly, structurally simple and widespread monoterpenes (citronellol, geraniol) showed the strongest activity among > 40 pure compounds tested. Of 28 essential oils tested so far, chamomile oil (German Chamomile, *Matricaria recutita* L.) showed the strongest effect, comparable with the reference compound, adamantylethanol, a potent MLE [1]. Activity-directed isolation led to the identification of E-ene-yne-dicycloether as the strongest MLE compound, about 3 times stronger than the Z-isomer. Bisabololoxides A and B were also significantly active but much less potent than the E-spiroether. These findings indicate that MHC-II loading enhancement might be involved in the immunological activities of essential oils and may also open new perspectives with respect to potential applications. **Keywords:** Major histocompatibility complex-II; MHC-loading enhancers MLE; essential oil, German Chamomile, Matricaria recutita **References:** 1. Höppner S et al. (2006) J Biol Chem 281: 38535 – 42. 2. Khadkodayan S et al. (2007) Assay Drug Dev Technol 5: 501 – 14. 3. HLA-DR1 (DRB1*0101) in house production S. Günther, MDC Berlin. 4. Lamb JR et al. (1982) Nature 300: 66 – 9.

PM175 Saponins from Astragalus pycnocephalus var. pycnocephalus FISCHER and their α/β-glucosidase inhibitory effects

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The genus *Astragalus* belonging to the Leguminosae family is widely distributed throughout the temperate regions of the world. In the flora of Turkey, this genus is represented by 445 species [1,2]. The roots of *Astragalus* are used in traditional medicine as an antiperspirant, diuretic and tonic drug. It has also been used in the treatment of diabetes mellitus, nephritis, leukemia and uterine cancer [3]. Astragalus species are known to be rich in two major classes of biologically active compounds, polysaccharides and saponins. Also the indolizidine alkaloids, the nitro compounds and flavonoids were isolated from the genus [4 – 6]. Inhibition of glycoside hydrolases has widespread application in the treatment of diabetes, viral infections, lysosomal storage diseases and cancers. As part of our ongoing research of new bioactive compounds from Turkish *Astragalus* species, we carried out a study on *A. pycnocephalus* Fischer var. *pycnocephalus*. In the present work, four known secondary metabolites namely trojanoside H, astragaloside IV, astragaloside VIII, and astrasieversianin X were purified by various chromatographic techniques and their inhibitory effects on α- and β-glucosidases were investigated. The compounds showed strong inhibition against α-glucosidase whereas they had moderate activity against β-glucosidase. This is the first phytochemical and biological activity investigation reported on *A. pycnocephalus* var. *pycnocephalus*.

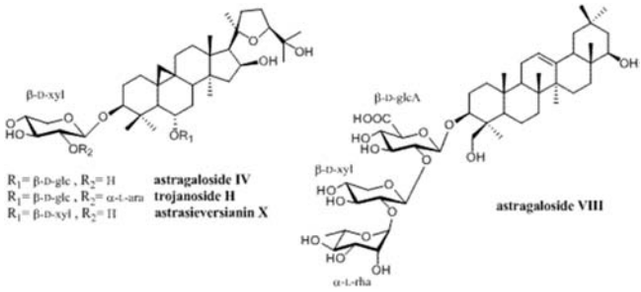

Figure 1

Keywords: Astragalus, Saponin, Cycloartane, alpha-glucosidase inhibition, beta-glucosidase inhibition **References:** 1. Davis PH (1970) Flora of Turkey and East Aegean Islands. University Press. Edinburg. 2. Aytaç Z Astragalus L.- In: Güner A, Ozhatay N, Ekim T, Başer KHC (eds.) (2000) Flora of Turkey and the East Aegean Islands. Edinburgh University Press. Edinburgh. 3.Tang W, Eisenbrand G (1992) Chinese Drugs of Plant Origin. Springer-Verlag, Berlin. 4. Rios LJ, Waterman PG (1997) Phytother Res 11: 411 – 418. 5. Bedir E et al. (2000) Biol Pharm Bull 23: 834 – 837. 6. Li XY (2000) Pharm Biol 38: 33 – 40.

PM176 An ethnopharmacological study on *Verbascum* species: From conventional wound healing use to scientific verification

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The leaves, flowers, and whole aerial parts of *Verbascum* L. (Scrophulariaceae) species are used to treat eczema and other types of inflammatory skin conditions for desiccating wounds in traditional Turkish medicine.The methanolic extracts prepared with thirteen *Verbascum* species growing in Turkey, including *V. chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., *V. dudleyanum* (Hub.-Mor.) Hub.-Mor., *V. lasianthum* Boiss., *V. latisepalum* Hub.-Mor., *V. mucronatum* Lam., *V. olympicum* Boiss., *V. pterocalycinum* var. *mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Heldr., *V. salviifolium* Boiss., *V. splendidum* Boiss., *V. stachydifolium* Boiss. & Heldr and *V. uschackense* (Murb.) Hub.-Mor. were assessed for their *in vivo* wound healing activity. *In vivo* wound healing activity of the plants were evaluated with linear incision and circular excision experimental models subsequently histopathological analysis. The healing potential was comparatively assessed with a reference ointment Madecassol®, which contains 1% extract of *Centella asiatica*. The methanolic extracts of *V. olympicum* (36.6%, 75.6%), *V. stachydifolium* (40.1%, 79.1%) and *V. uschackense* (37.4%, 70.5%) demonstrated the highest activities on the both wound models. Moreover, the methanolic extracts of *V. latisepalum* (32.7%, 40.3%), *V. mucronatum* (21.2%, 26.4%), and *V. pterocalycinum* var. *mutense* (26.7%, 56.6%) were found generally highly effective. On the other hand, the rest of the species did not show any remarkable wound healing effect. Results of the present study support the continued and expanded utilization of these plant species employed in Turkish folk medicine. The experimental study revealed that *Verbascum* species display remarkable wound healing activity.

PM177 Protective Effects of Astragaloside IV and Cycloastragenol in 6-hydroxydopamin (6-OHDA)-Induced Neurotoxicity in PC12 Cells

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Astragaloside IV (AST-IV), one of the bioactive constituents of Radix Astragali, was extracted from the roots of *Astragalus trojanus* Bunge (Leguminosae). Cycloastragenol (CG), which is a minor metabolite mostly found in its glycosidic form, was obtained from AST-IV via hydrolysis reaction. CG has been shown to extend T cell proliferation by increasing telomarase activity showing that it may also help delay the onset of cellular aging (1). Indeed, recently, CG has been introduced to the market as a new generation antiaging molecule. Moreover our studies proved CG as an extraordinary wound healing agent (2). Although AST-IV's neuroprotective effects on Parkinson's disease was reported previously, there has been no data for CG. The aim of this study was to investigate the protective effects of AST-IV and CG on neurotoxicity induced by 6-hydroxydopamin (6-OHDA) in PC12 cells, an excepted in vitro model for Parkinson's disease. The cells were seeded on tissue culture plates for 24 h. After 24 hours, they were incubated with AST-IV (0.1 μM-1 fM) and CG (0.1 μM-1 fM) for 30 min before the insults with 200 μM 6-OHDA. The cells were incubated for 24 h. Cell viability and cells death were assessed by (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) MTT assay and lactate dehydrogenase (LDH) assay kit, respectively. AST-IV and CG inhibited the apoptosis of PC12 induced by 6-OHDA at 0.001 and 0.0001 μM concentrations. On the basis of these results, we propose AST-IV and CG as potential neuroprotective agents in the treatment of Parkinson's disease.

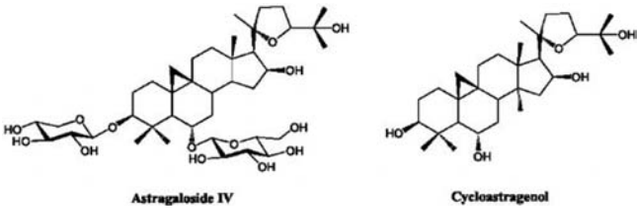

Figure 1

Keywords: Astragalus, Saponin, Cycloastragenol, Astragaloside IV, Neuroprotective effect, Parkinson's disease **References:** 1. Valenzuela HF et al. (2009) J Immun 182: 9 – 30. 2. Sevimli-Gür C et al. (2011) J Ethnopharmacol 134: 844 – 850.

PM178 A QSAR study of macrocyclic diterpenes with P-gp inhibitory activity isolated from *Euphorbia* species

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A set of 50 compounds, constituted mainly by macrocyclic diterpenes of the jatrophane and lathyrane-type, isolated from *Euphorbia* species, was used in the present study. These compounds were found to be P-glycoprotein-mediated multidrug-resistance (MDR) reversing agents in cancer cells. The reversal of MDR was investigated by flow cytometry, at two different concentrations, 4 and 40 μg/mL, by measuring rhodamine-123 accumulation, a fluorescent substrate analogue of doxorubicin [1, 2]. The quantitative structure-activity relationship (QSAR) methodology was applied to the whole set of diterpenes and to the jatrophanes subset at both concentrations studied. We used multiple linear regression (MLR) with forward features (from five classes of molecular descriptors: constitutional, topological, geometrical, electrostatic and quantum-chemical) selection to establish QSAR models. The best model obtained, at 4 μg/mL, for the diterpenes set was constructed using 4 descriptors with R^2 of 0.80 for a training set of 40 compounds and R^2_{pred} of 0.71 for a test set of 9 compounds. The best model obtained, at 4 μg/mL, for jatrophanes subset was constructed using 5 descriptors with R^2 of 0.82 for a training set of 28 compounds and R^2_{pred} of 0.54 for a test set of 6 compounds. All models were statistically valid with high predictability, and in both models the descriptors used (logP and quantum descriptor related to resonance energy) can easily be translated into the design of novel diterpene derivatives with a forecasted improved P-gp inhibitory activity. **Keywords:** QSAR, P-gp inhibitory activity, macrocyclic diterpenes **Acknowledgement:** *This study was supported by FCT (PTDC/QUI-QUI/099815/2008), Portugal.* **References:** 1. Molnár J et al. (2006) Curr Pharm Des 12: 287 – 311. 2. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 30.

PM179 Antidepressant mechanisms of action of willow bark extract STW 33-I

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Therapies in chronic musculoskeletal pain can be supplemented by antidepressants (1). For willow bark extracts, known for their anti-inflammatory and analgesic properties (2), additional antidepressant effects have been suggested (3). To elucidate the underlying potential mechanisms of action, the willow bark extract STW 33-I (Proaktiv®) and four of its fractions separated by polarity (4) have been studied. Male Sprague Dawley rats (n = 6/group) were treated for two weeks once daily per os with different doses of the extract and with fractions produced by liquid/liquid partition, in comparison to a control and reference group (imipramine 20 mg/kg b.w.). On day 15, a forced swim test according to Porsolt was performed and the locomotor activity was determined. Treatment was continued for further four days and neurotransmitter concentrations were determined in frontal cortex, hypothalamus, hippocampus and striatum. A significant shortening of the cumulative period of immobility was seen after treatment with 15, 30, 60 mg/kg b.w. of the extract, whereas the locomotor activity did not increase. Higher doses of STW 33-I were ineffective. Fractions were characterized analytically by HPLC and tested in doses resembling their yield in the extract, with best effects in doses equivalent to the lowest extract dose tested. 5-HT levels in the hippocampal tissue were increased significantly. STW 33-I and its fractions showed an antidepressant like effect. The serotonergic system seems to be involved. This central effect of the willow bark extract STW 33-I may contribute to its clinical efficacy in musculoskeletal pain. **Keywords:** Willow bark extract, pain, FST, analgesia, depression, hippocampus, serotonin, 5-HT **Acknowledgement:** *This contribution is dedicated in memoriam to Prof. Dr. Hilke Winterhoff, Institut für Pharmakologie und Toxikologie, Westfälische Wilhelms-Universität, Münster, Germany, and former Chair of the Permanent Committee on Biological and Pharmacological Activity of Natural Products of GA, who passed away on May 9, 2010* **References:** 1. Perrot S et al. (2008) Rheumatology doi: 10.1093/rheumatology/ken110; 2. Nahrstedt A et al. (2007) Wien Med Wochenschr 157:348 – 351; 3. Hegger S et al. (2005) Kongress Phytopharmaka Phytotherapie, Berlin 2005:S19, (4) Bonaterra GA et al. (2010) Phytomedicine 17: 1106 – 1113

PM180 Evaluation of diterpenic compounds as inhibitors of multidrug resistance on human colon adenocarcinoma cells

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One of the most promising strategies for overcoming multidrug resistance (MDR) is to use compounds that can modulate P-glycoprotein (Pgp) and restore the cytotoxicity of anticancer drugs. A large variety of compounds have been shown to be MDR-modulators, and some of them have undergone clinical trials. They are a large chemical and structurally diverse group that includes among others, natural products and their semi-synthetic derivatives. However there are currently no reversal agents clinically available. Therefore, a great need for new reversal

agents with higher specificity and efficacy still remains [1]. In previous studies, we have reported the isolation of several macrocyclic lathyrane and jatrophane-type diterpenes, which were found to have a potent inhibitory activity against P-glycoprotein of human MDR1 gene-transfected mouse lymphoma cells [2 – 4]. The purpose of this work was to study the ability of lathyrane and jatrophane derivatives to modulate the transport activity of P-glycoprotein on human colon adenocarcinoma cell lines (COLO 205 and COLO 320). The reversal of MDR was investigated by flow cytometry, measuring rhodamine-123 accumulation. Several of the compounds tested have shown to be strong Pgp inhibitors. Furthermore, some of these modulators, which presented a significant MDR reversal activity, were assayed, *in vitro*, for their antiproliferative effects in combination with doxorubicin. Some of the compounds synergistically enhanced the effect of the antitumor drug. According to these results, macrocyclic diterpenes may be valuable as lead compounds for the development of Pgp modulators in different multidrug-resistant cancer cells and to further study their effect in animal experiments. **Keywords:** Cancer, multidrug resistance, macrocyclic diterpenes, adenocarcinoma cells **Acknowledgement:** *This study was supported by FCT, Portugal (project PTDC/QUI-QUI/099815/2008; grant SFRH/BD/72915/2010) and Szeged Foundation for Cancer Research, Hungary* **References:** 1. Shukla S et al. (2008) Expert Opin Drug Metab Toxicol 4: 205 – 23 2. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 30. 3. Duarte N et al. (2007) Bioorg Med Chem 15: 546 – 54. 4. Madureira et al. (2006) J Nat Prod 69: 950 – 53.

PM181 Richness of extremophile plants in phenolics with interesting biological activities

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Extremophile plants are remarkable plants that tolerate severe environmental constraints which may trigger in plants an oxidative stress Able to overcome oxidative stress, these species have developed potent antioxidant systems. Among them, polyphenols constitute the main powerful compounds, owing to their strong biological activity. Therefore, the need exits for safe, natural antioxidants to replace synthetic ones. In this context, Tunisian extremophile species known for their ethno-pharmacological uses was making them good candidates for industrial application. This study aimed to investigate antioxidant activities, to estimate the antimicrobial capacities, to examine anti-inflammatory and anticancer activities, to identify phenolics, and to valorize extremophile plants in industry. Results showed that some halophytes (*Tamarix gallica* L. and *Limoniastrum monopetalum* Boiss.) exhibit 3 to 4-folds higher phenolics as compared to glycophyte medicinal plants (Mentha pulegium). Moreover, these species displayed an important antiradical activity, and β-carotene bleaching, and lipid peroxidation inhibition. In addition, halophyte extracts (*Tamarix gallica*) showed appreciable antibacterial properties against Human pathogen strains, and against *Botrytis cinerea* and have an interesting anti-amyloïdogenic activity, and have a cell renal protective effect against herpes simplex virus type 1. In addition, these tolerant plants (*Suaeda fruticosa* Forssk. ex J. F. Gmelin) showed an interesting in vivo antioxidant activity in fibroblast cell, anti-inflammatory ability in (LPS)-stimulated RAW 264.7 macrophage and an anticancer power against carcinoma cell lines. Indeed, for the contribution of these halophytes in the field of cosmetics, cell suspensions culture in bioreactors for the production of cells enriched in phenolic as active principle in cosmetic formulation was made. **Acknowledgement:** *This work was supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02).*

PM182 *Iberis amara* L. desensitizes low-threshold mechano-sensitive afferents of the colon

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Medicinal plants used in irritable bowel syndrome include *Iberis amara* L. (1). Its extract (STW 6) is component of the herbal combination preparation STW 5 (Iberogast®) (2), for which an antagonizing effect on gastrointestinal hypersensitivity has been shown (3). The colon of C57Bl6 mice was isolated and recordings of the mesenteric nerve were taken in an organ bath, while perfusing lumen and bath with Krebs solution (32 °C, 10 ml*min^{-1}) containing 1 μM nifedipine to eliminate contractility. STW 5 (lyophilized, 57.1 x 10^{-3}μg*ml^{-1}) or STW 6 (lyophilized, 21.2 x 10^{-3}μg*ml^{-1}) were applied 10 minutes prior to stimulation with bradykinin (0.5 μM), 5-HT (10 μM) or luminal ramp distension from 0 to 80 cm $H_2$0. Intestinal afferent nerve discharge to 5-HT was reduced to 0.2 ± 0.2imp*s^{-1} after STW 6, compared to 5 ± 2imp*s^{-1} following vehicle and 3 ± 1 imp*s^{-1} following STW 5 ($p < 0.05$ STW 6 vs. vehicle). The response to bradykinin was 36 ± 5 imp*s^{-1} after STW 6 and 38 ± 6 imp*s^{-1} after vehicle (n. s.). Following STW 5 it was reduced to 9 ± 2 imp*s^{-1} compared to vehicle ($p < 0.05$). Values at 80 cmH_2O were 14 ± 3 imp*s^{-1} after STW 6, 22 ± 3 imp*s^{-1} after vehicle and 4 ± 3 imp*s^{-1} after STW 5 ($p < 0.05$ for both versus vehicle). The *Iberis amara* extract STW 6 has a particular desensitizing effect in low-threshold mechano-sensitive afferents, while STW 5 acts on both low- and high-threshold afferents. Therefore the broader profile of STW 5, possibly mediating its therapeutic effects in irritable bowel syndrome, is in part, but not entirely, based on the effects of STW 6. **Keywords:** Iberis amara, Colon, Colon irritabile, Irritable bowel syndrome, Intestinal hypersensitivity, Bradykinin **References:** 1. Reichling J, Saller R (2002) Klass Naturheilkd, 9 (Suppl. 1): 21 – 32; 2. Kroll U, Cordes C (2006) Phytomedicine, 13 (Supp. V): 12 – 19; 3. Müller MH et al. (2006) Phytomedicine 13: 100 – 106

PM183 Larvicidal activity of Eugenia uniflora in Aedes aegyptii

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In Brazil, the leaves of *Eugenia uniflora* L. are crushed and spread on the floor for its flavour and fly-repellent property [1]. Insecticidal activity of the oil [2] and larvicidal activity of the extract has been reported[3]. The larvicidal activity of the leaf methanol extract was therefore investigated to determine the most active subfraction from which the active compounds could be isolated.The leaf methanol extract was partitioned into n-hexane, chloroform, ethylacetate, butanol and aqueous phase and tested for larvicidal activities, using the larvae of *Aedes aegyptii*, the most active n-hexane fraction (B_1) was sequentially subjected to Vacuum Liquid Chromatography (VLC) to yield 10 fractions C_1-C_{10} that were equally tested. The calculated percentage mortalities of the extract, fractions and subfractions of the leaf as well as of endosulphan, positive control, were used to determine their LC_{50} and LC_{90} values. The result showed that methanol extract had larvicidal activity that was statistically comparable ($P > 0.05$) to that of endosulphan. Fraction B_1 had LC_{50} and LC_{90} values that were insignificantly different from those of methanol extract and endosulphan. Fractions C_1 and C_2 had significantly greater activity than B_1 and comparable to that of endosulphan, making them to be the most active fractions. Work is in progress in order to isolate the active compounds from these most active fractions. **Keywords:** Eugenia uniflora, larvicidal, extract, leaf **References:** 1. Morton J (1987) Surinam Cherry. In: Fruits of Warm Climates, JF Morton, Miami Florida. 2. Gbolade AA, Adebayo T (1993) Insec Sci Appl 4(5/6): 631 – 636. 3.Luna JS et al. (2005) J Ethnopharmacol 97(2):199 – 206.

PM184 Effect of *Matricaria chamomilla* L. extract on fetal absorption, placenta structure and liver of diabetic pregnant rats.

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Diabetes mellitus (DM) results in severe metabolic imbalances and pathological changes in many tissues (1). Diabetes was induced by Streptozotocin in this research. Mating condition was prepared by putting male rats and diabetic female rats together and vaginal plaque was as a positive sign of pregnancy and treatment started with three doses:100,300,500, mg/kg chamomile extract extract or vehicle from 1th to 17th day of gestation by gastric gavages. Blood glucose was measured during 17 days. At 17th day, rats were scarified. The fetuses were released from the yolk sacs and surrounding deciduas and were examined for absorption rate. Results shows that level of blood glucose was reduced about 1.62 fold ($p < 0.00$) in compassion to vehicle treated diabetic rat group. In diabetic group that received no treatment feta's spontaneous abortion was 15%. Percentage of absorbed fetuses in chamomile groups received 100,300,500 doses and control group were 0%,2%,0% and 0%respectively. the percentage of absorptions was significantly elevated in vehicle-treated diabetic rats, in comparison with vehicle treated healthy rats and treated diabetic rats. Treatment with *Matricaria chamomilla* L. significantly reduced re-absorption rates in diabetic rats. also in placetas cause reduction of defects such as Artesia and immature trophoblast in treated diabetic rats. In the Diabetic group, all signs, such as separated necrosis of hepatocytes, anarchism of liver plates, and lymphocytic inflammation were improved. *Matricaria chamomilla* was found to have protective effects on spontaneous abortion and histopathological changes of placenta and liver associated with STZ diabetes in chammomile treated pregnant female rats. **Keywords:** *Matricaria chamomilla*, fetal absorption, placenta, liver, diabetes, pregnancy Ultrastructure, STZ **Acknowledgement:** *this project was a joint project between Ahvaz Jundishapur University of Medical Sciences and Pharmaceutical Research Network.* **References:** Aynes JW et al (1996) Curr Opin Endocrinol 3: 227 – 284.

PM185 Radical scavenging effects of fruit extracts from two *Ficus* species

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Ficus gnaphalocarpa L. and *Ficus dekdekena* (Miq.) A.Rich. belong to the well-known plants of Moraceae family growing in tropical regions. Their fruits are traditionally used as dietary wild fruits in West africa [1]. The aim of our study was to evaluate the *in vitro* radical scavenging activities (RSA) of these *Ficus* fruits hydroalcoholic extracts, using two different free radicals: hydroxyl radical ($HO^{\cdot}$) and superoxide anion ($O_2^{\cdot -}$). At doses of 100 and 25 $\mu g.mL^{-1}$ the percentage of inhibition of $O_2^{\cdot -}$values obtained for extracts were 61,41 and 24,02 for *F. dekdekena*, 23,3 and 31,77 for *F. gnaphalocarpa* respectively. At 10 and 2 $\mu g.mL$-1 the percentage of inhibition of $OH^{\cdot}$ values obtained for extracts were 88,37 and 52,51 for *F. dekdekena*, 87,09 and 50,28 for *F. gnaphalocarpa*, respectively Preliminary phytochemical investigation of the fruit extracts using HPLC-DAD and LC-MS showed the presence of protocatechuic acid, chlorogenic acid, ferulic acid, homo-orientin, rutin, hyperoside, catechin, epicatechin and other catechin derivatives well known as radical scavenging compounds [2]. These results suggest that *F. dekdekena* and *F. gnaphalocarpa* fruits possess radical scavenging activity, wich could be attributed to the presence of phenolic compounds. **Keywords:** Ficus, hydroxyl radical, superoxide anion, phenolic compounds **References:** 1. Lockett et al. (2000) J Food Sci Nutr 51(3): 195 – 208. 2. Nanjo F et al. (1999) Biosci Biotechnol Biochem 63(9):1621 – 1623

PM186 Polyacetylenes from *Notopterygium incisum* as a novel class of specific PPARgamma activators

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In the course of our search for PPARγ active anti-inflammatory natural products we have investigated the dichloromethane extract of the dried rhizome and root of *Notopterygium incisum* Ting ex H. T. Chang (Umbelliferae). PPARγ is one of the three Peroxisome Proliferator Activated Receptor (PPAR) subtypes and is involved in the regulation of glucose and lipid metabolism and therefore an important target for metabolic diseases. Additionally, PPARγ plays a role in other chronic diseases such as inflammation, cancer and atherosclerosis.[1] We have isolated six polyacetylenes, which were structurally characterized by means of multidimensional NMR and mass spectroscopy, and which were shown to inhibit NO production in RAW 264.7 macrophages.[2] Now, the potency of these polyacetylenes as PPARγ agonists has been evaluated. The EC_{50}s of 8-acetoxyfalcarinol, falcarindiol, 9-epoxy-falcarindiol, crithmumdiol, 9-heptadecene-4,6-diyn-1-ol and 2*Z*,9*Z*)-2,9-heptadecadiene-4,6-diyn-1-ol were determined as 2.36-fold activation (EC_{50} of 3.59 μM), 2.29-fold activation (EC_{50} of 4.25 μM), 1.88-fold activation (EC_{50} of 2.03 μM) and 2.29-fold activation (EC_{50} of 4.58 μM), 1.921 fold activation (EC_{50} of 11.31 μM) and 1.73-fold activation (EC_{50} of 4.18 μM), respectively, whereas the positive control pioglitazone exhibited 7.96-fold activation (EC_{50} of 0.31 μM). Therefore, these polyacetylene derivatives contribute to the anti-inflammatory activity of *Notopterygium incisum* by selectively activating PPARγ without affecting the other two PPAR subtypes.[3]

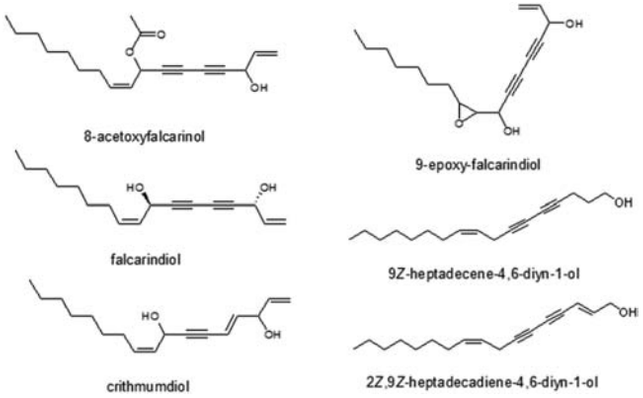

Figure 1: Chemical structures of the polyacetylenes from *N. incisum*.

Keywords: *Notopterygium incisum*, polyacetylenes, PPARγ, metabolic diseases, PPAR gamma activators **Acknowledgement:** *We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF) within project NFN S 10705-B13.* **References:** 1. Schmidt MV et al. (2010) Scientific World Journal 10: 2181 – 97. 2. Blunder M et al. (2011) Planta Med, in preparation 3. Blunder M et al. (2011) J Nat Prod, in preparation

PM187 Characterization of glycosidic triterpenoids of *Scilla litardierei* and investigation of its potential cytotoxic effects

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Scilla litardierei Breistr. (syn. *Chouardia litardierei* Speta) (Hyacinthaceae) is a bulbous geophyte, which is part of the original flora of southeast Europe and the Balkans. In this study the separation and purification of saponin-rich fractions obtained by partition of bulbous extracts from *S. litardierei* was carried out. The glycosidic natural products were elucidated by chromatographic and spectroscopic methods. Pure substances were gained by purification using preparative HPLC. Sugar moieties were identified after hydrolysation and derivatisation by gas chromatography. Several triterpenoids, which differ in the aglycons, were detected. African green monkey kidney cells (COS-7) and human embry-

onal kidney (HEK-293) cells were treated with isolated triterpenoids. In order to elucidate possible antiproliferative and cytotoxic effects of this substances the commonly used MTT assay was utilized. **Keywords:** Scilla litardierei, Glycosidic saponins, Hyacinthaceae, Triterpenoids **References:** 1. Müller B and Klar F (2010) Planta Med 76: 1254.

PM188 Effect of fumarprotocetraric acid isolated the Lichen *Cladonia verticillaris* on tracheobronchial phenol red excretion in mice

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The lichen *Cladonia verticillaris* is very common in the northeast of Brazil. It has as essential biologically active secondary metabolites the fumarprotocetraric (FUM) and protocetraric acid. Similar chemical composition is found in *Cetraria islandica* used in Turkish folk medicine for treatment of bronchitis and tuberculosis. This study shows the effect of FUM isolated from *C. verticillaris* on tracheobronchial phenol red excretion in mice. FUM administered by oral gavage (50 and 100 mg/kg) or intraperitoneally (25 and 50 mg/kg) and Ambroxol (1 mg/kg) were administrated in male Swiss mice (n = 6 animals/group) thirty minutes before the administration of phenol red (200 mg/kg; i. p.). Administration of vehicle (solution saline 0.9%) was used as control. Sixty minutes after the dye injection, the mice were euthanized and a bronchial lavage (BL) was realized with saline. The lavage fluid was mixed with NaOH 0,01N, and the quantification of phenol red in BL was analysed photometrically at 535 nm. The expectorant effect was determined by comparing the phenol red concentration (mg/mL) in BL of treated and control group. It was shown that oral gavage (50 mg/kg and 100 mg/kg) or intraperitoneal (25 and 50 mg/kg) administration of FUM increased phenol red excretion in BL in a dose-dependent manner in comparison to control group ($p < 0.05$). However, there was no statistical difference between phenol red excretion in the BL after Intraperitoneal (3.2 ± 0.38 µg/ml) or oral gavage (4.0 ± 0.68 µg/ml) administration of the same dose (50 mg/kg). The results suggest that expectorants action of FUM is not mediated by a vagal reflex initiated by stimulation of the gastric mucosa following oral administration. **Keywords:** Cladonia verticillaris, fumarprotocetraric acid, excretion **Acknowledgement:** *CAPES* **References:** [1] Dülger B et al. (1998) TJB 22:11 – 118. [2] Santos N. P, et al. (1997) RUASCB 1(2): 23 – 43.

PM189 Non-host interactions to detect anti-*Fusarium* substances

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Fusarium are ubiquitous filamentous fungi and a major threat for numerous plant or animal species [1]. They are often considered as highly resistant to conventional antifungal treatments. The *Fusarium* genus also contains isolates considered as endosymbiontic or saprotrophitic strains. These lifestyles are dispersed over a large phylogenetic spectrum [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or host. We've shown that *Fusarium* strains have genuine capacity to develop on various host and we made use of these adaptation capacities to establish non-host interactions on vine leaves. Some *Fusarium* strains developed efficiently as necrotrophic pathogens while others adopt less aggressive mode of interaction on susceptible vine varieties as Chasselas. Correspondingly, some *Vitis* species or varieties were more resistant and restrained efficiently fungal growth. To identify natural substances involve in the control of fungal development, we set-up a sensitivity test in 96 well plates to screen for anti-*Fusarium* activity of natural extracts. The test on solid growth medium enables to screen natural extracts on several filamentous fungi and we compared fungal susceptibility to these extracts using various human and plant pathogens. **Keywords:** Fusarium, antifungals **Acknowledgement:** *This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W. and K. G.)* **References:** 1. Schurch S et al. (2010) Agrarforschung Schweiz 1: 442 – 445. 2. Zhang et al. (2006) J Clin Microbiol 44: 2186 – 2190

PM190 *Coronilla varia* L. nitrotoxins as defensive secondary metabolite against heavy metals pollution

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Nitrotoxins or nitroglycosides are aliphatic nitro compounds, which chemically or structurally glucose esters of nitropropionic acid and nitropropanol, which were detected in some legumes (Papilionoideae). They are important due to mammalian toxicities; attraction of pollinators or seed disperses repulsion or inhibition of herbivores and microorganisms and has a role in plant defense [1]. In this study six weeks aged grown *Coronilla varia* L. plants in equal growth condition were treated with different concentrations of Zn and Ni for 24 and 72 h. The Qualitative test and quantitative determination for aliphatic nitro toxins of control and treated plants examined was done [2, 3]. Increasing nitrotoxins concentration ranging from 4 – 25 mg NO2 mg g-1plant in all treated plants were observed in compared to control (1 – 4 mg NO2 mg g-1plant). This study showed nitro compounds may have a protective defensive role against some environmental stresses such as heavy metals pollution [4, 5]. **Keywords:** Nitrotoxins, Coronilla varia, heavy metals, Zn, Ni, legumes **Acknowledgement:** *Authors wish to thank Biology Department of University of Arak.* **References:** 1. Majak M (2001) J Range Manage 54: 494 – 498. 2. Cooke AR (1955) Arch Biochem Biophys 55: 114 – 120. 3. Williams MC and Parker R (1974) Weed Sci 22: 259 – 262. 4. Noori M et al. (2007) Toxicol and Environ Chem 89 (3): 479 – 485. 5. Noori M et al. (2010) Toxicol and Environ Chem 92 (1): 97 – 105.

PM191 The Importance of Anthocyanins for Human and Animal Health

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Anthocyanins belong to the plant secondary metabolites causing pigmentation to flowers, fruits, seeds and leaves. They are abundant in red red berries and fruits. They are phenolic compounds belong to the flavonoids, and occur mainly as glycosides. They have antioxidant effects, and they protect cells against oxygen radical-related damage at the basis of various diseases. The concept evolves that the human and animal health condition could be partly controlled through the dietary intake of plant polyphenols. Plant polyphenols are recognized as naturally occurring antioxidants but also catalyze oxidative DNA degradation of cellular DNA either alone or in the presence of transition metal ions such as copper. In this paper we show that similar to various other classes of polyphenols, delphinidin is also capable of causing oxidative degradation of cellular DNA [1]. The antimicrobial activity and major anthocyanin pigments were determined of *Vitis vinifera* L. and the extracts of *Vitis vinifera* were studied with association of its antiradical activity [2]. Anthocyanin content varied from 85.7 to 1914.0 mg/kg fresh berry weight. Assessment of the antiradical activity of extracts suggesting that other constituents are likely to exert strong antioxidant effects in grapes. Our work is focused to study antimicrobial and antioxidant activity of anthocyanins from the selected plant species in Slovakia. **Keywords:** anthocyanines, antioxidant activity, health, animal **Acknowledgement:** *This research is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 26220120023, ITMS 26220120041, ITMS 26220220013.* **References:** [1] Hanif S et al. (2008) DNA Toxicology 249: 19 – 25. [2] Kallithraka S et al. (2005) J Food Comp Anal 18: 375 – 386.

PM192 Two matrix metalloproteinase inhibitors from *Scrophularia striata* Boiss

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Because the activation of metalloproteinases (MMPs) is a key factor in the metastatic process, compounds with the ability to inhibit MMP activity have a potential in treatment of tumor. The genus *Scrophularia*, consisting of about 300 Species, is one of the most important genera belonging to the Scrophulariaceae. In this study, two active substances of *S. striata* Boiss. were identified by using bioassay-guided fractionation and their chemical structures were deduced by nuclear magnetic resonance and mass spectrometry. Nepitrin at lower doses showed progressive MMPs inhibitory effect with negligible cytotoxicity, whereas acteoside 1 at higher doses (up to 80 µg/ml) revealed most MMPs inhibitory effect preserving low cytotoxicity. **Keywords:** Scrophularia striata, nepitrin, acteoside 1, wehi-164, zymoanalysis **References:** Hajiaghaee R et al. (2007) Phytother Res 21: 1127 – 1129.

PM193 Brazilian Amazon plant extract active against head-and-neck tumor cell-line tested for toxicity

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Plant extract obtained from *Pentachlethra macroloba* (Willd.) Kuntze [Pepm], Simaroubaceae, showed antiproliferative activity against head-and-neck human tumor cell line. Toxicity assays were carried out in order to obtain its toxic tendency, as well as its LD_{50}. Acute toxicity assay was carried out using groups of three young-adult Balb-c male mice. Animals received *i.p.* administration of Pepm suspended in almond oil, also used in the control group. Doses were tested starting up from 5 g/kg and subsequent ½-fold-decreasing doses until the observation of non-lethality. General activity including evaluation of more than 20 parameters was also accessed. Statistics was done using ANOVA. Parameters observed after ip administration of Pepm show that general activity was not significantly affected by treatment, as well as the surface-righting-reflex, body tone and grip reflex. Hindquarter-fall showed was significantly different from control after Pepm-administration of 5 and 2.5 g/kg. Significant differences between treatments were observed for Pepm administred at 5 g/kg for stimulation, tremor and ptosis, while cyanosis appeared in lower doses. Response to stimulation significantly decreased after administration of higher doses as well as the response to tail squeeze, and auricular reflex. Defecation significantly diminished after administration of higher doses, but micturition was elevated. LD_{50} for PEpm was 37 mg/kg (medium-toxicity). Toxicity of Pepm is being investigated and some degree of toxicity was found, although the effective cause of death in mice is unknown so far. Substances causing both the antiproliferative and the toxicity is being isolated and elucidated, as well as the hystopathological analysis of some organs as liver and kidneys. **Keywords:** toxicity, Pentachlethra macroloba, Amazon plant extract, general activity **Acknowledgement:** *FAPESP-grant#2008/58706 – 8, UNIP* **References:** Brito AS (1994) Manual de ensaios toxicológicos in vivo. Editora da UNICAMP. São Paulo. Botham PA (2004) Toxicol in vitro 18: 227 – 30.

PM194 Effect of an alpha – glucosidase inhibitor from the seeds of *adenanthera pavonina* on gonadal weight and function in mice

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The compound responsible for the alpha – glucosidase inhibitory activity of seeds of *Adenanthera pavonina* L. was isolated from ethanolic extract (70% v/v), purified by ion exchange chromatography and partially characterized by UV, ^{1}H NMR, and ^{13}C NMR to be an unresolved nitrogenous five- membered lactone structure, that is possibly pavonin. *In vivo* treatment of mice with isolate at sub acute levels (10 – 100 mg/kg body weight) for 28 days decreased significantly the relative weight (expressed as percentage of body weight) of the testes and ovaries of male and female rats respectively at $p < 0.05$. The glutathione-S-transferase activities of both organs also decreased significantly ($p < 0.05$) with time when compared with the control, suggesting reduced gonadal function. Histopathological studies on these organs revealed mild to moderate reduction of spermatogenesis in the testes, while in the ovaries there were mild reductions in follicular activities. However, resorption of foetus was observed in pregnant mice in the course of the experiment. Conclusively, the isolate may interfere with gonadal function by affecting spermatogenesis, oogenesis and possibly the production of sex hormones in both sexes. **Keywords:** *Adenanthera pavonina*, five membered lactone, gonadal weight, function **Acknowledgement:** *Dr A.I. Gray, Department of Chemistry, University of Strathclyde, Glasgow, for spectral elucidation of isolate* **References:** 1. Ali MS (2005) Nat Prod Res 19: 37 – 40 2. Awasthi S et al. (1993) Arch Biochem Biophys 301: 143 – 150. 3. Macedo MLR et al. (2010) Arch Insect Biochem Physiol 73: 213 – 231

PM195 Triterpenoids as inhibitors of Plasmodium liver-stage development

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Malaria is one of the foremost public health problems in Africa. It is endemic in 90 countries, affecting nearly 40% of the global population. The increasing prevalence of drug-resistant *Plasmodium falciparum* strains is one of the greatest challenges in malaria control. In order to overcome drug-resistance, new antimalarial drugs are urgently needed. Most of the available antimalarial agents kill blood stage parasites and only a limited number of drugs act on liver stages. In fact, the study of *Plasmodium* liver stage development has been hampered by limitations in the experimental approaches required to quantify hepatocyte infection by the parasite. Therefore, the development of new drugs targeting the *Plasmodium* liver stage represents an important and under-exploited site of intervention [1, 2]. Previously, bioassay-guided fractionation of the methanol extract of the aerial parts of *Momordica balsamina* L. led to the isolation of several cucurbitane-type triterpenoids. Several of those compounds and acylated derivatives displayed in vitro antimalarial activity against blood schizonts of chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* [3 – 5]. In this study some of the isolated compounds from *Momordica balsamina* and several alkanoyl and aroyl ester derivatives were evaluated for their in vitro tissue-schizontocidal activity on *Plasmodium berghei* infected hepatoma cells. Inhibition of liver stage infection was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* line, PbGFP-Luc_{con}, as previously described [1]. Most of the compound tested displayed a dose-dependent antimalarial activity with $IC_{50} < 5$ µM. **Keywords:** Malaria, *Plasmodium*, liver-stage, *Momordica balsamina* **Acknowledgement:** *This study was supported by FCT, Portugal (SFRH/BD/22321/2005).* **References:** 1. Ploemen et al (2009) PloS One 4: e7881. 2. Prudêncio et al. (2008) Cell Microbiol 10: 218 – 24. 3. Ramalhete et al. (2009) Bioorg. Med Chem 17: 6942 – 51. 4. Ramalhete et

al. (2010) Bioorg. Med Chem 18: 5254 – 60. 5. Ramalhete et al. (2011) Bioorg. Med Chem 19: 330 – 8.

PM196 Anthelmintic activity of *Cymbopogon schoenanthus* and *Cymbopogon martinii* essential oils evaluated by four different *in vitro* tests

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Anthelmintic resistance is a worldwide matter in small ruminant industry and new compounds derived from plants are being studied to be used as an additional tool to control nematodes [1,2]. *Cymbopogon schoenanthus* Spreng. and *Cymbopogon martinii* (Roxb.) J. F. Watson (family Poaceae) essential oils were chosen to be evaluated against development stages of trichostrongylids from sheep by Egg Hatch Assay (EHA), Larval Development Assay (LDA), Larval Feeding Inhibition Assay (LFIA) and Larval Exsheathment Assay (LEA). The essential oils were analyzed by gas chromatography and mass spectrometry, and their major constituents were geraniol (55.3%) and geranial (13.3%) for *C. schoenanthus* and geraniol (81.4%) and geranyl acetate (10.1%) for *C. martinii*. In all *in vitro* tests *C. schoenanthus* oil presented the best activity against ovine trichostrongylids. LC_{50} values are presented in Table 1. Considering these results, *C. schoenanthus* essential oil was selected for further experiments to evaluate its anthelmintic activity in *in vivo* models.

Table 1: CL 50 (µL/mL) and confidence limits of Cymbopogon schoenanthus and Cymbopogon martinii essential oils in egg hatch assay (EHA), larval development assay (LDA), larval exsheathment assay (LEA) and larval feeding inhibition assay (LFIA) against

	C. schoenanthus	C. martinii
EHA	0.05 (0.04 – 0.06)	0.15 (0.13 – 0.17)
LDA	0.07 (0.06 – 0.08)	0.18 (0.17 – 0.19)
LEA	27.10 (21.37 – 32.38)	32.02 (29.87 – 34.47)
LFIA	0.01 (0.01 – 0.02)	0.04 (0.04 – 0.05)

Keywords: Anthelmintic activity, essential oils, *Cymbopogon schoenanthus*, *Cymbopogon martinii* **References:** 1. Molan AC, Waghorn WC, McNabb WC (2002) Vet Rec 150: 65 – 69. 2. Brunet S, Hoste H (2006) J Agric. Food Chem 54: 7481 – 7487.

PM197 Anti-Zygomycetes activity of 7-hydroxycalamenene isolated from *Croton cajucara*

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The leaves and bark from *Croton cajucara* Benth. (family Euphorbiaceae), a shrub from the Amazon, have been used locally used in folk medicine to treat diabetes, malaria, gastrointestinal and liver disorders [1]. The essential oil from the leaves is rich in linalool [2] and presented antileishmanial and antimicrobial activities [3,4]. A chemotype of this species was found, with an essential oil rich in 7-hydroxycalamenene [5]. This substance is reported to have antifungal activity against *Botrytis cinerea*, *Cladosporium cucumerinum*, *Phythophthora infestans*, *Pyricularia oryzae* and *Septoria tritici* [6]. During our studies with *C. cajucara* oil, we isolated 7-hydroxycalamente by silicagel column chromatography followed by preparative TLC. The pure compound (+98% by GC) was tested against some zygomycetes. A minimum inhibitory concentration (MIC) of 9.76 µg/mL was found to *Absidia corymbifera*, *Cunninghamella elegans* and *Mucor circinelloides* f. *circinelloides*, while for *Rhizopus microsporus* and *Rhizopus oryzae* the MIC was 19.53 µg/mL. The reference drug used, amphotericin B, presented a MIC of 43.9 µg/mL for *C. elegans* and *M. circinelloides*, and 0.3 µg/mL for the other species tested. From these data, it was observed 7-hydroxycalamenene is a compound with good activity against zygomycetes. **Keywords:** Zygomycetes, *Croton cajucara*, essential oil, antifungal activity **Acknowledgement:** *CAPES, FAPERJ.* **References:** 1. Maciel MAM et al. (2000) J Ethnopharmacol 70: 41 – 45. 2. Lopes D et al. (2000) J Essent Oil Res 12: 705 – 708. 3. Rosa MSCS et al. (2003) Antimicrob Agents Chemother 47: 1895 – 1901. 4. Alviano WS et al. (2005) Oral Microbiol Immunol 20: 101 – 105. 5. Pereira AQ et al. (2010) J Essent Oil Res 23: 20 – 23. 6. Scher JM et al. (2004) Phytochemistry 65: 2583 – 2588.

PM198 Simultaneous determination of four auxins in cyanobacterial extracts using HPLC-ESI-MS

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The prokaryotic cyanobacteria are important source of structurally bioactive secondary metabolites[1]. Besides having considerable pharmacological impacts such as antibacterial, antifungal and also cytotoxicity, the potential biofertilizer activity of cyanobacteria make them an attractive alternative to chemical fertilizers. Most paddy soils have a natural population of cyanobacteria. Treatment of rice seedings with collected cyanobacteria from paddy fields of Iran showed positive growth effect in vitro [2] which could be partly attributed to the possible presence of phytohormons in their composition [3]. Herein, we report a new approach for the simultaneous determination of four important auxins including indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) with HPLC equipped with PDA detector at 220 nm after extraction of the micoalgae using ultrasonic and microwave assisted extraction. The identities of the auxins were further confirmed using liquid Chromatography-ESI-Mass spectrometry. Under the optimized conditions, a complete separation of four auxins was achieved within a short time with a good reproducibility. The comparison of auxin chromatographic profile of this cyanobacteria with others and also their concentration levels will be reported and further discussed. **Keywords:** Cyanobacteria, Auxin, HPLC-ESI-MS **References:** 1- Tan LT (2007) Phytochem 68: 954 – 979 2- Saadatnia H, Riahi H (2009) Plant Soil Environ 55:207 – 212 3- Sergeeva E, Liaimer A, Bergman B (2002) Planta 215: 229 – 238

PM199 Comparison of the cytotoxicity and antimicrobial activity of several isohexenylnaphthazarins

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Shikonins, alkannins and derivatives thereof are natural, lipophilic red pigments and found in many species of the Boraginaceae family. Since many centuries, they are traditionally used for the treatment of wounds and have been shown to possess wound-healing, anti-inflammatory, anti-microbial, anti-thrombotic and anti-cancer activities [1]. The cytotoxicity of several shikonins (shikonin, acetylshikonin, β-hydroxyisovalerylshikonin, isobutyrylshikonin, 2-methyl-n-butyryl-shikonin, deoxyshikonin, dimethylacrylshikonin, epoxyshikonin, and isovalerylshikonin) and alkannins (alkannin, acetylalkannin, β-hydroxyisovalerylalkannin, isobutyrylalkannin, α-methyl-butyryl-alkannin, dimethylacrylalkannin propionylalkannin and teracrylalkannin) was determined using the XTT viability assay and human CCRF-CEM leukemia, MDA-MB-231 breast cancer, U251 glioblastoma and HCT 116 colon cancer cells. Most IC 50 values of shikonins were in a range of 0.1 to 10 µM, whereby, the highest activity was found for shikonin. IC 50 values of alkannins varied from 0.4 to 70 µM indicating that shikonin derivatives possess a higher cytotoxic potential than alkannins. Dimethylacrylalkannin exhibited no

activity up to 100 μg/ml in contrast to dimethylacrylshikonin. Moreover, the anti-microbial activity of the alkannin derivatives and acetylshikonin against nine microorganisms (*Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. tropicalis* and *C. glabrata*) was examined by the disc diffusion method. The most active derivatives were alkannin, acetylshikonin, β-hydroxyisovalerylalkannin and isobutyrylalkannin. Also in this case, acetylshikonin exhibited higher activity than the respective alkannin derivative. **Keywords:** Isohexenylnaphthazarins, shikonins, alkannins, cytotoxicity, antimicrobial activity **Acknowledgement:** *This work was supported by the "FWF – Fonds zur Foerderung der Wissenschaftlichen Forschung" P21114.* **References:** 1. Papageorgiou VP et al. (1999) Angew Chem Int Ed. 38: 270

PM200 Evaluation of antioxidant capacity and L-ascorbic acid content in Brazilian tropical fruits acerola (*Malpighia emarginata*), mangaba (*Harconia speciosa*), siriguela (*Spondias purpurea*) and umbu (*Spondias tuberosa*)

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Lately in Brazil there is an appreciate increase in the consumption of non-traditional tropical fruits. However, very little information is available on the presence of bioactive compounds and antioxidant properties in these fruits. Some locally grown tropical fruits such as acerola (*Malpighia emarginata* DC.), mangaba (*Harconia speciosa* Gomez), siriguela (*Spondias purpurea* L.) and umbu (*Spondias tuberosa* Arruda) were analyzed for their antioxidant capacity and L-ascorbic acid content. Antioxidant capacity was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) standard and L-ascorbic acid content was determined by ultrafast liquid chromatography using a Shimadzu™, UFLC-20A chromatograph with a reversed-phase octadecylsilane column XR-ODS™, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Antioxidant capacity, expressed as concentration of antioxidant required to reduce the original amount of free radicals by 50% and values expressed as g of pulp per g of DPPH, was 7,257.6 for acerola; 15,163.9 for the mangaba; 9,145.3 for the siriguela and 14,100.4 for the umbu fruit pulp. Ascorbic acid was not detected in siriguela pulp, and its content (in mg per 100 g of pulp) was 1,719.63 in acerola; 22.62 in mangaba and 19.53 in umbu. These results indicate the nutritional and therapeutic potential of these tropical fruits for their antioxidants properties. Among the fruits evaluated, the decreasing order of antioxidant activity was ranked as mangaba followed by umbu, siriguela and acerola fruits. **Keywords:** Bioactivity, bioactive compounds, food phenolics **Acknowledgement:** *We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the fourth and fifth co-authors also thank CAPES for fellowships*

PM201 Allopathic impact of some medicinal plants on *Portulaca oleracea* L. and *Lepidium sativum* L. seed germination: a study to find natural herbicide

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Recently by expose the health requirement as primal needs of mankind and people interested to use of organic production, investigation to find out effective natural compounds to replace of synthetic materials is increasing [1, 2, and 3]. In this context, the use of plant extracts as natural materials is a new strategy for management of weeds. In present study the allelopathic effects of aqueous and hydro-alcoholic extracts of *Ruta graveolens* L., *Artemisia sieberi* Besser, *Allium sativum* L., *Tribulus terrestris* L. and *Peganum harmala* L. were evaluated on *Portulaca oleracea* L. and *Lepidium sativum* at plant germination stage (pre-emergence). Percentage, germination rate (GR) and mean days to germination (MDG) obtained in 1-day intervals for 12 days. The results showed that hydro-alcoholic were effective in prevention of seed germination in compare with aqueous extract. Aqueous extract of *R. graveolens* with (76%), *A. sieberi* (89.33%) and *A. sativum* (44%) showed preventive effect on *L. sativum* seeds germination in compared with control (100%). All aqueous extract had shown allopathic effect (64 – 73%) on *P. oleracea* in Compared with control (100%). Hydro alcoholic extract of plants had shown less than 40% germinated seed corresponding to both of evaluated plants. Generally both kinds of extracts cause to decrease (GR) and increase (MDG). In conclusion *T.terrestres* extracts is introduce as natural compound with high allopathic effect that can be used as herbicide. However other studies are necessary to fully understand the reasons by which some medicinal plants may affect as herbicide in order to commercial application. **Keywords:** natural herbicides, medicinal plants, germination, organic production, allopathic **References:** Bogatek R and Yaneccko A (2006) Journal of Chemical Ecology 32: 2569 – 2583 Chauhan B S et al. (2006) Weed Science 54: 854 – 860. Fattahi B et al. (2010) Planta Med 76: 1271.

PM202 Chemical composition, antioxidant and antimicrobial properties of *Frankenia thymifolia* Desf. shoot extracts

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Frankenia thymifolia Desf. is an endemic xero-halophyte species in the salted and arid region of Tunisia [1]. In this study, two shoot fractions (methanolic and chloroformic) of Frankenia were assessed on their polyphenol contents and biological activities [2]. Then, the main phenolic and fatty acid compositions were identified. Results showed that polar fraction contains a highest amount of polyphenol, flavonoïd and condensed tannin contents (14.2 mg GAE g^{-1} DW, 4.8 and 4.6 mg CE g^{-1} DW respectively). The higher phenolic content in this fraction reflect the best total antioxidant capacity (8.8 mg GAE g^{-1} DW), antiradical activity against DPPH, β-carotene bleaching and Fe-reducing tests with the lowest IC_{50} and EC_{50} values as compared to apolar fraction. However, chloroformic fraction was more efficient against human pathogen strains. In fact, this fraction was active against all strains. The HPLC analysis showed that salicylic and trans-cinnamic acids were the major phenolics. The major fatty acids identified by GC/MS were palmitic, elaïdic and linoleic acids. Such variability in biological capacities between the 2 fractions can be explained by different bioactive compounds contain in each fraction and might be of great importance in terms of valorizing this halophyte as a source of bioactive molecules for cosmetic and pharmaceutical industries. **Keywords:** biological activities, fatty acids, Frankenia thymifolia, phenolics, fractionation, HPLC **References:** 1. Harkat H, Haba H, Marcourt L, Long C, Benkhaled M (2007) Biochem Syst Ecol 35: 176 – 179. 2. Meot-Duros L, Le Floch G, Magné C (2008) J Ethnopharmacol 116: 258 – 262.

PM203 Solvent effects on Antioxidants and biological activities of the halophyte *Nitraria retusa* (Forssk.) Asch

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Nitraria retusa (Forssk.) Asch. is a traditional medicinal species widely used as anti-inflammatory and cicatrizing agent. In this work, two fractions (non-polar and polar) of Nitraria leaves, after fractionation by solvent mixture (chloroform/methanol/water, 12/5/3), were investigated on their phenolic content, antioxidant activity (using several tests) and antimicrobial capacity against human pathogen strains. Moreover, phenolic and fatty acid compositions were identified using RP-HPLC and GC/MS, respectively. Results showed that phenolic contents and antioxidant activities varied considerably as function of solvent polarity. Polar fraction leaves (methanol/water) showed the highest polyphenol (7.97 mg GAE/g DW) and tannin (1.78 mg CE/g DW) contents, while chloroform fraction (non-polar) exhibits the highest content of flavonoids (2.74 mg CE/g DW). Moreover, antiradical activity against DPPH, and β-carotene bleaching test (IC50 values were 39, 700 μg.ml-1, respectively) and Fe-

reducing power (EC 50 = 410 µg ml-1) were more important in leaf non-polar fraction as compared to polar fraction. Besides, chloroform fraction was more efficient against all human pathogen strains mainly *Escherichia coli* and *Staphylococcus aureus*. The HPLC analysis showed two major phenolic compounds: trans-4-hydroxy-3-methoxycinnamic acid (ferulic acid) and p-coumaric acid. The major fatty acids identified by GC/MS were palmitic acid (28.04%), and polyunsaturated acids (48.78%) are characterized by linolenic acid (29.69%) and α-linolenic acid (omega 3) (19.09%). These results indicate that selective extraction of bioactive molecules from natural sources as halophyte species, by appropriate solvents, is important for obtaining fractions with high biological activities which can be used as preservative ingredients in food, cosmetic and/or pharmaceutical industries. **Keywords:** Nitraria retusa, phenolic content, biological activities, phenolic composition, fatty acid, GC/MS, RP-HPLC **References:** 1. Ghaieb M, Boukhris M (1998) Association pour la protection de la Nature et de l'environnement. 178 – 179. 2. Meda A et al. (2005) Food Chem 91: 571 – 577.

PM204 The evaluation of *Teucrium persicum* methanolic extract and its fractions in Pavlovian Fear Conditioning

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In this research, the effects of total methanolic extract and related fractions (chloroform, ethyl acetate, aqueous) of *Teucrium persicum* Boiss. on conditional memory were evaluated. The methanolic extract of *T. persicum* aerial parts and related fractions were administrated orally in doses of 100 mg/kg, 300 mg/kg, 400 mg/kg every 24 hours for 10 days to male rats. 24 hours later animals were trained in Pavlovian Fear Conditioning. Training consists of 5 tone (30 s, 2.3 k Hz, 80 Db)-shock(2 s, 1 mA) pairings. Fear to the context and the tone were evaluated by measuring freezing in separate tests. The results showed that chloroform fraction of the methanolic extract had a positive effect on fear conditioning. According to positive effects of chloroform fraction on fear conditioning, possible cholinergic properties of the chloroform fraction (consisting of terpenes) was tested by inducing conditional memory *via* scopolamine (a non-selective cholinergic antagonist) before training. It was found that the chloroform fraction had a protective effect against cholinergic disruption. All the doses had more freezing duration(s) compared to control group and there was a significant difference between 400 mg/kg and control group (P value< 0.01 for contest test, P value< 0.05 for tone test). It is concluded that chloroform fraction of *T. persicum* has a memory enhancing effect according to cholinergic properties of the component. **Keywords:** Teucrium persicum, Alzheimer's disease, pavlovian fear conditioning, contextual conditioning, tone conditioning, chloroform fraction

PM205 Protective effect of (-) α-bisabolol on markers of oxidative stress in erythrocytes subjected to oxidative stress

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(-)-α-bisabolol is a sesquiterpene alcohol found as a major component of essential oil of chamomile (*Matricaria recutita* L., *Chamomilla recutita* L., *Matricaria chamomilla* L.; Family Asteraceae). Chamomile, one of the most ancient and widely recognized herbs to mankind, has been used traditionally for centuries as an anti-inflammatory, antispasmodic, carminative, mild astringent and healing medicine [1,2]. It is also known to be very helpful as an external agent for encouraging the rapid healing of ulcers and burns without infection, as well as persistent skin problems such as eczema and psoriasis [3]. Since clinical trials and human studies are limited, we have investigated the effect of (-)-α-bisabolol on markers of oxidative stress in human erythrocytes by incubating with hydrogen peroxide (2mM) and tert-butyl hydroperoxide (10 µM). Subjecting erythrocyte to oxidative stress caused a significant alteration in reduced glutathione GSH), malondialdehyde (MDA) concentration as well as superoxide dismutase and catalase activity compare to control. Presence of (-)-α-bisabolol as low as 0.1 µM in incubation medium protected the erythrocytes from oxidative stress and helps to maintain the basal level of GSH and MDA. The activity of superoxide dismutase and catalase were also restored in a concentration-dependent manner (0.01 – 100 µM). The effect was also compared with L-Ascorbic acid, quercetin and BHT. Our findings provide evidence for the protection of oxidative stress in erythrocytes by (-)-α-bisabolol that could be considered for further studies. **Keywords:** (-)-α-bisabolol, GSH, MDA, Superoxide dismutase, Catalase, Hydrogen peroxide, tert-butyl hydroperoxide **Acknowledgement:** *Council of Scientific and Industrial Research, New Delhi, Council of Science and Technology, Government of Uttar Pradesh, India.* **References:** 1. Srivastava JK, Shankar E, Gupta S (2010) Mol Med Report 3(6): 895 – 901. 2. McKay DL, Blumberg JB (2006) Phytother Res 20(7): 519 – 530. 3. Martens D (1995) J Chiropractic Acad Homeopathy 6:15 – 18.

PM206 Cytotoxic activity of selected plants extracts on normal and cancerous oral mucosal cells

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Oral cancer is associated with cells of the oral mucosa that have become malignant and destroys healthy tissues of the lips, tongue, gingiva and other intra-oral locations (1). Many plants from the tropical forest of Malaysia have been used in local medicaments in the prevention and treatments of various types of cancer. The active polar compounds are often obtained through vigorous heating and then consumed in the form of decoction. The objective of this study was to determine the toxic effect of extracts from three plants on oral mucosal cell lines. Fibroblast and two cancerous oral mucosal cell lines which include the KB (ATCC) and ORL-48 (CARIF, Malaysia) cells were tested for their responses to the extracts of *Brucea* sp., *Typhonium* sp. and *Azadirachta* sp. using the neutral red cytotoxicity assay (2). The effective concentrations (EC_{50}) of the extracts and the cytotoxic response of the cells at various concentrations of the extracts was observed using Giemsa staining procedure. Results revealed the extracts from all three plants to exhibit cytotoxic activity towards KB cells with EC_{50} at less than 100 µg/ml. Potent cytotoxic activity on ORL-48 cells at an EC_{50} of 6.67 ± 1.15 µg/ml was only displayed by *Brucea* sp. extract. Extracts of all plants did not produce toxic effect on normal fibroblast cells. Based on its strong cytotoxic activity, *Brucea* sp. extract is worthy of further investigation to isolate and identify the active compounds. **Keywords:** *Brucea* sp.; *Thyphonium* sp.; *Azaradichta* sp **Acknowledgement:** *The research was financial supported by the University of Malaya Research Grant (RG020/09HTM) and Post Graduate Grant (PS 164/2010B).* **References:** 1. Kumar V, Cotran RS, Robbins S.L (2006) Basic Pathology 6th Edn. W.B. Saunders and Company. USA. 2. Fathilah AR, Sujata R, Norhanom WA, Ilham MI (2010) J Med Plants Res 4(11):987 – 990.

PM207 Fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. fixed oil

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Pistacia lentiscus L. is known by its essential oil and its mastic. The fixed oil extracted from fruits is used in traditional medicine for stomach diseases and wound healing. This study aims to determine the fatty acid composition and the antioxidant activity of *Pistacia lentiscus* fixed oil extracted from different parts of mature fruits. The work was performed on three different parts of the fruit: the envelope, seed and whole fruit harvested from Nefza located in North West of Tunisia. The extraction was done by the Soxhlet Apparatus. The determination of the fatty acid composition, done by GC/MS, showed the presence of five principal fatty acids: oleic, palmitic, linoleic, palmitoleic and stearic acids. The major fatty acid is the oleic that present more than 53%, palmitic and linoleic acids present respectively 25 and 15%. The difference between fatty acid composition of oil extracted from envelope, seed and whole fruit was not significant. The antioxidant activity was determined by the DPPH test and the Trolox Equivalent Antioxidant Capacity (TEAC) for oils extracted from the different parts of fruit. A significant difference was

determined and the highest antioxidant activity value was reached by oil extracted from whole fruit with a percentage of inhibition of DPPH about 9.67% and about 7% for both envelope and seed oil. Similarly, the highest value of TEAC was reached by whole fruit oil with about 2.32 ng of Trolox/g of oil. **Acknowledgement:** *This study was financially supported by IRDC -Canada (105568 – 006) and WWF-Tunisia*

PM208 *In Vivo* Healing Potential of *Trifolium* L. species on Excisional And Incisional Dermal Wounds

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The genus *Trifolium* is represented by 96 species in Turkish Flora (1) Among these species *Trifolium pratense* L. is used in Turkish folk, for wound healing (6). Also it is used for chronic skin diseases in worldwide.In the present study, in order to evaluate the wound healing activities of the plants, extracts were prepared with methanol from the aerial parts of 11 *Trifolium* L. species growing in Turkey, including *Trifolium ambiguum* Bieb., *T. canescens* Willd., *T. hybridum* L. var. *anatolicum* (Boiss.) Boiss., *T. hybridum* L. var. *hybridum*, *T. pannonicum* Jacq., *T. pratense* L. var. *pratense*, *T. purpureum* Lois. var. *purpureum*, *T. repens* L. var. *repens*, *T. resupinatum* L. var. *microcephalum* Zoh., *T. spadiceum* L., *T. trichocephalum* Bieb. Results were also evaluated histopathologically. The wound healing effect was comparatively evaluated with a reference ointment Madecassol®. Noteworthy wound healing activity was observed for the ointment formulation prepared with 1% methanol extract. The results of histopathological evaluation supported the outcome of both incision and excision wound models. The methanol extract of *T. pratense* var. *pratense* and *T. canescens* exerted wound healing activity. *T. canescens* which contains % 0,13 genistein and % 0,05 biochanin A is found effective than *T. pratense var. pratense* which contains % 0,04 genistein and % 0,01 biochanin A, according to our previous studies (2). **Keywords:** Wound healing, *Trifolium* species, Medicinal plants, Isoflavonoids **References:** 1. Byfield A J (2000). Trifolium L. Güner A, Özhatay N, Ekim T, Başer KHC. (ed.) Flora of Turkey and East Aegean Islands Supll. II. (s.95). Edinburgh: Edinburgh University Press. 2. Gürhan G et al. (2010) Planta Med76 (12): 1335.

PM209 The medicinal plant "Velame" (*Macrosyphonia velame* (A. St.- Hil.) Müll. Arg. – Apocynaceae) in southwestern Mato Grosso, Brazil: aspects of occurrence, potential medical and popular use

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The "Velame" (*Macrosyphonia velame* (A. St.- Hil.) Müll. Arg. – Apocynaceae) has ornamental and medicinal potential. In the southwestern state of Mato Grosso (MT-sw), Brazil, this plant is native. Between the years 2005 – 2009, it was found in specific locations (savannah sparse tracks without the presence of cattle). Scientific studies have found that the "Velame" root extract intensely inhibits tyrosinase, an enzyme that is found in microorganisms, animals and plants. Inhibitors of this enzyme are important pharmaceuticals and cosmetics, being used for the treatment of skin hyperpigmentation. In the city of Cáceres (MT-sw) the "Velame" is sold, generally, to pieces of dried root or already prepared with other herbs (bottled). In a study in 21 municipalities of the MT-sw, in 2005, this plant was mentioned among the main medicinal use and indicated by popular culture, but only in three places (Gloria d'Oeste, Pontes e Lacerda, and Cáceres). In folk medicine is indicated to treat syphilis, rheumatism, colds, fevers, bleeding, peptic ulcer, and also used as purifying the blood. In MT-sw is also used for skin problems, kidney; to act as an aphrodisiac and anti-inflammatory. This is a species threatened with extinction, requiring intensification of research and encouraging the preservation and cultivation. The user of medicinal plants should be based on guidance from qualified health professionals in this area and careful about the dosage, and correct plant part, preparation methods and other appropriate aspects interfering. **Keywords:** Medicinal plant, Folk medicine, Mato Grosso State-Brazil, Velame, Macrosyphonia velame **Acknowledgement:** *For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances).*

PM210 Medicinal plant bioactivity – Catuaba (*Anemopaegma arvense* (Vell) Stellfeld JF & de Souza) on *Spodoptera frugiperda* (JE Smith) Lepidoptera: Noctuidae) in the larval stage

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Catuaba (*Anemopaegma arvense* (Vell) Stellfeld JF & de Souza (Bignoniaceae) is a medicinal plant used as aphrodisiac. Biocidal effect of *A. arvense*was evaluated on the larvae of *Spodoptera frugiperda*. Plant leaves were collected in Cáceres (MT, Brasil) to make a Catuaba crude methanoic extract (EMeC). Five 50 mL concentrations were used to make the treatments: (T0 = 0, distilled water; T1 = 2,250; T2 = 4,500; T3 = 9,000 and T4 = 18,000 ppm). It was used 120 third instar larvae of the second generation, raised at 30 ± 3 °C and relative humidity of 60 ± 10%. Under the effect of the treatments, the larvae were distributed in three classes of larval duration (Ci:days): C1:<= 10 (10.26%), C2:0 15 (64.10%), C3:> 15 (25.64%). The most significant differences between the observed and expected frequencies of larvae in C1, C2 and C3 occurred in T2 (50.00%), T1 (30.67%) and T1 (3.33%), respectively. Treatment 1 (T1) presented 95.83% of the larvae in C2 and 0 in C1 whereas the largest contribution to C1 was T2 (26.08%) and O by T1. The treatments which contributed the least to C3 class were T1 and T2. The mean larval duration in T4 (13.87 ± 1.512A) and T0 (13.70 ± 2.176A) are similar and longer than in T2(11.65 ± 2.308B) whereas those of T1 (12.62 ± 1.055AB) and T3 (12.77 ± 2.287AB) are among those. The results suggest that, at a certain concentration, the EMeC is bioactive, accelerating the larval cycle of *S. frugiperda*, because T1 and T2 tend to shorten (C1) and to center (C2) over the larval period, respectively, whereas T4 showed no equating effect for T0. **Keywords:** Anemopaegma arvense, Spodoptera frugiperda, biocidal effect

PM211 The plant known as "Quina" *Strychnos pseudoquina* A. St. Hil. (Loganiaceae) and its medicinal use in southwestern Mato Grosso, Brazil

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It was studied the occurrence and bioactive use of Quina (*Strychnos pseudoquina* A. St. Hil. (Loganiaceae) in southwestern MatoGrosso (MT-sw), Brazil from 2003 to 2008. Data from fieldwork were collected for characterization of occurrence and the environment and the medical use of the plant. Alternatives were found in seedling nursery. It was evaluated in the laboratory, the bioactivity of leaf crude methanol extract (EMeQ) on larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). It seems that Quina is in MT-sw, its native occurrence is in preserved savannah, but only in places with sparse vegetation. It also seems that Quina is threatened with extinction. The local population uses the bark of the Quina to treat diarrhea, worms, fever, ulcer, and as purifying and contraceptive. The plant parts are sold in boxes of herbs in fairs in the city. The knowledge of the healers on medicinal value and uses of Quina derived from the population ancestors. In the nursery, it was found that the seeds of Quina have low germination. The seedlings and plants show an extremely slow growth. Incentives to Quina growing are needed to prevent its regional extinction. Laboratory study showed that the extract EMeQ interferes with larval development of *S. frugiperda*, preventing the insect from completing its cycle, causing its death. In addition to the medicinal value of Quina, its biocidal effect is presented as an alternative for pest control. **Keywords:** Mato Grosso, Brazil, Bioactivity, Strychnos pseudoquina, Spodoptera frugiperda **Acknowledgement:** *For Fapemat – financial support, and for UNEMAT – institutional support; To the*

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PM212 Plant known as Sarsaparilla [*Herreria sarsaparilla* Mart. [Herreriaceae] and its medicinal use in southwestern Mato Grosso, Brazil

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The medicinal herb Sarsaparilla (*Herreria sarsaparilla* Mart [Herreriaceae]), native in Cáceres (MT, Brazil), was studied in our laboratory and the field, between 2003 – 2009. The species also has ornamental potential. It has roots thickened tuberiformes and elongated stems cylindrical. The roots and leaves of the plant, in tea form, are used in folk medicine for many different diseases, such as sweat, blood purifying, diuretic, and also for the treatment of skin diseases, gout, syphilis, gonorrhea, arthritis, fevers, coughs, and hypertension. The roots and leaves of the plant, in tea form, are used in folk medicine for many different diseases, such as sweat, blood purifying, diuretic, and also for the treatment of skin diseases, gout, syphilis, gonorrhea, arthritis, fevers, coughs, and hypertension. They mention that the root tea also help fight obesity; tea leaves and branches are used to aid digestion and relieve stomach pains. To treat influenza, colds and rheumatism use the infusion. The healers in the city of Cáceres sell the plant root, especially, for purifying and antirheumatic. There are few scientific studies of this species relationship with the indicated uses in folk medicine. In the laboratory, to evaluate the digestive tract in various concentrations, the effect of the raw methanol extract of leaves of the plant on mining of Spodoptera frugiperda not was no larvicidal effect "and also non-interference in the remaining duration of the larval stage. Alert treatments, that even though natural medicine, without guidance from qualified health professionals may harm due to inadequacies in its implementation. **Keywords:** Medicinal Plant, Bioactivity, Mato Grosso, Brazil **Acknowledgement:** *For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)*

PM213 Xanthine oxidase-inhibitory and hypouricemic action of Black poplar bud extract

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Our study aimed to investigate the effect of the extract from Black poplar (*Populus nigra* L.) buds on xanthine oxidase (XO) activity *in vitro* and its hypouricemic action in rats. Poplars have been traditionally used in gout- and arthritis-treatment practices in medieval Europe. Besides, phenolic-rich resins from poplar buds are usually the main costituents of honey bee propolis that has a wide traditional use. After preliminary *in vitro* evaluation, extract was administered for 3 consecutive days to potassium oxonate-induced hyperuricemic rats in concentrations of 100 and 500 mg/kg. Allopurinol (10 mg/kg), was used as a reference drug. Uric acid/serum urate, urea, creatinine and electrolytes Na^+, K^+, Cl^- were determined in daily collected urine and in serum at the end of the experiment. ALT, AST activities in serum and XO activities in the liver homogenate were determined. The extract inhibited XO activity in vitro, showing a mixed-mode inhibitory action and IC_{50} value of 8.2 µg/mL. In rats, poplar extract at 500 mg/kg significantly ($P < 0.05$) reduced serum urate levels by 27% compared to hyperuricemic control group which the effect similar to that of allopurinol at a dose 10 mg/kg. The mode of action still needs to be further elucidated as it did not exhibit effect on liver XO but the observed effect on Na^+, K^+, and Cl^- excretion suggest the uricosuric action. Further research is needed to fully elucidate the potential of poplar extract in management of hyperuricemia. **Keywords:** Xanthine oxidase, hypouricemic effect, hyperuricemia, gout, enzyme inhibition, *Populus nigra*, black poplar extract **Acknowledgement:** *This research was supported by MSM 6046070901 and GACR 525/08/P503*

PM214 n-Hexane extracted compounds of *Bromus inermis* with excellent anti-MRSA activity

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Bromus inermis Leyss. is a grass which belongs to family Poaceae. It is a potent weed. Weeds could be a potent source for finding antibacterial compound regarding their characteristics such as living in a divers conditions and situations. In a large screening program for antibacterial activity screening of some weed plants extracts, we finally find a great anti-MRSA activity in n-hexane extract of *B. inermis* (MIC of smaller than 100 µg/ml for crude extract in triplicate assays). We used HPTLC and a simple biographical assay in parallel for initial characterization and identification of effective substance(s) in the extract, and finally it was concluded that the effective anti-MRSA substance is a relatively polar substance which has MIC of about 3 µg/ml against our MRSA strain. Susceptibility tests with standard antibiotics had been shown the MIC of 35 for chloramphenicol against this strain. Further investigations with HPLC Fractionation and subsequent NMR analysis could reveal the identity of the effective substances in our continuing work.

PM215 The antioxidant activity and free radical scavenging potential of two medicinals plants *Salvia officinalis* L. and *Phlomis samia* L

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In recent years considerable attention has been devoted to medicinal plants with antioxidant properties. The properties are commonly postulated to play an important role in preventing diseases caused by oxidative stress, such as cancer, coronary arteriosclerosis, and the ageing process. And there is much literature concerning the antioxidant properties of many species of the genus Salvia, for their 1,1-diphenyl-2-picrylhydrazyl (DPPH.) free radical scavenging activities. Among them, *Salvia officinalis* L. leaf extracts have been shown to be the most active with effective dose (EC_{50}) of 17 µg/ml, followed by the genus *Phlomis* by (EC_{50}= 32 µg/ml). This is for the first time studied in our search. For its high antioxidant activity, *S. officinalis* besides rosemary is widely used commercially in foodstuffs (3). The air-dried and powdered overground parts of each plant (500 g) were macerated with MeOH (5000 ml) over night and successively extracted with MeOH at 40 °C. After filtration, the remaining plant material was then extracted with MeOH (2000 ml) at 40 °C for a second time. The combined methanolic extracts were evaporated under reduced pressure to give the crude methanolic extract.Total flavonoid compound amount in extracts was determined by Bhorun et al., 1996. The total phenolic compound amount in extracts was determined by Folin-Ciocalteu method, using the procedure of (Price and Butler, 1977). **Keywords:** antioxidant activity, DPPH. activity, Linoleic acid peroxidation, Salvia officinalis, Phlomis samia **References:** Price MP and Butler LG (1977) J Agric Food Chem 25: 1268 – 1273.

PM216 Bioassay guided fractionation of extracts from flowers of *Bellis perennis* L. for their anticancer activity

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Common daisy (*Bellis perennis* L.) is a member of the cosmopolitan family Compositae (Asteraceae). It is native to western, central, and northern Europe, but is commonly found as an invasive plant in North America (1). *B. perennis* has been used in the treatment of gastritis, enteritis, diarrhoea, bleeding, rheumatism, inflammation and infections of the upper respiratory tract (2 – 3). In this study, anticancer activity of crude hexane, dichloromethane, methanol, water extracts, also n-butanol and ethylacetate fractions (after separation of methanol extract) of flowers from *Bellis perennis* were investigated. Cytotoxic activities were carried out on human lung cancer (A549), human colorectal cancer

(DLD-1), and normal skin fibroblasts (WS1) using the resazurin reduction test. Following the bioassay guided fractionation, the most active methanolic extract, was treated by n-butanol and ethylacetate. The n-butanolic fraction was found to be the most active against A-549 lung carcinoma and DLD-1 colon carcinoma cells, with IC_{50} values of 16 ± 3 and 10 ± 2 μg/ml, respectively. The hexane, dichloromethane and water extracts did not show any significant activity against A-549 and DLD-1. **Keywords:** Bellis perennis, Common daisy, anticancer **References:** 1. Tutin TGet al. (1976) Flora Europaea Volume 4, Cambridge. Cambridge University Press. p. 111 2. Panda H (2004) Handbook on Medicinal Herbs with Uses. Asia Pacific Business Pres, India p. 188 – 189 3. Grieve M (1982) A Modern Herbal, Dover Publications, Inc., New York, Vol I, p. 247.

PM217 Antimicrobial activity of some *Teucrium* species from Serbia

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In the Serbian flora, genus *Teucrium* is represented by six species. In this work, for the first time, antimicrobial properties of methanol, ethyl acetate and acetone extracts of *T. scordium* L. subsp. *scordium*, *T. scordium* L. subsp. *scordioides* (Schreb.) Arcang. and *T. botrys* L. were examined [1]. Antimicrobial activity was tested by microdilution method determining minimum inhibitory (MIC) and microbicidal concentration (MMC) against standard and clinical strains of bacteria and fungi: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* ATCC 10231, *Candida albicans* and *Aspergillus niger* [2]. The activity of extracts varied depending on the microorganism species, plant species and type of extract. The extracts of *T. scordium* subsp. *scordium* and *T. scordium* subsp. *scordioides* showed inhibitory effects towards tested bacteria while *T. botrys* inhibit only *S. aureus* ATCC 25923. Antifungal activity was recorded for ethyl acetate extracts of *T. scordium* subsp. *scordium* and *T. botrys*. MIC and MMC values were in range from 0.3 mg/ml to > 20 mg/ml. Among tested extracts, the methanolic showed the greatest inhibitory effects. *S. aureus* ATCC 25923 was the most sensitive strain. It was inhibited by extracts of tested plants, except ethyl acetate extract of *T. botrys*. The inhibitory and bactericidal concentrations were lower than 1 mg/ml. Susceptibility of *S. aureus* and *P. aeruginosa* was moderate with MIC values between 5 mg/ml – 20 mg/ml. *E. coli*, clinical and standard strain, exhibited susceptibility to the extracts at higher concentration or resistance. Also, tested fungi showed low sensitivity to the extracts. **Keywords:** *Teucrium*, antimicrobial activity, MIC, MMC **Acknowledgement:** *Ministry of Science and Education, Republic of Serbia (III41010).* **References:** 1. Josifovic M (1972) The flora of SR Serbia. Serbian Academy of Science and Arts. Belgrade. 2. Sarker SD et al. (2007) Methods 42:321 – 324.

PM218 Cardioprotective effect of Algerian medicinal plant *Globularia alypum* extract against cardiotoxicity of Adriamycin in rats

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Adriamycin (ADR) is an efficient chemotherapeutic agent used against several types of tumors; however, its use is limited due to severe cardiotoxicity. Since it is accepted that ADR induced myocardiopathy is the consequence of Oxidative stress through the mediation of free radicals. The aim of this study was to investigate the effect of *Globularia alypum* L. butanolic extract on the acute cardiac toxicity induced by ADR in rats. GABE represent a significant source of phenolic compounds. It has been recognized that polyphenols and flavonoids show antioxidant activity. Oxidative stress in the heart tissue was estimated by measuring the glutathione (GSH) levels homogenate and malondialdehyde (MDA) in the cardiac cytosolic fraction. The pretreatment of rats with the GABE orally at a dose of 100 mg/kg for one month resulted in a decrease in cardiac cytosolic MDA and a maintenance of cardiac cytosolic GSH level as compared to ADR treated animals at a dose of 20 mg/kg intraperitoneally. In this study we have investigated the cardioprotective effect of GABE against ADR induced acute cardiotoxicity. We report here that the pretreatment of GABE is able to reduce the ADR induced acute cardiotoxic manifestations in GSH, MDA. Analytical chemical study revealed that GABE contains phenolics and flavonoids, which are responsible for its potent antioxidant activity. Our studies have shown that ADR induced considerable increase in lipid peroxidation In conclusion, the present study shows that chronic administration of butanolic fraction of *Globularia alypum* has cardioprotective potential against ADR induced cardiotoxicity. **Keywords:** Adriamycin, Globularia alypum, Cardiotoxicity, Antioxidants, Lipid peroxidation, Histopathology

PM219 *Breonadia salicina* (Rubiaceae) extracts are as effective as a commercial fungicide in post harvest protection of oranges against *Penicillium* infections

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After examining the antifungal activity of several medicinal and aromatic plants *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood was selected for in depth study. The main active compound (ursolic acid) was isolated and characterised. Acetone extracts had good *in vitro* antifungal activity against *P. janthinellum* (MIC 0.08 mg/ml.) but *P. digitatum* and *P. expansum* were more resistant (MIC 1.25 mg/m)l. *Penicillium* species cause serious post harvest problems in the citrus industry. To test the in vivo efficacy, oranges were infected with the fungi and treated with the extracts, ursolic acid and a commercial fungicide amphotericin B. The crude leaf extract had the same level of protection as ursolic acid indicating synergistic activities within the crude extract. The acetone extract had an MIC of 0.16 mg/ml compared to the MIC of 0.08 mg/ml of amphotericin B against P. digitatum. The acetone extract therefore had sufficient antifungal activity against these organisms to consider its use in the citrus industry especially since it could be produced at a very low cost. The extract was however more toxic to the kidney cells that to the fungi. The results show the potential use of plant extracts to combat plant fungal infections if extracts with lower cellular toxicity can be found or if the toxicity of the extract can be decreased without changing the antifungal activity. **Keywords:** antifungal extract, Breonadia salicina, Penicillium, post harvest, orange, antifungal compound **Acknowledgement:** *The National Research Foundation provided funding* **References:** 1. Mahlo SM, McGaw LJ and Eloff JN (2010) Crop Protection 29: 1529 – 1533

PM220 The Study on the Effect of Different Manure and Plants Density on the Growth and some Quantitative Characteristics of Milk Thistle (*Silybum marianum* L.)

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Milk thistle (*Silybum marianum* L.) is an annual medicinal plant. *Silybum marianum* has been recognized as an antihepatotoxic plant. The active constituents of *S. marianum* include a group of flavonolignans known collectively as silymarin. To investigation the effects of different manure and plant density and the intraction between manure and density on the grows and some quantitative characteristics of this plant an experiment were examined. The completely Randomized design was installed in the experimental field, College of Agricultural of Ferdowsi University located in Mashhad. The treatments were included of two factors. The first factor included 3 different manure: cow, ship and multiple manure (1.65 kg.m-2) and the second factor was three level of plant density (5, 7 and 10 pl.m-2) whit 3 replications. At the end of fullbloom stage, morphological characteristics included plant height, number of shoots, number and diameter of capitule, percentage of leaf dry matter, percentage of healthy and free seeds, mass of 1000 grains of main and secondary capitule and chlorophyll content of leaf. Mean comparison was carried out using LSD test (at 5% level). Results showed a significant effect

on number of capitula and shoots and plant height. By increase in plant density: plant height, number of shoots, number of capitule and mass of 1000 grains were decreased. The results showed that the best kind of manure was ship manure and the most suitable plant density was 40 x 40 cm (5 plant per m_2). **Keywords:** Milk thistle, Silybum marianum, Animal Manure, Plant Density

PM221 Chemical profile and biological activities of *Allium melanantherum* Panč. extracts

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Members of the genus *Allium* have been used and cultured for thousands of years for their medicinal properties and characteristic flavor. Only two species of genus *Allium* (*A. sativum* L. and *A. cepa* L.) are well researched, while data on chemical composition and biological activities of other species, including *Allium melanantherum* Panč. (subgen. *Allium*, sect. *Codonoprasum*) are very scarce. In the present study we investigated chemical composition, antioxidative and anti-inflammatory properties of methanolic extracts of *Allium melanantherum* wild growing in Serbia. Phytochemical profile was determined by measuring total phenolic, total flavonoid and total anthocyanin contents and by LC-MS/MS analysis of the extracts and headspace GC/MS analysis of fresh bulbs volatiles. The antioxidant activity was evaluated by measuring radical scavenging capacity towards 2,2-diphenyl-1-pycrylhydrazile (DPPH) and NO radicals and effect on lipid peroxidation (LP) [1]. In addition, the anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was observed [2]. High contents of total phenolics (5.13 – 5.31 mg gallic acid equivalents/g of dry extract), total flavonoids (1.15 – 2.82 mg quercetin equivalents/g of dry extract) and total monomeric anthocyanins (33 – 169 µg cyanidine-3-glucoside equivalents/g of dry extract) were found. The dominant phenolic compounds in the extracts are ferulic and p-coumaric acids and flavonoids rutin, quercetin-3-O-Glc and kaempferol-3-O-Glc. Dimethyl-disulphide was detected as only volatile compound. The extract inhibited production of 12-HETE, 12-HHT, PGE2 and TXB2 in a dose-dependent manner. Antioxidant activity was weak compared with synthetic antioxidants. **Keywords:** *Allium melanantherum*, antioxidant, anti-inflammatory, LC-MS/MS, GC-MS **Acknowledgement:** *Ministry of Science and Technological Development, Republic of Serbia, grant No. 172058* **References:** [1] Lesjak M et al. (2011) Food Chem 124:580 – 856. [2] Beara IN et al. (2010) J Pharm Biomed Anal 52: 701 – 706.

PM222 *Tectona grandis* Linn. (Verbenaceae) leaf ethanol extract in renal artery occluded hypertensive rats

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Hypertension is one of the principal health problems and leading cause of cardio-vascular deaths in various communities worldwide. An elevated arterial pressure is an important public health issue. Although it is common, asymptomatic and readily detectable, but it can often lead to lethal complications, if left untreated. Many new drugs have been introduced which may demonstrate better efficacy but posses side effects. Recently attention has been focused towards herbal preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases. Ethanol extract of *Tectona grandis* Linn. leaf (Family: Verbenaceae) was evaluated for its antihypertensive activity in renal artery occluded hypertensive rats. Wistar rats (160 – 250 g) were pretreated with ethanol extract of *T. grandis* for 6 weeks. Hypertension was induced in animals by clamping the renal artery with renal bulldog clamp for 4 h. Ischemia of the kidneys caused elevation of blood pressure by activation of the renin-angiotensin system. Elevated blood pressure of the animals was significantly ($p < 0.05$) decreased by the ethanol extract of *T. grandis* at the dose levels of 20, 40 and 80 mg/kg, *i. v.* Captopril, angiotensin converting enzyme inhibitor (ACE-I) at the dose of 1 mg/kg, *i. v.* showed significantly ($p < 0.05$) reduction in the elevated blood pressure. The antihypertensive activity of ethanol extract of *T. grandis* may be due to the action on rennin-angiotensin system. This result would tend to justify the traditional use of the herb for the management hypertension. **Keywords:** *Tectona grandis*, antihypertensive activity, rennin-angiotensin system

PM223 The use of metabolomics for the discovery of antimicrobial biomarkers from *Plectranthus* species indigenous to southern Africa

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For many years a reductionist approach has been followed in the evaluation of the bioactivity of medicinal plants and the subsequent isolation and identification of active compounds. Synergism and prodrug effects cannot be detected with this approach [1]. It is also a tedious and time consuming process involving isolation, dereplication of known compounds and structure elucidation [2]. A holistic approach is more appropriate in the study of herbal and traditional medicines [1]. This means that the phytomedicine is evaluated as one active ingredient or as a set of poly-phytochemicals acting synergistically [3]. The research method best suited for the holistic research concept of phytomedicine is metabolomics. *Plectranthus* is the largest genus of the Lamiaceae family in South Africa. The main recorded ethnobotanical use of these species is as traditional medicine for the treatment of various ailments such as digestive ailments, skin conditions and a range of infections [4]. A total of 93 leaf samples from different *Plectranthus* species, indigenous to southern Africa, were collected and extracted with dichloromethane followed by methanol. The antimicrobial activity of the samples was determined against various micro-organisms including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans* and *Cryptococcus neoformans*. One-dimensional NMR and LC-MS metabolomic fingerprints were acquired for all samples. Chemometric tools were employed to identify active and less active extracts by combining and correlating NMR and LC-MS metabolomic profiles of the different samples with the MIC assay results. Putative biomarkers responsible for the antimicrobial activity of active samples were also identified. **References:** 1. Verpoorte R, Choi YH, Kim HK (2005) J Ethnopharmacol100: 53 – 56. 2. Heinrich M (2008) Phytochemistry Letters 1: 1 – 5. 3. Li et al. (2008) Trends in Analytical Chemistry 1: 66 – 77. 4. Lukhobaa CW et al. (2006) J Ethnopharmacol 103:1 – 24. 5. Gibbons S (2004) Nat Prod Rep 21: 263 – 277.

PM224 Experimental evaluation of the potential of *Bridelia ferruginea* stem bark in wound management

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The methanol extract (ME) of *Bridelia ferruginea* Benth. stem bark obtained by 48 hr maceration, was subjected to solvent guided fractionation in a silica gel column using petroleum ether, dichloromethane and methanol successively to yield the petroleum ether (PF), dichloromethane (DCMF) and methanol (MF) fractions. The extract (ME) and fractions (DCMF and MF) were assessed for hemostatic activity using bleeding/clotting and coagulation time in rats. The effects of the extract and fractions on wound contraction and rate of epithelialization of excision wounds in rats were evaluated. Also the antimicrobial effects of the extracts and fractions were studied. Data collected were analyzed using one way ANOVA and further subjected to LSD Post Hoc tests. The extract and fractions significantly *($P < 0.05$)* reduced bleeding/clotting time in rats and also reduced the coagulation time of whole rat blood. They also significantly *($P < 0.05$)* increased rate of wound contraction and epithelialization of excision wounds compared to control rats; on day 17, extract treated rats showed 99.5 – 100% wound contraction. The extracts also exhibited antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Eschericia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumonia*. The results suggest that the stem bark of *Bridelia ferruginea* exerts beneficial effects in wound management through hemostatic, wound contraction and antimicrobial activities. **Keywords:** *Bridelia ferruginea*, hemostatic, wound contraction, epithelialization, antimicrobial

PM225 Biological properties of the berries of five *Juniperus* species in Juniperus section from Turkey

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In Turkish traditional medicine, the species under Juniperus section are frequently employed to treat several diseases [1,2]. This work was designed to define and compare the antiproliferative and antimicrobial activities of berries methanol extracts of five *Juniperus* species from Turkey: *J. communis* L. var. *communis* (Jcc), *J. communis* L. var. *saxatilis* (Pallas) A.E.Murray (Jcs), *J. drupacea* Lab. (Jd), *J. oxycedrus* L. ssp. *oxycedrus* (Joo), *J. oxycedrus* L. ssp. *macrocarpa* (Sibth. et Sm.) Ball (Jom). The effect of *Juniperus* extracts on cell proliferation was tested "in vitro" on human hepatocellular carcinoma (HepG2) cells. A decrease in HepG2 cells viability after 24-h exposure to Jcc, Jcs, and Jd extracts was observed. Based on IC_{50} values, the activity of the extracts decreased in the order Jcs > Jd > Jcc (IC_{50}= 6.62 ± 0.61 μg/mL, 7.61 ± 2.25 μg/mL, and 8.42 ± 1.32 μg/mL, respectively). Joo and Jom extracts inhibited the growth of HepG2 cells approximately of 40% at the lowest tested dose (1.25 μg/mL), while the activity diminished with increasing concentrations, resulting close to zero at the dose of 10 μg/mL. The antimicrobial activity was evaluated by standard methods on gram-positive, gram-negative bacteria and fungi.The efficacy was appreciable on gram-positive only. Among the extracts Jd showed the higher bacteriostatic activity (MIC: 78.12 – 312.50 μg/mL) than Jcs and Jcc (156.25 – 1250.00 μg/mL) followed by Joo and Jom (625.00 – 1250.00 μg/mL). The obtained results give support to the ethnopharmacological use of these Turkish *Juniperus* species and suggest their potential use in the prevention and/or treatment of infections and cancer. **Acknowledgement:** *The authors are grateful to Federfarma Messina for financial support* **References:** 1. Stanic G et al. (1998) Phytother Res 12: 494 – 497. 2. Sakar MK et al. (2002) Acta Pharm Turc 44: 213.

Topic N: Veterinary Applications

PN1 Traditional Ethnoveterinary Phytotherapies from Pakistan

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Present investigation was conducted to document plant based Traditional Ethnoveterinary Phytotherapies to cure various aliments in remote areas of Pakistan including Indo-Pak, Pak-Afghanistan, Pak-China, and Pak-Iran borders. Questionnaires based on semi-structured interviews and observations were used to collect data from traditional veterinary healers residing in these remote sites. Eighty seven medicinal plant species belonging to 46 families were recorded for their applications against veterinary diseases. All plant species were indigenous to the study areas. Dysentery, diarrhea, indigestion, gas trouble, constipation, colic, worms, ulcer, wounds, scabies, sores, infections of mouth, throat, lungs, foot, hooves; fever, cough, lactation, unequal memory glands, weakness, mastitis, arthritis and urethra prolepsis were frequently reported veterinary ailments. Forty one plant species were reported in more than two conditions. Commonly used routes of drug administration were oral and dermal. Validation of these ethnoveterinary practices for their quality, efficacy and standardization of doses and screening for active substances that may lead to the discovery of some new, safer and cost effective medicines.

PN2 The effects of the different levels of *Aloe vera* gel on oocysts shedding in broilers with coccidiosis

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Coccdiosis is the most important parasitic disease in poultry. The disease may result in losses, indigestion, and increased feed conversion ratio in chickens. Resistance against anti-coccidiosis drugs is among the major problems resulting from chemical therapy. Therefore, it seems necessary to replace chemical substances with herbs. Thus, the present study aims to identify the effects of different levels of *Aloe vera* L. gel on performance and oocysts shedding in broilers with coccidiosis. The study was carried out on 200 one-day-old male broilers from Ross 308 strain on a completely randomize design with four treatments each with five replicates each composing of ten chickens. The groups included control group (basal diet), three group with basal diet mixed different level of Aloe vera gel (1.5, 2, and 2.5%). On the day 28, all chickens with oocysts were challenged by *Eimeria maxima*. Feed conversion ratio was calculated for the whole farming period (42 days). The findings suggested that groups treated by *Aloe vera* gel had improved feed conversion ratio compared to the control group. The group treated by 2.5% *Aloe vera* gel showed significant difference from the control group. In addition, significant reduction was observed in oocysts per gram of feces in the *Aloe vera* gel groups in comparison with the control group. 2.5% *Aloe vera* gel group showed the lowest level of oocysts per gram of feces. The results of this study indicate that *Aloe vera* gel can improve feed conversion ratio in broilers with coccidiosis and reduces oocysts shedding. **Keywords:** Aloe vera, coccidiosis, broiler

PN3 Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Riphicephalus (Boophilus) microplus* (Acari; Ixodidae)

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This experiment was carried out to study the bioacaricidal activity of *Tetradenia riparia* (Hochst.) Codd essential oil against engorged females of *Rhipicephalus (Boophilus) microplus* (Acari; Ixodidae). For this purpose, nine serial concentrations (12.50, 6.25, 3.75, 1.80, 0.90, 0.45, 0.22, 0.11, and 0.056% w/v) of *T. riparia* were used for the adult immersion test (AIT). For the larval packet test (LPT), we used 14 serial concentrations (100.00, 50.00, 25.00, 12.50, 6.25, 3.65, 1.82, 0.91, 0.45, 0.228, 0.114, 0.057, 0.028, and 0.014% w/v). The results for AIT showed that the LC50 and LC99.9, calculated using the Probit test, were for mortality (%) 0.534 g/mL (0.436 – 0.632) and 1.552 g/mL (1.183 – 1.92); for total number of ovipositions were 0.449 g/mL (0.339 – 0.558) and 1.76 g/mL (1.27 – 2.248); and for hatchability inhibition were 0.114 g/mL (0.0 – 0.31) and 2.462 g/mL (1.501 – 3.422), respectively. The LPT showed that the LC_{50} and LC99.9 were 1.222 g/mL (0.655 – 1.788) and 11.382 g/mL (7.84 – 14.91), respectively. A positive correlation between *T. riparia* EO concentration and tick control, was observed by the strong acaricidal effects against *R. (B.) microplus*, and the mortality rate of ticks was dose-dependent. Our results showed that *T. riparia* is a promising candidate as an acaricide against resistant strains of *R. (B.) microplus*. **Keywords:** Tetradenia riparia, acaricide, Ixodidae, Tick; Rhipicephalus (Boophilus) microplus **Acknowledgement:** *The authors are grateful to CNPq for providing a research grant and fellowships* **References:** 1. Gazim ZC et al. (2010) Molecules 15: 5509 – 5524.

PN4 *In vitro* activity of different plants essential oils against the yeast-like alga *Prototheca*

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Species of the genus *Prototheca* (family *Chlorellaceae*) are unicellular achlorophyllous microalgae, spherical, oval or kidney shaped with diameters ranging from 3 to 30 μm. They are ubiquitous in nature and have a worldwide didtribution [1]. Of the five known species of the genus, *P. wickerhamii* causes human infection and *P. zopfii* is considered pathogenic for animals, particularly cows and dogs [2,3]. These algae do not respond to classic therapy so introduction of new therapeutic agents for

treatment or prophylaxis is an important goal. Therefore this study aimed to investigate the antimicrobial activity of *Mentha piperita* L. (peppermint), *Melaleuca alternifolia* Cheel (tea tree), *Origanum compactum* Benth. (oregano) and grape seed essential oils, compared with Amphotericin B (A2942, Sigma-Aldrich). Ten *P. zopfii* isolates from cow mastitic milk samples and two *P. zopfii* isolates from bovine feces were submitted to antifungal susceptibility testing by broth microdilution assay following the CLSI guidelines for yeasts. The inhibitory effects, depends on the plant species from which the product was obtained, on their chemical composition as well as on the tested concentration. Peppermint (MIC of 0,125 – 0,5 μg/mL) and tea tree (MIC of 1 – 2 μg/mL) essential oils demonstrated the strongest antifungal efficacy against all tested strains. In contrast Amphotericin B showed eficacity at MIC of 25 μg/mL. All tested isolates were resistant to oregano and grape seed essential oils. Difficulties in treating protothecosis and the potent *in vitro* activity of peppermint and tea tree essential oils demonstrated here raise the interest for further investigations on the therapeutic use of these natural products. **Keywords:** *Prototheca*, essential oil, *Mentha piperita*, *Maleleuca alternifolia*, *Origanum compactum*, grape seed **Acknowledgement:** *This work was supported by CNCSIS-UEFISCSU grant number PN II RU 175/2010.* **References:** [1]. Pore RS (1985) Mycopathologia 90: 129 – 139. [2]. Roesler U et al. (2006) Int J Syst Evol Microbiol 56: 1419 – 1425. [3]. Lass-Florl C et al. (2007) Clin Microbio. Rev 20: 230 – 242.

PN5 Antibacterial activity of the essential oil of Mountain Savory (*Satureja montana*) against *Arcanobacterium pyogenes*

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The pharmaceutical properties of aromatic plants are partially attributed to essential oils (EOs) which are widely used to prevent and treat human diseases [1]. However, little is known about the control of infective animal diseases with EOs. *Arcanobacterium pyogenes* (AP) is one of the important opportunistic pathogens of the upper respiratory and genital tracts of cattle, sheep, swine, and many other species [2]. Most frequently, this bacteria is isolated from inflamed lung lesions of pigs and cattle, in the samples of uterine mucos of sows and cows with endometritis and the milk from cows with clinical mastitis. Antimicrobial activity of Mountain Savory (*Satureja montana*) L. (SM) was detected *in vitro* conditions against *Clostridium perfringens* type A [3] and *Staphylococcus aureus* [4]. Therefore, it is assumed that SM can react against AP and therapeutic potential against bacterial infection in animals. The EO of the cultivated SM (Serbia), was extracted by hydrodistillation and analyzed by gas chromatography. According to compositional analysis of the SM EO, 27 chemical compounds were identified, and carvacrol (42.12%), linalool (24.57%) and p-cymene (19.85%) were found as predominant compounds in oil. Antibacterial sensitivity of AP (ATCC 19411) and other 18 isolates (field strain originating from swine-uterus and cattle-uterus and milk) were tested *in vitro* using an Agar Dilution Test to determine the minimal inhibitory concentration (MIC). The results obtained have shown that EO applied at a concentration of 0.78 μl ml-1, which was defined as the MIC, exhibited antimicrobial activity against all AP in the *in vitro* assays. **Keywords:** essential oil, *Satureja montana*, *Arcanobacterium pyogenes*, antibacterial MIC **Acknowledgement:** *This work was supported by a grant from scientific project TR 031071 of Ministry of Science and Technological Development of Republic of Serbia* **References:** 1. Edris AE (2007) Phytother Res 21: 308 – 323 2. Liu M-C et al. (2009) J Dairy Sci 92: 3659 – 3666 3. de Olivera TLC et al. (2011) Intern J Food Microbiol 144(3): 546 – 555 4. Donaldson J R et al. (2005) Pharmaceutical Biology 43: 687 – 695

PN6 Inhibition of chemically induced mammary and non-mammary carcinogenesis by astaxanthin in Wistar rats

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Astaxanthin is a fat-soluble, oxygenated pigment called a xanthophyll and a member of the carotenoid family. It has a unique molecular structure that gives it powerful antioxidant function. Astaxanthin is extracted from microalgae, salmon and *Pfaffia* (a yeast) (1, 2). The aim of the study is to follow up the effect of astaxanthin (ASTA) in chemoprevention of the chemically induced mammary carcinogenesis in immature Wistar female rats. There were established five groups of 37 days old Wistar rats: group I inoculated with the carcinogen MNU (N-methyl-N-nitrosourea) (n = 9), group II with MNU and ASTA in diet (n = 8), group III which received oil in diet (oil was used as solvent for ASTA) (n = 4), and group IV with ASTA in diet (n = 4). The ASTA was administered orally in a dose of 50 μg astaxanthin/rat/day, during 7 months. The experiment was finished at 14 months from MNU intake. Mammary tumor induction determined by MNU was reduced, representing 33,3% respectively 37,5% from all cases in groups I and II. There were diagnosed several other tumor types in several organs (nephroblastoma, liposarcoma, hemangiosarcoma, squamous carcinoma, pulmonary carcinoma, cholangiocarcinoma). Involvement of oxidative stress in (mammary and non-mammary) carcinogenesis was revealed by partial protection conferred by astaxanthin in cancer chemoprevention. Present study is one of the few long term experiments (420 days) that resemble the effect of astaxanthin in chemically induced carcinogenesis in rats. Concluding, a diet enriched in astaxanthin yield beneficial effects in cancer chemoprevention, minimizing the bad effects of oxidative stress induced by MNU. **Keywords:** astaxanthin, carcinogenesis, mammary, rat **Acknowledgement:** *This work was supported by CNCSIS-UEFISCSU grant number PN II RU 185/2010.* **References:** [1] Guerin M et al. (2003) Trends Biotechno. 21(5):210 – 6. [2] Hoyoku N et al. (1999) Pure Appl Chem. 71(12): 2273 – 2278.

PN7 Researches Regarding *in vitro* Antimicrobial Effect of Some Types of Honey from Transylvania on Staphylococci Isolated from Animals and Humans

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Despite the pharmaceutical industry development in recent years, resistance of microorganisms to antibiotics is increasing [1,2,3]. Under these conditions, the alternative of natural products with similar effect, must be considered. This study aimed to test *in vitro* antimicrobial activity of four types of honey obtained in Central Transylvania, on staphylococci isolated from lesions in both animals and humans, as well as to test comparatively the effect of the most frequently antibiotics used in treating the lesions caused by staphylococci. The products with potentially antimicrobial effect were represented by forest honey, multi-flower honey, lime honey and acacia honey. Tests were done on 38 strains of staphylococci form species *S. aureus* (8 strains), *S. intermedius* (10 strains), *S. xilosus* (7 strains), *S. hominis* (5 strains), *S. chromogenes* (4 strains) and *S. sciuri* (4 strains). Comparatively seven antibiotics commonly used to treat staphylococci were also tested. The sensibility was determinated using the microdillutions method obtaining mimimum inhibitory concentration (MIC) for each sample in accordance to CLSI standards. Forest honey had a good antimicrobial effect (MIC 15 μg/ml) on *S. intermedius* and *S. chromogenes* strains and multi-flower honey had good effect (MIC 15 μg/ml) against *S.sciurii*. Lime honey had a decreased

antimicrobial effect (MIC 60 µg/ml) on *S. xilosus* and acacia honey had good effect against *S. xilosus* (MIC 30 µg/ml). The most efficient antibiotics were ampicillin (MIC 8 µg/ml) on *S. aureus* and *S. intermedius*, and ceftiofur (MIC 4 µg/ml) against *S. xilosus*, *S. hominis*, *S. chromogenes* and *S. sciuri*. These results show that the antimicrobial effect of honey is variable it may depend on the type of honey used and tested microbial strains. **Keywords:** staphylococci, honey, antibiotics **References:** [1] Hancock EW (2005) Lancet Infect Dis 5(4): 209 – 218. [2] Levy SB et al. (2004) Nat Med 10: S122-S129. [3] Nascimento GGF et al. (2000) Braz J Microbiol 31: 247 – 256.

PN8 *In vitro* antimicrobial efficacy of honeydew honey and *Calendula officinalis* L. against *Pseudomonas aeruginosa*

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The complex therapeutic potential of honey and medicinal plants is well documented by the literature, but discrepancies may be observed while comparing research results [1, 2]. Twelve honeydew honey samples from different Transylvanian geographical locations were investigated for their antibacterial properties based on the results of two diffusion assays that included *Pseudomonas aeruginosa* (n = 10, strains isolated from bovine mastitis, and the reference strain *Ps. aeruginosa* ATCC 27853) as a relevant antibiotic resistant pathogen. Both screening tests indicated a strong inhibitory effect for eight samples when compared to the artificial honey and also that the development of growth inhibition zones was dose dependent. The most active samples were subjected alone and in combination with *Calendula officinalis* L. essential oil to minimum inhibitory concentrations assay using the broth microdilution method that pointed out values below those recorded for well or disc diffusion assays (MICs for honeydew honey ranged from 5% to 10% (v/v) and were even more reduced (up to 2 times) in association with *Calendula officinalis* essential oil). This study showed that Romanian honeydew honey manifested antibacterial activity against multi-drug resistant *Pseudomonas aeruginosa* isolates and also *Calendula officinalis* essential oil ability to enhance this potential. **Keywords:** honeydew honey, *Calendula officinalis*, antibacterial **References:** 1. Iauk L et al. (2003) Phytother Res 17: 599 – 604. 2. Majtan J et al. (2011) Phytother Res 25: 584 – 587.

PN9 Effects of dry plant extract (*Eleutherococcus senticosus* Maxim.) on the quality of eggs laying hens Hisex Braun

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The application of plant extracts into the feed of different farm animals has an importance in the health prevention of animals, in the aspect of immunostimulant effects and in the production of biofood, which is an important component of the food chain [1, 2]. In the model experiment a dry extract of *Eleutherococcus senticosus* Maxim. was applied to the layers of Hisex braun breed. The layers were divided into three groups, a control group (CG, n = 10), 1st experimental group (EGI, n = 10) with the addition of the extract in the concentration of 0.1%, the 2. experimental group (EGII, n = 10) with the addition of the extract in the concentration of 0.5%. The layers were bred in a three-storey terraced cage battery; feed and water were at disposal *ad libitum*. The dry extract contained Eleutheroside B (0.71%), Eleutheroside E (1.14%), and 30% ethanol was used as an extraction medium. The weight of the eggs and the layers was sampled weekly. The quality of the eggs was assessed on the basis of the weight and shape of the eggs, the strength and thickness of the eggshells. In the 2nd experimental group, statistically significant changes ($P < 0.05$) in the strength (EGII = 30.93 ± 5.00) and thickness (EGII = 0.41 ± 0.03) were detected in comparison with the control group (CG = 26.71 ± 4.42 strength, CG = 0.39 ± 0.02 thickness of the eggshells). **Keywords:** Hisex braun, Eleutheroside, Eleutherococcus senticosus, egg **Acknowledgement:** *This research is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 26220120023, ITMS 26220120041, ITMS 26220220013.* **References:** [1] Davydov M, Krikorian AD (2000)J Ethnopharmacol 72(3): 345 – 349. [2] Siwicki AK et al. (2004) Bull Vet Inst Pullawy 48: 489 – 492.

PN10 Comparison the anticoccidial effects of Artemisinin granule prepared from *Artemisia sieberi* extract with Monensin in experimental broiler chicken coccidiosis

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Coccidiosis is the most important parasitic disease of poultry production industry (4). Due to increasing resistance to conventional anticoccidial agents it is necessary to find out new anticoccidial compounds (1). Herbal compounds are promising weapons in this regard. One of them is *Artemisia sieberi* Besser in which primary studies have shown its possible anticoccidial effect (2 – 3). To compare the anticoccidial effect of artemisinin granule prepared form *Artemisia sieberi* extract vs. Monensin in experimental broiler coccidiosis, 120 Ross 308 broiler chickens in 4 groups each with 3 replicates (n = 10) were used. Group 1 was separated as uninfected negative control and received no drug. Form the first day group 3 received Artemisinin granule 5 mg/kg, group 4 Monensin 110 ppm till 42 days of age as feed additive. At 21 days of age groups 2, 3, 4 were inoculated with a mixed suspension of 200000 oocyst of *E. tenalla, E. maxima, E. acervulina* & *E. necartix*. Group 2 was kept as infected positive control which received no drug. One day after inoculation, oocyct per gram (OPG) of feces for 5 successive days and Mean Body Weight, Weigh Gain and Food Conversion Ratio (FCR) were determined weekly in each group. The data were presented as Mean ± SE and analyzed using Sigma stat (V 3.1) statistical software ($P < 0.05$). This study showed that both artemisinin granule and Monensin significantly ($P < 0.05$) decreased OPG (table1) also artemisinin granule improved performance included weight gain, mean body weight and FCR in infected broiler chickens which is comparable to Monensin. **Keywords:** Coccidiosis, Broiler chickens, Artemisinin granule, Monensin, Artemisia sieberi **References:** 1- Allen PC, Fetter RH (2002) Clin Microbiol Rev 15: 58 – 65. 2- Allen PC et al. (1997) Poul. Sci 76: 1156 – 1163. 3- Ara, HA et al. (2006) Trop Anim Health Pro, 38: 497 – 503. 4- Zhang Z, Zeng M (2005) Chin J Vet Parasitol 13: 29 – 36.

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