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- Quality control of MAP

IN VITRO PROPAGATION OF MEDICINAL PLANTS FOR QUALITY ASSURANCE AND PROTECTION OF ENDANGERED SPECIES

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Keywords: plant biotechnology, quality assurance, endangered species

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Plants are also the source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. More and more highly standardized, clinically approved phytopharmaceuticals are made available, and there is also a certain trend towards other systems like e.g. Ayurveda or Traditional Chinese Medicine. However, although there is a growing awareness of the necessity to intensify field cultivation of medicinal plants, the majority of the used species are still collected from the wild habitats. As a result many medicinal plants have become rare or even endangered. The domestication of concerned species is thus of high importance.

Plant tissue culture can help to make the whole process more effective. Especially the "classical" micropropagation techniques are of interest when certain criteria are fulfilled. In case the propagation of the plant species by conventional methods (seeds, cuttings etc.) is slow or otherwise inefficient, in vitro- (clonal) propagation would make it possible to produce large amounts of plants for subsequent field culture in a relatively short time. Provided that a suitable method is chosen, plantlets from tissue culture are genetically uniform and thus the crude drug material finally obtained is of homogenous quality. Hence, the integration of plant tissue culture can also result in quality enhancement of medicinal plants and preparations, be it high levels of desired secondary compounds, or the absence of toxic products. Finally, at the level of field culture a first standardization of the product is possible, and the required amounts of crude drug can be controlled. Problems with inhomogenous material and dependencies on the availability of medicinal plants can thus be avoided. Some examples from our Department on the use of in vitro-propagation for the production of defined medicinal plant material will be presented. In addition, improved multiplication rates have recently been achieved using Temporary Immersion Systems which will be briefly introduced, too.

The domestication of medicinal plants which at present are collected from the wild is increasingly necessary for reasons of plant protection and standardisation of the crude drug. In this the application of plant tissue culture can help to make the whole procedure more efficient when conventional propagation is a limiting factor.

GENETIC RESOURCES OF MEDICINAL, AROMATIC AND CULINARY PLANTS IN THE CZECH GENE BANK IN OLOMOUC

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Keywords: gene bank, ex situ collection, regeneration, conservation, biodiversity

Genetic resources of medicinal, aromatic and culinary plants (MAP) are maintained in the Research Institute of Crop Production (RICP) Prague - workplace Olomouc.

Collections of MAPs are in Czech Gene Bank carried out *ex situ* and the main attention is paid in collecting plants for their diversity, creation and enrichment of collections, conservation, plant characterisation and evaluation. The *ex situ* MAP collection in Olomouc is represented by 529 accessions distributed in 71 species. The highest species diversity is found in the families *Lamiaceae, Apiaceae* and *Asteraceae*. The wild species from both Czech Republic and abroad areas and also some regional and restricted varieties are included in collections. Most of this species are in collections represented also by Czech origin cultivars.

Huge parts of *Lavandula* (Dušek et al. 2005)*, Carum* and *Calamus* (Petříková et al. 2000; Dušek et al. 2005) collections have been studied for their diversity in pharmaceutically active substances content and another species (*Agrimonia, Betonica, Hypericum, Origanum, Plantago* and *Salvia*) forms a part of research project studying florid meadows re-creation at 5 protected landscape areas around the all country. A passport data of all the hold accessions and short information about availability for users are on view on-line at http://genbank.vurv.cz/genetic/resources.

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CULTIVATION OF MEDICINAL, AROMATIC AND SPICY PLANTS IN SLOVAKIA AFTER JOIN THE EUROPEAN UNION

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Keywords: cultivation, production, yields, medicinal, aromatic, spicy plants

European Union represents the biggest unique market with medicinal, aromatic and spicy plants (MASP) in the world. It was about 120,000 tons (e.g. 200 million USD) of this plant material during the years 1991-2004. Paper is orientated on the current status and situation in the field of MASP grown and produced in Slovakia. Growing areas of these plants were the lowest in 1999 (467.44 ha) and the highest in 2003 (851.85 ha). Global production varied between 222.2 (2000) and 1380.2 tons (2004). Average yields ranged from 0.32 (2000) to 2.60 $t.ha^{-1}$ (2004). Acute problem is to increase and stabilize the production and to obtain stronger position on global herbal market. The objectives of future medicinal plant strategy are: (1) To ensure the quality of MASP material used as the source for herbal medicine to improve the quality, safety and efficacy of finished herbal products; (2) To improve national and/or regional good agricultural practice, processing guidelines, publications about MASP and related standards for operating procedures;(3) To encourage and support the sustainable cultivation and collection of good quality MASP, in ways that respect and support the conservation of the environment. As an alternative for MASP producers can be the organic production of MASP. Demand for organic products is still increasing in EU as well as the consumption of natural substances.

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BIODIVERSITY AND PROTECTION OF THE MEDICINAL AND AROMATIC PLANTS IN BULGARIA

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Keywords: biodiversity, medicinal and aromatic plants, protection

The medicinal and aromatic plants (MAP) represent very important part of the biological resources in Bulgaria. According to the Medicinal Plants' Law 739 vascular plants are used as raw materials for the pharmaceutical industry, as spices, for cosmetics and technical purposes. Conservation importance have 61 taxa protected by Law and 76 were included in the Red Data Book of Bulgaria (Gussev, 2005).

The main threats for the MAP's biodiversity are the global climatic changes, destruction of the natural habitats and the overexploitation of the resources of some vulnerable MAP.

The scientific investigations and the activity of the Bulgarian government for the protection and conservation of the MAP's biodiversity are discussed in this paper.

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AN OVERVIEW ON THE ALBANIAN MEDICINAL AND AROMATIC PLANTS INDUSTRY

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*Keywords: Albania***,** herbs, *industry, spices, trade*

Albania offers a wide range of Aromatic and Medicinal plants which presently sell to the International markets mainly as bulk dried and essential oils. Over 95% of Albanian Medicinal and Aromatic plants are wild collections grown all over the country. The most important export items are Sage, Oregano, Juniper, Thyme, Savory and Laurel. Transitional developments in the country, over the last decade, contributed to a new design of this industry which in the meantime introduced several challenges. Within this challenging business environment, Albanian herb and spice industry businesses are making intensive and serious efforts to increase their

competitiveness capacities in order to maintain the existing trade links and/or capture new market segments. Considering the maxim "Don't put all your eggs in one basket!", it is important to search for new ventures although these are always risky. While Albania is on the way to become an EU member country, the Albanian herb and spice industry businesses should start preliminary preparations preceding the integration process, which means firstly an effective management system to ensure that MAP harvesting from the wild and protected areas are properly administered, and secondly more information on the international plant trade and regulation.

STUDY ON MEDICINAL AROMATIC PLANTS IN THE ZONE OF NATIONAL PARK OF LLOGORA

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Keywords: Llogora, medicinal and aromatic plants

The zone of National Park of Llogora, Massif of Rrëzë e Kanalit, Karaburun Peninsula, the Valley of Dukat, is distinguished for the rich flora incomparable to any other area in Albania.

The presence of some endemic, subendemic, relic, rare and threatened species improves the biodiversity values in this area.

About 1,400 species or 42,4 % of the Albanian flora inhabit the region. There are about 110 medicinal and aromatic plant species grown in this area, 22 medicinal plants are actually more used in the region.

Some medicinal and aromatic plants are affected by the phenomena of genetic erosion and 20 species of this region are "Threatened Medicinal plant species" included in the National Red Data Book" (Vangjeli et al., 1995).

A number of data exist on the use of aromatic plant on the area since ancient times. Famous authors as Nicander (physician and philosophies who lived one century before New Era), Theophrasti etc, mentioned as for example "Buxus of Oriku" (*Buxus sempervirens*), "Therebentin of Oriku" (*Pistacia terebinthus)* not only as tree of special characteristics but as aromatic medicinal plants as well (Baldacci, 1924).

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WILD AND CULTIVATED MEDICINAL PLANTS – AN IMPORTANT POTENTIAL FOR THE SUSTAINABLE ECONOMIC DEVELOPMENT OF ROMANIA

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Keywords: medicinal plants, traditional use, industrial processing, sustainable development

Romania has about 3500 species of higher plants; 700 of these are medicinal and aproximatelly 280 proved therapeutic properties [5]. Different classification criteria were used to define the specificity of local bioresources. Historical and ethnographic data show a long tradition of phytotherapeutic remedies [4]; this old activity got an industrial dimenssion when a national network of plant processing was set up (in '80 Romania became the 5th exporter of the world) [6]. The sustainable use of local resources (63 species mainly collected) asked the biodiversity conservation (297 species *ex situ* preservated, 179 species hold by Suceava Genebank, 13 species on Red List) but also the cultivation of 52 species until 41 000 ha [3]. All research & development projects (180 studied species)[1] and breeding activities-27 omologated cultivars, 31 certified local landraces [2]-supported scientifical point of view the successful economic and trade activity. After years '90, significant changes registered in agriculture (up to 84% of the farms are now private) and real progresses were registered by the processing industry (SME implemented new technologies, QMS, GMP, traceability, EU standards of raw material). A review of the last law and regulations as well as a presentation of the certification bodies and existing NGOs involved in medicinal plants field will be also presented.

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MEDICINAL SPECIES OF MACROMYCETES RECORDED IN THE REPUBLIC OF MACEDONIA

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Keywords: medicinal, macromycetes, R. Macedonia

Fungi have a long history of usage in traditional medicine. They represent a very heterogeneous group of organisms. However, studies to date have been concerned mainly with Ascomycota and Basidiomycota. The other phyla have been studied insufficiently. Thus far, in the Republic of Macedonia approximately 1,250 species of fungi have been recorded [1]. The majority of them belong to the phyla Ascomycota (130) and Basidiomycota (1,050). The Division of Mycology, within the Faculty of Natural Science (Institute of Biology), possesses a large collection of

macroscopic fungi called Fungi Macedonici. The collection contains approximately 10,000 specimens as well as a fungi database named MACFUNGI. This plentiful gene fond of fungi might be a potential source for isolation of active principles with antioxidative, antitumor, immunomodulative, antiinfective and other actions [2-5]. The content of medicinal species (about 200 species) has not been researched enough in Macedonia. Therefore, in the present study an attempt has been made to present the medicinal species of macromycetes recorded in the Republic of Macedonia together with the literature data for their effect in human therapy. Their large number facilitates the selection of those characterised by a significant medicinal quality.

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REVIEW OF SOME USEFUL METHODS IN TAXONOMICAL INTERPRETATION OF DIFFICULT TAXA OF MEDICINAL AND AROMATIC PLANTS. CASE: *THYMUS* L.

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Keywords: Thymus, morphology, anatomy, phytochemistry, molekular marker

Considering the fact that many taxa of medicinal and aromatic plants, including some genera, species and, especially, infra-species categories, are known to be complex and very difficult from the aspect of taxonomical interpretation, a survey of some useful methods and techniques was conducted, as proper determination of plant material is a starting point for further evaluation of raw quality, as well as development of range of descriptors needed for gene banks. Various methodological approaches were assessed and compared in order to establish their relevance in taxonomical description within genus *Thymus* L. Several populations of ten wild growing species of the genus *Thymus* in the region of Serbia were investigated through a study of their morphological, anatomical and phyto-chemical features. Additionally, results obtained by application of molecular marker techniques were discussed. It was shown that composition of essential oils varied significantly, whereas the ratio of their main components, such as thymol, geranial, carvacrol and neral could be treated as an important character in discrimination of some populations. Analysis of ITS DNA region performed on 41 populations of different *Thymus* species hasn't revealed any significant difference among them, and therefore the AFLP analysis could be recommended.

THE ROLE OF THE BOTANICAL GARDENS OF TARGU-MURES IN *EX-SITU* CONSERVATION OF RARE AND ENDANGERED SPECIES OF ROMANIA

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Keywords: plants, conservation

The development of human civilisation as an outcome of the scientifical and technical innovations puts a great presure on Nature and its resources. This generates ecological disorders which in time will develop into a severe ecological crisis. Because of this one of the most important duties of the contemporary world is the conservation of the species endangered due to the divers' impacts and masive exploitation.

We consider that due to the pertinent information we obtain through our research regarding threatened, rare and vulnerable plants they will be introduced in a crop sistem. Thus, by evidence contained by: The World Conservation Monitoring Centre, Globally threatened plants in Europe, Red List of Threatened Plants); Law 13/1993 for the Aderation of Romania to the Convention for the Preservation of Wild life and Natural Habitat in Europe, adopted in Bern at 19th of September 1979; The Red List of Superiour Plants in Romania drawn up by M.Oltean and contributors; Red List of extinct, threatened, vulnerable and rare vasculare plants in Romania's flora – N. Boscaiu and contributors – 1994; Red List of grass land plants in Romania including endemics and subendemics – G. Negrean – 2001, contains an inventory of 39 plants species.

This study imposed itself due to the fact that short term interest lead to the diminuation of plants populations

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MEDICINAL AND AROMATIC PLANT RESEARCHES AT THE CZECH AGRICULTURE UNIVERSITY IN PRAGUE

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Keywords: Hypericum perforatum L., Calendula officinalis L., Oenthera biennsis L., Matricaria recutita L.

Research in medicinal, aromatic and spicy plants at the Czech University of Agriculture in Prague is focused on these plant species:*Calendula officinalis, Hypericum perforatum L., Oenthera biennis L., Matricaria recutita L.,*

Hypericum perforatum L. and its duality

In our experiments we determined, e.g., hypericine content in above-ground parts of plant in different development stages in cultivar Topaz. The highest content of hypericine was found in buds and blooms (0,1% - 0,15 %), the lowest in stem. Based on acquired data we can

recommend that the length of harvested blooming haulm should be between 10–20 cm, which is the length of a blooming part of the plant.

Oenthera biennis L. and its biology

In our experiments dealing with biology of evening primrose we focused on long-day and short-day influence of generative parts formation. Short-day plants of Czech and English origin did not bloom from seedling and microseedling. The blooming initiation of long-day plants is determined in the first year by seed origin, weather conditions or locality and seedling size (achieved stage of development). Higher percentage of plants from the long day bloomed in the first experimental year. Higher percentage of blooming plants came from seedling.

Matricaria recutita L.- qualitative and quantitative evaluation

In experiments with organic and conventional methods of farming determined content of essential oil extended standard essential oil content according to Czech Pharmacopoeia. We can presume, that for the organic cultivation technique both varieties are suitable.

Calendula officinalis L. as an oil crop for industrial use

Recently we tested Marygold as an alternative oil crop for industrial use in experiments at the Czech University of Agriculture in marginal regions. This topic was presented on last conference (3rd CAMAPSEEC).

MEDICINAL AND AROMATIC PLANTS ON THE SERPENTINES IN THE VLAHINA MTS. (SW BULGARIA)

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Keywords: medicinal plants, serpentines, ethnobotany, Bulgaria

Three serpentine sites of 15 dka area in total found from 700 up to 790 m a.s.l. are investigated. The terrains are located between the West Frontier Mts. and the Struma valley floristic regions. The interest in the medicinal serpentine plants is provoked by the fact that screening of medicinal and aromatic plants (MAP) growing under stress conditions has provided another source for compounds with useful activities. The investigation has targeted plants with historical ethnobotanical use.

The paper presents data of the most popular herbs'main compounds with biological activity and their traditional medicinal use. Flavonoids, tanins, terpens, cardiac glycosides, mucous compounds and alkaloids are widely included and determine the biological activities which range from antimicrobial, diuretic, spasmolitic, stimulate appetite, antitumor, etc.

As poisonous herbs 26 species are determined. Ethnobotanical data about their effect under human and herbivorous animals are presented, the more so as the investigated terrains are used as pastures. The toxicity of the plants and their dry parts are caused of toxic agents such as alkaloids, cardiac glycosides and sapponins.

The presence of 223 taxa of vascular plants is established, 82 (36,8 %) of them being medicinal plants belong to 32 families and 72 genera. The predominant number of species is from Asteraceae, Rosaceae, Fabaceae, Lamiaceae and Aspleniaceae families. Three of the species are under special governmental control of protection and use - *dng0Sedum acre* L., *Asplenium trichomanes* L. and *Juniperus oxycederus* L. The species *Chamomilla recutita* (L.)Rausch. and *Rosa canina* L. are medicinal plants that are cultivated in Bulgaria.

The investigated populations as well as the spores and seed material collected represent a novel genetic resource of potential economical value.

MEDICINAL PLANT COLLECTION FROM THE BOTANICAL GARDENS OF THE STATE UNIVERSITY OF MEDICINE AND PHARMACY

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Keywords: medicinal plants, active principles, botanical gardens, collection

The State University of Medicine and Pharmacy „Nicolae Testemitanu" organised a collection of medicinal plants, which includes about 200 native and foreign taxa. This collection comprises species with different active principles: heterocides – 13, saponosides – 10, flavonoids – 10, tanins – 11, volatile oil – 30, alkaloids – 22, lipides – 10, cumarines – 6. Also there are species with sedative – 6, stimulating, tonic action, hepatoprotectors – 11, insecticide, vermifuges – 6, etc.

The main idea of the collection is to enlarge the theoretical knowledge and practical skills of the students – future pharmacists, as well as the scientifical researches in the direction of elaborating new medicines from autochthonous vegetal material.

The collection is also a base of semen and planting material for industrial plantations of medicinal plants.

THE SPECTRUM OF BIOFORMS AND THE AREAL GEOGRAPHIC STRUCTURE OF THE MEDICINAL PLANTS FROM THE WESTERN PART OF THE BANATIAN MOUNTAINS (ROMANIA)

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Keywords: medicinal plants, Banat region, bioforms, repartition

In today's Europe, where industrial development, pollution and urban agglomerations are continuously extending, the rich medicinal flora of Romania represents a priceless asset. In this concept, the knowledge of the flora of pharmaceutic interest becomes imperative, representing a prerequisite for its rational valorization and protection. The aim of the present study was to identify the medicinal plats (MP) from the Western part of the Banatian Mountains, known for their vegetal diversity, and to outline their spectrum of bioforms and areal-geographic structure. The researches pointed out the presence of 241 species employed in modern and traditional phytotherapy, belonging to 70 families. Considering the biologic type of the identified MP, the hemychryptophytes are dominating (44%), being followed by the phanerophytes (22%), geophytes (11%), therophytes (7,4%) and chamaephytes (6,6%). The analysis of the floristic elements outlines the predomination of the Eurasian species (43%), against their background intervened in different phytohistoric stages European (9,5%) and Circumpolar (9,1%) elements. The cosmopolite species (6,2 %) and the adventive ones (2,9%) also have a notable presence among the MP. This approach allowed the identification of the critical and/or valuable components of the genofond existing in the researched area.

RARE AND VULNERABLE SPECIES OF PHARMACEUTIC INTEREST FROM ANINEI MOUNTAINS (ROMANIA): RECENT DATA

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Keywords: rare and vulnerable taxons, medicinal plants, Angelica archangelica

The floristic diversity of the Aninei Mountains is imprinted by the peculiarities of the carstic relief, and the geographic position, at the interference of air masses with maritime and continental character. The present study aimed at identifying the endangered, vulnerable, rare and endemic taxons from the flora of the Aninei Mountains, establishing in accordance to the criteria of the World Conservation Union, the Red List of Vascular Plants (RLVP) from this area. A particular interest was paid to the present repartition of the medicinal plants included in this list. The mapping procedures were performed according to the TK25 system, used in the mapping of the Flora of Central Europe. Ninety-five taxons were included in the RLVP from the Aninei Mountains, among which: 1 taxon is endangered at European level; 5 taxons are vulnerable and 80 taxons are rare. Five endemic and six sub endemic species were noted. The RLVP also contains several medicinal plants, wild-growing in the region; among these *Angelica archangelica* is the most threatened one due to its gathering for pharmaceutical purposes. These observations suggest the necessity of supplementary measures for the protection of rare and vulnerable taxons at the level of the Agency of Environment Protection corresponding to the investigated region.

CONSERVATION OF MEDICINAL AND AROMATIC PLANTS IN EUROPE – A REVIEW OF CURRENT PROGRESS

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Keywords: conservation, medicinal and aromatic plants, Europe

Conservation programs, aimed at conserving of natural heritage, at improvement of the knowledge on the medicinal and aromatic plants (MAPs) genetic variability and improving biological knowledge as well as MAPs-related user safety should be promoted in the EU countries. Strategies of biodiversity conservation of MAPs in European region have been set up by ECP/GR MAP WG, consisting of 38 member countries. According to conservation strategies preparation of relevant descriptors (based on morphological characteristics, chemo-taxonomy and end-product quality requirements) and successive *ex situ* evaluation of ecotypes of MAPs will foster selection and breeding activities (e.g. cultivar/variety development). *In situ* conservation of MAP genetic resources in member countries will help to maintain the biodiversity of rare and vulnerable species. The collected seed material of endangered species will be used for multiplication/regeneration purposes in order to assure the MAP material for their characterization, evaluation and cultivation in the future. Development of agrosystems and cultivation of MAPs should be considered as the only way of protection of MAPs natural resources and their sustainable use in conditions of an increased market demand for raw materials. Sustainable use of MAPs in Europe can be achieved only by further introduction of wild plants into cultivation.

EPCA TOWARDS VALUE ADDING OF MAP'S IN ALBANIA

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Keywords: Albania, MAP's, essential oils, cultivation, EPCA

Aromatic and Medicinal Plants still continue to be a significant component of the Albania's overall agricultural exports. Blessed by mother nature, Albania represents a very diverse MAP's basin, most of which are basically harvested, processed and exported as bulk. However, along with MAP's utilization, it remains crucial the sustainable use of these natural resources. Within this framework, the Essence Producers and Cultivators Association (EPCA) has placed special emphasis on the preservation of MAP's as a guarantee for the present and the future of this industry in Albania which involves some thousands of rural families all over the country. EPCA promotes sustainable use of MAP's biodiversity through commercially viable cultivation alternatives particularly utilizing nonproductive lands. In the meantime EPCA is working towards further processing of these MAP's through increased quality and production of essential oils which in turn will bring in more dollar value in the country. In the challenging road of Industry towards progress, EPCA also recognizes as important increased awareness of its membership towards application of proper harvesting techniques and up to date processing technologies through continuous trainings and educational programs.

CONSERVATION OF EASTERN EUROPEAN MEDICINAL PLANTS: *ARNICA MONTANA* IN ROMANIA

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Keywords: Arnica montana, medicinal plants, conservation, land use management

The purpose of the project is to develop a model for the sustainable production and trade of *Arnica montana*, in Gîrda de Sus commune, Apuseni Mountains (Romania), resulting in benefits for biodiversity and livelihoods. The principles of which can be used to inform the development of conservation approaches and methodologies for other endangered medicinal and aromatic plants and their habitats. This model can be tested on other species and will have conservation benefits for habitats, extending beyond benefits for the targeted species. Ecological management and human management are equally challenging and essential for the success of the project. Based on experiences from a previous project and preliminary scientific data on arnica distribution, growth and reproduction in the area, key components for successful project implementation have been identified: research on arnica ecology, trade chain, socio-economic context and drying methods; training and capacity building; development of a local resource management and trade association; development and construction of arnica drying facilities.

CONSERVATION STRATEGIES AND CULTIVATION OF *BUNIUM PERSICUM* (BOISS) FEDTSCH – A POTENTIAL MEDICINAL HERBAL PLANT IN COLD DESERTS OF NORTH WESTERN HIMALAYAS

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Keywords: Bunium persicum (Boiss), ayurvedic medicines

Bunium persicum (Boiss) Fedstsch, Black caraway a perennial, aromatic spice and medicinal umbellifer (Apiaceae), growing naturally in dry temperate regions (1850-3100 m alms) of northern Himalayas and recently brought under domestication, yielding cuminaldehyde and p-mentheadiennals rich cumin oil and are in greatly demanded in ayurvedic medicines, condiments and perfumery. Recognizing the need for its conservation to save the plant from over exploitation and to meet the ever increasing demands. The author has studied its agro technology with respect to its phenology, phenotypic variability, methods of its plantation, intercropping, pests and diseases of crop. *Bunium persicum* exhibited a wide range of variation for primary, secondary umbels/plant, tuber weight and seed yield/plant. Yield/plant possessed significant positive correlation with plant attributes namely primary and secondary umbels/plant umbellets/secondary umbel and tuber weight /plant. Gram pod borer has been found to be the most serious pest of the region with 30-40 % infestation. Infested plants lost their umbels and were devoid of grains. Amongst the wide range of diseases like blight, powdery mildew and bulb rot infestation during sprouting and bolting stage of crop growth, bulb rot was a major disease with 15-35% infestation in comparison to others viz. powdery mildew (2-10%) and blight (7-25%).

INVESTIGATION OF THERAPEUTICAL PERSPECTIVE OF MEDICINAL PLANTS AND POSSIBILITIES OF THEIR INTRODUCTION INTO CULTURE

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Keywords: introduction of medicinal plants, cultivation, Teucrium, Salvia, Cota.

In the framework of research project GA SPU 705/02180 the investigation of localities with potential occurrence of perspective medicinal plant species was realized. Report is orientated to these genus: *Teucrium, Salvia and Cota* obtained from natural localities: Miksova – Benov, Bytca district; Cigel, Prievidza district; Krnca, Topolcany district; Liesek, Namestovo district. The soil samples for agri-chemical analyses were realized on these localities at the same time as the harvesting of a plant material. Chemical analyses of active ingredients in these drugs were made according to methods given in Ph.S. 1 (*Chalabala et al., 1997*). Results of the research were documented by a collection of harvested plant material, as well as by producing herbarium items, and photo documentations. Monitoring of selected localities with potential occurrence of perspective medicinal plant species according to preliminary results will be in progress in the future. The introduction of selected medicinal plant species to the suitable cultural agri-climatic conditions (warm and/or cold macro-climatic areas) will be proposed. Correct selection of suitable medicinal plant genotypes and their introduction to specific cultural growing condition predicts increasing of medicinal plants production and profitability of producers within domestic market of medicinal plants obtained from cultural agri-ecological conditions.

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NUMERICAL AND MORPHOSTRUCTURAL CHARACTERISTICS OF *TRIGONELLA FOENUM GRAECUM* (2N=16) MITOTIC CHROMOSOMES

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Keywords: Trigonella foenum graecum, karyotype, chromosomes

The *Trigonella foenum graecum* L species is very interesting one, for its pharmaceutical, industrial and alimentary valences. The cytogenetic analysis of this species is a part of complex investigations about the chromosomal features of medicinal and aromatic plants. The investigations have evidenced that the diploid chromosomal number of this species is 2n=16. In accordance with cytogenetical parameters resulted from measurements, was possible to establish eight pairs of homologous chromosomes (I – VIII). One pair of them is metacentric (I, with long arm/short arm = 1.0), three pairs are submedian (II, IV and V, with long arm/short arm = 1.77-2.00) and four pairs are median (III, VI, VII and VIII, with arms ratio = 1.17-1.58). The chromosomes length displays values between 2.10 μm and 2.79 μm. The relative length has values between 10.55 and 14.01 the haploid set length being of 19.91 μm.

The karyotype is nearly symmetrical, less evolved.

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STUDIES ON GENETICS AND BREEDING OF AROMATIC AND MEDICINAL PLANTS CARRIED OUT IN THE REPUBLIC OF MOLDOVA

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Keywords: varieties, medicinal and aromatic plants

New genotypes of aromatic and medicinal plants have been selected using various genetic methods; they were used as initial breeding material in the development of new varieties. Among them, there are *Salvia sclarea L.* varieties of hybride nature with different ripening time: early ripening (Dacia-50), late ripening (*Victor, Natali-Clary*) and medium ripening (*Dacia-99*). The producing capacity of new varieties is 34-48 kg/ha of essential oil at a high concentration of linalil acetate (65-78%) and sclareol (10-12%). These varieties are valuable in the *concrete* production with sclareol content of 60-70%. Polycross hybridization has resulted in new *Lavandula angustifolia Mill*. varieties named *Moldoveanca*, *Alba* and *Vis magic* that are

resistant to frost and drought and have a producing capacity of 130-200 kg/ha of essential oil. The studies carried out on *Anethum graveolens L*. Led to a variety named *Ambasador* that is characterised by an increased production of essential oil which comprises carvone (39.8%). New varieties, *Natalia* and *Diana*, have been developed in the *Calendula officinalis L.* species through complex hybridization. The producing capacity of these varieties is 10.7-10.8 q/ha of inflorescence containing 0.606-0.873% flavons and of 0.987-1.021 polyphenols %. A new, early-ripening variety of *Silybum marianum (L.) Gaert* named *Argintiu* with a producing capacity of 890-1000 kg/ha of fruits has also been developed. A new *Salvia officinalis L*. variety named Miracol has been developed, a single harvest of which provides for an yield of 18 kg/ha of essential oil or 8.4 M/C./ha of dry *Folium salviae*.

ECOPHYSIOLOGICAL RESEARCH ON SOME MEDICINAL PLANTS FROM BĂLTENI FOREST (VASLUI COUNTY)

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Keywords: ecophysiology, medicinal plants, forest ecosystems, active substances

The research reveals the physiological behavior of some woody and herbaceous medicinal plants from Bălteni Forest. The vertical structure of the plant community, in which light penetration through the forest canopy is an important factor, induces certain physiological aspects. Thus, in shade-loving species, the protoplasm has high water contents (76% in *Achillea millefolium* and 83% in *Tussilago farfara*) and high total mineral contents (17.15% in *Lamium album* and 19.45% in *Urtica dioica*), whereas in heliosciaphytes (*Rosa canina*, *Sambucus nigra*) and heliophytes (*Tilia tomentosa*) the protoplasm is rich total assimilatory pigments (3.60 – 4.97 mg/g of fresh material).

MORPHO-PHYSIOLOGICAL, PRODUCTION AND QUALITY TRAITS OF THE NAPOCA CULTIVAR OF *ECHINACEA PALLIDA* NUT.

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Keywords: Echinacea pallida, polysaccharides, volatile oil, phenil-propanic derivates

The Napoca cultivar of *Echinacea pallida* Nut. has been created at USAMV Cluj-Napoca, by the Research Centre for Hop and Medicinal Plants, through repeated mass selection from echinacea that was imported to our country and studied in comparative trials (with the temporary name of CN 11/992). The Napoca cultivar is semilate, with a vegetation period of 134-145 days in the second and third year; in the first year the root system is formed, and also a leaf rosette and flower stems with a reduced number of flowers, the complete flowering only beginning in the second year of vegetation. From the morpho-physiological point of view, the cultivar has been characterized with reference to type of root, stem, leaves, inflorescence and fruit and plant habitus. The raw root yield is of 4.5-5.2 t/ha (1.3-1.5 t/ha when dry). The content of active principles is high: imunostimulent polysaccharides (6.1-6.4g %), phenil-propanic derivates (0.5-0.6g %), volatile oil (0.9-1.2 ml/100g) etc., which enhances the immunity system, the raw material being used for obtaining anti-virus, imunostimulents, antitumorale products etc.The Napoca cultivar can be cultivated in all agricultural areas of Transylvania; for successful establishment it is suggested that the plantlets produced by the Research Centre for Hop and Medicinal Plants of USAMV Cluj-Napoca should be used.

MORPHOLOGICAL, ANATOMICAL, BIOCHEMICAL AND PHYSIOLOGICAL RESEARCHES UPON TAXA OF *ROSA* GENUS CULTIVATED IN IASI BOTANICAL GARDEN (NOTE II)

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Keywords: morpho-anatomical analysis, biochemical parameters, physiological parameters, essential oil

Three taxa of *Rosa* genera from the Romanian spontaneous flora have been studied: *R. canina* L., *R. glauca* Bourr. and *R. rubiginosa* L. All species have been taken from the Botanical Garden of Iasi, where they are cultivated under unprotected conditions. The material analyzed (leaves) has been taken in specific phenophases during the summer of 2004 (between June and September): for morpho-anatomical analysis during the opening of the first flowers, and for the biochemical and physiological analysis from the flowering point up to fructification point, senescence respectively. The morpho-anatomical characteristics, as well as the biochemical and physiological parameters vary strictly according to the analyzed taxon, according to the phenophase cultivation. The *R. rubiginosa* species is the only one which has secretory trichomes located especially on the lower epidermis of the leaves, as well as on the edge of stipels and rachis. Consequently, this is the only leaf origin essential oil producing species and the oil contains 19 components, among which predominant are eucalyptol (30,86%) and borneol (17,31%); these compounds give a persistent and pleasant odor. Aside from the abovementioned, acymen, γ-terminene, bornil acetate, β-cariophylene, α and β pinene and camphore have been found. The rest of the components are found in concentration of approximately or lower than 1%.

HISTO-ANATOMICAL RESEARCH REGARDING SOME SPECIES OF CUPRESSACEAE

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Keywords: anatomy, histological characteristics, pedo-climatic conditions

The authors investigate the structure of the leaf at 11 different species of *Cupressaceae*, belonging to the following genres: *Juniperus* (*J. communis* L., *J. sabina* L., *J. virginiana* L., *J. chinensis* L. Ph., J. *horizontalis* Moench.), *Thuja* (T. *orientalis* L., T. *occidentalis* L., T. *plicata* D.Don (syn. T. *gigantea* Nutt.), *Thuyopsis dolabrata* (L. f.) Sieb. et Zucc. and *Chamaecyparis* (*C. lawsoniana* (A. Murr) Parl., *C. pisifera* (S. et Z.) Endl.); some of them have, beyond any doubt, an important medical value.

We point out the histological characteristics used to differentiate more easily the species of the same genre under the circumstances when the plants don't have cones yet. Some of the characteristics of the structure are related to the geographical origin, the age of the photo-assimilator organ and to the pedo-climatic conditions in which these plants grow. We emphasis on the cyto-histological characteristics such as the cuticle, stomata, the secretory ducts, the endoderm and the mesophyll; depending on the structure of the mesophyll, we have destroyed bifacial isofacial leaves and bifacial heterofacial leaves, with normal or reversed dorsinventrality.

CONSIDERATIONS ON THE HISTO-ANATOMICAL STUDY OF THE LEAVES OF *CYNARA SCOLYMUS* L. TREATED WITH THIOPHANAT-METHYL (TOPSIN M)

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Keywords: Cynara scolymus L, thiophanat-methil, histo-anatomical modifications, leaf structure.

The purpose of this study was to investigate the histo-anatomical modifications of the leaves of *Cynara scolymus* L treated with thiophanat-methil compared with untreated sample. There were three applications and two variants of treatment: Topsin M70 0,1 %, a concentration used in agriculture and Topsin M70 0,4 %, because we wanted to observe the limits of concentration between the modifications induced by thiophanat-methil remain acceptable for the plant. It was used cross sections through the petiole and limb (the officinal product Cynarae folium) made at different levels and superficial sections for the upper and lower epidermis. The modifications observed were rather quantitative than qualitative, the general picture showing that the development of leaves was obviously stimulated.

For the treated plants, the comparative study relieved the following aspects: the thickness of petiole increase, the hypodermic walls of collenchymas are more developed, more specialized conducting bundles appear with a larger diameter of xylem vessels, the cambium layer's activity is more intensive; the form and dimensions of median nervure are modified, the mesophyll tend to thicken, palisadic tissue being much more developed; secretors and tector hairs are more numerous per unit area of leaf surface, epidemic cells are many more smaller and stomata more numerous per unit area, with guard cells of smaller dimensions.

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NECTARY STRUCTURE OF *OCIMUM BASILICUM* (L.)

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Keywords: Ocimum basilicum, nectary structure, modified stomata

Floral nectaries of *Ocimum basilicum*, during two different flowering stages were studied using light, fluorescence and scanning electron microscopy, in order to analyze position, size, appearance and anatomical characteristics of the gland, and the possible way of nectar exudation.

The floral nectary belongs to the toral type, developing on the surface of the receptacle along the ovary base, forming a four-lobed ring. In mature nectaries, according to the structural observations, three major zones were identified: the one-cell-layered epidermis, the sub-epidermal secretory tissue and the vascular tissue. In the epidermis, covered by a very thin

cuticle, many modified stomata involved in the exudation process are present,. The stomata are situated in the upper portions of the largest nectary lobe, exclusively along the brim and inner surface facing the ovary. They are diffusely distributed and lay at the same level of the epidermis. The secretory tissue is composed of small cells with thin walls, relatively large nuclei and dense granular cytoplasm. In nectariferous tissue, the majority of cells contain crystals of different size. The floral nectary is vascularized exclusively by phloem originating from vascular bundles destined for the gynoecium.

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MICROMORPHOLOGICAL ANALYSES OF *STACHYS SCARDICA* (GRISEB.) HAYEK TRICHOMES

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Keywords: Stachys scardica, Lamiaceae, trichomes, micromorphology

Stachys scardica (Griseb) Hayek is an erect hirsute, perennial herb widely distributed in Balkan peninsula, grows at subalpine meadows and shrub. Micromorphology and distribution of trichomes on stem, leaves and calyx of *Stachys scardica* (Griseb.) Hayek were investigated using LM and SEM. On the stems two types of trichomes were found: nonglandular 3-branched elongated trichomes were densely distributed, and glandular peltate trichomes with 4-celled heads. Leaf surfaces were covered with numerous multi-branched and elongated nonglandular trichomes and peltate trichomes with 4-celled heads. The outher surface of calyx was densely covered with numerous multi-branched nonglandular trichomes, among which the peltate glandular trichomes were distributed. From inner side of calyx very long simple trichomes were observed to emerge. Comparing to the results obtained in other *Stachys* species trichomes analyses, *S. scardica* was only one to possess branched nonglandular trichomes, while other species were covered with simple uniseriate nonglandular trichomes. Ecological and taxonomical aspect of trihome distribution is discussed.

FUNCTIONAL CORRELATIONS OF THE BIOTIC AND ABIOTIC FACTORS IN THE EVOLUTION AND THE PRODUCTIVITY OF THE *VERBASCUM* POPULATIONS

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Keywords: Verbascum sp., polyphenols, flavons, iridoids

The investigations on the extent of the *Verbascum* populations in the Moldavian Subcarpathian areal, established a vertical spreading of the species belonging to this genre: on three parallel altitudinal stripes with a north to south orientation. The etnophytoterapeutical investigations proved the traditional use of the Verbascum extracts in the treatment of the respiratory, digestive, neurological and dermatological affections. The numerical evolution and the biosynthesis of the active principles of the *Verbascum* populations have significant correlations with the ecological conditions, especially the climatic and those of the relief. In the biomass of the species, the

flavons are dominant followed by the polyphenols and iridoids. Among the species, from the point of view of the productivity: *Verbascum phlomoides* followed by *Verbascum lychnitis* and *Verbascum thapsus* are outstanding. Regarding the biometrical characteristics, there are significantly correlated in a positive way with the biosynthesis oh the polyphenols and flavons and significantly negative with the iridoid content. The optimum method to reconstructs the populations of these species was the transplantation while nature spontaneously offered us an example of a huge multiplication by means of seeds and vegetatively.

VARIATION OF SAGE HERB YIELD AND QUALITY REGARDING THE GENOTYPE AND AGROECOLOGICAL FACTORS

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Keywords: Salvia officinalis L., essential oil, herb drug quality

Data concerning aspects of sage yield regulation respecting required qualitative parameters. Our attention was aimed at the influence of selected agroecological factors on herba yield and chemical composition of sage. Observed factors were three sage genotypes (three varieties: Primorska, Comune, Krajova) and different sowing distances in rows. Sage essential oil quality was determined by date of harvest and differentiated highs of aboveground plat-parts. Field experiments were conducted at the Institute of Agroecology in Michalovce, point at the fact that on Eutric Fluvisol, without irrigation in 2003 - 2005. Yield of dried sage herb was statistically high significant influenced by climatic factors, variety and sowing distance in row. Comparison of observed varieties confirmed significant decrease of herb yield in order: Primorska – Comune – Krajova. The highest average yield was found at variety Primorska (3,18 $t.ha^{-1}$). Significantly the highest yields were obtained at variant with sowing distance in row 0,40 m (3,02 $t.ha^{-1}$). The most determining share of total production have been obtained at first date of harvest. Statistical evaluation confirmed high significant influence of differentiated highs of aboveground plat-parts, variety, climatic factors and date of harvest of essential oil content variability. The highest oil content was measured at Primorska variety 1.79 % dried drug. The most optimal quality parameters were determined at varieties Primorska and Krajová.

High yield and good quality of dried herb drug (optimal content of thujone and good relationship of various components of essential oil) determine potential for intensive cultivation in mono – agricultural conditions in Slovakia.

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QUANTITATIVE ANALYSIS OF SAGE (*SALVIA OFFICINALIS L.*) STAND STRUCTURE

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Keywords: sage, stand structure, horizontal and vertical level, density

Salvia spp. is very polymorphous and huge genus belongs to the *Lamiaceae* family and comprises of 900 species widespread all over the world. In the recent years sage (*Salvia officinalis* L.) represents mostly focused species. Our research was orientated on the application of modern ecobiological approach in the sage (*Salvia officinalis* L.) stand - structure study concerning the final production of plant biomass. The main observed factor was sowing spacing. Field experiments were conducted at the Institute of Agroecology in Michalovce (belonging to the Slovak Agricultural Research Centre in Nitra), point at the fact that on Eutric Fluvisol, without irrigation in 2003 - 2005. Resulting the data obtained from the study of quantitative analysis realized in the vertical and horizontal sage stand level, it can be stated, that different sowing distance under steady inter – spacing row distance influences production and distribution of sage biomass. Production of aboveground biomass was effected mostly by stand density which supported interspecies competition. Sowing spacing influenced the plant growth and development, mortality, total biomass amounts and has become the major reason of plant population structure. Obtained results confirmed, that the most optimum quantitative parameters in both horizontal and vertical stand levels, were found out at the spacing 0.45 m x 0.40 m.

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SELECTED PARAMETERS OF ECOLOGICALLY AND CONVENTIONALLY CULTIVATED MEDICINAL PLANTS

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Keywords: Peppermint, Pot Marigold, Ribwort, White Yarrow, Lemon Balm, Sage

In the period of 2003 – 2005 years of solution of the state research and development tasks we aimed at the possibilities of ecological cultivation of selected medicinal plants species: peppermint (*Mentha* x *piperita* L.), pot marigold (*Calendula officinalis* L.), ribwort (*Plantago lanceolata* L.), white yarrow (*Achillea millefolium* L.), lemon balm (*Melissa officinalis* L.), and sage (*Salvia officinalis* L.) in agri-ecological conditions of the testing area Rudnik, district of Myjava (Slovak Republic). In the 2003 – 2005 characteristics and specification of agri-ecological conditions of the testing area were worked out, culls of soil samples and biological

material were realized. Content of potential contaminants in soil samples did not exceed allowed limits for the content of heterogeneous substances in the soil. The yields of fresh above ground phytomass were: *Mentha* x *piperita* L. from 910 to 1 050 g.m^{-2} (e.g. 187 – 238 g.m^{-2} in the air-dried drug), *Calendula officinalis* L. from 253 to 472 g.m^{-2} (51 – 198 g.m^{-2}), *Plantago lanceolata* L. from 1 490 to 1 600 g.m^{-2} (278 – 298 g.m^{-2}), *Achillea millefolium* L. from 1 490 to 1 600 (580 – 630 g.m^{-2}), *Melissa officinalis* L. from 1 355 to 1 823 (271 – 344 g.m^{-2}), *Salvia officinalis* L. from 811 to 1 056 (133 – 192 g.m^{-2}) in correlation with different variants of the experiment.

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VARIABILITY OF PHARMACEUTICALLY ACTIVE SUBSTANCES WITHIN *ACHILLEA COLLINA* BECKER EX. RCHB. VAR. „ALBA"

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Keywords: Achillea collina, essential oil, flavonoids, tannins

Achillea collina Becker ex. Rchb., and especially the "Alba" variety, is the best species of *Achillea millefolium* s.l. complex (yarrow) used in pharmaceutics because of high essential oil yield and the presence of chamazulene in the oil. Not only essential oil is of therapeutic importance – but also flavonoids and tannins enact play a key role in the curative action of yarrow. The two-year study the variability of quantitative contents of these compounds in 35 resp. 44 random sampled plants was the main goal of this work.

The essential oil content in "Alba" variety is very high compared to the other yarrow species (Špinarová, Petříková 2003). The essential oil content was established to amount to 0,54 – 1,77% in constant dry material, which is the value several times higher than the limits set by Czech pharmacopoeia – 0,2% (CL 2002). The yarrow flavonoid content ranged between 2,04 – 3,99% and tannins content was established on the level 0,15 – 1,28%. Statistically significant differences in all these three substances were found between evaluated plants.

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CONTENT AND QUALITY OF ESSENTIAL OIL IN DIFFERENT MINT CULTIVARS

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Keywords: mint, essential oil, menthol, yield

Mint is one of the most important medicinal plant species. It's roll and significance in pharmaceutical, cosmetic and food industry is of great importance. This cosmopolitan plant

species is grown almost in all parts of the world. Demands for this precious medicinal row are growing. Simultaneously, genetic potential and farming measures providing increased yield in certain extent. To meet demands given by certain industries, along with higher yields, advancement in qualitative properties of mint cultivars (higher essential oil and menthol content) is required. To determine possibility of increasing yield of essential oil per area of cultivated plants, three different mint cultivars were tested, Mitcham, and two Moldavian cultivars: N-310 and U-2M. In experiments conducted in Obrenovac, interesting results were recorded. Content of the essential oil in cultivar Mitcham was 1.83%, in cv. U-2M – 2.97%, whilst far the biggest content was registered in cv. N-310, 5.70%. These data, along with those dealing with chemical composition of isolated oils (content of menthol and other important constituents), showed that cultivar N-310 deserves special treatment in the cultivar selection process. Final yield of essential oil, which could be achieved growing this cultivar, is 2-3 times higher in comparison with remaining two mint cultivars.

RESEARCH REGARDING THE ESSENTIAL OILS OF *AGASTACHE* GENUS (*LAMIACEAE*) CULTIVATED IN ROMANIA

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Keywords: essential oils, hydro distillation, phytochemical analysis

The researches were been performed on six species of *Agastache* genus, originally from America, cultivated by SCDL Bacau. The essential oils were obtained by hydro distillation, and analyzed by GC-MS. The main compounds were identified by mass spectra and retention-time correlations. The essential oils of *Agastache mexicana* held as main compounds: pulegone (36.78%), menthone (26.03%) and limonen (23.66%), and that of *Agastache rupestris*: estragol (methyl cavicol) (53.91%), pulegone (21.58%) and menthone (16.25%). The main oil constituents of *Agastache foenicullum* are menthone (36.63%), pulegone (28.31%), estragol (11.97%) and isomenthone (6.47%). Estragol is the main constituents of *Agastache anisata* (63.23%) and *Agastache hybrida* (89.19%). The essential oil extracted from *Agastache cana* held as main compounds: β-felandren (23.9%), β-cubeben (15.16%), limonen (12,57%), γ-terpinen (11.52%), β-pinen (7.49%) and cariofilen (6.75%). Pulegone and menthone are not present in the essential oils of this species. All investigated species have been studied for the first time in Romania from phytochemical point of view.

RESEARCH REGARDING THE CORRELATION BETWEEN TOTAL LIPIDS CONTENT AND BIOSYNTHETIC CAPACITY AT DIFFERENT ALKALOID TYPES STRAINS OF *CLAVICEPS PURPUREA* SCLEROTIA

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Keywords: lipids, alkaloids, Claviceps purpurea, biosynthetic capacity

The literature mentions that lipids, products of primary metabolism, record maximum values before the starting of the alkaloid biosynthesis. More, it is considered that the presence of lipids inclusion is typical of strains capable to produce alkaloids. After the release of alkaloids biosynthesis process the total lipids content is approximately constant.

In this paper we present the results concerning the correlation between total lipids content and biosynthetic capacity expressed by total alkaloid content. We have analyzed some *Claviceps purpurea* sclerotia of ergotamine and ergocristine types. At ergotamine strains the total lipids amount is higher than the ergocristine strains, behavior that is present at the biosynthetic capacity too. We find also the trend that studied strains present alkaloid biosynthesis capacity higher when the total lipids content is smaller.

ALTITUDINAL VARIATION OF SECONDARY METABOLITE PROFILES IN FLOWERING HEADS OF *ARNICA MONTANA*

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Keywords: chemical ecology, UV-B radiation, cultivation of medicinal plants, HPLC analysis, Asteraceae

The effect of intensified UV-B radiation in higher altitudes on secondary metabolites in flowering heads of *Arnica montana* L. was assessed. Plants of *A. montana* cultivar ARBO were grown in nine experimental plots at altitudes between 590 and 2230 m at Mount Patscherkofel near Innsbruck/Austria. Contents of sesquiterpene lactones, flavonoids and caffeoyl quinic acid derivatives were quantified by RP-HPLC. Total amounts of sesquiterpene lactones and flavonoids were not correlated with the altitude of the growing site. In contrast, the proportion of flavonoids with vicinal free hydroxy groups in ring B to flavonoids lacking this feature significantly increased with the elevation of the growing site. The level of caffeic acid derivatives and of 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid in particular also positively correlated with altitude. Samples from the summit region contained 85% more of the latter compound than samples from valley sites. As *ortho*-dihydroxylated flavonoids are more potent radical scavengers (by a factor of 3 to 4 times) than flavonoids without *ortho*-dihydroxy groups, the increase in the relative amount of 3',4'-dihydroxylated flavonoids in *Arnica* in combination with the increase of caffeic acid derivatives is considered to serve a UV-B damage protective function for the plants growing in higher altitude sites.

THE POSSIBILITY OF CAPITALIZATION OF SEA BUCKTHORN LEAVES AND COPSES FOR SEROTONINE AND MICROELEMENTS

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Keywords: seabuckthorn, serotonin, microelements

This paper presents results regarding the content of microelements and infra-microelements determined by the activation of neutrons in the seabuckthorn fruits and copses.

We also analysed the content of Zn, Cu, Mn, Fe, B, Mo, determined by fotometric of atomic absorbtion from fruits and leaves in 11 seabuckthorn bio-types.

The content in the dry substance has been analysed (macro, micro, semimicro and infra-microelements from the ashes of the sea buckthorn). There has been analysed the variation in content of micro-elements and infra-microelements in Serbanesti 1 biotypes, depending of the time of harvesting.

The statistics done on a number of analysed substances and serotonine show their limit in variation.

There has been analysed the variation in content of serotonine depending on the origin of the fruits (three geografic regions).

There have been done some estimations on the spectacular biological effects of seabuckthorn and some ways processing different plant organs and the perspective for serotonine as an imune-inductor with incredible results in different diseases especially if it is associated with the whole complex, „the seabuckthorne" a miraculous plant?

THE CAPITALIZATION OF SOME MEDICINAL SPECIES FROM THE WILD AND CULTIVATED FLORA IN THE FORM OF PROPHYLACTIC AND CURATIVE PHYTOPRODUCTS

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Keyword: the wild and cultivated flora, phytoproducts, nutrients and phytocompounds

The experimental investigations aimed the obtaining of some complex formulas in the form of tea, with a rich content of both nutrients and phytocompounds with curative and prophylactic potential.

We elaborated association formulas and studies from the point of view of their phytochemical compositions and the technological processing, the following vegetal mixtures:

- Sedative composition of medicinal plants rich in *flavonosides, saponins, biogene amins, volatile oils* that act sinergically in the therapy of psycho-neuro-vegetative sindrom with an anti-stress action;
- Tonic-fortifiable composition from medicinal plants with a rich content of *vitamins, oligoelements and flavonosides* with a tonic action on some nervous and annihilation of the free radicals;
- Immuno-stimulating and general tonic composition for the functions of the human body, composition from medicinal plants in *polyholosids, aminoacids, complex of vitamins, oligoelements*;
- Detoxifying composition from medicinal plants rich in flavonosids, saponosids, bitter principles, terpenoidic compounds that assure the elimination of the toxic substances from the human body and its rebalancing;
- Anti-cholitic composition from medicinal plants rich in mucilages, tannins, glycosides, flavonosides which act sinergically in the treatment of digestive affections.

THE CAPITALIZATION OF SOME MEDICINAL AND AROMATIC SPECIES FROM NATURAL MEADOWS OF THE EVERGLADE OF SIRET RIVER

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Keywords: natural meadows, Achilea sp., Mentha sp., Origanum sp., nutritionally and veterinary properties

Many of the active ingredients in chemically manufactured drugs were originally derived from plant compounds. Also, in many developing countries, medicinal plants are still being used on a regular basis. The use of plants and traditional methods for treating animals is called ethnoveterinary medicine. There is a renewed interest, especially in developed countries, in using

plants to treat livestock, pets, and humans. Of the hundreds of plants used in ethnopharmacology, very few have been researched for their efficiency and toxicity. It is difficult to assess the toxicity of the plant when used for medicinal purposes. Some phenolics are poisonous, while others are known to have antioxidant and anticancer properties. Very diverse biological activities are ascribed to saponins/triterpenes and they play important parts in food, animal feedstuffs, and have pharmaceutical properties. The essential oil has antibacterial, antiseptic, antifungal and larvicidal activity.

The vegetal material obtained from *Achilea*, *Mentha* and *Origanum* species, harvested from natural meadows, were chemically analysed. We evaluated, quantitatively and qualitatively, the biosynthesis of polyphenols and flavons, triterpens and phytosterols, essential oils. *Origani herba* contains the greatest quantities of polyphenols and flavons; raised quantities of polyphenols were isolated from *Mentha verticilata*. The identified triterpens and phytosterols were β-sitosterol (in all the samples); stigma-sterol (*Achilea setacea* and *Origanum vulgare*); oleanolic and ursolic acid (all the samples except *Achilea setacea*). The number of volatile fractions is different, the most numerous belonging to the samples of *Mentha verticilata* and *Achilea setacea*.

MORPHOLOGICAL AND MOLECULAR GENETIC APPROACH OF *ELYMUS REPENS* AND ITS RELATED POACEAE SPECIES

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Keywords: biodiversity, molecular genetic, Triticae tribe, red list

Taxonomic classification is a major task of the biodiversity domain which includes with great responsibility economic important plants, as medicinal species are. Classical, morphological methods proved frequently a poor potential for correct differentiation between related taxons; therefore, modern, molecular genetic approachess, such as random amplified polymorphic DNA (RADP) analysis [3] has been recently introduced for completing the peculiar information regarding genetic similarities of plant genomes.

A study case of a very common plant in our country, used as medicinal, *Elymus repens* respectively and its relatives [2], *E. farctus, E. elongatus, Agropyron brandzae* and *E. sabulossus*, is presented as a model for the complex approach in the domain of biodiversity, comprising morphological and molecular taxonomic methods.

Our choice is based on the frequent problems encountered with these taxons of *Triticae tribe* from *Poaceae* (*Gramineae*) family, which come obvious from their numerous synonims. Another motivation of this choice is based on the complex corological and sozological aspects regarding the endemic and endangered position of some of these taxons in the Red List [1].

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A COMPARATIVE STUDY ON THE EFFECT OF NATURAL BIOSTIMULATORS ON SEED GERMINATION OF MEDICINAL, AROMATIC AND HERBAL PLANT SEEDS

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Keywords: natural biostimulators, seed, germination, Echinacea purpurea, Ocimum basilicum, Salvia officinalis, Melissa officinalis

The study was conducted during 2005 in order to analyze the effect of natural biostimulators on seed germination potential of some medicinal, aromatic and herbal plant species. Different concentrations of domestic humates were used as biostimulators of germination.

Germination potential and total germination of *Echinacea purpurea*, *Ocimum basilicum*, *Salvia officinalis*, *Melissa officinalis* were analyzed using ISTA standards. Seeds were treated using humates (peat and brown coal dust-lignite) at rates 0.01% and 0.04%. A 0.02% solution of KNO_3 was used as control. Based on the results obtained it can be concluded that there was a statistically significant increase of seed germination potential of humate treated species in relation to the control. Germination potential was greater using 0.04% compared with the 0.01% humate.

Project TR-6900: "Application of slow disintegrating fertilizers and natural biostimulators in the commercial production of flowers, medicinal, aromatic and seasoning herbs seedlings". The realization of the project was financed by the Ministry of Science and Enviroment of the Republic of Serbia.

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STUDIES OF GERMINATIVE ENERGY AND FACULTY OF SOME SPECIES OF ECHINACEA (*ECHINACEA PALLIDA* NUTT. AND *ECHINACEA PURPUREA* L. MOENCH), DEPENDING ON SEEDS AGE

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Keywords: Echinacea pallida,Echinacea purpurea, seed, germination

Medicinal vegetal products of *Echinacea* radix and herba, are to be found with those having immunostimulating and antiviral effects, by action mode and, the active principles contained are phenyl-propionic derivates (echinacosid) and, imunostimulatory polysaccharides.

Variants under study with both species were : V1= 2 months of age (November 1997) – control ; V2= 4 months of age (January 1998); V3= 7 months of age (April 1998); V4= 8 months of age (May 1998);V5= 17 months of age (February 1998); V6= 21 months of age (June 2000); V7= 36 months of age (September 2000). Best germination was established on two germination layers (TP or BP); and at two different temperature (20 and 25.5° C).

Germinating energy and faculty at *Echinacea pallida* Nutt. increase with seeds ageing (after eight months), and decrease after seventeen months from harvest, both on TP and BP layer with

both temperatures, and loose germination almost completely within three years from harvest. Results on germinating faculty at *Echinacea purpurea* (L) Moench, depending on seed age, two germination layers (TP and BP) and two temperatures (20 and 25.5° C) reveals that germinating faculty has the highest values at two months after harvest, leading thus to the hypothesis that such species have a seminal pause shorter than two months.

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RESEARCH REGARDING *ECHINACEA PURPUREA* MOENCH (L.) SPECIES SEEDLINGS, BIOLOGY AND OPTIMAL NUTRITION SPACE

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Keywords: Echinacea purpurea, nutrition space, density, biology

The widest therapeutically utilization is in increasing non-specific resistance of the human body and in inhibiting viruses action.

The variants taken into consideration were: V1=planted at 50 cm (between row) x 20 cm (between plants on row), density 100 000 plants/ha (control);V2=planted at 50 x 30 cm, density 70 000 plants/ha; V3 =planted at 50 x 40 cm, density 50 000 plants/ha; V4 =planted at 50 x 50 cm, density 40 000 plants/ha; V5 =planted at 70 x 20 cm, density 70 000 plants/ha; V6 =planted at 70 x 30 cm, density 50 000 plants/ha; V7 =planted at 70 x 40 cm, density 40 000 plants/ha; V8 =planted at 70 x 50 cm, density 30 000 plants/ha.

In Cluj-Napoca climatic conditions, the average of the third experimental years (2001-2003) shows us that the vegetation period at *Echinacea purpurea* (L.) Moench specie was of 174 days. The phenophases of vegetation lasted, in average: start of vegetation-budding 40 days (23%), budding-flowering 36 days (21%) and fowering-seed ripening 98 days (56%).

We can appreciate that the highest yield of fresh matter at *Echinacea purpurea* (L.) Moench specie was registered at small distance planting, which implies high density on surface unit. Thus the best results were obtained for the distance of 50 x 30 cm (70.000pl/ha – 462 q/ha) for the herba yield; 50 x 30 cm (70.000 pl/ha – 115 q/ha) for the radix yield.

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BASIL SEED YIELD AND QUALITY

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Keywords: basil, seed yield, seed quality, greasy oil content

In Serbia until now basil was mostly used as a herb (*Basilici herba*) and for its etheric oil (*Basilici aetheroleum*). Investigations carried out so far were mostly focused on studying morphological and agronomic properties and chemical content of etheric oil (3). Basil seeds contain a certain amount of greasy oil (*Basilici oleum*) whose yield and quality is very similar to the quality of flax seed (1, 2).

The aim of the study was to assess the yield and quality of domestic basil seed. The investigation was conducted in 2004 and ten domestic basil populations were used. Seed yield and seed quality parameters (1000-seed weight, germination energy and total seed germination) were analyzed including greasy oil content (4). Based on the results obtained seed yield ranged from 312 kg/ha (population T-8) to 1300 kg/ha (population T-7). Seed quality of the basil populations studied was favourable. The content of greasy oil in the seed ranged from 12.48% (population T-9) to 25.29% (population T-2).

The conclusion which tends to emerge is the high seed yield and greasy oil content of domestic basil populations.

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SOME CONSIDERATIONS REGARDING THE *IN VITRO* BEHAVIOUR OF *LAVANDULA ANGUSTIFOLIA* L. SPECIES

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Keywords: lavander, callogenesis, caulogenesis, micropropagation

The biological material used to initiate the *in vitro* culture of *Lavandula angustifolia* L. was brought from Chalkidiki (Greece). Culture initiation took place in June 2003. We used shoot tips sterilised in chloramine-T (5%) and inoculated them on Murashige-Skoog (1962) medium – hormonefree or supplemented with 0.2-0.5 mg/l BAP.

The main reaction of explants (shoot tips, nodes, leaves) on varied hormonic formuli of MS medium was callogenesis. Nodes and shoot tips provided neoplantlets only on hormonefree MS and sporadically on MS with B_{02} (0.2 mg/l), A_2 (2 mg/l IAA) and KN_1 (1 mg/l kinetine and 0.5 mg/l NAA). A_2 hormonic formula induced a friable cream callus, high proliferative in light and also in darkness. Subcultivated on BG_1 (1 mg/l BAP and 0.5 mg/l GA), this callus displayed a lower proliferation on IB_2 (2 mg/l IBA) than on A_2, but on KN_1 and N_2 (2 mg/l NAA) it turned foamy and maintained high proliferative for 2 years. We ran biomass evaluations by growing it

on specific hormonic formuli in a certain period of time. Submitted to light, the highest biomass growth in 14 days was on BG_1 (14.35g) and the lowest on hormonefree MS (9.44g). In the absence of light the highest biomass was on BG_1 formula (14.99 g) and the lowest on B_{05} (0.5 mg/l BAP). Repeated subcultivation of this callus on B_{02} in light conditions improved its consistency and led to green isles of cells that provided many multiple shoots. Indirect caulogenesis for this type of callus was kept even after 2 years of transfer on MS. The shoots were resistant to root formation. Lavander neoplantlets obtained on MS and other hormonic formuli were easily accommodated in a hydroponic system then transfered to field.

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IN VITRO MICROPROPAGATION OF *HYPERICUM PERFORATUM L.* II. SAINT JOHN'S WORT CLONE SELECTION AND REGENERATION

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Keywords: Hypericum perforatum, callogenesis, caullogenesis, micropropagation

In this experiment, we focused on the micropropagation of *H. perforatum* plants selected in an experimental field in Piatra-Neamt. Plantlets were obtained by germination in aseptic conditions on agar medium and sterile segments represented the explants used. In order to initiate and cultivate callus tissues we used a 1:2 and 1:1 ratio exogenous auxins and cytokinines, respectively. MR basic medium was used which contained macro- and microelements, (Linsmaier and Skoog, 1965), 20g/l glucose, 100 mg/l meso-inositol, 2 mg/l glycine, and 7.5% agar.

Caulogenesis and rhizogenesis were carried out by placing the callus on agar-based MS and MR media variants suplimented with cytokinines (kinetin, 6-benzylaminopurine) and auxin (IAA, IBA and 2.4-D). The number of shoots obtained on the KB-medium inoculated callus within two weeks ranged between 79 – 172, whereas in 35 flasks an average of 136.10 regenerated shoots was obtained. A better caulogenesis was obtained when the callus was inoculated on MR medium where the number of regenerated shoots ranged between 145 – 581; the statistical analysis revealed an average of 330.70 shoots in 35 flasks. Regenerated shoots developed in significantly different ways on the rhizogenic media used to induce roots formation. Shoots on MS hormone free medium revealed the best vigour, although they needed a longer time (5 to 6 weeks), as this medium is richer in inorganic substances that the MR and MRI ones.

Plant accommodation was obtained in pots on sterile soil and in hydroponic system; in both cases plants were covered. After having restored the hydric balance of plants – which took 10 to 14 days – the plants were maintained in the hydroponic system. The adjustment efficiency of hydroponic cultures was 89.4%. A number of 57 fully adjusted plants were transferred to the experimental field with a view to monitoring active substance content.

THE *IN VITRO* MORPHOGENETIC REACTION OF *ECHINACEA PURPUREA* MOENCH

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Keywords: Echinacea purpurea, explants, callus, neoplantlets

Plantlets and cotyledons from germinated seeds were cultivated *in vitro* on media of initiation (MS, B_{02}, B_2) providing neoplantlets which represented the source of sterile explants – mainly shoot tips and nodes, but also leaf, internode, petiole and callus fragments. The best reaction obtained from shoot tips and nodes was on BN medium – multiple shooting with the most vigorous shoots and roots.

The leaf explants generally provided callus, but on media supplemented with auxins (IAA) very intense root genesis was induced. Nourishing media with BAP and IAA leaf fragments displayed the best caulogenetic reaction. The petiole explants were sporadically root-inducing.

We also tested the organogenetic capacity of the friable foamy callus that proved to be caulogenetic when it was transfered on B_2 hormonic formula (with cytokinins).

This species registered a low growth speed and development compared to other species that we have tested. We encountered some difficulties to accommodate the neoplantlets to septic environment in a hydroponic system.

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IN VITRO CULTIVATION OF HIPPOPHAE RHAMNOIDES

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Keywords: Hippophae rhamnoides, micropropagation, clone, caulogenesis

In vitro cultivation of *Hippophae rhamnoides* is an unconventional alternative to conserve and perpetuate an important resource for food and health.

Clonal micropropagation through adventitious and axillary shoots has begun to have impact on plants improvement.

It were cultivated *Hippophae rhamnoides* and tested their proliferative and regenerative capacity, depended on explants origin, type and concentration of the growth regulators from the culture media.

It was obtained viable plantlets from adventitious meristematic tissue induced by the action of a cytokinin on the cotyledons of mature *Hippophae rhamnoides* zygotic embryos.

The micro-shoots achieved by excising the terminal portion of an individual shoot grown on the inductive medium and formed normal shoots, while the basal portion formed axillary shoots. The axillary shoots developed into normal shoots when excised and transferred to fresh medium. The MS medium supplemented with 2mg/l BAP and 0,2 mg/l IAA stimulated shoot differentiation.

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ESTABLISHMENT AND MAINTENANCE OF CALLUS OF *STEVIA REBAUDIANA* UNDER ASEPTIC ENVIRONMENT

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The callus culture of leaves of *Stevia rebaudiana* was initiated and maintained on different strengths of Murashiage and Skoog (MS) medium supplemented with various phytohormones. Kinetin in combination with Naphthalene Acetic Acid (NAA) and 2,4-D showed better results in callus initiation whereas combined application of Benzyl adenine or 6-benzyl amino purine (BAP) and NAA showed most satisfactory results of callus maintenance in half strength of MS medium.

DIGITALIS PURPUREA IN VITRO CELL CULTURES FOR BIOTRANSFORMATION OF EXOGEN HYDROQUINONE INTO ARBUTOSID (I)

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Keywords: hydroquinone, arbutin, Digitalis purpurea (L.), in vitro cell suspensions

Digitalis purpurea (L.) or foxglove, is widely known for it's cardiac glycosides with a significant role in therapeutics. Cells of this plant hold a nonspecific enzyme called glycosiltransferase capable of biotransforming hydroquinone (a toxic compound) into arbutosid (with disinfecting and depigmentation properties). For a better understanding of how does this process naturally occur, we initiated *in vitro* cell suspensions of *Digitalis purpurea* (L.). The first step in preparing our dissociated cell suspension was to initiate the calus culture from different *Digitalis purpurea* explants cultured under sterile conditions on solidified media (Murashige-Skoog: 1mg/l 2,4-D+2mg/l BA).

Plant cell suspensions were induced from callus fragments grown in shaked liquid media (Murashige-Skoog: 0,2 g/l glutamin, 0,2 g/l cazein, 1 mg/l 2,4-D and 2 mg/l BA). These cell cultures were preserved in Erlenmeyer flasks of 200-400 ml, on a rotary shacker (100 rpm). We performed researches regarding plant cells at different stages in their growth curve. Furthermore we determined the optimum volume of inocula for an optimum growth of plant cell suspensions (2ml/40ml liquid media) as well as the required time period between subcultivations (14 days).

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USE OF JASMONATES TO INDUCE TERPENES IN *PICEA ABIES* TREES AND CELL CULTURES

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*Keywords: C*ell culture, *elicitor, monoterpene, Norway spruce*

Treatment of plants with jasmonates often shows that jasmonates seem to have a high potential in increasing level of biologically active compounds in plants. In this study, was employed methyl jasmonate (MJ) to increase levels of terpenes in intact trees and cell cultures of Norway spruce. Application of MJ caused a two to three-fold increase in mono-, sesqui-, and diterpenes in *Picea abies* sapwood, within a month following treatment. Although previous research have not shown terpenes in undifferentiated cell cultures, we found that *P. abies* cell suspension cultures constitutively biosynthesized small amounts of various monoterpene hydrocarbons *de novo* with a product profile similar to that of adult trees. Moreover, following application of MJ or a fungal elicitor, there was a three-fold increase in monoterpene accumulation, but no accumulation of sesquiterpenes or diterpenes was observed. The *in vitro* assays suggested that only monoterpene synthases were induced, not sesquiterpene or diterpene synthases. These results confirm that the cell suspension cultures might be a usefull system in producing high-value secondary metabolites in *P. abies*.

MANIPULATION OF BIOMASS AND BIOSYNTHETIC POTENTIAL OF *MORUS NIGRA* AND *GLYCYRHIZA GLABRA* TISSUE CULTURE BY *AGROBACTERIUM RHISOGENES* MEDIATED GENETIC TRANSFORMATION

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Keywords: rhisosphere, genetic transformation, biomass, biosynthetic potential, manipulation

Morus nigra and *Glycyrhiza glabra* are very known medicinal plants used for their active principles deoxynojirimycine and glycyrrhizic acid, respectively [1]. Most of the secondary metabolites with pharmaceutical importance are synthetized by the medicinal plants in their roots. Rhisosphere proved also numerous advantages for the stability of plant tissue culture, as compared to cell culture or other plant organ culture [2]. Therefore, much attention has been focussed recently in the domain of biotechnology for the manipulation of this type of biomass and, through it, the biosynthetic potential of active principles. *In vitro* tissue culture and root inducing (rol) genes transfer [3], as well as molecular genetic and biochemical (HPLC) analyses are described for the presentation of the *Agrobacterium rhisogenes* mediated genetic transformation system in *Morus nigra* and *Glycyrhiza glabra* species.

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COMPARATIVE PHYTOCHEMICAL STUDIES ON *IN VITRO* MULTIPLIED GENOTYPES OF *ORIGANUM*

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Keyword: Origanum vulgare genotypes, in vitro and conventional cultures, bioproductivity

Among the aromatic and medicinal plants studied all over the world, from the point of view of *in vitro* cultures, there is the group of the *Labiatae*. In case of the plants producing volatile oils, the cloning, through tissue cultures of the individuals with stable characters, is very important. This is due to the fact that its heritability degree is raised and the micropropagation creates the premise of reducing the amplitude of the variability. Using the regeneration techniques of the tissue cultures of *Origanum vulgare* L. aimed the micropropagation of valuable genotypes with well-defined biochemical characteristics.

For all the tested genotypes (4), we performed shoot regeneration by multiplying apical and axillary buds; shoot rooting, plantlet accommodation and the transfer into the experimental field did not raise special problems and we registered a 90% survival ratio. In 20-22 weeks one can obtain a convenient number of plantlets, the micropropagation technique being a multiplication alternative for the selected genotypes, androsterile and androfertile, in order to establish hybridation fields and a rapid promotion in the superior links of the melioration process.

The comparative studies, performed on *herba* from *in vitro* and conventional cultures, were achieved to identify the polyphenolic, flavonoidic, triterpenic components, as well as the volatile oil. The data obtained by phytochemical analysis assert that, by means of the micropropagation techniques used in the experiment, the bioproductivity features are preserved, the phenotypic expression reflecting the characteristics of the genotype.

RESEARCH ON TROPANE ALKALOID BIOSYNTHESIS IN *SCOPOLIA CARNIOLICA* JACQ. ADVENTITIOUS ROOT CULTURES

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Keywords: Scopolia carniolica Jacq., adventitious root cultures, atropine, scopolamine, elicitors

Adventitious root cultures of *Scopolia carniolica* Jacq., established from rhizogen callus, were analyzed for atropine and scopolamine biosynthesis. All cultures were maintained on B5 liquid medium containing 3% succrose. Four types of root cultures, selected by length and biomass development were maintained in medium containing 2.0 mg l^{-1} IBA, in the dark. The influence

of elicitors on tropane alkaloid biosynthesis was also studied. Root cultures induced in media containing 1mM putrescine or tropic acid were analyzed after 24, 48 and 72 hours contact with the elicitor-salicylic acid. The elicitor was used in two concentrations: 0.1 mM and 1.0 mM. The analyses were performed by HPLC-MS coupling system, using RP technique for separation and APCI mode for detection.

The four types of root cultures showed slight differences, but for all four it was detected an increasing amount of scopolamine in time, parallel with a slight decrease in atropine amount. The elicitor influence was considered optimal for 0.1 mM concentration, after 48 hours of contact. The salicylic acid in 1.0 mM concentration acted as inhibitor, in some cases the alkaloid amount being under detection limit. The presence of putrescine in culture media improved the alkaloid biosynthesis more than the tropic acid one.

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HISTO-ANATOMICAL INVESTIGATIONS ON *LAVANDULA ANGUSTIFOLIA* L CULTIVATED *IN VITRO*

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Keywords: in vitro, histo-anatomical, micropropagation, Lavandula angustifolia

Nodal explants of *Lavandula angustifolia* were cultured on Murashige and Skoog (MS) media supplemented with various concentrations of naphtyl acetic acid (NAA), benzylamino purine (BAP) and kinetine to induce adventitious shoots regeneration and micropropagation. Structural features of the callus, the presence or absence of meristematic and vascular centers was underlined for 12 culture variants. The influence of alternance of light and dark upon the callus growth was investigated. On some culture variants shoots were regenerated via callus; another one remained with undifferentiated cells, even after one year of culture.

HISTO-ANATOMICAL RESEARCHES REGARDING THE INFLUENCE OF TOPSIN M TREATMENTS ON *MENTHA LONGIFOLIA* (L.) HUDS. SPECIES. NOTE I.

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Keywords: Mentha longifolia, Topsin M, anatomy (stem, leaf)

The abusive and disordered use of pesticides have the potential to harm human health and the environment and pose ecological risk to ecosystems.

Topsin M is a common systemic fungicide used as protective/curative substance for alimentary and medicinal plants. That's why it's relevant to evaluate the influence of Topsin M on the anatomical features of *Mentha longifolia*, a volatile oil producing plant and parental species for

the hybrid *Mentha × piperita*. We analysed the anatomical features using superficial and cross sections through the vegetative organs (stems and leaves) belonging to treated and untreated plants. The epidermal features of the leaves were determined by electronic microscopy using S.E.M. method. Therefore, we established some data items of the stem and the leaves.

We observed, under antifungal treatment, more quantitative changes than qualitative changes, being modified especially the conductive and assimilatory tissues.

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MORPHOLOGICAL RESEARCHES REGARDING THE INFLUENCE OF TOPSIN M TREATMENTS ON *MENTHA LONGIFOLIA* (L.) HUDS. SPECIES. NOTE II.

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Keywords: Mentha longifolia, Topsin M, morphological features

The abusive and disordered use of pesticides has the potential to harm human health and the environment and pose ecological risk to ecosystems.

Topsin M is a common systemic fungicide used as protective/curative substance for alimentary and medicinal plants. That's why it's relevant to evaluate the influence of Topsin M on the morphological features of *Mentha longifolia*, a volatile oil producing plant and parental species for the hybrid *Mentha × piperita*. The morphological study was achieved through the analysis of parameters concerning aspect, colour and dimensions of the leaves (officinal product). The dimensional parameters data were statistically analysed through the *Oneway Anova* method using J.M.P. Programme 5.0.1.2. (SAS Institute, Cary N.C., S.U.A).

We noticed statistical significant variations (*Oneway Anova*) of the dimensional features of the leaves from Topsin M treated plants comparing to the control; therefore, we observed statistical significant variations (*Oneway Anova*) of the stomata length.

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QUALITATIVE-QUANTITATIVE CHARACTERISTICS OF PEPPERMINT OIL FROM DIFFERENT LOCALITY OF THIS PLANT CULTIVATION

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Keywords: Peppermint, essential oil, composition, the East Slovakia

Peppermint (*Mentha x piperita* L.) has been one of the oldest and traditionally used medicinal and culinary herbs until present. Peppermint herbal tea is very popular not only for its taste and

freshness but it is also valued for its phytotherapeutic effects. Peppermint teas can be used against nervousness, insomnia, cramps, cough, migraine, poor digestion, heartburn, nausea, abdominal pains, and various problems such as headache and vomiting due to nervous causes. Peppermint essential oil is chemically various mixture of substances, mostly monoterpenes which quality its strong aroma. Its main component is mentol. This contribution presents the results of essential oil content and its quality which are isolated from the Peppermint stands cultivated on different localities in the Estern Slovakia.

In regard to the obtained results the essential oil content was very high (from 1.80 to 2.30 %) in peppermint drug from cultivation in our conditions. A very interesting chemotype of peppermint with very high menthol was cultivated on the Presov University fields in Presov and in Caklov. The essential oil contains more than 72 % of menthol, 4 % of menthylacetate, from 2 to 16 % of menthone, from 1.3 do 4.2 % of iso-menthone. Regarding to comparison of essential oil quantity in different high of plants its biggest quantity was in 0.3 m of high plants. The biggest quantity of leaves and stem biomass were concentrated here. The menthol content into essential oil was going down from a lower plant parts to higher.

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THE ESSENTIAL OILS OF *NEPETA* L. GENUS (*LAMIACEAE, NEPETOIDEAE*) IN ROMANIA

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Keywords: Nepeta, Lamiaceae, essential oil, GT-FT systems, nepetalactone

The essential oils of four species of *Nepeta* L. genus have been examined. The oils of *N. cataria* L. (Sect. *Nepeta*) and *N. nuda* L. ssp. *nuda* (Sect. *Orthonepeta*) are characterized by the presence of nepetalactone isomers. The other two species represented by *N. parviflora* M. Bieb. and *N. ucranica* L. ssp. *ucranica* (Sect. *Oxynepeta*) have a rare status within The Vascular Plant Red List for Romania [3], [4]. Although *N. parviflora* has been studied in the literature, [1], [2], no previous data exist for *N. ucranica* ssp. *ucranica*. Is one of the objectives of this study to report the composition of *N. ucranica*. Composition of the essential oils of *Nepeta* species were obtained by hydro-distillation using neo-Clevenger extractor and investigated by capillary GC-FT systems. The main oil constituents of *N. cataria* and *N. nuda* are nepetalactone isomers, eucalyptol, β-caryophylene, α- and β-pinen, in different amounts. Germacrene-D (40,5%) is the main constituent of *N. parviflora* and the eucalyptol (65,8%) is the major compound of *N. ucranica* ssp. *ucranica* oil. Nepetalactone isomers are not present in the essential oils of the latest two species belonging to Sect. *Oxynepeta*. All examined species excepting *N. cataria* have been studied for the first time in Romania from phytochemical point of view.

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GERMAN CHAMOMILE (*CHAMOMILLA RECUTITA* L.), A SUITABLE CROP FOR CONVERSION PERIOD

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Keywords: Chamomile, conversion period, organic agriculture

In organic production conversion period is the most difficult time for producers. To overcome that period with minimum loss of income it is very important to choose appropriate crop. In this experiment 9 producers are chosen and they start with organic production on 11 ha. After two years of production it was obvious that chamomile is excellent crop for conversion period because does not need protection from pests and disease and does not need mineral fertilizers. Yield was between 1600 and 2500 kg/ha of pure chamomile flowers and between 1500 and 2200 kg/ha flowers with petals. After drying producers put on the market chamomile like conventional but anyway they earn more than with some cereal crops. This experiment shows that chamomile is for sure suitable crop for conversion period.

QUALITY EVALUATION OF CHAMOMILE *(MATRICARIA RECUTITA L.)* DEPENDENCE ON CULTIVATION TECHNOLOGY

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Keywords: organic agriculture, Matricaria recutita L., essential oils

Chamomile *(Matricaria recutita L.)* belongs to the oldest exploited drugs, which are planted and harvested in the Czech Republic. In relation to the possibility of exploitation this medicinal plant in organic agriculture, the varieties (Czech diploid variety Bohemia and Slovak tetraploid variety Goral.) are tested for quality and quantity characteristics with impact on the amount of essential oils of chamazulen and flavonoids for growing in organic agriculture. We compare accurate field experiments in conditions of both the traditional and the organic agricultures. As to the Czech Pharmacopoeia 2002 – a flower drug should contain at least 4 ml/1 kg drug of blue coloured essential oils. This standard is valid both for the traditional and the ecological cultivation of Chamomile.

In the experiments of both the ecological and the traditional cultivations - the content of essential oils was higher than the standard content of essential oils as stated in The Czech Pharmacopoeia. The highest measured value of the essential oils content was 8.25 ml/kg in the variety Goral at the 1st harvest in organic agriculture. The highest measured value of main substances (chamazulene, α-bisabolol, bisabololoxide A) of chamomile essential oils was also in the variety Goral at the 1st harvest in organic agriculture. When comparing the average content of essential oils in both varieties, the higher measured value of the content of essential oils and main substances in essentail oils were present in the tetraploid variety Goral. On the other hand also

the diploid variety Bohemia in the ecological part of experiments showed a permanent high content of essential oils. We can presume, that for the organic cultivation technique both varieties are suitable.

This problematic is solved within grant agencies Grant Agency of Czech University of Agriculture (CIGA): 213133, FRVŠ 2387/G4 (Grant Agency Ministry of Education Czech Republic).

COMPARATIVE INVESTIGATION OF ORGANIC CHAMOMILE [*C. RECUTITA* (L.) RAUSCH] PRODUCTION IN DIFFERENT AGRO-ECOLOGICAL REGIONS OF GREECE AND SERBIA

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Keywords: Chamomile, agro-ecological regions, organic fertilization

The influence of different agro-ecological factors on yield of the Serbian chamomile (*C. recutita* (L.) Rausch) cv "Banatska", and essential oil yield and quality was monitored throughout field experiments conducted in Serbia and Greece. The organic model of cultivation, in both countries, was applied. During fall 2004 and spring 2005 vegetation period, one experimental trial in Serbia, South Banat (Pančevo) and 4 experimental cultivations in Greece, two in Macedonia (Thermi and Kalindria), one in Thessaly (Stefanovikio) and one in Sterea Hellas (Ag. Konstatinos), were conducted. Sowing in Serbia was conducted on 05.10. 2004., at rate 10 kg/ha and the harvest took place 4.4.2005. Sowing in Greece was conducted during the period 1-7.11.2004, at sowing rate 6 kg/ha and harvest took place on 1-15.5.2005. Average yield of dry chamomile flowers obtained in Serbia was 778 kg/ha (ranging 725 – 931 kg/ha) and in Greece from 4 localities was 767 kg/ha (ranging 615 – 1027 kg/ha). The average content of essential oil in dry chamomile flowers cultivated in Serbia was 0,34 % (ranging 0,27 – 0,41%) and in Greece was 0,44 % (ranging 0,32 – 0,65%). The results showed that between two tested agro-ecological regions (Serbia and Greece), there are no significant differences regarding the obtained yield of dry chamomile flowers but the content of essential oil appeared to be significantly higher in chamomile cultivated in Greece.

SLOVAK VARIETY OF MAJORAN *„MARCELKA”*, ITS CULTIVATIONS AND ESENTIAL OIL CHARACTERISTICS

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Keywords: Sweet Marjoram, essential oil and its composition

Sweet Marjoram (*Majorana hortensis* L.) is grown for its strong aromatic drug. It is a mixture of dry leaves and herbal stems with a typical strong aroma. It comes either from North-western Afrika or an Eastern Mediterranean. Ancient Egyptians, Greeks and Romans cultivated it. The aim of our survey is the study of essential oil content, its quantity and quality and the aspect of Sweet Marjoram cultivation in the selected area of the Eastern lowland.

There is cultivated only one allowed variety called “*MARCELKA*” in Slovakia. The locality chosen for cultivation of this variety is called Streda nad Bodrogom. There is a soft ground and

rich in humus with neutral pH. The crops cultivated before Marjoram were potatoes. The planting of seedlings started at the beginning of May. Harvest of seeds began when the plant became getting dry and its lower third turned brown. General principles were applied with collection and drying of the aromatic herbs.

The content of essential oil into the dry herbs is 1.2%. A wide range of secondary metabolites (terpinen-4ol: 6%, α-terpinene: 28%, γ-terpinene: 16%, sabinene: 2%, limonene: 6%, cineole: 7% a linalool: 2%) presents its composition. They are giving to plants very nice and strong fragrance.

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METHODS USED FOR CARAWAY (*CARUM CARVI* L.) BREEDING IN THE CZECH REPUBLIC

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Keywords: caraway, Carum carvi L., breeding, dihaploids

Agritec Company is interested in breeding traditional biennial caraway and caraway with reduced vegetative period (winter caraway) for ten years. The breeding is focused on the stability of yield, good health state, the essential oil content and the non-shattering maintenance. The positive and negative selection and forced self-pollination are used for breeding biennial caraway. The genotypes with better quantitative characters then the current varieties were obtained by recurrent phenotypic selection. The formation of dihaploid plants is new method of caraway breeding. This process accelerates the creation of the homozygous and homogenous genotype. This method was not described for caraway yet. The procedure is finished and prepared for rutine usage now. The best results provided the dihaploid plants of winter form. The form with reduced vegetative period is bred for non-shattering maintenance, high essentials oil content and for antifreezing. When we obtain the homozygous and homogenous lines of caraway, it will be possible to think of breeding of new caraway varieties (line or hybride type). This work is supported by project of National Agency for Agricultural Research (Ministry of Agriculture of the Czech Rep.) QF 4056 “Utilization of current caraway varieties (*Carum carvi* L.) and new methods of its breeding for improvement of its qualitative and quantitative parameters”.

CULTIVATION DIFFERENCES BETWEEN POT MARINGOLD (*CALENDULA OFFICINALIS L.)* VARIETES

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Keywords: Pot Marigold, variety "PLAMEN PLUS", β-carotene, top cross, flower drug

Pot Marigold *(Calendula officinalis)* is an annual plant. It likes hot warm and dry climate with the lover moisture. It can be cultivated almost everywhere in Slovakian agricultural conditions. In the last five years its cultivation, collection and the sale of dry flowers became favorite and profitable activity in the Estern parts of Slovakia.

The variety *„PLAMEN"* is allowed and recommended variety of *Calendula officinalis*. In the last few years *Calendula officinalis* is dedicated not only to seedgrowing but also tried to put into effect a variety called *„PLAMEN PLUS"* in the Eastern Slovakia. The new *„PLAMEN PLUS"* variety was bred on plenty larger flower, the advantageof which is bigger weight.

According to this survey we can state that the Pot Marigold flower drug coming from different varieties has diferent weight of fresh and dry flowers furthermore it biosynthetises diferent amount of β-carotene and a percentage of phytotherapeutic explotiable substances. In comparism of 5 groupings containing 10 flowers we can say that they are heavier under fresh and dry conditions at „*PLAMEN PLUS*" variety which was not affected by higher proportion shrinkage near dry raw material. As to the quality, higher content of effective substances – carotenoides, the content of β-carotene is 0,090% at "*PLAMEN PLUS*" variety. With the second variety it is 0,030% more (0,120%).

In conclusion, we can say that the main differences between the production of flower heads and the content of active components between two Pot Marigold varieties were confirmed. We highly recommend choosing variety "*PLAMEN PLUS*" for production and collection. Harvest of dry flower heads can be 30% higher and the content of β-carotene can increase in 50%.

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RESEARCHES REGARDING THE CULTURE TECHNOLOGIES OF *WITHANIA SOMNIFERA* DUN. (SEA BUCKTHORN)

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Keywords: Withania somnifera, cultivation technologies

To obtain the main technological elements of *Withania somnifera* species, at SCAD Secuieni we initiated a series of experiments which observed the influence of the optimum epoch, of the nutrition space and of fertilization on the growth and plant development and on the *Withania somnifera* yield.

We noticed that:

- *Withania somnifera* is a plant sensible to warmth and highly needs humidity especially in the first vegetation stage;
- the *Withania somnifera* species may be sown between April, 20 – May, 10 only that in the dry and warmth springs it should be sown in the first part of the period so as the plants to

beneficiate of the humidity accumulated in winter and the beginning of spring fact that will assure a rapid start and a good further development. In the years with humid and cold springs the species will be sown at the end of the period because of the necessary thermic state;

- it does not need fertile soils; using organic manner will determine a reduction of the dry weight compared with the non-fertilized control;
- being a medium sized plant it prefers a greater nutrition space (50/50 cm or 70/25 cm) that will provide a good branching and necessary luminosity.

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INFLUENCE OF SOME HEAVY METAL CONTENT ON EXPLOITATION OF *HYPERICUM PERFORATUM* L.

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Keywords: Hypericum p erforatum, heavy metals, soil, leaves, recomendation.

For collecting and exploitating native medicinal and aromatic plants (MAP) ecological condition of the site are limited factors. Among them, the high content of haevy metals in the soil and serpentine parent rock are hazardous for this aim.

The results of analyses of heavy metals contents in soil and in leaves of *Hypericum perforatum* are presented. The field investigations were carried out in three localities in central and west Serbia, on serpentine parent rock.

Laboratory analyses of zinc (Zn), iron (Fe), lead (Pb), copper (Cu), nickel (Ni), chromium (Cr) and manganese (Mn) in the soil, shows that the content of Fe, Ni and Mn are above the allowed concentrations, and the other elements are below the critical limits.

In the leaves of *Hypericum perfotaum* contents of Cu and Cr are a little above the critical concentrations. Ni content is significantly above the critical concentration, and the other elements are within the critical limits.

Although *Hypericum perforatum* can be characterized as havy metal accumulator (Radanović & all. 2005), it cant be recommended to exploit St. John' s wort on these sites.

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EFFECT OF BIOFERTILIZATION ON THE INFLORESCENCES YIELD AND THE CONTENT OF CHLOROPHYLL AND CAROTENOID PIGMENTS IN MARIGOLD (*CALENDULA OFFICINALIS* L.)

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Keywords: biofertilization, marigold, chlorophyll, carotenoid pigments

Biofertilization is a technological method recommended in ecological agriculture, especially in medicinal plants culture. The experiments organized in field studied the effect of the microbiological compounds Extrasol and Azotofertil applied in soil and the Bionat, foliar fertilizer with natural extract of plants, on marigold culture. The dynamics of the inflorescences yield was determinated monthly, during vegetation. The content of chlorophyll and carotenoid pigments was analyzed in leaves and inflorescences spectrophotometrically, by the capacity to absorb light radiation in visible spectrum. The results reveal the stimulator effect of the biological compounds on the inflorescences yield, the most significant being in Azotofertil compound in July. The biological fertilization increases photosynthetic efficiency in leaves and the content of carotenoid pigments in inflorescences. These pigments are some of the most important bioactive compounds in marigold drug.

INTERACTION EFFECT BETWEEN PHOSPHORUS AND ZINC ON THEIR AVAILABILITY IN SOIL IN RELATION TO THEIR CONTENTS IN *STEVIA REBAUDIANA* GROWN IN INDIAN SUBTROPICS

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Keywords: Stevia rebaudiana, phosphorus, zinc

A greenhouse experiment was conducted at the Indian Institute of Horticultural Research (IIHR), Bangalore to study the interaction effect between phosphorus and zinc on their availability in soil in relation to their contents in stevia (*Stevia rebaudiana*). The results show that the amount of available P and Zn content in soil has been found to increase initially and, thereafter, the amount of the same decreased with the progress of plant growth up to 60 days irrespective of treatments. The amount of P and Zn in soils showed an increase with their separate applications either as soil or foliar spray while that of the same value significantly decreased both in soils and plants due to their combined applications, suggesting a mutual antagonistic effect between Zn and P affecting each other's availability in soil and content in the stevia plant.

INFLUENCE OF FYM FERTILIZATION ON CHAMOMILE [*C. RECUTITA* (L.) RAUSCH] AND PEPERMINT [*M. PIPERITA* (L.)] YIELDS AND QUALITY

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Keywords: chamomile, peppermint, FYM, organic fertilization

In one-year filed experiments conducted on chernozem in South Banat, comparative investigation of the efficacy of farmyard manure (FYM) and NPK application, in comparison to control treatment (no fertilization), on yield and quality of chamomile and peppermint was investigated. *Chamomile*: Application of FYM increased the obtained yield of flowers for 80 % in comparison to control. The obtained yield of flowers was 778 kg/ha with average essential oil content of 0.34 %. NPK fertilization increased yield of flowers for 34,8 % in comparison to control, with the lowest content of essential oil being 0.25 %. In control treatment the yields of flowers was 435 kg/ha, while the content of essential oils in flowers was 0.30 %. *Peppermint*: Application of FYM and NPK in the I growing year did not affect significantly yield of peppermint in comparison to control. The average obtained yields ranged 1038 – 1188 kg/ha in the first harvest and 1250 – 1440 kg/ha in the second one, with presence of great variability. The content of essential oil in peppermint leaves showed to be the highest with application of FYM and it was 2.24 % in the first harvest and 1.48 % in the second one. The application of NPK and control treatment had almost equal content of essential oil that ranged 1.11 – 1.16 % in the first harvest and 0.98% - 1.07 % in the second one. The preliminary results obtained so far point on conclusion that FYM may efficiently replace NPK in organic model of production of those two medicinal plants.

INSECTICIDAL EFFECT OF THE ESSENTIAL OILS AND EXTRACT FROM *APIACEAE* PLANTS

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Keywords: Essentials oil, aromatic plant extracts, insecticide effect, Tribolium confusum

In laboratory experiments the insecticidal effects of the essential oils and extract of plants from Apiaceae family were examined: *Daucus carota*, *Levisticum officinale*, *Foeniculum vulgare*, *Carum carvi*, *Pimpinella anisum* and *Coriandrum sativum*. *Tribolium confusum* (*Col., Tenebrionidae*) were used as experimental organisms. The doses of the essential oils and extracts were 1 ml or 2 ml. The essential oils were mixed with wetting agent Citowet in dose 0,01 ml. The insecticidal effect of extracts was observed after 24, 48 and 72 hours.

All of the examined extracts showed insecticidal effect. The best one (in point of concentration and dose) was *D. carota* IPM (isopropylmyristate) extract in dose 1 ml and diluted in ratio 1:1 (49% dead (D) and 51% tremorous (T) beetles), *Oleum foeniculi* in dose 1 ml and in ratio 1:1 a 2:1 (100% D, 93% D and 7% T respectivelly), *Oleum carvi* in ratio 1:1 (49% D and 51% T beetles). Concentrated extracts from *D. carota* killed the beetles in 24 h, the diluted extract in ratio 1:1 had no effect. Concentrated *Oleum carvi* killed 98 % beetles in 24 h, 100% in 48 h. Application of water solution in ratio 2:1 killed 12% beetles, others were tremorous. *Oleum anisi* showed insecticidal effect of 100% in dose 1 ml concentrated and diluted in ratio 1:1 and 3:1 respectivelly. *Oleum coriandri* showed 12% dead and 88 % tremorous beetles after 72 hours. This work is supported by project of National Agency for Agricultural Research (Ministry of

Agriculture of the Czech Rep.) QF 4056 "Utilization of current caraway varieties (*Carum carvi* L.) and new methods of its breeding for improvement of its qualitative and quantitative parameters".

INFLUENCE OF HERBICIDE PROTECTION ON QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF CARAWAY (*CARUM CARVI* L.)

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Keywords: caraway, herbicide, selectivity

Caraway is the plant which has very small competitive ability to the most of weed species. For this reason it is very important, especially what concerns cultivation of two-year caraway, to dispose of a convenient and well timing herbicide protection access. There were founded field trials both in the clean culture and as an undersowing of other crops, especially cereals (spring wheat, spring barley) in the course of solving herbicide protection of caraway. The only types of herbicide protection accesses were tested both on the varieties with standard length of vegetation (Kepron, Prochan, Rekord) and on the varieties with shortened vegetation length. The important part of the evaluating founded herbicide trials were not only effectivity assessments of comparable herbicides but also the selectivity assessment of the herbicides toward caraway and toward undersowing crops too. On the base of the results obtained in the previous vegetation seasons is possible to conclude, the best results provided preemergent herbicide treatment with active ingredients linuron and pendimethalin. This research is financially supported by the National Agency for Agricultural Research of the Czech Republic (project No. QF 4056).

CARROT FRUIT ESSENTIAL OIL AND SUPERCRITICAL FLUID EXTRACT – THE CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY

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Keywords: carrot fruit, essential oil, supercritical fluid extraction, antimicrobial activity

The composition of carrot fruit (*Daucus carrota*, cultivar Chanteney) essential oil obtained by hydrodistillation and its extract obtained with supercritical carbon dioxide at 313 K and 10 MPa were examined. The qualitative and quantitative analyses of the essential oil and supercritical extract were performed using GC and GC/MS methods. Antimicrobial properties of the oil and supercritical extract were compared using 10 species of microorganisms (*Staphylococcus aureus* ATCC 6538P, *Salmonella enteritidis* ATCC 13076, *Bacillus subtilis* ATCC 6633BB, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCFC 292121, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC, *Rhodococcus equi* CAPM 6312, *Bacillus cereus* ATCC 11778, *Listera monocytogenes* ATCC 19115). The MICs were determined by three methods: agar dilution method, broth dilution method and paper disc diffusion method. The supercritical extract of carrot fruit was the most effective against Gram-positive microorganisms, and then against fungi and Gram-negative microorganisms. Essential oil of carrot fruit didn't show antimicrobial activity on these microorganisms.

MEDICINAL AND AROMATIC PLANT DECAY CAUSED BY *SCLEROTINIA SCLEROTIORUM* IN SERBIA

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Keywords: medicinal and aromatic plants, marsh mallow, valerian, nettle, caraway, diseases, Sclerotinia sclerotiorum

Sclerotinia blight is a common disease of sunflower in Serbia. Recently (1999-2005), sudden wilt of marsh mallow (*Althea officinalis*), valerian (*Valeriana officinalis*), nettle (*Urtica dioica*), and caraway (*Carum carvi*) was observed in the spring and summer. Initial symptoms included stem necrosis at the soil line, yellowing and tan discoloration of leaves. As stem necrosis progressed, infected plants wilted and died before the end of vegetation. Necrotic tissues were covered with whitish cottony mycelia that produced dark, spherical to elongate spherical sclerotia (2-8 mm diameter). From symptomatic stem sections *Sclerotinia sclerotiorum* (Lib). De Bary was isolated on PDA at 22 C.

Pathogenicity of isolates was confirmed by inoculating seedlings of marsh mallow, valerian, nettle and caraway, which were grown in pots in the greenhouse. Inoculated plants developed similar symptoms, and characteristic mycelia and black sclerotia were formed within five and seven days, respectively. Control plants remained symptomless. The pathogen was reisolated from inoculated plants.

The best pathogen development *in vitro* and largest sclerotia formation was at potato dextrose agar. Pathogen grew well at all temperatures tested (5-30ºC) with optimum being at 20-25ºC. Apothecia with characteristic asci and ascospores were formed from sclerotia after 2 month incubation on wet filter paper at 4ºC. Sclerotia kept its viability for long time (over five years).

Sclerotinia sclerotiorum as a cosmopolitan, homothallic and necrotrophic, ascomycetous fungus (1) is an important plant pathogen of over 400 plant species. It is already described on marsh mallow and valerian (2, 3), but reported for the first time as pathogen of nettle and caraway in Serbia.

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FUNGI ON SAGE SEED IN SERBIA AND THEIR EFFECT TO SEED GERMINATION

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Keywords: seed, fungi, sage, Salvia officinalis, diseases

Fungi associated with garden sage (*Salvia officinalis* L.) seeds in field were studied in seed lots harvested from commercial production in Serbia, during five years, from 2001 till 2005, using blotter and agar plate methods (1). Thirteen fungal species were identified to be associated with

the seeds including *Alternaria alternata* (Fr.) Keissler, *Fusariium oxysporum* Schlechtend.,Fr. *Fusarium subglutinans* Wollenw. and Reinking, *Fusarium equiseti* (Corda) Sacc., *Aspergillus flavus* Link ex Fries., *Aspergillus niger* Van Tieghem., *Aspergillus* spp., *Epicoccum purpurescens* Ehrenb Ex Schlecht., *Cladosporium cladosporioides* (Snyder), *Chaetomium* spp. *Doratomyces* spp., *Verticillium* spp., *Penicillium* spp. and *Rhisopus* spp.(2). Determination of fungi was accomplished on the basis of their morphological, biometrics and growing properties, having in mind symptoms, whenever these were characteristics. The most abundant percentage of diseased plants was attacked by species belonging to the genus *Alternaria* (up to 78 %), and than to *Fusarium* species do (8%).

Testing was conducted to establish effect of phytopathogens in the phase of germination of seeds to germination energy. Experiment was set in Petri dishes, on wet filter paper, in four repetitions. While testing pathogenicity, mycelia of selected fungi were put directly to seeds. Control was set in the same manner, but without inoculation, as mentioned.

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CURRENT STUDY ON PARAZITIC MICROORGANISMS OF MEDICINAL AND AROMATIC PLANTS IN SERBIA

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Keywords: medicinal and aromatic plants, diseases, fungi, fastidious bacteria, phytoplasma

Pathogenic microorganisms on medicinal and aromatic plant species cultivated on plantation in Pančevo, Bela Crkva, Kačarevo, Gorobilje, Svrljig, Stara Pazova and Dubovac were studied during last ten years. The following plant species were included in study: mint, lemon balm, camomile, St. John's wort (*Hypericum perforatum* and *H. barbatum*), marshmallow (*Althaea officinalis*), mallow (*Malva silvestris*), fenugreek, elcampane, wormwood, colt'foot, sage, valerian, marigold, nettle, lovage, oregano, dandelion, yellow gentian, plantain, topinambou, dog-rose, fennel, and coneflowers (*Echinaceae purpurea* and *E. angustifolia*). The seeds of majority of these plants were contaminated with fungi from the genus *Fusarium, Alternaria, Verticilum, Colletotrichum, Penicillium, Aspergillus, Rhizopus* and *Phoma*, which significantly reduced seed germination and caused seedlings decay. On the stem and leaves the symptoms of powdery mildew, rust, fleckening and wilting were manifested. As causers the fungi from genus *Oidium*, *Puccinia*, *Coleosporium*, *Phragmidium, Alternaria, Fusarium, Phoma*, *Septoria, Sclerotinia*, *Verticilum* and *Botrytis* were isolated. The most intensive changes were recorded on St. John's wort and conflower (both species), where percentage of diseased plants was very high. In tissue diseased *Echinacea* species with symptoms of yellowing (witches brooms, virescence), presence of phytoplasma (Stolbur type) were established.(1). In xylem vessels of St. John's wort diseased plants, with symptoms of redness and premature decay, presence of fastidious bacteria (rickettsia- like organisms – RLO) was recorded.(2). Symptoms of redness also were registered in cultivated oregano and lovage, as well as in wild-growing dandelion.

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THE PROBLEMS OF CHAMOMILE PESTS AND DISEASES IN THE CZECH REPUBLIC

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Keywords: Matricaria chamomille, pests, diseases

Chamomile planted in the Czech Republic is damaged by the thrips, *Thrips tabaci* and *Thrips physapus*. They suck in flower heads and caused their disintegration. Thrips caused the importantant damages in 2005. *Trypanea stellata* is small fly whose larvae feed in flower heads of *Asteraceae*. The damaged flowers had brown spots on their surface. Chamomile plants are occasionally damaged by the aphids that suck on the leaves and stems. The most important species are *Aphis fabae, Brachycaudus helichrysi, B. helichrysi*, *B. cardui*. The bugs, *Lygocoris lucorum* and locusts, *Empoasca pteridis* and *E. vitis* suck on the young leaves, latest under flower head, but they are not important pests.

The diseases of the leaves of *Matricaria chamomille* are caused by the fungi *Alternaria* spp. and *Peronospora leptosperma.* Wilt of plants is caused by *Fusarium* spp. We detected fungus *Peronospora leptosperma* in the Czech Republic in 2005. Whitish mycelium, konidiophore and conidiums were determined on infected leaves.

ELECTRON BEAMS TREATMENT OF *SPIRULINA PLATENSIS* TO ENSURE MICROBIAL SAFETY

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Keywords: Spirulina platensis, electron beam treatment, microbial safety

Spirulina platensis has high protein content and therefore, a high nutritional value. Taking into account that nowadays it is used as substitute and/or supplement in human diets, it should have a proper hygienic quality. The treatment with accelerated electrons is already applied on various foods for decontamination and extension of shelf life, being considered an ecological and non-expensive one. The aim of the paper is to discuss the electron beams application on *Spirulina platensis* and the evolution of modifications during its storage. The results of our study showed that the treatment with accelerated electron beams on *Spirulina platensis* is suitable to ensure the microbiological safety.

THE INFLUENCE OF ELECTRON BEAMS ON THE SEA BUCKTHORN BERRIES

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Keywords: sea buckthorn, electron beams, microbial contamination

Sea buckthorn is well known for its quality with almost unlimited potential for the cure of some diseases. Due to the fact that it is necessary to harmonise the products across the international regulations regarding microbial contamination and active biologic compounds, we initiated a study with respect to the action of electron beam treatment on sea buckthorn berries. Sea buckthorn berries were treated with electron beams up to 40 kGy and microbial contamination, flavonoid derivates, carotenoid and protein contents as well as antioxidant activity were evaluated. The study revealed that after 3 kGy irradiation, the sea buckthorn microbial load was reduced under permissible level according European Pharmacopoeia without important alteration in active principles.

SUPERCRITICAL FLUID EXTRACTION OF VALERIAN ROOT

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Keywords: Valerian, Supercritical fluid extraction, Bornyl acetate, Valerenal, Isovaleric acid

Supercritical fluid extraction with carbon dioxide is powerful technique for extraction of medicinal and aromatic herbs since it has certain advantages over steam distillation and solvent extraction. Steam distillation can lead to thermal degradation and partial hydrolysis of some active compounds, while supercritical carbon dioxide extraction can be performed at lower temperatures. Solvent separation is easy and complite and obtained extracts are free of solvent resuduals. In this study supercritical carbon dioxide extraction from the root of two types of Valerian (*Valeriana officinalis* L.): cultivated sort (Arterner züctung) grown in northern Serbia, and wild grown valerian from central Serbia was investigated. Extractions were performed at temperatures of 313 and 323 K and pressures of 10 and 15 MPa. Obtained yields varied from 0.9% to 1.24%. Total extracts obtained by supercritical fluid extraction were analyzed by analytical GC (FID), GC-MS and HPLC. Higher extraction yields and higher quantities of active compounds (bornyl acetate and valerenal) were obtained in cultivated plant. Valeranone, borneol, valerianol, valerenic acid, isovaleric acid, β-caryophyllene, α-humulene, spathulenol, β-ionone and elemol were also present in extracts from both species. In the extract from wild valerian presence of kessanyl acetate was also detected.

INVESTIGATION OF OPTIMUM DRYING METHODS OF BAY LEAF (*LAURUS NOBILIS* L.)

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Keywords: Laurus nobilis L., Bay leaf, Essential Oil Content, Drying Methods, Solar Tunnel Dryer

Bay leaf is one of the most important aromatic plants exported from Turkey. Postharvest processing, especially drying, affect the essential oil content and quality of bay leaf. The aim of the present study was to determine the optimum drying method for obtaining the highest essential oil content in bay leaf. For this purpose, harvested fresh bay leaves from Southern Turkey (Silifke-Mersin) were dried in three different media (on shade, under sun and solar tunnel dryer) during 11 months, from October 2003 to August 2004. The essential oil contents were analyzed in dried leaf samples by hydrodistillation. Drying trials and laboratory analysis were conducted at Çukurova University, Agricultural Faculty, Field Crops Department, Medicinal and Aromatic Plants Laboratories. According to different drying methods the highest essential oil contents of dried bay leaf were determined on shade (2.89 %), in solar tunnel dryer (2.88 %) in October.

THE PRODUCTION OF ACTIVE DRUG CONSTITUENTS THROUGH BIOTECHNOLOGICAL METHODS

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Keywords: Shikonin, shikonin derivatives, Arnebia densiflora, Cucurbitacin B, Ecballium elaterium, active drug constituents, biotechnological methods.

Plants originated folk medicines have been widely used to cure diseases and disease symptoms for ages. Demand for naturally originated drug active constituents cause to decrease or even extinction of the source. These circumstances direct us to find different ways by using biotechnological methods. Production of desired compounds via tissue culture is one of the biotechnological methods.

Our main goal is to produce valuable natural drug constituents. We have several ongoing projects. Callus and suspension culture production of *Ecballium elaterium* and *Arnebia densiflora* are two of our projects.

Ecballium elaterium is known as squirting cucumber has been widely used to treat various diseases, such as hemorrhoids, otalgia and specifically sinusitis, in the Mediterranean region. Despite of the succes in treatment of sinusitis, some toxicities and edemas in upper respiratory tracks were reported. This brought deeper research on active compounds of the crude extracts. Yesilada *et al*. identified that triterpenic cucurbitacin B is the active phytochemical. Our studies focused on establishing callus and suspension cultures by using plant tissue culture methods to determine and increase the cucurbitacins. Cucurbitacin B was determined in our suspension cultures and some elicitors were applied to increase it. Succesful preliminary results were observed. We will continue trying different eliciters to obtain the best results.

Arnebia densiflora is one of the endemic plants that can produce shikonin and its derivatives. Shikonin is a phytochemical compound, which is valuable in pharmacy for its pharmacological effects and in food science as a food colorant because of its red color. Recently, it has been

found that shikonin derivatives have anti-HIV effects *in vitro*. We previously established callus cultures and we have just begun analyzing the cultures for their major phytochemical compounds.

ENEMIES AT THE GATE: TERPENOIDS AND DEFENSE AGAINST SPRUCE BARK BEETLE (*IPS TYPOGRAPHUS*)

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Keywords: Conifer, defense, jasmonates, Norway spruce, terpenes

The terpenoid and phenolic constituents of conifers have been implicated in protecting trees from infestation by bark beetles and phytopathogenic fungi, but it has been difficult to prove these defensive roles under natural conditions. In the present investigation, we studied the effect of chemical induced defense in host colonization process of the spruce bark beetle *Ips typographus* (L.) in mature Norway spruce. We used methyl jasmonate (MJ), a well-known inducer of plant defense responses, to manipulate the biochemistry and anatomy of mature *Picea abies* trees and test their resistance to attack by *Ips typographus*. Bark sections of *P. abies* treated with MJ had significantly less *I. typographus* colonization than bark sections in the controls and exhibited shorter parental galleries and fewer eggs deposited. In addition, fewer beetles were attracted to conspecifics tunneling in MJ-treated bark. Stem sections of *P. abies* treated with MJ had a significantly higher concentration of mono-, sesqui- and diterpenes than untreated sections. However, there were no qualitative differences in terpene composition in MJ_C vs. MJ treated bark or wood. The increased amount of terpenoid resin present in methyl jasmonate-treated bark could be directly responsible for the observed decrease in *I. typographus* colonization and reproduction.

SECTION II

TOPICS

- Phytochemistry
- Pharmacognosy
- Pharmacology, toxicology
- Quality control of map products
- Traditional medicine and map use

BUILDING OF DATABASE FOR THE FAST SCREENING OF FLAVOURS AND FRAGRANCES BY LS/MS TECHNIQUE

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Keywords: LS/MS technique, flavours, fragrances, HCA

In our previous contributions, dealing with the evaluation of liquid sampling mass spectrometry (LS/MS), detail description of the basic concept of the use of this technique for the characterisation and fast screening of essential oil was reported (1-4). Furthermore, usability of LS/MS technique for the fast quantification of certain major constituents in essential oils was also tested (5).

In the present article, the usage of LS/MS technique for the fast screening of commercial flavours and fragrances was tested. For this purpose, 49 samples of flavours and fragrances of different origin were analysed by LS/MS. Acquired mass spectra were processed and stored in MS library, whose usability for differentiation of samples was tested by common search engines (PBM/NIST). Clear differentiation of samples belonging to different groups of products (fruit flavours, flavours used in dentistry, and fragrances), as well as those within the same group was achieved. Further, MS data from created library were transformed, imported, and statistically processed to get better insight into differentiation power of LS/MS technique, applied to selected samples. Results obtained by hierarchical cluster analysis (HCA) approved LS/MS as a powerful tool for fast screening of tested flavours and products.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC QUANTIFICATION OF CAFEIC ACID FROM LEAVES OF *SALVIA OFFICINALIS L.*

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Using the HPLC method we identified and compared the concentration of cafeic acid from leaves of *Salvia officinalis* L., croped in the same period (July 2004) belonging to different geographic regions (Romania, Greece and Tunisia). Our results show that the highest concentration was found in the leaves from Tunise.

COMPARATIVE PHYTOCHEMICAL RESEARCH ON SOME INDIGENOUS SPECIES OF *VIOLA* (*VIOLACEAE*) FROM ROMANIA

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Keywords: Viola sp., polyphenolic compounds, essential oil, HPLC, GC-SM

The *Viola* genus contains many species, native in the temperate zones of Europe and Asia. *Viola tricolor* L. (wild pansy) is widely spread in Romania's spontaneous flora. In traditional medicine, the aerial parts are used for their anti-inflammatory, expectorant, diuretic properties, to treat skin conditions, bronchitis, cystitis, rheumatism. Its properties are ascribed to the presence of the following active principles: saponins, flavonoids, mucilages, salicylic derivatives, carotenoids, coumarins. *Viola arvensis* Murray and *Viola declinata* Waldst. et Kit. are closely related to *V. tricolor* L., but their chemical composition is less studied. We have analysed the polyphenolic compounds (flavonoids, phenolic carboxilic acids, anthocyanins, proanthocyanins) from the three *Viola species*. The qualitative analysis was performed by TLC and HPLC, whereas the quantitative determinations by spectrophotometric methods. We have identified quercetol and kaempferol as flavonoid aglycones, chlorogenic, gentisic, caffeic, caftaric and ferulic acids as phenolic carboxilic acids and delphinidin and cyanidin as anthocyanin aglycones. The essential oil of *V. tricolor* L., obtained by water steam distillation was analysed by GC-MS. The main components identified were: bisabolone-oxide, bisabolol-oxide A, B, methyl salicylate, trans-beta-farnesene, spathulenol.

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THE ANALYSIS OF *ARNICAE FLOS* FROM ROMANIA ACCORDING TO THE EUROPEAN PHARMACOPEA

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Keywords: Arnicae flos, helenaline, HPLC, TLC analysis

The blossoms of *Arnica montana* L. (*Asteraceae*, *Arnicae flos*) is one of the most important medicinal vegetal product for phytotherapy. In Romania the richest natural basins of *A. montana* are located in Apuseni Mts. at Poiana Horii (Cluj County) and Gârda de Sus (Alba County), from where the vegetal product is exported in the western European countries.

The growing interest for this product has determined us to perform an analysis by the stipulation of Eu. Ph.

The macro and microscopic investigation for the *Arnicae flos* monograph are stipulated, and also the loss on drying (max. 10%), foreign matter (max. 5%), the total ash (max. 10%), a TLC assay for the flavonoids and phenil-propanoid compounds, a HPLC analysis of a selective purified extract and than the identification and quantitative determination of helenaline, considered as the main active principle of *Arnicae flos*, using santonine like an internal test substance.

We determined the content of helenaline in *Arnicae flos* from Romanian (0.74%) and in the *T-ra Arnicae* (0.052%) by mean of this technique.

In addition, the content of flavonoids (0.45%) and essential oil (0.26%) was determined.

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BIOTECHNOLOGICAL AND PHYTOCHEMICAL RESEARCH ON *RUSCUS ACULEATUS* L.

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Keywords: Ruscus aculeatus L., biotechnology, steroidal saponins

A comparative study on steroidal saponins isolated from *Ruscus aculeatus* L. rhizoma with roots and biomass obtained by plant cell and tissue culture *in vitro* was done.

Ruscus aculeatus roots and rhizoma were harvested from Dealurile Lipovei (Arad County) spontaneous flora in October 2004. The *in vitro* obtained biomass is represented by callus with roots growth on Murashige and Skoog medium supplemented with different concentrations of auxins. The culture was initiated from roots explants sampled from plantlets of *R. aculeatus* obtained by aseptic *in vitro* seeds germination.

The steroidal saponins from *Rusci rhizoma et radix* were isolated with a 15.57% efficiency. Qualitative analysis was done by TLC and HPLC. Thus, three furostanol fractions and one spirostanol fraction, making a total number of four separated saponosidic fractions, was revealed by TLC analysis, the main two being also present in the *R. aculeatus* callus with roots. HPLC analysis showed six saponosidic fractions, five being identical with fractions obtained by *in vitro* biomass. This demonstrates that steroidal saponins specific to *Ruscus* rhizoma can be

synthesized by *in vitro* plant cell culture. As a result of acid hydrolysis, the TLC analysis marked two aglicons (ruscogenin and neoruscogenin).

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CHEMICAL COMPOUNDS IN BUTANOL EXTRACTS OF *SCLERANTHUS PERENNIS* L., *CARYOPHYLLACEAE*

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Keywords: Scleranthus perennis, flavonoids, sterols, sapogenins

Scleranthus perennis L., *Caryophyllaceae* is one of the seven species of this genus which are spread in Bulgaria. Together with *S. annuus* L. overground parts of the plants are commonly used in the traditional Eastern medicine and in Bulgarian folk veterinary medicine.

In previous studies we reported on separation of phenolic acids mixture in aqueous/alcoholic extracts after treating with various eluents, isolation of flavonoids from purified ethyl-acetate fractions and their identification. This work presents the results of our investigation on butanol extracts. It was found a presence of different chemical compounds: flavonoids, sterols, sapogenins.

COMPARATIVE PHYTOCHEMICAL STUDY ON *ERYNGIUM* SP. FROM ROMANIA

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Keywords: Eryngium, saponins, polyphenols

The aim of the present paper was to highlight the similarities and the differences between the chemical compositions of *E. planum* L., *E. campestre* L. and *E. maritimum* L., indigenous to Romania. Chemical assays on the aerial parts revealed in all three *Eryngium* sp.: triterpene saponins (as the major constituents) ranging from 3.7 to 10.1% as determined by gravimetric method, 0.32 to 0.56% flavonoids expressed as rutin, determined by spectrophotometry at 430 nm, 0.27 to 1.38% phenolic compounds as caffeic acid type assayed by spectrophotometry at 500 nm, 0.85 to 1.34% tannnins, 1.7 to 8.8% pectins determined by gravimetric method, coumarins, amino acids, sterols and essential oil.

A validated HPLC method for screening and for quantification of 18 polyphenols (flavonoids, flavonols and phenolic acids) revealed caffeic acid, quercitrine and rutin as common constituents to all tested species.

TLC-densitometry of the crude saponins yielded 4 saponosides in *E. planum*, 3 in *E. campestre* and 5 saponosides in *E. maritimum*. 2 saponosides (R_f 0.62 and 0.65) were common to all three species, while fraction at R_f 0.51 proved to be common to *E. campestre* and to *E. martimum*, as well.

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GC/EI-MS INVESTIGATION OF THE VOLATILE CONSTITUENTS OF *SIDERITIS SCARDICA* GRISEB FROM BULGARIA

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Keywords: Sideritis scardica, essential oil, volatile constituents, GS/MS analysis, anti microbial activity

Sideritis scardica Griseb is a perennial herbaceous plant and a Balkan endemic encountered in Southwest Albania, Bulgaria, Greece and Macedonia. The herb extracts of this plant have shown anti-inflammatory and antibacterial properties and are used as loosen agents in bronchitis, bronchial asthma, against common cold and gastro-intestinal inflammations. The oil yields (v/w) from the air-dried plant material collected at the flowering stage from three wild and four cultivated habitats were 0.04-0.07%. Three monoterpenes, thirteen sesquiterpenes, five diterpenes, one triterpene, five steroid components, four fatty acids and alcohols and five other volatile components were identified by GC/EI-MS analysis from over 66 found constituents. The essential oil possessed a slight anti-microbial activity against *Kleibsella* and *Bacillus cereus* from six investigated pathogenic microorganisms.

VOLATILE COMPOUNDS FROM *GYPSOPHILA TRICHOTOMA*

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Keywords: Gypsophila trichotoma, Caryophyllaceae, volatiles

In this study we investigate the volatile compounds in the roots and leaves of *Gypsophila trichotoma* Wend. (*Caryophyllaceae*).

The volatile compounds were obtained by hydrodistillation using fresh leaves (20g) and fresh roots (30g). The volatiles were extracted with diethyl ether and analyzed by gas

chromatography/mass spectrometry (GC/MS). The GC/MS investigation was based on the interpretation of the mass spectral fragmentation followed by comparisons of the obtained spectra with those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 were also applied.

The compounds identified in the roots were: ethyl 2-(phenylamino) acetate, dihydroxynaphtalene, methyl palmitate and methyl stearate. The main volatiles in the leaves were: β-damascon, 2,6-di-tert-butyl-4-methylenecyclohexa-2,5-dienone, ethyl 2-(phenylamino) acetate, dihydroxynaphtalene, isopropyl tetradecanoate, isopropyl palmitate, heneicosane, phytan, tricosane, octadecenic acid and bis (4-octylphenyl) - amine. The amount of the volatile compounds varied from traces to 66.4%.

The compounds are new for the species.

THE IDENTIFICATION AND QUANTITATIVE DETERMINATION OF ROSMARINIC ACID BY TLC AND HPLC-MS FROM MEDICINAL *LAMIACEAE* SPECIES

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Keywords: rosmarinic acid, Lamiaceae, TLC, HPLC

The rosmarinic acid (RA) is a depside common in *Lamiaceae* species, also named "tanin of *Lamiaceae*". It is the ester of caffeic acid with α-hydroxydihydrocaffeic acid. The interest for this compound has grown after the discovery of their antioxidant, antimicrobial, anti-inflammatory, anti-allergic, angiogenesis inhibitor, antiapoptotic effects, chemoprotective properties. Also RA stimulates prostaglandines synthesis, reduces the production of leucotriene and acts like an inhibitory compound of complement system.

Data about the content and the presence of RA in medicinal species of *Lamiaceae* are absent in Romanian literature, therefore we initiated a qualitative and quantitative research on 7 species of *Lamiaceae*: *Origanum vulgare* L. (1), *Rosmarinus officinalis* L. (2), *Melissa officinalis* L. (3), *Ocimum basilicum* L. (4), *Hyssopus officinalis* L. (5), *Salvia officinalis* L. (6) and *Ajuga genevensis* L. (7).

The identification of RA was made by TLC and HPLC-MS using RA (Fluka) like test substance. The identity of RA was attested, in addition, by MS.

The quantification of RA was made by HPLC coupled with mass spectrometry (LC/MS/MS) and the content of RA is: 1.24% (1), 0.78% (2), 0.72% (3), 0.35% (4), 0.28% (5), 0.21% (6) and 0.02% (7).

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ESSENTIAL OIL COMPOSITION OF *HYPERICUM AEGYPTICUM* L. GROWING IN GREECE

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Keywords: Hypericum aegypticum, essential oil composition

The genus *Hypericum* contains about 400 species, which occur in Europe, West Asia, North Africa and North America. A number of species have been shown to possess various biological activities. They are source of naphthodianthrones, flavonoids, xanthones, tannins and essential oil.

Hypericum aegypticum is a shrub, with leaves 3-10 mm, growing in rocky places near the sea in islands of C. and E. Mediterranean region.

Regarding the growing interest in constituents of this genus, in this study we investigated the chemical composition of *H. aegypticum* essential oil.

Aerial parts of *H. aegypticum* was collected during the flowering season in June 2004, from the island Kefalonia Greece. The essential oil was isolated by hydrodistillation and analyzed by GC and GC-MS.

The main components of the oil were α-pinene (41.6%), β-pinene (12.3%), sylvestrene (7.0%) and γ-himachalene (5.4%). The oil was characterized by a high amount of monoterpene hydrocarbons (69.2%).

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ESSENTIAL OIL OF *THYMUS ZYGIOIDES* GRISEB. FROM GREECE

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Keywords: Thymus zygioides; essential oil composition

The genus *Thymus* (Labiatae) includes c. 350 species, 66 of which growing wild in Europe (Mabberley 1997). *Thymus* L. species have been used for more than 2,000 years as medicinal herbs and many of them are still in use. The infraspecific variability of the essential oils of the genus *Thymus* is widely accepted. Thymol and carvacrol are the major compounds in most of the *Thymus* essential oils, while non-aromatic terpenes may also present as main constituents (Stahl-Biskup 1991, Stahl-Biskup 2002).

As a part of our work on the screening of odoriferous plants of Greece, we have investigated the essential oil composition of *T. zygioides* collected from island Lesvos. This species belongs to section Hyphodromi and is distributed in East part of Balkan peninsula, extending to S. E. Romania (Jalas 1972). The essential oil of this species was previously reported from Turkey (Baser et al. 1999).

The dried aerial parts were subjected to hydrodistillation for 3h and the obtained oil was analysed using GC/MS. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the NIST/NBS Wiley libraries. The oil was characterized by the abundance of p-cymene (20.3%), thymol (19.9%) and γ-terpinene (18.1%).

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THE INVESTIGATION OF THE BIOCHEMICAL POTENTIAL OF THE *MENTHA VIRIDIS* L. AND *MENTHA PIPERITA* L. EXPERIMENTAL VARIANTS

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Keyword: Folium Menthae, in vitro and conventional cultures, biosynthetic potential

The vegetal material obtained from *Mentha piperita* and *Mentha viridis* (variants that are in different vegetation years, controls and vitroclones) was phytochemically analysed to evaluate its biosynthetic potential. We evaluated the content of polyphenols and flavons, triterpens and phytosterols, essential oils. We analysed the etanolic extracts of the same concentrations (40, 50, 70%) obtained by the warm and cold procedure.

We noticed that the same extracting and biosynthetic model is respected for *Folium Menthae*, irrespective the species; the warm tinctures/extracts of 70% show the highest values for the flavons and plyphenols. The chromatograms for the triterpens and phytosterols show the presence, in all the types of the extracts, of β–sitosterol, stigmasterol, of oleanolc and ursolic acids in *Mentha piperita* and only β–sistosterol and ursolic acid in *Mentha viridis*.

The dominant fraction of volatile oil of *Mentha piperita* is L-(-)-menthol (40.33%), followed by menthone (27.50%). Among the fractions with aromatic and pharmaceutical importance is eucalyptol (1.41%) and limonene (0.65%). *Mentha viridis* is characterized by a composition that is richer in volatile fractions, some being found only in this species. The quantity of menthol is 2.56%, that of menthone of 2.62% and that of isomethone of 7.19%, fractions that are specific to the volatile oil from the Mentha viridis regenerants of the first vegetation year. Volatile fractions (dihydrocarvil acetate, β–burbonen and β–pinen) are in all the variants of *Mentha viridis*. We specify that the regenerants contain greater quantities of carvona (59.115 – in the first vegetation year and 59.16% in the second) than the control plants (34.46%).

THE COMPARATIVE PHYTOCHEMICAL ANALYSIS OF VOLATILE OILS IN *FOENICUM VULGARE* OF DIFFERENT ORIGINS

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Keywords: Foeniculum vulgare, volatile fractions, TLC, GC-MS

The fruit of *Foeniculum vulgare* (fennel) harvested from the two populations, Piatra-Neamt (PN) and Vanatori-Neamt (VN) were chemically analysed from both the quantitative and qualitative point of view to evaluate the biosynthesis of volatile oil and to decelate its fractions. The volatile oil obtained through water vapour distillation (Neoclevenger), the biosynthetic spectrum was determined by TLC and the chemical composition by means of the GC-MS (gas chromatography completed with mass spectrometry). In case of the populations the quantity of volatile oil was of 3.12 ml/100 d.w. for 1 PN and 3.50 ml/100 d.w. for 2 VN.

We noticed that there is a proportional variation among the volatile fractions of the oils obtained from the two populations. The Piatra-Neamt population although having a quality inferior to 3.12 ml/100 d.w., it presents a greater quality of trans-anethole of 46.95%, 17.54% fenchone and 3.88% limonene. This population is characterized by the presence of some fractions of anisil-acetone (3.44%), D-germacrene (0.76%) and 3.44% α-pinene. The fraction L-phelandren is not present in *Foeniculum fructus*, the PN population.

The analysis showed that the two populations are characterized by a good quality volatile oil as its main components (trans-anethole and fenchone) are present in high quantities. Together, the two fractions have the values of 64.49% for 1 PN and 61.68% for 2 VN.

ESSENTIAL OILS OF WILD GROWING *PISTACIA* SPECIES FROM MONTENEGRO

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Keywords: Pistacia lentiscus, Pistacia terebinthus, leaves, branches, essential oil

The members of the genus *Pistacia* are shrubs or little trees, very characteristic for the maquia type of vegetation in the Mediterranean region. *Pistacia lentiscus* and *P. terebinthus* are present in the vegetation of the peninsula Luštica at the Adriatic coast (Montenegro). There is no data about composition of the constituents of mastics resin or essential oil obtained from the plants from this aerie. This paper deals with the essential oil analysis. Plant material was collected in the July of 2004. From aerial dried leaves and branches essential oil was distilled in the Clavenger type apparatus. GC and GC/MS analyses of the oil were done.

Most abundant constituents of *P. lentiscus* leaves oil were: α-pinene (24.8 %), β-pinene (10,3%) and δ-cadinene (5.8 %). The oil obtained from the branches was reach in α-pinene (32.1 %), β-pinene (10.9 %) and sabinene (4.8 %).

Most abundant constituents of *P. terebinthus* leaves oil were: caryphylla-3,8(13)-dien-5-β-ol (10.9 %), τ-cadinol (7.1 %) and δ-cadinene (6.4 %). The oil obtained from the branches was reach in limonen (10.1 %), caryophyllen-oxide (6.5 %), α-pinene (5.5 %) and trans-β-ocymene (5.5 %).

THE ESSENTIAL OIL OF *HERACLEUM SPHONDYLIUM L.* (APIACEAE) FROM SERBIA

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Keywords: Heracleum sphondylium, essential oil, n-octyl acetate, cis-3-octen-1-yl acetate, n-octyl hexanoate

The essential oil of the species *Heracleum sphondylium* was isolated by hydrodistillation from shoots, inflorescence and fruits. The content of the oil was largest in fruits (1%), while only traces of essential oil were recorded in inflorescence and shoots. Chemical composition of these oils was determined by correlation of results obtained by GC/FID and GC/MS of isolated samples. Identification of essential oil constituents was accomplished by GC/MS on HP-5MS capillary column, comparing MS of peaks from samples with those from selected MS libraries (Wiley 275, NIST/NBS, Adams), using different search engines (PBM, NIST), and comparing experimentally determined values for appropriate retention data (AMDIS) with those from the literature. The most abundant constituent of fruit oil was n-octyl acetate 49.5% (3.5% in shoots; 5.0% in inflorescence). The content of cis-3-octen-1-yl acetate was determined to be 6.9% in fruits, but below 1% in the oils from other plant parts. Fruit oil also contained 7.1% of n-octyl hexanoate. The major constituents of the oil isolated from inflorescence were myrcene (12.4%), n-octyl hexanoate (10.8%) and cis-β-ocimene (7.0%). α-pinene (16.6%) and cis-β-ocimene (10.6%) were present in high concentration in the oil from shoots. Fruit oil, according to the content in samples of tested plant material and its composition, clearly differed from oils isolated from other parts of *Heracleum sphondylium*.

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS OF *THYMUS GLABRESCENS* WILLD., *LAMIACEAE* FROM BANAT

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Keywords: Thymus glabrescens, essential oils, GC, antioxidant activity, thymol

Chemical composition and antioxidant activity of essential oils of six *Thymus glabrescens* Willd., *Lamiaceae* populations from south and middle Banat (NE Serbia) were investigated in order to determinate the variability among populations from different habitats and correlation to its morphology. Essential oils were obtained by hydrodistillation of dried aerial parts of plants collected in blossoming stage (June 2004), with yields covering a range between 0.1 and 0.8 ml/100 g of plant material. Chemical analysis of essential oil samples was performed by GC-FID and GC-MSD. Investigated essential oils differed significantly to each other by the quantity of thymol, as its concentration covered a span between 0.65 and 55.12 % (w/w). In one sample, instead of thymol and/or carvacrol, high concentrations of geranial and neral were detected. Antioxidant activity of investigated oils has been estimated by the assay for DPPH free radical scavenging ability and compared to the same feature of BHT. The results of DPPH assay showed that investigated essential oils expressed moderate antioxidant activity, with IC_{50} 120-250 μg/ml, attributable to presence and concentration of thymol exclusively, as verified by dot-blot TLC analysis.

INFRASPECIFIC CHEMICAL TAXA OF *ACHILLEA DISTANS* W ET K

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Keywords: essential oil, Achillea distans, Achillea millefolium, azulene, taxa

We analysed the essential oils isolated from three subspecies of *Achillea distans* W et K (*mountain yarrow*) and we established that the taxa of subalpine belt does not contain of azulene, whereas the taxa of submountain belt has a very low concentrations of azulene (2.44%) while in *Achillea millefolium* L. the content of azulene is 25.26%.

The qualitative analysis of the essential oils, extracted in Neo-Clevenger apparatus by water steam distillation, was made by GC-MS and the quantitative analysis was made by spectrophotometric and GC methods.

	Taxa	Station	Essential oil content ml/100g	Content of azulene (%)
1	*Achillea distans* ssp. *alpina* with pink flowers	Rodnei Mountains, Iezer (1800 m)	0.25 colorless	-
2	*Achillea distans* ssp *distans* with white flowers	Rodnei Mountains, Iezer (1750 m)	0.40 colorless	-
3	*Achillea distans* ssp *distans* with white flowers	Apuseni Mountains, Tarniţa (750 m)	0.24 light blue	2.41
4	*Achillea millefolium*	Cluj-Napoca (320 m)	0.40 dark blue	25.26

From the data analysis it results out that the taxa of *Achillea distans* of subalpine belt at high altitude lacks azulene in essential oil, those of sub mountain belt have a very low content of azulene (2.41 %) whereas *Achillea millefolium* is rich in azulene (25.26%).

As a result, the flowers of *Achillea distans* are considered a substitution of *Millefolii flos*.

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ANALYSIS OF THE POLYPHENOLIC COMPOUNDS FROM *BASILICI HERBA*

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Keywords: Ocimum basilicum, Lamiaceae, polyphenolic compounds

Ocimum basilicum L. (*Lamiaceae*), sweet basil, is an annual species, original from Asia, being cultivated in all the Mediterranean and tropical countries. In Romania is frequently cultivated in alimentary, medicinal, ornamental and religious purposes. *Basilici herba* contains: essential oil, polyphenols, triterpenic acids, phytosterols. In phytotherapy is used for their antispasmodic, antiseptic, anti-inflammatory, antioxidant, adaptogen, antiulcer, antihelmintic properties. The

aim of this work was to analyze by TLC, HPLC/MS the flavonoids (F) and the caffeic acid derivatives (CAD) from four samples of *Basilici herba*. The samples are coming from the vegetal cultures initiated at the Faculty of Agronomy Cluj (sample I), south of the country (sample II) and two commercial samples of medicinal tees (III and IV). The quantitative determinations made by spectrophotometric methods showed different results: I - 2,28% CAD, 1,10% F, II - 1,19% CAD, 0,60% F, III - 0,71% CAD, 0,45% F, IV - 0,78% CAD, 0,35% F. We identified in all samples the rutoside, isoquerctroside and the caffeic, chlorogenic, rosmarinic, caftaric, ferulic, coumaric acids, in different concentrations, by TLC and HPLC/MS. After hydrolysis we emphasized the quercetol and kaemferol, like aglycons of the flavonoids.

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RESEARCHES ON A NEW ROMANIAN CHEMOVAR OF *ARTEMISIA DRACUNCULUS* L. *METHYLEUGENOLIFERUM* FOR ITS STANDARDIZATION AND USE IN AROMATHERAPY SECOND NOTE: STUDIES ABOUT CHEMOVAR'S STABILITY

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Keywords: Artemisia dracunculus, eugenol methyl-ether

The phenol methyl ethers are well known as essential oils components with remarkable antispastic properties. Among them, eugenol methyl-ether is the most valuable. It has higher antispastic activity than papaverine and has no adverse effects as anethole (estrogenic) and estragole (potential carcinogenic).

Because there are few sources of eugenol methyl-ether (oil of *Ocimum basilicum* L. var. *minimum* = 55 – 60%, *Melaleuca leucadendron* L. *methyleugenoliferum* = 99%), the authors of present work want to find through the species created at Fundulea Medicinal and Aromatic Plants Researches Resort, an eventual source which can be used in aromatherapy.

Among the chemovars that have been created here, we were interested in *Artemisia dracunculus* L., tarragon (*Asteraceae*), which was cultivated on many lots (8, 10, 16).

Tarragon is a plant known for its content in estragole.

We have studied the morph-anatomical characters and the chemical composition of the essential oil obtained through water steam distillation (in a Neo- Clevenger apparatus) of plants harvested at full blooming during 3 years (2003, 2004 and 2005). Using GC-MS we found a content of 1.1 – 2.55 % essential oil (the lot 16 is the richest), which contains between 51.15 and 69.73% eugenol methyl-ether.

We noticed little quantity variations of eugenol methyl-ether during those 3 years (59.71/2003, 51.91/2004, 57.47/2005 – the lot 8; 61.06/2003, 51.15/2004, 51.12/2005 - the lot 10 and 69.73/2003, 55.33/2004, 39.21/2005 - the lot 16). Though, there is a little decreasing of the quantity of the eugenol methyl-ether in the last 2 years, mostly on the lot 16.

FUNCTIONAL COMPONENTS OF COLD-PRESSED *AMARANTHUS SP.* SEED OIL

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Keywords: Oil from Amaranthus sp. seed, cold-pressing, α-tocopherol, squalene

Amaranthus sp. is a plant originating in America. Biological characteristics of these plants include low level of requirements towards environmental conditions proving easy introduction in non-traditional growing regions such as the province of Vojvodina.

Oil from *Amaranthus sp*. seed, obtained by cold-pressing has yellow-orange color and specific taste. Physical and chemical properties, oxidative stability of cold-pressed oil were compared with oils obtained by extraction with an organic solvent.

The content and quality of oil and specific functional components (α-tocopherol, squalen) were compared with other oils.

ESSENTIAL FATTY ACIDS AND NUTRITIVE VALUE OF COLD PRESSED HEMPSEED OIL, *CANNABIS SATIVA* L.

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Keywords: hempseed oil, gama-linolenic acid, tocopherols, shelf life

Certain vegetable oils are of special importance in human nutrition due to specific fatty acid composition. Namely, besides food value, these oils have a certain nutritive, and even therapeutical effect. Hempseed oil is very appreciated due to the content of specific gama - linolenic acid and ideal ratio of essential unsaturated fatty acids. The fatty acid composition, total tocopherols content and shelf life of cold pressed oil, obtained of seven different hemp cultivars, were investigated. The results show that the content of gama-linolenic acid in the oil depends on the cultivar, ranging from 0,80 to 2,46%. The ratio of essential omega-6 and omega-3 fatty acids in the oil was 3,9 to 4,2, satisfying the demands of modern healthy nutrition regarding lipids. The investigated cold pressed hemp oil samples are rich in tocopherols; their content is in the range 114 to 1422 mg/kg. However, due to high content of polyunsaturated fatty acids, the oxidative stability of hemp oil is very poor. The induction period at 100ºC, determined by accelerated oxidation method – Rancimat test – was 3,0 to 3,8 hours. Special protective measures are necessary to prevent oxidation in order to preserve the high nutritive value of this oil.

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PHYSICO-CHEMICAL STUDIES ON *ACER NEGUNDO* L. SEED OIL

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Key words: pinnated maple, seed, oil, essential fatty acid, gamma-linolenic acid

The physiological importance of essential fatty acids, especially gamma-linolenic acid in anti-inflammatory and anti-thrombotic eicosanoids biosynthesis and limited vegetable resources were the premises of this research. The objects of this study were the extraction, quantitative determination (gravimetric method) and physico-chemical analysis of pinnated maple seed fatty oil (*Acer negundo* L., *Aceraceae* family) in order to take it into account. The morpho-anatomical characteristics of seeds were established. The compositional data of the oil (fatty acids, phytosterols and monoglycerides profiles) were obtained using a gas chromatographic-mass spectrometric analysis.

Pinnated maple seeds were found to contain 20,76-21,55% oil rich in essential fatty acids: linoleic (32,44%), gamma-linolenic (8,39%) and alpha-linolenic acid (0,75%). Monoolein (4,36%) and one usual phytosterol, stigmasterol (3,33%) were identified. Although gamma-linolenic acid seed oil content was relative modest comparative to *Oenothera* species (13,75-15,70%), pinnated maple could be a convenient source for this acid because of its large spreading in Romanian spontaneous flora and important seeds production.

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LIPOPHILIC SUBSTANCES FROM FRUITS OF *PHYSALIS ALKEKENGI L.*

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Keywords: Physalis alkekengi L., carotenoids, phytosterols, acylglycerols

In the course of our phytochemical investigations of *Physalis alkekengi L.* we now report on the lipophilic constituents of the fruits. Ground fruits were exhaustively extracted with petroleum

ether in a Soxhlet apparatus. The concentrated lipid extract was separated by flash column chromatography using alumina as stationary phase and petroleum ether with 2% acetone followed by acetone as mobile phases. Fractions were monitored by TLC and reunited to give four fractions. Each fraction was analyzed by TLC with proper mobile phases and sprayed with reagents for lipids, terpenoids and flavonoids. The chromatographic properties of the separated compounds were compared with those of standards and literature data. Esters of phytosterols, acylglycerols, carotenoids, free fatty acids, terpenoids and unesterified phytosterols have been identified. No lipophilic flavons were found in the petroleum ether extract.

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CONCENTRATION AND PURIFICATION OF THE BIOLOGICAL ACTIVE COMPOUNDS FROM MEDICINAL PLANTS EXTRACTS USING MICRO- AND ULTRAFILTRATION TECHNIQUES

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Keywords: Cynara scolymus, Viscum album, polyphenols, flavonoids

This work is focussed on the study performed on 2 medicinal herbs (*Cynara scolymus* and *Viscum album)*, due to the complexity of the biological active compounds contained and to their pharmacodynamic activity.

The active biological components separation and concentration from *Cynara scolymus* and *Viscum album* extracts were performed by membranes processes: microfiltration and ultrafiltration.

The determinate values for the biological active compounds recovery degree from *Cynara scolymus* aqueous concentrated extract were over 77% for polyphenols and over 86% for flavonoids. The level of the proteins' recovery (such as lectine, viscotoxine) was over 92% for *Viscum album* aqueous extracts and over 94% for alcoholic extract. The experiments were done into the same concentrates.

In the conclusion, the quality and the high concentration of the final products (biological active compounds with pharmacodynamic effects) obtained by succession microfiltration – ultrafiltration were superior to the classical products.

SECONDARY METABOLITES FROM *ASPERULA LUTEA* SUBSP. RIGIDULA

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Keywords: Asperula lutea subsp. rigidula, Rubiaceae, flavonoids, iridoids, fatty acids

The genus *Asperula* (Rubiaceae) includes c. 90 species, 66 of which growing wild in Europe (Mabberley 1997). *A. odorata* L. (syn. *Galium odoratum*) is used in folk medicine as a diuretic, tonic and against diarrhea. Iridoid glycosides, cardenolides, flavonoids and anthraquinone

glycosides have been reported from several *Asperula* species. However, no work has been reported on the chemical constituents of *A. lutea* subsp. *rigidula* (Halacsy) Ehrend. an endemic of south eastern Greece (Ehrendorfer and Krendl 1976).

Aerial parts of *A. lutea* subsp. *rigidula* were collected in Megara (Perfecture Attiki), during the flowering period, in June 2000 [OT-12].

From the methanolic extract of the aerial parts were isolated by VLC, CC, preparative TLC and HPLC one sterol: β-sitosterol, one hydroxycinnamoylquinic acid: chlorogenic acid, three iridoids: geniposidic acid, deacetyl-asperulosidic acid, scandoside and the flavonoids: quercetin, hyperin, isoquercitrin and rutin. The structures of the isolated compounds were identified on the basis of their chromatographic behaviour, spectral characteristics and with the aid of one and two-dimensional NMR experiments, as well as by comparison with literature data.

A non polar fraction of the VLC was analyzed by gas chromatography-mass spectroscopy. Nineteen compounds were identified, mainly esters of fatty acids. The major components were ethyl ester of palmitic acid (32.7%), ethyl ester of linolenic acid (20%) and ethyl ester of linoleic acid (10.5%).

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THE ERGOPEPTIDE ALKALOIDS AND NUCLEIC ACIDS QUANTITY VARIATION FROM THE SCLEROTIA OF SOME *CLAVICEPS PURPUREA* STRAINS

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Keywords: DNA, RNA, ergo peptide alkaloids, ANOVA test

The research made on the sclerotia, that belong to *Claviceps purpurea* strains with different alkaloid type, have revealed interesting tendencies in expressing the nucleic acids and the relation between RNA/DNA, as well as the correlation between the DNA level and total alkaloid level. The variation level of the DNA quantity is wide enough (15.64 – 4.70), with an average of 7.82 mg/g DNA. The sclerotia, which have the ability of producing ergotamine and ergocristine, have the narrowest variation limits for AND in comparison to the sclerotia that predominantly produce one certain alkaloid. The variability coefficient (S%=27.55), reflects an increased variability of the DNA level from the *Claviceps purpurea* sclerotia. The minimum ARN level is situated at very close values for all analysed sclerotia types, obvious differences were present only by the higher level values.

The RNA/DNA relation, sensitive indicator of the growth rate and of the metabolic state, is relatively constant at the predominantly ergocristine producing sclerotia in comparison to the relation value that varies in wide limits at the ergotamine type sclerotia. The ANOVA test, created for the statistical significations analysis of the differences between the DNA content and the total alkaloid (CAT), but as well for the spectral composition level of them has revealed some interesting correlations concerning the *Claviceps purpurea* strains.

CHEMICAL RESEARCH ON SOME POLYPHENOLIC COMPOUNDS FROM *SALVIA SP.* (*LAMIACEAE*)

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Keywords: Salvia species, polyphenolic compounds

Salvia officinalis (sage) is a known medicinal plant, used for its carminative, stomachic, antispasmodic, antimicrobial, anti-inflammatory, antioxidant properties.

The polyphenolic compounds (flavonoids and caffeic acid derivatives) from *Salvia sp.* (*Lamiaceae*) were analysed, quantitatively by a spectrophotometric method and qualitatively by high performance liquid chromatography (HPLC), before and after hydrolysis. We studied 7 indigenous species of *Salvia: S. officinalis, S. pratensis, S. austriaca, S. nemorosa, S. sclarea, S. verticillata, S. glutinosa.* The quantitative analysis showed us that the richest species in caffeic acid derivatives were *S. officinalis* and *S. glutinosa* and high concentrations of flavonoids were found in *S. glutinosa, S. verticillata* and *S. officinalis*. We identified the following compounds: caffeic acid, luteolin and apigenin in all species, and coumaric acid, ferulic acid, sinapic acid, only in *S. officinalis* and *S. sclarea,* by HPLC, using extracts of the leaves of plants in ethylic ether, ethylic acetate and n-buthanol. The higher concentrations of these compounds after hydrolysis showed us that in plants they had formed glycosides or esters. Rosmarinic acid was detected in *S. officinalis* by HPLC coupled with mass spectrometry (LC/MS/MS) in a concentration of 0,21%.

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CONTRIBUTIONS TO THE STUDY OF *TAMUS COMMUNIS*

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Keywords: Tamus communis, anti-inflammatory properties, diosgenin, steroidal glycosides, calcium oxalate raphides

Plant used since ancient times in the traditional medicine, *Tamus communis* is recommended in the therapy of rheumatic conditions (arthralgias) and as anti-inflammatory agent primarily for external and less internal use.To the purpose of turning to good account a species so little investigated, a morphostructural and chemical study was carried out, completed with a test for anti-inflammatory potential of the plant and the formulation of a pharmaceutical form to exhibit the anti-inflammatory properties indicated. The microscopic examination of the powder of *Tamus communis* rhizomes identified the following characteristic elements: numerous calcium oxalate raphides, fragments of suberized tissue, abundant starch, isolated fibers or associations with suberized tissue and reticulate woody vessels, large woody vessels of the reticulate type. The chemical qualitative analysis and the chromatographic examination led to the identification

of many groups of active ingredients (e.g. phytosterols, fatty acids, resinic acids, tannins, reducing simple sugars, saponine, mucilages). Among these, the steroidal glycosides with diosgenin as the main aglycon moiety are the most distinctive.The tests performed showed a reduced toxicity and significant anti-inflammatory action. These prompted the preparation of an ointment with anti-inflammatory properties.

STUDIES FOR IDENTIFICATION OF SOME BIOLOGICAL ACTIVE COMPOUNDS FROM INDIGENOUS PLANTS WITH THERAPEUTICAL VALUE

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Keywords: Traditional medicine, indigene flora, some biochemical compounds

From the indigene flora are known plants which through traditional medicine was used for the overweighting prevention.

Some of these plants are: *Allium sativum, Crategus monogyna, Ribes nigrum, Avena sativa, Acer campestre*.

Our study was based on composition identification in amino acids, sythosterols, total sugar, microelements (selenium) and some vitamines for obtaining a bioproduct with antilypogenic and lipolytic profilactic effect from these plants.

Analytical comparative results of obtained extracts show that the *Allium sativum* extract contained 8 essential amino acids, 4,48 g% total sugars and *Acer campestre* extract contained 4 essential amino acids, 10 phytosterols and 0,38 g% total sugars respectively.

DETERMINATION OF IRIDOIDES FROM *MELAMPYRUM CRISTATUM* L. SPECIES BY CHROMATOGRAPHYC METHODS

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Keywords: column liquid-chromatography, Melampyrum cristatum L. , iridoide, aucubina, identification

The study consist in qualitative and quantitative analysis througt methods of thin layer chromatography coupled whit photodensitometry as a high performance liquid chromatographic coupled with mass spectrometry (LC/MS). In CSS case, the chromatographic plate was scanned with photodensitometer Shimadzu CS9000 after pulverizetion with iron clorure, then for HPLC it used analytical column Atlantis HILIC 100 mm x 3.0 mm i.d., 3.5 µm (Waters), then source of ions ESI (electrospray ionisation). After CSS analysis the iridoides concentation exprimate in aucubina is 0.23 mg/ml in flowers and 0.29 mg/ml in leafs, then as LC/MS method the concentration is 1.0928 mg/ml in flowers and 0.8675 mg/ml in leafs.

ARBUTIN CONTENT IN THE ABOVE-GROUND PARTS OF SOME SPECIES OF THE *ERICACEAE* AND *VACCINIACEAE* FAMILY

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Keywords: Arbutin, Ericaceae, Vacciniaceae, HPLC

Arbutin is the major active constituent of the *Uvae ursi folium*, a well-known herbal drug used in the treatment of uncomplicated infections of the urogenital tract. It has been shown that the other species of the *Ericaceae* and *Vacciniaceae* family also contain arbutin in smaller quantities (1).

In this work we compared two RP-HPLC methods (the official method in Ph.Eur.5, using isocratic elution, and modified method, using gradient elution) for the analysis of the arbutin and hydroquinone content in the above-ground parts of *Arbutus unedo*, *Bruckenthalia spiculifolia*, *Calluna vulgaris*, *Erica carnea*, *Erica arborea* (all members of the *Ericaceae* family) and two *Vaccinium* species (*V. vitis-idaea* and *V. myrtillus*), analysing as well an comercial sample of *Uvae ursi folium*. Aqueous extracts (deccoctions) of plant material were prepared according to the monograph of *Uvae ursi folium* in Ph.Eur.5 (2). Exept in *Uvae ursi folium*, a significant content of arbutin was found in the leaves of *Arbutus unedo* and *Vaccinium vitis-idaea*.

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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF SEROTONINE FROM SEA BUCKTHORN (*HYPPOPHAË RHAMNOIDES* L.)

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Keywords: Hippophaë rhamnoides, serotonine, phytochemistry

Seabuckthorn (*Hippophaë rhamnoides*) has been researched during the last five decades in Romania and still remains an inexhaustible source of phytochemical substances very important in point of its biochemical composition and therapeutical value. The presence of serotonine (important brain mediator) in the fruits of our indigenous sea buckthorn was evinced a few years ago in the frame of some extensive research pursued in collaboration with I.N.C.D.C.F. Bucharest.

These tests were continued by the authors and expanded, including the studies of leaves, stems and the bark of sea buckthorn stems, the results being revealed within this present paper.

The vegetal material was dried, ground, sifted and extracted in acidulated watery medium.

The methods we used were CSS, fluorimetrical and HPLC.

Our results indicate values between 30 and 100 μg of serotonine/gram of vegetal material, the highest amount was depicted in the stem bark, leaves and a lower one in fruits.

THE POTASSIUM / SODIUM RATIO OF MEDICINAL SPECIES

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Keywords: K/Na ratio, medicinal plants, K supplementation

The presence of an adequate K:Na ratio (surpassing 100:1) in medicinal plants is considered to contribute, beside the content in various saponins and flavonoids, to their diuretic-aquaretic effect. The aim of the present study was to assess this ratio in vegetal products originating from 56 medicinal species, used in modern and traditional phytotherapy, but also to point out some products that could complete the recommended daily intake of K, an essential mineral macro element. The content of Na and K was measured by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The plant material originated from plant populations growing wild in the Western part of Romania. The K/Na ratio proved to be beneath 100 in the case of underground parts; the majority of aerial parts (herba), leaves and flowers had an advantageous K/Na ratio. Remarkable in this regard were *Agrimoniae herba, Urticae folium, Origani herba, Equiseti herba, Genistae tinctoriae herba, Rubi idaei folium, Menthae longifoliae folium, Millefolii flos, Sambuci flos, Tiliae tomentosae flos cum bracteis, Verbasci flos*, and *Crataegi fructus. Conversely, Cerasorum stipites* and *Cichorii radix* showed a low K/Na ratio. *Taraxaci herba, Violae tricoloris herba* and S*ambuci flos possess* a high K content; as such their aqueous extracts could be used for K supplementation.

PIGMENTS FROM THE AERIAL PARTS OF *NIGELLA DAMASCENA* L. AND *NIGELLA SATIVA* L. SPECIES (*RANUNCULACEAE*)

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Keywords: carotenoids, Nigella sativa, Nigella damascena, herba, Ranunculaceae, HPLC

Nigella sativa L. and *Nigella damascena* L. are two herbal species from the *Ranunculaceae family*, original in the Magreb's: Morocco, Algeria, Tunisia and Egypt and also in the Middle Orient: Syria. In the originated countries the plants are very frequently used for their seeds, *Nigellae sativae (damascenae) semen,* venerated by all the Muslim peoples and thus quoted in the Koran for the multiple therapeutic effects. Thus the black caraway's seeds, the popular name of *Nigella* L. *(Ranunculaceae),* are appreciated for their anti-inflammatory, carminative, antihelmintec, ocitocic virtues and as one of the best Muslim traditional spices used for bread and cheese products.[1, 2, 3, 4]

Although very well knows, the seeds of the above-mentioned species were the only morphological part investigated unlike the vegetal product *Nigellae herba* that has never been investigated.

In this study is mentioned for the first time the presence of the carotenoids in the stems of the plants *Nigella sativa* L. and *Nigella damascena* L.*(Ranunculaceae)*. The analysed plants have been cultivated and then harvested in Romania, Vladimirescu locality, Arad County, starting from seeds originated in North Africa (Morocco and Tunisia). The part of the plant analysed from a pharmacognostic point of view was *herba*, harvested and dried in appropriate conditions in June 2004.

The object of this study is to investigate the presence of carotenoids pigments in the stems of above-mentioned species. The investigating technique used was Very High-Pressure Chromatography (HPLC), after the extracting and saponification operations. Another important objective was to determine the quantity of the carotenoid pigments.[5, 6]

After using this techniques proved the presence of the pigments: lutein, zeaxantin, β-criptoxantin, α-caroten, β-caroten, neoxantin şi violaxantin, and the percentage of these carotenoids. This were also determined the percentage concentrations of the carotenoids in the vegetal product *Nigellae herba.*

The big importance of these investigations consists in two facts. One fact is that for the first time attention was drawn to the stems of the *Nigella (Ranunculaceae)* which was not investigated from a medical point of view before. The second fact is the well-known antioxidant effect on the free radicals the carotenoids from the plants have.[4, 5, 6]

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COMPLETE SCREENING OF THE CATIONS PRESENT IN THE SPECIES OF *ADONIS, HELLEBORUS, RANUNCULUS* FROM THE *RANUNCULACEAE* FAMILY

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Keywords: cations, Ranunculaceae, diuretic effect, atomic absorption spectroscopy

The species of *Adonis, Helleborus, Ranunculus* are herbal plants of the *Ranunculaceae* family with therapeutic actions well determined, but little investigated from the cations presence in the different morphological categories point of view.[1, 2, 3]

Although the studies on the content of different cations responsible for the therapeutic properties are limited or completely missing, the utilisation of these plants in therapeutically purposes in widely practised.

The object of this study is the investigation and the comparing of the content of different cations in the roots, stems, leaves, flowers and fruit in the above mentioned species, this being able to point the part of the plant most useful to obtain the diuretic effect. [4, 5, 6, 7, 8, 9]

By wet disintegration followed by spectroscopy techniques of atomic absorption: K^+, Na^+, Mg^{2+}, Ca^{2+}, $Fe^{2+/3+}$, Mn^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} cations were investigated.

As a common characteristic of the above mentioned types is their diuretic effect. From this point of view the presence of the Potassium cations become very important, and the utilisation spectrum is considerably increased.[10, 11]

This study actually presents a complete screening of the main cations present in the different parts of the many species of the *Ranunculaceae* family and the statistic analysis of a great number of experimental data have concluded to very precise results.

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EVAPORATIVE LIGHTSCATTERING – A VERSATILE ALTERNATIVE DETECTION METHOD IN NATURAL PRODUCTS ANALYSIS

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Keywords: evaporative light scattering, natural products, low UV-absorption, saponin, terpene

The detection of HPLC analytes which show no or only weak UV absorbance is restricted to rather specific (fluorescence and electrochemical detector) or "universal" detectors. Among the latter are mass-spectrometry, refractive index and evaporative light scattering detector (ELSD). Despite the fact that the principles of ELSD have been developed more than 30 years ago, it is still regarded as a rather exotic detection alternative, with little practical use and few applications at hand. Over the last decade the technique steadily evolved, so that from today's point of view ELSD can be considered a very reliable, economic and versatile mode of detection.

In this presentation the impact of ELSD on natural products analysis will be discussed. After some introductory remarks regarding detection theory, and methodological pros and cons, a number of ELSD applications is presented, including the analysis of terpenes in ginkgo (*Ginkgo biloba*), and the determination of saponins in soy (*Glycine max*) and primula (*Primula veris* and *P. elatior*). These examples will indicate the benefits of ELSD in natural products research, and arouse further interest in a novel, yet old detection method.

AN EXAMINATION OF PATENTS ON SIGNIFICANT MEDICINAL PLANTS IN EUROPE USED FOR HERBAL MEDICINAL PRODUCTS

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Keywords: Patent, Intellectual Property, Medicinal Plants, Traditional Medicine

A patent is a legal right granted by a government to the original and first discoverer or "inventor" of an new intellectual property, to exclude others from making, using or selling the subject intellectual property "invention" for a defined period of time. A patent is allowed by the grantor, only if the claimed intellectual property is deemed useful, novel, and unobvious to others "skilled in the art"(1).

Traditional medicines play an important role in the provision of health care in many developing countries. Their use is also significant in developed countries, increasing their commercial value. Researchers or companies may also claim intellectual property rights over biological resources and/or traditional knowledge, after slightly modifying them. Pharmaceutical companies have shown an interest in acquiring and developing traditional medicines, since "promising species" can provide important leads for the discovery of new drugs (2). The fast growth of patent applications related to natural medicines shown this trend clearly (3).

In this study, patent applications within the scope of the Patent Cooperation Treaty (PCT) for 15 medicinal plants selected from among those with the highest market shares in Europe were examined and discussed in detail.

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THE AYURVEDIC PHYTOIATRY AND ITS LONG WAY, FROM THE VEDDIC CIVILIZATION TO THE ACTUAL SCIENTIFIC ACHIEVMENTS

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Keywords: Ayurveda, sophiatry, phytoiatry, indian iatrobotany, academic phytoiatric research

This proceeding is a presentation of the sofiatric ayurvedic conception, as well theoretical as praxiological, in its hypostasis of medical expression of the high culture of the veddic history. As "*science of life*", Ayurveda is marked from its start by the empirical traditional knwledge about the botanical medicinal patrimony of India and by the works of some outstanding scholars as *Charaka* (VI-th century b.C.): "*Materia Medica*", it became a genuine scientific domain of medicine, as a result of the manysided accomplishments of the medical phytoiatric indian school, in the last decades, having as slogan: "*The medicine based on facts, the single possible and acceptable*". Using the knowledge accumulated during several milleniums about the indian

medicinal species of plants, the indian phytoiatry developed an exhaustive phytotechnical, phytochemical, phytopharmaceutical research, and also a phytopharmacological, experimental and clinical too.

The authors refer also to other phytoiatric indian domains as Unani and Siddha and the actual "Star" programme in Romania.

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CONSIDERATIONS CONCERNING THE MANYSIDED TARGETS OF THE "STAR" AYURVEDIC PROGRAMME

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Keywords: "Star" programme, information, education, prophylaxy, therapy

The build up of the Ayurvedic Medical Center in Romania was followed soon by the establishment of a multidisciplinary and manysided targeted "Star" phytoiatric ayurvedic programme whose endeavour was, from the beginning, based on the promotion of several domains:

The settlement in Romania of a long lasting and extensive prophylactic and therapeutic programme of Ayurvedic medicine.

The settlement at a national scale and international too, of the "Star" programme with special regard to Southeastern Europe.

The organization of a multidisciplinary research of manysided ascertainment of more and more medicinal properties of the so rich indian flora and their use for the health of romanian patients, of all people, as well the elaboration of new ayurvedic "Star" remedies.

A manysided co-work with romanian phytoiatricians.

An educative and academic informative activity.

Intensive co-work with mass-media and various links with the various layers of the public opinion. The proceeding refers also to the endeavour of The Chamber of Commerce and Industry of the Prahova County to contribute, to promote phytoiaty by its activities in various fields.

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A PRELIMINARY TRIAL CONCERNING THE "STAR" PHYTOIATRIC REMEDIES WITH PSYCHOTHERAPEUTICAL EFFECTS: ASHWAGANDA AND POTENT POWER

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Keywords: phytoiatry, ayurveda, "Star" programme, neurotic complains, Ashwaganda, Potent Power

The phytoiatric treatments of patients suffering of various psychic complain belonging to its various nosological entities are the subject of this communication.

It concerns a trial carried out in *The Clinical Psychiatric Hospital "Prof. Obregia"* Bucharest, with 52 patients, aged 40 – 65, suffering of: anxiety, depressive reactive states, stress conditioned neurotic polymorphous states, disadaptiveness, in confrontation with existential harm.

The treatments were carried out with the ayurvedic phytoiatric *"Star"* remedies *Ashwaganda* and *Potent Power*. These remedies were applied with tablet conditioned remedies, 30 days, during an intermission of the treatment with the classical remedies; the patients were not in an acute state but in a relative stable state, but nevertheless with still persisting above mentioned symptoms, with neurotic intensity. Both ayurvedic "Star" remedies were applied alternatively during the 30 days of treatment, with an obvoious efficient control of reactive neurotic anxiety and depression. Two cases, aged 22 and 24, that followed an anti-stupefying drugs treatment, became more sedated and better minded, while 14 patients with premature ageing and sexual disabilities benefited too, from the treatments with the above mentioned remedies, and elicited a manysided improvment.

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GAS CHROMATOGRAPHIC ESTIMATION OF AROMATIC HERBAL DRUG CONTENT IN THEIR MIXTURES

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Keywords: herbal drug, mixtures, determination, essential oils, gas chromatography.

In this article a gas chromatographic method was developed for estimation of aromatic herbal drug content in their mixtures subjected to application in bakery industry. Subjects of the characterisation were two herbal mixtures. The first of these contained, along with one non-aromatic drug (*Frangulae cortex pulvis*), pulverised mint leaf (*Menthae piperitae foilum*), caraway fruit (*Carvi fructus*) and parsley fruit (*Petroselini fructus*). The second one also contained one non-aromatic drug (*Cynarae folium pulvis*), and pulverised oregano leaf (*Origani heracleotici folium*) and coriander fruit (*Coriandri fructus*). In the basis, method consists of several steps, isolation of essential oil from single herbal drugs and their mixtures (1°), GC analysis of isolated oils (2°), selection of reference (marker) constituent in GC profile of each single oil (3°), and calculation of the contents of constitutive herbal drugs in their mixtures (4°). In the first instance (1°) essential oils were isolated and quantified in three repetitions in a Clevenger type apparatus, according to Ph. Jug. IV. In the next phase (2°), each of isolated essential oil samples was analysed by GC/FID and GC/MS to have complete insight in their composition. While selecting marker constituents in each of single oils (3°), the aim was to select the most abundant and the most specific components, whenever it was possible. Although the final calculation (4°) could be theoretically accomplished taking from GC profile of selected oil component of free choice, for this purpose the major oil constituents were typically selected. Results obtained approved our assumption that this approach could be successfully applied in current quality control practice of such and similar products.

OXYGEN SPECIES SCAVENGING ACTIVITY OF *BASILICI HERBA* EXTRACT

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Keywords: Ocimum basilicum L., polyphenolic compounds, scavenger, reactive oxygen species

Ocimum basilicum L. is a medicinal plant containing polyphenolic compounds, namely flavonoids and phenolic acids. Since polyphenolic compounds have high antioxidant activity, the ability of a *Basilici herba* extract to act as a scavenger of reactive oxygen species was investigated. This paper reports the inhibitory effect of *Basilici herba* extract on the generation of reactive oxygen species by photochemiluminiscence technique. The scavenging activity of the extract on superoxide radical and hydroxyl radical were also evaluated *in vitro*. Superoxide radical was generated by phenazine methosulphate / NADH system; the extract – induced prevention of nitroblue tetrazolium reduction by superoxide radical was measured. Hydroxyl radical was generated by $FeSO_4$ and assayed by evaluating deoxyribose degradation using thiobarbituric acid method. The effect of *Basilici herba* extract on Fe^{2+} mediated lipid peroxidation was evaluated in liver homogenate of rats in terms of thiobarbituric acid reactive substances. A phytochemical study of the extract was also undertaken. *Basilici herba* extract acted as a scavenger of reactive oxygen species. This activity may be due, most probably, to the presence of polyphenolic compounds.

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THE CHEMICAL PROFILE OF *MENTHA LONGIFOLIA* (L.) HUDS. LEAVES UNDER ANTIFUNGAL TOPSIN M TREATMENT. NOTE III.

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Key words: Mentha longifolia, Topsin M, chemical composition

The effect of Topsin M treatments on metabolism products of *Mentha longifolia leaves* was investigated. For this purpose, we carried out both qualitative and quantitative studies of treated variants comparative to untreated ones (control). Investigation of chemical composition of *Mentha longifolia* leaves showed that although generally, both treated and control plants contain a similar array of constituents, there are quantitative changes regarding volatile oils, polyphenolic compounds and soluble proteins in treated plants comparative to control. In fact, the volatile fraction is the most affected, qualitative and quantitative, under antifungal Topsin M treatments.

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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF A SAMPLE TYPE EXTRASOL, OBTAINED FROM *OCIMUM SPECIES*

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Keywords: Ocimum species, antimicrobial, gingival disorders

Knowing the outstanding antimicrobial potential of many volatile oils, potential doubled by anti-inflammatory and healing properties, we considered that some vegetal products will act as remedies in the treatment of gums disorders. The bacterial plaque represents an important key in gingival disorders. *Ocimum basilicum* L. (*Lamiaceae* fam.) is an indigenous plant, used in vernacular Romanian medicine for the antimicrobial and anti-inflammatory effects; Ocimum sanctum L. (*Lamiaceae* fam.), known as Tulsi, is a plant considered to be sacred by the hindus and lately tends to be used more often in the modern medicine.

We pursued qualitative and quantitative studies of the most important chemical compounds in the extract. Then the extract has been pharmacologicaly evaluated with *in vivo* tests on rabits by

thermographic methods. In the clinical studies the same extract showed a good activity in the treatment of some gum disorder, not very complicated, normalizing a series of index: color, volume, the marginal outline of gums and also diminishing the bleeding.

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PRELIMINARY STUDIES ON THE *CRATAEGUS MONOGYNA* AND *CRATAEGUS OXYACANTHA* (L) SPECIES AND THEIR IMPORTANCE IN THE TREATMENT OF CARDIOVASCULAR DISEASES

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Keywords: Crataegus, dry-weight extract, cardiac insufficiency

Our goal is to conduct studies on *Crataegus monogyna* and *Crataegus oxycantha*, two species used in treatment of cardiovascular affections in many countries. The indigen species have been studied from a phyto-chemical point of view, by using techniques such as thin-layer chromatography, gas-chromatography and HPLC (High Performance Liquid Chraomatography) combined with mass spectrometry (LC/MS/MS). In the next step, we created an original Romanian product, standardized in flavonoids, called CRATECORD, which was the subject to pharmacological and preliminary clinical tests. The phyto-preparation was tested in the next step on different patients diagnosed as cardiac insufficiency level II and III (NYHA), using 3x2 pills of 0.37 g dry weight extract/day during 8 days associated to the previous treatment for cardio-vascular disease. The results consist of improvement of the clinical parameters, electro-cardiogram, eco-cardiogram and the decrease of Pro-BNP.

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PRELIMINARY RESULTS OF CLINICAL TRIALS WITH *ROSMARINI FOLIUMPULVIS* 0,5G/CAPSULE IN THE TREATMENT OF CHRONIC LOW BLOOD PRESSURE

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Keywords: Rosmarini, chronic low blood pressure

In medical literature, Rosemary is noted to efficiently increase blood pressure in chronic low blood pressure patients. Our goal was to create a set of standardized pharmaceutical products made from Rosemary, which were subjected to preliminary clinical testing in order to prove their therapeutic efficiency in chronic low blood pressure. Species of rosemary found in Cluj-Napoca were used during the trial.

We tested the product as operculated pills containing 0.5 g powder of *Rosmarini* leaves produced at Faculty of Pharmacy, Cluj-Napoca, on different clinical studies. Two groups of patients suffering of chronic low blood pressure were selected: the first group was treated with 2x1 pills of *Rosemary* per day during 8 weeks and the second group without treatment (control). For all the patients of the first group (treated) the results consist of an improvement of clinical parameters and an increase of blood pressure.

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THE ANTILEISHMANIOSICAL ACTION OF SOME DIFFERENT EXTRACTS FROM *NIGELLA DAMASCENA (RANUNCULACEAE)*

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Keywords: Nigella damascene, antileishmaniosical action

Nigella damascena L. is an herbal species from the *Ranunculaceae family*, original in the Magreb's: Morocco, Algeria, Tunisia and Egypt and also in the Middle Orient: Syria. In the originated countries the species is very frequently used for their seeds, *Nigellae damascenae semen,* venerated by all the Muslim peoples and thus quoted in the Koran for the multiple therapeutic effects. Thus the black caraway's seeds, the popular name of *Nigella* L. *(Ranunculaceae),* are appreciated for their anti-inflammatory, carminative, antihelmintic, ocitocic virtues and as one of the best Muslim traditional species used for bread and cheese products.

One important action of the black seeds is their antitermic effect.[1, 2, 3, 4]

Leishmania infantum is one parasite very frequently in the mediteranian countries.

Different kinds of extracts from *Nigellae damascenae semen* et *Nigellae damascenae herba* were tested on three types of cells:

- THP1 cells: human monocytes on which we evaluated the toxicity of the products tested;
- two different forms of *Leishmania infatum*: promastigotes and amastigotes cells.[5, 6]

We founded a rather interesting activity on the *Leishmania* promastigotes with the dichloromethanic extracts of *Nigellae damascenae semen* et *Nigellae damascenae herba.*

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PHARMACOLOGIC RESEARCH ON DIURETIC, SALURETIC AND URICOSURIC ACTIVITY OF SOME EXTRACTS FROM *HIERACIUM PILOSELLA* L. (*ASTERACEAE*)

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Keywords: Hieracium pilosella, diuretic activity, saluretic activity

Hieracium pilosella L., known as mouse-ear hawkweed, is a perennial plant, of little size, with a horizontal rhizome with numerous overground stolons. Although the plant's aerial part (*Pilosellae herba*) is used in traditional medicine as diuretic, the pharmacologic properties of this species were not studied before in Romania. In order to test the diuretic, saluretic and uricosuric properties of *Hieracium pilosella*, four lots of Wistar rats, weighing around 150g were used. The animals were administered orally an aqueous and an alcoholic extract from the plant. Furosemid was used as a control substance. The animals were placed in special diuretic cages, urine being collected after 24 hours. Diuresis was expressed in ml/kg/24h, and also the concentrations of Na^+ şi K^+ were determined by a potentiometric method in the collected urine, being expressed in mM/kg/24h. Uric acid concentration was also determined spectrophotometrically and expressed in mg/kg/24h.

All the extracts prepared from *Hieracium pilosella* showed a diuretic and saluretic activity superior to the wittness lot, but inferior to furosemide. On the other hand, only the alcoholic extract showed uricosuric properties.

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GENOTOXIC AND ANTIGENOTOXIC POTENTIAL OF BASIL (*OCIMUM BASILICUM* L.)

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Keywords: basil, E. Coli, S. cerevisiae, reversion tests, Comet assay

The genotoxic and antigenotoxic properties of essential oil (EO) of basil (*Ocimum basilicum* L.) and its major constituent linalool were examined in bacterial and yeast tests. In the Salmonella/microsome plate incorporation assay, performed in TA100, both derivatives showed no mutagenic effect, with or without metabolic activation. In E. Coli WP2 test, EO and linalool have significantly inhibited spontaneous and *t*-BOOH-induced mutagenesis in mutant deficient in induction of antioxidative enzymes ($OxyR^-$). In eukaryotic test on *S. cerevisiae* D7, EO and linalool showed co-mutagenic effect with *t*-BOOH. Antigenotoxic potential of linalool was tested in Comet assay on *S. cerevisiae*. Linalool exhibited protective capacity against H_2O_2-induced comets both in pre- and co-treatment experiments. Obtained results indicate that antimutagenic and antigenotoxic potential of basil derivatives could be attributed to their antioxidative properties. Nevertheless, they could also cause oxidative damage and mutagenic effect, depending on the dose and the test system. Regarding the detected antimutagenic potential against oxidative mutagenesis and protective effect against H_2O_2-induced DNA strand breaks, essential oil of basil and linalool could be recommended for further testing on higher organisms.

FOLIAR BUD EXTRACTS: CUTANEOUS TESTING REVEALS THEIR POTENTIAL IN DERMATOLOGY

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Keywords: foliar buds, coetaneous therapy, O/W basis, TEWA metry, corneometry

The present research evaluates the possibility to extend the therapeutic application of gemmotherapy in the field of local coetaneous therapies. Characterized by an intense metabolic activity, buds are rich in microelements, vitamins, enzymes, vegetal hormones, free amino acids, polyphenols etc. The presence of these substances argues in favor of their beneficial employment in dermatologic disorders and cosmetology. In order to evaluate the potential of buds in this regard, hydro-glycero-alcoholic extracts (1:1:1) were prepared from fresh buds of *Aesculus hippocastanum, Alnus glutinosa, Populus nigra* and *Ribes nigrum*, according to the provisions of the Xth French Pharmacopoeia. After the evaporation of ethanol, the fractions were incorporated in ointment basis of O/W type. The stability of the obtained ointments was assessed microscopically (Nikon Eclipse E60). Subsequently, dermatologic tests (corneometry, TEWA metry) were performed, using the ointment basis as control. The degree to which the preparations influence skin barrier function, skin texture, moisture, rugosity was evaluated. All analyzed parameters were positively influenced after the application of ointments containing bud extracts, uncovering novel possibilities for their utilization in dermo-cosmetology.

EFFICACY STUDIES ON VEGETAL EXTRACTS FROM *MAHONIA AQUIFOLIUM* AND *AESCULUS HIPPOCASTANUM* OF TOPICS FORMULATIONS

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Keywords: Mahonia aquifolium, Aesculus hippocastanum, dermatological formulations

Psoriasis and atopic dermatitis are chronic complex disorder of skin, genetically determined and characterized by inflammatory infiltration and vascular dilatation, xerosis, excariations, prurit.

Topical treatment of this dermatosis remains the most efficient therapy, free of risk and the most applied by medical practice. *Mahonia* extract (from bark of roots and stem), by its isoquinolinic alkaloids inhibits the cell proliferation and has on anti-inflammatory and immuno-stimulation activity. This alternative led to the preparation of two dermatological formulations with 1% *Mahonia* extract spiss. Clinical trials after 6 weeks of treatment on 30 patients (different forms of psoriasis) we observed an improvement of symptoms.

The horse chestnuts seed and bark extract (*Aescullus hippocastanum*) 4% in Synoderm basis (GMbH – Germany) reduces the erritation in 95% cases. The natural substances with antiradicals and anti-irritant effects can replace with success the topic treatment with corticosteroids.

IN VITRO SPECIFIC ANTIMICROBIAL PROPERTIES OF *HIBISCUS TRIONUM* L. EXTRACTIVE SOLUTIONS

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Keywords: antimicrobial, Hibiscus trionum, Kirby-Bauer diffusimetric method

Hibiscus trionum L. (Malvaceae) used in empirical treatment for the renal and hepatic disturbances [1], is an annual plant that originally grew to the east of the Mediterranean but it spread throughout Europe both as a weed and cultivated as a garden plant.

In the present study there were examined the antimicrobial activity of two aquose extractive solutions obtained from *Hibiscus trionum* leaf [2]. Analysis method chosen was Kirby-Bauer diffusimetric standardized method. As inocul was used standard references ATCC stems and isolated stems from biological products proceeded from patients. Antimicrobial action was confirmed for all tested stems: *Salmonella enteritidis*, *Shigella sonnei*, *Staphylococcus aureus*, *Bacillus proteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes.* The quantitative antibiotic sensitivity tests and concentration were performed on D.T.S. (QM 261 Oxoide) agar, 10% and 20% aquos extracts presenting inhibitions zone with close values.

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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *HELICHRYSUM ITALICUM* L., *ASTERACEAE*

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Keywords: Helichrysum italicum, essential oil, antimicrobial activity, diffusion technique, MIC

In this paper, antimicrobial activity of essential oil of *Helichrysum italicum* was investigated. Plant material was collected from three different locations. The essential oils were obtained by hydrodistillation and analyzed by GC and GC/MS using FID and MSD. The main components of the oil from Serbia were cyclofenchene (14,20%), ar-curcumene (9,88%), *β*-selinene (4,60%), cedrene (3.63%), caryophyllene oxide (3,54%) and neryl acetate (3,23%). The major components of the oil from Dalmatia were ar-curcumene (8,39%), cyclofenchene (7,33%) and *β*-selinene (4,88%), while the oil from Herzegovina contained *β*-selinene (5,89%), neryl acetate (3,42%) and ar-curcumene (3,01%), as the most abundant constituents. The antimicrobial properties of essential oil of *Helichrysum italicum* from Serbia were tested against the following bacterial species: *B. subtilis, B. cereus, Bifidobacterium* sp., *Corynobacterium* sp., *E. coli, Klebsiella* sp., *L. acidophilus, L. fermentum, L. plantarum, L. rhamnosus, Listeria monocytogenes, P. vulgaris, P. aerugenosa, S. enteritidis, Shigella* sp., *S. aureus,* and fungi *Candida albicans, Alternaria* sp. and *Aspergillus niger.* The diffusion technique was used for testing the antimicrobial activity, and the MIC was determined by broth dilution method. The essential oil of *H. italicum* showed high antimicrobial activity.

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ANTIBACTERIAL ACTIVITY OF THE OIL FROM *MENTHA VIRIDIS L.* AND *MENTHA PIPERITA L.*

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Keywords: Antibacterial activity, Mentha viridis L., Mentha piperita L.

In our studies on the efficacy of herbal extracts, we have been tested for in vitro antibacterial activity the volatile oils derived from the *Mentha piperita L* and *Mentha viridis L* (originate in vitro cultures). *Materials and methods*. We obtained three volatile oil samples from the aerial parts by steam distillation (named *M. viridis* II – oils obtained from the material harvested in

2004, from plants in their second vegetative year, *M. viridis* I, the corresponding oils from same vegetal material in first vegetative year, and *M. piperita*). *Antibacterial activity*. The qualitative and quantitative antibacterial assay of the oils were determined using the disc diffusion method and broth dilution technique. Minimum inhibitory concentrations (MICs) were recorded after 24 h of incubation at 35^0C, as the lowest concentration that inhibited the development of visible growth. *Tested microorganisms* were: *Staphylococcus aureus* ATCC 25923, *S. epidermidis*, *Sarcina lutea* ATCC 9341, *Bacillus cereus*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*. *Results*. The antibacterial activity of volatile oils is attributed to menthol and menthone. The sample of *M. viridis* II is superior in antimicrobial activity, amongst the oils tested, with a MIC values between 0,7- 47 mg/l against bacteria. The antifungal activity tested against *C. albicans* showed a pronounced activity with MIC of 0,4 mg/l for both *M. viridis* II and *M. piperita* samples and 1,5 mg/l for *M. viridis* I. *In conclusions*, the data obtained in the antibacterial assay indicate that the oil from *M. viridis* II was generally superior against tested strains when compared to the *M. viridis* I and *M. piperita*.

THE FUNGAL AUTOCHTHONOUS BIOPREPARATION ERGO-1, A NEW POTENTIAL ANTITUMORAL CHEMOTHERAPEUTIC AGENT

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Keywords: ergolinic extract, cytostatic agents, pharmacodynamic effect, action mechanisms

A major direction of the contemporary chemicopharmaceutical research is the discovery of new cytostatic agents for improving antineoplastic chemotherapy effectiveness. The purpose of the present paper is to investigate the reactivity of the tumoral cells to the action of the Ergo-1 autochthonous ergolinic biopreparation – obtained from submerged strains of *Claviceps purpurea* – for highlighting and appreciation of its antitumoral significance.

In vitro and respectively, in vivo treatment of HeLa or HEp-2p tumoral cells cultures and respectively, of rats bearing Walker 256 carcinosarcoma or Guérin-T8 lymphotropic epithelioma, has conditioned the expression of Ergo-1 cytostatic property, its evaluation being assured by comparative analyses of the evaluation indices values registered by us – on adequate experimental models – with those required by references screening programs.

The significance of the pharmacodynamic effect, the establishment of the action mechanisms –of membranary and metabolic type – implied in its induction, the estimation of moderate toxicity degree of the ergolinic extract upon healthy cells of the animal aggressed of neoplasm allow the preclinical pharmacological characterization of Ergo-1 biopreparation as cytostatic agent, its biomedical importance will be revealed by the clinical trial.

THE ANTIFUNGAL ACTIVITY OF *ALOË ARBORESCENS* FRESH LEAVES HYDROALCOHOLIC EXTRACT

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Keywords: Aloë arborescens, aloine, antifungal activity, chromatogram, phytopathogenic fungi

The total hydroalcoholic plant extract was obtained from fresh leaves of *Aloë arborescens* plants, cultivated in greenhouses, by the method described in literature (Anonymous, 1993).

The quantity of aloine in the plant extract was determined by a high-performance liquid chromatography method coupled with mass spectrometry, compared to Fluka aloine (Wu et al., 2005).

The antifungal activity of *Aloë arborescens* ethanolic plant extract was tested against the mycelial growth of *Aspergillus niger, Botrytis cinerea, B. gladiolorum, Fusarium oxysporum* f. sp. *gladioli* and *Penicillium gladioli* phytopathogenic fungi, by the agar dilution method.

The minimum fungicidal concentration (MFC) of *Aloë arborescens* plant extract varied between 60 – 100 µl/ml, depending on the fungal species. The total hydroalcoholic plant extract from fresh leaves of *Aloë arborescens* had a more powerful antifungal activity than aloine, at the same concentration.

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LICHEN METABOLITES – NEW THERAPEUTIC AGENTS. *IN VITRO* STUDY OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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Keywords: lichen metabolites, antimicrobial, antioxidants

The aim of our study was to determine some of the pharmacological properties of 3 lichen species from Romania's spontaneous flora: *Usnea barbata* (L) Wigg., *Parmelia physodes* (L) Ach. and *Evernia prunastri* (L) Ach. The 3 lichen species were extracted successively with chloroform and acetone using a microwave installation. By spectroscopic methods and TLC a total of 18 known compounds (terpenes, xanthones, depsides, depsidones, depsones, dibenzofurans, usnic acids, antrachinones) were identified in the 3 species.

In order to evaluate the antimicrobial activity, the lichen extracts were tested against *Staphylococcus aureus* ATCC 6538 P, *Bacillus subtilis* NCTC 2589, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. The protective effect against lipid peroxidation was investigated in rat liver homogenates.

The results showed that lichen extracts did not inhibit the Gram (-) bacteria but were active against Gram (+) bacteria and fungi. Also, the extracts exhibited antioxidant activity decreasing CCl_4-induced lipid peroxidation in a concentration-dependent manner.

The present study indicates that the unusual chemicals present in lichens and their biological activities represent a new challenge for the medicine.

CORELATION BETWEEN BIOCHEMICAL CHARACTERISTICS AND SPECIFIC ACTIONS OF AROMATIC AND MEDICINAL PLANTS USED IN SKIN THERAPEUTICS

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Keywords: skin, therapeutics, aromatic plant, medicinal plant

Our skin, as a monumental facade, is agressed in time, by different external agents, both abiotics (extreme temperatures, rain, wind) and biotics (infections, insect stings), which can release a numerous unhealthy affections.

The phytotherapeutical treatments are based on utilization of vegetal species with antibiotic, disinfectant, cicatrizing, antiseptic and antiinflammatory qualities.

In these treatments an important role is held by the aromatic species, which contain mainly oils composed of terpenes, aldehides, cetones, phenols, hydro carbons, alcohols etc, in various proportions, which give specificity to fragrances and physical, chemical and therapeutic properties.

Gradually, the use of aromatherapy has extended to most of the countries on all the continents, especially in Spain, Italy, France and England.

THE ANALYSIS OF SOME SEMISOLID PHARMACEUTICAL FORMULI WITH EXTRACTS FROM BUDS AND LEAVES OF *BETULA PENDULA* ROTH

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Keywords: birch tree, leaves, buds, and semisolid formula

The aim of the study was the formulation and the quality and stability analysis of some semisolid type (creams) pharmaceutical preparations, with vegetal active principles proceed from birch tree. The final applicability of these formulas is important in dermo-cosmetical field. The extracts were made from dry vegetal material (leaves, buds), by extraction with Soxhlet device and for the buds concordant with French Pharmacopoeia, X-th Edition, by maceration, as hydro-glycero-alcoholic forms (1:1:1). These were incorporated in formula with polymers and anionic surfactant (sodium lauryl sulphate). The stability was tested by microscopy, with Nikon Eclipse E60 microscope and Coolpix digital camera. The dermato-cosmetical effect of the formula was evaluated instrumentally, by "in vivo" moistening determination (Corneometry), the remake of the hydro-lypophile layer integrity, (Tewa-metry) and of the skin microrelief – roughness, velvety, texture parameters - (Skin – Visiometer coupled with Visioscan).

The main conclusion of the study was that the analysed preparations have presented the quality and stability demands which were satisfied and that indicates future cosmetically and therapeutically applications.

IN VITRO RADIOPROTECTIVE EFFECT OF A TOPICAL GEL WITH COLLAGEN-GREEN TEA MIXTURE

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Keywords: collagen, green tea, gels, antioxidant, radioprotection

Our study reports the radioprotective properties of a hydrogel made of collagen (COL) and a green tea (*Camellia sinensis*) extract (GT). Mixtures of COL-GT were prepared with increasing quantities of COL (50:1, 100:1, 200:1). Chemical analysis of COL-GT mixtures showed the presence of high polyphenol content (~30 mg % of cafeic acid). Their antioxidant potential was nearly 15 times higher than that of the standard (Trolox). The metal chelation activity exhibited by the COL-GT mixtures was found to increase in a concentration-dependent manner. The higher COL-GT concentration, the higher inhibition percent of iron-2,2'-bi-pyridyl complex formation was observed. The percent of *in vitro* adhered cells to these mixtures varied but was similar to the control. All these biochemical and biological tests indicated that the ratio of 100:1 COL:GT was optimum and hydrogels containing 10%, 15% and 20% mixture, respectively, were prepared. Their radioprotective effect was evaluated by the MTT assay on epithelial cells irradiated with UV light. Results indicated an increase in the percent of survival cells in case of cells treated with hydrogel containing 20% COL-GT mixture before radiation. In conclusion, COL-GT mixtures and the product conditioned as hydrogel could provide *in vitro* radioprotection, through various mechanisms that might synergistically act *in vivo*.

THE EFFECT OF *CHELIDONIUM MAJUS* L ON ACUTE EXPERIMENTAL INFLAMMATION

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Keywords: Chelidonium majus, inflammation, phagocytosis, nitric oxide

The present study evaluated *Chelidonium majus* L anti-inflammatory properties through the effect on the phagocytes activity and L-arginine-nitric oxide pathway, nitric oxide being an important inflammatory mediator. There have been used 5 groups of Wistar-Bratislawa male rats. The inflammation was induced by turpentine oil. *Chelidonium majus* L was administrated i.p. using two dilutions: 0,20 % and 0,02% expressed in chelidonine. The groups with inflammation and those treated with *Chelidonium majus* L were compared with animals that were treated with L-NAME or diclofenac. After 24 hours from turpentine administration blood samples were harvested for the *in vitro* phagocytosis test, total leukocyte count, differential leukocyte count expressed as a percentage and serum nitrite/nitrates determination (Griess). Conclusions: 1.*Chelidonium majus* L reduced phagocytes activity and nitric oxide synthesis; 2.The higer dilution of *Chelidonium majus* L had a stronger antiinflammatory effect; 3.The effect of *Chelidonium majus* L was smaller than that of L-NAME and diclofenac.

INFLUENCE OF *AJUGA GENEVENSIS* L. ALCOHOLIC EXTRACT ON RESERPINE'S GASTRIC EFFECT

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Keywords: Ajuga genevensis, gastric ulcers, reserpine, rat

There is evidence in popular medicine about antiulcerous effects of the plant *Ajuga genevensis* L. In order to test this, we studied *Ajuga genevensis* L. alcoholic extract (EAA) in gastric ulceration induced by reserpine in rat.

We performed an acute experiment on 5 groups of randomized albino Wistar_Bratislava rats, a control group, a reserpine (i.p.) treated group and 3 groups of reserpine and EAA (gavage) treated groups, in 1:2, 1:4, and 1:8 dilutions respectively. Animals were sacrificed and morphometric parameters and ulcerogenic index was determined. Statistical analysis using ANOVA test were considered significant at $p < 0.05$.

EAA does not influence body weight, morphometric parameters of rumen and glandular area. At high dilutions EAA doesn't act upon incidence, number and severity of gastric lesions. At high concentration EAA reduces number and severity of gastric ulcerations and diminishes muscular tonus of the stomach.

In conclusion, EAA at high concentrations has a mild gastro-protective effect against ulcerogenetic action of reserpine and extends gastric evacuation.

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THE INFLUENCE OF VARIOUS DOSSES OF DRIED EXTRACT *ELEUTHEROCOCCUS SENTICOSUS* MAXIM. ON WEIGHT OF THE LAYING HENS

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Keywords: weight, laying hens, Eleutherococcus senticosus, dried extract

It was observed changes in hen weight after administration of dried extract of Eleutherococcus senticosus Maxim. The laying hens Hisex braun (n=35) were divided into 3 groups. The control group (CG) with 13 birds. For the 1. experimental group 1. EG (n=10) was administered extract in dose of 0.1 % concentration in food. In 2. experimental group (2.EG) was administered extract in dose of 0.5 % concentration in food. The weight of hens was observed every week during 8 weeks. Concerning weight hens the results of this study indicate that addition of dried extract of Eleutherococcus senticosus were not statistically significant. Despite of this the higher weight hens in the 2. experimental group during all experiment was discovered.

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THE INFLUENCE OF *ELEUTHEROCOCCUS SENTICOSUS* MAXIM. ON EGGS PRODUCTION OF THE LAYING HENS HISEX BRAUN

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Keywords: Eleutherococcus senticosus, adaptogene, poultry, eggs production, laying hens

In this study was examined changes in hen eggs production after administration of dried extract of *Eleutherococcus senticosus* MAXIM. Laying hens (n=35) were divided into 3 groups. The control group (CG) consisted of 13 birds. In the 1. experimental group (1.EG, n=10) was administered extract of *Eleutherococcus senticosus* in dose of 0.1% concentration in food. In experimental group (2.EG, n=12) was administered extract of *Eleutherococcus senticosus* in dose of 0.5 % concentration in food. The laying of eggs were observed every week during all experiment - 8 weeks. Concerning eggs production the results of this study indicate that addition of *Eleutherococcus senticosus* extract were not statistically significant. Despite of this the higher eggs production in the 2. Experimental group during all experiment was discovered.

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RECENT BIOACTIVITY ASPECTS ON TURKISH *PISTACIA VERA* L.

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Keywords: Pistacia vera, Anacardiaceae, pistachio, bioactivity

Pistacia vera L., a member of Anacardiaceae family, is native to Asia minor and widely distributed throughout the Mediterranean region. *P. vera*, among eleven species of *Pistacia* genus, is by far the most economically important species. As a Mediterranean country, Turkey is one of the major pistachio producers in the world, with the share of 19.5%. On the other hand, pistachio nuts are well-known for their rich essential fatty acid (linoleic and linolenic acids) content, vital for human health.

Pistacia species have been so far reported to have a number of biological activities such as anti-atherogenic, hypoglycemic, anti-oxidant, anti-inflammatory, and anti-insect activities. Besides, the oleoresin of *P. vera* was recorded to be used against asthma in Turkish folk medicine.

In this presentation, the recent results of biological activity studies, including antibacterial, antifungal, antiviral, anti-inflammatory, antinociceptive and antiprotozoal activities, carried out on *P. vera* growing in Turkey will be extensively given.

HYPOGLYCEMIC ACTIVITY AND ANTI-DIABETIC ACTIVITY OF *CASSIA FISTULA* FLOWERS IN NORMAL AND DIABETIC RATS

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Keywords: Cassia fistula, anti-diabetic, streptozotocin, rats

Aqueous extract of flowers of *Cassia fistula* (Leguminous) was evaluated for anti-diabetic activity in normal and streptozotocin induced diabetic rats. Results showed significant ($p<0.001$) reduction in blood glucose levels in both normal and diabetic rats. Further the extract also caused significant change in antioxidant enzyme levels and histopathological findings. It was concluded that aqueous extract of *Cassia fistula* flowers was found to possess anti-diabetic and anti-oxidant activity.

RESEARCHES TO OBTAIN SOME PHYTOTHERAPEUTICAL PRODUCTS FROM FLAVONOIDIC COMPOUNDS TO TREAT MENOPAUSAL OSTEOPOROSIS. NOTE 2. STUDIES REGARDING FLAVONOIDS EXTRACTION

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Keywords: extraction, flavonoid compounds, osteoporosis

The aim of our study was to establish the optimum extraction parameters (extraction type, extraction solvent, the ratio vegetal product/ solvent, extraction time) and to obtain selective extracts which are standardized, enriched with flavonoid compounds (flavones, polyphenols, isoflavones) and aminoacids.

The vegetal extracts from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos* were quantitatively and qualitatively analyzed by phytochemical methods.

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RESEARCHES TO OBTAIN SOME PHYTOTHERAPEUTICAL PRODUCTS FROM FLAVONOIDIC COMPOUNDS TO TREAT MENOPAUSAL OSTEOPOROSIS. NOTE 3. FLAVONOID – BASED PHYTOMEDICINES TO TREAT MENOPAUSAL OSTEOPOROSIS

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Keywords: flavonoids compound, phytotherapeutical products, osteoporosis

The aim of our study was to obtain phytotherapeutical products comprising flavonoids-containing plant extracts from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos*, and vegetal powders from *Spirulina platensis*, *Equiseti herba* and *Medicago herba*, and methods of manufacturing them.

We have made the phytochemical quantitative and qualitative analysis by HPTLC, UV-VIS spectrophotometry and atomic absorption spectrophotometry in order to determine the active principles (flavonoids compounds, aminoacids and minerals) from the phytotherapeutical products with an important impact on the treatment of menopausal osteoporosis.

CHEMICAL AND MICROBIOLOGICAL COMPARATIVE STUDY OF THREE PROPOLIS EXTRACTS

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Keywords: Propolis, phytochemical study, antimicrobial activity

A natural mixture of bee pollen and other substances produced by honey bees, propolis offers significant anti-inflammatory and antimicrobial benefits. Not surprisingly, researchers find propolis, or "bee glue," another medicinal marvel from the beehive. Chemically speaking, propolis is a very complex mixture; its chemical elements vary according to its source. Initially our study was focused on phytochemical evaluation of three different extracts of propolis provided by specialised production laboratories. We characterized the main groups of active compunds in the three samples from a qualitative (for flavonoids, polyphenolcarboxilic acids – TLC analysis) and quantitative (for polyphenols and flavonoids) point of view. The extracts were

further submitted to microbiological tests that showed strong antimicrobial properties against various gram positive and gram negative bacterias.

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EFFECTS OF ANTHRACYCLINE – COMBINED THERAPY ON LIVER METABOLIC FUNCTION IN MICE

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Keywords: Animal models, antineoplastic agents, liver, biochemical parameters

It was investigated the way in which anthracycline-combined therapy changes the metabolic function of liver in experimental animals. Doxorubicin causes formation of free oxygen radicals (ROS) leading to lipid peroxidation of biological membranes, resulting in cardio- and hepatotoxicity. Our research was aimed to establish if and how sellenium ion, N-acetylcysteine (NAC), sodium salt of monoketocholic acid (MKH) and superoxide-dismutase (SOD), affect the possible cytotoxic consequences of anthracycline- combined therapy with doxorubicin (D), vincristin (V) and prednisolone (P), all together (DVP). The following biochemical parameters were investigated: activities of peroxidase (Px), catalase (CAT), xanthine-oxidase (XOD), in the mice liver as experimental model. All used agents statistically reduced Px activity, mostly pronounced by DVP+MKH.. The tendency to reduce the activity of this enzyme by SOD, MKH and DVP+MKH, have been recorded. Treatments with DVP+SOD increased the activity of DVP, SOD, MKH, but statistically insignificantly, while treatments with DVP+SOD, DVP+SE and DVP+MKH reduced XOD activity, mostly pronounced in case of MKH also statistically insignificantly. It was found that NAC and Se reduced activity of this enzyme. NAC, SE, DVP, MKH and DVP+MKH caused reduction in CAT activity, while DVP+SOD and DVP+SE increased its activity.

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MEDICINAL PLANTS FOR WOUND HEALING OBTAINED BY CELL CULTURE TECHNOLOGIES

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Keywords: Achillea millefolium extract, Equisetum arvense extract, wound healing, collagenous gel

In vitro tests are now widely employed in ethnopharmacological research because of ethical and their usefulness in bio-active guided fractionation and determination of active compounds. Since the biochemical mechanisms underlying disease and healing processes are now better understood, a variety of *in vitro* tests can be employed for many disease conditions. In the present work we tried to demonstrate the positive role of *Achillea millefolium* (L.) and *Equisetum arvense* (L.) on *in vitro* wound healing models. These plants may be good potential agents for wound healing. It has been proven that they exhibit remarkable antiinflammatory, antioxidant, antimicrobial properties. We checked the activity of normal human cells (fibroblasts, endhotelial cells) involved in wound healing. *In vitro* studies of the aqueous extracts from the aerial parts of these plants demonstrated enhance viability, adhesivity and spreading of fibroblasts grown on collagenous gel. Enhanced proliferation and diferentiation of endothelial cells grown on Matrigel in an *in vitro* angiogenesis model were also detected. By SDS-PAGE zymography we observed that the plant extracts modulated a slight stimulation of some specific proteinases (gelatinases) expression during the wound healing process. The scientific approaches used for the study of the two traditional plants for wound healing provide us an important platform for rigorous testing and evaluation of their clinical efficacy based on accepted rules of evidence.

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CYTOTOXIC LIGNANS IN PLANTS AND *IN VITRO* CULTURES FROM *LINUM* SPECIES

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Keywords: lignans, Linum, cytotoxicity

In our research on lignans in *Linum* species, *Linaceae*, we have investigated the Balkan and Krim endemic species *L. tauricum* Willd. ssp. *linearifolium* (Podp.) Petrova and *L. tauricum ssp. tauricum* (Willd.) Petrova, belonging to the Section Syllinum. *In vitro* cultures of the both species were initiated. Two main lignans - MPTOX and 4'-demethyl-6-methoxypodophyllotoxin, which differed from MPTOX in the absence of the 4'-O-methyl group, were isolated from the plant and in vitro cultures of *ssp. tauricum*. Podophylotoxin (PTOX) and 6-methoxypodophylotoxin (MPTOX) were identified from *ssp. linearifolium*. The compounds were identified by comparison of HPLC retention time and UV spectra with those of authentic

samples - MPTOX (retention time 11.95 min), 4'-demethyl-MPTOX (Rt = 8.68 min) and PTOX (Rt =9.32 min).

The antitumor effect of extracts by the MTT-dye reduction assay on human leukemic cell lines were tested. The cytotoxic activity of extracts correlated with the content of podophyllotoxin derivatives, which were determined by HPLC. 4'-DM-6-Mptox, isolated from *L. tauricum ssp. tauricum* was found to exert prominent cytotoxicity, with IC_{50} values being several-fold lower than those of the referent antineoplastic agent etoposide.

ALLIUM URSINUM L.: A POTENTIAL SOURCE FOR COMPLETING THE RECOMMENDED DAILY INTAKE OF ESSENTIAL MINERAL ELEMENTS

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Keywords: Allium ursinum, content in mineral elements

Wood garlic (*Allium ursinum*) enjoys a large popularity in Romania, its leaves being consumed fresh, as salad (in spring) or dried, as a spice. It also possesses therapeutic qualities, due to its content in various sulfur-containing compounds. The aim of the present study is represented by the extensive investigation of the inorganic components of Alii ursini folium, little researched up to the present. Forty-seven elements were analyzed in samples from five populations of wild-growing wood garlic. The mineral content in the vegetal product was measured by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma - Mass Spectroscopy (ICP-MS). Mercury was assessed by atomic fluorescence. Of the analyzed elements, K concentration is the highest (29.3 g/kg dry matter), followed by Ca (14.6 g/kg), Mg (2.3 g/kg), Na (163.6 mg/kg) and Mn (93.6 mg/kg). The content of toxic metals in the samples varies, being below the admitted limit for medicinal plants in case of mercury (0.02 mg/kg), lead (2.75 mg/kg), and cadmium (0.19 mg/kg). The data encourage the consummation of wood garlic leaves, as they also complete the daily intake of essential mineral elements; 5 g dried leaves contribute with 15% of the normative daily intake (NDI) for Ca, 10% of the NDI for K, and 6% of the NDI for Mg.

SOME ASPECTS CONCERNING THE USE OF CERTAIN TOXIC PLANTS IN PHYTOTHERAPY

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Keywords: phytoterapy, spontaneous flora, toxic plants

In the spontaneous flora of Romania, there are numerous species featuring phytoterapeutical properties. Some of them are regarded as toxic and some their use in phytoterapy needs quite careful precautions, especially when they are empirically used.

Poisonous species could be used generally for infusion, decoct, tinctures, forms etc all alone or mixture but the quantity must be very few for avoid intoxication. The paper aims to present the most important herbs that could have toxic effect, the poisoning symptoms and their medicinal use.

ULTRASTRUCTURAL EFFECTS OF *NIGELLA SATIVA* TOTAL ALKALOIDS EXTRACT AT LIVER LEVEL (*MUS MUSCULUS*)

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Keywords: Alkaloids, Nigella sativa, deuterium-depleted water, Mus musculus, radio protective effect

The researches regarding the effect of the Depleted Deuterium Water (DDW) on animals (mice) reveal its protective action to X-rays (Bild et al., 1999). Somlyai reported in 2001, the successful treatment with DDW in cancer patients in Hungary. The alkaloids present a radioprotective effect in *Mus musculus*, established by cytogenetics and biochemical investigations (Corneanu et al., 2004).The single or combined effect of a bioactive substance (a total acid alkaloid extract obtained from *Nigella sativa* seeds), administered in deuterium-depleted water (DDW), against a stress factor (the whole body X-irradiation), on the liver ultrastructure of *Mus musculus*, was analysed. The single action of X-rays induced alteration of the hepatocyte structure. DDW induced some minors modifications, especially the increase of the lysosome numbers, and the cell metabolic activity. The total acid alkaloid extract from *Nigella sativa* seeds, in DDW, had a protective effect. The cell organites (mitochondria, endoplasmic reticule, a/o), as well as the nucleus present the ultrastructural features a little disturbed. In the cells are present many peroxisomes and mitochondria. These results can be applied in the cancer treatment, based on the ascertainment that the total acid alkaloid extract, in DDW, has a protection effect on the cells situated in a normal mitotic activity.

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NATURAL PRODUCTS WITH ANTI-INFLAMMATORY ACTION FOR TOPICAL USE

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Keywords: anti-inflammatory, natural products, essential oils

Two anti-inflammatory products (hydrosoluble gel consistency) were achieved, comprising natural extracts with features that complete one another by association, increasing the desired effect.

The first product – "Supliform"- contains a combination of alcoholic and watery extracts from: chilli pepper, ivy, chestnut tree and essential oils and the second one – "Arnicol"- comprises: hydroalcoholic extract of Arnica, gemoderivate extract of willow bark, hydrolysed collagen and essential oils.

The "Supliform" product with an important revulsive and vessel dilatative effects also stimulated the adipose deposits; apart from its anti-inflammatory and analgesic properties it proved to be anticellulitic.

The "Arnicol" product showed an analgesic and anti-inflammatory intense action due to its natural salicilic compounds. The products from Arnica and the collagen intervene through their topic troffic regenerative effect.

The essential oils of the two formuli activate topic blood circulation favouring the active substances entrance within tissues.

Both products represent very efficient combinations reducing pain and inflammatory processes after a few doses.

PHYTO-PRODUCTS WITH POLYPHENOLIC AND MUCILAGE COMPOUNDS TO TREAT AFFECTIONS OF THE MOUTH CAVITY: NOTE 2. RESEARCH TO OBTAIN MUCOADHESIVE TABLETS WITH VEGETAL EXTRACTS FOR ORAL ADMINISTRATION

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Keywords: mucoadhesive tablets, vegetal extracts, mouth cavity affections, polyphenols

The aim of the present study was preparation and characterization of mucoadhesive formulations with polyphenolic compounds (flavones, polyphenol acids) and mucilages for treatment of the mouth cavity affections (gingivitis, stomatitis, parodontosis, tonsillitis, pharingitis, laryngitis etc).

Three types of tablet containing *Melissa, Ocimum* and *Hyssopus* standardized extracts and four mucoadhesive components: hydroxypropylmethyl cellulose (Methocel K4M), carboxyvinyl polymer (Carbopol 974P, 71G), and polycarbophyl (Noveon AA1) were developed. Tablets were manufactured by direct compression. Technological controls tablets (thickness, diameter, friability, hardness, uniformity of content, weigh uniformity and dissolution kinetic) were carried out.

The new mucoadhesive tablets, which release natural active agents with antimicrobial and antiinflamatory properties have been chemical characterized.

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PHYTO-PRODUCTS FROM *TRIGONELLA FOENUM-GRAECUM*. NOTE 1. RESEARCHES FOR OBTAINING FENUGREEK SELECTIVE STANDARDIZED EXTRACTS AS FUNCTIONAL FOOD INGREDIENTS

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Keywords: fenugreek, diosgenin, amino acids, vegetal extracts, functional food

The aim of the present study was to establish the technical conditions for an integral capitalization by selective and successive extractions of fenugreek seeds.

Depending of seed crumbling grade, extraction solvent, temperature and rate vegetal product/extraction solvent, three types of extracts containing: sapogenins (expressed as diosgenin), glucomannans, amino acids and a vegetal residue consisting in insoluble dietetical fibres were developed.

The selective standardized extracts contain natural active agents with hypoglycaemic, anticholesterolemic, anti-inflammatory, carminative, emollient, expectorant, galactogogue, laxative, restorative and uterine tonic properties and may be use as valuable functional food ingredients.

All extracts have been physico-chemical characterized and some chemical classes were qualitative (HPTLC) and quantitative (UV-VIS spectrophotometry) determining.

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AQUAPLANT-LINE – BALNEARY USE PRODUCTS BASED ON A PHYTOCOMPLEX FROM MEDICINAL AND AROMATIC PLANTS FOR BALNEARY USE

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Keywords: balneary products, phytocomplex, potentiating agents

The aim of our study was to bring up the scientific and applicative research results of our specialists, with an important application in the elaboration of some balneary use products from medicinal and aromatic plants. Their main purpose is a systemic effect which can lead to amelioration of some chronic affection, and to improve the organism tonus. We have combined the phytocomplex with essential oils as potentiating agents. The phytocomplex has a selective

efficiency for the answered affection due to the active principles- synergic action. The potentiating agent is very important for its specific action combined to those of phytocomplex, and for the increased penetration of active principles through skin.

AQUAPLANT-line is effective in the improvement of rheumatoid ailments (*ALGIN*), of renal and bladder ailments and organism re- mineralization (*RENAL*), in genital apparatus affection and menopausal problems (*FEMINA*), in the reduction of circulatory disturbances (*VENOL*) and in sensorial and cerebral nervous disorders (*SEDIN*), as an adjuvant therapy.

EFFECT OF HERBAL MIXTURE "GASTROHERB®" ON THE QUALITY OF PIZZA CRUSTS

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Keywords: medicinal and aromatic herbs, bakery products, sensory properties

In order to obtain novel bakery products with specific biological attributes, medicinal and aromatic herb mixtures have been included in product formulations. Researches from the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade have formulated herbal mixture "Gastroherb®" in pulverized form and extract that enhances the functionality of bakery products. Medicinal herbs and aromatic components of the mixture (sage, artichoke, coriander and oregan) contain a complex of biologically active components exhibiting numerous activities (choleretic, carminative, antioxidative, antimycotic) that positively influence digestion.

Herbal mixture "Gastroherb®" doses varried in dough formulations depending on the form: 2% (flour basis) for pulverized mixture and 8% (flour basis) for extract. The addition of herbal mixture to pizza crust contributed to the development of new sensory profile and functional properties (improves digestion). In the paper, characterization of herbal raw materials and the mixture, process technology as well as the sensory properties of pizza crusts have been accented.

ALKALOID PLANTS AS INDICATORS OF THE SOIL CONTAMINATION

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Keywords: alkaloid, Datura stramonium, Atropa belladonna, Hyosciamus niger

Influence on humans to the plants is so specific and important that should be considered as special entirety. Because of the close relationship with the environment where they lives, certain plant species and communities are excellent indicators of given habitats. Subsequently, the aim of this contribution was usage of alkaloid plants as indicators anthropogenic contamination of their habitats. Selected plant species, rich in tropane alkaloids, expresses positive correlation between the content of alkaloids and the level of the soil contamination. Thus, higher degree of contamination of organic origin (anthropogenic) leads to the higher content of tropane alkaloids in such plants, similar as in the case of application of nitrogen containing fertilisers. Experimental design was directed to the study of three prominent indicator species interested as a source of tropane alkaloids: thorn apple (*Datura stramonium* L.), belladonna (*Atropa belladonna* L.), and henbane (*Hyosciamus niger* L.). Samples of these species were collected at localities Vratna and Ploče (Southern Serbia), and subjected to determination of atropine and

scopolamine contents. After drying of crude herbal drugs at room temperature, isolation of alkaloids was accomplished by chloroform-ammonia extraction. Contents of atropine and scopolamine in different plant parts of tested wild growing plants were finally determined by GC/MS and considered as reference values.

RESEARCHES CONCERNING THE IMPROVEMENT AND SIMPLIFYING THE *TRITICUM* BIOTEST

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Keywords: bioassays, Triticum test

At the Faculty of Pharmacy in Bucharest there is a long tradition of a bioassay using wheat caryopses (*Triticum vulgare* or *Triticum aestivum*), bioassay known as phytobiological method Constantinescu, Triticum test. The classical test suppose the germination of wheat caryopses in Linhart vessels, the selection of a minimal number of caryopses with embryonary radicles of 1 cm for each solution and concentration tested, then measuring of the radicular elongation (length of main radicle) for each germinated caryopse, for 5 days. This working technique involves certain difficulties and disadvantages, especially tha fact that there is a waiting period for the radicle to reach 1 cm (which could happen during daytime but equally in the most unfit hours of the night), that macroscopical measurements must be carried out for 5 days and that the inter-individual variability is relatively high. We have proposed therefore the improvement of this technique by measurng another parameter than radicle elongation and by shortening the study period for at most 3 days. We propose to measure not the absolut radicle length, but a surogate parameter, the report of the radicle length in two consecutive days, for each caryopse, which may decrease the inter-individual variability. In the case of main radicle measurements, the relative standard deviation (as a measure of results dispersion) varied between 12.7 and 14.0%, in the 5 days, while in the case of the proposed parameter, the relative standard deviation was within 6.4% and 9.2%, which proves a lower variability of results. On the other hand, the study on more test solutions, quantifying the inhibitory effect through a parameter called inhibition index, shows that generally the results of the first 3 days are sufficient to obtain conclusions, two extra days not bringing significant information in comparison with the first three days.

a reality check

SPRUCE TERPENES AND OXIDATIVE STRESS: A REALITY CHECK

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Keywords: Lipid-peroxidation, reactive oxygen species, spruce, *volatile terpenes*

The chemical composition and basic physiology of conifer resin has been studied for years, but the functions of resin in the life of the tree itself are still only poorly understood. While the non-volatile resin components have often been suggested to have a role in protecting the tree against herbivores and pathogens, the function of the volatile portion of the resin is less clear. Based on the fact that certain monoterpenes and sesquiterpenes react readily with reactive oxygen species (ROS) in laboratory experiments, it has been hypothesized that they could protect plant cells against oxidation *in vivo*. However, this hypothesis has never been studied. A series of experiments was set up to evaluate the role of volatile plant terpenes in protecting conifers against ROS. The experiments were designed to measure the quantity of volatile terpenes emitted from Norway spruce after different treatment and meanwhile to measure the extent of oxidative damage [in the form of lipid-peroxidation and malondialdehyde (MDA) accumulation].

Treatments caused a dramatic increase in terpene emission as well as reduction on MDA which support the hypothesis of the protective role of volatile terpenes against oxidative stress, but more experiments are needed to confirm it.

POLYPHENOLS CONTENTS OF SOME VEGETAL EXTRACTS AND THEIR RADIOPROTECTIVE EFFECT

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Keywords: polyphenols; antioxidant activity; radioprotective effect.

It is well known that free radicals are responsible for a large number of human diseases evolution. Polyphenols are natural antioxidants and the interest for the utilization of these compounds in green products is highly increased in the last years. Our goal was the determination of polyphenol content from some vegetal extracts (*Vitis vinifera, Salvia officinalis*), in order to use them in radioprotective formulas. Extraction of polyphenols from *Vitis vinifera* (peels) was made in two ways: firstly using aqueous acidified acetone (70%), secondly using acidified methanol (abs.). The extracts of *Salvia officinales* were hydroalcoholic ones (30-80% v/v). The reproducible data were obtained by purification of the extracts using solid-phase extraction (SPE). In our experiments, for polyphenol content evaluation there were used qualitative and quantitative methods: thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and electrochemical methods. It was determined the vegetal extract contents in phenolic acids, flavonoids and anthocyans with a sensitivity of ppm. It was also measured the antioxidant capacity (DPPH-Trolox) of the extracts using square wave voltammetry. The concentrated extracts were tested on a Vero cell line to evaluate the radioprotective effect.

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