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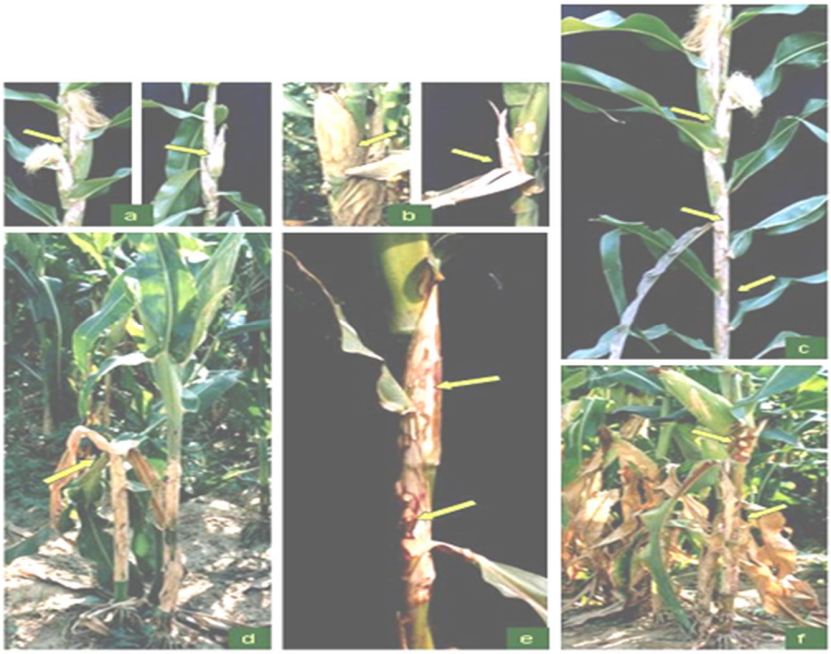

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Genetic Analysis of Banded Leaf and Sheath Blight Resistance (*Rhizoctonia solani*) In Maize

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Line × tester analysis involving 12 inbred lines and 5 inbred testers were carried to study the genetics of resistance to banded leaf and sheath blight in maize incited by *Rhizoctonia solani* in Kharif 2010 at B.A.U. research farm. Out of seventeen inbreds including five testers, three lines were resistant, twelve lines were moderately resistant and two lines were moderately susceptible. Both additive and dominance components were important in the inheritance of this disease with the predominant role of additive gene action. The inbreds, BAUIM-3, BQPM-2 and BQPM-4 were good general combiners for disease resistance as well as yield. Among the testers, BAUIM-2 was found to be the best general combiner for disease resistance as well as yield. The estimate of specific combining ability effect revealed that the cross combinations, BAU1M-3× CM-150, BAU1M-1×BAU1M-2, BAU1M-4×HKI 193-1, BAU1M-4×HKI163, V341× CM150, BQPM-2× BAU1M-2 and CML161× HKI 193-1 were identified as best cross combinations in respect of disease resistance as well as yield

Keyword: Banded Leaf and Sheath Blight, Combining Ability, Line X Tester, *Rhizoctonia solani*

1. Introduction

Maize plant is affected by as many as 61 diseases, out of which 16 have been identified a major ones which occur both in tropical and temperate regions of India (Sharma and Payak, 1986). Among these, banded leaf and sheath blight (BLSB) incited by *Rhizoctonia solani* is gaining economic importance. Grain yield loss, depending on severity varies between 11 to 40 per cent (Singh and Sharma, 1976). Lal et al. (1985) reported that the losses in grain yield may vary to the extent of over 90.0 per cent. Now BLSB is considered to be one of the most serious problem threatening cultivation of maize in India. It appears on plants before flowering, which is highly favoured by warm humid weather, and it causes severe damages to leaves, leaf sheaths as well as cobs (Fig. 1). For an economic and effective control of this disease, development of resistant genotypes is of primary importance. Hence, the present investigation was carried out to study the inheritance of resistance of this disease.

2. Materials and Methods:

The genetics of resistance in banded leaf and sheath blight disease of maize was studied in 80 genotypes in Kharif 2010 using line x tester (L × T) method. The basic material for the present study comprised twelve inbred lines of diverse, vigorous and productive nature and five well adapted testers of varying genetic base. These were crossed in line x tester mating design to generate 60 hybrids. These 60 hybrids and

seventeen parental lines with three standard checks viz., HQPM-1, Vivek Hybrid-9 and Suwan were sown in a randomized block design in three replications. Each entry was sown in two rows having 60 cm × 20 cm crop geometry. The plot size was single row of 3m length.

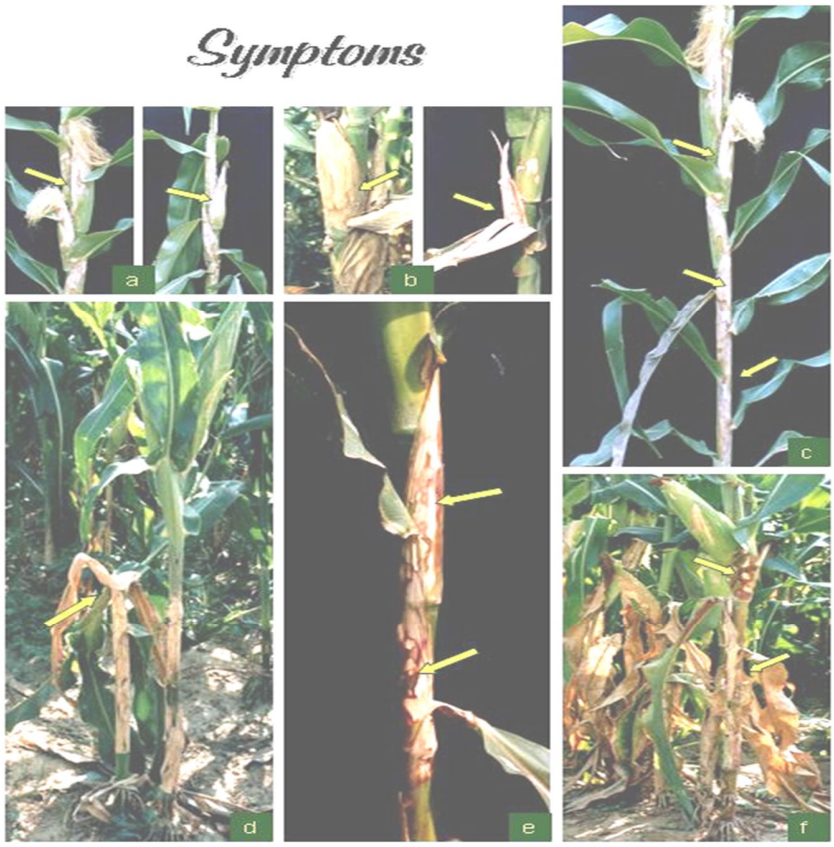

Fig. 1. Banded Leaf and Sheath Blight on maize caused by *Rhizoctonia solani* f. sp. *sasakii*
a &b- Ear infection and ear rot; c- Infection full plant; d- Toppling of plant;
e &f - Infection with discolouration on stem and sheath

For artificial inoculation, original cultures of the organisms were isolated by collecting leaf lesions and placing in moist chamber. Four to five days later, newly formed spores on the surface of the lesions were picked up with a fine flattened needle under a microscope, placed in a droplet of sterile water and streaked across the surface of hardened, acidified water agar in Petri plates. They were cut out of the agar and transferred to hard acidified potato dextrose agar and incubated for one week at 24°C. The inoculum was increased on whole sorghum grains following the method of Lim (1975 B). The inoculation was made by placing 10-20 grains into the individual leaf whorls at 7 to 9 leaf stage, in evening.

A second inoculation was made two weeks later. Required humidity was maintained by frequent watering. Observations in respect of disease intensity were recorded on the basis of five randomly selected plants from each plot in each replication. Disease severity was recorded in thirty days after inoculation using 1-5 scale where, 1 indicates no disease and 5 indicates maximum disease incidence given by Ahuja and Payak (1983) and Vimla and Mukherjee (1987).

Combining ability analysis was carried as per procedure given by Kempthorne (1957).

Table 1. ***Per se*** **performance of Disease scoring against Banded leaf and sheath blight and Grain yield**

Parents	Disease score	Disease reaction	Grain yield(q/ha)
BAUIM-3	1.13	R	34.26
CM111	2.33	MR	35.09
CM151	2.26	MR	31.23
CM152	2.13	MR	36.35
BAU1M-1	2.24	MR	36.44
BAU1M-4	2.47	MR	37.54
V341	3.30	MS	31.95
1025	2.47	MR	31.49
BQPM-2	1.18	R	36.99
BQPM-4	2.22	MR	30.73
CML161	2.26	MR	34.10
V351	2.23	MR	26.85
CM-150 (T1)	2.17	MR	28.89
BAU1M-2 (T2)	1.26	R	39.61
K1105 (T3)	3.10	MS	29.18
HKI 193-1 (T4)	2.38	MR	39.91
HKI-163 (T5)	2.33	MR	39.32
Mean	2.20		28.76
Checks			
HQPM-1	2.40	MR	41.97
Vivek Hybrid-9	2.53	MR	48.77
Suwan	2.58	MR	44.59
Mean	2.50		45.11
CD at 5%	0.14		5.85

3. Results and Discussion

The variances due to GCA as well as SCA were highly significant for disease severity. The ratio of GCA and SCA variance indicated the importance of both additive and non-additive types of gene actions involved in the inheritance of this character with the predominant role of additive gene action. The results were supported by Bhavana and Gadag (2009) and Vivek *et al.* (2010).

The disease reaction of inbreds to banded leaf and sheath blight and grain yield is presented in Table 1. The parental mean value for disease scoring ranged from 1.13 (BAUIM-3, resistant) to 3.30 (V 341, moderately susceptible) with an overall parental mean value of 2.20 (moderately resistant). Three parents (BAUIM-3, BQPM-2 and BAUIM-2) were found to be significantly resistant to the check HQPM-1. The parental mean value for grain yield ranged from 26.85 q/ha (V 351) to 39.91 q/ha (HKI 193-1). The parents, BAUIM-3, BQPM-2 and BAUIM-2 were good yielders with less disease infestation.

The estimates of gca effect (Table 2) indicated that the inbred lines BAUIM-3, BQPM-2 and BQPM-4 were good general combiners for disease resistance as well as yield. Among the testers, BAUIM-2 was found to be the best general combiner for disease resistance as well as yield.

The estimate of specific combining ability effect (Table 3) revealed that the cross combinations BAU1M-3 × CM-150, BAU1M-1 × BAU1M-2, BAU1M-4 × HKI 193-1, BAU1M-4 × HKI-163, V341 × CM150, BQPM-2 × BAU1M-2 and CML161 × HKI 193-1 were identified as best cross combinations in respect of disease resistance as well as yield. The F_1 progeny of the two crosses (BAU1M-3 × CM-150 and BAU1M-1 × BAU1M-2) involving the resistant parent and moderately resistant parent were resistant indicating dominance of resistance, the other cross (BQPM-2 × BAU1M-2) where both the parents were resistant showed resistant F_1 while others were moderately resistant (Table 4). Thus these hybrids may be utilized further for commercial cultivation to develop resistant and high yielding varieties and the identified inbreds may be further exploited in hybrid breeding programme towards development of resistant lines in maize.

Table 2. Estimates of general combining ability (gca) effects for Disease (BLSB) and Grain yield

Parents	Disease (BLSB)	Grain yield
BAUIM-3	-0.22**	2.48 **
CM111	0.10**	-8.45 **
CM151	-0.02	-3.04 **
CM152	-0.02	-1.20
BAU1M-1	0.01	-1.20
BAU1M-4	0.01	-9.84 **
V341	0.18 **	5.38 **
1025	0.06**	6.08 **
BQPM-2	-0.08**	7.99 **
BQPM-4	-0.08**	3.80 **
CML161	-0.02	1.49*
V351	-0.02	1.49*
CM-150 (T1)	-0.01	0.26
BAU1M-2 (T2)	-0.03*	2.10 **
K1105 (T3)	0.02*	-1.14**
HKI 193-1 (T4)	0.01	-0.40
HK-163 (T5)	-0.01	-0.82
CD at 5% (Lines)	0.05	1.31
CD at 5% (Testers)	0.03	0.85

***, ** Significant at p = 0.05 and P = 0.01 levels, respectively**

Table 3. Estimates of specific combining ability (sca) effects of promising hybrids for Disease (BLSB) and Grain yield

Crosses	Disease (BLSB)	Yield
BAU1M-3 × CM-150	-0.19**	6.15**
BAU1M-1 × BAU1M-2	-0.09**	7.89**
BAU1M-4 × HKI 193-1	-0.13**	6.39**
BAU1M-4 × HKI-163	-0.09**	13.26**

V341 × CM150	-0.10**	7.35**
BQPM-2 × BAU1M-2	-0.09**	6.31**
CML161 × HKI 193-1	-0.10**	6.90**

***, ** Significant at p = 0.05 and P = 0.01 levels, respectively**

Table 4. Disease reactions of Inbred combinations and their crosses

Crosses	Disease reaction of Inbred combinations	Disease reaction of Crosses
BAU1M-3 × CM-150	R x MR	R
BAU1M-1 × BAU1M-2	MR x R	R
BAU1M-4 × HKI 193-1	MR x MR	MR
BAU1M-4 × HKI-163	MR x MR	MR
V341 × CM150	MS x MR	MR
BQPM-2 × BAU1M-2	R x R	R
CML161 × HKI 193-1	MR x MR	MR

4. Acknowledgement:

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Preliminary Phytochemical Studies and Evaluation of Antimicrobial Property of the Methanol Extract of the Roobark of *Ritchiea longipedicellata* Gilg Family *Capparidacaea*

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Purpose: The root of *Ritchiea longipedicellata* was claimed to have antimicrobial properties. The people of Idemili area in Anambra State of Nigeria use the decoction of it to treat wounds, running stomach, aches and pains. It is because of this background that this investigation was carried out to ascertain the veracity of the claim.
Methodology: The root of *Ritchiea longipedicellata* was collected and dried at ambient temperature. It was pulverized into powder. 500gm of the powdered drug was placed into a 2litre beaker containing 1litre of methanol. It was allowed to stand with occasional shaking for 48hrs. The content was filtered and the filtrate was concentrated using rotary evaporator. The extract contains the following secondary metabolites – alkaloids, flavonoids, terpenoids, saponins and glycosides. Agar diffusion method was used to investigate antimicrobial activity.
Result: The root of *Ritchiea longipedicellata* exhibited antimicrobial property.
Conclusion: The claim of Idemili people of Anambra State Nigeria on the use of *Ritchiea longipedicellata* appears to be obvious in line with the results of the investigation

Keyword: *Ritchiea longipedicellata*, Agar Diffusion

1. Introduction:

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade (Sofowura A. 2008). Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations (Srinivas *et al,* 2007). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some

case) and the cultural and spiritual point of view of the people of the countries (Srinivas *et al)*.

In Western developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited (Satyejji and lutfun*, 2007)*. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over - prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary. (Fagbohun *et al,* 2010).

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people. Nowadays, it has been accepted that infectious diseases are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries (Iwu *et al*., 1999). In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents.

The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier et al. 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control. Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis. This work is therefore a preliminary work to prove that there is scientific evidence to the use of the root of *Ritchiea longipedicellata* in the treatment of diseases.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health.

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials (Farms Worth and Moris, 1976).

2. Taxonomy of the Plant and Its Description

Kingdom	: Plantae
Division	: Angiospermae
Class	: Dicotyledonae
Subclass	: Archichlamydae
Order	: Papaverale/Brassicales
Suborder	: Capparineae
Family	: Capparidaceae
Genus	: *Ritchiea*
Species	: *Ritchiea longipedicellata Gilg.*

Fig1: Plant of *Ritchiea longipedicellata*

3. Plant's Description:

The plant is an evergreen climber but when alone it is a self-supporting shrub with compound palmate leaves. The leaves can be collected all - year round as the plant can stand dry season. The roots are tuberous and with strong pungent odours when perceived. Like other capparidaceae, this herb is indigenous to the tropics, found mostly in the lowland area of rain forest, especially beside water body and virgin up -lands. As a shrub it grows to a height of few meter(s) and as climber can grow a considerable length of about 5 meters with several branches (**Local source**).

4. Distribution of *Ritchiea Longipedicellata Gilg*.

The plant *Ritchiea longipedicellata G*. is virtually all over the tropical land of Africa and particularly West Africa. In Nigeria, the plant is found in the south east where the plant is used locally for various indications. The local name springs from the number of leaves (three) present in a leaflet hence it is called Nchi-ato [3-ears] by the Ibo people (Ikwo).

5. Ethnobotanical Uses of *Ritchiea Longipedicellata G*

The plant is used in Nigerian local villages (particularly in Ikwo L. G. A. in Ebonyi State and Idemili in Anambra State) where the root and the leaves are used for treatment of various illness.– small quantity of the root can be chewed (with closed mouth) to relieve pain in the head, cold, upper respiratory tract infections. Local palm wine extract of the plant is used for the treatment typhoid fever and malaria and general illness that prove resistance to modern therapies **(Local users and traditionalist**).

6. Review of Activities of Some Capparidaceae Plants

The results of screening of Petroleum ether, chloroform, ethanol and water extracts of roots of *Capparis grandiflora* Wall. ex Hook's &Thomson showed inhibitory activities against 24-hour cultures of *Staphylococcus aureus, Bacillus subtilis, Bacillus pumillu, Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* but none showed antifungal activity. The phytochemical screening showed the presence of tannins, saponins, sesquiterpenes, alkaloids, and phlobatannins *(*Karanayil *et al, 2011*).

The *in vitro antibacterial screening of the extracts of Boscia angustifolia roots (*family Capparidaceae*) showed that the crude water and chloroform extracts possess significant (P< 0.05) inhibitory activities against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pneumonia with the exception of S. typhi. Phytochemistry showed absence of flavonoids, steroids, free anthraquinones balsams and resins (*Hassan *et al,* 2oo6*)*

Cappari tomentosa used traditionally as spices and for healing a lot of complaints ranging from cough to infertility and impotence reportedly showed no activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*, With no effect on fibroblast growth.

Leaf extract of *Capparis zeylanica* L. (CZ) is locally taken with black pepper powder is taken twice daily for treatment of dysentery. Leaf juice of CZ taken orally with cup of fresh goat milk for curing cough and cold. For the treatment of diabetes, ripe fruits are consumed twice for fortnight. The screening of water extract (200 mg/kg) significantly (P<0.01) reversed yeast-induced fever in rodents. The aqueous extract

from total aerial parts of the plant has been used for its antifungal, anti-inflammatory, antidiabetic, and antihyperlipidemic activities and is among the constituents of polyherbal formulations to treat liver ailments; preliminary phytochemical screening of the leaf extracts show the presence of alkaloids, flavonoids, saponins glycosides, terpenoids, tannins, proteins and carbohydrates . The roots of C. zeylanica contain alkaloid, phytosterol, acids and mucilage (Sunil et al, 2011).

Gynandropsis gynandra commonly called spider flower is member of the family capparidaceae. It has demonstrated high activity against both bacteria, fungi and helminthes .It has steroidal nucleus, alkaloid, reducing sugar, and cyanidin (Ajaiyeoba, 2000).

7. Materials and Method

A. Materials

1. Chemicals and Solvent

The chemicals used for this experiment include methanol (Qualichem pvt ltd), dimethyl sulphoxide (DMSO), Nutrient Agar. The reagents used were – concentrated sulfuric acid, naphthol solution in ethanol (Molisch reagents) picric acid, ammonium solution, nitric acid, Aluminum chloride solution, Fehling solution A and B, Wagner's reagents (iodine and potassium iodide), Hager's reagent (saturated solution of picric acid).

2. Sources of Microorganisms

The microorganisms used were both bacteria obtained from laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka. The organisms include bacteria (*Staphylococcus aureus*, *pseudomonas aeruginosae , Escherichia coli, Bacillus subtilis*, *Salmonella typhi*

3. Equipment

Weighing Balance [Scout pro u401 made in China], Beakers, measuring cylinder, test tubes**,** incubators (GentLab UK), autoclave, test tubes, test tube racks, syringes and needle, Pasteur's pipette, conical flask, glass rod, inoculation loop, Tripod stand, filter paper (Whatman No 1), Mortar and pestle , water bath, muslin- cloth, reagent bottles, Bunsen burner, and permanent marker.

B. Methods

1. Source and Identification of Plant Materials

The root of *Ritchiea Longipedicellata* was obtained from Echialike in Ikwo local Government Area, Ebonyi state in January 2012. The plant was identified by Mr Ozioko – a Taxonomist of University of Nigeria, Nsukka. The root was air dried in the Pharmacognosy Laboratory and then were pulverized to produce 500g of powder.

2. Extraction Process

Extraction was done with methanol. The 500g of powdered dried root was macerated with one Liter of methanol in a 2liter beaker for two days with occasional agitations. At the end it was strained using white muslin cloth and then filtered using Whatman No 1 filter paper. The process was repeated using the marc. The combined filtrates were concentrated using rotary evaporator under reduced pressure. Aliu AB et al (2008).

Qualitative assay for the presence of secondary plant metabolites were carried out on the methanol extract of the root of *Ritchiea longipedicellata* using the standard procedures (Harborne 1991), (Trease and Evans, 1989).

8. Phytochemical Screening of the Plant

Standard screening tests were carried out on powdered root for various phytochemical constituents. The procedure used was obtained from Evans (2002) and Departmental Laboratory Manual (2009), Awe et al (2003) and Beena et al (2010).

a. Test for Protein

Xanthoproteic reaction test: 5 ml volume of the filtrate obtained from boiling few grams of powdered plant is heated with few drops of concentrated nitric acid; yellow colour that

changes to orange on addition of alkali indicates the presence of protein.

b. Test for Carbohydrates

0.1g of the powdered leave was boiled with 2mL of distilled water and was filtered .To the filtrate, few drops of naphthol solution in ethanol (Molish reagent) were added. Concentrated sulphuric acid was then poured gently down the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrate (starch).

c. Test for Alkaloids

About 5 g of powdered root placed in the test tube and 20ml methanol added to the tube, the mixture was heated in water bath and allowed to boil for two minutes. It was cooled and filtered. 5ml of the filtrate was tested with two drops Wagner's reagent (solution of iodine and potassium iodide).

To another 5mL portion of the extract 2 drops of Hager's reagent (saturated picric acid solution) was added. The presence of precipitate indicates alkaloid.

d. Test for Steroids

About 9ml of ethanol was added to 1g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath. 5ml of hot distilled water was added to the concentrated solution. The mixture was allowed to stand for 1 hour and the waxy matter was filtered. The filtrate was extracted with 2.5ml of the chloroform using separating funnel. To 0.5ml of the chloroform extract in a test tube, 1ml of concentrated sulfuric acid was added to form a lower layer. A reddish brown interface shows the presence of steroids.

e. Tests for Saponins

About 20ml of water was added to 0.25g of crude extract and boiled gently in a hot water bath for 20 minutes. The mixture was filtered hot and allowed to cool and the filtrate was used for the following tests.

I. Frothing test: 5ml of filtrate was diluted with 20ml of water and vigorously shaken. The test tube was observed for the presence of stable foam upon standing.
II. Emulsion test: To the frothing solution, 2 drops of olive oil was added and the content shaken vigorously and observed for the formation of emulsion.
III. Fehling's test: To 5ml of the filtrate was added 5ml of Fehling's solutions (equal parts of A and B) and the content was heated in a water bath and a reddish precipitate which turns brick red on further heating with sulphuric acid indicates the presence of saponins (general test for glycosides).

f. Test for Flavonoids

About 10ml of ethylacetate was added to 0.2g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and used for the following test.

I. Ammonium test: 4ml of filtrate was shaken with 1ml of dilute ammonium solution. The yellow colour in the ammonical layer indicates the presence of the flavonoids.
II. Aluminum chloride solution (1% test) another 4 ml portion of the filtrate was shaken with 1ml of 1% aluminum chloride solution. The layers were allowed to separate; a yellow colour in the aluminum chloride indicates the presence of flavonoids.

g. Fixed Oil

Whole extract solution (0.5ml) with two drops of 1M alcoholic $K_2Cr_2O_7$ and 3 drops of phenolphthalein were added in a clean test tube. Soap formation shown by frothing indicated the presence of fixed oil.

h. Phenolic Group

Alcoholic plant extract (0.5ml) was taken in a test tube. Two drops of 1M ferric chloride was added. Appearance of intense color indicated the presence of phenolic groups.

i. Cyanogenetic Glycosides

About 1 g of powdered sample was boiled with distilled water and moist sodium picrate paper held inside the tube with a cork. A colour change from yellow to Brick-red of the picrate paper is positive for cyanogenetic glycosides.

9. Pharmacological Tests

A. Antimicrobial Assay

a. Microorganisms: 24hour Cultures of five human pathogenic bacteria made up of both gram positive (*S. aureus,* and *B.subtilis*) and and gram negative (*P. aeruginosa, E. coli* and *S. typhi*) bacteria were used for the *in-vitro* antibacterial assay. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka.

b. Preparation of Media

Nutrient agar was used in the assays. Dimethylsulphoxide (DMSO) was used in solublising the extracts and drugs and as a negative control in the study. The media was prepared by dispersing the weighed amount in water and then sterilized in autoclave. The plates of nutrient agar were poured and allowed to solidify after the appropriate organisms were seeded (Majorie Murphy Cowan 1999).

c. Antimicrobial Agents: Ampicillin, 20ug/ml (Mecure indusrial ltd lagos Nigeria.); was used in the study as standard reference drug.

d. Antimicrobial Activity Determination

An overnight broth culture used to obtain 0.5 **Marcfarland** standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45°C. Seven holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified; 400mg/ml, 200mg/ml,100mg/ml,50mg/ml,25mg/ml12.5mg/ ml and 6.25mg/ml of extract were prepared in dimethylsulphoxide (DMSO) by preparing a stock solution and carrying out double fold dilutions on it. And with the aid of a Syringe, the wells were filled with 0.25 ml (5drops) of different dilutions of the extract while the centre well was filled with 20µg/ml of ampicillin (also dissolved in DMSO). Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h for bacteria. This test was conducted first on the crude extract and the solvent dimethylsulphoxide was used as negative control while ampicillin was used as positive control.

10. Results and Analysis

The results of phytochemical screening showed presence of alkaloid, glycosides and flavonoid. Also present were saponins and flavonoids Lather Amit et al (2010).

Table 1: Result of Phytochemical Screening of *Ritchiea longipedicellata Gilg*

Secondary metabolites	Results
Alkaloid	+
Tannin	-
Flavonoids	+
Saponins	+
Steroid	-
Terpenoid	+
Glycoside	+

Key: + = present; - = absent

Table 2: The Results of Antimicrobial Screening

Bacteria	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Amp20ug/ml
Staphilococcus aureus	8mm	6mm	4mm	2mm	1mm	-	-	6mm
Escherichia coli	-	-	-	-	-	-	-	9mm
Bacillus subtilis	-	-	-	-	-	-	-	5mm
Pseudomonas aeruginosa	6mm	4mm	2mm	1mm		-	-	6mm
Samonela typhi	6mm	4mm	2mm	1mm	-	-	-	6mm

The extracts displayed various activities against bacteria inhibiting it at various concentrations ranging from 400 to 6.25 mg/ml. The inhibition zone of the extract at 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml are 8mm, 6mm, 4mm and 2mm respectively against *Staphiloccocus aureus*. At 400mg/ml and 100mg/ml the activity of the extract is comparable to the standard antibiotic ampicillin with inhibition zone of 6mm. it has no activity against both E. coli and Bacillus subtilis. But it is effective against Pseudomonas aeruginosa and Salmonella typhi with inhibition zones of 6mm, 4mm, and 2mm at concentration of 400mg/ml, 200mg/ml and 100mg/ml respectively. At 400mg/ml the extract is comparable with the standard drug with inhibition zone of 6mm.

11. Discussion, Conclusion and Recommendation

The results of phytochemical screening showed presence of simple sugar and flavonoid, essential oil, phenolic group, glycoside, and saponin in the methanol root extract screened for secondary metabolites. Some of these active principles (secondary metabolites) have been reported to have activity against micro-organisms. Flavonoid, phenolics, Alkloids, triterpenes and essential oils have been shown to have activities (Majorie, 1999). The Presence of alkaloids, cyanogenetic glycosides, steroidal nucleus and reducing sugars, phenolic group and essential oil are normal with the plants of this family capparidaceae (Kjaer and Thomson, 1973; Lakshimi and Chanhan, 1977) Ajaiyeoba E. O., 2000)..

The extract displayed various activities against bacteria inhibiting it at various concentrations ranging from 400 to 6.25 mg/ml. The inhibition zone of the extract at 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml are 8mm, 6mm, 4mm and 2mm respectively against Staphylococcus aureus. At 400mg/ml and 200mg/ml the activity of the extract is comparable to the standard antibiotic ampicillin with inhibition zone of 6mm. It has no activity against both E.coli and Bacillus subtilis. But it is effective against Pseudomonas aeruginosa and Salmonella typhi with inhibition zones of 6mm, 4mm, and 2mm at concentration of 400mg/ml, 200mg/ml and 100mg/ml respectively. At 400mg/ml the extract is comparable with the standard drug with inhibition zone of 6mm.

12. Conclusion

The present study shows that the root of Ritchiea longipecellata has a lot of potential as an anti-microbial agent. These observed activities appear to justify the ethmopharmacological uses of the plant.

13. Recommendation

There is need for further study and characterization of the plant to ascertain the active constituent of the drug for easy design and synthesis.

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Pharmacognostical Studies of the Pericarp of *Lagonychium farctum* (Banks & Sol.) Bobr. Growing in Egypt

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Lagonychium farctum (Banks and Sol.) Bobr. is an erect prickly trees or shrubs. It belongs to family Leguminosae. It is used in traditional system of medicine as an astringent as well as anti-dysenteric. The present study investigates various standardized parameters such as macroscopic and microscopic characters which could be helpful in authentication of the pericarp of *Lagonychium farctum*. (Banks & Sol.) Bobr

Keyword: *Lagonychium farctum*, Prosopis, Acacia, Mimosa, Leguminosae, Pericarp, Pharmacognostical Study

1. Introduction

Genus Lagonychium (Prosopis) includes 45 species (tropical and subtropical). *Lagonychium farctum* (Banks & Sol.) Bobr. belongs to family Leguminosae and sub-family Mimosoideae[1-4]. This plant has many other synonyms as *L. stephaniana* M. B., *Prosopis farcta* (Banks & Sol.) (Orth. Err. farcata), *P. stephaniana* (Willd Kunth ex Spreng, *Acacia stephaniana* (M. B.), *A. heterocarp* Del., *Mimosa farcta* (Banks & Sol.) and *M. stephaniana*[2-6]. It is used in Palestine as an astringent and anti-dysenteric[5]. Ingestion of large amount of this plant is toxic due to presence of saponins that cause inflammation to the digestive tract and quick peristalsis[7]. Sensor and motor nerve fibers are also affected in a serious way [7]. It is an erect prickly trees or shrubs. Leaves: compound, bipinnate. Stipules: small or absent. Leaflets: small, narrow. Stem: with or without spines. Spines solitary or in pairs. Flowers: pentamerous, usually sessile in narrow spikes, regular, actinomorphic, hermaphrodite. Calyx: five-shortly toothed or subentire sepales. Corolla: five petals, valvate. Stamens: 10, free, short. Ovary: sessile or stalked, multiovulate. Style: slender, filiform. Stigma: minute, terminal. Pod: turgid, cylindrical or oblong, straight, variously twisted, septa between the seeds. Mesocarp: thick and spongy. Seeds: usually ovoid compressed, albuminous[8-10].

Fig 1 : Photo of *Lagonychium farctum*

Fig2: Picture of the fruit

Fig .3 : Diagram of the herb 0.3X

2. Taxonomy:

Lagonychium farctum (Banks & Sol.) Bobr. belongs to[2,1]1:
Phylum: Angiospermae, Subphylum: Dicotyledonae, Class: Magnoliopsidae, Subclass: Rosidae, Order: Fabales, Family: Leguminosae, Subfamily: Mimosoideae, Genus: *Lagonychium*, Species: *farctum* (Banks & Sol.) Bobr.

3. Materials and Methods:

A. Plant Material: The fruits of *Lagonychium farctum* (Banks & Sol.) Bobr. were collected from sandy area around Kharga Oasis, Egypt, in June 1996. The sample was identified by Prof. A. Fayed (Professor of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt). It was dried at room temperature, then reduced to fine powder. The materials used for botanical study were taken from the samples preserved in 70% ethanol containing 5% glycerin.

B. Microscopic Studies : Surface preparation, transverse section as well as powder of the pericarp were used for observation of various microscopic features.

4. Results and Discussion

A. Macroscopical Characters:

The Fruit (Fig. 2, 3, 4A-C):

It is a stalked dry indehiscent pod, derived from a superior monolocular ovary with marginal placentation. The fruit is true, simple, dry, lomentum or sometimes legume. It is swollen curved or kidney-shaped, cylindrical usually 2-valved. It breaks transversely into one-seeded segments and contains up to 10 seeds. The surface is smooth, showing fine reticulations. It has a round distal end bearing a small point marking the position of the style. It is born on a short stalk arising from the ventral suture. The outer epicarp is thin, leathery to woody. Dorsal suture carries single vascular bundle and the ventral one carries two closely applied strands. Internally the fruit shows a spongy pulp diffused with the endocarp and the seeds are embedded in the pulp. It has constrictions and divided internally with weak false septa resulting in unequal compartments. The spongy pulp is pale-

brown to buff in colour. The stalk is straight, cylindrical with a somewhat rough surface, pale brown colour and measures from 7 to 10 mm in length and 1 to 3 mm in diameter. The fruits measure from 3 to 6.5 cm in length and 1 to 2.5 cm in diameter with dark brown colour, faint characteristic odour and astringent lastly sweet taste.

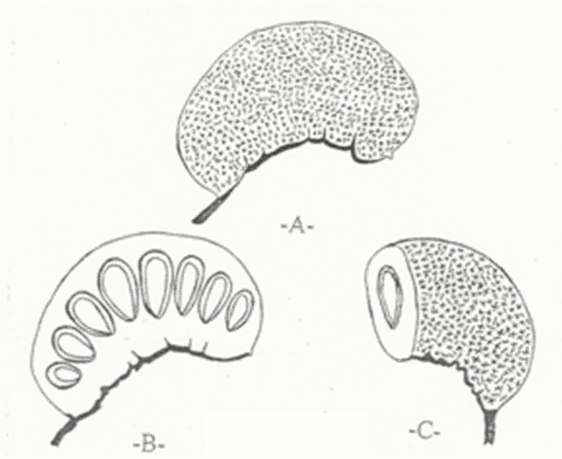

Fig .4: Morpholgy of the fruit; **A**-the fruit, **B**-Longtudinal section, **C**-Transverse section. (All 0.6 X)

B. Microscopical characters of the pericarp:

1. I-The Pericarp (Fig. 5B, C):

A transverse section through the pericarp shows an epicarp of one layer and an inner parenchymatous endocarp enclosing in between a wide region of pulpy mesocarp traversed by vascular bundles. The outer zone of mesocarp is formed of parenchyma followed by a zone of schlerenchyma. The latter is lined internally by a row of collateral vascular bundles. The rest of the mesocarp shows a wide region of thin walled parenchyma lined internally by a narrow zone of schlerenchyma accompanied by a lower layer of collapsed parenchyma. The endocarp is represented by one layer of tangentially elongated cellulosic cells covered with smooth and thin cuticle.

2. A-The epicarp (Fig. 5A, C, 6A):

It is formed of one row of tangentially elongated cells as seen in the transverse section. In surface view (Fig. 5A, 6A): The cells are polygonal, isodiametric with straight anticlinal walls, covered with smooth, thin cuticle and measure from 10 to 12 to 14 µ in diameter. They show few paracytic stomata. Hairs are not observed.

3. B-The Mesocarp (Fig. 5B, C, 6B, C):

The outer zone of the mesocarp is formed of oval to rounded parenchyma with large intercellular spaces and arranged in 5-12 rows which are brown due to presence of numerous tannin bodies which stain olive green with ferric chloride T.S. They measure from 24 to 42 to 60 µ in diameter. The second layer of the mesocarp is formed of schlerenchyma layer which is arranged in about 8 to 12 rows of sclereids with pitted lignified walls and wide lumina with numerous pits.

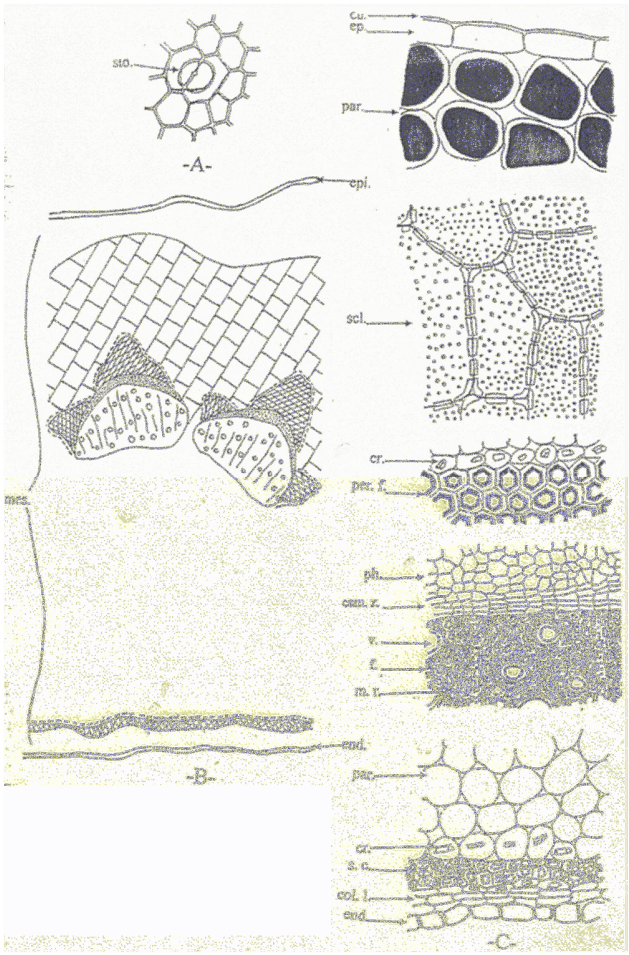

Fig .5 : The pericarp; A. Surface preparation, **B**. Diagrammatic transverse, **C**. Detailed transverse section (**A**, **C** X 500 and **B** X 50)

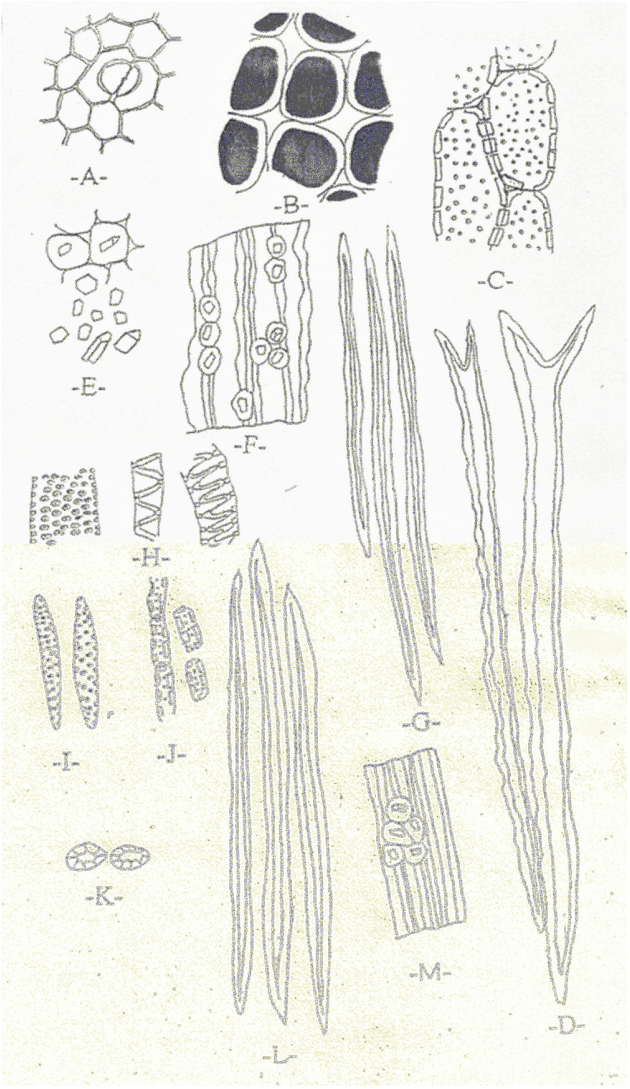

Fig .6 : The pericarp powder; **A.** Epicarp**, B.** Mesocarpal parenchyma**, C.** Mesocarpal schlerenchyma**, D.** Pericycle fibers**, E.** Prisms of calcium oxalate crystals**, F.** Crystal sheath of the pericycle**, G.** Wood fibers**, H.** Xylem vessels**, I.** Tracheids**, J.** Medullary rays**, K.** Stone cells**, L.** Fibers of the innermost layer of the mesocarp**, M.** Crystal sheath of the mesocarp **(All X 500)**

They are oval or subrectangular to somewhat isodiametric in shape and measure from 60 to 110 to 180 µ in length and 35 to 50 to 60 µ in width. The vascular bundles show an outer pericycle formed of interrupted schlerenchyma. This schlerenchyma consists of lignified fibers and parenchyma. The pericylic fibres consist of 2 to 4 arms of fibers over each bundle and arranged in 2 to 10 rows. They have moderately thick, slightly lignified tortuous walls, wide lumina and forked apices. They are surrounded by parenchyma containing prisms of calcium oxalate forming a crystal sheath. Calcium oxalate prisms measure from 10 to 14 to 18 µ in diameter. The pericycle fibers measure from 280 to 360 to 420 µ in length and 8 to 10 to 16 µ in width. The phloem is formed of soft elements viz. sieve tubes, companion cells and phloem parenchyma. It is lined internally by a narrow cambial zone. The xylem consists mainly of spiral, pitted and reticulate lignified vessels being about 10 to 23 to 36 µ in diameter. Few tracheids have lignified walls, elongated with blunt apices. They show simple and bordered pits and measure from 60 to 70 to 80 µ in length and 8 to 11 to 14 µ in diameter. Wood fibers have thick, lignified, walls, narrow lumina and acute apices. They measure from 120 to 180 to 190 µ in length and 6 to 8 to 10 µ in width. The medullary rays are uniseriate to biseriate with lignified walls and pitted lumina. They measure from 20 to 30 to 40 µ in length and 8 to 10 to 12 µ in width. The rest of the mesocarp shows a wide region of thin walled parenchyma which are oval to rounded in shape with thin cellulosic walls. They measure from 14 to 27 to 40 µ in diameter. The schlerenchyma layer is formed of 1 to 4 rows of fibers with thick lignified walls, narrow lumina and acute apices. They are interrupted by few stone cells which are oval to rounded in shape with moderately thick lignified walls and moderately wide lumina. They measure from 16 to 20 to 24 µ in diameter. The fibers are surrounded by parenchyma containing prisms of calcium oxalate forming a crystal sheath. Calcium oxalate prisms measure from 6 to 9 to 12 µ in diameter. The fibers measure from 220 to 260 to 300 µ in length and 8 to 10 to 12 µ in width. The innermost layer of the mesopcarp consists of 1 to 2 layers of thin walled collapsed parenchyma abutting on the endocarp.

C-The Endocarp (Fig. 5B, C):

It is formed of one layer of cubic to rectangular cellulosic cells with slightly thick radial and outer tangential walls. They measure from 14 to 18 to 22 µ in length and 8 to 12 to 16 µ in width.

D-The Powdered Pericarp (Fig. 6):

The dried powdered pericarp is yellowish-brown in colour with faint characteristic odour and astringent lastly sweet taste. The following are the most diagnostic microscopic features of the powder (Fig. 6):

1. Fragments from the epicarp consist of polygonal or isodiametric cells with straight anticlinal walls and covered with thin, smooth cuticle. The fragments bear few paracytic stomata. Hairs are absent.
2. Fragments from the mesocarpal parenchyma which are oval to rounded with large intercellular spaces.
3. Fragments from mesocarpal schlerenchyma which are elongated, oval or subrectangular, to somewhat isodiametric in shape. Sclereids have pitted lignified walls and wide lumina with numerous pits.
4. Lignified pericycle fibers with moderately thick, slightly lignified tortuous walls, wide lumina and forked apices which are accompanied by a crystal sheath.
5. Few tracheids which are elongated showing lignified pitted walls and blunt apices. The pits are both simple and borded.
6. Spiral, pitted and reticulate lignified xylem vessels.
7. Wood fibers have lignified, thick walls, narrow lumina and acute apices.
8. Medullary rays showing lignified walls and pitted lumina.
9. Fibers of the mesocarp with thick lignified walls, narrow lumina and acute apices. They are accompanied by a crystal sheath.
10. Few stone cells oval to rounded in shape, with moderately thick lignified walls and moderately wide lumina.
11. Free and scattered prisms of calcium oxalate crystals.
12. Absence of trichomes and starch grains.

5. Conclusion

From over present study entitled, Pharmacognostical Studies of the Pericarp of *Lagonychium farctum* (Banks & Sol.) Bobr. Growing in Egypt, it could be helpful in authentication of the pericarp. Moreover, it is helpful in the identification of powdered drug prior using in any herbal formulations.

6. Acknowledgement

We would like to express our deep thanks to Prof . A.M. El-Moghazy Shoaib (Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Assuit Uni., Egypt) for the identification of the pharmacognostical characters of the pericarp.

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Molecular Target-Oriented Phytochemical Database and Its Application to the Network Analysis of Action Mechanisms of Herbal Medicines

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Kampo medicines, the Japanese adaptation of traditional Chinese medicines, are formed by combining several herbs containing multiple phytochemicals. The considerable ambiguity of pharmacological profiles of Kampo medicines is expected to be clarified by identifying the molecular targets of constituent phytochemicals and analyzing the combined effects of the phytochemicals on the pharmacological pathways formed by those targets. To facilitate this line of study, we constructed paired databases named PhytodamaTarget and PhytodamaTaxon DBs which treat molecular targets of phytochemicals and constituent phytochemicals of plant taxa, respectively, by utilizing information from the literature. We then used the databases to explore possible mechanisms of synergism in analgesic activity between *Glycyrrhiza globra* and *Paeonia lactiflora*

Keyword: Phytochemicals, Database, Molecular Target, Kampo Medicines, Synergism, Analgesic

1. Introduction

Kampo and traditional Chinese medicines are characterized by a complex composition with multiple pharmacologically active phytochemicals[1]. A tremendous number of these medicines have been developed by combining various specified herbs to achieve the greatest possible pharmacological effect[2]. However, most of the mechanisms of pharmacological action of Kampo medicines have yet to be fully elucidated at a molecular level[3]. To elucidate these molecular mechanisms, it is necessary to identify the molecular targets of the herbal ingredients and to clarify the synergism or antagonism of molecular actions. The purpose of this study is to construct a phytochemical database that facilitates identification of the molecular targets of phytochemicals contained in Kampo formulations and to examine its usefulness for exploring the molecular mechanisms behind the medicines actions.

2. Methods

2.1 Database generation

The data for molecular targets and the taxonomy of producers of phytochemicals were compiled from both primary and secondary sources, particularly Gideon Polya's Biochemical Targets of Plant Bioactive Compounds[4]. The chemical

information on phytochemicals was also obtained from primary and secondary sources, the latter of which included databases such as PubChem (http://pubchem.ncbi.nlm.nih.gov/) and NikkajiWeb (http://nikkajiweb.jst.go.jp/nikkaji_web/pages/top_e.html). PhytodamaTarget DB and PhytodamaTaxon DB were constructed by using Microsoft Office Access 2003.

2.2 Graphical presentation of relationship between phytochemicals and their molecular targets

CellDesigner 4.2, a tool for modeling biochemical networks with a graphical user interface developed by H. Kitano *et al.*[5], was used to present graphically the relationship between phytochemicals and their molecular targets.

2.3 Screening of molecular targets involved in analgesic activity

A list of 371 genes involved in pain was obtained from the database PainGenesdb (http://www.jbldesign.com/jmogil/enter.html).

The molecular targets of phytochemicals involved in analgesic activity were identified among the targets of either *Glycyrrhiza globra* or *Paeonia lactiflora* by checking whether they were included in the pain gene list.

3. Results and Discussion

3.1 Construction of PhytodamaTarget and PhytodamaTaxon Databases

We constructed two databases named PhytodamaTarget and PhytodamaTaxon DBs, the former covering information on molecular targets of phytochemicals together with their chemical properties, and the latter providing information on the taxonomy of the producers of phytochemicals together with herbal information.

The basic information in PhytodamaTarget DB consists of phytochemical names, phytochemical synonyms, target names, target subfamilies, target categories, modes of action, IC_{50}/EC_{50}, *Kd/Ki*, chemical structure, chemical formulae, smiles strings, molecular weights, biosynthetic pathways, links to other databases such as PubChem, KEGG (http://www.genome.jp/kegg/) and GeneCards (http://www.genecards.org/) and references (Fig.1) whereas that in PhytodamaTaxon DB consists of taxonomic names including genus, family, order, clade, Japanese, Chinese and English herb names, and phytochemical names (Fig.2).

Fig.1. Representative form of PhytodamaTarget DB.

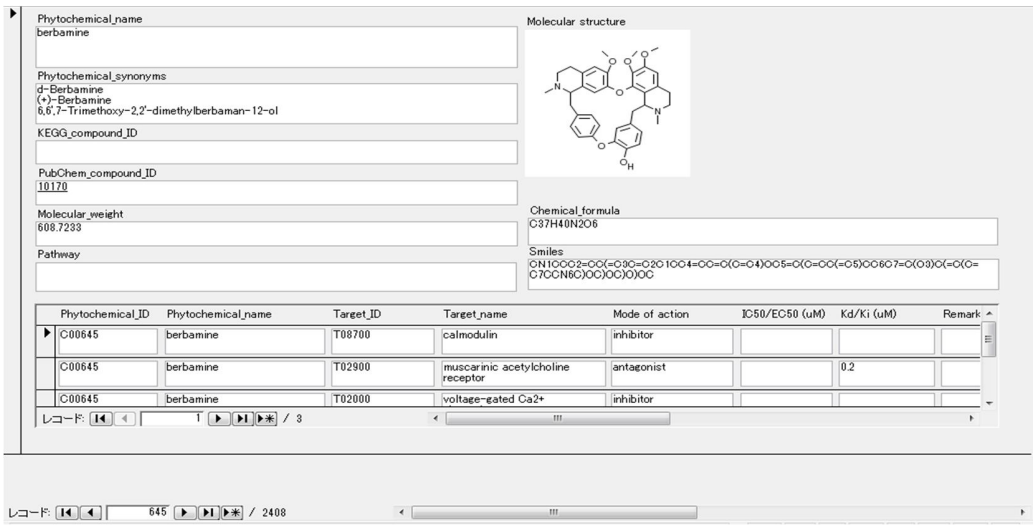
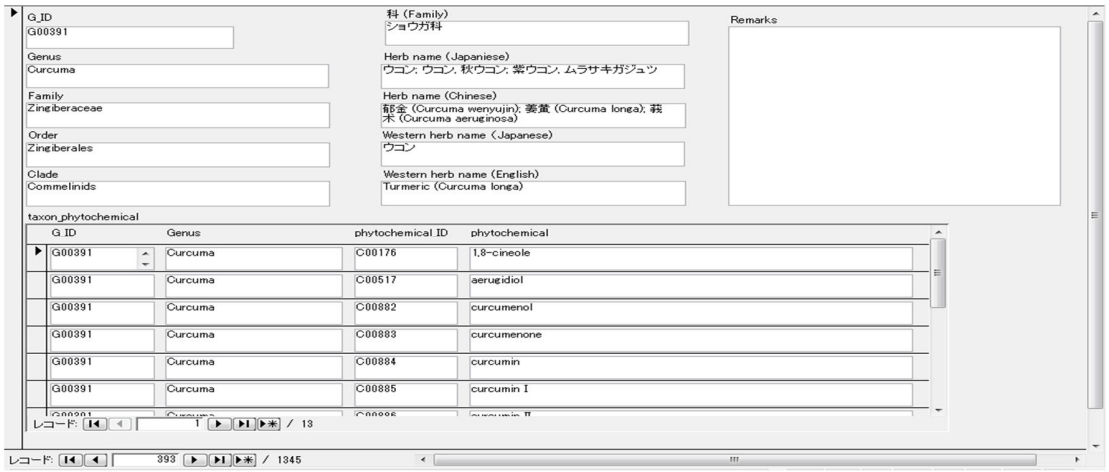


Fig. 2. Representative form of PhytodamaTaxon DB.



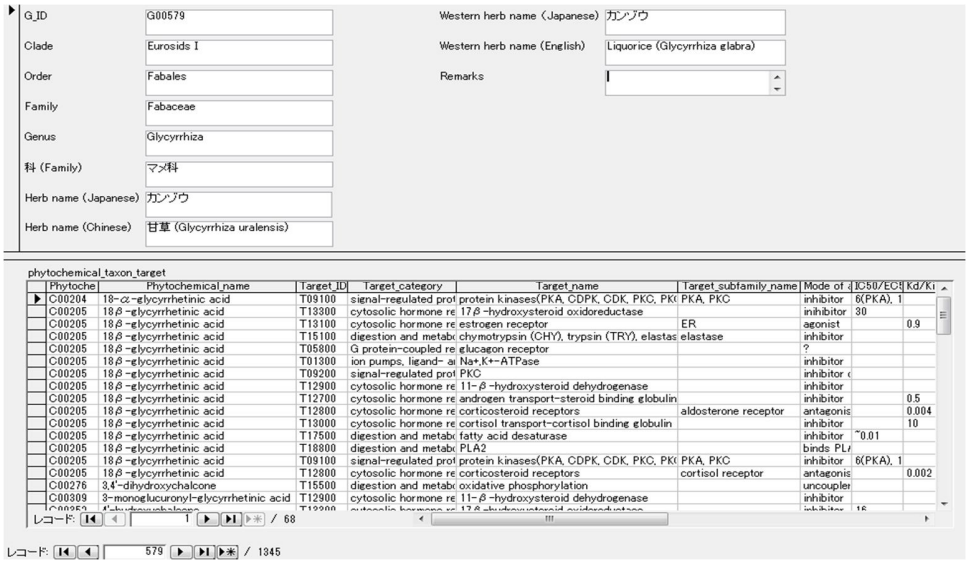
The data set used in the classification of targets: target category, target name and target subfamily designates pharmacologically distinct groups such as G protein-coupled receptors, gene product families such as receptor families, and gene product subfamilies such as receptor subtypes, respectively. The plant classification was performed according to the APG III system[6].

Registered numbers of phytochemicals, targets, plant genuses and herbs in the databases are 2408, 210, 1345 and 159, respectively.

3.2 Retrieval of Targets of Herbs

Identification of molecular targets of phytochemicals contained in herbs is helpful in elucidating the pharmacological effect of herbs. This identification is easily accomplished by combined use of PhytodamaTarget and PhytodamaTaxon DBs. Namely, data on plant genus can be obtained instantly together with that on its constituent phytochemicals and their targets by using the "subform" and "query" functions of Microsoft Access (Fig. 3).

Fig.3. Presentation of phytochemicals and their targets corresponding to a plant genus record.

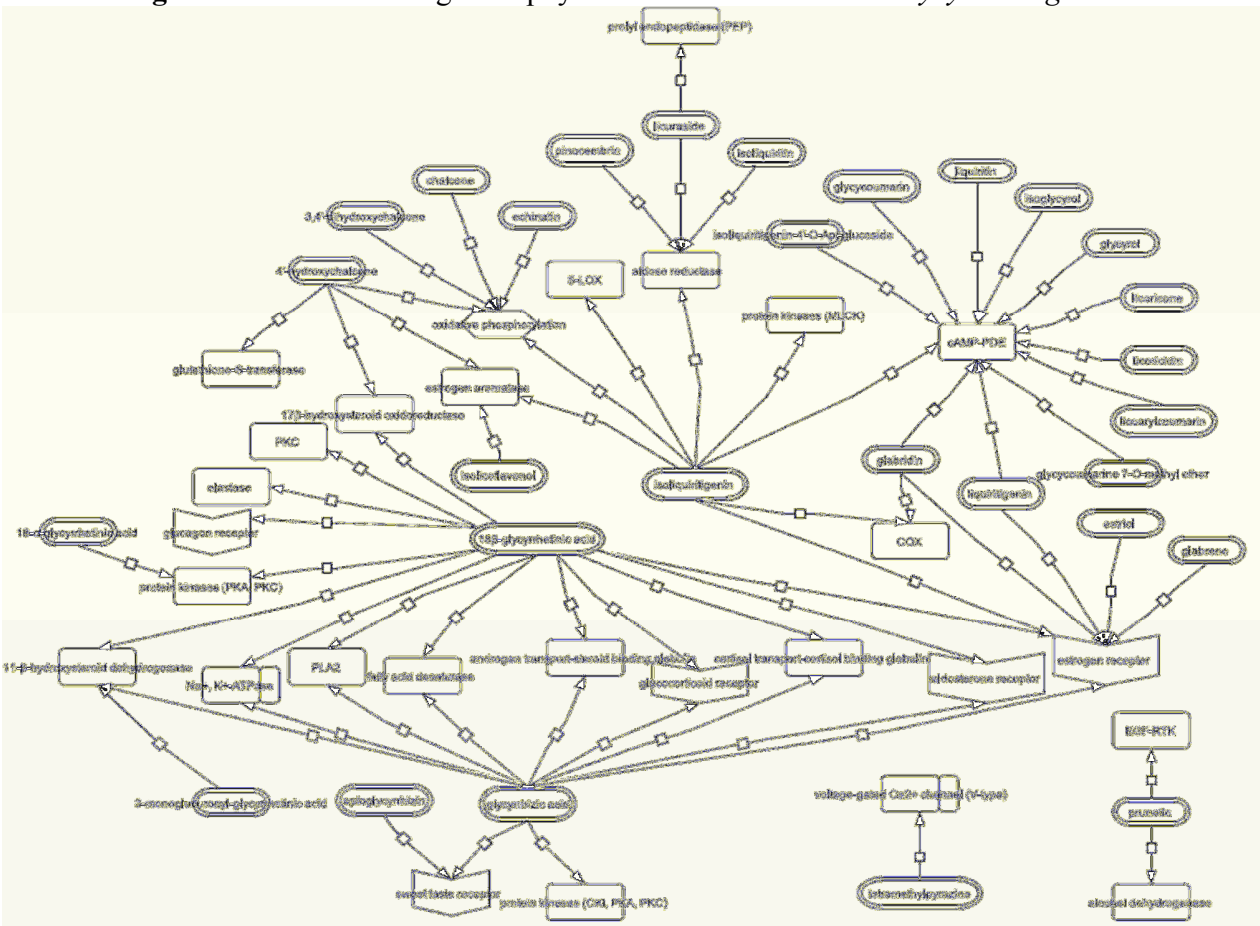


3.3 Possible mechanism of synergism in analgesic activity between herbal medicines derived from *Glycyrrhiza globra and Paeonia lactiflora*

To validate the usefulness of our databases for exploring the molecular mechanisms responsible for the pharmacological activities of Kampo medicines, we used them to analyze the synergistic analgesic activity between the herbs from *Glycyrrhiza globra* and *Paeonia lactiflora*.

Synergism in analgesic activity between the herbs from *Glycyrrhiza globra* and *Paeonia lactiflora* was first described in a Chinese book named Treatise on Cold Damage Disorders collated by Zhang Zhongjing. Presently *Shakuyakukanzoto*, a Kampo formulation composed of *Glycyrrhiza globra* and *Paeonia lactiflora*, is mostly used to relieve muscle pain[7]. The pharmacological mechanism of this analgesic activity has been investigated by several groups. Kimura *et al.* found that a combination of paeoniflorin and glycyrrhizin, major ingredients of *Paeonia lactiflora* and *Glycyrrhiza globra*, respectively, synergistically inhibited twitch tensions of indirectly stimulated diaphragm muscles of mice[8]. Satoh and Tsuruo also found that *Shakuyakukanzoto* relaxed carbachol-induced contractions of rat intestinal smooth muscles and assumed that the relaxation was caused by anti-cholinergic and phosphodiesterase inhibitory actions[9]. However, anti-cholinergic activity of the ingredients of both *Glycyrrhiza globra* and *Paeonia lactiflora* has yet to be shown directly although phosphodiesterase inhibitory activities of several compounds of *Glycyrrhiza globra* were reported.

Fig.4. Network of the targets of phytochemicals contained in *Glycyrrhiza globra.*

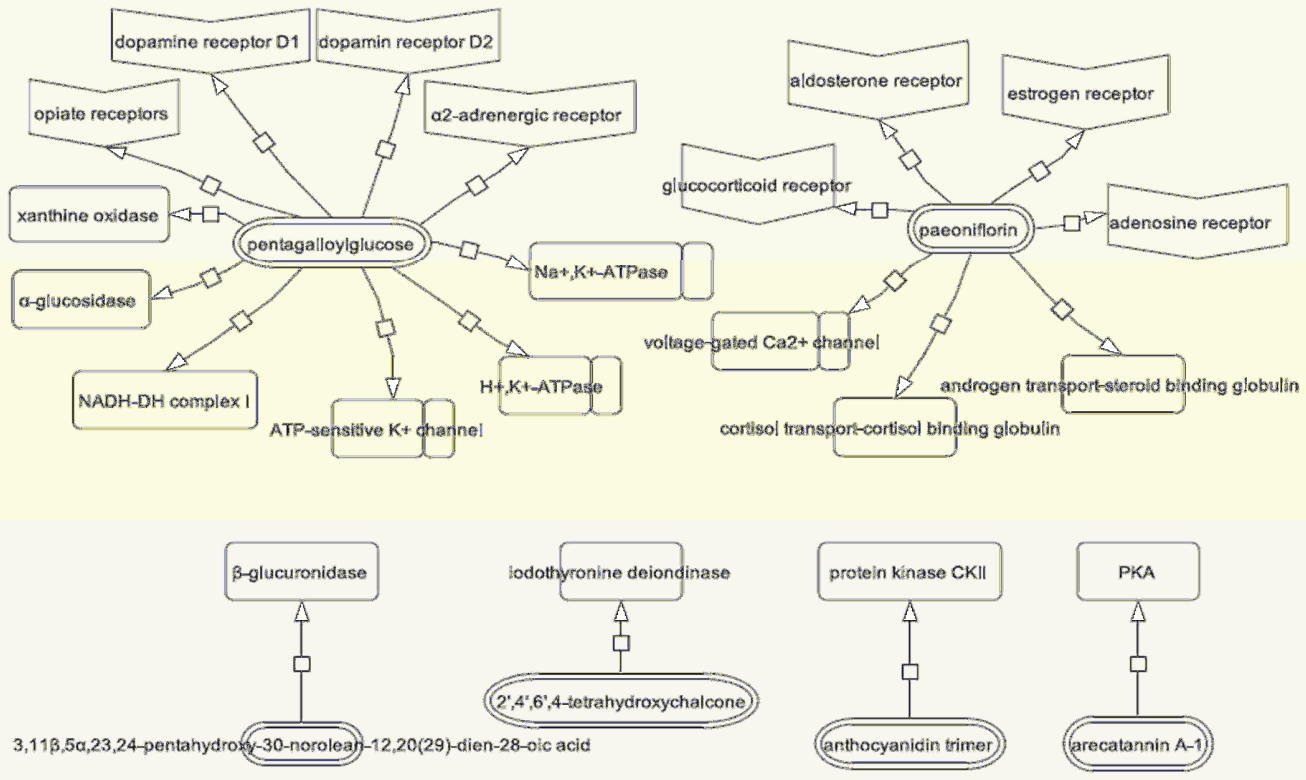


Meanwhile, other mechanisms of the analgesic activity of *Shakuyakukanzoto* and its constituents have been reported. Omiya *et al.* found that *Shakuyakukanzoto* showed antinociceptive activity in diabetic mice and assumed the activity to be caused by activation of the descending noradrenergic neurons[10]. Liu *et al.* ascribed the antinociceptive effect of paeoniflorin in mice to its ability to increase the binding and antinociceptive effect of an adenosine A1 agonist by binding with A1 receptors[11]. According to our database, *Paeonia lactiflora* contains a constituent with α2-adrenergic agonist activity, namely β-1,2,3,4, 6-penta-O-galloyl-D-glucose (PGG), together with paeoniflorin. Further studies are necessary to conclude whether PGG, paeoniflorin or same as yet unidentified ingredient is responsible for the analgesic activity of *Shakuyakukanzoto* in diabetic mice.

The molecular mechanism behind the synergistic analgesic effect of *Shakuyakukanzoto* remains largely unclear despite extensive studies. To elucidate it, it is necessary to clarify the interactions among constituents whose molecular targets are involved in the analgesia. Accordingly, the molecular targets of phytochemicals contained in *Glycyrrhiza globra* and *Paeonia lactiflora* were first retrieved by combined use of our databases. The number of hits for phytochemicals in *Glycyrrhiza globra* and *Paeonia lactiflora* was 29 and 6, respectively and the total number of hits for the molecular targets of these phytochemicals was 28 and 21, respectively (Fig.4 and Fig.5). The number of molecular targets common to both genuses was 6. Furthermore, some molecular targets were shared by multiple phytochemicals within the genus (Fig.4).

The symbols used to represent molecules and actions are as follows; ovoids, concave hexagons, and divided and simple round-cornered squares represent phytochemicals, receptors, ion channels and generic proteins, respectively. Arrows represent actions of phytochemicals against targets.

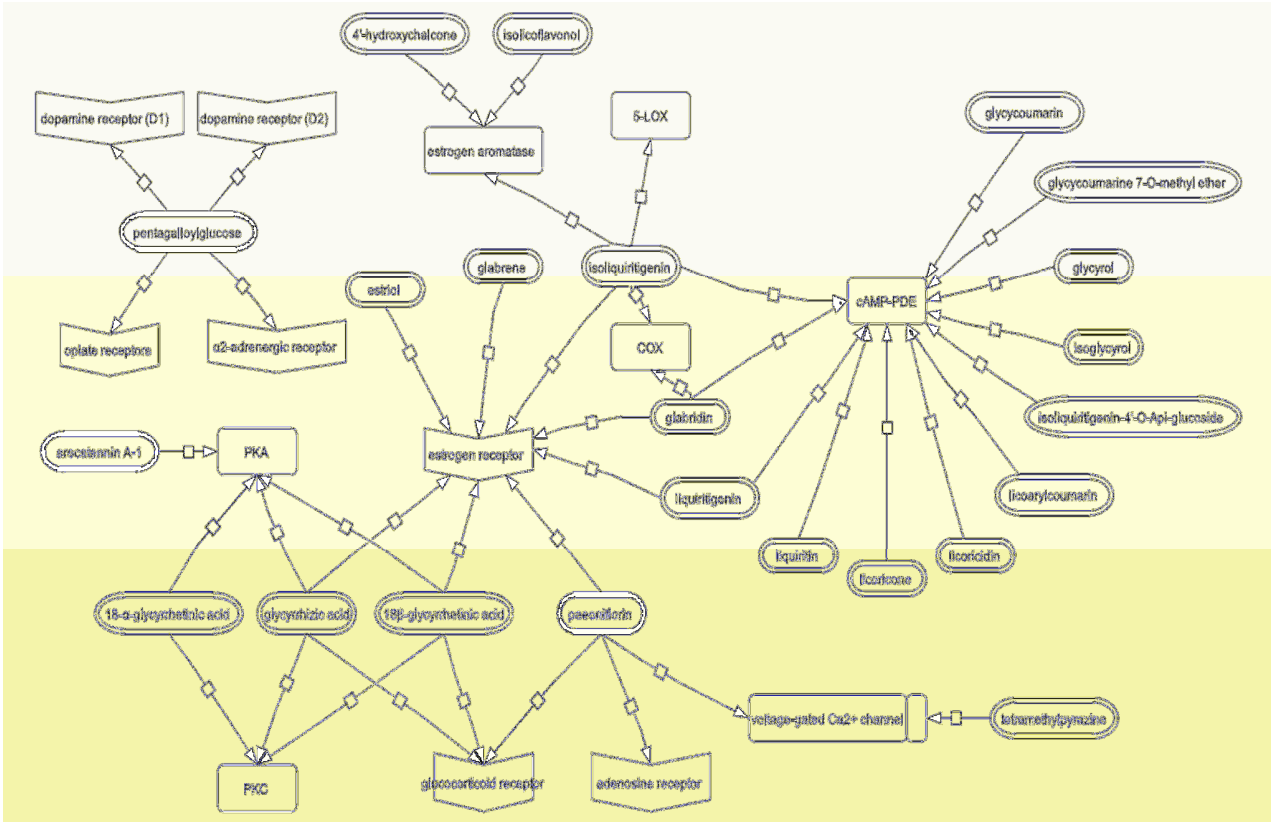
Fig.5. Network of the targets of phytochemicals contained in *Paeonia lactiflora.*



The symbols used are explained in the legend of Fig. 4.

To identify the molecular targets involved in analgesic activity among those listed, those coinciding with the gene products registered in the pain genes database[12] were screened. Eight out of 28 targets for *Glycyrrhiza globra* and 8 of 21 targets for *Paeonia lactiflora* were hit. They are cyclooxygenase, estrogen aromatase, the estrogen receptor, 5-lipooxygenase, protein kinase A, protein kinase C, cAMP phosphodiesterase and voltage-gated Ca^{2+} channels for *Glycyrrhiza globra* and the adenosine receptor, dopamine receptors, the estrogen receptor, opiate receptors, protein kinase A, voltage-gated Ca^{2+} channels and the α2-adrenergic receptor for *Paeonia lactiflora*. Although the glucocorticoid receptor, a target for both genuses, is missing in the pain genes database, it may be added as a pain gene, referring to the literature[13,14]. Among the targets identified as pain-related, cyclooxygenase, 5-lipooxygenase and estrogen aromatase are involved in the biosynthesis of algesic and analgesic substances[15,16], the adenosine, estrogen and glucocorticoid receptors are receptors for algesic or analgesic substances[13,14,17,18], the α2-adrenergic, dopamine and opiate receptors are receptors for neurotransmitters[19-21], protein kinases A, C and cAMP phosphodiesterase are involved in postreceptor signaling pathways[22] and voltage-gated Ca^{2+} channels are involved in neural transmission including regulation of the release of neurotransmitters[23]. Fig. 6 summarizes the interaction between the herbal ingredients and their molecular targets involved in analgesia.

Fig.6. Network of the pain-involved targets of phytochemicals contained in either *Glycyrrhiza globra* or *Paeonia lactiflora.*



The symbols are the same as those in Fig.4 except that ovoids with double outlines represent phytochemicals contained in *Glycyrrhiza globra* whereas ovoids with a thick outline represent those in *Paeonia lactiflora*.

The network of herbal ingredients and their pain-involved molecular targets shows that there are hubs, targets highly connected with ingredients, such as cAMP phosphodiesterase and the estrogen receptor, suggesting their possible greater roles for analgesic activity of the herbal medicine. Cyclic AMP phosphodiesterase catabolizes cAMP which is a pain-mediating second messenger.[24] Hence, the inhibitory activity of phytochemicals against cAMP phosphodiesterase would lead to pain enhancement. However, contrary to this speculation, cAMP phosphodiesterase inhibitors were shown to elevate nociceptive thresholds in the central nervous system by increasing antinociceptive natural epoxy-fatty acids[25]. The estrogen receptor was shown to be involved in lowering nociceptive thresholds by using the estrogen receptor knockout female mice although its mechanism has yet to be deciphered[26]. The phytochemicals would exert analgesic activity not by their agonistic activity but by their antagonistic activity against the estrogen receptor in the presence of endogenous estrogen[27].
Meanwhile, among targets shared by multiple components, those shared by components from different herbs, such as voltage-gated Ca^{2+} channels, the glucocorticoid receptor, the estrogen receptor and protein kinase A, are particularly noteworthy since one possible mechanism of synergism between agonists/antagonists or inhibitors is differential activities against a shared target. However, targets other than those mentioned above could be involved in the synergism, because simultaneous activities against targets closely related to analgesic activity is another possible mechanism of synergism. Though the ideas discussed above need to be examined further in wet experiments, the information provided by our database should help to elucidate the molecular mechanism of synergism in the analgesic activity of *Shakuyakukanzoto*. Furthermore, PhytodamaTarget and PhytodamaTaxon DBs should prove useful for analyzing the synergism or antagonism of pharmacological activities among herbal components of Kampo and other traditional medicines.

4. Conclusion

PhytodamaTarget and PhytodamaTaxon DBs were shown to facilitate identification of the molecular targets of phytochemicals contained in Kampo formulations and to be useful for exploring the molecular mechanisms of the synergistic pharmacological actions of these medicines.

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Antioxidant Activity of *Centella asiatica* (Linn.) Urban: Impact of Extraction Solvent Polarity

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In vitro antioxidant activity of *Centella asiatica* (Linn.) and the impact of extraction solvent polarity on the antioxidant potential were investigated in the present study. 100% ethanol, 50% ethanol and water were chosen as extraction solvent due to arithmetic progression of their polarity. Total polyphenol, flavonoid, β-carotene, tannin and vitamin C content of these three extracts were determined while their antioxidant potentials were assayed by total reducing power assay and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity. 50% ethanol extract of *C. asiatica* contained significantly higher amount of polyphenol, flavonoid while moderate amount of carotene and tannin but the lowest amount of vitamin C compared to 100% ethanol and water extract. All the phytochemicals showed solvent polarity specific extraction pattern. Total reducing power and DPPH-radical scavenging activity of 50% ethanol extract also were significantly higher when compared to those of the 100% ethanol and water extracts. Significant variations of antioxidant potentials of *C. asiatica* due to differences in the extraction solvent polarity were demonstrated in this study

Keyword: Antioxidant, Flavoniod, *Centella asiatica*, DPPH, Polarity Index.

1. Introduction:

Oxidative stress occurs when the generation of free radicals or reactive oxygen species (ROS) exceeds the antioxidant capacity of a biological system[1]. Excess free radicals and ROS attack biological molecules such as lipids, proteins and nucleic acids that lead to tissue or cellular injury[2,3]. Oxidative stress has already been implicated in atherosclerosis, cancer, diabetes, arthritis, reperfusion damage and inflammation[4]. Antioxidants are free-radical scavengers that provide protection to living organisms from damage caused by ROS. Although almost all organisms possess antioxidant defense and repair systems but these systems are insufficient to cope over entire damage. So, dietary antioxidant supplementation is a promising mean to strengthen the antioxidant defense and repair systems. However, antioxidants from natural source are of great value as most commonly used synthetic antioxidants (e.g. butylatedhydoxyanisole, butylatedhydoxytoluene and propylgallate) have health hazardous side effects like liver damage and carcinogenesis[5].

Centella asiatica (Linn.) Urban is a prostrate stoloniferous plant that belongs to the family Apiaceae and endogenous to Bangladesh[6]. The therapeutic use of C. *asiatica* with its wide range of application has been documented in South East Asia and Bangladesh for centuries. C. asiatica is effectively being used in the treatment of fever, jaundice, dysentery, diarrhea, mental illness

within the frame of traditional medicine of Bangladesh[7]. Though several investigations showed antioxidant activity of C. *asiatica* but none of these explained the role of extraction solvent polarity on antioxidant potential[8,9,10]. We, therefore, investigated in vitro antioxidant activity of C. *asiatica* and the impact of the extraction solvent polarity on antioxidant potential in this present study.

2. Materials and Methods

2.1 Collection of Plant Materials and Extraction

Wild *Centella asiatica* was collected from the Jabra, Manikgang. The plant was identified and authenticated by Department of Botany Jahangirnagar University, Savar, Dhaka (NoJUBD: 1206). The fresh leaves with petioles were then air dried in shadow and grinded by mechanical grinder. Fine plant powder was then used for the exhausted extraction by Soxhlet apparatus for four repeated cycle using 100% ethanol, 50% ethanol and water as extraction solvent. During extraction solute-solvent ratio was 10:1 and extraction temperature was 45º±2ºC. The extracts were then filtered, evaporated using oven to a thick residue at 45°C and stored at 4°C. This crude extract was used for further analyses.

2.2 Total Polyphenol Content

Total polyphenol content of extracts was determined following Amin *et al.* (2006) using pyrogallol as standard[11]. The concentration of total phenol compounds in extracts was determined as pyrogallol equivalents (µg of PE/mg of extract).

2.3 Total Flavonoid Content

Total flavonoid content of extracts was estimated following aluminum chloride colorimetric assay described by Chang *et al.* (2001)[12]. Quercetin was used as standard. The concentration of total flavonoid in the extract was determined as quercetin equivalents (µg of QE/mg of extract).

2.4 β-carotene Content

ß-carotene content of extracts was determined by the method described by Nagata and Yamashita (1992) with slight modification[13]. The dried extract of *C. asiatica* (100 mg) was vigorously shaken with 10 ml of acetone - hexane (4:6) for 1 min and filtered through filter paper (Whatman No. 4). The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm spectrophotometrically. ß-carotene content was calculated according to the following equations:

$$\textit{ß-carotene (mg/100 ml)} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

The concentration of ß-carotene in the extracts was expressed as µg of ß-carotene /mg of extract.

2.5 Total Tannin Content

Total tannin content of extracts was determined by the Folin-Ciocalteu's method using tannic acid as standard[14]. The concentration of total tannin in extracts was expressed as tannic acid equivalents (µg of TE/mg of extract).

2.6 Vitamin-C content

Vitamin C content of extracts was estimated by the method of Omaye *et al.* (1994)[15]. Vitamin C was used as standard. The vitamin C content of extract was calculated as ascorbate equivalents (µg of AE/mg of extract).

2.7 Total Reducing Potential

The reducing power of extracts was estimated following Oyaizu (1986) using vitamin C as standard[16]. The reducing power of extract was calculated as ascorbic acid equivalents (µg of AE/mg of extract).

2.8 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging Activity

DPPH-free radical scavenging activity of extracts was measured following Braca *et al.* (2002)[17]. DPPH-free radical scavenging activity of *C. asiatica* was calculated as % of radical inhibition by following equation:

% Radical Inhibition = {(Control OD – Sample OD)/ Control OD)} X 100

DPPH-free radical scavenging activity was calculated and expressed as IC_{50} that is the concentration of *C. asiatica* required to scavenge 50% of DPPH used.

2.9 Statistical Analysis

The results are expressed as mean ± SEM (Standard error of mean). The statistical programs used were StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA) and GRAPHPAD PRISM® (version 4.00; GraphPad Software Inc., San Diego, CA, USA). Intergroup variation was analyzed by one way ANOVA followed by Tukey's least square differences test for post hoc comparisons. A level of $P<0.05$ was considered statistically significant.

3. Results and Discussion

Antioxidant activity of a plant extract is a complex attribute of its phytoconstituents. In the present study we have estimated total polyphenol, total flavonoid, β-carotene, tannin and vitamin C content in three different extracts namely 100% ethanol extract, 50% ethanol extract and water extract of *C. asiatica*. The results of the phytoconstituents content of these three extracts have been summarized in Table 1.

Table 1. Antioxidant phytoconstituents of 100% ethanol extract of *C. asiatica* (100% E Ex), 50% ethanol extract of *C. asiatica* (50% E Ex) and water extract of *C. asiatica* (H_2O Ex).

Antioxidant Phytoconstituents	Content (µg/mg of extract)		
	100% E Ex	50% E Ex	H_2O Ex
Polyphenols (PE)	21.1±0.1[a]	45.2±0.3[b]	35.6±0.5[c]
Flavonoids (QE)	9.3±0.3[a]	14.6±0.2[b]	11.7±0.2[c]
ß-Carotene	1.1±0.4[a]	0.7±0.1[a]	0.2±0.1[a]
Tannin (TE)	85.7±3.3[a]	59.7±0.9[b]	60.7±1.8[b]
Vitamin C (AE)	12.5±0.7[a]	9.5±0.2[b]	13.3±0.4[a]

Results are mean±SEM (n=3). PE, Pyrogallol Equivalent; QE, Quercetin Equivalent; TE, Tannic acid Equivalent; AE, Ascorbic acid Equivalent. Intergroup variation was analyzed by one-way ANOVA followed by Tukey's least square differences test for post hoc comparisons. Values in the same row with different subscription are significantly different at P< 0.05.

Polyphenols are available plant secondary metabolites and a critical index for determining the antioxidant capacity[18]. The antioxidant activity of polyphenols are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators[19]. The mechanisms of action of flavonoids are exerted through scavenging or chelating process[20]. Vitamin C directly interacts with a broad spectrum of ROS and terminates chain reaction initiated by these free radicals through electron transfer while involved in the regeneration of vitamin E[21]. ß-carotene is an excellent scavenger of singlet oxygen[22].

In the present investigation, total reducing potential assay and the DPPH scavenging activity were performed to evaluate *in vitro* antioxidant potential of three different extracts of *C. asiatica* including 100% ethanol extract, 50% ethanol extract and water extract. Total reducing power of 50% ethanol extract of *C. asiatica* (63.4±1.7 µg of AE/ml) was significantly higher than total reducing power of 100% of ethanol extract of *C. asiatica* (40.4±0.7 µg of AE/ml) and water extract of *C. asiatica* (56.3±0.6 µg of AE/ml) (Table 2). DPPH is a relatively stable nitrogen centered free

radical that easily accepts an electron or hydrogen when reacts with suitable reducing agents as results of which the electrons become paired off and the solution losses color depending on the number of electrons taken up[23]. In case of DPPH-scavenging activity, IC_{50} values of 100% ethanol extract of *C. asiatica*, 50% ethanol extract of *C. asiatica* and water extract of *C. asiatica* were 35.6±1.3 µg/ml , 7.1±1.5 µg/ml and 10.3±1.2 µg/ml respectively. IC_{50} values of 50% ethanol extract of *C. asiatica* and water extract of *C. asiatica* were nearly similar and not significantly (P>0.05) different. But, IC_{50} value of 50% ethanol extract of *C. asiatica* was significantly higher than that of 100% ethanol extract and water extract (Table 2).

Table 2. Comparative *in vitro* antioxidant activity of 100% ethanol extract of *C. asiatica* (100% E Ex), 50% ethanol extract of *C. asiatica* (50% E Ex) and water extract of *C. asiatica* (H_2O Ex).

Extracts	100% E Ex	50% E Ex	H_2O Ex
Reducing power (AE)	40.37±0.73[a]	63.4±1.72[b]	56.3±0.57[c]
DPPH scavenging Activity (IC_{50})	35.56±1.24[a]	7.08±1.54[b]	10.23±1.20[b]

Results are mean ± SEM, n= 3. AE, Ascorbic acid Equivalent; IC50 =Concentration required to inhibit 50% of DPPH radical. Intergroup variation was analyzed by one way ANOVA followed by Tukey's least square differences test for post hoc comparisons. Values in the same row with different subscription are significantly (P<0.05) different.

The presences of reductive phytoconstituents are reflected by reducing potential and DPPH-scavenging activity of corresponding extract[24]. We therefore, speculated that observed total reducing power and DPPH scavenging activity of the different extracts may be the contribution of one or more antioxidant phytoconstituents.

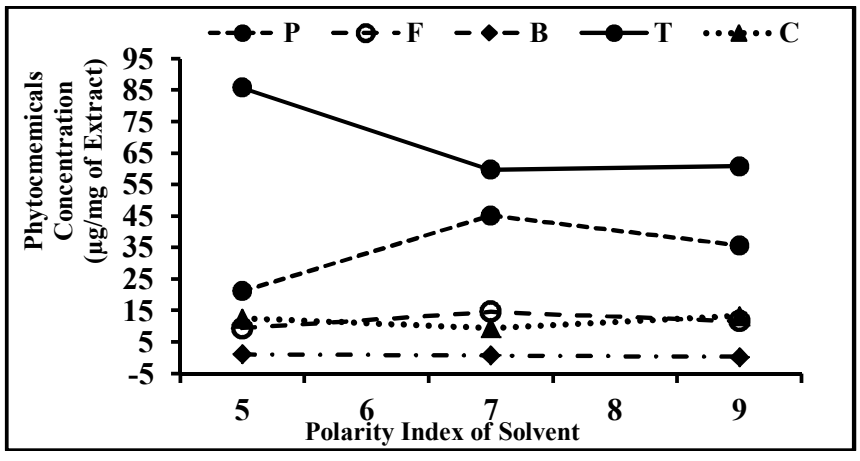

Fig 1: Impact of solvent polarity on the phytochemicals extracted. Here, P= total polyphenols content; F= total flavonoids content; B= ß-Carotene content; T= tannin content and C=vitamin C content. The polarity index value for 100% ethanol, 50% ethanol and water are 5.2, 7.1 and 9 respectively.

We chose 100% ethanol, 50% ethanol and water as extraction solvent in the present study. The polarity index value for 100% ethanol, 50% ethanol and water are 5.2, 7.1 and 9, respectively[25]. We took the arithmetic progression pattern (9-7.1=1.9; 7.1-5.2=1.9) of polarity as advantage to select the solvent of interest. Maximum extraction of both polyphenols and flavonoids occurs within a selective polarity range. Any deviation from that range either to higher polarity or to lower polarity decreases the extraction yields. The extraction yield for tannin was observed to decrease up to a steady state with the increase of polarity. ß-carotene showed a gradual decrease in extraction yield with the increase of polarity. Interestingly, maximum extraction of vitamin C was found in both higher and lower solvent polarity (Fig1). During extraction, organic solvents diffuse into the solid material and solubilize the compound with similar polarity. The nature of the solvent used will determine the type of chemicals likely extracted from plant materials[26]. Polarity is the relative ability of a molecule to engage in strong interactions with other polar molecules[27]. Polarity therefore represents the ability of a molecule to enter into interactions of all kinds. Polar solvents have property of dipole interaction forces, particularly hydrogen-bond formation for which solvating molecules become soluble and leads to the solubility of the compound[28]. Most of the bioactive components of plant matrices are medium-sized molecules. Due to the presence of aromatic delocalized µ-electrons, the molecules are highly polarizable[29]. Therefore, difference in the polarizability makes the phytochemicals liable to a variety of specific interactions with polar solvents that lead to polarity dependent extraction yield variation. This can explain the present observation though further studies are essential to explain the phenomenon minutely.

4. Conclusion

Besides the food value C. *asiatica* is used in a wide range of pharmacological activity within the ethnbotanic frame worldwide. Emphasis should be paid to the extraction solvent property as variation of pharmacological activity might be attributed through extraction solvent differences. Thus, significant variation of antioxidant potential of C. *asiatica* due to extraction solvent polarity difference was demonstrated in this study.

5. Conflict of Interests

Authors have declared that no competing interests exist.

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Screening of Analgesic and Immunomodulator activity of *Artocarpus heterophyllus* Lam. Leaves (Jackfruit) in Mice

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Adaptability is probably the most distinct characteristics of life which may be defined as sum of all nonspecific response of the body to any demands made upon it; fundamentally it was a physiological response; primary object of which was to maintain life and to re-establish the normal state. Analgesic and Immunomodulator activity of leaves of *Artocarpus heterophyllus* Lam. was evaluated by using Eddy's hot plate method and Swimming endurance test at the dose levels of 250 and 500mg/kg in Swiss albino mice respectively. The methanolic and aqueous extracts of leaves of A. *heterophyllus* were administered to the experimental animals among which the methanolic extract of A. *heterophyllus* leaves have shown to be exhibit significant analgesic and immunomodulator effect by paw licking and increasing the swimming or survival time (P<0.001) respectively in mice

Keyword: *Artocarpus heterophyllus* Lam., Eddy's hot plate, Swimming endurance test, Analgesic, Immunomodulator

1. Introduction:

Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens. Several plant products have been reported for immunomodulatory activity and many formulations of these plant products are available to enhance the immune system. Plants are the essential and integral part in complementary and alternative medicine. Plants have the ability of the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases [1, 2].

Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. There are many plants, which are having immunostimulatory where as other have immunosuppressant activity [3]. The *Artocarpus heterophyllus* Lam. is a species of tree of the mulberry family Moraceae, known by other names jackfruit. It is a large, evergreen tree, 10-15m in height, indigenous to the evergreen forests at altitude of 450-1,200m and cultivated throughout the hotter parts of India. *Artocarpus heterophyllus* Lam. is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, cycloheterophyllin ($C_{30}H_{30}O_7$), artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophylol [4, 5] which are useful in fever, boils, wounds, skin diseases, convulsions, diuretic, constipation, ophthalmic disorders and snake bite etc. The leaves are useful in fever, ulcers, boils wounds, skin diseases, antidiarrhoeal, analgesic and as immunomodulator. The ripe fruits are sweet,

cooling, laxative, aphrodisiac, and tonic. The seeds are sweet, diuretic, aphrodisiac and constipating[6]. The plant is reported to possess antibacterial[7], anti-inflammatory [8], antidiabetic [9], antioxidant[10], antifungal [11] and immunomodulatory properties[12].

Artocapus heterophyllus Lam. is used as a traditional medicine as analgesic and immunomodulator. Immunomodulator activity has been scientifically proved in fruits whereas analgesic activity is not scientifically proved. The aim of the present study is to investigate its analgesic and immunomodulator activity on leaves of *A. heterophyllus* in a scientific manner which has not been carried out so far.

2. Materials and Methods

2.1 Collection of Plant Materials

The leaves of *Artocarpus heterophyllus* Lam were collected from local area of Lucknow and authenticated from Taxonomic division of National Botanical Research Institute, Lucknow and a voucher specimen was deposited for future references (ref. no.: NBRI/CIF/Re./08/2008/32).

2.2 Preparation of Extracts

The leaves were washed with tap water and then with distilled water and dried in shade. Leaves were comminuted to powder and extracted with methanol and water. The extracts were concentrated under reduced pressure using rotavapour.

2.3 Animals

Adult male Swiss albino mice weighing 20 ± 5g, six animals per group were used for the study. The animals were housed under standard laboratory conditions in polypropylene cages. Ambient temperature of 25±4°C, 55±2% relative humidity and 12h dark and light cycle was maintained. They were supplied with food and water *ad libitum*. All groups of animals were treated with normal saline water, standard and test drugs for 21 days. Acute toxicity of all the extracts was determined by LD_{50} values by staircase method which was more than 3000mg/kg b.w. for the methanol and the aqueous extracts of leaves [13]. All pharmacological activities were carried out as per CPCSEA norms, after obtaining the approval (Ethical committee No. BBDNITM/IAEC/Clear/03/2009) from the Institutional Animal Ethical Committee of Babu Banarasi Das National Institute of Technology & Management, Lucknow, India.

2.4 Analgesic Activity

a. Eddy's Hot Plate Method

The animals were divided into six groups of 6 animals each. Group I served as control. Group VI served as standard and were injected Diclofenac sodium (9.5mg/kg) intraperitonially. Group II and III were treated orally with methanolic extract of 250 and 500mg/kg body weight respectively. Group IV and V were treated orally with aqueous extract at a dose level of 250 and 500mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds [14, 15].

2.5 Immunomodulatory Activity

a. Swimming Endurance Test

Swimming endurance test was carried out on a 21st days according to method described by standard monograph. Precaution was taken that mice should not be at rest at any particular place and should swim continuously. End point of the test is considered to be the point of exhaustion, when the animal remains floating passively in water in an upright position, making only small movements to maintain the head just above the water level [16, 17].

3. Results and Discussion

The data reveals that the methanolic extract of *A. heterophyllus* leaves at a dose of 500mg/kg showed significant activity ($P<0.001$*, $P<0.05$**) after 60 minutes. The results showed significant analgesic activity against thermal stimuli. The analgesic studies revealed that the methanolic extract of *A. heterophyllus* leaves exhibited

potent analgesic (central analgesic activity) and also revealed that the extracts shows dose dependent analgesic effect. On the basis of swimming endurance test, the effect of methanolic and aqueous extract of *A. heterophyllus* Lam. leaves was compared respectively. It was concluded that the methanolic extract of leaves were having higher values with respect to aqueous extract in increasing swimming or survival time, hence methanolic extract were found to exert more immunomodulator effect than aqueous extract. Thus, the results obtained in the present study suggested that *Artocapus heterophyllus* exhibited analgesic and immunomodulator activity and thus supports the folk usage, it is highly desirable to explore its mechanism of action further for pharmacological justification to the use of the plant extract by traditional medicine practitioners as analgesic and immunomodulator action.

Table 1: Effect of Extracts on Pain Induced By Hot Plate

S. No.	Groups	Eddy's hot plate, paw licking time in seconds after (Mean±SEM)				
		0 Min.	30 Min.	60 Min.	90 Min.	120 min
I.	Control (DMSO) 1 ml per oral	6.0±0.56	6.20±0.34	5.72±0.28	5.24±0.87	5.12±0.34*
II.	Methanolic extract (250mg/kg per oral)	6.2±0.36	12.55±0.34*	16.34±0.67*	15.44±0.18*	11.55±0.65*
III.	Methanolic extract (500mg/kg per oral)	6.0±0.58	19.67±0.86*	21.78±1.59*	20.53±0.78*	13.98±0.37*
IV.	Aqueous extract (250mg/kgper oral)	6.4±0.76	10.67±0.98*	11.56±0.34*	8.98±0.67*	6.59±0.45**
V.	Aqueous extract (500mg/kg per oral)	6.5±0.46	15.89±0.45*	15.34±0.27*	11.54±0.39*	8.72±0.70*
VI.	Diclofenac Sod. (9.5 mg/kg intra peritoneally)	5.5±0.89	23.45±0.98*	24.52±0.65*	21.26±0.64*	18.40±0.45*

Values are expressed as mean±SEM, (n=6) (compared to control group) by using One Way Analysis of Variance (ANOVA) followed by Newman-Keuls test P<0.001*, P<0.05**.

Table 2: Effect of Jackfruit Extracts (Methanolic and Aqueous) On Swimming Endurance

Treatment groups (oral)	Dose (on the basis of body weight) by oral route	Mean Swimming time (in min.)
Control (Normal Saline)	2 ml	280.60±1.39
Standard (AP-3000)	30 mg/kg	355.50±1.4**
Methanolic extract	250 mg/kg	318.70±0.88***
Methanolic extract	500mg/kg	329.0±0.97***
Aqueous extract	250 mg/kg	307.0±0.51***
Aqueous extract	500mg/kg	318.0±1.16***

Values are expressed as mean±SEM (n=6) One Way Analysis of Variance (ANOVA) followed by Newman-Keuls test P* <0.05, P**<0.01, P***<0.001.

4. Conclusion

The above studies indicate that the methanolic extract of the leaves of *Artocapus heterophyllus* possess analgesic and immunomodulator activity up to significant level which is justified by Eddy's hot plate and Swimming endurance test. Further studies are desirable to isolate the active constituents responsible for this activity.

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Pharmacognostical Studies of the Seed of *Lagonychium farctum* (Banks & Sol.) Bobr. Growing in Egypt

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Lagonychium farctum (Banks & Sol.) Bobr.is a wild plant growing in the deserts of Upper Egypt as a prickly perennial shrub with monopodial branches. It belongs to family Leguminosae. The plant is usually branching from the base and attains up to 2.5 m in height. It is used in traditional system of medicine as an astringent as well as anti-dysenteric. The present study investigates various standardized parameters such as macroscopic and microscopic characters which could be helpful in authentication of the seed of *Lagonychium farctum*. (Banks & Sol.) Bobr.

Keyword: *Lagonychium farctum*, Prosopis, Acacia, Mimosa, Leguminosae, Seed, Pharmacognostical Study

1. Introduction

Genus Lagonychium (Prosopis) includes 45 species (tropical and subtropical). *Lagonychium farctum* (Banks & Sol.) Bobr. belongs to family Leguminosae and sub-family Mimosoideae[1-4]. This plant has many other synonyms as *L. stephaniana* M. B., *Prosopis farcta* (Banks & Sol.) (Orth. Err. farcata), *P. stephaniana* (Willd Kunth ex Spreng, *Acacia stephaniana* (M. B.), *A. heterocarp* Del., *Mimosa farcta* (Banks & Sol.) and *M. stephaniana*[2-6]. It is used in Palestine as an astringent and anti-dysenteric[5]. Ingestion of large amount of this plant is toxic due to presence of saponins that cause inflammation to the digestive tract and quick peristalsis [7]. Sensor and motor nerve fibers are also affected in a serious way[7]. It is an erect prickly trees or shrubs. Leaves: compound, bipinnate. Stipules: small or absent. Leaflets: small, narrow. Stem: with or without spines. Spines solitary or in pairs. Flowers: pentamerous, usually sessile in narrow spikes, regular, actinomorphic, hermaphrodite. Calyx: five-shortly toothed or subentire sepales. Corolla: five petals, valvate. Stamens: 10, free, short. Ovary: sessile or stalked, multiovulate. Style: slender, filiform. Stigma: minute, terminal. Pod: turgid, cylindrical or oblong, straight, variously twisted, septa between the seeds. Mesocarp: thick and spongy. Seeds: usually ovoid compressed, albuminous[8-10].

Fig .1: Picture of the fruit

2. Taxonomy:

Lagonychium farctum (Banks & Sol.) Bobr. belongs to[2,11]:

Phylum	:	Angiospermae,
Subphylum	:	Dicotyledonae,
Class	:	Magnoliopsidae,
Subclass	:	Rosidae,
Order	:	Fabales,
Family	:	Leguminosae,
Subfamily	:	Mimosoideae,
Genus	:	*Lagonychium*,
Species	:	*farctum* (Banks & Sol.) Bobr.

3. Materials and Methods:

3.1 Plant Material: The seeds of *Lagonychium farctum* (Banks & Sol.) Bobr. were collected from sandy area around Kharga Oasis, Egypt, in June 1996. The sample was identified by Prof. A. Fayed (Professor of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt). It was dried at room temperature, then reduced to fine powder. The materials used for botanical study were taken from the samples preserved in 70% ethanol containing 5% glycerin.

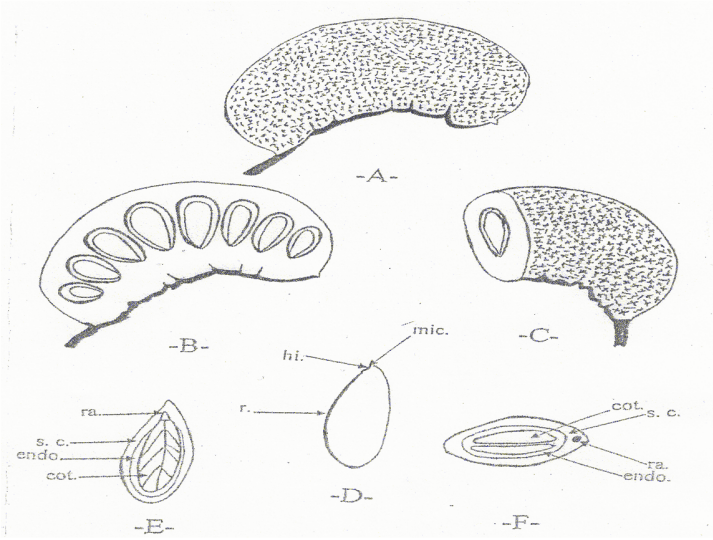

Fig .2: Morpholgy of the fruit and seed; **A.** the whole fruit, **B.** Longtudinal section of the fruit, **C.** Transverse section of the fruit, **D.**The whole seed, **E.** Longtudinal section of the seed and **F.** Transverse section of the seed.(All X 2)

3.2 Microscopic studies:
Transverse sections as well as powder of the seed were used for observation of various microscopic features.

4. Results and Discussion
4.1 Macroscopical Characters:
The Seed (Fig. 2):
The fruit breaks transversely into one-seeded segments and contains up to 10 seeds. It is oval, flattened having a shiny, smooth surface and both sides are convex. It has a narrow or somewhat frequently pointed end at which the hilum and micropyle are situated in a shallow depression and another rounded broad one. The raphé is running from the micropylar end along the seed on one of its edges. The seed arises from anatropus ovule. A transverse and differently directed longitudinal cuts show two planoconvex, yellowish, fleshy, flattened cotyledones which are surrounded by whitish endosperm and a thick seed coat. The embryo is straight, composed of two large cotyledons and a cylindrical short radicle directed towards the micropyle. The seed is albuminous. 100 seeds weigh from 8 to 14 g. The seeds measure from 8 to 15 mm in length, 4 to 7 mm in width and 2 to 5 mm in thickness. They vary in colour from dark brown in fully ripe seeds to a whitish green in the unripe ones, with faint characteristic odour and slight bitter, astringent mucilaginous taste.

4.2 Microscopical characters of the Seed:
The Seed (Fig. 3 and 4):
The seed is more or less oval in cross section. The testa shows an outer epidermis accompanied with a hypodermal layer. The latter consists of about 15 to 20 rows of thin walled parenchyma which is followed internally by about 10 to 13 rows of thin walled and collapsed cells forming a hyaline layer. The endosperm is narrow and mucilaginous followed internally by the oily dicotyledonous embryo.

A-Testa:
1-The epidermis (Fig. 3 and 4A-C):
It is formed of one row of radially elongated cells as seen in the transverse section. The cells are closely packed showing no intercellular spaces. They have unevenly thickened striated cellulosic walls and narrow to somewhat wide lumina forming a palisade like layer. Therefore, refraction line appears due to the difference in thickness. They are covered with smooth and thick cuticle. In surface view (Fig. 7C, D, 8B, C): The cells appear very small, polygonal, mostly isodiametric with straight anticlinal walls and narrow (outer 2/3 part of the epidermis) or wide (inner 1/3 of epidermis) radiating lumina. They measure from 90 to 100 to 110 µ in length and 6 to 10 to 14 µ in width.

2-The Hypodermis (Fig. 3A, 3B and 4D):
It is formed of nearly oval to rounded cells as seen in transverse section. They have thin cellulosic walls, narrow intercellular spaces and contain brown tannin bodies which stain olive green with ferric chloride T.S. They measure from 14 to 16 to 24 µ in diameter.

3-The hyaline later (Fig. 3A and 3B):
It is formed of collapsed cells with thin cellulosic walls and free from contents.

B-The endosperm (Fig. 3A, 3B and 4E):
It is formed of isodiametric, usually hexagonal cells with thin cellulosic walls with rare intercellular spaces. They contain neutral mucilage which stains blue with methylene blue. They measure from 14 to 22 to 28 µ in diameter.

C-The Embryo (Fig. 3A, 3B and 4F):
The cells of the cotyledons are surrounded by outer epidermis formed of parenchymatous square or tabular cells. They enclose parenchymatous palisade like cells. The embryo contains granular protein contents which stain yellow with picric acid and fixed oil globules which stain red with sudan III. The outer cells measure from 22 to 31 to 40 µ in diameter and

the inner one measures from 40 to 48 to 56 µ in length and 6 to 8 to 10 µ in width.

D-The Powdered Seed (Fig. 4):

It is pale yellowish-brown in colour with faint characteristic odour and slight bitter astringent mucilaginous taste. Microscopically, it is characterized by the following:

1. Fragments of epidermis which appear in side, top or basal views. In side view they are elongated, closely packed showing no intercellular spaces, having unevenly, thickened, striated, cellulosic walls, narrow to somewhat wide lumina and covered with smooth and thick cuticle. Top or basal view, they appear small polygonal, mostly isodiametric with straight anticlinal walls, narrow (top view) or wide (basal view) radiating lumina.

2. Numerous scattered cells from the hypodermis which are oval or rounded in shape with thin cellulosic walls, narrow intercellular spaces and contain brown tannin bodies which stain olive green with ferric chloride T.S.

3. Few fragments of endosperm which appear as isodiametric, usually hexagonal cells with thin cellluosic walls with rare intercellular spaces. They contain neutral mucilage which stain blue with methylene blue.

4. Fragments from the embryo showing parenchymatous palisade like cells. They contain granular protein contents which stain yellow with picric acid and fixed oil globules which stain red with sudan III.

5. Few spiral and annular lignified xylem vessels from the vascular bundle of the raphé.

6. Absence of stone cells or hairs.

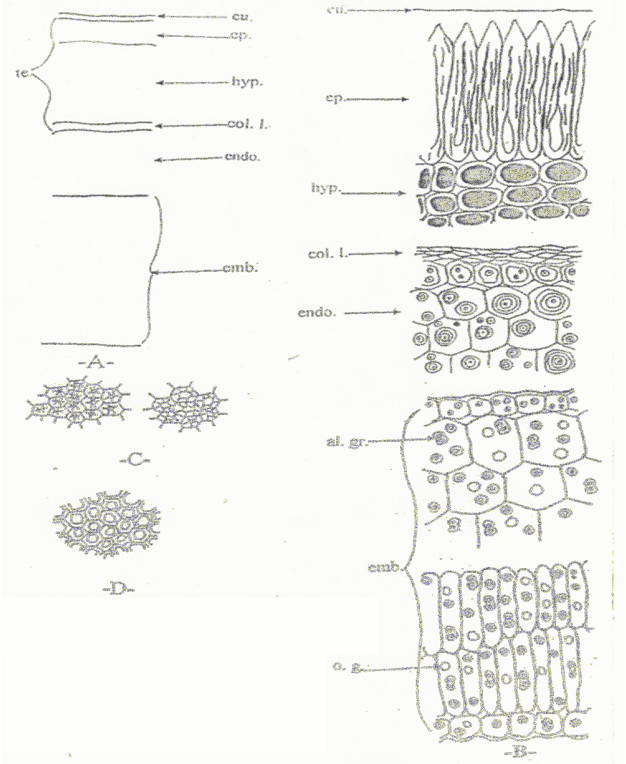

Fig .3 : The seed; A. Diagrammatic transverse section, **B.** Detailed transverse section, **C.** Top view of the epidermis and **D.** Basal view of the epidermis (A X 125 and B, C, D X 50)

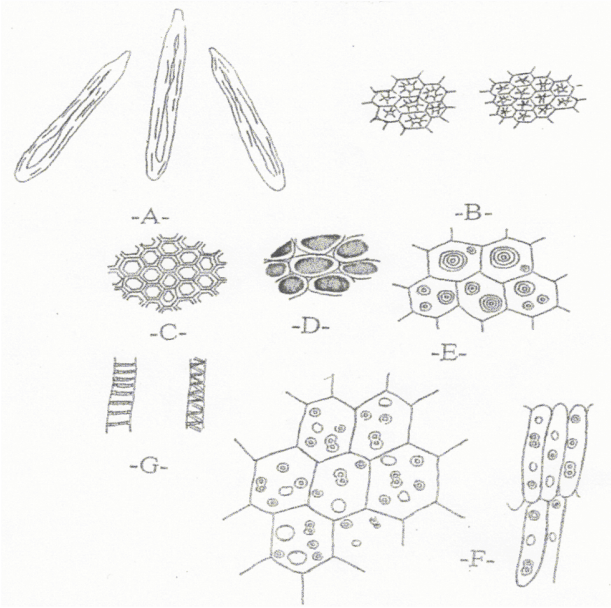

Fig .4: The seed powder**; A.** Side view of the epidermis, **B.** Basal view of the epidermis, **C.** Top view of the epidermis, **D.** Hypodermis, **E.** Endosperm, **F.** Embryo and **G.** Raphe xylem vessels (All X 500)

5. Conclusion

From the present study, the microscopic studies on the seed of *Lagonychium farctum* (Banks & Sol.) Bobr. can assist as a relevant source of information and contribute towards the standards to dispose the quality and identity of this plant in future exploration.

6. Acknowledgement

We specially wish to express our sincere thanks and gratitude to Prof . A.M. El Moghazy Shoaib (Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Assuit Uni., Egypt) for the identification of the pharmacognostical characters of the seed.

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Phytosteroids from tissue culture of *Allium cepa* L. and *Trachyspermum ammi* S prague.

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Production of secondary metabolites by cultured cells provides a particularly important benefit to manipulate and improve the production of desired compounds; thus biotechnological approaches to increase the concentrations of the metabolites are discussed. Present study deals with the production, isolation and identification of phytosterols from tissue culture of *Allium cepa* and from plant parts and tissue culture of *Trachyspermum ammi*. Steroidal analysis of plant parts showed the maximum amount of stigmasterol (0.240 mg/gdw) which was comparatively little less than that of the amount of β- sitosterol (0.295 mg/gdw) in the seeds of T. *ammi* . The maximum amount of stigmasterol was present in four weeks old tissue of T. *ammi* (0.249 mg/gdw) whereas the highest content of β-sitosterol was observed in six weeks old tissue of A. *cepa* (0.315 mg/gdw) However, lanosterol, was present only in the tissue of A. *cepa* which was maximum in six weeks old tissue (0.039 mg/gdw)

Keyword: *Allium cepa*, Liliaceae, *Trachyspermum ammi* Umbellifereae, Stigmasterol, Beta sitostewrol

1. Introduction

Phytosterols (referred to as plant sterol and stanol esters) are a group of naturally occurring compounds found in plant cell membranes. Because phytosterols are structurally similar to the body's cholesterol, when they are consumed they compete with cholesterol for absorption in the digestive system. As a result, cholesterol absorption is blocked, and blood cholesterol levels reduced. Throughout much of human evolution, it is likely that large amounts of plant foods were consumed. In addition to being rich in fiber and plant protein, the diets of our ancestors were also rich in phytosterols—plant-derived sterols that are similar in structure and function to cholesterol. There is increasing evidence that the reintroduction of plant foods providing phytosterols into the modern diet can improve serum lipid (cholesterol) profiles and reduce the risk of cardiovascular disease.

In the present study the callus of *Allium cepa* (Liliaceae) and Trachyspermum ammi (Umbellifereae) were successfully maintained in the laboratory and evaluated for their phytosterol content which is not well documented for these plants.

2. Material and Methods

Plant parts (seeds, stem and leaves) of *T. ammi* were collected from Haffkine campus. Plant parts were washed in running water, cut into small pieces, dried at 100^0 to inactivate enzymes and then dried at 60^0 till a constant weight was achieved. The tissue of *A. cepa* and *T. ammi* were

raised from the seedlings, grown and maintained by frequent subculturings of 6-8 weeks for twenty four months on fresh RT medium. The growth indices were calculated at the transfer age of the tissue at two, four, six and eight weeks. Three replicates in each case were examined and mean values taken into consideration.

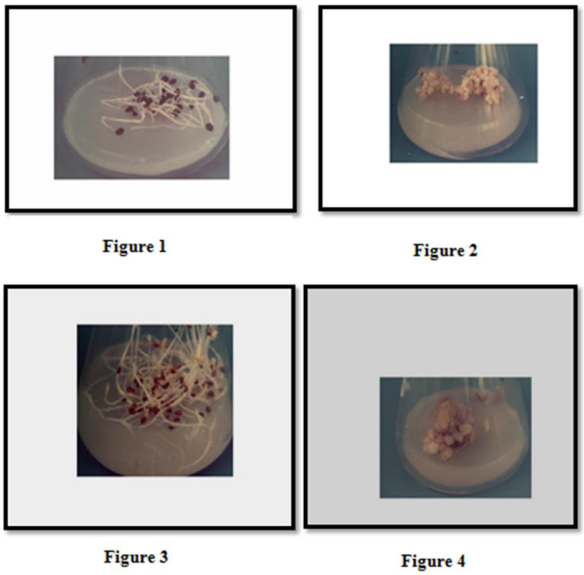

Fig 1-2: Showing the seed germination and developed callus of *Allium cepa* L.

Fig 3-4: Showing the seed germination and developed callus of *Trachyspermum ammi*. Sprague

S. No.	Age in weeks	Growth Index	
		A.cepa	*T. ammi*
1	2	0.76	0.91
2	4	1.5	1.4
3	6	2.1	2.7
4	8	1.7	1.8

Table 1. Showing the growth index of *A.cepa* and *T. ammi*

2.1 Extraction Procedure

Each of the plant parts (seeds, stem and leaves) as well as the various tissue samples (2,4,6 and 8 weeks old)were dried, powdered, weighed and then separately subjected to soxhlet extraction in petroleum ether for 24 hr on a water bath for removing fatty acids. Each of the mixture was filtered and residual tissue masses were hydrolysed with 15% ethanolic hydrochloric acid (w/v) for 4 hr (Tomita et al., 1970)Each of the hydrolysed samples was filtered, the filtrate extracted in ethyl acetate separately and given continuous washings of distilled water till the pH was 7. The extract was passed over Na_2SO_4, for removing moisture, dried, taken up in chloroform and then analysed for steroidal content.

2.2 Qualitative Analysis

a. Thin-layer Chromatography (TLC)

Each of the extracts was applied on silica gel G coated and activated glass plates along with the standard samples of sterols (cholesterol, lanosterol, stigmasterol and B-sitosterol) The glass plates were then developed in an organic solvent misture of Hexane and acetone (80:20: Fazil and Hardman, 1968) and air dried. On spraying the developed plates with 50% H_2SO_4 three spots corresponging. to those of the standard of these of the standard samples and a stigmasterol (Rf 0.91; color grey) lanosterol (Rf 0.89; color, brown) and B-sitosterol (Rf. 0.85; color, purple) were observed in *A. cepa*. whereas two spots coinciding with those of the standard samples of β-sitosterol and stigmasterol were marked in case of *T. ammi* (Table 6.2) The developed plates were also sprayed with anisaldehyde reagent but 50% H_2SO_4 gave excellent results.

A few other solvent systems (Benzene : ethyl acetate: 85:15: Heble et al., 1968a; Benzene : ethylacetate 3:1: Kaul and Staba, 1968) were also use but Hexane and acetone gave excellent results in the present study. Ten replicates in each case were examined and the mean Rf values calculated.

b. Preparative Thin-layer Chromatography (PTLC)

Each of the extracts as also the standard samples of B-sitosterol, stigmasterol and lansterol were also applied on thickly coated silica gel and activated glass plates. The plates were developed as described above and a portion of the plates was sprayed with 50% H_2SO_4. Three spots corresponding to the standard samples of B-sitosterol, lanosterol and stigmasterol in case of A.cepa and two spots coinciding with those of the standard samples of B-sitosterol and stigmasterol were scrapped off alongwith silica gel from about 150 unsprayed plates. The isolated mixtures were separately extracted with chloroform. The various isolates were rechromatographed separately to test their purity. Each of the isolated purified compounds was crystallized by adding saturated acetone solution to which a few drops of methanol were added (Kaul and Staba. 1968). The crystals formed were removed from the mother liquor, washed twice and cold menthol and dried in vacuo. Each of crystallised compounds of all the samples was subjected to colorimetry (for quantitative estimation). mp (Thoshniwal Melting Point Apparatus, India) Infra-red spectral (Perkin-Elmer 337 Infra-red Spectrophotometer) studies and Gas-liquid chromatography (Perkin-Elmer OV-11 Gas Chromatograph) along with their respective standard reference sterols.

c. Gas-Liquid Charomatography

The Steroidal extracts of both the plant species were analysed along with their standard samples of β-sitosterol, stigmasterol and lanosterol by GLC equipped with a flame lionization detector and a stainless steel column containing SE-30, coated with 3% Gas chrom P. The operating temperature used for analysis was 300^0 Hydrogen was used as the carrier gas at a flow rate of 0.5 cm/min to ascertain the concentration of various steroidal compounds in the tissue.

2.3 Quantitative Analysis

Quantitative estimation of various identified sterols was carried out colormetrically with the help of a Spectrophotometer, following the method of Das and Benerjee (1980), which includes the preparation of a regression curve for each of the standard reference compound. A stock solution of each of the reference compounds (lanosterol, B-sitorsterol and stogmasterol) was prepared (in chloroform 500 mg/1) separately. From this six concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml) were prepared and spotted on silica gel G coated and activated glass plates. The plates were developed as described above and the plates were exposed to iodine vapours. Iodine positive spots were marked and heated to evaporate the excess of iodine.

The spots were separately scrapped off along with silica gen and such eluates were taken up in 5 ml of chloroform in test tubes. Each of the tubes was centrifuged, supernatants taken and evaporated to dryness. To each of the dried samples, 3 ml of glacial acetic acid was added

and shaken vigorously at room temperature for 1 min, then placed in freezer. To this frozen sample, 2 ml of freshly prepared chromogenic reagent (0.5 ml of 0.5% anhydrous ferric chloride in glacial acetic acid and 100 ml of concentrated H_2SO_4 ; Klyne, 1965) was added drop wise at 0^0 and mixed thoroughly. Each of the reaction mixtures was incubated at 40^0 for 30 min and optical density was read on a Spectronic-20 Colorimeter (Bausch and Lomb) set as 540 nm against a blank (3 ml of glacial acetic acid and 2 ml of chromogenic reagent). Five such replicates were run for each of the concentrations and average optical density was plotted against their respective concentration to compute a regression curve which followed the Beer's law.

Each of the extracts was dissolved in chloroform and was spotted along with the standard reference samples of βsitosterol, stigmasterol and lanosterol on silica gel coated and activated glass plates which were developed as described earlier. Three spots coinciding with those of the authentic sample of β-sitosterol, stigmasterol and lanosterol were marked. Each of these eluates were dried taken up in 5 ml of chlololform and were worked out as described above. Concentrations of β-sitosterol, stigmasterol and lanosterol were calculated (mg/gdw) by computing the optical density of the experimental sample with the regression curve of the standard reference sample of β-sitosterol, stigmasterol and lanosterol. Three such replicates were examined in each case and mean values calculated. Six weeks old tissues of *A. cepa* and *T. ammi* were extracted and analysed with reference samples of B-sitosterol, stigmasterol and lanosterol by GLC.

PLANT	PHYTOSTEROL	RF VALUE IN HAXENE: ACETONE 8:2	COLOR AFTER SPRAYING WITH 50%H_2SO_4	COLOR IN UV
A.CEPA	βSITOSTEROL	95	PURPLE	DL RED
	STIGMASTEROL	85	GREY	DKBROWN
	LANOSTEROL	89	YELLOW	GREEN
T.AMMI	βSITOSTEROL	95	PURPLE	DL-RED
	STIGMASTEROL	85	GREY	DKBROWN

PLANT	PLANT PARTS USED	AGE IN WEEK	GROWTH INDEX	STIGMASTEROL	LANOSTEROL	βSITOSTEROLMG/GDW
T.AMMI	SEED	-		0.240	-	0.295
	LEAVES	-		0.014	-	0.012
	STEM	-		0.224	-	0.019
		2	0.91	0.13	-	0.012
		4	1.4	0.249	-	0.027
		6	2.7	0.17	-	0.107
		8	1.8	0.061		0.09
		2	0.76	0.045	0.015	0.081
A.CEPA		4	1.5	0.075	0.025	0.12
		6	2.1	0.025	0.039	0.315
		8	1.7	0.018	0.023	0.14

Table. 2: showing the steroidal content in *Allium cepa* and *Trachyspermum ammi*

3. Results and Discussion

The maximum growth index was observed in six weeks old tissues of *T. ammi* (2.7) followed by six weeks oldtissue of *A. cepa* (2.1) The growth index increased in a linear fashion up to a period of six weeks which then gradually declined (Table 6.2; Fig. 6.1). The chromatographic analysis showed the presence of two phytosterols corresponding to those of the standard samples of β-sitosterol (Rf. 0.85: colour, purple) and stigmasterol (Rf. 0.91; colour, grey) in *T. ammi*, whereas the presence of three spots coinciding

with those of the standard samples of β-sitosterol (Rf. 0.85; Colour, Purple) Stigmasterol(Rf. 0.91; Colour, grey) and lanosterol (Rf. 0.87; Colour, Purple) were observed in *A. cepa* (Table 6.1). Further confirmation of the isolated compounds was done by mp (B-sitosterol, 139-140^0; stigmasterol 114-115^0; and lanosterol 143-144^0), superimposable IR spectra and Gas-liquid chromatography of the isolated and their respective standard compounds standard compounds (Fig. 6.2) Steroidal analysis of plant parts showed the maximum amount of stigmasterol (0.240 mg/gdw) which was comparatively little less than that of the amount of β- sitosterol (0.295 mg/gdw) in the seeds of *T.ammi* (Table 6.2; Fig. 6.1) The maximum amount of stigmasterol was present in four weeks old tissue of *T.ammi* (0.249 mg/gdw) whereas the highest content of β- sitosterol was observed in six weeks old tissue of *A. cepa* (0.315 mg/gdw) However, lanosterol, was present only in the tissue of *A. cepa* which was maximum in six weeks old tissue (0.039 mg/gdw).

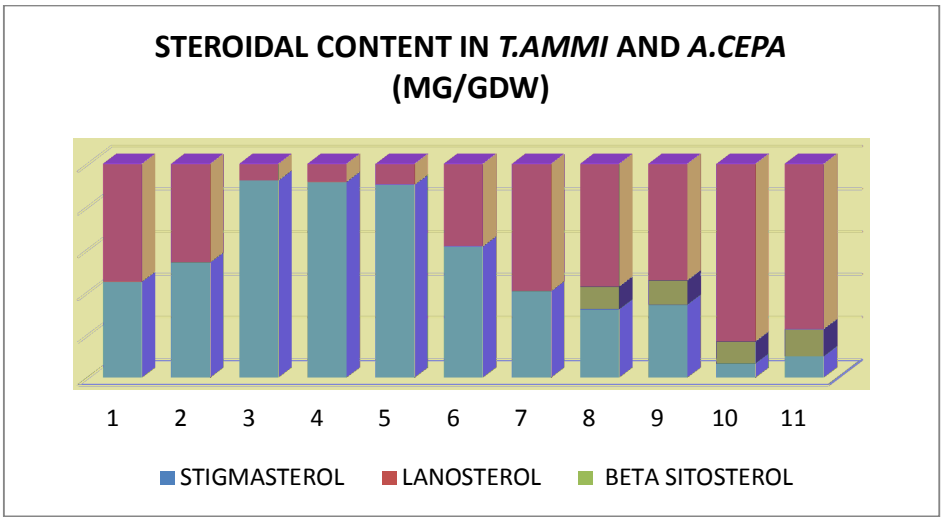

FIG. 2: Graph showing the steroidal content in *A.cepa* and *T.ammi*

Phytosterol such as β-sitosterol and stigmasterol have been reported in tissue cultures of Artemisea scoparia, *Datura metel*, *Emblica officinalis* and *Trigonella foenum-graecum* (Khanna, 1987) and βsitosterol and stigmasterol in the tissue of *C. arietinum*, *S. indicum* and *Momordia charantia* (Khanna, 1984). Only β-sitosterol and stigmasterol.It has been reported in tissue culture of *S. xanthocarpum* (Heble et al., 1968) *Helianthus annus* (Sharma, 1975), *S. aviculare* (Gaur, 1978) and *Trigonella corniculata* (Jain, 1979) .Lanosterol, stigmasterol and β-sitosterol along with cholesterol have been observed in tissue of *Sesamum indicum* (Jain and Khanna, 1973) and *M. Charantia* (Khanna and Mohan, 1973) Smoczklewicz et. al., (1982) have reported βsitosterol from bulbs of *Allium cepa*. Whereas Cholesterol, campesterol. β-sitosterol and stigmasterol have been identified from the bulbs of *A. sativum* (Stoyamo et.al., 1981) Claus et. al., (1980) and Catalano et. al., (1983) have shown the presence of β-sitosterol and stigmasterol in umbelliferous vegetables.

In the present study, however, three phytosterols as β-sitosterol , stigmasterol and lanosterol have been observed in the tissue of *A. cepa* whereas only two phytosterols, β-sitosterol , stigmasterol have been identified from plant parts and tissue culture of T. *ammi*.

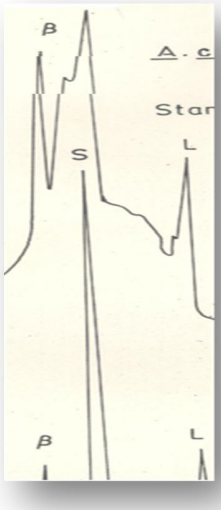

Fig 3: GLC curve of isolated and standard β-sitosterol , stigmasterol and lanosterol from *Allium cepa* L. tissue culture.

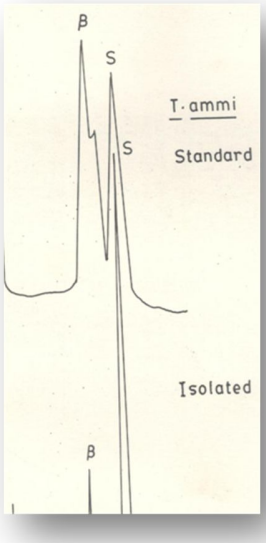

Fig 4: GLC curve of isolated and standard β-sitosterol , stigmasterol from *Trachysoermum ammi* Sprague.

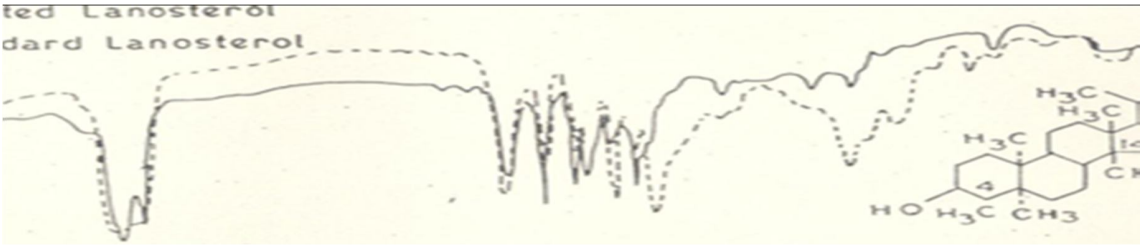

Fig 5: Infra-red spectra of isolated and standard lanosterol

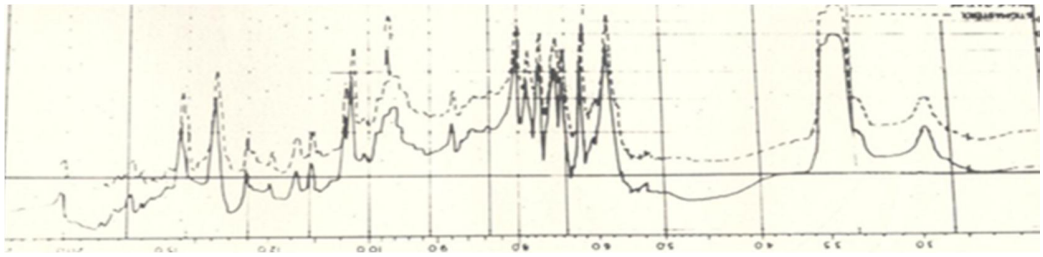

Fig 6. Infra-red spectra of isolated and standard stigmasterol.

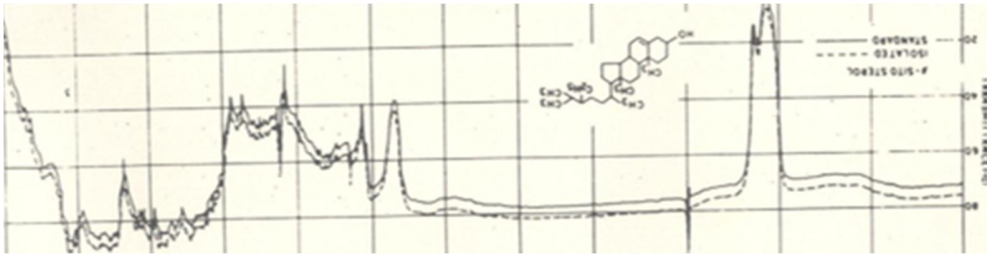

Fig.7. Infra-red spectra of isolated and standard β-sitosterol.

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Isolation and Structure Elucidation of Two Triterpene Acids from the Leaves of *Perilla frutescens*

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Purification of the dichloromethane (CH2Cl2) fraction of the aqueous alcoholic extract of the leaves of *Perilla frutescens* resulted in the isolation of two triterpene acids namely corosolic acid and tomentic acid. The structures of isolated compounds were characterized on the basis of extensive spectral data (1D and 2D NMR; and MS) and in comparison with their physical and spectral data reported earlier

Keyword: *Perilla frutescens*, Triterpene acids, Isolation and purification, NMR, MS, Structure elucidation.

1. Introduction

Perilla frutescens Britt. var. *acuta* Kudo is an annual herbaceous plant native to Asia. The leaves of *P. frutescens* are used in Asian gourmet food is a medicinal herb of Iabiatae[1]. *P. frutescens* (Japanese name; Shiso) is important in Japanese cooking as one of the popular garnishes of that country and also used as a food colorant. The leaves are treated with table-salt to remove the harshness prior to the application as a colorant, suggests the existence of some water-soluble principles in addition to flavonoids. The major constituent of *P.frutescens* is perillaldehyde and several other constituents were reported [2-4]. As a part of our research to discover natural sweeteners, we have recently reported several diterpene glycosides from *S. rebaudiana* and *R. suavissimus*; triterpene glycosides from *Siraitia grosvenorii*; phenolic glycosides and sterols from *R. suavissimus*[5-12].

This paper describes the isolation and structure elucidation of two triterpene acids namely corosolic acid (**1**) and tomentic acid acid **(2)** (Figure 1) on the basis of extensive NMR and mass spectroscopic data and in comparison of their physical and spectral properties reported from the literature.

2. Materials and Methods

2.1 General Instrumentation

Melting points were measured using a SRS Optimelt MPA 100 instrument and are uncorrected. Optical rotations were recorded using a Rudolph Autopol V at 25°C and NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. HRMS data was generated with a

Thermo LTQ Orbitrap Discovery mass spectrometer in the positive positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples.

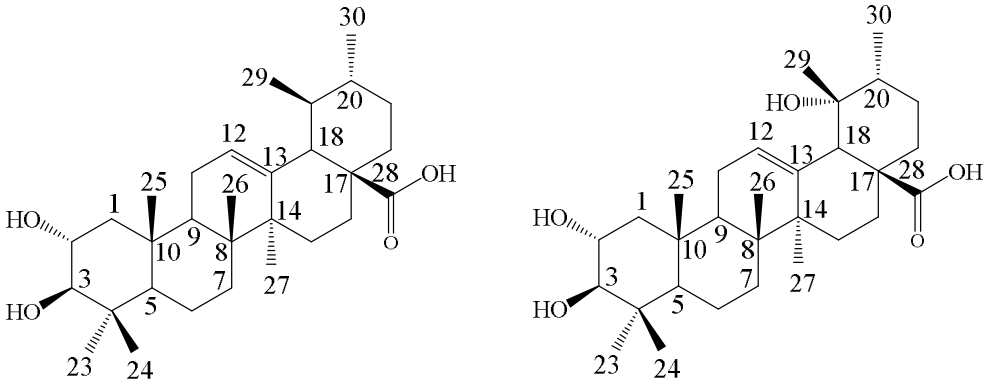

Figure 1: Structures of Corosolic acid (1), and Tomentic acid (2)

Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the onboard syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 µm). TLC was performed on Baker Si-C_{18}F plates and identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80 ^oC.

2.2 Plant Material

The commercial extract of the aqueous/alcoholic (4:1) extract of the leaves of *P. frutescens* was purchased from Naturomic LLC, Anaheim, CA, USA. A voucher specimen was deposited at The Coca-Cola Company, No: VSPC-3166-165.

2.3 Isolation and purification of Aromatic phenol acids (1-2)

The aqueous extract of the leaves of *P. frutescens* (15 g) was suspended in 100 ml water and extracted successively with *n*-hexane (3 x 100 ml), CH_2Cl_2 (3 x 100 ml) and *n*-BuOH (2 x 100 ml). The CH_2Cl_2 layer was concentrated under vacuum furnished a residue (2.6 g) which was purified on a Biotage flash chromatography system using C-18 (100 g) column (solvent system: gradient from 60-40 MeOH-water to 100% MeOH at 50 ml/min, detection at UV 210 nm) for 60 min by collecting 80 fractions. Fractions 54-60 were combined to get a residue 0.36 g respectively, which on repeated purification using the gradient 90% MeOH-water to 100% MeOH on a C-18 (10 g) column at 10 ml/min for 40 min resulted corosolic acid (**1**, 35 mg), and tomentic acid (**2**, 63 mg), respectively.

2.4 Identification of Corosolic acid (1), and Tomentic acid (2)

Corosolic acid (1) White powder; mp 250-253^oC; EIMS m/z: 473 $[M+H]^+$; ^1H NMR (pyridine-d_5, 600 MHz): δ 5.42 (1H, br s, H-12), 4.04 (1H, ddd, *J* = 10.8, 8.6, 4.2 Hz, H-2), 3.36 (1H, d, *J* = 9.1 Hz, H-3), 2.62 (1H, d, *J* = 11.1 Hz, H-18), 1.26 (3H, s, H-23), 1.24 (3H, s, H-27), 1.11 (3H, s, H-24), 1.04 (3H, s, H-25), 1.02 (3H, d, J = 6.6 Hz, H- 30), 0.98 (3H, s, H-26), 0.94 (3H, d, *J* = 6.4 Hz, H-29); ^{13}C NMR (pyridine-d_5, 150 MHz), see Table 1 [13].

Tomentic acid (2) White powder; mp 273-274°C; ESIMS m/z: 489 $[M+H]^+$; ^1H NMR (pyridine-d_5, 600 MHz): δ 5.56 (1H, br s, H-12), 4.82 (1H, br s, 19-OH), 4.06 (1H, ddd, *J* = 11.4, 9.4, 4.6 Hz, H-2), 3.36 (1H, d, *J* = 9.3 Hz, H-3), 3.04 (1H, s, H-18), 1.68 (3H, s, H-27), 1.42 (3H, s, H-29), 1.31 (3H, s, H- 23), 1.15 (3H, d, *J* = 6.4 Hz, H-30), 1.10 (3H, s, H-26), 1.07 (3H, s, H-24), 1.02 (3H, s, H-25); ^{13}C NMR (pyridine-d_5, 150 MHz), see Table 1 [14].

3. Results and Discussion

Compound **1** was isolated as a white powder. The mass spectral data of compound **1** gave a molecular ion peak at m/z 473 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{30}H_{48}O_2$, which was supported by the ^{13}C NMR spectral data. Liebermann-Burchard reaction indicated compound **1** is having a terpenoid skeleton[15-16]. The ^1H NMR spectra of compound **1** showed the presence of five methyl signals at 0.98, 1.04, 1.11, 1.24, and 1.26; two methyl doublets that appeared at δ 0.94 and 1.02. The ^1H NMR spectra of compound **1** also showed two oxymethine protons resonating at δ 3.36 and 4.04; and a trisubstituted olefinic proton at δ 5.42. The presence of five methyl singlets and two methyl doublets suggested that compound **1** belongs to ursane type triterpenoid having two secondary hydroxyl groups and a trisubstituted double bond between C-12/C-13. The ^{13}C NMR values for all the protons and carbons were assigned on the basis of HMQC and HMBC correlations as reported and were given in Table 1.

The appearance of a carbonyl group resonating at δ 180.1 in the ^{13}C NMR spectral data of **1** suggested the presence of an acid functional group and its location was identified at C-28 by the key COSY and HMBC correlations as shown in Figure 3. A close study of COSY and HMBC spectrum of **1** indicated that the two oxymethine groups are adjacent to each other. A search in literature found that the physical and spectral characteristics of **1** were consistent to the reported literature values of corosolic acid that confirmed by the key COSY and HMBC correlations as shown in Figure 2.

Table 1. C NMR chemical shift values for Corosolic acid[13] (**1**) and Tometnic acid (**2**) recorded in C_5D_5N [a-c].

Position	1	2
1	47.8	47.7
2	69.3	69.3
3	84.4	84.6
4	39.8	40.1
5	56.0	56.3
6	18.9	19.1
7	33.8	33.7
8	40.3	40.8
9	48.6	48.1
10	38.4	38.8
11	24.9	24.7
12	125.6	125.8
13	139.4	140.1
14	42.9	42.3
15	29.3	29.8
16	24.0	26.3
17	48.2	48.8
18	53.1	55.1
19	39.1	73.2
20	39.5	42.5
21	31.6	27.6
22	37.5	39.1
23	29.4	29.6
24	17.1	18.2
25	18.1	17.3
26	18.3	18.0
27	24.8	24.9
28	180.1	180.2
29	17.3	17.2
30	22.1	27.3

a. assignments made on the basis of COSY, HMQC and HMBC correlations; **b.** Chemical shift values are in δ (ppm); **C.** Coupling constants are in Hz.

Compound **2** was also isolated as a white powder and its mass spectral data suggested the molecular formula as $C_{30}H_{48}O_3$, 16 amu more to **1** Compound **2** also showed positive Liebermann-Burchard reaction for terpenes as in **1**. The ^1H NMR spectra of compound **2** also showed the presence of five methyl signals, two methyl doublets, two oxygenated methine signals, and a trisubstituted olefinic proton as in **1**. The presence of an additional hydroxyl group together with the absence of any primary and secondary hydroxyl protons in the ^1H NMR spectral data of **2** suggested the presence of a tertiary hydroxyl group in its structure.

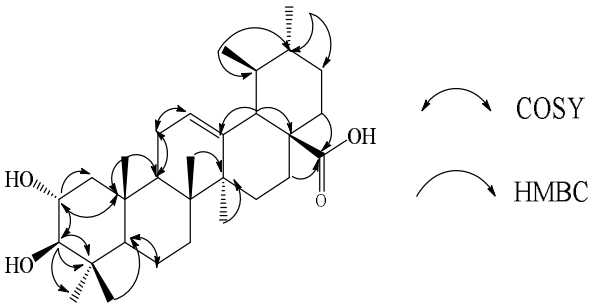

Figure 2: Key COSY and HMBC correlations of Corosolic acid (1)

This indicates the presence of tertiary hydroxyl group resonating at δ 73.2 in the ^{13}C NMR spectral data of **1** at either C-19 or C-20 positions. The ^{13}C NMR values for all the protons and carbons were assigned on the basis of HMQC and HMBC correlations as reported and were given in Table 1. The key COSY and HMBC correlations as shown in Figure 3 suggested the presence of tertiary hydroxyl group at C-19 position as in tomentic acid. Therefore, the structure of **2** was suggested to be tormentic acid (2α,3β,19α-trihydroxy-urs-12-en-28-oic acid) and its NMR spectral showed a good agreement with reported data.

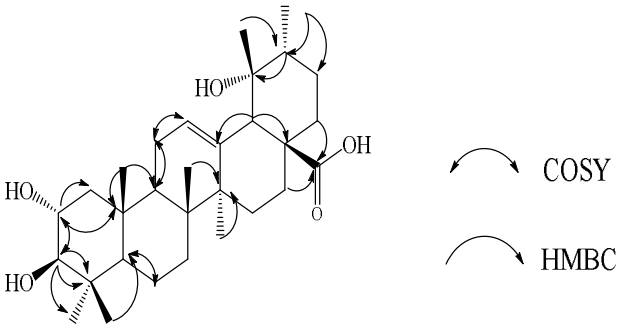

Figure 3: Key COSY and HMBC correlations of Tomentic acid (2)

4. Conclusion

Two triterpene acids were isolated from the commercial aqueous alcoholic extract of the leaves of *P. frutescens*. The structures of the two isolated compounds were identified as corosolic acid (**1**), and tomentic acid (**2**), on the basis of extensive spectroscopic studies and by comparing their physical properties and spectral data reported in the literature.

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Platelet-Aggregation Inhibitory Activity of Oleanolic Acid, Ursolic Acid, Betulinic Acid, and Maslinic Acid

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Platelet aggregation is the process by which platelets adhere to each other at sites of vascular injury. This process has long been recognized as critical for hemostatic plug formation and thrombosis. Until relatively recently, platelet aggregation was considered a straightforward process involving the non-covalent bridging of integrin αIIbβ3 receptors on the platelet surface by the dimeric adhesive protein fibrinogen. Naturally occurring triterpenes are endowed with a broad range of useful pharmacological properties. In our search for new and potent ethnopharmaceuticals, oleanolic acid **(OA) (1)**, ursolic acid **(UA) (2)** betulinic acid **(BA) (3)**, and maslinic acid **(MA) (4)** isolated from Syzygium aromaticum, Eucalyptus grandis, Callistemon viminalis, and Syzygium aromaticum, respectively, were evaluated in vitro for anti-platelet aggregation activity on thrombin, adenosine diphosphate (ADP), and epinephrine-induced rat platelet aggregation. The triterpenes exhibited a dose dependent inhibitory activity on platelet aggregation induced by the three platelet agonists. The compounds exhibited more potency mostly at the highest concentration (10.0 mg/ml) except for **UA (2)** on thrombin-induced platelet aggregation. The percentage inhibitory activity of **UA (2)** on throbine-induced platelet aggregation was found to decrease with increase in concentration (86.8±1.23, 53.0±0.43, 46.2±0.23) 1-10.0 mg/ml, this invariably suggests an optimal concentration (≤ 1.0 mg/ml). The IC50s of the compounds are remarkably better than that of heparin (IC50 of 2.80mg/ml). The highest activity by **OA (1)** (IC50 of 0.84 mg/ml) and mixture of **BA/OA** (IC50 of 2.61 mg/ml) was observed on thrombin-induced platelet aggregation. **BA/OA** (IC50 of 2.57 mg/ml) also showed a significant platelet aggregation inhibitory activity on epinephrine-induced platelet aggregation. These preliminary findings show that these pentacyclic triterpenoic acids possess pharmacological activity and could be potential templates for development of new anti-platelet agents

Keyword: Pentacyclic Triterpenes, Anti-Platelet Aggregation, Atherothrombotic Disorders, Ethnopharmaceuticals, Drug Discovery

1. Introduction

Platelets play an important role in hemostasis during tissue injury. They interact with activated plasma clotting factors at the site of blood vessel injury, forming a mechanical plug which blocks the defect and terminates blood loss (Harker & Mann, 1992). Platelet aggregation is induced by the action of endogenous agonists such as arachidonic acid (AA), adenosine diphosphate (ADP), platelet activating factor (PAF), thrombin,

and collagen (Arita et al., 1989). The propensity of platelets to clump together at sites of vascular injury was first recognized more than 100 years ago (Bizzozero, 1882). This phenomenon was described as platelet cohesion although more commonly referred to as platelet aggregation, and it was later identified as important for hemostatic plug formation. It was also well understood at the time that abnormal platelets aggregation played a key role in the development of thrombosis, but, it was not until almost a century later that it became widely accepted that platelets hyperactivity play a pivotal role in development of cardiovascular diseases. As a result, inhibitors of platelet aggregation have become increasingly important parts of the armamentarium for the prevention and treatment of many atherothrombotic disorders (Jackson, 2007).

Aspirin has been the drug of choice for long-term treatment of platelet hyperactivity, especially to reduce the risk of serious ischemic events in several cardiovascular disease states including stroke, myocardial infarction, unstable angina and following coronary artery bypass surgery. Aspirin usage is associated with resistance and serious side effects such as gastric hemorrhage (Lloyd &Bochner, 1996).

The search for new generation of effective and safer non-aspirin platelet inhibitors from natural sources has been emphasized (Amrani et al., 2009). It is widely accepted that natural products are proven template for the development of new scaffolds of drugs (Cragg et al., 1997). In recent years, alkaloids of diverse structures, phenolic compounds, prenylflavonoids and a diterpene isolated from different plant sources, have shown potent anti-platelet activity (Jantan et., 2006, Nurtjahja-Tjendraputra et al., 2003, Lin et al., 1993, Shen et al., 2000). Recently a number of naturally occurring triterpenes and their derivatives have been reported to possess remarkable anti-platelet aggregation (Mosa et al., 2011; Habila et al., 2011). This paper reports on the platelet aggregation inhibition of **OA** (**1**), **UA** (**2**), mixture of **OA** (**1**) and **BA** (**3**), as well as **MA** (**4**).

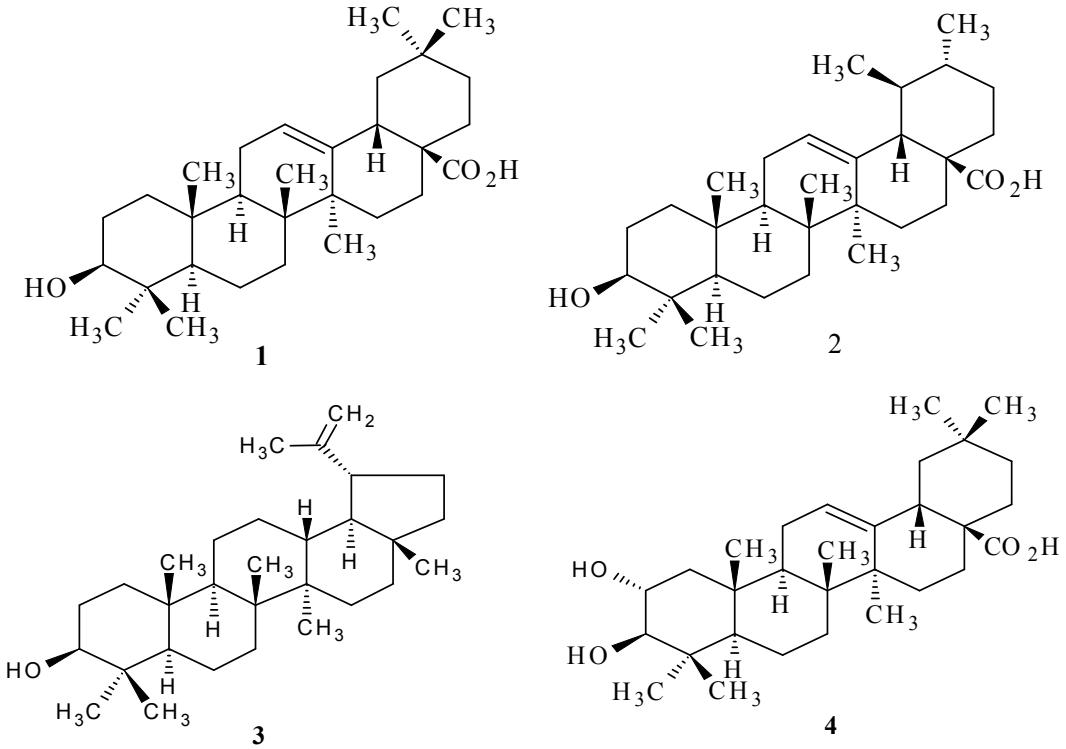

Fig 1: Structure of **OA** (**1**), **UA** (**2**), **BA** (**3**) and **MA** (**4**).

2. Material and Methods

2.1 Plants

The plants used in this study were collected from different areas within KwaZulu-Natal province, Republic of South Africa. The buds of *S. aromaticum* were purchased from the spice market in Durban and were authenticated by Mr. Pravin Poorun a senior plant taxonomist, of the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus. A voucher specimen (voucher number OO4) was deposited at the University Herbarium.

Leaves of *Eucalyptus grandis* were collected from Mondi plantation in Empangeni, KwaZulu-Nata Province and authenticated by Mrs NR Ntuli of Botany Department, University of Zululand, a collector's number (Shode/01) was assigned and the leaf sample was kept at the University of Zululand, Herbarium.

Fresh leaf samples of *Callistemon viminalis* were collected in July 2010 from University of Zululand, KwaDlangezwa campus, Empangeni (KwaZulu-Natal Province) in South Africa. The fresh plant materials were authenticated by Dr. S.J. Siebert, a plant taxonomist, of the Department of Botany, University of Zululand, where voucher specimens already existed [OOO 13 (ZULU)].

2.2 Extraction and Isolation of OA (1) and MA (4) from *S. aromaticum*

The buds (500g) were milled using an industrial blender. The milled plant sample was extracted on a Labcom shaker Dichloromethane (2 L) overnight and the solvent removed under reduced pressure to yield 18.4 g (3.8%) of plant extract. The extract was treated with hexane to yield crude mixture of **OA (1)** and **BA (3)**. 5.0 g of the crude extract was subjected to normal phase column chromatography using gradient solvent system of hexane-ethyl acetate (5% stepwise increase). Fractions were collected, like fractions were pooled together, further purification led to isolation of a white amorphous compound coded FS02, and a colourless crystalline substance FS04. These compounds were subjected to NMR spectroscopic analysis for structural elucidation and confirmed to be **OA (1)** and **MA (4)**. The spectra data are in concordant with literature value (Werner et al., 2003).

2.3 Extraction and Isolation UA (2) from *Eucalyptus grandis*

The fresh leaves were dried under shade, pulverized into coarse form (600 g) for extraction using a protocol that is still being developed in our lab. The method selectively extracts various classes of acids including pentacyclic triterpenoic acids**.** The crude extract was chromatographed using gradient elution of hexane–ethyl acetate (5% stepwise increase). A total of 220 fractions (20mL, each) were collected and monitored by TLC. Like fractions were pooled together on the basis of TLC analysis to give a total of 11 fractions (A-K). Fractions 93-105 coded (I) gave light yellowish-green powder which was confirmed spectroscopically using ^{1}H, ^{13}C, DEPT, HSQC and FT IR to be **UA (2)**.

2.4 Extraction and Isolation of BA/OA Mixture

Fresh matured leaves (500 g) of *Callistemon viminalis* were air-dried and pulverized into coarse form. It was extracted by cold maceration in ethyl acetate (2L x 3) for 60h, filtered and concentrated under reduced pressure at 40 $^{\circ}C$ using rotary evaporator and allowed to dry under air. The concentrated extract was then deffated with n-haxane to afford (21.6 g) of creamy solid material. A portion of the solid material (12.0 g) was subjected to column chromatography using gradient solvent system of hexane-ethyl acetate (8:2) to give a white solid suspected to be mixture of **BA (3)** and **OA (1)**. The structures were confirmed by NMR spectra. Repeated attempts to separate the solid mixture were unsuccessful.

2.5 Biological Studies

Animals

Ethical clearance for the use of animals in this study was obtained from the institutional (University of Zululand) research animal ethics committee. Adult rats (*Sprague-Dawley*) of either sex were collected from the Department of

Biochemistry and Microbiology, University of Zululand. The animals were housed under standard conditions and had free access to standard pellet feed and drinking water *ad libitum*.

2.6 In vitro anti-platelet aggregation study

2.6.1 Preparation of Blood Platelets

The blood platelets were prepared according to the method described by Tomita et al., (1993).The rat was sacrificed by a blow to the head and blood was immediately collected by cardiac puncture. The blood was mixed (5:1 v/v) with an anticoagulant (acid-dextrose-anticoagulant, 0.085 M trisodium citrate, 0.065 citric acid, 2% dextrose).

The platelets were obtained by a series of centrifugation at 1200rpm for 15min and at 2200 rpm for 3min consecutively. The supernatant was collected and centrifuged at 3200rpm for 15 min. The resulting supernatant was discarded and the sediment (platelets) was re-suspended in 5ml washing buffer (pH 6.5). This was centrifuged again at 3000 rpm for 15 min after which the supernatant was discarded and the platelets were finally suspended in a small volume of re-suspending buffer (pH 7.4; containing 0.14 M NaCl, 15 mM Tris–HCl, 5 mM glucose). The platelets were further diluted with the re-suspending buffer (1:10) and the resulting solution was mixed with calcium chloride (0.4 ml: 10 µl $CaCl_2$).

Table1. Percentage (%) Inhibitory activity of the compounds on thrombin- induced platelet aggregation.

Compound	Concentrations			IC_{50} (mg/ml)
	1.0 mg/ml	3.0 mg/ml	10.0 mg/ml	
OA (1)	60.2 ± 0.42	60.2 ± 0.42	88.8 ± 0.42	0.84
UA (2)	86.8 ± 1.23	53.0 ± 0.43	46.2 ± 0.23	2.82
BA/OA	21.0 ± 0.85	56.6 ± 0.39	81.3 ± 0.73	2.61
MA (4)	37.2 ± 0.90	53.0 ± 1.23	69.9 ± 0.35	2.81
Heparin	11.9±0.24	22.1±0.09	66.3±1.01	2.80

Table 2. Percentage (%) Inhibitory activity of OA (1), UA (2), BA/OA, and MA (4) on ADP-induced platelet aggregation.

Compound	Concentrations			IC_{50} (mg/ml)
	1.0 mg/ml	3.0 mg/ml	10.0 mg/ml	
OA (1)	21.1±0.26	27.5±0.31	80.8±0.79	5.98
UA (2)	0.00±0.61	60.1±4.38	76.7±0.76	3.00
BA/OA	0.00±0.85	0.00±0.91	49.0±0.96	ND
MA (4)	0.00± 0.90	0,00±1.23	8.80±0.53	ND

ND- Not determined

Table 3. Percentage (%) inhibitory activity of OA (1), UA (2), BA/OA, and MA (4) on epinephrine-induced platelet aggregation.

Compound	Concentrations			IC_{50} (mg/ml)
	1.0 mg/ml	3.0 mg/ml	10.0 mg/ml	
OA (1)	0.00±0.26	0.00±0.71	47.8.±0.42	ND
UA (2)	0.8±0.68	0.0±0.16	17.4±0.56	ND
BA/OA	27.0 ±0.58	57.9±0.29	67.7±0.50	2.57
MA (4)	0.00± 0.90	35.9±1.32	87.0±1.53	4.99

ND- Not determined

2.6.2 Anti-Platelet Aggregation Evaluation

Anti-platelet aggregation activity was determined by the method described by Mekhfi et al., (2004) with some modifications. The compounds were separately solubilized in dimethyl sulfoxides (DMSO) before making up the volume with 50MmTris–HCl buffer (pH 7.4; containing 7.5 mM ethylenediaminetetra-acetic acid (EDTA) and 175mM NaCl) to a final 1% DMSO concentration. Different concentrations (1, 3 and 10 mg/ml) were used in the assay. The platelet aggregation inhibitory activity of the triterpenes was separately evaluated on thrombin (5 U/ml), ADP (5 mM), and epinephrine (10 mM) induced aggregation. The platelets (100 µl) were pre-incubated for 5 min with different concentrations of the compounds before introduction of platelets agonist (20 µl) to the mixture.

Aggregation was determined with the Biotek plate reader (ELx 808 UI, Biotek Instrument Supplies) using Gen5 software by following change in absorbance at 415 nm. DMSO (1%) was used as negative control and heparin was used as positive control.

Statistical analysis

All assays were done in triplicate and the mean slope (A) ± standard error of mean (SEM) reported. The inhibitory effect of the compounds on each parameter was calculated as: Inhibition (%) = $[(A_o - A_1)/A_o \times 100]$. Where A_o is the mean slope of the control and A_1 is the mean slope of the test compound. IC_{50} values were determined using statistical package origin 6.1

3. Results and Discussion

Aberrant platelet aggregation is central to the development of atherothrombotic disorders. As a result, inhibitors of platelet aggregation have become increasingly important in the prevention and treatment of many atherothrombotic disorders (Jackson, 2007). The dissatisfaction on the efficacy and/or potential undesirable side-effects of the current anti-platelet agents has fuelled the search for new generation of effective agents from natural sources.

A number of naturally occurring triterpenes and their derivatives have been reported to possess remarkable anti-platelet aggregation activity (Mosa et al., 2011; Habila et al., 2011). In this study, the anti-platelet aggregation of four pentacyclic triterpenoic acids namely (**OA**) (**1**), (**UA**) (**2**), (**BA**) (**3**)/**OA** (**1**), **MA** (**4**) was evaluated against thrombin, ADP and epinephrine induced rat platelet aggregation. The triterpenes exhibited a dose dependent inhibitory activity on platelet aggregation induced by the three platelet agonists (Tables 1, 2 and 3). The compounds exhibited more potent activity, mostly at the highest concentration (10.0 mg/ml). The highest activity by **OA (1)** (IC_{50} of 0.84mg/ml) and **BA**/**OA** (IC_{50} of 2.61mg/ml) was observed on thrombin induced platelet aggregation. The IC_{50s} of **OA** (**1**) and **BA**/**OA** mixture were remarkably better than that of heparin (IC_{50} of 2.80mg/ml) (Table 1). A high anti-platelet activity by **BA**/**OA** (IC_{50} of 2.57mg/ml) was also observed on epinephrine induced platelet aggregation (Table 3). The inhibitor activity of **UA** (**2**) on thrombin-induced platelet aggregation was optimal at 1.0 mg/ml, and insignificantly low on epinephrine-induced platelet aggregation. This result is concordant with what was reported in literature (Habila et al., 2011).

The anti-platelet aggregation activity of **UA** (**2**) and **OA** (**1**) from the leaves of *Acanthopanax senticosus* and the fruits of *A. sessiliflorus* has been reported (Jin et al., 2004). Furthermore, the platelet aggregation inhibitory activity of **BA** (**3**) has also been recently explored (Tzakos et al., 2012). Therefore, the potent activity of **BA**/**OA** could be attributed to the synergistic activity of both **BA (3)** and **OA (1)**.

The results obtained from this study revealed that **OA** (**1**), **UA (2)**, **BA**/**OA**, as well as **MA** (**4**) have anti-platelet aggregation properties. The results suggest that these triterpenes as potential templates for development of new anti-platelet agents.

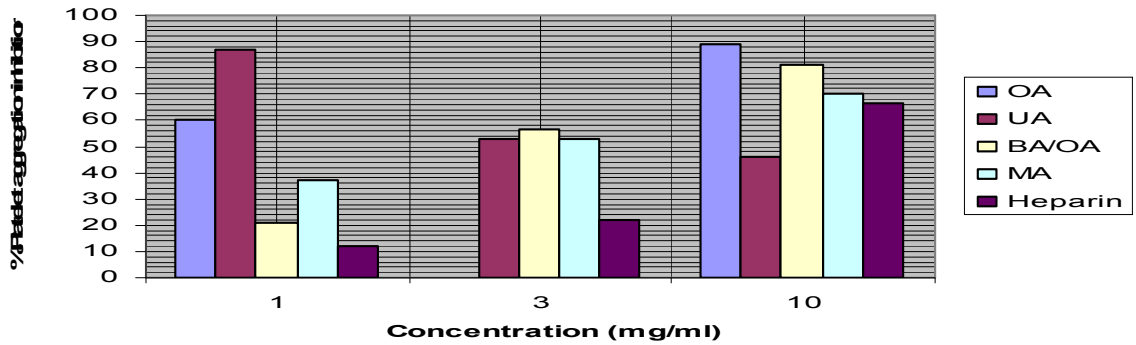

Fig 5. Graphical presentation of Inhibitory activity of the compounds on thrombin-induced rat platelet aggregation

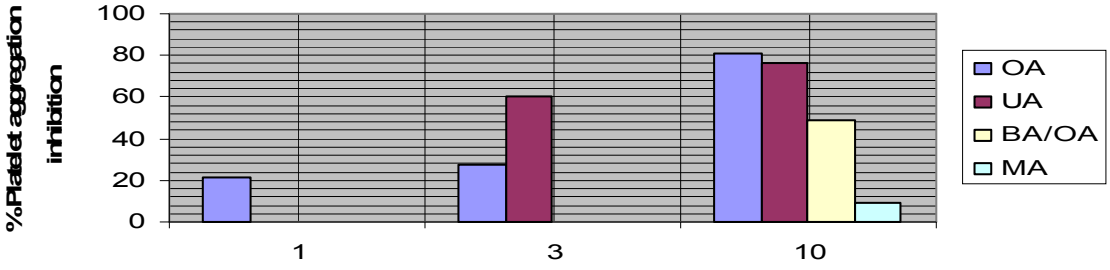

Fig.6 Graphical presenartion of inhibitory activity of the compounds on ADP-induced platelet aggregation

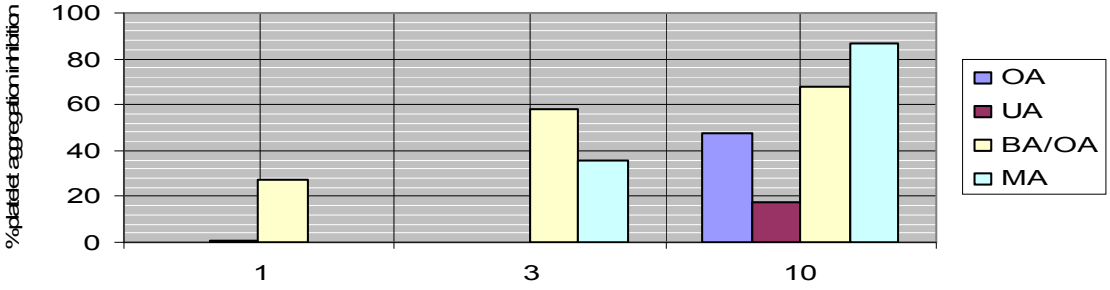

Fig. 7 Graphical presentation of inhibitory activity of the compounds onepinephrine-induced platelet aggregation

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Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia*

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The study of heavy metal contamination in plants, serve to indicate the metal concentration status of the site, where plants have grown. This is an important tool for phytoremediation. Proximate analysis in plants gives valuable information and help to access the quality of the sample. So studies were carried out on the roots of *Anogeissus latifolia* which is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. for heavy metal contents and proximate analysis of the root sample was also done. The root showed the absence of heavy metals when it was subjected to analysis by Inductively Coupled Plasma Atomic Spectrometer. The ash values, extractive values, moisture content, total solid content and crude fiber content of the root sample were also determined

Keyword: Heavy Metals, Proximate Analysis, Inductively Coupled Plasma Atomic Spectrometer, *Anogeissus latifolia*

1. Introduction

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plants. Medicinal plants play a major role in the health care sector of developing nations for the management of diseases. Thus herbal medicines have a prominent role to play in the pharmaceutical markets and health care sector of the 2st century[1]. *Anogeissus latifolia* one of the important medicinal plant since Ayurveda in cardiac disorder. The plant is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. The plant is rich in pharmacologically active phenolic phytoconstituent-ellagic acid[2]. It possess healing potential, microbicidal activities, antiulcer potential, hypolipidemic activities and hepatoprotective potential. The root is particularly useful in haematuria, urethral discharges and urinary tract infections[3]

Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning and pesticides used in agriculture[4] Human beings, animals and plants take up these metals from the environment through air and water. Heavy metals have the tendency to accumulate in both plants and human organs[5]The accumulation of heavy metals can have middle-term and long term health risks, and strict periodical surveillance of these contaminants is therefore advisable[6]. Lead accumulation results first in reduced functioning

of kidney, liver and brain cells and later in complete breakdown of the tissues. Cadmium and its compounds are also toxic to humans[7]
Proximate analysis in plants gives valuable information and help to access the quality of the sample. It provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug. Minerals are not destroyed by heating and they have a low volatility as compared to other food components. Total ash may vary with in wide limits for specimen of genuine drugs due to variable natural or physiological ash. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect[8]. So the present studies were done on the useful root of *Anogeissus latifolia*. The proximate analysis as well the heavy metal analysis was conducted for the drug respectively.

2. Materials and Methods

2.1 Plant Collection:

The root was collected from Dang Forests near Waghai Botanical Garden, Saputara. The fresh roots were dried in shade and were crushed and then powdered in an electric grinder and were used for the proximate analysis as well as for heavy metal determination.

2.2 Proximate Analysis[9,10,11,12]

The parameters determined for proximate analysis include ash value, moisture content, extractive value, total solid content and crude fibre content of the drug.
Determination of Ash values:

(a) Total ash:

Accurately weighed 2 gm of the powdered drug was taken in a tarred silica dish and it was incinerated at a temperature not exceeding 450°C until free from carbon. The sample was cooled and weighed. If a carbon free ash cannot be obtained in this way, the charred mass was exhausted with hot water. The residue was collected on ash less filter paper was incinerated and then filtrate was evaporated to dryness, and ignited at a temperature not exceeding 450°C. The percentage of ash was calculated with reference to the air dried drug.

(b) Acid-insoluble ash:

The ash obtained described as total ash was boiled for 5 min. with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

(c) Water-soluble ash:

To the ash obtained as total ash 25 ml water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of total ash. The content of water-soluble ash with reference to dried drug was calculated.

2.3 Extractive Values:

(a) Water soluble Extractives:

5gm of powdered drug was taken in conical flask. Then added 100ml of water was added in the flask containing powdered drug. 5% solution in water was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs.(with regular shaking). After the period of 24 hours the solution was filtered out with the help of the whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed the dish and noted down. Then 25ml of 5% solution of each of drug was taken in evaporating dish. Then the evaporating dish was heated until the damp mass was formed. Then cooled the evaporating dish and weighted it. Difference of evaporating dish containing damp mass and empty

evaporating dish was taken and directly calculated the water soluble extractive value.

(b) Alcohol soluble extractives:

5gm of powdered drug was taken in conical flask. Then 100ml of methanol was added to each of the flasks powdered drug. 5% solution in methanol was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs. After the period of 24 hours the solution was filtered out with the help of the whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed all dishes and noted down. Then 25ml of 5% solution of each drug was taken in evaporating dish. Now all evaporating dishes were heated until the damp mass is formed. Then cooled the evaporating dish and weighted it. Difference of evaporating dish containing damp mass and empty evaporating dish was taken and directly calculated the extractive value.

2.4 Moisture Content and total solid content:

Powdered roots of *Anogeissus latifolia* (W_2, 2g) was placed in a weighed petridish (W_1). The petridish was kept in a hot air oven at 60°C till constant weight (W_3) was achieved. The sample was placed in a desiccator after it had achieved constant weight and then weighed to determine the moisture content and total solids using the following formula:

$$\text{Moisture (\%)} = \frac{(W_1+W_2)-W_3}{W_2} \times 100$$

Where, W1 = Weight of petridish, W2 = Weight of sample, W3 = Weight of dried sample + weight of petridish

Total solid content was determined by the following formula:

$$\text{Total solids (\%)} = 100 - \text{Moisture (\%)}$$

2.5 Crude fibre content:

Extract 2 g of ground material with petroleum ether to remove fat. If fat content is below 1 % extraction may be omitted. After extraction with petroleum ether boil 2 g of dried material with 200 ml of sulphuric acid for 30 mins with bumping chips. Filter and wash with boiling water until washings are no longer acidic. Boil with 200 ml of sodium hydroxide for 30 mins. Filter and wash again with sulphuric acid, three 50 ml portions of water and lastly 25 ml alcohol. Remove the residue and transfer to ashing dish (preweighed dish W1). Dry for two hours at 130± 2. Cool the dish in a dessicator and weigh W2. Ignite for 30 mins, at 600°C. Cool in a dessicator and reweigh (W3).

$$\text{Crude fiber content} = \frac{\text{Loss in weight on ignition}(W2-W1)-(W3-W1)}{\text{Weight of sample}} * 100$$

3. Heavy Metal Analysis:[13,14]

3.1 Preparation of Sample:

2 gm of dried powder of roots of *Anogeissus latifolia* was weighed and subjected to dry-ash in a well cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/ HCl /H_2O (1:2:3) and gently heated on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transformed into a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis and concentration of element in the sample was calculated as the percentage of dry matter.

3.2 Preparation of Blank:

To 5 ml of HNO_3/ HCl /H_2O (1:2:3), 5 ml of deionized water was added and the volume was made upto 100 ml in a volumetric flask. This was used as blank.

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Table.1 : The Results Of Proximate Analysis

Sr. no	Experimental Studies	Observations for powdered roots of *Anogeissus latifolia*
1	Total ash value	11.75%
2	Acid insoluble ash value	7.45%
3	Water soluble ash value	7.85%
4	Water soluble extractive value	73.68%
5	Alcohol soluble extractive value	43.21%
6	Moisture content	7.80%
7	Total solid content	92.2%
8	Crude fibre content	28.85%

4. Instrument used:

Inductively coupled plasma atomic spectrometer (Perkin Elmer, USA, 3300RL)

5. Results and Discussions:

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash value, acid insoluble ash, water soluble ash was found to be 11.75%, 7.45% and 7.85%. This percentage clearly indicates that the root is best for drug action and effects. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The water soluble extractive value proved to be higher than alcohol soluble extractive value. It was found to be 73.68%. This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The moisture content of the crude drug was found below 7.80. Crude fibre is the fraction of carbohydrate that remains after treatment with acid and alkali. The crude fibre content of the root was found to be 28.85%.

The element content of roots of *Anogeissus latifolia* was determined by inductively coupled plasma. The results are shown in Table 2

Table 2: Heavy Metal Analysis

Sample	Element	Wavelength(nm)	Instrument Detection Limit (ppm) mg/L	Sample results (ppm) mg/L
Anogeissus latifolia roots	Lead (Pb)	220.353	0.0420	Not detected
	Arsenic(As)	193.696	0.0530	Not detected
	Zinc (Zn)	206.200	0.0059	Not detected
	Cadmium(Cd)	228.802	0.0027	Not detected

The roots showed the absence of heavy metal contamination (arsenic, lead, cadmium, zinc) and so considered safe for use if the drug is used in making of any formulation.

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Screening a Mediterranean Sponge *Axinella verrucosa* For Antibacterial Activity in Comparison to Some Antibiotics.

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The antibacterial effect of crude extract of marine sponge *Axinella verrucosa* at room temperature against seven nosocomial bacteria and one fungal isolates including, Staphylococcus aureus, *Pseudomonas aeruginosa*, *Acinetobacter septicus* and *Proteus vulgaris*, *Acinetobacter meningitis*, *Klebsiella pneumonia*, *E. coli* and the fungal pathogen *Candida albicans* were studied by Kirby-Bauer disc diffusion assay and, the findings of antibacterial activity of crude methanolic extract sponge *A. verrucosa* were compared to the efficiency of some marketed antibiotics that were tested against the same bacteria at given concentrations. In result, it was found that MeOH crude extract of *Axinella verrucosa* is effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter septicus* and *Proteus vulgaris* and except for its ineffectiveness against *Acinetobacter meningitis*, *Klebsiella pneumonia*, *E. coli* and the fungal pathogen *Candida albicans*. It was more active than Azithromycin and Gentamicin Against Proteus vulgaris and more efficient than ciprofloxacin against *Acinetobacter septicus*. Hexane and Ethyl acetate crude extracts derived from *A. verrucosa* revealed no activity against all bacterial and fungal pathogen. Sponge *Axinella verrucosa* remains an interesting source of new antibacterial metabolites with better activity than some antibiotics

Keyword: Antimicrobial Activity, *Axinella verrucosa*, Nosocomial Pathogens

1. Introduction

The marine environment comprises of complex ecosystem with a plethora of organisms are known to possess bioactive metabolites compounds a common means of defense. Predominantly microorganisms and plants, have provided mankind with many of the therapeutic agents currently on the market. These natural products have been used directly as drugs, or have provided leads for the synthetic preparation of pharmaceutical products[1]. Members of the phylum *Porifera* are renowned for their medicinal applications, as important sources of chemicals used in traditional medicine[1][2]. The sponges represent a diverse and ancient group of multicellular animals. Basic sponge body plans consist of multiple cell layers, each with individual specialization, functioning in the place of discrete organs. Due to porous structure and filter feeding sponges produce such a wide array of bio-active chemicals may be to defend themselves against the effects of potentially pathogenic organisms inadvertently ingested by the sponge[3]. Many biologically active substances have been successfully isolated from sponges especially demosponges.

It's been found that that antimicrobial activity is influenced by sponge morphology; branched and massive sponges showed higher bioactivity comparing to encrusting forms[4][5]. Marine sponges of genus *Axinella* are a well-known source of brominated pyrrole alkaloids ,the genus *Axinella* (class Demospongiae, order Halichondrida, family Axinellidae) contains approximately 20 species, distributed world-wide is known to be a source of a variety of secondary metabolites such as bromocompounds, cyclopeptides, polyethers, sterols, and terpenes[6][7]. Antimicrobial peptides were isolated from many marine sponges[6][8], the sponge genus Axinella is one of them due to the densely tiny pore- structure of *Axinella* on one hand it provides a unique environment for microorganisms which is supposed to play a role in producing proteins on the other hand[9][10]. Nosocomial infections are those that originate or occur in a hospital or hospital-like setting. They are responsible for high morbidity. Nosocomial infections are primarily caused by opportunists. The sites of nosocomial infections, in order from most to least common, are as follows: urinary tract, surgical wounds, respiratory tract, skin (especially burns), blood (bacteremia), gastrointestinal tract, central nervous system. Nosocomial pathogens may tend to be more resistant to antibiotics[11]. Members of the phylum *Porifera* are renowned for their medicinal applications, as important sources of chemicals used in traditional medicine[1][2]. The sponges represent a diverse and ancient group of multicellular animals. Basic sponge body plans consist of multiple cell layers, each with individual specialization, functioning in the place of discrete organs. Due to porous structure and filter feeding sponges produce such a wide array of bio-active chemicals may be to defend themselves against the effects of potentially pathogenic organisms inadvertently ingested by the sponge[3]. Many biologically active substances have been successfully isolated from sponges especially demosponges. It's been found that that antimicrobial activity is influenced by sponge morphology, branched and massive sponges showed higher bioactivity comparing to encrusting forms[4][5]. Considering all the above facts the present study has been undertaken to test the crud extracts derived from branched form sponge *Axinella verrucosa* against some nosocomial pathogens in comparison to some antibiotics that are in use, then, to identified some compounds found in MeOH extract.

2. Material and Methods

2.1 Sampling

At depths between 25-35 m, namely*: Axinella verrucosa*, *was* collected by scuba diving during the first half of February 2007 from ***Ibn- Hani*** (35°35'37"N 35°45'20"E) of **Latakia** coast. The sponges were sorted, cleaned from associated biota, placed in zipper freezer bags, and then frozen at -20°c before extraction. The sponge *Axinella verrucosa* was classified depending on (shape, color and type of spicules).

2.2 Preparation of Pathogens

The pathogens were obtained from university hospital lab, nosocomial pathogens taken from hospitalized patients were identified using API20E. The test microorganisms were *Staphylococcus aureus, Escherichia coli, Acinetobacter meningitis, Klebsiella pneumonia, Acinetobacter septicus, Proteus vulgaris, Pseudomonas aeruginosa and one fungal pathogen Candida albicans,* they were from different clinical specimens (table 1).

Table 1: The Test Microorganisms and Their Sources

Clinical specimen	Test microorganism
Pharyngeal swab(neonates)	*Candida albicans*
Umbilicus swab	*Staphylococcus aureus*
CSF	*Acinetobacter meningitis*
CSF	*Klebsiella pneumonia*
Urea	*Escherichia coli*
Gastric secretion(neonate)	*Pseudomonas aeruginosa*
Umbilicus swab	*Acinetobacter septicus*
Blood	*Proteus vulgaris*

2.3 Obtaining of Crude Extract

2.3.1 Preparation of Sponge

Frozen sample of two Sponge species were left to thaw and gently washed with distilled water to remove salts and epibionts. Wet samples of both

species were weighed, and placed in a fume hood for 48 hours to be dried, in order to remove as much water as possible. The wet sponges were found to weigh approximately 150g. The final weight of the dry sponges was approximately 130g. The dry sponges were 1cm cube chopped using sterile scissors

2.3.2 Extraction Procedure

The extraction procedure was based on **Larsen's** method[3] with minor modifications. The dry sponge pieces were successively processed using solvents with different polarities namely: methanol, ethyl acetate, and hexane. The amount of each solvent was approximately 130 ml. The sponges were soaked in methanol three times overnight, and filtered, and then they were immersed in ethyl acetate three times overnight and filtered .In addition the sponges were soaked once in hexane for 24 hours and filtered. Following each soak the sponges were allowed to dry in fume hood to remove any remaining solvent. As a final step, the sponge pieces were homogenized with 130 ml of ethyl acetate using a macerator and left at room temperature for 24 hours, then filtered. The filtrate was collected and combined with ethyl acetate extract collected from the initial extractions. In the end, three sets of immersion extracts one each of methanol, ethyl acetate and hexane were obtained. Individually, the extract mixtures were rotavapped under vacuum. The temperature of the water bath was set at -30°c and the rotation rate was medium. The crude extracts of both species were placed in small vials and kept at - 4°c for the susceptibility testing using Kirby-Bauer disc diffusion assay[8].

2.4 Seeding the Plates

Petri dishes were filled with approximately 4 mm thickness of previously sterilized Mueller-Hinton agar for bacteria and YPD for the yeast. Using sterilized swab, both media were inoculated with the pure isolates of bacteria and the yeast. The inoculum density was standardized for a susceptibility test, a $BaSO_4$ turbidity standard, was equivalent to a 0.5 McFarland standard**,** suspension contained approximately 1 - 2 x 10^8 CFU/ml

2.5 Kirby-Bauer Antimicrobial Assay

Punched sterilized 6mm disks (Whatman, no,1) were impregnated with 20µl of the obtained crude extracts from each sponge species, left to dry and then placed on the inoculated media. A sterilized blank disk was used as a control[8]. The bacterial cultures were incubated at 37°c for 24 hours and the yeast cultures were incubated at 27°c for 24 hours.

2.6 Collection of Data

The day after, inhibition zone diameters were measured and the results were scored as follow:

1. - (no zone of activity),
2. + (8-10 mm diameter zone of activity),
3. ++ (11-15 mm diameter zone of activity)
4. +++ (>15 mm diameter zone of activity).

On the other hand, the results were compared to reference values currently used antibiotics that belong to different groups

- Ceftriaxone (30micro grams per disk) β-lactam group, Cephalosporin 3rd generation.
- Azithromycin (15 micro grams per disk) a macrolide
- Gentamicin (10 micro grams per disk) an aminoglycoside.
- Ciprofloxacin (5micrograms per disk) an Fluroquinolone

The antibiotics were chosen according to the pathogen, disease and age.

2.7 Identification of Chemical Structures of Some Compounds

Spectral analyses were carried out and three compounds tentatively identified as being present in the crude extract of the sponge *Axinella verrucosa*. The crude methanol extract was partitioned by Dr. Inman at UCSC between methylene chloride (coded DCM) and water. The solvent from DCM layer was rotary evaporated. A portion of the DCM dried material was dissolved in methanol, concentration approx. 1 mg/mL This crude solution was analyzed by LC-

ESI-MS (in positive mode, equipped with a 150 x 4.6 mm, 5 micron, C18 column (Luna, Phenomenex Inc.) with a solvent gradient consisting of solvent A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile. The gradient began with 10% of the 0.1% formic acid in acetonitrile to 100% over 20 minutes, 10 µl injection. The molecular ions of several of the most important peaks were obtained from a full mass spectrum scan and this information was dereplicated using standard databases at UC Santa Cruz including the Dictionary of Natural Products and Marine Lit**.**

3. Results

3.1 Sponge Description

Mediterranean sponge *Axinella verrucosa*, belongs to the class of demosponges. It is medium sized, branching, ophidian like shape. Dark brown in color, the branches are regular in diameter. The sponge has no stem, the surface is rough textured and elastic, pores are numerous and obvious. Table 2 Fig1

Fig. 1**:** Morphological Description of Sponge *Axinella verrucosa*

Table. 2: The Classification and Description of the Sponge Samples

Sponge species	**Sub Class**	**Order**	**Family**	**Description**
Axinella verrucosa (Esper,1794)	Tetractinomorpha	Halichondrida	Axinellidae	Medium sized ,branching, ophidian like shape. Dark brown in color. The branches are regular in diameter. the sponge has no stem, the surface is rough textured and elastic, pores are numerous and obvious

3.2 The Antimicrobial Activity

The antimicrobial activity of crude methanolic, ethyl acetate, and hexane extracts of sponge *Axinella verrucosa* was evaluated against some hospital-acquired pathogens: *Staphylococcus aureus, Escherichia coli, Acinetobacter meningitis, Klebsiella pneumonia, Acinetobacter septicus, Proteus vulgaris, Pseudomonas aeruginosa and fungal pathogen Candida albicans*. Different concentrations of various commercial antibiotic discs, (Ceftriaxone, Gentamicin, Azithromycin, and Ciprofloxacin) were used to assay their antimicrobial activity against the test pathogens. Hexane and Ethyl acetate crude extracts derived from *A. verrucosa* revealed no activity against all bacterial and fungal pathogens. Crude methanolic extract of *Axinella verrucosa* revealed strong activity

against Gram positive *Staphylococcus aureus*, the zone of inhibition was found more than 21mm Table 3 Fig 2. Gram negative *Acinetobacter septicus*, was extremely sensitive to *Axinella verrucosa*, the zone formed was more than 21mm Table 4 fig 4. Crude methanolic extract of *Axinella verrucosa* revealed strong activity against Gram negative *Pseudomonas aeruginosa* the zone of inhibition was found more than 20mm Table 5 Fig 6 Gram negative *Proteus vulgaris* revealed high susceptibility against MeOH extract of *Axinella verrucosa* the zone of inhibition was found more than 20mm Table 6 Fig8. Comparatively ,as shown in Table3 Fig 3, the antibiotics Ceftriaxone 30µg , Azithromycin 15µg showed maximum activity against *S.aureus* (20mm) ,Gentamicin 10µg exhibited potent activity against *S.aureus*(16mm).
Ceftriaxone30µg, Azithromycin 15µg showed maximum activity against *A. septicus* (20mm) , Ciprofloxacin 5µg exhibited medium activity against *A. septicus* (13mm) Table 4 Fig 5. Ceftriaxone 30µg, Azithromycin 15µg were strongly active against *P. aeruginosa* (20mm), Ciprofloxacin 5µg exhibited high degree of antibacterial activity against *P. aeruginosa* (17mm) Table5 Fig7. While, Ceftriaxone30µg, Azithromycin 15µg showed maximum activity against *P. vulgaris* (20mm), Ciprofloxacin 5µg and Gentamicin 10µg exhibited moderate activity against *P. vulgaris* (12mm and 13mm respectively) Table 6 fig 9.

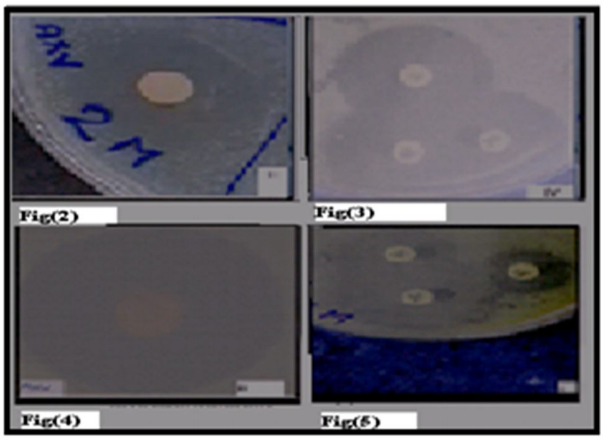

Fig (2): *S.aureus* susceptibility to crude MeOH extract of *A.verrucosa*
Fig (3): *S.aureus* susceptibility to Ceftriaxone ,Azithromycin and Gentamicin
Fig (4): *A.septicus* susceptibility to crude MeOH extract of *A.verrucosa*
Fig (5): *A.septicus* susceptibility to Ceftriaxone ,Azithromycin and Ciprofloxacin

Table 3: In vitro antimicrobial activity of MeOH crude extracts of *Axinella verrucosa* against *S.aureus* in comparison to Ceftriaxone, Azithromycin and Gentamicin.

	A. verrucosa	**Ceftriaxone 30 µg**	**Azithromycin 15 µg**	**Gentamicin 10µg**
S. aureus	>21 +++	20 +++	20 +++	16 +++

Table 4: In vitro antimicrobial activity of MeOH crude extracts of *Axinella verrucosa* against *A.septicus* in comparison to Ceftriaxone, Azithromycin and Ciprofloxacin.

	A. verrucosa	**Ceftriaxone 30 µg**	**Azithromycin 15 µg**	**Ciprofloxacin 5µg**
A.septicus	**>21** +++	**20** +++	**20** +++	**13** ++

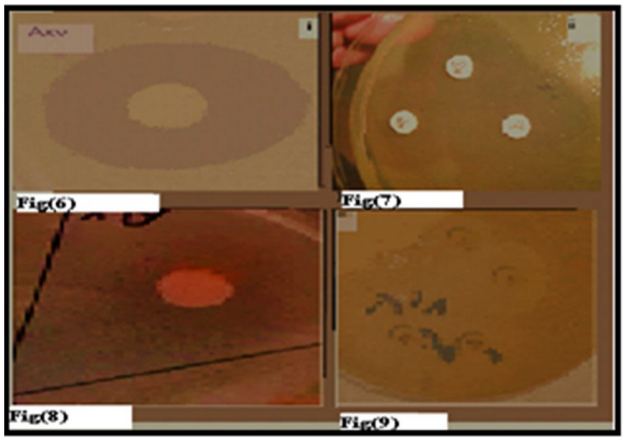

Fig (6): *P.aeruginosa* susceptibility to crude MeOH extract of *A. verrucosa*
Fig (7): *P.aeruginosa* susceptibility to Ceftriaxone ,Azithromycin and Gentamicin
Fig (8): *P.vulgaris* susceptibility to crude MeOH extract of *A. verrucosa*
Fig (9): *P.vulgaris* susceptibility to Ceftriaxone, Ciprofloxacin , Azithromycin and Gentamicin

Table 5: In vitro antimicrobial activity of MeOH crude extracts of *Axinella verrucosa* against *P. aeruginosa* in comparison to Ceftriaxone, Azithromycin and Ciprofloxacin.

	A. verrucosa	**Ceftriaxone 30 µg**	**Azithromycin 15 µg**	**Ciprofloxacin 5µg**
P.aeruginosa	20 +++	20 +++	20 +++	17 +++

Table 6: In vitro antimicrobial activity of MeOH crude extracts of *Axinella verrucosa* against *P.vulgaris* in comparison to Ceftriaxone, Azithromycin, Gentamicin and Ciprofloxacin.

	A.verrucosa	**Gentamicin 10µg**	**Ceftriaxone 30 µg**	**Azithromycin 15 µg**	**Ciprofloxacin 5µg**
P.vulgaris	>21 +++	13 ++	20 +++	20 ++++	12 ++

3.3 Identification of Bioactive Compound Present in Sponge

The MS m/z data and UV data were consistent with that of three compounds: Hymenialdisine, and two derivatives, 10- E-Hymenialdisine and Spongiacidine B, whose structures are shown below. However none of these compounds were isolated in pure form; consequently additional data such as NMR properties were not obtained. Their positive antibacterial actions are synergistic, and so more powerful, as compared with those from terrestrial origins.

a) Hymenialdisine:

4-(2-Amino-4-oxo-2-imidazolidin-5-ylidene)-2-bromo-4, 5, 6, 7-tetrahydropyrrolo [2, 3-c] azepin-8-one.molecular formula $C_{11}H_{10}BrN_5O_2$ molecular weight 324.136 melting point160-164ºC rates of elements C 40.76%; H 3.11%; Br; 24.65%; N 21.61%; O 9.87% this compound belongs to Pyrrole Imidazole alkaloids .Fig 10 shows LCMS chromatogram that illustrates molecular ions $\{M^+H\}$ m/z 324.08, 326.07, 327.10 at retention times (4.26-4.27-4.29 mins) ,peaks of UV absorbance : max λ nm 238.00, nm 260.00, 345.00 nm and chemical structure .

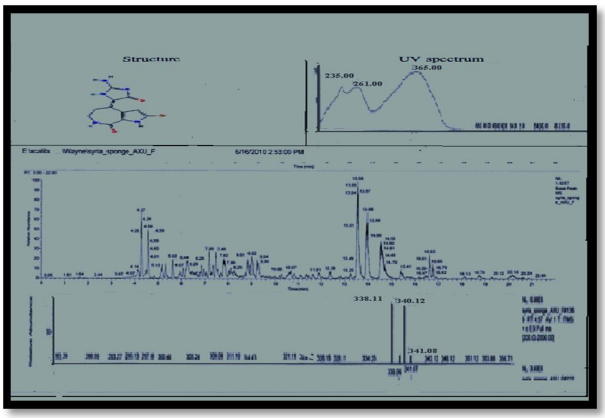

Fig 10: UV, ESI_MS spectra and chemical structure of the elucidated compound Hymenialdisine : peaks of UV absorbance **:** max λ nm 238.00, nm 260.00, 345.00 molecular ions $\{M^+H\}$ m/z 324.08, 326.07, 327.10 at retention times (4.26-4.27-4.29 mins)

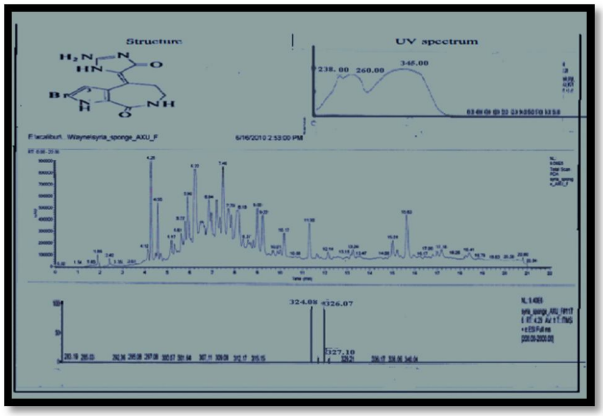

Figure 11: UV, ESI_MS spectra and chemical structure of the elucidated compound Hymenialdisine *10E:* peaks of UV absorbance**:** max λ nm235.00, nm261.00, nm 355.00 molecular ions $\{M^+H\}$ m/z 338. 11, 340.12, 341.08 at retention times (4.57-4.58-4.59 mins)

b) E Hymenialdisine

Molecular formula $C_{11}H_{10}BrN_5O_2$ molecular weight 324.136 melting point160-164ºC rates of elements C 40.76%; H 3.11%; Br; 24.65%; N 21.61%; O 9.87% this compound belongs to Pyrrole Imidazole alkaloids.Fig11 reveals LCMS chromatogram that shows molecular ions $\{M^+H\}$ m/z 338. 11, 340.12, 341.08 at retention times (4.57-4.58-4.59 mins), peaks of UV absorbance: max λ nm235.00, nm261.00, nm 355.00 and the chemical structure.

c) Spongiacidine B:

Molecular formula $C_{11}H_{10}BrN_5O_2$ Molecular weight 324.136 Melting point160-164ºC rates of elements C 40.76%; H 3.11%; Br; 24.65%; N 21.61%; O 9.87% This compound belongs to Pyrrole Imidazole alkaloids. Fig 12 represents LCMS Chromatogram that shows molecular ion$\{M^+H\}$ m/z 339. 09, 341.07, 342.13 at retention times (7.46-7.48-7.49 mins), peaks of UV absorbance: max λ nm239.00, nm265.00, nm 359.00 and the chemical structure.

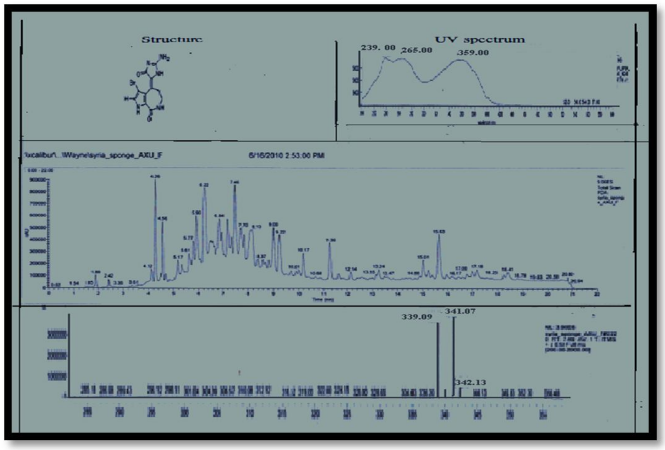

Fig 12: UV,ESI_MS spectra and chemical structure of the elucidated compound spongiacidin B :peaks of UV absorbance **:** max λ nm 238.00, nm 260.00, 345.00 nm molecular ions $\{M^+H\}$ m/z 324.08, 326.07, 327.10 at retention times (4.26-4.27-4.29 mins)

4. Discussion

In this study, sponge *Axinella verrucosa* was selected to be evaluated for its antimicrobial activity against some human pathogens due to its erected branch shape known to have potent antimicrobial activity[4][5]. Tthis could be attributed wide exhibition to water currents and to predators in comparison to massive shaped sponges. We specifically focused on Methanol extracts since (MeOH) has the potential to penetrate cell membranes on one hand[12] .Previous studies indicate to the surface origin of polar metabolites which are easily extracted and first used in sponge defense before non-polar metabolites or even metabolites with moderate polarity on the other hand[13]. Pyrrole Imidazole alkaloids in sponges are of a wider variety. Majority of them show antimicrobial, antitumor and anticancer properties[14][15][16]. References show that sponge antimicrobial extract attacks some bacteria and retain its activity against resistant bacteria to conventional antibiotics[6]. This activity encourages us to compare the antimicrobial capacity of the crude extracts to that for Antibiotics such as β-lactam group that comes first. Some antimicrobial activities can be lost in the process of fractionation of sponge crude extract the activity of the crude extract, and chromatographic procedure could affect the activity of the compounds by reducing the solubility of the bioactive compounds, this leads us to postulate that an extract may contain a number of pharmacologically active components

acting simultaneously or synergistically to affect bacterial pathogens[8]. MeOH crude extract of *Axinella verrucosa* is effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter septicus* and *Proteus vulgaris* and except for its ineffectiveness against *Acinetobacter meningitis*, *Klebsiella pneumonia*, *E.coli* and the fungal pathogen *Candida albicans*. Comparatively, MeOH extract of *Axinella verrucosa* had stronger activity than (Ceftriaxone Azithromycin and gentamicin respectively) against *S.aureus*.

MeOH crude extract of *Axinella verrucosa* was as efficient as Azithromycin, ceftriaxone and Ciprofloxacin against *P. aeruginosa*.

While, crude MeOH extract, Azithromycin and ceftriaxone showed the same degree of activity, they were more active than ciprofloxacin against Gram negative Acinetobacter septicus. In addition, Gram negative *Proteus vulgaris* revealed high susceptibility against MeOH extract of *Axinella verrucosa*. Comparatively, Ceftriaxone and Ciprofloxacin had stronger activity than Azithromycin and gentamicin against *p. vulgaris*.

The compounds present in the extract as well as three compounds that were identified: Hymenialdisine, and two derivatives, 10-E-Hymenialdisine and Spongiacidine B of Pyrrole Imidazole alkaloids and others of various chemical classes presumably showed synergistic antibacterial activity.

5. Conclusion

Based on the findings, promising methanolic extract from Mediterranean sponge *Axinella verrucosa*, hence this information may help to develop potential purified bioactive compounds in the pharmaceutical industry for the development of drugs. In future, this may lead the way towards large scale profitable production of antimicrobials from *Axinella verrucosa.*

6. Acknowledgments

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Evaluation of the Main Polyphenolic Compounds in Aromatic Plants of Asteraceae and Solanaceae Families of Bulgarian Origin

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Bulgarian specimens of Asteraceae and Solanaceae families are very scarce studied concerning the polyphenolic compounds. The aim of this investigation was to obtain fingerprint chromatographic profiles and to quantify the main polyphenols. HPLC–PDA on Kromasil and Purospher columns was employed for the separation of the components by applying two methods (TFA and AcAc). Better separation and reliable quantification of the polyphenols in *Achillea* species were obtained by the TFA method on Kromasil column. Chromatographic profiling and quantification of polyphenols in A. *campestris* was successful by using the TFA method on both columns. The polyphenols of A. *vulgaris* were better separated by AcAc method on Purospher column. Caffeoylquinic acids and rutin were present in higher quantities in *Achillea asplenifolia* and tobacco (Djebel). Fingerprint chromatographic profiles and reliable quantification of the polyphenolic compounds were achieved by considering the differences in the polyphenol complex and the specificity of the plant matrix

Keyword: Polyphenols, *Achillea*, *Artemisia*, tobacco, HPLC- PDA

1. Introduction

The aromatic plants of Asteraceae family are well known all over the world because of their different therapeutic applications[8,9]. Tobacco (Solanaceae family), especially the oriental types are used in the traditional medicine [5]. In many cases the healthy effect of the plants has been related with their polyphenolic components. For this reason, in the last years a great number of works has been aimed at finding active polyphenol components in a variety of different plants[2,14,16].

Polyphenols are one of the most widely occurring groups of phytochemicals of considerable physiological and morphological importance in plants. As a large group of bioactive chemicals, they have diverse biological functions. The most abundant polyphenols in plants are the phenolic acids and the flavonoids. The phenolic acids are presented mostly by the caffeoylquinic acids, which are ubiquitous, long recognized as powerful antioxidants. The chlorogenic acid family may provide significant health benefits by protecting against the damaging effects of free radicals. Growing evidence indicates the contributions of dicaffeoyl derivatives to the antioxidant activity of the plants of Asteraceae family[10,12]. Flavonoids are the other most commonly found phytochemicals in the medicinal plants of Asteraceae family [1]. Typically these compounds help to protect the plant against UV radiation, fungal parasites, herbivores, pathogens and oxidative cell injury. The ability of these natural antioxidants to scavenge several oxygen and nitrogen free

radicals has been associated to their health benefits[18]. The polyphenols of *Achillea* specimens include predominantly chlorogenic acid, dicaffeoylquinic acids as well as the flavonoids apigenin, luteolin, quercetin and their glycosides[3,15,17]. Most abundant are the 7-glycosides of the flavonoids apigenin and luteolin. In the polyphenolic profiles of *Artemisia* species a great number of flavonoids have been identified, namely quercetin, kaempferol, patuletin, apigenin, luteolin and their glycosides[4,11,13].

Djebel is a tobacco cultivar traditionally cultivated in Bulgaria. This cultivar is characterized by specific, pleasant aroma of the leaves. The Djebel plants have low nicotine content and are rich in polyphenols[6,7]. In tobacco, the polyphenols are presented with the so called typical five, chlorogenic acid and its isomers- neochlorogenic acid and 4-0-caffeoylquinic acid, and the flavonoids rutin and kaempferol-3-rutinoside[6,19]. Hence, the tobacco polyphenol profile is relatively simple and well defined.

HPLC has become the preferable analytical technique for determination of polyphenols providing qualitative and quantitative data. HPLC especially coupled with photodiode array detector (PDA, DAD) produces multi-wavelength detection. HPLC- PDA is used to facilitate the identification of compounds and to control the peak purity. The separations and the detection modes differ one from other aiming at identifying or quantifying preferable components of the polyphenol complex. Good separation is absolutely necessary to achieve fingerprint chromatographic profile and reliable quantification of the components. It depends on the distribution of the polyphenols and the complexity of the plant matrix. Most of the investigations are directed to identify and quantify the representatives of the phenolic acids or the flavonoid components, taking into account only the chlorogenic acid[3,10]. Hence, studying the polyphenol complex (phenolic acids and flavonoids) requires most complicated technique, such as the application of different chromatographic columns and mobile phases. There are no data on the separation of polyphenol components of *Achillea* and *Artemisia* obtained by different chromatographic conditions of HPLC- PDA method. Fingerprint chromatographic profiles and quantitative results on the polyphenol distribution in these species and Djebel cv. originating from Bulgaria are very scarce.

The aim of the present work was to obtain fingerprint chromatographic profiles of the polyphenolic compounds in *Achillea* and *Artemisia* species and Djebel tobacco cv. and to quantify the main compounds.

2. Materials and Methods

a. Plant Material

For the aim of the investigation two wild species of genus *Achillea* - *A. collina* and *A. asplenifolia* (members of *A. millefolium* group) were studied, each represented by one population, namely *A. collina* 102 and *A.* a*splenifolia* 9602, two wild species of *Artemisia, A. campestris* and *A. vulgaris*, and Djebel tobacco cv. Seedlings of *Achillea* plants were produced in a greenhouse and then transferred to the experimental field of Institute of Plant Physiology and Genetics, Sofia, in 2009. These species were identified by Assoc. Prof. Dr. Antonina Asenova Vitkova (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria). The specimens of *Artemisia* species were collected in the region of Shipka town, Balkan Mountain and determined by Assist. Prof. Dr. Katia Uzundjalieva (Institute for Plant Genetic Resources "K. Malkov", Town of Sadovo, Bulgaria). The plant material was collected in July at full flowering stage. Flos (*Achillea*) and herbal (*Artemisia*) material were taken to the purpose of this study. Dry leaves of Djebel cv. were purchased from Tobacco and Tobacco Products Institute, Plovdiv, Bulgaria and identified by Assoc. Prof. Dr. D. Dimanov. To prepare representative samples the material was dried at room temperature, avoiding direct sunlight, then milled, packed into multilayer

paper bags and stored in a dark room at ambient temperature.

b. Reagents

Methanol and acetonitrile used for HPLC analyses were of chromatographic grade (VWR, Austria). Water for HPLC was prepared with Millipore purifier (Millipore, USA). Trifluoroacetic acid (TFA) and acetic acid (AcAc), HPLC grade, Merck, Germany were used to prepare the mobile phases. Neochlorogenic acid (Cas Number 906-33-2), chlorogenic acid (Cas Number 327-97-9), rutin (Cas Number 250249-75-3), quercetin (Cas Number 117-39-5) and apigenin (Cas Number 520-36-5) were purchased from Sigma Aldrich, HPLC standards, purity >95%. Standard solutions with concentrations of 0.1, 0.05, 0.025, 0.0125, 0.0062 mg ml^{-1} were obtained by diluting the stock solution of each component in methanol.

c. Extraction of Polyphenols

The milled plant material was weighed with 0.0001 g precision, taking 0.2 g samples for *Achillea* and *Artemisia* and 0.1 g for Djebel cv. Two replications of every sample were prepared. The flavonoids were analyzed in their glycoside form and therefore nonhydrolysed plant extracts were prepared. The powdered *Achillea* and *Artemisia* samples were extracted with 10 ml 70% (v/v) aqueous methanol. The tobacco powder was extracted with 5 ml, 60 % (v/v) aqueous methanol. All extracts were sonicated for 30 min and then they were filtrated under vacuum. The extract from tobacco was purified by passing it through cartridge C18 according to the method described by Dagnon & Edreva[6]. The volume of all samples was adjusted to 10 ml and passed through a membrane filter 0.45 µm prior to HPLC analysis.

d. HPLC –PDA Analysis

The instrumentation used for HPLC analysis consisted of quaternary mixer Smartline Manager 5000, pump Smartline 1000 and PDA 2800 detector (Knauer, Germany). Two chromatographic columns were used: a Purospher ^{R}star RP-18e 25cm x 4.6mm i.d., 5µm particle size (Merck, Germany) and a Kromasil C18, 15 cm x 4.6 mm i.d., 5 µm particle size (Supelco, USA). Mobile phase flow rate was set by 1.0 ml min^{-1}; sample volume was 20 µl.

d.1 Separation Modes

Two different methods were employed to separate the polyphenols. In the TFA method 0.1% (v/v) trifluoroacetic acid in acetonitrile (AcN) was used as solvent B. A mixture from 90 parts water and 10 parts 0.1% TFA in acetonitrile was used as solvent A with the following gradient elution program: 0-10 min, 100%-90% A (0-10% B), 10-18 min, 89% A (11% B), 18-25 min, 85% A (15% B), 25-40 min, 45% A (55% B).

In the AcAc method the mobile phase composition was A - CH_3OH: H_2O: CH_3COOH= 5: 93: 2; B - CH_3OH: H_2O: CH_3COOH= 86: 12: 2. The elution followed the gradient profile: 0-15 min, 100% - 80% A (0-20% B); 15-35 min, 45% A (55% B); 35-50 min to 0% A (100% B). By this method maximum separation of chlorogenic acid from its isomer 4-0-caffeoylquinic acid was achieved. All samples (*A. collina* 102, *A. asplenifolia* 9602, *A. campestris*, *A. vulgaris* and Djebel tobacco cv.) were analyzed on the two chromatographic columns by using the described methods of separation (TFA and AcAc).

d.2 Detection

The polyphenols were monitored at 320 nm (maximum absorption for caffeoylquinic acids), 352 nm (maximum absorption for rutin and quercetin) and at 340 nm (maximum absorption for apigenin).

d.3 Identification and qualitative analysis

The identity of the chromatographic peaks was confirmed by comparison of the retention times of the samples with those of the standard compounds. The spectral characteristics of the eluting peaks, scanned with diode-array detector (λ=200-400 nm) were compared with those of the authentic standards. Peak assignments for those components where no references were available (4-0-caffeoylquinic acid and kaempferol-3-rutinoside) were based on data for retention time according to the literature and the quantification

was done by using the calibration curves of chlorogenic acid and rutin. The content of the components was determined by the external method using a calibration curves established with five dilutions of each standard with correlation coefficients between 0.997 and 0.999. The recovery of the polyphenols by the established procedures was from 95% to 100%. The relative standard deviation (RSD) of the methods varied between 3% and 18% by the different components. The limit of detection of the polyphenols pointed to 0.5 µg/ml. The limit of quantification differed from 1.0µg/ml to 3.0 µg/ml. Each sample was analyzed several times (min 3), and the mean value was used for calculation. The results are expressed as mean ±SD.

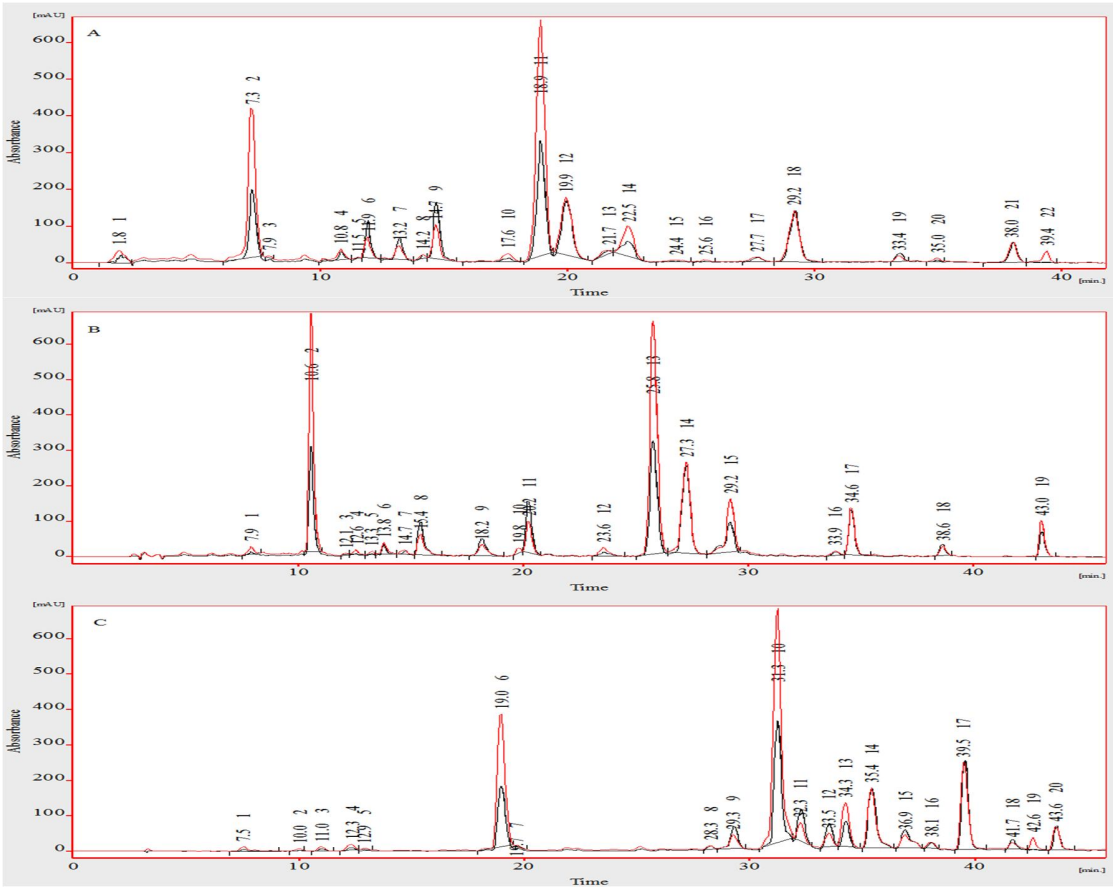

Fig. 1: Chromatographic profiles of polyphenols of *A. asplenifolia* 9602 on Kromasil column, TFA method (A), Purospher column, TFA method (B) and Purospher column, AcAc method (C). Peaks 2, 7, 19 and 21 were identified as chlorogenic acid, rutin, quercetin and apigenin, respectively (A); Peak 2, 9, 18, 19 as chlorogenic acid, rutin, mixed, mixed, respectively (B); Peaks 4, 6 and 7 as chlorogenic acid and isomers, peak 12 as rutin, peak 18 as quercetin and peak 20 as apigenin (C). Red line represents absorbtion at 320 nm. Black line represents absorbtion at 340 and 352 nm.

3. Results and Discussion

3.1 Separation of Polyphenols

In this study, Bulgarian aromatic plants (*Achillea, Artemisia* and *N. tabacum* species) were studied for the distribution and quantification of the main polyphenol compounds. Two chromatographic methods (TFA and AcAc) were employed to separate the polyphenols. They were designed to obtain fingerprint profiles of the polyphenol complex in the studied plants. The qualitative and quantitative differences in the polyphenols of the three groups of plants, the specificity of the plant

matrix and the need to obtain reliable data for the Bulgarian specimens motivated the development of this study. The use of chromatographic column Kromasil RP 18 and trifluoroacetic acid in the mobile phase were the most applied chromatographic conditions for the determination of flavonoids in *Achillea* species[3,15]. The application of RP 8 or RP 18 columns with other acids in the mobile phase was successful for the analysis of caffeoyl derivatives[10, 12]. Thus, there are few data concerning the simultaneously separation and quantification of the phenolic acids and flavonoids in *Achillea* and *Artemisia* species. Such data obtained by employing different chromatographic conditions will contribute to increase the reliability and accuracy of the phytochemical methods.

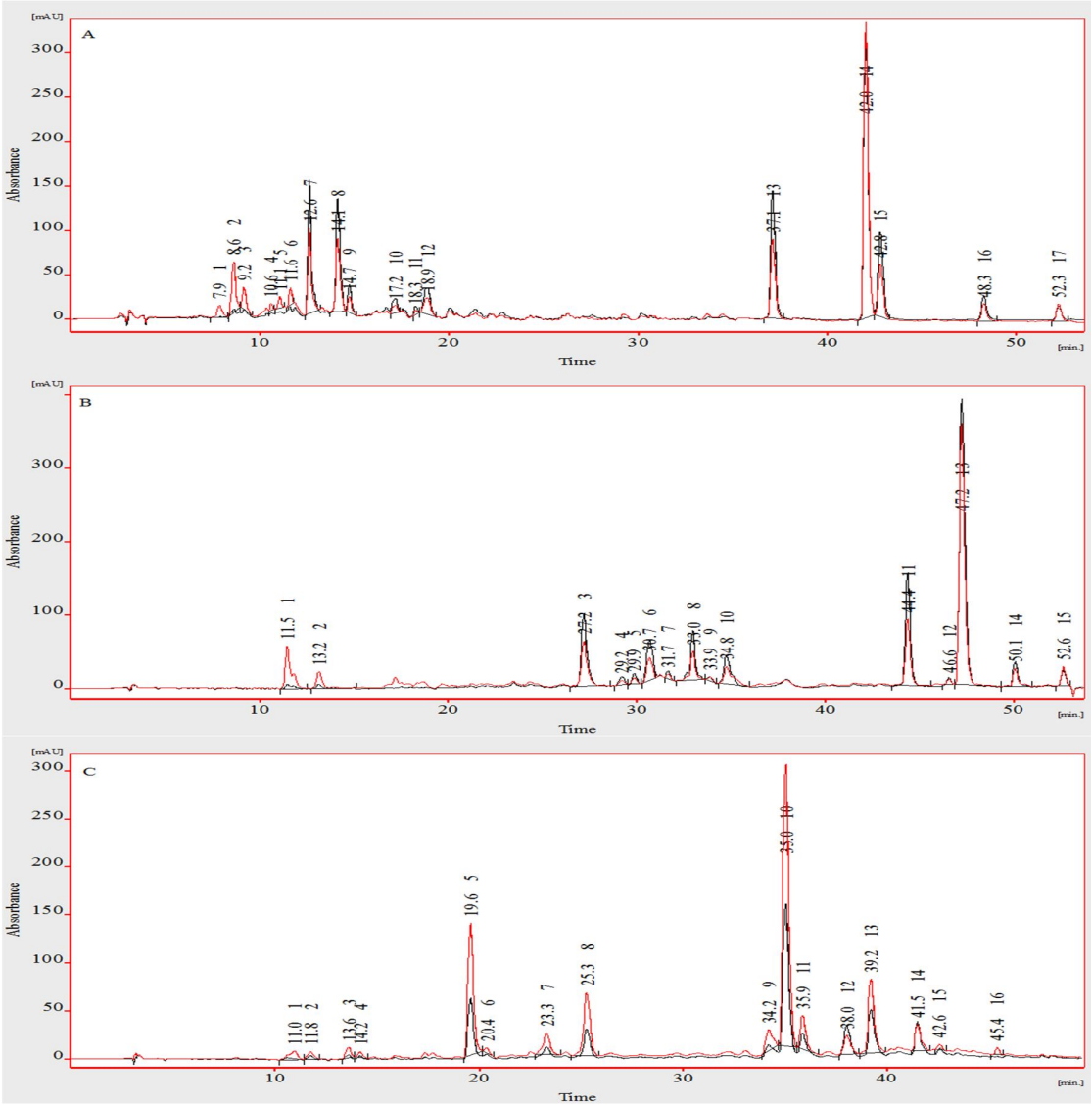

Fig. 2: Chromatographic profiles of polyphenols of *Artemisia* species on Purospher column, TFA method (A), AcAc method (B) and AcAc method (C); *A. campestris* (peak 9-rutin, peak 13-quercetin, peak 14-apigenin) (A); *A. campestris* (peak 2- neochlorogenic acid, peak 8- rutin, peak 11-quercetin, peak 13-mixed) (B); *A. vulgaris* (peaks 3, 5 and 6-chlorogenic acid and isomers, peak 12- rutin) (C). Red line represents absorbtion at 320 nm. Black line represents absorbtion at 340 and 352 nm.

The chromatographic profiles of the polyphenols in *A. asplenifolia* 9602 are presented on Figure 1. Figures 1A and 1B represent the separation of the polyphenol components by the chromatographic conditions of the TFA method on Kromasil and Purospher columns, respectively. They reveal some differences in the chromatographic shape, mostly in the area of flavonoid aglycones and one of them was critical for the quantification of apigenin. As shown on Figure 1A (TFA method, Kromasil column) the peak of apigenin (21) is followed by a well-defined peak(22) with an absorption maximum at 320 nm. This component was not separated from the peak of apigenin (19) by the same mobile phase conditions on Purospher column (Figure 1B). Figure 1C represents the chromatographic profile of polyphenols of *A. asplenifolia* 9602 obtained by using AcAc method on Purospher column. By this method the peak of apigenin (20) and the peak with an absorbtion maximum at 320 nm (19) were clearly separated, which allowed the successful quantification of the flavonoid apigenin (Figure 1C).

The chromatographic profile of *A. asplenifolia* 9602 obtained by the TFA method, Kromasil column compared to the profiles of *Achillea* species in previous studies differ in relation of quercetin, obviously presented in the Bulgarian specimens[3, 15]. The chromatographic patterns are similar and they confirm the presence of the three main flavonoids – luteolin, quercetin and apigenin in the studied *Achillea* specimens.

On Figure 2 the chromatographic profiles of *Artemisia* species are shown. They were obtained on Purospher column by using the TFA (2A) and AcAc (2B) methods. The separation of the polyphenols in the specimen of *A. campestris* pointed to the predominant presence of two flavonoid aglycones. The highest peak on the profile of *A. campestris* was identified as apigenin (14) and the peak 13 as quercetin (Figure 2A). The peak of apigenin (14) is well separated from the following peak (15) as shown on Figure 2A. The same shape of the polyphenols was obtained by the TFA method on the Kromasil column. On the contrary both peaks overlapped in the conditions of AcAc method, Purospher column, as shown on Figure 2B. Hence the quantification of the flavonoid apigenin in *A. campestris* was possible by using TFA method on both columns.

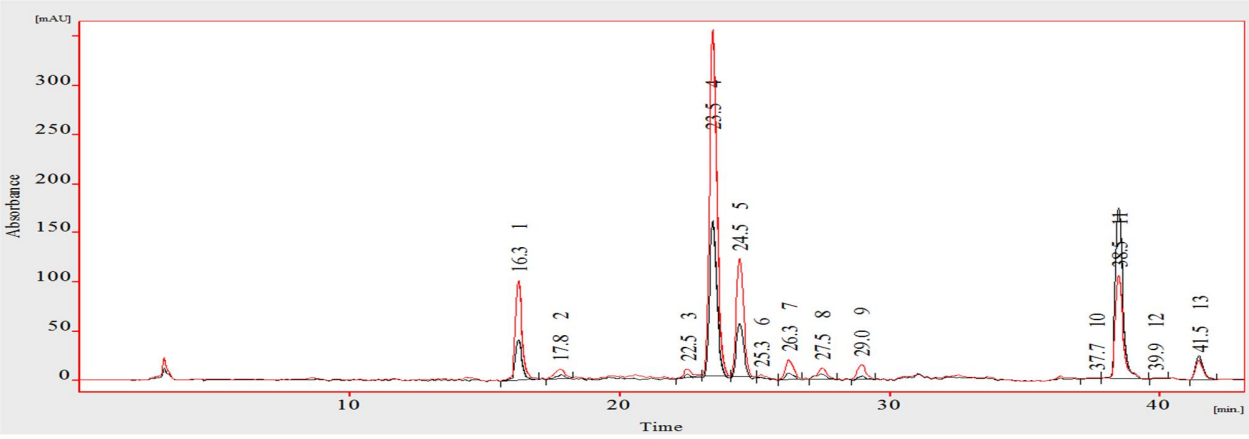

Fig. 3: Chromatographic profile of polyphenols in Djebel cv. tobacco on Purospher column, AcAc method. Peaks 1, 4 and 5 were identified as neochlorogenic acid, chlorogenic acid and 4-caffeoylquinic acid, peak 11 as rutin and peak 13 as kaempferol-3-rutinoside, respectively. Red line represents absorbtion at 320 nm. Black line represents absorbtion at 340 and 352 nm.

In the chromatographic profile of the specimen of *A. vulgaris* (Figure 2C) many differences in comparison with those of *A. campestris* obtained by the same conditions (AcAc method, Purospher column) have been observed. Obviously, most of the polyphenol components (peaks 3, 5, 6, 8, 10, 13) have absorption maximum at 320 nm (Figure 2C). This fact points to the predominant presence of the phenolic acids in the specimen of *A. vulgaris* (Figure 2C) in contrast to *A. campestris* (Figure 2B).

The chromatographic profile of Djebel tobacco cv. is presented on Figure 3. It shows the clear separation of the chlorogenic acid from its isomer 4-caffeoylquinic acid achieved by AcAc method on Purospher column. Moreover, it reveals the abundant presence of the caffeoylquinic acids (neochlorogenic, chlorogenic and 4-0-caffeoylquinic acids) and the flavonoid glycosides (rutin and kaempferol-3-rutinoside) (Figure 3).

3.2 Quantification of Polyphenols

The content of the main polyphenols in the specimens of *Achillea*, *Artemisia* and Djebel tobacco cv. was determined. The amount of the identified compounds is given in Table 1. Chlorogenic acid was presented in all specimens in being of lowest amount in *A. campestris* (Table 1). The neochlorogenic acid was the major caffeoylquinic acid in the specimen of *A. campestris*. Quercetin and apigenin were the most abundant flavonoid aglycones in the polyphenol complex of *A. campestris,* 2.12 mg g^{-1} DM and 2.81 mg g^{-1} DM, respectively. In comparison, these flavonoids were not found in the specimen of *A. vulgaris* (Table 1).

Table 1: Content of main polyphenolic compounds (mg g^{-1} DM) in flos of *Achillea*, in herbal material of *Artemisia* species and in dry leaves of Djebel tobacco cv. Caffeoylquinic acids and flavonoid glycosides are calculated by using method AcAc. Flavonoid aglycones are calculated by using method TFA. Data are means of three replicates ±SD.

Sample description	Caffeoylquinic acids		Rutin	Kaemp-ferol-3-rutinoside	Quercetin	Apigenin
A. collina 102	Neochloro-genic Chlorogenic 4-0-caffeoyl-quinic	0.19±0.013 4.49±0.22 0.02±0.009	n.d.	n.d.	1.03±0.12	0.29±0.03
A. asplenifolia 9602	Neochloro-genic Chlorogenic 4-0-caffeoylquinic	0.27±0.03 6.54±0.18 0.07±0.009	2.42±0.22	n.d.	0.42±0.08	0.55±0.02
A. campestris	Neochloro-genic Chlorogenic 4-0-caffeoylquinic	0.33±0.05 0.078±0.008 n.d.	1.26±0.20	n.d.	2.12±0.15	2.81±0.15
A. vulgaris	Neochloro-genic Chlorogenic 4-0-caffeoylquinic	0.13±0.014 1.70±0.10 0.05±0.009	0.87±0.06	n.d.	n.d.	n.d.
Djebel cv.	Neochloro-genic Chlorogenic 4-0-caffeoylquinic	2.41±0.15 10.27±0.28 3.23±0.25	9.20±0.32	1.62±0.20	n.d.	n.d.

The caffeoylquinic acids were the dominating components of the polyphenol complex in the *A. vulgaris* (Table 1 and Figure 2C). The *Achillea* species and Djebel cv. were rich of caffeoylquinic acids (Table 1). The data in Table 1 reveal that the highest quantities of the three isomers of the caffeoylquinic acid (neochlorogenic 2.41 mg g^{-1}, chlorogenic 10.27 mg g^{-1} and 4-0-caffeoylquinic 3.23 mg g^{-1}) and the highest amount of rutin, 9.20 mg g^{-1} DM were determined in the sample of tobacco. In lower amount rutin was quantified in the two *Artemisia* species and in *A. asplenifolia* 9602. The content of rutin was 1.26 mg g^{-1} DM (*A. campestris*), 0.87 mg g^{-1} DM (*A. vulgaris*) and 2.42 mg g^{-1} DM (*A. asplenifolia* 9602) (Table 1). Rutin was not detected in the specimen of *A. collina* 102.

The results of this study show undoubtedly that for reliable quantification of the polyphenol compounds it is necessary to perform good separation considering the different distribution of the polyphenols in the plant species. The separation and quantification of flavonoid aglycones were better achieved by using method TFA. Method AcAc was most suitable to separate the caffeoylquinic acids and flavonoid glycosides. Thus, it is advisable to analyze the polyphenols in the species of *A. millefolium* by using method TFA on Kromasil column. The polyphenols of *Artemisia campestris* can be analyzed successfully employing the same method on Kromasil or Purospher column. Chromatographic profiling of polyphenol components in *Artemisia vulgaris* requires applying the method AcAc to achieve good separation of the phenolic acids. Changing the mobile phase composition and monitoring the UV spectrum of the peaks are very helpful analytical practice to improve the separation and for accurate quantification.

4. Conclusions

The data presented in this investigation have revealed a considerable variation in the chromatographic profiles of the polyphenols in the studied *Achillea* and *Artemisia* species, and in the Djebel cv. Thus, the separation and quantification of the polyphenolic compounds in the different plant species require employing a specific chromatographic approach. Tobacco polyphenol profile may be regarded as a model for caffeoylquinic acid distribution in the plant kingdom and can help to distinguish the chlorogenic acid isomers.

5. Acknowledgements

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Syzygium Cumini (L.) Seed Extract Improves Memory Related Learning Ability of Old Rats in Eight Arm Radial Maze

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Oxidative stress is implicated in age-related deterioration of memory and learning ability. In this study, we evaluated whether oral administration of *Syzygium cumini* seed extract (SE) ameliorates the learning-related memory of old rats by eight-arm radial maze task. After 12-weeks oral administration of SE extract, learning-related memory was ameliorated, concurrently with decreases in the corticohippcampal levels of lipid peroxide (LPO) and increases in the catalase and AChE activities in the cortex. The levels of LPO were positively correlated with the RMEs. In carotid artery occlusion-induced hypoxia study, the SE-fed rats displayed significantly less microglial invasion, cell swelling and rupture in the cortico-hippocampal brain slices, thus suggesting an antioxidative defense of SE. Chemical analyses of SE revealed substantial amounts of polyphenols and free-radical scavenging activities. Finally, our results show that the S. *cumini* seed extract improves learning-related memory in old rats, probably, by instigating the antioxidant defense of the brain

Keyword: Antioxidants, *Syzygium cumini*, Memory, Alzheimer's Disease, Aacetylcholine Esterase and Catalase Enzyme

1. Introduction

Oxidative injury reflects imbalanced equilibrium between reactive oxygen species (ROS) generation and inadequate antioxidative defense of the biological system[1]. According to the free radical theory of aging, an excess generation of ROS relates to age-associated neurodegeneration and related disorder[2]. Neurodegenerative disorders are associated with various degrees of memory impairments[3]. Neuro-behavioral impairments are cumulative effects of oxidative damage to proteins, lipids, and nucleotides[4]. Age-related memory deficits and poor antioxidant defenses can be reversed by foods enriched with the antioxidant[5,6,7]. Therefore much attention is being paid toward dietary antioxidant, particularly those found in fruits and vegetables to prevent age-related deterioration of brain performance such as memory.

Syzygium cumini is a seasonal fruit of Bangladesh and it is considered as a rich source of antioxidative and redical scavenging phytoconstituents[8]. The seeds of this fruit retain extraordinary medicinal properties, which have remained largely undiscovered. We have previously reported that *S. cumini* seed powder possesses anti-diabetes activity[9] and protects the liver against the lipid peroxidation in association with a concurrent amelioration of hepatocellular status of alcoholic rats[10]. *S. cumini* seed extract

also exhibited stronger *in vitro* free radical scavenging activity than that of the synthetic antioxidant, such as butylated hydroxy toluene and natural antioxidant vitamin C. Furthermore, the oral administration of *S. cumini* seed extract restored the levels of lipid peroxides (LPO) in the cerebral cortex of alcoholic rats to those of the normal rats[11]. All these results thus suggest that the *S. cumini* seed extract might play an important role in the oxidation-related deterioration of brain activities. Notably, consumption of alcohol severely impairs memory and confers massive oxidative stress to brain, thus again indicating that *S. cumini* causes an improvement of the brain functions. In addition, we produced *in vivo* hypoxia-induced oxidative stress in the brain. The *S. cumini* seed extract-pretreated rats had an augmented antioxidative defense in the brain. We, therefore, were very much interested to study whether the *S. cumini* seed extract affects the memory-related learning ability of old rats and whether the outcome relates with to the *in vivo* antioxidant activity of *S. cumini* seed extract.

2. Materials and Methods

a. Animals

Twenty ~100 weeks old male rats (210-240g) were used in the present study. The rats were housed in an animal room at 23± 2ºC, under 12 h dark-light cycles (light 8.00-20.00 h; dark 20.00-8.00 h). The rats were randomly divided into two groups: The control group [Control, vehicle (saline) administered rat group]; the *S. cumini* seed extract-administered group (SE). The SE rats were intragastrically administered to seed extract of *S. cumini* at a dose of 400 mg/Kg of body weight per day. Administration of the seed extract was continued until the completion of behavioral study.

b. Eight- Arm Radial Maze Tusk

Learning-related behavior was assessed by four-arm baited eight-arm radial maze paradigm using standard eight-arm radial maze as described previously[12]. The radial maze is an octagonal central platform surrounded by eight equally spaced radial arms with a food cup at the end of each arm. The maze was placed in a closed room decorated with some fixed visual cues. The rats were first adapted to radial maze. Rats were then trained to collect food reward at the end of each of four arms of the eight-arm radial maze under food deprivation (~10-15%) schedule. A trial was terminated after either all the bait was consumed or after 4 min had elapsed, whatever occurred first. The performance in this situation involved two parameters (i) reference memory errors (RME), entry into unbaited arms; and (ii) working memory errors (WME), repeated entry into arms that had already been visited and obtaining the rewards within a trail. The testing continued for six days weak. Each rat was given one daily trial for six days/week for a total of 10 weeks.

c. Preparation of Brain Tissue

After the behavioral studies were completed, the rats were anaesthetized with sodium pentobarbital (65 mg/kg BW, i.p.) to collect blood, and the hippocampus and cerebral cortex were separated on ice as described previously[12]. Tissues were either first stored at −30 ºC or immediately homogenized in ice-cold 0.32 M sucrose buffer (pH 7.4) containing 2 mM EDTA, protease inhibitor cocktails and 0.2 mM phenylmethylsufonyl fluoride using a Polytron homogenizer (PCU 2–110; Kinematica GmbH, Steinhofhale, Switzerland). The homogenates were immediately subjected to the assays described below or stored at −80 ºC.

d. Measurement of Acetylcholine Esterase Activity of Cerebral Cortex

Acetylcholine esterase activity was measured by the method of Ellman et al. (1961) [13] at 25ºC. The method is based on the hydrolysis of the substrate acetylthiocholine. The standard 1.0 ml reaction mixture for the assay contained 100 mM phosphate buffer (pH 8.0), 1 mM $MgCl_2$, 0.50 mM acetylthiocholine, 0.125 mM 5,5'-dithiobis-2-nitrobenzoic acid and 100 □g homogenate protein. The blank consisted of solutions without the protein. The reaction was recorded at 412 nm by using a Hitachi U-2000 spectrophotometer and the rate was calculated as □moles of substrate

hydrolyzed per min per mg protein. Acetylcholine esterase activity was expressed as U/mg of protein. One unit was the amount of enzyme that hydrolyzes 1 µmol of acetylthiocholineiodide/min/mg protein.

e. Measurement of Catalase Activity of Cerebral Cortex

Catalase activity was assayed according to the method of Claiborne (1985)[14]. Briefly, the assay mixture consisted of 1.90 ml of phosphate buffer (50 mM, pH 7.0), 1 ml of H_2O_2 (0.02 M) and 0.1 ml of cortex homogenate in a final volume of 3 ml. Control cuvette contained all the components except substrate. Change in absorbance was recorded at 240 nm. Catalase activity was expressed as U/mg protein by using molar absorption coefficient of H_2O_2 as 43.6 M-1cm-1 (one unit was the amount of enzyme that utilized 1 µmol of H2O2/ min/mg protein).

f. Lipid Peroxidation (LPO) Assay

Lipid peroxide content (LPO) was estimated by the thiobarbituric acid reactive substances (TBARS) test of Ohkawa et al. (1979)[15]. Tissue homogenate (0.1 ml) was mixed with 0.1 ml of 8.1% (w/v) sodium dodecylsulphate, 2 ml of 0.4% thiobarbituric acid in 20% acetic acid (pH 3.5) and 0.1ml distilled water. Each tube was tightly capped and heated in a heating block at 95 °C for 1h. After cooling the tubes with tape water, 1.5 ml of n-butanol-pyridine (15:1, v/v) was added and shaken vigorously for 10 min. The tubes were then centrifuged at 2500 rpm for 10 minutes at room temperature and the absorbance of the supernatant fraction was measured at 532 nm. The levels of LPO were expressed as nmol/mg of protein of the tissue homogenate against 1, 1, 3, 3-tetraethoxypropane as standard.
Total protein was estimated by the method of Lowry *et al.* (1951)[16].

g. Surgery for Unilateral Common Carotid Artery Occlusion to Produce Hypoxia-Induced Oxidative Stress

In parallel set of experiments, hypoxia was induced by unilateral occlusion of the common carotid arteries of both the *S. cumini* seed extract-pretreated and control rats. Under pentobarbital anesthesia (0.2 ml for induction, 0.1ml for maintenance), a midline neck incision was made and the left common carotid artery was occluded with surgical thread, the incision was closed and left for 4h on a heating pad (35°C to 37°C) for recovery. After 4h, the carotid arterial blocks were released and allowed further 1h in order to allow re-perfusion. Afterwards, the rats were killed and their hippocampus and cortex were separated from the skull for oxidative stress assay and/or perfused with saline followed by 10% formaldehyde for brain histology.

h. Histology

The brains were paraffin processed and excised for coronal blocks from the cortex and hippocampus tissue. Sections with a thickness of 10 m were cut and stained with hematoxylin and eosin for examining the overall morphology (Leica CM1850, Leica Microsystems GmbH, Wetzlar, Germany). Sections were mounted on gelatin–chrome–alum coated slides. The microscopic observation was done by fluorescent microscope normal spectra (Nikon eclipse E200) in 100X and 10X. The picture was taken by digital camera attached to it.

i. Total Polyphenol Contents and In *Vitro* Free Radical Scavenging Activity of *S. cumini* Seed Extract

To estimate the polyphenolic content by modified Folin- Ciocalteu's method[17] gallic acid was used as an analytical standard, as described previously[11], but with slight modification. Briefly, the calibration curve was generated using different concentrations of gallic acid (0 - 0.2 mol) in the reaction mixture of Folin-Ciocalteu's phenol (FCP) reagent in total volume 200 l in microwell plate. These solutions were incubated at room temperature for 40 min. The absorbance was recorded at 710 nm using ELISA plate reader.

Antioxdative power of the *S. cumini* seed extract was analyzed by its DPPH radical scavenging activity, as described previously[11]. The free radical scavenging effect was compared with that

of the quercetin and BHT using equimolar concentrations with equal reaction volumes. Antioxidant activity was expressed as the concentration of the seed extract (IC50) required to decrease the absorbance of the DPPH by 50%. To directly visualize the antioxidant activity of the extract, aliquots of 8 µL 4 mM DPPH solution were subjected to thin layer chromotagraphic (TLC) plate. After air dry, 8 µL of the extract and reference standard was re-applied onto the DPPH spots. After 30 min of incubation, the spots were photographed and analyzed by ImageJ. The references were equimolar concentrations of vitamin C and BHT.

3. Statistical Analysis

Results are expressed as mean ± SEM (standard error of means). Behavioral data were analyzed by a 2-factor (group and session) randomized block factorial ANOVA, and all other parameters were analyzed for intergroup differences by unpaired student's *t*-test. Correlation was determined by simple regression analysis. Statistical programs used were GB-STAT^{TM} 6.5.4 (Dynamic Microsystems, Inc., Silver Spring, MD, USA), and STATVIEW v4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA). A level of $P < 0.05$ was considered statistically significant.

4. Results

4.1 Body Weight and Food Intake

No significant differences were observed in body weight or food intake, measured after 12 wk, between control and *S. cumini* seed extract groups.

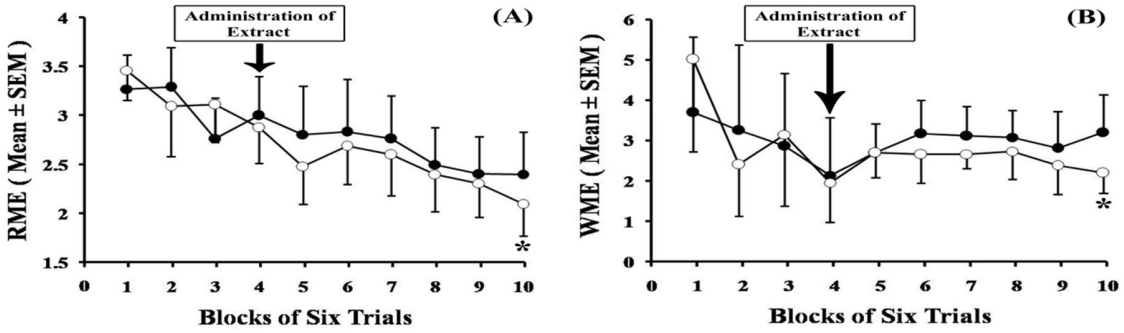

Fig 1. Effects of oral administration of S. *cumuni* extract on reference memory error (RME) (A) and working memory error (B). Data are the mean ± SEM for each block of six trials showing the number of reference memory error until the rat acquired all the rewards. Data were analyzed by randomized block two-way (block and group) ANOVA.

4.2 Eight- Arm Radial Maze Task

Figure 1A shows the effect of chronic administration of DHA on reference memory-related learning ability in aged rats. The score is expressed as mean number of reference memory errors (REMs) with data averaged over blocks of six days. A randomized block two-way ANOVA (block and group) was conducted based on the scores. The analysis revealed a significant main effect of blocks of trials ($F_{9,59} = 2.81$; $P<0.0085$) and group ($F_{1,9} = 7.68$; $P<0.022$) on the number of RME. The analysis also revealed a significant block-group interaction ($F_{9,59} = 2.86$; $P < 0.0075$). The effect of oral administration of *S. cuminii* on working memory related learning abilities of the old rats is shown in Figure 1B. The score is expressed as mean number of working memory errors (WMEs) with data averaged over blocks of six days. A randomized block two-way ANOVA (block and group) was conducted based on the scores. The analysis revealed a significant main effect of blocks of trials ($F_{9,59} = 5.59$; $P < 0.0001$)

on the number of WME but not of group ($F_{1,9}$ = 5.57; P<0.043). The analysis also reveals a significant block-group interaction ($F_{9,59}$ = 2.11; P < 0.048).

4.3 Brain Lipid Peroxidation

The levels of LPO significantly decreased both in the cerebral cortex and hippocampus of the *S. cumini* seed extract-administered rats (Table 1), as compared to those of the control rats.

Table 1. Effects of oral administration of *S. cuminii* extract on the levels of LPO in cerebral cortex and hippocampus

	Lipid Peroxidation (nmol/mg of protein)	
	Cerebral cortex	**Hippocampus**
Control	3.10 ± 0.10 [a]	2.58 ± 0.20 [a]
S. cumini	1.60 ± 0.35 [b]	1.55 ± 0.10 [b]

Data are the mean ± SEM, n = 5. Values in the same column that do not share common superscripts are significantly different at P < 0.05. Data were analyzed by unpaired student's *t*-test.

4.4 Enzyme Activity of Cerebral Cortex

Acetylcholine esterase activity of cerebral cortex of *S. cumini* seed extract-administered rats increased significantly, as compared to those of the control rats (Fig 2A). Catalase activity also significantly increased in the *S. cumini* seed extract-administered rats as a result of oral administration of *S. cumini* extract (Fig 2B).

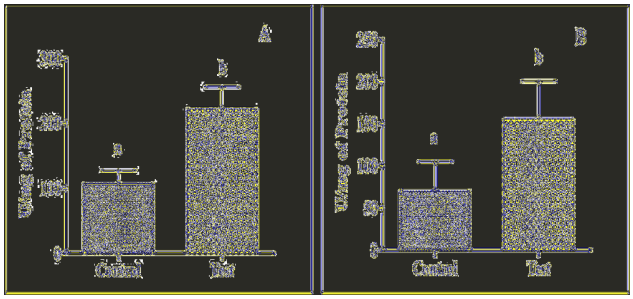

Fig 2. Effects of oral administration of *S. cumini* seed extract on cerebral cortex acetylcholine esterase (A) and catalase (B) activities. Results are mean ± SEM for six rats each with duplicate determinations. Bars with different alphabets are significantly different at P < 0.05.

4.5 Correlation Between Learning Ability Vs. The Corticohippocampal Levels of LPO

Regression analysis showed a significant positive relationship between the corticohippocampal levels of LPO and mean number of RMEs in the final block (Fig 3). Regression analysis also revealed significant negative relationship between acetylcholine esterase activities in the cerebral cortex and mean number of RMEs in the final block (r = - 0.63; P < 0.05).

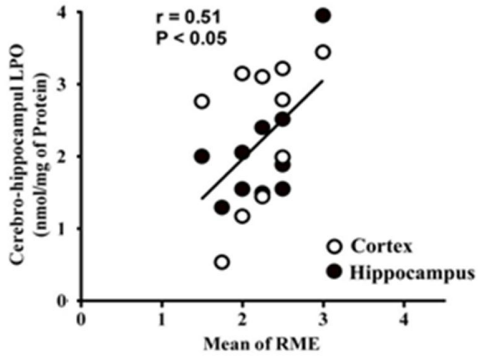

Fig 3. Correlation between learning ability and the levels of lipid peroxide in cortex and hippocampus. Learning ability is expressed as the mean of reference memory error (RME) in the final block. Data were subjected to linear simple regression analysis.

4.6 Histopathology

In hypoxic rat brain cortex we found cell swelling characteristic in most of the cells and many cell membrane was found broken in contrast with those in the non-hypoxic control brain slice. Normal characteristic of the CA region of the hippocampus also was found damaged in the hypoxic rats. The pyramidal layer cell layer was scattered and showed high inflammation of that region due to the presence of many inflammatory cells. In the hypoxic group treated with *S. cumini* extract, the pyramidal cell layer of the hippocampus was visually normal and there was much less inflammation than that of the untreated control rats. Therefore, these results suggest that carotid artery occlusion caused hypoxia in the brain tissues and consequently oxidative stress-mediated brain cell damage. Moreover, the hypoxia-induced oxidative stress was evident by the increased levels of LPO both in the cortex and hippocampus (data not shown). However, seed extracted-pretreated hypoxic rats treated with seed extract exhibited very few damaged cells and more over cells with less-swelling characteristics. Most of the cells retained their normal cellular structure, thus demonstrating that an extract-mediated protective effects against cellular damages in the corticohippocampal brain tissues.

4.7 Antioxidant Properties of *S. cumini* Extract

Since fruits grown in different seasons vary in its antioxidant capacity, we re-determined the total polyphenol contents of *S. cumini* seed extract. This method revealed that *S. cumini* seed extract contained ~11 µmol GAE/100 mg of dry powder, which was slightly higher than that our previous result (~ 8 µmol GAE/100 mg of dry powder). The result is consistent with the seasonal variation of the antioxidants in the seed powder of *S. cumini* fruits. Accordingly, the IC_{50} values were: 9.0, ~ 200 and ~ 7.0 µM GAE, respectively, for quercetin, BHT and *S. cumini* seed extract. The results were also consistent with the dose dependent disappearance of the DPPH's purple color spots of the thin layer chromatographic plate in the presence of *S. cumini* seed extract (Fig. 1B).

5. Discussion

The present study demonstrates that the oral administration of S. *cumini* seed extract improves radial arm performance in old rats concomitantly with the amelioration of the levels of LPO in the cerebral cortex and hippocampus. Radial maze behavior allows the simultaneous measurement of reference memory and working memory without any harmful effects on the rats while most widely used to study the spatial memory performance[12].

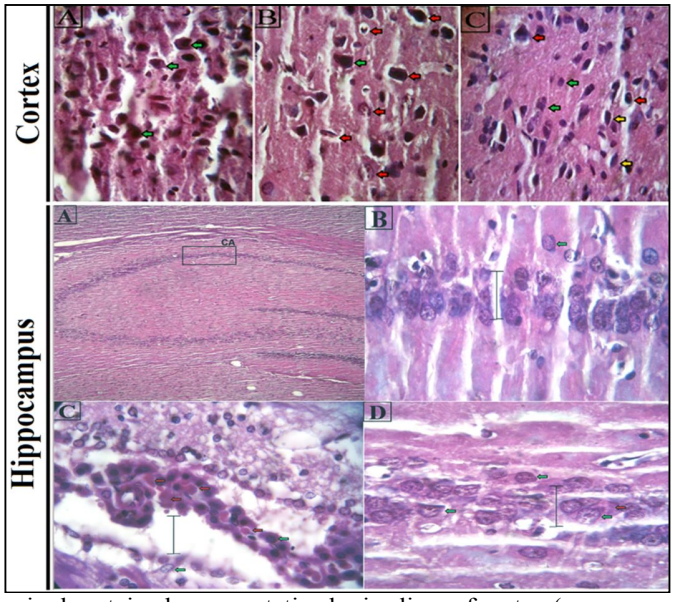

Fig 4. Hematoxylene and eosin dye stained representative brain slices of cortex (upper panel) and hippocampus (lower panel). **Cortex**: Normal (A), hypoxic (B) and hypoxic treated with seed extract (C). **Hippocampus**: Normal (A, B), hypoxic (C) and hypoxic rats treated with seed extract (D). Here, green arrows showed normal cell with normal physiological characteristics, red arrows showed cell swelling and cell rupture, yellow arrows showed cells with less damage showing improvement in characteristics. Picture (A) (B) (C) was taken at 100×magnification.

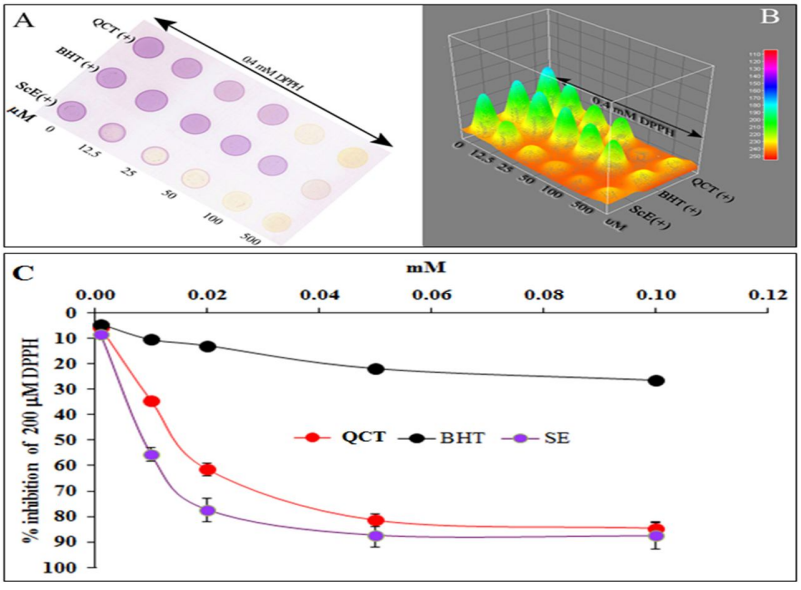

Fig 4. **A**: DPPH staining of TLC plate (A) in the absence or presence of quercetin (QCT), butylated hydroxyl tolune (BHT) and *S. cumini* seed extract (SE).The scavenging effects were dose dependent. **B**: The color of the DPPH spot was 3-D digitized by using ImageJ analyzer. **C**: Scavenging of stable DPPH free radicals by varying concentrations of *S. cumini* seed extract (SE), QCT and BHT. Each symbol represents the mean ± SEM of triplicate determinations. Data were subjected to nonlinear regression analysis with the hyperbola) equiation [$Y = B_{max}*X=/(K_i + X]$, where B_{max} is the maximal inhibition and K_i is the concentrations required to reach half maximal inhibition (IC50).

Prior to oral administration of S. *cumini* seed extract, the rats did not show any significant changes in the RME and WME scores between the control and the test rat groups. However, administration of S. *cumini* seed extract significantly decreased the numbers of RME and WME scores, thus demonstrating the improvement of spatial memory related-learning ability in SE-administered old rats (Fig 4). There was a significant positive correlation between the cerebrohippocampal levels of LPO and RME scores.

Hippocampus is considered as the key structure for the memory formation[18]. Furthermore, impairment of cognitive performance in older rats relates with increases of oxidative stress[19]. It is thus speculated that the improvement of memory-related learning ability was due to the ameliorating effect of seed extract on the oxidative stress, i.e. lipid peroxide. We previously demonstrated that the production of ROS exceeds the capacity of the cellular antioxidant defense system during aging[20]. Excessive ROS causes oxidative stress that leads to cellular damage and subsequent cell death mainly by apoptosis in neurodegeneration because the ROS oxidize vital cellular components such as lipids, proteins, and DNA[21,22]. In the present study, the administration of SE significantly decreased oxidative stress on cerebral cortex and hippocampus, as indicated by the significant reductions in the levels of LPO in these brain regions of the S. *cumini* seed extract-fed rats than those of the control rats. Regarding this, we also observed a significant augmentation of the cortical catalase activity in the test group rats than that of the control rats. Catalase activity of cerebral cortex is known to undergo an age-dependent decrement that may make polyunsaturated fatty acids (PUFA)-rich brain more susceptible to oxidative damage by free radicals in aging[1,23]. Malondialdehyde is a major product of free radical attack to membrane polyunsaturated fatty acids and it is, probably, the most widely used biomarker of lipid peroxidation[24]. Thus, the level of LPO rises as aging marker. The reductions in the levels of LPO in cerebral cortex and hippocampus of the S. *cumini* seed extract-administered rats were, thus, at least partially, attributable to the increased catalase activity.

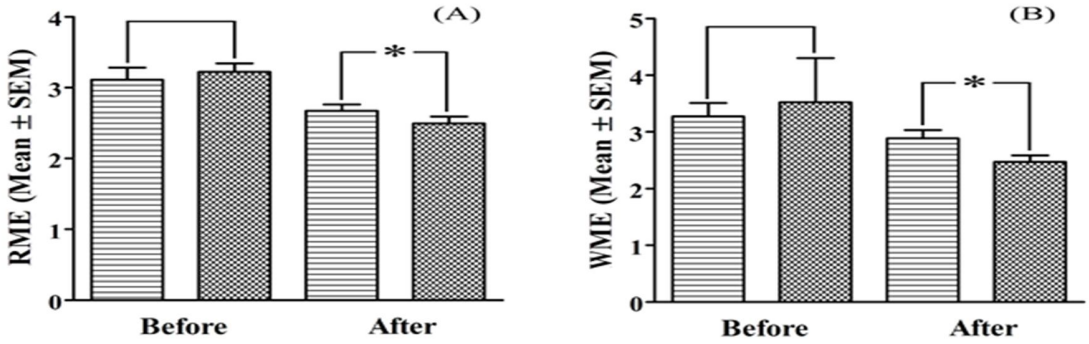

Fig 4. Effect of *S. cumini* seed extracts on of RME (A) score and WME (B) before and after oral administration of SE. Data are the mean ± SEM, n = 5. Bars with different notions are significantly different at P<0.05. Data were analyzed by student's *t*-test.

Aging is associated with a decline in cognitive function that can, in part, be explained by neurotransmission dysfunction that leads to degeneration of the cholinergic neurons in the hippocampus and cortex[25]. Hippocampus is crucially involved in spatial memory while the cortex is necessary for working memory and executive function[17,26]. In the present study, the

activity level of acetylcholine esterase enzyme significantly increased in the seed extract-administered rats than that of the control rats.

Aging is associated with changes in term of both morphological atrophy and reduction of the number of synapses that could affect the mechanism of plasticity[17,27,28]. Increased level of acetylcholine esterase activity passively indicates increased synthesis and/or release of acetylcholine. A rise in the acetylcholine esterase activity corresponds to modulation of the dendritic branching pattern or dendritic aborization and thus functionally reflects plasticity related synaptogenesis[29,30,31]. It was indirectly speculated that seed extract-induced amelioration in the cognitive function was either due to increased or restoration of age-related decline of the cholinergic system or the slowdown of the cholinergic degeneration. Long lasting change in synaptic efficacy (e.g. long-term potentiating; LTP) is the cellular basis of the memory[32]. Age-related alterations in hippocampal synaptic efficacy correlate with impairments in hippocampal-dependent learning and memory tasks[33]. Declining of spatial memory in aged rats, such as RME in the present study, is due to a decline of the synaptic efficacy of the hippocampus[34].

6. Conclusion

Finally, the results of the present investigation indicate that the memory enhancement occurs in the *S. cumini*-administered rats. Antioxidants such as vitamin E, vitamin C have are amply reported to improve the cognitive impairments of Alzheimer's disease model rats. Accordingly, we have been testing whether the oral administration of S. *cumini* seed extract to the amyloid β peptide-infused Alzheimer's disease model rats ameliorate the cognitive memory. However, further investigations are required to evaluate the mechanism(s), such as, what component(s) of S. *cumini* seed extract provide the exact beneficial effects on the learning-related memory of the rats.

7. Acknowledgement

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Extraction and Identification of Compound Derived from *Ipomoea palmata* Through Various Spectroscopic Techniques

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Extraction and separation of constituents from *Ipomoea palmata* have carried out adopting various chemical routes. Extracted and separated compounds have been identified by various spectroscopic techniques such as UV, and ^{1}HNMR.Thease techniques revel that that two compound can be as 4-hydroxy-2-(3, 7- dimethyl-octa, 2-6, diphanyle) 6-methoxy acetophenones and 4,6-dihydroxy, -2-0-(4 hydroxy butyl) acetophonone, whose melting points are 225^{0}C and 280^{0}C respectively. Elemental analysis and molecular weight determination (M^+ 302) of compound –I (M.P. 225^{0}C) have established that compound has molecular formula as $C_{19}H_{26}O_3$ while compound II (MP. 280^{0}C) depict its molecular formula as $C_{12}H_{16}O_5$. These compound have potential application in various industries like medicine, beverages etc.

Keyword: *Ipomoea palmata*, UV-Vis Spectroscopy, NMR, Mass Spectra, Molecular Weight.

1. Introduction

Ipomoea palmata belongs to a *Ipomoea cairica* (convoluulace) family. It is a climbing herb and is found abundantly in tropical and subtropical region. It has many common name and is also knows as railroad creeper[1]. This vining perennial has palmate leaves and large, showy white to lavender flowers. Each fruit matures at about 1 cm across and contain hairy seeds .Its native range is uncertain , through it is believed to originate from a rather wide area, ranging from Cape Verde to the Arabian Peninsula , including northern Africa. Because of human disperser, it occurs today on most continents as an introduction species and is sometimes a noxious weed . It is a major problem along the coast of New South Wales. In the united State it occurs in Hawaii, California, all the gulf coast states, as well as Arkansas and Missouri[2]. Some plant nurseries sell this plat as an ornamental. It can grow as a separate plant if snapped during attempted removal process[3].

The genus ipomoea has 400 species all over the world from ipomoea palmata forsks or ipomoea caircia L. grow abundantly in Egypt. Ipomoea palmate is used in treatment of various disease[4]. The major bioactive constituents previously isolated from the genus ipomoea were lipoidal matters[5] and Phenolic compounds[6]. The genius ipomoea has been reported to have many biological activities. Pongprayoon et.al[7]. showed a significant analysis effect attributed to the petroleum ether, chloroform and ethanol extract

of ipomoea palmate in albino rats. The structure of compounds have been established although spectroscopic techniques such as NMR, UV etc. These two compounds have been reported for the first time from this plant.

2. Experimental

The air dried and grounded seed (1 Kg) of Ipomoea Palmata were first defatted by extraction with petroleum ether. The defatted plant – material was then extracted with ethanol and concentrated. The extract was then separated into water soluble and insoluble fractions.

Water soluble portion was loaded over flash column and then eluted with different solvents of increasing polarity. Characterization has been carried out with the help of NMR of Bruker 400 MHz FT-NMR and UV spectrophotometer of Varian UV Visible Spectrophotometer.

3. Elution of column

1. Elution with chloroform : Methanol (8:2, v/v) yielded compound I (900 mg)
2. Elution with chloroform: Methanol (6:14, v/v) yielded compound II (100 mg)

Thus two different compounds were isolated from the seeds of Ipomoea Palmata. The process flow charts are as follows:

Fig 1: Flow Diagram of Extraction and Purification of Compounds from Ipomoea Palmata

The seeds of Ipomoea Palmata were collected from F.R.I. Dehradun (a herbarium specimen of the plant on file in Botanical survey of India) and was dried in air circulated oven at 80^0 C and crushed in mixer at 500 rpm. The air dried and crushed seeds of Ipomoea Palmata (1Kg) were extracted with petroleum ether ($60\text{-}80^0$C) in soxhlet extractor till the appearance of colour. The defatted seeds were subsequently extracted under reflux in soxhlet with ethanol. The extract was concentrated under reduced pressure. Now concentrate (100 ml) was poured into ice cold

water and kept for overnight. Then it was filtered and water insoluble and soluble fractions were obtained.

4. Analysis of Water Insoluble Fractions:

Water insoluble portion (40 gm) was then loaded over flash column of silica gel G. then it was eluted with hexane, benzene, chloroform, ethyl acetate, ethanol and methanol and different ratio of solvents in increasing polarity. From the chloroform: Methanol (8:2, v/v), a red coloured compound, m.p. 225^{0}C was separated and marked as I, it was recrystallised from ethanol. The chloroform: Methanol (6:4, v/v) elute, on concentration gave a red coloured compound, m.p. 280^{0}C. It was labeled as II and recrystallised from ethanol. Both these compounds isolated are new and not reported earlier in literatures.

5. Acetylation of Compounds

The compound (40 mg) was dissolved in acetic anhydride (5ml) and pyridine (2ml). It was left for 48 hours at room temperature. The reaction mixture was poured into ice cold distilled water and dried. The residue obtained was crystallized from methanol and was analyzed for one acetyl group.

6. Physical Data

A: Compound I

Compound was crystallized from methanol as red granular compound.

Solubility	:	Soluble in Chloroform, ethylacetate, acetone, methanol and ethanol.	
M.P.	:	225 ºC	
Rf	:	0.71 (Ethyl acetate)	
		0.72 (Methanol: Chloroform) (1: 1, v/v)	
		Found	Calculated for $C_{19}H_{26}O_3$
Elemental Analysis	:	C – 75.49% H – 8.60%	C – 75.50% H – 8.62%

nm : 220, 285; +$AlCl_3$ / HCl: 220, 287; +NaOAc; 230, 29.

cm^{-1} : 3200, 2
900, 2825, 1660, 1600, 1530, 1450,
1380, 1105, 115, 880, 825, 780.

^{1H}NMR [90MHz, $CDCl_3$] : 5.70 (d, 2.4Hz), 5.85 (1H, d, J = 2.4 Hz, H-5), 2.45 (s, 3H, H-8), 3.30 (d, 2H, J = 6.2 Hz, H-1), 5.10 – 5.28 (brm, 2H, H – 2, 6'), 2.05 (brm, 4H, H-4' & H-5'), 1.60 (s, 3H), 165 (s, 3H), 1.79 (s, 3H), 7.50 (Ph-OH), 3.59 (s, 3H) ppm.

Mass Spectra, m/z (%) : 302 [M^+], 287, 271, 233, 210, 165, 104, 91, 76.

Spectral data after Acetylation

		Found	Calculated for $C_{21}H_{28}O_4$
Elemental Analysis	:	C = 73% H = 8%	C = 73.25% H = 8.13%

cm^{-1} : 1760, 1260 (Acetate), 1655 (C=0), 1600, 1520 (aromatic), 1180, 1010, 880, 820 cm^{-1}

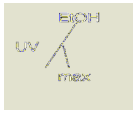
^{1H}NMR [90 MHz, $CDCl_3$] : δ – 6.02 (d, 1H, J = 2.5 Hz, H – 3), 6.20 (d, 1H, J = 2.5 Hz, H – 5), 2.40 (s, 3H), 3.31 (d, 2H, J = 6.2 Hz, H-1), 5.10 – 5.28 (brm, 2H, H – 2', H - 6'), 2.06 (brm, 4H, H-4' & H-5'), 1.62 (s, 3H), 1.65 (s, 3H), 1.80 (s, 3H), 3.16 (s, 3H), 2.16 (s, 3H, 1XOAC) ppm.

Mass Spectra, m/z (%) : 344 [M^+]

B: Compound II

Compound was crystallized from methanol as red granular compound.

Solubility	:	Chloroform, DMSO, methanol.	
M.P.	:	280ºC	
Rf	:	0.25 Meo, $CHCl_3$, (1:1, v/v)	
		0.72 MeOH : $CHCl_3$ (1 : 1, v/v)	
		Found	Calculated for $C_{12}H_{16}O_5$
Elemental Analysis	:	C – 60%	C – 60%
		H – 6.7%	H – 6.66%



nm : 230, 290, +$AlCl_3$ / HCl : 251, 285, +NaOAc- 242, 287.

cm^{-1} : 3380, 3200, 2918, 2850, 1650, 1540, 1450, 885, 825, 780.

^{1}HNMR [90MHz, $CDCl_3$] : 5.65 (d, 1H, J = 2.5 Hz, H-3), 5.80 (d, 1H, J=2.5 Hz, H-5), 2.48 (s, 3H), 4.30 (t, 2H, H-1′),1.79(s, 4H, H-2′ and H-3′), 3.65 (dd, 1H, H-1′), 3.58 (dd, 1H, H-4′), 7.90 (ph-OH), 13.10 (1H, ph-OH), 2.62 (brt, J=6.2 Hz, 1H) ppm.

MS m/s : 240 [M^+]

Spectral Data after Acetylation

Elemental Analysis	:	C: 59.2%	C : 59%
		H: 6.01%	H : 6%

cm^{-1} : 1760, 1680, 1600, 1580, 1370, 880, 810

^{1}H NMR[90MHz, $CDCl_3$]: 6.00 (d, 1H, 2.5 Hz, H - 3), 6.31 (d, 1H,J = 2.5 Hz, H – 5), 2.35 (s, 3H), 4.31 (t, 2H, H-1'), 1.80 (s, 4H, H-2', H-3') 3.65 (dd, 1H, H-4') 3.59 (dd, 1H, H-4') 2.11 (s, 3H, - $OCOCH_3$), 211(s, 3H, - $OCOCH_3$), 2.02(s, 3H, - $OCHOCH_3$) ppm.

Maas Spectroscopy, m/z : 366 [M^+]

7. Results and Discussion

A. Compound I

The compound I, m.p. 225^{0}C, on elemental analysis and molecular weight determination (M^+300) established its molecular formula as $C_{19}H_{26}O_3$. The compound showed UV spectrum characteristic of acetophenone derivatives. Appearance of peaks at 3200 cm^{-1} in its IR spectrum showed the presence of hydroxyl group. The peak at 1660 cm^{-1} was found due to presence of α-β- unsaturated keto group.

On acetylation with acetic anhydride and pyridine, it formed monoacetato, showing the presence of one hydroxyl group in the molecule. The compound induced bathochromic shift of 10 nm in its UV spectrum, on addition of sodium acetate, to the ethanolic solution of compound and no shift with $AlCl_3$. This showed the presence of free hydroxyl group at para position with respect to keto group.

The ^{1}HNMR spectrum of the compound showed singlet at δ2.45 (3H) and δ3.59 (3H) ppm for CH_3 Co- and – OCH_3 protons respectively. ^{1}HNMR displayed signals at δ 1.60 (s, 3H), 1.65 (s, 3H), 1.79 (s, 3H), 2.05 (br m4h) 3.30 (d, 2H, J=6.2 Hz), 5.10 – 5.28 (2H, br) ppm for the presence of 3, 7 dimethyl octadienyl chain[3] (see compound II). ^{1}HNMR also gave two signals for aromatic protons of acetophenone ring at δ 5.70 (d, ^{1}H, J=2.4 Hz) and 5.85 (d, ^{1}H, J = 2.4 Hz). These protons are meta, coupled as indicated by J value. NMR values are shown in compound II discription.

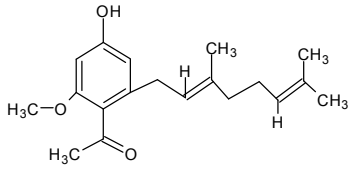
Thus, ^{1}HNMR of the compound is consistent with p-hydroxy acetophenone having methoxy and dimethyl octa dienyl group as substituents.

As two aromatic protons are meta coupled so the two substituents either must be present at position C-2 and C-6 or position C-3 and C-5.

The protons at position C-2 and C-6 are deshielded (ortho to carbonyl group), so they should absorb at higher δ value (δ 7.57 and 7.73 ppm) and proton at C-3 and C-5 are shielded by –OH group, so they should absorb at lower δ value (δ 5.70 and 5.85 ppm) so the substituent must be present at position C-2 and C-6.

Position of substituent at these positions was further confirmed by the ^{1}HNMR data of acetate (J') of compound, which suggested that these two aromatic protons must be present at position C-3 and C-5 because aromatic protons showed a considerable down field of phenolic–OH group.

Compound I was thus established as 4-hydroxy-2-[3', 7'-dimethylocta-2',6' diphanyle] 6-methoxy acetophenone.



The compound is new and not reported earlier from any other plant source, but some natural compound having this type of side chain are reported earlier [8-10].

B. Compound II

The compound IInd, m.p. 280ºC was analyzed for $C_{12}H_{16}O_5$. The compound gave characteristic UV spectrum of acetophenones. It showed IR absorption corresponding to α, β – unsaturated and hydroxyl group (1650, 3200, and 3380 cm^{-1}).

The compound induced shift in UV spectrum on addition of NaOAc (12 nm) as well as on addition of $AlCl_3$ / HCl (21 nm), showing the presence of free hydroxyl group at ortho and para position with respect to – COMe group (see compound III). Acetylation of compound IInd yielded triacetate (IIIrd) showing the presence of three hydroxyl groups.

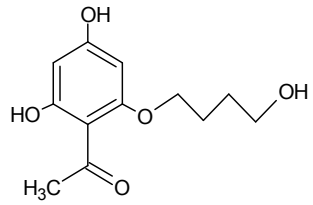
NMR values of IInd and IIIrd compounds showed the presence of –O-hydroxy-butyl side chain. The structure of hydroxyl butyl side chain was established unambiguously by ^{1}H NMR. There are two –OCH_2 groups present in the side chain. One shows up as a triplet of 2H and the other is represented by two diostereotopic protons with different chemical shifts. The letter must be associated with –CH_2OH group, since in very

pure NMR a direct coupling of the two diastereomeric oxymethylene protons with the hydroxyl protons is observed. The other $–OCH_2$ group was therefore directly linked to acetophenone ring with aromatic ether linkage.

The broad singlet for $–CH_2-CH_2-$ group was observed at δ 1.79 (4H, Br, S) so sequence in side chain is given as Ar – OCH_2 CH_2 CH_2 – CH_2 OH.

The ^{1}H NMR also showed signals for two phenolic hydroxyl group, so the ^{1}H NMR of compound was consistent with a tetra substituted aromatic ring with $–COCH_3$ group, one –O-hydroxyl butyl residue and two phenolic hydroxyl groups as substituents.
^{1}H NMR also showed presence of two aromatic protons at δ 5.65 (d, J = 2.5 Hz) and 5.80 (d, J = 2.5 Hz) ppm. The protons are meta coupled as indicated by J value. So -O- hydroxyl butyl residue must be present at meta position to hydroxyl group. Thus substituents must be present at C-2 and C-6.
Position of substituents at these positions was further confirmed by ^{1}H NMR. As aromatic protons at C-3 and C-5 are shielded by –OH group so they should absorb at lower value (δ 5.65 and 5.80).

Thus compound was established as 4, 6 dihydroxy, 2-O-(4' hydroxy butyl) acetophenone.



The compound is not reported earlier from any other plant source.

8. Conclusion

Extracted and separated compounds have been identified as 4-hydroxy-2-(3, 7- dimethyl-octa, 2-6, diphanyle) 6-methoxy acetophenones and 4, 6-dihydroxy, -2-0-(4 hydroxy butyl) acetophonone, whose melting points are 225^{0}C and 280^{0}C respectively. Elemental analysis and molecular weight determination have established that compound I has molecular formula as C_{19} $H_{26}O_3$ while compound II has molecular formula as $C_{12}H_{16}O_5$.

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Comparative Growth Inhibitory Assay of the Methanol Extract of the Leaf and Seed of *Persea americana* Mill (Lauraceae)

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Persea americana is one of the recipes used by traditional healers in parts of Edo State of Nigeria for the treatment of cancer. This claim was investigated using predictive bench-top assay method of germinating seeds radicle of guinea corn (*Sorghum bicolor*). The powdered leaves and seeds were extracted by Soxhlet using methanol. The growth inhibitory effect was examined using radicle lengths of germinating seeds of *Sorghum bicolor* at 1-30 mg/ml. The methanol extract of the leaf of *Persea americana* was subjected to solvent partitioning. Phytochemical screening showed the presence of saponins, flavonoids, tannins and cardiac glycosides with no traces of anthracene derivatives. The aqueous fraction was more active than the chloroform fraction and completely inhibited the germination of the seeds at 20mg/ml. The results suggest the probable use of the plant in preparing recipes for tumor-related ailments. However, using appropriate human cell lines will further justify this claim

Keyword: *Persea americana*, Growth Inhibitory, Phytochemical, *Sorghum bicolor*

1. Introduction

The use of medicinal plants as sources of drugs in treating various forms of cancer diseases has attracted the attention of scientist worldwide. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. This is due to the fact that they are relatively cheap, safe and easily available compare to orthodox medicines. Since the beginning of life, man has depended on plants for food, drinks, shelter, clothing, equipment, dental care and medicine[1]. In fact, the early men depended on plants, herbs for treatment of various diseases before the introduction of orthodox medicine.

Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations[2]. Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack[3].

According to the WHO, 80% of the world's population, primarily those of developing countries rely on plant derived medicines for their healthcare[4]. More than a third of people in the developing countries have no access to essential medicines. Across the cultures, traditional medicine is mainly derived from plant. In deed about 25% of the modern drugs are derived from plants first used traditionally. The widespread use

of herbal medicine is mainly due to its ready availability and affordability.

The search for antitumor agents of natural origin continues to increase due to the non-selective pattern in the activities of the conventional drugs coupled with their high costs and scarcity, particularly in rural areas. Research into antitumor agents usually involves a series of complex procedures that sometimes produces non-encouraging results after much material and time has been expended. In addition to these, paucity of research funds has made the development and acceptance of simple bench-top assays imperative[5].

Ethnodicinal uses of plant are usually obtained from the direct users of the plants in the society most especially from traditional medical practioners. *Persea americana* have previously been reported to be used among the Owan people in Edo State in Nigeria in treating tumour-related ailments[6]. In local Nigerian languages, the plant is known as *Pia*-Yoruba, *Orumwu*-Bini, ube oyibo, Efik-*eban mbakara*[7] and *ewo ebo in* Owan speaking tribes of Edo State.

The plant *Persea americana* is a multipurpose used plant which has diverse applications in ethnomedicines ranging from treatment of diarrhoea, dysentery, toothache, intestinal parasites to the area of skin treatment and beautification[8]. It has also been reported to be used as an emmenagogue (hot water extract, decoction), for asthma (bark), used for cough, fever, kidney and liver troubles, for diabetes, as food, for skin blemishes, as an abortifacient, for female disorders among others[9].

2. Materials and Methods

The leaves and seeds of *Persea americana* were collected in March 2009 at a playground at Okhoro, a suburb in Benin City. The identity of the plant was confirmed by Dr. Shasanya Olufemi, the Plant Taxonomist at the Forest Research Institute of Nigeria (FRIN), Ibadan. A herbarium specimen number FHI 109574 was deposited at the institute for reference. The plant material was air dried in the laboratory for 5 days at room temperature followed by oven drying at 40° C followed by grinding to powder form using an electric mill. The powdered sample was kept in an air tight container until required.

2.1 Extraction of the Plant Material

1kg of the powdered leaves and seeds were extracted by Soxhlet extractor using methanol in 2.5 L of methanol. The liquid extracts obtained were concentrated using a rotary evaporator maintained at 40°C and were kept in the refrigerator.

2.2 Phytochemical Screening of the Plant Material

This was carried out to test for the presence or otherwise of tannins, flavonoids, saponins, alkaloids, steroidal glycosides and anthracene derivatives using standard methods[10,11].

2.3 Source and Preparation of the Guinea Corn *Sorghum bicolor*

Seeds of *Sorghum bicolor* (Guinea corn) were purchased from Oba market in Benin City. A simple viability test was carried out by placing a handful of the seeds in distilled water. The viability of the seeds was determined by their ability to remain submerged in water. The viable seeds were washed with 95% ethanol for sterilization for 1 minute and were finally rinsed with distilled water and dried for use.

2.4 Determination of The Growth Inhibitory Effects of the Extracts on Guinea Corn (*Sorghum bicolor*)

10 ml different concentrations of the leaf methanol extract (1-30 mg/ml) containing 5% DMSO was poured into the petri-dish of about 9cm wide containing filter (Whatman No.1) underlay with cotton wool, after which twenty of the sterilized seeds were spread on each of the petri-dishes. The petri-dishes were incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured at 24, 48, 72 and 96 hours. The control seeds were treated with 10ml distilled water containing 5% DMSO (*13*).The experiment was carried out in triplicates for all concentrations and controls while the radicle lengths were measured to the nearest millimetre.

The procedure was repeated for the seed methanol extract.

2.5 Partitioning of the Methanol Extract of the Leaf of *Persea americana*

97g of the crude methanol extract of the leaves were re-dissolved in methanol-water (1:1) and partitioned exhaustively with chloroform (200 ml ×4) volumes in a separating funnel. The aqueous and the chloroform fractions were concentrated to dryness on a rotary evaporator and their respective yields noted. The growth inhibitory effects of the two fractions were separately carried out as earlier described above.

3. Statistical Analysis

All data were expressed as mean ± SEM and one way Analysis of Variance Anova statistical test using Graph pad Instant R version 2.05 (UK) was used to test for significance. P< 0.05 was considered Significant.

4. Result

The 2kg of the powdered leaf and seeds of *Persea americana* were observed to yield **95.55** and 76.32 g of the methanol extracts corresponding to 4.78 and 3.82 % respectively.

Partioning of the methanol extract of the leaf gave 42.57g (2.13%) of the aqueous and 28.55g (1.43%) of the chloroform fraction respectively.

The leaf and seeds of *Persea americana* were observed to contain alkaloids, saponins, tannins, flavonoids, tannins and cardiac glycosides while anthracene derivatives were absent (Table 1).

Table 1: The results of the preliminary phytochemical screening of the methanol extracts of the leaves and seeds of *Persea americana.*

CONSTITUENTS	LEAF EXTRACT	SEED EXTRACT
Alkaloids	++	+
Anthraquinone	-	-
Cardiac glycosides	+++	+
Flavonoids	+++	+
Saponin	+++	++
Steroids	+	+
Tannins	+++	+
Terpenoids	+	+

- = not detect; + = low; ++ = moderate; and +++ = strong.

4.1 Results of the Extracts on Radicle Growth.

The results of the growth inhibitory effects of the extracts of the plant parts on guinea radicle length showed a concentration dependent reduction in length of the radicle that emerged from the guinea corn seeds. At 24 h, control seeds showed an average radicle length of 4.61±0.99 mm compared to average length of 0.65 ± 0.27 mm(86% inhibition) shown by the seeds pre-treated with 20mg/ml of the leaf extract. Almost complete inhibition of germination (100% inhibition) was observed in seeds pre-treated with 30 mg/ml of the extract. After 96 hour incubation period, the control seeds produced an average length of 34.72 7.37 mm whereas the seeds pre-treated with 20 and 30 mg/ml of the extract produced average lengths of 1.73 ± 0.67 and 1.38 ± 0.66 mm respectively (Fig. 1). These implied 95% and 97% reduction in length compared to the control seeds. These variations in length were found to be significant at P<0.01

Similarly for the seed extract, the control seed at 24 hr gave an average length of 3.87 ± 0.59 mm compared to 1.63 ± 0.29 mm (57.88 %

inhibition) and 0.67 ± 0.03 mm (82.69 % inhibition) showed by seeds pre-treated with 20 and 30 mg/ml of the seed extract. Also, at 96 hr an average length of 61.85 ± 7.83 mm was recorded for the control seeds while 8.62 ± 1.92 mm (86.06 % inhibition) and 6.25 ± 1.44 mm (89.89 % inhibition) as shown in Fig. 2. These variations in length were found to be significant at $P<0.01$

The aqueous and the chloroform fractions of the methanol extract of the leaf were also observed to suppress the growth of the radicle length with increase in concentrations with the aqueous fraction observed to be a little more effective than the chloroform fraction. At 24 hours, the average radicle length of the control seeds was 5.00 ± 0.77 mm while seeds pre-treated with 2, 5 and 10mg/ml of the aqueous fraction produced average lengths of 2.97 ± 0.31, 2.32 ± 0.51, and 0.9 ± 0.06 mm which implies 41, 54 and 87 % reduction in radicle length compare to the control seeds. Those pre-treated with 20 and 30 mg/ml concentrations produced no signs of germination. After 96 hours, the control had an average length of 47.07 ± 6.05 mm while seeds pre-treated with 2, 5 and 10mg/ml showed an average lengths of 16.52 ± 2.84mm (65 % inhibition) 10.85 ±1.67 mm (77 % inhibition) and 2.92 ± 0.52 mm (94 % inhibition) respectively. Throughout the period of incubation, 20 and 30mg/ml completely inhibited the germination of the seeds (Fig. 3). The variations in the growth inhibition were observed to be significant at $P<0.05$

The chloroform fraction also produced remarkable inhibition of the radicle growth but germination of seeds and hence radicle growth were not absolutely inhibited as seeds pre-treated with 20 and 30 mg/ml of the fraction produced 0.38 ± 0.08 and 0.15 ± 0.03 mm respectively compared to 54.97mm produced by the control after 96 h incubation period (Fig. 3).

5. Discussion

The medicinal value of any plant is measured by the extent to which it is able to remove or mitigate the harmful effects of diseases or organisms causing them. The therapeutic effect of such plant is also a direct function of the various constituents it contains naturally, which may be acting synergistically with one another. Close examination of the results of the phytochemical screening of the two morphological parts used showed that the various groups of phytochemical constituents such as reducing sugars, saponins, alkaloids, tannins, flavonoids, cardiac glycosides were more on the leaf but fairly distributed in the seeds.

The activity of the leaf extract was remarkably pronounced in the inhibition of the growth of radicles of *Sorghum bicolor* seeds. At 30 mg/ml, the extract produced an average length of 1.38 ± 0.66 mm at 96 hr of incubation period while the seeds produced 6.25 ± 1.44 mm. These observations which obviated the choice of the leaf extract for further work implied that the constituents responsible for the activities were mostly concentrated in the leaves. It could be that the constituents were biosynthesized in the leaves, after which some quantities are latter translocated to the other parts of the plants including the seeds.

The use of guinea corn seeds (*Sorghum bicolor*) was necessitated by the fact that meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions and the extent of proliferation is reflected in the increase in the length of the radicles produced in 96 hrs in the control seeds. Although any seeds such as cowpea (*Vigna unguiculata*), maize (*Zea mays*) and other seeds can be used, that of *S. bicolor* was found to be most convenient because of its relatively small size. The experiment can be carried out in plates or in small glass containers; hence the amount of solution required is small. Also, the availability is high and up to 90% can germinate within 24hours.

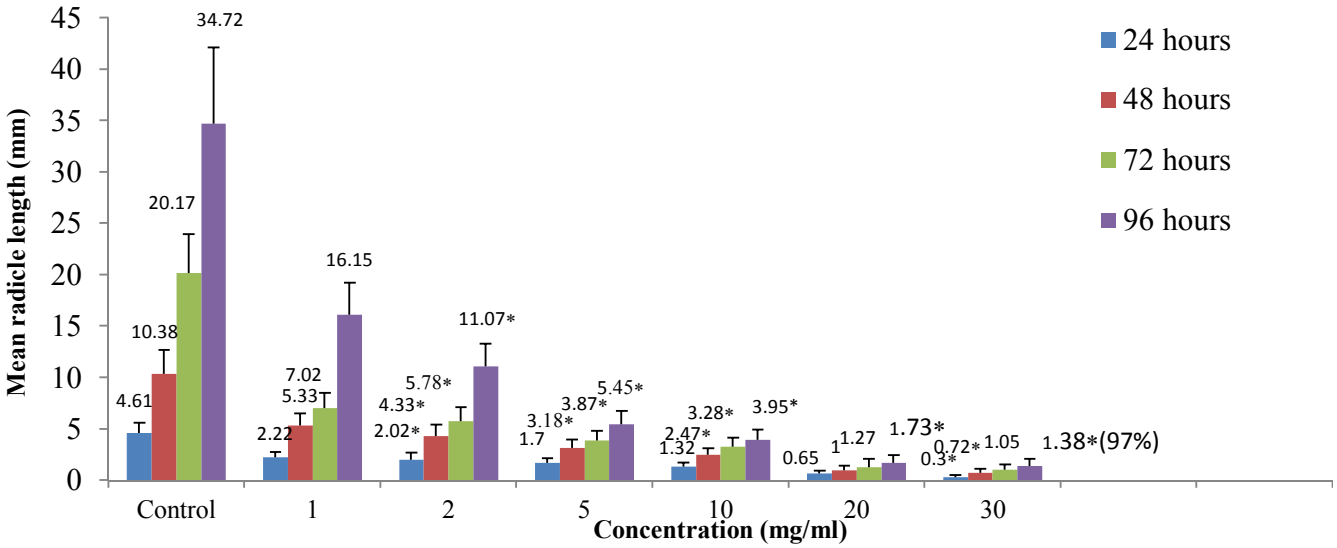

Fig. 1: The growth inhibitory effects of the methanol extract of the leaf of *P. americana* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20. ∗Significantly different from control. P<0.05

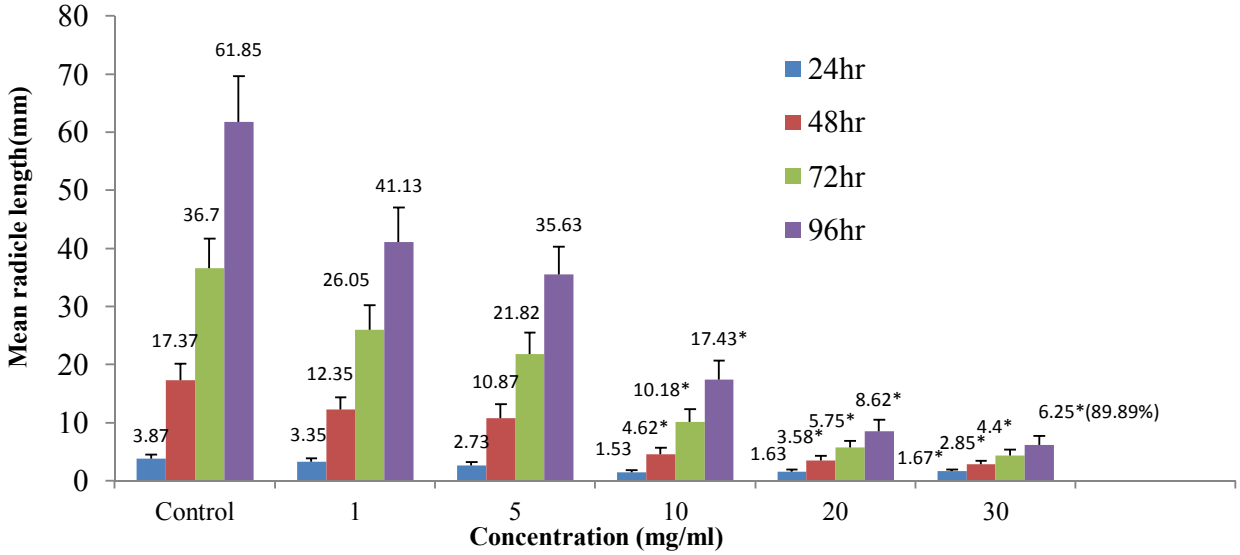

Fig. 2: The growth inhibitory effects of the methanol extract of the seeds of *P. americana* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.∗Significantly different from control. P<0.05

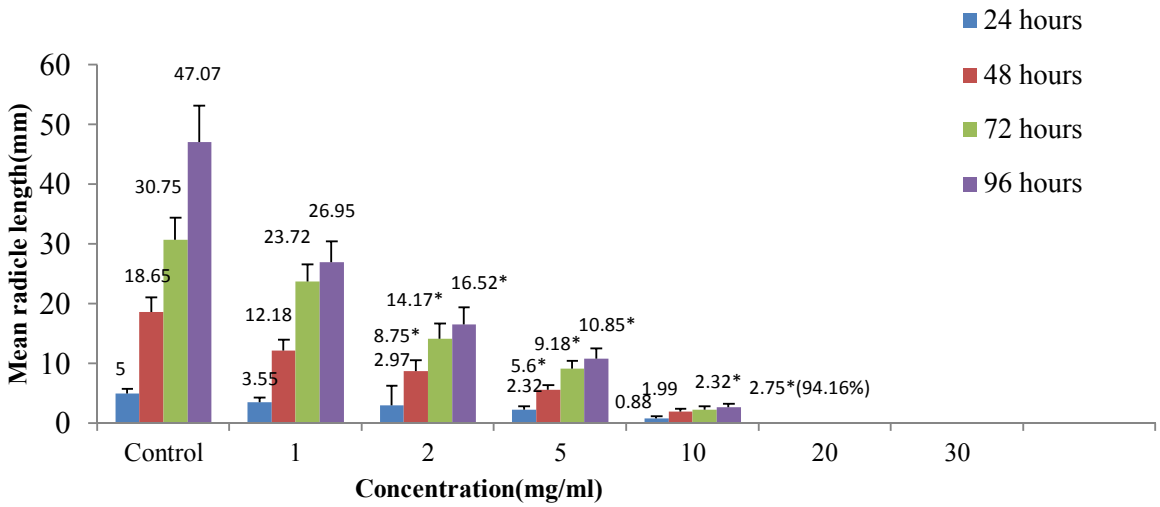

Fig 3:Inhibitory effects of the aqueous fraction on guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

*Significantly different from control.P<0.05

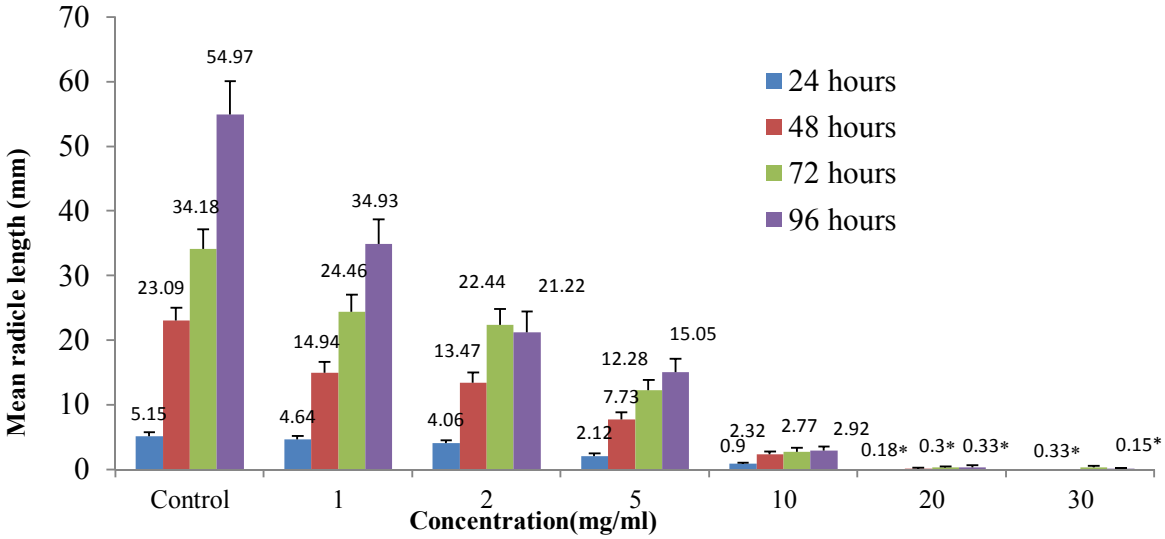

Fig 4: Inhibitory effects of the chloroform fraction on the growth of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

*Significantly different from control. P<0.05

As plants are known to contain many constituents of varying polarities, molecular mass and concentrations, using two appropriate immiscible solvents ensures the separation of the constituents based on their relative solubilities in the solvents. As stated earlier, the choice of methanol extract of the leaf for partitioning was based on the higher activities it exhibited over the seeds.

Partitioning of leaf extracts into chloroform and aqueous phases remarkably improved the growth inhibitory effects of the constituents. For instance, the aqueous fraction completely inhibited seed germination and subsequent growth of the guinea radicle at 20 mg/ml compared with the 30 mg/ml of the extract which produced 1.38 ±0.66 mm at the end of 96 h incubation period. For the aqueous fraction, it is possible that some of the constituents may have affected water and consequently osmotic potential thus preventing the development of turgor pressure in the seed, which has been considered as one of the key factors required for the initiation of radicle growth during seed germination[12]. This goes to show that the constituents responsible for this effect are polar in nature.

Comparing the activities of *Persea americana* with other plants like *Struchium sparganophora*, it was observed that the latter is more potent than the former. The methanol extract of *S. sparganophora* at 4mg/ml reduces the growth of radicle to 47.16 % compare to the control while the aqueous and chloroform fractions reduces the growth of radicle to 43.81 and 32.51% % respectively[13].

From this study, it can be inferred that *Persea americana* leaf may likely have effects on tumour-producing cells as claimed in ethnomedical uses of the plant among the Owan people of Edo State. However, further investigation using tumour cell lines *in vitro* or *in vivo* may be necessary to confirm the folkloric anti-tumour activity of *Persea americana.*

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Antidiabetic and Antihyperlipidemic Activity of *Cucurbita maxima* Duchense (Pumpkin) Seeds on Streptozotocin Induced Diabetic Rats

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The objective of the present study was to evaluate the antidiabetic and antihyperlipidemic effect of petroleum ether, ethyl acetate and alcohol extract of seeds *of Cucurbita maxima* for its purported use in diabetes. The antidiabetic and antihyperlipidemic activity of different extracts of *Cucurbita maxima* seeds was evaluated in wistar albino rats against streptozotocin (50 mg/kg i.p.) at dose of 200 mg/kg p.o. for 21 days. Glibenclamide (500µg/kg) was used as reference drug. Fasting blood glucose (FBG) levels were measured on day 0, 7, 14 and 21. It was found that blood glucose concentration was significantly ($P<0.05$) decreased compared to control. In addition, oral administration of *Cucurbita maxima* significantly ($P<0.05$) decreased serum total cholesterol, LDL, VLDL, triglycerides and at the same time markedly increased serum insulin and HDL-cholesterol levels. Administration of glibenclamide, a reference drug also produced a significant ($P<0.05$) reduction in blood glucose concentration in streptozotocin-induced diabetic rats. Thus, the results of this experimental study shows that *Cucurbita maxima* possess antidiabetic and antihyperlipidemic effect and is able to ameliorate the diabetic state and is a source of potent antidiabetic agent

Keyword: *Cucurbita maxima*, Glibenclamide, Streptozotocin, Antidiabetic Activity, Antihyperlipidemic Activity.

1. Introduction

Diabetes mellitus, a complex syndrome is characterized by the imbalance in blood glucose homeostasis leading to hyperglycemia (high blood glucose) and a series of secondary complications caused by an absolute or relative lack of insulin. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetes[1]. Diabetes induction causes increase in cholesterol, triglycerides, LDL and VLDL[2]. The levels of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease[3]. Besides drugs classically used for the treatment of diabetes (insulin, sulfonylureas, biguanides and thiazolidinediones) several species of plants have been described in the scientific and popular literature as having a hypoglycemic activity[4]. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown[5]. The present study investigate the effect of oral administration of petroleum ether, ethyl acetate and alcoholic extract of *Cucurbita maxima* on blood glucose and lipid profile in diabetic rats.

The pumpkin, *Cucurbita maxima* Duchense belongs to family cucurbutaceae. It is a trailing annual herb with somewhat prickly or hairy stem and axillary tendrils, leaves simple, alternate; flowers large, yellow, unisexual, solitary; fruits fleshy, round or oval, brown; seeds ovoid or oblong, compressed[6,7] . *Cucurbita maxima* is widely cultivated throughout India and in most warm regions of the world, for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable. This plant has been traditionally used in many countries such as India, China, Brazil, Yougoslavia and America as antidiabetic, antitumor, antihypertensive, anti-inflammatory, immuno-modulatory and antibacterial agents[6,8,9,10,11,12,13]. Popularity of pumpkin in various traditional system of medicine for several ailments focused the investigator's attention on this plant.

A daily supplement of pumpkin in fruit powder was found to reduce blood glucose levels significantly ($P<0.05$) in the 20 NIDDM diabetics[14]. Although yet more scientific validation of the hypoglycemic and hypolipidemic activity of the seeds needs to be established. Therefore, the present study was undertaken to evaluate the antidiabetic and antihyperlipidemic activity of seeds of *Cucurbita maxima* in streptozotocin-induced diabetic rats.

2. Material and Methods

2.1 Plant Material

The plant used in this study, *Cucurbita maxima* seeds were collected from local market of Jaipur and were identified and authentificated by the Department of Botany, University of Rajasthan, Jaipur (Rajasthan), India. A voucher specimen (No. RUBL-20941) were kept in the herbarium department for future reference.

2.2 Preparation of Extract

The seeds were cleaned well with water and dried in a shadow place. After complete drying, the seeds were powdered and were extracted by using soxhlet apparatus with petroleum ether, ethyl acetate and alcohol as solvents for extraction. Solvents elimination under reduced pressure afford a solid residue (% yield). The yield of petroleum ether, ethyl acetate and alcohol are 7.80%, 8.62% and 9.84% respectively. The dry residue of the crude extract obtained were stored at 4^0C for further use.

The pumpkin extracts were analyzed for the presence of phytochemicals[15,16]. The phytochemical screening gave positive tests for carbohydrates, flavonoids, tannins, phenolic and saponins.

2.3 Experimental Animals

Healthy wistar albino rats of either sex weighing 150-170g were used for this study. Before starting the experiment, the animals were acclimatized to the laboratory conditions for a period of 2 weeks at an ambient temperature (24±2 ºC) and relative humidity (40-60%). The light - dark cycle was followed. The animals were fed with standard laboratory diet and water *ad libitum*. The animals were fasted for overnight before the study but had free approach to water. All the experimental procedure and protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and all the experiments were carried out by following the guidelines of CPCSEA.

2.4 Drug Administration

Various extracts (Petroleum ether, Ethyl acetate, Alcohol) of *Cucurbita maxima* was suspended in distilled water and administered orally through ingastric tube at dose of 200 mg/kg body weight. The administration of the herbal extracts and standard drugs were carried out every day for 21 days. Blood samples were collected through the tail vein just prior to and on days 0,7,14 and 21 after the drug administration.

2.5 Acute Oral Toxicity Study

The rats were treated with graded dose of powered seed extracts of *Cucurbita maxima* (5, 50, 300, 2000 mg/kg body wt./rats/day) to find out any possible toxic effects and/or changes in behavioral pattern, and were kept under close

observation. All symptoms including changes in awareness, mood, motor activity, posture activity & mortality were recorded and no changes were observed in behavior and mortality as well as in toxicity or death for these given dose levels in the selected and treated animals. The LD_{50} of the pet ether, ethyl acetate and alcoholic extracts was found more than 2000 mg/kg whereas as per OECD guidelines-423 it is 2000 mg/kg so it was prove that extracts were not having any toxic effects. One-tenth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment. Hence, On the basis of above observations the biological dose was fixed 200 mg/kg Body weight for each the extracts for further treatment[17,18].

3. Biochemical Analysis
3. Antidiabetic Activity
3.1 Experimental Design
Evaluation of antidiabetic effect of test plant extracts was done on six groups of rats by randomly selecting six rats for each group. The groups are as following.

3.2 Induction of Diabetes in Rats
Rats were made diabetic by single administration of streptozotocin (50 mg/kg) dissolved in 0.1M citrate buffer, pH 4.5 was intraperitoneal injected to overnight fasted rats. The blood samples were collected from tail vein using capillary tubes. The blood glucose level was measured and the rats were having blood glucose level more than 200 mg/dl were considered as diabetic and used for the study[19,20,21].

3.3 Recording of Body Weight
The change body weight was recorded during the study period. Body weight was measured before and after the streptozotocin administration on the 0, 7th, 14th and 21st study days during the treatment in normal control, diabetic control, standard glibenclamide, petroleum ether, ethyl acetate and alcoholic extracts[22].

Table 1: Grouping of animals [19,20,21]

Group No.	Description
1	Served as normal control (Received normal saline 0.5 ml/kg body weight).
2	Served as diabetic control (treated with STZ dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 50 mg/kg body weight).
3	Served as reference standards (glibenclamide, 500 µg/kg body weight orally).
4	Diabetic rats given pet ether extract of *Cucurbita maxima*, 200 mg/kg body weight which is prepared in 1 % CMC and was given orally.
5	Diabetic rats given ethyl acetate extract of *Cucurbita maxima*, 200 mg/kg body weight which is prepared in 1 % CMC and was given orally.
6	Diabetic rats given alcoholic extract of *Cucurbita maxima*, 200 mg/kg body weight which is prepared in 1 % CMC was given orally.

3.4 Collection of Blood Sample
Blood samples for estimation of blood glucose was collected from each animal from the tip of the tail under mild ether as an anesthesia on 0th day (before treatment) and 7th, 14th, 21st days (during treatment). The blood samples for measuring lipids profile, liver functions tests were collected on 21st day from each animal by retro-orbital route in Eppendroff's test tubes and serum was separated by centrifuge at 3000 rpm[20,21].

3.5 Estimation of Blood Glucose Level
Blood sugar estimation was done by using a glucometer (Accu-check® sensor, Roche Diagnostics GmbH, Mannheim) and strips. Blood glucose level was measured on the 0th, 7th, 14th and 21st study days during the treatment in normal control, diabetic mice standard glibenclamide, pet ether, alcoholic and aqueous extracts[23,24].

3.5. Estimation of Lipid Profile
Lipid profile (Total cholesterol, Triglyceride, LDL, HDL and VLDL) were estimated by using Star 21 bio auto analyzer (E114947) at 505 nm by standard kits (Span diagnostics Ltd. India).

3.6. Estimation of Liver Physiological Profile

Liver function parameters (ALP, SGOT, SGPT & Total bilirubin) estimations were carried out by Star 21 bio auto analyzer (E114947), using standard kits (Span diagnostics Ltd. India)

3.7 Statistical Analysis

The results were expressed as mean ± SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests; Diabetic control was compared with control rats. Diabetic + Glibenclamide, diabetic + Pet ether extract, diabetic + Ethyl acetate extract and diabetic + alcoholic extract were compared with diabetic control.

4. Results

The results of body weight, blood glucose level, lipid profile and liver function parameters of normal control group, diabetic control group, standard group (Glibenclamide 500 µg/kg) and three different extracts (Petroleum ether, Ethyl acetate and Alcohol) of herbal drug *Cucurbita maxima* were summarized in table no.1,2,3,4.

4.1 Effect of extracts on body weight

Table-1 shows that a significant decrease was observed in the body weight of diabetic rats compared with control rats. Treatment with extracts of seeds of *Cucurbita maxima* and glibenclamide, the body weight gain was improved but the effect was more pronounced in alcoholic extracts of *Cucurbita maxima* treated rats than glibenclamide on 14, 21st day of study.

Table-1: Effect of *Cucurbita maxima* extracts on body weight recorded before & after streptozotocin administration

Groups	Before STZ	Body weight after streptozotocin (gm)			
		0th	7th	14th	21st
Control	161.2±5.963	160.1±5.833	160.8±5.961	161.7±6.382	162.4±7.729
Diabetic control	164.3±7.657	164.2±7.158*	162.8±6.725*	160.8±5.725*	158.4±5.329*
GLB	153.9±7.232	153.2±7.441*	154.6±6.146*	155.2±5.689*	156.8±5.146*
Pet. ether extract	159.2±7.696	158.5±8.369*	159.1±8.382*	160.2±8.462*	161.6±7.314*
Eth. acetae extract	158.8±8.221	157.7±8.408*	158.6±8.284*	159.3±7.343*	160.8±7.145*
Alcoholic extract	160.5±7.146	159.6±8.375*	160.9±7.354*	161.7±7.151*	163.2±7.014*

The results are expressed as mean ± SEM; n=6 animals in each group; Values are statistically significant at *P<0.05; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests; GLB = Glibenclamide

4.2 Estimation of blood glucose level

Table-2 shows that treatment with oral glibenclamide & various extracts of seeds of *Cucurbita maxima* diminished blood glucose level on day 0, 7th, 14th & 21. The untreated diabetic control rat group showed increase in blood glucose level throughout the entire study period. Initially blood glucose level of untreated diabetic control group was 288.6±2.376 and after 21 days of trial period the blood glucose level was increased to 318.6±4.471. For trial drugs *Cucurbita maxima* (200 mg/kg) blood glucose were studied in three different groups of animals. All the three groups showed a significant decrease of blood glucose level on streptozotocin-induced diabetic rats when compared to control group. The initial readings of blood glucose level of Petroleum ether, Ethyl acetate and Alcoholic

extract were 289.7±3.375, 287.5±3.402 and 285.3±3.753 respectively. After the trial period, there was marked reduction in blood glucose levels 264.1±1.815, 230.8±2.712 and 189.9±1.896 in 21 days. However alcoholic extract of *Cucurbita maxima* has shown maximum effect than petroleum ether and ethyl acetate. In standard group initial blood glucose was 270.6±1.783 and the post test was 141.6±1.113 which showed that the standard drug produced maximum hypoglycemic effect and the statistical analysis was extremely significant and slightly higher than that of trial drug group.

Table-2: Effect of *Cucurbita maxima* on blood glucose level

Groups	Blood glucose (mg/dl)			
	0 day	7th day	14th day	21th day
Control	86.2±1.713	86.5±1.317	86.8±1.610	86.5±0.992
Diabetic control	288.6±2.376	292.7±4.502	301.8±3.120	318.6±4.471
Glibenclamide	270.6±1.783*	168.3±1.332*	152.8 ±1.878*	141.6±1.113*
Pet. ether extract	289.7±3.375*	281.3±2.232*	272.4±1.793*	264.1±1.815*
Eth.acetate extract	287.5±3.402*	263.4±2.282*	242.3±2.343*	230.8±2.712*
Alcoholic extract	285.3±3.753*	230.5±2.354*	209.7±1.835*	189.9±1.896*

The results were expressed as mean ± SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests; Diabetic control was compared with control rats. Diabetic + Glibenclamide, diabetic + Pet ether, diabetic + Ethyl acetate extract and diabetic + alcoholic extract were compared with diabetic control.

4.3 Estimation of Lipid Profile of *Cucurbita maxima*

Table-3 shows the levels of Total Cholesterol (TC), Triglycerides (TG's), Low Density Lipids (LDL), High Density Lipids (HDL) and Very Low Density Lipids (VLDL) levels in liver of control and experimental rats. The results showed that increased levels of TC, TG's, LDL and VLDL in diabetic rats when compared with normal rats. In rats treated with different extracts of *Cucurbita maxima* and Glibenclamide there was a significant decrease in content of TC, TG's, LDL and VLDL levels and significantly increase in HDL levels when compared with diabetic control rats.

4.4 Estimation of Liver Physiological Profile of *Cucurbita maxima*

Table-4 shows the levels of ALP, SGOT, SGPT and Bilirubin in liver of control and experimental rats. The results showed that increased levels of ALP, SGOT, SGPT and Bilirubin in diabetic rats when compared with normal rats. In rats treated with different extracts of *Cucurbita maxima* and Glibenclamide there was a significant decrease in content of ALP, SGOT, SGPT and Bilirubin levels when compared with diabetic control rats.

Table-3: Effect of extract of seeds of *Cucurbita maxima* on lipid profile

Drug	Total cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Control	94.79±1.376	62.72±1.011	47.62±1.182	38.66±0.874	11.48±0.672
Diabetic Control	145.03±1.979	93.25±2.191	82.47±1.252	23.72±1.439	19.47±0.479
GLB	98.21±1.928*	65.91±1.285*	42.31±1.505*	39.13±0.871*	12.24±0.187*
PE	131.27±2.738	88.16±1.983*	69.77±1.186*	24.55±2.681	23.37±1.232*
EAE	125.13±1.732*	81.26±1.872*	66.89±1.831*	32.46±1.667*	19.24±1.341*
AE	111.47±1.765*	77.13±1.516*	57.46±0.987*	29.88±0.816*	17.28±1.211*

The results were expressed as mean ± SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control;Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests; GLB = Glibenclamide; PE=Pet. ether extract; EAE=Ethyl acetate extract; AE= Alcoholic extract.

Table-4: Effect of *Cucurbita maxima* on liver function parameters

Groups	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Bilirubin (IU/L)
Control	156.08±3.532	65.6±2.356	51.52±2.651	0.72±0.011
Diabetic control	267.81±3.114	125.51±1.744	105.41±2.312	2.12±0.094
Glibenclamide	172.18±2.063*	58.32±1.511*	63.20±2.451*	0.65±0.515*
Pet. ether extract	242.84±1.934*	116.52±1.662*	93.56±2.843*	2.11±1.015*
Eth. acetate extract	217.32±1.707*	102.18±1.432*	84.28±1.856*	1.81±0.842*
Alcoholic extract	204.28±1.662*	94.24±1.464*	72.08±1.498*	1.57±0.040*

The results were expressed as mean ± SEM; n=6 animals in each group; * P<0.05:Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03); One way ANOVA was used, followed by Bonferroni multiple comparison tests.

5. Discussion

Diabetes mellitus is one of the most common chronic disease and is associated with hyperglcaemia, hyperlipidemia and co-morbidites such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes. Streptozotocin, an analogue (2-deoxy-2-(3-methyl-3-nitrosouredia)-D-glucopyranose), is a potent diabetogenic agent and widely used for inducing diabetes in a variety of animals by the selective degeneration and necrosis of pancreatic cells[26,27]. Streptozotocin selectively destroys pancreatic insulin secreting β-cells causing diabetes close to type-2 diabetes of humans[28]. Streptozotocin induces a wide variety of animals species by damaging the insulin secreting pancreatic β-cells, resulting in a disease in endogenous insulin release, which

paves the ways for the decreased utilization of glucose by the tissues+. Glibenclamide has been used for many years to treat diabetes to stimulate insulin secretion from pancreatic β-cells[30]. The present data indicated that different extracts of *Cucurbita maxima* significantly reduced the elevated fasting blood glucose with respect to those of diabetic control animals. The maximum result obtained from alcoholic extract of *Cucurbita maxima.* The possible mechanism by which *Cucurbita maxima* brings about its hypoglycemic action may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of islets of langerhans or its release from bound insulin or increased peripheral utilization of glucose.

Lipids play a vital role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor coronary heart disease[3]. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factor[31,32]. The abnormal high concentration of serum lipids in the diabetic subjects is due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia[33,34]. Significant lowering of total cholesterol and rise HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions[35]. The various extracts (petroleum ether, ethyl acetate and alcohol) of *Cucurbita maxima* brought down the elevated levels of TC, LDL, VLDL-cholesterol and TG's in diabetic animals to nearly normal levels. There was increase in HDL-cholesterol also, was a desirable feature. The alcoholic extract of *Cucurbita maxima* had shown significant reduction in TC, LDL, VLDL and TG's and significant rise in HDL-cholesterol among the three different extracts.

Serum enzymes including, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphate (ALP) and bilirubin are used in the elevation of hepatic disorders. Serum enzyme levels were significantly raised to high values in diabetic control animals, reflecting active liver damage or inflammatory hepatocellular disorders[36,37]. The various extracts (petroleum ether, ethyl acetate and alcohol) of *Cucurbita maxima* caused significant reduction in the activities of ALP, SGOT, SGPT and bilirubin to normal levels, showing the protective effect of the extract. The elevated levels of SGPT, SGOT reduced by the treatment of *Cucurbita maxima* which might be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by streptozotocin. The alcoholic extract of *Cucurbita maxima* had shown significant reduction in serum ALP and bilirubin, indicated an improvement in secretory mechanism.

The phytochemical and pharmacological studies performed indicated the different extracts (petroleum ether, ethyl acetate and alcohol) of *Cucurbita maxima* contain carbohydrates, flavanoids, tannins, phenolics and saponins. Saponins appear to involve stimulation of pancreatic β-cells and subsequent secretion of insulin[38].

6. Conclusion

From the present investigation, it can be concluded that oral administration of *Cucurbita maxima* seed extracts produces significant antidiabetic effect in controlling the blood glucose level. In addition it possesses potent antihyperlipidemic effect, lowers both total cholesterol and triglycerides and at the same time increases HDL-cholesterol in STZ-induced diabetic rats and have a protective role on complications associated with diabetes. Hence, the seed extracts of *Cucurbita maxima* can be considered as a potent source of antidiabetic and antihyperlipidemic agents, which may be due to presence of flavanoids, phenols or saponins in the extract. Further studies to isolate and to

characterize the active compound and to further elucidate the mechanism involved in the antidiabetic effect are underway.

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Estimation of Quercetin, an Anxiolytic Constituent, in *Elaeocarpus ganitrus*

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Having established quercetin as the anxiolytic constituent of *Elaeocarpus ganitrus*, it was decided to use it as marker to standardize the plant material. Quercetin was used as an external standard for determining its content in E. *ganitrus* beads by TLC densitometry. An HPTLC densitometric method has been developed to estimate quercetin in E. *ganitrus* beads so that plant can be standardized on the basis of its bioactive marker. Two methods were followed for preparing the test samples for determining the quercetin content. Initially, quercetin was determined in the ethanol extract of the plant material. It was also determined in the acid hydrolyzed ethanol extract, in order to free quercetin from its O-glycoside. Quercetin content in the hydrolyzed ethanol extract (0.11% w/w) of E. *ganitrus* beads was found to be about 4 times more than in the ethanol extract prepared by direct method (0.03% w/w). Results showed that quercetin occurs in E. *ganitrus* beads in the form of glycoside

Keyword: Anxiolytic, Quercetin, HPTLC Densitometry, *Elaeocarpus ganitrus*

1. Introduction

Genus *Elaeocarpus*, which has about 360 species, occurs throughout Australia, East Asia, Malaysia and the Pacific Islands. About 120 species belonging to this genus have been reported from different parts of Asia and out of this, 25 species occur in India alone[1]. *Elaeocarpus ganitrus* Roxb. (Syn. *E. sphaericus* Gaertn., *E. angustifolius* Blume) is an evergreen tree, ripe fruits of which contain a hard and highly ornamental stony endocarp known as bead or nut, and is commonly termed as Rudrakasha in India. It holds popular belief reinforced by experiments that it has confirmed medicinal uses apart from its attractive stones[2].

Ethnic people use fruit of *E. ganitrus* to treat various ailments. The flesh or pulp of drupe in green and fresh state is sour in taste, stimulates appetite[3] and is given in epilepsy, diseases of the head and mental illness. The fruit stone (seed kernel) is sweet, cooling and emollient. Externally the stone (fruit or drupe) is rubbed with water (like sandalwood) and then it is applied to small-pox eruptions. Similarly, it is applied on organs having burning sensation and in other conditions i.e. eruptions, measles, fevers, etc[3].

E. ganitrus fruits contain glycosides, steroids, alkaloids and flavonoids. Apart from this, it has been found that the exocarp of the fruit supplies a nutritious reward to consumers, particularly rich in carbo-hydrates (21.0% dry mass, or 0.58 g per fruit) and proteins (4.3% dry mass, or 0.12g per fruit), but lacking in lipids[4].

The leaves of *E. ganitrus* contain quercetin, gallic

and ellagic acids[5]. Constituents such as (+)-elaeocarpiline, isoelaeocarpiline, (-)-epi-elaeocarpiline, (+)-epi-isoelaeocarpiline, (+)-epialloelaeocarpiline, (-)-alloelaeocarpiline, elaeocarpidine, and pseudoepi-isoelaeocarpiline having a dihydro-γ-pyrone chromophore in their molecules have also been reported. A minor non-aromatic indolizidine alkaloid, rudrakine ($C_{16}H_{23}NO_3$, mp 159-160°C), was also isolated from the leaves of *E. ganitrus* Roxb.[6].

In most biological studies undertaken, extracts of *E. ganitrus* exhibited wide range of pharmacological activities and are identified as active against specific biological targets during large scale screening of multiple plant extracts. Literature reports are available on various pharmacological activities which include analgesic and anti-inflammatory[7], CNS activities, typical behavioral actions[8], sedative, tranquillizing[8], hypnosis potentiation, antidepressant, antiasthmatic, hydrocholeretic[8], antidiabetic[9], cardiostimulation, antihypertensive[10], anticonvulsant[11], etc.

Despite a long history of use of *E. ganitrus* as a traditional medicine for the treatment of various ailments, especially CNS disorders, the plant has never been subjected to detailed antianxiety activity studies. Recently, authors have reported that among various extracts, viz., petroleum ether, chloroform, ethanol, and water, of *E. ganitrus* fruits, only the ethanol extract (200 mg/kg, *p.o.*) exhibited significant antianxiety activity on elevated plus maze model[12]. An anxiolytic constituent quercetin was isolated from ethanol extract of *E. ganitrus* beads using antianxiety activity guided fractionation.

Present investigation was undertaken with an objective to develop an HPTLC densitometric method to estimate quercetin in *E. ganitrus* beads so that plant can be standardized on the basis of its bioactive marker.

2. Materials and Methods

2.1 Plant Material

Plant material was procured from Rati Ram Nursery, village Khurrampur, district Saharanpur, U.P, India, in the month of September-October 2007. The taxonomic identity of the plant was confirmed by Mr. Ram Prasad, Department of Botanical and Environmental sciences, Guru Nanak Dev University, Amritsar. A voucher specimen *Elaeocarpus ganitrus* (S.R. Bot Sci/0348) has been deposited in the same department's herbarium.

2.2 TLC densitometry

TLC densitometer system comprised of LINOMAT – 1V applicator and CAMAG TLC SCANNER – III with CATS 4 software. Chromatographic analyses of the standard and extracts were performed on pre-coated silica gel aluminum-based plates (20×20 cm, 0.2 mm, E Merck). Aliquots of standard and extracts were applied on TLC plates using 1.0 or 2.0 µl CAMAG capillaries. TLC plates were developed using toluene:ethyl acetate:formic acid (10:3:1) as the mobile phase. The development distance was around 8 cm. The plates were dried and scanned at 372 nm.

2.3 Preparation of standard solution

Stock solution of quercetin (8 mg/10 ml) was prepared in ethanol. From this stock solution, five working standard solutions (6, 4, 3, 2 or 1 mg/10 ml) were obtained by appropriate dilution with ethanol.

2.4 Preparation of test samples

2.4.1 Direct method

Dried powdered beads of *E. ganitrus* (2 g), packed in a filter paper sachet, were defatted by refluxing in 250 ml round bottom flask on boiling water bath with 3×50 ml quantity of petroleum ether (1h each). The marc obtained was air dried and refluxed under similar conditions with 3×50 ml quantity of ethanol. The ethanol extracts were pooled, filtered and concentrated under reduced pressure. Dried ethanol extract was reconstituted in ethanol, in a volumetric flask, and its volume was made upto 10 ml.

Table 1: Quercetin content in *E. ganitrus* beads as determined by TLC densitometry.

Method	BS_I content (% w/w) ($Mean^n \pm$ S.D.)
Direct extraction with ethanol	0.03± 0.001
After acid hydrolysis of ethanol extract	0.11± 0.001

n = 3

Table 2: Intra-day and Inter-day precision of Quercetin.

Marker	Concentration (ng/spot)	Intra-day precision (% RSD)	Inter-day precision (% RSD)
Quercetin	200	0.48	0.41
	400	0.53	0.65
	800	0.52	0.56

Table 3: Recovery study of Quercetin.

Marker	Amount of marker present (µg)	Amount of marker added (µg)	Amount of marker found (µg) (Mean ± S.D.)	Recovery (%)	Average recovery (Mean ± S.D.)
Quercetin	400	200	591.2 ± 5.64	98.53	98.32 ± 0.1
	400	400	786.2 ± 6.22	98.28	
	400	500	883.4 ± 7.84	98.16	

2.4.2 Acid Hydrolysis

Following the above procedure (Section 2.4.1), ethanol extract of *E. ganitrus* beads was prepared. Dried ethanol extract was heated in a 50 ml round bottom flask with 6% aqueous hydrochloric acid (25 ml) for 45 min on water bath. Aglycones, precipitated on cooling the solution, were removed by filtration, and dissolved in ethanol. The filtrate was fractionated with 3×20 ml quantity of diethyl ether; the fractions were combined, dried over anhydrous sodium sulphate, and the solvent was removed completely under reduced pressure. The diethyl ether extract, thus obtained, was pooled with ethanol solution of aglycones. Finally, volume was made up to 10 ml with ethanol in a volumetric flask.

2.5 Preparation of Standard Curve

Two µl each of the stock solution and the four working standard solutions of quercetin were applied, in triplicate, on 20 cm × 20 cm TLC plates. The plates were developed using toluene:ethyl acetate:formic acid (10:3:1) as mobile phase. The developed plates were scanned at 372 nm using TLC densitometer. A standard graph was plotted against absorbance and quercetin amount (µg).

2.6 Estimation of Quercetin Content in Test Samples

Aliquots (2 µl) of test samples were applied, in triplicate, to the TLC plates. The plates were developed using toluene:ethyl acetate:formic acid (10:3:1) as mobile phase, and the chromatograms were scanned at 372 nm using TLC densitometer. The area under the curve of every sample was recorded, and quercetin content was determined from the regression equation of the standard graph.

2.7 Validation of TLC Assay

ICH guidelines were followed for the validation of the analytical method developed (CPMP/ICH/281/95).

2.7.1 Instrument Precision

Instrumental precision was checked by repeated scanning (n=7) of the same spot of quercetin (400 ng/spot), and was expressed as relative standard deviation.

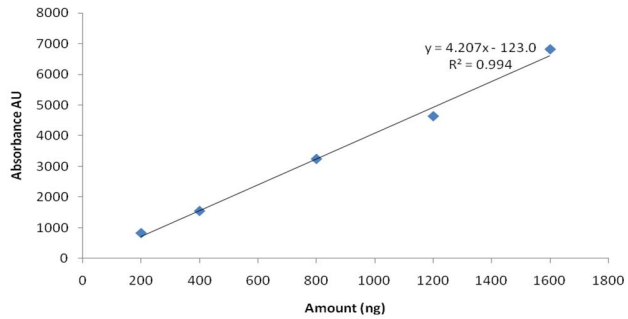

Figure 1: Standard curve of absorbance against amount of Quercetin.

2.7.2 Repeatability

The repeatability of the method was confirmed by analyzing 400 ng/spot of quercetin individually on a TLC plate (n=5) and expressed as %RSD.

2.7.3 Intra-Day and Inter-Day Variation

Variability of the method was studied by analyzing aliquots of standard solution containing 200, 300 or 400 ng/spot of quercetin on the same day (intra-day precision) and on different days (inter-Day precision).

2.7.4 Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by applying different concentrations of the standard solutions of quercetin along with ethanol as blank and determined on the basis of signal-to-noise ratio (S/N). LOD was determined at S/N of 3:1 and LOQ at S/N of 10:1.

2.7.5 Recovery Studies

The accuracy of the method was assessed by performing recovery studies at three different levels (50, 100 and 125% addition of quercetin). The percent recovery and average percent recovery were calculated.

2.7.6 Specificity

This was ascertained by analyzing the standard compound and sample. The band for quercetin from sample solutions was confirmed by comparing the R_f and spectra of the bands to those of the standards. The peak purity of quercetin was analyzed by comparing the spectra at three different levels, i.e., start middle and end positions of the bands.

3. Results and Discussion

The developed plates were scanned at 372 nm using HPTLC densitometer. Figure 1 shows standard plot of quercetin. Figures 2 and 3 show TL chromatogram of ethanol extract of *E. ganitrus* and that of acid hydrolyzed ethanol extract of *E. ganitrus*. Table 1 shows quercetin content in *E. ganitrus* beads as determined by TLC densitometry. Results of intra-day and inter-day precision, and recovery studies of quercetin are shown in tables 2 and 3 respectively.

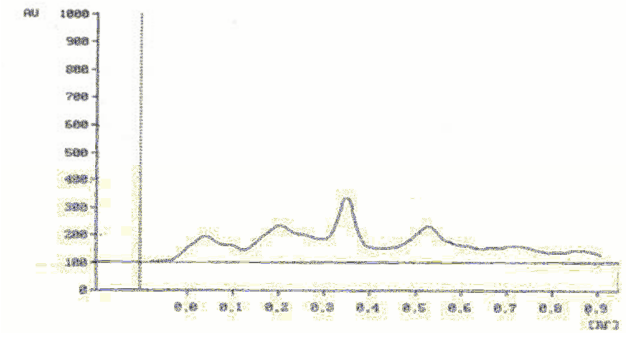

Figure 2: TL chromatogram of ethanol extract of *E. ganitrus* beads

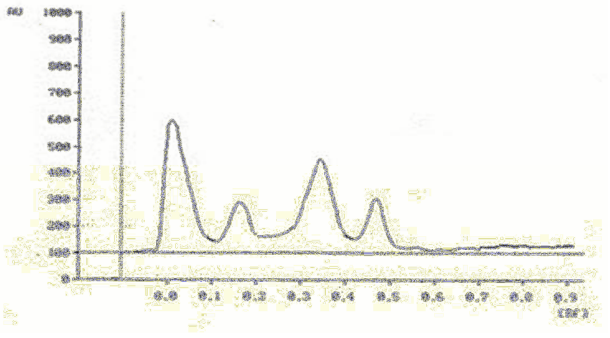

Figure 3: TL chromatogram of acid hydrolyzed ethanol extract of *E. ganitrus* beads.

Having established quercetin as the anxiolytic constituent of *E. ganitrus*, it was decided to use it as marker to standardize the plant material. Quercetin was used as an external standard for

determining its content in *E. ganitrus* by TLC densitometry. TLC was used as its method of development is easy, cost effective, efficient and requires not much clean-up of the test samples.

TLC studies revealed that quercetin resolved well in test samples using toluene:ethyl acetate:formic acid (10:3:1) as the mobile phase. The chromatograms of quercetin and acid hydrolysed ethanol extract of *E. ganitrus* beads were scanned under UV at 372 nm (Figures 2 and 3).

It is a well-known fact that quercetin occurs in plants mainly as glycoside. Quercetin has been isolated, in the present investigation, as an aglycone. Keeping in view the possibility of its occurrence in the plant as glycoside, extraction procedure was modified for preparing the extract for determining quercetin content.

Standard procedures were adopted to obtain ethanol extract of *E. ganitrus* beads. Two methods were followed for preparing the test samples for determining the quercetin content. Initially, quercetin was determined in the ethanol extract of the plant material. It was also determined in the acid hydrolyzed ethanol extract, in order to free quercetin from its *O*-glycoside. Quercetin content in the hydrolyzed ethanol extract (0.11% w/w) of *E. ganitrus* beads was found to be about 4 times more than in the ethanol extract prepared by direct method (0.03% w/w) (Table 1). From this observation, it can be concluded that most of the quercetin is present in O-glycosidic form in *E. ganitrus* beads.

Further, the TLC assay was validated in terms of precision, repeatability and accuracy. The linearity of the calibration curve was achieved between 200-1600 ng for quercetin (r^2 = 0.994; Figure 1). The TLC assay was found to be precise with % RSD for intra-day in the range of 0.48-0.52 and inter-day in the range of 0.41-0.56 for different concentrations of quercetin (Table 2). This indicates that the proposed method is precise and reproducible. The LOD and LOQ values for quercetin were found to be 60 and 140 ng/spot respectively. The average recovery at three different levels of quercetin was observed to be very high (98.32%) (Table 3) indicating that the assay procedure developed, in the present investigation, is quite reliable.

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Antimicrobial Activity of Volatile Oil of *Artemisia capillaris* Growing Wild in Uttrakhand Himalaya

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The genus *Artemisia* (family: Asteraceae) is a source of valuable drugs and essential oils because of its intricate chemical composition comprising several chemotypes. The in vitro antibacterial activities of the essential oils were evaluated against a total of six bacteria, viz, *Salmonellatyphi, Klebsiellapneumoniae, E.coli, Staphylococcusaureus, Streptococcusmutans, Bacillussubtilis* and antifungal activity tested against Candida *albicans* and Candida *glabrata*

Keyword: Asteraceae, *Artemisia capillaris*, Essential Oil, Antibacterial.

1. Introduction

Uttrakhand Himalaya is bestowed with a rich flora of aromatic plants. The climatic variations from subtropical to alpine have favored the growth of many aromatic plants. The genus *Artemisia* (family: Asteraceae) is a source of valuable drugs and essential oils because of its intricate chemical composition comprising several chemotypes. Reports on chemical composition of various *Artemisia* species from different origins show the presence of 1, 8-cineole, α-thujone, β-thujone, chamazulene, davanone, artemisia ketone, germacrene D, β-caryophyllene and caryophyllene oxide.[1,2] In recent years, there has been increasing interest in healthy lifestyles and healthy aging. As a result, many people are involved in searches for natural compounds that can improve health, especially those of plant origins. A great number of aromatic, spicy, medicinal, and other plants belonging to the family Asteraceae contain chemical compounds exhibiting antimicrobial and antioxidant properties[3]. Antimicrobial and antioxidative plant oils and extracts have been used for many purposes, including raw and processed food preservation, pharmaceuticals, alternative medicines, and natural therapies. Natural products are perceived as having fewer negative impacts than synthetic agents; natural products may be effective, selective, biodegradable, and less toxic to the environment. The genus *Artemisia* is one of the most important genera in the family Asteraceae and is widespread throughout the world[4]. Previous reports on *A.capillaris* showed that it has antifungal effects[5] and allelopathic effects[6] stimulates immune activities in human cells, and has anticancer activity[7]. The major active components of *A. capillaris* are scoparone and capillarisin, and the concentrations of these compounds are related to the season of harvest[8]. Many members of the genus *Artemisia* (Asteraceae) are important medicinal plants. For example, *A. vulgaris* (mugwort), native of Britain and Europe, has been used as a tonic, febrifuge, anthelmintic, women's menstrual troubles and

infertility, anti-nervous disorders, against complaints of the gastrointestinal tract (e.g. stomach ulcers and indigestion). The essential oil shows the antimicrobial activity and contains 1,8-cineole, camphor and thujone as major constituents[9]. We have already reported the phenyl alkynes rich essential oil from leaves and roots of *A.capillaris*[10]. Present communication reveals the antimicrobial activity of leaf volatile oil of *A.capillaris*.

2. Materials and Methods

2.1 Plant Material

The plant material was collected from Milam glacier at an altitude of 3,600 m in the month of August. The identifications were done separately from Botany Department, Kumaun University, Nainital and Botanical Survey of India (BSI) Dehradun. The voucher specimens (No.CHEM/DST/06/01) have been deposited in the Phytochemistry laboratory of the Chemistry Department, Kumaun University, Nainital.

2.2 Oil Isolation

The fresh leaves (2.5 kg) were subjected to steam distillation in a copper electric still fitted with spiral glass condensers obtaining 5L water distillate each. The distillates were saturated with NaCl and the oils were extracted with hexane followed by dichloromethane. The organic phases were then dried over anhydrous Na_2SO_4 and the solvent distilled off in a thin film rotary vacuum evaporator at 30^0 C.

2.3 Antimicrobial Activity

The *in vitro* antibacterial activities of the essential oils were evaluated against a total of six bacteria, *viz Salmonella typhi,* (Clinical isolated), *Klebsiella pneumoniae* (MTCC 109), *E. coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 96), *Streptococcus mutans* (MTCC 890), *Bacillus subtilis* (MTCC 121).The antifungal activity of the oils was performed against *Candida albicans* (MTCC 1637) and *Candida glabrata* (MTCC 3019). The test strains were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh. MTCC (Microbial Technology Culture Collection) numbers represents the standard strain numbers assigned to these microorganisms. The cultures of bacteria and fungi were maintained on their appropriate agar slants at 4^0 C throughout and used as stock cultures.

2.4 Determination of Zone of Inhibition (ZOI)

The antimicrobial activity of the essential oils was investigated by the disc diffusion method using 24-48 h grown strains reseeded on Nutrient Broth (bacterial strains) and Potato Dextrose Agar (PDA, fungal strains)[11]. The cultures were adjusted to 5 × 106 CFU/mL with sterile water. 100 µL of the suspensions were spread over Nutrient agar and PDA plates to obtain uniform microbial growth. Filter paper discs (6.0 mm in diameter) were impregnated with 20 µL of the oils and then placed onto the agar plates which had previously been inoculated with the test microorganism. The petri dishes were kept at 4^o C for 2 h. The plates were incubated at 37^o C (24 h) and at 30^o C (4 h) for bacterial and fungal strains, respectively. The diameter of the inhibition zones (mean values) were measured in millimeter and considered as the zone of inhibition (ZOI). All experiments were performed in triplicate.

2.5 Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined using a modified agar-well diffusion method[11]. In the agar-well diffusion technique, two-fold serial dilutions of the essential oils were prepared by diluting oil with hexane to achieve a decreasing concentration range from 50 to 1.26 µL/mL (for the fungi) and 50 µL/mL to 2.20 µL/mL (for the bacteria), using 100 µL of a suspension containing 5 × 106 CFU/mL of bacteria spread on nutrient agar plates, whereas the fungal strains were reseeded on PDA. The wells were filled with 20 µL of essential oil solutions in the inoculated Nutrient PDA agar plates. The bacterial cultures were incubated at 37°C for 2 hrs, while fungal cultures were incubated at 30°C for 48 hrs. The least concentration of each essential oil showing a clear zone of inhibition

was taken as the MIC. Hexane was used as the negative control. Chloramphenicol and amphotericin B were used as positive controls for bacteria and fungi, respectively. Antimicrobial (antibacterial and antifungal) activity of *A. capillaris* leaf oil by disc diffusion assay (10µL of oil/disc) against different microorganisms shown in the table 1 and 2 respectively.

3. Results and Discussion

Antibacterial activity against *Staphylcoccus aures,* ZOI 25 mm, (MIC, 4.25) with respect to standard, *viz* chloramphenicol, ZOI 22 mm showed very good activity. Also antifungal activity showed by leaf oil of *A. capillaris* against *Candida albicans* and *Candida glabrata,* exhibit largest ZOI 29 mm and 20 mm (MIC, 1.26, 2.08) with respect to standards viz amphotericin B (20 µg), ZOI 16 mm and 11 mm respectively. Earlier work on *A. capillaris* showed antiplatelet and anti-Hiv activity, insecticidal activity against *Sitophilus zeamais*, and antimicrobial activity[12]. This species was recorded to control cabbage white butterfly (*Pieris rapae*), cotton aphids (*Aphis gossypii*), cucurbit leaf beetle (*Aulacophora femoralis*) and other vegitable pests in china[13].

Table: 1 Antibacterial activity of leaf essential oil *A. capillaris* (by disc diffusion assay (10 µL of oil/disc) (Zone of inhibition and MIC)

Oil/antibiotic	***S. typhi***	***K. pneumoniae***	***E. coli***	***S. aureus***	***S. mutans***	***B. subtilis***
A. capillaris	9.0 mm (15.54)	10 mm (14.16)	9.0 mm (10.2)	25 mm (4.25)	11 mm (3.20)	14 mm (2.20)
Cp.(10µg/disc)	25 mm	25 mm	21 mm	22 mm	30 mm	24 mm

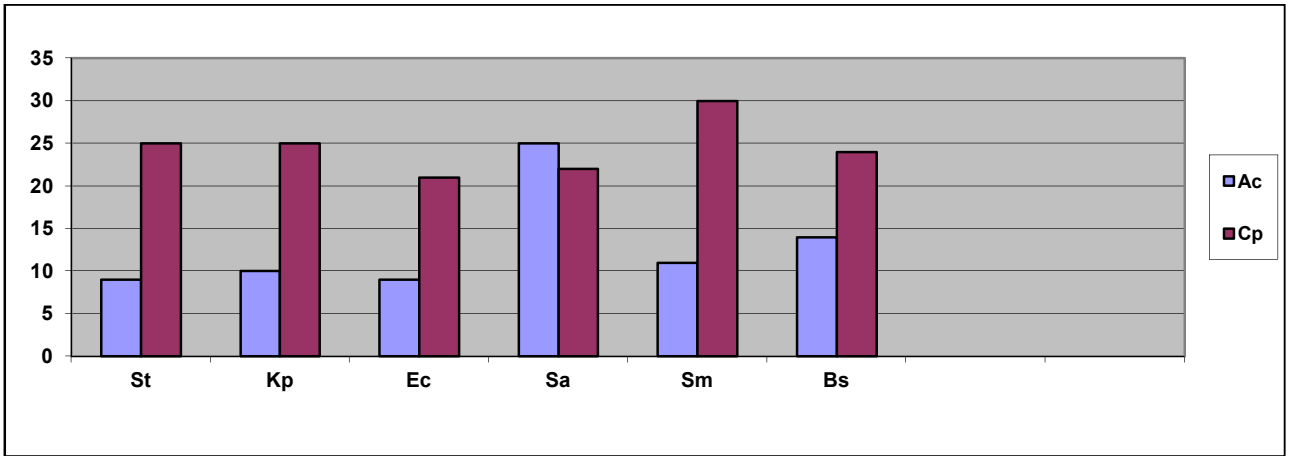

Fig: 1 Antibacterial activity of leaf essential oil of *A. capillaris* (Ac=*Artemisia capillaris*, Cp= Chloramphenicol) Bacteria: St, Salmonella typhi; Kp, Klebsiella pneumoniae ; Ec, Escherichia coli ; Sa, Staphylococcus aureus; Sm, Streptococcus mutans ; Bs Bacillus subtilis No inhibition zone. Chloramphenicol (10 µg/disc)

Table: 2 Antifungal activity of leaf essential oil *A. capillaris* (by well diffusion assay (40µL of oil/well) (Zone of inhibition and MIC)

Oil /antifungal	*Candida albicans*	*Candida glabrata*
A. capillaris	29 mm (1.26)	20 mm (2.08)
Amphotericin B (20µg)	16 mm	11 mm

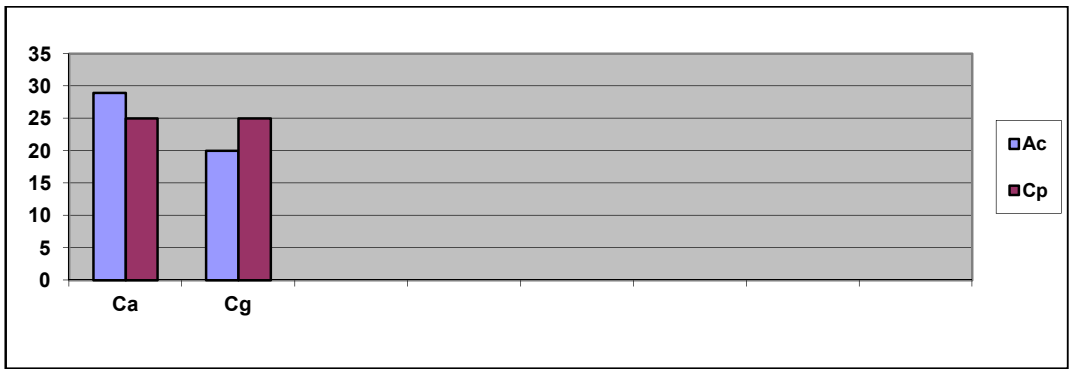

Fig: 2 Antifungal activity of leaf essential oil of *A. capillaris*

(Ac =*Artemisia capillaris*, Am= Amphotericin B) Fungal strains: Ca, Candida albicans; Cg, Candida glabrata; No inhibition zone. Amphotericin B 20µg

4. Conclusion

The present investigation reveals that the essential oil of *Artemisia capillaris* is found to be good natural antibacterial and antifungal agent. Attempts will be made in future to isolate the huge amount of oil to use this purpose.

5. Acknowledgement

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Antimicrobial Properties and Characterization of Phytoconstituents of the Leaf Extracts of Some Medicinal Plants

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Background: This study was designed to determine and compare the in-vitro antimicrobial effects of these plants phyto preparations. **Materials and Methods:** The leaves of O. *basilicum*, C. *citrates*, C. *roseus* and A. *paniculata* were collected from Bhopal (MP) India and leaves extract were examined to evaluate the phytoconstituents and antimicrobial activity by Agar well diffusion antimicrobial bioassay. Preliminary evaluation of the solvent fractions showed a broad spectrum of activity since the extracts inhibit the growth of both gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Enterococcus faecalis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial isolates. **Results:** In the present study the plant parts contain levels of alkaloids, terpenoids, flavonoids and other phytochemical. In antimicrobial assay 100%, 75% and 25% methanolic extracts shows very potent antimicrobial agent on both gram positive E. *faecalis* (highest inhibition zone 3.6 mm) and gram negative bacterial isolates. The result from this study provides suitable support for the use of A. *paniculata* and C. *rosea* in the control and treatment of diabetes and use of O. *basilicum*, C. *citrates* for the control of infectious disease caused by both gram positive and gram negative bacteria. **Conclusion:** Among all the four investigated plants C. *citrates*, C. *roseus* and A. *paniculata* was found to be efficient against most of the investigated pathogens. This findings suggest that there is a potential in the discovery of novel antimicrobial agents from medicinal plants and further study should be made in order to identify the active phytochemical constituents and on toxicity of active plant principles to determine their safety use

Keyword: Antimicrobial Activity, Medicinal Plants, Phytoconstituents and Zone of Inhibition

1. Introduction

The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers[1].

The steadily increasing bacterial resistance to existing drugs is a serious problem in antimicrobial therapy and necessitates continuing

research into new classes of antimicrobials[2,3]. One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents[4]. It is anticipated that phytochemicals with adequate antimicrobial efficacy could be used for the treatment of bacterial infections[5]. One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects[6]. There are several reports of antibiotic resistance of human pathogens to available antibiotics[7-11].

Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human and plant pathogens and hence in the present investigation, leaves of *Ocimum basilicum*, *Cymbopogen citrates*, *Catharanthus roseus* and *Andrographis paniculata* was tested for its efficacy to inhibit against human pathogens. These plants are well known for its medicinal value as antiviral, antidiabetic, antioxidants and antimicrobial properties. In order to elucidate such a phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their bioactivity by various in vitro model systems. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in tropical countries. Therefore the main objectives of the present study were to determine and compare the in-vitro antimicrobial effects of these plants phyto preparations.

2. Materials and Methods

2.1 Bacterial Isolates and Sampling Procedure

Bacterial isolates were obtained from clinical samples belonging to different sources such as skin infection, throat sample and water samples. These samples were collected with sterile cotton swabs from the actual infection site aseptically into the sterile container containing normal saline. These swabs will capture the causative organism in most cases and the culture will allow the specific organism to be grown in the microbiology laboratory under certain conditions. Samples were taken to the laboratory in cold condition within 4 hr for further microbiological analysis.

Table 1. Phytochemical constituents of leaf extract of *O. basilicum*, *C. citrates*, *C. roseus* and *A. paniculata*

Phytochemical constituent	Methanolic extract			
	O. basilicum	***C. citrates***	***C. roseus***	***A. paniculata***
Terpenoids	+	+	+	+
Alkaloids	-	-	+	+
Coumarins	+	+	+	+
Flavonoids	-	-	-	+
Tannins	+	+	+	-
Saponins	-	+	-	+

Negative (-) absent, Positive (+) present

2.2 Bacterial Isolation and Identification

The samples were inoculated on nutrient agar and MacConkey agar media plate and incubated at 37°C for 24 hr. The colonies of isolated organism have been sub culture on nutrient agar plate and pure culture were obtained in various selective media such as Mannitol Salt agar, Blood agar and Chocolate agar and further identified by biochemical tests for confirmation of bacteria. For the confirmation of the isolated bacteria and

their species specific biochemical tests such as IMViC test, catalase test, coagulase test, urease test, oxidase test, bile solubility test, Deoxyribonuclease (DNAse) test and carbohydrate fermentation test were performed. *S. aureus*, was identified by the positive catalase, from IMViC-MR positive and acid-gas production by carbohydrate fermentation test and other biochemical characters. *S. pneumoniae,* was isolated on Streptococcus selection agar and identified positive for IMViC-VP and bile solubility test. *Enterococcus faecalis* isolated on Brain Heart infusion agar and Blood agar, showed positive results of Methyl red and nitrate reduction.

Escherichia coli, isolated on Nutrient agar media and identified positive for IMViC-Indole, MR, catalase and carbohydrate fermentation. *P. aeruginosa,* isolated on Pseudomonas agar base and identified positive for catalase, oxidase and citrate utilization shown in Figure 1.

Table 2. Results of the antimicrobial activity of the selected plants

Test organisms	A	B	C	D
S. aureus	+	+	+	+
S. pneumonia	+	-	-	-
E. faecalis	-	+	+	+
E. coli	-	+	+	-
P. aeruginosa	-	-	-	+

(A) *Ocimum basilicum*, **(B)** *Cymbopogen citrates,* **(C)** *Catharanthus roseus* and **(D)** *Andrographis paniculata*

Negative (-) absent, Positive (+) present

2.3 Collection of Plant Material

(A) *Ocimum basilicum*, (B) *Cymbopogen citrates,* (C) *Catharanthus roseus* and (D) *Andrographis paniculata* plants are known for their medicinal importance. These plants were collected from different localities in Bhopal (MP), India. Dirt was removed from the plant parts by rinsing in clean water. The leaves were air-dried at room temperature for 2 weeks. All dried material were chopped into small fragments and reduced into fine powder with mortar and pestle, which can pass through 0.5 mm pore size sieve.

2.4 Preparation of Solvent Extracts

The methanol extracts were prepared by soaking each of the dry powdered plant materials in methanol at room temperature for 48 h. This step was repeated several times till the extraction was complete. The total extracted volume of each plant subjected to filtration after 48 h using Whatman No.1 filter paper and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40° C. All the dried samples were subjected to antimicrobial activity assay and phytochemical analysis.

3. Phytochemical Analysis

Phytochemicals screening of active plant extracts was done by following the standard method of Khandelwal[12] for the qualitative analysis of various phytochemicals studies such as terpenoids, alkaloids, coumarins, flavonoids, tannins and saponins.

3.1 Terpenoids Determination

A portion of 0.5 gm of each of the extract was taken, 2 ml of chloroform and few drops of conc. H_2SO_4 were added. A reddish brown colouration of the interface indicates the presence of terpenoids.

3.2 Alkaloids Determination

a. Mayer's test: Few drops of Mayer's reagent (Prepared by dissolving 1.36 gm $HgCl_3$ in 60 ml double distilled water and 5 gm KI in 20 ml

double distilled water) added in 2-3 ml test sample, creamy precipitate observed indicates presence of alkaloids.

b. Wagner's test: Few drops of Wagner's reagent (Prepared by dissolving 1.27 gm of Iodine and 2 gm KI in 100 ml double distilled water) added in 2-3 ml test sample, reddish brown color observed indicates presence of alkaloids.

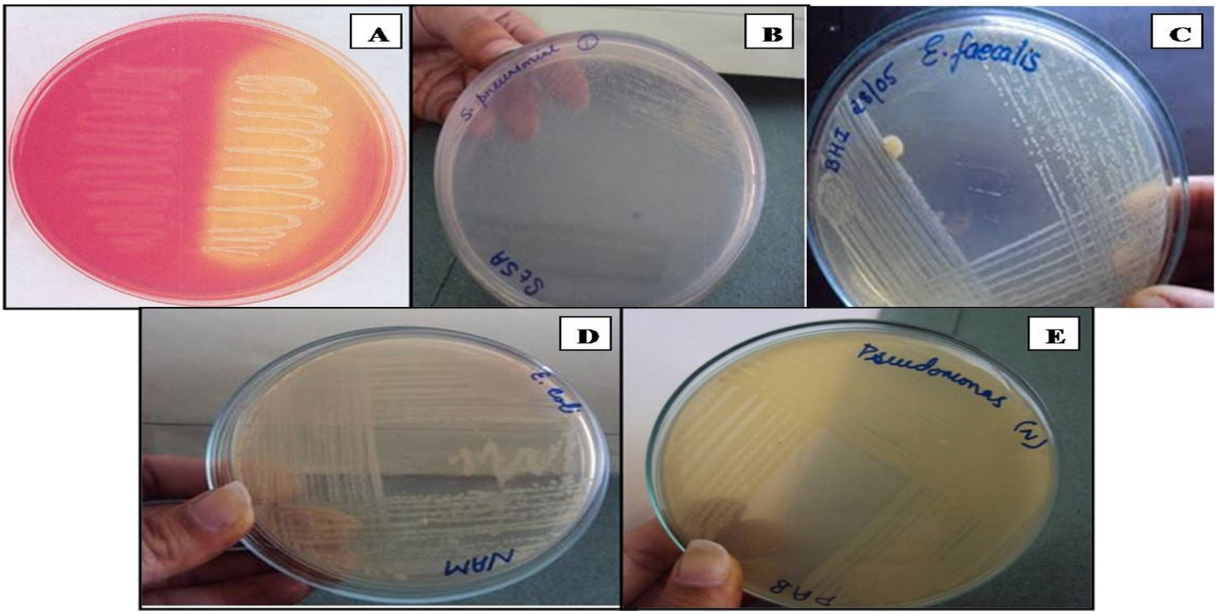

Figure 1. Pure Bacterial cultures of Gram Positive Bacteria; (A) *S. aureus* on Mannitol Salt agar, (B) *S. pneumonia* on Streptococcus Selection agar, (C) *E. faecalis* on Brain Heart Infusion agar and Gram Negative Bacteria; (D) *E. coli* on Nutrient agar media, (E) *P. aeruginosa* on Pseudomonas Agar Base.

3.3 Coumarins Determination

a. Aromatic odor: Coumarin given aromatic odor.

b. Filter Paper Test: Test samples taken in test tube and covered with filter paper soaked in dilute NaOH and kept in hot water bath, after some time filter paper gives yellowish green fluorescence.

3.4 Flavonoids Determination

A portion of each extract solution was taken in a test tube and 5 ml of dilute ammonia solution and 1 ml conc. H_2SO_4 were added. A yellow color appears that disappear on standing indicating the presence of flavonids.

A portion of each extract solution was taken in a test tube and 10 ml of ethyl acetate was added and heated over the steam bath for 3 minutes. Mixture was filtrated, on 4 ml filtrate 1 ml of dilute ammonium solution were added. A yellow coloration indicates the presence of flavonoids.

3.5 Tannins Determination

A portion of 0.5 gm extract was boiled in 10 ml of water in attest tube and then filtered. Few drops of 0.1% ferric chloride were added. Brownish green or a blue-black color develops confirms presence of tannins.

3.6 Saponins Determination

Shake the plant extract vigorously with water, persistent foam observed, indicates the presence of saponins.

3.7 Glycoside Determination

A portion of 0.5 gm extract was diluted in 5 ml of distilled water and 2 ml of glacial acetic acid and 1% of ferric chloride solution were added with few drops of conc. H_2SO_4. A violet ring appear below the brown ring or a greenish ring form just above the brown ring and gradually spread throughout this layer which confirms the presence of glycoside.

4. Antimicrobial activity

The antimicrobial assay was performed by Agar well diffusion method[13, 14] for solvent extract. Petri plates were prepared with 20 mL of sterile brain heart infusion agar (BHI) (Himedia, Mumbai). The test cultures (100 µL of suspension containing 10^8 CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. For agar well diffusion method, a well was prepared in the plates with the help of a cork borer (6.0 mm). The active extract fractions were serially diluted in the respective solvent used for its extraction. The active extract fractions were diluted and used at concentrations of 0%, 25%, 50%, 75% and 100%. Normal saline was used as the negative control while Ampicillin of 30 µg/ml was used as the positive control. Into the well, 100 µl of the test compound was introduced. The plates were incubated overnight at 37°C. The positive antimicrobial growth was determined by measuring the diameter of the zone of inhibition after 24h in which square radius of the clear zone around each well (y^2) were measured and divided over the square well radii (x^2) to obtain absolute unit (AU) for the inhibition zone[15]. Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism.

5. Results

5.1 Phytochemical analyses

The phytochemical screening of the selected plants showed the presence of terpenoids and coumarins in all the four plants. Alkaloids were present in *C. roseus* and *A. paniculata.* Flavonoids were absent in all plants except for *A. paniculata.* Tannins were present in all the plants except *A. paniculata.* Saponins were present in *C. citrates* and *A. paniculata* as presented in Table 1.

5.2 Glycoside determination

O. basilicum, *C. citrates* and *C. roseus* methanolic extract showed the presence of glycoside whereas *A. paniculata* showed negative result.

5.3 Antimicrobial assay

The antimicrobial efficacy of *O. basilicum*, *C. citrates, C. roseus* and *A. paniculata* extracts of 100%, 75% and 50% Methanol, plants against bacterial isolates showed varied level of inhibition. Almost all the selected plants showed antimicrobial activity against the investigated five human pathogens, the presence and absence of antimicrobial activity is summarized in the Table 2. The zone of inhibition ranged from 0.6 mm to 3.6 mm. The highest inhibition zone 3.6 mm (100 %) was formed by the extract of *A. paniculata* against *E. faecalis* at the highest concentration, followed by 3 mm (75%), 1.3 mm (50% and 25%) whereas, 3.3 mm (100%), 3 mm (75%) and 1.3 mm (50% and 25%) zone recorded in *S. aureus*. *C. roseus* plant extract showed 2.5 mm (100%), 1.8 mm (75%) and 0.8 mm (50%) inhibition zone against *E. faecalis* followed by 2.6 mm (100%) and 1.3 mm (50% and 25%) inhibition zone against *S. aureus*, 1.3 mm (100% and 50%) and 0.6 mm (25%) inhibition zone against *E. coli. C. citrates* plant extract showed 3.3 mm (100%), 1.6 (75% and 50%) and 1.3 mm (25%) inhibition zone against *S. aureus* followed by 3 mm (100%), 1.6 mm (75%) and 0.6 mm (50%) inhibition zone against *E. faecalis.* The positive control (Ampicillin) exhibited far stronger antimicrobial activity with inhibition diameters 10.0 mm while no zone was observed by negative control. The rest of the zones of inhibition (mm) are given in the Table 3. None of the investigated plants showed activity against *P. aeruginosa.* All plant extracts showed anti-microbial activity against *S. aureus* (Figure 2). There was no significant difference in the activity of the various extracts suggesting that, maybe none of the extraction modes used affected the bioactive components of the plant extracts or that

none of the extraction modes adequately extracted the bioactive components from the raw plant materials.

Table 3. Inhibition of bacterial isolates exposed to selected plant extracts using agar well diffusion technique at different concentration dose

Microorganisms	% Conc. (µg/ml)	Zone of inhibition (mm)											
		A		B		C		D		PC		NC	
		Y	AU	Y	AU	Y	AU	Y	AU	Y	AU	Y	AU
S. aureus	100	12	2	20	3.3	16	2.6	20	3.3	60	10	-	-
	75	9	1.5	10	1.6	8	1.3	18	3	NT	NT	NT	NT
	50	8	1.3	10	1.6	8	1.3	8	1.3	NT	NT	NT	NT
	25	4	0.6	8	1.3	6	1	8	1.3	NT	NT	NT	NT
S. pneumonia	100	15	2.5	-	-	-	-	-	-	53	8.6	-	-
	75	10	1.6	-	-	-	-	-	-	NT	NT	NT	NT
	50	-	-	-	-	-	-	-	-	NT	NT	NT	NT
	25	-	-	-	-	-	-	-	-	NT	NT	NT	NT
E. faecalis	100	-	-	18	3	15	2.5	22	3.6	50	8.3	-	-
	75	-	-	10	1.6	11	1.8	18	3	NT	NT	NT	NT
	50	-	-	4	0.6	5	0.8	8	1.3	NT	NT	NT	NT
	25	-	-	-	-	-	-	8	1.3	NT	NT	NT	NT
E. coli	100	-	-	10	1.6	8	1.3	-	-	60	10	-	-
	75	-	-	8	1.3	8	1.3	-	-	NT	NT	NT	NT
	50	-	-	-	-	-	-	-	-	NT	NT	NT	NT
	25	-	-	6	1	4	0.6	-	-	NT	NT	NT	NT
P. aeruginosa	100	-	-	-	-	-	-	-	-	48	8	-	-
	75	-	-	-	-	-	-	-	-	NT	NT	NT	NT

Y: diameter of inhibition zone (mm); **AU**=y/x µl of plant extract where **x**: Diameter of well (6mm), **NT**: Not tested, **-** : Absent

A: *Ocimum basilicum*, **B**: *Cymbopogen citrates,* **C**: *Catharanthus roseus,* **D**: *Andrographis paniculata*, **PC**: Positive control (Ampicillin), **NC**: Negative control (Normal saline)

Among all the five investigated plants *C. citrates, C. roseus* and *A. paniculata* was found to be efficient against most of the investigated pathogens.

6. Discussion

Medicinal plants are used in traditional medicine for several purposes. The secondary metabolites produced by plants constitute a source of bioactive substances and nowadays scientific interest has increased due to the search for new drugs of plant origin[16,17].

Considering the evolution of resistance genes to antibiotics of microbial origin and non-antibiotic Chemicals[18], plant materials have become the subject of public attention and therefore the pharmaceutical industry is moving away from drug discovery or screening towards compounds isolated by medicinal plants.

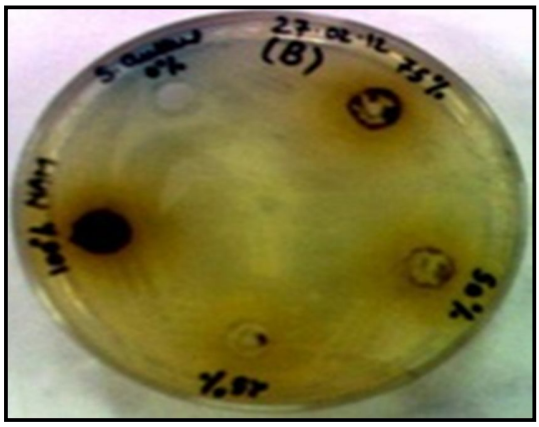

Figure 2. Anti-microbial activity of selected plant extracts against *S. aureus* by the well diffusion method.

Standardization of herbal drugs is most desirable part of plant based drugs designing that includes macroscopic, microscopic and phytochemical studies of investigated plant parts. Macroscopic characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. The microscopical studies (anatomical and histochemical) are often necessary to establish the botanical identity of commercial samples of medicinal plants, timbers, fibers etc. and may play an important part in checking adulteration and substitution.

The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes, etc., are effective antimicrobial substances against a wide range of microorganisms. It can be seen from the above results that the leaf extract contains alkaloids and other phytochemicals. These compounds could be used as substitutes for synthetic antibiotics for the treatment of chronic kidney infection, bacterial endocarditis and carrier conditions of typhoid[19].

The in vitro antimicrobial activity of *O. basilicum*, *C. citrates, C. roseus* and *A. paniculata* against Gram positive *S. aureus* and *E. faecalis* and efficient result was observed against *S. aureus*. Very less or no activity was observed against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). This can be explained because the outer membrane of Gram-negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are able of breaking down foreign molecules introduced from outside.

Further investigation is required for the isolation of the active principle which could serve as a broad-spectrum antimicrobial agent for treating bacterial infections.

7. Conclusion

The phytochemicals that have efficient antimicrobial activity could be screened, isolated and used as substitute for antibiotics. In the present study almost all the plants showed antimicrobial activity but *C. citrates*, *C. roseus* and *A. paniculata* were found to be more effective against most of the investigated pathogens.

In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines. However, problems may arise due to intrinsic toxicity, adulteration, substitution, contamination, misidentification, drug-herb interactions and lack of standardization[20]. This unfavourable fact urges the study of medicinal plants and plant derived compounds used in medicine and food industry.

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Martynia annua L.: A Review on Its Ethnobotany, Phytochemical and Pharmacological Profile

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Martynia annua L. is a well-known small herbaceous annual plant in Ayurveda as kakanasika, which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis. This weed has been known to possess anthelmintic, analgesic, antipyretic, antibacterial, anti-convulsant, antifertility, antinociceptive, antioxidant, CNS depressant and wound healing activity. A wide range of chemical compounds including oleic acid, arachidic acid, linoleic acid, palmitic acid, gentisic acid, stearic acid, pelargonidin-3,5-diglucoside, cyanidin-3-galactoside, p-hydroxy benzoic acid, apigenin, apigenin-7-o-glucuronide have been isolated from this plant. The aim of this review article was to summarize the information related to botany, phytochemistry, traditional and pharmacological activity of the *Martynia annua* plant.

Keyword: *Martynia annua*, Martyniaceae, Kakanasika, Antifertility, Antibacterial, Wound healing.

1. Introduction

1.1 Occurrence, Botanical Description and Ethnopharmacology:

Martynia annua L. (Family: Martyniaceae), is native of Mexico and also found throughout India, in waster places, rubbish heaps and road sides. The plant is commonly known as Devil's claw (English), Bichu (Hindi), kakanasika (Sanskrit) and Vichchida (Gujarati)[1].

It is a small herbaceous, erect, branched, glandular hairy annual herb growing upto 0.9-1.2 m in hight. Leaves are large, simple, opposite, green in color, broadly ovate to triangular-ovate, glandular hairy, 9–22 × 9–20 cm, cordate at base with sinuolate-dentate margin and acute apex, sticky as often covered with glutinous dew-like substance[2].

The scientific classification of the plant is given below:

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Angiosperms
Order:	Lamiales
Family:	Martyniaceae
Genus:	Martynia
Species:	*Martynia annua*

Glandular hairs exude a slimy sap which gives the plant a clammy feel. Stems are green, robust,

branched and covered with glandular hairs. Flowers are drooping, large, pale mauve or lavender in short spikes at the end of branches. They are tubular shaped 4-6 cm long, pink and dark purple blotched with yellow inside, foxglove shaped, ill-smelling and terminate in 5 spreading lobes with a prominent spot between each lobe[3]. Fruits are oblong, green and fleshy when young, becoming black and woody when mature, 3-4 cm long, 1-1.5 cm wide tapering into a long beak (claw), which splits into two sharp re-curved hooks when dry. Claws are shorter than the body of the fruit. Seeds are flat, brown to black, elongated, two seeds to each pod, usually remaining inside the pod[3]. Racemes are long, erect and terminal. Corolla are glandular hairy with very oblique mouth lobes[4-5].

In Ayurveda, the plant is known as kakanasika, which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis[6]. The leaves and fruits are biologically active part of this plant[7-8]. The leaves of the *Martynia annua* are edible and used as antiepileptic and antiseptic, applied locally to tuberculous glands of the neck, the juice of the leaves as a gargle for sore throat and the leaf paste for wounds of domestic animals[9-10]. The fruit is considered alexiteric and useful in inflammations while ash of fruit mixed with coconut oil applied on burns[6]. Seed oil applied on abscesses and for treating itching and skin affections[11]. The Ayurvedic Pharmacopoeia of India recommended the seed of *Martynia annua* for arresting of graying of hair[6]. The fruits of *Martynia annua* used as local sedative and also used as antidote to scorpion stings to venomous bites and stings[12]. A detailed view of the ethno-medicinal uses of different parts of the plant is given in **Table 1**.

2. Phytochemistry:

Qualitative phyto-chemical analysis of whole plant extracts of *Martynia annua* reveals the presence of glycosides, tannins, carbohydrates, phenols, flavonoids and anthocyanins[13]. GC-MS studies on both aqueous and alcohol extract shows the presence of twenty eight compounds, out of which oleic acid, constitutes the major part. Other major biological compounds include pelargonidin-3-5-diglucoside, cyanidin-3-galactoside, p-hydroxy benzoic acid, gentisic acid, arachidic acid, linoleic acid, palmitic acid, stearic acid, apigenin, apigenin-7-o-glucuronide[14].

Table -1. Ethanobotanical information on *Martynia annua* L.

S. No.	Traditional uses	Plant part	Preparation	Reference
1	Antidote to venomous stings	Leaves	Paste	[12]
2	Antifertility	Root	Extract	[16]
3	Alexiteric	Fruit	Paste	[20]
4	Applied to tuberculous glands	Leaves	Paste	[20]
5	Epilepsy	Leaves	Paste	[21]
6	Gargle	Leaves	Juice	[20]
7	Inflammations	Fruit	Paste	[20]
8	Scabies	Ripe fruit	Oil	[22]
9	Scabies	Leaves	Eaten as such	[21]
10	Sedative	Root	Paste	[22]
12	*Tinea corporis*	Fruit	Oil	[22]
13	To kill bugs	Leaves	As such	[22]

The methanolic extract of *Martynia annua* leaves shows the presence of higher amount of terpenoid, alkaloids, glycosides, steroids, tannins and saponins and moderate amount of cardiac

glycosides, phenols and anthroquinones. While, it doesn't shows the presence of flavonoids and resins[15]. The leaves mainly contain chlorogenic acid, p-hydroxy benzoic acid, snapic acid[16] and fatty acids such as palmitic acid and stearic acid[13]. The flowers of the plant mainly contain cyanidin-3-galactoside and pelargonidin-3, 5-diglucoside whilst gentisic acid are present in fruits[16]. The seeds show the presence of arachidic acid, cyclopropenoid, linoleic acid, malvalic acid, oleic acid, palmitic acid and stearic acid[4]. A detailed view of the phytoconstituents present in different parts of the plant is given in Table 2 and chemical structures of some of the phytoconstituents were shown in Figure 1.

Table 2: Phytoconstituents of *Martynia annua* L. plant

S. No.	Phyto-constituents	Type	Plant part	Reference
1	Arachidic acid	Lipid	Seed	[23]
2	Chlorogenic acid	Phenolic acids	Leaves	[13]
3	Cyanidin-3-galactoside	-	Whole plant, Flowers	[16], [14]
4	Cyclo propenoid	-	Seeds	[23]
5	HCN	Cyanogenic group	Seed	[23]
6	Linoleic acid	Lipid	Seed	[23]
7	Malvalic acid	-	Seed	[23]
8	Oleic acid	Lipid	Whole plant, Seed	[23]
9	Palmitic acid	Lipid	Seed, leaves	[13], [23]
10	Pelargonidin-3,5-diglucoside	-	Whole plant, Flowers	[16], [14]
11	Stearic acid	Lipid	Seed, leaves	[13], [23]
12	Gentisic acid	Lipid	Fruits	[16]
13	p-hydroxy benzoic acid	Phenolic acids	Leaves	[13]
14	Apigenin, Apigenin-7-O-beta- D- glucuronide	Flavonoids	Whole plant, Seed	[14], [24]

3. Pharmacological Activities:

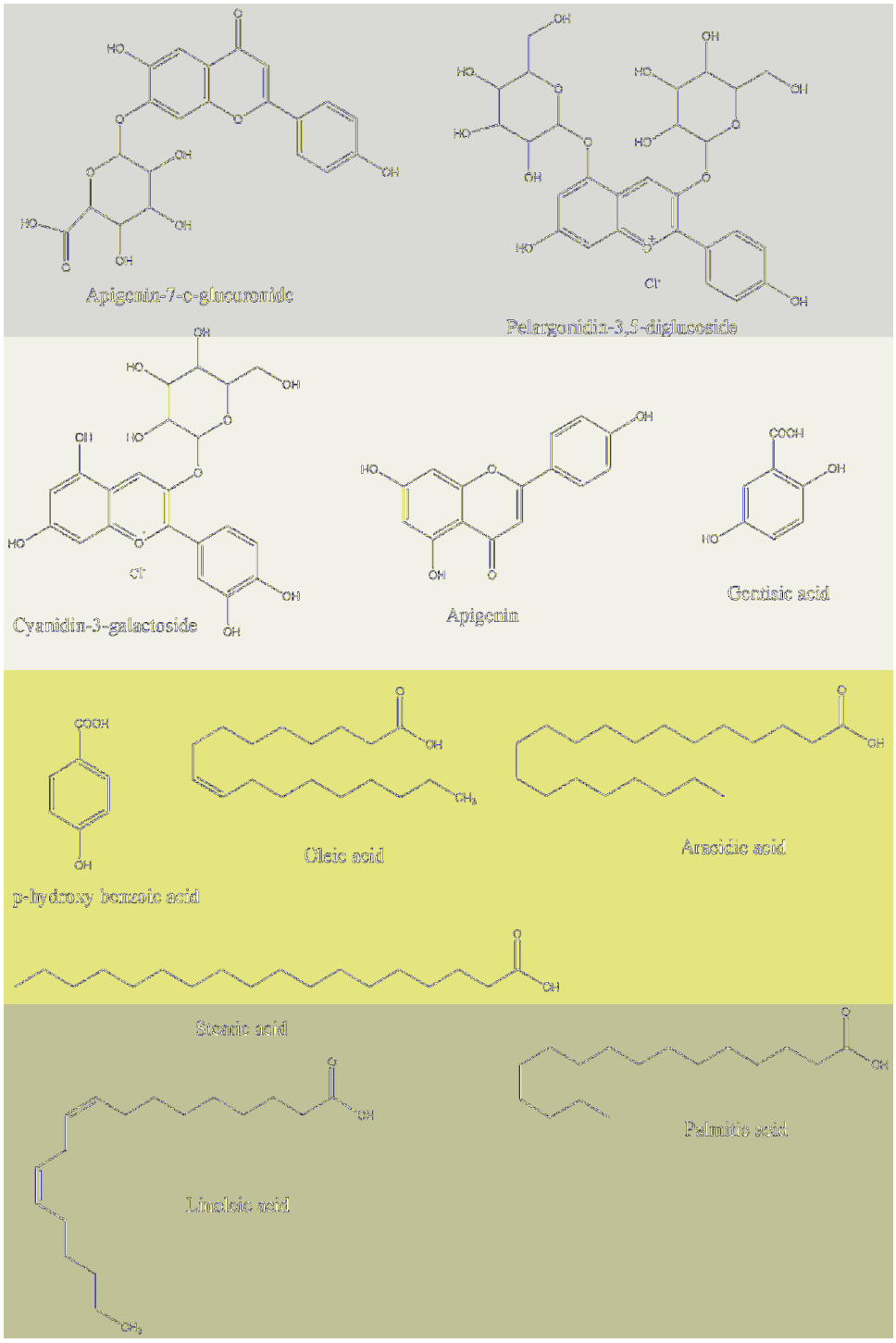
The use of *Martynia annua* as medicine is fairly large, yet, its curative efficacy have been assessed only for few cases. The present review explores the pharmacological potential of the plant.

3.1 Anthelmintic Activity: The petroleum ether extract of *Martynia annua* roots showed potent antihelmintic activity against earthworms *Pheritima posthuma* as compared with the reference drug albendazole[17].

3.2 Analgesic and Antipyretic Activity: The petroleum ether, chloroform, ethanol and aqueous extracts of *Martynia annua* fruits were evaluated for analgesic effect in Swiss albino mice using hot plate and tail flick methods and for antipyretic effect against brewers-yeast- induced hyperpyrexia in adult Wistar rats. The extracts show significant analgesic and antipyretic activity at 20 mg/kg. It was also observed that the petroleum ether and chloroform extracts exhibits greater analgesic and antipyretic activities as compared to ethanol and aqueous fruit extract of the plant[18].

3.3 Antibacterial: The chloroform, ethyl acetate and methanol extract of *Martynia annua* leaves were found antibacterial when evaluated against gram positive and gram negative bacteria. All the solvent extracts show antibacterial action respective to different bacterias. Chloroform extract shows higher antibacterial activity against *Proteus vulgaris*, Bacillus *subtilis* and *B. thuringensis*. Ethyl acetate extract was potentially effective against *Salmonella paratyphi A, Salmonella paratyphi B*, *Proteus mirabilis*, *P. vulgaris* and *Klebsiella pneumonia*, whereas the methnol extract, shows greater antibacterial potential towards *Proteus vulgaris*, *B. subtilis, S. paratyphi B* and *Pseudomonas aeruginosa*. The antibacterial activity was carried out by Disc Diffusion method in which all solvent extract were used as 100% concentration alone[15].

Figure 1: Chemical Structure of the Phytoconstituents



3.4 Anti-Convulsant: The methanol extract of *Martynia annua* (MEMA) leaves at doses of 200 mg/kg and 400 mg/kg were significantly reduced the duration of tonic hindleg extension and protect the animals from seizures. On comparing with, the standard drug phenytoin (100%) the

MEMA 200 mg/kg and 400 mg/kg have shown 66.31 % and 82.731 % protection respectively against maxima electroshock (MES) induced seizures whereas on comparison with the standard drug diazepam (100%) the MEMA 200 mg/kg and 400 mg/kg have shown 70.33% and 82.88% protection of convulsion and 83.33% and 100% protection of mortality respectively against pentylenetetrazol (PTZ) Induced epilepsy. However, the anticonvulsant activity of MEMA was due to the potentiation of neurotransmitter in brain[6].

3.5 Anti-Fertility:

The 50% ethanol extract of *Martynia annua* L. root at dose of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight po, showed significant decreases in the weights of testes, epididymides, seminal vesicle and ventral prostate on male rats. Moreover, the antifertility effect was found to be dose dependent without altering general body metabolism[16].

3.6 Antinociceptive Activity and CNS Depressant activity:

The petroleum ether, ethyl acetate and methanol root extracts of *Martynia annua* were evaluated for antinociceptive and CNS depressant activity. Amongst all extracts petroleum ether extract at the dose of 50 mg/kg, i.p. showed significant increase in reaction time in hot plate method and also showed more inhibitory effect on writhing induced by acetic acid against all extracts and standard drug pentazocine and paracetamol respectively. Apart from this the petroleum ether extract at similar dose also showed significant reduction in the locomotor activity when compared with standard drug diazepam and at the dose of 30 mg/kg, i.p. it potentiates pentobarbitone sodium induced sleeping time up to 215.34%[19].

3.7 Antioxidant Activity: The methanol and aqueous extract of *Martynia annua* Linn. leaves were evaluated for antioxidant activity by *in vitro* methods, namely, reducing power assay, DPPH radical-scavenging activity, nitric oxide scavenging activity, H_2O_2 radical scavenging activity, superoxide radical scavenging assay, hydroxyl radical-scavenging activity, and total antioxidant capacity. The methanol extract was found to have higher antioxidant activity than the aqueous extract[1].

3.8 Wound Healing:

The methanol fraction of ethanolic extract of *Martynia annua* leaves shows significant wound healing effect by stimulating of wound contraction as well as epithelialization. Moreover, phytochemical studies demonstrated that the methanol fraction mainly contains flavanoid luteolin responsible for enhancement of wound healing process due to the free radical scavenging mechanism[13].

4. Conclusion:

Although the plants grown in rubbish heaps and in waste places, the herb is being used as an important medicinal plant since a long period of time. In view of the wide-ranging medicinal value of *Martynia annua* plants as described in Ayurvedic literature, it is imperative that more clinical and pharmacological trials are needed to investigate the unexploited potential of this plant.

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Herbal Plants Used For the Treatment of Malaria- A Literature Review

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Malaria is one of the most common major health problems all over the world. Pharmacotherapy is the most common treatment strategy for the disease. But, the main obstacle behind this is emergence of resistance for many of these drugs. Hence, to overcome this problem, the major option available since ancient times is medicinal plants all over the world. Mankind is blessed with wide range of herbal plants for various ailments with lot of those useful for malaria treatment also. In this review, we have highlighted many of those plants all over the world which are being used since ancient times for the treatment of malaria

Keyword: Herbal plants, Malaria, Anti-malarial activity, Plasmodium

1. Introduction

Malaria is one of the most common major health problem in tropical and developing countries of sub-Saharan Africa and south East Asia including India[1]. It is major killing disease responsible for the death of millions of children, pregnant women and adults[1]. Malaria is caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected *Anopheles* mosquitoes, called "malaria vectors"[2]. There are four parasite species that cause malaria in humans which are Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale.[2] P.falciparum and *P.* vivax are the most common species clinically but P. falciparum is the most deadly leading to many fatal complications including cerebral malaria[2]. Clinical symptoms of malaria ranges from acute febrile illness with fever associated with chills, headache, and vomiting to deadly complications like severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria which can eventually lead to death[2].

In 2010, malaria caused an estimated 6,60,000 deaths, mostly among African children[2]. As per the latest WHO estimates, there are 300-500 million new clinical cases globally and >1 million deaths occur due to malaria each year[3].

Treatment strategies of malaria aim to terminate the acute blood infection, to cure the clinical symptoms, to clear hypnozoites from the liver to prevent future relapses and to prevent the spread of infection[4]. Radical treatment and curative treatment comprise main aspects of treatment. Various pharmacological options available for this purpose are *Chloroquine, Mefloquine, Quinine, Primaquine, Pyremethamine, Artemisinin derivatives like artesunate, artemether, arteether* and *amino alcohols like Lumefantrine* and *Halofantrine along with tetracycline, Doxycyclines* and *Sulfadoxime* etc[3]. The greatest problem associated with this treatment is emergence of drug resistance which

leads to treatment failure in significant number of cases[4].

2. Use of herbal plants in the treatment of malaria

Apart from the pharmacological treatment, various options are being used since ancient times for many health ailments. Nearly 80 % of the global population still depends upon the herbal drugs for their health care. In India, the use of several medicinal plants to cure specific ailments has been practiced since ancient times. Various cultural traditions are associated with use of wild plants as medicinal herbs. This medico-lore is passed over generations traditionally all over the world. Reliance on plants is primarily due to their safety, effectiveness, cultural preferences, inexpensiveness and abundant availability all the time. The medicinal virtues of plants are identified by instinct/intuition or by trial and errors. Globally, traditional healers are using various medicinal plants for the treatment of malaria; however, this practice is not really completely recognized by modern medicine.

Knowledge about traditional medicinal practices and plants is currently transmitted from generation to generation principally by word of mouth. Large number of plant species has been identified as anti-malarial medicinal plants. In the present review, extensive literature review was done on the plants, which have been identified as anti-malarial plants and the work done so far in evaluating their anti-malarial potential. A wide variety of plants belonging to several families have been identified through ethnobotanical and ethno pharmacological studies as anti-malarial medicinal plants.

This review is an attempt to present a comprehensive account of numerous medicinal plants used in the treatment of malaria in either forms. A thorough literature survey highlights that plant kingdom has a tremendous resources which can be exploited for unidentified novel compounds anti-malarial activity.

Table 1: Plants Having Anti- Malarial Activities

Sr. No	Botanical Name	Common name/ Vernacular name	Family	Parts used
1	*Clerodendrum viscosum* Vent[5]	Viti, Bhat Pata, Bhati, Vaita, Foksha, Baadbagora.	Lamiaceae	Whole plant
2	*Duranta repens* L[5]	Kata-mehandi, Kata-mehendhe	Verbenaceae	Whole plant
3	*Lantana camara* L[5]	Chaturaangi, Jangoli-janglog	Verbenaceae	Leaf, root, flower
4	*Nyctanthes arbor tristis* L[5]	Shefali, Sheuli, Sheuly-phang	Oleaceae	Leaf
5	*Dracaena reflexa* Lamk[6].	Hasina Pleomele, Song of India	Asparagaceae	Leaf and bark decoction
6	*Cinnamosma fragrans* H[6].	Sakarivohazo,	*Canellaceae*	A decoction of the leaf and bark
7	*Andropogon schoenanthus/nardis* L[6].	veromanitra	*Gramineae*	The leaf decoction
8	*Desmodium mauritianum D.C*[6].	Bean of the hare oganana	*Leguminosae*	A decoction of the leaf and bark
9	*Desmodium hirtum Grill* and Perr[6].	Tsilavindrivotro	*Leguminosae*	A decoction of the leaf and bark
10	*Tristellateia madagascariensis* Poir[6].	Menahelika	*Malpighiaceae*	The leaf decoction
11	*Ficus megapoda* Bak[6].	Mandresy	*Moraceae*	A decoction of the leaf and bark
12	*Nymphaea lotus* L[6].	Voahirana or retsimilana	*Nymphaeaceae*	A decoction of the leaf and bark
13	*Vepris ampody* H. Perr[6].	Ampody	*Rutaceae*	A decoction of the leaf and bark
14	*Zanthoxylum tsihanimposa* Bak[6].	Tsihanihimposa	*Rutaceae*	A decoction of the leaf and bark
15	*Peddiea involucrata* Bak[6].	Montana	*Thymelaeaceae*	A decoction of the leaf and bark

16	*E. angolense*[7]	Mukus, Edinam	*Meliaceae*	Plant extract
17	*P. nitida*[7]	waboom, blousuikerbos	*Apocynaceae*	Plant extract
18	*T. hensii*[7]		*Thomandersiaceae*	Plant extract
19	*Shumanniophyton magnificum*[7]	Sierra Leone Mend	*Rubiaceae*	Plant extract
20	*Rauvolfia vomitoria Afzel.*[8]	Omuatabusinde/Kinyabusinde	*Apocynaceae*	The leaves decoction
21	*Canarium schweinfurtii* Engl[8]	Muubani	*Burseraceae*	The bark scent
22	*Zehneria scabra* Sond[8]	Akabindizi	*Cucurbitaceae*	The leaves decoction
23	*Bridelia micrantha* (Hochst.) Baill[8]	Mshamako	*Euphorbiaceae*	Root decoction
24	*Tragia furialis* Bojer[8]	Omugonampili	*Euphorbiaceae*	The leaves decoction
25	*Abrus precatorius* L[8]	Kaligaligo	*Fabaceae*	The leaves decoction
26	*Dolichos kilimandscharicus* Taub[8]	Khat	*Fabaceae*	The leaves
27	*Sesbania microphylla* E.Phillips & Hutch[8]	Msenga, Mbondo	*Fabaceae*	The leaves
28	*Senna occidentalis (*L.) Link[8]	Omwetanjoka	*Fabaceae*	The leaves decoction
29	*Tetradenia urticifolia* (Baker) Phillipson[8]	Lwamo	*Lamiaceae*	The leaves decoction
30	*Solanum aculeastrum* Dunal[8]	Omulembezi, Entobatobe	*Solanaceae*	Fruits
31	*Caesalpinia nuga* (L.)W. T. Aiton[9]	Krung-khai	*Fabaceae*	Seed
32	*Adansonia digitata* L[9]	Kattio-daghor	*Bombacaceae*	Leaf, root, Flower
33	*Jatropha gossypifolia*[9]	Titto-long	*Euphorbiaceae*	Seed
34	*Rauwolfia serpentina* Benth[9]	Sharpagandha	*Apocynaceae*	Root
35	*Hodgsonia macrocarpa* Cogn[9]	Keha-pang	*Cucurbitaceae*	Fruit
36	*Erythrina variegata* L[9]	Mada-kamiama-fang	*Fabaceae*	Bark
37	*Streblus asper* Lour[9]	Sarwa	*Moraceae*	Bark
38	*Clerodendrum viscosum* Vent[9]	Kung-sroi-ma	*Verbenaceae*	Leaf
39	*Amaranthus spinosus*[9]	Kang-chuo	*Amaranthaceae*	Root
40	*Mussaenda corymbosa* A.L. de Jussieu[9]	Mok-ae	*Rubiaceae*	Leaf
41	*Scoparia dulcis*[9]	Tapra-amkanlu	*Scrophulariaceae*	Leaf
42	*Ocimum sanctum* Linn.[10]	Tulsi	*Lamiaceae*	Plant extract
43	*Cryptolepis Sanguinolenta*[11,12]	Nibima	*Apocynaceae*	aqueous extract
44	*Artemisia annua*[11,12,13]	sweet wormwood	*Asteraceae*	aqueous extract
45	*Dichroa Febrifuga*[11]	Gigil,Tataruman	*Hydrangeaceae*	aqueous extract
46	*Kalanchoe pinnata*[12]	Air Plant, Life Plant	*Crassulaceae*	Leaf
47	*Esenbeckia febrifuga* (A.St.-Hil.) A.Juss. ex Mart[13]	Três folhas	*Rutaceae*	Hexane/ethanolic extracts
48	*Boerhavia hirsuta*[13]	Pega Pinto	*Nyctaginaceae*	Hexane/ethanolic extracts
49	*A. austral*[13]	Carrapicho	*Cerambycidae*	Hexane/ethanolic extracts
50	*Tachia guianensis*[13]	Caferana	*Gentianaceae*	Hexane/ethanolic extracts
51	*Cecropia glaziouvii*[13]	Umbauba	*Urticaceae*	Plant
52	*Bidens pilosa*[13]	Cobbler's Pegs or Spanish Needle	*Asteraceae*	Extracts and fractions of plants
53	*A.amazonicus Ducke*[13]	Indian beer	*Rhamnaceae*	Dried Ground roots
54	*Alstonia scholaris* (L.) R.Br[14]	Yaknalae's Ita	*Apocynaceae*	Leaves and bark
55	*Aristolochia indica* L. [14]	Yaki'Itchale	*Meliaceae*	Leaves and bark decoction
56	*Maclura* sp. [14]	Iveriate	*Moraceae*	Leaves
57	*Polygala paniculata* L. [14]	Nuva gihi	*Polygalaceae*	Leaves and fruits
58	*Polyscias filicifolia*[14]	Iriduki'Imetchale	*Araliaceae*	Leaves
59	*Setaria sp.* [14]	Nomu suva	*Poaceae*	whole plant
60	*Tasmannia piperita* (Hook. f.) Miers.[14]	Iridukichale	*Winteraceae*	Leaves
61	*Tristiropsis sp.* [14]	Longola	*Sapindaceae*	Soft Leaves
62	*T. herba-barona*[15]	Caraway thyme	*Lamiaceae*	Aerial parts

63	*S. thymbra*[15]	Thyme-leaved savory	*Lamiaceae*	Aerial parts
64	*M. communis*[15]	Myrtle	*Myrtaceae*	Aerial parts
65	*Cymbogon citratus*[15]	Lemon grass, oil grass	*Poaceae*	Vapours of a decoction
66	*Ocimum gratissimum*[15]	Clove Basil, African Basil	*Lamiaceae*	Vapours of a decoction
67	*Cuviera longiflora*[16]	-	*Rubiaceae*	Leaves
68	*Dacryodes edulis*[16]	Zo'o	*Burseraceae*	Leaves
69	*Eucalyptus globules*[16]	Klatusse	*Myrtaceae*	Leaves
70	*Kotschya speciosa*[16]	Hepper	*Leguminoceae*	Whole plant
71	*Coula edulis*[16]	Walnut	*Olacaceae*	Stem bark
72	*Vernonia amygdalina*[16]	Bitter leaf	*Asteraceae*	Leaves, root bark
73	*Vismia guinensis*[16]	-	*Asteraceae*	Stem bark
74	*Nauclea latifolia*[17]	Pin cushion tree	*Rubiaceae*	stems and roots
75	*Pseudocedrela kotschyi*[17]	Dry zone cedar; Hard cedar-mahogany	*Meliaceae*	Leaves
76	*Prosopis africana*[17]	African mesquite	*Fabaceae*	Plant
77	*Trichilia emetica*[17]	Natal Mahogany	*Meliaceae*	Plant
78	*Diospyros quaesita* Thw. [18]	Calamander	*Ebenaceae*	Leaf and stem
79	*Gongronema napalense* (Wall.) Decne.[18]	-	*Asclepiadaceae*	Whole flower(wine)
80	*Nauclea orientalis* (L.)[18]	yellow cheesewood	*Rubiaceae*	Dried plant/stem
81	*Rourea minor* (Gaertn.) Aubl. [18]	A woody vine	*Connaraceae*	Dried stem
82	*Amaranthus hybridus*[19]	Slim amaranth	*Amaranthaceae*	Leaves
83	*Uvaria scheffleri* Diels[19]	Mukukuma	*Annonaceae*	Leaves
84	*Carissa edulis* (Forssk.)[19]	Simple-spined num-num	*Apocynaceae*	Root bark
85	*Landolphia buchananii* (Hallier f.)Stapf[19]	Apricot vine	*Apocynaceae Juss*	Leaves
86	*Rauwolfia* Cothen. [19]	-	*Apocynaceae Juss.*	Root bark
87	*Vernonia amygdalina* A. Chev. [19]	Ewuro	*Asteraceae*	Leaves
88	*Tridax procumbens* L. [19]	lilac tassel flower	*Asteraceae*	Whole plant
89	*Commiphora schimperi* (O.Berg)Engl. [19]	Glossy-leaved corkwood	*Burseraceae Kunth*	Roots, stem bark
90	*Combretummolle* Engl. & Diels[19]	Velvet bush willow	*Combretaceae*	Leaves
91	*Gerrardanthus lobatus* C. Jeffrey[19]	Cogniaux	*Cucurbitaceae*	Roots
92	*Momordica foetida* Schumach. [19]	Concombre sauvage	*Cucurbitaceae Juss*	Leaves
93	*Ricinus communis* L. [19]	Castor Bean	*Euphorbiaceae*	Roots, leaves
94	*Suregada zanzibariensis* Baill. [19]	Prota	*Euphorbiaceae Juss*	Root bark
95	*Albizia anthelmintica* Brongn. [19]	Kyoa	*Fabaceae*	Stem bark
96	*Acaciaseyal* Delile[19]	Shittimwood	*Fabaceae*	Roots
97	*Dichrostachys cinerea* (L.) Wight&Arn. [19]	Sickle bush	*Fabaceae*	Roots
98	*Tamarindus indica* L. [19]	Tamarind	*Fabaceae Lindl*	Roots, leaves
99	*Harungana madagascariensis* Lam. Ex Poir.[19]	Praying hands	*Hypericaceae Juss.*	Root bark, stem bark
100	*Hoslundia opposita* Vahl[19]	Orange bird berry	*Lamiaceae*	Roots
101	*Ocimum gratissimum* L.[19]	African basil	*Lamiaceae*	Leaves
102	*Clerodendrum myricoides* R. Br. [19]	Bandamuchenene	*Lamiaceae*	Root bark
103	*Adansonia digitata* L. [19]	Baobab, Dead-Rat Tree	*Malvaceae*	Leaves
104	*Grewia hainesiana* Hole[19]	Phalsa	*Malvaceae Juss.*	Leaves
105	*Grewia trichocarpa* Hochst.exA.Rich. [19]	Ecol. Status	*Malvaceae Juss.*	Roots
106	*Azadirachtaindica* A. Juss. [19]	Neem	*Meliaceae*	Roots, stem bark, leaves
107	*Cissampelos mucronata* A. Rich. [19]	Heart-leaved vine	*Menispermaceae Juss*	Root bark
108	*Ficus bussei* Warb.ex Mildbr.& Burret[19]	Rudraksha	*Moraceae*	Roots
109	*Securidaca longifolia* Poepp. [19]	-	*Polygalaceae*	Roots
110	*Canthium glaucum* Hiern[19]	Bluish-Green, Glaucous	*Rubiaceae*	Fruits
111	*Pentas longiflora* Oliv. [19]	Golden Shrimp Plant	*Rubiaceae*	Root bark
112	*Clausena anisata* (Willd.) Hook. f.	Horsewood	*Rutaceae*	Leaves

	ex Benth[19]			
113	*Zanthoxylum chalybeum* Engl. [19]	Bemba	*Rutaceae*	Root bark
114	*Toddalia asiatica* (L.) Lam. [19]	Orange climbe	*Rutaceae*	Root bark
115	*Fagaropsis angolensis* (Engl.) Dale[19]	Caper-bush	*Rutaceae Juss.*	Leaves
116	*Teclea simplicifolia* (Engl.) I. Verd. [19]	Teclea nobilis	*Rutaceae Juss.*	Roots
117	*Flacourtia indica* (Burm.f.)Merr. [19]	Governor plum	*Salicaceae*	Roots
118	*Lantana camara* L. [19]	Shrub verbenas	*Verbenaceae*	Leaves
119	*Aloe vera* L. ex Webb[19]	English musambra aloe	*Xanthorrhoeaceae*	Leaves
120	*Hypericum lanceolatum*[20]	Curry bush	*Hypericaceae*	Stem bark
121	*Caesalpinia pluviosa*[21]	Sibipiruna	*Fabaceae*	Crude extract
122	*Icacina senegalensis* A. Juss.[22]	Bankanas, False yam	*Icacinaceae*	Leaf extracts
123	*Holarrhena antidysenterica*[23]	Bitter Oleander	*Apocynaceae*	Plant extracts
124	*Viola canescens*223]	Viola serpens var.	*Violaceae*	Plant
125	*Aframomum* sp[24]	Grains of paradise	*Zingiberaceae*	Plant
126	*Vernonia guineensis*[24]	Ginseng	*Asteraceae*	Plant
127	*Spilanthes oleracea*[24]	Toothache Plant	*Asteraceae*	Plant
128	*Alstonia boonei*[24]	Alstonia, Cheesewood	*Apocynaceae*	Plant
129	*Ambrosia Maritima*[25]	Damsisa	*Asteraceae*	Whole plant
130	*Aristolochia Bracteolata*[25]	Um Galagel	*Aristolochiaceae*	Leaves
131	*Citrullus colocynthis*[25]	El-Handal	*Cucurbitaceae*	Seed
132	*Croton zambesicus*[25]	Um-Geleigla	*Euphorbiaceae*	Fruit
133	*Nigella sativa*[25]	Kamun-Aswad	*Ranuneulaceae*	Seed
134	*Solenostema argel*[25]	El-Hargel	*Ascepiadaceae*	Leaves
135	*Allium sativum L.*[26]	Sunkurtae	*Alliaceae*	Fresh or dry fruits
136	*Artemisia afra* Jack. ex Wild[26]	Chugughee	*Asteraceae*	Fresh/dry leaves
137	*Lepidium sativum* L. [26]	Feaxxo	*Brassicaceae*	Seeds
138	*Croton macrostachyus* Del. [26]	Bissano	*Euphorbiaceae*	Fresh/dry leaves
139	*Phytolacca dodecandra* L'Herit[26]	Phytolaceae	*Indoodae*	Fresh/dry leaves

3. Discussion

The present article brings out information on different medicinal plants used in various parts of world for the treatment of malaria. One hundred thirty nine medicinal plants have been recorded here in for their use as anti-malarials. Although traditional remedies are widely used to treat malaria, and are often more available and affordable than Western drugs, they are not without limitations. Some of the limitations include unpredictable efficacy, non-established dosage and the short and long term safety are not known.

The development of new antimalarials from the highly active natural products, which have already been discovered, is crucial in order to overcome the increasing resistance of *Plasmodium* to available antimalarial drugs. Therefore, there is a need to advance the work on plants which have already been shown to have antimalarial activity through further *in vitro* and *in vivo* testing in animal models of malaria followed by sub-acute and chronic toxicity tests. This is likely to reveal suitable candidate molecules which may serve as leads which can be optimized followed by development into new antimalarials. This task will require capacity building in the various facets of such an approach, which capacity is inadequate at the moment. This strategy if pursued from drug discovery research on to preclinical followed by clinical studies will certainly yield the much desired highly efficacious and safe antimalarials.

However, further studies including controlled clinical trials are necessary before specific traditional remedies can be recommended on a large scale.

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Golden Heart of the Nature: *Piper betle* L.

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The heart shaped betel leaves are found in ancient Sanskrit texts, including Charaka, Sushruta Samhita and Astanga Hradayam. *Piper betle* L. have been used in chinese, Indian folk medicine for centuries. In this review, different research works related to Ayurvedic consequence, geographical distribution and cultivation, morphoanatomy, phytochemistry, biological activities, along with tradomedicinal uses which signify the tremendous potential of "*Piper betle* L." to come out as *Green medicine*. The objective of it is to revels the potential effect of this plant in the development of therapeutically active herbal drugs against different microbial infections especially for oral cavity, which also gives the opportunity to pharmaceutical companies interested in formulation and production of natural product based drugs targeted towards specific aliments

Keyword: *Piper betle* L., Morpho-anatomy, Antimicrobial Activity.

1. Introduction

Piper betel is blessed as evergreen and perennial plant, that has God created and have given the shape of his own heart. Anthropologists have found traces of betel in the spirit caves in Northwest Thailand dating back as to 5500-7000BC, which is even before systematic and organised agriculture came to be practiced. There have been similar findings in Timor in Indonesia going back to 3000 BC and in the blacked teeth of a human skeleton in Palawan in a Philippines going back to 2600 BC. It had found a place in the most ancient Sri Lanka Historical Book "Mahawamsa" written in *palli*. Even today some hardened betel chewers in Thailand, Myanmar and Indonesia with black teeth as result of long years of chewing[1]. There is archaeological evidence that the betel leaves have been chewed along with the Arica nut since very ancient times it is not known when these two different stimulants substances were first put together. It may difficult to clearly ascertain the period when the tradition of paan chewing was started. However, its mention in the Vatsyayana's Kamsutra and Kalidas's Raghuvamsa in itself reflects the antiquity of this practice. Social status of pan can also be appreciated from the fact that it was considered to be a great honour to receive *paan bida* [A pair of leaves with Churna (Lime), kattha (catechu) and supari (areca nut)] from kings and nobles. Such was the status of pan in ancient India. During this period (Circa 600AD)

words like Tambuladhikara, Tambuladyaka, Tambuladayini and Tambulika etc. used in different texts. Some of the common usages are mention in Kadamberi[2]. Paan has been referred to in Sakta-tantra as one of the means of achieving siddhi. It was believed that without betel chewing and offering pan to Guru no siddhi can be gained. Tambool has also been referred to as facilitating the sadhak in chewing dharma, yasha aisvarya, Srivairagya and mukti. Tambool find frequent mention in writings from the fifth century onwards especially, Reetikaaleen Hindi poetry[3, 4, 5].

2. Ayurvedic Significance:

Piper betel is a Vedic plant and its Vedic name is Saptasira[6] and in sankrit it known as Tambool, Nagvelleri, Nagani[7] were used as remedy against various diseases. In most of these texts were various medicinal properties has been highlighted. Reference to Tambool occurs right from Vatsyayana's Kamasutra & Panchatantra down to Kalhan's Rajatarngni (which may perhaps be the last of the recognise old Sanskrit writing of historical significance). Tambool has been referred to, thus roughly across a period of about 2000 year.

In Ayurveda medicine system, the properties of betel leaf describe as given below[8]:

Guna (Quality)	:Laghu, Ruksha, Tikshan
Rasa (Taste)	:Tikta
Vipak (Metabolism)	: Katu
Virya (Potency)	: Ushan
Prabhav (Impact)	: Hridya

In Ayurveda betel leaf extract is frequently used as an adjuvant & mixed with different medicines possibly for better effects beside its independent use as medicine. In Susrta Samhita (Ch 28-46, 279-280) tambool leaves have been described as aromatic, sharp, hot, acrid and beneficial for voice, laxative, appetizer, beside this they pacify vata and aggravate pitta. Similar characteristics have been described in Bhabaprakash (Sloka180-183)[2].

ताम्बूलवल्ली तम्बूली भागिनी नागवल्लरी I ताम्बूलं विशदं रूच्यं तीक्ष्णोष्णं तुवरं सरमू II११II
वश्यं तिक्तं कटु क्षारं रक्तपितकरं लघु I बल्यं श्लेष्मास्यदौर्गन्ध्यमलवातश्रमापहमू II१२II
(भा. प्र. गुडूच्यादिवर्ग ११-१२)

In addition to these, the aphrodisiac effect of betel chewing has been indicated in ancient texts. Pan also believed to provide strength to heart and regulate blood. Its utility as anti-inflammatory and anti-microbial is emphasized at several place. In ayurveda it acts as vata and kapha suppressant. It also helps in expelling out the mucus from the respiratory tract because of its hot potency[7]. According to Yunani system the leaf has a sharp taste and good smell improves taste and appetite, tonic to brain, heart and liver, lessens the thirst, clear the throat and purify the blood[9].

3. Plant Profile:

I. Botanical name and taxonomic classification[10,11]:

- Kingdom: Plantae
- Division: Magnoliphyta
- Class: Magnolipsida
- Order: Piperales
- Family: Piperaceae
- Genus: Piper
- Species: *betle*
- Binomial name*: Piper betle* L.

II. Vernacular name[7, 12, 13]:

- Sanskrit: Nagavallari, Nagini, Nagavallika,Tambool, Saptashira, Mukhbhushan, Varnalata
- Malaysia: Sirih, Sirih melayu, Sirih cina, Sirih hudang, Sirih carang, Sirih kerakap
- English: Betel, Betel pepper, Betel-vine
- Tamil: Vetrilai
- Telugu: Nagballi, Tamalpaku
- Hindi: Pan

- Gujurati: Nagarbael
- Marathi: Nagbael
- Bengali: Pan
- Arabic: Tambol, Tambool
- Semang: Serasa, Cabe
- Jakun: Kerekap, Kenayek
- Sakai: Jerak
- Javanese: Sirih, Suruh, Bodeh
- Thai: Pelu

III. Geographical distribution:

Piper betel is native to central and eastern Malaysia and was taken into cultivation more than 2500 years ago throughout Malaysia and tropical Asia. It reached Madagascar and East Africa much later and was also introduced into the West Indies. Written Chinese sources from the period of the Tang dynasty (A.D. 618-907) described Southeast Asia as a region of betel users. Betel chewing was widespread in South India and South China when the first Europeans arrived in the 15th century. With known ethno medicinal properties, this plant is widely use in India, Indonesia and other countries of the Indo-China region (Malaysia, Vietnam, Laos, Kampuchea, Thailand, Myanmar, Singapore)[2,12] as shown in Fig no. 01.

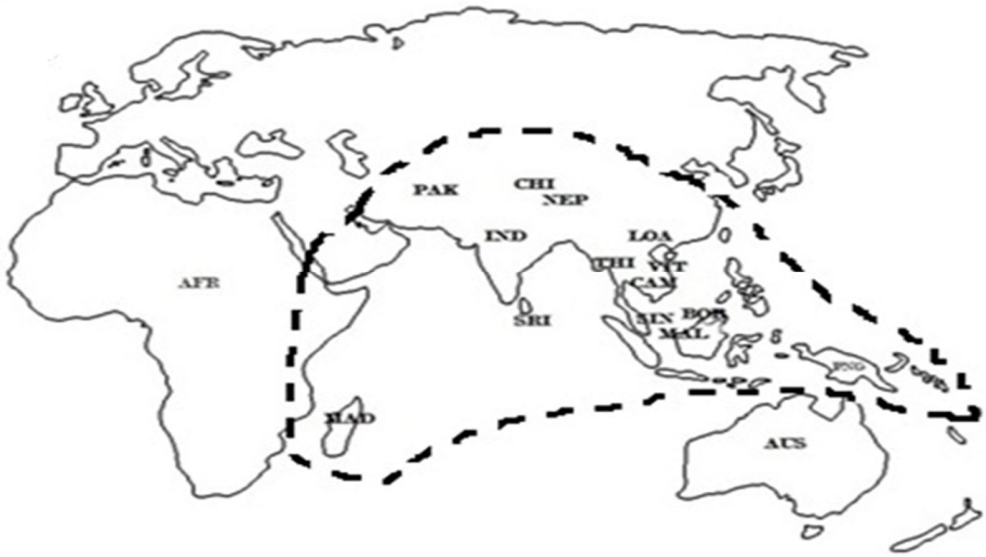


Fig.no.1. Region within the dotted lines shows major areas of *Piper betel* consumption.

Where, AFR: Africa, MAD: Madagascar, PAK: Pakistan, IND: India, NEP: Nepal, SRI: Sri Lanka, THI: Thailand, CAM: Cambodia, MAL: Malaysia, PNG: Papua New Guinea, BOR: Borneo, SIN: Singapore, LOA: Laos, VIT: Vietnam

Fig.no.1. Region within the dotted lines shows major areas of piper betel consumption. Barring the areas where the climatic conditions (high or low temperature accompanied by very low humidity) do not support its cultivation. Betel vine is widely distributed in all over the state of India except northern regions (Jammu and Kashmir, Haryana, Punjab, Himachal Pradesh), due to sever winter and northern west (Rajasthan, Gujarat) due to hot dry summer[14,15] as shown in Fig No. 02.

IV. Habitat & Ecology

Betel vine thrives under humid forest conditions with high relative humidity. It prefers deep, well-drained, friable loamy and clayey soils, rich in organic matter with a pH of about 7-7.5. It flourishes in areas with 2250-4750 mm annual rainfall and is cultivated at altitudes up to 900m[12].

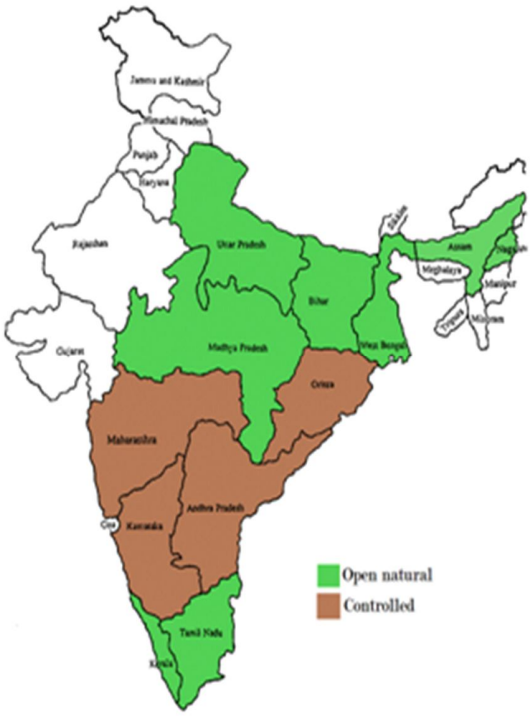


Fig no.2 Major Betel vine growing areas in India.

V. Cultivation and propagation

Cultivation

It is cultivated in soil, which is black, friable, clay loam resembling tank earth, contains large portion of organic matter, but the best pans are grown in Bengal on light loam sightless reddish in colour. The plant grown from sea level to1000mt, rainfall of more than 179cm is necessary. It thrives best under tropical forest condition with shade considerable humidity & plenty of soil moisture. Broadly there are two cropping system under natural conditions and controlled conditions. The open system of cultivation under natural conditions is practiced in regions where conditions of high humidity and moderate sunshine prevail throughout the year. The type is basically a climber which required supporting tree like areca nut (*Areca catechu*) and coconut (*Cocos nucifera*) and generally attain 10-15 meters height with profuse branching at the top and lot of foliage. Another type of cropping is partially controlled cultivation which is common in regions where high humidity and low sunshine conditions do not prevail round the year and the plants are to be protected from excessive sunlight dry air. Vines are trained on live support of plants such as *Sesbania grandiflora*, *Sesbania sesban, Erythrina indica* and *Moringa oleifera* which also provide shade and contribute to the increase in humidity. The close planting of vines also helps in moisture retention and creation of microclimate conducive to growth. Unlike the practice in north-east where the vines are allowed to attain height of the supporting tree, it is just 1-2 metre only. This change in plant habitat is achieved by suppressing the linear growth and promoting profuse branching. The plant type in partially controlled cultivation is modified to suit the prevailing conditions. Such weather conditions with adequate sunshine (photo synthetically active radiations 1200-1800µ mole $m^{-2}S^{-1}$) are hardly conducive to good growth. With the advancement in greenhouse/ glasshouse construction technology, now it is quite easy to shift plants from their natural habitat and grow them under controlled conditions by regulating precisely the humidity incident of light and temperature. Cultivation under controlled conditions is practiced in subtropical regions where relative humidity is often low and temperature remaining high (max. temp. above 40°C) in summer and low (min. temp. below 10°C) in winters. The cultivation of betel vine under controlled conditions is also a case of creating indigenous system of "environmental chamber" with the materials available in nature. The covered structure is known as bareja. The bareja structure is made up of locally available materials such as wooden poles, bamboos stalk and a variety of grasses as thatching material. Barejas are generally rectangular in shape and normal workable size often 50×30mt. When a bareja is erected on slopes, west to east gradient is preferred. It is mandatory to keep the east and west side shorter than north and south sides. This shape carries sound logic in terms of humidity and temperature control. Westerly winds are not only dry but also high or low temperature depending on the season. In order to minimise the wind impact, thatching on the west side is thickest so that the wind velocity entering the

bareja is reduced. Thatching on the north side of the bareja is thicker than on the eastern side. The side facing south is kept thinnest, possibly to allow some light from that direction. The top of the bareja is covered by leaves of locally available grasses in order to reduce the light incident on the plant and soil surface. Height of the mandap varies from 2-3 meters or little more depending on the season and growth of the vine. Thus this practice of cultivation in the bareja may be older than 600-400 BC. If the proper irrigation and sunlight controlling cannot be maintained it gets some negative impact in the size, shape, colour, and taste of its leaves. Some of the impacts are given below:

1) Too much light, but adequate soil moisture: leaf becomes darker green, harder, rough taste.
2) Too less light, but adequate soil moisture: leaf becomes light green, thinner leaf, short lasting as chewed, taste becomes lighter, smaller leaf sizes.
3) Adequate light, but less soil moisture: hard leaf, smaller size, rough taste, early falling leafs, turn leafs yellow, weak plant stem, less number of leafs per plant, broken-uneven edges.
4) Adequate light, but too much soil moisture: rotten roots, dying plants, slowdown in leaf maturity, weaker taste, leaf uneven surfaces[2,6].

Propagation

Propagation is easy by root division or cuttings, preferably taken in spring or summer. Betel leaf requires a rich soil and prefers a semi-shade position. Regular feeding and watering will keep it growing very lush.

VI Morpho-Anatomy.

a. Macroscopic characters:

- Colour: Yellowish green to dark green in colour with glossy upper surface.
- Odour: Characteristic and Pleasant.
- Taste: The betel leaves are aromatic with varied taste, ranging from sweet to pungent due to the presence of essential oils.
- Shape and Size: The betel leaf is a heart shaped with different size. The size of the leaf varies with different cultivar from 7-15cm in length and 5-14cm in width. Betel leaves are simple alternate stipulate petiolate with 0.75 to 3.8cm, ovate oblong broadly ovate cordate or obliquely elliptic entire glabrous coriaceous 10 to 18 cm long and 5 to 10 cm broad acuminate oblique and rounded base[16,17,18].

b. Microscopic characters:

Transverse section of leaf through midrib shows four layered upper and two layered lower epidermis. The cuticle is thick on the upper epidermis and thin on the lower epidermis. The cells of the outer epidermal layers on both sides of the leaf are small, that possess tannins and oils. The sub epidermal cells on the abaxial side are enlarged and they store water. Crystal and oil reserves are found in the sub epidermal cells on both sides. The palisade layer are well distinguished they are double layered short wide compact cells and mesophyll cells are 3-4 layered and small lobed. Thick walled irregular secretory cells are seen with dense contents of probable an essential oil as shown in fig no. 3 and 4.

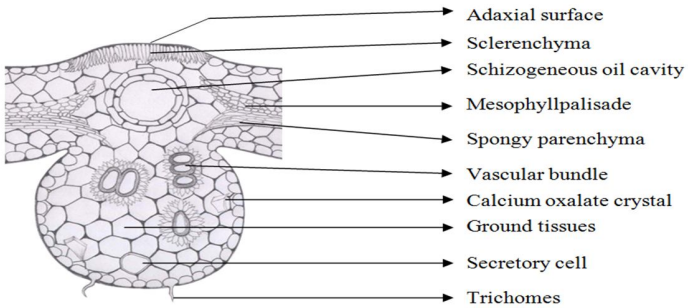


Fig no. 3 Transverse section of Betel leaf with midrib

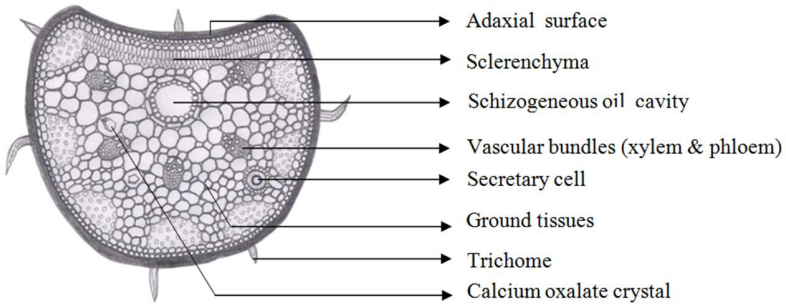
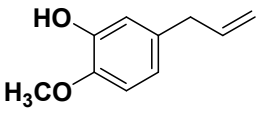


Fig no. 4 Transverse section of petiole of Betel leaf

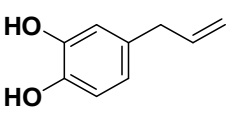
The leaves are hypostomatic, tetra-cytic stomatal complexes are common which is the characteristic feature of the Piperaceae. The trichomes are glandular which have unicellular apical cell and a short pedicel. The pedicle has thicker wall, surrounded by 5 or 6 epidermal cell arranged in a rosette disc like manner. The apical cell of trichome is slightly pointed or clavate shaped. The vascular bundles located at the centre of midrib portion single ovate collateral cells with destea of xylem elements and a thick phloem was observed. The stems are dichotomous, articulate, swollen and rooted at nodes 3mm in diameter, woody and with 2.5 to 4cm long internodes. Stem stout with pinkish-stripe along node dilated and rooting. The inflorescence is an axillaries spike which is 5.5 cm long. The fruits are drupaceous, orange, and 3mm in diameter[8,16,17,18].

VII. Phyto-constituents:

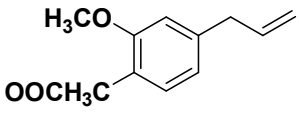
The leaf contains Water (85-90%), Proteins (3-3.5%), Carbohydrates (0.5-6.1%), Minerals (2.3-3.3%), Fat (0.4-1%), Fibre (2.3%), Essential oil (0.08-0.2%), Tannin (0.1-1.3%), Alkaloid (arakene). It also contains different vitamins like Vitamin-C (0.005-0.01%), Nicotinic acid (0.63-0.89mg/100gms), Vitamin-A (1.9-2.9mg/100gms), Thiamine (10-70µg/100gms), Riboflavin (1.9-30µg/100gms) beside this it contains minerals such as Calcium (0.2-0.5%), Iron (0.005-0.007), Iodine (3.4µg/100gms), Phosphorus (0.05-0.6%), Potassium (1.1-4.6%)[14]. Leaves contain bitter compounds that are about (0.7-2.6%). The specific strong pungent aromatic flavour in leaves is due to phenol and terpene like bodies[19]. The total phenol contain is vary on the gender. The male plant contains three fold higher total phenols content and two fold higher thiocyanate content as compare to female plant. The quality of the leaf depends upon the phenolic content, i.e., more the phenolic content betters the leaf quality[20]. Recently many researches works shows the betel leaves contains starch, diastases, sugars and an essential oil composing of safrole, allyl pyrocatechol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, etc. as the major components[21,22]. Phytochemical investigation on leaves revealed the presence of Alkaloids, Carbohydrate, Amino acids, Tannins and Steroidal components[23]. The middle part of the main vine contains largest quantity of Tannin. The terpenoids include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, pinene, Chavicol, ally pyrocatechol, carvacrol, safrole, eugenol and chavibetol are the major phenols found in betel leaf. Eugenol was identified as the antifungal principle in the oil. The fresh new leaves contain much more amount of essential oil diastase enzyme and sugar as compare to old leaves. Chavicol is four times potent as antiseptic agent as compare to carbolic acid. Some of the phyto-constituents of the plant are given below[15,24-31].



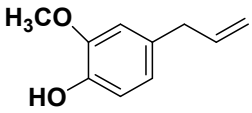
Chavibetol



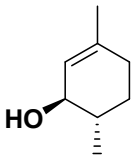
Allypyrocatechol



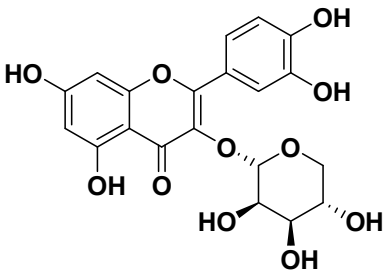
Chavibetol acetate



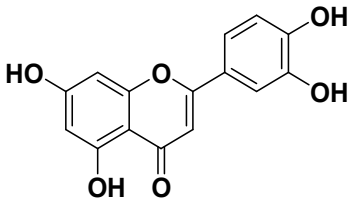
Eugenol



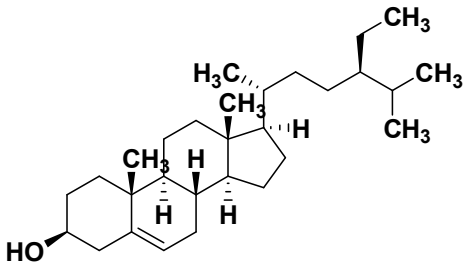
Piperitol



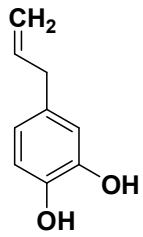
Quercetin



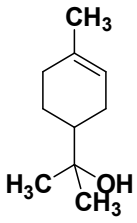
Luteolin



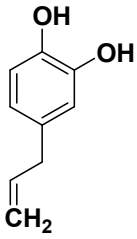
β- sitosterol



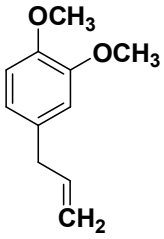
Hydroxychavicol



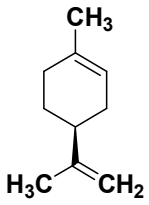
α-terpineol



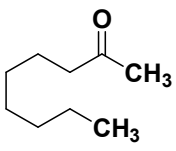
Allyl catecol

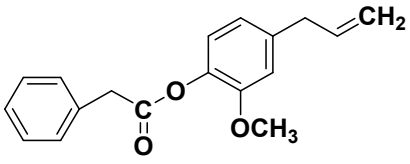


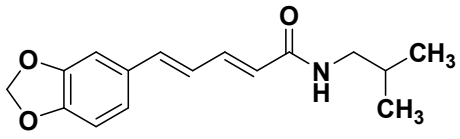
Eugenol methyl ether



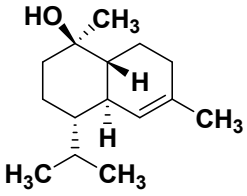
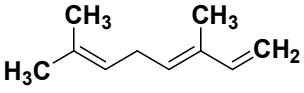
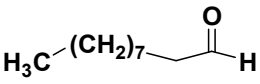
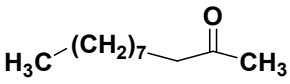
D- limonene



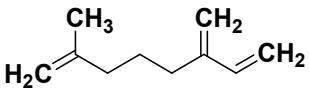
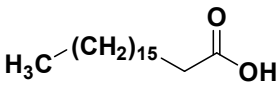
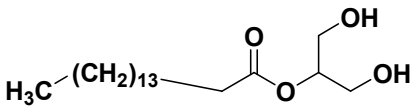




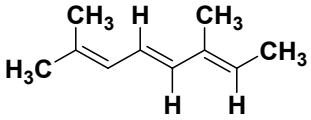
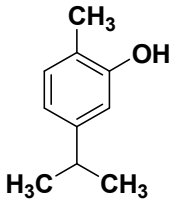
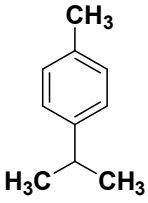
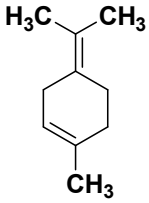
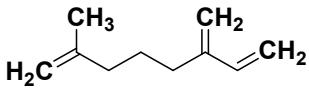
2-noanone 4-allyl phenyl acetate (db) Piperlonguminine

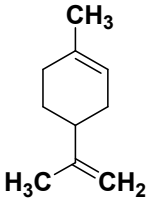
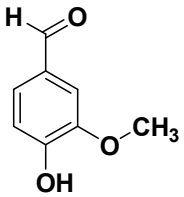
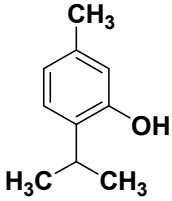
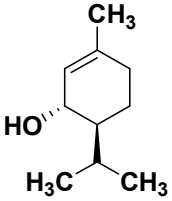
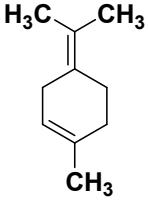
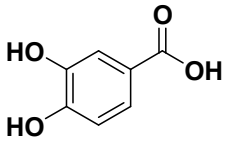
α-cadinol Ocimene N-decanal 2-undecanone

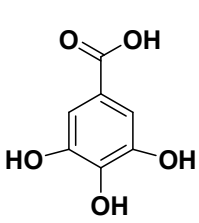
Myrcene Stearic acid 2- Mono palmitin

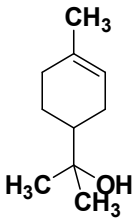
Allo ocimene Cavacrol Cymene Terpinolene α-Myrcene

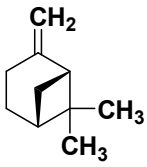
Limonine Vanillin Thymol Cis-piperitol Terpinolene Procatechuic acid



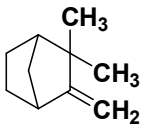
Gallic acid



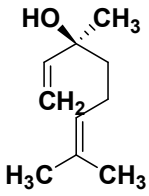
α-terpineol



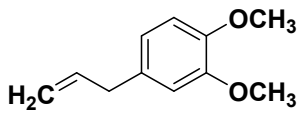
β-pinene



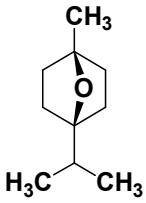
Camphene



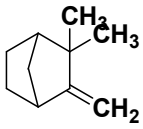
Linalool



Allyl diacetoxy benzene



Eucalyptol



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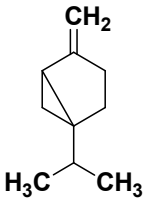
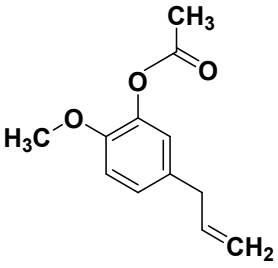
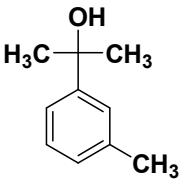


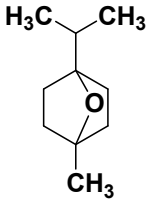
Sabinene



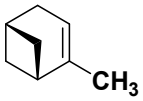
3-allyl-6-methoxyphenolb



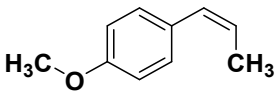
m-Cymen-8-ol



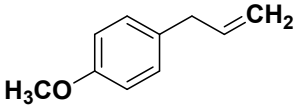
1, 4 cineole



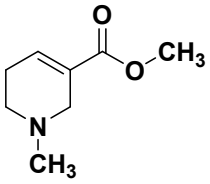
α-pinene



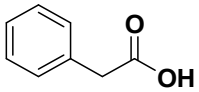
Anethole



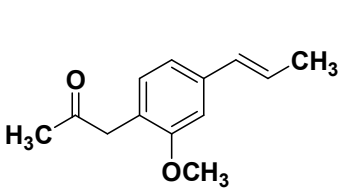
Estragol



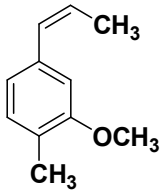
Arecoline



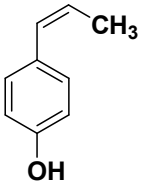
Benzene acetic acid



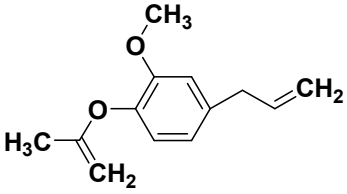
Iso eugenyl acetate



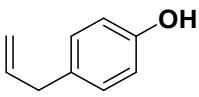
Isoeugenol



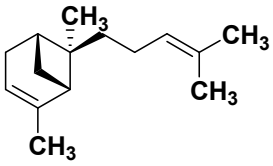
Chavicol



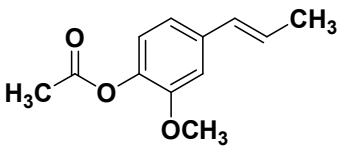
Eugenyl acetate



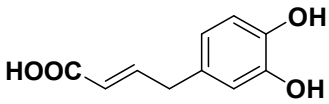
4-allyl phenol



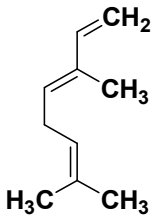
α-bergamotene



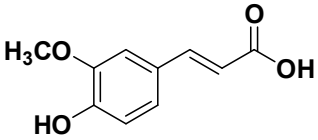
Isoeugenyl acetate



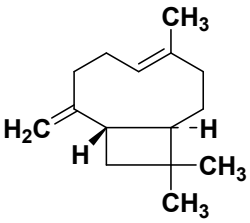
Caffeic acid



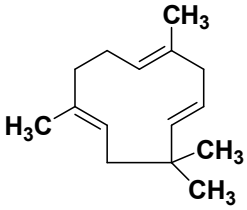
(E)-ß-ocimene



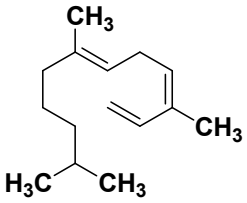
Ferulic acid



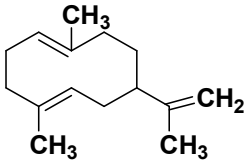
Carryophyllene



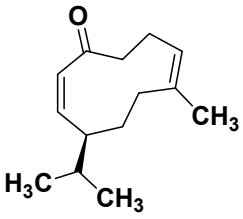
Humlene



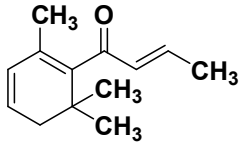
α-farnesene



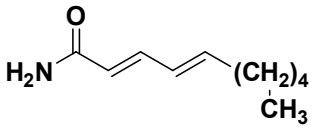
Germacrene-A



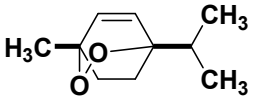
Germacrene-D



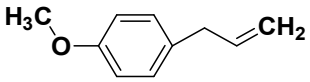
(E)-ß- Damascenone



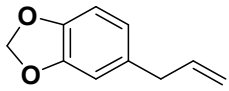
4E-decadienamide



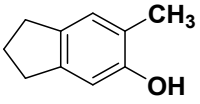
Isoascaridole



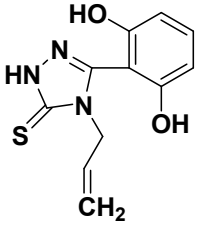
4-Allyl anisole



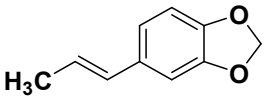
Safrole



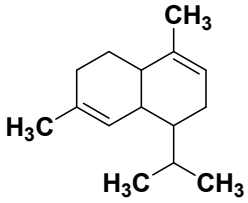
5-Indanol



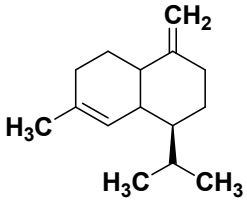
4-allyl resorcinol



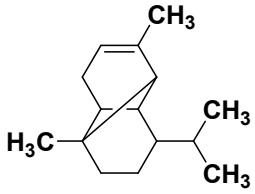
ß-iso safrole



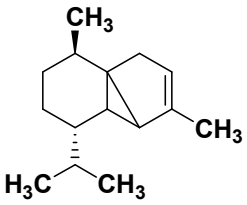
α-muurolene



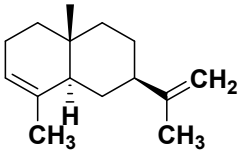
Cadinene



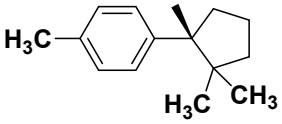
α- copaene



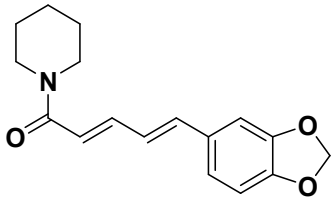
α-cubebene



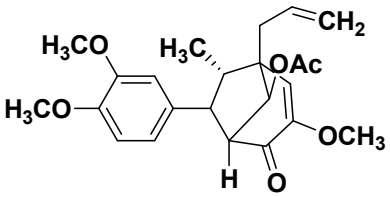
α-selinene



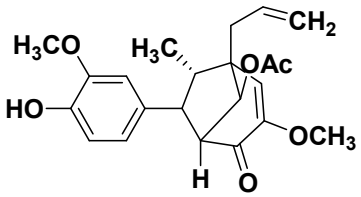
Cuparene



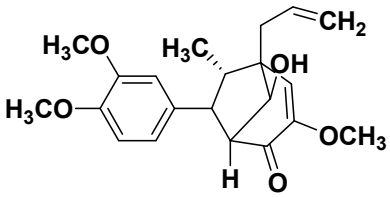
Piperine



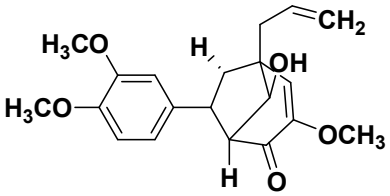
Piperbetol



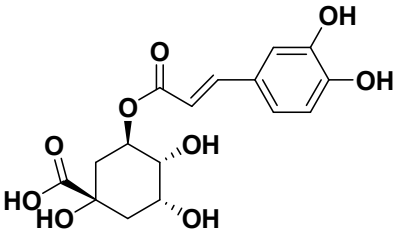
Methylpiperbetol



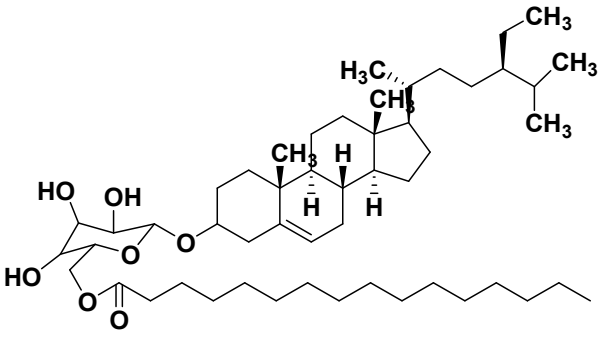
Piperol-B



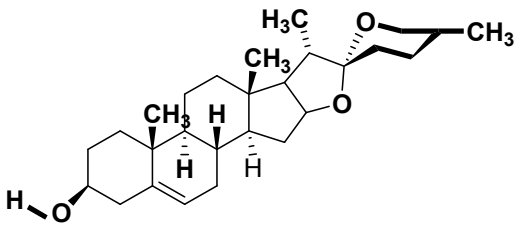
Piperol-A



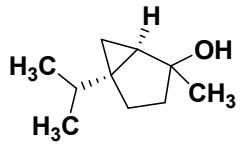
Chlorogenic acid



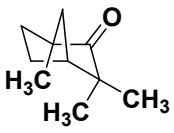
ß-sitosteryl palmitate



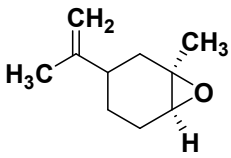
Diosgenin



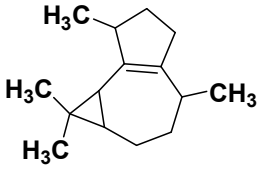
Cis Sabinene hydrate



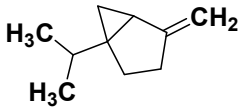
Fenchone



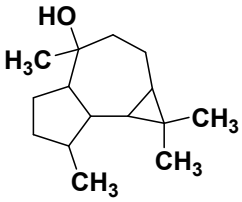
Cis-Limonene oxide



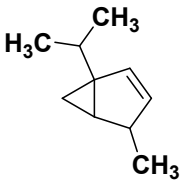
Isoledene



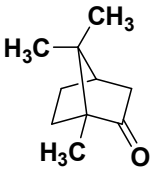
Sabinene



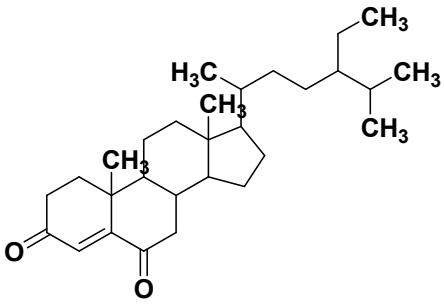
Ledol



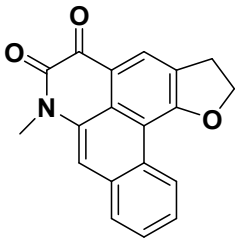
Thujene



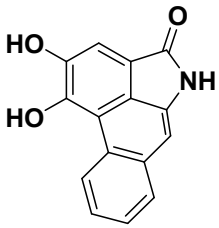
Camphor



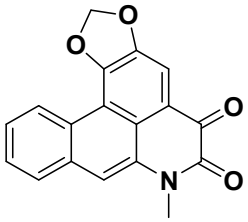
Stigmast-4-en-3,6-dione



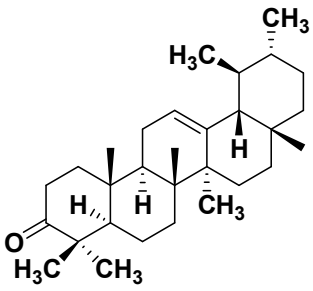
Cepharadione A



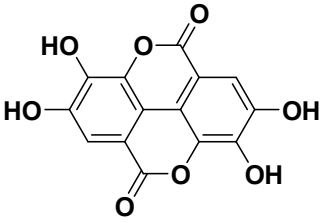
Aristololactame (A-II)



Cepharadione

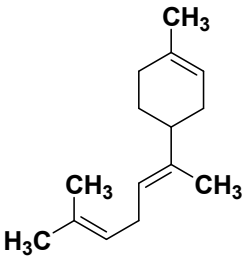


Ursonic acid



Ellagic acid

Cepharadione-A



α-Bisabolene

VIII. Therapeutic ethics:

A. Traditional Use

Betel leaf has been described from ancient times as an aromatic, stimulo-carminative[32] (katu), astringent and aphrodisiac (kamagnisandipanam)[33,34]. The leaves are credited with wound healing property[12,35]. The Indian traditional system of medicine has identified the leaves with digestive and pancreatic lipase stimulant activities[36-41]. Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries[42]. Fresh juice of betel leaves is used in many ayurvedic preparations[43]. Leaves considered being useful in treating bronchitis and dyspnea[37]. The leaves were chewed by singers to improve their voice[44]. The fruit of Piper betel employed with honey as a remedy for cough[45]. The fresh betel leaves possess antimicrobial, ringworm, antifungal, antiseptic and antihelminthic effects[46]. Leaves are used in eye drops for eye injury/infection as a baby lotion for the new born, for coughs, asthma, constipation and to arrest milk secretion[47]. Essential oil from leaves of this plant has been used for the treatment of respiratory catarrhs and antiseptic[45,48]. Leaf extract is reported to inhibit male reproductive competence[49-51]. The leaves possess antifertility on male rats[51] and anti-motility effects on washed human spermatozoa[49]. In folk medicine root is used as long lasting female oral contraceptive[42, 52]. The users believe that chewing the *'paan'* improves their efficiency and stamina[44]. Piper betel showed hypotensive, cardio tonic, smooth and skeletal muscles relaxant actions[53-55].

B. Biological Activities

i. Antimicrobial activity

The leaf has a significant antimicrobial activity against broad spectrum of micro-organisms[56]. The betel shows the antimicrobial activity against *Streptococcus pyrogen, Staphylococcus aureus, Proteus vulgaris, E.coli, Pseudomonas aeruginosa* etc., beside this the leaf extract also poses the bactericidal activity against the urinary tract pathogenic bacteria such as *Enterococococcus faecalis, C.koseri, C.fruendi, Klebsiella pnemoniae* etc[57,58]. The bioactive molecule thought to be responsible for anti-bacterial activity is sterol, which has been obtained in large quantities in betel leaf extracts. The mode of action may be due to surface interaction of sterol molecule present in the extracts with the bacterial cell wall and membrane leading to alteration in the primary structure of cell wall, ultimately lead to pore formation and degradation of the bacterial components. It is reported that sterol act through the disruption of the permeability barrier of microbial membrane structures[58]. Gram-positive bacteria were more susceptible to the inhibitory effects of the plant extract because of single layer and lack the natural sieve effect against large molecules, whereas gram negative bacteria are multi layered and complex cell wall structure[57,59]. The leaf has also poses the antifungal activity against many fungal infections[60]. One of them is dermatophytosis. Dermatophytosis is a disease of the keratinized parts of the body (skin, hair, and nail) caused by a three genera (*Trichophyton, Microsporum,* and *Epidermophyton*) of highly specialized fungi called the Dermatophytes[61]. The chloroform extract of piper betel shows the much more efficiency than the methanol fraction against dermatophytes because of presence of non-polar components in the fraction[62].

ii. Gastroprotective activity

The hot water extract significantly increased the mucus content adhering to the wall of the gastric mucosa. Mucus layer is considered to be important in mucosal defences against endogenous aggressors, e.g., acids, and also as an agent in facilitate the repair process. It is generally believed that enhanced acid secretion is the most important factor for the induction of gastric lesions. The higher dose of hot water extract does not cause significant inhibition in acidity or pH of gastric fluid. Therefore, gastro-protective effect of piper betel was not mediated

via inhibition of acid secretion in the gastric mucosa but by increasing its mucus content. The gastroprotective activities of the higher dose of hot water extract significantly greater than Misoprostol[63]. The extensive research has been proven that anti-oxidants might be effective mechanism not only in protecting against gastric mucosal injury, but also inhibiting progression of gastric ulceration. Ulceration progression is caused by free radical-induced chain process. Consequently, its arrest by radical scavengers helps in the faster healing[64,65]. Allylpyrocatacol has shown a powerful anti-oxidant potential in various in-vitro models. Treatment with Allylpyrocatacol significantly accelerated the ulcer-healing process, which increases the mucus production usually assist the healing process by protecting the ulcer crater against irritant stomach secretion (HCl and Pepsin) thereby enhancing the rate of local healing process[66].

iii. Antioxidant activity

Oxidative damage is an important effect of ionizing radiation on biological membranes. It is a chain reaction[67]. Free radicals generated from the radiolytic decomposition of water can attack fatty acid chains of membrane lipid. A free radical that has sufficient energy to abstract an allylic hydrogen from the methylene carbon of polyunsaturated fatty acids can initiate the peroxidative process. Here the presence polyphenols compounds like chatecol, allylpyrocatecol etc. in betel leaf extract inhibited the radiation induced lipid peroxidation process effectively. This could be attributed to its ability to scavenge free radicals involved in initiation and propagation steps[68]. The extracts reduced most of the Fe^{3+} ions and possess strong reductive ability[69]. The extract also showed strong hydroxyl radical and superoxide anion radical scavenging property when compared with different standards such as ascorbic acid and BHT[70-73].

iv. Antidiabetic activity

The aqueous extract of betel leaves possess marked hypoglycaemic activity when tested in fasted normoglycaemic rat[27]. In glucose tolerance test, both extracts markedly reduced the external glucose load. The leaf suspension, significant reduces the blood glucose level, glycosylated haemoglobin and decreased activities of liver glucose-6-phosphatase and fructose-1, 6- bisphosphatase, whereas liver hexokinase increased in Streptozocin (STZ) diabetic rats compared with untreated diabetic rats. The ability of lowering blood glucose level of Streptozocin (STZ) induced diabetic rat gives a suggestion that the extract have the insulinomimetic activity[74,75].

v. Radio protective activity

The ethanolic extract of betel leaf shows the radioprotective activity and it has been studied using rat liver mitochondria and pBR 322 plasmid DNA as two model in vitro systems. The extract effectively prevented γ-ray induced lipid peroxidation as assessed by measuring thiobarbituric acid reactive substrates, lipid hydroperoxide and conjugated diene. Likewise, it prevented radiation-induced DNA strand breaks in a concentration dependent manner. The radioprotective activity of betel leaf could be attributed to its hydroxyl and superoxide radicals scavenging property along with its lymphoproliferative activity. The radical scavenging capacity of betel leaf was primarily due to its constituent phenolics, which were isolated and identified as chavibetol and allyl pyrocatechol[76,77].

vi. Effect on the cardiovascular system / Platelet Inhibition activity

The heart shape of betel leaf makes it a suitable candidate for heart-related curative properties/medicine[15]. Leaf is considered to provide strength to the heart (cardio tonic) and regulates irregular heart beat and blood pressure[78]. Cardiovascular response of acquires great significance by the fact that it is consumed globally, making it a feasible substitute for *Digitalis purpurea*[79]. The effect chewing can be observed within minutes[80], which includes cardio-acceleration, sweating and salivation. It induces catecholamine secretion from the adrenal cortex contributing to increase in stamina, heart

rate, blood pressure, blood glucose levels and sympathetic neural activity. The effect of on vasorelaxation has been studied on isolated perfuse mesenteric artery rings, wherein it was observed that the vasorelaxant effect of PB was mainly endothelium-dependent and nitric oxide (NO)-mediated, as the effect was prevented by pretreatment with N(omega)- nitro-L-arginine (NOLA), a nitric oxide synthase (NOS) inhibitor, or by removal of endothelium[81]. Platelet hyperactivity is important in the pathogenesis of cardiovascular diseases due to intravascular thrombosis. Piperbetol, ethylpiperbetol, piperol A and piperol B isolated from leaves, selectively inhibited platelet aggregation induced by platelet activating factor (PAF) in a concentration-dependent manner[82].

vii. Antifertility activity

As the structural and functional integrity of reproductive organ depend on circulating level of estrogen, any small change in estrogen level may lead to altered structural and functional activity of reproductive organs[83]. The plant extract may brought about its effect through pituitary-gonadal axis, which resulted in diminished gonadolrophine release, in turn reduced reproductive organ weights and estrogen level affecting ovarian cyst. Serum biochemistry revealed that glucose level was declined but cholesterol and Vitamin C concentrations were elevated beyond control value; indicate non-utilization of cholesterol by the system, hence decrease in estrogen level. The data suggests that betel extract brought about antifertility and antiestrogonic effects in female rats and these effects were reversible on cessation of treatment[84,85].

viii. Immunomodulatory activity

Many of the disorders today are based on the imbalances of immunological processes. This necessitates the search for newer and safer immunomodulators. The methanolic extract has lymphocyte proliferation, interferon-C receptors and the production of nitric oxide were measured in vitro. Further, the extract at different dose levels was studied in vivo for the humoral and cellular immune responses on mice immunized with sheep red blood cells. The result showed that it significantly suppressed haemaglutinin stimulated peripheral blood lymphocyte proliferation in a dose-dependent manner. The decrease in antibody titre and increased suppression of inflammation suggests possible immunosuppressive effect of extract on cellular and humoral response in mice[86]. From literature it conclude that betel leaf a novel candidate for immunosuppressive activity. The same could be further evaluated for its anticancer activity or as a potential candidate in the treatment of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythomatous or emphysema[68].

ix. Cholinomimetic effect

Betel leaf rise in body temperature due to cholinergic responses. Aqueous and ethyl acetate extracts were evaluated for their cholinergic responses using isolated guinea-pig ileum[15]. It was observed that the spasmogenic activity was more in water than ethyl acetate extract. In isolated rabbit jejunum K+-induced contraction was inhibited by both extract, suggesting blockade in calcium channel. Thus, leaves contain cholinomimetic and possible calcium channel antagonist constituents which may provide the basis for several activities shown by this plant[87].

x. Hepato-protective activity

The antihepatotoxic effect of betel leaf extract was evaluated on ethanol and carbon tetrachloride (CCl4) induced liver injury in a rat model[88]. Fibrosis and hepatic damage, as revealed by histology and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were induced in rats by CCl_4. The extract significantly inhibited the elevated activities of AST and ALT and also attenuated total glutathione S-transferase (GST), which led to a rise in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT)[15]. The histological examination showed that the betel leaf extract protected liver from the

damage induced by CCl_4 by decreasing alpha-smooth muscle actin (alpha-sma) expression, inducing active matrix metalloproteinase-2 (MMP2) expression through the Ras/Erk pathway, and inhibiting TIMP2 level that consequently attenuated the fibrosis of liver. These findings support a chemo preventive potential of betel leaf against liver fibrosis[89].

xi. As an Oral care agent

Dental caries is a chronic endogenous infection caused by the normal oral commensally flora. The carious lesion is the result of demineralization of enamel and later of dentine by acids produced by plaque microorganisms as they metabolize dietary carbohydrates[90,91,92]. The bacteria primarily responsible for dental decay in man are *Streptococcus mutans*. *Streptococci* belong to four main species groups: *mutan, salivarius, anginosus and mitis*. In addition to *Streptococcis mutans, Lactobacillus acidophilus* bacteria probably also pay a minor role in acid production in the plaque[93]. The stickiness of the plaque is caused by dextran, which is produced by the fermentation of dietary sucrose by *Streptococcus mutans*. The plaque bacteria, particularly *Streptococcus mutans*, act on dietary fructose to produce lactic acid, which causes enamel decalcification (at below or above 5.5pH)[94]. The plaque and dietary carbohydrates are in the initiation of enamel caries. Certain cariogenic and highly acidogenic strains of streptococci, especially *S. mutans* have the ability to metabolize dietary sucrose and synthesize glucan by cell-surface and extracellular glucosyl transferase. This enzyme is considered to be of special importance in the establishment of *S. mutans* in the dental plaque[95, 96, 97]. The aqueous extract inhibit the different acid-producing oral pathogens which changes in the ultra-structure of the enamel and its properties like *Streptococci, Lactobacilli, Staphylococci, Corynebacteria, Porphyromonas gingivalis* and *Treponema denticola*. So it is best natural substance and its rating as second most popular daily consummation item in Asia, which contribute the best oral hygiene to oral cavity[98].

xii. Neuropharmacological profile

Hydroalcoholic extract of betel leaves exhibited improvement in the discrimination index, potentiating the haloperidol induced catalepsy, reduction in basal as well as amphetamine induced increased locomotors activity and delay in sodium nitrite induced respiratory arrest. These results from review suggest possible facilitation of cholinergic transmission and inhibition of dopaminergic as well as nor adrenergic transmission by the extract[96,99].

4. Conclusion

The medicinal importance of the herb as discussed above evidently prove that betel leaf is one of the most promising commercial botanical with earlier reported to possess a lot of therapeutic values. The leaf has the great potency to act as natural antioxidant. The anti-oxidant property is correlated with different biological activities like hepatoprotective, antidiabetic, antiarthritis, anti-stroke and anticancer properties, since free radicals are involved in all these diseases. The leaf poses the broad spectrum antimicrobial activity against various bacterial strains including *Bacillus cereus, Enterococcus faecalis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Salmonella Enteritidis, Streptococcus mutans, Streptococcus pyogenes, Enterococcus faecium, Actinomycetes viscosus, Streptococcus sanguis, Fusobacterium nucleatum, Prevotella intermedia* beside this, the leaves also poses the antifungal and antiprotozoal activity against pathogen, which causing typhoid, cholera, tuberculosis, etc. The leaf extract shows the gastroprotective activity by enhancing the mucus rather than decrease the acid production. Chewing of betel leaf not only accelerating the salivation but also enhances the gastric juice, pancreatic lipase secretion which aids in digestion process. This may the reason for which traditionally pan was chewed after eating. The aqueous extract possess oral protective action against different acid-producing oral pathogens which changes in the ultra-structure of the enamel and its properties like *Streptococci, Lactobacilli,*

Staphylococci, Corynebacteria, Porphyromonas gingivalis and *Treponema denticola*. Chewing of betel leaf increase salivation which increases the contains of peroxidase, lysozyme and antibodies to combat bacterial growth in the oral cavity. It is the best choice for oral hygiene because while chewing bio-active phytochemicals are released into the oral cavity. The betel leaves are also reported to possess anti-mutagenic and anti-carcinogenic properties particularly against the tobacco carcinogens due to presence of phytoconstituents like hydroxychavicol and chlorogenic acid, the latter compound is also reported to kill the cancerous cell without affecting the normal cells unlike the common anticancer drugs. The chewing of betel leaf results cardio-tonic action by accelerating catecholamine from adrenal cortex contributing to increase the stamina of the cardiac muscles, heart rate, blood pressure and sympathetic neural activity. It has also the platelet inhibition activity leads vasorelaxation effect mediated through endothelium dependent and nitric oxide pathways. Hence, betel leaf is beneficial for different cardiovascular disorders like Congestive heart failure, Coronary artery disease, acute myocardial infarction, atherosclerosis etc. The aqueous leaf suspension has significant reduction in blood glucose level, act same as insulinomimetic. The methanolic extract of the betel leaf decrease the antibody titre and increase the suppression of inflammation suggests possible immunosuppressive effect of extract on cellular and humoral response. Considering the above properties, it comes to conclusion that betel leaf place its position in nature same as our heart in our body and role the same with lots of biological activities and has a tremendous strength to come out as a *future green medicine*, hence *Piper betle.* L. leaf regard as "Golden heart of Nature".

5. Future Perspectives

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. These problems stress a transformed attempt to find the antimicrobial agents effective against the pathogenic microorganisms resistant to current antibiotics. Therefore there is an extensive requirement to establish alternative antibacterial molecules for the treatment of infectious diseases from other sources. From this review it concluded that phenolic antibacterial from betel leaf cause suppression of bacterial activity in the oral cavity and prevents halitosis. Activity-directed purification led to the identification of allylpyrocatechol (APC) possessing antimicrobial activity against oral bacteria, *Staphylococcus aureus*. Crude aqueous extract of Piper betel was found effective against other oral microbes, which causes changes in the ultra-structure and its acid-producing properties like *Streptococci, Lactobacilli, Staphylococci, Corynebacteria, Porphyromonas gingivalis* and *Treponema denticola*. Gram positive bacterial strains were found to be more susceptible. It may be due to that cell wall of gram positive bacteria is less complex and lack natural sieve effect against large molecules due to small pores in their cell envelope. So, essential oil obtained from leaf extract may be commercialized by used as active pharmaceutical ingredient in different oral care product like toothpaste, mouthwash, mouth fresheners to get maximum result against dental pathogens. Due to high phenolic content in the leaf, the plant poses high antioxidant activity. Other pharmacological activities like antiulcer, antidiabetic, immunomodulatory, cardiovascular and anticancer were demonstrated in last two decade. Piper betel also offers a possibility for use in drug delivery though buccal mucosa by passing the gastric route, where drug has to endure gastric acidic pH. This route of administration may be beneficial for those Pharmaceutical drugs which acid labile i.e., degrading in the acidic medium. The betel leaf may enhance the bioavailability of a drug because of its nature as described by "Bhabaprakash", (Virya: Ushan). It supposed to elevate the temperature of plasma fluid which may ultimately fasten the drug absorption, hence the betel leaf may be used as natural bio enhancer. The leaves are very nutritive and contain substantial amount of vitamins and minerals. The leaves also contain the enzymes like diastase and catalase besides a

significant amount of all the essential amino acids except lysine, histidine and arginine, which are found only in traces; hence it has great potency to entry to the nutriceuticals industry as food additives.

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Phytochemistry of Medicinal Plants

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Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. Studies carried out during the past 2–3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The major classes of phytochemicals with disease-preventing functions are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals have more than one function. There is, however, much scope for further systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases

Keyword: Phytochemicals, Alkaloids, Terpenoids, Flavonoids, Saponins, Tannins and Phenolics.

1. Introduction

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients[1]. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals[2,3]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged[4] and are classified by protective function, physical characteristics and chemical characteristics[5] and About 150 phytochemicals have been studied in detail[4].

In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices[3]. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources[6]. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds[7]. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions[8]. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals[4].

These compounds are known as secondary plant metabolites and have biological properties

such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases[9].

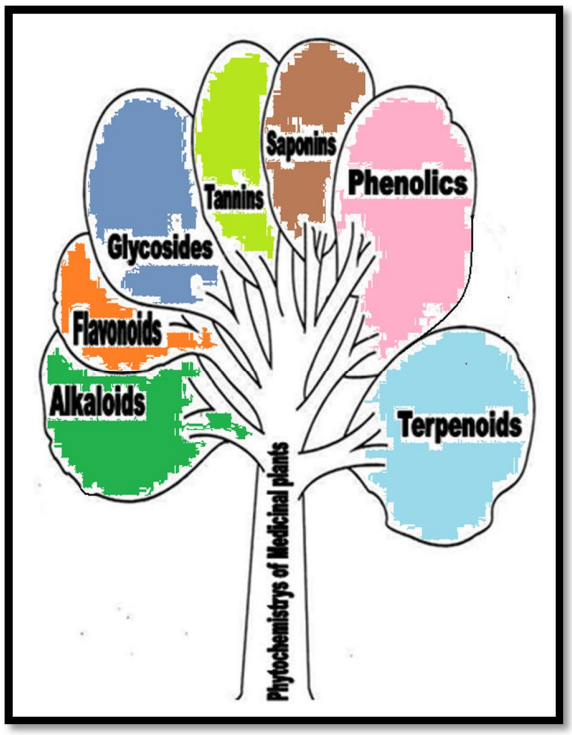


Fig.1: Phytochemistry of medicinal plants.

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, Because of this property; many researchers have been performed to reveal the beneficial health effects of phytochemicals. The purpose of the present review is to provide an overview of the extremely diverse phytochemicals presents in medicinal plants.

2. The Journey of Medicinal Plant Research

An assessment of the previous trends and impact of research into the phytochemistry on medicinal plants of the world is quite desirable before considering recent trends. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period. Over the last decade, however, interest in drugs of plant and probably animal origin has grown steadily[10]. Utilization of medicinal plants has almost doubled in Western Europe during that period. Ecological awareness, the efficacy of a good number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian and increased interest of major pharmaceutical companies in higher medicinal plants as sources for new lead structures has been the main reasons for this renewal of interest. With the development of chemical science and pharmacognosy physicians began to extract chemical products from medicinal plants. A few examples of the products extracted from medicinal plants are - in 1920, quinine was isolated from Cinchona by the French pharmacist, Peletier & Caventou. In the mid-nineteenth century, a German chemist, Hoffmann obtained Aspirin from the bark of the willow. With the active principles in medicinal plants identified and isolated, plant-based prescriptions began to be substituted more and more with pure substances, which were more powerful and easier to prescribe and administer[11]. Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be

as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases[12].

3. Biological Activities of Phytochemicals

The phytochemicals present in plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man. Study findings suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity[3,13]. Phytochemicals may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. For example, according to data summarized by Meagher and Thomson, genistein prevents the formation of new capillaries that are needed for tumor growth and metastasis[5]. The physiologic properties of relatively few phytochemicals are well understood and more many research has focused on their possible role in preventing or treating cancer and heart disease[3]. Phytochemicals have also been promoted for the prevention and treatment of diabetes, high blood pressure, and macular degeneration[4]. While phytochemicals are classified by function, an individual compound may have more than one biological function serving as both an antioxidant and antibacterial agent[13]. Bioactive and Disease-preventing phytochemicals present in plant are shown in Table 1.

Table 1. Bioactive Phytochemicals In Medicinal Plants.

Classification	Main groups of compounds	Biological function
NSA (Non-starch poly-saccharides.)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, Flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti- oxidants, cancer chemoprevention

4. Classification of Phytochemicals

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In resent year Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides[14]. Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytocontituents. (Figure 2).

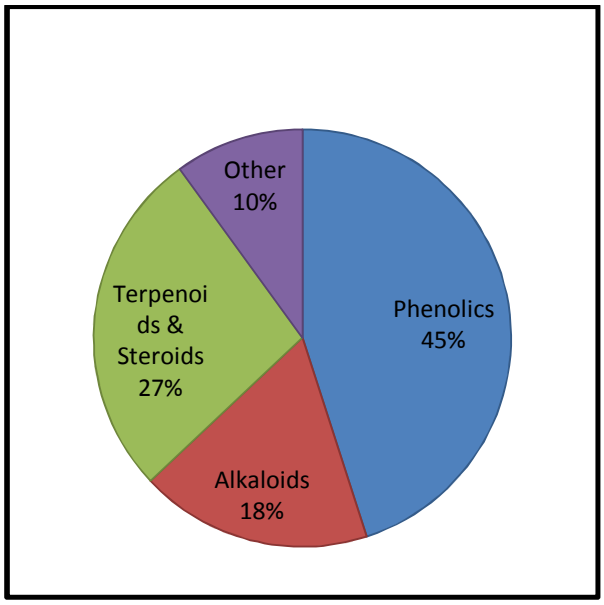


Fig.2: Pie chart representing the major groups of plant Phytochemicals.

5. Phenolics

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants[15]. They are plant secondary metabolites, and they have an important role as defence compounds. phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. Flavonoids are the largest group of plant phenols and the most studied[16]. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins.

6. Phenolic acids

The term "phenolic acids", in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures (Figure3). Hydroxycinnamic acid compounds are produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings[17]. These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukaemia cells, typically have higher levels of reactive oxygen species (ROS) than normal cells so that they are particularly sensitive to oxidative stress[18]. Many papers and reviews describe studies on bioavailability of phenolic acids, emphasizing both the direct intake through food consumption and the indirect bioavailability deriving by gastric, intestinal and hepatic metabolism[19].

Table 2: The Major Classes of Phenolic Compounds in Plants

S.N.	Number of carbon atom	Basic skeleton	Class
1.	6	C_6	Simple phenols Benzoquinones
2.	7	C_6-C_1	Phenolic acids
3.	8	C_6-C_2	Acetophenones Tyrosine derivatives
4.	9	C_6-C_3	Hydroxycinnamic acid, Coumarins
5.	10	C_6-C_4	Naphthoquinones
6.	13	C_6- C_1-C_6	Xanthones
7.	14	C_6- C_2-C_6	Stilbenes
8.	15	C_6- C_3-C_6	Flavonoids
9.	18	$(C_6$- $C_3)_2$	Lignans
10.	30	$(C_6$- C_3-$C_6)_2$	Bioflavonoids
11.	N	$(C_6$- C_3-$C_6)_n$	Condensed tannins

In addition Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical and medical studies. In recent years, the importance of antioxidant activities of phenolic compounds and their potential usage in processed foods as a natural antioxidant compounds has reached a new level and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity[20].

5.1 Activity of Phenolic Acids

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical and medical studies. Phenolic compounds in many plants are polymerized into larger molecules such as the proanthocyanidins (PA; condensed tannins) and lignins. Moreover, phenolic acids may arise in food plants as glycosides or esters with other natural compounds such as sterols, alcohols, glucosides and hydroxyfatty acids. Varied biological activities of phenolic acids were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as staphylococcus aureus are some of biological activities of phenolic acids[21]. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti- inflammatory, antioxidant[22], cytotoxic and antitumor, antispasmodic, and antidepressant activities[23].

6. Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks[24]. The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. Flavonoids are ubiquitous

among vascular plants and occur as aglycones, glucosides and methylated derivatives. More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known[25]. Small amount of aglycones (i.e., flavonoids without attached sugar) are frequently present and occasionally represent a considerably important proportion of the total flavonoid compounds in the plant.

Figure 4, represents major flavonoids' structures. The six-membered ring condensed with the benzene ring is either -pyrone (flavones and flavonols) or its dihydroderivative (flavanones and flavan-3-ols). The position of the benzenoid substituent divides the flavonoids into two classes: flavone (2-position) and isoflavone (3-position). Most flavonoids occur naturally associated with sugar in conjugated form and, within any one class, may be characterized as monoglycosidic, diglycosidic, etc. The glycosidic linkage is normally located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose[26].

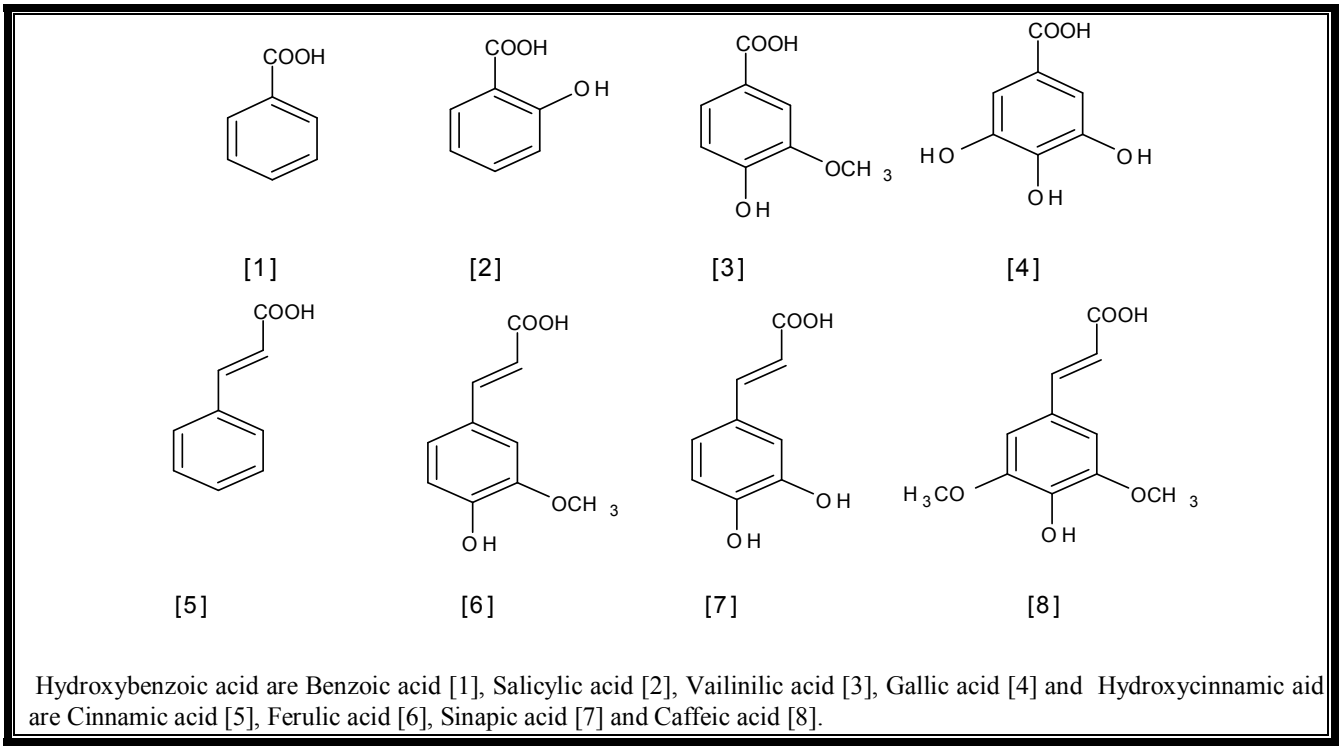


Fig. 3. Structures of the important naturally occurring phenolic acids.

6.1 Activity of Flavonoids

Flavonoids have gained recent attention because of their broad biological and pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as antioxidants

depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand flavonoids such as luteolin and cathechins, are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity[27]. Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA[28].

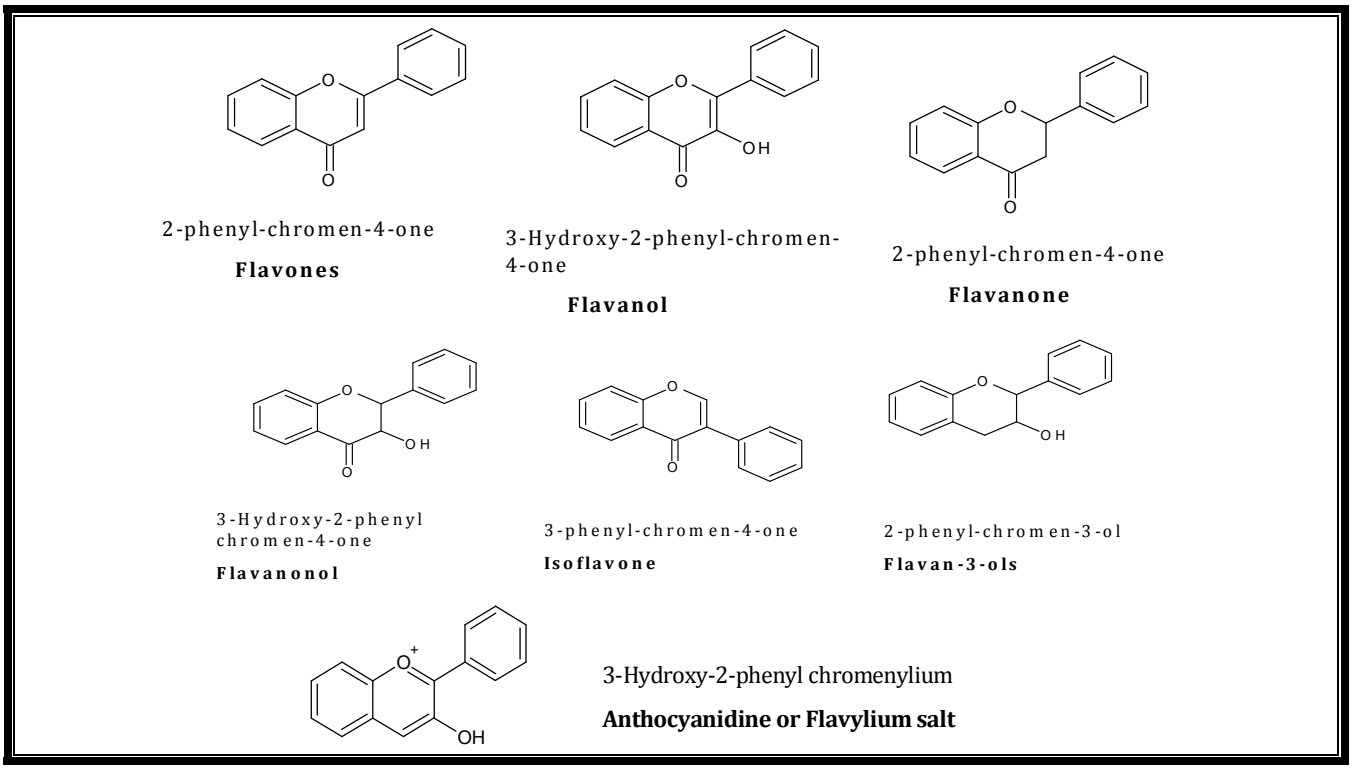


Fig.4. Chemical structures of some representative flavonoids.

7. Tannin

From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers[29,30]. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc[31,32,33]. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: *Gallotannins, ellagitannins, complex tannins, and condensed tannins*[34,35,36] (Figure 5).

(1) Gallotannins are all those tannins in which galloyl units or their *meta*-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.
(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.
(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of *C*-4 of one catechin with *C*-8 or *C*-6 of the next monomeric catechin.
Tannins are found commonly in fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages, legume trees like *Acacia* spp., *Sesbania* spp., in grasses i.e; sorghum, corn, etc[37]. Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established[38].

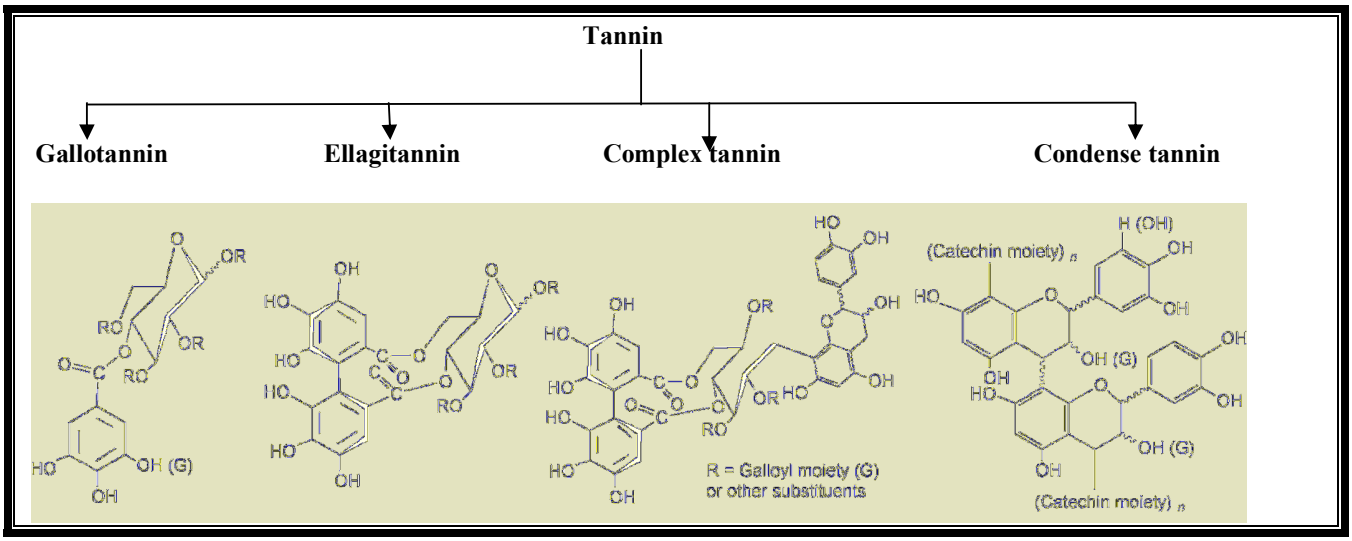


Fig.5. Classification of tannins.

7.1 Activity of Tannins

In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours[39], and as antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals[40]. Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber Production[41]. Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers+[42]. The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented[43,44].

8. Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the "alkaline" and it was used to describe any nitrogen-containing base[45]. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Some of the fires natural products to be

isolated from medicinal plants were alkaloids when they first obtained from the plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid. Hence they were known as the vegetable alkalis or alkaloids and these alkaloids are used as the local anesthetic and stimulant as cocaine+[46]. Almost all the alkaloids have a bitter taste. The alkaloid quinine for example is one of the bitterest tasting substances known and is significantly bitter ($1x10^{-5}$) at a molar concentration[47].

Alkaloids are so numerous and involve such a variety of molecular structure that their rational classification is difficult. However, the best approach to the problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule[48]. For historicxal reasons as also because of their structural complexities, the nomenclature of alkaloids has not been systematized. The names of individual members are, therfour, generally derived from the name of the plant in which they occur, or from their characteristic physiological activity.the various classes of alkaloids according to the heterocyclic ring system they contain are listed below.

Pyrrolidine alkaloids: they contain pyrrolidine (tetrahydropyrrole) ring system. E.g Hygrine found in *Erythroxylum coca* leaves.

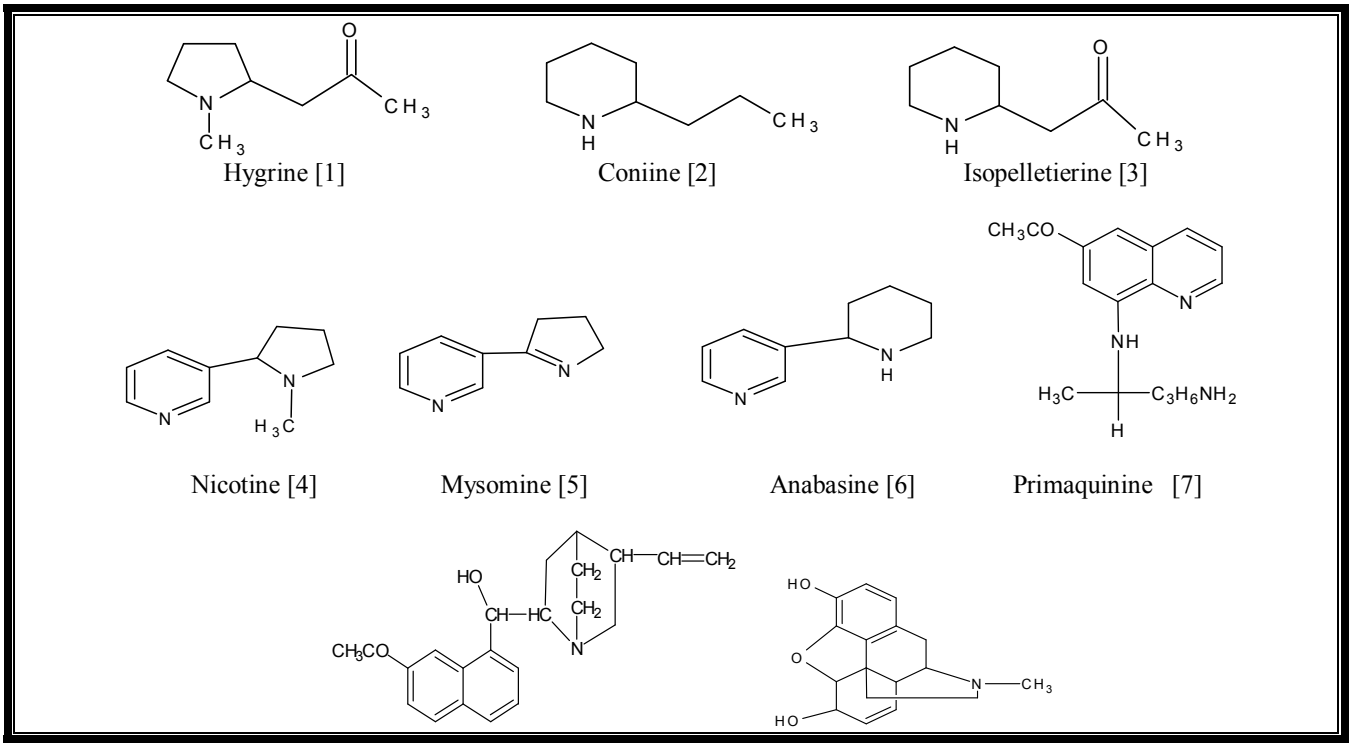
Pyridine alkaloids: they have piperidine (hexahydropyridine) ring system. E.g Coniine, piperine and isopelletierine.

Pyrrolidine-pyridine alkaloids: the heterocyclic ring system present in there alkaloids is Pyrrolidine-pyridine.E.g Myosmine, Nicotine alkaloid found in tobacco (*Nicotiana tabacum*) plant.

Pyridine-piperidine alkaloids: This family of alkaloids contains a pyridine ring system join to a piperidine ring system the simplest member is Anabasine alkaloid isolated from poisonous Asiatic plant anabasis aphyllan.

Quinoline Alkaloids: These have the basic heterocyclic ring system quinoline .E.g Quinine occurs in the bark of cinchona tree.It has been used for centuries for treatment of malaria.Synthetic drugs such as primaquinine have largely replace quinine as an anti-malarial.

Isoquinoline alkaloids: They contain heterocyclic rig system isoquinoline. E.g Opium alkaloids like narcotine, papaverine, morphine, codeine, and heroine.



Quinine [8]	Morphine [9]

Fig. 6. Structures of the important naturally occurring alkaloids.

8.1. Activity of Alkaloids

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrens) and also against other plants by means of allelopathically active chemicals[49]. The useof alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), andanticancer actions (dimeric indoles, vincristine, vinblastine). These are just a few example illustrating the great economic importanceof this group of plant constituents[50]. Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug[46].

9. Terpenoids

The terpenoids are a class of natural products which have been derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products[51]. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and cosmetics examples menthol and sclareol or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers like linalool[52]. Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_2$. Terpene hydrocarbons therefore have molecular formula (C_5H_8) n and they are classified according to the number of isoprene units[53].

9.1 Hemiterpenoids: Consist of a single isoprene unit. The only hemiterpene is the Isoprene itself, but oxygen-containing derivatives of isoprene such as isovaleric acid and prenol is classify as hemiterpenoids.

9.2 Monoterpenoids: Biochemical modifications of monoterpenes such as oxidation or rearrangement produce the related monoterpenoids. Monoterpenoids have two isoprene units. Monoterpenes may be of two types i.e linear (acyclic) or contain rings e.g. Geranyl pyrophosphate, Eucalyptol, Limonene, Citral, Camphor and Pinene.

9.3 Sesquiterpenes: Sesquiterpenes have *three isoprene* units e.g. Artemisinin, Bisabolol and Fernesol, oil of flowers, or as cyclic compounds, such as Eudesmol, found in Eucalyptus oil.

9.4 Diterpenes: It composed for four isoprene units. They derive from geranylgeranyl pyrophosphate. There are some examples of diterpenes such as cembrene, kahweol, taxadiene and cafestol. Retinol, retinal, and phytol are the biologically important compounds while using diterpenes as the base.

9.5 Triterpenes: It consists of *six* isoprene units e.g. Lanosterol and squalene found in wheat germ, and olives.

9.6 Tetraterpenoids: It contains eight isoprene units which may be acyclic like lycopene, monocyclic like gamma-carotene, and bicyclic like alpha- and betacarotenes.

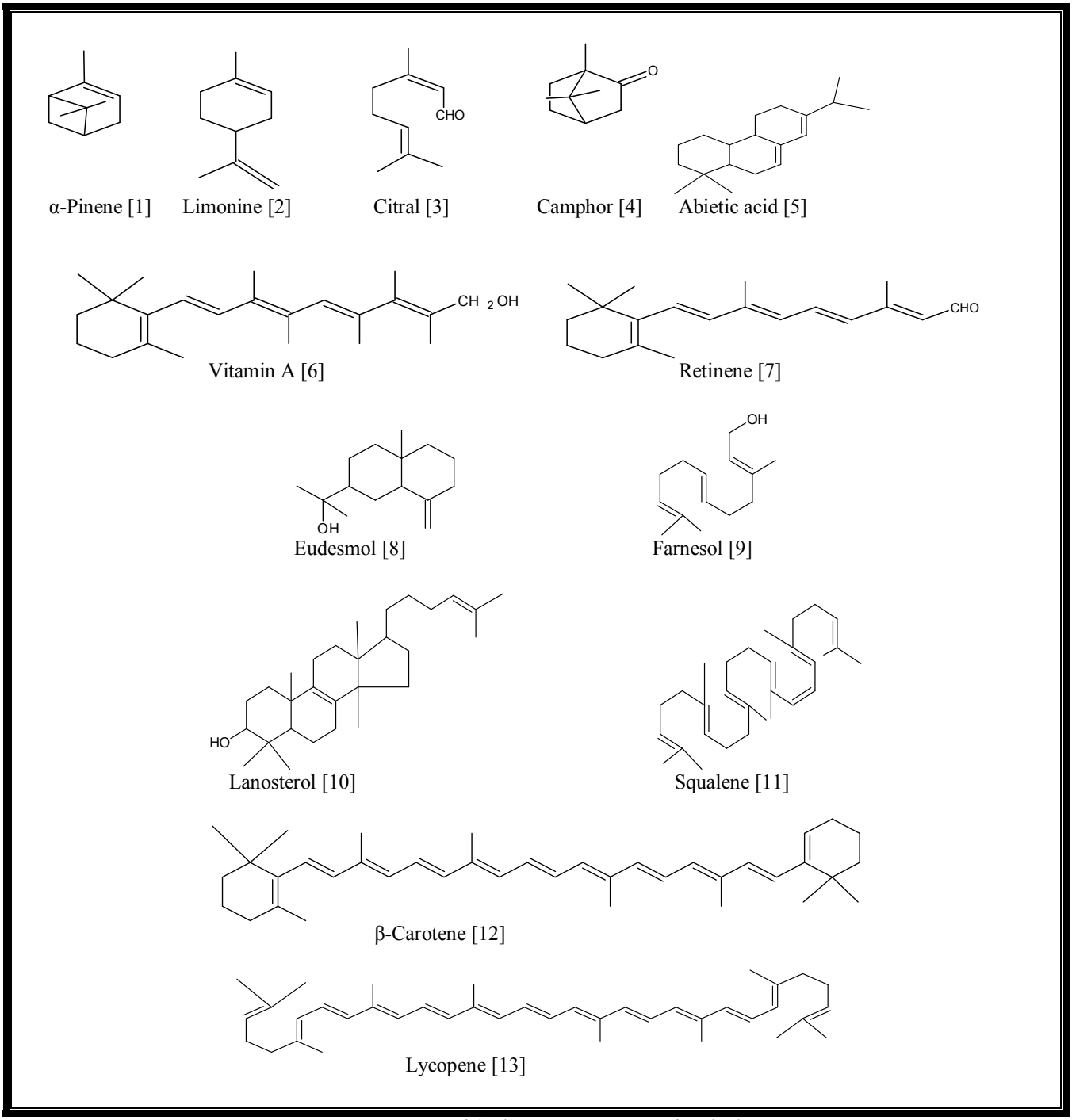


Fig. 7: Structures of the important terpenes of each class.

9.7 Activity of Terpenes

Among plant secondary metabolites terpenoids are a structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses which involves herbivores and their natural enemies[54]. Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plants from being eaten by animals (antifeedants)[55]. Last, but not least, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. In addition, terpenoids can have medicinal properties such as anti-carcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol.[53,56].

10. Saponin

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom They form a stable foam in aqueous solutions such as soap, hence the name "saponin". Chemically, saponins asa group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives (Figure 8A,B, respectively). The main triterpene aglycone is a derivative of oleanane (Figure 8C)[57]. The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). Saponins that have one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins[58].

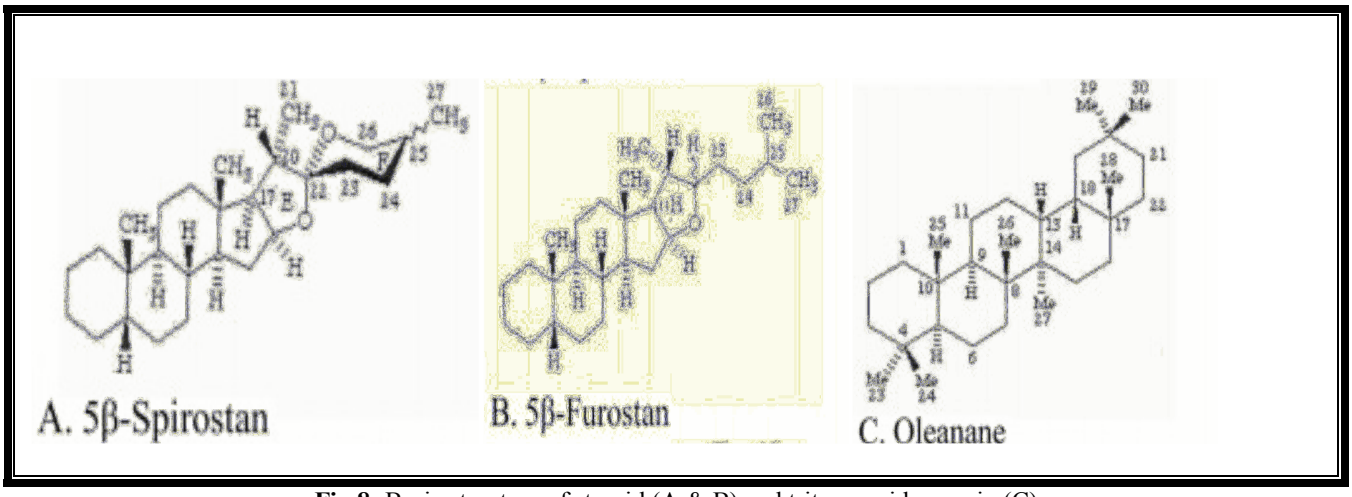


Fig.8: Basic structure of steroid (A & B) and triterpenoid saponin (C)

10.1 Activity of Saponins

The physiological role of saponins in plants is not yet fully understood. While there is a number of a publication describing their identification in plants, and their multiple effects in animal cells and on fungi and bacteria, only a few have addressed their function in plant cells. Many saponins are known to be antimicrobial, to inhibit

mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants[59]. Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral[60,61,62].

11. Conclusion

Nature is a unique source of structures of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. In the context of the worldwide spread different diseases such as AIDS, chronic diseases and a variety of cancers, an intensive search for new lead compounds for the development of novel pharmacological therapeutics is extremely important. With the present information are reported in this review, it is difficult to establish clear functionality and structure–activity relationships regarding the effects of phytochemicals in biological systems activity. This is largely due to the occurrence of a vast number of phytochemicals with similar chemical structures, and to the complexity of physiological reactions. Moreover, given the number of phytochemicals isolated so far, nature must still have many more in store. With the advances in synthetic methodology and the development of more sophisticated isolation and analytical techniques, many more of these phytochemicals should be identified.

12. Acknowledgement

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Effect of Phytogenic Growth Promoter on Broiler Birds

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The present experiment was conducted to study the biological effect of phytogenic growth promoter supplemented with the diet or added in the drinking water in the broiler birds. In the experiment, two proven phytogenic growth promoters, Digestarom 1317 (dosage 150 ppm) and Digestarom 1440 (dosage 800 ppm) AC were fed to the broiler chickens against an antibiotic growth promoter, Bacitracin Methylene Disalicylate (BMD). The experimental birds were distributed into four equal groups viz., T1, T2, T3 and T4. Birds of the negative control group (T1) were supplemented with basal diet but no growth promoter and birds of the positive control group (T2) were supplemented with basal diet with BMD. Birds of T3 and T4 groups were supplemented basal diet along with two different doses of phytogenic feed additive, Digestarom 1317 @ 150 ppm and Digestarom 1440 @ 800 ppm. Studies on different biological parameters revealed that phytogenic growth promoters significantly ($P<0.001$) enhance the productive performance of treatment group

Keyword: Biological Parameters, BMD, Phytogenic Growth Promoter.

1. Introduction

Phytogenic feed additives (often also called phytobiotics or botanicals) are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals' production performance, and improving the quality of food derived from those animals, such as herbs (flowering, non woody, and non-persistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by non-aqueous solvents). Within phytogenic feed additives, the content of active substances in products may vary widely, depending on the plant part used (e.g. seeds, leaf, root or bark), harvesting season, and geographical origin. The technique for processing (e.g. cold expression, steam distillation, extraction with non-aqueous

solvents etc.) modifies the active substances and associated compounds within the final product.

The present investigation was conducted on broiler birds to study the biological studies of growth performance, to assess bacteriological load in different parts of digestive tract and to study the morphological studies of gut and villus height in different segments of small intestine mainly in duodenum.

Table 1: Grouping Pattern of Experimental Chick.

S. No.	Treatment	Code	Number of birds
1	Negative control	T1	48
2	Positive control	T2	48
3	Digestarom 1317	T3	48
4	Digestarom 1440	T4	48

2. Materials and Methods

In the present study the experiment was conducted for evaluating the efficiency or effect of the phytogenic growth promoter. The phytogenic growth promoter was active throughout the gastrointestinal tract and as a consequence, it will exert broad spectrum antimicrobial action, will enhance nutrient utilization by improving gastrointestinal histo-morphology and will augment the host immunity. In the experiment, two proven and approved phytogenic growth promoters, Digestarom 1317 (dosage 150 ppm) and Digestarom 1440 (dosage 800 ppm) AC were fed to the broiler chickens against an antibiotic growth promoter, Bacitracin Methylene Disalicylate (BMD).

Table 2: Effect of phytogenic growth promoter on body weight per week basis (gm).

T1	T2	T3	T4	Polled SEm	P value
52.57	53.78	51.95	53.79	0.34	0.48
126.90	127.75	126.10	129.50	0.85	0.62
318.35	340.20	321.24	339.50	1.26	0.31
587.66^{a}	649.85^{b}	635.92^{b}	650.50^{b}	3.87	0.043*
915.80^{a}	1120.82^{b}	1047.82^{b}	1152.7^{c}	8.65	0.007**
1253.52^{a}	1511.50^{b}	1449.02^{b}	1534.50^{c}	8.94	0.003**
1457.75^{a}	1916.7^{b}	1886.52^{b}	1976.25^{c}	8.97	0.006**

*P<0.005, **P<0.001 Values bearing different superscripts within a row differ significantly

Digestarom AC is a combination of phytogenic components with glycerides of short chain fatty acids. Basically, Digestarom AC is a complex of plant extracts and plant essential oils along with monoglycerides, lactic acids and multiglyceride complexes. Being a complex of plant extracts and essential oils, Digestarom AC is hypothesized to stimulate feed intake, intestinal secretion of enzymes and enhance digestibility of nutrients. Additionally, Digestarom AC is anticipated to act as a broad spectrum antimicrobial substances throughout the gastrointestinal tract and promote development of the villus structure of the gut.

a. Experimental broiler birds and their management

The experiment was conducted in the Poultry shed at Faculty of Veterinary & Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India. Few days prior to the arrival of the chicks, the experimental house and cages were fumigated with potassium permanganate ($KMnO_4$) and formaldehyde solution (40 ml formaldehyde + 20 gm $KMnO_4$ per 100 cu.ft). Before fumigation all the ventilators, windows and doors were closed and sealed. The house was kept dry, clean, disinfected, protected and well ventilated throughout the experimental period.

Table 3: Effect of phytogenic growth promoter on body weight gain per week basis (gm.)

T1	T2	T3	T4	Polled SEm	P value
74.33	73.97	74.15	75.71	0.87	0.25
191.45	212.45	195.14	210	2.67	0.73
269.31	309.65	314.68	311	4.76	0.38
328.14^{a}	470.97^{b}	411.9^{b}	502.20^{b}	10.43	0.002***
337.72^{a}	390.68^{b}	401.20^{b}	368.75^{b}	6.78	0.035*
204.23^{a}	405.20^{b}	437.5^{b}	441.75^{b}	12.89	0.007**

***P<0.001, Values bearing different superscripts within a row differ significantly

One hundred and ninety two day-old broiler chicks of Vencobb-400 strain were procured from a commercial hatchery. Water with glucose and electrolytes were administered to the chicks on their arrival which was followed by weighing of the individual chicks and their random distribution into the respective groups (treatment and control).Each groups comprised 4 replicate of 12 birds each. The chicks were maintained under standard conditions of hygiene and management. The birds were vaccinated against Newcastle Disease (La Sota) on 7^{th} and 28^{th} day and against infectious bursal disease orally on 14^{th} day of experiment through drinking water.

The experimental birds were distributed in four groups as maintained in Table I. Each group offered basal diet as mentioned in Table 2. T1 group offered only basal diet without any growth promoter .T2 group offered basal diet with B.M.D. (Bacitracin Methylene Disalicylate) @ 500mg/kg body weight; whereas T3 and T4 group offered basal diet with two different dosages of phytogenic growth promoter @150 ppm and @ 800 ppm respectively.

Table 4: Microbial population in the crop.

Attributes	*E.coli*	*Salmonella*	*Lactobacillus*
T1	35	29	183^{b}
T2	N.D.	N.D.	63.8^{c}
T3	N.D.	N.D.	709.2^{a}
T4	N.D.	N.D.	714.5^{a}
Polled SEm	-	-	89.07

Figures in parenthesis indicate the count of bacteria expressed as log $_{10}$CFU/g of content. ND: Not detectable, (P<0.001)

b. Studies on growth performance

Body weight of individual bird of each group was recorded at the commencement of the experiment (day 0) and subsequently at weekly interval in the morning before offering feed water to the birds till the termination of the experiment at 6 weeks of age. Then the body weight gain was calculated.

c. Bacteriological studies of gastrointestinal tract content

The entire gastrointestinal tract along with crop, proventriculus, gizzard, part of small intestine was severed from two birds of a replicate in each treatment group and was separated. The content present inside these segment were gently expelled into sterile Petri dishes and 1g of content from each segment was taken in sterile test tubes and diluted to 10 ml with triple distilled deionized water (autoclaved at 15 lbs pressure for 15 min).The contents were then serially diluted and the final dilution of 10^5 was used for enumeration of *Escherichia coli*, *Salmonella sp*. *and Lactobacillus sp*. 0.1 ml from the diluted contents was spread with a sterile platinum loop on the surface of specific culture media and incubated aerobically at 37^{o}C for 24 to 48 h and the colonies were enumerated. The bacterial counts were expressed as $\log_{10}$ CFU (colony forming units) per gram of content.

Table 5: Effect of phytogenic growth promoter on villus height in different parts of small intestine.

T1	T2	T3	T4	Polled SEm	P value
904.70^a	1118.46^b	1182.54^c	1238.45^c	8.29	0.037*

Values bearing different superscripts within a row differ significantly, (*P<0.001)

d. Morphological studies of gut and measurement of villus height

Slides were prepared by sectioning and staining of tissues from different segment of small intestine of sacrificed birds of each group. Height of intestinal villus was measured by ocular micrometer under 10X objective of the compound microscope. Average of six readings was taken. The reading obtained from ocular micrometer was multiplied by conversion factor, (calculated with the help of stage micrometer) to obtain the actual height in millimeter (mm). Then the length was converted into micron (μ).

e. Statistical analysis

All data were analyzed in the general linear model (GLM) of SPSS version 10.0 (1999). Dietary combinations were used as the fixed factors and the results were expressed as mean and pool standard error mean. Probability value of $p<0.05$ was described as statistically significant and $P<0.01$ was described as highly significant.

3. Results

a. Biological studies of growth performance

The effect of phytogenic growth promoter on body weight and body weight gain (per week basis) is presented in Tables 2 and 3 respectively. From the Table 2 it is evident that after the end of 2nd week of experiment, there was no significant difference in body weight of the birds of the control (T1 and T2) and two treatment groups. (T3 andT4).

At the end of 3rd week there was significant difference between the negative control (T1) and the rest groups ($p<0.05$).The highest body weight was observed in the birds of T4 group followed by T2, T3 and T1 groups respectively. Birds of the negative control group revealed the lowest body weight.

From the Table 3 it was obvious that after the end of 3rd week of experiment, there was no significant difference in body weight gain of the birds of the control(T1 and T2) and two

treatment groups.(T3 and T4) (P>0.05). At the end of 4th week of the experiment significant difference in body weight gain among the birds of the T1 and rest of the groups ((T2, T3 and T4) were noticed (P<0.001) though there was subtle difference among the birds of T2, T3 and T4 treatment groups. Birds of T4 group revealed highest body weight gain followed byT2, T3 and T1 groups respectively. Birds of the negative control group revealed lowest body weight gain. At the end of 5th week there was significant difference (P<0.05) in body weight gain among the birds of T1 and rest of groups. Birds of T3 group exhibited highest body weight gain and birds of negative control group revealed lowest body weight gain. Supplementation of phytogenic growth promoter results in much more body weight gain than that of BMD group.

b. Bacteriological studies

Pathogenic bacteria like *Escherichia coli* and *Salmonella sp* were not detected in the crop content of birds of T2, T3, and T4 group. T1 group showed highest population of the previously mentioned pathogenic bacteria. *Lactobacillus sp* was present in the crop of birds of all groups (Table 4).

c. Effect on villus height

Villus height of different parts of small intestine of the experimental broiler birds of control and treatment group is presented in Table 5. Average villus height of small intestine of group T1, T2, T3 and T4 were 904.70, 1118.46, 1182.54 and 1238.45 micron (µ) respectively. Statistical analysis revealed significantly (P<0.001) higher villus height in the birds of treatment group (T3 and T4 group) in comparison to that of T2 group. No significant difference in villus height was detected among birds of T3 andT4 group. Highest villus height was detected in the small intestine of the birds of T4 group. The villus height of T2 group is lower than that of T3 and T4 group.

4. Discussion

The results of present study are in accordance with the findings of Jamroz and Kamel (2002) who observed improvements in daily weight gain (8.1%) and in feed conversion ratio (7.7%) of chickens when feed with diets supplemented (300mg/kg) with a plant extract containing capsaicin, cinnamaldehyde and carvacrol. The present investigation also reveals similar results. Biavatti *et al*. (2003) reported *Alternanthera brasiliana* extracts (180 ml/200 kg feed) improved broiler performance from 14 to 21 days. The findings of the present investigation corroborates with that of the previous worker. Hernandez *et al*. (2004) studied that blend of essential oils of cinnamon, pepper and oregano compounds improved digestibility of nutrients in chicken. Jang *et al*. (2006) in chicken is the benefit of some natural substances on gastro intestinal enzymatic activity, most likely improving nutrient digestibility.

Burt (2004) stated microbial analysis of minimum inhibitory concentration (MIC) of plant extracts from spices and herbs, as well as of pare active substances, revealed levels that considerably exceeded the dietary doses when used as phytogenic feed additive. Aksit *et al.* (2006) reported antimicrobial action of phytogenic feed additive may be in be improving the microbial hygiene of carcass. The present study is in accordance with the earlier findings.

Batal and Parsons (2002) indicated that micronutrients also influenced the morphology of intestines. They observed an increased height of villi of jejunum in broilers at 28th day of age when fed with 5g BioMos/Kg from 7 to 28 day. Jamroz *et al.* (2006) have conducted a study that phytogenic formulations contained pungent principles (e.g. capsaicin) significantly increased intestinal mucus production.

5. Conclusion

From the findings obtained from the present investigation it can be concluded that phytogenic growth promoter enhance productive performance of the broiler in terms of body weight gain with minimum alteration of gut morphology and the possibility of bacterial invasion is much less. Phytogenic growth promoter can be used as a potent replacer of antibiotic growth promoter if used at optimum level.

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Plant based native therapy for the treatment of Kidney stones in Aurangabad (M.S)

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Plants have been the major source of therapeutic agents for curing the human diseases. Tribals as well as the rural people depend for all their medicinal and other necessities on the surrounding plant wealth. The urinary bladder or kidney stone has posed a challenge to the medicinal world. The disease is found occurring in both young and old persons. Even the advanced method and technology for the treatment of urinary calculi is available in the Allopathic system of medicine, it has own limitation as in some cases, several side effects as even periodical reoccurrence of stones in a few. The alternative system of medicine which usually employs natural sources-green medicine with a minimum or no side effects. During the ethnobotanical survey of plants from Aurangabad region, a few medicinal plants used in the treatment of kidney stones have been recorded which are discussed in this paper

Keyword: Therapeutic Agents, Kidney Stones, Allopathic System, Aurangabad Region.

1. Introduction

Kidney acts as a filter for blood, removing waste products from the body and helping to regulate the levels of chemicals which are important for body functions. The urine drains from the kidney into the bladder through a narrow tube called the ureter. When the bladder fills and there is an urge to urinate, the bladder empties through the urethra, a much wider tube than the ureter.

In some people, the urine chemicals crystallize and form the beginning, or a nidus of a kidney stone. These stones are very tiny when they form, smaller than a grain of sand but gradually they can grow to a quarter inch or larger. The size of the stone doesn't matter as much as where it is located.

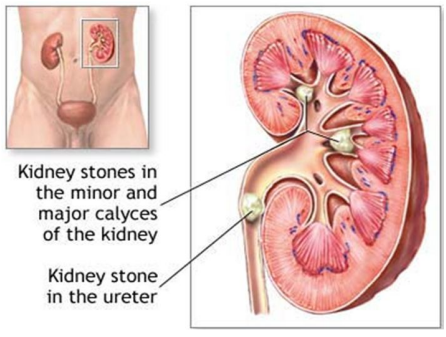

Fig 1: Diagram Showing Kidney Stones

When the stone sits in the kidney, it rarely causes problems, but when it fall into the ureter, it acts like a dam. Kidney continues to function and make urine, which backs up behind the stone,

stretching the kidney. This pressure build up causes the pain of a kidney stone but it also helps to push the stone along the course of the ureter. When the stone enters the bladder, the obstruction in the ureter is relieved and the symptoms of kidney stone are resolved.

Perusal of literature revealed that though a lot of work has been done on the medicinal plants (Chopra *et al.,* 1956, 1965; Dastur, 1962, Dey, 1980, Jain, 1996; Kirtikar and Basu, 1935, Natesh and Mohan Ram, 1999 Sivarajan and Indira Balachandran, 1994). Naik (1998) has attempted the study of medicinal plants of Marathwada. Naser (2002), Naser and Vaikos(2005) has described some plants for human diseases. No account on this aspect pertaining to Aurangabad is available. The present study has been undertaken with the aim of recording of various plants used for treatment of kidney stones by the tribals and non tribals.

2. Study Area

Aurangabad district, a soul of Marathwada region, from the part of Maharashtra state. It is situated in the upper Godavari basin to the extreme north west of the Marathwada. In general, the district slops down towards the south and southeast. The district lies, between the parallels of 19° 20' and 20° 40' 10" north and between 70° 40' and 75° 50' east. The general elevation above the sea level varies between 665 and 735 meters on the north and between 565 and 635 metres towards south. Aurangabad a major district of Marathwada on Deccan plateau, has 440 sq. km forest cover, that is almost 4.35 percentage of the districts total area. The agriculture occupies considerable land. Inhabitants in these areas particularly the rural and tribal people still dwell in and depend on the agricultural and remaining piece of surrounding forests for their needs like shelter, food, fuel, fodder, medicine, animal treatment and farm implements.

The tribal people and ethnic races have developed their own cultures customs, religions rites, legends and myth, folk tales, songs, food and medicinal practices. Many wild and cultivated plants play a very important and vital role among these cultures and these relationships have evolved over generations of experience and practices. The modern civilization which is penetrating into most of the region of the district still holds primitive societies. The consequent divorcement of aboriginal people from dependence upon their vegetal environment for the necessities of life has been set in motion resulting in the disintegration of knowledge of plants and their properties. There appears a steady decline in human expertise capable of recognizing the medicinal plants.

During the study period from December 2008 to July, 2009 several botanical tours have been conducted in various areas of the district in different seasons. Emphasis has been given to visit the area where more and diverse tribal belts and rural people inhabiting different villages are studied.

Fig 2: Maps of India, Maharashtra and Aurangabad

3. Materials and Methods

The methodology of collection of voucher specimens, their preservation in herbaria and technique for the collection of ethnomedicinal information is adopted for the study as recommended by Jain (1989).

During this investigation, ethano-medico botanical data was gathered by interviewing tribals, Bhagats (Tribal vaidyas) Vaidyas, Hakims, village men, even farmers, milkman, senior citizens, knowledgeable man and practitioners. The information was verified by repeated enquiries in different areas of the district. The plants were identified with the help of related literature. The voucher specimen are collected. These specimens are preserved and made into herbarium specimens by conventional method, giving suitable voucher specimen numbers and deposited in the herbaria of Department of Botany, Maulana Azad College, Aurangabad.

The data collected on a particular ailment or species were verified by discussing about these aspects with other tribal facilities. This helped to document quite reliable information not only on the species but also dosages. During the dialogues, care was taken not to contradict with the informants on any point.

4. Preservation of Data

While writing the text, plants were mentioned alphabetically followed by family within parenthesis and vernacular name or local name, voucher specimen number. Followed by the utility of plants for kidney treatment and method of administered doses are narrated.

Biophytum sensitivum. (L.) Dc. (Oxalidaceae), Lajalu, Jharera MACH0057

- Decoction of root is given 3 times a day for removal of kidney stone (Lithiasis).
- Fresh leaves decoction is taken in morning and evening.

Bombax ceiba *L (Salmalia malabaricum Dc.)* (Bombacaceae), Kate sawar MACH0059

- Dry fruit is used in the form of extract or powder before breakfast daily.
- Bark powder 5gms 3 times a day is useful for urination.

Butea monosperma *(Lamk.) Taub.* (Fabaceae), Palas, MACH0063

- Leaves juice or decoction is useful as per requirement.
- Takes seed powder in one teaspoon after meals

Celastrus paniculata Willd. (Celastraceae), Mal kanguni, MACH0087

- Fresh leaves crushed and mixed with curd, gives before breakfast-no intake except water up to 3pm.
- Releases stone in the form of powder.
- ***Celosia argentea*** L. Var. *argentea* L.Sp. (Amaranthaceae), Kurdu, MACH0088
- Root decoction if taken in morning gives good results.
- Seed powder taken in a gap of 4 hours, gives very good results.

Crateva adansonii Dc.Subsp. odora(Buch. Ham.) (Capparaceae), Waiwarna MACH0301

- Bark is used in the form of powder or decoction-before breakfast.

Drimia indica (Roxb.)Jessop. *(Urginea indica)* (Roxb.) (Liliaceae), Jungli piyaz. MACH0302

- Bulb extract is useful in morning before breakfast.

Hemidesmus indicus (l.) (Periplocaceae), Anant mul. MACH0167

- Gives root powder daily morning, afternoon and evening.
- Leaf decoction is used in morning and evening.

Hollarrhena pubescens. *(Buch. Ham).Wall. ex. G. Don* (Apocynaceae), Pandhara kuda, Indrajaw. MACH0172

- Gives Internal bark powder one teaspoon in morning and evening.
- Stem powder used before meals.
- Seed powder takes before breakfast.

Kalanchoe pinnata (Lamk.) (*Bryophyllum calycinum*) (Craussulaceae), Panphuti. MACH0303

- Gives Fresh leaves juice at any time.

Macratyloma uniflora (Lamk.) verde. *(Dolichos biflorus Linn.)*(Fabaceae), Kulthi, Hulge, Kulith. MACH0304

- Fruits boiled in water and cold water gives to patient thrice a day.

Mentha spicata *L.* (Lamiaceae), Pudina. MACH0305

- Fresh leaves should be taken with salt after a particular intervals throughout the day.

Mimosa pudica *L.* (Mimosaceae), Lajalu, Lajwanti MACH0198

- Leaf juice is added in tea and used time to time.
- Root powder gives before breakfast.

Ocimum tenuiflorum L. (Lamiaceae), Tulsi, Tulas. MACH0209

- Entire plant should burn, ash of the plant mixed with water and given thrice a day.

Punica granatum L. (Punciaceae), Anar, Dalimb. MACH0306

- Seed juice is given before breakfast.

Raphanus sativus L. (Brassicaceae), Mula. MACH0307

- Root juice is given after meals.
- Leaf juice is given before breakfast- after this no intake up to lunch.
- Seed powder is useful before breakfast.

Tamarindus indica L. (Caesalpiniaceae), Imli, Chinch. MACH0237

- Dry exocarp of the pod is boiled in the water, this filtered water is given twice a day.

Tephrosia purpurea L. (Fabaceae),Sharapunkha, Unhali MACH0236

- Root powder or juice is useful if taken morning and evening.
- Gives leaf decoction-one glass before breakfast .
- Entire plant boiled and juice is given after particular intervals.

Terminalia arjuna (Roxb.) Wt**.** (Combretaceae), Arjun sadoda. MACH0237

- Gives bark powder after breakfast, lunch and dinner.

Tribulus terrestris L**.** (Zygophyllaceae), Goakru, Sarata. MACH0308

- Leaves decoction is taken in morning and evening.
- Fruit juice or extract is used in morning and evening.

5. Mixture

- Anantmul **(***Hemidesmus indicus***)** + Goakru (*Tribulus terrestris*) + Kurdu (*Celosia argentea*) + Unhali (*Tephrosia purpurea)*.

Mix in water, boil-Filter and cool water should be given as per the requirement.

6. Results and Discussion

This investigation presents a role of plants of this district for kidney stone treatment. Present investigation reveals that the entire plant or their different organs are utilized by tribal people residing at different corners of the district and also by rural and urban persons. After the survey it is observed that in Aurangabad district there is lot of traditional utility of plants for diseases but it is very dishearten to observe that some plants mentioned in this present work, many of them are gradually diminishing. Authors fears that the changing environment, increasing population, government planning may be responsible for this. In this present survey of investigation for Aurangabad district based on ethnobotanical information, a humble attempt is made in this work by way of gathering information. This has proved one fact that some of the plants are quite versatile having varied uses and secondly the knowledge about the ethnobotanical plants has developed independently and parallel, where cross linkage and mutual integrations have been very rare.

It is observed that in the studied area, high population density and urbanization have led to intensive exploitation of plant resources, which in turn brought about the depletion of forest areas. Many drug plants have disappeared and few have reached the brink of extinction and still others facing similar stress. There is urgent need for conservation of some important drug plants.

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Bioprospecting of *Moringa* (Moringaceae): Microbiological Perspective

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Plants produce primary and secondary metabolites which encompass a wide array of functions. Some of these have been subsequently exploited by humans for their beneficial role in a diverse array of applications. However, out of 750,000 species available on earth, only 1 to 10 % is being potentially used. *Moringa* is one such genus belonging to the family of Moringaceae, a monotypic family of single genera with around 33 species. Most of these species have not been explored fully despite the enormous bioactivity reports concerning various potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepato protective; antibacterial and antifungal activities. They are claimed to treat different ailments in the indigenous system of medicine. Surprisingly, some of the species have been reported to be extinct from the face of earth before their exploration and exploitation for economic benefits. This review focuses on the bio-prospects of *Moringa* particularly on relatively little explored area of their microbiological applications

Keyword: Applied microbiology, Antimicrobials, *Moringa* species.

1. Introduction

Plants have been and will remain vital to mankind, animals as well as environment. They produce primary and secondary metabolites which encompass a wide array of functions[1] many of which have been subsequently exploited by humans for their beneficial role in a diverse array of applications[2]. The most important of these bioactive constituents of plants are the secondary metabolites which include alkaloids, phenolic compounds, tannins, phytosterols, and terpenoids. Infectious diseases are the leading cause of death worldwide in the current scenario where clinical efficacy of many conventional antibiotics is being threatened by the emergence of multidrug resistant pathogens[3] and for this, phytomedicine is becoming popular in developing and developed countries owing to its natural origin and lesser[4] side effects. Plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs[5,6]. The list of benefits of plants' bioactive compounds to human health such as anti- cancer, anti-hypertension, anti-hypoglycaemia, anti-oxidants and antimicrobial activities have been reported[7-14].

The search for plants as a source of potential candidate for drug development is still unsound. Out of 250,000 to 500,000 species available on earth only 1-10% percent are being potentially used[15]. *Moringa* is one such genus whose various species have not been explored fully despite the enormous reports concerning the various parts of a few species' potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepato- protective; antibacterial and antifungal activities. These are also being used for treatment of different ailments in the indigenous system of medicine[16-21]. The indigenous knowledge and use of *Moringa* is referenced in more than 80 countries and it's known in over 200 local languages. *Moringa* has been used by various societies including the Roman, Greek, Egypt, India and many others for thousands of years with writings dating as far back as 150 AD. The history of *Moringa* dates back to 150 B.C. where ancient kings and queens used *Moringa* leaves and fruit in their diet to maintain mental alertness and healthy skin. Ancient Maurian warriors of India were fed with *Moringa* leaf extract in the warfront. The Elixir drink was believed to add them extra energy and relieve them of the stress and pain incurred during war. The *Moringa* species are currently of wide interest because of their outstanding economic potential. Amongst these species, *M. oleifera* is the most prevalent for its nutritious and numerous medicinal uses that have been appreciated for centuries in many parts of its native and introduced ranges[22-24].Recently, a few others like *M. stenopetala, M. peregrina* and *M. concanensis* have been discovered to be having equal potential such as nutritious vegetables, high-quality seed oil, antibiotics and water clarification agents just like the *M. oleifera*. In this review we focus on the bio-prospects of *Moringa* species particularly on relatively little explored area of their microbiological applications and ascertain the prevailing gaps.

2. Moringaceae Family

The family Moringaceae is a monotypic family of single genera with around 33 species (Table 1) of which 4 are accepted, 4 are synonym and 25 are unassessed[25]. Out of these, 13 species, native of old world tropics[26] are documented. (Table 2, Figure1 a, b, c)

2.1. Taxonomic classification: [27]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Eudicots
Subclass	:	Rosids
Order	:	Brassicales
Family	:	Moringaceae
Genus	:	*Moringa*

Table 1: Different species of *Moringa*

Species	Authorship	Taxonomic status
Moringa amara	Durin	Unresolved
M. aptera	Gaertn.	Unresolved
M. arabica	Pers.	Unresolved
M. arborea	Verdc.	Unresolved
M. borziana	Mattei	Unresolved
M. concanensis	Nimmo ex Dalzell & A.Gibson	Unresolved
M. concanensis	Nimmo	Unresolved
M. domestica	Buch.-Ham.	Unresolved
M. drouhardii	Jum.	Unresolved
M. edulis	Medik.	Unresolved
M. erecta	Salisb.	Unresolved
M. hildebrandtii	Engl.	Unresolved
M. longituba	Engl.	Unresolved
M. Moringa	(L.) Millsp.	Synonym
M. myrepsica	Thell.	Unresolved
M. nux-eben	Desf.	Unresolved
M. octogona	Stokes	Unresolved
M. oleifera	**Lam.**	**Accepted**
M. ovalifolia	**Dinter & A.Berger**	**Accepted**
M. ovalifolia	Dinter & Berger	Unresolved
M. ovalifoliolata	Dinter & A. Berger	Synonym
M. parvifolia	Noronha	Unresolved
M. peregrine	**(Forssk.) Fiori**	**Accepted**
M. polygona	DC.	Unresolved
M. pterygosperma	Gaertn.	Synonym
M. pygmaea	Verdc.	Unresolved
M. rivae	Chiov.	Unresolved
M. robusta	Bojer	Unresolved
M. ruspoliana	Engl.	Unresolved
M. stenopetala	**(Baker f.) Cufod.**	**Accepted**
M. streptocarpa	Chiov.	Unresolved
M. sylvestris	Buch.-Ham.	Unresolved
M. zeylanica	Burmann	Synonym

Source data[25]

Table 2: Geographic distribution of documented 13 *Moringa* species and their morphotypes

Species	Geographical location
Bottle trees	
M. drouhardii Jum	Madagascar
M. hildebrandtii Engl.	-do-
M. ovalifolia Dinter & A. Berger	Namibia and S.W. Angola
M. stenopetala (Baker f.) Cufod	Kenya and Ethiopia
Slender trees	
M. concanensis Nimmo.	India
M. oleifera Lam.	-do-
M. peregrina (Forssk) Fiori	Red Sea, Arabia, Horn of Africa
Tuberous shrubs and herbs of North Eastern Africa	
M. arborea Verdc.	North Eastern Kenya
M. borziana Mattei	Kenya and Somalia
M. longituba Engl.	Kenya, Ethiopia, Somalia
M. pygmaea Verdc.	North Somalia
M. rivae Chiov.	Kenya and Ethiopia
M. ruspoliana Engl	Kenya, Ethiopia, Somalia

Source data[28]

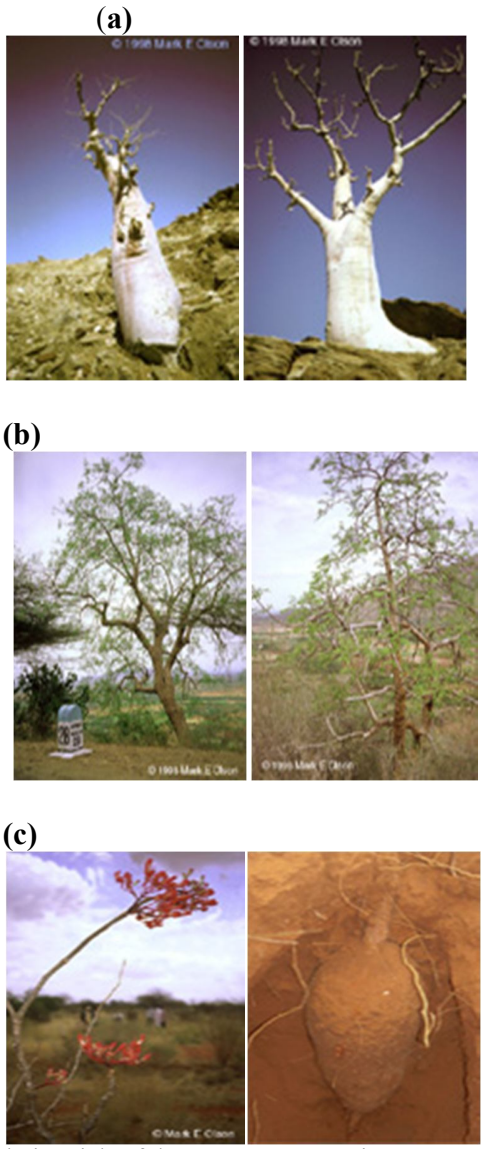

Figure 1: Morphological pictorials of three *Moringa* species; **a):** Bottle Trees; **b):** Slender Trees; **c):** Tuberous shrubs. Reprinted with permission from[28]

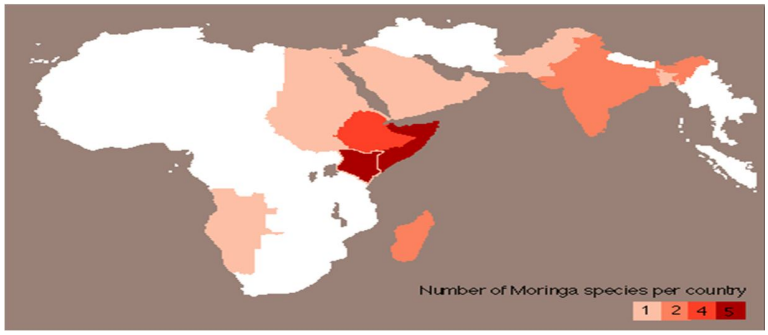

Figure 3. Distribution of *Moringa* in the old world tropics. Reprinted with permission from[28]

3. Bioactivity feasibility of *Moringa*

3.1. Indigenous claims of unexplored Moringa species

Out of the 33 species listed in Table 1, only documentation of 13 species is available. Though the information is not of scientific background, the claims by various indigenous people on their medicinal uses are an indication of the untapped potential (Table 3).

Table 3: Unexplored species of *Moringa*

Species	Morphological characteristics	Indigenous claims	Geographic location
M. drouhardii Jum.	Bloated bright white trunk	Scented bark used for Colds and coughs	Madagascar
M. ovalifolia Dinter & A. Berger	Bloated white trunk	Not documented	Namibia
*M. Pygmaea**	Tuberous shrub with tiny leaflets and yellow flowers	Not documented	Somalia
M. rivae Chiovenda*	Large spray of pale pink & wine Red flowers	Not documented	Kenya, Ethiopia
M. ruspoliana Engler	A small tree with thick, tough & large Leaves, large flowers, thick taproot which becomes more globose as the plant ages	Not documented	Somalia, Ethiopia, Kenya
M. longituba Engler	Bright red flowers, large tuber	Root extracts used for treating intestinal infections of domestic animals	Kenya, Ethiopia, Somalia
M. arborea Verdcour	Large sprays of pale pink & wine red flowers	Unspecified medicinal uses	Kenya, Ethiopia
M. borziana Mattei	Bears one or two stems which die back to the tuber every few years, sometimes due to variation of environmental parameters the plant grows into a small tree, Greenish cream to yellow flowers with brown Used for treatment of smudges on the petal tips	Used for treatment of different ailments	Kenya and Somalia
M. hilderbrandtii Engler	A massive water storing bloated trunk, deep red stem tip of young plants, large spray of small whitish flowers[29,30]	Unspecified medicinal uses	Madagascar
M. Peregrine (Forssk) Fiori	Erect trunk and white bark, small long and remote leaflets, pendulous pods with angled nut like white seeds	Used as an analgesic in the ancient world[31]	Egypt

*: Extinct species; Source data[28]

3.2. Documented Moringa species and their known bioactivities

Moringa oleifera Lamarck (Lam.) also known by different common names as per the different countries' vernacular names and can be found in the following link: http:/www.treesforlife.org/our-work/our-initiatives/*Moringa*. It is a small, fast growing evergreen or deciduous tree that usually grows up to 10 or 12m in height. It has a spreading, open crown of drooping, fragile branches, feathery foliage of tripinnate leaves and thick corky, whitish bark [21,22,32]. The uses of its roots, root bark, stem bark, exudates, leaves, flowers and seeds in the treatment of a wide variety of ailments have been discussed in the Sanskrit texts on medicinal plants[24] and the tree continues to have an important role particularly as counter-irritant in the

indigenous medicine in Asia and West Africa[33,21,34]. Based on the indigenous claims, *Moringa oleifera* (the drumstick tree) has been prevalently the subject of much research and development. *M. stenopetala* (Baker f.) Cufodontis is another species in focus due to its equal potential as *M. oleifera*. It is an important food plant in southwestern Ethiopia, where it is cultivated as a crop plant. *Moringa concanensis,* Nimmo. Tree commonly known as Horseradish tree, Drumstick tree, Never die tree, West Indian ben tree, and Radish tree which is native through the sub- Himalayan tracts of India[35,36]. It has a very strong central trunk that is covered with an extremely distinctive layer of very furrowed bark. The flowers also have distinctive green patches at the tips of the petals and sepals. It is commonly known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamil Nadu, India, and widely used since the Ayurveda and Unani medicinal systems for the treatment of several ailments[37,24]. In Microbiological perspective, the various parts of these plants have been explored and reported for their potential in medical, food industry, agriculture and the environment as explained further;

3.2.1. Moringa in Water Treatment

Water is vital to life; however, due to indiscriminate human activities its quality has deteriorated causing about 80% of diseases which plague the human race especially in many developing countries. Promising water treatment techniques are far much costly for the 'have nots' and many disinfectants currently used can be harmful. For instance, it has been indicated that the chemicals used for water purification can cause serious health hazards if mishandled in the course of treatment process[38,39]. These reports suggested that a high level of aluminium in the brain may be a risk factor for Alzheimer's disease. Several researchers have raised doubts about the advisability of introducing aluminium into the environment by the continuous use of aluminium sulphate as a coagulant in water treatment[39,40,41]. This has aggravated the search for safer organic alternatives. *Moringa* seeds have been commonly known and used in many rural areas for clarification of drinking water to reduce the health risks associated with excessive turbidity. Turbidity has also been found to have a significant effect on the microbiological quality of drinking water and can interfere with the detection of bacteria and viruses[42].Though not a direct indicator of health risk, a strong relationship between turbidity removal and protozoa removal has been established[43].

A simplified, low cost, point of use, low risk drinking water treatment protocol using *M. oleifera* seeds has been invented for use by the rural and pre – urban people living in extreme poverty who are presently using highly turbid and microbiologically contaminated water. Systematic research has shown that *M. oleifera* seeds acts as an effective water clarifying agent across a wide range of various colloidal suspensions[44]. They yield water soluble organic polymer also known as natural cationic (net positive charge) poly-electrolyte[45]. It was confirmed later to be a low molecular weight water soluble protein with a positive charge acting as a coagulant responsible for binding with predominantly negative charged particulate matter that make raw water turbid[46]. In an investigation on *Moringa oleifera* (Drumstick) seed as natural absorbent and antimicrobial agent for river water treatment, a reduction of microbial colonies on plate with increase in concentration of sample was observed[47]. This endorsed the previous study[34,48] as 4 alpha rhamnosyl oxybenzyl isothiocyanate and presently known as glucosidal mustard oil which coagulates the solid matter in water and removes a good portion of suspended bacteria. Similar studies

show that *Moringa* flocculants are basic polypeptides with molecular weight between 6 and 16kDa with an isoelectric pH of 10 to11[49].The natural poly-elecrolytes released from the crushed seed kernels function as natural flocculating agents, binding suspended particles in a colloidal suspension, forming larger sedimenting particles (flocs) in which microorganisms are generally attached. Hence, treatment employing *M. oleifera* seed (press cake) can remove 90% to 99% of fecal coliforms bacterial load[50]. However, it has to be noted that after several hours of storage, temperature-induced bacteria might regrow within the storage container and there's no guarantee for 100% virus and /or bacteria-free water immediately after treatment or storage hence additional disinfection process may be required. Similarly, a group of researchers in their study on traditional water purification using *Moringa oleifera* seeds discovered a steroidal glycoside – strophantidin as a bioactive agent which was more efficient in the clarification and sedimentation of inorganic and organic matter in raw water and reduced 55% and 65% microbial and coliform load respectively after 24 hours whereas alum achieved 65% and 83% reduction under similar conditions[51]. The difference in efficacy as shown in both cases above may be attributed to the effect of oil on the bioactive agent. It forms an emulsion of film coating which may inhibit its contact with the surface of reaction and thus reduce floc formation[46]. Similarly, the difference in location of cultivation as reported[52,53] may cause the variation.

The seeds of *Moringa stenopetala* have been found to have flocculating and anti-microbial properties. The active substances are found only in the cotyledons of the seeds[54]. In a recent study on the antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seeds extracts exhibited highest inhibition of *E. coli, S. typhi* and *V. cholera* and the samples were active in low doses[55]. This could be due to less oil in low concentrations and it goes well with observations made[46] regarding the effect of oil on the bioactive agent which forms an emulsion of film coating that inhibits the reaction. *Moringa* seeds contain an antibiotic principle known as pterygospermin which is responsible for destruction of micro-organisms in water[56]. In Sudan though not proven scientifically, the seeds of *M. peregrina* have been used as coagulant to purify water[57].

3.2.2. Moringa a Source of Pharmacological Products

3.2.2.1. Alternative Medicine for Human Pathogens

An escalating antibiotic resistance by the pathogenic bacteria has been observed since last decade and the adverse effects of conventional antibiotics calls for a friendly alternative. Out of the 250,000 to 500,000 species of plants on earth[15], *Moringa* is one of the 10% which have a profound potential in pharmaceutical industry as a source of bioactive constituents for drug development. Chen[58] in her studies on the synergistic effect of *M. oleifera* seeds and chitosan (an essential and abundant component of exoskeletons – the mucoadhesive polymer which is derived from chitin) on antibacterial activity against *Bacillus subtilis* and *Pseudomonas putida* found the individual samples to be more effective than the two combined. However, in another study to determine antimicrobial potential of different plant seed extracts against Multidrug Resistant Methicillin Resistant *Staphylococcus aureus* (MDR – MRSA), it was established that *Moringa oleifera* seeds had a synergistic potential to restore the effectiveness of B – lactam antibiotics against MRSA[59]. The synergistic properties could be attributed to β – lactamase inhibition by the Flo peptide (a specific polypeptide

found in *Moringa oleifera* that is both a flocculent and a biocide). The cationic Flo peptide supposedly serves as a highly efficacious immunity response, interacting with the anionic cell membranes of bacterium[60]. This interaction destabilizes the bacterial membrane, causing leakage of cytoplasmic content and killing the bacterial cell. Antimicrobial peptides, such as the Flo peptide, have been reported to act directly and non-specifically upon bacterial membranes, thus hindering their ability to develop resistance. However, antimicrobial peptides rarely affect the membranes of cells in multicellular species[61] an indication that they are ineffective against eukaryotes especially fungal pathogens. Recently, an *in vitro* antimicrobial activity of *Moringa oleifera* L. seed extracts prepared in aqueous and organic solvents against *Staphylococcus aureus*, *Bacillus subtilis*, *Escheriachia coli, Pseudomonas aeruginosa, Aspergillus niger and Candida albicans* exhibited antimicrobial properties[62]. However, this is partly contradicted by the findings in our laboratory using up to 20% aqueous extracts of the same plant (unpublished data) where one of the seeds sample collected from a different locality exhibited less active and to a few test organisms among Gram positive, Gram negative and yeast pathogens used which may be due to differences in source of the samples as depicted earlier[52,53]. The variation may also be brought about by environmental changes such as effect of pathogens[63], allelopathy and herbivory[64] which may trigger production of high levels of secondary metabolites. On the other hand, water availability, exposure to soil pathogens and variations of soil pH and nutrients affect the accumulation of secondary metabolites[65]. Similarly, environmental factors such as temperature, rainfall, day length and edaphic factors affect the efficacy of the medicinal properties of different plants[66].

Urinary tract infections are the second most common type of infection in the world. It is mainly a bacterial infection that affects peoples throughout their lifespan[67]. These are more common in women than men, leading to approximately 8.3 million doctor visits per year. *Proteus mirabilis* is a small Gram negative bacillus, facultative anaerobe belonging to the family of Enterobacteriaceae that commonly cause urinary tract infections and formation of stones[68].

In a study on the antibacterial effect of *Moringa oeifera* leaves extracts prepared in different solvents, petroleum ether extracts demonstrated the highest activity against clinical samples and environmental samples of *Proteus mirabilis*[69]. However, in a separate study chloroform extracts[70] showed broad spectrum potential than that of petroleum ether an indication of the presence of different active principles. *In vitro* studies on different extracts of the root bark of *Moringa oleifera* against *Staphylococcus aureus, Echerichia coli, Salmonella gallinarum, Pseudomonas aeruginosa* among others showed that ethyl acetate and acetone extracts exhibited maximum activity as compared to other solvents[71,72] which shows that active compounds are polar in nature. Antimicrobial activity studies of stem bark of *Moringa oleifera* against some human pathogens demonstrated methanolic extracts to be the most effective among other solvents used[73,74]. In most recent study on *in vitro* antibacterial and antifungal potential of *Moringa oleifera* stem bark against ten bacterial strains and six fungal strains, petroleum ether extract was reported inactive[75]. To this point, it emanates that the different plant parts of *Moringa* contain a vast array of bioactive constituents of varying polarity which can be potential candidates as drug leads/ development.

Still within this genus, a project on development of pharmaceutical products from medicinal plants was carried out in Ethiopia by the institute of Pathobiology, Addis Ababa University in 1996, *Moringa Stenopetala* was one of the plants assessed. It was reported that the biological active compounds isolated from both leaves and seeds of the plant by a bioassay guided fractionation exhibited antimicrobial activity against *Staphylococcus aureus, Salmonella typhi, Shigella* and *Candida albicans.* A number of Laehiums prepared by herbal venders in South India was tested for antimicrobial activity. It was reported that ethanol, petroleum ether, hexane (prepared in 1000 ppm) and aqueous extracts (20%) resins of *Moringa concanensis* (which were traditionally used for treatment of fire burns) exhibited antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*[76].

3.2.2.2. Synthesis of Nanoparticles

In addition to above searches, the possibility of using *Moringa* in nanotechnology is being explored for useful products. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. Metal nanoparticles which have a high specific surface area and a high fraction of surface atoms have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties[77]. Nanoparticles have a long list of applicability in improving human life as well the environment and among this drug delivery technology. The technology has come into spotlight due to its benefits such as shorter development periods and lower costs compared to the development of a new drug[78-80]. The ideal nanoparticle materials are those which do not undergo chemical changes, satisfy the conditions of biodegradability, bio-compatibility and delivery speed of the drug[81,82]. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained[83,84]. Generally metal nanoparticles are synthesized and stabilized by using chemical methods such as chemical reduction[85,86] which are too costly and hazardous. For this reason, cheaper and environmental friendly biological methods are sought for. Synthesis of nanoparticles within the biological means using bacteria and fungi[87,88], has been proved efficient and safer. However, it has been established that use of plant leaves extract is cheaper[89] as it reduces the costs and does not require any special culture preparation and isolation techniques. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provides advancement over chemical and physical method as it is cost effective and eco-friendly. It can be easily scaled up for large scale synthesis and there is no need to use high pressure, energy, temperature and toxic chemicals. In this regard, investigations in the biofabrication of Ag nanoparticles using *M. oleifera* leaves extract revealed the leaves to have the potential of producing Ag nanoparticles extracellularly by rapid reduction of silver ions (Ag^{+} to Ag^{0}) which were quite stable in solution[90]. In subsequent testing for antimicrobial activity against a number of pathogens, this Ag nanoparticles suspended hydrosol showed considerable antimicrobial activity in comparison to chloramphenicol and ketoconazole antibiotics.

3.2.2.3. Bio- Enhancing Properties

Some parts of this multipurpose genus have also been associated with bio-enhancing properties. Bio-enhancers are molecules which do not possess drug activity of their

own but promote and augment the biological activity or bioavailability or the uptake of drugs in combination therapy, resulting in reduced drug associated toxicity, reduced cost and duration of chemotherapy. Isolated plant biomolecules or their semisynthetic derivatives have provided useful clues in the production of medicines[91]. Recently, in a pre-clinical study on the influence of *Moringa oleifera* pods on Pharmacokinetic disposition of rifampicin using HPLC- PDA method established that the active fraction isolated from air dried pods of the plant when mixed with rifampicin and administered to the experimental animals enhanced systemic availability of the drug and suppression of the drug metabolizing cytochrome P-450[92]. In another study, a bioenhancing property of *M. oleifera* pods extract was reported. It was found that niaziridin rich fraction of *M. oleifera* pods enhances the bioactivity of commonly used antibiotics such as rifampicin, tetracycline and ampicillin against Gram positive and Gram negative bacteria. It also facilitated the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bio-availability[91]. This lowering of the dosage level and shortened treatment course of rifampicin as an anti-tuberculosis drug minimize its associated side effects to the advantage of the patients.

3.2.2.4. Other Pharmacological Potentials

Though the review focuses on the economic potentials of *Moringa* from the Microbiological perspective, it will be worth listing the prospects of this genus in other pharmacological fields (Table4)

3.2.3. Moringa in Food Preservation

Protection of food from microbial or chemical deterioration has traditionally been an important concern in the food industry. Chemically synthesized preservatives have been classically used to decrease both microbial spoilage and oxidative deterioration of food[106]. However, in recent years; consumers are demanding partial or complete substitution of chemically synthesised preservatives due to their possible adverse health effects. This fact has led to an increasing interest in developing more "natural" alternatives in order to enhance shelf-life and safety of the food[107]. Though not so extensive work in this field in regard to the plant under review, a recent study indicated that seeds exhibited the potential as sanitizers/preservatives by inhibiting the growth of organisms such as *E. coli, S. aureus, P. aeruginosa, S. typhi, S. typhimurium* and *E. aerogenes* which range from pathogenic to toxigenic organisms liable to cause food – borne illnesses to spoilage-causing organisms liable to spoil food products[108].

3.2.4. Moringa in Agriculture

The practice of using plant derivatives or botanical insecticides in agriculture dates back to at least two millennia in ancient China, Egypt, Greece, and India [109,110]. What is clear from recent history is that synthetic insecticides effectively relegated botanicals from an important role in agriculture to an essentially trivial position in the marketplace among crop protectants. However, history also shows that overzealous use of synthetic insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, farm workers, and even consumers; destruction of fish, birds, and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening human and environmental health; and the evolution of resistance to pesticides in pest populations[111-114]. Trials on the potential of *Moringa oleifera* for agricultural and

Table 4: Scientific / Traditional claims of pharmacological potentials of Moringa species

Species	Therapeutic Effects	Plant Part	Referenc
a	b	cL,F,B,R,P,S,O,G,	35, 93- 97
d	Leishmaniasis trypanosomiasis	LR	98,99
	Anti-fertility, oxytocic, activity, hypoglycaemic activity, cholesteral reduction	L	99-101
e	Cosmoceutical	O	
	Fire burn wounds	G	102
	Ophthalmic, goitre, venereal infections, glycosuria, lipid disorder	S	76
	Anti-inflammatory, analgesic, Antipyretic,	F	103
	Cholesterol reduction * and body weight, ophthalmic, menstrual pains, women fertility, aphrodisiac, jaundice, fatigue, diabetes blood pressure*,constipation* ,skin tumor	L	
	Thyroid disorders, leucorrhea, abortion	F	
	Spleenomegaly*	L_T	
	Bloat	B_s	
	Intestinal worms	S	
	Head ache*	G	
	Spinal cord pain	R	104
f	Analgesic Abdominal Burns Constipation Febrifuge Laxative Headache	NS**	31
	Cosmoceutical , abdominal pain	O	
	GNS***	S	57
g	GNS***	W	
h	GNS***	W	30
****	Cosmoceutical Colds and coughs	O BW	105
	-	-	-

a: *M. oleifera;* **b**: Comprehensively covered; **c**: L- leaves, L_T- tender leaves, F-flowers, B-bark, Bs- stem bark, R-roots, P-pods, S-seeds, O-oil (from seeds), G-gum, W-wood; **d**: *M. stenopetala*; **e**:M. *concanensis*; **f**:*M. peregrina*; **g**: *M. hildebrandtii*; **h**: *M. drouhardii*;*: Combination therapy; **NS: Not specified; ***GNS: General not specific; ****: Other species not documented

industrial use have shown that the leaves of this plant contains a bioactive substance which when sprayed on crops indicates accelerated growth of young plants, which become more resistant to pests and diseases[115]. Though no further literature is available in this regard, the potential of *Moringa* in agriculture is obvious in this study and needs extensive exploration.

4. Toxicological reports on some *Moringa* species

Toxicological evaluation of medicinal plants has often been neglected by many traditional healers with the notion that plants are harmless and therefore historical usage of such products cannot always be a reliable guarantee of safety. It is difficult for these practitioners to detect or monitor delayed effects (e.g. mutagenicity), rare adverse effects arising from long-term use[116] as in food supplements and nutraceuticals e.g. *Glycyrrhiza glabra*, which is used for conditions like bronchitis and peptic ulcers causes not only hypertension, weight gain and hypokalaemia but also low levels of aldosterone and anti-diuretic hormone on excessive or prolonged usage[117]. Many widely used medicinal plants have been implicated as possible causes of long-term disease manifestations such as liver and kidney diseases for instance widespread use of *Scenecio, Crotalaria* and *Cynoglossum* has been implicated in the occurrence of liver lesions and tumours, lung and kidney diseases in certain areas of Ethiopia [118]. Similarly, reports are available on accidents due to mistakes of botanical identification, plants that interfere with pharmacological therapy (such as those containing coumarinic derivatives, high tyramine content, those containing oestrogenic compounds, those that cause irritation and allergy, those containing photosensitive compounds)[119-123].

In light of the above, there is scanty literature on *Moringa* genus in regard to toxicological studies. However, the few reports on *M. oleifera* and *M. stenopetala* available are not exhaustive. Adedapo *et al*.[124] established in their study that aqueous extract of *Moringa oleifera* leaves was nontoxic in rats both oral and sub-acute on haematological biochemical and histological parameters. In a similar study, Ashong and Brown[125] observed no impact and short term toxicity of aqueous leaf extracts of various concentration on feed intake of poultry. The ethanolic and aqueous extracts of *M. oleifera* bark were found to have no adverse effect on growth related and biochemical parameters in rats, an indication that neither steroids, triterpenoids, saponins, alkaloids nor carbohydrates phytoconstituents identified were toxic [126]. Kasolo *et al.*[127] in their *in vivo* study established that acute toxicity tests with aqueous and ethanol extracts of M. *oleifera* roots exhibited a safe range where the LD_{50} for aqueous extracts was 15.9mg/kg and for ethanolic extract was 17.8mg/kg. However, in the most recent studies on the effect of methanolic extracts of *M. oleifera* roots on histology of kidney and liver on Guinea Pigs was found to distort the histo-architecture of both the organs and the effects were time as well as dose dependent[128]. Several *in vivo* studies indicate aqueous extracts of M. *oleifera* seeds to be safe[129,130]. However, Oluduro and Aderiye[131] contradicted these findings in their study on the effect of *M. oleifera* aqueous seed extracts on vital organs and tissue enzyme activities of male albino rats where their findings suggested that prolonged consumption of water treated with $\geq$ 2mg/ml of *M. oleifera* seeds may lead liver infarction. Similar observations though with methanolic extracts were made in a different study which confirmed that administration of these seeds extracts appears relatively nontoxic to animals at low

doses. However, at high dosages, the alterations observed in various parameters tested suggested a dose sensitive toxicity when repeatedly consumed on a daily basis for a prolonged time[132].

The cytotoxicity of extracts from a widely used species of plant, *Moringa stenopetala*, was assessed in HEPG2 cells, by measuring the leakage of lactate dehydrogenase (LDH) and cell viability. The functional integrity of extract-exposed cells was determined by measuring intracellular levels of ATP and glutathione (GSH). The ethanol extracts of leaves and seeds significantly increased ($p < 0.01$) LDH leakage in a dose- and time-dependent manner. However, aqueous extract of leaves and ethanol extract of the root did not increase LDH leakage. A highly significant ($p < 0.001$) decrease in HEPG2 viability was found after incubating the cells with the highest concentration (500µg/ml) of the ethanol leaf and seed extracts. The water extract of the leaves did not alter GSH or LDH levels or affect cell viability, suggesting that it may be non-toxic. This was an indication that not all compounds of these morphological parts tested were toxic but only those extractable by ethanol[98]. In another study to establish the effects of *M. stenopetala* on Blood parameters and histopathology of liver and kidney in mice, it was reported that the extract did not show any morphological changes in the liver cells as well as no histopathological changes in the kidneys of the treated mice with all doses used (600,750,900mg/kg) [101]. From these studies we cannot make a haste conclusion of safety or toxicity of the sample under study since different solvents are responsible for extracting different compounds. Therefore, a compound of interest should be tested on its own or a systematic extraction (using all possible solvents) and subsequent toxicity testing of the compound of interest is of paramount importance. However, it calls for intervention of more rapid and less expensive approaches to work at the *in vitro* as well as *in vivo* toxicity.

5. Phytoconstituents of *Moringa*

Out of the 13 species of *Moringa, Moringa oleifera* has been given much publicity including its phytoconstituents. A few others such as *M. stenopetala, M. peregrina, M. concanensis* have been reported. Nevertheless, the various studies reported (Table 5) are not exhaustive and much work is needed to establish the comprehensive phytoconstituents of these and other *Moringa* species, and further explore and exploit their antimicrobial properties not forgetting to ascertain the safety of the active principles.

In light of the limitations in the various studies reported herein, looking at different parameters in a systematic way in this context and not just a single assay for determining the biological efficacy of plants is of essence based on the following facts:

- Some compounds which show good activity *in vitro* may be metabolized *in vivo* into inactive metabolites. Alternatively, extracts may only show *in vivo* activity due to the metabolism of inactive compounds into active forms [140].
- Similarly, the pharmacological investigation of drug interactions in multi-compound preparations is difficult due to the presence of several constituents where some may show less specific activity and some may camouflage the toxicity and activity of the more therapeutically effective compounds.
- There is no one solvent which can extract all the phyto-constituents and thus several of them should be used for better comparison.
- Some of the most common side effects are difficult to recognize in animal models e.g. nausea, nervousness, lethargy, heartburn, headache, depression, stiffness, etc.
- *In vitro* findings may not extrapolate into *in vivo* models such as animals and humans. Therefore a thorough investigation and trials

to authenticate such findings is the need of tomorrow.

- Toxicological studies are mandatory at the initial stages of pharmacological studies to avoid waste of resources on unsuitable compounds.
- Efforts are required for wholesome research including elucidation of structure of responsible compound/s and establishing the mechanism of action.

Table 5: Phytoconstituents of various *Moringa* species

Plant Species	Phytoconstituents and their bioactivity		Reference
[a]	**Stem bark**		
	Methanol	NR***	
	Moringly linoleneate,	-do-	
	β- sitosterol, epilupeol,	-do-	
	oleiferyl capriate,	-do-	
	glyceropalmityl phosphate,	-do-	
	glycero-oleiostearyl phosp.	-do-	133
	Standard methods		
	Alkaloids	-	
	Saponins	-	
	Triterpenoids	Antimicrobial	
	Diterpenoids	-do-	
	Flavonoids	-do-	
	Cardiac glycoside	-do-	
	Tannins	-	
	Phytosterols	-	Arora and Onsare (unpublished)
	Ethanol		
	Steroids	NR***	
	Triterpenoids	-do-	
	Saponins	-do-	
	Alkaloids	-do-	
	Carbohydrates	-do-	
	Distilled Water		
	Saponins	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	126
	Seeds		
	Ethanol		
	Sterols	NS*	
	Glycosides	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Flavonoids	-do-	
	Petroleum ether		
	Sterols	-do-	
	Carbohydrates	-do-	
	Flavonoids	-do-	
	Chloroform		
	Carbohydrates	-do-	
	Distilled water		
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Flavonoids	-do-	134
	Roots		
	Ether		
	Gallic tannins	NR***	
	Steroids	-do-	
	Triterpenoids	-do-	
	Anthraquinones	-do-	

Ethanol		
Catechol Tannins	-do-	
Steroids	-do-	
Triterpenoids	-do-	
Anthraquinones	-do-	
Alkaloids	-do-	
Carbohydrates	-do-	
Distilled Water		
Saponins	-do-	
Alkaloids	-do-	
Carbohydrates	-do-	127
Leaves		
Petroleum ether		
Alkaloids	NS**	
Flavonoids	-do-	
Tannin	-do-	
Phenolic compounds	-do-	
Carbohydrates	-do-	
Acetone		
Tannin	-do-	
Phenolic compounds	-do-	
Carbohydrates	-do-	
Isopropyl alcohol		
Alkaloids	-do-	
Flavonoids	-do-	
Tannins	-do-	
Phenolic compounds	-do-	69
Ethanol		
Flavonoids	NS**	
Tannin	-do-	
Glycoside	-do-	
Terpenoids	-do-	
Chloroform		
Alkaloids	-do-	
Saponins	-do-	
Tannin	-do-	
Distilled Water		
Flavonoids	-do-	
Saponins	-do-	
Tannin	-do-	
Glycoside	-do-	
Terpenoids	-do-	135
Seeds' coat		
Standard methods		
Alkaloids	-	
Triterpenoids	Antimicrobial	
Flavonoids	-do-	
Diterpenoids	-do-	
Cardiac glycoside	-do-	
Phytosterols	-	
Tannins	-	
Pods' husks		
Standard methods		
Alkaloids	-	
Triterpenoids	Antimicrobial	
Flavonoids	-do-	
Diterpenoids	-do-	
Cardiac glycoside	-	
Phytosterols	-	
Tannins	-	Arora and Onsare (unpublished)
Leaves		
Standard methods		

b	Chromophers	Hypotensive	
	Polyphenols	-do-	
	Saponins	-do-	
	Physteroides and withanoids	-do-	
	Flavonoids	-do-	
	Tannins	-do-	
	Alkaloids and anthraquinone	-do-	
	Glycosides	-do-	136
c	**Seeds**		
	4-(α-L-Rhamnosyloxy) benzyl-isothiocyanate	NR***	48
	4-(4'-*O*-Acetyl- α-L-rhamnosyloxy)-benzyl isothiocyanate	-do-	
	2-Propyl isothiocyanate	-do-	
	2-Butyl isothiocyanate	-do-	
	2-Methylpropyl-isothiocyanate	-do-	
	5,5-Dimethyl-oxazolidine -2-thione	-do-	137
	Roots		
	4-(α –L- Rhamnosyloxy) Benzyl-isothiocyanate	-do-	138
	Stem bark		
d	**Hydroalcoholic**	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Saponins	-do-	
	Terpenoids	-do-	
	Flavonoids	-do-	
	Proteins	-do-	
	Chloroform		
	Alkaloids	-do-	
	Tannins	-do-	
	Terpenoids	-do-	
	Ethyl acetate		
	Carbohydrates	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Flavonoids	-do-	
	Petroleum ether		
	Steroids	-do-	
	Terpenoids	-do-	
	Ethanol		
	Carbohydrates	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Saponins	-do-	
	Flavonoids	-do-	
	Proteins	-do-	139
e*****			

a: *M. oleifera*; **b:** *M. stenopetala*; **c:** *M. peregrine*; **d:** *M. Concanesnsis*; **e:** Other species; NS*: Anti-inflammatory reported but not specified; NS**: Antibacterial reported but not specified; NR***:Not reported; NT**** :Not yet tested; *****: Literature not available.

6.0. Strength, Opportunities and Threats

Out of the 33 species of *Moringa,* 13 have been documented where *M. oleifera, M. concanensis, M. peregrina* and *M. stenopetala* and the unexplored ones have been proven scientifically as well as untapped indigenous claims to have prospects as potential candidates for development of useful products. Hence, the establishment of the location as well as continual documentation of information of the various species of this family is a breakthrough towards exploration and exploitation of their potentials. However, amid the much work on the Indian *Moringa oleifera,* gaps in comprehensive microbiological studies in this as well as the other unexplored species of the genus still exist. A systematic research of this prospective Moringaceae family will lead to potential bio-products of vast array applications in fields such as; water, pharmaceutical, food and agricultural industries thus,

1. A great hope to the two million people who die from diseases caught from contaminated water every year, with the majority of these deaths occurring among children under five years of age.
2. Safer and eco-friendly products.
3. Empowerment to the 'have nots' and hope to unemployed.
4. It should be noted with concern that some of the species are reported to be extinct from the face of the earth. Human activities and the aggravating effects of climate change may lead to loss of the remaining species unless appropriate measures are taken into consideration. Similarly from the technical point of view, due to lack of systematic research in addition to limitations already heighted, the lives of many may be at stake especially those who rely on herbal products whose toxicity has not been established.

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8. Conflict of Interest

The authors declare no conflict of interest in the contents of this paper.

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Pharmacognostial Characterization of Some Selected Medicinal Plants of Semi-Arid Regions.

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Pharmacogonasy is mainly concerned with naturally occurring substances having a medicinal action. It also includes the study of other material used in pharmacy such as flavoring and suspending agents, disintegrants, filtering and support media and so on. It is closely related to both botany and plant chemistry. During present investigations studies were conducted on some selected plants of semi-arid regions

Keyword: Phamarcognostical Studies, Rajasthan, Medicinal Plants.

1. Introduction

Several studies have been conducted on medicinal plants of Rajasthan (Kumar and Sopory, 2008). Kumar (2000) studied traditional Indian Ayurvedic Medicines and some potential plants for bioenergy, medicine from India. Sharma, Agrawal and Kumar (2003) characterized Medicinal plants for skin and hair care. Mahlawat and Kumar (2005) studied some traditional medicinal plants used by tribal people of Rajasthan in Human ailments. Sharma and Kumar, (2006, 2007), studied traditional medicinal practices of Rajasthan. Parveen et al., (2007) studied Traditional use of medicinal plants among the rural communities of Churu District in the Thar Desert, India have been reported by Parveen et al., (2007). Plant based veterinary medicine from traditional knowledge of India has been recorded in Bulletin of Botanical Survey of India (Sharma, Dadhich and Kumar, 2005). Ethnobotanical survey of medicinal plants from Baran District (Meena and Kumar, 2012:

The word "Pharmacognosy" derives from the Greek words *pharmakon* (drug), and *gnosis* or knowledge. The term pharmacognosy was used for the first time by the Austrian physician Schmidt in 1811. A "crude drug" means a dried unprepared natural material of plant, animal or mineral origin, which is used for medicine. The term "Pharmakognosie" and it discipline developed in German speaking areas of Europe - where it is a synonym of "Drogenkunde" ("science of the crude drugs").

The different fields within today's pharmacognosy include: (i) Ethnobotany or ethnopharmagocology; study of the traditional use of plants in the society. Ethnobotany refers to any use of the plants, whereas ethnopharmacology refers more specifically to the madical use of the plants. (ii) Phytochemistry, or natural product chemistry; a field closely

related to organic chemistry, studying the chemical composition of living organisms. It is also closely connected to the process of finding new drug candidates from natural sources. (iii) Phytotherapy study of crude drugs, i.e. extracts from natural sources in medical use .

Pharmacognosy includes the study of the proper horticulture, harvesting and uses of the raw medicinals found in nature. Its scope includes the identification or authentication of crude drugs (using macroscopical, microscopical, radiological or chemical methods), and their bio-pharmacological and clinical evaluations. Sharma, and **Kumar,** (2012) carried out pharmacognostical studies on medicinal plants of semi-arid regions. Upadhyay, Singh and **Kumar** (2011) studied Ethno-veterinary uses and informants consensus factor of medicinal plants of Sariska region, Rajasthan. Sharma and **Kumar** (2011) recorded ethnobotanical uses of medicinal plants of Rajasthan. Although today pharmacognosy is still taught in a small number of university pharmacy schools in US and in the UK, this subject is still obligatory within the pharmacy curricula in all universities of continental Europe.

Pharmacogonasy is mainly concerned with naturally occurring substances having a medicinal action. It also includes the study of other material used in pharmacy such as flavouring and suspending agents, disintegrants, filtering and support media and so on. It is closely related to both botany and plant chemistry.

During earlier investigations studies have been conducted on ethnobotanical and pharmacognostical characterization of medicinal plants (Kumar, 2000; **Cordell and Colvard, 2005 ;** Parveen et al., Upadhyay et al, Kumar and Sharma, Sharma et al, Sharma et al., 2012
Pharmacognosy has been generally pursued for utilitarian ends and may thus be called an applied science. It has played an important role in the development of the pure sciences, e.g. in descriptive botany, plant classification (taxonomy) and plant chemistry (phytochemistry). Chemical plant taxonomy, genetical studies, involving secondary metabolites are now attracting the attention of more and more botanist and chemists.

Vegetable drugs are usually arranged for study in one of the following five ways:

1. ***Alphabetical*:** The drugs are arranged in alphabetical order using either Latin or English names.
2. ***Taxonomic**:* The drugs are arranged according to the plant from which they are obtained in phyla, orders, families, genera and species.
3. ***Morphological*** : Drugs are divided into different groups such as organized drugs which include root, wood, bark, flowers, fruits and leaves and unorganized drugs such as oil, fats, extracts and gums.
4. ***Pharmacological or therapeutic:*** Classification of drugs is according to the pharmacological action of their most important constituent.
5. ***Chemical:*** Drugs are classified according to their most important constituent that is alkaloids, volatile oils etc.

Pharmacy starting from medicine, separated and materia medica the science of material medicines describing collection, preparation and compounding emerged.

Even up to the begining of 20^{th} century pharmacognosy was more a descriptive subject mainly of botanical science and consisted of identification of drugs both in entire and powdered condition and their history, commerce, collection, preparation and storage.

1.1 Period 1934-1960:

The development of modern pharmacognosy book place later during the period 1934-1960 by simultaneous application of disciplines like organic chemistry, biochemistry, biosynthesis, pharmacology and modern methods and techniques of analytic chemistry including paper, thin layer and gas chromatography and spectrophotometry.

The substances from the plants were isolated, their structure elucidated and pharmacologically active constituents studied. The development was

mainly due to following four events:

1. Isolation of penicillin in 1928 by Fleming and large scale production in 1941 by Florey and Chain.
2. Isolation of reserpine from Rauwolfia roots and confirming its hypotensive and tranquillising properties.
3. Isolation of Vinca alkaloids especially vincristine and vinblastine. Vincristine was found useful in the treatment of leukeemia. These alkaloids have also anticancer properties.
4. Steroid hormones like progesterone were isolated by partial synthesis from diosgenin and other steroid saponins by Marker's method. From progesterone by microbial reactions, cortisone and hydrocortisone are obtained.

1.2 Progress from 1960 onwards:

During this period only a few active constituents mainly antibiotics, hormones and antitumour drugs were isolated or new possibilities for their production were found. From 6- amino penicillianic acid which has very little antibiotic action of its own but from which important broad spectrum semi-synthetic penicillins like ampicillin and amoxycillin were developed.

From ergocryptine alkaloid of ergot, bromocryptine has been sybnthesised. Bromocryptine is a prolactine inhibitor and also has activity in Parkinson's disease.

By applications of several disciplines pharmacognosy from a descriptive subject has developed into an integral, important discipline of pharmaceutical sciences.

Diseases are born with man and drugs came into existence since a very early period to remove the pain of diseases and to cure them. Thus, the story or history of drugs is as old as mankind.

Drugs used in medicine today are either obtained from nature or are of synthetic origin. Natural drugs are obtained from plants, animals or mineral kingdom. Drugs made from micro-organisms like antibiotics were not known in the early period. Synthetic drugs (or syntheticals) like aspirin, sulpha drugs, some vitamins and some antibiotics are synthesized in laboratories from simple chemical (or chemicals) through various chemical reactions.

Natural drugs obtained from plants and animals are called drugs of biological origin and are produced in the living cells of plants or animals.

Each drug is always obtained from the same plant or animal. The Latin name of the plant or animal is called its botanical or zoological source. The family to which this plant or animal belongs is also mentioned, e.g. Vasaka leaves are obtained from *Adhatoda vasica* plant; family Acanthaceae. Vasaka leaves are included in the Indian pharmacopoeia and are called official leaves. Their botanical source is called official source. Geographical source or habitat gives us information about the country or place where the drug is produced. Ginger is produced in Jamaica and nux vomica and ispaghula in India. In some cases the original native place of a drug is not the same as the present geographical source, e.g. cinchona is a native of South America and is at present cultivated in Indonesia, India and Congo. History of the drugs gives us useful information about how the drug was known, where it was growing originally and how it was introduced into the modern medicine. History of some drugs like cinchona bark, coca leaves, rauwolfia root and opium is very interesting. Politics play its part in the drugs also. Thus there is restriction on the import of buchu leaves growing in South Africa because of our political relations with that country.

Sometimes crude drugs are adulterated. An adulterant is the drug resembling the original or authentic drug but usually quite different or inferior, less effective, containing less percentage of active constituents and sometimes containing more extraneous matter than permitted.. Nature of adulteration can be determined by the study of pharmacognosy.

In organoleptic evaluation macroscopical and sensory characters are mentioned. In microscopical evaluation, microscopic characters of drugs are described. In biological, physical and chemical evaluation quality or activity of the drug is determined.

In chemical classification as the medicinal action

of the drug is due to active chemical constituents, drugs are classified according to the chemical nature of active constituents. Thus alkaloid containing drugs like opium or solanaceous drugs or rauwolfia are arranged under alkaloidal drugs and even according to the chemical nature of alkaloids. Drugs containing anthraquinone glycosides like senna, cascara, rhubarb and those containing cardiac glycosides like digitalis, strophanthus and scilla are grouped together. Similarly, drugs containing volatile oils like clove, cardamom and umbelliferous fruits are put together.

2. Material and Methods

The plant material was collected from Department of Botany, U.O.R. Jaipur. Efforts were made to collect this plant in flowering and fruiting conditions for correct botanical identification. For macroscopical studies free hand sections of the tuberous roots were taken from fresh material. These were further dehydrated, stained in alcoholic safranin and light green and finally mounted in Canada balsam.

3. Pharmacognosy of Some Plants

Some of the commercially available plants based pharmaceuticals are presented below.

3. 1 *ALOE VERA*

3.1.1 History of *Aloe vera*:

There is historical documentation that shows *Aloe vera* being used as far back as 1500 B.C. and *Aloe vera* is often referred to as the "Miracle plant." *Aloe* was also mentioned in the Natural History of Pliny the Elder (23-79A.D) and in the Materia Medica of Dioscorides (first century A.D.) as well as by later Greek and Arabian physicians.

Mahatma Gandhi said that "You ask me what were the secret forces which sustained me during my long fasts. Well it was my unshakeable faith in God, my simple and frugal lifestyle and the *Aloe*, whose benefits I discovered upon my arrival in South Africa at the end of the 19th century."

The word *Aloe* is derived from the Arabic word 'alloeh' which means 'bitter and shiny substance.' *Aloe* describes more than three hundred fifty closely related plants that grow all over the world.

Aloe has been used in folk remedies around the world for over 2,000 years. The ancient Chinese used it to relieve stomach ailments. The South American and Central American Indians use *Aloe* gel to treat kidney and bladder problems, and to increase longevity. In other cultures, *Aloe* is used to treat a variety of skin diseases. In India, *Aloe* medications are used for a variety of conditions, particularly for their cathartic, stomachic, emmernagogic and anthelminthic properties. Whole leaves, the exudate, and the fresh gel are all used.

Evolving historical patterns of the use of plant substances in traumatic conditions and in systemic illnesses have revealed that man may have first observed animals who were injured or ill to be eating or rolling in patches of certain plants. Subsequently, early man found that these plants would aid in healing human illnesses. Initially, *Aloe* substances were mainly used as healing aids for topical skin problems and conditions and this has remained their most wide spread use to the present. However, over the years the use of these and some other plant substances has been extended into scientific experimental treatments for internal upsets and conditions.

Aloe vera is known to contain well over 100 separate ingredients or constituents between those found in the leaf and those found in the mucilaginous gel inside the leaf. It is also known that some of the ingredients found in the leaf such as aloin, or the emodins are recognized as having laxative and anti-microbial properties respectively, which are not present to any significant degree in the gel itself.

There are many other areas of research imvolving *Aloe* that are being conducted around the world. There is a tendency in today's pharmaceutical

and cosmetic markets to move away from synthetic ingredients and return to natural botanicals whenever possible. *Aloe vera* is certainly one of the more complex and widely recognized natural botanicals yet discovered. Much more controlled, scientific research must be conducted by respected research entities on *Aloe* before the many secrets associated with its benefits are unlocked. It is gratifying to see that this unique botanical is finally getting the scientific attention it deserves.

3.1.2 Vernacular names:

Sanskrit :	Kumarirasambhava, Ghritkumari
Gujarati :	Kunwar
Hindi:	Ghikanvar
Malyalam:	Kattavazha
Telgu:	Kalabanda

3.1.3 Distribution:

Aloe are member of the Liliaceae and are mostly succulents with a whorl of elongated, pointed leaves. There are over 360 accepted species. Some species are tree-like with long stems, while others are small, with their leaves at ground level. They occur over most of Africa, Southern Arabia and Madagascar, but not in rain forest regions or dry deserts. A few species have been carried in cultivation around the Mediterranean and from there have reached as far as Japan in the east and America in the west. The plant is distributed throughout India. It has become completely naturalised, especially in the hot dry valley of North-Western Himalayas.

3.1.4 Cultivation and Collection:

For cultivation in West Indies young offsets are planted in the soil after the rainy season in rows situated at distance of 60 cm. (Fig. 5.1, 5.4). In the second year leaves are collected by the natives. Because of the spiny nature of leaves the natives protect their hand and feet, cut the leaves near the take them to a central place for the preparation of aloe.

Juice of aloe is present in parenchymatous cells of pericycle, surrounding which are mucilage cells. On incision of leaves, juice exudes from pericyclic cells and mucilage cells exert pressure on pericyclic cells and by a single incision juice of the entire leaf is drained out. Although it is cultivated and harvested in large areas in Rajasthan but due to lack to factories in Rajasthan it is exported to Haryana where factories make huge profit from this plant.

3.1.5 Nomenclature:

The nomenclature of *Aloe vera* has been very confused and the plant has been known under a variety of names, most notably *Aloe barbadensis* Mill., *Aloe vera* Tourn. ex. Linn., and *Aloe vulgaris* Lam. While *Aloe barbadensis* Mill. was until recently the official name, the plant was still popularly known as *Aloe vera,* and Newton (1979) argued that the scientific name should also be *Aloe vera.*

3.1.6 Botanical source:

Aloe is the dried juice collected by incision from the bases of the leaves of various species of aloe.

3.1.7 Geographical origin:

The geographical origin of *A. vera* is not known for sure, since it has been introduced and naturalised throughout most of the tropics and warmer regions of the world, including the West Indies and Bahamas, Southern U.S.A., Mexico, Central America, Arabia, India and other parts of Asia.

3.1.8 Description:

3.1.8.1 Macroscopic: Leaves large, succulent, subulate 20-50 cm long and 5-10 cm wide. Apex in the form of a sharp and acute spine. Both the surfaces are strongly cuticularized. Dried leaf juice dark chocolate brown to black in colour and of irregular masses. Odour, characteristic; taste very bitter.

3.1.8.2 Microscopic: Thin walled, large pericyclic cells contain yellow fluid. The fluid under microscope shows crystals in the form of innumerable needles, varying in size and shape. The odour of each variety is characteristic, and taste is nauseous and bitter.

3.1.9 Chemical Constituent:

Aloe contains about 30% aloin and is a mixture of three isomers: barbaloin, β-barbaloin and isobarbaloin. Barbaloin present in all the four varieties is slightly yellow-coloured, bitter water-soluble crystalline glycoside. β-barbaloin is amorphous and present in Cape aloe and can be produced from barbaloin on heating. Isobarbaloin is crystalline, present in Curacao aloe and in traces in Cape aloe and absent in Socotrine and Zanzibar aloe. Barbaloin is a cardiac glycoside compared to common glycosides and cannot be hydrolyzed by acid or alkali but only by oxidative hydrolysis with ferric chloride and hydrochloric acid or periodate. Barbaloin on hydrolysis yields aloe-emodin anthrone and arabinose. Besides aloe-emodin anthrone, aloe-emodin anthranol and aloe-emodin are also present. *Aloe* contains a resin which is ester of *p*-coumaric acid or *p*-hydroxy cinnammic acid esterified with aloeresinotannol. According to recent work Cape aloe-resin consists of aloe-resin B is chromone C. glycoside with glucose and aloe-resin A is P. coumaric acid ester of aloe-resin B esterified at one of the OH groups of glucose.

3.1.10 Uses: *Aloe* and aloin are strong purgatives and in higher doses may act as abortificient. If used alone, aloe causes griping and is usually combined with carminatives or antispasmodics like belladonna of hyoscyamus. Recently, a preliminary study suggests that the *Aloe vera* may mimic Zidovudine without toxicity. A substance in *Aloe vera* show signs of boosting the immune systems of AIDS patients and blocking the human immune- deficiency virus spread without the toxic side effects.

3.2 *Acacia nilotica* Linn.

3.2.1 Vernacular Names :

Arabic : Saelam; Bengal : Babur, Babul, Kikar; Bombay : Babhula, Kikar Kalikikar; Central Provinces : Babul; English: Black Babool, Indian Gum Arabic Tree ; Gujrat : Babalia, Bavan; Hindi : Babla, Babul, Babur, Kikar; Punjab : Babla, Babul, Babur, Kikar; Rajasthan : Babul, Bambun, Bawal; Sanskrit: Pitapushpa, Sukshmapatra, Babbula; Tamil : Iramangandan, Karuvelam; Telgu : Barburamu, Nellatuma.

3.2.2 Family: Mimosaceae.

3.2.3 Distribution: It is found in Delhi, Rajasthan, Punjab and Saurashtra.

3.2.4 Morphology: A ever green moderate sized tree with terete, pubescent and slender branches when young. Bark grey or brown. Leaves bi-pinnate, pinnae 4-10 pairs, leaflets 10-25 pairs and subsessile, linear oblong, glabrous and sub-obtuse. Flowers yellow coloured (Fig. 3.16). Calyx campanulate with short teeth. Corolla 3 mm long and lobes triangular. Pods compressed , persistently grey-downy, moniliform and contain 8-12 black-brown, sub orbicular, compressed and smooth seeds (Bhandari, 1978).

3.2.5 Part Used: Bark, Leaf, Fruit, Gum, Pod and Flowers.

3.2.6 Uses: Bark is a powerful astringent, demulcent used for remedy in relaxed condition of mucous membranes, in leucorrhoea used as a poultice for ulcers attended with sanious discharges. Decoction of bark is used as an astringent in diarrhoea, dysentery, in gleet and leucorrhoea, as a wash for haemorrhagic ulcers, also as a gargle in affection of mouth and throat. A decoction of leaves with a piece of tamarind pod is used to cure a disease of eye which causes the lashes to fall out. Leaves are beaten in pulp are given in diarrhoea as an astringent. These leaves mixed with leaves of pomegranate is given in gonorrhoea. Leaves bruised and mixed with human milk are used in inflammation of conjunctiva as a poultice or juice mixed with milk is dropped into eye. Fruit is a powerful astringent and used in cases of fever, diarrhoea, dysentery, ophthalmia. Gum is useful in diabetes mellitus. A strengthening sweet meat is nade by frying the gum with spices and butter, is given to recently delievered women as a tonic. Gum resin is used for troubles of throat and the chest. Gum alone is used for dysentery. An infusion of pod is used in ophthalmia. A decoction of pod is given for fever, venereal disease and leprosy. Fresh pods are sucked as a specific for cough, pulverized and mixed with water and drunk every morning for

cough and chest complaints. The juice of pods and bark or astringent extract is used to arrest bleedings of circumcision wounds and pulverized pods are applied to sores of mouth or to hasten cicatrisation of syphilitic ulcers Tender leaves and bark are burnt to ashes and are sprinkled daily over wounds. Leaves, Bark and Pod are chewed as antiscorbictic and infusion of pod and bark alone is used for dysentery. Juice from flowers is recommended as a wash for sore eyes.

3.3 *Cassia senna* Syn. *Cassia angustifolia*

It is known by different names:

Hindi: Bhuikhakhasa, Hindisana and Sonamukhi; English : Alexandrian, Bombay, or Tinnevelly senna ; Sanskrit : Bhumiari, Pitapushpi, Swarnamukhi, swarnapatrika ; Rajasthan : *Senna*.

The plant is a variable, branching, erect shrub, upto 1.8 m in height (Fig. 5.5). Leaves pinnate pubescent, leaflets pale green to bluish green, 3 to 9 pairs, lanceolate or elliptic, varying on the same plant, 1.5 to 5 cm. X 0.4 to 2 cm. flowers brilliant yellow, in erect, terminal racemes ; pods light green when young to dark brown or black when mature, flat, thin, oblong pubescent, 3.5 to 7 cm. x 0.2 to 2.5 cm. ; seeds dark brown, obovate-oblong, 5 to 7 (Fig. 5.6).

It is highly drought resistant crop and suitable for desert. It is largely cultivated on marginal lands in a 10,000 ha. , both as rain-fed and irrigated crop, mainly in Tamil Nadu, where it is now grown principally as a cash-crop in Tirunelveli, Ramanathapuram, Tiruchchirappalli and Madurai districts and to a lesser extent in Salem district (Gupta *et al.*, 1977). Recently, it has been observed occuring wild in Cuddapah district of Andhra Pradesh, and Bhuj district of Gujarat. At present, it is also being cultivated in Andhra Pradesh, Karnataka and Maharashtra (Pune). About 2,700 ha. is under cultivation in Cuddapah, Mysore and Anand and Mehsana districts of Gujarat. Trials conducted at Jammu, Jhodhpur district of Rajasthan and Delhi have given very encouraging results ; in Jammu, it can be grown at lower altitudes in drier regions. It was successfully introduced into West Bengal, and recommended for Tripura (Gupta *et al.*, 1977).

The plant requires dry and warm climate, bright sunshine, and occasional drizzle for good growth. It can grow in places where the average minimum and maximum temperatures fluctuate between 10 and 42^0 C. A rainfall of 60-70 cm per year is sufficient for a good crop however, 25-40 cm of rainfall was reported to be sufficient in arid areas.
The plant is stripped three times during the season, first picking is in March and others before May ; more picking can be taken later in October and December. Immediately after picking the leaves are dried in sun. Quick drying ensures excellent green colour. The method of drying affects the percentage of sennosides in the leaves. In sun drying-sennosides are 2.78% ; moisture , 70.70% and in oven drying (40^0 to $\pm 2^0$) – sennosides are 3.13% ; moisture 72.90% .

Among different methods of drying, freeze drying was found to be the best method of drying. The *senna* is well known drug in Unani, Ayurvedic and Allopathic systems of medicines and is also a house hold medicine. The drug from India is known as Tinnevelly senna. The dried leaves and pods comprise the drug, the former known as Senna leaf and later Senna fruit as pod. The commercial drug consists of dried green leaves and shells of nearly dried and ripe pods. The flowers are reported to contain considerable quantity of sennoside (2.6%). The commercial samples of pod (shells) contain sennosides 3 to 5% and the foliage 2.5 to 5%.

The Senna leaves and pods contain sennasoides A, B, C, D, G, rhein, aloe-amine, Kaempferein and iso-rhein in the free and compound glycoside forms. The leaves, pods and roots of *Cassia senna* contains rhein, chrysophenol, imodin and aloe-imodin.

The leaves and pods (shells) are usually administered in the Ayurvedic and Unani systems of medicine as infusion, and considered a great tonic. The milk of nursing women acquires purgative properties after the use of senna. The

drug is contra indicated in spastic constipation and colitis. The senna is an efficient purgative either for occasional use or for habitual constipation. It is free from astringent action of rhubarb (*Rheum* sp.) type but has a tendency to cause gripe ; hence it is combined with carminatives, aromatics and other saline laxatives ; the pods, however, cause less gripe. The disagreeable odour is masked by the addition of ginger or cloves. In India several household preparations such as decoction, powder, syrup, infusion and confection are made with senna. It enters into a compound Nilaavarai Churnam used for treating distention of stomach, hiccups, vomiting and biliousness.

Besides being an excellent laxative, the senna is used as a febrifuge, in splenic enlargements, anaemia, typhoid, cholera, biliousness, jaundice, gout, rheumatism, tumours, foul breath and bronchitis and probably in leprosy. It is employed in the treatment of amoebic dysentery, as an anthelmintic and as a mild liver stimulant. The leaf is one of the constituents of a patented drug reported to have protective effects on the liver. The leaves in the form of confection of senna are used in treating haemorrhoids. They are externally used for certain skin diseases and the powdered leaves in the vinegar are applied to wounds and burns, and to remove pimples. However, it has been known to cause a severe and painful dermatitis in sensitized persons. The leaves along with those of hina are used to dye the hair black.

3.4 *Tinospora Cordifolia*

3.4.1 Botanical origin: *Tinospora cordifolia* (Willid.) Miers (Fig. 4.1).

3.4.2 Family: Menispermaceae

3.4.3 Sanskrit synonyms

Amrita, Amritalata, Amritavallari, Amritavalli, Bhishakapriya, Chakralakshna, Chakrangi, Chandrahasa, Dhira, Guduchi, Jivanthika, Madhuparni, Pittaghni.

3.4.4 Regional names

Bengali: Gadancha, Giloe, Gulancha, Guluncho, Nimgilo.
Bombay: Ambarvel, Gharol, Giroli, Guloe, Gulwel.
Gujarati: Gado, Galo, Gulo, Gulvel.
Hindi: Giloe, Gulancha, Gulbel, Gurach, Ambarvel.
English: Gulancha, Tinospora
Marathi: Ambervel, Gharol, Giroli.

3.4.5 Parts used: Root, stem and leaves.

3.4.6 Properties and Uses:

Tinospora cordifolia is mentioned in Ayurvedic literature as a constituent of several compound preparations used in general debility, dyspepsia, fever and urinary diseases. The plant attracted the early notice of European physicians in India. Fleming remarked on its use as a febrifuge and as a drug in gout. Ainslie described the root as a powerful emetic.

The drug Guduchi has been in use in the indigenous system of medicine since remote past. The leaves of Guduchi are mentioned under Tikta-saka varga which is claimed to be salutary and useful in treating kushta, meha jwara, svasa, kasa and aruchi (Susruta). It has been indicated in Ayurvedic treatises in various ailments like kamala (Jaundice), Jvara(fever), vatarakta and so on. The fresh plant is said to be more efficacious than the dry and the stem is the part which is mostly used, from which a kind of starch is prepared known as Giloe-Ke-Sat or Guduchi-Satva.

Guduchi is considered as bitter, tonic astringent, diuretic and a potent aphrodisiac and curative against skin infections, jaundice, diabetes and chronic diarrohea and dysentery. Dhanvantari Nighantu mentions other properties and uses such as cure for bleeding piles, promoting longevity, curing itching and erysipelas. Its use has been indicated in heart diseases, hypertension, leprosy, helminthiasis and rheumatoid arthritis.

It has been in extensive use in India as a valuable tonic, alterative and antipyretic. It caught the notice of European physicians in India as a specific tonic, antiperiodic and diuretic. The drug itself as well as a tincture prepared from it are now official in the Indian Pharmacopoeia.

Gulancha was included in the Bengal Pharmacopoeia of

1844 and the Indian Pharmacopoeia of 1868. Gulancha which grow on Neem trees is considered to be most efficacious for remedial purpose.

3.4.7 Botanical description:

This is a glabrous, succulent, climbing shrub, often attaining a great height and sending down long thread like aerial roots. The plant seems to be particularly found climbing up the trunks of large Neem trees. The aerial roots that arise from the mature branches or cut bits of stems grow downward and by continuously lengthening sometimes reach the ground. They thicken gradually and resemble the stems, except for the absence of nodal swellings. The fresh or tender stems are greenish, longitudinally striated ribbed. The bark is grey of creamy-white in colour, deeply cleft with spiral and longitudinal clefts, the space between the clefts being usually dotted with large rosette like lenticels. The branches bear smooth heart shaped leaves.

3.4.8 Distribution:

Indigenous and found distributed throughout most parts of tropical India from Kumaon to Assam, in north extending through Bengal, Bihar, Deccan, Konkan, Karnataka and Kerala. It is a fairly common wild plant of the deciduous and dry forests of most districts growing over hedges and small trees.

3.4.9 Cultivation:

The plant is sometimes cultivated for ornament and is propagated by cuttings. It is perfectly suited to and grows well in almost any type of soil and under varying climatic conditions. It is specially trained to grow on Neem and mango trees, thereby it is supposed to possess increase in its medicinal virtue.

a. **Stem:** The drug occurs as long, cylindrical, glabrous, soft wooded pieces which show characteristic nodal swelling. The fresh stems are greenish with a smooth surface but the older stems have a warty surface due to the presence of circular lenticels. Fracture is fibrous, taste bitter and odourless.
b. **Leaves:** Simple, alternate, ex-stipulate, fairly long petiolate and are articulated to short tumid nodal projections on the stem, petiole slender, rounded, basal part pulvinate for a short length and this thickened portion is slightly twisted. Lamina broadly ovate to roundish cordate, thin, entire, glabrous on both surfaces, tip acute or shortly acuminate and base with a broad sinus and five to seven –nerved.
c. **Inflorescence:** The plants are dioecious, male and female developing on separate plants. Racemose, simple or panicled. Staminate inflorescences are usually dropping, longer than the leaves and bear the flowers in fascicles of two to six. The pistillate inflorescence is often shorter with flowers borne singly but densely packed on the rachis.
d. **Flower:** Small, numerous, very early deciduous, greenish-yellow with short slender pedicels. Bract lanceolate, subulate, the lower ones occasionally somewhat leafy.
e. **Sepals:** Six , free, deciduous in two series of which the outer are small, ovate, oblong, acute and the inner three larger and membraneous.
f. **Petals:** Six, in one whorl free, smaller than the sepals being about half the length of the calyx. In staminate flowers each petal loosely embraces or encloses a stamen and its limb finally gets reflexed. In pistillate flowers, petals are cuneate, oblong with the limb entire and not getting reflexed.
g. **Staminate flowers:** Stamens six, filaments free, spreading, slightly longer than and wrapped in petals, anther cells oblong.
h. **Pistillate flower:** Have six clavate staminodes in addition to the gynoecium. Gynoecium superior of three free carpels. Styles very short, simple, stigma dilated and forked.
i. **Fruit:** An aggregate of one to three sessile drupelets. Seed, solitary in each drupelets.

Aerial root:
In commerce the aerial roots are usually seen associated with the pieces of stem. The young roots are thread like, whereas the mature ones resemble the young stem except for the presence of nodal swellings. The surface is light grey, fracture short, taste bitter, odourless.

3.5 *Ricinus Communis*

3.5.1 Botanical origin : *Ricinus communis*

3.5.2 Family : Euphorbiaceae

3.5.3 Sanskrit synonyms:
Erandah, Rubukah, Urubukah,Tribijah,Gandharva hasta.

3.5.4 Regional names:

Assamese	:	Eri
Bengali	:	Bherenda
Gujarati	:	Diveligo,Diveli
Hindi	:	Arand, Arend, Erend, Rendi
Rajasthani	:	Edia, Arend
English	:	Castor oil plant
Trade name	:	Castor oil plant

3.5.5 Parts used:
Root, Root bark, leaves and flower, fruit.

3.5.6 Botanical description
It is a tall glabrous glaucous branched shrub of almost a small tree, 2-4 m high, the stem and branches green when young but turning grey and getting covered with thin greyish brown bark, when mature (Fig. 5.7). A similar plant *Jatropha curcas* also has great medicinal potential.

3.5.7 Distribution:
Ricinus communis is believed to be a native of tropical Africa. Its occurrence in the scrubby jungles of the outer Himalayas in what would appear to be a truly wild state, together with the undoubted antiquity of the knowledge of its use as a drug, as revealed by Sanskrit literature are held to point to its being a native of India as well as of Africa. It is said to be under cultivation from ancient times in both these areas.
It is indigenous to India and Africa but is diffused now over all tropical and sub-tropical countries. In India, the plant is found throughout the hotter parts of the country, cultivated in the fields and gardens, also frequently found wild near habitations, roadsides and on waste lands.

3.5.8 Macroscopical characters:

a. **Root:** The tap root gives off profuse branches. Branches are straight, tortuous at the end greyish brown in colour and become lighter on drying. Dry root breaks with a granular fracture. Entire root surface bears longitudinal corrugations which unite at their ends. The corrugations are long and appear running parallel in the case of young roots but are quite separate and convergant on old roots. Well-developed lenticels occur irregularly on old roots. They are globular on younger parts geeting elongated on older regions. Secondary thickening results in the formation of annual growth rings. The colour of the cut end is light cream and hence is easily distinguished from the external surface of the root.

b. **Root bark:** The drug occurs generally in thin curved pieces, 4 to 7mm thick, while a few pieces from upper portion of the root are much thicker. Outer surface is light dirty yellowish brown with five longitudinal wrinkles. Scars are present due to the removal of rootlets. At some place the cork exfoliates leaving lighter patches. Inner surface is light yellowish brown and somewhat smooth. Fracture is soft and fracture is slightly fibrous. Odour is not characteristic and taste is slightly astringent.

c. **Fruits and seeds:** The fruit is dry three-celled three-seeded, ovoid and thorny capsules about 3.4 cm long. The seeds are about 8 to 15 mm long, 6 to 9 mm. wide and 4 to 8 mm thick. The testa is hard, glossy and smooth, grey to red brown and marbled with reddish brown or black

spots and strips. They are oval and slightly flattened, the flattened ventral surface being slightly ridged, along the rounded ridge, the rapture extends from the caruncle at the hilar end of the seed to the chalaza at the other extremity. Within the testa is a delicate colourless membrane surrounding the kernel, this membrane is the remains of nucellus. The kernel consists of an abundant oily endosperm having a wide, oval disc shaped central cavity in which lie the two colourless leafy cotyledons of the embryo, the radicle of which pierces the layer of endosperm and points towards the micropyle immediately beneath the caruncle. The seeds have a slight odour and a weakly acrid taste.

3.5.9 Cultivation and collection:

India occupies the second place among castor seed producing countries in the world, being Brazil the leading country. Russia, Thailand, USA and Rumania are some of the other countries which produce sizable quantities of castor seed. Castor is one of the five major oilseed crops cultivated in India and a major part of the production is exported in the form of oil. Though it is grown in almost all states Andhra Pradesh, Gujarat and Mysore are the three major areas which include about 64.12 and 7.5 per cent respectively of the total area under the crop in the country.

The plant is essentially a crop of the tropics and it can withstand dry arid climates as well as also heavy rains and floods. It is generally grown on sandy or clayey deep red loams and on good alluvial loams. It is frequently grown as a mixed crop, the time of sowing is June-July or September-October.

The method of cultivation appears to be almost the same everywhere. The best soil for the cultivation of castor oil plant is red loam. It also grows well on alluvial earths. The land is ploughed in May and in July or August and after the first showers, the seeds are sown. The seeds are first soaked in water and then sown in well and deeply tilled soil, in holes about 15 cm. apart three or four seeds being put in each hole. The plants are finely thinned out so as to leave only the strongest which are spaded about 1.5 to 2 meters apart.

The plants begin to bear when four to six months old and the number of spikes of flowers on each plant is increased by tipping back the main stem. When the capsules begin to turn brown the spikes of fruits are collected and exposed to the sun or concrete floors in layer about 15 cm. deep.

The harvested spikes are stacked in heaps till the capsules blacken and they are then spread out in the sun or dry. Once or twice daily they are turned over with a rake and after three or four days the capsules burst suddenly and the seeds are flung out violently. The husks are removed by winnowing. The average yield of castor seed for all India is about 200-290 kg/ha.

3.5.10 Properties and uses:

The root of *Ricinus communis* and the oil obtained from the seeds have been used in medicine by the Hindus from a very remote period. They are mentioned by Susruta. In Ayurveda, root bark is very much reputed for its anti-inflammatory activity. Root and root bark are used as carminative, alternative in asthma, bronchitis and skin diseases. It is also recommended as a febrifuge, purgative, in eye diseases, jaundice, nervous disease, rheumatic affections such as lumbago, pleurodynia and sciatica. Eranda is sweet, bitter, light and hot. It is purgative, cures dysponea, hydrocele flatulance, piles, cough, lumbago, headache, leprosy, arthritis, calculus and dysuria.

3.5.11 Economic uses:

Castor oil is frequently employed by the Indian dyers as an auxillary in certain dye preparations. The oil is used for lubricating all kinds of machinary, clocks and also considered as the best lamp oil in India. The dried stems and husks after the extraction of the seeds constitute a highly combustible fuel, which is largely used in boiling sugar-cane juice.

a. **Leaves:** Large, alternate, long petiolate, stipulate, peltate, palmately veined, broad,

nearly orbicular 7-10 or more lobed. Lobes membraneous, oblong to linear acute of acuminate, gland serrated. The petioles vary from 4 to 20 cm. in length and 2.5 to 7.5 cm in breadth. A number of saucer shaped glands 1 to 2 mm in diameter are present on the petiole, two prominent glands occur close together at the top of the petiole.

b. **Inflorescence:** Stout, erect, sub-panicled racemes that terminate the main axis and branches. Flowers very large, monoecious. The staminate flowers are usually located in the distal or upper leaf of the inflorescence in a crowded manner and the pistillate at the basal part. Stamens apparently many, anther cells distinct, sub-globose and divergent on the rather large connective. Pistillate flowers large, perianth spathaceous, ovary superior, three chambered with one ovule in each chamber. Styles three, short or long spreading often very large and brightly or lightly coloured, entire, two fid or two partite. Stigmas feathery or papillose.

c. **Fruit:** A globose or globular-oblong, explosively dehiscent three seeded capsule 1.2 to 2 cm. long, septicidally dehiscent or splitting into three two-valved cocci. The fruit when young is green and usually covered with fleshy prickles.

d. **Seeds:** Carunculate, oblong, 1-1.5 cm, long with smooth hard mottled crustaceous testa and oily or fleshy endosperm. Embryo thin with flat broad cotyledons. *Ricinus communis* said to be a very variable species. It has been divided into a number of varieties and forms based on the characteristics of capsule, seed and inflorescence.

3.5.12 Preliminary phytochemical studies

The root powder shows presence of phenolic constituents. Anthraquinone derivatives are absent in the water extract of the root powder. Powder mixed with water, shaken at left to stand for sometimes, yields a mucilaginous substance. This substance is light yellow in colour and turn dark on heating. The powder does not froth when mixed with water indicating the absence of saponins.

3.5.13 Diagnostic characters:

The roots are greyish brown in colour, rough due to the presence of irregular lenticels. Fracture is granular. The root bark occurs in thin curved pieces, outer surface dirty yellowish brown with fine longitudinal wrinkles. Taste is slightly astringent. The root comprises of periderm, cortex and stele. Highly branched latex canals are present in the cortex. Xylem is pentarch. Root bark shows lignified cork cells, groups of fibres in the phelloderm and cork and a broad zone of phloem. Resin and tannin are present in the cortex and phloem.

3.5.14 Chemistry:

Castor seeds contain about 50% of the fixed oil and about 26% of proteins. The cake leaf after the expression of the oil contains about 0.2% of ricinine, a crystalline principle (m.p. 201.5°) ricin, a toxin similar in nature to the bacterial toxins, a very active lipase, and other enzymes.
Castor oil consists of the glycerides of ricinolecis, isoricinoleic, stearic and dihydroxy- stearic acids. Root bark shows the presence of steroid having m.p. 159°. It also contains considerable amount of gallotannins along with many inorganic ions. Ricinine is a water soluble alkaloid present in the seed coat, leaves and stems.

4. Conclusion

Rajasthan is rich in vegetation and pharmcognostical studies provide valuable insight into identification and characterization of some of the plants of semi-arid and arid regions.

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Preliminary Phytochemical Screening and Physico-Chemical Parameters of *Artemisia absinthium* and *Artemisia annua*

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The family Asteraceae or compositae known as the ester, daisy or sunflower family is the largest family of flowering plants. *Artemisia* is a large diverse genus of plants with between 100 to 150 species belonging to the family asteraceae (compositae). It comprises hardy herbs and shrubs known for their volatile oils. They grow in temperate climate of the northern hemisphere and southern hemisphere usually in dry or, semidry habitats. The collected herbs were authenticated, dried and extracted to calculate the percentage of yield. Phytochemical studies of the Hexane and alcoholic extracts showed the presence of various phytoconstituents i.e. carbohydrate, saponins, phytosterol, proteins and amino acid, tannin, phenolic compounds and flavonoids. It was observed that all the extracts show more important chemical constituents for various pharmacological activities. The determination of these characters will aid future investigators in their Pharmacological analysis of this species.

Keyword: *Artemisia*, Phenol, Phytochemical screening, Tannin.

1. Introduction

The life cycle of *A. absinthium* has been reported[1]. Growth begins in late April, and new plants are 4-12 inches tall by mid-May. Flowering begins in late July to early August. During late fall, the aboveground portion of the plant dies. Seedlings may emerge at any time from late spring to early fall. Seedlings may be unnoticed for some time as they are low with small leaves before the upright flowering stems emerge[2]. The leaves and flowering tops are gathered when the plant is in full bloom, and dried naturally or with artificial heat. Its use has been claimed to remedy indigestion and gastric pain, it acts as an antiseptic, and as a febrifuge. For medicinal use, the herb is used to make a tea for helping pregnant women during pain of labor. A wine can be made by macerating the herb. It is also available in powder form and as a tincture. The oil of the plant can be used as a cardiac stimulant to improve blood circulation. Pure wormwood oil is very poisonous, but with proper dosage poses little or no danger. Wormwood is mostly a stomach medicine[3]. Safe in moderation, but large doses are toxic. Poisoning leads to seizures, delirium, hepatoprotective, and hallucinations and death[4]. The whole plant had shown various pharmacological activity viz. antihelmintics[5], stomachache, appetizer, diabetes, uterus cyst, tuberculosis,

antihypertensive, and leaves may also showed Antihypertensive, Wounds, Diabetes,[6] anti-microbial activity[7], antimalarial[8], and antifertility[9].

Artemisinin is a sesquiterpene lactone with an endoperoxide bridge and has been produced semisynthetically as an antimalarial drug. The efficacy of tea made from *A. annua* in the treatment of malaria is contentious. According to some authors, artemisinin is not soluble in water and the concentrations in these infusions are considered insufficient to treat malaria[10,11]. In 2004, the Ethiopian Ministry of Health changed Ethiopia's first line antimalaria drug from sulfadoxine/pyrimethamine (Fansidar), which has an average 36% treatment failure rate, to artemether/lumefantrine (Coartem), a drug therapy containing artemisinin which is 100% effective when used correctly, despite a worldwide shortage at the time of the needed derivative from *A. annua*[12]. Artemisinin has no reported toxicity if taken in recommended doses for short periods in the treatment of malaria[13]. The plant shows more pharmacological activity viz. treatment of malaria[14], immunosuppressive[15], antifungal activity[16] and antipyretic activity[17].

2. Materials and Methods:

2.1 Plant Materials:

The plant (*Artemisia absinthium* and *Artemisia annua*) used for this study was collected from around dehradun and identified at Department of Pharmacy, Kumaun University Nainital. A voucher specimen has been deposited in the herbarium of the institute for future references. All parameters were studied as per Ayurvedic Pharmacopoeia of India[18] and Fluorescence analysis; primary and secondary plant metabolites were also investigated. All the reagents used were of the analytical and highest purity grade from standard companies.

2.2 Fluorescence Analysis:

The fluorescent method is adequately sensitive and enables the precise and accurate determination of analyze over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples. To check the fluorescent property of crude drug powder is used for analysis under ultra violet light. Powder as such, Powder + Water, Powder + Conc. HCl, Powder + Conc. H_2SO_4, Powder + Conc. HNO_3, Powder + 5% NaOH, Powder + methanol, Powder + acetone, Powder + NaOH (0.1N) and Powder + HCl (0.1N) are used to perform for fluorescence analysis[19].

2.3 Extraction:

The air dried both plant parts were cleaned and reduced to powdery form with the help of mechanical grinder after which each 250 gm of powdered sample was exhaustively extracted with 2.0 lt of alcohol (analytical grade), for 3 days (by soxlet apparatus). The extracted plant materials were separated by filtration technique and the hexane and alcoholic extracts were concentrated under reduced pressure (by Rotavapour, Büchi, Switzerland) and lyophilized to preserve it. The residues was found to be 5.4%, 21.52% w/w and 6.4%, 20.46 % w/w respectively for *Artemisia absinthium* and *Artemisia annua* and stored in a refrigerator at 4^0C for further investigation.

2.4 Phytochemical screening:

The various extract of *Artemisia absinthium* and *Artemisia annua* was subjected to preliminary phytochemical screening using standard screening method. The molish's test and fehling's test were carried out for carbohydrate, foam test for saponins, salkowiski test & libermann burchard test for phytosterol, sodium hydroxide test, concentrated sulphuric acid test and shinoda's test for flavonoids, biuret test, ninhydrin test and million's test for proteins and amino acid[20].

3. Results and Discussion:

3.1 *Artemisia absinthium*

Fresh plant material (*Artemisia absinthium*) was collected and subjected to various physicochemical parameters such as moisture content and foreign matter was determined. The total ash of the plant sample, acid insoluble ash

and water-soluble ash values were also determined. Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily, estimated by any other method. Further, these values indicate the nature of the constituents present in a crude drug. The percentage of alcohol soluble extractive value and water-soluble extractive value were also determined (Table: 01).

The percentage of starch calculated in leaves was found to be 11.66%. The percentage of sugar calculated in leaves was found to be 6.38%.The percentage of tannin calculated in leaves was found to be 0.20%. The percentage of total phenol calculated in leaves was found to be 2.78% (Table: 01).

Table 1: Quantitative Physico-Chemical Analysis of *Artemisia absinthium*.

S.No.	Parameters	Range(%)	Mean(%)	S.D
1	Moisture content (w/w)	19.8-16.1	17.2	±0.8124
2	Foreign matters (w/w)	0.2-0.8	0.5	± 0.0702
3	Total ash (w/w)	2.42-2.52	2.50	±0.1714
4	Acid insoluble ash (w/w)	0.22-0.27	0.25	±0.3762
5	Water soluble ash (w/w)	0.35-0.44	0.39	±0.0327
6	Alcohol soluble extractive (w/w)	11.37-13.59	12.67	±0.2731
7	Water soluble extractive (w/w)	10.60-11.57	10.98	±0.3521
8	Starch	11.55-11.73	11.66	±0.1622
9	Sugar	6.15-6.43	6.38	±0.0023
10	Tannin	0.20-0.21	0.20	±0.0132
11	Total Phenolic	2.75-2.86	2.78	±0.0520

Fluorescence study is an essential parameter for first line standardization of crude drug. In fluorescence, the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight. (Table: 02).

Table: 02 Fluorescence analysis of *Artemisia absinthium*

S.No.	Drug	Short ultra violet (256nm)	Long ultra violet (365nm)	Visible/Day light
1.	Powder as such	Black	Black	Light green
2.	Powder+water	Brown	Black	Light green
3.	Powder+conc.HCl	Black	Black	Brown
4.	Powder+conc.H_2SO_4	Black	Dark brown	Brown
5.	Powder+conc.HNO_3	Pale green	Black	Dark green
6.	Powder+5% NaoH	Pale brown	Black	Black
7.	Powder+Methanol	Light green	Light black	Pale green
8.	Powder+Aetone	Brown	Black	Green
9.	Powder+NaoH(0.1N)	Pale yellow	Black	Light yellow
10.	Powder+HCl(0.1N)	Brown	Black	Brownish black

Table: 03 phytochemical screening of *Artemisia absinthium*

S. No.	Constituents	Tests	Hexane extract	Methanolic extract
1.	Carbohydrate	Molish's test	_	+
		Fehling's test	_	_
2.	Glycoside	Borntrager's test	_	_
		Keller killanis test	_	+
3.	Fixed oil & fats	Spot test	+	–
		Saponification test	+	–
4.	Proteins & amino acids	Million's test	_	+
		Ninhydrin test	_	_
		Biuret test	_	+
5.	Saponins	Foam test	_	+
6.	Phenolic compunds & tannins	$FeCl_3$ test	_	+
		Lead acetate test	_	+
7.	Phytosterol	Salkowiski test	_	+
		Libermann burchard test	_	+
8.	Alkaloids	Dragendroff's test	_	_
		Mayer's test	_	_
		Hager's test	_	–
10.	Resin	Resin	_	+
11.	Flavonoids	Aq. NaOH test	+	+
		Conc. H_2SO_4 test	+	+
		Shinoda's test	_	+

(+) = Presence, (−) = Absence

3.2 *Artemisia annua*

Fresh plant material (*Artemisia annua*) was collected and subjected to various physicochemical parameters such as moisture content, foreign matter, total ash, acid insoluble ash, water-soluble ash values were determined. The percentage of alcohol soluble extractive value and water-soluble extractive value were also determined (Table: 04).

Table: 04 Quantitative Physico-Chemical Analysis of *Artemisia annua*.

S.No.	Paramaters	Range(%)	Mean(%)	S.D.
1	Moisture content (w/w)	15.52-16.43	15.70	±0.6545
2	Foreign matters (w/w)	0.14-0.5	0.313	±0.1803
3	Total ash (w/w)	13.68-13.95	14.18	±0.6464
4	Acid insoluble ash (w/w)	0.21-0.26	0.25	±0.0360
5	Water soluble ash (w/w)	0.18-0.19	0.17	±0.0152
6	Alcohol soluble extractive (w/w)	7.65-7.44	7.30	±0.4315
7	Water soluble extractive (w/w)	9.36-10.56	10.2	±0.7299
8	Starch	14.26-14.21	13.95	±0.4942
9	Sugar	9.25-10.68	9.69	±0.8527
10	Tannin	0.16-0.19	0.18	±0.0167
11	Total Phenolic	1.72-1.83	1.83	±0.1100

The percentage of starch calculated in whole plants was found to be 10.52%. The percentage of sugar calculated in whole plants was found to be 7.23%. The percentage of tannin calculated in whole plants was found to be 0.21%. The percentage of total phenol calculated in whole plants was found to be 2.14% (Table: 04).

Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. Fluorescence is a type of luminescence in which the molecule emits visible radiation passing from higher to lower electronic state. The molecules absorbs light usually over a specific range of wavelength, get excited from ground state to a high energy level and many of them emit such radiations while coming back to the ground state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". For fluorescence analysis, powdered drug was sieved through 60 mesh and observations were made following (Table: 05).

Table: 05 Fluorescence analysis of *Artemisia annua*

S.No.	Drug	Short ultra violet (256nm)	Long ultra violet (365nm)	Visible/Day light
1.	Powder as such	Yellowish	Black	Pale green
2.	Powder+water	Light green	Brown	Pale green
3.	Powder+conc.HCl	Brown	Black	Black
4.	Powder+conc.H_2SO_4	Brown	Dark brown	Light brown
5.	Powder+conc.HNO_3	Dark green	Black	Dark green
6.	Powder+5% NaoH	Brown	Black	Light black
7.	Powder+Methanol	Brown	Light black	Pale brown
8.	Powder+Aetone	Light yellow	Black	Yellowish
9.	Powder+NaoH(0.1N)	Pale yellow	Black	Pale yellow
10.	Powder+HCl(0.1N)	Light brown	Black	Brownish black

3.3 Phytochemical screening:

The results (*Artemisia absinthium* and *Artemisia annua*) of the phytochemical screening carried out on two extracts was recorded as shown in Table: 03 and Table: 06 respectively. Preliminary phytochemical studies revealed the presence of saponins, phytosterols, carbohydrates, proteins, amino acid, and flavonoids in alcoholic extract of *Artemisia absinthium* and *Artemisia annua*. Phytoconstituents in the various part of the plant vary significantly.

Ascorbic acid and phenolics contains plants are showing powerful antioxidants. The presence of saponins protects plant from microbial pathogens[21].

Flavonoids act as an anti-inflammatory agent in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins[22]. Further studies may reveal the extract mechanisms of action responsible for the analgesic, anti-inflammatory and hepatoprotective activity of *Artemisia absinthium and Artemisia annua.*

Results reveal that the all the extracts have large class of phytoconstituents, which may be responsible for many pharmacological activities; further work is required to investigate all extracts of plant parts of *Artemisia absinthium and Artemisia annua* for various pharmacological activities.

Table: 06 Phytochemical screening of *Artemisia annua*

S. No.	Constituents	Tests	Hexane extract	Ethanolic extract
1.	Carbohydrate	Molish's test	_	+
		Fehling's test	_	+
2.	Glycoside	Borntrager's test	_	_
		Keller killanis test	_	+
3.	Fixed oil & fats	Spot test	+	–
		Saponification test	+	–
4.	Proteins & amino acids	Million's test	_	+
		Ninhydrin test	_	+
		Biuret test	_	_
5.	Saponins	Foam test	_	+
6.	Phenolic compunds & tannins	$FeCl_3$ test	_	+
		Lead acetate test	_	+
7.	Phytosterol	Salkowiski test	_	+
		Libermann burchard test	_	+
8.	Alkaloids	Dragendroff's test	_	_
		Mayer's test	_	_
		Hager's test	_	–
10.	Resin	Resin	_	+
11.	Flavonoids	Aq. NaOH test	+	_
		Conc. H_2SO_4 test	+	+
		Shinoda's test	_	+

(+) = Presence, (−) = Absence

4. Conclusion:

Preliminary phytochemical screening of the alcoholic extracts shows the presence of various phytoconstituents i.e. flavonoids saponins etc. These bioactive agents (flavonoids and saponins) have the ability to inhibit pain perception and they can also serve as antiinflammatory agents[23]. Flavonoids act as an antiinflammatory response in the same way as the nonsteroidal antiinflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins. Further studies may reveal the mechanisms of action responsible for the analgesic, antiinflammatory and hepatoprotective activity of *Artemisia absinthium and Artemisia annua.*

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