Pharmacognostical and phytochemical investigation of whole plant of *Blepharis maderaspatensis* (L.) Heyne ex Roth.

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ABSTRACT

Blepharis maderaspatensis (L.) Heyne ex Roth. is used for treatment of a number of ailments like dysuria, headache, diseases of nervous system, diuretic and aphrodisiac. The present investigation deals with the pharmacognostic studies of the whole plant of the said plant. Pharmacognostic studies include microscopic, physicochemical constant (ash & extractive valves), fluorescence analysis and preliminary phytochemical evolutions. The results of the study could be useful for the identification and preparation of a monograph of the plant.

Key words: *Blepharis maderaspatensis*, Pharmacognostic, Preliminary phytochemical

INTRODUCTION

Medicinal plants constitute an effective source of traditional and modern medicines. Standardization of plant products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential.[1] Therefore, active interests in the pharmacognostic and phytochemistry of the plant drugs have been gaining momentum. Scientists of modern era often ask for scientific validation of herbal remedies. So that herbals are standardized for active constituent. Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals. Medicinal plant materials are characterized according to sensory microscopic and macroscopic characteristics. Taking into consideration the variation in sources of crude drugs and their chemical nature, they are standardized by using different techniques of chief active constituent. Organoleptic evaluations can be done by means of organs of sense. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample. A number of different bases are used for morphological studies and a natural variation in these characteristics plays an important role for preliminary evaluation of crude drugs.

Blepharis Maderaspatensis is used for headache.[2] Seeds are used as dysuria, diseases of nervous system, diuretic, aphrodisiac.[3] In spite of its use in herbal formulation no systematic pharmacognostic studies have been reported and hence our efforts were devoted in this direction.

MATERIALS AND METHODS

The whole plant of *Blepharis maderaspatensis* were collected from the well grown healthy plants inhabiting the natural forests of Saduragiri hills, Virudhunagar District, South-Eastern slopes of Western Ghats, Tamil Nadu, India. For anatomical investigations standard microtome techniques[4] were followed. T.S. of 10 to 12 µm thickness were prepared. These microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) and adjusted to pH 4.7.[5] For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by

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partial maceration employing Jeffrey's maceration fluid[6] were prepared. Pieces of leaves measuring 0.5 cm^2 and thin fragments of stem were subjected to maceration. The stem fragments were immersed in hot water to remove air and then it was immersed in Jeffrey's Maceration fluid (5% chromic acid and 5% nitric acid, mixed in equal volumes) and the vials containing the material in the fluid were kept in the thermostat for several hours. For the epidermal peeling, partial maceration of the leaf was obtained after a few hours of treating with the maceration fluid. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with 5% NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured. Photomicrographs were taken with NIKON trinocular photo micrographic unit. The most accepted descriptive terms were being used to describe the root and stem anatomy.[7]

Physicochemical constant and fluorescence analyses

These studies were carried out as per the standard procedures.[8] In the present study, the whole plant powder was treated with 1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide, acids like 1N hydrochloric acid, 50% sulphuric acid, nitric acid, picric acid, acetic acid and nitric acid with ammonia. These extracts were subjected to fluorescence analysis in visible/daylight and UV light (254nm & 365nm). Various ash types and extractive values were determined by following standard method.[10,11]

Preliminary phytochemical analysis

Shade dried and powdered whole plant samples were successively extracted with petroleum ether, benzene, chloroform and methanol. The extracts were filtered and concentrated using vaccum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure.[8,12]

RESULTS AND DISCUSSION

The leaf is distinctly dorsiventral, with mesophyll differentiation and prominent midrib. The midrib portion has fairly wide adaxial epidermal layer of rectangular cells. The epidermis of the abaxial hump has thick walled rectangular cells. The ground tissue of the midrib is parenchymatous and vascular strand is single, semicircular in outline and collateral. It consists of a few clusters of vessels which are circular, thick walled. Thick walled fibres also occur along with the xylem elements. Phloem occurs in a thin arc beneath the xylem. A thick vertical pillar of parenchyma cells forms the adaxial extension between the vascular bundle and the upper epidermis (Plate I A).

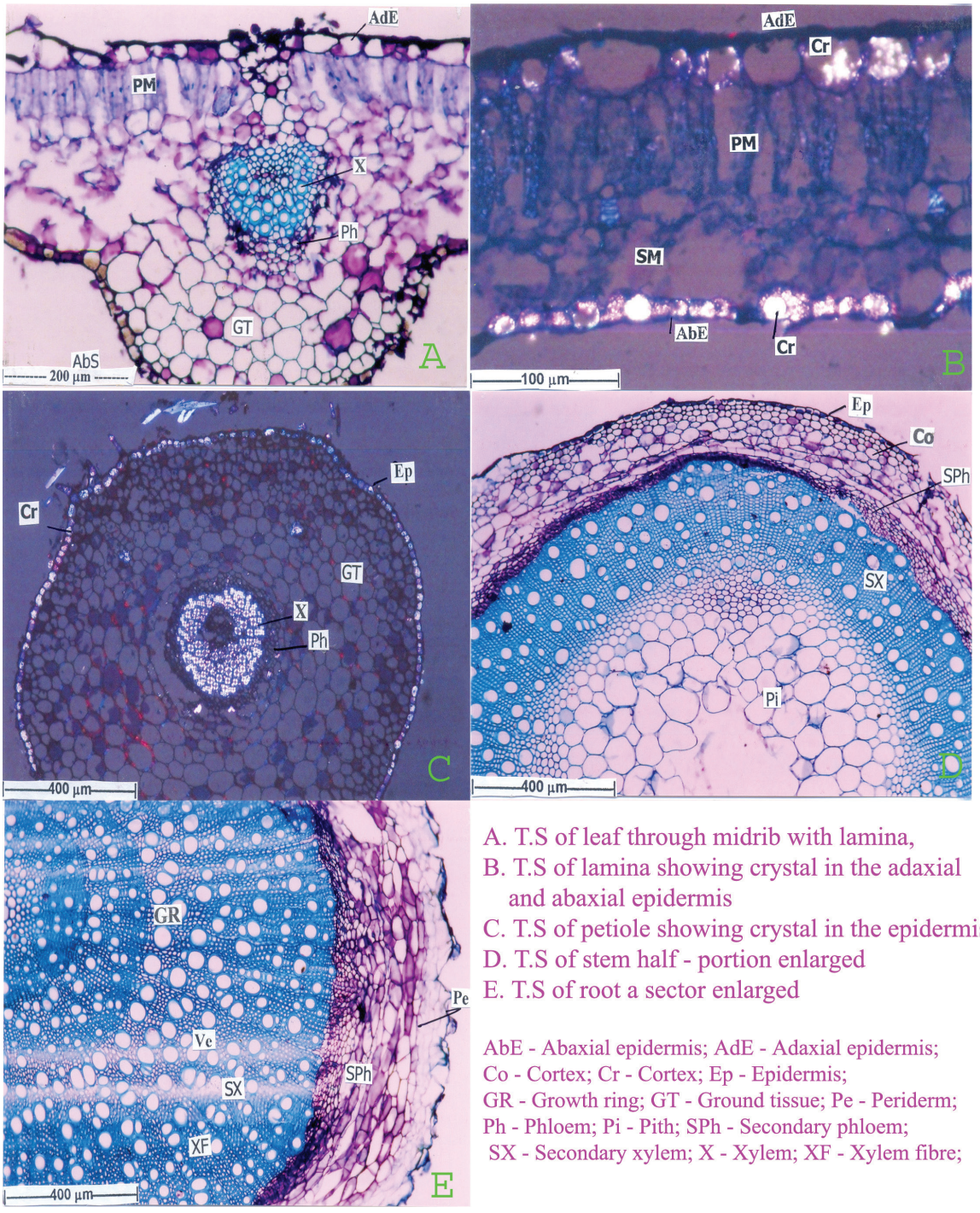
The lamina is bifacial due to distinction of thicker adaxial epidermis, wide palisade zone and stomatiferous abaxial epidermis. The adaxial epidermis is 30 – 35 μm thick; the cells are semicircular with flat, thickly cuticularized adaxial walls (Plate I B). The palisade zone has a single row of narrow, cylindrical loosely arranged cells. The spongy mesophyll has three or four lobed arenchyma cells. The major lateral vein project slightly on the abaxial side. It has collateral vascular bundle with thick mass of xylem and thin arc of phloem; the bundle is placed upon an abaxial pad of parenchyma cells. The leaf – margin is blunt and gradually tapering. The structure of the leaf margin does not differ from the remaining part of the lamina. The lateral vein and vein lets are thin and straight. Distinct vein islets are formed by the lateral veins. The vein islets are rectangular to squarish in outline. The vein terminations are simple slender, straight or curved.

Proximal part of the petiole is circular and it has a prominent epidermal layer of squarish cells with dark inclusions. The ground tissue is differentiated into three or four layers of collenchymatous cells and thin walled less compact parenchyma cells. The vascular system consists of a central, prominent, circular, solid main vascular strand and two small, less prominent accessory adaxial strands. The main strand has several radial, compact parallel lines of xylem elements which encircle a small central core of parenchyma cells. Phloem occurs as thin sheath around the xylem cylinder.

The cross sectional outline of the stem is circular. It consists of an intact epidermal layer, cortex wide, hollow vascular cylinder and broad pith. The epidermis is 13 μm thick. The cortex is 110 μm wide. The outer part of the cortex has two or three layers of collenchyma cells and the inner portion has compact 5 or 6 layers of parenchyma cells. The vascular cylinder has outer phloem of 60 μm width; the cells are large and arranged in radial files. Intermixed with the phloem tissues, there are sparse and solitary sclerenchyma elements. Secondary xylem is 200 μm thick. It consists of diffusely distributed, circular, solitary, thick walled vessels and radial rows of thick walled fibres. The vessels are 30 μm wide (Plate I D).

The root has narrow, superficial, less distinct periderm comprising of two or three layers of cells. The epidermis (rhizodermis) has disintegrated. The cortical zone is 60 μm wide. It has about eight layers of tangentially elongated and compressed parenchyma cells. Secondary phloem is narrow cylinder and consists of randomly oriented sieve elements and other phloem cells. Secondary xylem cylinder is wide and dense. It exhibits fairly distinct growth rings, which has semi–ring porous distribution of vessels within a growth ring, the early wood vessels are wider than the

Plate I-*Blepharis maderaspatensis (L.) Heyne ex Roth.*



A. T.S of leaf through midrib with lamina,
B. T.S of lamina showing crystal in the adaxial and abaxial epidermis
C. T.S of petiole showing crystal in the epidermis
D. T.S of stem half - portion enlarged
E. T.S of root a sector enlarged

AbE - Abaxial epidermis; AdE - Adaxial epidermis;
Co - Cortex; Cr - Cortex; Ep - Epidermis;
GR - Growth ring; GT - Ground tissue; Pe - Periderm;
Ph - Phloem; Pi - Pith; SPh - Secondary phloem;
SX - Secondary xylem; X - Xylem; XF - Xylem fibre;

later wood vessels. In the outer portion of the root, the growth rings are less distinct. However, the vessels of uniform diameter occur in regular concentric rings in successive layers. The narrow vessels are 10 µm wide and the wide ones are 40 µm wide (Plate I E).

Calcium oxalate crystals are fairly abundant in the leaf. The crystals are sphaerocrystals. Their distribution is specific and characteristic. They are present in almost all cells of the lower epidermis and the epidermal cells of the petiole (Plate I C). They are lacking in the ground tissues of the leaf and petiole as well as in the stem. The druses are up to 15 µm in diameter. In the epidermal cells that occur around the basal cell of the trichome, the crystals are seen as dense granules. These cells form a rosette pattern.

Powder microscopic observations

Powder/maceration of the leaf and stem consists of the three types of trichomes of epidermis, such as glandular trichomes, non-glandular short trichomes and non-glandular long trichomes. These trichomes are 3 or 4 celled, uniseriate, unbranched, thin walled and pointed. They are also bent or curved. They are diffuse in distribution. They are 250 μm in length and 20 μm thick at the base.

Rosettes cells

Circular group of 2 – 5 celled rosettes are also seen in the powder. They are epidermal cells from which the epidermal trichomes arise. The epidermal cells around the point of origin of the trichome form a circle of rosette as seen in surface view. These rosette cells are 60- 80 μm in diameter.

Stem powder (maceration)

In the macerated preparation of the stem, vessel elements, xylem fibres and xylem parenchyma were observed.

Vessel elements are narrow, cylindrical and elongated. Some of the vessel elements have short or long tails or more frequently they are tailless. They have simple, wide, circular perforation which may he horizontal or oblique. The lateral walls are densely pitted. The length of the vessel elements is 320 - 750 μm.

Xylem fibres are abundant in the powder. They are narrow, long and pointed. They have thick walls and narrow lumen. Lateral walls pits are not evident. They are 500 – 800 μm long. Xylem parenchyma cells are less frequent. They are wide, thin walled cells. They may be square shaped or rectangular. They are 170 × 60 – 110 μm in size.

In *Blepharis maderaspatensis* (L.) Heyne ex Roth a member of Acanthaceae, has planoconvex midrib and single stranded collateral cup shaped bundle. The lamina has quite high palisade cells in a single row. Presence of distinct vein islets, elaborate vein termination and amoeboid epidermal cells with wavy anticlinal walls are diagnostic features.

Powder analysis of the drug

The results of the ash and extractive values of *Blepharis maderaspatensis* whole plant drug powder are depicted in Table-1. The total ash content of the powdered whole plant is 6.43% and extractive value in water is more than in ethanol.

The results of fluorescence analysis of whole plant powder of *Blepharis maderaspatensis* are shown in Table-2. The whole plant powder shows the characteristic fluorescent colour, when treated with IN alcoholic NaOH and 50% Sulphuric acid under short UV light. Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples.[13,14]

The results of preliminary phytochemical screening of whole plant extracts of *Blepharis maderaspatensis* are presented in Table-3. The methanol extracts of the whole plant shows the presence of alkaloid, catachin, flavonoid,

Table 1: Ash and extractive values of the powdered whole plant of *Blepharis maderaspatensis*

Type of Ash	% of Ash
Total ash value of powder	6.43 ± 0.06
Water soluble ash	1.98 ± 0.03
Alkalinity of wate r soluble ash	2.45 ± 0.01
Acid insoluble ash	1.34 ± 0.02
Nature of extract	**Extractive value (%)**
Alcohol (Ethonolic)	6.93 ± 0.07
Water (Aqueous)	8.24 ± 0.03

Table 2: Fluorescence analysis of the powdered whole plant of *Blepharis maderaspatensis*

Experiments	Visible/Day light	UV Light	
		254nm	365nm
Drug powder as such	Pale green	Pale green	Dark green
Powder + 1N NaOH (aqueous)	Pale brown	Pale green	Green
Powder + 1N NaOH (alcohol)	Brown	Fluorescent green	Green
Powder + 1N HCL	Pale brown	Yellowish green	Brown
Powder + 50% H_2So_4	Reddish brown	Fluorescent yellow	Dark green
Drug powder + Nitric acid	Yellowish green	Pale green	Dark green
Drug Powder + Picric acid	Reddish brown	Pale brown	Fluorescent green
Drug Powder + Acetic acid	Pale brown	Brown	Brown
Drug Powder + HNO3 + NH3	Reddish brown	Pale brown	Dark brown

Table 3: Preliminary phytochemical screening of whole plant extract of *Blepharis maderaspatensis*

Test	Petroleum ether	Benzene	Chloroform	Methanol
Alkaloid	–	–	–	+
Anthraquinone	–	+	–	–
Catachin	+	+	–	+
Coumarin	–	+	+	–
Flavonoid	–	–	–	+
Phenol	+	+	+	+
Quinone	–	–	+	–
Saponin	–	+	–	+
Steroid	+	–	+	–
Tannin	+	–	+	+
Terpenoid	–	+	+	+
Sugar	–	+	+	+
Glycosides	+	–	–	–
Xanthoprotein	–	+	+	+
Fixed oil	+	–	+	–

phenol, saponin, terpenoid, tannin, sugar, glycosides and xanthoprotein. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance saponin is used as mild detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, etc. it is also known to have antifungal properties.[15]

From this study presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for detailed photochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds such as alkaloids, terpenoids, coumarin, tannin, saponin, flavonoids, quinones, anthraquinones, phenols and glycosides are detected in *Blepharis maderaspatensis* whole plant extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

In this dimension pharmacognostic studies on *Blepharis maderaspatensis* is a substantial step and it further requires a long term study to evaluate pharmacological action as well as therapeutic efficacy and toxicity of said plant to establish as the drug. The pharmacognostic study of the *Blepharis maderaspatensis* has been carried out for the first time. This could be useful in the preparation of the herbal section of proposed pharmacopoeia.

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Determination of Physicochemical parameters and DPPH radical scavenging activity of *Chenopodium album* Linn

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ABSTRACT

Aim: The existing study was conceded out to offer requisite detail of Phytochemical and Physicochemical parameters and radical scavenging activity of the plant *Chenopodium album* Linn. **Materials and Method:** In the present work, different extracts of *Chenopodium album* Linn (*C.album*) were prepared and tested for the presence of secondary metabolites and various physico-chemical parameters for the phytochemical analysis of plant. All polar extract were tested for the free radical scavenging activity. **Results:** The pharmacognostical results exposed the presence of flavonoids, tannins and alkaloids in the plant and significant physico-chemical values. Utmost free radical scavenging activity was found to be in butanol fraction and lowly in dichloromethane extract. **Conclusion:** It may be concluded that *C. album* Linn could be a source of therapeutic and natural scavenging agent.

Key words: *Chenopodium album;* Extractive values; Sulphated ash; Antioxidant activity.

INTRODUCTION

Natural products have played a significant role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates and its importance in modern medicine has been discussed in different reviews and reports.[1-6]

Freshly there has been a move in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Nature has bestowed our country with a huge prosperity of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world. In the series of potent medicinal plant, *Chenopodium album* Linn (family- Chenopodiaceae) is an herbaceous plant commonly known as Bathua Sag in Hindi, Vastukah in Sanskrit, and Wild Spinach in English. The plant is small odorless herb up to 3.5 m in height, erect or ascending. The leaves are variable in size and shape, Oblong, rhombic and deltoid or lanceolate. The seeds are 1.5 mm in diameter, orbicular and compressed with an acute margin.[7] According to traditional literature, *Chenopodium album* Linn is sweet, acrid, digestive, carminative, laxative, anthelmintic, diuretic, aphrodisiac and tonic. The plant is also useful in vitiated conditions of pittic, peptic ulcer, helminthiasis, dyspepsia, flatulence, strangury, seminal weakness, haemorrhoids, cardiac disorders and general debility.[8] A thorough survey of literature indicates that a good number of pharmacological studies have been explored on *Chenopodium album* Linn *(C.album)*. The present study is therefore undertaken to recognize physicochemical parameters and free radical scavenging activity using standard procedures.

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MATERIALS AND METHODS

Plant material

The plant of *C. album* Linn were identified and collected from the local market of Bhopal. The plant was authenticated by Dr. A.S Yadav, Professor in Department of Botany, M.V.M Government College Bhopal (M.P.), where a voucher

specimen is deposited. The plant was washed, shade dried, pulverized into moderately coarse powder and stored in airtight container for further studies.

PREPARATION OF C.ALBUM EXTRACTS

Physicochemical analysis

The pulverized plant material was packed in soxhlet apparatus for successively hot continuous extraction with petroleum ether, methanol, ethanol and water as solvent. All the extracts finally reduced to dryness at 40° C by Rotavapour. The traces of the solvents were removed by keeping the dried extracts in to desiccators. The concentrated extracts were weighed and percentage of yield (w/w) was calculated.

DPPH radical scavenging assay

To the methanolic crude extract 500 ml of cold acetone was added and the mixture was placed on a stir plate overnight in a cold room. The acetone extraction produced heavy precipitation consisting mostly of proteinaceous material, which was removed by centrifugation. The remaining solution was concentrated under reduced pressure and was sequentially extracted with a series of organic solvents ranging from least polar to most polar (hexane, diethyl ether, dichloromethane, ethyl acetate and n-butanol). The polar extract was partitioned five times with 250 ml (5 × 250 ml) of five solvents. Solvents from each fraction were evaporated to dryness and lyophilized. Each polar fraction was tested for free radical scavenging activity.

Phytochemical analysis

Phytochemical screening means to examine the plant material in terms of its active constituents. All the extracts obtained from *Chenopodium album* Linn were subjected to various qualitative tests for the identification of phytoconstituents by using standard phytochemical procedures.[9-10]

Physicochemical parameters

Physicochemical parameters such as percentage of total ash, acid insoluble ash, water soluble ash and sulphated ash were calculated based upon standard procedures.[11, 12]

Total ash

About 2 gm accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450° C until free from carbon. It was then cooled and weighed. The percentage w/w of ash with reference to the air-dried drug was calculated.

Acid insoluble ash

Ash is boiled with 25 ml dilute HCL (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450° C to a constant weight.

Water soluble ash

Ash is dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450° C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash is obtained. Percentage of water soluble ash was calculated with reference to the air dried drug.

Sulphated ash

2 g of powdered samples are taken in crucibles and ignited at 450° C in a muffle furnace until the material gets thoroughly charred. The crucibles along with ash are taken out in desiccators and cooled. 1 ml H_2SO_4 is added to each crucible in order to moisten the residue. Heat gently until white fumes was no longer evolved and ignites at 800° C until black particles were disappeared. The crucibles are removed from the muffle furnace to desiccators, cooled and weighed to give the sulphated ash content.

DPPH radical scavenging assay

The free radical scavenging potential of extracts was evaluated by spectrophotometric assay, quantifying their capacity to bleach a purple solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol.[13] Different concentration of *C.album* extract were prepared in methanol and 3ml of each solution was mixed with 1ml of 0.1mM methanolic DPPH solution. After a 30 min incubation period at room temperature, each absorbance (A) was determined at 517 nm. Inhibition percentage of DPPH (I %) was calculated as follows:

$$I\ \% = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of a control solution containing only the DPPH reagent, and A_{sample} is the absorbance of the sample reaction. The effective dose of 50% inhibition (ED_{50}) was obtained from a plot of percentage inhibition verses extract concentration. These determinations were carried out in triplicate and mean values were calculated. Ascorbic acid and Butylated hydroxyl toluene (BHT) was used as positive control.

Statistical analysis

Experimental data are expressed as mean ± SEM (n = 3), and were compared using one-way analysis of variance (ANOVA), followed by Dunnet's pairwise test, with p values < 0.05 being considered significant.

RESULTS

Extractive values

Extractive values obtained from *C. album* Linn using different solvents were recorded in table 1. The ethanolic extract has maximum yield (16.56g) as compared to other fractions.

Table 1: Extractive values obtained from *C.album* Linn using different solvents

Solvent	Time of extraction	Colors of extract	Yield	% Yield
Petroleum Ether	48 hours	Light green	2.8 g	3.21 %
Methanol	28 hours	Dark green	16.56 g	19.03 %
Ethanol	12 hours	Light green	2.10 g	2.41 %
Water	12 hours	Light brown	10.1 g	11.60 %

Table 2: Preliminary phytochemical analysis of various extracts of *C.album* Linn

Phytoconstituents	Petroleum ether Extract	Methanolic Extract	Ethanolic Extract	Aqueous Extract
Alkaloids	–	+	+	+
Carbohydrates	–	+	–	–
Flavonoids	–	+	–	+
Glycosides	–	–	–	–
Proteins	–	+	–	–
Resins	+	–	–	–
Steroids	–		–	–
Tannins	–	+	+	+

(+) Present (-) Absent

Table 3: Physicochemical parameters of *C.album* Linn

Type of ash	Results (%)
Total ash	10.5
Acid insoluble ash	1.5
Water soluble ash	4.5
Sulphated ash	2.5

Table 4: Effect of *C.album* extract and its fractions on free radical generation *in vitro*

Tested material	ED_{50} (µg/ml) ± S.E.M
	DPPH method
Methanolic extract	129.20 ± 2.10
Dichloromethane fraction	285.62 ± 3.85
Ethyl acetate fraction	87.52 ± 1.98
Butanol fraction	29.30 ± 1.46
Ascorbic acid	10.85 ± 1.38
BHT	13.50 ± 1.79

Phytochemical analysis

The results of phytochemical analysis of different extracts and fractions of plant were reported in table 2. From this analysis, methanol extract found to have more constituents compare to other extracts. Flavonoids and Tannins are determined more significant in this extract. So this extract was further sequentially extracted with a series of organic solvents and tested for radical scavenging activity.

Physico-Chemical Parameters

The results of physicochemical parameters were investigated and reported in table 3. The above studies assist the credit of the plant material for future investigation and form an important aspect of drug studies.

DPPH radical scavenging activity

Percentage inhibition (ED_{50}) of crude methanolic extract and its polar fractions towards DPPH radicals is tabulated in table 4. Butanol fraction has showed utmost free radical scavenging activity with DPPH ED_{50} values of 29.30 µg/ml, showing almost half of the BHT free radical scavenging potential, while it was least in dichloromethane extract with ED_{50} values of 285.62 µg/ml. These values were found to be less than those obtained for the reference standards, Ascorbic acid and BHT.

DISCUSSION

A large no. of medicinal plants is currently being used in the treatment of various disease conditions in the form of herbal medicines containing crude drugs. The standardization of a crude drug is an essential part of establishing its proper identity. Before any crude drug can be integrated in the herbal pharmacopoeia, pharmacognostic parameters and standards must be established.[14]

Herbal medicines are medicinal products that contain plant materials as their pharmacologically active components.[15] For many herbal medicines; the specific ingredients that determine the pharmacologic activity of the product are as yet incompletely characterized. Preliminary phytochemical analysis of different extracts of *C. album* revealed the presence of alkaloids, flavonoids, and tannins. These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, and tannins. These phytoconstituents may be responsible for the many pharmacological actions of the plant like hypolipidemic[16] antipruritic,[17] and anthelmintic activity.[18] Additional work could also be possible to examine the specific phytoconstituents responsible for these activities.

The physico-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs.

Free radicals and other reactive oxygen species are considered to be important causative factors in the development of diseases of aging such as neurodegenerative diseases cancer, and cardiovascular diseases.[19] Antioxidant activity was found in the order of butanol fraction> ethyl acetate fraction> crude methanolic fraction> dichloromethane fraction. The result found clearly demonstrates that the bioactive compounds contained in this plant have a polar character since they are soluble in water and polar organic solvents, and it is evident by many studies that polar constituents of many medicinal plants such as falvonoid and phenolic compounds, are able to scavenge radicals readily.[20-21] So it is proposed in this study that the radical scavenging activity of extract could possibly be related to phenolic compounds and flavonoid as the given *c. album* extract has shown affirmative outcome for flavonoid compound.

CONCLUSION

The current work was taken up with an idea to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. This analysis may serve in providing preliminary basis for exploration of the potential of plant for different pharmacological activities. The findings of the present study suggest that it can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application, moreover it could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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In Vitro Antioxidant Activity of Extracts from Fruits of *Muntingia calabura* Linn. from India

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ABSTRACT

Introduction: In the present study, hexane, chloroform, ethyl acetate, butanol and methanol extracts of fruits of *Muntingia calabura L* (Elaeocarpaceae) were examined total phenolics (TP) and *in vitro* antioxidative capacity. **Methods:** For the determination of total phenolics (TP) and *in vitro* antioxidative capacity, established assay methods such as 1, 1 - diphenyl – 2- picryl hydroxyl (DPPH) radical assay, reducing power, ferric ion chelating assay, superoxide anion, and nitric oxide scavenging activity assays were used with reference to synthetic antioxidant butyl hydroxyl toluene (BHT). One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were carried out. **Results:** The TP ranged from 1486 ± 0.028 mg GAE /100 of fresh mass (FW) to 358 ± 0.020 mg GAE/100 of fresh mass (FW). The data obtained from the study showed high levels of antioxidant activity of the fruit extracts. **Conclusion:** From the findings, it was observed that there was a well correlation between antioxidant activity and total phenolic/flavonoid contents. These results may be useful to further analyze wild edible fruits that contain most antioxidant activity in order to identify the active constituents.

Key words: Antioxidant activity, butyl hydroxyl toluene, Elaeocarpaceae, *Muntingia calabura* L., phenolic content

INTRODUCTION

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community since epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer.[1,2] These benefits are thought to result from the antioxidant components of plant origin, vitamins, flavonoids, and carotenoids.[3-5] Fruits contain many different antioxidant compounds i.e., vitamin C and E, carotenoids and phenolic compounds, that serve as radical scavengers. Recent studies have demonstrated the antioxidant activities and health benefits of the phenolic compounds occurring in fruits and vegetables.[6-9] This lends support to the hypothesis that fruits and vegetables high in antioxidant potentially exert a protective effect against degenerative diseases.

Muntingia calabura L. (Elaeocarpaceae) commonly known as Jamaican cherry, Panama berry, Singapore cherry, is the sole species in the genus *Muntingia*. The fruits are antinociceptive, antioxidant and antipyretic.[10,11] Aqueous extract of leaves of *M. calabura* showed protective effect against isoproterenol-induced biochemical alterations in rats.[12] Methanol extract of fruits of *M. calabura* exhibited potent DPPH quenching capacity.[13] Volatile compounds were isolated from the fruits of *M. calabura*.[14] In order to supply more scientific evidence for research and development of wild edible fruits in the field of functional foods, the current study was focused to determine total phenolic content, and to examine the potential antioxidant activities of different extracts from fruits of *M. calabura* using a range of indices of antioxidant activity.

MATERIALS AND METHODS

Chemicals

The solvents used in the present work were purchased from Qualigenes. Folin-Ciocalteu reagent, DPPH, Gallic acid and quercetin were procured from Sigma, USA. Ammonium molybdate tetrahydrate, aluminium chloride, TCA were acquired from E. Merck (INDIA) Limited. Mumbai, India. Nitro blue tetrazolium (NBT), Ferrozine were purchased from HI-MEDIA, Pvt. Ltd, India. All other reagents were of analytical grade.

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Fruits

The fully ripened fruits of *M. calabura* were collected from Erode District, Tamil Nadu during May 2008 and June 2008. The fruits were pooled and were kept in cold (–4° C) dark storage until further analysis. Voucher specimens were prepared and deposited at the herbarium of Kongu Arts and Science College, Erode (T.N), India.

Preparation of fruit extracts

Firstly, the fruits (100 g) were removed from bunch and homogenized in a blender with 500 ml of hexane, chloroform, ethyl acetate, butanol and methanol, and extracted exhaustively. Then, the extracts were centrifuged thrice (3000 g, 15 min) and the clear supernatants were collected separately from the corresponding solvents and filtered over Whatman No. 1 filter paper. The extracts were then condensed to dryness by rotary flash evaporator (Buchi type Rotavapor). Various concentrations of the extracts were prepared from the resultant extracts to determine *in vitro* antioxidant activity.

Determination of total phenolics

Total phenolic contents in the extracts were determined by Folin-Ciocalteu method.[15] An aliquot of the extracts was mixed with 5 ml of Folin- Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml of Na_2CO_3 (7.5g/l). The tubes were allowed to stand for 15 min and the total phenols were determined using UV/VIS Spectrophotometer (Elico SL 164 Double Beam, Elico Ltd.) at 765 nm. Total phenol content was expressed in terms of gallic acid equivalent in mg/100 g of fresh material (FM). All experiments were performed in triplicate.

Assessment of Free Radical Scavenging and Antioxidant Activity

DPPH radical scavenging activity

The quenching effect of extracts on DPPH radical was determined using the method of Blois.[16] Briefly, to 1 ml of different concentrations (500, 400, 300, 200, 100 µg/ml) of extracts, 5 ml of methanol solution of DPPH (0.1 mM) was added, vortexed, followed by incubation at 27° C for 20 min. The control was prepared without any extract and absorbance of the sample was measured at 517 nm using UV/VIS spectrophotometer. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) $= \frac{[(Abs_{control} - Abs_{sample})]}{(Abs_{control})} \times 100.$

Reductive ability

Total reducing power was determined as described previously.[17] One ml of sample solution at different concentration was mixed with 2.5 ml of phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50° C for 20 min. To this, 2.5 ml of trichloroacetic acid (TCA, 10%) was added and centrifuged at 3000 *g* for 10 min. The supernatant (5 ml) was mixed with 1 ml of ferric chloride (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity was based on the method described previously.[18] Superoxide radicals were generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In brief, 3 mL of sample solutions at different concentrations were mixed with 1 ml of NBT (156 µM) and 1 ml of NADH (468 µM). The reaction started by adding 0.1 ml of phenazine metho sulphate (PMS) solution (60 µM) to the mixture. The reaction mixture was incubated at 25ºC for 5 min, and the absorbance at 560 nm was measured against blank samples. The percentage inhibition of superoxide anion generation was calculated using the following formula: Inhibition of superoxide generation (%) $= \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100.$

Scavenging capacity towards hydroxyl ion (˙OH) radicals

The ˙OH scavenging activity of the fruit extracts was determined according to the method described previously.[19] Different concentrations of extracts were added with 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was started by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90° C for 15 min in a water bath. The reaction was then terminated by the addition of 1.0 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The intensity of the color formed was measured at 412 nm against reagent blank. BHT was considered as the reference standard. The hydroxyl radical scavenging activity is calculated by the following formula: HRSA (%) $= 1 - \left(\frac{\text{difference in absorbance of sample}}{\text{difference in absorbance of blank}}\right) \times 100.$

Scavenging activity against nitric oxide

Nitric oxide interacts with oxygen to produce stable products, nitrite and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution was assayed spectrophotometrically by using the Greiss reagent, with which nitrite reacts to give a stable product absorbing at 546 nm.[20] Nitric oxide radicals were generated from sodium

nitroprusside (SNP) solution at physiological pH. Sodium nitroprusside (1 ml of 10 mM) was mixed with 1 ml various concentrations of sample extracts in phosphate buffer (pH 7.4). The mixture was incubated at 25ºC for 150 min. To 1 ml of the incubated solution, 1 ml of greiss reagent (1% sulphanilamide, 2% ortho phosphoric acid and 0.1 % napthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition was calculated using the formula: inhibition (%) = $\left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$.

Iron chelating activity

The iron chelating activity was measured by the decrease in absorbance at 562 nm of the iron (II) –ferrozine complex.[21] The reaction mixture contained 0.5 ml of various concentrations of the fruit extracts, 0.1 ml of ferric chloride (0.6 mM) and 900 µl methanol. The mixture was shaken and left at room temperature for 10 min. To this, 0.1 ml of ferrozine (5 mM) in methanol were added, mixed and left for 5 min to complex the residual Fe^{2+}. The absorbance of the resulting solution was measured at 562 nm. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula: Chelating effect (%) = $\frac{[(Abs_{control} - Abs_{sample})]}{(Abs_{control})} \times 100$.

Reduction of lipid peroxidation

Inhibition of lipid peroxidation (LPO) in rat lever homogenate was determined in terms of formation of thiobarbituric acid reactive substances (TBARS) with minor changes.[22] In brief, different concentrations of fruit extracts and standard were individually added to 0.2 mL of liver homogenate (10%) extracted with KCl (15%). To the above mixture, 0.1 mL of $FeSO_4$ (10 mM) solution was added to initiate LPO. The volume of the mixtures was finally made up to 2 mL with phosphate buffer (0.1 mM, pH 7) and incubated at 37º C for 30 min. At the end of the incubation period, reaction mixture (0.3 mL) was added with 1 mL of TBA (0.8%, w/v) and 0.1 mL of TCA (20%) solution. The mixture was then heated on a water bath at 100º C for 60 min. After cooling, *n* – butanol (4 mL) was added in each tube and centrifuged at 3000 × *g* for 10 min. The absorbance of the organic upper layer was read at 532 nm. BHT was used for comparison. The percentage reduction of LPO was calculated as follows: Reduction of TBARS (%) = $\frac{1 - Sample_{532nm}}{Control_{532nm}} \times 100$ where, $Sample_{532nm}$ was absorbance of the sample and $Control_{532nm}$ was absorbance of control.

Statistical analysis

The experimental data were mean ± standard deviation of three measurements ($n = 3$). Linear regression analysis was used to calculate the efficient concentration (IC_{50}) values. One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were carried out. The *P* values of less than 0.05 were adopted as statistically significant.

RESULTS AND DISCUSSION

In this investigation, contents of total phenolics as well as the *in vitro* antioxidant activities of extracts from fruits of *M. calabura* were determined. The IC_{50} values were obtained for tested assays and are given Table 2.

Phenolic content

In our study, the contents of the total phenolics in different fractions of *M. calabura* (PE, $CHCl_3$, EA, BuOH and MeOH) determined using Folin–Ciocalteau method was expressed as gallic acid equivalents (GE). The estimation of TP amongst different fractions of fruits revealed that the

Table 1: Total phenolics content of *M. calabura* fruit

M. calabura	Total phenolics (mg/100g)[a]
PE	358 ± 0.020
$CHCl_3$	447 ± 0.025
EA	1140 ± 0.02
BuOH	940 ± 0.03
MeOH	1486 ± 0.028

Each value is presented as Mean ± Standard Deviation ($n = 3$). [a]mg GAE/100g FW

Table 2: Antioxidant capacities of different extracts of *M. calabura* fruit

	IC_{50} (g/mL)					
Extracts	**DPPH·**	**O_2^-**	**OH·**	**NO**	**Metal chelating**	**LPO**
PE	98 ± 0.62	310.2 ± 0.04	79.46 ± 0.08	207 ± 0.02	480.6 ± 0.02	240.2 ± 0.04
$CHCl_3$	245.42 ± 0.22	378.2 ± 0.08	280.4 ± 0.8	250 ± 0.08	91.2 ± 0.64	490.23 ± 0.24
EA	100.24 ± 0.24	240.5 ± 0.2	198.2 ± 0.02	497.2 ± 0.08	81.4 ± 0.04	190.2 ± 0.62
BuOH	350.12 ± 0.88	250.5 ± 0.48	52 ± 0.4	189 ± 0.26	290.2 ± 0.24	540.1 ± 0.02
MeOH	90 ± 0.04	79.2 ± 0.04	49.98 ± 0.2	187 ± 0.04	80.26 ± 0.08	110.4 ± 0.64
BHT	26.0 ± 0.65	83.0 ± 2.35	41.5±2.23	33.5 ± 2.12	ND	ND

The data are presented as mean value ± standard deviation SD ($n = 3$). Values with different letters in same column were significantly different at $P > 0.05$. ND – Not Determined. PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxy Toluene.

MeOH extract rendered highest phenolic content (1486 ± 0.028 mg GAE /100g FW) followed by EA extract (1140 ± 0.02 mg/100g), BuOH extract (940 ± 0.03 mg/100g), $CHCl_3$ fraction (447 ± 0.025 mg/g GAE). The PE extract has the lowest content of phenolics (358 ± 0.020 mg/100g). The result of the study implied that total phenolics were in MeOH extract, and MeOH extract was expected to be the most active one.

DPPH scavenging activity

The dose-response DPPH radical-scavenging activity of various extracts studied in this study is shown in Figure 1. With regard to the estimated IC_{50} values, all the extracts of *M. calabura* displayed significant DPPH radical quenching property. Among the extracts studied, the most active extract was found to be MeOH (IC_{50} = 90 ± 0.04 µg/mL). The MeOH extract significantly quenched DPPH radical though it showed lesser activity than the standard BHT. The BuOH extract showed the weakest quenching capacity with an IC_{50} value of 350.12 ± 0.88 µg/mL. The effectiveness of antioxidants as DPPH radical scavengers ranged in the following descending order: MeOH (90 ± 0.04 µg/mL). > PE (98 ± 0.62 µg/mL) > EA extract (100.24 ± 0.24 µg/mL) > $CHCl_3$ (245.42 ± 0.22 µg/mL) > BuOH (350.12 ± 0.88 µg/mL).

Reducing power

The reducing power of the crude extracts of the sample was examined as a function of their concentration. The concentration dependent reducing capacity for investigated extracts of *M. calabura* is illustrated in Figure 2. Out of five extracts, the EA displayed the highest reductive capacity (OD of 0.732 ± 0.04 at 1000 µg/mL) followed by BuOH, MeOH, PE and $CHCl_3$ extracts. The reference standard BHT showed 1.634 ± 0.02 at 1000 µg/mL. For the determination of reducing capacity, "Fe^{3+} – Fe^{2+} transportation" in the presence of extract was observed. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.[23] The reducing power of the extracts might be due to the di and mono hydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities.

Superoxide scavenging activity

The $O_2^{\cdot -}$ radical is one of the most dangerous free radicals in humans and also the source of hydroxyl radical ($OH^{\cdot}$).[24] The fruit extracts from *M. calabura* were screened for their $O_2^{\cdot -}$ scavenging activity using PMS-NADH-NBT assay. In the PMS/NADH-NBT system $O_2^{\cdot -}$ derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The effect of extracts (PE, $CHCl_3$, EA, BuOH and MeOH) on superoxide anion radicals in PMS-NADH/NBT system are shown in Fig. 3. The effect of MeOH and EA extracts was the highest with IC_{50} value of 79.2 ± 0.04 µg/mL and 240.5 ± 0.2 µg/mL respectively. The PE and BuOH extracts exhibited moderate superoxide anion radical scavenging activity with IC_{50} µg/mL values of 310.2 ± 0.04 and 250.5 ± 0.48 µg/mL respectively and the $CHCl_3$ extract showed least activity (IC_{50} 378 µg/mL).

OH scavenging activity

Hydroxyl radical ($^{\cdot}OH$) which is the most reactive free radical, has the capacity to conjugate with nucleotides in

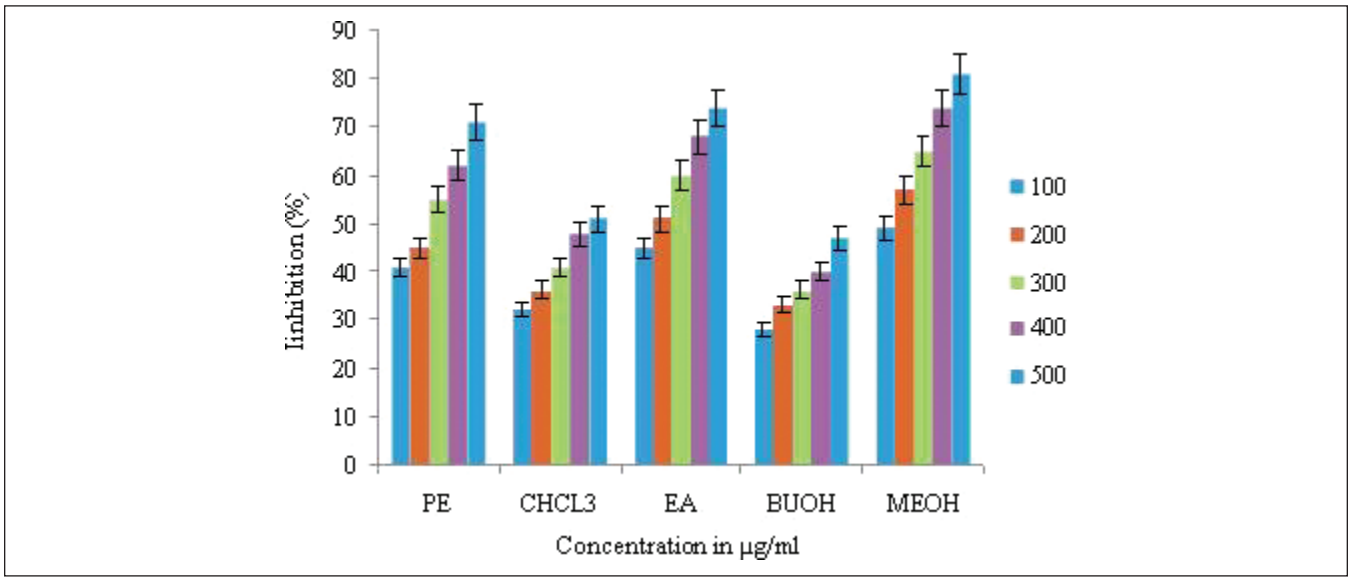

Figure 1: DPPH· scavenging activity of extracts from *M. calabura*.
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity.[25] The scavenging activity of the fruit extracts on ·OH is presented in the figure 4. The extracts from the fruit displayed dose dependent scavenging activity against the ·OH species, of which, MeOH and BuOH extracts were the most effective (IC_{50} 49.98 ± 0.2 µg/mL and 52 ± 0.4 µg/mL respectively). The PE (IC_{50} 79.46 ± 0.08 µg/mL), $CHCl_3$ (IC_{50} 280.4 ± 0.8 µg/mL), and EA (IC_{50} 198.2 ± 0.02 µg/mL) extracts displayed moderate scavenging capacity.

Nitric oxide radical scavenging activity

In addition to reactive oxygen species, nitric oxide (NO) is also implicated in inflammation, cancer and other pathological conditions. The NO generated from sodium nitroprusside reacts with oxygen to form nitrite. From the results obtained, the extracts dose-dependently inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The nitric oxide (NO) scavenging activity of the extracts of fruit of *M. calabura* is depicted in figure. 5. NO scavenging activity of MeOH extract from

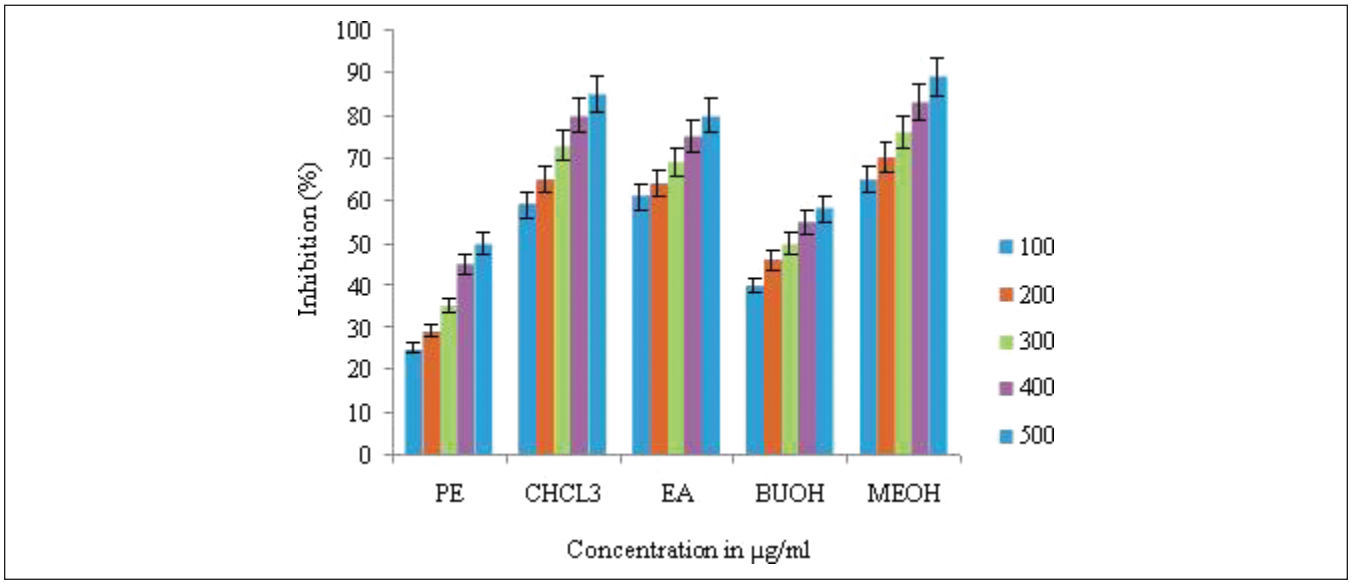

Figure 2: Reductive ability of extracts from *M. calabura.*
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

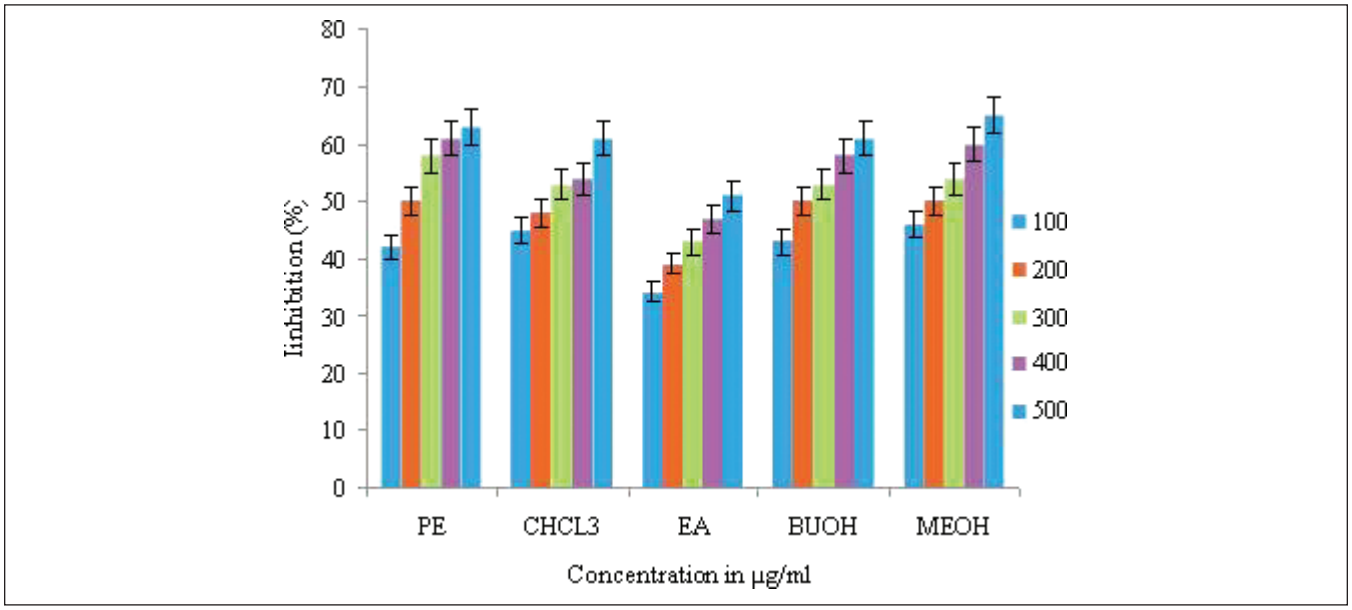

Figure 3: O_2^- scavenging capacity of extracts from *M. calabura.*
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

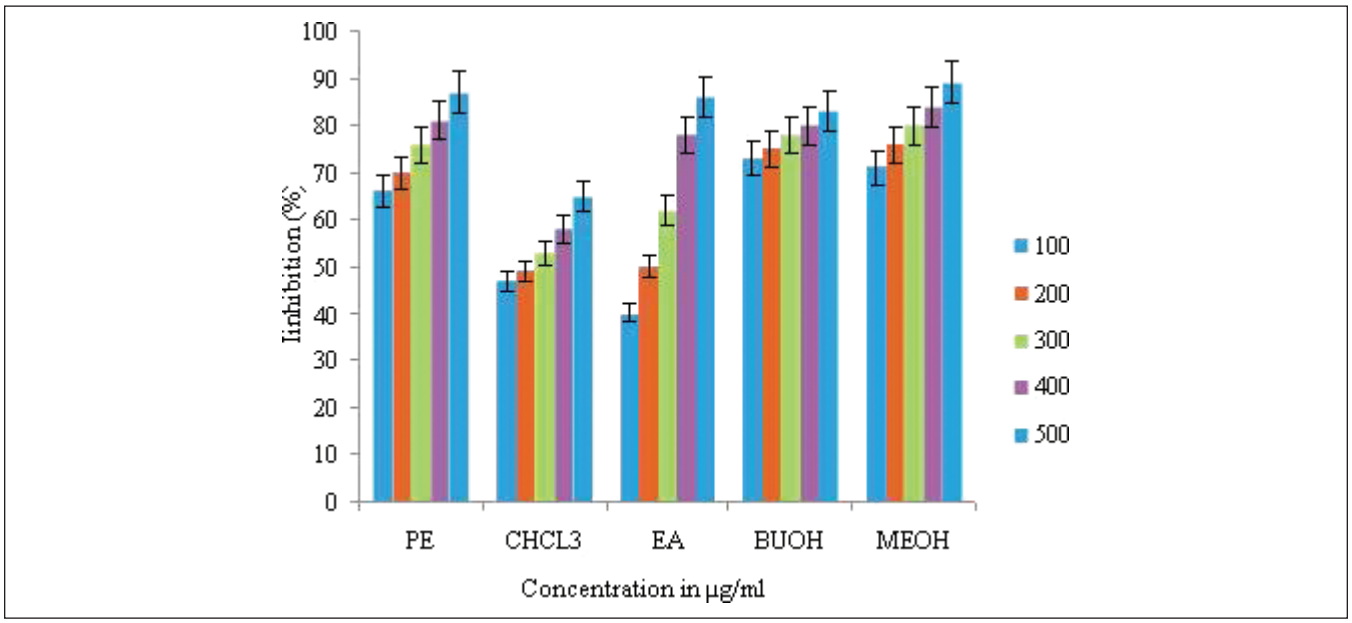

Figure 4: ˙OH scavenging capacity of extracts from *M. calabura*.
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

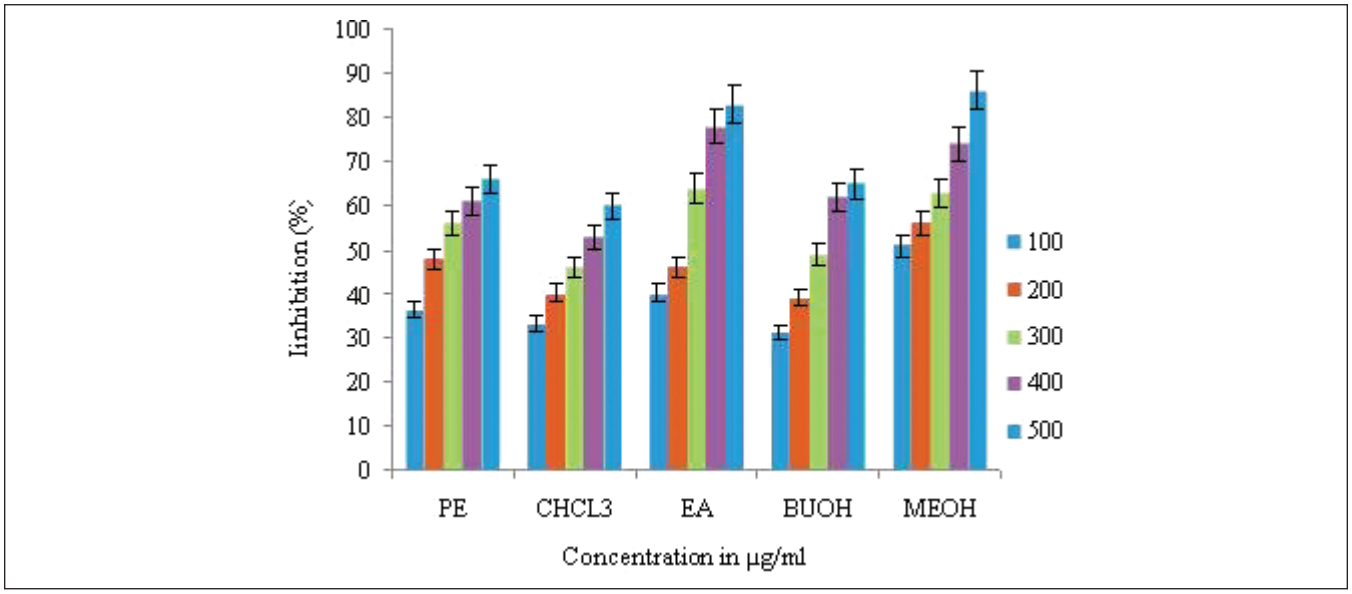

Figure 5: NO radical scavenging capacity of extracts from *M. calabura*.
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

the fruits was higher (IC_{50}187 ± 0.04 µg/mL) compared to other extracts. The NO scavenging activity ranged in the following descending order: MeOH (IC_{50}187 ± 0.6 µg/mL) > BuOH (IC_{50}189 ± 0.26 µg/mL) > PE (IC_{50} 207 ± 0.02 µg/mL) > $CHCl_3$ (IC_{50} 250 ± 0.08 µg/mL) > EA extract (IC_{50} 497.2 ± 0.08 µg/mL).

Ferrous ions chelating activity

Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation.[26] Figure 6 shows concentration dependent chelating effects of fruit extracts from *M. calabura* on the Fe^{2+} - ferrozine complex. The MeOH extract displayed the highest chelating activity with an IC_{50} value of 80.26 ± 0.08 µg/mL followed by the EA (IC_{50} 81.4 ± 0.04 µg/mL), $CHCl_3$ (IC_{50} 91.2 ± 0.64 µg/mL), BuOH (IC_{50} 290.2 ± 0.24 µg/mL) and PE (IC_{50} 480.6 ± 0.02 µg/mL) extracts. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion. Accordingly, it is suggested

that the low to moderate ferrous ions chelating effects of these fractions would be somewhat beneficial to protect against oxidation damage.

Inhibition of lipid peroxidation

Lipid peroxidation plays an important role in causing oxidative damage to biological systems and its by-product malondialdehyde (MDA) induces damage to other biomolecules.[27] The inhibitory effect of fruit extracts on lipid peroxidation (LPO) is shown in Figure 7. In this study, a variation of inhibitory effect of the extracts on LPO was observed (Table 2). The MeoH (IC_{50} 110.4 ± 0.64 µg/mL) extract was able to inhibit the generation of LPO efficiently. The EA and PE extracts exhibited moderate LPO inhibitory activity with the IC_{50} values of IC_{50} 190.2 ± 0.62 µg/mL and IC_{50} 240.2 ± 0.04 µg/mL respectively. $CHCl_3$ and BuOH extracts showed least activity (IC_{50} 490.23 ± 0.24 µg/mL and IC_{50} 540.1 ± 0.02 µg/mL respectively).

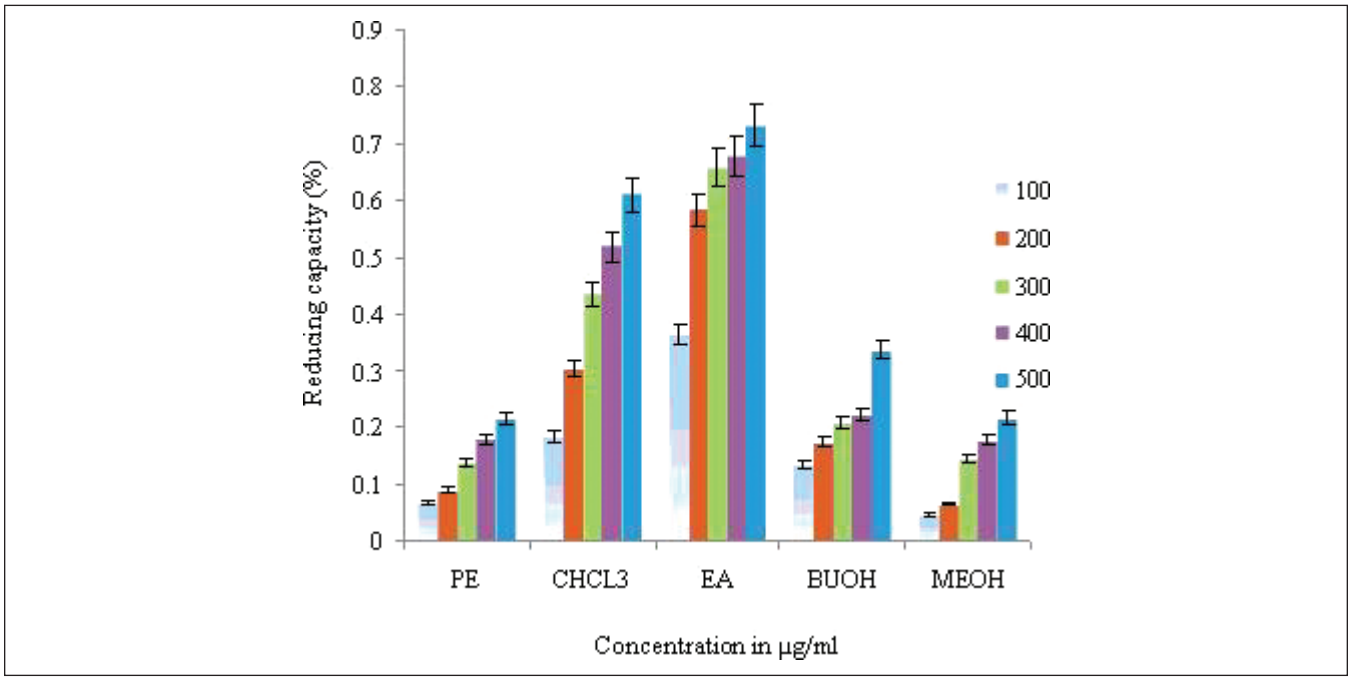

Figure 6: Metal chelating capacity of extracts from *M. calabura.*
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

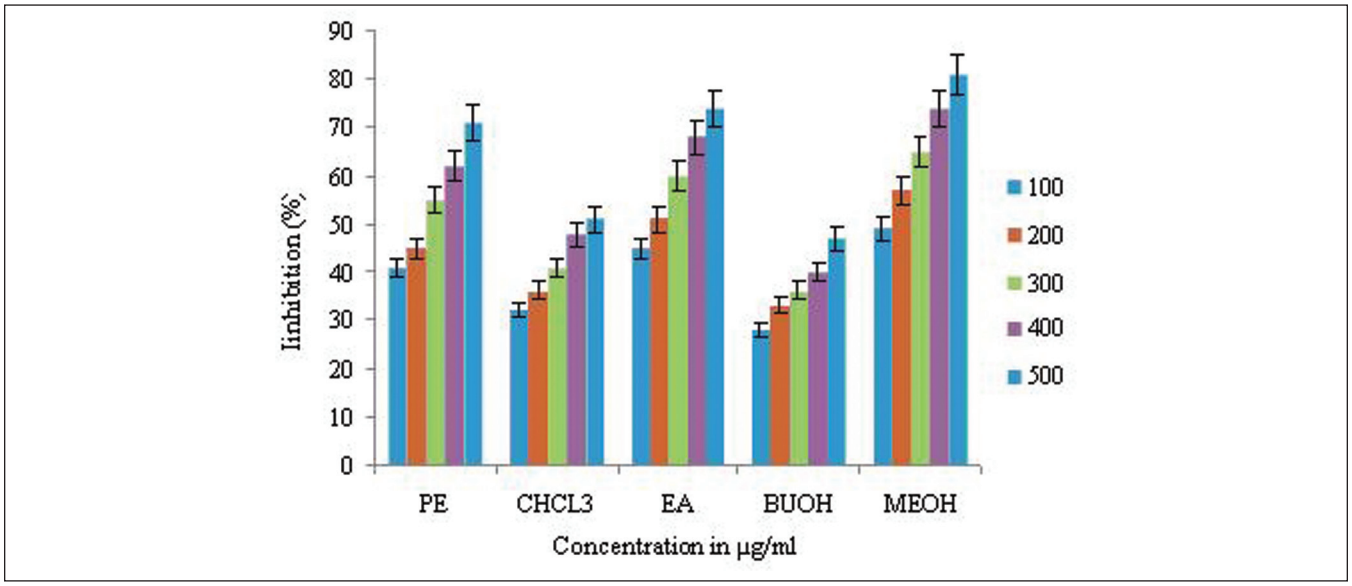

Figure 7: LPO inhibitory activity of extracts from *M. calabura.*
The data are presented as mean value ± standard deviation SD (n = 3). PE – Petroleum ether, $CHCL_3$ – Chloroform, EA – Ethyl acetate, BuOH – Butanol, MeOH – Methanol, BHT – Butyl Hydroxyl Toluene.

CONCLUSION

Recent years have seen an exponential increase in research antioxidant properties of fruits and vegetables. If it is accepted that higher intakes of natural antioxidants containing phenolics are associated with long-term health benefits, then the results presented in this paper offer possible avenues toward health promotion by identifying those compounds. The health promoting properties of fruits of *M. calabura* may be due to its antioxidant properties and is also attributed to its multitherapeutic characteristics. Thus, *M. calabura* might be useful in the development of raw materials of medicine.

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Evaluation of Antibacterial Activity and Phytochemical Screening of *Wrightia tinctoria* L.

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ABSTRACT

Wrightia tinctoria L. leaf hexane, methanol, chloroform, ethyl acetate and water extracts were screened against plant pathogenic bacteria by *in vitro*. The extracts were tested using disc diffusion method and minimum inhibitory concentration. Ethyl acetate and methanol extracts showed significant antibacterial activity against gram negative bacteria; the MIC was 50 µg/ml for *Xanthomonas campestris* and *Erwina* sp. Study shows *Wrightia tinctoria* leaves possessed potent antimicrobial properties against plant pathogenic bacteria suggesting that the active principles may be useful in the control of plant disease.

Key words: *Wrightia tinctoria*, antibacterial, phytochemicals, MIC

INTRODUCTION

Medicinal plants are an important therapeutic aid for various ailments. Around 15% of angiosperms have been investigated chemically and from that nearly 74% of pharmacologically active bioactive substances were identified.[1] This evidence contributes to support and quantify the importance of further screening of medicinal plants.

Medicinal plants are known to contain innumerable biologically active substances which possess antibacterial properties.[2] Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. These natural plant products, known as biopesticides, have long been used in the control of microorganisms causing plant diseases.

Plant pathogenic bacteria cause many serious diseases of plants throughout the world. Most plants, both economic and wild, have innate immunity or resistance to many pathogens but certain food crops and ornamental plants are susceptible to diseases caused by bacteria which are difficult to control and often result in sudden, devastating financial losses to farmers. Synthetic pesticides are commonly employed in agriculture to control phytopathogenic microorganisms.[3] Although it is highly effective, synthetic pesticide often have undesirable side effects such as toxicity to mammals and causing environmental pollution.[4-6] Most importantly, many phytopathogenic bacteria have acquired resistance to synthetic pesticides.[7]

Considering the harmful effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms.[8]

The use of plant-derived alternative pesticides seems to be regaining popularity and could play a vital role in meeting the demand for organically produced plants. Biopesticides has been suggested as an effective substitute for chemicals.[9] Many authors have contributed only on the use of several plant by-products, which posses antimicrobial properties, on several human pathogenic bacteria and fungi;[10-15] but information is obscure on the inhibitory activity of plant extract on phytopathogenic bacteria which are known to cause many diseases in a wide variety of crops, causing considerable losses in yield and quality.

Wrightia tinctoria is a well known potential medicinal plant distributed in tropical region belongs to the family

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Apocynaceae. The leaves of this plant are traditionally used in the treatment against Psoriasis and non-specific dermatitis in Siddha and Ayurvedic systems of medicine. This plant is good for treatment of dandruff, various scalp and skin disorders.[16-17] In the present study an attempt has been made to evaluate the antibacterial activity and to test the efficacy of the leaf solvents extracts on important plant pathogenic bacteria with the aim of discovering new bioactive substances that can be used as for controlling diseases caused by them in economically important crops.

MATERIALS AND METHODS

Source of the Plant material

Fresh healthy leaves of *Wrightia tinctoria* L, were collected from the locality near the Chengalpattu, Tamil Nadu, India.

Sample Preparation for Phytochemical analysis

Healthy leaf samples were washed thoroughly 2-3 times with running tap water and followed by sterile water, shade-dried and ground into uniform powder, extracted in distilled water and organic solvents like ethyl acetate and methanol (1:2 w/v) for 12 hr. The extract was filtered using Whatman No. 1 filter paper and used for the screening of phytochemicals. Qualitative tests were carried out using solvent extract and powdered sample according to standard procedures for identification of major secondary metabolites such as alkaloids, glycosoides, flavonoids, tannins, phlobatannins, saponins and terpenoids.[18-20]

Preparation for water and solvent extractions

Fifty grams of shade dried, leaf powder of *W. tinctoria* was extracted in 200 ml of each hexane, ethyl acetate, chloroform, methanol and water using a Soxhlet extractor for 48 hours. The collected extracts were concentrated using rotary flash evaporator and stored in refrigerator at 4° C for further use.

Plant Pathogens

Pure isolates of phytopathogenic bacteria, *Erwinia* sp. MTCC 2760, *Xanthomonas campestris* MTCC 2286, *Xanthomonas citri* and *Xanthomonas oryzae* pv. *oryzae* MTCC 5156 were obtained from Institute of Microbial Technology, Chandigarh, India.

Antibacterial activity assay

Antibacterial activity of water and solvent extracts were determined by Disc diffusion method. Leaf powdered extract was added with 5% Dimethyl Sulfoxide (DMSO) and 100 µg of each was loaded on to sterile disc (Himedia) and placed assay plates containing on Muller Hinton Agar spread with over night grown bacterial pathogens.[21] Inhibition zones around the discs were measured after 24h. Discs loaded with 5% DMSO served as negative control and Ampicillin, a synthetic antibiotic served as positive control. Triplicates were maintained and the results were expressed in mean ± standard error, using statistical software SPSS 10.1.

MIC assay

Minimum Inhibitory Concentration was determined by the broth microdilution method.[22-24] All tests were performed in Mueller Hinton agar broth (Himedia). The concentrations of maximum active leaf extract of *W. tinctoria* was dissolved in water + DMSO (95: 5) and serial double dilutions were prepared that were added to a 96-well microtiter plate over the range of 7 – 3,125 µg/ml. Overnight broth cultures of each strain were prepared and the final concentration of the microbe in each well was adjusted to 2×10^3 cfu/mL. Plates were incubated at 37° C for 24 h. The MIC was determined by reading the absorbance of each well using an automatic ELISA tray reader adjusted at 630nm (SLT Spectra). The samples were analyzed in duplicate and the assay was repeated twice. The wells showing complete absence of growth were identified and 10 µL of each well were transferred to Mueller Hinton agar plates and incubated at previously mentioned times and temperatures. Values are expressed as mean ± standard error and statistical significance was set at $P < 0.05$.

RESULTS

Phytochemical Screening

The preliminary phytochemical screening indicated ethyl acetate, methanol and water leaf extracts of *Wrightia tinctoria* showed the presence of bioactive constituents such as alkaloids, terpenoids, glycosides, flavanoids, saponin and phlobatannin (*Table 1*). These substances are said to be responsible for the antimicrobial activity of this plant.

Antibacterial activity of leaf extract of *W. tinctoria*

The result of the antimicrobial screening crude leaf extracts is shown in the *Table 2*. The maximum inhibitory activity was recorded in methanolic extract followed by ethyl acetate extract against the growth of all tested gram-negative plant pathogenic bacteria such as *Erwinia* sp., *Xanthomonas campestris*, *X. citri* and *X. oryzae*. Hexane and water extracts

Table 1: Phytochemical analysis of different solvent leaf extracts of Wrightia tinctoria

Phytochemicals	Ethyl acetate	Methanol	Water
Alkaloids	+	+	–
Terpenoids	+	+	+
Glycosides	+	+	+
Flavanoids	+	+	+
Saponin	+	+	+
Tannin	–	–	–
Phlobatannin	+	+	+

(+); Present, (–); Absent

Table 2: Antibacterial activity of Wrightia tinctoria against the plant pathogenic bacteria

Plant pathogen	Zone of inhibition* (mm in diameter)						
	Hexane	Ethyl Acetate	Chloroform	Methanol	Water	Control (5% DMSO)	Ampicilin
Erwinia sp.	0.00 ± 0.00	26.59 ± 0.00	0.00 ± 0.00	29.59 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.33 ± 0.69
Xanthomonas campestris	0.00 ± 0.00	24.00 ± 0.59	0.00 ± 0.00	25.67 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	12.33 ± 0.33
Xanthomonas citri	0.00 ± 0.00	8.00 ± 0.33	0.00 ± 0.00	8.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	12.69 ± 0.69
Xanthomonas oryzae	0.00 ± 0.00	10.00 ± 0.00	8.33 ± 0.33	18.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00

*Disc potency 100 µg.
Mean ± Standard error; Standard deviation $P \leq 0.05$

did not show activity. The commercial antibiotic ampicilin used as positive control also showed activity against all the tested plant pathogens.

Minimum Inhibitory Concentration (MIC) of the leaf extract of *W. tinctoria*

Minimum Inhibitory Concentrations of the methanol and ethyl acetate leaf extracts showed maximum activity against all the four pathogens are depicted in *Table 3*. The MIC of the leaf extracts studied ranged between 50 µg/ml and 180 µg/ml. Minimum concentrations of ethyl acetate and methanol extracts were effective in controlling the growth of *Erwinia* sp and *Xanthomonas campestris*.

DISCUSSION AND CONCLUSION

Natural products are considered an important source of new antibacterial agents.[25] Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development.[26] Recently, researches have attempted in the screening of diverse plants for the antimicrobial potential in the management of disease caused by various phytopathogens.[27-31] These evidences contributes to support and quantify the importance of screening natural products for exploitation of naturally available chemicals from plants, which would be an effective and eco-friendly approach for plant protection. It will also have a prominent role in the development of future commercial pesticides.[32]

Table 3: MIC of Wrightia tinctoria against the plant pathogenic bacteria

Pathogens	Extracts	MIC (µg/ml)[a]	Ampicilin MIC (µg/ml)[a]
Erwinia sp.	Ethyl acetate	50	170
	Methanol	50	
Xanthomonas campestris	Ethyl acetate	50	160
	Methanol	50	
Xanthomonas citri	Ethyl acetate	180	160
	Methanol	180	
Xanthomonas oryzae	Ethyl acetate	170	170
	Methanol	75	
	Chloroform	180	

[a] Values are the mean of 3 replicates using 1×10^3 cells of each culture

In the present study, results show that antibacterial activity of methanol and ethyl acetate leaf extracts exhibited more inhibitory activity indicating the effective extraction than the other extracts. The absence of antibacterial activity in the Hexane and aqueous extracts indicates the insolubility of the active substances in these solvents.

In conclusion, the present investigation forms a good basis in the selection of *W. tinctoria* for further TLC separation and characterization of bioactive substances. This plant could be successfully exploited for management of the diseases of plant pathogenic bacteria preventing considerable losses in yield and quality in an eco-friendly way for commercially important crops.

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Analgesic and Anti-inflammatory Activities of the Essential oil from *Cymbopogon flexuosus*

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ABSTRACT

Cymbopogon flexuosus (D.C) Stapf.(Graminae) commonly known as lemon grass is used as indigenous medicine. Externally applied, it forms an excellent embrocation in chronic rheumatism, neuralgia and other painful affections. Hence The essential oil from leaves of *Cymbopogon flexuosus* (D.C) Stapf (Graminae) was subjected to analgesic and anti-inflammatory screening using various animal models. The anti-inflammatory activity was studied using acute or chronic treatment in rats. Analgesic effect of the essential oil was evaluated in acetic acid-induced writhing and tail flick model.The essential oil exhibited significant anti-inflammatory activities in the acute carrageenan-induced rat paw edema and the chronic granuloma pouch models. However, it was devoid of analgesic activity in tail flick model.On the basis of these findings, it may be inferred that *Cymbopogon flexuosus* is an anti-inflammatory and analgesic agent and the results are in agreement with its traditional use.

Key words: Carrageenan, writhing, aspirin

INTRODUCTION

Cymbopogon flexuosus (D.C) Stapf.(Graminae) commonly known as lemon grass is used as indigenous medicine. The grass is laxative appetizer, aphrodisiac, anthelmintic and antiepileptic. Externally applied, it forms an excellent embrocation in chronic rheumatism, neuralgia and other painful affections. This is also said to be a good cure for fever.[1] Commercially important essential oils derived from these grass are: East Indian lemon grass oil obtained from Cymbopogon flexuosus (D.C) Stapf and West Indian lemon grass oil obtained from *Cymbopogon citratus* (D.C) Stapf.[2] Citral and myrcene have been reported from the essential oil.[3] To our knowledge, no previous studies have been undertaken on analgesic and anti-inflammatory activities of *C. flexuosus*. In the present investigation, essential oil from the leaves of *C. flexuosus* was used for analgesic and anti-inflammatory activity studies.

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MATERIALS AND METHODS

Plant material

The leaves of *C. flexuosus* were collected from Udupi district. They were identified by Dr. K. Gopalkrishna Bhat by comparision with standard specimens deposited at the Department of Botany, Poorna Prajna College, Udupi. A voucher specimen was deposited in NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore, India. Fresh leaves were subjected to steam distillation. 0.8% of essential oil is obtained. The essential oil was maintained protected from light and heat until use. This essential oil was subjected to pharmacological screening.

Experimental animals

Swiss albino mice and albino rats (HA strain) of either sex weighing 20 – 25 g and 100 – 150 g, respectively, were obtained from animal colony of NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore, India. They were housed in polypropylene cages in an air conditioned area at 25+/–2° C with 10:14 h light and dark cycle, and maintained on Amrut brand balanced animal feed and water ad libitum. In all experiment sets, 6 rats and 10 mice were used for each treatment.

LD_{50} studies

Mice were treated in graded doses up to 2000 mg/kg per oral (p.o) and were observed for any behavioral changes or mortality up to 7 days. There were few changes in the behavioral response like alertness, touch and restlessness. Therefore 1/10th of maximum tolerated dose i.e 200 mg/kg body weight was chosen for the studies.

ANALGESIC ACTIVITY

Acetic acid induced writhings

Male mice were injected intraperitonially with 1 ml/kg of 3% aqueous acetic acid 30 min after oral administration of essential oil (50, 100, 200 mg/kg) or aspirin (50 mg/kg) orally to various groups of mice. The number of writhing episodes of individual mouse were recorded for 30 min after acetic acid treatment.

Tail flick

Male mice were administered orally with 50 or 200 mg/kg doses of essential oil or aspirin (50 mg/kg) to various groups of mice. The mouse was held firmly to immerse its tail in a water bath maintained at the constant temperature of 58° C. The time required for the typical reaction, a violent jerk of the tail, was recorded to assess response to noxious stimulus.[4]

Antiinflammatory activity

This activity was studied using acute or chronic treatment in rats.

Acute. Carrageenan-induced rat paw edema

Essential oil was administered in 50, 100 or 200 mg/kg doses or diclofinac sodium (100 mg/kg), 0.5 h prior to Carrageenan subcutaneous (s.c) n the planter region of the rat hind paw to induce inflammation.[5] The paw volume was measured initially and 1, 2, 3, 4 and 5 h after Carrageenan injected by the plethysmographic method.[6]

Chronic. Cotton pellet-induced granuloma in rats

Four sterilized cotton pellets, each weighing 10 mg, were implanted s.c., one in each axilla and groin in an anoesthetized rat, using the method.[7] After treatment with essential oil at 10 or 50 mg/kg or diclofinac sodium (5 mg/kg) for 7 days, the rats were sacrificed next day. The pellets were dissected out and granuloma was dried at 60° C overnight to determine the dry weight.

Statistical Analysis

The statistical analysis was carried out to calculate mean (SEM). Further analysis was carried out by Student's *t*-test to calculate significance of results. *P* values >0.05 were considered as non-significant.

RESULTS

Acetic acid-induced writhing

There was significant reduction in acetic acid–induced writhing due to essential oil treatment at various doses and with aspirin pre-treatment as shown in Table 1.

Tail Flick

There was no effect on tail flick response due to the hot water-induced noxious stimuli after extract pre-treatment. However, this response was significantly altered due to Aspirin pre-treatment.

Carrageenan-induced Rat Paw Edema

The extract as well as diclofinac sodium showed antiphleogestic activity. This anti-inflammatory was dose dependent and significant at 3 and 4 h after carrageenan injection, as shown in Table 2.

Cotton pellet-induced granuloma in rats

There was statistically significant reduction in weight of granuloma in extract and diclofinac sodium treated rats as shown in Table 3.

Table 1: Effect of essential oil of *Cymbopogon flexuosus* leaves on acetic acid induced writhing in albino mice.

Pre-treatment		Mean number of writhing ± SEM
Control		83.1 ± 0.44
Essential oil	50 mg/kg	70.3 ± 0.51*
	100 mg/kg	41.3 ± 1.63*
	200 mg/kg	27.4 ± 1.25*
Aspirin	50m g/kg	22.9 ± 0.76

*$P < 0.05$ = Significant as compared to control

Table 2: Antiinflammatory activity of essential oil of *Cymbopogon flexuosus* in Carrageenan-induced rat paw edema.

Treatment		% Inflammation ± SEM at (hours)				
		1	2	3	4	5
Control		20.01 ± 1.14	31.71 ± 1.12	45.20 ± 1.13	60.21 ± 2.11	78.12 ± 2.14
Essential oil	50 mg/kg	14.03 ± 1.44	28.01 ± 2.12	33.89 ± 1.24*	41.11 ± 1.61*	64.12 ± 4.76*
	100 mg/kg	13.02 ± 0.70	25.15 ± 1.76	32.03 ± 1.55*	41.41 ± 3.13*	63.21 ± 5.53*
	200 mg/kg	12.55 ± 1.35	21.60 ± 1.33*	25.40 ± 1.65*	33.15 ± 1.66*	61.82 ± 3.17*
Diclofinac	100 mg/kg	14.50 ± 1.14*	23.75 ± 1.15	29.55 ± 2.35*	46.13 ± 3.28*	62.62 ± .12

*$P < 0.05$ = significant as compared to the control.

Table 3: Effect of essential oil of *Cymbopogon flexuosus* on cotton pellet-induced granuloma in albino in albino rats.

Treatment		Mean weight of granuloma in mg ± SEM
Control		36.02 ± 3.55
Extract	100 mg/kg	21.15 ± 0.44*
	200 mg/kg	17.16 ± 0.42*
	400 mg/kg	16.58 ± 1.77*

*$P < 0.05$ = Significant as compared to control.

DISCUSSION

LD_{50} of the was more than 2000 mg/kg body weight. Hence a dose of 50, 100 and 200 mg/kg body weight were chosen for the study.

The essential oil demonstrated significant reduction in acetic acid–induced writhing, however, there was no effect on tail flick response due to noxious stimuli. It is worthwhile to note that central nervous depressants, antihistaminics, are known to reduce the number of writhings, thus indicating above active relates to central nervous system depressant activity of essential oil. There was significant and dose-dependent anti-inflammatory activity of the extract in the acute carrageenan-induced rat paw edema model. Further, the essential oil also showed significant reduction in weight of granuloma after chronic treatment indicating activity of extract on granular tissue formation. It is worthwhile to investigate this important lead.

CONCLUSIONS

To conclude, the results showed anti-inflammatory and analgesic activities of essential oil of *Cymbopogon flexuosus*. These activities were related to dose and these results corroborate the traditional use of the plant in inflammatory conditions.

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Antidiabetic Activity and Phytochemical Screening of Crude Extract of *Stevia Rebaudiana* in Alloxan-induced Diabetiis Rats

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ABSTRACT

Results of phytochemical tests showed presence of different kinds of phyto-constituents in aqueous, ether and methanol extract of *Stevia rebaudiana* leaves. Daily single dose (2.0 g/kg) administration of aqueous extract (A.E.) , ether extract (E.E.) and methanol extract (M.E.) for 28 days of *S. rebaudiana* could not show any significant change in ALT and AST levels in rats. Blood sugar level was found to be decreased on day 28 in groups of rats treated with A.E., E.E. and M.E. of *S. rebaudiana*.

Key words: *Stevia rebaudiana*, Aqueous Extract, Ether Extract, Methanol Extract, Diabetes, Sub-acute toxicity, Rats

INTRODUCTION

Stevia rebaudiana Bertoni commonly known as sweet leaf is a perennial shrub and is a member of *Asteraceae* family. It is native to the valley of the Rio Monday in highlands of Paraguay, between 25 and 26 degrees south latitude where it grows in sandy soils near streams. Its medicinal use includes regulating blood sugar, preventing hypertension, treatment of skin disorder and prevention of tooth decay. It also possesses antibacterial and antiviral properties. Standerd extracts of *S. rebaudiana* are used as natural sweetner or dietry supplements in different countries for their content of stevioside or rebaudioside A. These compounds possess upto 250 times the sweetness intensity of sucrose and they are non-calorigenic.

The principles of *S.rebaudiana* is due to natural sweet active components present in the leaves that is stevioside and rebaudiosides A, B, C, D and E; Dulcoside A; and steviolbioside. Stevioside has a slight bitter aftertaste and provides 250 to 300 times the sweetness of sugar.[1] The sweet diterpenoid glycoside, rebaudioside F has been isolated from leaves and its structrure was established by chemical and spectral studies.[2,18]

In Japan, cultivation of stevia is done as an alternative to artficial sweeteners such as cyclamates saccharine which are suspected carcinogen. The plants leaves, the aqueous extract of leaves and purified steviosides are used as sweeteners. Japan currently consumes more stevia than any other country, with stevia accounting for 40% of the sweetener market. Today stevia is cultivated and used in food elsewhere in East Asia, including in China, Korea, Thailand and Malaysia. China is the world's largest exporter of stevioside.

In US stevia is mostly employed as sugar substitute. About ¼ teaspoon of the natural ground leaves is equivalent to 1 teaspoon of sugar. In South America, a standard infusion is sometimes used as a natural aid for diabetes and hypertension. The difference between stevia and sugar is that stevia does not cause tooth decay. It has been reported that stevia kills the bacteria *streptococcus mutans*, which is prime factor in caution of teeth plaque.

Active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in

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the treatment of diabetes mellitus. For diabetes treatment, before the discovery of insulin, the only options were those based on traditional practices.[15] Till today metformin is the only ethical drug approved for the treatment of non insulin dependent diabetes mellitus patients, which is derived from a medicinal plant *Galega officinalis*. Among those plants used traditionally for the treatment of diabetic complications is *Stevia rebaudiana Bertoni*.[8] Hence, the present experiment was undertaken to study the antidiabetic effect of *S. rebaudiana* in rats.

MATERIALS AND METHODS

Experimental animals

Rats of Wister strain (180-200 gm) of both sex and guinea pigs were used in this experiment after approval of the protocol by Institutional animal ethics committee. Rats were kept in cages (2-3 rats per cage) under standard laboratory conditions (light period of 12 hour per day and temperature 27 ± 2° C). They were fed standard pelleted feed and access to water *ad lib*. The rats were acclimatized to the animal house conditions. Prior to each study, the animals were made to fast for 12-14 hours but had free access to water.[14]

Plant materials

Fresh mature leaves of authenticated *S. rebaudiana* leaves were obtained from Directorate of Research, BAU, Kanke, Ranchi. It was air dried under shed at room temperature and finely powdered with the help of grinder. Leaves powders were always prepared fresh for aqueous, ether and methanolic extraction.

Preparation of extracts

(A) **Aqueous extract:** 50 gm of powdered leaves were kept in a beaker to which 250 ml of distilled water was added. The mixture was shaken properly and kept at room temperature for 24 hours. It was stirred 2-3 times a day. After 24 hours, mixture was filtered through ordinary filter paper and the filtrate was evaporated using rotary vaccum evaporator at 40-45° C. The extractability percentage was determined as per the method suggested by Rosenthaler.[16]

(B) **Ether extract:** 50 gm dried powdered leaves of *S. rebaudiana* was taken into thimble and 750 ml of petroleum ether was added into the soxhlet and boiled on the water bath. After 10-15 cycles it was decanted into the beaker and was evoporated using rotary vaccum evaporator at 40-45° C.

(C) **Methanol extract (ME):** 50 gm dried powdered leaves of *S. rebaudiana* was taken into thimble and 750 ml of methanol was taken into the flask of soxhlet apparatus and cycled 10-15 times. After that it was decanted into the beaker and was left open, so that the methanol evaporated using rotary vaccum evaporator at 40-45° C.

Preliminary phytochemical screening

Standard screening tests of three extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures.[10]

i. Test for alkaloids

a) **Preliminary test**: A 100 mg of an extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff's and Mayer's reagents. The treated solutions were observed for any precipitation.

b) **Confirmatory test**: Five grams of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated *in vacuo* to about 5 ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendroff's spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidence for presence of alkaloids.

ii. Test for steroidal compounds

a) **Salkowski's test**: 0.5 g of the extracts were dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

b) **Lieberman's test**: 0.5 g of the extracts were dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A colour change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of the cardiac glycosides.

iii. Test for phenolic compounds

a) To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green color was taken as positive.

b) The dried EE and ME extracts (100 mg) were dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

iv. Flavonoids

a) **Test for free flavonoids**: Five milliliters of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow colour in the organic layer which is taken as positive for free flavonoids.
b) **Lead acetate test**: To a solution of 0.5 g of the extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.
c) **Reaction with sodium hydroxide**: Dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The mixture was inspected for the production of yellow color which considered as positive test for flavonoids.

v. Test for saponins

Froth test: 0.5 g of the extracts were dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If a "honey comb" froth above the surface of liquid persists after 30 min. the sample is suspected to contain saponins.

vi. Test for tannins

a) **Ferric chloride test:** A portion of the extracts were dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.
b) **Formaldehyde test**: To a solution of about 0.5 g of the extract in 5ml water, 3 drops of formaldehyde and 6 drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 minute and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.
c) **Test for Phlobatannins**: Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as an evidence for the presence of phlobatannins.
d) **Modified iron complex test:** To a solution of 0.5 g of the plant extract in five milliliter of water a drop of 33% acetic acid and 1 g sodium potassium tartarate was added. The mixture was warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of colour is obtained and then boiled. Purple or blackish precipitates which is insoluble in hot water; alcohol or dilute ammonia denotes pyrogallol tannin present.

vii. Test for Anthraquinones

a) **Test for free anthraquinones (Borntrager's test)** The hydro - extracts of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.
b) **Test for O-anthraquinone glycosides (Modified Borntrager's test)**
For combined anthraquinones, 5 g of the plant extracts were boiled with 10 ml 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract

a. **Toxicity study**
   i. ***Acute oral toxicity***

The acute oral toxicity studies of all the three extracts were undertaken as per the Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals by Up-and-Down Procedure. The rats were fasted overnight and the weight of each rat used was recorded just before use. Animals were divided randomly into a control and three treatment groups for each extract, each group consisting of four mice (2 male and 2 female). Control group received only the vehicle and each treatment group received orally the EE, ME and AE of the studied plant in the limit test @ 2000 $mg.kg^{-1}$ body weight was conducted and terminated after 4 survivals out of 4 animals.

Again a higher dose of 5000 mg/kg of all extracts were given to three groups of rats. Animals were kept under close observation for 4 hours after administering the extracts,[3] and then they were observed daily for three days for any change in general behaviour and other physical activities

ii. ***Subacute toxicity***

Subacute toxicity of A.E., E.E. and M.E. of *S. rebaudiana* leaves was studied in albino rats of either se × ($n = 24$). Rats were divided randomly into four groups. Group I ($n = 6$) served as control and the other three groups were used as experimental groups. Group II, A.E. ($n = 6$) III, E.E. ($n = 6$) and IV, M.E. ($n = 6$) were given 2g/kg, i.p. of *S. rebaudiana* leaves per day for four weeks. The blood samples were collected on day 0, 14th and 28th by heart puncture after anaesthetizing the rats by ethyl alcohol. The biochemical parameters (ALT and AST) were measured by kit supplied by ERBA chemicals on semiauto-analyzer.

b. **Antidiabetic effect**

Diabetes in rats was induced by a single dose of 5% alloxan monohydrate (125 mg/kg, i.p.) after 24 hour fasting. Induction of diabetes was confirmed after a week of alloxan treatment by estimation of fasting blood glucose level.Only those rats with blood glucose level between 200-300 mg/ dl were included in the study. These rats were further divided into seven groups (I- non diabetic control, II- diabetic control, III, IV, V, VI & VII) of six rats each. Group III, IV & V were sub-grouped (IIIA, IIIB, IVA, IVB, VA, VB). The group I & II (control) received comparable volume of NSS. Group III, IV, V received lower and higher daily doses of A.E., E.E. and M.E. @ 50 and 100 mg/kg p.o. respectively once daily for four weeks. VIth group was administered hypoglycemic drug glibenclamide (5 mg/kg, p.o.) once daily for 4 weeks and group VII was administered daily dose of glibenclamide (50 mg/kg) and 100 mg/kg AE, p.o. respectively. The blood glucose levels were measured by glucometer on day 0, 1, 5, 7, 14 and 28. The blood samples were collected from tail vein puncture and blood glucose levels were analysed.

RESULTS AND DISCUSSION

Phytochemical studies

In order to determine the presence of chemical constituents, phytochemical teste were performed which revealed the presence of phytoconstituents in aqueous, ether and methanol extracts (Table 1), which is in consonance with the report of.[7]

Acute toxicity (Determination of ALD_{50})

A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. Acute toxicity studies conducted revealed that the administration of graded doses of three crude aqueous,ether and methanol extracts (up to a dose of 5000 mg/kg) of *S.rebaudiana* did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 5 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 5 g/kg body weight in mice. Search for the available literature revealed the non-toxic effect of the leaves of *S. rebaudiana* in mice.[7]

Table 3 shows the mean concentration of alanine transaminase and aspartate transaminase. ALT was estimated

Table 1: Results of phytochemical screening of the extracts of *S. rebaudian* Leaves

Tests	Reagents	A.E	E.E	M.M
Test for alkaloids	Dragendroff's	++	–	–
	Mayer's	++	–	–
Test for steroidal	Acetic anhydride and	+++	+	+
Compounds	conc. sulfuric acid Chloroform and conc. Sulfuric acid	+++	++	++
Test for phenolic	Ferric chloride and	+	++	++
Compounds	potassium ferrocyanide 10 % lead acetate	+	++	++
Test for flavonoids	sodium hydroxide	++	+	+
Test for saponins	Froth Test	trace	+	+
	Ferric chloride	trace	+	+
	Aqueous hydrochloric acid	trace	+	trace
Test for tannins	Formal dehyde	trace	+	–
	Modified iron complex	trace	+	–
Test for anthraquinones	Test for free anthraquinones	–	–	–
	Test for o-anthraquinone	–	–	–
	Glycosides			

+++; Highly positive.
++; Moderately positive
–; negative

Table 2: Effect of treatment (mean ± S.E.) of *S. rebaudiana* extracts on blood glucose level in alloxan induced diabetic rats

Treatment	Dose mg/kg	n	Day 0	Day 1	Day 5	Day 7	Day 14	Day 28
Normal control(I)	–	6	90.83 ± 3.56^{a}	89.00 ± 3.64^{a}	83.33 ± 2.40^{a}	85.66 ± 5.45^{a}	77.66 ± 3.42^{a}	82.00 ± 3.18^{a}
Diabetic control(II)	–	3	214.5 ± 2.61^{bc}	219.16 ± 2.53^{bc}	224.66 ± 2.18^{de}	230.66 ± 3.39^{e}	234.33 ± 4.11^{f}	239.33 ± 3.59^{e}
A.E.(III)	A) 100	3	220.16 ± 8.63^{bc}	217.16 ± 9.16^{bc}	213.83 ± 10.24^{cde}	212.00 ± 10.21^{cde}	193.50 ± 6.73^{de}	137.66 ± 4.12^{b}
	B) 50	3	220.00 ± 11.20^{bc}	219.50 ± 11.93^{bc}	218.66 ± 11.27^{cde}	216.50 ± 11.29^{de}	209.83 ± 11.89^{de}	161.83 ± 7.72^{c}
E.E.(IV)	A) 100	3	209.66 ± 4.15^{b}	209.50 ± 4.11^{bc}	206.50 ± 3.92^{cd}	230.33 ± 3.64^{cd}	197.33 ± 3.42^{de}	168.00 ± 5.50^{cd}
	B) 50	3	220.83 ± 9.24^{bc}	221.16 ± 9.16^{c}	219.83 ± 9.14--	216.83 ± 9.15--	203.16 ± 8.58^{ef}	171.83 ± 3.04^{d}
M.E.(V)	A) 100	3	218.66 ± 4.93^{bc}	218.33 ± 4.83^{bc}	217.50 ± 4.85^{cde}	216.83 ± 3.15^{de}	202.00 ± 2.20^{de}	181.16 ± 3.55^{cd}
	B) 50	3	232.00 ± 11.81^{c}	231.50 ± 11.96^{bc}	230.50 ± 11.82^{e}	226.83 ± 11.32^{de}	210.33 ± 8.87^{de}	163.33 ± 13.35^{c}
Glibenclamide(VI)	5	3	211.00 ± 5.10^{bc}	192.16 ± 6.93^{b}	124.83 ± 1.19^{b}	119.33 ± 0.33^{b}	108.16 ± 30.2^{b}	101.83 ± 0.30^{a}
Glibenclamide + A.E.(VII)	5+100	3	208.16 ± 9.23^{bc}	205.83 ± 8.81^{bc}	200.50 ± 8.15^{c}	193.00 ± 6.70^{c}	154.66 ± 4.72^{c}	100.50 ± 0.22^{a}

Table 3: Effect of A.E, E.E. and M.E.(2.0g/kg,oral) of *S. rebaudiana* on serum enzyme activity (Mean ± S. E.) of albino rats after once daily administration for 28 days

Days	ALT (Unit/ml)				AST (Unit/ml)			
	C	A.E.	E.E.	M.E.	C	A.E.	E.E.	M.E.
0	40.33 ± 1.43^{NS}	40.00 ± 1.40^{NS}	40.33 ± 1.39^{NS}	40.21 ± 1.23^{NS}	20.25 ± 1.56^{NS}	20.15 ± 1.33^{NS}	20.26 ± 1.35^{NS}	20.75 ± 1.15^{NS}
14	40.25 ± 1.50^{NS}	39.50 ± 0.97^{NS}	40.12 ± 1.10^{NS}	40.25 ± 1.22^{NS}	20.11 ± 1.32^{NS}	20.33 ±0.95^{NS}	20.10 ± 1.20^{NS}	20.25 ± 1.10^{NS}
28	40.15 ± 1.65^{NS}	40.75 ± 1.42^{NS}	39.75 ± 0.97^{NS}	40.15 ± 1.00^{NS}	20.25 ± 1.36^{NS}	20.12 ±1.00^{NS}	20.22 ± 1.11^{NS}	20.16 ± 1.10^{NS}

to be 40.00 ± 1.40, 40.33 ± 1.39, 40.21 ±1.23 unit/ml in A.E., E.E. and M.E. treated groups respectively. On 28th day it was found to be 40.75 ± 1.42, 39.75 ± 0.97 and 40.15 ± 1.00 unit/ml in all the three groups. No significant difference in the mean concentration was found.

The aspartate transaminase level was recorded as 20.15 ± 1.33, 20.26 ± 1.35, 20.75 ± 1.15 in A.E., E.E. and M.E. respectively. On day 28 the level of AST was recorded as 20.12 ± 1.00, 20.22 ± 1.11 and 20.16 ± 1.10 units/ml in AE, EE and ME respectively. Mean values did not show significant difference on day 0 and 28.

Anti-diabetic Effect

The blood glucose levels were 220.16 ± 8.63, 220.00 ± 11.20 in A.E., 209.66 ± 4.15, 220.83 ±09.24 in E.E., 218.66 ±4.93, 232.00 ± 11.81 mg/dl in M.E. treated rats on day 0 (Table 2). In glibenclamide treated group the blood glucose was 211.00 ± 5.10 mg/dl on day 0 where as in glibenclamide + A.E. treated rats the blood glucose level on day 0 was found to be 208.16 ± 9.23 mg/dl. It may be noted in the above table that a significant decrease in the mean blood glucose levels was found on day 28 in A.E., E.E. and M.E. treated rats, both after 50 and 100 mg/kg daily dose administration. The results obtained in this study for extracts of *S. rebaudiana* showed decrease in the mean blood glucose levels which were in agreement with the observations of Abdula *et al.*[1]

The study also showed that the rats which had been given the extracts of A.E. and E.E. at higher dose (100 mg/kg) exhibited greater decrease in mean blood glucose level as compared to those given @ 50 mg/kg b.w on day 28. Therefore, it is obvious from the results obtained in this study that anti-hyperglycemic activity of A.E. and E.E. were dose dependent. The findings obtained in this investigation are similar to that of Jeppesen *et al.*, 2000 who pointed that stevioside and steviol dose dependently enhanced insulin secretion. The data showed that there was significant decrease in the mean blood glucose level (100.50 ± 0.22) in the group VII where A.E. was given with glibenclamide. However it did not differ with the blood glucose level on day 28 (101.83 ± 0.30) as compared to that group which received only glibenclamide.Therefore, it is obvious that glibenclamide and A.E. both are working differently in rats.

The Stevia leaves powder has also been reported to reduce the blood glucose concentration of diabetic rats.The findings of this experiment are similar to the reports of Chang *et al.*[6] However, it was observed that as hypoglycemic drug, Glimepiride was better, though powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves @ 250 mg/kg body weight showed very potent hypoglycemic efficacy, but comparatively less effective than Glimepiride.It is known that sulphonylureas like Glimepiride, produce hypoglycemia by increasing the secretion of insulin from the pancreas and these compounds are active in mild Streptozotocin-induced diabetes whereas they are inactive in intense Streptozotocin diabetes (nearly all b-cells have been destroyed).[9,19] Since our results showed that

Glimepiride reduce the blood glucose levels in hyperglycemic animals, so it can be postulated that the state of diabetes was not severe. It may be mentioned that stevioside regulates blood glucose level by enhancing insulin secretion and also enhances glucose utilization in peripheral tissues and muscles in diabetic rats.[5]

It was concluded that the extracts of *Stevioside rebaudiana* could decrease the blood glucose level in diabetic rats in time dependent manner. The antidiabetic effect might be due to steviosides counteracting the glucotoxicity in β cells or also by suppressing the glucagon secretion by α cell of pancreas, both the mechanisms have been depicted by Shibata *et al.*[17] and Chen *et al.*[4]

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Phyto-Pharmacology of *Caralluma Adscendens* Roxb: A Review

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ABSTRACT

Many herbal remedies have been employed in various medicinal systems for the treatment and management of various diseases. The plant *Caralluma adscendens* has been used in different system of traditional medication for the treatment of disease and aliments of human being. It is reported to contain various glycosides, flavonoids and steroids. It has been reported as an anti-inflammatory, antioxidant, anti-diabetic, analgesic, anti ulcers, antibacterial, hypoglycemic activities. There are also reports available for traditional uses of this plant for its dermatitis, anti-obesity, as a bloat, wound healing activities. Many isolated constituents from *Caralluma adscendens* lack the reports of pharmacological activities, which support its further pharmacological activities.

Key words: *Caralluma adscendens*, Phytochemistry, Pharmacological activities.

INTRODUCTION

Plants have played a significant role in maintaining human health. Herbal medicine is based on the premise that plant contains natural substance that can promote health and alleviate illness. In recent times, focus on plant research has increased all over world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Herbal drugs or medicinal plants, their extracts and their isolated compound have demonstrated biological activities. Such have been used and continued to be used as medicine in folklore for various disorders. Ethno pharmacological studies on such herbs important plant continued to interest investigators throughout the world.

Herbal drugs have been used since ancient times as medicines for the treatment of range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants have become the focus of intense study In terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore.[1] On such plant, *Caralluma adscendens* invites attention of the researcher worldwide for its pharmacological activities ranging from anti-inflammatory to anticancer activities. *Caralluma adscendens* Roxb. Belongs to family Asclepiadaceae.[2]

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Synonym: - *Caralluma fimbriata,* Comman Name: - Ranshbar, Maked Shenguli, Shindala Makadi, Vernacular Name**:** Q Kullee moofiyan, Kallimudayan (Tamil), KaraIlamu (Telegu), Yugmaphallottatna (Sanskrit), shindala makadi (Marathi).[1]

This plant grows throughout India, in deciduous and hilly areas. The latex cell usually contains latex rich in triterpines, other constituents includes alkaloids of Indole, Phenanthrine, Indozolidine, Glycosides, Saponin, Tannins. Many members are used in folk medicine in their countries.[3]

Is a variable herb, up to 1m in height with fleshy, almost leafless stem, deep purple, brown or yellowish white flower and 10-20 cm long slender follicles, distributed in India from Andhra Pradesh up to 600 m, a few varieties have been reported. The herb is consumed as a vegetable and also made into a pickle. The herb contains hydrocarbons N- pentatriacontane and glycosides.[4] The genus Caralluma

(Asclipiadaceae), which are comprises about 200 genera and 2500 species. The member of the genus is small plant, erect, fleshy. They have four grooved stems, round shape devoid of leaves and small flowers in several varieties of dark Colors. The species of Caralluma found in India are edible and form part of the traditional medicine system of the country.[5] The key phytochemical constituents of the herb are pregnane glycoside (25%), Flavone glycoside (chemotaxonomic marker), Saponin glycoside (10%), and Megastamine glycoside, Bitters (3%), Sitosterol and Tomentogenin.[6-7]

TRADITIONAL USES

Wild plant species *Caralluma adscendens* (Kundaetikommulu) used as food by tribal people by Andhra Pradesh. For chutney purpose.[10] A few varieties have been reported that herb is consumed as a vegetable and also made into a pickle.[2, 4]

In addition to Caralluma species commonly used in treatment of rheumatism, diabetes, leprosy, antipyretic and anthelmintic, for tumor, fungal diseases, snake, scorpion bite and antinociceptive activity.[11] Caralluma species have shown anti-inflammatory, anti-nociceptive,[12] antidiabetic,[13-14] gastric mucosa protecting, anti ulcer and cytoprotective properties. The species of Caralluma found in India are edible, their medicinal properties includes anti-inflammatory, anti-nociceptive, antioxidant, antiulcer, ant diabetic, carminative, antipyretic. Caralluma extract have also found to be appetite suppressant a properties which is well known to Indian tribals and hunters. Indian folklore record their records used as a potent appetite suppressant and weight loss promoter. The *Caralluma fimbriata* extract, in the form of capsules has been released under trade name GENASLIM for body weight control.[15-16] Bloat: Farmers believe that feeding leaves of *Caralluma adscendens* R. Br. (*muyal kathu, muyal, kurabu*) in odd numbers. i.e. 3, 5, 7 or 9, can relieve bloat. Treating Mastitis: The farmers in the study locale adopted the mixture of paste from ghee and leaves of Caralluma species to cure mastitis. It was found that 68% of the scientists could neither say the practice was valid nor invalid. The validity score of 81 was secured by the practice. Few scientists agreed upon the fact that the contents may have anti-inflammatory effect.[17] The plant is eaten as Vegetables, throughout India.[18] *Caralluma adscendens* (periyasirmankeerai) belongs to family Asclepiadaceae, wild edible plants used by palliyars of western ghat.[19]

Table 1: Taxonomic Classification of *Caralluma adscendens* Roxb.[8-9]

Taxonomy	Caralluma adscendens
Domain	Eukaryota
Kingdom	Plantae
Sub kingdom	Viridaeplantae
Phylum	Magnoliophyta
Subphylum	Spermatophytina
Infraphylum	Angiospermae
Class	Magnolliosida
Subclass	Lamiidae
Super order	Gentiananae
Order	Gentianales
Family	Asclepiadaceae
Genus	Caralluma
Specific epithet	adscendens
Variety	Gracilis
Botanical name	*Caralluma adscendens* var. *gracilis*

PHYTOCHEMICAL STUDY

In 2008, the Eleven novel pregnane glycosides, 2-7 and 9-13, of which four, i.e., 10-13, comprised a new pregnane- type genin exhibiting a hydroxymethylene instead of a Me group at C (19,) and the Known pregnane glycoside stalagmoside V were isolated from whole plants of *Caralluma adscendens* var. *fimbriata,* a native Indian succulent plant. Their structures were elucidated by extensive 2D-NMR spectroscopic studies.[20]

In 1982, the pentatriacontane which is the saturated hydrocarbon isolated from *Caralluma fimbriata* and confirmed it by its spectral data and elemental analysis.[21]

In 1976, dry plant material from *Caralluma fimbriata* was extracted with petroleum ether, benzene, ethanol; Pet ether extract gave a waxy solid which on spectral analysis. The benzene extract gives three comp. After chromatography a glycoside was partially identified.[22-23]

In1999, flavone glycoside isolated from three Caralluma species.[24]

In 2009, Phytochemical studies of botanicals: Hoodia gordonii and *Caralluma* species. They describe a phytochemical study of a species of Caralluma genus. *Caralluma adscendens* var. *fimbriata*, which is also known as "Indian Hoodia", Phytochemical investigation of a commercially available Caralluma sample was undertaken in order to generate better understanding of the chemical constitution of this species and to develop marker constituents for development of quality control methods. The chromatographic separation of methanolic extract of Caralluma species resulted in isolation of one new pregnane glycoside (Carallumoside A) and seven known steroid derivatives namely, Caraumbellogenin, Carallumoside B, boucerin, Caraumbelloside I, II, and III, and boerharigenin B. Most of the known compounds were previously reported from other species of Caralluma genus.[25]

In 1983, Qualitative chemical test was performed for the presence of different class of constituents in *Caralluma adscendens* plant extracts; these include alkaloids, flavonoids, saponins, tannins, etc.[26]

In 2002, the key phytochemical constituents and potentially active molecules in *Caralluma fimbriata* include pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, saponins and various flavanoids.[27]

In 2007, Pregnanes and Pregnane Glycosides isolated from *Caralluma fimbriata* shows Appetite Suppressant Dietary Supplement. Plant reported to be rich in steroidal glycosides. Several other species of *Caralluma* have been reported to contain a wide range of pregnane glycosides; however there has not been any phytochemical study on *C. fimbriata* till today. As a part of our ongoing phytochemical investigation, we have isolated pregnanes and pregnane glycosides from *C. fimbriata*. Isolation and structure elucidation of these compounds using various spectroscopic techniques (HR-MS, FT-IR, 1D and 2D NMR).[28]

In 2010, *Caralluma fimbriata* (Slimaluma®) is an edible succulent cactus that belongs to the family Asclepiadaceae. Its key ingredients are pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, saponins and various other flavonoids. The appetite suppressant action of *C. fimbriata* could be mainly attributed to the pregnane glycosides. These compounds seem to have peripheral and central effects. In the adipose tissue, pregnane glycosides reduce lipogenesis.[29]

In 2006, an oxypregnane steroidal glycoside, known as P57, is the only reported active constituent from hoodia. Extract of Hoodia gordoni (*Caralluma fimbriata*) is one of the most popular herbal supplements claimed to possess appetite suppressant properties.[30]

PHARMACOLOGICAL STUDY

Antibacterial activity

In 2003 Tatiya A.U. et al observed Antibacterial activity of various extracts of stem of *Caralluma adscendens* was studied against B. Pumilus, E. coli, and S. aureus. Pet ether extracts shows antibacterial activity.[31]

Anti-inflammatory activity

In 2009 D.R. Jadge et al observed screening of *Caralluma adscendens* for Anti-inflammatory activities. Anti-inflammatory activity of aqueous and ethanolic extracts of whole plant of *Caralluma adscendens* in rats. The anti inflammatory activity was evaluated by using Digital Plethysmometer. The study was carried out by using dose of 250 mg /kg of ethanolic and aqueous extract orally. All extract showed significant activity for all dose as compared to diclofenac sodium.[11]

Hypoglycemic activity

In 2009 Mali K et al observed screening of *Caralluma adscendens* for hypoglycemic activity.[32]

Antioxidant and Hypolipidemic activity

In 2010 Tatiya A.U. observed various extracts of *Caralluma adscendens* were evaluated by alloxan induced (150 mg kg-1 i.p.) diabetic rats and oral glucose tolerance test. Fasting blood glucose estimation was done at 0, 30, 90 and 150 min after treatment. Lipid profile and body weight measurements were done on day 0, 7, 14 of the study. Antioxidant effects were also evaluated using Diphenyl-1-Picrylhydrazyl (DPPH), *in vitro* lipid peroxidation and reductive ability methods. The treatment showed significant lowering of blood glucose in the treated diabetic rat from 273.1±4.01 to 82.1±1.4* mg dL-1 by butanolic extract of *C. adscendens*, 150 min after the treatment *(*P < 0.01)*. It also showed significant decrease in total cholesterol, LDL, triglyceride and TC /HDL and an increase in HDL in the treated diabetic animal group. Glucose tolerance was also improved. *In vitro* antioxidant activity showed that the butanolic extract exhibited potent free radical scavenging effects. All the results were compared with standard drug Glibenclamide.[33]

Effect of Caralluma Fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women.

In 2007 Rebecca Kuriyana et al observed Effect of *Caralluma Fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women. Waist circumference and hunger levels over the observation period showed a significant decline in the experimental group when compared to the placebo group. While there was a trend towards a greater decrease in body weight, body mass index, hip circumference, body fat and energy intake between assessment time points in the experimental group, these were not significantly different between experimental and placebo groups. Caralluma extract appears to suppress appetite, and reduce waist circumference when compared to placebo over a 2 month period.[34]

Clinical trial Research

In 2006 A. Jagtap et al observed Toxicological evaluation of *Caralluma Fimbriata* extract in wistar rats. Assessment of acute and sub chronic oral toxicity and mutagenicity of hydro alcoholic extract of *Caralluma fimbriata* (CFE). Acute and sub chronic oral toxicity studies were performed in accordance with OECD guideline 423 and 407 respectively. The Salmonella typhimurium reverse mutation test (Ames test) was performed in accordance with OECD guideline.[35]

Report on the Safety of Caralluma fimbriata and its Extract

A famine food – suppressing appetite and quenching thirst. Legend has it that hunting tribes chewed chunks of the Caralluma cactus to suppress hunger and thirst when on a long hunt. Most importantly to determine safety, there are

no adverse event reports on the Indian subcontinent over the centuries of use.[36] *Caralluma fimbriata* is listed as a vegetable in The Wealth of India, the Indian Health Ministry's comprehensive compilation on medicinal plants.[2] Key phytochemical ingredients include pregnane glycosides,[37-38] flavone glycosides,[39] megastigmane glycoside,[40] bitter principles, saponins, various flavonoids.[41]

Proposed Mechanisms of Action for Weight Reduction and Safety

It is postulated that the pregnane glycosides and perhaps other constituents in *Caralluma fimbriata* prevent fat accumulation via blocking citrate lyase. This would be similar to the mechanisms proposed for another product from India, *Garcinia cambogia.*[42] This is important for two reasons. In addition, clues as to how *Caralluma fimbriata* works to reduce weight may emanate from our knowledge of *Garcinia cambogia*. The active component in *Garcinia cambogia* is hydroxycitrate (HCA),[42] and HCA has been reported to cause weight loss in humans without stimulating the central nervous system.[43] Because it is a competitive inhibitor of ATP-citrate lyase, an extra mitochondrial enzyme involved in the initial steps of *de novo* lipogenesis.[42] Consequently, HCA reduces the transformation of citrate into acetyl coenzyme A, a step necessary for the formation of fatty acids in the liver. In addition to its effect of citrate lyase, the postulated blocking of malonyl Coenzyme A by *Caralluma fimbriata* could further lead to a decrease in fat formation in the metabolic pathway. Again similar to *Garcinia cambogia,*[42] *Caralluma fimbriata* is reported to suppress appetite hypothesized to be secondary to effects on the appetite control center of the brain. HC has been demonstrated to reduce food intake in animals suggesting its role in the treatment of obesity and has been demonstrated to increase the availability of serotonin in isolated rat brain cortex that could affect satiety.[44-49] More specifically, it is believed that the pregnane glycosides in *Caralluma fimbriata* inhibit the hunger sensory mechanisms of the hypothalamus.

GENERAL STUDY

In vitro propagation of pharmaceutically valuable varieties of Caralluma adscendens from nodal explants

In 2009 Aruna V et al observed a procedure for *in vitro* propagation of pharmaceutically valuable varieties of *Caralluma adscendens* from nodal explants is described. The highest shoot multiplication with 80% frequency was achieved within one month on Murashige and Skoog's medium supplemented with 8.87 μM BA. Shoot multiplication occurred in subsequent subcultures in culture bottles on MS medium. Regenerated shoots were rooted on half strength MS medium supplemented with NAA (0.54 μM) in all the three varieties.[50]

Appetite suppressant

In 2006 Mary Shomon et al observed Caralluma for Weight Loss Indian Cactus is Promising Appetite Suppressant and Diet Aid. *Caralluma fimbriata* is a succulent plant, in the cactus family, that has been used as a natural appetite suppressant in India for centuries. It's another new arrival in the family of various cactii that are being used for their appetite suppressant, blood sugar lowering, and weight loss properties, much like the increasingly popular hoodia gordonii from the Kalahari Desert in Africa.[51]

In 2007 Jen Cully et al observed *Caralluma fimbriata* is the newest all- natural appetite suppressant to the global market and Millennium Health supplement is thrilled to add this great new product to its line of bulk ingredients.[52]

Other Actions of Caralluma

In folklore medicine, plants of the Caralluma species have been used to treat diabetes. In a study using streptozotocin diabetic mice, acute or sub acute treatment with *C. Arabica* caused a statistically significant lowering of circulating blood glucose levels.[53] Streptozotocin-induced diabetes is a model for Type I diabetes mellitus. Accordingly, in these insulin deficient mice, the Caralluma species was able to lower blood glucose suggesting an "insulin-like" action, an increase in insulin release, and/or an ability to sensitize the animal to lesser amounts of insulin. However, one oddity was that in this particular study the glucose tolerance to a glucose challenge appeared better in the control animals even though baseline sugars were higher in control. Similar to *C. arabica*, extracts from C. *attenuata* were found to be antihyperglycemic in alloxan-diabetic rats.[54] Animal studies suggest that *C. arabica* extract is anti-nociceptive and anti-inflammatory.[55-56] Using the hot plate and writhing methods in albino mice and the tail-flick method in Wistar rats, the nociceptive properties of *C. arabica* were shown. This occurred when the extract was placed on the skin indicating transdermal absorption. The lesser accumulation of edema in the in paws injected with carageenan indicated anti-inflammatory properties as well. This property was localized to a specific pregnane glycoside from *C. umbellate,*[57] *C. Arabica* also has been shown to possess anti-gastric ulcer and cytoprotective properties against damage produced by phenybutazone, indomethacin, ethanol, sodium hydroxide, and/or cold restraint stress.[58] The protective effect was postulated to be via multi mechanisms, including increased gastric production of prostaglandins and mucin and reduced gastric acidity.

Overall View of Caralluma fimbriata and Extract

All current evidence points to the safety of *Caralluma fimbriata* extract at the recommended doses. Believe that *Caralluma*

fimbriata is safe to consume at recommended doses based on the following:

1. The cactus has been in the food chain of India for years and has not been associated with any significant adverse side effects.
2. *Caralluma fimbriata* is listed in the Wealth of India as a famine food and by various individuals on the internet as a safe-to-consume food
3. Various testimonials by doctors and scientists confirm to its safety.
4. Testimonial by individuals who regularly consume the product describes its safety.
5. The daily dose of the extract contains the same concentration of ingredients as commonly eaten daily in the raw vegetable
6. A study to determine LD50 did not disclose toxicity, and it was reported that the LD50 exceeded 5g/kg
7. Two clinical studies composed of 44 individuals consuming the extract failed to reveal any significant adverse events.

A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity[59]

Waist and hip circumference Caralluma fimbriata and CQ with or without IG significantly decreased waist size, Food intake: Decreases in appetite or amount of food or energy intake with a supplement containing ephedra and caffeine and *Caralluma fimbriata* were shown (not Significant) but hydroxycitric acid (HCA-SX) with or without Gymnema sylvestre decreased the amount of food intake efficiently. A natural compound containing Capsicum and other lipotropic nutrients did not significantly change energy intake.

CONCLUSION

Caralluma adscendens is commonly found in hilly areas throughout India. The plant is used as anti-inflammatory, analgesic, anti-oxidant, anti-pyretic, anti-diabetic. It is reported to contain glycosides, flavonoids, saponins, steroids, tannins etc. The pharmacological and clinical studies reported in the review confirm the therapeutic value of *Caralluma adscendens*. Less information available regarding the chemical constituents of this plant. There is lack of phytochemical and phytoanalytical studies of this plant with the availability of primary information, further studies can be carried out like phyto-pharmacology of different extract, identification and pharmacological studies of isolated compound.

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Peristrophe bicalyculata - A Review

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ABSTRACT

Peristrophe bicalyculata (Acanthaceae) is upto 60-180 cm in height and found almost throughout India, Afghanistan and Africa. It is commonly known as kali aghedi in Hindi and kakajangha in Sanskrit. The herb is used for its anti-bacterial property (tuberculostatic), snake poison, in bone fracture, sprain, fever, cold, cough and for ear and eye treatments. The chemical composition of the dried aerial parts showed 14-methyl-tritriacont-14-en-15-ol and 35-hydroxynonatriacontanal. In this review we have explored the detailed pharmacognostical study of stem and leaf as well as physicochemical parameters, phytochemical screening and leaf constant.

Key words: *Peristrophe bicalyculata*, Pharmacognostical study, Phytochemical screening

INTRODUCTION

Peristrophe bicalyculata (Acanthaceae) is upto 60-180 cm in height and found almost throughout India, Afghanistan and Africa. It is commonly known as kali aghedi in Hindi and kakajangha in Sanskrit. The herb is used for its anti-bacterial property (tuberculostatic), snake poison, in bone fracture and sprain. Leaf extract is used for fever, cold and cough. Mucilage medicines are used for ear and eye treatments.[1-7] The chemical composition of the dried aerial parts of *P. bicalyculata* reveals that is comprised of 14-methyl-tritriacont-14-en-15-ol and 35-hydroxynonatriacontanal (Singh et al., 2000). The essential oil shows tuberculostatic activity in vitro against the growth of various strains of *Mycobacterium tuberculosis* (Chopra and Chopra, 1959).

COMMON NAME[1,2,8]

Hindi : Atrial, Itrelal masi, Nasbhanga
Bengal : Nasabhaga
Marathi : Ghati pitta papada, Rankirayat
Gujarati : Kali-adhedi, Kari-adhedi, Lisi-adhedi
Sanskrit : Kakajangha
Telugu : Chebira
Kan. : Cheebeegida, Cheebeera soppu
Santal : Bange khode baha, Bargekhodebaha
Mundari : Huring mara chuta, Luputian mara chuta
Bombay : Pitpara
Sind : Nazpat
Sinhalese : Mahanelu

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DISTRIBUTION[3,8,9]

Throughout in India in forest as undergrowth, hedges and wasteland, Afghanistan, Tropical Africa. Flowers and fruits: Oct-Apr. Flowered at the end of rains and died early in the dry season

DISCRIPTION

Macroscopy of plant (Figure 1)[1,8-11]

An erect, hispid herb or undershrub, 60-180 cm high, found in forest undergrowth, hedges and wasteland almost throughout India. Leaves: Ovate or elliptic ovate, appressed-hairy, acuminate, pubescent, densely lineolate, more or less hairy above, somewhat densely so on the nerves and veins beneath, base usually rounded, main nerves 4-6 pairs. Petioles: 6-15 cm long Stems and Branches: Usually 6- angled, more or less hairy, usually rough on the angles. Flowers: In trichotomous cymes in large lax divaricate pubescent panicles, rose, purple or pink, in lax panicles, panicles axillary and terminal, trichotomously branched. Bracts: Beneath the calyx 2, opposite, often very unequal, the longer of the pair sometimes 1 cm, the shorter 6 mm long, linear, acute, micronate with white membranous margins. Bracteoles: 4,

Figure 1: Plant of *Peristrophe bicalyculata*

similar to the bracts but shorter, subequal or sometimes unequal. Calyx: 3-4 mm, long, divided to within about 1.2 mm of the base. Segments are lanceolate- subulate with ciliolate margins. Corolla: Rosy, nearly 1.3 cm long, pubescent outside, upper lip 6 by 3 mm, elliptic oblong, obtuse, entire, lower lip slightly longer, oblong with 3 acute lobes about 1 mm deep. Androceium: Filament hairy, Anther one almost entirely above the other, muticous. Gynoecium: Ovary pubescent at the tip, Stlye mearly glabrous. Fruit: Capsules 0.8-1 cm long, pubiscent in upper half, shortly beaked, pointed, narrowed into cylindric stalk. Seeds: 4, orbicular, papillose, slightly rugose, minutely hairy.

ROOT

Macroscopy[10]

Roots: Occur upto 0.7 cm thick and upto 4 cm long cylindrical with branched lateral roots, dirty brown. Fracture: Fibrous Odor and Taste: Not characteristic

Microscopy[10]

Cork: Poorly developed, consisting of 2-4 layers of tangentially elongated, thin walled cells. Epidermis: Consisting of single layered cells. Cortex: 2° cortex narrow, consisting of 5-7 layers of elliptical or tangentially elongated, thin walled, parenchymatous cells. 2° phloem narrow, consisting of sieve elements and parenchyma, Phloem rays not distinct. 2° xylem consisting of pitted vessels, fibres, tracheids and parenchyma. Vessels occur singly or in groups of 2-4 or more and arranged radially throughout 2° xylem, vessels with simple pits, tracheids thick-walled and lignified.

Powder characteristics[10]

Dirty brown, shows parenchymatous cells, aseptate fibres and pitted vessels.

IDENTITY, PURITY AND STRENGTH[10]

Foreign matter: NMT 2% Total Ash: NMT 9% Acid insoluble Ash: NMT 2% Alcohol – soluble extractive: NLT 3% Water soluble extractive: NLT 7%

Dose[10]

1-5 g in powder form.

Properties and action[10]

Rasa: Tikta, Kasaya Guna: Sara, Picchila Virya: Sita Vipaka: Katu Karma: Pittahara, Kaphahara, Varnya

Important formulation[10]

Aragvadhadi Kvatha Curna.

STEM

Microscopy (Figure 2)[12]

The transverse section of stem shows epidermis which is single layered, flattened tangentially and fitted closely along their radial walls with a cuticle extending over it. It is followed

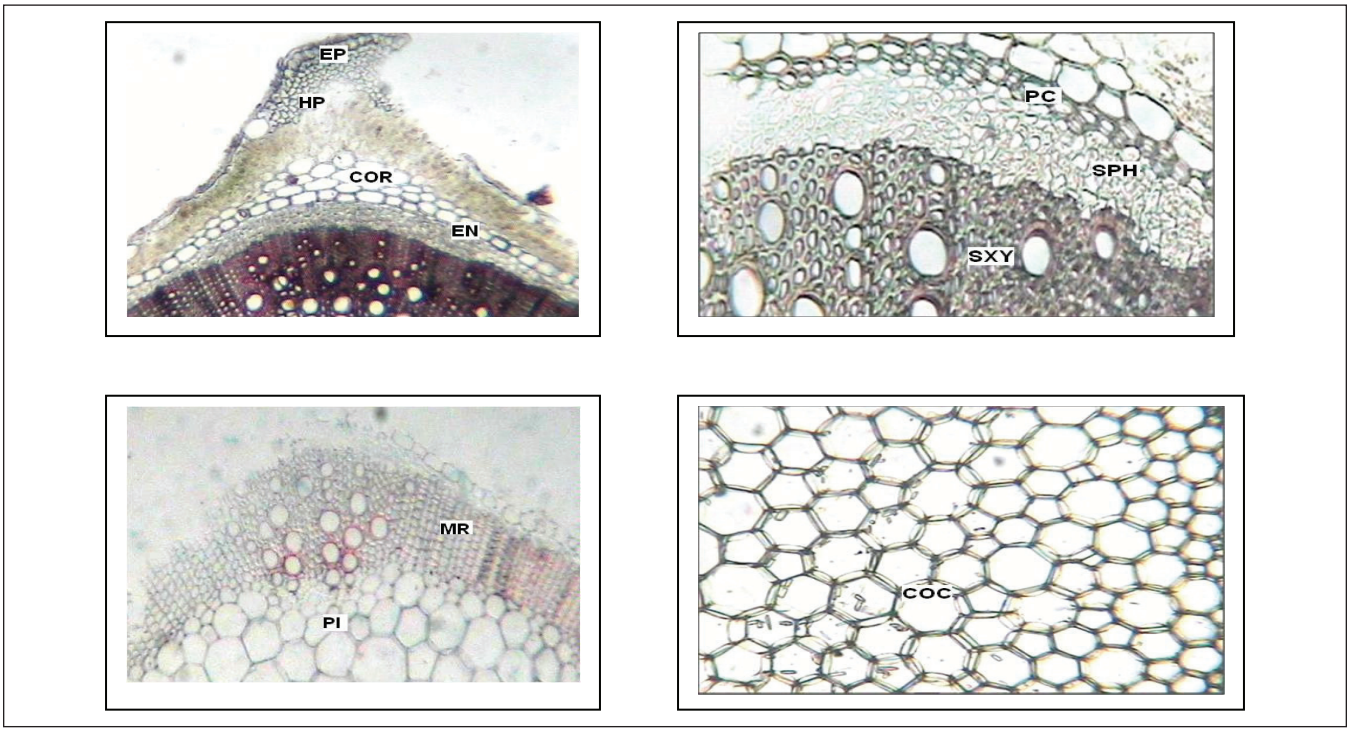

Figure 2: Microscopy of stem: EP- Epidermis, HP-Hypodermis, COR-Cortex, EN-Endodermis, PC-Pericycle, SPH-Secondary phloem, SXY-Secondary xylem, MR-Medullary rays, PI-Pith, COC-Calcium oxalate crystals

by cortex consists of hypodermis, general cortex and endodermis. Hypodermis is angular and in each angle it shows 6-7 layers of collenchymas and in furrow 2-3 layers of collenchyma. It shows brownish green colored cortex. General cortex lies internal to the hypodermis and consists of a few layers of thin walled, large, rounded or oval, parenchymatous cells. Endodermis is conspicuous and consists of single layer of closely fitted parenchymatous cells. It is followed by pericyclic region made up of discontinuous lignified pericyclic fibre. Stelar region shows secondary growth in which 2° phloem lies externally and is composed of only thin and cellulose-walled elements. It consists of sieve tubes, companion cells and phloem parenchyma. Inner to secondary phloem well developed secondary xylem is present. It consists of xylem vessels, tracheides, xylem fibres and xylem parenchyma. It is lignified. Xylem vessels are more distinguished below ridges. Xylem is transversed by well developed multiseriate lignified medullary rays. In the centre pith is wide, parenchymatous and showing presence of acicular crystals.

LEAF

Microscopy (Figure 3)[12]

Transverse section of the leaf is dorsiventral and transcurrent. Lamina portion shows upper epidermis which is single layered having rectangular cells and cuticularized. Surface framing uniseriate multicellular 2-3 celled trichomes. Lower epidermis is identical to upper epidermis but having less trichomes and has numerous stomatas of caryophyllaceous type. Mesophyll consists of single layer of palisade parenchyma and 4-5 layers of loosely bound spongy parenchymas. Midrib portion shows both upper and lower epidermis which are continuous. Upper convex surface is grooved and below each groove, it shows 5-7 layers of collenchyma which is followed by single layer of embedded palisade parenchyma. While lower midrib shows 3-4 layers of collenchyma above epidermis. It shows many trichomes which are uniseriate 2-3 celled. Vascular bundles are arc shaped and on each side of vascular bundle one one small vascular bundles are also present. Xylem is lignified and phloem is non-lignified.

PHARMACOGNOSTICAL STUDIES

Anuradha U., Kumbhojkar M.S. and Vartak V.D. have repored six species, viz. *Glossocardia bosvallea. Justicia procumbens, Haplanthus verticillaris, Oldenlandia corymbosa, Peristrophe bicalyculata* and *Rungia repenes* used as 'Pittapapada' by local people of Pune and neighbouring destricts. Their taxonomical status, vernacular names and precise uses are given. For easy identification of the market drug samples, an artificial key is devised on the basis of exomorphic characters of leaves.[13]

Kumar K.A. and Nisteswar K. have described the folk uses of *Tribulus terrestris, Viscum orientale, Rivea ornata, Coldenia*

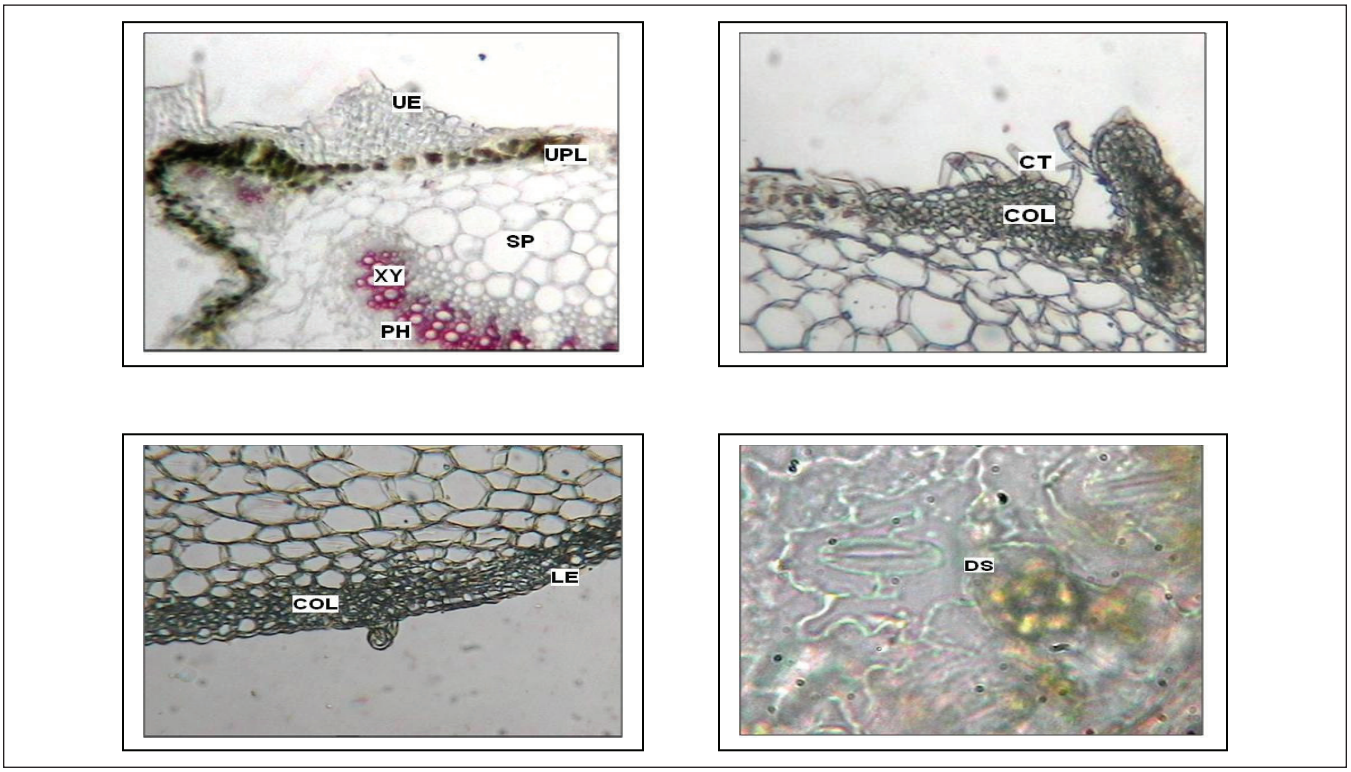

Figure 3: Microscopy of leaf: UP-Upper epidermis, UPL-Upper palisade layer, XY-Xylem, SP-Spongy parenchyma, CT-Covering trichome, COL-Collenchyma, LE-Lower epidermis, DS-Diacytic stomata

procumbens, Moringa oleifera, Abutilon indicum, Borassus flabellifer, Sphaeranthus indicus, Alangium salvifolium, Peristrophe bicalyculata and *Cryptolepis buchanana.*[14]

Sabu P.M. and Ghouse A.K.M. have showed the morphological variations of *Peristrophe bicalyculata* Nees as induced by pollutants resulting from coal burning. It is a common wasteland weed, which is widely used as a cattle fodder. The noxious substances and gases emitting from the thermal power plant change the environmental conditions towards the worse and affect the vegetation in more than one way. The plant studied, shows significant reduction in overall plant growth, leaf number, floral organs and biomass.[15]

Begum M. and Sarker A.K. have found the cystolith as a parameter in delimiting different taxa of the family Acanthaceae (in bangladesh). The characteristics of cystolith (shape, size, occurrence, etc.) are considered to be important taxonomic criteria for the identification of various angiospermic taxa. Thus, the study of 30 species of Acanthaceae from Bangladesh revealed that cystolith of various sizes is present in the following taxa: *Andrographis paniculata, Peristrophe bicalyculata, Beloperone guttata, Cardenthera difformis, Gendarussa vulgaris, Lepidagathis incurva, Rungia pectinata, Ruellia tuberosa, R. prostrata*, etc; whereas, it is absent in the following: *Nelsonia canescens, Adhatoda zeylanica, Hypoestes triflora, Thunbergia grandiflora, T. erecta* and *T. laevis*. The occurrence, distribution, shape, size and other aspects of cystolith studied in the family Acanthaceae have been presented.[16]

Table 1: Quantitative Leaf Microscopy of *Peristrophe bicalyculata*

Parameter	Range (per sq mm)
Palisade Ratio	40-44
Stomatal Number	277.78-300.92-347.22
Stomatal Index	20.0-26.32
Vein-islet Number	18.75-25.00
Veinlet Termination Number	12.5-18.75

Quantitative microscopy of leaf[12]

The palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number of leaves are given in Table – 1.

Physicochemical parameters[12]

The results of the physicochemical parameters are given in Table – 2.

CHEMICAL CONSTITUENTS[10,17-21]

Flowers contain petunidin-3-rhamnoglucoside, roots contain volatile oil, stem and root contains sterols and fatty acids, in stem methanolic extract shows free amino acids and sugars,

seed contain mucilage, leaf and stem contains alkaloids and aerial parts contains14-methyltritriacont-14-en-15-ol and 35-hydroxynonatriacontanal. (yields – 40 mg and 601 mg respectively, from 4 kg of air-dried aerial parts.)

PHYTOCHEMICAL STUDIES

Sharma B., Lukka K.V. and Baxi A.J. have done the chemical investigation of stem and root of *Peristrophe bicalyculata* (Retz.) Nees. which showed the presence of at least 4 sterols and 3 fatty acids isolated by TLC and reverse phase chromatography respectively. From methanolic extract of stem 5 free amino acid and 4 free sugars were isolated.[22]

Singh R.S., Pandey R.P., Singh B.K. and Singh R.G. have done the isolation and spectral data of new 14-methyl-tritriacont-14-en-15-ol and 35-hydroxynonatriacontanal from aerial parts of *Peristrophe bicalyculata*.[23]

Phytochemical screening[12]

The results of preliminary phytochemical screening of petroleum ether, chloroform, acetone, methanol and water extracts of aerial parts of *Peristrophe bicalyculata* are presented in Table – 3 and 4. The qualitative chemical tests revealed the presence of phytosterols, carbohydrates, protein, amino acids, flavanoid and phenolic compounds.

Table 2: Numerical Data for Aerial Parts of *Peristrophe bicalyculata*

Parameter	Mean (%w/w)
Total Ash	14.78% ± 0.1591
Acid Insoluble Ash	1.68% ± 0.0530
Water Soluble Ash	10.15% ± 0.0353
Alcohol Soluble Extractive Value	4.42% ± 0.0368
Water Soluble Extractive Value	9.65% ± 0.7634

TLC (root)[10]

TLC of Alcoholic extract on Silica Gel 'G' plate using Toluene: Ethyl acetate (93:7).

→ Shows under U.V. (366 nm) five fluorescent zones at Rf 0.15, 0.30, 0.52, 0.90 and 0.98 (all light blue).

→ On exposure to 12 vapor 6 spots appear at Rf 0.07, 0.15, 0.30, 0.43, 0.57 and 0.98 (all yellow).

→ On spraying with Vanillin – H_2SO_4 reagent and heating the plate for 10 min at 110'c 5 spots appear at Rf 0.07, 0.30, 0.43, 0.57 and 0.98 (all violet)

PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES

Qureshi S., Rai M.K. and Agrawal S.C. have evaluated the effect of extract of 18 plant species, viz., *Acorus calamus, Adhatoda vasica, Amomum subulatum, Andrographis paniculata, Boerhaavia diffusa, Cassia occidentalis, Centella asiatica, Cymbopogon citratus, Hemidesmus indicus, Hyptis suaveolens, Malvestrum sp., Passiflora edulis, Pergularia daemia, Peristrophe bicalyculata, Shuteria hirsuta, Solanum nigrum, Tecoma stans* and *Verbascum chinense* on the growth of *Microsporum gypseum, Chrysosporium tropicum* and *Trichophyton terrestre*. The sensitivity of the keratinophilic fungi was evaluated by dry-weight method. The maximum inhibition of mycelial growth was shown by *M. gypseum* (86.62%) followed by *T. terrestre* (81.86%) and *C. tropicum* (74.06%) when treated with *S. hirsuta* whereas the minimum inhibition was exhibited by *M. gypseum* (0.29%), *C. tropicum* (0.16%) and *T. terrestre* (1.76%) when tested with the extract of *P. edulis, A. vasica* and *B. diffusa* respectively.[24]

Table 3: Qualitative Chemical Examination of the Plant Extracts Obtained by Successive Solvent Extraction

Sr. No.	Constituent	Petroleum Ether	Chloroform	Acetone	Methanol	Water
1	**Alkaloids**					
	a) Dragendorff's Reagent	*	–ve	–ve	–ve	*
	b) Mayer's Reagent	*	–ve	–ve	–ve	*
2	**Carbohydrate**					
	a) Molisch's Test	*	*	–ve	+ve	+ve
	b) Fehling's Solution Test	*	*	–ve	+ve	+ve
3	**Glycosides**					
	a) Anthraquinone Borntrager's Test	*	*	*	–ve	–ve
	b) Steroids Liebermann-Burchard's Test	+ve	+ve	+ve	–ve	*
	c) Saponin Foam Test	–ve	–ve	–ve	–ve	–ve
	d) Flavanoid Shinoda Test	–ve	–ve	–ve	+ve	–ve
	e) Cardiac Legal's Test	*	*	*	–ve	–ve
4	**Fixed Oil and Fats**					
	a) Spot Test	–ve	–ve	–ve	–ve	–ve
5	**Phenolic Compounds and Tannins**					
	a) FeCl3 Solution	–ve	–ve	–ve	+ve	–ve
6	**Protein and Amino Acids**					
	a) Biuret's Test	*	*	*	+ve	+ve
	b) Ninhydrin Test	*	*	*	+ve	+ve

* Test was not performed

Khan and Shahid Shaukat S. have carried out the Bioassays with *Achyranthes aspera* L. and demonstrated that aqueous root and shoot extracts against *Triticum aestivum*, a cultivated species and four field associates viz. *Cenchrus pennisetiformis, C. setigerus, Chloris barbata* and *Peristrophe bicalyculata* impeded or reduced germination of test species. The suppression of germination in shoot extract was in the order: *C. barabata* < *T. aestivum* < *C. setigerus* < *C. pennisetiformis* < *P. bicalyculata* and root extract suppressed germination in order: *C. barbata* < *T. aestivum* < *C. setigerus, P. bicalyculata* < *C. pennisetiformis.* Shoot extract was inhibitorier than root extract. Reduction in seedling growth was species specific.[25]

Kumar Sushil, Bagchi Gurudas and Darokar Mahendra Pandurang have identified the seeds of coprophilous plants (*Peristrophe bicalyculata*) growing in bovine cattle dung (fresh or decomposing upto 15 days old) for antimicrobial potency and abiotic stress.[26,27]

Rathi A., Rao C.V., Khatoon S., Rawat A.K.S. and Mehrotra S. have examined ethanolic (50%v/v) extract of *Peristrophe bicalyculata* for anti inflammatory and analgesic activity in experimental animal models like 1% carrageenin, cotton pellet granuloma, tail flick reaction time, analgesy-meter induced pain and acetic acid induced writhing in doses of 50, 100, 200 mg/Kg body weight. The experimental results revealed the % suppression 12.25-24.49% in carrageenin induced inflammation and decreased 16.62-39.44% granulation in cotton pellet induced granuloma.[28]

Hoda S., Afaq S.H. and Tajuddin have shown that the drug 'Chaksini' (*Peristrophe bicalyculata*) is a lesser known drug. It is used in wound healing in traditional Unani medicine. Pharmacological screening of analgesic and anti inflammatory effect of drug are reported. The aqueous extract exhibited wound healing effect.[29]

Hoda S., Mohammad A., Afaq S.H. and Tajuddin have carried out the pharmacological studies of "Chaksini" (*Peristrophe bicalyculata* Nees) in relation to psychosomatic disorders. The gross behaviour, spontaneous motor activity, pentobarbitone sodium induced narcosis and supramaximally electric shock seizure tests were carried out. The aqueous extract has CNS depressant activity. The effect is probably due to water soluble glycoside present in drug.[30]

CLINICAL STUDIES

Tajuddin, Afaq S.H. and Hoda M.S. have reported that *Peristrophe bicalyculata*, an erect spreading shrub of controversial identity, is very effectively being used by Unani physicians for the treatment of nervous disorders like hysteria and leucoderma. A multidisciplinary research oriented study has been carried out on the plant which include establishment of its botanical identity after cultivation in the department as well as physico chemical standards for purity of drug. The details of results of pharmacognostical and phytochemical studies along with some pharmacological effects are discussed.[31]

USES[1-5,21,32,33]

Infusion is used as insecticidal. Poultice is used in skin troubles. Essential oil shows tuberculostatic activity in vitro. Plant macerated in an infusion of rice said to be antidote to snake poison. Paste of whole plant along with common salt is used externally in bone fracture and sprains. Leaf paste is externally used to treat bone fractures and pains. 2 Drops of juice of freshly collected and washed leaves is poured into eyes twice daily in cases of conjunctivitis for 2-3 days. Leaf extract is used for fever, cough and colds. It is also used in malarial fever, flatulence of cattle. Mucilage medicines are used for ear treatments, eye treatments. It is also used as fodder for horses and as green manure. The honey collector applies the fresh juice of whole herb on body and collects the honey. According to them, its specific smell and taste repel away the bees. Astringent decoction mixed with honey destroys worms originating and affecting teeth. Roots are used as bandaging and as sedative. Roots of the plant applied to ulcerative wounds for 3 days to remove their suppuration, pain and fetid odor. The seeds carry a certain amount of mucilage which on drying can be stretched out into a fine thread. This is used in Senegal for fishing out foreign bodies from eyes and ears.

CONCLUSION

We conclude from vast literature study and experimental result analysis that *Peristrophe bicalyculata* is a traditional remedy for tuberculosis, antidote to snake poison, in bone fracture and sprains, for treatment of hysteria and lecoderma, anti-inflammatory and analgesic activity and in psychomotor disorder. Taking great concern of useful benefits of the plant, it can be advocated as a safe, highly important, medicinal plant for general mankind.

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