Pharmacognostic Investigation of Cynodon dactylon Pers Roots

Sumit R Deore and Ajay G Namdeo*

Department of Pharmacognosy, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune- 411038

ABSTRACT

Background: *Cynodon dactylon* (L.) Pers. family (Graminae/poaceae) occupies its unique place and key position in ethnomedicinal practices and traditional medical (Ayurvedic, Unani, Nepalese, and Chinese) knowledge systems but according to best of our knowledge lack is done on its standardization of the herb for its quality control and authenticity. **Objective:** To evaluate the morphological and microscopical characters of *Cynodon dactylon* Pers roots collected from Maharashtra region and its phytochemical and physicochemical analysis. **Methods:** Microscopic, macroscopic characters and fluorescence analysis of roots samples were analyzed. The physicochemical properties such as loss on drying, total ash value, acid insoluble ash, water soluble ash value and extractive values of *Cynodon dactylon* were carried out. **Results:** The detailed microscopy revealed the presence wide cortex, intact epidermis, wide circular metaxylem and parenchymatous cells loaded with starch grain. Preliminary phytochemical investigation revealed the presence of carbohydrates, flavonoids, phenols and tannins. **Conclusions:** This is first report on the pharmacognostic studies of *Cynodon dactylon* and is helpful in laying down identification, standardization and pharmacopeial standards.

INTRODUCTION

The proper identification of plants and drugs is the most important aspect of any crude drug research. The problem of identification is more pronounced in Ayurveda since much controversy exists due to one or more than several botanical species for the same drug.[1]Since many of the drugs in Ayurveda are sold as crude drugs, it is essential to study and understand the tissue systems which hold key in arriving at the correct identification. Thus, this original classical approach towards Pharmacognosy is essential for the proper characterization and identification of the drug and the plant.

Bermuda grass, Doob Ghas, or Durva or taxonomically the *Cynodon dactylon* (L.) Pers. family (Graminae/poaceae) occupies its unique place and key position in ethnomedicinal practices and traditional medical (Ayurvedic, Unani, Nepalese, and Chinese) knowledge systems. The herbal preparations of this grass are being based on folklore and traditional wisdom.[2]

***Corresponding author.**
Phone: 91 9881299129
Fax:

E-mail: agnamdeo@gmail.com

DOI: 10.5530/pj.2014.1.1

It is an inseparable part of religious rituals and is a valuable herbal medicine used as first aid in minor injuries.[3] In Indian households it is a common practice to place strands of this grass on eatables during eclipse. Folk wisdom claims it to be water purifier.[4] The juice of the plant is astringent and the fresh juice is used in the treatment of chronic diarrhoea and dysentery.[5] The plant occupies a renowned position in Ayurveda, Unani and Homoeopathic systems of medicine.[6] It possesses various medicinal properties such as antimicrobial, antiviral activity[7] and has significant application in treating dysentery, dropsy and secondary syphilis.[8]

MATERIAL METHODS

Plant collection and extraction

Fresh roots of *Cynodon dactylon* Pers were collected from the local area of Pune District of Maharashtra, India in month of June 2011. The plant specimen was identified and authenticated as *Cynodon dactylon* Pers by BSI, Pune, India. The voucher specimen (No. SRD-1) is preserved in the herbarium of Dept. of Pharmacognosy. The air-dried roots of C.dactylon were made into coarse powder. The powdered material was extracted with Soxhlet apparatus using different solvents like petroleum ether (60°–80°C), chloroform, methanol and water as per their polarity

successively. The extract was dried using rotary evaporator and was kept in a dessicator till experimentation.[9,10]

PHARMACOGNOSTIC STUDIES

Macroscopy evaluation

Different sensory parameters of the root material (like colour, odour, size, shape and taste) were studied.

Microscopic analysis

Microscopic studies were done by simple microscope. Free hand section of root was taken and stained by safranine to confirm its lignifications. Powder microscopy was also carried out and the specific diagnostic characteristics were recorded.[11]

Physicochemical parameters

Physicochemical parameters of powdered drug such as total ash, water soluble ash, and acid-insoluble ash were determined. Alcohol and water soluble extractives values were determined to find out amount of alcohol and water soluble constituents. Loss on drying method was employed to find moisture content.[12]

Phytochemical analysis

Consistency, color, appearance of the extracts and their percentage yield were noted. The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, and phytosterols using reported methods.[13,14]

Fluorescence analysis

Powdered root material was analysed under visible light, short ultra-violet light, long short ultra-violet light after treatment with various organic/inorganic reagents like NaOH, HCl, HNO_3 and H_2SO_4. The colors observed by application of different reagents in different radiations were recorded.[15, 16]

RESULTS

Pharmacognostic studies

Macroscopy evaluation

Root-Fibrous, cylindrical, upto 4mm thick, minute hair-like roots arise from the main roots; cream coloured.

Microscopic analysis

Transverse section of roots revealed the presence of smooth and even surface. It is nearly 4mm thick. It consists of continuous intact epidermis, fairly wide cortex and wide dictyostele (Fig 1.1). Epidermal layer includes small thick walled cells with heavy cuticle (Fig 1.2). Cortex has about 6 layers of circular or angular, compact parenchyma cells.

Inner to the cortex is a thick and continuous cylinder of fibres which posses thick lignified walls and narrow lumen (Fig 1.2, Fig 2.1, Fig 2.2). Some of the vascular strands are included within the sclerenchyma cylinder either in outer part or in the inner part. (Fig 1.2, Fig 2.1, Fig 2.2). The vascular strand located with outer part of the sclerenchyma cylinder are small and somewhat circular with tangential band of xylem elements and small cluster of phloem found on the outer part of the strand. (Fig 2.2).

The inner vascular strand and central strands are larger and more prominent (Fig 2.1, Fig 3.1). They are collateral

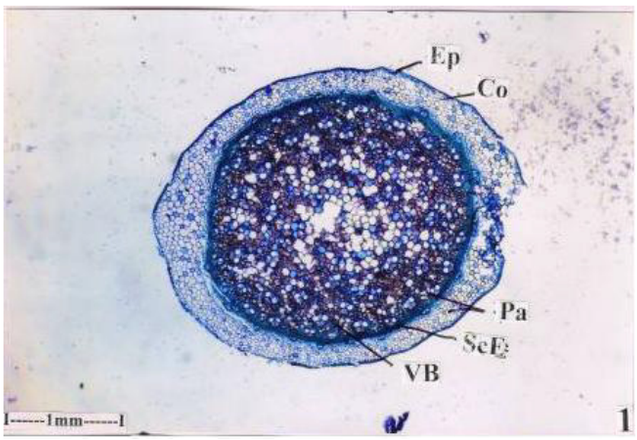


Figure 1.1 TS of root-Entire view.

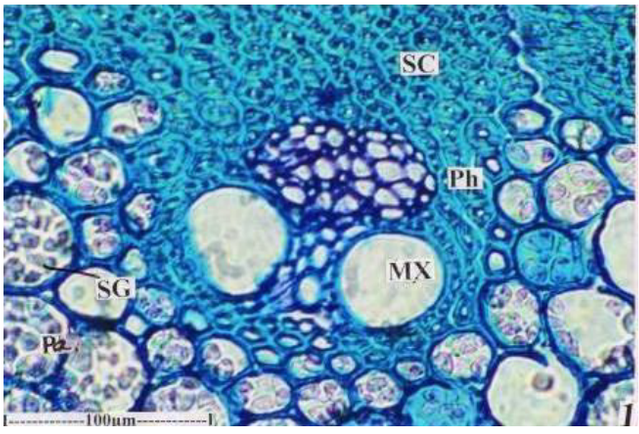


Figure 2.1 Vascular bundle on the inner part of the sclerenchyma cylinder.

and closed. There are two wide circular metaxylem elements and narrow vertical row of protoxylem elements lying in between the metaxylem cells. Some of the protoxylem cells disintegrate form a small protoxylem lacuna (Fig 3.1). The medullary bundles which are free from the cortical sclerenchyma cylinder are surrounded by thin bundle sheet fibres.

The ground tissue is parenchymatous. The cells are large, circular and thin walled. The cells are heavily loaded with simple or compound starch grains (Fig 3.2).

Powder of the root consists of following elements.

Parenchyma cells: (Fig 4.1) wide and parenchyma cells are frequently seen in the powder. The cells are rectangular or squarish in shape. Most of the cells are seen in strands attached end to end. The cell walls are thin. The cells posses abundance of starch grains. The cells 40 × 50 μm in size. Fibres: Fibres are predominant elements in the powder. The fibres are of two types. Some are narrow, long and pointed at the ends. (Fig 4.1, 5.1) They have thick wall and narrow lumen. They are 650μm and 12μm

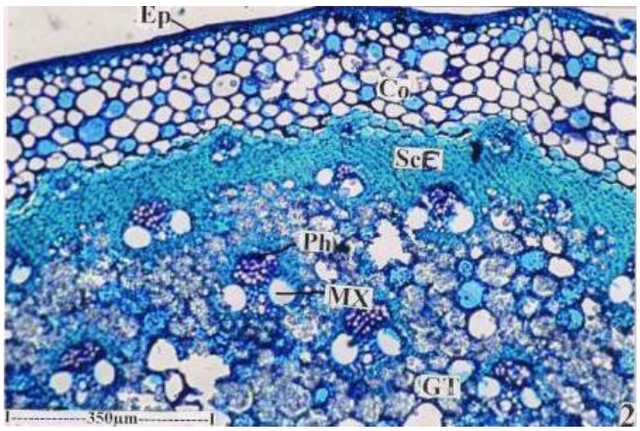


Figure 1.2 TS of root-A sector (co–cortex, Ep-epidermis, GT- ground tissue, MX-metaxylem, Pa-parenchyma, Ph-Phloem, ScE-sclerotic endodermis, VB-vascular bundle).

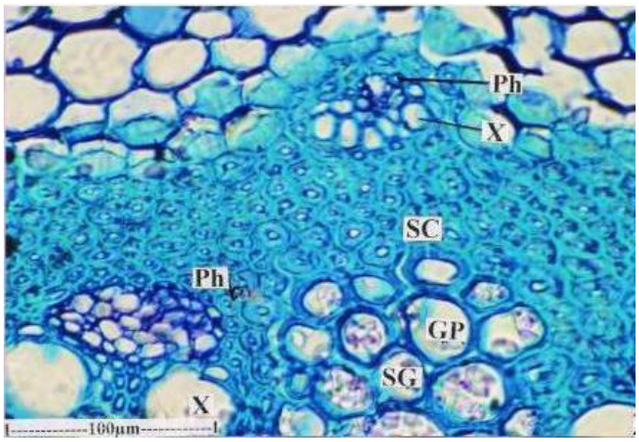


Figure 2.2 Vascular strand situated in the outer part of the sclerenchyma cylinder. (Gp-ground plan, Mx-meta xylem, Ph-Pholem, Pa-Parenchyma, Sc-sclerenchyma, SG-starch grain, X-xylem).

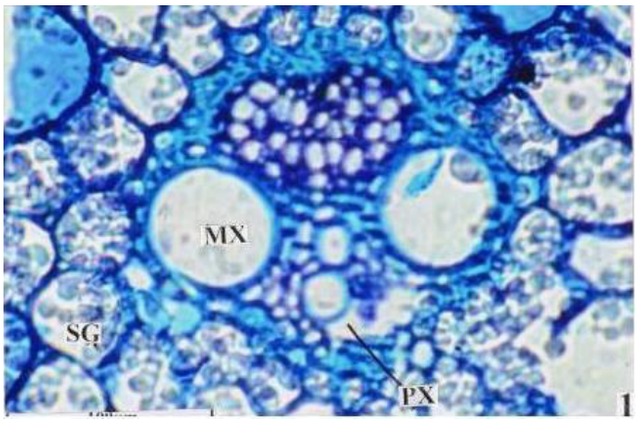


Figure 3.1 Medullary vascular bundle-enlarged.

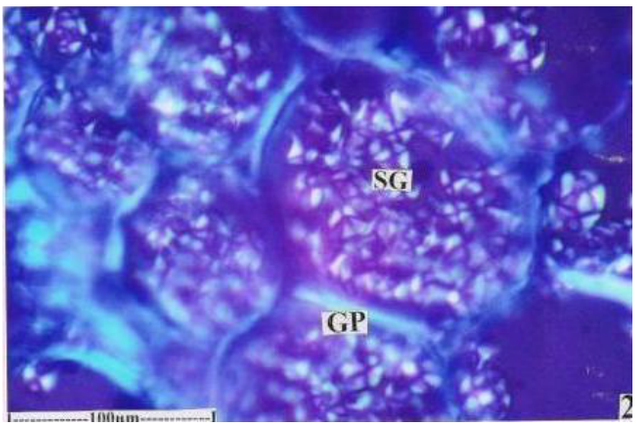


Figure 3.2 Ground parenchyma cells with starch grains (Gp-ground plan, Mx-meta xylem, SG-starch grain, Px-proto xylem) Powder microscopy.

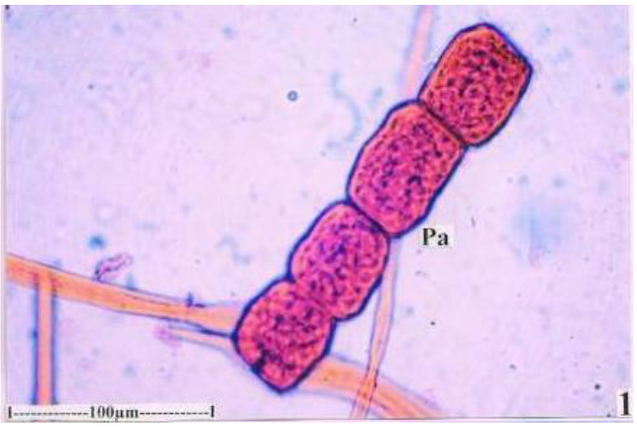


Figure 4.1 Parenchyma strand.

thick. The second types of fibres are wide fibres. They are thin walled with wide lumen. The largest of the wide fibres ranges from 500–700µm, the thickness of the cells is 15–22µm (Fig 5.2) Vessel elements are long narrow and cylindrical (Fig 6.1, 2). The end wall perforation is simple, circular and horizontal in orientation. The lateral wall pits are horizontal elliptic, multiseriate and dense. The vessel elements are 430µm long and 30µm wide.

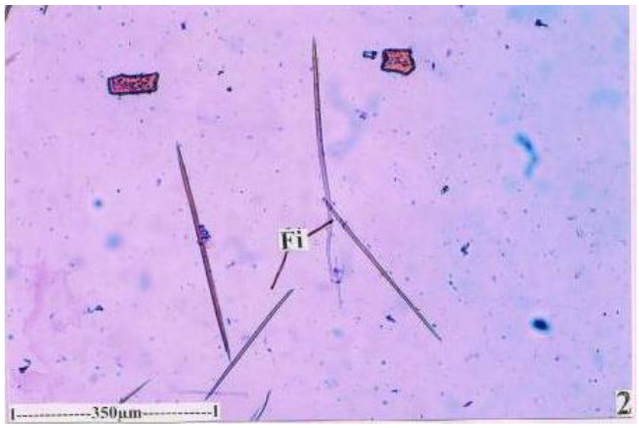


Figure 4.2 Narrow fibers (F-fibers, Pa-parenchyma).

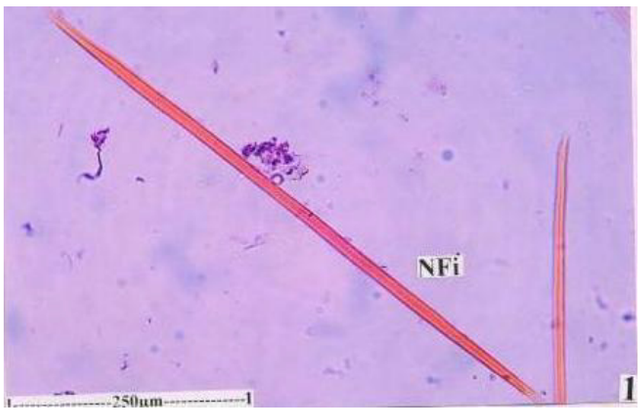


Figure 5.1 Narrow fiber-enlarged.

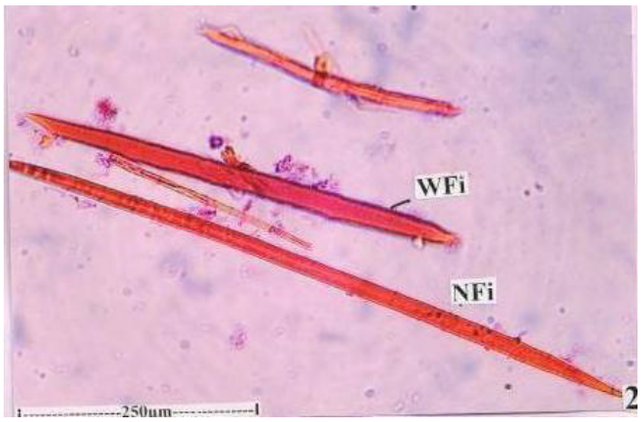


Figure 5.2 One narrow fiber and one wide fiber (NFi-narrow wide fiber, WFi-wide fiber).

Physicochemical parameters

Ash values of the drug give idea about earthy matter or inorganic composition and other impurities present along with the drug. Various physicochemical parameters such as total ash, water soluble ash and acid insoluble ash of *C.dactylon* root was found to be 7.15, 5.35 and 3.18% w/w, respectively.

Moisture content in the root was found to be 8.11% w/w. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Various extractive values such as petroleum ether soluble extract, chloroform soluble extract, methanol soluble extract and water soluble extract of *C.dactylon* root was found to be 3.61, 1.83, 8.11 and 7.85% w/w respectively given in Table 1.

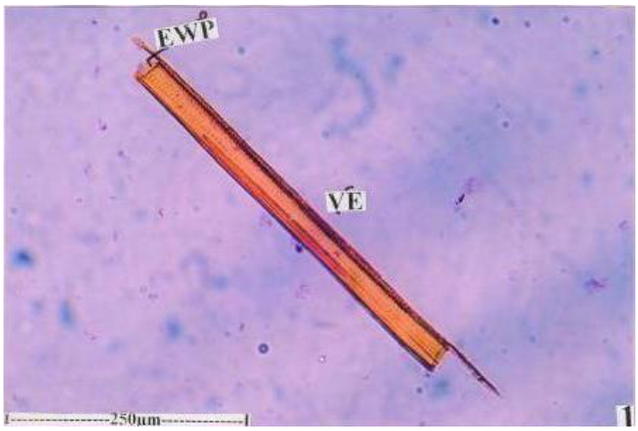


Figure 6.1 Two vessel elements with simple end wall perforation (EWP-end wall perforation, Pi-pits, Ve-vessel elements).

Figure 6.2 Two vessel elements with simple end wall perforation (EWP-end wall perforation, Pi-pits, Ve-vessel elements).

Phytochemical analysis

Successive solvent extracts of root was studied for their phytochemical profile. Their % yield, color and consistency are recorded in Table 2. Preliminary phytochemical screening mainly revealed the presence of phenol and tannins in petroleum ether extract; carbohydrates, phenols and saponins in chloroform extract; carbohydrates, tannins and flavonoids in methanol extract and carbohydrates, tannins, flavonoids and carbohydrates in aqueous extract are mentioned in Table 3.

Fluorescence analysis

The fluorescence analysis of the root powder with different chemical reagents is summarized in Table 4.

DISCUSSION

Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. It is necessary that standards have to be laid down to control and check the identity of the plant and ascertain its quality before use. According to World Health Organization (WHO) the macroscopic and microscopic description of medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[17]

Microscopical evaluation is simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents.[18–20]

Total ash values and extractive values are useful in identification and authentication of the plant material.[21,22] Extractive values are useful to evaluate the chemical constituents of crude drug.[23]

Table 1. Physicochemical constant of root of Cynodon dactylon.

Sr. No	Parameters	Mean % w/w
1.	Loss on drying	8.11
2.	Total ash value	7.15
3.	Acid–insoluble ash value	3.18
4.	Water soluble ash value	5.35
5.	Alcohol soluble extractive value	2.48
6.	Water soluble extractive value	8.21

Table 2. Preliminary phytoprofile of root of Cynodon dactylon.

Sr. No	Type of extract	Day light	UV short light	UV long light
1.	Pet ether (60°–80°C)	Yellow	Brown	Greenish
2.	Chloroform	Green	Dark green	Black
3.	Methanol	Brown	Deep brown	Black
4.	Aqueous	Brown	Deep brown	Black

Table 3. Qualitative chemical test on extracts of roots of Cynodon dactylon.

Sr. No	Phytoconstituents	Pet ether (60o–80o C)	Chloroform	Methanol	Aqueous
1.	Alkaloids	-	-	-	-
2.	Carbohydrates	-	+	+	+
3.	Glycosides	-	-	-	-
4.	Flavonoids	-	-	+	+
5.	Phenol & tannins	+	+	+	+
6.	Steriods	-	-	-	-
7.	Triterpenoids	-	-	-	-
8.	Saponins	-	+	-	-
9.	Proteins	-	-	-	+
10.	Amino acids	-	-	-	-

– Negative; + Positivew

Table 4. Fluorescence analysis of root powder of Cynodon dactylon with various reagents.

Sr. No	Reagent + Drug	Colour of powder at Day light	UV Light Short	UV Light Long
1.	Untreated powder	Yellowish brown	Brown	Black
2.	Powder + saturated Picric Acid	Pale green	Green	Dark brown
3.	Powder + Nitric acid	Brown	Brown	Blackish
4.	Powder + 1 N HCl	Brownish	Greenish brown	Dark green
5.	Powder + conc. H2SO4	Light brown	Blackish brown	Black
6.	Powder + Glacial Acetic Acid	Brownish	Brownish	Blackish
7.	Powder + 1N NaOH	Brownish green	Light green	Blackish green
8.	Powder + Iodine	Brownish	Blackish brown	Blackish
9.	Powder + Ferric chloride	Yellowish brown	Dark green	Black

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products, which do not fluoresce in daylight. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is important parameter of pharmacognostic evaluation.[21,22]

Preliminary phytochemical screening mainly revealed the presence of phenol and tannins in petroleum ether extract; carbohydrates, phenols and saponins in chloroform extract; carbohydrates, tannins and flavonoids in methanol extract and carbohydrates, tannins, flavonoids and carbohydrates in aqueous extract. T.S of the root confirmed the presence of wide cortex, intact epidermis, wide circular metaxylem and parenchymatous cells loaded with starch grain.

In conclusion the detail study was undertaken with an aim of pharmacognostic standardization and preliminary phytochemical analysis of *C.dactylon* roots established in the present study will useful in identifying the genuine drug and will also be useful in development of pharmacopeial standards for further studies.

ACKNOWLEDGEMENT

The authors acknowledge the grant received from All India Council of Technical Education (AICTE) for this project under Quality Improvement Programme (QIP) Scheme for teachers.

REFERENCES

1. Subramanyam K, Yoganarasimhan SN. Open dichotomous venation in the leaves of *Utricularia striatula* (Lentibulariaceae). *Plant Syst Evol*, 1979; 131:133–42.
2. Mishra MP. Succession of fungi and their eco-microbial involvement in the decay of *Cynodon dactylon* Pers. Ph.D. Thesis. 2006; 14–21.
3. Oudhia P. Medicinal weeds in groundnut fields of Chattisghar (India). *Int. Arachis News Lett*, 1999; 19:62–64.
4. Artizzu N, Bonsignore L, Cottiglia F, Loy G. Studies on the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil. *Fitoterapia*, 1996; 67:174–76.
5. Anonymous, The Wealth of India Vol. 2, A Dictionary of Indian Raw Materials and Industrial Products, First Supplement Series (Raw Materials), Council of Scientific and Industrial Research, New Delhi; 2001:331–32.
6. Chopra RN, Nayar SL, Chopra IC, Glossary of Indian Medicinal Plants. Council for Scientific and Industrial Research, New Delhi; 1956:88.
7. Dhar ML, Dhawan M, Melhrotra, Screening of Indian plants for biological activity. Part I. *Ind J Exp Bio*, 1968; 6:232.
8. Chopra RN, Handa KL. Indigenous Drugs of India, 2nd ed. Academic Publishers, Calcultta; 1982:504.
9. The Ayurvedic Pharmacopoeia of India. Part-I, vol. III, 1st ed. Government of India, Ministry of Health & Family Welfare, Dept. of Indian Systems of Medicines & Homeopathy New Delhi; 2001:233.
10. Government of India. Indian Pharmacopoeia Vol-2. Ministry of Health and Family Welfare, Controller of Publications, New Delhi, India; 1996:A-54.
11. Khandelwal KR. Practical Pharmacognosy, 19th ed. Nirali Prakashan,Pune, India; 2008:49–70.
12. Mukherjee PK. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. 2nd ed. New Delhi, India: Business Horizons; 2007.
13. Harborne JB. Phytochemical methods. 2nd Ed. Chapman and Hall London;1998:60.
14. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biomed Res*, 2007; 10:175–181.
15. Pratt RJ, Chase CR. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharm Assoc*, 1949; 38:324–333.
16. Kokoski CJ, Kokoski RJ, Slama FJ. Fluorescence of powdered vegetable drugs under with particular reference to devlopment of ultraviolet light radiation. *J Am Pharma Assoc*, 1958; 47:715–717.
17. WHO. Quality control methods for medicinal plants. Geneva. WHO; 2002:28–31.
18. Iqbal Ahmad, Farrukh Aqil, Mohammad Owais. Modern Phytomedicine: Turning Medicinal Plants into Drugs, John Wiley and Sons; 2007:34.
19. Wallis TE. Textbook of Pharmacognosy 5th Ed. New Delhi: CBS Publications; 1985:111–117.
20. Pratima H and Pratima Mathad. Pharmacognostic Evaluation and Phytochemical Analysis of Leaves of *Cajanus cajan* L. *J Adv Dev Res*, 2011; 2(2):181–185.
21. Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. *Int J Pharm Tech Res*, 2010; 2(1):140–143.
22. Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leaf. *Asian Pac J Trop Biomed*, 2011; 1(3):177–181.
23. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. *J Herb Med Toxicol*, 2008; 2(2):51–54.

Pharmacognostic studies of *Lagenaria siceraria* (Molina) standley fruits

C.V. Panchal[a,*], Jyotiram A. Sawale[b], B. N. Poul[a] and K.R. Khandelwal[c]

[a]*Maharashtra College of Pharmacy, Nilanga, Dist. Latur (M.S.) PIN-413521*
[b]*IES College of Pharmcy, Kalkheda Ratibad Main Road, Bhopal (M.P.) PIN-462001*
[c]*Rajarshi Shahu College of Pharmacy and Research, Tathwade, Pune-33 (M.S.)*

ABSTRACT

Background: *Lagenaria siceraria* (LS) fruits belonging to Cucurbitaceae family is widely used in Indian traditional medicine for its various medicinal values. As per best of our knowledge there were no pharmacognostical reports, specifically to determine anatomical and other physicochemical standards required for its standardization. **Material and Methods:** In this study various standardization parameters like macroscopic and microscopic studies, physicochemical constants, extractive values and preliminary phytochemical screening were studied and reported. **Results:** Different standardization parameters were reported, which would be of immense use to identify and establish the authenticity of the plant. **Conclusion:** Preliminary pharmacognostic evaluation of *Lagenaria siceraria* fruits can give some useful information, which will be further used for standardization.

Keywords: Pharmacognostic, Bhopla, Cucurbitaceae, standardization, Lagenaria siceraria.

INTRODUCTION

Lagenaria siceraria (LS) locally called as 'Bhopla' is a large softly pubescent annual climber distributed in Asia, America and tropical Africa, wild or cultivated in all warmer regions as a vegetable. The fruit is reported as diuretic and antipyretic. Traditionally a decoction of leaves mixed with sugar is given in jaundice. The seeds are being used to cure cough, fever, earache, as brain tonic and anti-inflammatory.[1–4] The fruit contains saponins and some researchers have already reported saponins plays a vital role as hepatoprotective.[4–8,15]

In the present study, various standardization parameters of LS fruits like macroscopical and microscopical characters, extractive values, ash values and preliminary phytochemical screening were carried out to identify the major phytoconstituents.

***Corresponding author.**
Chandrawadan V. Panchal (Assistant Professor)
Maharashtra College of Pharmacy
Nilanga, Dist. Latur (MS) PIN-413 521.
Mob.No. 9860786596

E-mail: cvpanchal.mcpnilanga@gmail.com

DOI: 10.5530/pj.2014.1.2

MATERIALS AND METHODS

Plant material

The fruits of LS were collected in the month of October, 2005 from local areas of Pune region and authenticated by Botanical Survey of India, Pune and voucher specimen number, deposited as PCV 1. *Lagenaria siceraria* (Mol.) Standl., belonging to family Cucurbitaceae

Chemicals and instuments

All the chemicals were used of analytical grade. Compound microscope, watch glass, cover slip, glass slide and other common glass wares were used in this experiment. Paraffin embedded blocks were sectioned with help of rotary microtome. Photographs were taken with Nikon Labphot 2 microscopic unit. Various solvents used mainly Ethyl alcohol (70%), tertiary butyl alcohol and reagents used for staining different sections like formalin, acetic acid, toluidine blue, safranin, fast-green and IKI were procured from CDH, Mumbai, India.

Macroscopic and microscopic studies

Macroscopic study of fruit such as size, shape, color, odor and taste was performed.

Microscopic study: The fruit of LS was taken and cut into small pieces. It was fixed in FAA (formalin – 5mL + Acetic acid – 5mL + 70% Ethyl alcohol – 90mL). After 24 hrs. of fixing, the specimen were dehydrated with graded series of tertiary- butyl alcohol as per the schedule given.[9] Infiltration of specimen was carried out by gradual addition of paraffin wax until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

The paraffin embedded blocks were sectioned with help of rotary microtome. The thickness of the section was 1–12µm. Dewaxing of the sections was by customary procedures.[10] The sections were stained with Toluidine blue as per the standard method.[11] Since Toluidine blue is a polychromatic stain, the staining was remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells and protein bodies, dark green to suberin, violet to the mucilage etc., wherever necessary sections were stained with safranin and fast-green and IKI (for starch).

Photomicrographs

Photomicrographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For the study of lignified cells polarized light was employed. Magnification of the figures is indicated by the scale bars. Descriptive terms of the anatomical features are as given in standard anatomy books.[12]

Determination of physicochemical constants

Ash value[1,13]

Total ash

The total ash is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Method

About 2.003g of powdered material was accurately weighed and taken separately in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried drug.

Acid-insoluble ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This ash value particularly indicates contamination with silicious material such as earth and sand.

Method

The ash obtained as described above was boiled with 25mL of 2N HCl for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible, ignited, and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water-soluble ash

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Method

The ash obtained as described in the determination of total ash was boiled for 5 minutes with 25mL of water. The insoluble matter was collected on ashless filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 minutes, and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive values[1,13]

Alcohol soluble extractive value

About 5g each of coarsely powdered material was accurately weighed and macerated with 100mL of alcohol of specified strength, separately in a closed flask for 24 hours. Shaking was done frequently during first 6 hours and then allowed to stand for 24 hours. This was filtered rapidly taking precaution against loss of alcohol. Then, 25mL each of these alcoholic extracts were evaporated to dryness in a tared flat bottom shallow dish and were dried at 105°C and weighed. Percentage of alcohol

soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive value

About 5g each of coarsely powdered material was accurately weighed and macerated with 1000mL of chloroform water, separately in a closed flask for 24 hours. Shaking was done frequently during first 6 hours and then allowed to stand for 24 hours. This was filtered and then, 25mL each of these chloroform water extracts were evaporated to dryness in a tared flat bottom shallow dish and were dried at 105°C and weighed. Percentage of water soluble extractive was calculated with reference to the air-dried drug.

Preliminary phytochemical screening[1,13–15]

Preliminary phytochemical screening for different constituents was carried out in petroleum ether extract and ethanolic extract.

RESULTS

Macroscopic study:

Macroscopic and sensory characters of fruits are as shown below

Length: 25–35 cm.

Shape: Bottle, dumbbell, cylindrical shaped.

Width: 5–8 cm.

Surface: Densely hairy to glabrous.

Weight: 400–800 gm.

Colour: Faint green.

Odour: Pleasant.

Taste: Sweet.

Microscopic study

The histological findings of LS fruits are given as follows:

Fruit

The pericarp is the rind of the fruit. The pericarp has epicarp and mesocarp. Epicarp is unistrstose forming the epidermal layer of the fruit. The epidermis is 20mm thick; the cells are radically oblong and wide. The outer and inner tangential walls are thin; stomata are sporadically seen in the epidermis.

The mesocarp is differentiated into outer zone and inner zone (fig. 1.1). The outer zone is 120mm wide and 10 to 15 layered. The cells are small, tangentially elliptic, compact and darkly staining. As the fruit matures, the epicarp and the outer mesocarp combined together develop into the hard rind. Inner and outer mesocarp is the inner mesocarp, which fills the entire pulp of the fruit. This tissue has large, polygonal compact cells. A few layers of the cells in the peripheral region are thick walled and lignified. The lignified cell zone is gradually transformed into thin walled soft parenchyma cells. Sporadically, one or two cells in between the two zones of mesocarp have dark amorphous contents.

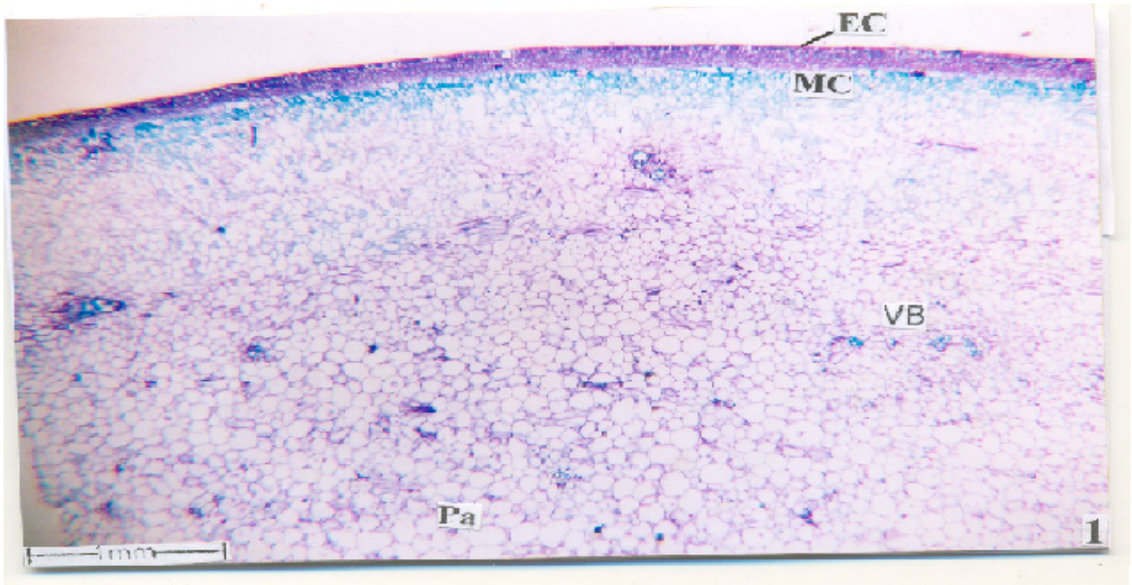


Figure 1.1 T.S. of fruit under low magnification Where: VB – Vascular bundle, MC – Mesocarp, EC – Epicarp, Pa – Palisade cells.

Vascular strands are scattered within the thin walled parenchymatous tissue. The vascular strands consist of one or two wide, thick walled meta xylem elements and proto xylem elements. Small groups of phloem elements occur on the outer part of the meta xylem elements (fig. 1.2). The meta xylem elements are 70µm wide.

When the section of the fruit wall is viewed under the polarized light microscope the lignified cells of the inner mesocarp tissue exhibit scalariform piths, their walls also appear bright denoting the lignin content of the cellwalls.

Physicochemical constants

Table 1.1 Various Ash values of crude drug.

Sr. No.	Particulars	Dried fruit pulp (% w/w)
1.	Total Ash	4.40*
2.	Acid-insoluble Ash	0.70*
3.	Water soluble ash	2.30*

* = Average of three values

Table 1.2 Various Extractive values of crude drug.

Sr. No.	Particulars	Dried fruit pulp (% w/w)
1.	Alcohol soluble extractive	6.42*
2.	Water soluble extractive	18.59*

* = Average of three values

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to know the presence of various phytochemical constituents. Sterols, flavonoids, phenolics and tannins found present in Petroleum ether (PE) and Ethanolic (ETH) extract, saponins present was noted in ETH extract.

DISCUSSION

Macroscopic, microscopic characters and various physicochemical constants such as total ash, acid insoluble ash, water soluble ash, water and alcoholic extractive value of LS fruits were studied and it may be important tools for the identification, authentication and standardization of the plant. Ethanolic extract of LS fruits contain saponins, which may be responsible for various biological activities right from anti-inflammatory to anticancer.

CONCLUSION

Preliminary pharmacognostic evaluation of *Lagenaria siceraria* fruits reveals the presence of flavonoids, saponins and steroids which play vital role in the manifestation of different disease conditions. The further study will be done for isolation and characterization of different phytoconstituents and its probable biological activities.

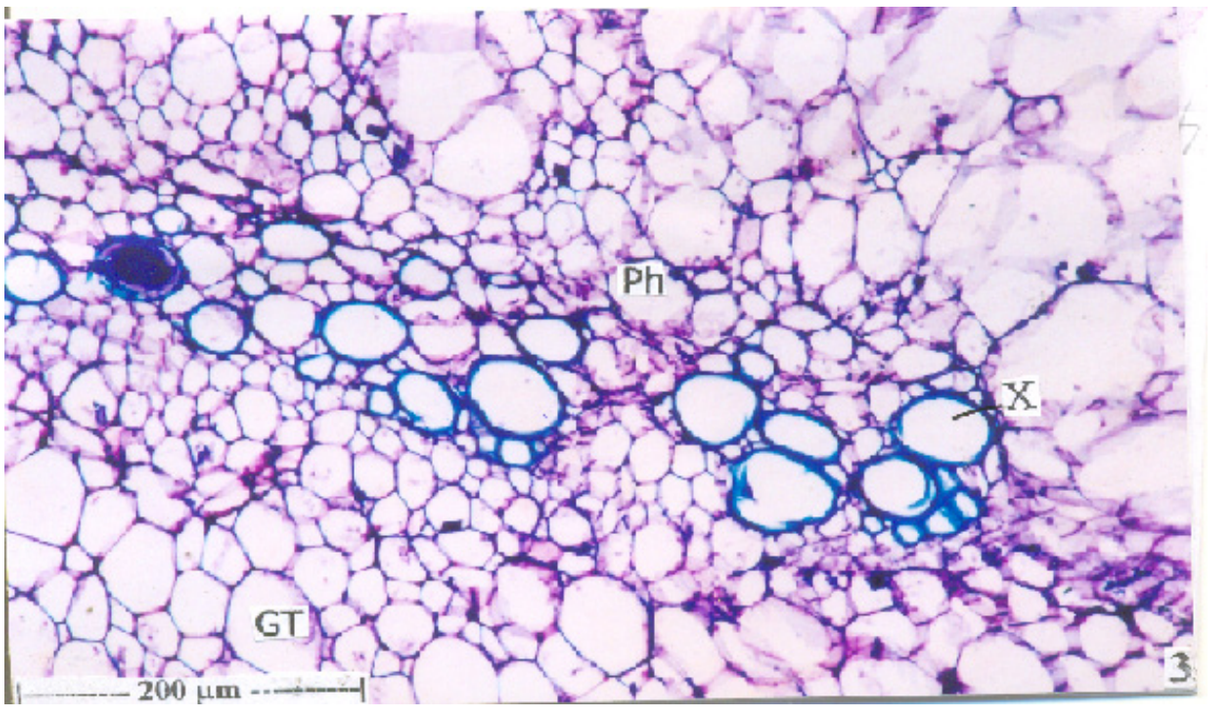


Figure 1.2 T.S. Showing Vascular strands in the parenchymatous tissue in mesocarp Where: Ph – Phloem, X – Xylem, GT – ground tissue.

REFERENCES

1. Anonymous, *Indian Pharmacopoeia*, *Ministry of Health and family welfare, Govt. of India.* Controller of Publications, New Delhi; 1996. II A:53–54.
2. Chopra RN, Chopra IC, Verma BS. *Suppliment to Glossary of Indian Medicinal Plants*. NISC, CSIR; 1998. 51.
3. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. IInd Edn., Bishen Singh and M.P. Singh Publishers, Dehradun; 2003.1116–1119.
4. Rastogi RP. *Compendium of Indian Medicinal Plants.* 1970; 2: 403.
5. Longmann O. *Indian Medicinal Plants*: *Compendium of 500 Medicinal Plants.* 2003. 292–296.
6. Maheshwari JK. *Ethnobotany and Medicinal Plants of Indian Subcontinent.* Scientific Publisher (India); 2003; 273–281.
7. Nadkarni KM. *Indian Materia Medica*, *I.* Bombay Popular Prakashan, IIIrd revised Edition; 1982; 721–723.
8. Nadkarni KM. *Indian Plants and Drugs with their Medicinal Properties and uses.* Srishti books Distributors, New Delhi; 2002; 216.
9. Sass JE. *Elements of Botanical Michrotechnique*. Mc Graw Hill Book Co; New York; 1940; 222.
10. Johansen DA. *Plant Michrotechnique.* Mc Graw Hill Book Co., New York; 1940; 523.
11. O'Brien TP, Feeder N, Mc Cull ME. *Polychromatic staining of plant cell walls by Toluidine Blue-O., Protolasm*. 1964; 59:364–367.
12. Easu K. *Plant anatomy*, John Wiley and Sons, New York; 1940; 767.
13. Khandelwal KR. *Practical Pharmacognosy Techniques and Experiments.* 10th Edn. Nirali Prakashan, Pune; 2003; 149–158.
14. Trease and Evans, *Pharmacognosy*, 15th Edn, W.B. Saunders Publishers, London; 2002.
15. Shirwaikar A, Shrinivasan KK. Chemical investigation and antihepatotoxic activity of the fruits of *Lagenaria siceraria. IJPS*.1996; 58(5):197–202.

Pharmacognostical studies on stem bark of *Canarium strictum* Roxb

Ragunathan Muthuswamy*[,a] and R. Senthamarai[b]

[a]*Department of Pharmacognosy, Nehru College of Pharmacy, Pampady, Thiruvilwamala, Thrissur Dt. Kerala-680 597, India*
[b]*Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences, Periyar Centenary Educational Complex, K. Sathanoor Main Road, Tiruchirappalli-620021, Tamilnadu, India*

ABSTRACT

Aim & Background: Resin of *Canarium strictum* Roxb., is an imperative commodity in traditional medicine in South and South East Asia. The current study aims to establish the quality control parameters for the bark as it secreted more useful resin. **Methods:** Anatomical studies and physiochemical evaluation of the bark was carried out according to the standard procedure was given in WHO/QCMMP guidelines and Indian Ayurvedic Pharmacopoeia. The anatomical studies of tissues were taken as photographs with different magnifications by using Nikon lab photo 2 microscopic Unit. The elemental analysis was done by using Perkin Elmer 5000 an atomic absorption spectrophotometer. **Results:** The different cell components were studied and measured quantitatively. The calcium oxalate prismatic crystals were estimated about 10×10 or 10×5μm in size. The sclereids were very long of unlimited length and 10μm in thickness. The long narrow lignified fibers has been found and estimated about 210–260μm long and about 10μm thick. The height of the ray is up to 350μm in height and 60μm in breadth. The physiochemical parameters such as total ash and acid insoluble ash (5.52% w/w, 2.66% w/w, respectively), extractive values (aqueous 4.55% w/w and alcoholic 6.05% w/w), foreign organic matter (2.4%) and loss on drying (7.09% w/w) were also estimated. An elemental analysis result shows the quantity of elements (μg/g) were present in the bark powder. Among the elements Mn-73.6, Cu-65.4, Cr-49.5 were major contents, while Pd-25.6 and Zn-35.4 were the minor contents. **Conclusion:** The current study report will be unique finger print for microscopical evaluation of bark of this tree and also used to differentiate the plant species among *Canarium* L.

Keywords: Burseraceae, Western Ghats, Quality control, Siddha medicine, Black dammer, Rheumatism.

INTRODUCTION

Canarium L. belongs to the family of Burseraceae Kunth., in the order Sapindales Juss. ex Bercht. & J. Pearl. This family comprises of 18 genera and estimated about 700 species of tropical trees.[1] *Canarium strictum* species producing resin, is a rich source for making fragrance smoke (Sambrani or Black dammer) and it is given for the treatment of bronchial diseases and orally given resin powder used to cure rheumatism and it is one of the major drug in Siddha medicine.[2] Black dammer resin is also used as an alternate for burgundy pitch in making medical plasters.[2,3] Extreme usage of resin in industry as well as in traditional medicine, the species currently positioned on the IUCN red list as endangered species in the regions of Tamilnadu, Kerala and Karnataka state in South India.[2]

Canarium strictum Roxb. Syn *C.* sikkimense King (Burseraceae) yields the resin, which is harvested from evergreen forest Nilgiri Biosphere Reserve (NBR) and throughout Western Ghats of Kerala, a biodiversity centre.[4] *Canarium sikkimense* King is called as gogul dhuup in Nepal.[5] Black dammar is being used traditionally to treat rheumatism, asthma, coughs, fever, epilepsy, chronic skin diseases and hemorrhage.[6]

The black dammer resin which is collected from wounded trunk of the tree contains triterpenoids such as α-amyrin β-amyrin, β-amyrin acetate, (+) junenol, canarone, *epi*-khusinol and -Ψ-taraxasterol and epi-Ψ-taraxastane diol.[7] Resin and its isolated compounds exhibited anti

***Corresponding author.**
Prof. Ragunathan Muthuswamy, Department of Pharmacognosy, Nehru College of Pharmacy, Pampady, Thiruvilwamala-680597, Thrissur district, Kerala state, India,

E-mail: ragunathranilmonica@gmail.com

DOI: 10.5530/pj.2014.1.3

inflammatory, analgesic and anti-bacterial and anti-fungal activities.[8,9]

Unlike many other *Canarium* species; the fruits of *C. strictum* are not edible. The harvest of black dammar is permitted for trade in Kerala, but in Tamil Nadu, as a conservation measure, harvest is permitted only for personal or home use. The resin is important worship item for tribal groups of nilgiris district, particularly baduga they hold 3 to 4kg in home and they collect resin only from female tree rather than male.[4]

No studies were carried out on the synthesis of black dammar, synthesis of most of the constituents of resin in *Canarium* species, is considered to be produced by making incision over the bark.[10] As an alternative of selecting other part of this plant for setting quality control parameters, choosing stem bark was found to be significant as it is secreted more useful resin. As a result the current study has decided to investigate the anatomical, physicochemical characters and presence of elements in much used bark of the *C. strictum*.

MATERIALS AND METHODS

Collection of specimens

The fresh bark was collected early in the morning during the rainy season in July 2011 from the tall tree of *Canarium strictum* in Rayairath garden, Pattikadu, Thrissur district, Kerala. The plant material was taxonomically identified by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai. The voucher specimen (No.PARC/2010 /1475) was deposited in medicinal plant documentation unit in pharmacognosy and phyto chemistry department, Nehru College of Pharmacy, Pampady, Thiruvilwamala-680597, Thrissur district, Kerala state, India as future reference. For anatomical studies, the collected bark was fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Alcohol-90ml). After 24 hrs of fixing, the specimen was dehydrated with graded series of tertiary–Butyl alcohol (TBA).[11] Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58–60 C) until TBA solution attained super saturation.[12] The specimen was cast into paraffin blocks.

Sectioning

The paraffin embedded specimen was sectioned with the help of Rotary Microtome. The thickness of the sections was 10–12µm. De waxing of the sections was done by standard procedure.[13] The sections were stained with toluidine blue,[4] as it is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary, sections were also stained with safranin and fast-green and iodine (for Starch). Glycerine mounted temporary preparations were made for cleared materials. Powdered materials of different parts were cleared with sodium hydroxide solution and mounted in glycerine medium after staining.[11] Different cell components were studied and measured quantitatively.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have finger print property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Anatomical features of description were as given from the standard anatomy book.[15,16]

Histochemical colour reactions

The micro-chemical tests for histological region were performed according to the standard methods.[13,17–19]

Behaviour of powder with different chemical reagents

The powder material was treated with different chemical reagents to detect the phytoconstituents with colour changes under ordinary daylight by the standard method.[20]

Colour and consistency of extracts

Colour and consistency of extracts were observed by standard method.[20]

Estimation of inorganic constituents

To calculate the inorganic metal content, 1g of the completely dried powder sample was digested with concentrated nitric acid and perchloric acid (3:1) until a clear solution was obtained. After cooling, the solution was made up to a specific volume with demineralized water and analyzed in an atomic absorption spectrophotometer (Perkin Elmer 5000).[21]

Fluorescence analysis of powder and extracts

The bark extracts were examined and analyzed in daylight, short and long UV light for fluorescence, according to the standard methods.[22]

Determination of physicochemical parameters

Loss on drying, Water soluble extractive value, Alcohol soluble extractive value, Foreign organic matter, Total ash value, Determine the pH of 1% crude drug solution. Crude fiber content of bark of *Canarium strictum* Roxb., were evaluated according to the standard procedures.[23–25] Preliminary phytochemical screening evaluation was carried out by using standard procedure.[26–28]

RESULTS

Physicochemical evaluation report was depicted in table 1&2. The coarse dried bark powder was extracted with hexane, chloroform and ethyl acetate and ethanol by successive solvent hot percolation method. The percentage of yield of each extract was calculated and its color, consistency was noted. Elemental analysis was carried out by standard method. The percentages of water and alcohol soluble matter were determined and all these results were depicted in table 1.

Qualitative phytochemical analysis of successive solvent extracts was performed; the report shows the presence of triterpenoids in hexane, chloroform and ethanol extracts and flavanoids phenolic compounds and tannins and saponins present in ethyl acetate and ethanol extracts. Almost all the successive solvent extracts exhibited yellowish green colour fluorescence both in short as well as long UV. The dried bark powder shows the pale yellow colour in day light. Histochemical colour reaction of the bark powder of *Canarium strictum* shows the presence of lignin and tannins and calcium oxalate. The starch and proteins were absent.

In florescence analysis, both in long and short UV, pale yellow colour was exhibited by hexane, chloroform and ethanol extracts but in contrast ethanol extract shows the yellowish black in short UV and blackish brown in long UV. The aqeous extract shows the pale yellow colour in short UV and yellowish brown in long UV.

Table 1. Physicochemical values of bark of Canarium srictum Roxb.

	Parameters	Results
I	Organoleptic characteristics	
	Appearance	Powder
	Colour	Pale yellow
	Odour	Characteristics odor
	Taste	No taste
II.	Loss on drying	7.09%
III.	pH values	
	pH of 1% aqueous solution	6.56
IV.	Ash values	
	Total ash	5.52 %w/w
	Water soluble ash	2.66 %w/w
	Acid insoluble ash	1.76%w/w
	Sulphated ash	2.63 %w/w
V.	Alcohol soluble matter	6.05%w/w
VI.	Water soluble matter	4.55%w/w
VII.	Successive extractives	
	Hexane extract	0.19%w/w
	Chloroform extract	0.39%w/w
	Ethanol extract	6.95%w/w
	Aqueous extract	4.23%w/w
VIII	Crude fiber content	53.5%w/w
IX.	Foreign organic matter	2.4 %w/w
X.	Inorganic constituents present in dried bark of *Canarium srictum*	Quantity of elements (µg/g) in dried powder
	Zn	35.4
	Mn	73.6
	Cu	65.4
	Cr	49.5
	Pd	25.6

N-Hexane, chloroform and ethyl acetate extracts were shows solid consistency where as alcohol extract was in semi solid consistency. Table 2 depicted the behavior of the bark powder with different chemical reagents.

Microscopical characters of transverse sections and powder microscopical characters of bark of *Canarium strictum* were studied and the findings were described here under with related photographs were shown in different panel of figures.

Microscopical characters of Canarium strictum stem bark

The surface of the bark is rough and irregularly fissured. The periderm peels off in to irregular pieces. (Fig. 1) Inner to the periderm occur thick zone of collapsed phloem which includes thick tangential blocks of sclereids and long darkly stained thick tangential lines of collapsed phloem. Inner to the collapsed phloem zone is a narrow

Table 2. Fluorescence characteristics of powdered bark of *Canarium srictum* Roxb.

S.No	Treatment with Reagents	Under Ordinary light	Under UV light	
			Long wavelength	Short wavelength
I	Powder as such	Pale yellow	Pale yellowish	Pale Yellowish
II	Dry powder was placed on glass slide affixed with nitrocellulose	pale Yellow	Yellow	Pale Yellow
I II	Powder treated with 1M NaOH in Methanol	Pale Reddish	Dull reddish brown	reddish brown
IV	Powder treated with 1N NaOH in Methanol, dried and then mounted in Nitrocellulose in Amylacetate	Pale reddish	Reddish black	Reddish black
V	Powder treated with 1M HCl	No change	No change	No change
VI	Powder treated with 1M HCl, dried and then mounted in nitrocellulose in amylacetate	No change	No change	No change
VII	Powder treated with 1M NaOH in water	Pale Reddish	Dark Reddish	Dark reddish
VIII	Powder treated with 1M NaOH in water, dried and then mounted in nitrocellulose in amylacetate	Reddish brown	Pale Reddish	Reddish black
IX	Powder treated with 50% HNO3	Yellow	Yellowish green	Yellowish green
X	Powder treated with 50% H2SO4	No change	No change	No change

Figure 1. T.S. outer bark showing flakes of periderm and collapsed phloem with sclereids and crushed tangential bands of sieve elements. Cph Collapsed phloem, NCPh Non collapsed phloem, Pe- Periderm, SC- Secretary cavity, Scl- Sclereids.

zone of non collapsed phloem. In this zone there are only limited numbers of small sclereid masses. The phloem cells are intact and fairly well preserved. (Fig. 1)

In the collapsed phloem the parenchyma cells are dilated, while the sieve elements are compressed into thick tangential lines. The sclereids are branchy sclereid type with thick lignified walls and dense simple pith.

Calcium oxalate crystals are fairly abundant in the collapsed phloem zone. The crystals are either prismatic type or druses. The crystals are mostly associated with the sclereids bands of the collapsed phloem.

In tangential longitudinal sectional view TLS the characters of the phloem rays were studied. The phloem rays are mostly biseriate or three seriate, short and spindle shaped. The rays are hetrocellular (Fig. 2.1, 2 & 3). The ray consists of long conical upright cells and upper and lower ends.

The middle portion consists of circular or angular compact procumbent cells. The rays are not storied. The height of the ray is up to 350µm in height and 60µm in breath. The axial parenchyma cells are seen in vertical row of long cylindrical cells. The sclereids are also visible in TLS.

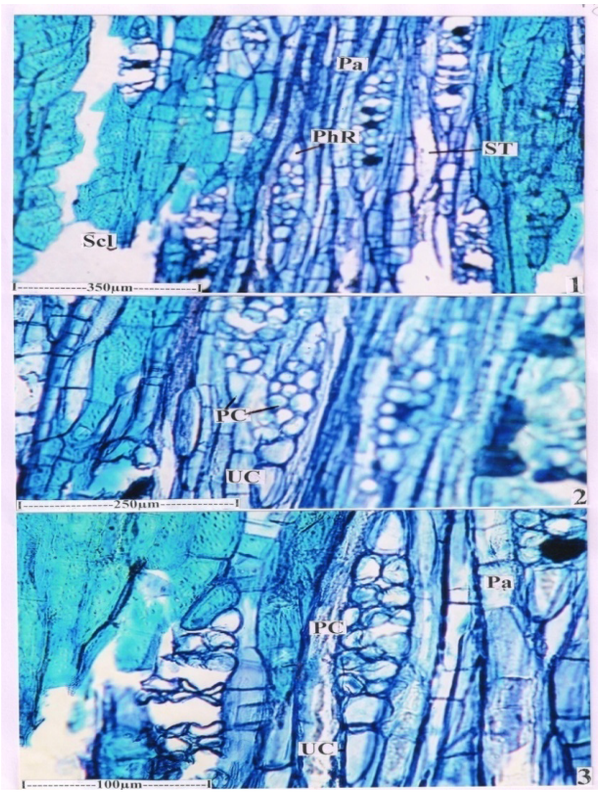


Figure 2. Panel 2.1&2.2 shows TLS view of the phloem showing hetero cellular rays and PhR- Phloem ray, SCl- Sclereids, ST- Sieve tube, Panel 2.3 shows one ray enlarged Pa-Parenchyma, PC- Procumbent cells, UC-Upright cells.

In Radial longitudinal section RLS view the rays appear in horizontal bands. The rays have upright cells on the upper and lower ends and horizontally oriented procumbent cells with wavy cell walls. The parenchyma cells are seen in vertical strands and the cells are vertically elongated and thin walled. Sclerenchyma elements are also seen in vertical bundles.

Calcium oxalate prismatic crystals are abundant in the collapsed phloem. They are mostly located within the lumen of sclerenchyma cells. (Fig. 3.1 & 2) The crystals are 10×10 or 10×5µm in size.

Powder microscopy

In the powder of the bark the following elements were observed.

Fibre sclereids

These are fiber like in length and thickness. However the cell walls are very thick and the cells lumen is very narrow. The sclereids are very long of unlimited length and 10µm in thickness.

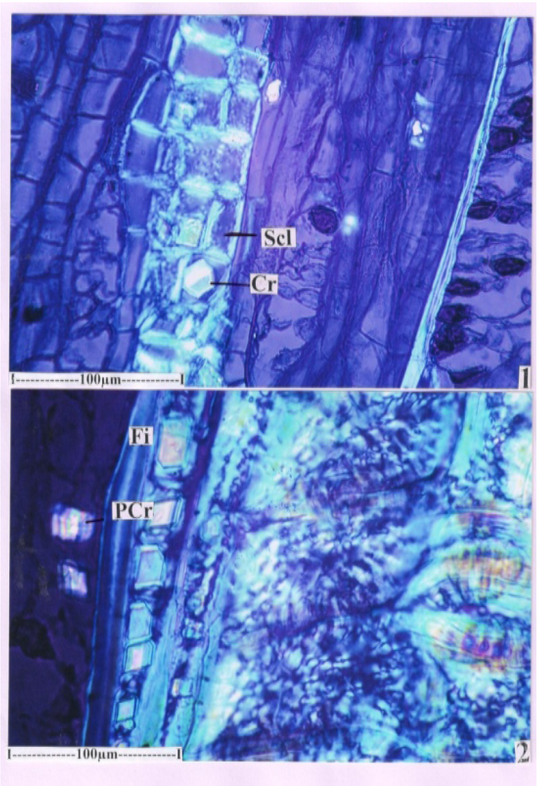


Figure 3. Prismatic calcium oxalate crystals located within the lumen of the sclerenchyma cells.Cr- Crystal, Fi- Fiber, PCr- Prismatic crystal, Scl- Sclerieds.

Crystals

Prismatic crystals of calcium oxalate are seen associated with fibers of sclereids. The crystals are located within the lumen of the fibers or outside the fiber, located in the axial parenchyma cells. These crystals are either cuboidal or rectangular in shape. The crystals are also seen away from the cells and scattered in the medium. The free crystals are different type of prismatic crystals.

Sclereids

Branchy sclereids are abundant in the powder. They are cuboidal or rectangular; they have thick lignified cell walls and narrow lumen. Dense simple pits are seen on all walls of the sclerieds.

Fibers

Long narrow lignified fibers are also seen commonly in the powder. The fibers are all narrow type. They have thick lignified walls. The fibers are all narrow type. They have thick lignified walls and pointed ends. Pits are not evident in the fibers. The fibers are 210–260μm long and about 10μm thick.

DISCUSSION

In the view of fact that resin is created by trees to defend against possible damage from abiotic or biotic stress. According to the type of species resin might be gathered in resin canals or resin pockets in the wood or the bark. In number of species peripheral rows of distressing resin canals are induced after wounding. Resin canals (axial or radial) are extended extracellular structures that allow long distance resin transport. Resin usually secreted through secretory cells known as 'epithelium' that surrounds resin canals or resin pockets.[29] Black dammer resin can be collected after making injury of outer bark shown that resin secretory structure may be in radial resin canals also near xylem and phloem and sclerenchyma part of the inner bark. Bark and wood of the tree found to be fibrous has 53.5% of crude fiber content.

During our Nilgiri Biosphere Reserve (NBR) visit we found that the dark brown colour resin was collected from the wounded trunk of the tree was given orally in the form of decoction, twice a day used to treat body pain and arthritis and the resin in oily preparation named as "Sivappu kukkil" is applied externally to treat skin inflammation. In eastern Arunachal Pradesh, Khamti tribal were applied melted resin over the skin to reduce the inflammation induced by contact poison of brown hairs of caterpillar.[30]

Entire plant excluding root of *Canarium strictum* alcoholic extracts was evaluated biologically for various activities such as Antibacterial; Antifungal; Antiprotozoal; Anthelmintic; Antiviral; Anti-implantation in rats; Hypoglycemic; Effect on cardiovascular system; Effect on respiration; Effect on blood pressure; Effect on preganglionically stimulated nictitating membrane; Heart rate; Effect on acetylcholine; Effect on adrenaline; Effect of histamine on guinea pig ileum; Effect on central nervous system and gross behavior Gross effects, analgesia; Supramaximal electroshock seizure pattern test.[31] Apart from root the whole plant was not exhibited any potent biological activity.

Literature shows that the genus *Canarium* L. contains terpenes which include monoterpenes, triterpenes and tetraterpenes like carotenoids, sesquiterpenes, cyclohexane and sterols and carboxylic acids, coumarins, furans, lipids and phenolic compounds such as flavonoids, tannins, phenolic acids.[1] Our studies found through the qualitative analysis the presence of flavonoids, phenolic acids saponins, lignin and tannins and terpenoids and calcium oxalate in the bark.

The cheapest way to identify the plant and to develop monograph is to studies of anatomical characters including macro and microscopical characters are fundamental of Pharmacognostic evaluation of crude drugs.[32]

CONCLUSION

The current study has been established the anatomical character and physicochemical characters and quantitative measurement of cell components and elemental analysis of the bark. The data we provided can be considered as finger print towards correct identification of *Canarium strictum* from other plant species of the same genus *Canarium* L. By using GCMS, the current study will be extended to investigate the presence of terpenes in the essential oil which is distilled from the resin of the tree. The major terpene of the essential oil will be isolated and screened for antinociceptive activity to prove folkloric report. Optimistically this study provided the information which is helpful to develop the official monograph of *Canarium strictum* Roxb.

ACKNOWLEDGEMENT

We thank Mr. Rayairath, Rayairath herbal garden, Pattikadu, Thrissur, Kerala, India to provide all the facilities to

collect plant parts and the resin from his herbal garden. We are grateful to Dr.Sivakumar General Manager, SKM Ayurveda, Siddha Pharmaceuticals, Erode for providing ancient palm script literature and encouragement all the way through the work.

ABBREVIATIONS

IUCN- International Union for Conservation of Nature, WHO-World Health Organization, QCMMP-Quality control methods for medicinal plant materials, PARC-Plant anatomy research centre, Zn- Zinc, Mn-Magnesium, Cu-Copper, Cr-Chromium, Pd-Lead, UV-Ultraviolet, NBR-Nilgiri Biosphere Reserve, GCMS- Gas chromatography mass spectra, RLS-Radial longitudinal section, TLS-Transverse longitudinal section, FAA-Formalin Acetic acid Alcohol, TBA tertiary–Butyl alcohol, Cph Collapsed phloem, NCPh-Non collapsed phloem, Pe- Periderm, SC- Secretary cavity, Scl- Sclereids PhR- Phloem ray, ST-Sieve tube, Pa-Parenchyma, PC- Procumbent cells, UC-Upright cells, Cr- Crystal, Fi- Fiber, PCr-Prismatic crystal, Scl- Sclerieds, g-gram, µg-Microgram.

REFERENCES

1. Mogana R, Wiart C. *Canarium* L. A Phytochemical and Pharmacological Review. J Phar Res. 2011; 8:2482–9.
2. Desha Meena, Nagarajan Binaibabu, Jesubalan Doss. Future prospects for the critically endangered medicinally important species, *Canarium strictum* Roxb. A Review. Int J Conser Sci. 2012; 3(3):231–7.
3. Edson S. Bastin. Botanical medicine monographs and sundry structure of Cimicifuga. Amer J Pharmacy.1895; 67(3):8.
4. Anita Varghese, Tamara Ticktin; Resilience Alliance. Regional Variation in Non-Timber Forest Product Harvest Strategies, Trade, and Ecological Impacts: the Case of Black Dammar (*Canarium strictum* Roxb.) Use and Conservation in the Nilgiri Biosphere Reserve, India. Ecology and Society.2008;13(2):11. Available from: http://www.ecology and society.org/ vol13/iss2/ art11/
5. Khare CP. Indian Medicinal plants, an illustrated dictionary. New York: Springer; 2007.
6. Ravikumar K, Ved DK. Illustrated field guide: 100 Red Listed Medicinal Plants of Conservation Concern in Southern India. Bangalore: Foundation for the Revitalization of Local Health Traditions (FRLHT); 2000.
7. Hinge VK, Wagh SK, Bhattacharyya SC. Constituents of Indian black dammer resin. Tetrahedron.1965; 21:3197–203.
8. Suruse PB, Duragkar NJ, Shivhare UD, Kale MK, Bodele SB. Study of antibacterial activity of *Canarium strictum* gum resin. Res J Pharmacog Phytochem. 2010: 2:435–7.
9. Suruse PB, Bodele SB, Duragkarn J, Kale MK. Anti inflammatory and analgesic activities of isolated compounds from *Canarium strictum*. J Cell Tissue Res.2008; 8(3):1481–4.
10. Langenheim, JH. Plant resins: Chemistry, Evolution, Ecology, Ethno botany. Portland, Oregon, USA: Timber Press; 2003.
11. Sass JE. Elements of botanical micro technique. New York: Mc Graw Hill; 1940.
12. Kraus JE, Arduin M. Manual basico de metodos em morfologia vegetal. Editora da universidade rural, Seropedica; 1997.
13. Johansen DA. Plant Micro technique. New York: Mc Graw-Hill; 1940.
14. O'Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell walls by toluidine blue-O.Protoplasma.1964; 59:364–73.
15. Easu K. Plant Anatomy. New York: John Wiley and sons: 1965.
16. Easu K. Anatomy of seed Plants. New York: John Wiley and sons; 1979.
17. Kay LA. The Microscopical Studies of Drugs. 1st ed. London: Bailliere and Cox: 1938.
18. Trease GE, Trease WC. Pharmacognosy. 10th ed. London: Bailliere Tindall; 1972.
19. Wallis TE. Text Book of Pharmacognosy. 5th ed. London: J & A Churchill Ltd; 1967.
20. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc. 1949; 38:324–33.
21. Vogel AIC. A Text Book of Macro and Semi-Micro Qualitative inorganic analysis. London: Longman, Green- Co.Ltd; 1953.
22. Kokoski J, Kokoski R, Salma FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Am Pharm Assoc. 1958; 47:715–7.
23. John SS, Joseph S, James M, Joseph L, Surendran CN, Dhanabal SP, Suresh B, et.al. Pharmacognostical studies on Costus mexicanus (Costaceae).Hamdard Medicus.2009; 52(1):147–52.
24. WHO. Quality Control Methods for Medicinal Plant Materials. Organization Mondiale De La Sante. Geneva; 1992.
25. Government of India. The Ayurvedic Pharmacopoeia of India. 1st ed. Ministry of Health and Family Welfare, Department of Indian Systems of Medicines and Homeopathy, New Delhi, p. A53–A55; 1996.
26. Kokate CK. Practical Pharmacognosy, 1st ed. New Delhi (India): Vallabh Prakashan; 1994.
27. Harbone JB. Methods of extraction and isolation, In: phytochemical methods. London: Chapman and Hall, p. 60–66: 1998.
28. Mukherji PK. Quality control of herbal drugs-An Approach to evaluation of botanicals.1st ed. New Delhi: Business Horizons; 2002.
29. Motuma T, David M, Ute SK, Frank JS, Paul C, Frans B. Resin secretory structures of *Boswellia papyrifera* and implications for frankincense yield. Annals of Botany.2013; 111:1–8.
30. Hui tag, Das AK, Hari loyi. Anti-inflammatory plants used by the khamti tribe of lohit district in eastern Arunachal Pradesh, India. Nat prod radiance. 2007; 6(4):334–40.
31. Goel AK, Kulshreshtha DK, Dubey MP, Rajendran SM. Screening of Indian plants for biological activity Part XVI. Ind J Exp Bio.2002; 40:812–27.
32. Samuelsson G. Drugs of Natural Origin. Sweden: Swedish Pharmaceutical Press; 1999.

Morphological and Anatomical Investigations of *Ecbolium viride* (Forssk.) Alston

Francina Cecilia Kalaimaran and Ravindhran Ramalingam*

T.A.L. Samy Unit for Plant Tissue Culture and Molecular Biology, Department of Plant Biology and Biotechnology, Loyola College, Chennai 600 034, India

ABSTRACT

Background: *Ecbolium viride* commonly known as green shrimp is a medicinal plant that grows well in the tropical and temperate regions of the world. International criteria for validation and standardization of herbal product as phytomedicine include the integration of microscopic characteristics of herbal drug to guarantee its authenticity. **Objective:** The present study was therefore undertaken to evaluate the macroscopic and microscopic characters of the leaves and roots of *E. viride*, used in traditional medicine for treating jaundice. **Methods:** Leaf and root sections of *E. viride* were sectioned with rotary microtome and microscopical features were examined using bright field microscope. **Results:** Anatomical characteristics such as crystal distribution in leaf, secondary xylem and vessel elements in root, glandular and non-glandular trichome in powder microscopy are some of the unique features of the plant with taxonomic value. The most distinguishing anatomical feature of this plant is the presence of cystolith calcium carbonate crystals. **Conclusion:** Morphological and anatomical parameters presented in this paper would serve as a useful gauge in ensuring quality formulations of drug preparation and in determining the genuineness of the drug.

Keywords: *Acanthaceae, Cystolith, Ecbolium viride, Leaf microscopy, Trichome*.

INTRODUCTION

Plants form the basis for traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind.[1] Plants are rich in bio-active constituents with potential therapeutic activities. The foremost step in using plant based drug involves their correct identification based on macroscopic and microscopic characters. Today this simple approach in establishing the identity of plants is highly neglected in the validation of plant drugs.

Ecbolium viride (Forssk.) Alston is a perennial woody shrub which belongs to the family, Acanthaceae. It is commonly known as Green Shrimp, Green Ice Crossandra, Turquoise Crossandra, Sahachara and Blue Fox Tail Nail Dye. In Tamil, it is called as Nilambari and in Sanskrit, Neela Sahachara. It is distributed in India and also in Arabia, Malaysia, Sri Lanka and Tropical Africa. The whole plant is used in traditional medicines of Siddha, Ayurveda, Unani and Folk. Leaf has been used for the treatment of fever,[2] gout, dysuria[3] and stricture.[4] In traditional medicine, the aqueous extract of dried roots of the plant has been used in treating jaundice,[5] rheumatism[2] and menorrhagia.[6,7] Phytochemicals such as luteolin, orientin, isoorientin and vitexin with unique biological activity were isolated from the plant. Flavones and glycoflavones have also been reported from this plant.[8] The plant has been screened for various pharmacological activities like antioxidant,[9] anti-inflammatory,[10] antimicrobial,[11,12] anti-hepatotoxicity,[13,14] antiplasmodial, antitrypanosomal and antimalarial.[15] Therefore, the present study was undertaken to elucidate the macroscopical and microscopical features of the leaf and root of *E. viride*. These investigations will be useful in establishing the authenticity of *E. viride* in herbal

***Corresponding author.**
R. Ravindhran
Tel.: +91 44-28178304; Fax: 044-28175566
E-mail: raviloyola1998@gmail.com

DOI: 10.5530/pj.2014.1.4

drugs and in monitoring the quality parameters for the formulation of new drugs.

MATERIALS AND METHODS

Plant material

Healthy plants for the study were collected during the summer (June) of 2011 from Srirangam, Trichy, Tamil Nadu, India. The plant material was authenticated by Dr. P. Jayaraman, Scientist, Plant Anatomy Research Centre (PARC), Tambaram, Tamil Nadu, India. A voucher specimen of the plant (PARC/2012/1152) was deposited in the herbarium of Department of Plant Biology and Biotechnology, Loyola College, Chennai, India.

Macroscopic evaluation

Macroscopical characters like shape, size, colour and odour of the plant parts were examined.

Microscopic evaluation

Collection and preparation of specimens

The plant material was collected freshly and care was taken to select healthy and disease free plant parts. The leaf and root samples were fixed in FAA (Formalin 5ml + Acetic acid 5ml + 70% ethyl alcohol 90ml). After 24h of fixing, the plant specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA) as per the protocol described by Sass.[16] Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58–68°C) until TBA solution attained super-saturation. The specimens were then cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned (10–12µm thick) using rotary microtome (Wesvox Indian rotary microtome, India). The sections were dewaxed according to Johansen[17] and stained with toluidine blue polychromatic stain.[18] Other standard staining solutions like safranin, fast-green and iodine-potassium iodide were also used wherever necessary. To study the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections parallel to the leaf surface) were taken by hand peeling. Clearing of leaf was done using 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid.[16] The cleared materials were mounted on glycerin for observation.

Quantitative microscopical analysis

Quantitative leaf microscopical parameters like palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number were determined on epidermal strips as described by Wallis[19] and Trease and Evans.[20]

Powder microscopy

Leaves and roots of the plant were powdered well and passed through sieve No. 60. The powdered materials were treated with NaOH, mounted on glycerin after staining and different cell components were observed.

Photomicrographs

Photomicrographs were taken using bright field microscope attached with Nikon Labophot-2 camera under different magnifications. Polarized light was employed for the study of crystals, starch grains and lignified cells.

RESULTS AND DISCUSSION

Macroscopic evaluation

Ecbolium viride (Forssk.) Alston is an erect glabrous shrub which grows up to 2.5m in height (Fig. 1a,b). Leaves were dark green, about 14.5cm long, 6.5cm broad and elliptic to ovate in shape (Fig. 1c). Leaf apex was acute with thin and coriaceous lamina. Inflorescence was terminal or axillary. Bracts had brownish margins, imbricate, rotund, 3cm, apiculate, and bracteoles were 2, lanceolate and smaller (Fig. 1d). Calyx was pentalobed, subequal, shortly decurrent, valvate, lanceolate, 5mm, glandular pubescent and acuminate. Corolla was bluish green, 4 × 0.3cm across, tube narrowly cylindric, five lobes, imbricate, glandular pubescent with 2 lipped, upper lip linear, 1.2cm, shortly forked, lower lip spreading with 2 wing like lateral lobes, 1.5cm and acute (Fig. 1e,f). Stamens two, attached at the base of upper lip, exerted, filaments flattened, 2mm, anther cells oblong, parallel, unequal, 2.5mm and muticous. Ovary oblong to globular, bicarpellary, syncarpous, 3 × 1mm, 2 ovuled, style filiform, hairy below, 3.5cm and stigma capitate. Capsule ovoid compressed, 2 × 0.8cm with a basal peak, seeds 2, orbicular, tuberculate and retinacula curved. Roots were circular with dark fissured surface and measured 4mm in diameter (Fig. 1g,h).

Microscopic evaluation

The photomicrographs of leaf and root sections were remarkably good with staining by rendering pink colour

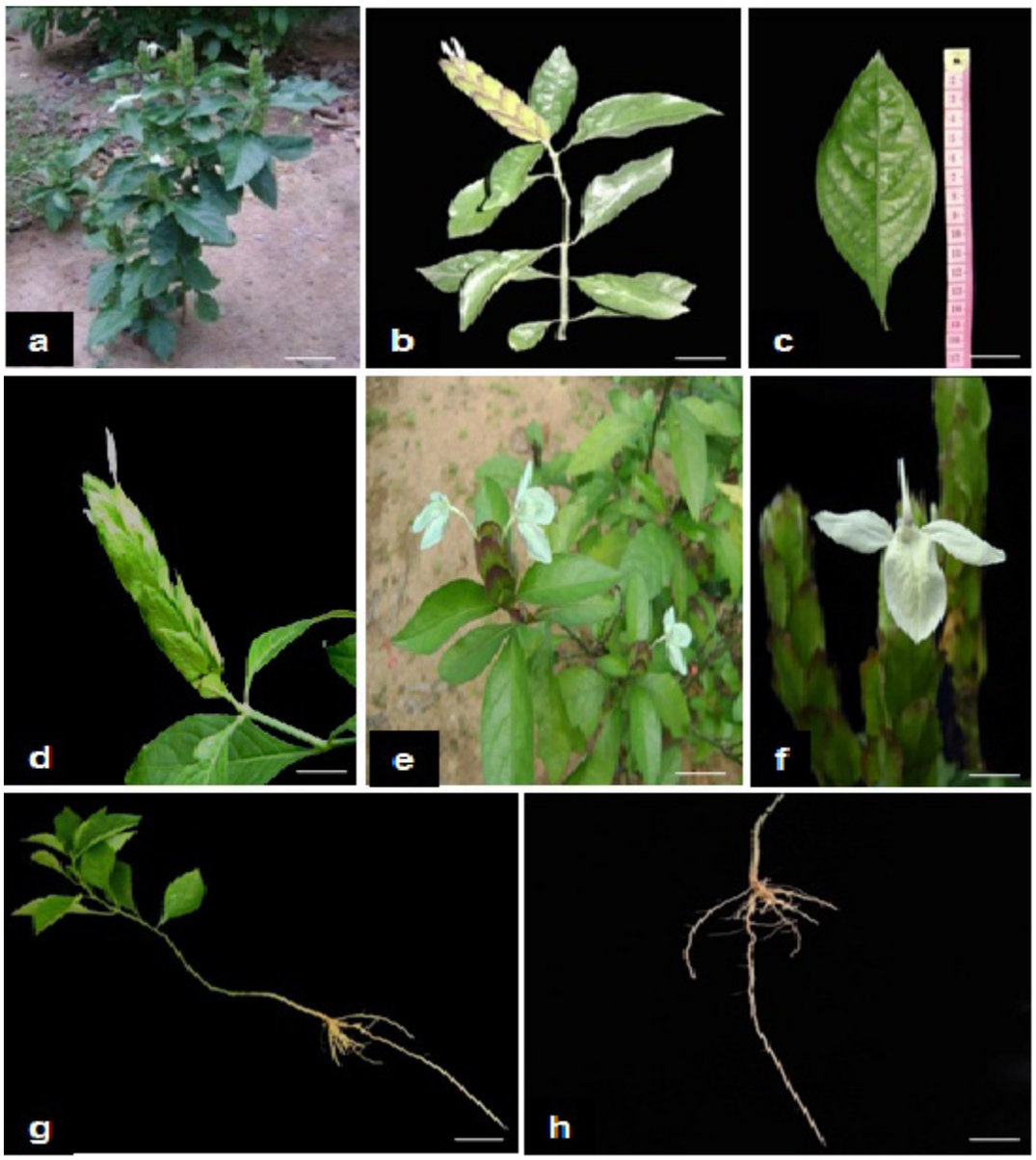


Figure 1. Morphological features of *Ecbolium viride* (Forssk.) Alston. a: Habitat. b: Plant twig. c: Individual leaf. d: Inflorescence bearing flower bud. e: Flowers. f: Enlarged portion of flower. g: Plant showing underground roots. h: Enlarged portion of root. a–h scale bar-1cm.

to the cellulose walls, blue to lignified cells, dark green to suberin and blue to the protein bodies.

Transverse section of leaf

Midrib

The leaf consisted of fairly prominent midrib which has broad semi-circular adaxial part and thick hemispherical abaxial part (Fig. 2a). The epidermal layer of the midrib was thinner than the epidermis of the lamina. The epidermal cells of the midrib were small and thick walled. Inner to the epidermis, 3 or 4 layers of small angular collenchyma cells were observed. On the adaxial part, a band of the palisade cells was transcurrent beneath the collenchyma tissue. The remaining ground tissue was parenchymatous comprising circular, compact thin walled wide cells. The vascular strand was wide and deeply bowl shaped and comprised several long, narrow files of small angular thick walled xylem elements. Phloem elements were located in small groups in close layer along the lower end of the xylem strand (Fig. 2b).

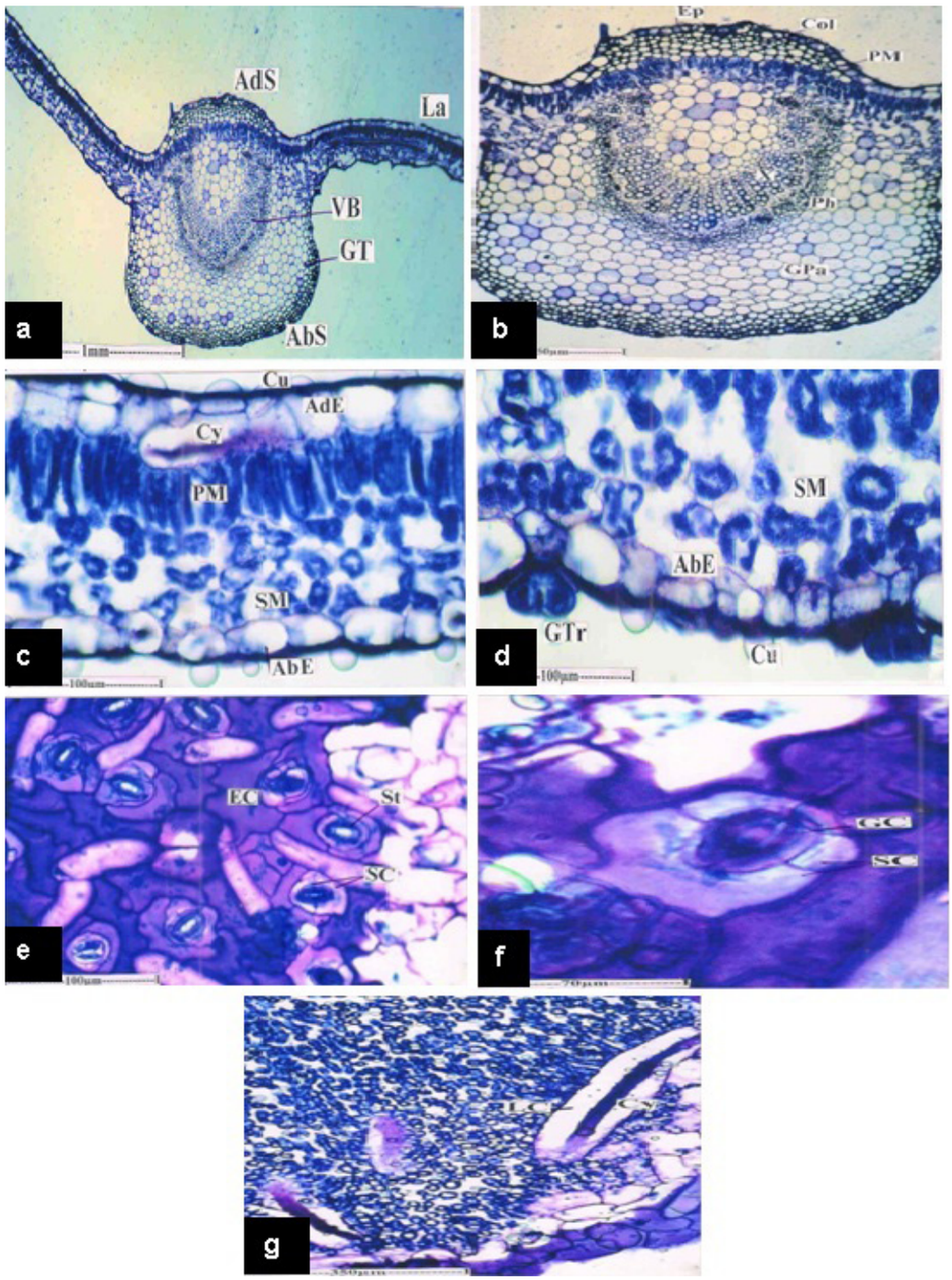


Figure 2. Transverse section of Leaf. a: T.S. of leaf through midrib. b: T.S of midrib enlarged. c: T.S of lamina showing cystolith. d: T.S of lamina showing glandular trichome. e: Paradermal section of epidermis showing stomata. f: Diacytic stoma enlarged g: Cystolith inside lithocyst. Abaxial Epidermis (AbE), Abaxial side (Abs), Adaxial Epidermis (AdE), Adaxial side (Ads), Collenchyma (Col), Cuticle (Cu), Cystolith (Cy), Epidermal cells (EC), Epidermis (Ep), Guard cell (GC), Ground Parenchyma (GPa), Ground Tissue (GT), Glandular trichome (GTr), Lamina (La), Lithocyst (LC), Phloem (Ph), Palisade Mesophyll (PM), Subsidiary cell (SC), Spongy Mesophyll (SM), Stomata (St), Vascular Bundle (VB), Xylem (X).

Lamina

The lamina was 170µm thick and distinctly dorsiventral. The adaxial epidermal cells (30µm thick) were quite thick and squarish to rectangular in shape. The cuticle was very thick with smooth surface. Some of the epidermal cells were dilated into wide horizontally elongated cells called lithocysts, which possess cylindrical cystolith (Fig. 2c),

characteristic of the family Acanthaceae as reported by Kumar and Paliwal.[21]

Glandular epidermal trichomes were occasionally seen on the abaxial epidermis (Fig. 2d). The glands (20µm high and 40µm wide) were sessile and they arise from small epidermal cells. The glands were capitate type and consist of 2–4 celled spherical darkly stained body. Such glandular trichomes were observed in *Justicia brandegeana* on the abaxial surface of leaf.[22,23]

Epidermal cells and stomata

The epidermal cells and stomata were studied from the surface view of paradermal sections of the lamina. The epidermal cells were amoeboid in outline due to waxy anticlinal walls (Fig. 2e). Stomata were dense and random in distribution. The guard cells were 12 × 15–20µm in size. The stoma was diacytic type. A stoma had two subsidiary cells; the common wall of the subsidiary cells was at right angle to the long axis of the guard cells (Fig. 2f). Lithocysts were long, wide and possessed elongated thin cylindrical calcium carbonate cystolith which was 550µm long and 35µm thick (Fig. 2g). Similar observations were made in *Trichanthera gigantea*.[24]

Venation pattern

Due to dense growth of the epidermal trichomes, the veins were not distinctly visible. The veins were thin, straight and densely reticulate with wide vein islets (Fig. 3a). The vein islets were wide, variable in size and shape and fenced by thin vein boundaries. The vein terminations were present in almost all islets. They were unbranched, long and slender. Some terminations were branched once or twice (Fig. 3b).

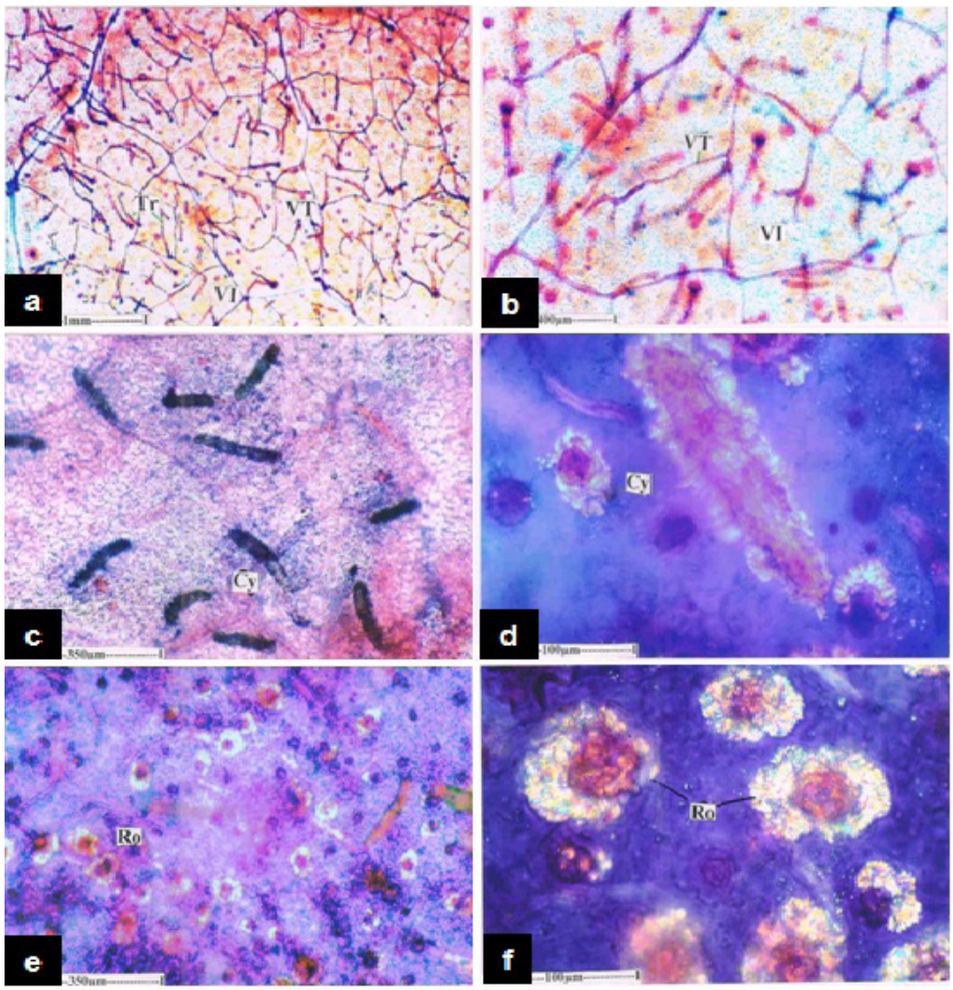


Figure 3. Venation pattern and crystal distribution of leaf. a: Surface view of lamina showing venation. b: Vein Termination and Vein Islet. c: Cystolith bodies in the mesophyll tissue. d: Single cystolith seen under polarized light. e: Lamina showing distribution of rosette type of crystals. f: Rosette crystals enlarged showing central core of organic substance surrounded by calcium oxalate crystals. Cystolith (Cy), Rosette crystals (Ro), Trichomes (Tr), Vein Islet (VI), Vein Termination (VT).

Crystal distribution

The family Acanthaceae is known for its epidermal cells that develop cystolith calcium carbonate crystals as a defense mechanism.[25] Calcium carbonate crystals were viewed under polarized light microscope. Cystoliths turned black (Fig. 3c) and the surface of the cystolith appeared echinate (Fig. 3d). Calcium oxalate crystals of rosettes were also observed. The rosettes have central core of organic substances (Fig. 3e). Calcium oxalate prismatic particles were found surrounding the central core (Fig. 3f). Crystals appeared bright under dark background. This is due to the birefringent property of crystals. Calcium oxalate crystals in the leaf cells reflect light toward photosynthetic cells, especially in shaded conditions and may aid in the survival of the plants.[26]

Quantitative microscopical analysis

The leaf constants, *i.e.* stomatal number, stomatal index, palisade ratio, vein termination and vein islet number are presented in Table 1.

Table 1. Quantitative microscopical parameters of the leaf of *Ecbolium viride* (Forssk.) Alston.

Leaf constants	Values obtained
Stomatal number in adaxial epidermis	3/mm^2
Stomatal number in abaxial epidermis	2/mm^2
Stomatal index in adaxial epidermis	40–60
Stomatal index in abaxial epidermis	40–60
Palisade ratio	6.15/mm^2
Vein islet number	22/mm^2
Vein termination number	10/mm^2

Transverse section of root

The surface of the root (1.6µm thick) was covered with thick dark crust formed by disintegrated epidermis and periderm. Periderm was one or two layered, located visibly inner to the dark surface layer (Fig. 4a). The cortex was 7–9 layered. The cortical cells were tangentially elongated and elliptical. Some of the cortical cells were dilated into elongated elliptical lithocysts. Secondary xylem (2.3µm thick) was wide and solid dense cylinder in appearance. Secondary xylem had no growth rings. The vessels were diffuse in distribution. They were distributed in several radial lines spreading from the centre towards the periphery. The vessels (10–15µm wide) were solitary, circular and thick walled. Xylem rays were fairly distinct. The ray cells were thick walled and lignified. Xylem fibers were heavily thick walled and lignified (Fig. 4b). The cell lumen was narrow. Secondary phloem was in narrow continuous zone. The phloem elements were small, compact and random in arrangement (Fig. 4c).

Powder analysis

Powder microscopy of leaf

The powder was green in colour with no characteristic odour and taste. Leaf powder was examined under the microscope and it showed the presence of fragments of lamina, glandular trichomes and epidermal tissue.

Fragments of lamina

Fragments of lamina bearing non-glandular and glandular trichomes were common in the powder. The non-glandular trichomes were dense and diffuse in distribution. The trichomes (130–300µm long and 15µm thick) were 2 or 4 celled, unbranched, narrow, long and tapering at the end. The cell walls were thick and the surface was finely echinate (Fig. 5a). Trichomes help to protect against direct sun exposure and insulate the plant from wind which further reduces evapotranspiration.[27] It also prevents small animals and insects from reaching the plant surface.

Glandular trichomes were found to be less frequent. The glands were peltate type. They have short, one-celled stalk and circular plate of multicellular head. The head (30µm in diameter) consists of four triangular cells which radiate from centre, forming a compact circular disc (Fig. 5b). The presence of glandular trichomes in many of the medicinal plants is considered indicative of the concentration of secondary metabolites with pesticidal, pharmacological and fragrant properties.[28] The presence of different types of trichomes in Acanthaceae and Asteraceae are used as an aid for taxonomical identification.[29,30]

Epidermal tissue

Epidermal tissue showing diacytic stomata were frequently seen in the powder (Fig. 5c). The epidermal cells have highly waxy anticlinal walls and the cells were amoeboid in outline. Cylindrical worm like cystolith was found diffusely distributed in the mesophyll. The surface of the cystolith was echinate. The occurrence of cystoliths in various parts of the plant has always been considered as a useful character in identification and it has often proved to be of systematic value.[31–34]

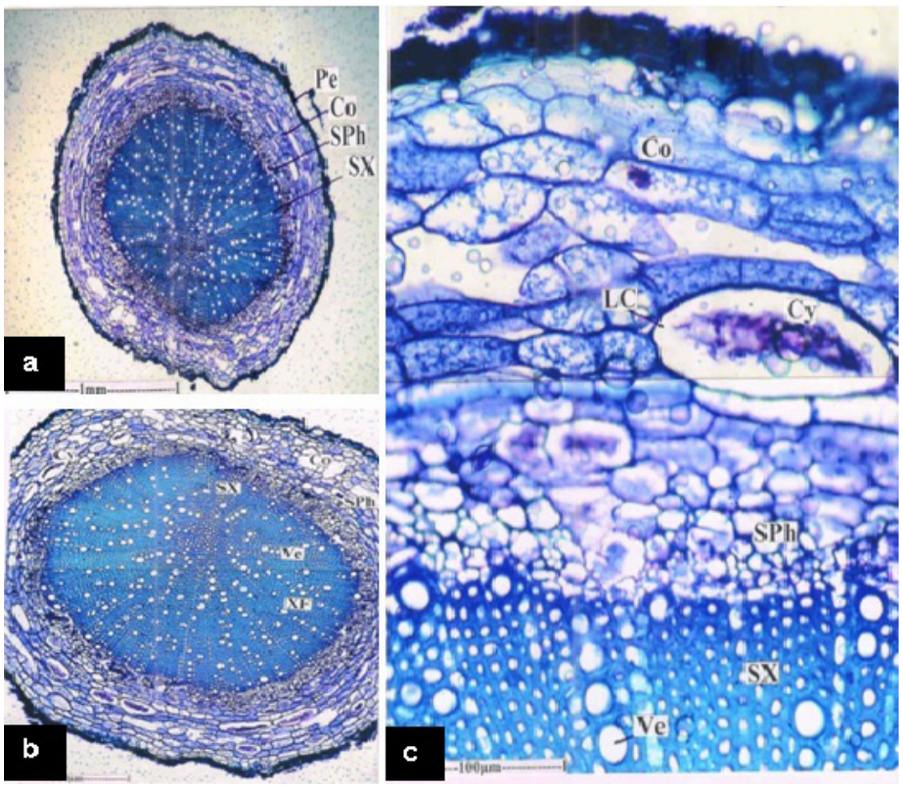


Figure 4. Transverse section of root. a: Ground plan. b: A portion enlarged showing xylem fibre. c: T.S of root, a section enlarged showing secondary xylem and phloem. Cortex (Co), Cystolith (Cy), Lithocyst (LC), Periderm (Pe), Secondary Phloem (SPh), Secondary Xylem (SX), Vessel (Ve), Xylem fibre (XF).

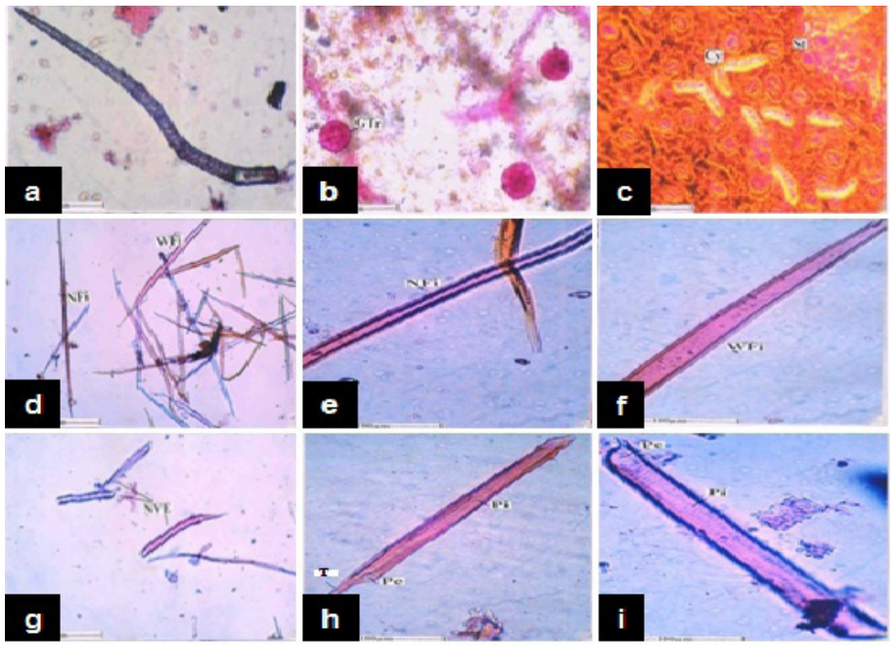


Figure 5. Powder microscopy of leaf and root. a: Non-glandular epidermal trichome. b: Glandular trichomes in surface view. c: Surface view of lamina showing cystolith and stomata. d: Root powder showing narrow and wide fibres. e: Enlarged narrow fibre. f: Enlarged wide fibre. g: Powder showing long and narrow vessels. h: Vessel element showing the tail. i: Enlarged vessel element. Cystolith (Cy), Glandular Trichome (GTr), Narrow Fibre (NFi), Narrow Vessel Element (NVE), Perforation (Pe), Pits (Pi), Stomata (St), Tail (T), Trichomes (Tr), Wide Fibre (WFi).

Powder microscopy of root

The powder was light green in colour with no characteristic odour and taste. Isolated xylem elements were seen in the root powder when viewed under microscope. The elements include fibers and vessel elements. This result is in support with the findings of Balakrishnan *et al.*[35]

Fibres

There were two types of fibres seen in the powder. The narrow fibres (700µm long and 10µm thick) have thicker walls and narrow cell-lumen (Fig. 5d,e). Wide fibres (550µm long and 20µm thick) have thin walls and wide lumen. Their ends are more or less abruptly tapering (Fig. 5f).

Vessel elements

The vessel elements (310–330µm long) were invariably narrow, long and cylindrical (Fig. 5g). Some of them have long or short tails. The end wall perforation was simple, circular and oblique (Fig. 5h). The pits on the lateral walls were minute, multi-seriate and bordered (Fig. 5i).

CONCLUSION

In conclusion, standardization is an essential measure for quality, purity and sample identification. Leaf anatomy and powder microscopy of *E. viride* have been studied for the first time. Macroscopical and microscopical evaluations reported in the present study are sufficient enough to identify and authenticate the plant material. Thus pharmacognostical evaluation of *E. viride* will provide useful information for its identification and for further isolation of valuable secondary metabolites from this important medicinal plant.

ACKNOWLEDGEMENTS

The authors are thankful to Loyola College management, for providing the necessary facilities.

REFERENCES

1. Patel SS. Morphology and Pharmacology of *Passiflora edulis*. J Herbal Med Toxicol, 2009; 3:1–6.
2. Shanmugam S, Gayathri, Sakthivel B, Ramar S, Rajendran K. Plants used as medicine by Paliyar tribes of Shenbagathope in Virudhunagar district of Tamil Nadu, India. Ethnobot Leaflets, 2009; 13:370–378.
3. Vollesen K. A revision of *Megalochlamys* and *Ecbolium* (Acanthaceae: Justicieae). Kew Bull, 1989; 44:601–680.
4. Elumalai A, Chinna EM, Lakshmi M. A short review on unexplored medicinal plant *Ecbolium viride*. Int J Res Ayur Pharm, 2011; 2: 1539–1540.
5. Ebana RUB, Madunagu BE, Ekpe ED, Otung IN. Microbiological exploitation of cardiac glycoside and alkaloids from *Garcinia kola, Borreriao cymoides, Kola nitida* and *Citrus aurantifolia*. J Appl Biotechnol, 1991; 71:398–401.
6. Datta PC, Maiti RK. Pharmacognostic study on *Ecbolium linneanum* var. *dentata*. J Crude Drug Res, 1968; 8:1189–1192.
7. Kirtikar KR, Basu BD. Indian Medicinal Plants, Dehradun. International Book Publishers, 1987; p. 915.
8. Nair RAG, Ranesh P, Subramanian SS. Occurrence of glycoflavones in the Acanthaceae. Phytochemistry, 1975; 14: 1644.
9. Ashoka BVL, Arunachalam G, Jayaveera KN, Madhavan V, Shanaz B. Free radical scavenging activity of methanolic extract of *Ecbolium viride* (Forssk.) Alston roots. Der Pharmacia Lettre, 2011; 3:285–288.
10. Lalitha KG, Sethuraman MG. Anti-inflammatory activity of roots of *Ecbolium viride* (Forsk) Merrill. J Ethnopharmacol, 2010; 128: 248–250.
11. Francina CK, Ravindhran R, Duraipandiyan V. Evaluation of antimicrobial efficacy of *Ecbolium viride* (Forssk.) Alston root extracts. Asian J Pharma Clin Res, 2012a; 5:239–241.
12. Francina CK, Ravindhran R, Duraipandiyan V. Ecbolin A: A bioactive compound from the roots of *Ecbolium viride* (Forssk.) Alston. Asian J Pharma Clin Res, 2012b; 5:99–101.
13. Jayaprakash NJ, Janorious WJ, Rajkumar M, Senthil KKL. Study of hepatoprotective activity of *Ecbolium viride* (Forssk.) Alston. J Pharm, 2012; 2:157–161.
14. Preethi PSP, Satyanarayana T, Ganga RB, Rajesh K. Hepatoprotective activity of *Ecbolium viride* (Forssk.) Alst. (Acanthaceae) on experimental liver damage in rats. Int Res J Pharm Appl Sci, 2011; 1:27–33.
15. Abdel SE, Harraz FM, Soliman MA, Al A, El MS, Ichino C, Kiyohara H, OtoguroK, Omura S, Yamada H. Antiplasmodial and antitrypanosomal activity of plants from the Kingdom of Saudi Arabia. J Nat Med, 2009; 63:232–239.
16. Sass JE. Elements of Botanical Microtechnique, ed, New York. Mcgraw Hill Book Company, 1940 p. 222.
17. Johansen DA. Plant Microtechnique, New York and London. McGraw-Hill Book Company, 1940 p. 523.
18. O'Brien TPN, Feder, McCully ME. Polychromatic staining of Plant cell walls by toluidine blue O. Protoplasma, 1964; 59:367–373.
19. Wallis TE. Textbook of Pharmacognosy, London. J and A Churchill Limited, 1997 p. 652.
20. Trease GE, Evans W. Pharmacognosy, East Bourne. ELBS Publication, 1996 p. 344–539.
21. Kumar S, Paliwal GS. Foliar anatomy of Scrophulariancеae and Acanthaceae. Geophytology, 1982; 12:22–29.
22. Caitlin ONS. Anatomy of the shrimp plant, *Justicia brandegeana* (Acanthaceae). Stud Undergraduate Res Guelph, 2010; 3:41–47.
23. Dipa C, Daniel M. Foliar trichomes of some members of the family Acanthaceae and their taxonomic utility. Int J Pharm Bio Sci, 2011; 2:231–235.
24. Ben JHTW. Cystoliths in the Secondary Xylem of *Sparattanthelium* (Hernandiaceae). IAWA Bull, 1980; 1:43.
25. Katherine E. Plant anatomy, London, Wiley; 1953 p. 735.
26. Ling LKH, Maurice KSB, Vincent FR. Correlations between calcium oxalate crystals and photosynthetic activities in palisade cells of shade adapted *Peperomia glabella*. Bot Stud, 2007; 48: 155–164.
27. Graham L, Graham J, Wilcox W. Arid terrestrial ecosystems, in Plant Biology, eds. Trueheart C, New Jersey. Pearson Prentice Hall, 2006 p. 482–511.

28. Duke SO. Glandular trichomes a focal point of chemical and structural interaction. Int J Plant Sci, 1994; 155:617–620.
29. Hayat MQ, Asraf M, Khan MA, Yasmin G, Shaheen N, Jabeen S. Diversity of foliar trichomes and their systematic implications in the Genus *Artemisia* (Asteraceae). Int J Agric Biol, 2009; 11: 542–546.
30. Parveen A, Abid R, Fatima R. Stomatal types of some dicots within flora of Karachi, Pakistan. Pak J Bot, 2007; 39:1017–1023.
31. Solereder H. Systematic anatomy of the Dicotyledons, Oxford. Clarendron press, 1908 p. 642.
32. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons, Oxford. Clarendron press, 1950 p. 1500.
33. Fahn A. Plant anatomy, Oxford. Pergamon Press, 1967 p. 534
34. Pireyre N. Contributions to the morphological, histological and physiological study of Cystoliths. Rev Cyt Plt Bio, 1961; 23: 93–320.
35. Balakrishnan M, Dhanapal R, Laksmi MVM, Chandra SKB. Pharmacognostical and preliminary phytochemical evaluation of root of *Ecbolium viride* (Forssk.). Int J Pharm. Therap, 2010; 1: 27–33.

Pharmacognostical and phytochemical standardization of *Houttuynia cordata* Thunb.: A potent medicinal herb of North–Eastern India and China

Manish Kumar, Satyendra K. Prasad, Damiki Laloo, Apurva Joshi and Siva Hemalatha*

Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi–221005, India

ABSTRACT

Aim: *Houttuynia cordata* Thunb. (Saururaceae) is one of the perennial herb indigenous to North-East India and China. Despite the popular utilization of this herb as medicine, still no study has been reported so far regarding the pharmacognostical standardization. Thus, the aim of the present study was to scientifically establish a standard monograph on the basis of pharmacognostical and phytochemical aspects. **Methods:** The quality control standardization of *H. cordata* was done as per the methods described in the World Health Organization guidelines (2002). **Results:** The diagnostic characters of the *H. Cordata* leaf and rhizome portion were evaluated based on the macroscopical and microscopical characters. Determination of various physicochemical parameters such as water soluble ash (1.12% w/w), acid insoluble ash (4.02% w/w), sulphated ash (3.15% w/w), alcohol soluble extractive (12.8% w/w), water soluble extractive (14.9% w/w), loss on drying (3.42% w/w) and crude fibres content (13.10% w/w) was ascertained. Heavy metal, microbial load, fluorescence drug analysis, and preliminary phytochemical screening of different fractions were also carried out. Total phenols (45.74 mg/g tannic acid equivalent, TAE), tannins (33.29mg/g TAE), flavonoids (104.55 mg/g rutin equivalent, RE), and flavonols (17.16mg/g RE) were quantified from the ethanolic extract of the whole plant. Quantification of quercetin in the ethanolic extract was assessed by HPTLC analysis and was found to contain 4.39%, w/w. **Conclusion:** The obtained qualitative and quantitative standards will provide referential information for correct identification and standardization of this medicinal plant.

KEYWORDS: *Houttuynia cordata*, pharmacognosy, quercetin, HPTLC

INTRODUCTION

The relevance of pharmacognosy in standardization of herbal drugs was long been stressed. Many monographs based on pharmacognostical studies have emerged as an aid in the taxonomical and botanical identifications.[1] The process of standardization can be achieved by stepwise pharmacognostic studies. These studies will probably helps in the identification and authentication of the plant material.

***Corresponding author.**
Dr. (Mrs) S. Hemalatha
Department of Pharmaceutics, Indian Institute of Technology
Banaras Hindu University, Varanasi, (U.P.) 221005, India
Phone No: +919415256481

E-mail: shemalatha.phe@itbhu.ac.in

DOI: 10.5530/pj.2014.1.6

Houttuynia cordata Thumb. is a perennial herb with heart–like leaf and stoloniferous rhizome native to Japan, South–East Asia, and the Himalayas. It is a single species of the genus *Houttuynia* belonging to Saururaceae family and is restricted only to specialized moist habitats. *H. cordata* has been identified as one of the most potential medicinal and edible wild plant resources in China. Its young plants, including the aerial stems, leaves and underground stems are consumed as wild vegetable and its mature plants are commonly used as a kind of traditional medicinal herb in China, Korea, India, Vietnam and Thailand. In North–East India, this plant is eaten raw as a medicinal salad for lowering the blood sugar level and is commonly known by the name "Jamyrdoh" (Khasi and Jaintia tribes of Meghalaya). *H. cordata* contains six major classes of phytochemicals *viz*. essential oils, flavonoids, alkaloids, fatty acids, sterols and polyphenolic acids and these compounds exhibit a variety of pharmacological activities like anti–cancer,[2] anti–oxidant,[3] anti–diabetic,[4] anti–hypertension,[5] anti–inflammatory,[6] anti–mutagenic[7]

and anti–bacterial.[8] It is effective in treating pneumonia, severe acute respiratory syndrome, human immunodeficiency virus, influenza virus and refractory haemoptysis.[3,9,10] In view of the importance of *H. cordata* in traditional and modern system of medicine, it was thought worthwhile to develop quality standard for the same. As far as chemistry and pharmacology of this plant is concerned, large number of scientific data is available but a systematic standardization study is still lacking. Hence, in the present investigation, an attempt has been made to standardize the *H.cordata* herb which would help in the identification as well as in checking possible chances of adulteration, if any. Further, the study will also help in quality assurance of finished herbal products from this plant.

EXPERIMENTAL METHODS

Plant material

Whole plant of *H. cordata* was collected during the month of June–September (2012) from various areas of the West and East Jaintia's Hills district (*viz.* Jowai, Mihmyntdu, Khliehriat, Ladrymbai) of Meghalaya, North–East, India. Voucher herbarium specimen (COG/HC/011-2012), was prepared and preserved along with sample of crude drug in the Pharmacognosy research laboratory of Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi (U.P), India. The plant material was identified and authenticated by Dr. B.K. Sinha (Scientist In–charge), Botanical Survey of India, Shillong, Meghalaya.

Pharmacognostical studies

For pharmacognostical studies, samples of plant in fresh condition were preserved in 70% alcohol. Pharmacognostical study including morphological, histochemical and powder studies were carried out by taking the sections with the help of rotary microtome (York Scientific Industries Pvt. Ltd.).[11] Safranin (1%), fast green and toludine blue (0.2%) were used as staining reagents. Reagents like ferric chloride, ruthenium red, iodine solution and mixture of concentrated HCl and alcoholic pholoroglucinol solution (1:1, v/v) were used for histochemical tests. Photographs of different magnification were taken with Nikon trinocular microscopic unit, Model E–200, Japan. For normal observation bright field was used whereas for the study of crystal, fibers and lignified cells, polarized light was employed. Since many plant characters have bi–refringent property as seen under polarized light, hence they appear bright against dark background.

Physicochemical evaluation

Physicochemical constants such as foreign matter, loss on drying, extractive values, microbial count, crude fibre content, heavy metal analysis, total ash, acid–insoluble ash, water–soluble ash and sulphated ash values were carried out on shade–dried powdered drug following methods described in WHO guidelines and Indian Herbal Pharmacopoeia.[12,13] Fluorescence analysis of powdered drug was carried out under visible/daylight and UV light (254nm & 365nm) as per the standard procedures.[14]

Phytochemical evaluation

Preliminary phytochemical screening

The coarsely powdered plant material of *H. cordata* was exhaustively extracted for 24h by soxhlation using 95% ethanol (3L v/v) as solvent for extraction. The resulting extract was filtered and concentrated under reduced pressure to obtain the crude ethanolic extract of *H. cordata* (EHC). The ethanol extract (EHC) was then subjected to successive fractionation by suspending in aqueous media and then partitioning with solvents of varying polarity such as hexane, chloroform and ethyl acetate in order of their ascending polarity. Further, the extract (EHC) and its successive fractions were subjected to preliminary phytochemical tests to check the presence of various phytochemical classes.[15]

Quantitative estimation of various classes of phytoconstituents

Total phenolic and tannin contents were estimated spectrophotometrically in EHC according to the method described by Makkar *et al.*, 2000 using tannic acid as standard.[16] Estimation of total flavonoid and flavonol content were also ascertained using rutin as the external standard.[17] Total alkaloid content in the plant material was estimated using the usual gravimetric analysis in which the plant material was first extracted with H_2SO_4 and was further given successive washes with chloroform and diethyl ether.[18]

Thin layer chromatography (TLC) and High performance thin layer chromatography (HPTLC) analysis

Thin layer chromatography of EHC was analysed on pre–coated aluminium silica gel plates 60 F–254 as stationary phase. Mobile phases used for developing the chromatogram were composed with mixtures of solvents having varying polarity. Different reagents were sprayed

to confirm the presence of various phytoconstituents such as Dragendroff's reagent for alkaloids, vanillin sulphuric acid for the presence of terpenoidal class and sodium metaperiodate followed by benzidine for glycosides or glycoside containing sugars.

HPTLC is a sophisticated and automated technique, which is useful in separation of phytochemical mixtures present in the sample. Pre–coated plates and auto sampler were used for precision and to achieve significant separation. A stock solution of both EHC and standard quercetin in methanol was prepared in concentration of 5mg/mL and 0.2mg/mL respectively. Mobile phase for developing the chromatogram was composed of chloroform, methanol and formic acid mixture in the ratio 7.5:1.5:1 (v/v/v). The study was carried out using Camag–HPTLC instrumentation equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software for data interpretation. The R_f values were recorded and the developed plate was screened and photo–documented at ultra violet range with wavelength (λ_{max}) of 254nm.

RESULTS

Morphological, histological and powder evaluation

The leaves of the plant are green, 4–8cm in length, 3–4cm in width, simple and are broad with a long petiole, ovate–cordate with shortly acuminate apex bearing entire margin and are pubescent due to presence of trichomes. The leaves are slightly pungent in taste with a characteristic aromatic odour (Fig. 1).

The Petiole, in transverse sections through the distal end, exhibits a single U-shaped vascular strand with incurved ends. Histologically, the section also showed the presence of single layered epidermis followed by a wide zone (3–4 layers) of cortex comprised of parenchymatous cells. It is followed by continues ring of pericycle composed of sclerenchymatous cells. There are 4–6 vascular bundles arranged in ring form just below the pericycle. The central region shows the presence of large pith composed of parenchymatous cells with abundant simple and compound starch grains and raphides of calcium oxalate crystals (Fig. 2).

The transverse section of the leaf showed the epidermal layer (upper and lower) which is made up of single layer of parenchymatous cells (10–15µm in length and 5–10µm in width) covered with a thick cuticle. The leaf is of isobilateral nature bearing a single layered

Figure 1. *Houttuynia cordata* thunb.

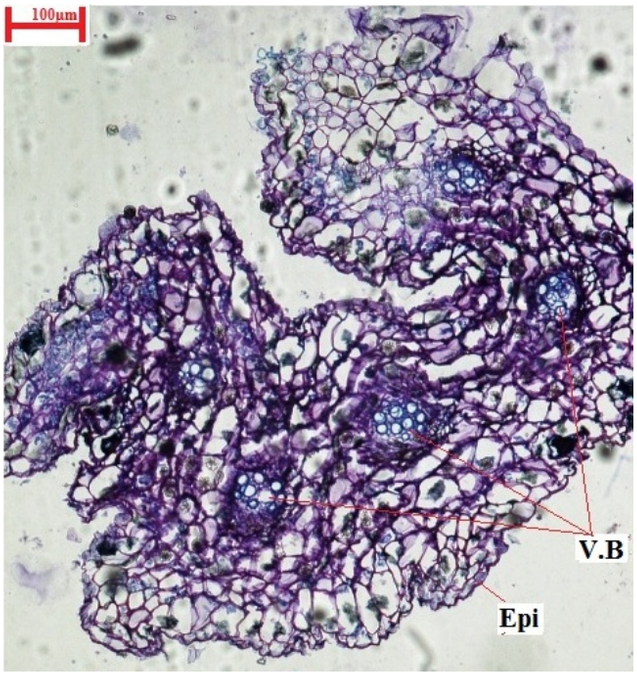


Figure 2. Histological study through petiole of *H. cordata*.

of wide palisade cells (arm palisade type) in both upper and lower epidermis continued over the midrib region. The lower epidermal layer of the lamina region showed the presence of two distinct types of trichomes *viz.* multicellular covering trichomes (2–4 celled) bearing pointed or blunted ends (90–150µm in length and 10–15µm in width) and glandular trichomes (3–4 celled glandular head) bearing unicellular stalk (70–120µm in length and 25–40µm in width). The spongy parenchymatous cells at mesophyll of the lamina when viewed under polarised light showed the presence of raphides of calcium oxalate crystals. The central region of the midrib

contains the vascular bundle bearing phloem and lignified xylem vessels with spiral thickenings (Fig. 3). In addition, the leaf at several places of the lamina bear the presence of ranunculaceous type of stomata on lower surface of the leaf and the values of stomatal number and stomatal index were found to be 104–119.4–132/mm^2 and 11–17 respectively.

The rhizomes are hairy, cylindrical in shape, 2–6mm in diameter with prominent ridges and are green in color having characteristics odour and slight pungent in taste. The transverse section of rhizome shows the presence of single layer outer epidermis composed of thick walled rectangular shaped epidermis (7–12µm in length and 5–8µm in width) which is followed by single layer of thin walled collenchymatous cell (10–15µm in length and 8–12µm in width). Below the collenchymatous layer appears the cortex region showing 10–25 layers of irregular shaped parenchymatous cells with sizes ranging from 10–25µm in length and 10–15µm in width. The parenchymatous cells of the cortical region showed the presence of numerous simple and compound starch grains along with raphides of calcium oxalate crystals. The cortex region is followed by a single continuous layer of lignified pericyclic fibers which are made up of sclerenchymatous cells (6–10µm in length and 2–4µm in width). Below the pericyclic layer is the vascular bundle layer (18–20 in number) which are conjoint in nature and are collateral. Phloem is represented by sieve elements while xylem is represented by vessels, tracheids, fibres and xylem parenchyma all showing strong lignifications on their walls. The innermost layer of the rhizome is made up of wide central pith which is composed of irregular shape parenchymatous cells (15–30µm in length and 12–25µm in width) filled with numerous starch grains (Fig. 4).

Powder microscopy of whole plant shows the presence of slender shaped fibers with size ranging from 250–350µm in length and 5–8µm in width and in many places it also shows raphides of calcium oxalate crystals. The trichomes (150–250µm in length and 10–15µm in width, and xylem vessels with spiral thickening (203–381µm length and 15–20µm in width) were also found (Fig. 5).

Physicochemical evaluation and Quantitative estimations

Evaluation of physicochemical parameter is important in the determination of adulterants and improper handling of drugs. The ethanol soluble and water soluble extractive values were found to be 12.8% w/w and 14.9% w/w

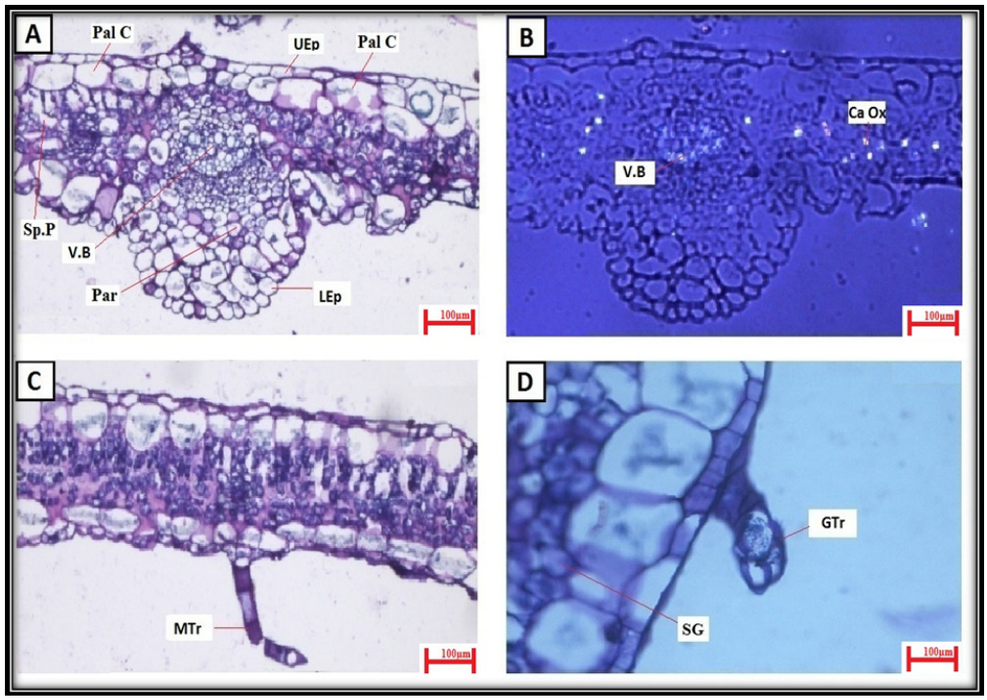
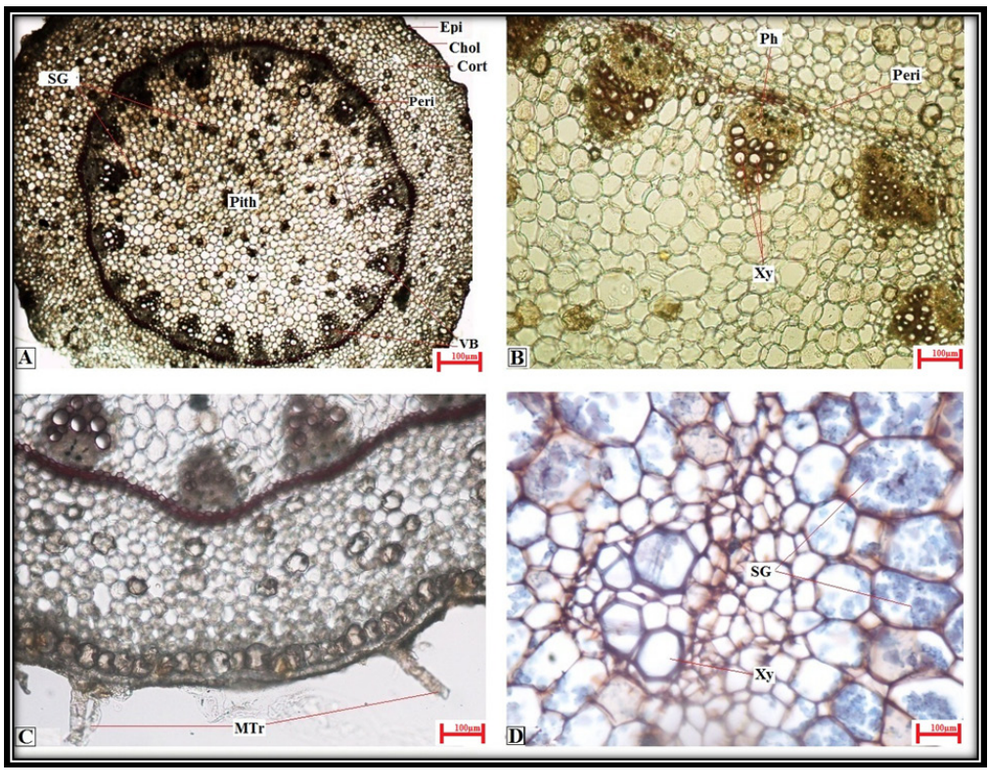


Figure 3. Histological study of *H. cordata* leaf. [A]: transverse section (TS) of midrib portion of leaf (10 X), [B]: TS showing vascular bundles (V.B) and calcium oxalate crystals (Ca.Ox) under polarized light, [C–D]: TS of lamina of leaf showing multicellular (MTr) and glandular (GTr) trichomes, [UEp – upper epidermis; LEp – lower epidermis; PalC – palisade cell; Sp.P – spongy parenchyma; Par – parenchymatous cells; V.B – vascular bundels; SG – starch grain].



Fgure 4. Histological study of *H. cordata* rhizome. [A]: TS of rhizome (10X), [B]: TS of rhizome (100X) showing arrangement of vascular bundles (VB), [C]: TS showing multicellular trichomes (MTr), [D]: TS showing iodine stained starch grains (SG). [Epi – epidermis; Chol – chollenchyma; Cort – cortex; Peri – pericycle; Pith – pith; Xy – xylem; Ph – phloem].

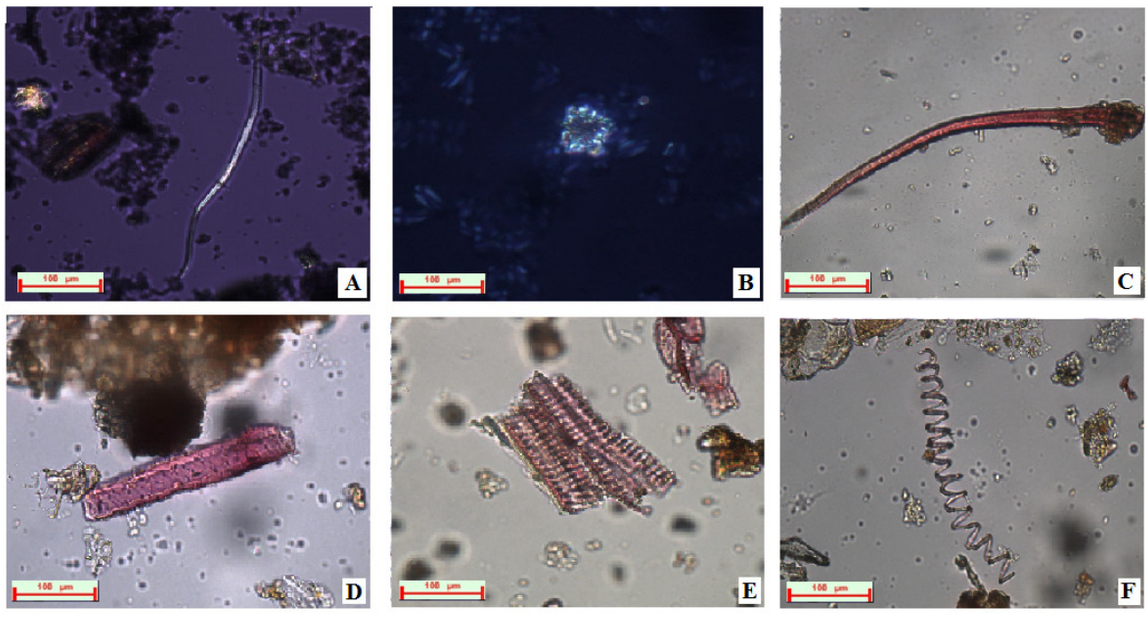


Figure 5. Powder characteristics study of *H. cordata.* [A]: Slender shaped fiber, [B] Raphides of calcium oxalate crystals, [C]: Lignified trichome, [D–F]: Vascular bundles with spiral thickening.

respectively. The total ash value of the crude drug was found to be 14.13% w/w, while water soluble ash, acid insoluble ash and sulphated ash values were determined as 1.12%, 4.02% and 3.15% w/w, respectively. Loss on drying at 105°C is determined since the presence of excess moisture is conclusive to the promotion of mould and bacterial growth, and subsequently to deterioration and spoilage of the drug. Loss on drying content was

determined to be 3.42% w/w. Crude fibre content was found to be 13.10% w/w. Microbial contamination of powder was confirmed to be within limits as shown in Table 1. Heavy metal analysis revealed that each element was present within specified limits as per WHO guideline 2002 as results are shown in Table 2. Color reaction of powdered drug and their fluorescence analysis with different reagents were studied (Table 3).

Phytochemical evaluation

Yield of sub fractions and preliminary phytochemical screening

The percentage yield of the EHC obtained by soxhlation was found to be 13.2% w/w, whereas, the percentage yield of various fractions *viz.* successive fractionation of EHC gave hexane (5.3% w/w), chloroform (3.9% w/w), ethyl acetate (2.2% w/w) and aqueous fraction (6.1% w/w). The results for the preliminary phytochemical screening of EHC and its successive fraction are represented in table 4. The phytochemicals present were found to be polyphenolics (tannins, phenolic and flavonoids), steroids, proteins, amino acids, carbohydrates and alkaloids. Whereas, glycoside, sapponin and mucilage components seem to be absent. Ethyl acetate fraction was found to be rich in phenolics and flavonoids (Table 4). Preleminary phytochemical screening gives a brief idea about the qualitative nature of active phytoconstituents present in the *H. cordata* extract/fractions.

Quantitative estimation

Preliminary phytochemical analysis of the extract revealed the presence of phenols, flavonoids, tannins and alkaloids as a major component. Total phenolic content of *H. cordata* was reported to be 45.74mg/g tannic acid equivalent while total tannin content was estimated to be 33.29mg/g tannic acid equivalent. Total flavonoid and flavonol content were found to be 104.55 and 17.16mg/g rutin equivalent. Total alkaloid content estimated in the plant material were reported to be 0.13% w/w. HPTLC studies revealed well resolved peaks of EHC containing quercetin. The spots of the entire chromatogram were visualized under UV 254nm and the percentage of

Table 1. Microbial contamination test.

Parameter	Specified limit	Value
Total plate count	<1000 cfu/g	102 cfu/g
Yeast & Mould	<100 cfu/g	13 cfu/g
E. Coli	Negative	Negative
Salmonella	Negative	Negative
Staphylococcin	Negative	Negative

CFU: colony forming unit per gram

Table 2. Heavy metal content.

Heavy metal	Specified limit (ppm)	Result (ppm)
Arsenic (As)	< 2	0.091
Lead (Pb)	< 2	0.0189
Cadmium	< 2	0.0003
Mercury	< 2	0.078
Zinc	< 2	0.019

ppm: parts per million

Table 3. Fluorescence powder drug analysis of *Houttuynia cordata* Thunb.

S. No.	Powder + Chemical	Fluorescence in day light	Fluorescence at λ_{max}254nm	Fluorescence at λ_{max}365nm
1	Powder as such	Brown	NF	NF
2	Powder + 1N NaOH in methanol	Dark olive green	NF	Corn silk
3	Powder + 1N NaOH in water	Maroon	NF	Light green
4	Powder + 1N HCL in methanol	Dark red	NF	Corn silk
5	Powder + 1N HCL in water	Khaki	NF	Pale green
6	Powder + 1N HNO_3 in methanol	Brown	NF	Blanched almond
7	Powder + 1N HNO_3 in water	Orange	Golden red	Yellow green
8	Powder + Iodine (5%)	Orange red	NF	NF
9	Powder + $FeCl_3$ (5%)	Dark olive green	NF	NF
10	Powder + KOH (50%)	Maroon	Lawn green	Spring green
11	Powder + NH_3 (25%)	Dark red	NF	Green yellow
12	Powder + Picric Acid	Yellow	NF	NF
13	Powder + Acetic Acid	Dark orange	NF	Ivory

NF: No Fluorescence

Table 4. Preliminary Phytochemical screening of *Houttuynia cordata* Thunb.

Phytochemical class	Ethanolic extract (EHC)	Successive fractions from EHC			
		Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction
Alkaloids	+	–	+	+	–
Phytosterol	–	+	+	+	–
Phenolic	+	–	+	+	+
Tannins	+	–	–	–	+
Flavonoids	+	–	–	+	+
carbohydrates	+	–	–	–	+
Reducing sugars	+	–	–	+	+
Hexose sugars	+	–	–	+	+
Saponins	–	–	–	–	–
Proteins	+	–	–	+	–
Amino acids	+	–	–	–	+
Cardiac Glycosides	–	–	–	–	–
Anthraquinon glycosides	–	–	–	–	–

(+) indicates present, (-) indicates absence

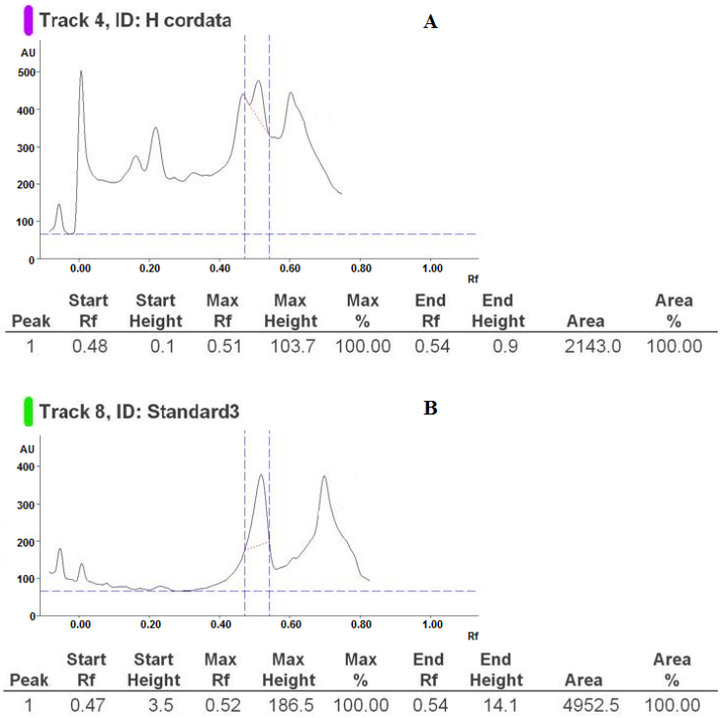


Figure 6. HPTLC densitogram of quercetin in ethanol extract of *H. Cordata* (HC). In figure A: Peak of quercetin present in HC and B: Standard peak of quercetin.

quercetin (R_f 0.51) in *H. cordata* extract (EHC) was found to be 4.39% (w/w) (Fig. 6).

DISCUSSION

Currently, there is a prominence on the standardization of medicinal plant materials for their therapeutic potentials. The modern pharmacognostic techniques available make the identification and evaluation of crude drugs more reliable, accurate and inexpensive. According to the World Health Organisation (WHO), pharmacognostical standards are considered to be the primary step for diagnosis of the herbal drug, which includes macroscopic and microscopic evaluation of the particular plant/plant parts.[19] Further, the evaluation of crude drugs on the

basis of histological studies helps to broaden the views about the type of characters and their occurrence in plant tissues, which in turn is necessary in proper identification of plant/plant parts.[20] Thus, the present work was carried out on *H. cordata* to lay down the standards which could be useful for establishing its authenticity and maintaining its quality, safety and reproducibility. On the basis of the pharmacognostical studies it was observed that there are various diagnostic features present in the *H. cordata* plant which can serve as useful information in maintaining the genuine nature of the drug. The specific diagnostic character of leaf showed the presence of arm palisade cells in both below the upper and lower epidermis which confirms its isobilateral nature. Moreover, another important features are the presence of trichomes which are multicellular and glandular types, raphides of calcium oxalate crystals and stomata of ranunculaceous type. However, the rhizome showed the presence of lignified pericyclic fibers, ring of collateral vascular bundles, compound starch grains and raphides of calcium oxalate crystals.

The physicochemical evaluation is an important parameter which helps in detecting adulteration or improper handling of the drug. The ash values are quantitative standards that represent the presence of various impurities like carbonate, oxalate and silicate which may be naturally occurring or deliberately added to crude drug as a form of adulterant. Total ash includes both physiological as well as non–physiological ash, while acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs.[21,22] Extractive values are useful to assess the amount of active chemical constituents present in the plant/plant parts using different solvents.[12] Loss on drying indicates that the drug is safe regarding any growth of bacteria, fungi and yeast**.** Quantitative determination of pharmacognostical parameters is efficient to set up standards for crude drugs. Heavy metal (As, Pb, Ca, Hg and Zn) and microbial load for powder drug were found to be present within the limit of WHO guidelines, indicating that the plant is safe to be used free from any unwanted contaminations. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and steroids in different fractions of *H.cordata*. Such phytochemical screening is helpful in the prediction of nature of phytoconstituents present in the tested drugs since phytochemicals are proven to be responsible for the activity of the drugs. Moreover, the chemical standardization of EHC was also performed with the help of HPTLC and the amount of quercetin was quantified as a chemical marker.

Recently, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics.[23] In order to avoid the misuse of harmful plant material pharmacognostic studies and phytochemical screening can serve as a basis for proper identification, collection and investigation of the plant. These parameters are to be useful in the preparation of the herbal monograph for its proper identification. Any crude drug which is claimed to be *H. cordata*, but whose characters significantly deviate from the above accepted standards would then be rejected as contaminated, adulterated or downright fake. Hence the present study will serve as useful information with respect to the identification, authentication and standardization of *H. cordata* herb.

CONFLICT OF INTEREST

No conflict of interest has been reported by all the authors.

AKNOWLEDGEMENT

Financial assistance provided by Rajiv Gandhi National Fellowship Scheme (RGNFS) to Mr. Manish Kumar is greatly acknowledged. Authors also wished to acknowledge Dr. B.K. Sinha, Botanical Survey of India, Shillong, Meghalaya for the identification and authentication of the plant material.

REFERENCES

1. Kalidass C, Shanmugasundaram R, Mohan VR. Pharmacognostic studies of *Capparis separia* (L.) R. *Br Pharmacognosy Journal*. 2009a; 2:121–125.
2. Lai KC, Chiu YJ, Tang YJ, Lin KL, Chiang JH, Jiang YL, Jen HF, Kuo YH, Agamaya S, Chung JG, Yang JS. *Houttuynia cordata* Thunb extract inhibits cell growth and induces apoptosis in human primary colorectal cancer cells. *Anticancer Res*. 2010; 30: 3549–56.
3. Nuengchamnong N, Krittasilp K, Ingkaninan K. Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC–ESI–MS coupled with DPPH assay. *Food Chem*. 2009; 117:750–756.
4. Liu Y, Wang H. Mechanism of herba *Houttuyniae* on relieving renal impairment in streptozotocin-induced diabetic rats. *Tradit Chin Drug Res Clin Pharmacol* 2010; 02:107–110.
5. Lu HM, Liang YZ, Yi LZ, Wu XJ. Anti–inflammatory effect of *Houttuynia cordata* injection. *J Ethnopharmacol*. 2006; 104: 245–249.
6. Li W, Zhou P, Zhang Y, He L. *Houttuynia cordata*, a novel and selective COX–2 inhibitor with anti–inflammatory activity. *J Ethnopharmacol*. 2011; 133:922–7.

7. Chen YY, Liu JF, Chen CM, Chao PY, Chang TJ. A study of the antioxidative and antimutagenic effects of *Houttuynia cordata* Thunb. using an oxidized frying oil–fed model. *J Nutr Sci Vitaminol*. 2003; 49:327–33.
8. Kim GS, Kim DH, Lim JJ, Lee JJ, Han DY, Lee WM, Jung WC, Min WG, Won CG, Rhee MH, Lee HJ, Kim S. Biological and antibacterial activities of the natural herb *Houttuynia cordata* water extract against the intracellular bacterial pathogen *salmonella* within the RAW 264.7 macrophage. *Biol Pharm Bull*. 2008; 31:2012–2017.
9. Lau KM, Lee KM, Koon CM, Cheung CSF, Lau CP, Ho HM, *et al*. Immunomodulatory and anti–SARS activities of *Houttuynia cordata*. *J Ethnopharmacol*. 2008; 118:79–85.
10. Hayashi K, Kamiya M, Hayashi T. Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV–1, influenza virus, and HIV. *Planta Med*. 1995; 61:237–241.
11. Prasad SK, Sahu AN, Hemalatha S. Cytomorphological and physicochemical evaluations of *Cryptocoryne spiralis. J Herbs Spices Med Plants*. 2012; 18:304–317.
12. Anonymous. Quality Control Methods for Medicinal Plant Materials (WHO), Geneva A.I.T.B.S. Publishers & Distributors (Regd.), Delhi, pp. 28–30; 2002.
13. Anonymous. Indian Herbal Pharmacopoeia, Revised edition published by Indian drug manufacturer, pp. 495–6; 2002.
14. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharm Assoc*. 1949; 38:324–331.
15. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments, 17th ed. Nirali Prakashan, Pune, India, pp. 149; 2007.
16. Makkar HPS. Quantification of Tannins in Tree Foliage: A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on 'Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminants of Tanniniferous Tree Foliage. Working document. Vienna: FAO/IAEA; 2000:3–5.
17. Kumaran A, Karunakaran J. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT–Food Science and Technology*. 2007; 40:344–352.
18. Prasad SK, Laloo D, Kumar M, Hemalatha S. Quality control standardization and antioxidant activity of roots from *Eriosema chinense*. *Phcog J*. 2013; 5(4);149–155.
19. Woratouch T, Thatree P, Suchada S. Pharmacognostic evaluations of *Lagerstroemia speciosa* leaves. *J Medicinal Plants Res*.2011; 5(8):1330–1337.
20. Shanmugakumar SD, Amerjothy S, Balakrishna K. Pharmacognostical, antibacterial and antifungal potentials of the leaf extracts of *Pithecellobium dulce* Benth. *Phcog Mag*. 2006; 2:0973–1296.
21. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 34th ed. Nirali Prakashan, Pune, India. 2006.
22. Evans WC. Trease and Evans, Pharmacognosy, 15th ed. W.B. Saunders, Edinburgh. 2002.
23. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. *The Ind Pharm*. 2006; 5(45):26–30.

15-Lipoxygenase inhibition of selected Philippine medicinal plants

Noemi D. Paguigan[1] and Christine L. Chichioco-Hernandez*[,2]

[1]Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City, Philippines 1101
[2]Institute of Chemistry, College of Science, University of the Philippines, Diliman, Quezon City, Philippines 1101

ABSTRACT

Several extracts from Philippine medicinal plants used for asthma and other inflammatory diseases were evaluated for their ability to inhibit the action of 15-lipoxygenase. The inhibitory activity was tested spectrophotometrically using quercetin as positive control. Eleven species belonging to 11 families displayed varying inhibitory activities. *Commelina diffusa* and *Euphorbia hirta* showed the highest inhibitory activity at 51.3% and 48.5%, respectively. These plants may contain new 15-lipoxygenase inhibitors.

Keywords: Asthma, inflammation, lipoxygenase, medicinal plants, plant extracts.

INTRODUCTION

Lipoxygenases (LOX) constitute a heterogeneous family of lipid peroxidizing enzymes which catalyze the dioxygenation of polyunsaturated fatty acids containing the 1,4-cis,cis system to their corresponding hydroperoxy derivatives.[1] The primary lipid targets of LOX activity are arachidonic acid(AA) and linoleic acid(LA). The metabolism of LA and AA by LOXs produces biologically active pro-inflammatory mediators implicated in the development of asthma.[2] Hence, inhibition of LOX activity and identification of bioactive extracts are of pharmacological interests. In this paper, we evaluated the 15-lipoxygenase inhibitory action and analyzed the phytochemical contents of plants that are traditionally used to treat inflammatory conditions like asthma in the Philippines. The use of different herbal preparations is still being used in the Philippines. The country is rich in folklore that attributed medicinal benefits to quite a number of plants.[3] However, a considerable number of plants still needs to be scientifically validated, hence, much work is still needed to investigate the bioactivity and phytochemicals of these plants. The present research was undertaken to determine the potential scientific basis for the use of these plants in traditional medicine.

***Corresponding author.**
Phone: +632-981-8500 local 3652;
Fax: +632-920-5427

E-mail: cchernandez@up.edu.ph

DOI: 10.5530/pj.2014.1.7

MATERIALS AND METHODS

Plant material

Fresh leaves of *Amaranthus viridis*, *Bambusa spinosa*, *Commelina diffusa*, *Crataeva religiosa*, *Eclipta alba*, *Euphorbia hirta*, *Isotoma longiflora*, *Monochoria vaginalis, Pistia stratiotes, Plumeria rubra* and *Premna odorata* were collected from the University of the Philippines, Diliman Campus and submitted to the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman for authentication. Voucher specimens for each plant were also deposited.

Plant extraction

The leaves were washed with running water and allowed to drip dry. The air-dried samples were weighed then homogenized for overnight soaking in methanol using clean glass jars. The crude methanolic extracts were concentrated *in vacuo* using a rotary evaporator (Heidolph).

Phytochemical analysis

The phytochemical screening methods used were based on Edeoga[4] and Harborne.[5] Qualitative test

for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed.

15-Lipoxygenase inhibitory assay

The spectrophotometric assay used is generally based on the 15-LOX catalyzed reaction between oxygen and a polyunsaturated fatty acid with a 1,4-diene-type structure. Briefly, inhibition of 15-LOX was carried out as described by Wangensteen[6] with modifications using a reaction medium containing 237 U/mL of soybean 15-LOX-1 (Sigma), 33 uM LA , 2 M borate buffer at pH equal to 9 at ambient temperature. The metabolism of LA by 15-LOX-1 leads to the formation of 13-S-hydroperoxyoctadecadienoic acid (13-HPODE) which is hydrolyzed to 13-S-hydroxyoctadecadienoic acid (13-HODE). The enzyme inhibitory effect was tested by adding the crude methanol extract of the sample (dissolved in 10% DMSO in borate buffer) to the incubation mixture. The LOX activity was monitored as an increase of the absorbance at 234 nm, which reflects the formation of 13-HPODE from LA, every 30 seconds for 5 minutes and done in triplicates.

The value for % inhibition of enzyme activity was calculated as:

$$Activity = \frac{(\Delta A_1/\Delta t) - (\Delta A_2/\Delta t)}{\Delta A_1/\Delta t} \times 100\%$$

where $DA_1/\Delta t$ and $DA_2/\Delta t$ are values for increase in A_{234} for sample without test sample and with test sample, respectively.

RESULTS AND DISCUSSION

Acute inflammation in the lung and airway is fundamentally important to host defence, but chronic or excessive inflammation leads to several common respiratory and airway diseases, including asthma[7]. Asthma has the highest morbidity amongst inflammatory lung diseases[8] and has become the most common chronic disease among children according to the World Health Organization. Although asthma cannot be cured, appropriate management can control the disease. Current therapeutic targets of this inflammatory disease focus on blocking the initiating mediators of the inflammation. One of the therapeutic strategies being studied for asthma management involves controlling the activity of lipoxygenases.

The *in vitro* inhibition of soybean 15-LOX activity was used to evaluate the activities of the plant extracts in this study. Inhibition of soybean 15-LOX is generally regarded as predictive inhibition of the mammalian enzyme.[9,10] Based on the result, most tested methanolic plant extracts showed LOX inhibitory activity with *C. diffusa* showing the highest activity at 51.3% followed by *E. hirta* with 48.5% inhibitory effect at a concentration of 167µg/ml. All samples however did not exhibit higher activity compared with the positive control quercetin at 62.4% at a concentration of 17µg/ml as shown in Table 2. The preliminary result of this study validates the traditional use of the plant extracts as remedy for respiratory inflammation specifically asthma.[3]

Table 1. Details of the plants used in the study.

Local name	Scientific name	Family	Traditional uses[3]	Plant part used
Kolitis	*Amaranthus viridis*	Amaranthaceae	Poultice for inflammation, boils and abscesses	Leaves
Kawayan	*Bambusa spinosa*	Gramineae	Emmenagogue, anthelminthic, antispasmodic, coughs, asthma, food	Roots, Leaves, young shoots
Alikbangon	*Commelina diffusa*	Commelinaceae	Swelling, burns, boils	Leaves
Salingbobog	*Crataeva religiosa*	Capparaceae	Stomach ache, swelling, rheumatism	Leaves, bark
Tinta-tinta	*Eclipta alba*	Asteraceae	Hepatitis, wound healing, asthma, skin diseases	Leaves
Gatas-gatas	*Euphorbia hirta*	Euphorbiaceae	Asthma, sedative, hemostatic	Leaves
Estrella	*Isotoma longiflora*	Campanulaceae	Asthma, wounds	Plant
Gabing uwak	*Monochoria vaginalis*	Pontederiaceae	Boils, asthma, coughs, toothache	Leaves, roots
Kiapo	*Pistia stratiotes*	Araceae	Diuretic, coughs, asthma skin disease	Leaves
Kalachuchi	*Plumeria rubra*	Apocynaceae	Swelling, asthma, purgative	Leaves
Alagaw	*Premna odorata*	Verbenaceae	Coughs, headache	Leaves

Table 2. 15-Lipoxygenase inhibitory effects of the various methanol extracts.

Sample	Percent inhibition
B. spinosa	4.4
P. stratiotes	2.3
I. longiflora	8.6
M. vaginalis	12.5
P. rubra	11.1
E.hirta	48.5
A.viridis	2.7
C.diffusa	51.3
E. alba	40.8
P. odorata	12.3
C. religiosa	4.4
Quercetin	62.4

C. diffusa exhibited the highest activity at 51.3%. It has shown antifungal activities against Trychophyton species and evaluated for its wound healing action.[11] It was evaluated against 5-LOX where it exhibited 27% inhibition.[12] The difference between the three mammalian lipoxygenases, 5-, 12-, and 15- is the carbon position where they catalyze arachidonic acid oxygenation.[13]

E. hirta showed moderate inhibition at 48.5%. This plant has been previously evaluated against a wide range of activities which includes anti-diabetic,[14] mutagenicity,[15] antiviral,[16] antibacterial and antifungal.[17]

In the literature, natural compounds reported as 15-LOX inhibitors comprise of flavonoids isolated from orange peels, and from the leaves of *Orthosiphon spicatus;*[18] terpenoids as 15-LOX inhibitors have also been isolated from sponges.[19] The 15-LOX inhibitory activity of extracts from the leaves and seeds of *Coriandrum sativum* have been shown to be positively correlated to its phenolic content.[6] In order to check for the presence of flavonoids, terpenoids, and other secondary metabolites in the plant samples a phytochemical screening was done. The result for the phytochemical screening is shown in Table 3. Phytochemical screening of plant extracts reveals the presence of flavonoids, and terpenoids, which have been proven in previous studies to be responsible for the suppression of LOX activity. Qualitative test for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed. *C. diffusa* and *E. hirta* were both positive for the presence of flavonoids and terpenoids. It is possible that the compounds responsible for the observed bioactivity belongs to the said family and specifically inhibits 15-LOX.

To the best of our knowledge, this is the first report of the evaluation of the 15-lipoxygenase inhibitory activity of the tested medicinal plant extracts. This work has scientifically validated the use of the plant extracts in folkloric medicine and *C. diffusa* and *E. hirta* could contain new 15-lipoxygenase inhibitors.

ACKNOWLEDGEMENT

This project was funded by the Natural Sciences Research Institute of the University of the Philippines Diliman.

Table 3. Phytochemical screening of crude methanol extracts.

Sample	Tannin	Saponin	Terpenoid	Flavonoid	Cardiac Glycoside	Phenolics	Steroids	Alkaloid
B. spinosa	–	–	+	–	–	–	–	–
P. stratiotes	–	+	+	+	–	–	–	–
I. longiflora	–	+	–	–	+	–	–	–
M. vaginalis	–	+	+	+	–	–	–	–
P. rubra	–	+	+	–	+	–	–	–
E.hirta	+	+	+	+	+	+	+	–
A.viridis	–	+	–	–	+	–	–	–
C.diffusa	–	+	+	+	+	+	–	–
E. alba	+	–	+	+	+	+	–	–
P. odorata	–	+	+	+	+	+	+	–
C. religiosa	–	+	+	–	+	–	+	–

(-) absent (+) present

REFERENCES

1. Borngraber S, Browner M, Gillmor S, Gerth C, Anton M, Fletterick R, et al. Shape and Specificity in Mammalian 15-Lipoxygenase Active Site. The Functional Interplay of Sequence Determinants for the Reaction Specificity. J Biol Chem, 1999; 274:37345–37350.
2. Claesoon HE, Dahlen SE. Asthma and Leukotrienes: Antileukotrienes as Novel Anti-Asthmatic Drugs. J Intern Med, 1999; 245:205–227.
3. Quisumbing E. Medicinal Plants of the Philippines. Katha Publishing Incorporated. Quezon City Philippines; 1978. p 90, 144, 149, 152, 268, 341, 504, 800, 958, 985.
4. Edeoga HO, Okwu DE Mbaebie. Phytochemical Constituents of Some Nigerian Medicinal Plants. Afr J Biotechnol, 2005; 4: 685–688.
5. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. Chapman and Hall, USA. 1984.
6. Wangensteen H, Samuelsen A, Malterud KE. Antioxidant activity in extracts from from coriander. Food Chem, 2004; 88:293–297.
7. Haworth O, Levy BD. Endogenous lipid mediators in the resolution of airway inflammation. Eur Respir J, 2007; 30:980–992.
8. Busse WW, Lemanske RF. Asthma. New Eng J Med, 2001; 344: 350–362.
9. Gundersen LL, Malterud KE, Negussie AH, Rise F, Teklu S, Ostby OB. Indozilines as Novel Potent Inhibitors of 15-lipoxygenase. Bioorgan Med Chem, 2003;11:5409–5415.
10. Maiga A, Malterud KE, Diallo D, Paulsen BS. Antioxidant and 15-lipoxygenase inhibitory activities of the Malian medicinal plants *Diospyros abyssinica* (Hiern) F. White (Ebenaceae), *Lanella velutina* A. Rich (Anacardiaceae) and *Crossopteryx febrifuga* (Afzel) Benth. Rubiaceae. J Ethnopharm, 2006; 104:132–137.
11. Mensah AY, Houghton PJ, Dickson RA, Fleischer TC, Heinrich M, Bremner P. *In vitro* evaluation of effects of two Ghanaian plants relevant to wound healing. Phytother Res, 2006; 20:941–944.
12. Braga FC, Wagner H, Lombardi JA, de Oliveira AB. Screening Brazilian plant species for in vitro inhibition of 5-lipoxygenase. Phytomedicine 2000; 6:447–452.
13. Samuelsson, B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis and biological effects. Science 1987; 237:1171–1176.
14. Subramaniam SP, Bhuvaneshwari S, Prasath GS. Antidiabetic and antioxidant potentials of *Euphorbia hirta* leaves extract studied in streptozotocin-induced experimental diabetes in rats. Gen Physiol Biophys, 2011; 30:278–285.
15. Loh DSY, Er HM, Chen YS. Mutagenic and antimutagenic activities of aqueous and methanol extracts of *Euphorbia hirta*. J Ethnopharm, 2009; 126:406–414.
16. Gyuris A, Szlavik L, Minarovits J, Vasas A, Molnar J, Hohmann J. Antiviral activities of extracts of *Euphorbia hirta* L. against HIV-1, HIV-2 and SIVmac251. In vivo 2009; 23:429–432.
17. Rajeh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Assessment of *Euphorbia hirta* L. leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality. Molecules 2010; 15:6008–6018.
18. Lyckander I M, Malterud KE. Lipophilic flavonoids from *Orthosiphon spicatus* as inhibitors of 15-lipoxygenase. Acta Pharm Nordica, 1992; 4:159–166.
19. Amagata T, Whitman S, Johnson TA, Stessman CC, Loo CP, Lobkovsky E, et al. Exploring sponge-derived terpenoids for their potency and selectivity against 12-human, 15-human, and 15-soybean lipoxygenases. J Nat Prod, 2003; 66:230–235.

Cytotoxicity and Oral Acute Toxicity Studies of β-mangostin Isolated from *Cratoxylum arborescens*

Suvitha Syam[*,1], Ahmad Bustamam[1,*], Rasedee Abdullah[2], Mohamed Aspollah Sukari[3], Najihah Mohd Hashim[4], Maizatulakmal Yahayu[4], Pouya Hassandarvish[4], Syam Mohan[5] and Siddig Ibrahim Abdelwahab[5]

[1]UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, University Putra Malaysia, Serdang, Selangor, Malaysia, Tel: +60389472120

[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary, University Putra Malaysia, Serdang, Selangor, Malaysia, Tel: +60386093887

[3]Department of Chemistry, Faculty of Science, University Putra Malaysia, Serdang, Selangor, Malaysia, Tel: +60389466775

[4]Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, Tel: +60379674909

[5]Medical Research Centre, Jazan University, P.O. Box 114 Jazan, Kingdom of Saudi Arabia, Tel: 00966506612390

ABSTRACT

Introduction: The objective of this study was to investigate the cytotoxicity and oral acute toxicity of β-mangostin isolated from *Cratoxylum arborescens*. **Material and methods:** Healthy male and female ICR mice (8 weeks) were fed orally with 250 and 500mg/kg of β-mangostin. Body weight of each animal was measured and any gross behavioral change was observed daily. Hematological and clinical biochemical parameters as well as histopathological analysis were carried out on 15th day. The level of oxidative stress was analyzed using MDA and GSH measurement. **Discussion:** The results showed that oral administration of the β-mangostin had no adverse effect on the growth rate, hematological and clinical biochemical parameters. Histological studies showed that the treatments did not induce any pathological changes in the liver and kidney. The compound at both the doses did not alter the oxidative stress biomarkers. The *in vitro* cytotoxicity of β Mangostin was investigated in HepG2, A549, MCF-7, MDA-MB-231 and PC3 cells. There was significant cytotoxicity in both type of breast cancer cells (MCF-7 and MDA-MB-231). In conclusion, our results show that there was no treatment-related acute toxicity in mice following 14-days oral administration of 250 and 500mg/kg of β-mangostin. **Conclusion:** The results showed that the compound can be selected for detailed *in vitro* and *in vivo* breast cancer research.

Keywords: *Cratoxylum arborescens*; β-mangostin; acute toxicity; anti-cancer.

INTRODUCTION

Throughout the history of mankind plants have played a unique role in providing new remedies for various diseases in the world and served to be the foundation of many traditional medicine systems. Plants were served in various formulations including tinctures, teas, poultices, cream, powders etc., and have become the basis of novel drug discovery.[1] They are also the rich source of secondary metabolites or phyto-pharmaceuticals used in pharmaceutical industry. These secondary metabolites behold single or multiple pharmacological properties. Anti-fungal, anti-bacterial, anti-cancer, anti-viral, anti-inflammatory, HIV-inhibitory, anti-diabetioecdc and hepatoprotective properties are some of them. Xanthone, Mangostin, α-Mangostin, γ-Mangostin, Cowaxanthone F, 1,6-dihydroxyxanthone, 1,3,8-trihydroxyxanthone, 1,6- and 3,5-dihydroxyxanthones are few examples of such xanthones.[2–5]

Cratoxylum arborescens (Family: Guttiferae) is a well-known tropical tree from which several xanthones were isolated and these xanthones are present in the leaves, twigs and bark of the tree.[6] From this plant the compounds namely α-Mangostin, β-mangostin (BM) and γ-Mangostin were isolated previously; of which, α-Mangostin and γ-Mangostin where extensively studied pharmacologically.[7,8] β-mangostin (Figure 1), now being focused for its pharmacological benefits, including

***Corresponding author:**
UPM-MAKNA
Cancer Research Laboratory, Institute of Bioscience, University Putra Malaysia, Serdang
Phone: +60389472120
Fax: +60 38 9472101

E-mail: suvithaavs@gmail.com

DOI: 10.5530/pj.2014.1.8

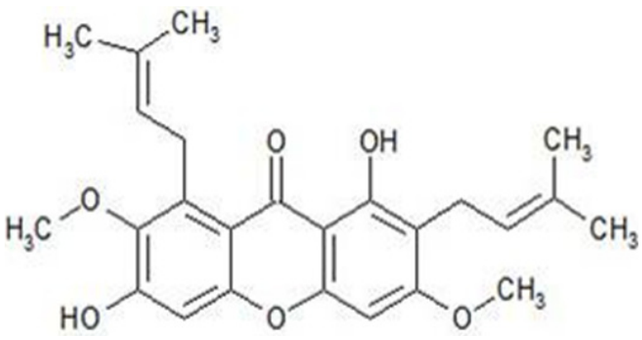


Figure 1. Structure of β-Mangostin [1,6-dihydroxy-3,7-dimethoxy-2,8-bis(3-methylbut-2-enyl)-9H-xanthen-9-one].

anticancer activity,[9,10] however, lacks some important informations with respect to toxicity. As part of a permanent screening program for searching natural products with beneficial biological properties (especially anti-cancer), the current investigation herby reports the toxicity of β-mangostin *in vivo* and its possible effects on various cancer cells *in vitro*. We have designed this study in such a way, the outcome of this study will give better understating of this compound with respect to its suitability in being studied as an anticancer agent, and the safety range that could be used for *in vivo* anticancer mechanisms.

EXPERIMENTAL SECTION

Plant sample

The ground air-dried stem bark of *Cratoxylum arborescens* (1.0kg) was soaked at room temperature in hexane for three days and repeated thrice. The extract was filtered and then concentrated by using rotary evaporator under reduced pressure to give dark gummy semisolid residue. The plant material was then sequentially extracted with chloroform, and methanol. The weights of hexane, chloroform, and methanol crude extracts obtained were 6.12g, 28.18g, and 40.27g, respectively. The hexane extract (6.12g) was separated by vacuum column chromatography and eluted with hexane and followed by mixtures of solvents, hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol to give 26 fractions of 200ml each. Similar fractions based on TLC and observed under UV light were combined. Fraction 14 was further separated by mini column chromatography to give 56 fractions. Fractions 16–52 (eluted with 50% ethyl acetate: 50% methanol) was similarly further purified by preparative thin layer chromatography as well as chromatotron to give yellowish solid and was identified as β-mangostin. Similar separation and fractionation of the chloroform and methanol extracts with series of column chromatography led to the isolation another batch of β-mangostin.

Acute oral toxicity study of β-mangostin

The experimental protocol with animals in the study was in compliance with the regulations set by the Institutional Animal Care and Use Committee, Faculty of Medicine, UM. Healthy male and female ICR mice (8 weeks) used for the acute oral toxicity study were bred and reared at the Animal House, University of Malaya, Malaysia. The animals were housed in polypropylene cages with stainless steel grill tops and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for 1-week prior to treatment. The temperature in the animal room was maintained between 25±2 °C with a relative humidity of 30–70% and illumination cycle set to 12h light and 12h dark. The mice were fed with commercial pellet diet. All animals had free access to tap water and food except for an overnight fasting before treatment. The mice were then allowed to take food after an hour of treatment.

Treatment

Being a dietary constituent from fruits, we expected β-mangostin to be relatively safe.[11] Thus, two doses (low and high), as recommended by Organisation for Economic Co-operation and Development[12] guidelines of 250 and 500mg/kg of compound was suspended in 10% Tween20 and administered by gavage to mice weighing between 25–30g (n=5). The control group received tween 20 alone. The general behaviour of mice was observed every one hour for six hours and thereafter every 24 hour until 14 days. Animals were observed for any sign of toxicity and for mortality up until 14 days and daily weight were noted as described earlier.[13] Other features observed included changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern. Upon completing 14 days, the animals were sacrificed and vital organs (lungs, heart, spleen, liver, kidneys) were carefully examined macroscopically and weighed. Hematological parameters and histological (liver and kidney) parameters were determined by standard methods.[14] Lipid peroxidation was also performed on fresh liver and kidney necropsies.

Assessment of kidney and liver functions

All biochemical assays were performed spectrophotometrically using Hitachi-912 Autoanalyser (Mannheim,

Germany) with kits were supplied by Roche Diagnostics (Mannheim, Germany). The common indicators of kidney function such as serum creatinine, blood urea nitrogen, sodium, potassium, chloride, carbon dioxide and anion gap levels were measured. Serum Alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total protein, albumin, globulin, total bilirubin and conjugated bilirubin levels were measured so as to evaluate the liver function. To establish a good sensitivity and validity of the data, serum samples were analyzed in triplicates and blindly.

Assessment of lipid profile

Analysis of total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were performed with the aid of commercial kits of Span Diagnostics as described previously.[15] GPO-PAP end point assay was used to assess the triglycerides concentration.

Histopathological examinations

Fixed (10% formalin) renal and hepatic tissues were embedded in paraffin, sectioned at 5μm and stained with haematoxylin-eosin. All sections were examined using a photomicroscope (Olympus BH-2, Japan). Samples were blindly analysed for histopathology by an independent histopathologist.

Lipid peroxidation

Lipid peroxidation was assessed using malondialdehyde (MDA) as indicator (Kei, 1978). Briefly, 10% (weight/volume) homogenates of kidney and liver were made by 0.1mol/l phosphate buffer. The homogenates were centrifuged at 3500 rpm (4ºC) for 10 min. 0.2ml supernatant was mixed with 0.67% 2-thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532nm. The results were expressed as MDAnmol/mg protein. Protein concentration of the supernatant was measured by Lowry's method.[16]

Measurement of tissue glutathione

The tissue samples were homogenized in 10 volumes of ice-cold 10% trichloroacetic acid and centrifuged at 1000g (4˚C) for 15 min. The supernatant was discarded and centrifuged at 35,000g (4˚C) at for 8 min. GSH was determined spectrophotometrically by Ellman procedure with slight modification.[17]

Cytotoxicity study

Cell culture

MCF-7 (oestrogen receptor-positive human breast adenocarcinoma cell line), MDA-MB 231 (oestrogen receptor-negative human breast adenocarcinoma cell line), HepG2 (human liver hepatocellular cells), A-549 (human lung cancer cell line) and PC3 (Human prostate cancer cell line) were obtained from American Type Culture Collection (ATCC, Va, USA). All cells were maintained in RPMI-1640 medium that is supplemented with 10% Fetal Bovine Serum (FBS) and incubated at 37°C. Celll viability assay was determined by MTT assay as previously described by Mossman et al.[18,19] Briefly, 1×10^6 cells/ml were treated with the compound at different concentration in 96-well plate and incubated for 24h. The colorimetric assay is measured and recorded at absorbance of 570nm. Results of cell viability were expressed as percentage of control. The potency of cell growth inhibition for test agent was expressed as IC_{50} value.

STATISTICAL ANALYSES

Data was presented as mean ± standard deviation. Significant differences were determined by using the Student's t-test and ANOVA, where $*p < 0.05$ denotes a statistically significant difference. All the samples were measured in triplicates.

RESULTS

Isolation of β-Mangostin

β-mangostin was initially obtained as yellowish solid and yellowish needle-shaped crystals were obtained after recrystallized with hot chloroform. IR $\nu_{max}cm^{-1}$ (KBr): 3407 (OH), 2923 (CH), 1642 (C=O) and 1596 (C=C); UV MeOH $\lambda_{max}nm$ (log ε): 374 (1.89), 350 (3.99), 344 (2.19), and 340 (3.29); EIMS *m/z* (% intensity): 424 (53.79), 409 (5.20), 393 (1.19), 3.81 (19.45), 368 (31.93), 353 (100.00), 335 (20.79), 310 (7.59), 299 (23.66) and 169 (8.41); ^{1}H-NMR (500 MHz, acetone-d_6): δ 13.61 (OH-1), 9.62 (OH-6), 6.82 (*s*, 1H, H-5), 6.47 (*s*, 1H, H-4), 5.25 (*t*, *J* = 6.9 Hz, 1H, H-12), 5.18 (*t*, *J* = 6.9 Hz, 1H, H-17), 4.10 (*d*, *J* = 6.9 Hz, 2H, H-11), 3.94 (OMe-3), 3.77 (OMe-7), 3.29 (*d*, *J* = 6.9 Hz, 2H, H-16), 1.80 (*s*, 3H, Me-14), 1.75 (*s*, 3H, Me-19), 1.63 (*s*, 3H, Me-15) and 1.61 (*s*, 3H, Me-20); ^{13}C-NMR (125 MHz, acetone-d_6): δ 186.8 (C-9), 168.5 (C-4a), 164.4 (C-1), 161.5 (C-10a), 160.2 (C-6), 160.1 (C-3), 148.5 (C-7), 142.0 (C-8), 135.4 (C-18 and C-13), 128.6 (C-12), 127.2 (C-17), 115.9 (C-8a), 115.7 (C-2),

108.0 (C-9a), 106.6 (C-5), 93.8 (C-4), 65.2 (OMe-7), 60.4 (OMe-3), 30.8 (C-11), 29.8 (C-15), 29.8 (C-20), 25.8 (C-16), 22.2 (C-14) and 21.7 (C-19).

The structure of β-mangostin was established due to significant correlations in Heteronuclear Multiple Bond Connectivity, Heteronuclear Multiple Quantum Coherence, Distortionless Enhancement by Polarization Transfer together with the signals displayed by ^{1}H and ^{13}C-NMR spectra. The fragmentation pattern and molecular mass of the compound were further confirmed by the Electron Impact Mass Spectrometry and the typical absorption bands of the functional groups were displayed from the Infrared spectroscopic data.

The purity of the compound was analyzed by Shimadzu UFLC system equipped with a PDA UV detector and Ion Trap TOF mass spectrometer. Column: Waters Xbridge 50 × 2.1mm 2.5µM. Mobile phase: H_2O (0.1% formic acid):MeCN (0.1% formic acid). Flow rate: 0.50mL/min. Column temperature: 40°C. Gradient: 10–100% MeCN over 7 min (Figure 2), and the compound was also identified by comparison of its spectral data to those reported in the literature.[8]

Acute Oral Toxicity Study

General observations

There was no mortality, abnormal behaviour or physical signs of toxicity observed after oral administration of β-mangostin at any tested doses. In all cases the faeces of the experimental animals were dry and dark coloured for all groups. No differences were observed in average daily food and water intake among the groups.

Effect of β-mangostin on animal weight and relative organ weight

β-mangostin had no significant ($p>0.05$) effect on the body weight of mice in any group throughout the experiment. Figure 3 suggests that based on differential analysis on body weight, animals of both the sexes showed normal weight gain.

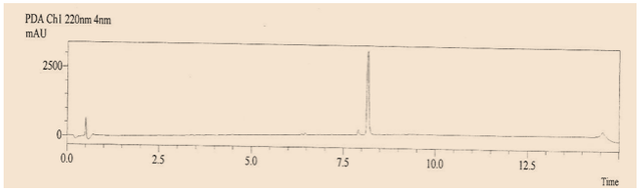


Figure 2. Chromatogram of β-Mangostin at 220nm. Analysis was performed using a Shimadzu UFLC system equipped with a PDA UV detector and Ion Trap TOF mass spectrometer. Column: Waters Xbridge 50 × 2.1mm 2.5µM. Mobile phase: H2O (0.1% formic acid): MeCN (0.1% formic acid). Flow rate: 0.50mL/min. Column temperature: 40°C. Gradient: 10–100% MeCN over 7 min.

It is common to have reduction in organ weight in toxicity conditions; hence, the liver, kidney, lungs, spleen and heart were collected and weighed after the sacrificing animals. It was observed that there was no significant difference in the relative organ weight among all the treated groups as compared to the control (Figure 4).

Serum biochemical parameters

As observed from the serum biochemistry data presented in Table 1, there were no treatment-related significant differences in various parameters including serum electrolytes such as sodium, potassium and chloride. The effect of β-mangostin on liver function parameters such as ALT, AST, ALP, conjugated bilirubin and total bilirubin in serum were also investigated. No significant differences in these markers were noted with treatment of 250 and 500mg/kg β-mangostin. Data in relation to effects of β-mangostin on triglyceride, high density lipoprotein

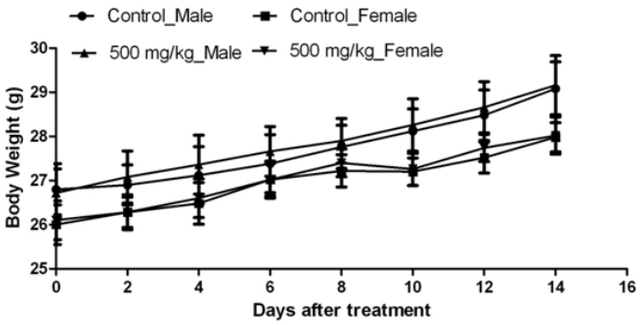


Figure 3. Comparison of the changes in body mass across sexes in control and treatment groups during observation based on acute toxicity test of β-mangostin on ICR mice. β-mangostin was given to the treated group and 10% Tween-20 was given to the control group.

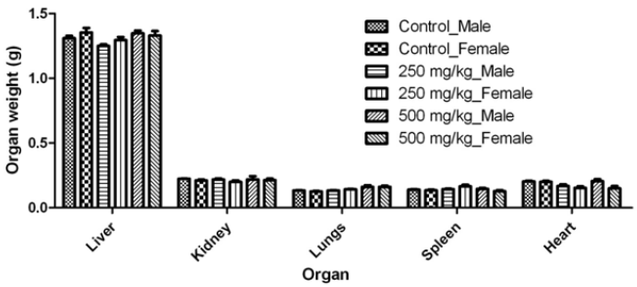


Figure 4. Comparison of organ weight across sexes in control and treatment groups observed during acute toxicity test of β-mangostin on ICR mice. β-mangostin was given to the treated group and 10% Tween-20 was given to the control group.

cholesterol HDL and total cholesterol levels are shown in Table 1. Animals of both sexes that received high dose (500mg/kg) showed significant decrease in triglyceride, HDL cholesterol and total cholesterol levels. This effect was however not evident in low dose treatment group.

Histopathological evaluation

No significant differences were observed in gross examination during autopsy and histopathological evaluations of liver and kidney stained with hematoxylin and eosin. Histological profile of the normal and treated animals revealed normal hepatocytes with well-preserved cytoplasm, prominent nucleus, nucleolus and central vein. No sign of inflammation, fatty change and necrosis were seen in these animals (Data not shown).

Effect of b-mangostin on oxidative stress biomarkers

Oxidative stress in the tissues was evaluated quantitatively using measurements of MDA and GSH (Table 2). β-mangostin (500mg/kg) treated animals showed slightly higher MDA and GSH levels regardless of their gender compared to normal control, but was not significant

Table 1. The effects of β-mangostin on the biochemical parameters in serum of ICR mice.

Parameter	Sex	Normal control	β-Mangostin 250mg/kg (b.w)	β-Mangostin 500mg/kg (b.w)
Sodium (mmol/l)	Male	146.4±11.2	141.2±10.3	144.2±10.0
	Female	147.2±13.5	149.1±11.2	146.2±11.5
Potassium (mmol/l)	Male	5.1±0.4	6.2±0.3	6.2±1.0
	Female	4.8±0.5	3.6±0.2	5.9±1.1
Chloride (mmol/l)	Male	111.0±10.2	114.3±11.0	119.1±11.5
	Female	110.7±13.0	120.1±12.4	114.2±12.2
Carbon Dioxide (mmol/l)	Male	15.1±1.1	14.2±3.1	16.7±3.1
	Female	13.9±1.4	16.1±2.0	15.0±0.8
Anion Gap (mmol/l)	Male	24.8±1.6	29.8±2.9	26.0±0.7
	Female	26.3±1.1	21.2±2.8	24.1±2.2
Urea Nitrogen (mmol/l)	Male	12.9±2.0	14.1±3.1	13.1±3.1
	Female	9.9±1.3	10.3±1.2	11.1±2.0
Creatinine (µmol/l)	Male	21.7±2.6	22.1±3.4	24.0±1.3
	Female	28.3±2.8	29.1±1.8	30.0±1.2
Total Protien (g/l)	Male	65.2±3.8	64.2±1.9	66.2±7.8
	Female	56.25±4.1	60.21±2.4	55.11±1.1
Albumin (g/l)	Male	17.1±1.9	18.1±2.1	18.3±1.1
	Female	14.5±2.7	15.0±1.8	15.1±9.0
Total Protein (g/l)	Male	58.2±2.1	62.4±4.2	56.6±4.0
	Female	59.5±3.2	64.3±3.0	61.0±4.1
Albumin (g/l)	Male	14.0±1.0	13.2±2.1	12.2±2.7
	Female	11.1±1.0	10.2±2.5	16.6±3.3
Globulin (g/l)	Male	44.7±1.5	49.6±1.4	49.2±3.0
	Female	49.0±3.1	45.5±2.7	55.5±4.6
Total Bilirubin (µmol/l)	Male	2.0±0.6	2.4±0.1	2.5±0.2
	Female	1.5±0.9	1.1±0.5	1.4±0.01
Conjugated Bilirubin (µmol/l)	Male	0.7±0.01	1.1±0.4	0.6±0.02
	Female	1.0±0.2	1.3±0.7	1.1±0.09
Alkaline Phosphatase (IU/l)	Male	48.4±1.2	45.7±1.3	39.7±2.8
	Female	52.6±2.5	59.0±2.1	61.2±1.7
Alanine Aminotransferase (IU/l)	Male	75.0±2.5	81.1±2.2	68.8±1.8
	Female	70.0±1.9	76.1±1.0	81.1±4.1
AST (IU/l)	Male	404.2±13.1	396.3±21.2	421.5±16.0
	Female	411.8±19.9	410.4±19.4	401.9±30.1
G-Glutamyal Transferase IU/l	Male	2.6±0.2	2.0±0.4	3.1±0.8
	Female	1.5±0.3	1.7±0.3	2.2±0.9
Triglyceride (mmol/l)	Male	0.7±0.01	0.9±0.01	0.1±0.01*
	Female	1.1±0.5	1.8±0.01	0.2±0.01*
Total Cholestrol (mmol/l)	Male	1.5±0.07	1.1±0.01	0.3±0.03*
	Female	1.3±0.02	1.2±0.03	0.5±0.01*
HDL Cholestrol (mmol/l)	Male	1.59±0.5	1.09±0.2	0.19±0.6*
	Female	1.8±0.1	1.1±0.9	0.2±0.3*

Data are expressed as mean ± SD of five mice for each group. '*' indicates statistical difference at $p<0.05$.

Table 2. Tissue malondialdehyde levels and glutathione content of β-mangostin on the ICR mice.

Parameter	Organ	Sex	Normal control	β-Mangostin 250mg/kg (b.w)	β-Mangostin 500 mg/kg (b.w)
MDA (nmol/g)	Liver	Male	0.05±0.005	0.06±0.010	0.07±0.000
	Kidney	Female	0.04±0.007	0.05±0.000	0.06±0.008
GSH (nmol/g)	Liver	Male	0.05±0.003	0.05±0.001	0.06±0.001
	Kidney	Female	0.05±0.005	0.05±0.007	0.06±0.006

Data are expressed as mean ± SD of five mice for each group.

($p>0.05$) while treatment with the dose of 250mg/kg of β-mangostin did not show any abnormal levels of MDA and GSH in male and female treated animals.

In vitro cytotoxicity

To evaluate the cytotoxic activity, β-mangostin was tested with a series of different concentrations on A549, PC-3, HepG2, MCF-7 and MDA-MB-231 cells (Fig. 5). Cell viability was determined using MTT assay. As shown in Fig. 5, β-mangostin induced cytotoxicity in a concentration dependent manner to both breast cancer cells with low IC_{50} value. The data from MTT assay presents that β-mangostin has different degrees of cytotoxicity on different cell types. Amongst the cells, the liver cells, HepG2 showed had very low cytotoxicity with an IC_{50} of 43.5μg/ml.

In the MCF-7 and MDA-MB-231 cells, the morphological features as observed under normal inverted microscope was having slight alterations in cellular components after 24h post-treatment (Fig. 6 A and C) as compared to non-treated cells (Fig. 6 B and D). Cells exposed to β-mangostin showed more prominent growth inhibition and shrinkage of the cells. On the contrary, untreated cells remained confluent throughout the incubation period.

DISCUSSION

A survey by Word Health Organization stated that 70–80% of the people around the world count on non-conventional medicine for primary health care, which is predominantly of herbal source.[20] Especially in the developing nations, where most people who cannot afford a consultation with western style doctor or the cost of medication, this becomes a common practice. This knowledge of man's dependency on traditional remedies is the sole backbone of journey from ethnomedicine to modern medicine.[21] The novel drug development process begins from the secondary metabolite that the plants produce; often ending up to produce successful medications based on new compounds. This becomes a boon where these new compounds are proven clinically useful for deadly diseases including cancer or sometimes, simply inflammation or other diseases for which the scientific community is constantly in hunt for new targets to harness such diseases. However, always not all active secondary metabolites reach the final stage of drug development due to their adverse effects or toxicity.[22]

In the preclinical stage of drug investigation, it is mandatory to have the data showing the safety of that drug using preclinical animal toxicology studies. The *in vivo* toxicity studies using animal models are much reliable because of the similarity in mechanisms of toxicity across species, which are often based on similarity in anatomical, physiological, metabolic, pathological and pharmacological characteristics.[23] A good correlation has been reported between toxicological insults in mice and humans[24] and hence, the vast number of studies demonstrating the acute effects of different test substance (compounds/drugs) in mice; sometimes, with the doses potentially usable in humans. In the mice model, various organ level toxicities and biochemical parameters including the overall condition of experimental animals are studied post-treatment to the compound.[25,26] A common toxic effect noted is the reduction in body weight, which could be primarily attributed to diminished food intake which may be the consequence of toxins released and absorbed in the gastrointestinal tract. As such, the body weight of all experimental animals was noted and there were no significant changes in body weight throughout the study across all groups and both sexes. Accompanied by variation in body weight due to toxicity, is very common that various body organ weights also decrease. β-mangostin could be said safe with this aspect as no significant changes in the organ weight was observed with the mice being treated with this compound.

Similarly, exposure of various groups of mice to β-mangostin had no adverse effect on the usual markers of liver and kidney toxicity (the plasma levels of liver enzymes, ALT and AST, bilirubin and creatinine). The transaminases (AST and ALT) are well-known enzymes which are good indicators of liver function as biomarkers in predicting toxicity.[27] Any kind of insult to the parenchymal liver cells results elevated level of both transaminases in the blood.[28] The serum AST is of both mitochondrial and cytoplasmic origin and hence any rise of its level

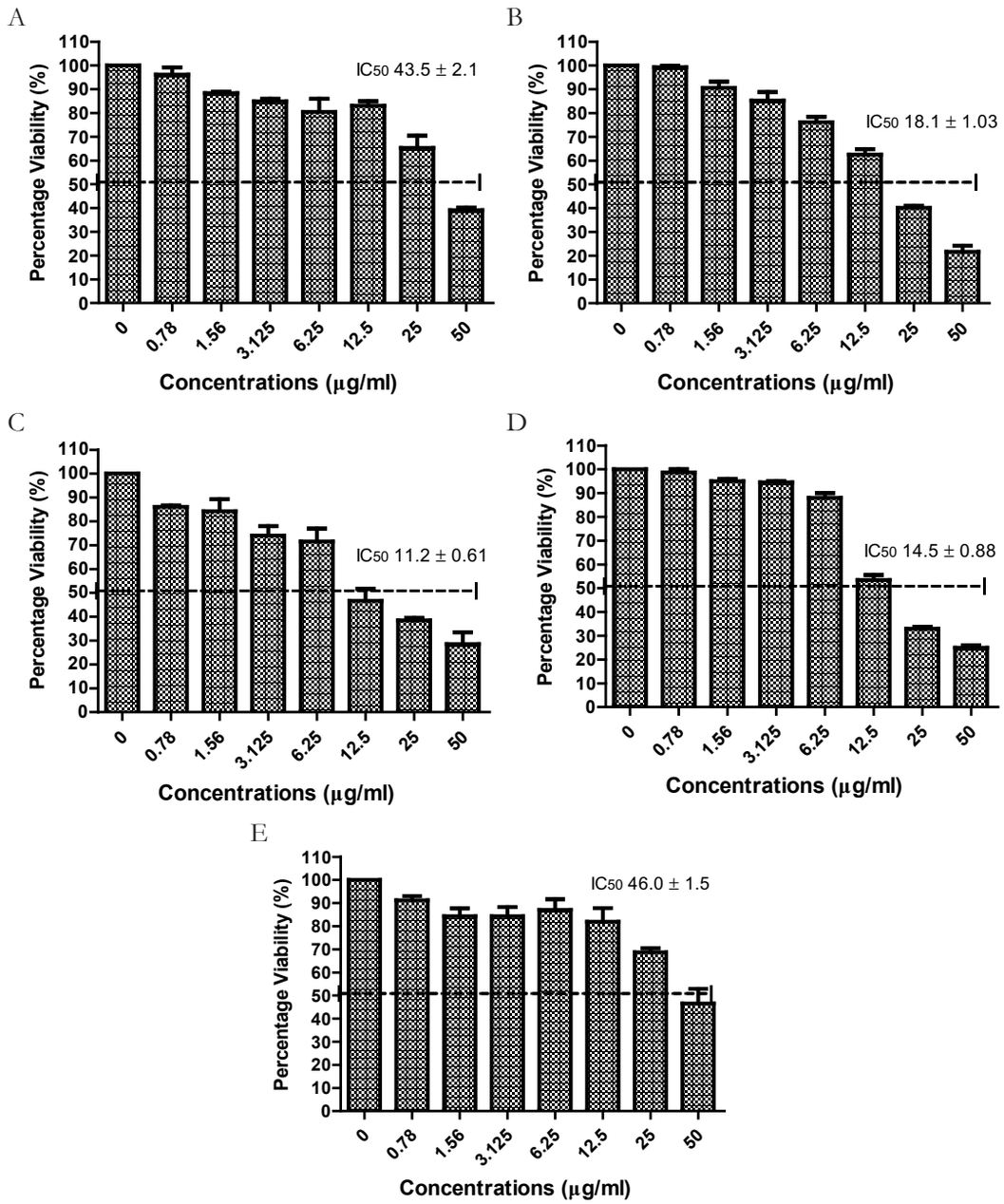


Figure 5. Effect of β-mangostin on different cells (A: HepG2 cells, B: A549 cells, C: MCF-7 cells, D: MDA-MB-231 cells and E: PC3 cells) type expressed as IC50 values in MTT assay.

is considered first sign of cell damage that leads to the outflow of the enzymes into the serum. Therefore, the acute administration of β-mangostin did not affect the hepatocytes function and metabolism. Further, there was also no remarkable change in creatinine level upon administration of any of the doses of β-mangostin in the tested animals when compared to the control. As increased creatinine level in serum is characterized in association to renal damage, this indicator is used to identify renal toxicity during drug administration.[29,30] Thus, the results recorded in this study suggest that β-mangostin did not affect the renal function.

Other biochemical parameters analyzed in this study such as the total protein and albumin levels also had no significant changes in the treated groups when compared to control. Even though the aforementioned parameters of treatment group were at par with that of the control group, the cholesterol levels were observed to have decreased with administration of high dose of β-mangostin. Thus

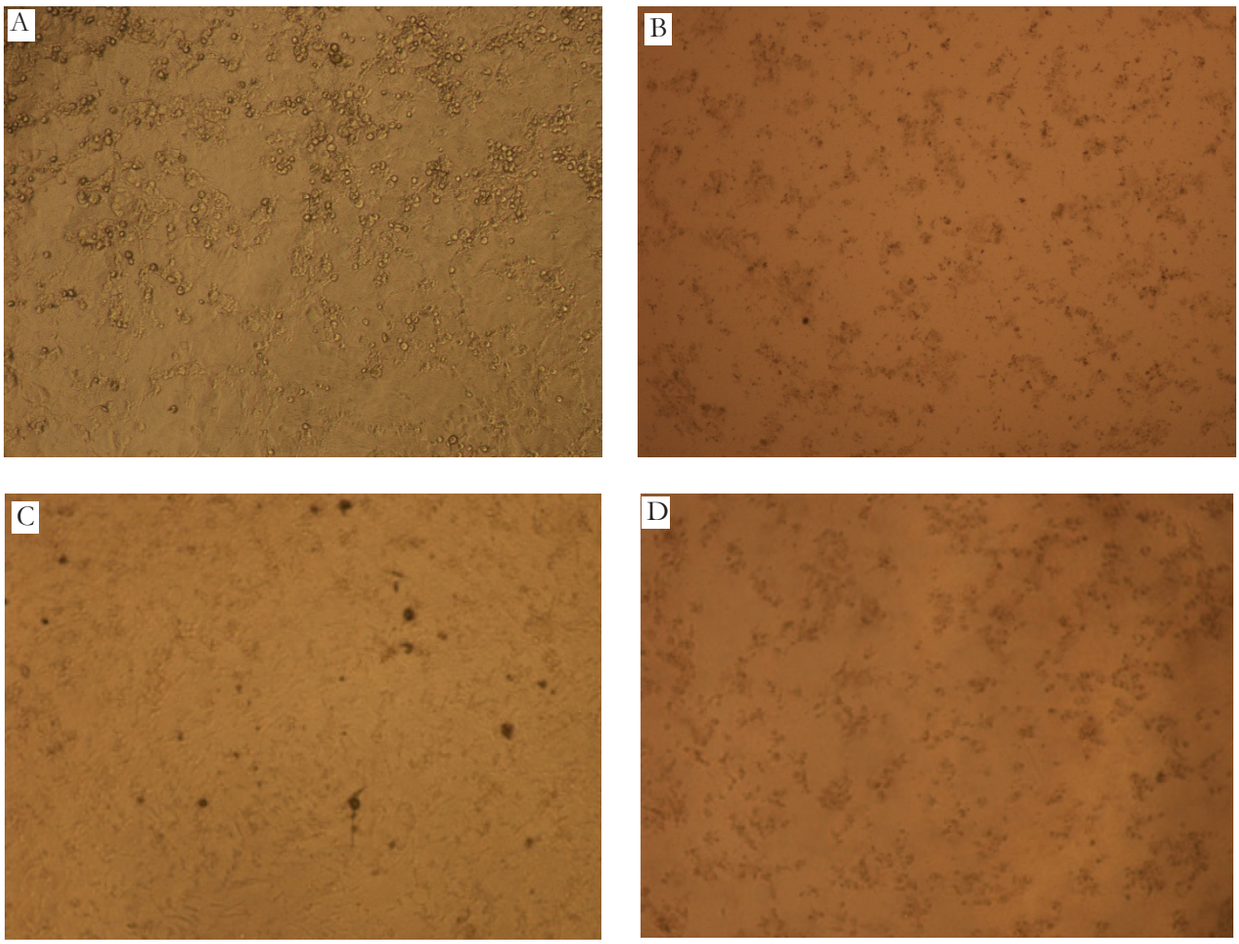


Figure 6. Morphological features of MCF-7 and MDA-MB-231 at IC50 concentrations of β-mangostin. Phase contrast micrographs of MCF-7 and MDA-MB-231cells treated at IC50 of β-mangostin 24h, where (A) MCF-7 control, (B) MCF-7 treated, (C) MDA-MB-231 control and (D) MDA-MB-231 treated (10 × magnifications).

β-mangostin had some effect on the cholesterol metabolism of the mice. The levels of electrolytes that maintain the body fluid equilibrium; however, had no significant changes. This suggests that β-mangostin had not affected the function and equilibrium of body fluids. Except for a significant decrease in the levels of triglycerides and cholesterol, there was no noticeable changes in hematological and biochemical profile upon administration of β-mangostin. Thus it is observed that β-mangostin is relatively low or non-toxic natural compound under the experimental conditions of this study. This is, so far, the first study to report the suitability of *in vivo* administration of β-mangostin, a major phytochemical present in the fuctional food. In support to these findings the gross examination during autopsy and histopathological evaluations of the various organs stained with hematoxylin and eosin revealed no significant differences in the kidney and liver. Toxicity of compounds normally induces the cellular stress by increasing the oxidative stress.[31,32] This may attribute to generation of free radicals and later kill the cells. In the current study, we also intended to confirm the possibility of evoking any cellular stress associated to the administration of β-mangostin. Therefore, we have measure the two main biomarkers of oxidative stress: MDA and GSH. The non-significant levels of these biomarkers post-treatment of β-mangostin ruled out the involvement of oxidative stress of the compound at the mentioned doses.

In vitro behavior of cells in response to various compounds are little different than that of the *in vivo* environment. Since we observed no sign of toxicity in animal model, then we focus to study the behavior of different cancer cells upon exposure to β-mangostin *in vitro*. Subsequently, our intention is to study the effect of β-mangostin in treating cancer *in vitro* and *in vivo*. Herein, we explore its anti-proliferative capacity towards various cancer cell lines. As shown in Fig. 5, β-mangostin exhibited different

level of cytotoxicity in the selected cell lines. HepG2 is a well-known *in vitro* model for assessing toxicity of compounds. Previously, many researchers have used this cell line to establish the safety of compounds[33–35]. Comparable to *in vivo* effect, there was no significant cytotoxicity in HepG2 cells produced by β-mangostin. As per the results obtained in the current study, the compound was significantly toxic to two types of breast cancer cells used (MCF-7 and MDA-MB-231). The antipoliferative result thus highlights the need to study the compound for its anticancer action against breast cancer cells.

CONCLUSIONS

In conclusion, the present evaluation had showed the potential of β-mangostin to be selected for further studies. There was no significant toxicity observed in the studied parameters; moreover, the compound showed cell specific cytotoxicity towards breast cancer cells *in vitro*. Hence, currently the mechanism of cytotoxicity of β-mangostin on breast cancer *in vitro* an *in vivo* is being studied in our lab.

ACKNOWLEDGEMENTS

The authors would like to express their utmost gratitude and appreciation to University of Malaya and Ministry of Higher Education (HIR grant F00009-21001) for providing grant to conduct this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. *Life sciences.* 2005; 78(5):431–441.
2. Bumrungpert A, Kalpravidh RW, Chitchumroonchokchai C, et al. Xanthones from mangosteen prevent lipopolysaccharide-mediated inflammation and insulin resistance in primary cultures of human adipocytes. *The Journal of Nutrition.* 2009; 139(6):1185–1191.
3. Gopalakrishnan C, Shankaranarayanan D, Kameswaran L, Nazimudeen S. Effect of mangostin, a xanthone from *Garcinia mangostana* Linn. in immunopathological & Inflammatory reactions. *Indian Journal of Experimental Biology.* 1980; 18: 843–846.
4. Panthong KPK, Hutadilok-Towatana NH-TN, Panthong APA. Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from Garcinia cowa. *Canadian Journal of Chemistry.* 2009; 87(11):1636–1640.
5. Tchamo Diderot N, Silvere N, Etienne T. Xanthones as therapeutic agents: chemistry and pharmacology. *Advances in Phytomedicine.* 2006; 2:273–298.
6. Pattanaprateeb P, Ruangrungsi N, Cordell GA. Cytotoxic constituents from *Cratoxylum arborescens*. *Planta Medica.* 2005; 71(2):181–183.
7. Sidahmed HM, Abdelwahab SI, Mohan S, et al. α-mangostin from *Cratoxylum arborescens* (Vahl) Blume demonstrates anti-ulcerogenic property: a mechanistic study. *Evidence-Based Complementary and Alternative Medicine.* 2013; 2013 (Article ID 450840):10.
8. Sim WC, Lim CJ, Lian GEC, Md.Sukari MABH. *Cratoxylum glaucum* and *Cratoxylum arborescens* (Guttiferae)-two potential source of antioxidant agents. *Asian Journal of Chemistry* 2011; 23(2):569–572.
9. Ee GCL, Daud S, Izzaddin SA, Rahmani M. *Garcinia mangostana*: a source of potential anti-cancer lead compounds against CEM-SS cell line. *Journal of Asian Natural Products Research.* 2008; 10(5):475–479.
10. Chitchumroonchokchai C, Thomas-Ahner JM, Li J, et al. Anti-tumorigenicity of dietary α-mangostin in an HT-29 colon cell xenograft model and the tissue distribution of xanthones and their phase II metabolites. *Molecular nutrition & food research.* 2013; 57(2):203–211.
11. Angell M, Kassirer JP. Alternative medicine-the risks of untested and unregulated remedies. *New England Journal of Medicine.* 1998;339:839-840.
12. Pour BM, Latha LY, Sasidharan S. Cytotoxicity and oral acute toxicity studies of *Lantana camara* leaf extract. *Molecules.* 2011; 16(5):3663–3674.
13. Lee J, Park C, Kim H, Hwang S, Chung W. Single dose toxicity study of Hwangjaegongjinbo, an invigorator, in mice and rats. *Journal of Toxicology and Public Health.* 2002; 18:73–77.
14. Lorentz K. IFCC methods for the measurement of catalytic concentration of enzymes part 9. IFCC method for α-amylase (1–4-α-D-Glucan 4-Glucanohydrolase, ec 3.2. 1.1). *Indian Journal of Clinical Biochemistry.* 1997; 12(1):1–24.
15. Wybenga D, Pileggi V, Dirstine PH, Di Giorgio J. Direct manual determination of serum total cholesterol with a single stable reagent. *Clinical Chemistry.* 1970; 16(12):980–984.
16. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proceedings of the National Academy of Sciences.* 1984; 81(12):3883–3887.
17. Ellman GL. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics.* 1959; 82(1):70–77.
18. Yang H, Bocchetta M, Kroczynska B, et al. TNF-α inhibits asbestos-induced cytotoxicity via a NF-κB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. *Proceedings of the National Academy of Sciences.* 2006; 103(27):10397–10402.
19. Singh S, Ishar MPS, Saxena AK, Kaur A. Cytotoxic Effect of *Anthocephalus Cadamba* Miq. Leaves On Human Cancer Cell Lines. *Pharmacognosy Journal.* 2013; 5(3):127–129.
20. Santé Omdl. *Legal Status of Traditional Medicine and Complementary-alternative Medicine: A Worldwide Review*: Worlds health organization; 2001.
21. Parray SA, udin Bhat J, Ahmad G, Jahan N, Sofi G, Iqbal SF. Ruta graveolens: from Traditional System of Medicine to Modern Pharmacology: an Overview. *American Journal of PharmTech Research.* 2012; 2(2):239–252.
22. Nielsen KF, Mogensen JM, Johansen M, Larsen TO, Frisvad JC. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Analytical and Bioanalytical Chemistry.* 2009; 395(5):1225–1242.
23. Nickoloff BJ, Kunkel SL, Burdick M, Strieter RM. Severe combined immunodeficiency mouse and human psoriatic skin chimeras. Validation of a new animal model. *The American Journal of Pathology.* 1995; 146(3):580–588.
24. Sutton V, Sherwin B. Toxicological Animal Studies Disparate Treatment as Scientific Evidence. 2012.

25. Afzan A, Abdullah NR, Halim SZ, et al. Repeated dose 28-days oral toxicity study of *Carica papaya* L. leaf extract in sprague dawley rats. *Molecules.* 2012; 17(4):4326–4342.
26. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S. Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. *Molecules.* 2011; 16(6):5268–5282.
27. Mariappan G, Saha B, Sutharson L, et al. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. *Saudi Pharmaceutical Journal.* 2011; 19(2):115–122.
28. Streetz KL, Tacke F, Leifeld L, et al. Interleukin 6/gp130-dependent pathways are protective during chronic liver diseases. *Hepatology.* 2003; 38(1):218–229.
29. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney International.* 2002; 62(1):237–244.
30. Sayed-Ahmed MM. Role of carnitine in cancer chemotherapy-induced multiple organ toxicity. *Saudi Pharmaceutical Journal.* 2010; 18(4):195–206.
31. Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology.* 2003; 189(1):147–163.
32. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *Journal of Biological Chemistry.* 2004; 279(41): 42351–42354.
33. Dehn P, White C, Conners D, Shipkey G, Cumbo T. Characterization of the human hepatocellular carcinoma (HepG2) cell line as an *in vitro* model for cadmium toxicity studies. *In Vitro Cellular & Developmental Biology-Animal.* 2004; 40(5-6): 172–182.
34. Nerurkar PV, Dragull K, Tang C-S. *In vitro* toxicity of kava alkaloid, pipermethystine, in HepG2 cells compared to kavalactones. *Toxicological Sciences.* 2004; 79(1):106–111.
35. Kawata K, Osawa M, Okabe S. *In vitro* toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. *Environmental Science & Technology.* 2009; 43(15): 6046–6051.

Evaluation of anti-cancer potential of aqueous extract of *Pandanus odoratissimus* (Y.Kimura) Hatus. forma *ferreus*, by *in vivo* ascitic tumor model in swiss albino mice

Gunti Gowtham Raj[a], Hyma Sara Varghese[b], Sarita Kotagiri[c] and Vrushabendra Swamy B.M[c]

[a]*Department of Pharmacology, Gautham College of Pharmacy, Sultanpalya, Bangalore, Karnataka – 560 032, India*
[b]*Department of Pharmacology, Hillside College of Pharmacy and Research centre, Raghuvanahalli, Bangalore, Karnataka – 560 062, India*
[c]*Department of Pharmacology, East point College of Pharmacy, Bangalore,Karnataka – 560 049, India*

ABSTRACT

Background: India is a rich source of medicinal plants and number of plant extracts are used against diseases in various systems of medicine such as ayurveda, unani and siddha where only a few of them were scientifically explored. **Objective:** The objective of the present study was undertaken to perform dose dependent anti-cancer effect of aqueous and methanolic extracts of *P. odoratissimus* roots and leaves whose scientific documentation for anti-tumor agent is lacking despite using traditionally. **Materials and Methods:** The anti-cancer activity of methanolic extract of *P. odoratissimus* (MEPO) and aqueous extract of *P. odoratissimus* (AEPO) were tested against Ehrlich ascites carcinoma induced liquid tumors in swiss albino mice. The degree of protection was determined by change in body weight (gm), tumour volume (ml), packed cell volume (ml), cell viability (%), hematological parameters (R.B.C, W.B.C and hemoglobin content), mean survival time (MST), % increase in lifespan (% ILS) and histopathological observation of part of peritoneal layer. **Results:** The treatment with AEPO 400mg/kg, p.o. in EAC treated mice reduced tumor volume, packed cell volume, body weight, cell viability and improved all hematological parameters, mean survival time and life span. Histopathological changes showed degenerative changes of tumor cells in peritoneal layer. The anti-cancer effects of AEPO 400mg/kg, p.o. are equally more with that of the standard drug cisplatin. **Conclusion:** The results suggested that aqueous extract of roots and leaves of *P. odoratissimus* possess *in vivo* anti-cancer activity comparable to cisplatin and this study scientifically validated the traditional use of this plant.

Keywords: Anticancer, *Pandanus odoratissimus*, Ehrlich ascites carcinoma.

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in indian traditional systems of medicine. *Pandanus odoratissimus* (Pandanaceae) is one of such plants, distributed commonly throughout india. In ayurveda, unani and siddha the leaves are used for treating back ache, rheumatic diseases, epilepsy, wound healing, nervous disorders, loss of appetite, indigestion, constipation, diabetes, infertility, skin diseases, urinary disorders and fever.[1] The plant is known to possess a broad spectrum of medicinal, pharmacological and therapeutic properties. Tribals believe that this herb is an effective remedy for wide range of illnesses.[2] Leaves of *Pandanus* plants contain alkaloids such as pandanamine, pandamerilactones with pyrroline derived structures as major chemical constituent[3] and was found to possess anti-oxidant, anti-inflammatory and anti-diabetic activities.[4–6] In ayurveda paste of *P. odoratissimus* with sugar is used for treating cancers.[7] Active principles of methanolic extract of whole plant are 3-(4-(dimethylamino)cinnamoyyl)-4-hydroxycomarin, 3,3'-methylenebis (4-hydroxycomarin), erythro-9,10-dihydroxyoctadecanoic acid, octadecanedioic acid and dihydroagathic acid.[8] Recently, the acute and subacute toxicological studies on methanolic extract of *P. odoratissimus* did not produced

***Corresponding author.**
Mobile No: +918019691049
E-mail: raj.gowtham43@gmail.com

DOI: 10.5530/pj.2014.1.9

any significant changes in hematological, biochemical parameters and histopathology of vital internal organs.[9] The scientific evidence for supporting anti-tumor activity of *P. odoratissimus* is lacking, despite it is being used as potential anti-tumor agent in traditional system. Hence it was decided to illustrate the ethnobotanical use of this plant by examining dose dependent anticancer effects of methanolic and aqueous extracts from roots and leaves against Ehrlich ascites carcinoma induced liquid tumors in swiss albino mice.

MATERIALS AND METHODS

Chemicals

Cisplatin was obtained from sigma chemicals ltd., India. All other chemicals and solvents were obtained from reachem laboratory and Sd fine chemicals mumbai, India and were of analytical grade with highest purity.

Collection, authentication and extract preparation of *pandanus odoratissimus* (Y.Kimura) Hatus. forma *ferreus*

The plant *Pandanus odoratissimus* used for the present study was collected from the forest near punalur at kollam district, Kerala during mid winter season of 2012. The plant was identified, confirmed and authenticated by Dr. M. D. Rajanna, professor and head, department of botany (No. 3/proj/B-Garden), university of agricultural sciences, GKVK, bangalore, karnataka, India. A voucher specimen was deposited in department of botany GKVK for future reference.

The roots and leaves of the plant were shade dried, chopped into small pieces and powdered by a mechanical mixer. Coarse material of 500gm was extracted with two different solvents i.e., methanol (2.5L) and distilled water (2.5L) separately using soxhlet extraction apparatus. The solvents were evaporated using rotary vacuum evaporator (YamatoRE300, Japan) at 50°C and dried in dessicator.[10]

Phytochemical analysis

The qualitative and the quantitative analysis of the plant's constituents were examined by the methods described by Trease and Evans, El-Olemmy and Harbone[11–13] and were later used for assessment of *in vivo* anti-cancer activity.

Experimental animals

Swiss albino mice of 25–30g were procured from biogen, bangalore. They were maintained in the animal house of Gautham college of pharmacy for experimental purpose. The study conducted was approved by the Institutional Animal Ethics Committee (IAEC) of Gautham college of pharmacy, bangalore (REF-IAEC/03/05/2011) according to prescribed guidelines of CPCSEA (Reg No: 491/01/c/CPCSEA), Govt. of India.

Determination of acute toxicity (LD_{50})

Acute oral toxicity studies were performed according to OECD 423 (acute toxic class method) (Organization for Economic Co-operation and Development).[14] Swiss albino male mice (n = 6/each dose) selected through random sampling technique were employed in this study. The general behavior such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin color were observed for the first hour and after 24 h of test drug administration.

In vivo Anti-tumor activity

Ehrlich ascites carcinoma (EAC) cell lines used for the study was supplied by amala cancer research center, thrissur. Cell lines were aspirated aseptically from the mouse peritoneum of a fully grown tumor using syringe with 18 gauge needle, washed thrice with 0.9% saline and suspended in phosphate buffer saline and about 1×10^6 cells in 0.3ml of PBS was injected intraperitoneally into a new healthy mouse.[15]

Treatment protocol

- Group I : Normal saline control (10ml/kg p.o)
- Group II : EAC control (10ml/kg i.p.)
- Group III : EAC (0.3ml) + cisplatin (25mg/kg i.p.)
- Group IV : EAC (0.3ml) + aqueous extract of *P.odoratissimus* (200mg/kg p.o.)
- Group V : EAC (0.3ml) + aqueous extract of *P.odoratissimus* (400mg/kg p.o.)
- Group VI : EAC (0.3ml) + methanolic extract of *P.odoratissimus* (200mg/kg p.o.)
- Group VII : EAC (0.3ml) + methanolic extract of *P.odoratissimus* (400mg/kg p.o.)

Determination of anti-tumor activity

The anti-tumor potential of MEPO and AEPO were assessed for change in body weight, determination of survival time, % ILS, cell viability by trypan blue dye exclusion method, total ascites fluid volume, packed cell volume, hematological parameters and histopathological evaluation.[16–19] Formulae used for calculating above parameters are mentioned below.

$$\%\,\text{decrease in body weight} = \frac{\begin{pmatrix}\text{gain in body weight}\\ \text{of control group}\\ \text{-gain in body weight}\\ \text{of treated group}\end{pmatrix}}{\begin{pmatrix}\text{gain in body weight}\\ \text{of control group}\end{pmatrix}} \times 100$$

$$\%\,\text{viability} = (\text{no. of viable cells}) / (\text{no. of total cells}) \times 100$$

$$\%\text{ILS} = \frac{\begin{pmatrix}\text{MST of treated group}\\ \text{- MST of control group}\end{pmatrix} \times 100}{(\text{MST of control group})}$$

Statistical analysis

The values are expressed as mean ± SEM. The data were analysed by one way ANOVA followed by Dunnett's test using Graph pad prism software. p value < 0.05 was considered statistically significant.

RESULTS

The results of phytochemical analysis showed the presence of alkaloids (1.2%), flavonoids (4.6%), glycosides (2.6%) and phenolic content (3.1%) in aqueous extract of *P.odoratissimus* and presence of alkaloids (1.7%), flavonoids (1.3%) and carbohydrates (2.8%) in methanolic extract of *P.odoratissimus.*

In both phase I and phase II procedures, none of the animals had shown any toxicity up on single administration of MEPO and AEPO (2000mg/kg p.o.). Thus, 1/10th and 1/5th doses (200, 400mg/kg p.o.) from maximum dose were selected to study the dose dependent response.

Treatment with AEPO at dose of 400mg/kg, p.o. showed the maximum decrease in body weight, tumor volume, packed cell volume, cell viability more than standard cisplatin and greater than other treatment groups. There was a significant reduction in mean body weight 1.14 ± 0.31, mean tumor volume 1.25 ± 0.30, mean packed cell volume 0.75 ± 0.17 (p<0.001) and percentage cell viability 18%, in AEPO treatment group at a dose of 400mg/kg, p.o. over standard cisplatin and MEPO. Mean survival time in EAC control group was found to be 14 days which increased significantly to 23 days (62.06%) with AEPO 200mg/kg p.o. and to 31 days (113.79%) with AEPO 400mg/kg p.o., which showed maximum increase in the life span over standard cisplatin 26 days (79.31%) and different doses of MEPO on comparison with EAC control animals. There was a gradual decrease in percentage of body weight in EAC inoculated animals treated with AEPO, cisplatin and MEPO respectively. The percentage cell viability was found to be 92% in EAC control group which was reduced gradually by different treatment groups like AEPO, MEPO and cisplatin, respectively.

Table 1 shows the effect of cisplatin, AEPO and MEPO on EAC inoculated mice for all the above parameters including hematology profile. AEPO 200, 400mg/kg p.o. showed better improvement in the hematological parameters and reverted to normal levels. The total WBC, RBC and hemoglobin count in EAC control group were found to be 20.86 ± 0.36, 3.21 ± 0.058 and 7.63 ± 0.08. There was a significant decrease in WBC count in EAC inoculated animals treated with AEPO (p < 0.001), MEPO (p < 0.001) and cisplatin (p < 0.001) respectively. RBC count and hemoglobin content in AEPO treated animals showed significant increase (p < 0.001) equally more than cisplatin (p < 0.001). Whereas, MEPO at 400mg/kg p.o. (p < 0.01) treated animals showed slight improvement in RBC and hemoglobin (p < 0.01).

Histological section of peritoneum (Fig. 1) showed skeletal muscle with mesothelial proliferation (small arrows). The mesothelial proliferation consists of tumor cells having round to pleomorphic vesicular nucleus with prominent nucleoli and scant cytoplasm. Some of these tumor cells showed degenerative changes (large arrows) in AEPO at 400mg/kg p.o treated animals and cisplatin 25mg/kg i.p.

DISCUSSION

Flavonoids have been reported to act as anti-cancer agents *via* regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, modulation of enzyme activity related to detoxification, oxidation and reduction, stimulation of the immune system and DNA repair, and regulation of hormone metabolism.[20] Polyphenols have protective role in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high antioxidant capacity.[21]

In ascitic model following the inoculation of EAC tumor cell lines a marked decrease in life span and increase in body weight of mice were observed. Ascites fluid is the direct nutritional source to tumor cells and faster increase in ascites fluid with tumor growth could possibly means to meet more nutritional requirement of tumor cells. A rapid increase in ascites tumor volume was noted in tumor bearing mice was an indication of increase in body weight.[22]

Table 1. Effect of AEPO, MEPO and Cisplatin on following parameters.

Parameters	Normal control	EAC + Solvent 10 ml/kg i.p.	EAC i.p. + cisplatin 25 mg/kg i.p.	EAC i.p. + AEPO 200 mg/kg p.o.	EAC i.p.+ AEPO 400 mg/kg p.o.	EAC i.p. + MEPO 200 mg/kg p.o.	EAC i.p.+ MEPO 400 mg/kg p.o.
Change in b. w (gm)	0.40 ± 0.11	10.43 ± 0.13	1.80 ± 0.23***	2.39 ± 0.20***	1.14 ± 0.31***	6.97 ± 0.35***	5.33 ± 0.31***
TV (ml)	–	10.15 ± 0.14	1.66 ± 0.24***	2.13 ± 0.21***	1.25 ± 0.30***	6.80 ± 0.35***	5.20 ± 0.33***
PCV (ml)	–	5.55 ± 0.31	0.95 ± 0.12***	1.03 ± 0.20***	0.75 ± 0.17***	3.9 ± 0.21***	3.06 ± 0.24***
% Decrease in b.w	–	–	82.69	77.05	89.00	33.09	48.89
% CV	–	92	24	33	18	62	45
MST (days)	–	14.50 ± 0.50	26 ± 1.00***	23.50 ± 0.50**	31 ± 1.00***	16.50 ± 0.50^{ns}	17.50 ± 0.50^{ns}
% ILS	–	–	79.31	62.06	113.79	13.80	20.68
WBC ($x10^6$/ml)	7.26 ± 0.25	20.86 ± 0.36	10.20 ± 0.24***	10.52 ± 0.23***	8.20 ± 0.08***	15.42 ± 0.11***	16.32 ± 0.13***
RBC ($x10^9$/ml)	5.56 ± 0.06	3.21 ± 0.058	5.18 ± 0.04***	5.23 ± 0.05***	5.58 ± 0.07***	3.36 ± 0.12^{ns}	3.80 ± 0.04**
Hb (gm %)	12.53 ± 0.13	7.63 ± 0.08	12.07 ± 0.12***	11.38 ± 0.19***	12.19 ± 0.11***	7.68±0.07^{ns}	8.61 ± 0.12**

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** $P<0.001$, ** $P<0.01$, * $P<0.05$ and ns represents not significant. All values were compared with EAC control. Where, AEPO = Aqueous extract of P. odoratissimus; MEPO = Methanolic extract of P. odoratissimus; b.w = body weight; TV = Tumor Volume; PCV = Packed Cell Volume; CV = Cell Viability; MST = Mean Survival Time, ILS = Increase in Life Span.

The reliable criteria for judging the efficacy of any anti-cancer drug is prolongation of lifespan of the animals and the decrease of leukemic cells from blood.[23] The EAC control group was marked by significant increase in packed cell volume, WBC and viable tumor cell count whereas RBC, hemoglobin, lymphocytes, neutrophils and monocytes showed pronounced decrease. It is understood that the significant rise in WBC in EAC induced group, might be a defensive mechanism against cancer cells. As the progression of cancer was brought under control by *P. odoratissimus*, the WBC count got reduced in treated groups. In cancer chemotherapy, the major problems are myelosuppression and anemia.[24] The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions.[25] Interestingly, the present study showed that antitumor activity of *P.odoratissimus* was associated with the remarkable restoration of various hematological parameters including RBC and hemoglobin content, which was comparable with the standard anti-cancer drug cisplatin.

The mesothelial proliferation consisting tumor cells showed degenerative changes in aqueous extract treated animals at a dose of 400mg/kg p.o. Control of the cell cycle is accomplished via the coordinated interaction of cyclins with their respective cyclin-dependent kinases (CDKs) to form active complexes and drive cells into the next phase at the appropriate time. Disordering the cell cycle may result in genomic instability and apoptosis. Bcl-2 family proteins are important regulators of apoptosis. The family comprises both anti-apoptotic (e.g., Bcl-2) and pro-apoptotic proteins (e.g., Bax) with opposing biological functions.[26] Apoptosis in cells might happen through very complex functions. It has been suggested that apoptosis may happen by disruption mitochondrial function and induces lysosomal damage as the first target which leads to other cellular events including ROS production and oxidative damage[27] lysosomal damage, lipid peroxidation, DNA strand breaks, gene expression, chromosomal aberrations, inhibition of DNA repair processes and induction of apoptosis.[28]

CONCLUSION

AEPO at the dose of 400mg/kg p.o. had shown significant prolongation of lifespan, reduction in tumor volume, packed cell volume, cell viability, improvement in the hematological parameters, changes in histopathological observation when compared to the rest of the groups. There by it can be concluded that AEPO at 400mg/kg p.o. possess better anti-cancer activity than rest of the dose and doses of other drug.

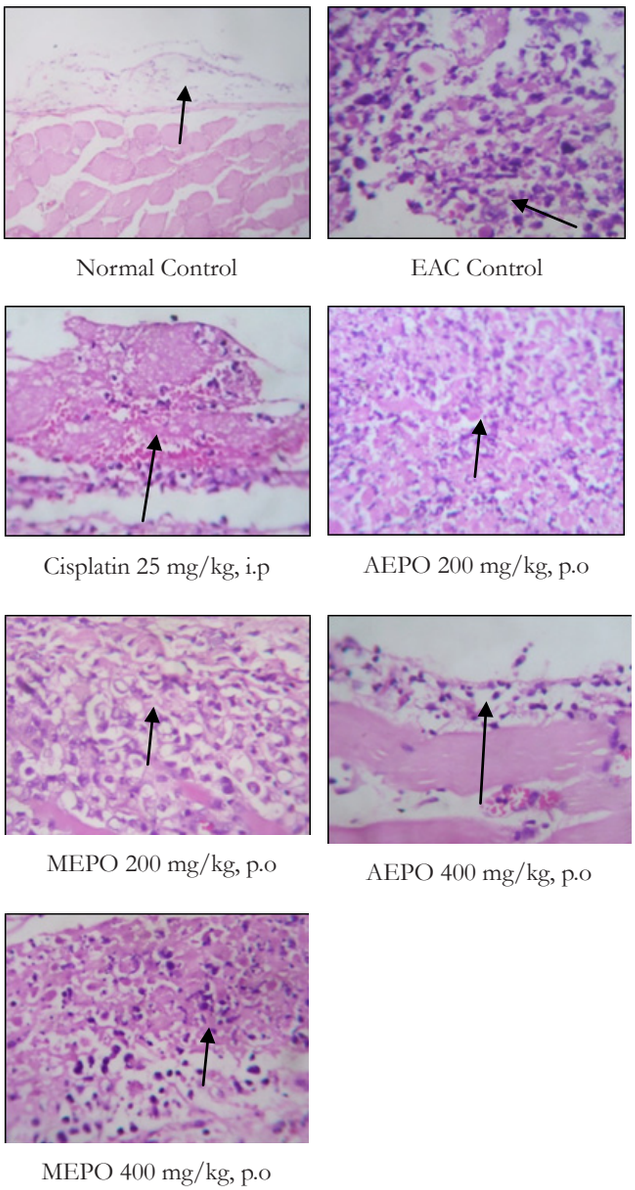


Figure 1. Histopathological study of peritoneum.

ACKNOWLEDGEMENT

The authors are thankful to Mrs. P. Archana swamy, principal of Gautham college of pharmacy, for providing necessary facilities and Mr. K.M Vishwanath swamy, for his valuable support, Mr. Varghese Thomas for helping in plant collection and Green Chem Laboratories for carrying extraction process.

REFERENCES

1. Majumdar K, Saha R, Datta BK, Bhakta T. Medicinal plants prescribed by different tribal and non-tribal medicine men of Tripura state. *Ind J Trad Know*. 2006; 5:559–562.
2. Del Rosario AG, Esguerra NM. Medicinal Plants in Palau. Palau: Palau Community College; 2003.
3. Nonato MG, Garson MJ, Truscot RJW, Carver JA. Structural characterization of piperidine alkaloids from *Pandanus amaryllifolius* by inverse-detected 2DNMR techniques. *Phytochemistry*. 1993; 34:1159–1163.
4. Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, et al. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu India. *J Med Plants Res*. 2010; 4:1089–1101.
5. Rajeswari J, Kesavan K, Jayakar B. Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam. *Asian Pac J Trop Med*. 2011; 4:649–653.
6. Jothimni Rajeswari, Karthikeyan Kesavan, Balasundaram Jayakar. Antidiabetic activity and chemical characterization of aqueous/ethanol prop roots extracts of *Pandanus fascicularis* Lam in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed*. 2012; S170–S174.
7. Premalatha Balachandran, Rajgopal Govindarajan. Cancer – an Ayurvedic perspective. *Pharmacol Res*. 2005; 51:19–30.
8. Bharathidasan R, Mahalingam R, Ambikapathy V, Panneerselvam A. Phytochemical compound analysis of *Pandanus odoratissimum. Asian J Plant Sci and Res.* 2012; 2:228–231.
9. Malligeswari Krishnamurthi Senthil kumar, Lakshmy Suseela, Venugopalan Rajesh. Neutralization potential of *Pandanus odoratissimus* Linn. leaf extract against snake venom poisoning. Tamilnadu; 2012.
10. Sokeng SD, Rokeya B, Mostafa M, Nahar N, et al. Antihyperglycemic effect of *Bridelia ndellensis* ethanol extract and fractions in streptozotocin-induced diabetic rats. *Afr J Trad*. 2005; 2:94–102.
11. In: Evans WC editors. 'Trease and Evans' Pharmacognosy. 14th ed.. London: WB Saunders; 1999.
12. El-Olemmy MM, Muhtadi FA, Affifi AA. Experimental Phytochemical. Riyadh: King Saudi University Press; 1994.
13. Harbone JB. Phytochemical methods. 68th ed.. London; 1993.
14. Ecobichon DJ. The Basis of Toxicology Testing. New York: CRC Press; 1997.
15. Nataraj Loganayaki, Sellamuthu Manian. Antitumor activity of the methanolic extract of *Ammannia baccifera* L. against Dalton's ascites lymphoma induced ascitic and solid tumors in mice. *Journal of Ethnopharmacol*. 2012; 142:305–309.
16. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumor activity of methanol extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. *Journal of Ethnopharmacol.* 2000; 72:151–156.
17. Da Mota, Mariana Flavia. Investigation of Ehrlich ascites tumor cell death mechanisms induced by *Synadenium umbellatum* Pax. *Journal of Ethnopharmacol.* 2012; 139:319–329.
18. Sreelatha S, Padma PR, Umasankari E. Evaluation of anticancer activity of ethanol extract of *Sesbania grandiflora* (Agati Sesban) against Ehrlich ascites carcinoma in Swiss albino mice. *Journal of ethnopharmacol*. 2011; 134:984.
19. Christina AJM, Gladwin Joseph D, Packialakshmi M, Kothai R, et al. Anticarcinogenic activity of *Withania somnifera* Dunal against Dalton's Ascitic Lymphoma. *Journal of Ethnopharmacol.* 2004; 93:359–61.
20. Aron PM, Kennedy JA. Flavan-3-ols: Nature, occurrence and biological activity. *Mol. Nutr. Food Res*. 2008; 52:79–104.
21. Tapiero H, Tew KD, Nguyen Ba G, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies. *Biomed Pharmacother*. 2002; 56:200–207.
22. Prasad SB, Giri A. Antitumor effect of Cisplatin against murine ascites Dalton's Lymphoma. *Ind J Exp Biol*. 1994; 32:155.
23. Gupta M, Mazumder UK, Sambath kumar R, Sivakumar T, Vamsi MLM. Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascites carcinoma in Swiss albino mice. *J Pharmacol Sci*. 2004; 94:177–184.

24. Maseki M, Nishiagaki I, Hagishara M, Tomoda Y, et al. Lipid peroxidation levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia. *J Clinica Chimica Acta*. 1981; 41:424–426.
25. Fenninger LD, Mider GB. Energy and nitrogen metabolism in cancer. *Adv Cancer Res*. 1954; 9:95–102.
26. Miyashita T, Krajewski S, Krajewska M. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression *in vitro* and in *vivo*. *Oncogene*. 1994; 9:1799–805.
27. Watjen W, Beyersmann D. Cadmium-induced apoptosis in C6 glioma cells: influence of oxidative stress. *J Biometals*. 2004; 17:65–78.
28. Biesalski HK, de Mesquita B, Chesson A, et al. European consensus statement on lung cancer: risk factors and prevention. *Cancer J Clin*. 1998; 48:167–176.

Alpha glucosidase inhibitory activity of hydro-methanolic (2:3) extract of seed of *Swietenia mahagoni* (L.) Jacq

Tushar Kanti Bera,[1,2] Kausik Chatterjee[1] and Debidas Ghosh[1,*]

[1]Department of Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapur-721 102, West Bengal, India
[2]Department of Pharmaceutical Division, Southern Health Improvement Samity (SHIS), South 24 Paraganas, Bhangar-743 502, West Bengal, India

ABSTRACT

Objectives: Present study investigated the effect of hydro-methanolic extract of seed of *Swietenia mahagoni* (HMESM) on α-glucosidase inhibition in normal and streptozotocin-induced diabetic rats. **Methods:** Oral carbohydrate tolerance tests were performed in 16h fasted normal and diabetic rats loaded with starch or sucrose or glucose at the dose of 3g/kg, 15min after administration of 250 (S1), 500 (S2), 1000 (S3) mg/kg of HMESM, vehicle (control),or pretreatment at the dose of 10 mg/kg of acarbose (Acar). Blood samples were analyzed for glucose levels at 0, 30th, 60th, and 120th min after respective treatments and the peak blood glucose (PBG) levels and area under the curves (AUC) were determined. **Results:** Results demonstrated that 500mg, 1000mg/kg of HMESM reduced and prolonged the PBG and decreased AUC simultaneously after starch and sucrose loading in normal and diabetic rats. Similarly acarbose also reduce the sucrose and starch induced blood glucose excursion, whereas it had no peak blood glucose suppressive effect after exogenous glucose load in both normal and diabetic rats. On the other hand, phytochemical study of the said extract revealed that it is rich in phenolic compounds (46.25 mg of gallic acid equivalent/g of extract) and flavonoids (231.72mg of quercetin equivalent/g of the extract), which may may responsible for pharmacological activities. **Conclusion:** The HMESM may have PBG suppressive effect in post-carbohydrate challenged state as evidenced by reduced PBG and AUC. This suggest that HMESM may be used effectively as a safer alternative to control postprandial hyperglycemia especially in pre-diabetic and diabetic patients.

Keywords: Streptozotocin, α-glucosidase, Postprandial hyperglycemia, Total flavonoids.

INTRODUCTION

Diabetes is one of the oldest known human diseases whose devastating effects not only increase day by day but its severity is also almost at epidemic level. The number of cases for diabetes that is currently at 171 million is predicted to reach 366 million by the year 2030, and around 3.2 million deaths every year are attributable due to complications of diabetes and it results six deaths every minutes.[1]

***Corresponding author:**
Department of Bio-Medical Laboratory Science and Management
Vidyasagar University
Midnapur-721 102
West Bengal, India

E-mail: debidas_ghosh@yahoo.co.in

DOI: 10.5530/pj.2014.1.10

The recent investigation projected that postprandial hyperglycemia (PPH) could induce the nonenzymatic glycation of different proteins, resulting in the development of chronic complications related with cerebrovascular disorder, retinopathy, nephropathy, and neuropathy etc.[2] Hence, the control of PPH is an important strategy for the management of diabetes mellitus, especially type-1 diabetes and to minimize the chronic complications associated with the disease.[3] In this regard, synthetic drugs such as acarbose, miglitol and voglibose are widely used to reduce the PPH by retarding the absorption of glucose through inhibition in the activity of carbohydrate hydrolyzing enzyme i. e. alpha-glucosidase in the digestive tract, but these drugs might induce the onset of symptoms such as abdominal distention, diarrhea, and soft feces etc.[4, 5] Plant extract have long been used for the ethnomedical treatment of diabetes in various system of medicine and are currently accepted as an alternative for diabetic

therapy. However, for many plant extracts, there is no clear understanding of the mechanism of action. Though some preliminary screening studies on α-glucosidase inhibitors from various plant extracts have been reported, the in vitro α-glucosidase inhibitory activity may not always correlate with the in vivo α-glucosidase inhibitory activity.[6] So, it is necessary to investigate the in-vivo action after oral administration on whole live animals, which is an important step in screening plant extracts for physiological and pharmacological effects.

The plant, *Swietenia mahagoni* (L.) Jacq. (Family- Meliaceae) is a beautiful, lofty, evergreen large tree, native to tropical America, Maxico and South America as well as India. *S. mahagoni* is a large medicinally and economically important timber tree native to West Indies. Seed extract of *S. mahagoni* is widely used in Indonesia as folk medicine to cure diabetes.[7] Thus, the current study was designed to determine the possible effect of graded doses of hydro-methanolic (2:3) extract of seeds of *S. mahagoni* after an oral carbohydrate (starch, sucrose, glucose) load on the peak blood glucose (PBG) level and area under the curve (AUC) value in normal and diabetic rats.

MATERIALS AND METHODS

Plant materials

The seeds of *Swietenia mahagoni* (L.) Jacq. (Family- Meliaceae) were collected from Medinipur, District of Paschim Medinipur, West Bengal, India, in the month of December and the materials were identified by Prof. R. K. Bhakat, Department of Botany and Forestry, Vidyasagar University, Medinipur. The voucher specimen was deposited in the Department of Botany, Vidyasagar University (Ref. No. *Swietenia mahagoni* (L.) Jacq./ VU/01/09).

Preparation of hydro-methanolic (2:3) extract from the seeds of Swietenia mahagoni

Fresh seeds were dried in an incubator for 2 days at 40 ^{0}C, crushed separately in an electric grinder and then pulverized. Fifty gm of pulverized powder of the seeds of Swietenia *mahagoni* was dissolved in 250ml of hydro-methanolic (2:3) solution and then kept in incubator at 37^{0}C for 36h. The slurry was stirred intermittently for 2h and left overnight. The mixture was then filtered and filtrate was dried by low pressure of rotary evaporator apparatus (Hahnvapor Scientific Co.). From that amount, 9gm light brown semi-solid residue was collected. The residue was dissolved in water and three separate doses (250mg, 500mg and 1000mg/kg body weight) were used for the treatment.

Chemicals

Streptozotocin (STZ) was purchased from Sigma, USA. Other chemicals were purchased from Sigma –Aldrich Diagnostic Ltd. USA. Blood glucose levels were measured using a one touch electronic glucometer of Ascensia Entrust, Bayer Diagnostics Ltd., Borada, India.

Animals' treatment

Study was conducted on Wistar strain male albino rats, weighing about 190 ± 10g. Animals were acclimatized for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages, at an ambient temperature of 25 ± 2^{0}C with 12h light: 12h dark cycle. Rats have free access to standard food and water *ad libitum*. The principle of laboratory animal care was followed throughout the duration of experiment and instruction given by our Institutional Animal Ethical Committee (Ref. No. VU/IAEC/BMLSM/18/12) was considered regarding injection and relevant treatment of the experimental animals. Diabetes was induced in the animals by a single intramuscular injection of streptozotocin (STZ) at the dose of 4mg/0.1ml of citrate buffer (pH 4.5)/100g body weight/rat. Blood glucose levels were constantly monitored using Ascensia® Entrust® blood glucometer (Bayer Diagnostics India LTD., Baroda, India) and rats showing blood glucose level around 300mg/dl were included in this study as diabetic animal. Acarbose (Bayer Pharmaceuticals, Leverkusen, Germany) was used as a positive control at a dose of 10mg/kg body weight.

The oral carbohydrate tolerance test was carried out in normal and diabetic groups of rats and was equally divided into various treatment groups as mentioned below.

Oral starch tolerance test

Rats were divided into five groups consisting of six rats in each group. The rats were fasted overnight for 16 h but had free access to water. In treatment group I, rats were treated orally with 250mg/kg body weight of HMESM, in treatment group II, rats were treated orally with 500mg/kg body weight of HMESM, and in treatment group III, rats received orally 1000mg kg body weight of HMESM. The groups were designated as S1, S2, S3, respectively. Rats under treatment group IV were treated orally with only distilled water (control) and finally, rat treatment group V, were treated orally with positive control acarbose (Acar) 10mg/kg body weight. After 15 min, all rats were given starch at the dose of 3g/kg (R & M Chemicals, Essex, UK) body weight orally and the tail

was snipped for blood glucose estimation before (0 min i.e. starch loading) and at 30th, 60th, and 120th min post loading state.

Blood glucose levels were recorded and PBG and AUC values were determined. The maximum blood glucose levels found during blood glucose determination was taken as the PBG. The formula for AUC determination is as follows.[8]

$$AUC(mmol/L) = \frac{(BG_0 + BG_{30})}{2} \times 0.5 + \frac{(BG_{30} + BG_{60})}{2} \times 0.5 + \frac{(BG_{60} + BG_{120})}{2} \times 1$$

Oral sucrose tolerance test

The oral sucrose tolerance test was carried out in the same way, but in this test sucrose (R & M Chemicals, Essex, UK) at a dose of 3g/kg body weight was used.[8]

Oral glucose tolerance test

The oral glucose tolerance test was conducted in the same manner where glucose loading (R & M Chemicals, Essex, UK) was performed at a dose of 3g/kg body weight.[8]

Determination of total phenolic compounds (TPC) and total flavonoid compounds (TFC)

The total phenolic content present in the extract was determined using Folin-Ciocalteu reagent.[9] The reaction mixture was prepared by adding 1ml of extract with 0.5ml of Folin-Ciocalteu reagent, 3ml of 20% Na_2CO_3 and 10ml of distilled water. After incubation for 2 h at ambient temperature, the absorbance was measured spectrophotometrically at 765nm. The total phenolic contents were calculated from a gallic acid standard curve and the result was expressed in terms of mg gallic acid equivalents (GAE)/g of extract.

The total flavonoid content was determined by aluminium chloride ($AlCl_3$) using colorimetric method with quercetin as standard.[10] The reaction mixture was prepared using 1ml of extract, 4ml of distilled water, and 0.3ml of $NaNO_2$ and after 5 minutes 3ml of 10% $AlCl_3$ was added to the reaction mixture. After incubation for further 5 minutes the reaction mixture was treated with 2ml of 1 M NaOH and the absorbance was measured at 510nm. The flavonoid content was calculated from a quercetin standard curve and the result was expressed in terms of mg quercetin equivalents (QE)/g of extract.

Statistical analysis

Statistical difference in PBG and AUC values between control and various treatment groups were determined using software in computer (Origin 6.1). One-way analysis of variance (ANOVA) followed by multiple comparison two-tail 't' test was conducted to find out the significant of the result.[11] Values were expressed as mean ± SEM.

RESULTS

Effect of hydro-methonolic extract of seeds of *Swietenia mahagoni* on oral starch tolerance tests in normal and diabetic rats

The results of the oral starch tolerance tests on normal rats demonstrated an inhibition in the increment of blood glucose levels and AUC values at 30th min after HMESM administration followed by oral starch loading in control, S1, and S2 groups. The same nature of curve up to 30th min of HMESM administration was noted in control, S1, and S2 groups in diabetic condition but the blood glucose levels and AUC values were decreased from the starting period in S3 and acarbose treated groups both in normal and diabetic condition(Fig. 1, 2 and Table 1).

In normal condition, blood glucose levels and AUC values were remain stable beyond 30th min up 120th min in the control, S1, and S2 groups but in the S3 and acarbose groups, the levels of blood glucose and AUC values were significantly decreased in that period in respect to control (Fig. 1 and Table 1).

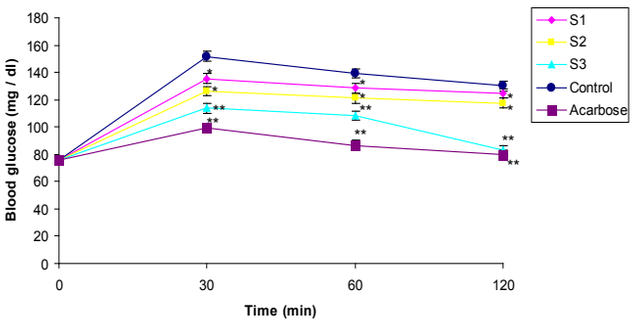


Figure 1. Blood glucose response in oral starch tolerance test after loading with starch at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

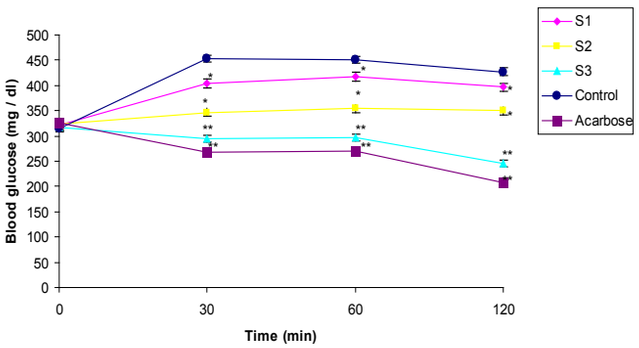


Figure 2. Blood glucose response in oral starch tolerance test after loading with starch at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

Table 1. Effect on PBG and AUC after starch loading at the dose of 3g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg of hydro-methanolic (2:3) extract of seeds of Swietenia mahagoni, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	135.53±3.2*	245.07±3.1*
S2	126.5±2.5*	231.8±2.4*
S3	115.65±2.1**	199.54±2.5**
Control	151.8±3.7	264.26±3.3
Acarbose	99.39±2.3**	173.39±2.2**
Diabetic rats		
S1	404.8±4.1*	792.29±4.3*
S2	346.97±3.7*	692.25±3.8*
S3	294.56±3.1**	573.28±4.1**
Control	453.59±4.1	857.75±5.2
Acarbose	267.45±3.2**	520.41±4.2**

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

In diabetic condition, blood glucose levels and AUC values were remained in flat in S1, and S2 groups in respect to control though the above levels were significantly lower than the control. In contrast, in S3 and acarbose treated groups, the blood glucose levels and AUC values were continuously decreased up to 120th min which were significantly differ in respect to the control (Fig. 2 and Table 1).

Effect of hydro-methonolic extract from seeds of *Swietenia mahagoni* on oral sucrose tolerance tests in normal and diabetic rats

Results of oral sucrose tolerance tests on normal rat's demonstrated an elevation in blood glucose levels and AUC values at 30th min after HMESM administration followed by oral sucrose loading in all the groups as the curve noted in control. The blood glucose levels and AUC values were decreased in normal condition beyond 30th min up to 120th min in S1, and S2 groups. There was no significant difference in the levels of above parameters among S1, S2, and control groups at 120th min of the test. In S3 and acarbose treated groups results were significantly decreased in the levels of said biosensors in that period in respect to control (Fig. 3 and Table 2).

In diabetic condition, blood glucose levels and AUC values were remained in flat in S1, and S2 groups in respect to control though the levels were significantly lower than the control. In contrast, the blood glucose levels and AUC values was continuous decreased up to 120th min in S3 and acarbose treated groups which were significantly less in respect to the control (Fig. 4 and Table 2).

Effect of hydro-methonolic extract of seeds of *Swietenia mahagoni* on oral glucose tolerance tests in normal and diabetic rats

Both in normal and diabetic condition, the blood glucose levels and AUC values attained their peak values at 30th min after the HMESM administration followed by glucose loading in all groups (Fig. 5 and Table 3). After 30th min up to 120th min, the blood glucose levels and AUC values in all the groups remained in flat state which were insignificant differ from the respective control values (Fig. 6 and Table 3).

TPC and TFC compound

The total phenolic compound (TPC) was 46.25mg of gallic acid equivalent and total flavonoid content (TFC) was 231.72mg of quercetin equivalent were present per gram of the hydro-methanolic extract of seeds of *Swietenia mahagoni* (Table 4).

DISCUSSION

The effective management of postprandial hyperglycemia (PPH) in diabetes mellitus is a key problem because high blood glucose levels may cause the stimulation and / or progression of diabetic complications through activation of the polyol pathway, elevation in protein glycation and promotion of hyperinsulinemia.[12–14] A prominent pathway for glucose production from food is the breakdown of carbohydrates by intestinal amylases or glycoside hydrolyses in the intestine. Thus, blood glucose management, prevention in the elevation in postprandial blood

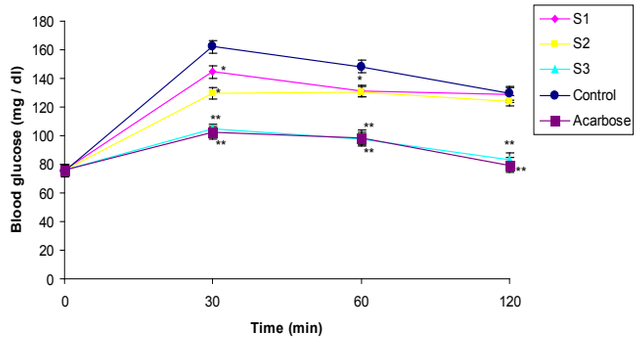


Figure 3. Blood glucose response in oral sucrose tolerance test after loading with sucrose at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10 mg/kg. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

Table 2. Effect on PBG and AUC after sucrose loading at the dose of 3 g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	144.52±3.1*	254.22±3.4*
S2	129.34±3.5*	243.83±2.8*
S3	104.8±2.6**	186.09±2.5**
Control	162.02±3.3	275.88±3.2
Acarbose	102.1±2.7**	183.67±2.6**
Diabetic rats		
S1	421.2±4.2*	790.7±4.8*
S2	364.5±3.7*	691.52±5.1*
S3	297.6±3.8**	527.67±4.6**
Control	448.5±4.4	864.15±5.3
Acarbose	284.3±3.6**	502.37±4.7**

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

glucose level and for maintaining blood glucose level within the normal range by controlling glucose production from food sources using an oral α-glucosidase inhibitor would be an idealistic and effective management for NIDDM patients.

Acarbose-like drugs, those inhibit α-glucosidase activity present in the epithelium of the small intestine, have been demonstrated to decrease postprandial hyperglycemia (PPH) and improve impaired glucose metabolism without promoting insulin secretion in NIDDM patients.[15] Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase

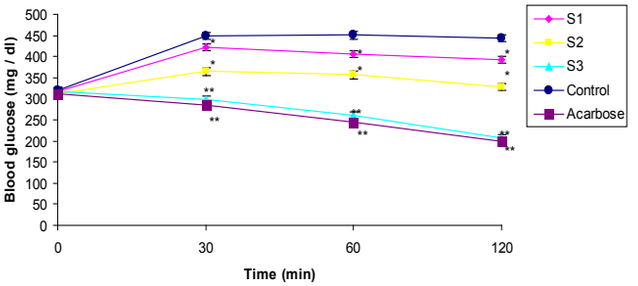


Figure 4. Blood glucose response in oral sucrose tolerance test after loading with sucrose at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

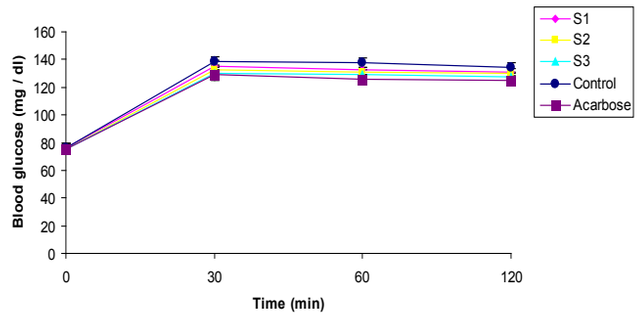


Figure 5. Blood glucose response in oral glucose tolerance test after loading with glucose at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

Table 3. Effect on PBG and AUC after glucose loading at the dose of 3g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	133.63±3.3	251.72±4.4
S2	132.81±3.2	249.16±4.2
S3	130.42±2.9	247.62±3.8
Control	136.75±3.6	253.59±4.6
Acarbose	129.31±3.2	245.41±3.7
Diabetic rats		
S1	370.66±4.1	719.11±4.4
S2	369.21±3.8	718.42 ±4.1
S3	367.93±3.6	716.88±3.9
Control	372.47±4.7	721.03±4.8
Acarbose	365.23±3.4	714.12±3.7

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

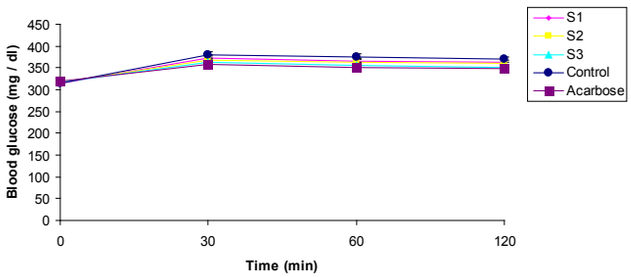


Figure 6. Blood glucose response in oral glucose tolerance test after loading with glucose at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

Table 4. Total phenolic and total flavonoid content of hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni* (L.) Jacq.

Samples	Total phenolic content (TPC) (mg GAE / g dried extract)	Total flavonoid content (TFC) (mg QE / g dried extract)
Hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni* (L.) Jacq.	46.25	231.72

GAE: gallic acid equivalents; QE: quercetin equivalents.

inhibitor offers a prospective therapeutic approach for the management of type -2 diabetes mellitus.[16] We found in the above experiments that S2 and S3 doses of the HMESM reduced the blood glucose excursions and decrease the PBG and AUC after sucrose and starch loading in normal and diabetic rats. The tendency of HMESM to suppress the PBG at 30min in both normal and diabetic rats demonstrates the α-glucosidase inhibitory activity and interfere the PBG. The HMESM seems to delay the quick digestion of starch as well as sucrose and lengthen the duration of carbohydrate absorption and thus reducing the PBG and AUC values. The above results show a striking similarity to the effect of acarbose.

On the other hand phenolic and flavonoids compounds of the extract are responsible for the inhibition in α-glucosidase activity and thereby inhibit glucose absorption in connection with the management of postprandial hyperglycemia may be proposed here as phytochemical analysis of the extract focused the presence of phenolic compounds, i.e., 46.25mg gallic acid equivalents (GAE) and flavonoids 231.72mg quercetin equivalents (QE) per gram of the extract. This proposal is consistent with the findings of others covering other plants.[17] In the field of phytochemistry and herbal medicines, there has been an enormous interest in the development of alternative medicines for type-2 diabetes, especially screening for natural bioactive compounds with the ability to delay or prevent glucose absorption. This is because any control of PPH by these alternative medicines would be much safer and will improve the quality of life of persons with borderline NIDDM. α-glucosidase inhibitors, α-amylase inhibitors, or glucose transport inhibitors have been screened to flatten postprandial blood glucose rise and are in the process of further development.[18–20] At present, only synthetic α-glucosidase inhibitors have been clinically used for management of type 2 diabetes. It is already known that ingestion of α-glucosidase inhibitors like acarbose or voglibiose regularly is more effective in moderating hyperglycemia in borderline NIDDM.[21]

CONCLUSION

The HMESM seems to have α-glucosidase inhibitory activity in diabetic rats because it suppresses the PBG and reduces AUC after simultaneous starch and sucrose loading and significantly affects the absorption of starch and sucrose. It may be used a safer alternative treatment to control PPH particularly in type 2 diabetic patients and also in borderline patients not properly controlled through diet alone. These medications are most useful for people who have just been diagnosed with type-II diabetes. The HMESM are also useful for people taking oral antidiabetic agents who need an additional medication to keep their blood glucose levels within a safe range. However, it is still early to suggest its use in humans, and only a thorough in-depth study can warrant its clinical use.

ACKNOWLEDGEMENT

This research work funded by Indian Council of Medical Research (ICMR), Govt. of India, for financial assistance (Project No. 59/30/2008/BMS/TRM).

REFERENCES

1. Wild SG, Folic A, Green R, King H. Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030. Diabetes Care, 2004; 27:1047–1054.
2. Ortiz-Andrade RR, Garcia-Jimenez S, Castillo-Espana P, Villalobos-Molina R, Estrada-Soto S. Alpha-glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana:* An antihyperglycemic agent. J Ethnopharmacol, 2007; 109: 48–53.
3. Subramanian R, Asmawi MZ, Sadikun A. In vitro alpha-glucosidase and alpha amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. Acta Biochimica Polonica, 2008; 55: 391–398.

4. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effects of pine extract on alpha-glucosidase activity and post prandial hyperglycemia. Nutrition, 2005; 21:756–761.
5. van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C. α-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta analysis. Diabetes Care, 2005; 28:154–163.
6. Gowri PM, Tiwari AK, Ali AZ, Rao JM. Inhibition of alpha-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Roxb. seeds. Phytotherap Res, 2007; 21: 796–799.
7. Li DD, Chen JH, Chen Q, Li GW, Chen J, Yue JM. *Swietenia mahagoni* extract shows agonistic activity to PPAR (gamma) and gives ameliorative effects on diabetic db/ db mice. Acta Pharmacol Sin, 2005; 26:220–222.
8. Rammohan S, Zaini A, Amirin. In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. Acta Biochimica Polonica, 2008; 55:391–398.
9. Mariod AA, Ibrahim RM, Ismail M, Ismail N. Antioxidant activities of phenolic rich fractions (PRFs) obtained from black mahlab (*Monechma ciliatum*) and white mahlab (*Prunus mahaleb*) seedcakes. Food Chem, 2010; 118:120–127.
10. Kumar TS, Shanmugam S, Palvannan T, Kumar VMB. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. leaves. Ira J Pharmacol Res, 2008; 7:211–215.
11. Sokal RR, Rohle FJ. Introduction to Analysis of Variance. In: Biometry. New York. WH Freeman and Company, 1997 p 179–206.
12. Dewi RT, Iskandar YM, Hanafi M. Inhibitory effect of koji *Aspergillus terreus* on α-glucosidase activity and postprandial hyperglycemia. Pak J Biol Sci, 2007; 10:3131–3135.
13. Yuhao L, Wen S, Prasad-Kota B, Peng G, Qian-Li G, Yamahara J. *Punica granatum* flower extract, a potent alpha glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. J Ethnopharmacol, 2005; 99:239–244.
14. Leach MJ. *Gymnema sylvestre* for diabetes mellitus: a systematic review. J Alt Com Med, 2007; 13:977–983.
15. Abesundara K J, Matsui T, Matsumoto K. Alpha-glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to therapeutic drug acarbose. J Agricultural Food Chem, 2004; 52:2541–2545.
16. Scheen AJ. Is there a role for a-glucosidase inhibitors in the prevention of type 2 diabetes mellitus? Drugs, 2003; 10: 933–951.
17. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. J Nutri Sci Vitaminol, 2006; 52:149–153.
18. Kim KY, Nam KA, Kurihara H, Kim SM. Potent α- glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. Phytochem, 2008; 69:2820–2825.
19. Funke I, Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of *α*-amylase activity. Bra J Pharmacol, 2006; 16:1–5.
20. Carrascosa JM, Molero JC, Fermin Y, Martinez C, Andres A, Satrustegui J. Effects of chronic treatment with acarbose on glucose and lipid metabolism in obese diabetic wistar rats. Diabetes Obes Metab, 2001; 3:240–248.
21. Lee HW, Park YS, Choi JW, Yi SY, Shin WS. Antidiabetic effects of chitosan oligosaccharides in neonatal streptozotocin-induced noninsulin-dependent diabetes mellitus in rats. Biol Pharm Bull, 2003, 26:1100–1103.