Assessment of quality of *Manilkara hexandra* (Roxb.) Dubard leaf (Sapotaceae): Pharmacognostical and Physicochemical profile

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INTRODUCTION

Pharmacognosy has always been a translational or multidisciplinary science, most recently emphasized in the discussion of modern pharmacognosy, as described by Bohlin and co-workers.[1] According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[2]

Manilkara hexandra (Roxb.) Dubard (Syn: *Mimusops hexandra* Roxb.) (Sapotaceae) is a large evergreen tree widely distributed throughout the greater parts of India and other tropical countries. The stem bark is astringent, febrifuge, sweet, tonic, and is used traditionally to treat a wide range of gastrointestinal symptoms.[3]

The therapeutic activity of herbs is because of various constituents present in them. The therapeutic effects of herbal products is inconsistent and varies because the chemical constituents vary; they depend on various factors and one of them is the source. In some plants toxic constituents are also present therefore it is essential to evaluate their quality, safety and efficacy. Correct identification and quality assurance of the starting material is, therefore an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy.[4]

ABSTRACT: The term pharmacognosy, a constituent scientific discipline of pharmacy, has been in use for nearly 200 years and it refers to the studies on natural product drugs. The present study deals with the macroscopic and microscopic studies of *Manilkara hexandra* (Roxb.) Dubard leaf. The anatomy of the leaf was studied by taking transverse section which showed anomocytic stomata, unicellular trichomes, etc. Powder microscopic examination showed presence of pitted pericyclic fibres, spiral xylem vessels and calcium oxalate crystals. Physicochemical parameters and heavy metal analysis of the powder were also carried out. The present investigation on *M. hexandra* leaf might be useful to supplement information in regard to its identification parameters. Such studies are important in the way of acceptability of herbal drugs in present scenario of lacking regulatory laws to control quality of herbal drugs.

Keywords: *Manilkara hexandra* leaf, Pharmacognosy, Physicochemical profile.
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The present work is aimed at the pharmacognostical and physicoochemical analysis of *Manilkara hexandra* (Roxb.) Dubard leaf.

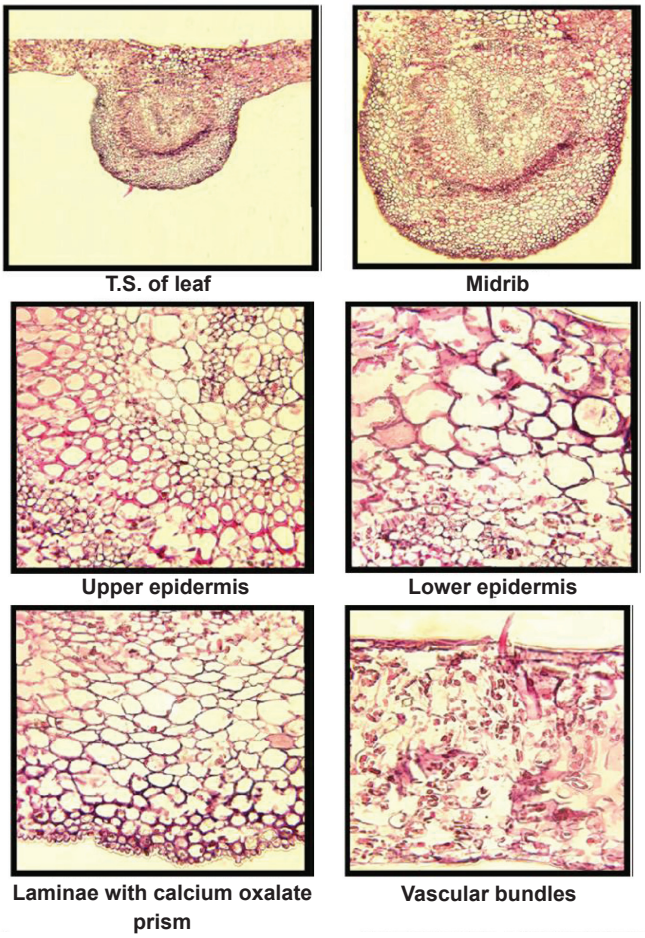
MATERIALS AND METHODS

Plant material

Fresh leaves of *Manilkara hexandra* (Roxb.) Dubard were collected in the month of August 2005, from Anand Agricultural University, Anand. The identity of the plant was confirmed by Dr. Sriram, Research Scientist, Anand Agricultural University, Anand. The plant was compared with voucher specimen (voucher specimen No. PSN428) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. For further confirmation, the microscopic characteristics of this plant were studied and compared with available literature. The fresh plant material collected was thoroughly cleaned by washing under running tap water and air-dried. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

FIGURE 1: Macroscopic characteristics of *Manilkara hexandra* (Roxb.) Dubard leaf.

FIGURE 2: Photomicrographs of the microscopic characteristic of *Manilkara hexandra* (Roxb.) Dubard Leaf.



Pharmacognostic Studies

Macroscopic study: Macroscopic observation of *M. hexandra* leaf was done. It comprised of shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.[5]

Microscopic study: Transverse sections of the leaves of *M. hexandra* were taken by using a microtome.

Permanent mount of leaf was prepared using paraffin method.[6]

Physicochemical parameters: Physicochemical parameters were determined as per guidelines of WHO.[2] Total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility, melting point, pH, heavy metal analysis, petroleum ether soluble extractive, alcohol soluble extractive value and water soluble extractive value were determined.

RESULT AND DISSCUSION

Pharmacognostic study

The pharmacognostic study is the major and reliable criteria for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostic evaluation would give valuable information for future studies.

Macroscopically leaves are alternate, coriaceous, elliptic, oblong or obovate, oblong, rounded or emarginated at apex, glabrous, dark green and polished above, paler beneath. It measures about 2.5–11 × 1–6 cm. (Fig. 1). Its taste is slightly bitter. The microscopic studies of leaves showed following tissue systems:

Epidermis

Upper Epidermis: The single layered epidermal cells were straight walled, rectangular in shape and covered with thin cuticle. The simple, unicellular, conical and thick trichomes with single covering were present. Lower Epidermis: Similar to upper epidermis. The lower epidermis had more number of trichomes than the upper epidermis. The anomocytic stomata were present in lower epidermis. Prisms of calcium oxalate were present in the lower epidermis. (Fig. 2).

Mesophyll

Leaf was dorsiventral. Therefore, mesophyll was divided into single layered elongated palisade cells under upper epidermis and a loose tissue of irregular spongy parenchymatous cells having large intercellular spaces above lower epidermis.

Midrib

The T.S. of midrib showed meristele in the form of an arc which contained xylem towards the dorsal side and phloem towards the ventral side. Epidermal layers were continuous. Below upper epidermis and above lower epidermis, the thick walled cellulosic collenchyma cells were present. The vascular bundle was collateral i.e. xylem and phloem were present on the same radius side by side. The xylem was lignified while phloem was non-lignified. Thick walled pericyclic fibers containing sclerenchyma cells were present between the phloem and cortical region and surrounded the vascular bundle. (Fig. 2).

Powder characteristics

The crude powder of *M. hexandra* (Roxb.) Dubard leaf was dark green in color with characteristic odour and slightly bitter in taste. Microscopy study of powder showed the presence of upper epidermis in surface view which showed straight walled cells, lower epidermis in surface view showed somewhat wavy walled cells with anomocytic stomata, simple covering unicellular trichome, group of pericyclic fibers, prisms of calcium oxalate crystals and xylem vessels and longitudinal sectional view showed spiral thickening. (Fig. 3).

The quantitative determination of some pharmacognostic parameters is useful for setting standards for

FIGURE 3: Photomicrographs of the specific characteristics determined from the powder study of *Manilkara hexandra* Dubard leaf.

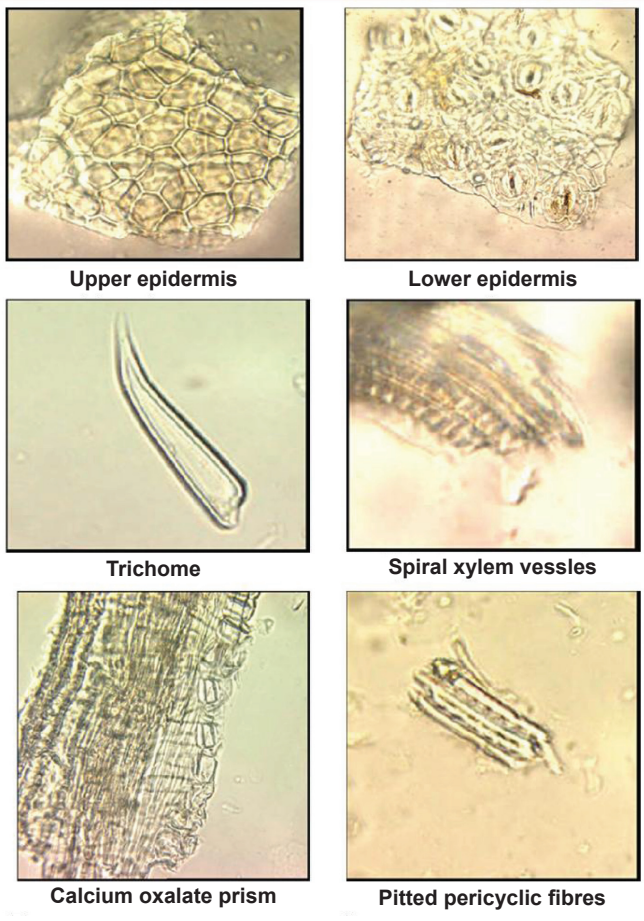


TABLE 1: Determination of proximate parameters of crude powder of *Manilkara hexandra* (Roxb.) Dubard leaf.

PROXIMATE PARAMETERS	AVERAGE VALUE % w/w
Loss on drying	4
Total ash	6
Acid insoluble ash	1
Petroleum ether soluble extractive	3.74
Alcohol soluble extractive	10.6
Water soluble extractive	12.3

crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.[7]

The results of physicochemical parameter analysis of crude powder of *M. hexandra* leaf (MCR) were shown in Table 1. The average values are expressed as percentage of air-dried material. MCR showed 4% of loss on drying. It contained 6% of total ash and about 1% of acid insoluble ash. The percentage extractive yield of crude powder extracted in petroleum ether, alchohol and water were 3.74, 10.6 and 12.3 respectively.

The three extracts MPE (Petroleum ether extract), MAC (Acetone extract) and MME (Methanol extract) of *M. hexandra* were evaluated for qualitative solubility test for 29 solvents with varied polarities (Table 2). MME and MAC were almost soluble in all the solvents while MPE showed comparatively less solubility due to its hydrophobic nature. The results of melting point and pH of the crude powder and different extracts of *M. hexandra* are shown in Table 3. The melting point of crude powder (MCR), acetone extract (MAC) and methanol extract (MME) was > 300°C and that of petroleum ether extract (MPE) was 206–210°C. All the samples were acidic in nature. The methanol extract (MME) was the most acidic in nature.

TABLE 2: Determination of solubility of different extracts of *Manilkara hexandra* (Roxb.) Dubard leaf.

SOLVENTS	SOLUBILITY		
	MPE	MAC	MME
Hexane	++	+	+
Heptane	++	+	+
Benzene	+++	++	++
Diethyl ether	++	+++	++
Petroleum ether	++	+	+
1-4 dioxan	++	+++	++
Tetrahydrofuran	-	++	+++
Ethyl acetate	++	++	++
Chloroform	+++	++	++
Acetone	++	++	++
Dimethylformamide	+	+++	+++
Dimethylsulphoxide	++	+++	+++
1-Butanol	++	++	++
1-Propanol	++	+++	+++
Acetic acid	++	++	++
Ethanol	++	+++	+++
Methanol	+	+++	+++
2-methoxy ethanol	++	+++	+++
Triacetin	+	+	+
Toluene	-	++	+++
Distilled water	-	+	+
Tap water	-	++	++
2-methyl Propanol	++	++	+
Dichloromethane	+++	++	++
Amyl alcohol	++	++	+
Benzyl alcohol	++	+++	++
Benzaldehyde	+++	+++	++
Orthophosphoric acid	+	++	+++
Formic acid	++	+++	+++

(-): Not Soluble, (+): Sparingly soluble, (++): Soluble, (+++): Highly soluble, MPE: Petroleum ether extract, MAC: Acetone extract, MME: Methanol extract

TABLE 3: Determination of melting point and pH of the crude powder and different extracts of *Manilkara hexandra* (Roxb.) Dubard leaf.

PLANT EXTRACT	MELTING POINT (°C)	pH
MCR	> 300	5.51
MPE	206–210	ND
MAC	> 300	5.01
MME	> 300	4.82

MCR: Crude powder, MPE: Petroleum ether extract, MAC: Acetone extract, MME: Methanol extract, ND: Not determined

The crude leaf powder of *M. hexandra* and its extracts (MPE, MAC and MME) were analyzed for the presence of heavy metals. The results (Table 4) showed that

TABLE 4: Determination of heavy metals in crude powder and different extracts of *Manilkara hexandra* (Roxb.) Dubard leaf.

PLANT EXTRACT	HEAVY METALS (ppm)					
	MERCURY (Hg)	LEAD (Pb)	CHROMIUM (Cr)	COBALT (Co)	ARSENIC (As)	NICKEL (Ni)
MCR	0.071	NDT	NDT	2.07	NDT	NDT
MPE	0.09	NDT	NDT	2.36	0.045	NDT
MAC	0.046	NDT	NDT	2.12	0.027	NDT
MME	0.094	NDT	NDT	2.02	NDT	NDT

MCR: Crude powder, MPE: Petroleum ether extract, MAC: Acetone extract, MME: Methanol extract, NDT: Not detected

lead, chromium and nickel were not present in any of the samples; mercury and cobalt was present in all the four samples with varying values. The presence of arsenic was found only in two samples (MPE and MAC). Although, there was minor presence of some heavy metals but the extracts did not exceed the limit given according to the WHO guidelines.[8] Therefore, the samples investigated were free from heavy metal contamination.

CONCLUSION

The micromorphological investigations have given useful pharmacognostic elements for the correct identification of the drugs both in scraped and powdered forms and this is of great interest for quality control in basic research and drug production, especially for imported items and for raw material sold by traditional herbalists.

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Evaluation of Various Natural Gums as Release Modifiers in Tablet Formulations

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INTRODUCTION

Oral drug delivery system is the most convenient way for drug delivery. Release of water soluble drug from the matrix system is not easy to control due to different factors such as high solubility and hence dose dumping of drug. This may results in toxicity of drug. Matrix tablets are easy to prepare and they are cost effective and exhibit predictable release behavior.[1] Sustained released products designed to reduce the frequency of dosing by modifying the rate of drug absorption have been available for many years.[2] Natural polymers are generally considered to be stable and safe as release retardant excipients in the development of oral controlled release dosage forms.[3] They are non-toxic, cost effective as well as easily available. The varied structure and chemistry of natural polymers provide ample opportunity for their use in the formulation of sustained release drug delivery systems. Guar gum, a polysaccharide derivative with glycoside linkage has been used as matrix former for controlled release of isoniazide and diltiazem.[4] Gum acacia or gum arabic is often

ABSTRACT

Background and purpose of study: In the present investigation, sustained release tablets of diclofenac sodium were formulated using guar gum and gum arabic (gum acacia) as release modifier.

Methods: Six batches of sustained release matrix tablets of diclofenac sodium were prepared by using different drug:polymer ratios viz., 1:1, 1:1.5, 1:2, 1:2.5, 1:3, and 1:3.5 for both guar gum and gum acacia. The tablets were evaluated for physical characteristics like hardness, weight variation, fraibilty, and drug content. *In vitro* release of drug was studied in phosphate buffered saline (pH 7.4) for twenty four hours.

Results: All the physical characters of the formulated tablets were found to be within acceptable limits. The tablets with guar gum exhibited greater swelling index than those with gum acacia. A better controlled drug release (98.7%) was obtained with the matrix tablet (F1) made-up of the guar gum.

Conclusion: It is clear through the dissolution profile of matrix tablets prepared using guar gum and gum acacia that these polymers have ability to retard drug release for 24 hrs.

Keywords: Diclofenac sodium, guar gum, gum acacia, sustained release matrix tablets.

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TABLE 1: Formulation composition of matrix tablets.

INGREDIENTS	FORMULATIONS					
	BATCH F1	BATCH F2	BATCH F3	BATCH F4	BATCH F5	BATCH F6
Diclofenac sodium	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
Polymer [a]	50 mg	75 mg	100 mg	125 mg	150 mg	175 mg
Microcrystalline cellulose	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg
Total weight	300 mg	300 mg	300 mg	300 mg	300 mg	300 mg

[a] *guar gum and gum acacia for their respective batches.*

used in the preparation as plasticizer and as a tablet binder. Gum acacia has been recognized as an acidic polysaccharide containing D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid.[5] Sustained release of ferrous sulphate was achieved for 7 h by preparing gum arabic pellet. All the gums are hydrophilic polymers, which until recently had been limited for use in gelation, thickening, suspending and water based emulsifying properties.[6–9] Drug release from hydrophilic matrices is mainly based on complex interaction involving swelling, diffusion and erosion mechanisms.[10–13] Diclofenac sodium is sodium 2-[(2, 6-dichlorophenyl)-amino] phenyl acetate. Diclofenac is an acetic acid nonsteroidal antiinflammatory drug (NSAID) with analgesic and antipyretic properties. Diclofenac is used to treat pain, dysmenorrhea, ocular inflammation, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and actinic keratosis.[14,15] The present investigation is aimed to formulate the matrix tablet of diclofenac sodium with guar gum and gum arabic using no other varying parameter.

MATERIAL AND METHODS

Diclofenac sodium was obtained as gift sample from Alchem Laboratories, Baddi, India. The pharmacopoeial grade of guar gum and gum acacia were obtained from Ranbaxy Fine Chemicals Limited, New Delhi, India. Other materials used were of analytical grade, and procured from commercial sources.

Preparation of sustained release matrix tablets

Sustained release matrix tablets of diclofenac sodium were prepared by using different drug:polymer ratios viz., 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5 for Batch 1, Batch 2, Batch 3, Batch 4, Batch 5 and Batch 6 respectively (Table 1). Guar gum and gum acacia were used as matrix forming material, while microcrystalline cellulose was used as filler to maintain the tablet weight. All ingredients were passed through a # 20 sieve, weighed, and blended. The granulated formulations (which were obtained after wet granulation, using water as granulating agent) were compressed by a direct compression technique, using KBr press (IR press), with the help of 8 mm flat faced punches.[16–19]

Evaluation of Fabricated Matrix Tablets

Weight variation: All batches of matrix tablets were evaluated for weight variation as per USP XXIV monograph. Twenty tablets of each batch were used to evaluate weight variation among tablets and standard deviation was calculated.[18,19]

Friability: Tablets of all batches were used to evaluate friability as per USP XXIV monograph. Friability testing was done by Roche friabilator with triplicate readings.[18,19]

Hardness: Hardness of all batches was determined using Digital Force Gauge (Model: EL = 500 N, Electrolab). The test was carried out in triplicate for all batches as per USP XXIV monograph for uncoated tablets.[18,19]

TABLE 2: Physical parameters for fabricated guar gum tablets.

PARAMETERS	FORMULATIONS					
	BATCH F1	BATCH F2	BATCH F3	BATCH F4	BATCH F5	BATCH F6
Weight variation (g)	0.301 ± 0.01	0.303 ± 0.01	0.302 ± 0.01	0.301 ± 0.01	0.299 ± 0.01	0.299 ± 0.01
Friability (%)	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Hardness (N)	20.27 ± 0.06	20.67 ± 0.06	20.83 ± 0.06	21.27 ± 0.12	21.5 ± 0.10	22.07 ± 0.06
Thickness (mm)	4.18 ± 0.03	4.15 ± 0.01	4.32 ± 0.05	4.38 ± 0.08	4.32 ± 0.08	4.31 ± 0.05

TABLE 3: Various evaluation parameters for fabricated gum acacia tablets.

PARAMETERS	FORMULATIONS					
	BATCH F1	BATCH F2	BATCH F3	BATCH F4	BATCH F5	BATCH F6
Weight variation (g)	0.301 ± 0.01	0.293 ± 0.02	0.299 ± 0.01	0.298 ± 0.01	0.301 ± 0.01	0.302 ± 0.01
Friability (%)	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Hardness (N)	20.07 ± 0.06	20.20 ± 0.0	20.37 ± 0.06	20.53 ± 0.06	20.63 ± 0.06	20.83 ± 0.06
Thickness (mm)	3.503 ± 0.02	3.560 ± 0.08	3.740 ± 0.04	3.683 ± 0.03	3.777 ± 0.04	4.04 ± 0.07

Thickness: Thickness was measured by vernier caliper as per USP XXIV monograph. The readings were carried out in triplicate and average value was noted.[18,19]

Drug content: The tablets were powdered, and 50 mg equivalent weight of diclofenac sodium in tablet powder was accurately weighed and transferred into a 100 ml volumetric flask. Initially, 10 ml of phosphate buffer (pH 6.6) was added and shaken for 10 min. Then, the volume was made up to 100 ml with buffer. Subsequently, the solution in volumetric flask was filtered, and 1 ml of the filtrate was diluted and analyzed at 276 nm using UV-visible spectrophotometer (Shimadzu UV-2450, Japan). The drug content of the each sample was estimated from the standard curve.[18–21]

Swelling Behavior of Sustained Release Matrix Tablets

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behavior of all formulations was studied. One tablet from each formulation was kept in a petridish containing phosphate buffer (pH 7.4). At the end of 1 h, the tablet was withdrawn, wiped with tissue paper, and weighed. Then for every 2 h, weights of the tablets were noted, and the procedure was continued till the end of 8 h. Percentage weight gain by the tablets (swelling index, S.I.) was calculated by using the formula:

$$S.I. = \{(M_t - M_0) / M_0\} \times 100,$$

Where, S.I. = Swelling index, M_t = weight of tablet at time t (in sec) and M_0 = weight of tablet at time 0.[18,19,22,23]

In vitro drug release study

In vitro drug release was studied using LabIndia dissolution apparatus, with 900 ml of dissolution medium maintained at 37 ± 1°C for 24 h, at 50 rpm. 5 ml of sample was withdrawn after every hour, and was replaced by an equal volume of fresh dissolution medium of same pH. Collected samples were analyzed spectrophotometrically, at wavelength of 276 nm, and cumulative percentage drug release was calculated. The study was performed in triplicate and results were recorded.[18,19,24]

The data obtained in the *in vitro* dissolution study is grouped according to two modes of data treatment as follows:

1. Percentage drug released Vs time in hrs.
2. Cumulative percentage drug released Vs time in hrs.

In these two methods, drug release profile can be better studied using cumulative percentage drug release Vs time (h) plot.

RESULTS AND DISCUSSION

The formulated matrix tablets met the pharmacopoeial requirement of uniformity of weight. All the tablets confirmed to the requirement of assay, as per I.P. Hardness, percentage friability and thickness were within the acceptable limits (Table 2, Table 3).[18,19] It has less absorption through gastric fluid due to less solubility at the pH studied. Sustained, but complete drug release was displayed by all the formulations in phosphate buffer (pH 7.4). Thus it can be concluded, that drug dissolution was a function of drug solubility, at various pH ranges. When pH rises above pKa, rapid increase in solubility occurs.

FIGURE 1: Swelling index profile of guar gum based tablet.

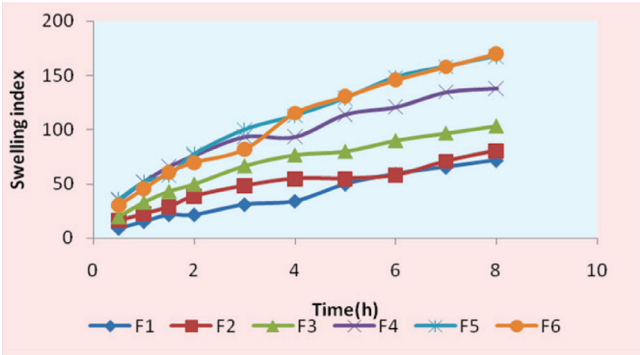


FIGURE 2: Swelling index profile of gum acacia based tablet.

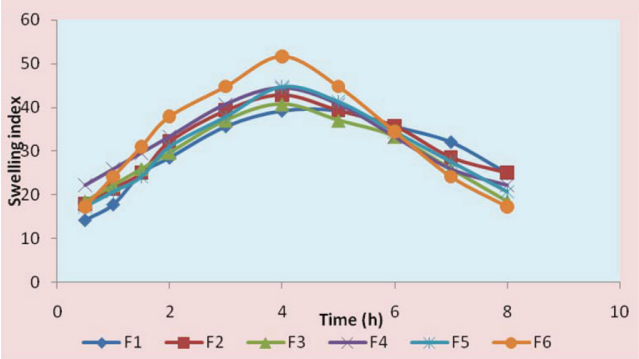
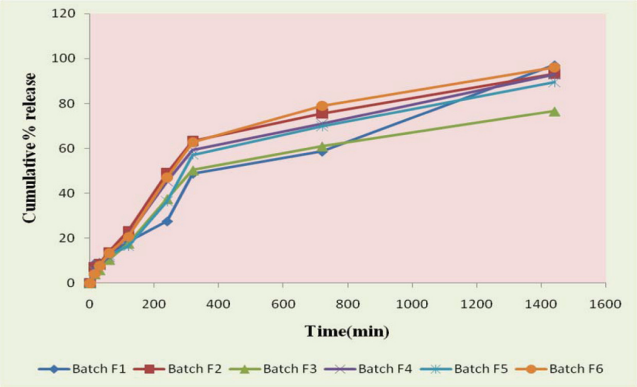


FIGURE 4: Release profile of drug from guar gum based matrix tablets.



The swelling index was calculated with respect to time. As time increases, the swelling index was increased, because weight gain by tablet increased proportionally with rate of hydration up to a certain limit. Later on, it decreases gradually due to dissolution of outermost gelled layer of tablet into dissolution medium (Figure 1, Figure 2, Figure 3). A direct relationship was observed between swelling index and gum concentration, and as gum concentration increases, swelling index was increased.[22,23] It has been observed that the cumulative percentage drug release decreases with increasing concentration of gum and swelling index. The reason attributed to this fact is the slow erosion of the gelled layer from the tablets containing higher amount of guar gum. This slow release is because of the formation of a thick gel structure that delays drug release from tablet matrix.

It has been observed that the cumulative percentage drug release decreases with increasing concentration of polymer and swelling index. The reason attributed to this fact is slow erosion of the gelled layer from the tablets containing higher amount of natural polymer. This slow release is due to the formation of a thick gel structure that delays drug release from tablet matrix.[24–26] From the findings, obtained so far it can be concluded that guar gum in the concentration ratio of 1:4 (G4) was promising concentration for oral controlled release tablet of diclofenac (Figure 4, Figure 5).

The drug release profile including the cumulative drug release for six batches of each polymer can be elucidated by plotting the graph of cumulative release vs. time. This can help in determining the release characteristics and matrix forming ability of natural polymers under study.

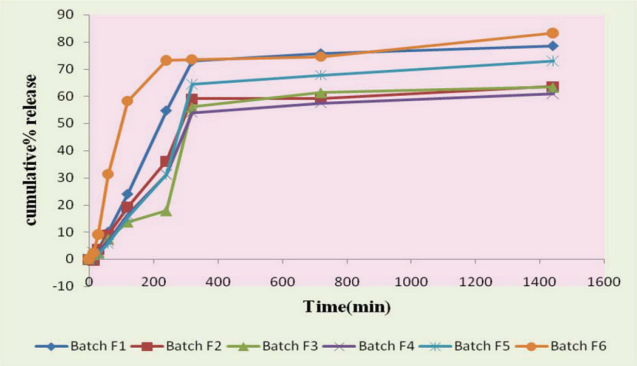
CONCLUSION

Natural polymers when used as release retardent exhibits uniform release over longer period of time. Hence it can be concluded that, guar gum which is a

FIGURE 3: Different stages during swelling study of guar gum tablet (Batch F6).

t = 2h t = 2h t = 8h

FIGURE 5: Release profile of drug from gum acacia based matrix tablets.



natural polymer can be used as a promising drug release retardent in comparison to the estabilized gum acacia in a particular concentration range.

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Pharmacognostic Studies on Leaves of *Flacourtia ramontchi* L.'Herit

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INTRODUCTION

Flacourtia ramontchi L.'Herit (Flacourtiaceae), commonly called Vikankata in Sanskrit, Kankata in Gujrati, Bilangra in Hindi and Governor's Plum or Madaraskara Plum in English, is a native of tropical Africa and Asia.[1–2] It is found in the Sub-Himalayan tract and Outer Himalayas, up to 4,000 ft. from the Indus eastwards and in the adjacent plains, Upper Gangetic Plain, common in the Peninsula, Western Ghats and forests of the North Circars and Deccan up to 3,000 ft. It is common throughout Chhota Nagpur, Deccan and South India in mixed deciduous or scrub forests. It is very variable and includes a number of varieties or forms, some of which are considered as separate species, e.g. var. *latifolia* Hook. F. & Thomas. and var. *occidentalis* Hook. F. & Thomas. [3] In Ayurveda, the most ancient traditional system of Indian medicine, the plant is used against jaundice, oedema and diseases due to vitiated blood.[1] Leaves and young shoots are astringent and stomachic. Leaf is used in inflammation, jaundice and as blood purifier. The bark is considered astringent and diuretic. Bark is used as an antidysenteric and as tanning material. Stem bark is used in jaundice. Fruit of this plant is red or dark brown, inky when ripe, fruits are sweet, appetizing and it is given in jaundice and enlargement of spleen. After child birth among the poor, the seeds are grounds to powder with turmeric and rubbed all over the body to prevent rheumatic pains from exposure to damp winds. Gum is administered along with other ingredients in cholera and also used as a gargle.

ABSTRACT: *Flacourtia ramontchi* L.'Herit (Family: Flacourtiaceae), commonly called Vikankata in Sanskrit, Governor's Plum or Madaraskara Plum in English, is found in the Sub-Himalayan tract and Outer Himalayas in India. The leaves of this plant have different indications in Ayurveda and are used traditionally for several ailments. Considerable confusion exists regarding the identification of this species. Hence, the present work was undertaken to establish the requisite pharmacognostic standards for evaluating the plant material. Although some pharmacognostic characters were already reported in Ayurvedic Pharmacopoeia of India, present work re-investigates some of them and reports other necessary pharmacognostic parameters of leaves of *Flacourtia ramontchi* L.'Herit.

Keywords: *Flacourtia ramontchi* L.'Herit., Preliminary phytochemical screening, Jaundice.

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Gum can be applied to eczema and skin diseases. Root is applied externally in skin diseases.[2,4–6]

Considerable confusion exists regarding the identification of this species. It has been included, along with *F. sepiara*, under *F. indica* by Merill. Later researchers, however, do not agree with this. Indian workers prefer to keep the two species separate.[3]

Pharmacognostic parameters serve to identify the crude plant material and to ensure its quality. Some pharmacognostic parameters of *F. ramontchi* leaves were already reported in Ayurvedic Pharmacopoeia of India (API). However, the present study was carried out to re-evaluate some reported pharmacognostic parameters for comparative study and to report some other useful parameters of leaves of *F. ramontchi*; with an aim to enrich the existing pharmacognostic data which may serve as a measure of authentication and quality control for commercial samples of the crude drug.

MATERIALS AND METHODS

Plant material

Fresh *F. ramontchi* leaves were collected from the Anand Agriculture University, Anand, Gujarat, India, in the month of August–September 2009 and were authenticated by Dr. A. S. Reddy, Professor and Head of Botany Department, Sardar Patel University, Vallabh Vidyanagar. The herbarium of the plant was deposited in Department of Pharmacognosy, A.R. College of Pharmacy, Vallabh Vidyanagar with voucher specimen number HHP/Fr-1/10/ARGH-09. For macroscopical and microscopical studies, the fresh leaves were used; whereas rest of the studies were carried out using dried powdered material. The leaves were dried under shade and powdered to 60 # size separately and stored in airtight containers.

PHARMACOGNOSTIC EVALUATION

Macroscopical studies

The fresh intact leaves of the plant were spread on a clean dry plastic sheet and investigated for different organoleptic features by repeated observation using hand magnifying glass and ruler (where required) and the observations were recorded.

Microscopical studies

The transverse sections (TS) of fresh leaf were obtained by usual techniques.[7] Good sections were collected and placed on a grease free microscopic slide. Furthermore, microscopical study of the plant was also carried out.

Histochemical studies

Transverse sections were taken as mentioned above and different histochemical reagents were applied suitably over the slide and the specific colours produced due to presence/absence of putative constituents were observed under the compound microscope. The presence/absence of different chemical constituents in different tissues was recorded.

Determination of leaf constants

As a part of quantitative microscopy, vein islet number, veinlet termination number, stomatal number, stomatal index and palisade ratio of the leaves was determined by usual techniques.[8]

Physicochemical evaluation

Physicochemical parameters includeing proximate analysis such as the total ash, acid insoluble ash, water soluble ash and loss of moisture content were determined as per reported methods.[9–10] Considering the diversity of chemical nature and properties of contents of drugs, six different solvents were used for determination of extractive values as per reported methods.[11] All determinations were performed in triplicate and the results are presented as mean ± standard error of mean (SEM).

Preliminary phytochemical screening

The dried leaf powder was subjected to preliminary phytochemical screening for qualitative detection of the nature of phytoconstituents. The dried leaf powder (100 g) was extracted successively with petroleum ether (40–60°C), toluene, chloroform, acetone and methanol in a Soxhlet extractor. Finally, the marc was macerated with chloroform water at room temperature (24–26°C). Each time before extracting with the next solvent of higher polarity, the powdered material (marc) was dried in a hot air oven below 50°C for 10 minutes. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The concentrated extracts were evaporated to dryness and the extracts obtained with each solvent were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material.[11–12]

Fluorescence analysis

A small amount of powdered leaves was treated with different types of chemical reagents as mentioned in Table 6 and the colour characteristics were observed under ultraviolet light (254 nm and 365 nm) and day light.[8,13]

FIGURE 1: Leaves of *Flacourtia ramontchi* L.'Herit.

RESULTS

Macroscopical studies

The arrangement of leaves is simple, usually alternate, rarely opposite or verticillate, sometimes crowded at apices of branches. Shape of leaf is broadly elliptic, obovate or suborbicular and size is variable, 2–9 cm. long and 2–5 cm. broad. Petiole generally present, sometimes with apex, 2 glandular and/or with additional glands along petiole length; leaf blade usually pinnate-veined, sometimes 3–5 veined from base or palmate-veined, with or without pellucid dots or lines. Texture is glabrous or pubescent above, more or less pubescent

FIGURE 2: Microscopy of leaf of *Flacourtia ramontchi* L.'Herit.

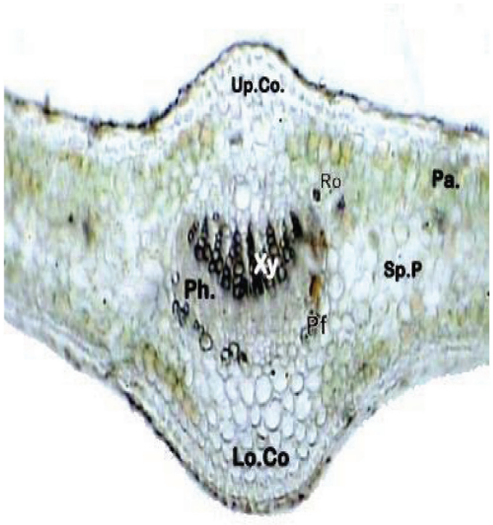

Fig. 2A

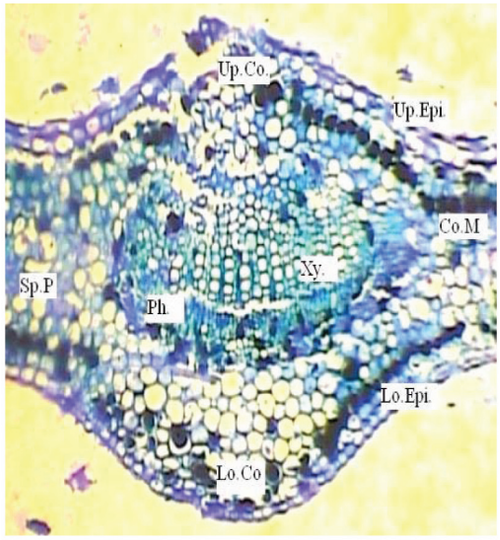

Fig. 2B

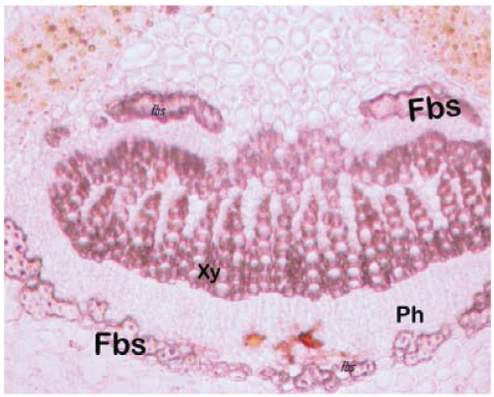

Fig. 2C

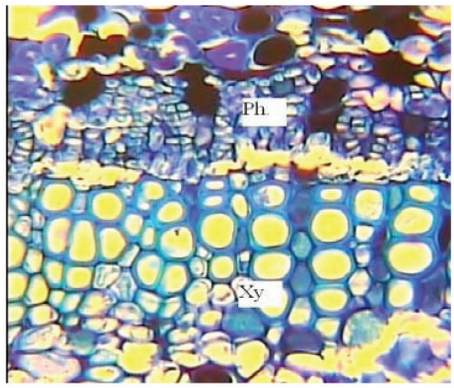

Fig. 2D

2A: Transverse section of leaf of Flacourtia ramontchi (Stained with phloroglucinol and Conc. HCl.); 2B: transverse section of leaf of Flacourtia ramontchi (Stained with Toluidine Blue); 2C: Midrib Enlarged-10X (Stained with phloroglucinol and Conc. HCl); 2D: Vascular bundle (Stained with Toluidine Blue.)

FIGURE 3: Microscopy of leaf powder of *Flacourtia ramontchi* L.'Herit.

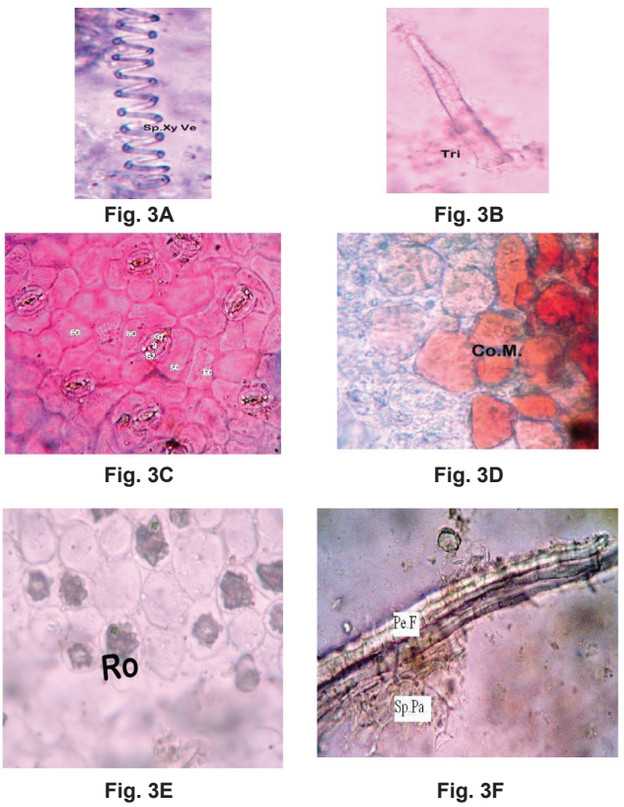

Fig. 3A | Fig. 3B | Fig. 3C | Fig. 3D | Fig. 3E | Fig. 3F

3A: Spiral xylem vessel; 3B: Unicellular covering trichome; 3C: Anomocytic stomata; 3D: Colouring matter; 3F: Spongy parenchyma with pericyclic fibre

beneath and hairless. Margin is entire or toothed, teeth glandular or not. Colour of leaf is deep green in fresh condition (Figure 1), otherwise it is reddish brown in dried condition. Odour of leaf is distinct and slightly aromatic with bitter and acrid taste.

TABLE 2: Leaf constants of *F.ramontchi.*

SR. NO	DETERMINATION	VALUE
1.	Stomatal number	Lower epidermis : 3–5 Upper epidermis : 4–6
2.	Stomatal index	Lower epidermis : 16–19 Upper epidermis : 14–17
3.	Palisade ratio	2 or 3
4.	Vein-islet number	9 per sq. mm.
5.	Veinlet-termination number	11 per sq.mm.

Microscopical studies

Transverse section of the leaf of *Flacourtia ramontchi* was found to be dorsiventral. The lamina portion consisted of upper epidermis, mesophyll region and lower epidermis. Upper epidermis and lower epidermis showed the presence of a single layer of tubular cells covered with thin layer of cuticle. Coloring matter was found to be scattered in entire section of leaf. Mesophyll region consisted of double layer of palisade parenchyma and 2–3 layer of spongy parenchyma. In the midrib region upper and lower epidermises were continuous. 2–3 layers of collenchyma were present below the upper epidermis and above the lower epidermis. In the centre collateral vascular bundle was present. Phloem was followed by lignified pericyclic fibres. In spongy parenchyma calcium oxalate crystal were present. (Figure 2).

TABLE 1: Histochemical colour reactions of *Flacourtia ramontchi* leaves.

REAGENT	CONSTITUENT	COLOUR	HISTOLOGICAL ZONE
Phloroglucinol + Conc. HCl	Lignin	Pink	Vascular bundles
Aniline sulphate + H_2SO_4	Lignin	Yellow	Vascular bundles
Iodine-KI solution	Starch	–	–
Alc. Sudan III solution	Oil globules, suberin	Pink	Vascular bundles
Caustic alkali, mineral acid treatment	Calcium oxalate	–	–
Aq. Ferric chloride	Tannins	Black	Lamina
Potassium iodobismuthate	Alkaloids	_	_
Millon's reagent	Proteins	–	–
Libermann-Burchard's reagent	Steroids	Greenish	Lamina
Ruthenium red	Mucilage	Red	Epidermis
Conc. Sulphuric acid	Cellulose	Green	Mesophyll
Tincture alkanna	Fixed oils and fats	Red	Lamina and midrib portion

TABLE 3: Proximate analysis of leaf powder.

SR. NO	DETERMINATION	PERCENTAGE w/w ± SEM*
1.	Total ash	8.877 ± 0.035
2.	Acid insoluble ash	0.572 ± 0.008
3.	Water soluble ash	0.155 ± 0.006
4.	Alcohol soluble extractive	9.772 ± 0.142
5.	Water soluble extractive	5.645 ± 0.120
6.	Loss of moisture content	39.45 ± 2.90

** SEM = Standard Error of Mean (n = 3)*

When the powder of leaf of *Flacourtia ramontchi* was observed under microscope, identifying characters observed were spiral xylem vessels, unicellular covering trichome, anomocytic stomata, coloring matter, rosette of calcium oxalate and pericyclic fibre with spongy parenchyma (Figure 3).

Histochemical studies

The findings of histochemical colour reactions were reported in Table 1.

Determination of leaf constants

The leaf constants values were shown in Table 2.

Physicochemical evaluations

The physicochemical values of all determinations are summarized in Table 3 and Table 4. The colour of the extracts in different solvents was also summarized in Table 4.

Phytochemical screening

The results of *F. ramontchi* showed the presence of lipids, flavonoids, saponins, alkaloids, tannins, amino acids, triterpenoids, and steroids in the leaf of *F. ramontchi*. It was observed that saponins, sterols and triterpenoids are present in petroleum ether and methanol extracts. Fixed oils and fats are present in petroleum ether and toluene extracts. Furthermore, methanol extract contains carbohydrates, tannins, phenolic compounds and coumarins. Acetone and water extracts showed the presence of flavonoids, phenolic compounds and tannins. In addition to this, acetone extract showed triterpenoids and sterols, and water extract showed coumarins, saponins and carbohydrate tests positive.

Fluorescence analysis

The fluorescence characteristics of leaf powder as treated and observed under two different wave length of UV light and day light are summarized in Table 5.

DISCUSSION

Pharmacognosy is the study of natural substances, principally plants, those which are used in medicine. Pharmacognosy also encompasses the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of organoleptic, physical, chemical and structural characters and uses of crude drugs. The objective of Pharmacognosy is to contribute to rational relationship between chemical moieties of naturally occurring drugs and their biological and therapeutic effects.[14]

From the present macroscopical studies, it was found that characters like arrangement, shape and colour of leaf of plant as mentioned in API were observed in the present study. The additional diagnostic features observed in our sample are texture and margin which are not mentioned in API can be of immense help in authentication of plant material.

The microscopic and histochemical studies and fluorescence analysis of powdered drug were reported and discussed in the present paper. Stomata were found to occur on both surfaces. In the present study stomatal number and stomatal index were determined which will

TABLE 4: Preliminary phytoprofile of leaf of *Flacourtia ramontchi* L.'Herit.

SR. NO	SOLVENT	COLOR AND CONSISTENCY AFTER DRYING	% w/w ± SEM*
1.	Petroleum ether (40–60°C)	Black greenish sticky mass	6.55 ± 0.28
2.	Toluene	Dark greenish sticky mass	1.77 ± 0.19
3.	Chloroform	Green non sticky mass	2.15 ± 0.19
4.	Acetone	Yellowish green	1.15 ± 0.22
5.	Methanol	Green non sticky mass	9.13 ± 0.20
6.	Chloroform Water	Reddish brown	9.88 ± 0.07

** SEM = Standard Error of Mean (n = 3)*

TABLE 5: Behaviour of powdered leaves of *F. ramontchi* with different chemical reagents.

REAGENTS	DAY LIGHT	UV LIGHT (254 nm)	UV LIGHT (365 nm)
Powder as such	Brownish green	Light green	Brown
Cold water	Brown	Dark green	Brown
Hot water	Light brown	Green	Green
Picric acid	Light yellow	No color	No color
Ammonia solution	Dark brown	Green	Brown
1N NaOH (Aq.)	Dark brown	Light brown	Green
1N NaOH(Alc.)	Light green	Light green	Light green
1N HCl	Brown	Brown	Brown
5% Iodine soln.	Light brown	Light brown	Light green
5 % $FeCl_3$	Dark green	Green	Green
1N H_2SO_4	Reddish brown	No color	Light brown
Acetic acid	Light green	Green	Green
HNO_3	Light brown	Light brown	Light brown
Aq. Silver Nitrate soln.	No change	--	--

serve as an important parameter for microscopical evaluation of the crude drug. Stomatal index is very important leaf constant and quantitative parameter of diagnostic significance of the species. Preliminary phytochemical screening of successive extracts indicated presence of lipids, flavonoids, saponins, alkaloids, tannins, carbohydrates, terpenoids, and steroids in *F. ramontchi* leaves. A vast array of compounds were found to be present in the plant material under the present study. The physical evaluation furnished three different ash values and extractive values in six different solvents. Total ash and acid insoluble ash values were found to be within the API limits. Water soluble extractive value was found to comply with the API limit.

In addition, other properties *viz*., behavioral pattern of powdered crude drug with different chemical reagents and fluorescent characteristics of powdered crude drug on exposure to UV light are reported for identification and authentication of the crude drug for the first time. This information may be useful as diagnostic parameters. After present investigation it can be concluded that the pharmacognostic study of leaves from *F. ramontchi* grown in Sub-Himalayan tract and Outer Himalayas of India, have furnished a set of qualitative and quantitative parameters that can serve as an important source of information which may substantiate the existing pharmacopoeial (API) data to ascertain the identity and to determine and track the quality and purity of the commercial plant material in future studies.

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Phyto-Physicochemical Investigation of Leaves of *Plectranthus amboinicus* (Lour) Spreng

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INTRODUCTION

Plectranthus amboinicus (Lour) Spreng belongs to family Lamiaceae and known as country borage in English.[1] It is large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose. The leaves are 2.5–2 cm long, simple, opposite, petioled, broadly ovate or cordate, crenate, fleshy and very aromatic.[2] It is found throughout India, Sri Lanka and Moluccas.[3] Earlier claims showed that the leaves are bitter, acrid, thermogenic, aromatic, anodyne, appetizing, digestive, carminative, stomachic, anthelmintic, constipating, deodorant, expectorant, lithontriptic, diuretic and liver tonic. They are useful in cephalalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea and cholera especially in children, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccough, bochitis, renal and vesical calculi, strangury, hepatopathy, malarial fever, antispasmodic and cathartic.[2] Upon literature review it was found that the plant contains butylanisode, β-caryophyllene, quercetin, ursolic acids, triterpenic acids, α-pinene, β-pinene, thymol, eugenol, carvacrol, 1,8-cineole, β-phellandrene, p-cymene, salvigenin, crisimaritin and chrysoeriol.[4–9] Many pharmacological properties have been reported including its use in urolithiasis,[10–11] as antiepileptic,[12] as antitumour and antimutagenic,[13] and for neuropharmacological,[14]

ABSTRACT: *Plectranthus amboinicus* (Lour) Spreng belongs to family Lamiaceae and known as country borage in English. It is large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose. It is found throughout India, Sri Lanka and Moluccas. The leaves of the plant are bitter, acrid and were being widely used traditionally for various purposes. The present study was therefore carried out to provide requisite pharmacognostic details about the leaf. The leaf was characterized for its physico-chemical properties. The shade dried powder of leaves of *Plectranthus amboinicus* (Lour) Spreng was subjected to successive extraction using the solvents (petroleum ether, chloroform, ethanol and water) in the increasing order of polarity. The preliminary phytochemical analysis and Thin Layer Chromatography has been performed. It was found that the leaves revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinone, tannins, phenolic compounds and terpenoids. The findings will be useful towards establishing pharmacognostic standards for identification, purity, quality and preparation of monograph of the plant.

Keywords: *Plectranthus amboinicus*, physico-chemical, phytochemical.

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radioprotective,[15] antioxidant,[16] anti-microbial,[17–18] anti-bacterial and anti-fungal properties.[19–20] Hence, the present cram was focused to investigate physico-chemical and phytochemical properties of the plant.

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Plectranthus amboinicus* (Lour) Spreng were collected from the fields of Kanchipuram, Tamil Nadu. It was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen (No. PARC/2007/89) has been deposited in the institute.

Physicochemical standards

Physicochemical parameters of the powdered drug such as ash value, extractive value, loss on drying were performed.[22] Extracts were prepared by various solvents by maceration and percentage of dry extract was calculated in terms of air dried leaf powder.

Determination of volatile oil

The determination of volatile oil in plant material of *Plectranthus amboinicus* Lour. was carried out by steam distillation. 250 gm of leaves of the plant were taken into round bottomed flask with water and the Clevenger's apparatus was assembled. The round bottom flask was heated on a mantle for 2 hrs and the oil was collected in the graduated tube. The oil obtained was 1.3 ml from which percentage of oil in the leaves of *Plectranthus amboinicus* Lour. was calculated.[22]

Reactions of powdered leaf with different reagent

The powdered drug was treated with different reagents and the colour shown is noted.[23]

Fluorescence analysis

Fluorescence characteristics of the powdered drug with different chemicals were observed in daylight and ultraviolet light. Various solvent extracts were also subjected to daylight and ultraviolet light for its fluorescence characteristics. The powder was treated with neutral solvents like methanol, water and acids like 1N hydrochloric acid, 50% hydrochloric acid, 50% sulphuric acid, 50% nitric acid, alkaline solutions like 1N sodium hydroxide and alcoholic 1N sodium hydroxide.

Preparation of extracts

The leaves of *Plectranthus amboinicus* (Lour) Spreng were dried in shade. Then the shade dried leaves were powered to get coarse powder. The powdered material was successively extracted with petroleum ether, chloroform, ethanol and water by cold maceration in increasing order of their polarity.[21] In addition the fresh powder was defatted with petroleum ether and extracted with 95% ethanol (72 hours) and water (24 hours) separately. The extracts were filtered with muslin cloth and the solvent was distilled off. Final traces of solvent were removed under vacuum.

Phytochemical investigation

The petroleum ether (PEPA), chloroform (CEPA), ethanol (EEPA) and aqueous (AEPA) extracts of *P. amboinicus* were subjected to further preliminary qualitative phytochemical investigation.[24] The percentage yield of PEPA, CEPA, EEPA, AEPA were found to be 2.47, 3.69, 12.2 and 18.1 respectively.

Thin Layer Chromatography

Ethanolic and aqueous extracts and oil of leaves of *Plectranthus amboinicus* Lour. were subjected to thin layer chromatography studies, to find the presence of number of compounds on support of chemical test.[25,26]

High Performance Thin Layer Chromatography[27]

Thin layer chromatography has progressed so rapidly that in many field it has surpassed paper chromatography, chiefly because it is usually quicker and gives better separations. The method is used for separation of the components present in the mixture both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is still a better means to separate the various components of a mixture. In the present study by chemical test and thin layer chromatography the ethanolic and aqueous extracts and volatile oil of *Plectranthus amboinicus* Lour. were found to have a number of phytoconstituents. Hence further attempt was taken to separate the individual components of both extracts and oil by HPTLC.

Sample :	1. The ethanolic extract (3 mg) was dissolved in 1ml ethanol. 2. The aqueous extract (3 mg) was dissolved in 1ml of ethanol:water (3:1) 3. Volatile oil.
Stationary phase :	HPTLC precoated plates with silica gel G 60 F_{254} as adsorbent (Merck, Germany)
Mobile phase :	1. Ethanolic extract—n-hexane :acetone:chloroform (5:3:3) 2. Aqueous extract—acetone: water:acetic acid (5:5:0.5)

3. Volatile oil—n-Hexane:Ethyl acetate:Acetic acid (7:3:0.5)

Sample concentration : 10 mg/ml
Size of the plate : 10 × 10 cm
Developing chamber : Twin trough glass chamber, 20 × 10 cm
Mode of application : Band
Band size : 5 mm
Separation technique : Ascending
Temperature : 20 ± 5ºC
Saturation time : 15 min.
Scanner : CAMAG TLC scanner-3
Scanning wavelength : 254/280 nm
Scanning mode : Absorbance/Reflectance

RESULTS AND DISCUSSION

Physicochemical analysis

The physicochemical constants like ash value, extractive value and loss on drying, determination of volatile oil and reaction with different reagents of coarse powderof plant were estimated and the values are shown in Table 1, 2, 3, 4 and 5 respectively.

The results revealed that the leaf of the plant was having 7.5% w/w total ash, 2.85% w/w acid insoluble ash and 4.6% w/w water soluble ash. The water extractive value is higher (19.2% w/w) as compared to alcohol soluble extractive value (15.2%). The moisture content was 6.24% w/w and the volatile oil content was found to be 0.005% v/w. The powdered drug reaction with different reagents showed brown colour with conc. nitric acid, greenish brown with glacial acetic acid, yellowish brown with iodine solution and green colour with conc. hydrochloric acid, conc. sulphuric acid and alcoholic sodium hydroxide. These data are helpful to identify and ascertain the quality of the crude drug.

TABLE 1: Ash values of the leaf of *Plectranthus amboinicus* Lour.

PARAMETERS	AVG. VALUES OF ASH
Total ash	7.5% w/w
Acid insoluble ash	2.85% w/w
Water soluble ash	4.6% w/w

TABLE 2: Extractive values of the leaf of *Plectranthus amboinicus* Lour.

PARAMETERS	AVG. EXTRACTIVE VALUES	COLOUR OF EXTRACT
Alcohol	15.2% w/v	Dark green
Water	19.2% w/v	Dark brown
Petroleum ether	2.79% w/v	Greenish
Chloroform	4.14% w/v	Greenish

TABLE 3: Moisture content of the leaf of *Plectranthus amboinicus* Lour.

PARAMETER	AVG. VALUE
Loss on drying	6.24% w/w

TABLE 4: Volatile oil content of the leaf of *Plectranthus amboinicus* Lour.

PARAMETER	VALUE
Volatile oil	0.005% v/w

TABLE 5: Reactions of powdered leaf with different reagent.

TREATMENTS	OBSERVATIONS
Conc. HCl	Green
Conc. HNO_3	Brown
Conc. H_2SO_4	Green
Glacial acetic acid	Greenish brown
Iodine solution	Yellowish brown
Alcoholic NaOH	Green

Fluorescence analysis

Fluorescence analysis of powdered leaf, ethanolic and aqueous extracts of leaf had been carried out in day light and under UV light, with different chemicals and

TABLE 6: Fluorescence Analysis of the leaf of *Plectranthus amboinicus* Lour.

TREATMENTS	DAY LIGHT	UV LIGHT
1N HCl	Light green	Green
50% HCl	Pale green	Green
50% H_2SO_4	Green	Deep green
50% HNO_3	Yellowish brown	Green
1N NaOH	Yellow	Yellowish green
Alcoholic NaOH	Dark green	Brown
Water	Green	Green
Methanol	Dark green	Green
Ethanolic extract	Dark green	Dark green
Aqueous extract	Dark brown	Green

TABLE 7: Percentage yield of leaf extracts of *Plectranthus amboinicus* Lour.

EXTARCTS	PERCENTAGE YIELD	COLOUR
Ethanolic extract	12.2% w/w	Dark green
Aqueous extract	18.1% w/w	Dark brown

results revealed green colour with hydrochloric acid, sulphuric acid, nitric acid, water and methanol, yellowish green with 1N sodium hydroxide and brown colour with alcoholic sodium hydroxide. Ethanolic and aqueous extracts showed dark green and green colour respectively in UV light, which are reported in Table 6. From the above data it was found that the collected crude drug possesses all the quality parameters which are enough to take it for other evaluated studies.

Phytochemical investigation

Extraction of dried leaf of *Plectranthus amboinicus* Lour. was carried out by cold maceration and percentage yield was found to be 12.2% w/w with ethanol and 18.1% w/w with water which has been tabulated in Table 7. The preliminary phytochemical tests was carried out to identify the constituents to support literature review and the results have been tabulated in Table 8 which revealed the presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinones, tannins, phenolic compounds and terpenoids.

Thin Layer Chromatography

Further an attempt has been made to separate the individual chemical constituents of both extracts and volatile oil by chromatography. First they were subjected to TLC. A number of solvent systems of low to high polarity were tried. The solvent system which shows good resolution was used. The results are tabulated in Table 9 and Figures 1, 2, 3, 4, 5 and 6. The ethanolic extract showed 6 spots at R_f values 0.07, 0.22, 0.83, 0.89, 0.93 and 0.96, aqueous extract showed 3 spots at R_f values 0.36, 0.72 and 0.82 and volatile oil showed 1 spot at R_f value 0.72 in solvent system n-butanol:acetic acid:water (10:1:1) whereas in

TABLE 8: Preliminary Phytochemical Analysis of extracts of the leaf of *Plectranthus amboinicus* Lour.

S. NO	CONSTITUENTS	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	Alkaloids	+	+
2	Sugar and Carbohydrates	+	+
3	Glycosides	+	+
4	Protein	+	+
5	Amino acid	+	+
6	Steroids	-	-
7	Saponin	-	-
8	Flavonoids	+	+
9	Quinone	+	+
10	Tannins	+	+
11	Anthocyanin	-	-
12	Phenolic compounds	+	+
13	Terpenoids	+	+
14	Fixed oil and fats	-	-
15	Gums & mucilage	-	-
16	Resins	-	-

TABLE 9: Thin Layer Chromatography of extracts and oil of the leaf of *Plectranthus amboinicus* Lour.

S. NO	EXTRACTS	SOLVENT SYSTEMS	NUMBER OF SPOTS	R_f VALUES
1	Ethanolic extract	n-Butanol:Acetic acid:Water (10:1:1)	6	0.07, 0.22, 0.83, 0.89, 0.93, 0.96
2	Ethanolic extract	n-Hexane:Formic acid:Ethyl acetate:Water (5:4:3:1)	4	0.67, 0.72, 0.76, 0.83
3	Aqueous extract	n-Butanol:Acetic acid:Water (10:1:1)	3	0.36, 0.72, 0.82
4	Aqueous extract	n-Hexane:Formic acid:Ethyl acetate:Water (5:4:3:1)	1	0.79
5	Volatile oil	n-Butanol:Acetic acid:Water (10:1:1)	1	0.72
6	Volatile oil	n-Hexane:Formic acid:Ethyl acetate:Water (5:4:3:1)	1	0.69

FIG. 1: TLC of ethanolic extract in n-Butanol:Acetic Acid:Water (10:1:1).

FIG. 2: TLC of ethanolic extract in n-Hexane:Formic acid: Ethyl acetate:Water (5:4:3:1).

solvent system n-hexane:formic acid:ethyl acetate:water (5:4:3:1) ethanolic extract showed 4 spots at Rf values 0.67, 0.72, 0.76 and 0.83, aqueous extract showed 1 spot at R_f value 0.79 and volatile oil of the leaf showed 1 spot at R_f value 0.69.

FIG. 3: TLC of aqueous extract in n-Butanol:Acetic Acid:Water (10:1:1).

FIG. 4: TLC of aqueous extract in n-Hexane:Formic acid: Ethyl acetate:Water (5:4:3:1).

High Performance Thin Layer Chromatography

Further both extracts and oil were subjected to HPTLC for qualitative analysis of phytoconstituents in support of TLC. The results have been reported in Figs. 7, 8 and 9. The ethanolic extract showed 12 spots at R_f values 0.08, 0.15, 0.21, 0.31, 0.47, 0.54, 0.66, 0.68, 0.75, 0.81, 0.85 and 0.90 in solvent system

FIG. 5: TLC of volatile oil in n-Butanol: Acetic Acid:Water (10:1:1).

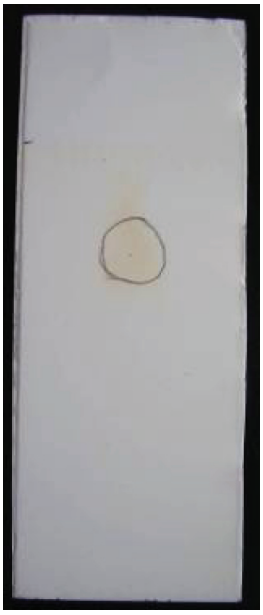


FIG. 6: TLC of volatile oil in n-Hexane: Formic acid: Ethyl acetate:Water (5:4:3:1).

n-hexane:acetone:chloroform (5:3:3) at 280 nm. The aqueous extract showed 5 spots at R_f values 0.15, 0.17, 0.26, 0.65 and 0.81 in solvent system acetone:water: acetic acid (5:5:0.5) at 280 nm. Volatile oil showed 6 spots at R_f values 0.04, 0.26, 0.32, 0.41, 0.49 and 0.61 in solvent system n-hexane:ethyl acetate:acetic acid (7:3:0.5) at 254 nm.

FIG. 7: HPTLC fingerprint profile of ethanolic extract of *Plectranthus amboinicus* Lour. at 280 nm.

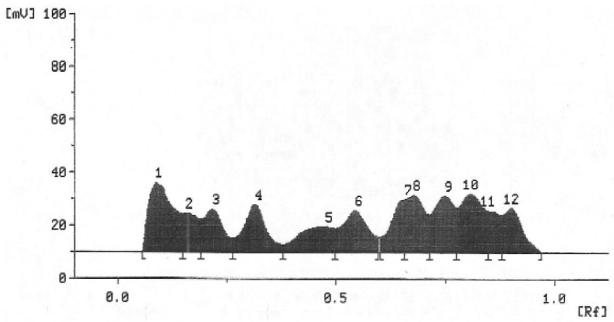


FIG. 8: HPTLC fingerprint profile of aqueous extract of *Plectranthus amboinicus* Lour. at 280 nm.

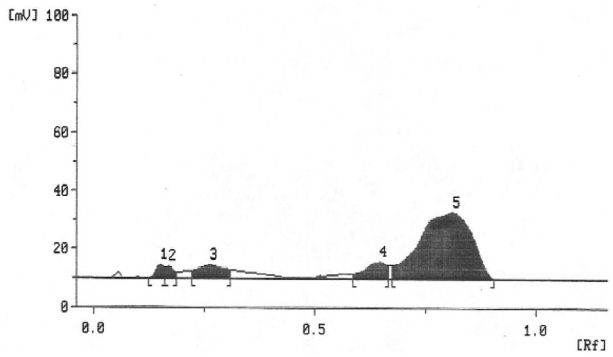
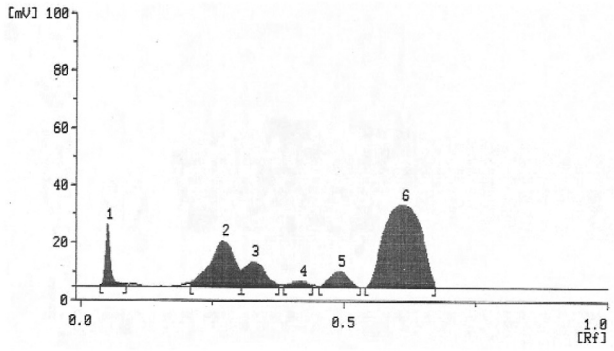


FIG. 9: HPTLC fingerprint profile of volatile oil of *Plectranthus amboinicus* Lour. at 254 nm.



CONCLUSION

The traditional plant *Plectranthus amboinicus* Lour. belongs to family Lamiaceae. The physicochemical analysis such as ash values, extractive values, moisture content, volatile oil content, powdered drug reaction and fluorescence analysis of the powder and extracts of the *Plectranthus amboinicus* Lour. were studied for ascertaining the quality of the crude drug. Phytochemical investigation of the extracts revealed the presence of various constituents. By using TLC and HPTLC, a number of phytoconstituents were separated and their respective R_f values have been reported. These revealed that ethanol gives better extraction of the phytochemicals than water since the ethanolic extract resolved into maximum number of bands as compared to aqueous extract. The present study will provide useful information for its correct identity and may enable those who handle this plant to maintain its quality. In addition the results of the present study could be useful for preparation of a monograph of the plant.

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HPTLC Fingerprint Profile of Extracts from Gum, Bark and Leaf of *Boswellia serrata* Linn. in Different Solvents

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INTRODUCTION

Boswellia serrata Linn. (Family: Burseraceae) is commonly used in Indian system of medicine (Ayurvedic) as anti-inflammatory, analgesic and anti-arthritic.[1–3] *Boswellia seratta* gum resin was first mentioned in the ancient Ayurvedic treatises, Sushruta Samhita and Charaka Samhita. *Boswellia serrata* is also known as Salai Guggal or Indian Frankincense and has been available as a high quality extract in India for approximately 25 years and marketed under the name Sallaki. *Boswellia serrata* is mainly used in rheumatic disorders, to improve appetite and general weakness.[4–6] Extract of gum resin of *B. serrata* containing 60% acetyl 11-keto beta boswellic acid (AKBA) along with other constituents such as 11-keto beta-boswellic acid (KBA), acetyl beta-boswellic acid and beta-boswellic acid has been evaluated for anti-anaphylactic.[7] Preparation from the gum resin of *Boswellia serrata* have been used as a traditional remedy in Ayurvedic medicine in India for the treatment of inflammatory diseases. Compounds from the gum with anti-inflammatory effects are pentacyclic

ABSTRACT

Introduction: *Boswellia serrata* Linn. tree is commonly found in India. The therapeutic value of its gum (guggulu) has been known to possess good anti-inflammatory, anti-arthritic, anti-proliferative and analgesic activities. Oleo-gum resins from *Boswellia* species are used in traditional medicine in India and African countries for the treatment of a variety of diseases.

Methods: Chromatographic techniques were used for separation of components from different extracts of plant parts. This study was planned to develop a fingerprint profile of drug extracts from different parts of *Boswellia serrata* Linn. i.e., bark, gum and leaf in different solvents such as petroleum ether, chloroform and water.

Results: A high-performance thin layer chromatography (HPTLC) method for the separation of the active constituents in *Boswellia serrata* extracts has been developed and TLC of these extracts on silica gel precoated aluminium plates of Merck by automatic TLC applicator and using solvent gradient system was performed.

Conclusion: The HPTLC method for routine quality control of present species can be carried out using this method for different extracts of plant parts and serve in standardization of the drug.

Keywords: Chromatography, quality control, standardization.

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triterpenes of the boswellic acid type.[8] *Boswellia serrata* extract or boswellic acid shows inhibitory effect on human leukocyte elastase. It blocks leukotriene biosynthesis and exerts potent anti-inflammatory effects. It causes inhibition of 5-lipoxygenase and human leukocyte elastase simultaneously.[9] *Boswellia serrata* gum resin contains different sugars like D-galactose, D-xylose and D-mannose. *Boswellia serrata* gum resin also contains volatile oil and uronic acids. A range of triterpene acids such as β-boswellic acid, acetyl-β-boswellic acid and keto-β-boswellic acid responsible for medicinal actions can be found in *Boswellia serrata* gum resin. Its exudates is either naturally obtained from reservoirs in barks or is produced from human-made incisions in the cortex of the tree.[10] *Boswellia serrata* gum resin is fragrant transparent and golden yellow, and solidifies to give a brownish yellow color. The yield per tree may vary from 0.9 to 2.5 kgs/tree/year. *Boswellia serrata* gum resin burns with an agreeable odour and is chiefly used as incense. *Boswellia serrata* gum resin is widely used in Ayurvedic formulations for treating asthma and arthritis.[11]

Chemical constituents and components present in the crude drug: Main chemical components are boswellic acids, essential oils, gum, tannins, beta sitosterol, lignin and terpenoids.

These studies show that there is a vital need to study the extracts of *Boswellic serrata* from different parts of the plants. Here an attempt was made to develop the fingerprint method for the separation and identification of components with the help of HPTLC profile. HPTLC analysis was done for different drug extracts of *Boswellia serrata* Linn from different parts of the plants such as bark, gum and leaf; which can further lead to provide a beneficial information towards the quality of the drug and also standardization of the drug.

TABLE 1 : TLC profile of different extract of different plant parts of *Boswellia serrata* along with R_f values and detection system.

S.NO	NAME OF THE EXTRACT	SOLVENT SYSTEM	DETECTION	R_f VALUES
1.	B.S. gum petroluem ether extract.	Tol: $CHCl_3$ = 7:3	spraying with 5% methanolic sulphuric acid and observed at 366 nm	0.03, 0.17, 0.24, 0.31, 0.36, 0.40, 0.53, 0.63, 0.72 and 0.97
2.	B.S. gum chloroform extract	Tol: $CHCl_3$ = 7:3	spraying with 5% methanolic sulphuric acid and observed at 366 nm	0.04, 0.16, 0.32, 0.37, 0.49, 0.59, 0.69 and 0.95
3.	B.S. gum petroleum ether extract.	Tol: $CHCl_3$ = 7:3	spraying with 5% methanolic sulphuric acid and observed at visible range	0.03, 0.17, 0.23, 0.30, 0.34, 0.39, 0.50, 0.60, 0.70, 0.91 and 0.94
4.	B.S. gum chloroform extract	Tol: $CHCl_3$ = 7:3	spraying with 5% methanolic sulphuric acid and observed at visible range	0.04, 0.16, 0.32, 0.37, 0.48, 0.59, 0.69 and 0.95
5.	B.S. leaf petroluem ether extract	Tol: EA = 9:1	Observed under UV range at 366 nm	0.01, 0.08, 0.25, 0.41, 0.45, 0.67, 0.73 and 0.93
6.	B.S. leaf chloroform extract	Tol: EA = 9:1	Observed under UV range at 366 nm	0.01, 0.09, 0.26, 0.41, 0.49, 0.75 and 0.87
7.	B.S. leaf petroleum ether extract	Tol: EA = 9:1	spraying with 5% methanolic sulphuric acid and observed at 366 nm	0.01, 0.25, 0.40, 0.55, 0.68 and 0.93
8.	B.S. leaf chloroform extract	Tol: EA = 9:1	spraying with 5% methanolic sulphuric acid and observed at 366 nm	0.01, 0.09, 0.26, 0.74 and 0.95
9.	B.S. bark petroleum ether extract	Tol: EA = 9:1	observed at 366 nm	0.02, 0.30, 0.39, 0.46 and 0.55
10.	B.S. bark petroleum ether extract	Tol: EA = 9:1	spraying with 5% methanolic sulphuric acid and observed at 366 nm	0.01, 0.10, 0.30, 0.41, 0.60, 0.77 and 0.93
11.	B.S. bark aqueous extract	Toluene: ethyl acetate : formic acid = 5:4:1	Observed under UV range at 366 nm	0.03, 0.24, 0.35 and 0.90

MATERIALS AND METHODS

Boswellia serrata gum, leaves and bark were collected from the authorized agent for herbal drugs in the local market of Hyderabad. The drug was authenticated by botanist from Central Research Institute of Unani Medicine (CRIUM), Hyderabad. Different plant parts were powdered separately with the help of pestle and mortar. Desaga HPTLC system with Proquant 1.6 version software system was used for the analysis of extracts from CRIUM, Hyderabad. Rotavapor (Equitron make) was used for evaporation of the solvents from extracts.

Preparation of extract of the sample drug

The powdered plant parts of *Boswellia serrata* were taken in a stoppered conical flask separately and macerated with the particular solvent as stated in the Table 1. Then the contents were filtered using Whattmann filter paper no 42 and evaporated to dryness by Rotavapor. From each extract, 100 mg was taken and dissolved in the corresponding solvent from which the extract was prepared and made up to 20 ml and solution obtained was applied on the TLC plate as sample solution.

Development and determination of the solvent system

A highly sensitive and accurate HPTLC method was developed and used for *Boswellia serrata*. Chromatographic separation was carried on 10 cm × 10 cm aluminum plates precoated with silica gel $60F_{254}$ (Merck) as the stationary phase for different extracts prepared from *Boswellia serrata*. 10 µl of the sample was applied and different solvent systems were selected for different extracts. The scan was performed at a wavelength of 366 nm and also at visible range. A saturation time of 25 minutes was allowed before chromatographic run.

The sample was spotted on the TLC plate in triplicate with the help of automatic TLC applicator system of the DESAGA Sarstedt Gruppe on the Merck precoated aluminum sheets of silica gel $60F_{254.}$ After trying with various solvent systems with variable volume ratio, the suitable solvent system selected is as stated in the Table 1 in proportional ratio and developed in the twin through chamber of TLC to the maximum height of the plate so that it can be able to separate all the components on the polar phase of silica gel and that of mobile phase of solvent system. The components get separated by the principle of adsorption, having differential migration rates of individual component towards the phases.

Development of HPTLC technique

After the development, TLC plate is then removed, dried completely and detected with the suitable detection system as 5% methanolic sulphuric acid system or UV cabinet system for detection of spots. Further it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 395 nm. A corresponding densitogram was then obtained in which peaks are appeared for the corresponding spots being detected in the densitometer while scanning, and the peak area under the curve corresponds to the concentration of the component in the sample for the concentration applied on the TLC plate is given in the Table 2–12 for different extracts.

RESULTS AND DISCUSSION

Boswellia serrata extract solution was spotted as 8–10 mm on the precoated HPTLC silica gel $60F_{254}$ plates. The R_f value of the corresponding component as obtained through the software system attached with the instrument i.e., ProQuant 1.6 version and the area

TABLE 2 : Peak list and R_f values of the chromatogram of gum extracted with petroleum ether at UV 366 nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	9.7	3974.74	34.7	937.80	0.03
2.	20.9	548.49	4.8	174.31	0.17
3.	26.5	432.53	3.8	124.23	0.24
4.	31.8	781.56	6.8	193.89	0.31
5.	35.5	783.45	6.8	220.26	0.36
6.	39.4	670.03	5.9	207.93	0.40
7.	49.2	464.53	4.1	91.43	0.53
8.	57.3	1421.96	12.4	272.28	0.63
9.	64.9	991.58	8.7	195.89	0.72
10.	84.7	1375.45	12.0	188.83	0.97

TABLE 3 : Peak list and R_f values of the chromatogram of gum extracted with chloroform at UV 366 nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.1	2248.58	52.9	797.89	0.04
2.	19.9	84.42	2.0	36.94	0.16
3.	33.5	508.40	12.0	99.10	0.32
4.	37.4	215.27	5.1	71.52	0.37
5.	47.6	122.97	2.9	30.58	0.49
6.	55.7	424.06	10.0	90.85	0.59
7.	64.2	352.05	8.3	77.69	0.69
8.	85.7	294.69	6.9	56.47	0.95

TABLE 4 : Peak list and R_f values of the chromatogram of gum extracted with petroleum ether at UV 390 nm after spray treatment.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	9.7	5092.12	38.3	911.53	0.03
2.	21.0	668.14	5.0	194.96	0.17
3.	26.1	506.00	3.8	134.49	0.23
4.	32.0	914.34	6.9	214.16	0.30
5.	35.5	919.74	6.9	238.59	0.34
6.	39.3	768.87	5.8	226.93	0.39
7.	48.8	503.21	3.8	97.98	0.50
8.	57.2	1523.53	11.5	286.77	0.60
9.	64.8	1067.23	8.0	206.30	0.70
10.	82.5	744.14	5.6	172.66	0.91
11.	85.1	591.19	4.4	173.04	0.94

TABLE 5 : Peak list and R_f values of the chromatogram of gum extracted with chloroform at UV 390 nm after spray treatment.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.0	2124.76	54.8	750.64	0.04
2.	19.9	70.32	1.8	32.05	0.16
3.	33.6	453.68	11.7	91.69	0.32
4.	37.3	162.54	4.2	59.59	0.37
5.	47.2	80.41	2.1	23.21	0.48
6.	55.6	338.00	8.7	81.07	0.59
7.	64.1	346.75	8.9	81.19	0.69
8.	85.7	301.36	7.8	56.32	0.95

TABLE 6 : Peak list and R_f values of the chromatogram of leaf extracted with petroleum ether at UV 366 nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	9.9	1369.47	30.0	340.77	0.01
2.	15.8	354.76	7.8	104.30	0.08
3.	29.7	1289.33	28.3	334.82	0.25
4.	43.3	465.40	10.2	119.55	0.41
5.	46.5	576.57	12.7	124.59	0.45
6.	64.9	14.65	0.3	4.34	0.67
7.	70.0	3.35	0.1	1.51	0.73
8.	86.1	484.19	10.6	91.83	0.93

TABLE 7 : Peak list and R_f values of the chromatogram of leaf extracted with chloroform at UV 366 nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.1	1668.02	85.8	584.57	0.01
2.	16.3	25.48	1.3	12.75	0.09
3.	30.7	85.13	4.4	27.01	0.26
4.	42.9	71.26	3.7	23.15	0.41
5.	49.3	4.25	0.2	2.29	0.49
6.	71.6	27.66	1.4	6.67	0.75
7.	81.2	62.74	3.2	6.98	0.87

TABLE 8 : Peak list and R_f values of the chromatogram of leaf extracted with petroleum ether at UV 366 nm after spray treatment.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.0	4123.09	25.5	674.33	0.01
2.	29.9	1888.27	11.7	296.55	0.25
3.	42.0	2539.05	15.7	195.62	0.40
4.	54.9	1079.77	6.7	175.41	0.55
5.	65.2	1226.35	7.6	164.48	0.68
6.	86.6	5281.69	32.7	693.02	0.93

TABLE 9 : Peak list and R_f values of the chromatogram of BS leaf extracted with chloroform at UV 366 nm after spray treatment.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1	9.9	3546.97	69.7	1021.98	0.01
2	16.1	459.44	9.0	100.36	0.09
3	30.6	156.69	3.1	35.49	0.26
4	70.2	58.59	1.2	14.59	0.74
5	88.0	870.82	17.1	171.58	0.95

TABLE 10 : Peak list and R_f values of the chromatogram of BS bark extracted with petroleum ether at UV 366nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.6	818.14	81.2	150.98	0.02
2.	33.4	129.36	12.8	20.37	0.30
3.	41.2	1.23	0.1	0.66	0.39
4.	47.0	54.01	5.4	12.07	0.46
5.	54.2	5.12	0.5	1.71	0.55

TABLE 11 : Peak list and R_f values of the chromatogram of BS bark extracted with petroleum ether at UV 366 nm after spray treatment.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	10.0	2398.29	34.4	372.58	0.01
2.	17.2	681.36	9.8	132.20	0.10
3.	33.2	1030.51	14.8	106.44	0.30
4.	42.6	764.53	11.0	99.16	0.41
5.	58.2	1037.51	14.9	167.34	0.60
6.	72.0	457.24	6.5	60.93	0.77
7.	85.0	612.45	8.8	97.53	0.93

TABLE 12 : Peak list and R_f values of the chromatogram of bark aqueous extract at UV 366 nm.

PEAK	Y-POS (mm)	AREA	AREA (%)	HEIGHT	R_f
1.	12.5	1773.81	87.2	516.76	0.03
2.	27.7	27.84	1.4	8.90	0.24
3.	36.4	181.59	8.9	41.54	0.35
4.	77.2	50.29	2.5	15.05	0.90

corresponds to each peak for the corresponding spot or component determines the concentration of the component in the solution were shown in the Table 2–12.

Boswellia serrata gum extracted with petroleum ether and chloroform separately was subjected to HPTLC analysis by specific solvent system as toluene and chloroform (7:3) and after spraying with 5% methanolic sulphuric acid and heated the plate at 105°C for 5 minutes and then detected under UV 366 nm and also at UV 390 nm as shown in the Figure 1 and 4. The densitogram obtained upon scanning under the densitometer were shown in the Figure 2 and 3 at UV 366 nm whereas at UV 390 nm were shown in the Figure 5 and 6 provided Rf values for the peaks and the peak list along with R_f values at UV 366 nm were given in the Table 2 and 3 whereas at UV 390 nm were given in the Table 4 and 5.

Boswellia serrata leaves extracted with petroleum ether and chloroform separately was subjected to HPTLC analysis by specific solvent system as toluene and ethyl acetate (9:1) and detected under UV 366 nm before and after spray treatment as shown in the Figure 7

FIGURE 1 : *Boswellia serrata* gum extract Petroleum ether extract applied in triplicate Track 1, 2, 3. Chloroform extract applied in triplicate Track 4, 5, 6. Solvent system: Toluene: Chloroform = 7:3. Detection system: spraying with 5% methanolic sulphuric acid and observed at 366 nm.

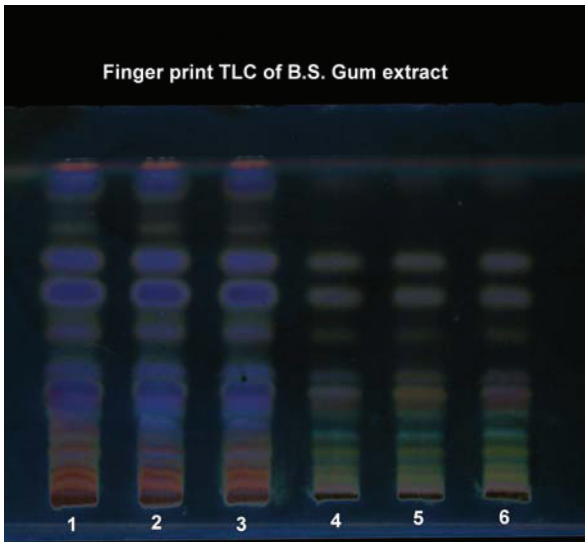


FIGURE 2 : Densitogram of *Boswellia serrata* gum extracted with petroleum ether at UV 366 nm.

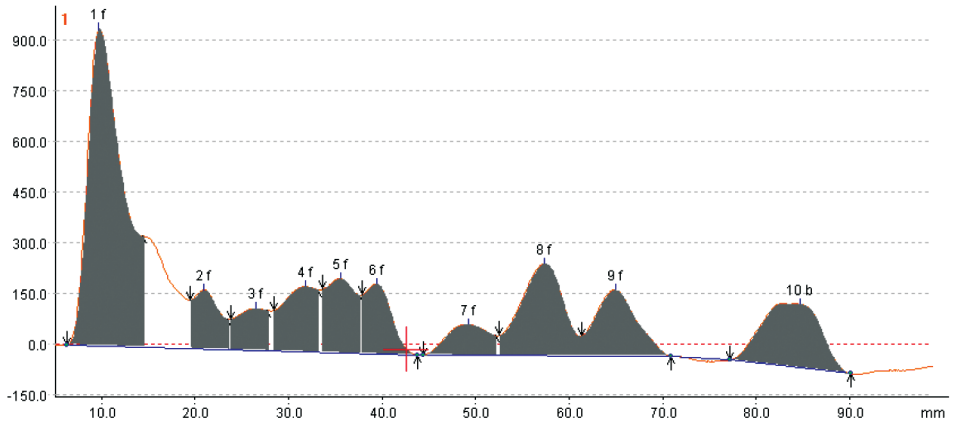


FIGURE 3 : Densitogram of *Boswellia serrata* gum extracted with chloroform at UV 366 nm.

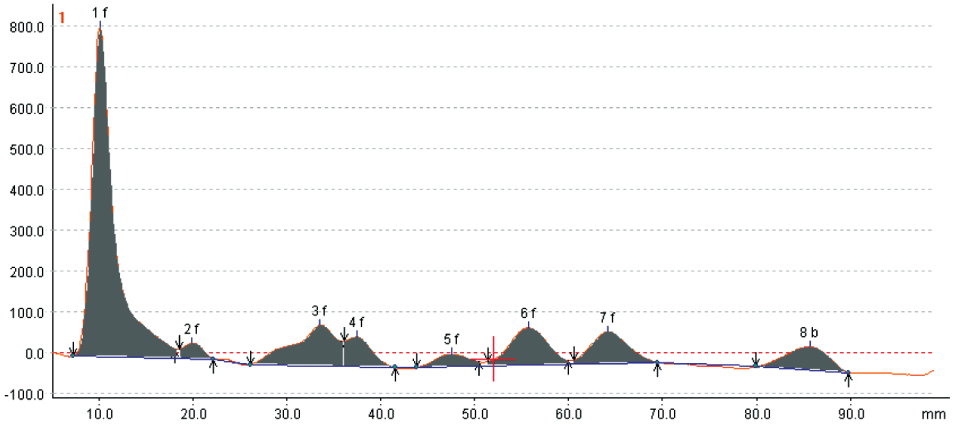


FIGURE 4 : *Boswellia serrata* gum extract. Petroleum ether extract applied in triplicate Track 1, 2, 3. Chloroform extract applied in triplicate Track 4, 5, 6. Solvent system: Toluene:Chloroform = 7:3. Detection system: spraying with 5% methanolic sulphuric acid and observed at visible range.

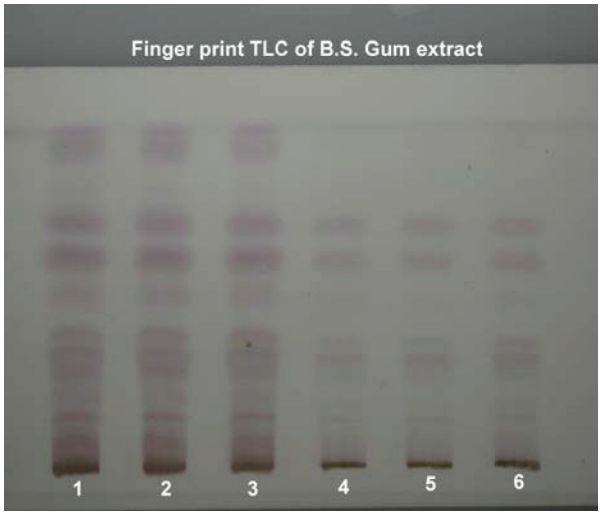


FIGURE 5 : Densitogram of *Boswellia serrata* gum extracted with petroleum ether at UV 390 nm after spray treatment.

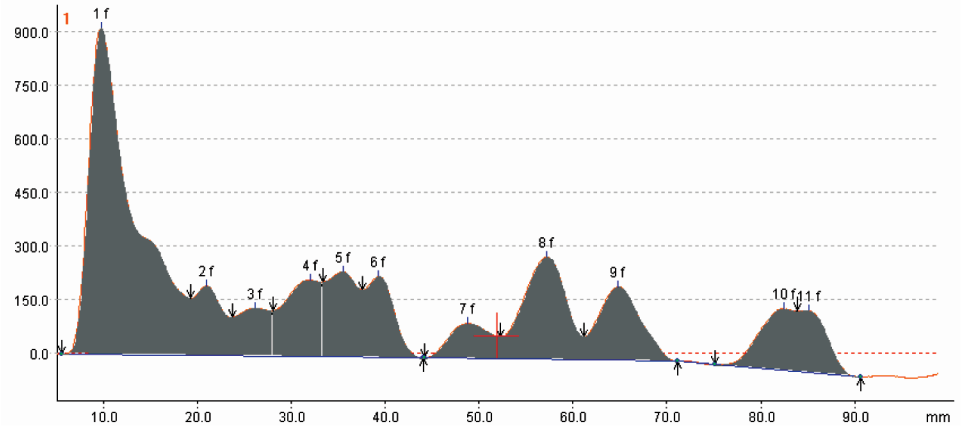


FIGURE 6 : Densitogram of *Boswellia serrata* gum extracted with chloroform at UV 390 nm after spray treatment.

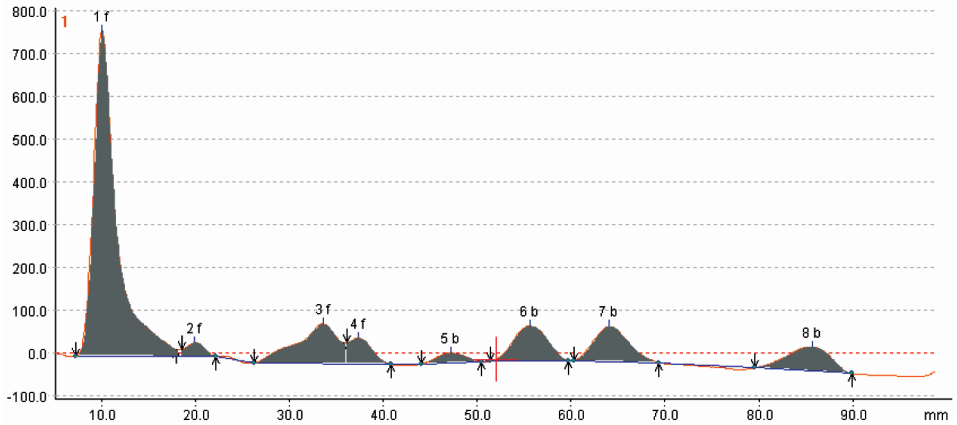


FIGURE 7 : *Boswellia serrata* leaf extract. Petroleum ether extract applied in triplicate Track 1, 2, 3. Chloroform extract applied in triplicate Track 4, 5, 6. Solvent sytem: Toluene:Ethyl acetate= 9:1. Detection System:Observed Under UV range at 366 nm.

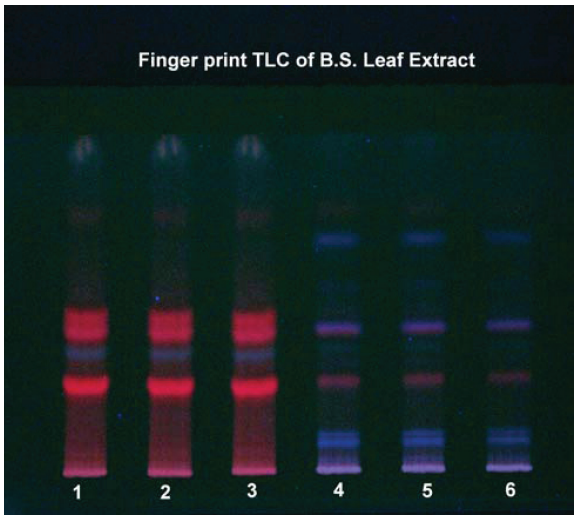


FIGURE 8 : Densitogram of *Boswellia serrata* leaf extracted with Petroleum ether at UV 366 nm.

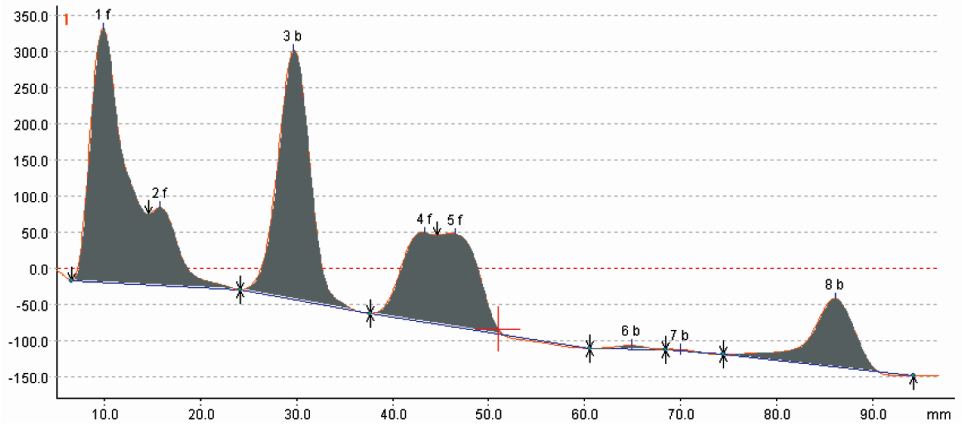


FIGURE 9 : Densitogram of *Boswellia serrata* leaf extracted with chloroform at UV 366 nm.

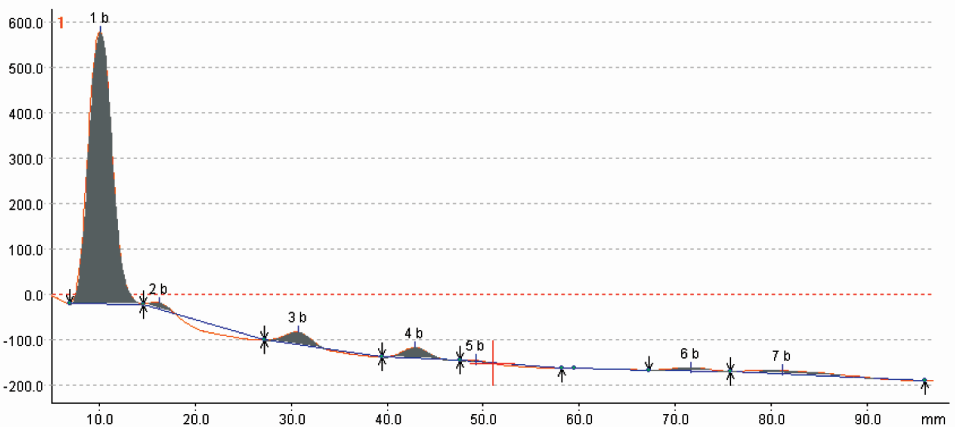


FIGURE 10 : *Boswellia serrata* leaf extract. Petroleum extract applied in triplicate Track 1, 2, 3. Chloroform extract applied in triplicate Track 4, 5, 6. Solvent sytem:Toluene:Ethyl acetate = 9:1. Detection system:spraying with 5% methanolic sulphuric acid and observed at 366 nm.

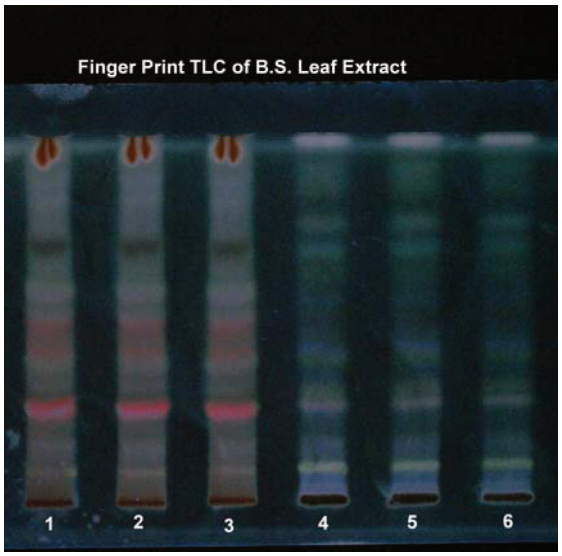


FIGURE 11 : Densitogram of *Boswellia serrata* leaf extracted with petroleum ether at UV 366 nm after spray treatment.

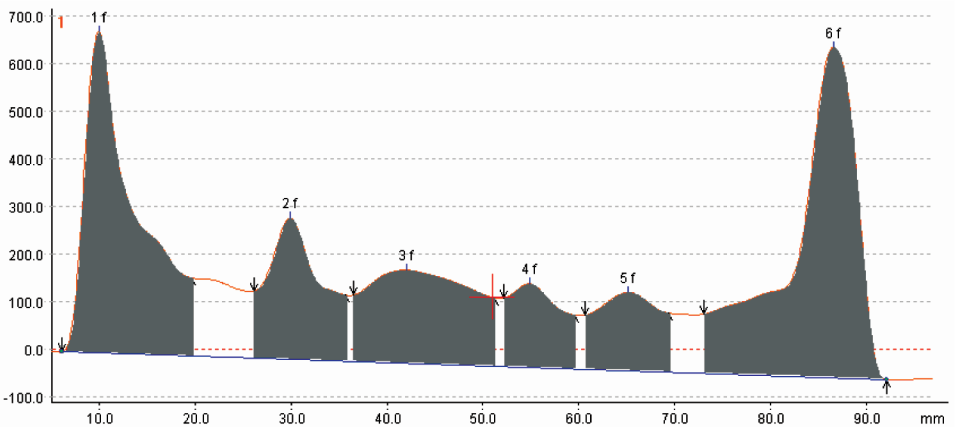


FIGURE 12 : Densitogram of *Boswellia serrata* leaf extracted with chloroform at UV 366 nm after spray treatment.

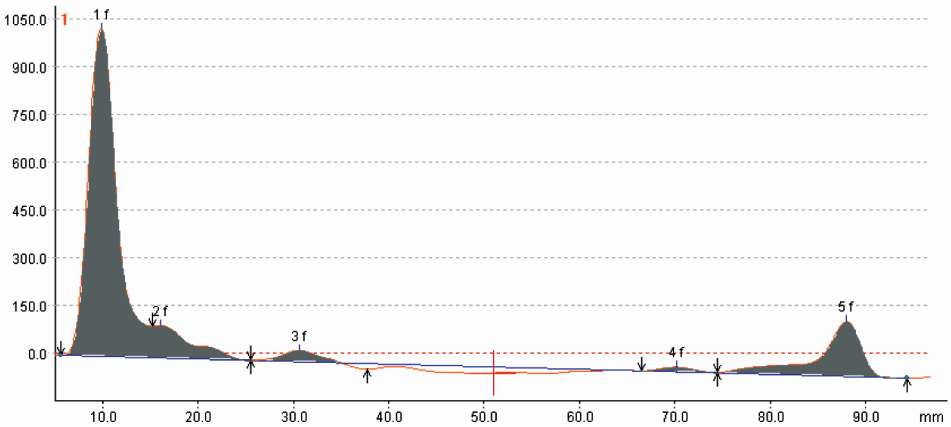


FIGURE 13 : *Boswellia serrata* bark extract. Petroleum ether extract applied in triplicate Track 1, 2, 3. Solvent sytem: Toluene:Ethyl acetate = 9:1. Detection system: observed at UV 366 nm

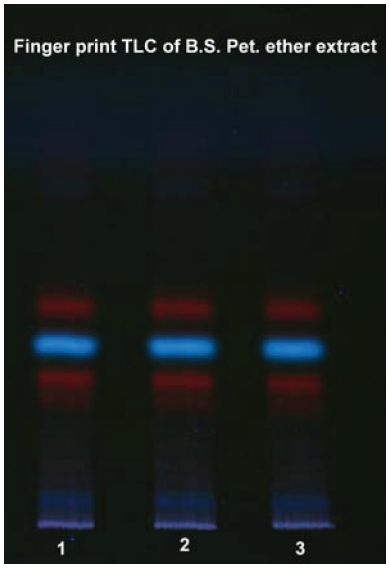


FIGURE 14 : Densitogram of *Boswellia serrata* bark extracted with petroleum ether at UV 366 nm.

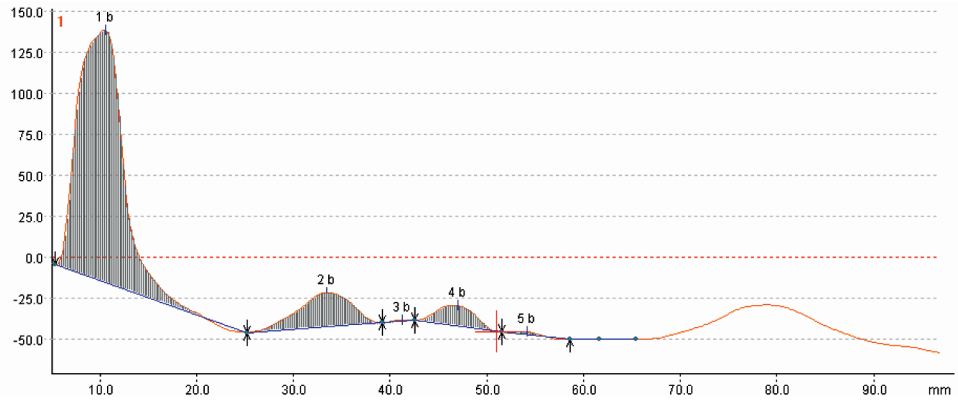


FIGURE 15 : *Boswellia serrata* bark extract. Petroleum ether extract applied in triplicate Track 1, 2, 3. Solvent sytem: Toluene:Ethyl acetate = 9:1. Detection system: spraying with 5% methanolic sulphuric acid and observed at 366 nm

FIGURE 17 :*Boswellia serrata* bark aqueous extract. Aqueous extract applied in triplicate Track 1, 2, 3. Solvent sytem: Toluene:Ethyl acetate:Formic acid = 5:4:1. Detection System:Observed under UV range at 366 nm.

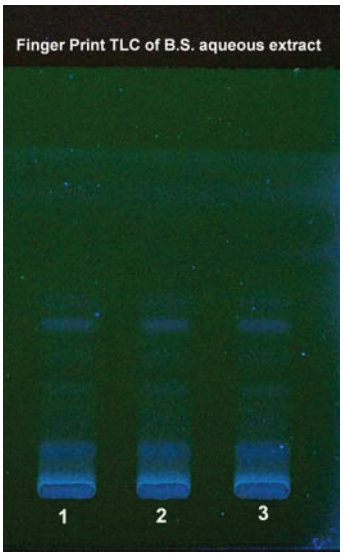


FIGURE 16 : Densitogram of *Boswellia serrata* bark extracted with petroleum ether at UV 366 nm after spray treatment.

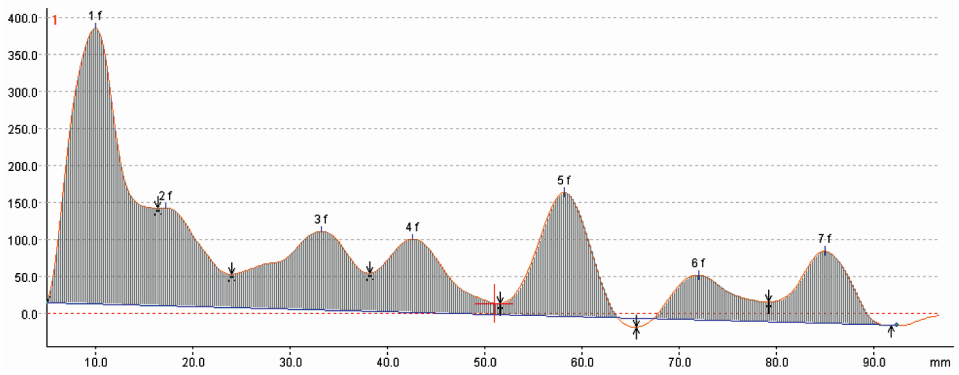
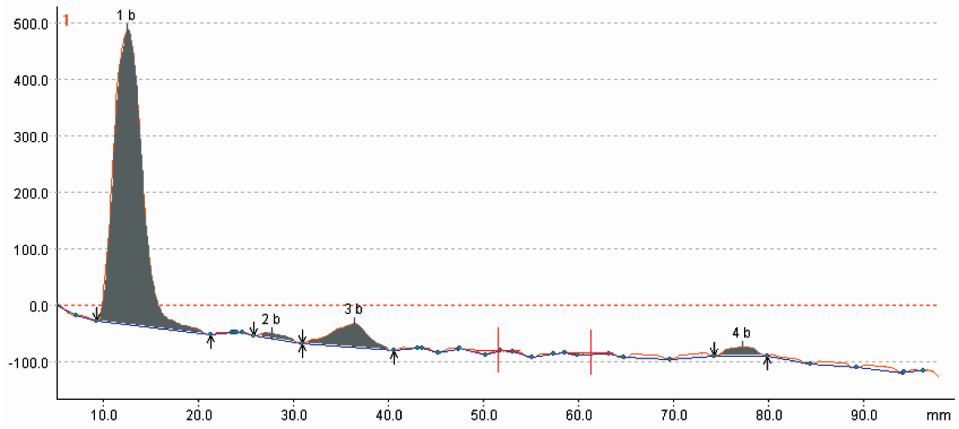


FIGURE 18 : Densitogram of *Boswellia serrata* bark aqueous extract at UV 366 nm.



and 10. The densitogram obtained upon scanning under the densitometer at UV 366 nm before spray treatment were shown in the Figure 8 and 9 whereas at UV 366 nm after spray treatment were shown in the Figure 11 and 12 provided R_f values for the peaks. The peak list along with the R_f values at UV 366 nm were given in the Table 6 and 7 for before spray treatment whereas in Table 8 and 9 for after spray treatment.

Boswellia serrata bark extracted with petroleum ether was subjected to HPTLC analysis by specific solvent system as toluene and ethyl acetate (9:1) and detected under UV 366 nm as shown in the Figure 13. The chromatogram obtained upon scanning under the densitometer at UV 366 nm was shown in the Figure 14 provided R_f values for the peaks as shown in the Table 10. Later the chromatogram was sprayed with 5% methanolic sulphuric acid and heated the plate at 105°C for 5 minutes and observed at UV366 nm as shown in the Figure 15. Densitogram obtained upon scanning with densitometer shown in the Figure 16 and the peak list and R_f values were given in the Table 11.

Boswellia serrata aqueous bark extract was subjected to HPTLC analysis by specific solvent system as toluene: ethyl acetate:formic acid (5:4:1) and detected under UV at 366 nm as shown in the Figure 17. The densitogram obtained upon scanning under the densitometer was shown in the Figure 18 provided R_f values for the peaks and the peak list was shown in the Table 12.

CONCLUSION

This method of HPTLC for the different extracts of *Boswellia serrata* plant parts was very much helpful in determining the quality of the crude drug and also helps to separate and isolate the components using other chromatographic techniques which can be used for further studies.

ACKNOWLEDGEMENTS

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Chemical Standardization of *Cassia angustifolia* Vahl seed

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INTRODUCTION

The most popular natural remedies have recently re-immerged after disastrous effects of synthetics in health and economy. *Cassia* species is known from time immemorial for its diversified medicinal and economic properties. Anthraquinone derivatives are the main active constituents of senna, which are responsible for its laxative properties. Senna contains sennosides A, B, C & D,[1–3] kaempferol,[4] phytosterols,[5–7] glycosides of rhein and chrysophanic acid. Tinnevelin glycoside is also identified in *C. angustifolia* which differentiates similar species *C. auriculata*.[8] Apart from their leaves, seeds also have immense potential and contain useful protein and water-soluble polysaccharides.[9] Different forms of senna are being used in medicine *viz.*, senna fruit extract, senna leaf powder, senna pod, senna syrup and sennosides A & B. Although a number of synthetic laxatives are available, sennoside-containing preparations are still the most widely used.[10]

ABSTRACT: *Cassia angustifolia* Vahl, commonly known as *'Sanaai'*, is employed in various indigenous systems of medicine against several diseases. Almost every part of the plant has diverse medicinal properties. The seeds are used as anthelmintic, digestive, to treat piles, skin diseases and abdominal troubles.

The present communication deals with the chemical standardization of the seeds of *C. angustifolia*. The study includes physicochemical studies and development of chemical markers by HPTLC fingerprinting of seed samples from different geographical zones.

The study revealed that the seed samples procured from different zones have similar physico-chemical values and chemical profile. However it was noted that the percentage of active principles (sennosides A & B) varied significantly in samples procured from different parts of the country.

Detailed chemical studies are useful in establishing physicochemical standards and biologically active chemical markers for correct identification and authentication. Studies also better characterize the plant matrix through componential analysis of active principles for feasible, acceptable, specific and sustainable value added utilization of plant or their individual components. Studies not only explore the possibility of utilization but also co-relates/authenticates the materials with the commercial formulation for market acceptability and competency.

Keywords: *Cassia angustifolia*, HPTLC, Seeds, Sennoside A, Sennoside B.

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Active components (sennosides A & B) available singly or in combination with other plants in different forms are Panchasakar churna, Correctol Herbal Tea, Senna concentrate, Senokot, Spolax and Periderm granules, Virechni, Ex-lax gentle, Herblax and Delcolex tablets. Since sennosides are the most important bioactive principles, most of the studies were elaborated in order to determine sennosides componentially, precisely and accurately. Though different methods and systems have been proposed for the separation and estimation of sennosides which seems to be good for identification, their identification in crude senna seed extracts still remains a difficult task and methods suffer from inconvenience. Hence an attempt is made to standardize and analyse free and combined anthraquinones (both are required for specific functional activity), through simplest technique for higher feasibility, flexibility and acceptability. Quantitative estimation of sennosides in important laxative commercial formulation Delcolex has also been compared with seed samples to establish the quality of laxative principles commercialized at international level. Seeds possess diversified medicinal and economic importance and rigourous chemical standardization for quality and quantity is mandatory. In this context, a detail phytochemical standardization of *C. angustifolia* seeds has been performed. The samples procured form different parts of the country has been studied for the geographical variations if any. All the seeds are compared with the pods of the plant which are commercial sources of sennosides as laxative principle. Hydrolysis of the seed and its component endosperm was undergone to standardize them on the basis of aglycones separated after hydrolysis. Their separation in hydrolyzed product may be used as identifying marker for the seed.

FIGURE 1: ***C. angustifolia*: Whole plant.**

FIGURE 2: Seeds of *C. angustifolia*.

MATERIALS AND METHODS

Plant Material

The plant material was collected from the Hyderabad (Andhra Pradesh), India. The specimens were authenticated by Dr. AKS Rawat and a voucher specimen was deposited in the Institute's herbarium (LWG 221242).

Procurement of commercial samples: The market samples were procured from important herbal drug markets of the country, *viz*. Dehradun, Hyderabad, Jodhpur, and Mumbai.

Separation of endosperm as crude gum and isolation of purified gum (Fig. 2): Separation of endosperm from the seeds of *C. angustifolia* was done by cleaning the seeds from dust and other impurities by manual process and subjecting it to mild roasting in microwave oven for 2 min. The roasting process caused loosening of the seed coat from endosperm which facilitated separation of the seed components. The endosperm was separated from hull and germ by repeated treatments in mixer using suitable sieves by dry milling process. The endosperm as crude gum of #50 mesh was obtained by crushing it in a high speed hammer mill. This was purified by procedure of fractional precipitation and barium complexing.

Hydrolysis of seed and endosperm: 0.5 g of the sample was heated under reflux for 15 minutes with 25 ml of 7.5% hydrochloric acid mixture was extracted after cooling with 20 ml ether. The ether phase was separated, concentrated and used as test solution for application.

Physicochemical standardization: Physicochemical studies *viz.,* moisture percentage, total ash, acid insoluble ash, water and alcohol soluble extractives of different parts of seed (seed, endosperm, meal) were also calculated according to methods described in the Indian Pharmacopoeia.[11] Screening tests for the seed and endosperm were performed to detect the chemical constituents qualitatively.[12] The percentage of fibre and mucilage.[13] protein,[14] sugar/starch[15] and tannin[16] were also calculated.

TABLE 1: Physicochemical parameters of *Cassia angustifolia* seeds and endosperm.

S.N.	PARAMETERS WITH PARTS	MEAN (%)	SD
1.	**Moisture**		
	Seed	89.053	± 1.256
	Endosperm	11.450	± 0.237
2.	**Total ash**		
	Seed	4.223	± 0.117
	Endosperm	2.350	± 0.145
3.	**Acid insoluble ash**		
	Seed	0.200	± 0.000
	Endosperm	0.010	± 0.000
4.	**Alcohol soluble extractive**		
	Seed	9.416	± 0.376
	Endosperm	2.250	± 0.121
5.	**Water soluble extractive**		
	Seed	32.417	± 1.49
	Endosperm	-	-

Chemical standardization

Chemicals and standard compounds: Reagents used were from Merck (Germany) and standards *viz.,* sennosides A & B were from Sigma-Aldrich (Steinheim, Germany).

Extraction of plant material: Air dried (45–50°C) powdered sample of 0.5 g of powdered *Cassia angustifolia* seeds of different geographical regions viz. Dehradun, Hyderabad, Jodhpur and Mumbai were refluxed in 50% methanol for 15 min and filtered to obtain test solutions. Extracts were concentrated under vacuum, re-dissolved in methanol, filtered and finally made up to 100 mL volume with methanol prior to HPTLC analysis.

Preparation of reference solutions: 0.1 mg of sennoside Aand 0.55 mg of sennoside B were dissolved separately in 1 ml each of isopropyl alcohol for formation of reference solution.

75 mg of Delcolex™ powder was dissolved in 5 ml of 50% methanol for formation of reference solution.

Application procedure: Test solutions (10 µl) of each sample along with reference solutions of sennoside A and sennoside B were applied in different tracks on precoated silica gel GF254 Merck plate by Linomat Camag Applicator.

Test solutions of 10 µl each along with reference solutions of anthraquinones were applied in band form with a band width of 6 mm, band space of 6 mm and flow of 10 sec/µL on precoated silica gel G F254 Merck plate of 20 × 10 cm by Linomat Camag Applicator.

Test solutions of each sample along with reference solutions of commercial formulation Delcolex were applied in different tracks on precoated silica gel G F254 Merck plate by Linomat Camag Applicator.

Development procedure: The development of TLC plate was performed with butanol: acetic acid: water (6:1:2 v/v), propanol: ethyl acetate: water (4:4:3) and hexane: ethyl acetate: formic acid (7.5:2.5:0.1) as mobile phase. Chamber saturation period was 20 min at 25°C, humidity was 50% and development distance was 9 cm. After development, the plate was dried through hair dryer.

Detection: Detection of active constituents and chemical components were made in UV254 and visible light after spraying with anisaldehyde sulphuric acid and heating at 120°C for 10 min on Camag heater.

Qualitative estimation: Different bands were qualitatively estimated and documented.

Quantitative estimation of chemical markers: Quantitative estimation was done using Camag Scanner 3.

RESULTS AND DISCUSSION

Physicochemical standardization

The physicochemical parameters of the seed *viz.*, moisture, total ash, acid insoluble ash were found to be 89.05%, 4.2% and 0.2% respectively while in the endosperm sample it was estimated as 11.45%, 2.35% and 0.01% respectively. Percentage alcohol extractive was 9.4 and 2.25 in the seed and endosperm respectively but the percentage of water extractive was found to be significantly high i.e. 32.4% in the seed (Table 1).

Fiber was found to be 0.217% in the seed and 0.158% in the seed endosperm respectively. Seed and endosperm both had enough mucilage i.e. 17.62% and 13.79%, respectively. Total sugar ranged from 3 to 6% in the seed and endosperm samples while starch content was significantly high i.e., upto 62% in both the samples. Percentage oil in the seed and endosperm was also determined which was found to be 3.22% and 0.544%, respectively. Percentage protein is an important parameter for standardization of seed. Hence, protein has been estimated in all the components of seed. 6.5% of protein was found in whole seed while endosperm contained 3.1% only. Meal, comprising of testa along with germ possessed 8.6% of protein. It is noteworthy that cotyledons contained an appreciable amount of protein of upto 18.7% hence it could be used as cattle feed. (Table 2).

Chemical standardization through HPTLC

Determination of gross HPTLC finger print profile of Cassia angustifolia seeds: HPTLC studies were performed to develop characteristic gross HPTLC

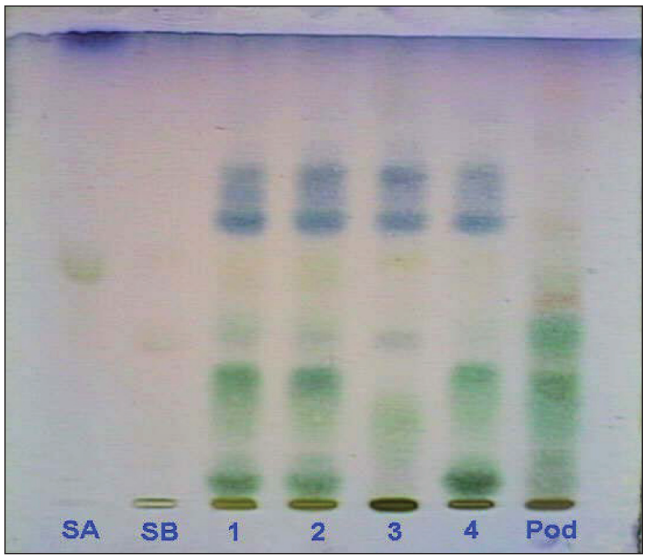
TABLE 2: Physico chemical parameters of *Cassia angustifolia* seeds and endosperm.

S.N.	PARAMETERS WITH PARTS	MEAN (%)	SD
1.	**Fiber content**		
	a. Seed	0.158	± 0.002
	b. Endosperm	0.217	± 0.004
2.	**Mucilage content**		
	a. Seed	13.789	± 0.093
	b. Endosperm	17.620	± 0.095
3.	**Tannins**		
	a. Seed	0.366	± 0.005
	b. Endosperm crude	0.271	± 0.001
4.	**Sugar content**		
	a. Seed	3.093	± 0.175
	b. Endosperm	6.037	± 0.311
5.	**Oil content**		
	a. Seed	3.220	± 0.036
	b. Endosperm	0.544	± 0.030
6.	**Protein content**		
	a. Seed	6.518	± 0.033
	b. Endosperm	3.112	± 0.018
	c. Meal	8.625	± 0.032
	d. Cotyledons	18.699	± 0.043
7.	**Starch content**		
	a. Seed	62.065	± 0.205
	b. Endosperm	62.781	0.501

finger print profile, which may be used as marker for quality evaluation and standardization of the drug.

In the present study, eight chemical markers were developed at R_f values of 0.10, 0.21, 0.27, 0.32, 0.52, 0.58, 0.62 and 0.64 of characteristic colour in all the seed samples procured from different geographical zones of the country (Table 3 & Fig. 3).

FIGURE 3: HPTLC studies of *Cassia angustifolia* seeds and pod.



(SA; Sennoside-A; SB; Sennoside-B; 1-Bombay; 2-Dehradun; 3-Hyderabad; 4-Jodhpur; Pod- Pod sample from Mumbai)

Solvent system: *Butanol: Acetic acid: Water (6:1:2)*

Determination of sennoside A and B in pods and its comparison with collected seeds: This estimation was done to authenticate and chemically standardize the seed samples on the basis of presence of common and identifying markers in reference to commercially utilized pods as a source of laxative drugs. Blue coloured identifying markers at R_f values of 0.58, 0.62 and 0.64

TABLE 3: Quantitative HPTLC estimation of sennosides in *C. angustifolia* seeds of different zones in visible light after spraying.

REFERENCE STANDARDS	MUMBAI		DEHRADUN		HYDERABAD		JODHPUR	
	R_f value	Colour	R_f value	Colour	R_f value	Colour	R_f value	Colour
	0.1	Green	0.1	Green	0.1		0.1	Green
	0.21	Yellowish green	0.21	Yellowish green	0.21	Yellowish green	0.21	Yellowish green
	0.27	Green	0.27	Green	0.27	Green	0.27	Green
Sennoside A	0.32	Greyish blue	0.32	Greyish blue	0.32	Greyish blue	0.32	Greyish blue
Sennoside B	0.52	Light green	0.52	Light green	0.52	Light green	0.52	Light green
	0.58	Blue	0.58	Blue	0.58	Blue	0.58	Blue
	0.62	Blue	0.62	Blue	0.62	Blue	0.62	Blue
	0.64	Blue	0.64	Blue	0.64	Blue	0.64	Blue

TABLE 4: Percentage concentration of sennoside A and B in seed samples of different geographical regions.

	DEHRADUN	HYDERABAD	JODHPUR	MUMBAI	PODS MUMBAI
Sennoside A	1.000%	2.134%	1.856%	0.219%	1.247%
Sennoside B	3.951%	7.446%	8.526%	1.348%	3.793%

are separated in seeds which are absent in pods. In addition, characterstic green band at R_f value of 0.30 and red band at R_f value of 0.40 are identifying markers developed in pods. (Table 3 and Fig. 3).

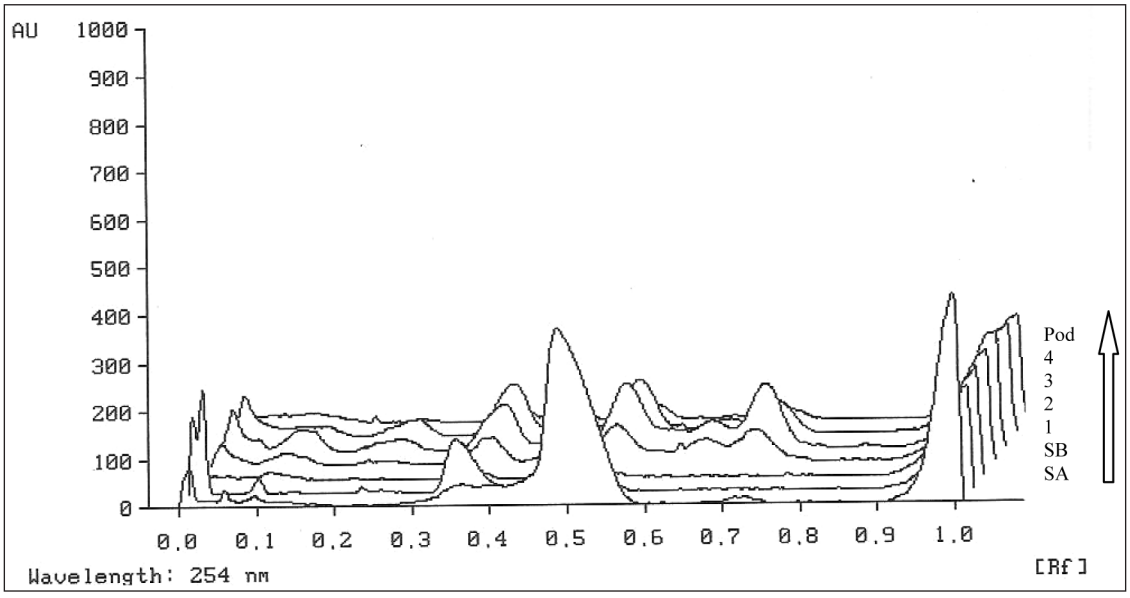
Determination of specific active components (sennosides A & B) in C. angustifolia seeds of different geographical zones: The concentration of sennosides (A & B) was also studied in samples from different geographical zones. Various workers have estimated their concentration in the leaves and pods of the fruit, but no such record is available for seeds. Since in the present studies, the main emphasis was on commercial value of seeds, the percentage of sennosides was determined in seeds and it was found that the concentration of sennoside B was significantly high in majority of the samples. The plate in UV254 and visible light after spraying with detecting reagent showed the presence of sennoside A and sennoside B at R_f of 0.52 and 0.32 in all the seed samples. Sennosides A and B when qualitatively estimated, were found to be present in all the seed samples though their quantity varied from one region to another (Fig. 3 and 4).

The concentration of sennoside A varied from 0.21–2.13 percent in seeds of different regions while sennoside B showed a wide range of variation from 1.34–8.52 percent. Hyderabad seeds had maximum sennoside A (2.13%) which was least in the Mumbai seeds, i.e., 0.219%. On the contrary, sennoside B was found maximum in Jodhpur seeds (8.52%) which were about 7 times more than the seeds of Mumbai i.e., 1.34%. The seeds from Hyderabad too had appreciable concentration of sennoside B, i.e., 7.4% however in Dehradun seeds it was limited to 3.95% (Table 4).

These variations in the seed samples may be due to difference in ecological conditions as soil climate, soil nature, seed maturity and changes in agricultural practices at different places where they are cultivated.

Determination of specific active components (sennoside A and B) in commercial drug Delcolex and its comparison with the authentic source: Identifying markers has also been determined through HPTLC to determine the individual sennoside A and B in the seed and endosperm with reference to sennosides present in Delcolex tablet (Fig. 5).

FIGURE 4: Densitometric chromatograms of different samples of *Cassia angustifolia* seeds procured from different regions along with the active constituents sennoside A & B.



(SA; Sennoside-A; SB; Sennoside-B; 1-Mumbai; 2-Dehradun; 3-Hyderabad; 4-Jodhpur; Pod- Pod sample from Mumbai)

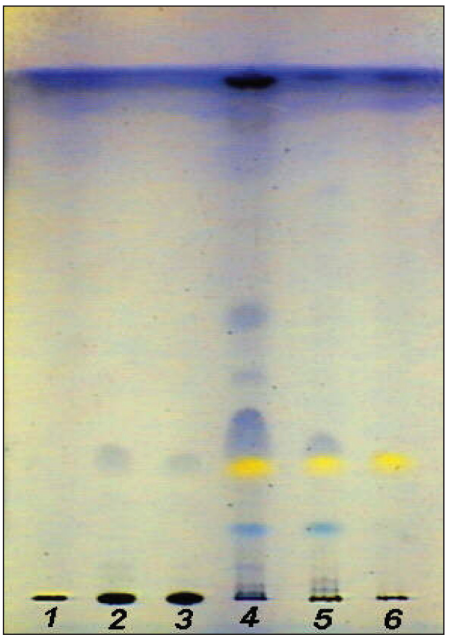
Solvent system: *Butanol: Acetic acid: Water (6:1:2)*

FIGURE 5: HPTLC finger print profile of *C. angustifolia* seeds and endosperm for identification of marker components with reference to Delcolex in UV254.

(1-Delcolex; 2-Crude seed; 3- Endosperm; 4-Purified endosperm)

Solvent system: *Propanol: Ethyl Acetate: Water (4:4:3)*

FIGURE 6: HPTLC profile of crude and hydrolyzed products of *C. angustifolia* seeds and endosperm in visible after spraying.



(1-Unhydrolyzed crude seed; 2&3- endosperm; 4- Hydrolyzed seed; 5- Endosperm; 6- Purified endosperm)

Solvent system: *Hexane: Ethyl acetate: Formic acid (7.5:2.5:0.1)*

Both seed and endosperm extracts in UV254 nm showed 5 common marker components at R_f values of 0.05, 0.10, 0.19, 0.36 and 0.61 respectively. A brown coloured band at R_f value of 0.19 was found in the seed, and endosperm sample corresponding to that in the tablet which can be identified as commerciable marker possessing specific functional property (Table 5).

Development of specific HPTLC profiles by detection of markers in crude and hydrolyzed products of seed and endosperm: Un-hydrolyzed crude seed, crude endosperm and purified endosperm were compared with their hydrolyzed samples through TLC, unhydrolyzed seed and endosperm showed three marker bands of grey colour at R_f values of 0.06, 0.11 and 0.28. Seed after hydrolysis showed additional characteristic markers at R_f values of 0.01, 0.03, 0.13, 0.25, 0.41, 0.53 and 0.90 of characteristic colours. In acid hydrolysed crude endosperm, five identifying markers as compared to seeds are obtained at an R_f values of 0.01, 0.03, 0.13, 0.25 and 0.28 of which blue (R_f, 0.13), yellow (R_f, 0.25)

TABLE 5: HPTLC finger print profile of *C. angustifolia* seeds and endosperm for identification of marker components with reference to Delcolex in UV254.

REFERENCE (DELCOLEX)		SEED		CRUDE ENDOSPERM		PURIFIED ENDOSPERM
R_f	Colour	R_f	Colour	R_f	Colour	
-	-	0.05	Brown	0.05	Brown	No band separation.
-	-	0.10	Brown	0.10	Brown	
0.19	Dark Brown	0.19	Dark Brown	0.19	Dark Brown	
0.36	Brown	0.36	Brown	0.36	Brown	
0.42	Brown	-	-	-	-	
-	-	0.61	Brown	0.61	Brown	
0.700	Brown	-	-	-	-	

TABLE 6: HPTLC profile of crude and hydrolyzed products of *C. angustifolia* seeds and endosperm in visible after spraying.

CRUDE SEED		CRUDE ENDOSPERM		CRUDE SEED AFTER HYDROLYSIS		CRUDE ENDOSPERM AFTER HYDROLYSIS		PURIFIED ENDOSPERM AFTER HYDROLYSIS	
R_f	Colour	R_f	Colour	R_f	Colour	R_f	Colour	R_f	Colour
-	-	-	-	0.01	Greyish blue	0.01	Greyish blue	-	-
-	-	-	-	0.03	Brown	0.03	Brown	-	-
0.064	Grey	-	-	-	-	-	-	-	-
0.110	Grey			-	-	-	-	-	-
-	-	-	-	0.13	Blue	0.13	Blue	-	-
0.250	Grey			0.25	Yellow	0.25	Yellow	0.25	Yellow
		0.280	Grey	0.28	Grey	0.28	Yellow		
-	-	-	-	0.410	Grey	-	-	-	-
-	-	-	-	0.530	Grey	-	-	-	-
-	-	-	-	0.900	Grey	-	-	-	-

and blue (R_f, 0.28) markers were the major ones identified. Hydrolysed purified endosperm showed only a single yellow coloured characteristic spot at R_f value of 0.25 (Table 6 and Fig. 6).

It can hence be inferred that band at R_f value of 0.25 was a characteristic common identifying marker of seed and endosperm in the hydrolysed product and the band at R_f value of 0.28, as identifying marker for both hydrolysed and unhydrolysed seed and endosperm (Table 6).

CONCLUSION

Detailed chemical studies are useful in establishing physicochemical standards and biologically active chemical markers for correct identification and authentication. Studies also better characterize the plant matrix through componential analysis of active principles for feasible, acceptable, specific and sustainable value added utilization of plant or their individual components. Studies not only explore the possibility of utilization but also co-relates/authenticates the materials with the commercial formulation for market acceptability and competency.

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Extraction of Saponins from Safed Musli

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INTRODUCTION

Safed musli is extensively used in Ayurveda as a vitalizer and health tonic, a curative for pre-natal and post-natal problems, and a restorative for immunity improvement and as a remedy for diabetes and arthritis. Safed musli represents the dried tuberous roots obtained from *Chlorophytum borivilianum* belonging to family Liliaceae. In last few years extensive cultivation has been undertaken in India of *Chlorophytum borivilianum* considering the increasing demand both from domestic as well as overseas market. In spite of good demand for the drug it is still continued to sell in the raw form. There is hardly any effort made to add value to this product. Studies indicate that saponins are the active principles of therapeutic importance. In order to develop formulations based on safed musli, it becomes necessary to extract the saponins in an efficient way. The total extractives so obtained from the plant can then be used for the preparation of phytopharmaceuticals or subjected to further purification to get pure active chemical that offer greater stability and precisely known potency (Laddha, 2002). Thus this study was undertaken to setup parameters for extraction of safed musli.

ABSTRACT:

Introduction: Research studies on *Chlorophytum* conducted in India and elsewhere indicate that saponins are responsible for medicinal properties. Thus, this study was undertaken in order to setup optimum parameters for the effective extraction of saponins from *Chlorophytum borivilianum* tubers.

Methods: Particle size of the material for extraction; the effect of time, temperature, choice of solvent on extraction were studied. The effect of different drying techniques was also studied.

Result: It was observed that mesh size 30/60, at 60°C for 4 h using either water or methanol as solvent was giving appreciable good yield of saponins in the extract

Conclusion: The extraction is best accomplished at a temperature of 60ºC for four hours. The extract so obtained can be dried using either of the techniques, depending on the type of formulation required to be made out of the extract.

Keywords: *Chlorophytum borivilianum* (Santa Pau & Fernandes), extractive value, saponin content.

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TABLE 1: Effect of particle size on extraction efficiency.

SR. NO.	PARTICLE SIZE (MESH SIZE)	% EXTRACTIVE VALUE (AVERAGE ± STD.DEV., N = 3)	% SAPONINS (AVERAGE ± STD. DEV., N = 3)
1.	10/22	46.88 ± 0.138	0.75 ± 0.005
2.	22/36	60.72 ± 0.138	0.76 ± 0.010
3.	36/60	66.43 ± 0.212	0.77 ± 0.005
4.	60/80	64.58 ± 0.201	0.77 ± 0.005

TABLE 2: Effect of time on extraction efficiency.

SR. NO.	TIME (HOURS)	% EXTRACTIVE VALUE (AVERAGE ± STD. DEV., N = 3)	% SAPONINS (AVERAGE ± STD. DEV., N = 3)
1.	1	36.43 ± 0.252	0.64 ± 0.0265
2.	3	44.23 ± 0.252	0.72 ± 0.01
3.	6	53.66 ± 0.153	0.753 ± 0.0152
4.	8	61.46 ± 0.153	0.76 ± 0.01
5.	17	68 ± 0.2	0.763 ± 0.0057
6.	20	68.2 ± 0.173	0.763 ± 0.0057

MATERIALS AND METHODS

Methanol and butanol were obtained from Merck Chemicals, Mumbai. The water and blood reagent were prepared on the day of analysis. Anthrone and Folin-Ciocalteau reagent were obtained from Sigma Chemicals, Mumbai

The effect of following parameters were studied for better yield of the extract in shortest period of time for saponins from safed musli (1) Particle size (2) Time (3) Temperature and (4) Solvent.

Effect of particle size was studied for four fractions of safed musli root powder. Particle sizes of # 10/22, 22/36; 36/60 and 60/80 and the extraction was carried out for 24 hours at room temperature. Drug of particle size 22/36 was chosen for the study of rest of the parameters because it gives greater surface area exposed to the solvent, and in turn facilitate extraction. The time parameter was undertaken to ensure less time consumption for extraction and to find out saturation time, the extraction was performed at room temperature. The temperature effect was studied at different temperatures e.g., room temperature (RT), 37°C, 60°C and 75°C for a period of eight hours. Different solvents were used to choose the best solvent for complete extraction of saponins and the extraction was carried out at room temperature for eight hours.

Efforts were also made to study the effect of the type of drying on the extract. The extract was dried in three ways: spray drying, freeze drying and tray drying. The various physical parameters were further studied on the extracts according to Martin (1983).

Estimation of saponins was done using the hemolytic method (Barve and Laddha, 2006) wherein the saponins react with blood reagent to release oxy-hemoglobin, which in turn is estimated colorimetrically. The total extractive value was calculated by gravimetric method as per the WHO guidelines (1998). The physical

TABLE 3: Effect of temperature on extraction efficiency.

SR. NO.	TEMPERATURE (°C)	% EXTRACTIVE VALUE (AVERAGE ± STD. DEV., N = 3)	% SAPONINS (AVERAGE ± STD. DEV., N = 3)
1.	R.T.	61.46 ± 0.153	0.74 ± 0.010
2.	45	62.26 ± 0.378	0.76 ± 0.010
3.	60	67.83 ± 0.153	0.84 ± 0.036
4.	75	69.93 ± 0.153	0.71 ± 0.011

TABLE 4: Effect of solvent on extraction efficiency.

SR. NO.	SOLVENT	% EXTRACTIVE VALUE (AVERAGE ± STD. DEV., N = 3)	% SAPONINS (AVERAGE ± STD. DEV., N = 3)
1.	Water	65.63 ± 0.203	0.74 ± 0.010
2.	Methanol	9.36 ± 0.125	0.76 ± 0.017
3.	Butanol	0.50 ± 0.064	0.45 ± 0.030

properties of the extract were evaluated as per the Indian Pharmacopoeia, 1985. Total sugars (Fairbairn, 1953) and proteins (Plummer, 1978) were determined by colorimetric method.

RESULTS

It was observed that the powder having a particle size range of 36/60 is the most suitable for complete extraction of safed musli roots as shown in Table 1, but subsequent clarification is difficult and also fine dry grinding may affect the properties of the phytoconstituents and thereby initiate chemical reactions in which the active principle forms complexes with other cell constituents from which the pure compound becomes difficult to separate. Next it is observed that the powder having a particle size range of 60/80 shows a decrease in the extractive value. This may be because the crude drug swells in water producing a bed of low permeability making it difficult for proper extraction.

The study of time factor for extraction indicates that longer the crude drug stays in contact with the solvent; more effective is the extraction as observed from Table 2. As the study was carried out on water extract, prolonging the time period may also result in fungal growth or microbial contamination and hence is limited only to 20 hours.

The temperature effects on extraction shows that raising the temperature of the solvent during extraction facilitates the release of phytoconstituents owing to the increased rate of diffusion, stronger convection currents, and better solubility. This in turn increases the extractive value simultaneously decreasing the time of extraction. In this case, it was found that as the temperature was increased above 60°C, the extractive value decreased indicating the degradation of saponins (Table 3).

TABLE 5: Physical properties of spray dried, tray dried and freeze dried extracts.

SR. NO.	PARAMETER	SPRAY DRIED EXTRACT (AVERAGE ± STD. DEV., N = 3)	TRAY DRIED EXTRACT (AVERAGE ± STD. DEV., N = 3)	FREEZE DRIED EXTRACT (AVERAGE ± STD. DEV., N = 3)
1.	Density	0.195 ± 0.014	0.7692 ± 0.02	0.094 ± 0.000
2.	Color	Cream color	Light brown	White
3.	pH (1 % w/v)	5.96 ± 0.030	5.76 ± 0.0264	5.12 ± 0.0264
4.	Loss on drying (% w/w)	10.6 ± 0.050	9.83 ± 0.072	8.93 ± 0.651
5.	Ash value (% w/w)	3.85 ± 0.001	4.06 ± 0.012	3.1 ± 0.1
6.	Acid insoluble ash value (% w/w)	0.33 ± 0.0005	0.23 ± 0.0005	0.223 ± 0.0152
7.	Carbohydrate content (% w/w)	60.32 ± 0.0984	59.01 ± 0.2	61.13 ± 0.325
8.	Protein content (% w/w)	9.18 ± 0.050	9.24 ± 0.085	11.05 ± 0.875
9.	Saponin content Hemolytic method (% w/w)	1.21 ± 0.002	0.97 ± 0.001	1.22 ± 0.002
10.	Gravimetric method (% w/w)	3.43 ± 0.057	3.10 ± 0.100	3.86 ± 0.057

The saponins are highly polar phytochemicals, hence use of polar solvents is justified for its extraction. According to the literature, it was found that saponins are more soluble in water, methanol and butanol. Table 4 shows that water and methanol both could be used as extracting solvents, but the total extractive value is more for water. indicating that this extract has many other phytochemicals apart from saponins. This other phytochemical is the mucilage which is not extracted in methanol. Thus if a nutraceutical is to be prepared, one must use the water extract as it will have the therapeutic quality of the mucilage and if a separation/isolation of saponin is to be carried out, one must use the methanol extract as it will be less of other constituents.

Making use of different methods of drying for the extract alters the physical characteristics of the extract but doesn't have any effect on the chemical parameters as seen in Table 5. It was observed that all the three types of water extracts showed positive tests for carbohydrates, proteins, phytosterols, glycosides, saponins, and mucilage.

CONCLUSION

From the various parameters studied for the extraction of safed musli it was observed that root powder of particle size range 30/60 may be used because it gives greater surface area exposed to the solvent, and in turn facilitate extraction. The extraction is best accomplished at a temperature of 60°C for four hours. The total extractive is more when water is used as a solvent indicating, this extract has many other phytochemicals apart from saponins. This other phytochemical is the mucilage which is not extracted in methanol. Thus if a nutraceutical is to be prepared, one must use the water extract as it will have the therapeutic efficacy of the mucilage as well and if a separation/isolation of saponin is to be carried out, one must use the methanol extract as it would be devoid of other constituents. Knowing the physical parameters of both the extracts, it becomes easier for choosing the type of drying based on the formulation to be developed.

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Pharmacognostical Standardization of Fruits of *Elaeocarpus sphaericus* (Gaertn). K. Schum

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INTRODUCTION

The World Health Organization estimates that 80% of the world's population relies on herbal medicine. Meanwhile, the use of herbs in the United States is expanding rapidly, to the point where herbal products are readily found in most pharmacies and supermarkets. From 1990 to 1997, as the use of complementary and alternative medicine rose from 34 to 42%, herbal use quadrupled from 3 to 12%. It is worth remembering that these rapid changes have come not through the medical profession, but by popular demand. The public has discovered that natural medicines often provide a safe, effective, and economical alternative to pharmaceuticals, and research validates this finding. The majority of those who use herbal and high-dose vitamin products fail to tell their physicians. Either they assume that these products are harmless and not worth mentioning or they fear being ridiculed by doctors skeptical about their use. These same doctors, however, must begin to familiarize themselves with the subject. Aside from the advantages of the natural products, herb– drug interactions are a growing concern: almost one in five prescription drug users also using supplements.[1] India has a rich heritage of traditional medicines and the traditional health care systems have been flourishing for many centuries. It mainly consist of three major systems namely Ayurveda, Siddha and Unani systems of Medicine.[2] In almost all the traditional systems of medicine, the quality control aspect has been

ABSTRACT: *Elaeocarpus sphaericus* fruits have been used, as anticonvulsant and analgesic. It is also used in the treatment of epilepsy and hysteria. *Elaeocarpus sphaericus* fruits are used in treatment of head related troubles, epileptic fits, rheumatism, typhoid fever, dysentery and diarrhea. The present study will assist in standardization for quality, purity and sample identification. Various standardization parameters like morphological characters, microscopic evaluation, physicochemical evaluations (loss on drying, ash values, extractive values), preliminary phytochemical screening and TLC chromatographic profile of the extract were carried out and the qualitative parameters were reported. These studies provide referential information for correct identification and standardization of this plant material.

Keywords: *Elaeocarpus sphaericus,* standardization, microscopic evaluation, TLC.

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considered from its inspection itself by the Rishis and later by the Vaidya and Hakims. However, in modern concept it requires necessary changes in their approach. Quality control and quality assurance is an integral part of traditional medicines, which ensures that it delivers the required quantity of quality medicament.[3]

Elaeocarpus sphaericus fruit belongs to the family Elaeocarpaceae, also called Rudraksha, used in Ayurveda for mental diseases, epilepsy, asthma, hypertension, arhthiritis, and liver diseases. Rudraksha is very useful for the patients of high blood pressure. It does not let it go up or down. It keeps the blood pressure normal. It pacifies Vaata (air), Pitta (bile) and cough automatically. It is also useful for women in pregnancy. Rudraksha are a good medicine for skin diseases and for leprosy. Hysteria, coma, leucorrhoea and female diseases related to genital organs can be cured by Rudraksha. Rudraksha increase memory also. Rudraksha beads are dielectric as they store electrical energy and they have permanent magnetic properties that change with the different mukhi beads, e.g., one mukhi bead boosts the concentration and will power of the wearer. According to Vedas it prevents diseases of head such as stroke, paralysis, etc. The complete list of all the properties found inherent in the Rudraksha beads includes electromagnetism, paramagnetism, diamagnetism and dynamic polarity or the ability to change polarity. Wearing of Rudraksha controls heart beat and has a positive effect on blood pressure, stress, anxiety, depression, palpitations and lack of concentration. It was also found that Rudraksha beads have antiageing properties based on their electromagnetism. Rudraksha beads users have repeatedly confirmed that they have experienced considerable relief from the debilitating effects of high blood pressure, stress, hypertension, depression and other mind related problems including neurotic conditions.[4] Moreover, *Elaeocarpus sphaericus* fruits are also useful in lowering the body temperature and act as an antipyretic agent during fever. *Elaeocarpus sphaericus* are effective in treatment of malaria.[5] According to Ayurveda, *Elaeocarpus sphaericus* fruits are used in treatment of typhoid fever, dysentery and diarrhea. The leaves are used in rheumatism. The bark is useful in vomiting of blood and in biliousness.[6] Alkaloid are reported to be the major phytoconstituents of *E. sphaericus.* These include, elaeocarpidine, elaeocarpine[7] and rudrakine.[8] Flavonoids are also reported to be the phytoconstituents of *E. sphaericus.* It includes quercetin.[9] Earlier pharmacological activities of *Elaeocarpus sphaericus* showed that the crude extract has antiasthmatic, antidepressant[10] anti-inflammatory,[11] antimicrobial,[12] anticonvulsive, antiepileptic and antihypertensive properties.[13]

However, no scientific standards or pharmacognostic parameters are yet available to determine the quality of this crude drug. The present work therefore, attempts to report necessary pharmacognostic and standardization parameters for fruits of *Elaeocarpus sphaericus.*

MATERIALS AND METHODS

Description of plant of Elaeocarpus sphaericus

Tree: The tree is large evergreen broad-leaved tree.

Leaves: Leaves are 10–15 by 2.4–4.5 cm., oblong-lanceolate, acute or acuminate, obscurely and irregularly crenate-serrate or sub entire, decurrent into the petiole, glabrous; petioles 6–10 mm. long**.**

Bark: The bark is grayish white and rough in texture with small vertical lenticels and natural horizontal furrow.

Trunk: The main trunk of Rudraksh tree is cylindrical.

Flowers: Racemes 5–7.5 cm. long, from the old wood; buds ovoid-conical, pointed; pedicels 6 mm. long. Sepal 6 mm long, oblong, acuminate, pubescent outside. Petals 8 mm. long, oblong, laciniate about half way down and ciliolate. Stamens about 40, in groups opposite each petal; filaments very short; anthers linear, one valve tipped at the apex with a small tuft of glistening hairs.[14]

Fruits: Fruits 2.5 cm in diameter globose somewhat obovoid purple drupes stone tubercled longitudinally grooved generally five celled and five seeded. The ovary of Rudraksh is five celled. Thus, an ovary may produce 1, 2, 3, 4 or 5 seeds depending upon the abortion of ovules during development into seeds.[15]

Collection and authentication

Elaeocarpus sphaericus fruits were collected in the month of February 2007 from Hari Har Ashram Haridwar, India. The taxonomic identity of the plant was confirmed by Dr. P. Jayaraman, Head, Plant Anatomy Research Center (PARC), Pharmacognosy Institute, Chennai 60045. A voucher specimen (No. PARC 2007/8) has been deposited in the same herbarium.

Preparation of fruit extract

Fruits of *Elaeocarpus sphaericus* were separately dried in shade and powdered. One hundred grams of powdered fruits and seeds were subjected separately to successive Soxhlet extraction by solvents in increasing order of polarity viz., petroleum ether (60–80°C), chloroform and methanol. Before each extraction, the powdered material was dried in hot air-over below

50°C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. The three extracts were weighed and the percentage was calculated in terms of air-dried weight of the plant material.

Phytochemical screening

The various extracts of *Elaeocarpus sphaericus* of both fruits were subjected to qualitative chemical examination.[16,17]

Thin layer chromatographic profile

TLC glass plates (5 × 15 cm), 0.25 mm thick were prepared using silica gel G. The plates were activated at 110°C for 30 minutes. The TLC profiles of the extracts were studied using different solvent systems. TLC plates were developed in TLC chamber. Thin layer chromatograms were visualized under 254/366 nm UV light and in iodine chamber. Spraying reagent 5% methanolic-sulphuric acid is used.

Organoleptic evaluation

Organoleptic evaluation of fruits and seeds was done by observing fruits and seeds with naked eyes.

Microscopic and histological technique of fruits

Study of transverse sections: The fruits of *Elaeocarpus sphaericus* were boiled with water until soft. Free hand sections of both fruits and seeds were cut transferred on slides cleared by warming with chloral hydrate and mounted in glycerin. The lignified and cellulosic tissues were distinguished using differential staining techniques.[18]

Photomicrography: Microscopic evaluation of tissues was supplemented with micrographs. Photographs of different magnifications were taken with Nikon Labpot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grain and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.[19]

Powder microscopy: A few drops of chloral hydrate solution was added to a sample of powered plant material on a slide, covered with a glass slip and heated gently over a microbunsen. Vigorous boiling was avoided. The slide was examined under the microscope. When the clearing process is completed a drop of glycerol solution was added which will prevent crystallization of the mounting agent on cooling.

Physicochemical analysis: Physicochemical analysis i.e., alcohol (90% ethanol) and water soluble extractive values, total ash, acid-insoluble ash, and loss on drying of the powdered drug were determined.[20,21]

RESULTS

Organoleptic features of fruit

Colour - Blue

Taste - Pungent

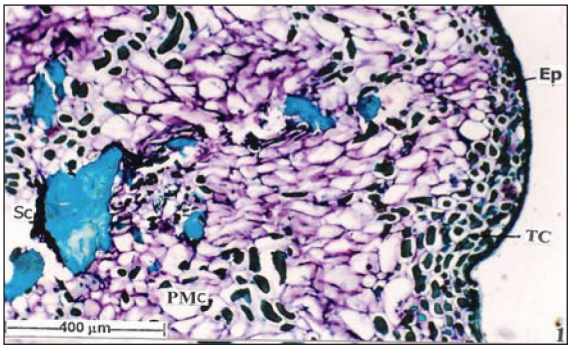
Description - The fruit is spherical with smooth surface. It is a single seeded drupe with thick, soft epicarp and mesocarp and hard stony endocarp. The surface of the endocarp is nodulated with irregular tubercles (Figure 1). Inside the fruit occurs a single seed with endosperm surrounding the embryo.

FIGURE 1: Single fruit showing soft epicarp and hard stony endocarp.

Microscopic features of the fruit

In the cross sectional view, the epicarp is represented by a thin dark epidermal layer of small rectangular cells (Figure 2).

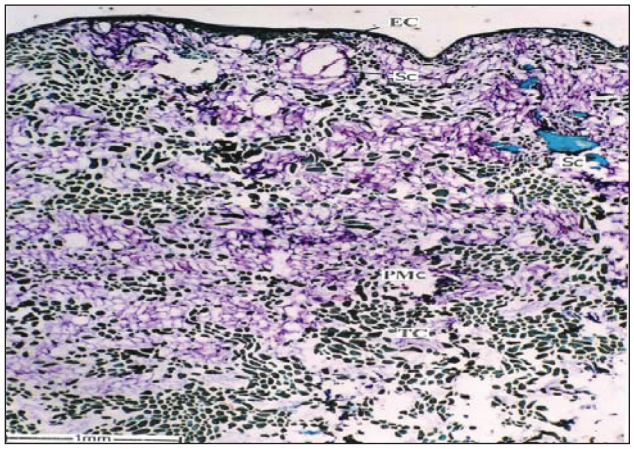
FIGURE 2: T.S. of fruit, outer portion enlarged (anatomy of fruit).



[Ep-Epidermis; PMc-Parenchymatous Mesocarp; Sc-Sclereids; TC- Tanniniferous cells; X- xylem]

Inner to the epidermis is a wide fleshy mesocarp. The cells of mesocarp are parenchymatous, thin walled and compact (Figure 3).

FIGURE 3: T.S. of fruit, a sector enlarged.



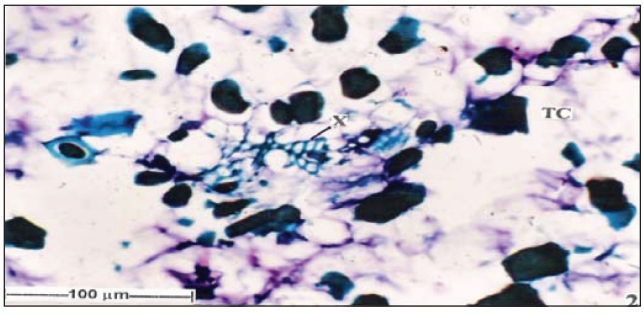
[EC – Epicarp; PMc-Parenchymatous Mesocarp; Sc-Sclereids; TC-Tanniniferous cells; SC – Secretary cavity]

Major portion of the mesocarp cells have dense tannin content, especially, four or five layers of mesocarp cells, inner to the epidermis have dense tannin content. There are also irregular masses of sclereids especially in the outer zone (Figure 2 and Figure 3). The sclereids are polyhedral with thick walls and narrow lumen. Scattered in mesocarp there are vascular strands comprising of few clusters of xylem elements (Figure 4).

Powder microscopy

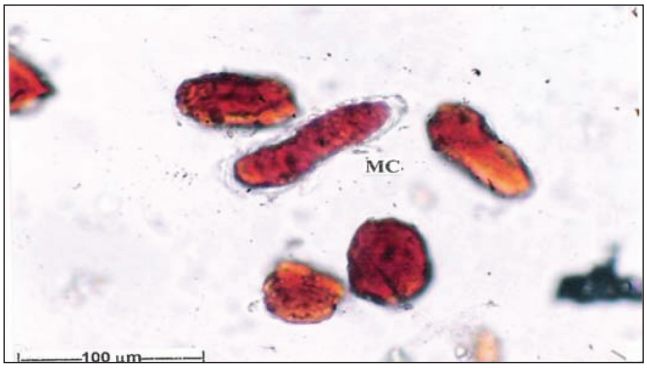
The powder of the mesocarp, stony endocarp and seed were studied under the microscope. The following components are observed: -

FIGURE 4: T.S. of fruit, vascular system and tanniniferous cells.



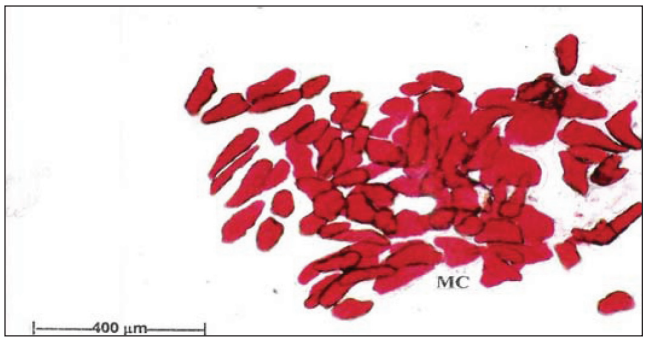
[X-xylem; TC-Tanniniferous cells]

FIGURE 5: Dense parenchymatous mesocarp.



[MC- Mesocarp]

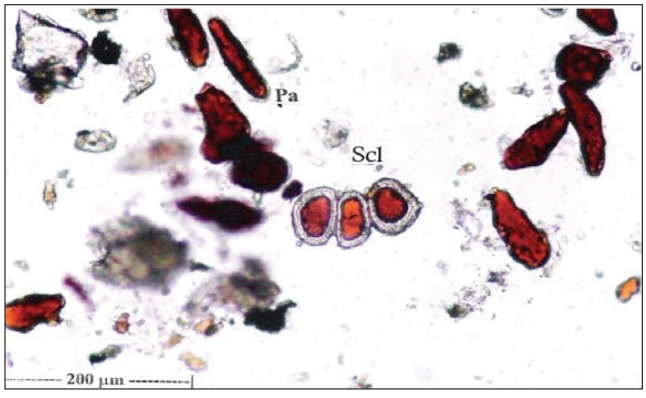
FIGURE 6: Elongated, cylindrical parenchymatous cells, enlarged.



[MC-Mesocarp]

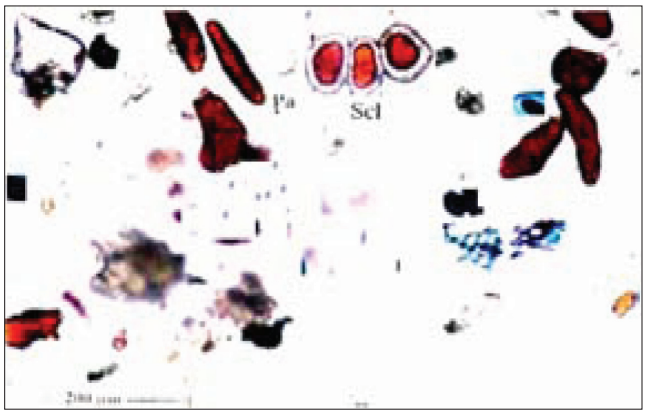
(a) The cells of mesocarp are parenchymatous. They are elongated, cylindrical or spherical in shape (Figures 5, 6). They have thin cellulose walls; the cells content in dense and darkly staining. The cylindrical cells are 150 µm long and 30

FIGURE 7: Powder microscopy showing stony endocarp.



[Pa-Parenchymatous mesocarp; Scl-Sclereid]

FIGURE 7.1: Parenchymatous mesocarp and stony sclereids of endocarp.



[Pa-Parenchymatous mesocarp; Scl-Sclereid]

μm thick. The square shaped cells are 70 μm in diameter.

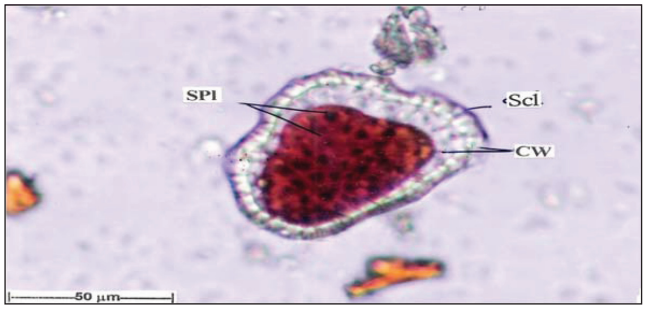
(b) Cells of stony endocarp (Figure 7 and Figure 8). The major component of the endocarp is the stone cells. This is diametric in shape. They have thick, lignified and smooth walls. The cell's lumen is wider. The cell walls have abundant canal like thickenings (Figure 7.1). The sclereids are 70 μm long and 30–40 μm wide.

(c) Crystal bearing cells (Figure 9–10). Thick mass of cells of the powder, when viewed under the polarized light microscope, showed the presence of calcium oxalate crystals in the cells. They are large and diffuse in distribution. They are 20–40 μm in diameter.

Physical parameters

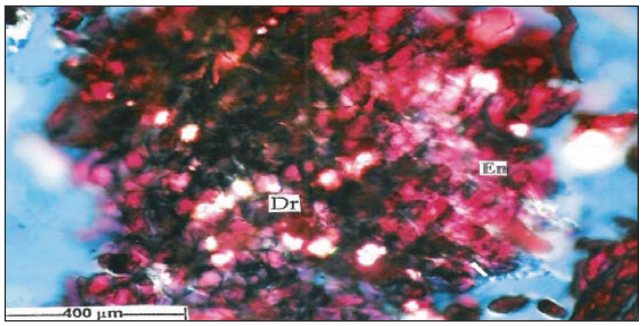
The physical parameters of powdered fruits of *Elaeocarpus sphaericus* were evaluated Table 1.

FIGURE 8: Stone cells enlarged.



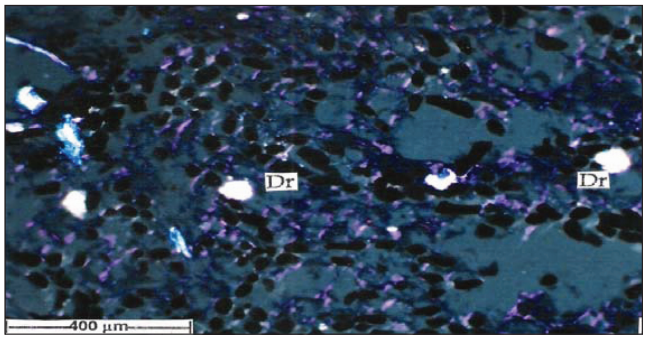
[Cw-cell wall; Pa- Parenchymatous mesocarp; Spl-sieve plate; Scl-Sclereid]

FIGURE 9: Druses in the endocarp under polarized light microscope.



[Dr- Druses; En- Endocarp]

FIGURE 10: Druses and tannjferous cells under low magnification in polarized light microscope.



[Dr- Druses].

Thin layer chromatography

TLC of the methanolic extract on silica gel G using n-butanol : acetic acid :water (4:1:5) under UV (366 nm) shows one fluorescent zone at R_f value of 0.91 (violet). On exposure to iodine vapour, three spots appeared at R_f values of 0.18, 0.29 and 0.50 (all yellow). On spraying with 5% methanolic-sulphuric acid reagent and heating the plate at 105°C for ten minutes a single spot appears at R_f 0.89 (grey).

TABLE 1: Physical parameters of powdered fruits of *Elaeocarpus sphaericus*.

PHYSICAL PARAMETER	% W/W (AIR DRIED DRUG)
Ethanol-soluble extractive	2.4
Water soluble extractive	3.2
Total ash	1.0
Acid insoluble ash	0.24
Loss on drying	8.2

TABLE 2: Results of phytochemical screening of *Elaeocarpus sphaericus* fruit extracts.

PHYTOCHEMICALS	PETROLEUM ETHER	CHLOROFORM	METHANOL
Alkaloids	–	+	+
Carbohydrate	–		+
Proteins and aminoacids	–	+	+
Phytosterols	–		+
Phenolic compounds and tannins	–	+	+
Saponins	–	–	+
Triterpenoids	–	–	
Flavonoids	–	–	+
Fixed oils and fats	–	–	–

Preliminary phytochemical screening

The preliminary phytochemical investigation of the methanol, petroleum ether and chloroform extracts of *Elaeocarpus sphaericus* fruits shows the presence of alkaloids, carbohydrates, steroids, flavonoids, phenolic compounds and tannins (Table 2).

DISCUSSION

As a part of standardization, the macroscopical examination of fruits of *Elaeocarpus sphaericus* was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the fruits of plant can serve as diagnostic parameters. The microscopic evaluation of fruits of *Elaeocarpus sphaericus* and extractive values, ash values and loss on drying of the powdered drug and phytochemical screening of the extract have been carried out which would be of considerable use in the identification of this drug. Percentages of the extractive values, ash value and loss on drying were calculated with reference to the air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. Thin layer chromatography (TLC) was examined in short UV (254 nm) and long UV (366 nm) which is particularly valuable for the preliminary separation and determination of plant constituents. This finding is useful to supplement the existing information with regard to identification and standardization of *Elaeocarpus sphaericus* even in the powdered form of the plant drug to distinguish it from drug and adulterant. These studies also suggest that the observed pharmacognostic and physiochemical parameters are of great value in the quality control and formulation development.

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in Ayurvedic system of medicine.

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Pharmacognostic Studies and Physicochemical Properties of the *Polyalthia longifolia* var. *pendula* Leaf

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INTRODUCTION

Pharmacognosy basically deals with the standardization, authentication and study of natural drugs. It is closely involved with allied fields, viz., phytochemistry and toxicological screening of natural products. Pharmacognosy has been involved in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, histological, physicochemical and toxicological parameters, especially heavy metal estimation and radiobiological contamination in plants, prescribed by an authoritative source. The importance of Pharmacognosy has been widely felt in recent times.[1]

Polyalthia longifolia (Sonn.) Thw. var. *pendula* (Annonaceae) is a tall evergreen tree, is cultivated all over India. The plant has been used in traditional systems of medicine for the treatment of fever, skin diseases and hypertension,[2] for hepatoprotective, anti-inflammatory,[3] antimicrobial,[4] hypoglycemic and antihyperglycemic[5] and antiulcer activities.[6] The objective of the present study was to evaluate various pharmacognostic parameters like macroscopic and microscopic characters, powder microscopic features and physicochemical properties of *P. longifolia* leaf.

ABSTRACT: The leaves of *Polyalthia longifolia* (Sonn.) Thw. Var. *pendula* (Annonaceae) are reported to have great medicinal value. The present investigation was therefore undertaken to determine the requisite pharmacognostic standards for evaluating the plant material. Pharmacognostic studies including examinations of macroscopic and microscopic characters, and powder analysis were determined on leaf of *Polyalthia longifolia*. The physiochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble ash value, pH, boiling point, melting point and extractive values of leaf were carried out. The solubility of methanolic extract of leaves was carried out in various polar and nonpolar solvents. These studies provided crucial information for correct identification and standardization of this plant material.

Keywords: *Polyalthia longifolia*, Pharmacognostic, Physicochemical, Solubility.

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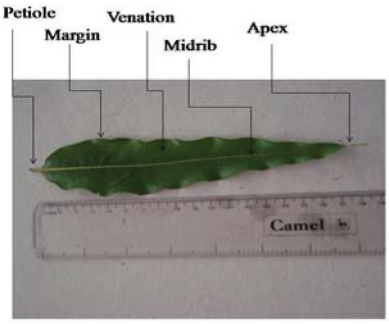
FIGURE 1: Macroscopic study of *P. longifolia* leaf.

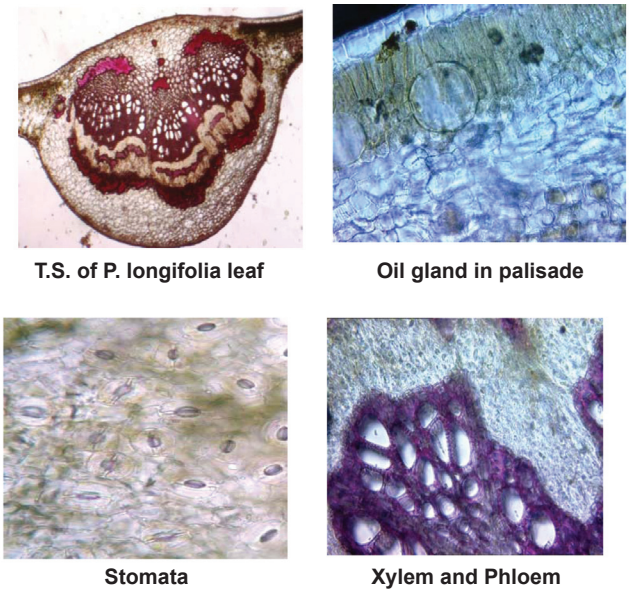


MATERIAL AND METHODS

Plant collection and extraction

Fresh leaves of *P. longifolia* were collected in 2007, Rajkot, Gujarat, India. The plant was compared with voucher specimen (No. PSN4) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The fresh and mature leaves were used for pharmacognostic study. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles. The powder was defatted in hexane and extracted in methanol for 24 h on a rotary shaker by cold percolation method.[7] The methanolic extract was concentrated using rotary vacuum evaporator to get a solid mass. The yield obtained was 8.62%. The methanolic extract was used for the solubility study.

FIGURE 2: Microscopic study of *P. longifolia* leaf.



Pharmacognostic studies

Macroscopic characteristics: The plant was macroscopically examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.[8]

Microscopic characteristics: Microscopic studies were done by preparing a thin hand section of midrib and lamina region of leaf of *P. longifolia*. The section was stained by safranine. The powder of the dried leaf was used for observation of microscopic characters.[8]

Physicochemical properties: Physicochemical parameters were determined as per guidelines of WHO.[9] Total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility of the extract in different solvents, melting point, boiling point, pH, heavy metal analysis, petroleum ether soluble extractive, hexane soluble extractive, ethyl alcohol soluble extractive, methanol soluble extractive and water soluble extractive values were determined.

RESULTS AND DISCUSSION

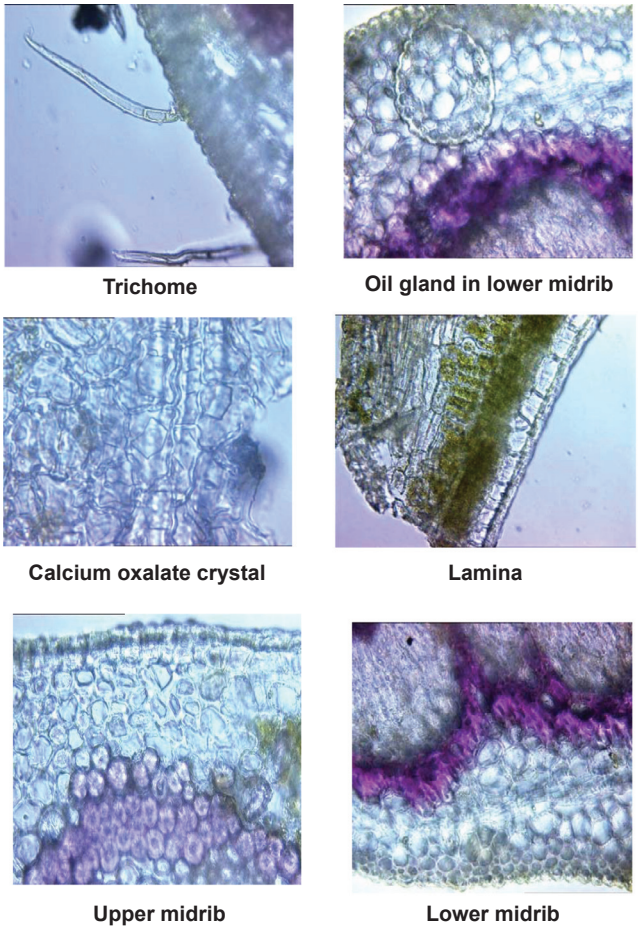
Macroscopic characteristics

P. longifolia is a tall tree up to 25 m tall, with a straight trunk and horizontal branches, dense crown and hairless twigs. The leaves are ovate-oblong to narrowly lance-shaped, 11–22 × 2–5 cm size, wedge-shaped to round at base, wavy at margins and with 4–8 mm long petiole (Fig. 1). As reported earlier,[10] the leaf primordia in *P. longifolia* arise in an alternate pattern, the initiation of the appendage taking place in the second tunica layer. The venation pattern of the leaf is pinnate, the secondary veins traversing obliquely from the midrib and anastomosing with adjacent ones by tertiary veins near the margin.[11] Flowers are in axils of leaves or fallen leaves and usually numerous. The leaves of the *P. longifolia* are bright green in color and polished above, paler beneath.

Microscopic characteristics

Transverse section of leaf is shown in Fig. 2 Leaf is dorsiventral. The upper and lower epidermis are single layered. The palisade was two layered. Prismatic crystals of calcium oxalate are found. The mesophyll is small, circular and spherical celled with plenty of air spaces. The collenchyma is 2–3 layered. Oil glands are found in palisade and in lower midrib (Figs. 2, 3). The lower epidermis had more number of trichomes than the upper epidermis. The anomocytic stomata were present in lower epidermis. The stomata were surrounded by small subsidiary cells, whereas the guard cells were comparatively larger in size and each stoma was surrounded by

FIGURE 3: Microscopic study of *P. longifolia* leaf.



4–5 subsidiary cells and hence the type of stomata is anomocytic. There is a large vascular bundle covered with sclerenchymatous ring.

The crude powder of *P. longifolia* leaf was light green in color with characteristic odour. The powder characteristics are shown in Fig. 4. The specific characteristics determined from the powder study were epidermis, mesophyll, multicellular blunt tip trichome, phloem and spiral xylem vessel.

Physicochemical properties

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of dry powder of leaves of *P. longifolia* was 12% which is not very high, hence it would discourage bacteria, fungi or yeast growth. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different forms viz., total ash, acid-insoluble ash, and water-soluble ash. The total ash is employed 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 adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. [12,13] These ash values are important quantitative standards. Total ash of crude powder of leaves of *P. longifolia* was 5.8%, acid insoluble ash was 0.4% and water soluble ash was 2.5%. Low amount of total ash, acid insoluble ash and water soluble ash indicates that the inorganic matter and non-physiological matter such as silica is less in leaves of *P. longifolia*. The boiling and melting point of methanolic extract of *P. longifolia* was <100°C. The methanolic extract of *P. longifolia* was acidic in nature. Extractive yield of leaves of *P. longifolia* was highest in

FIGURE 4: Microscopic characteristics of powder of *P. longifolia* leaf.

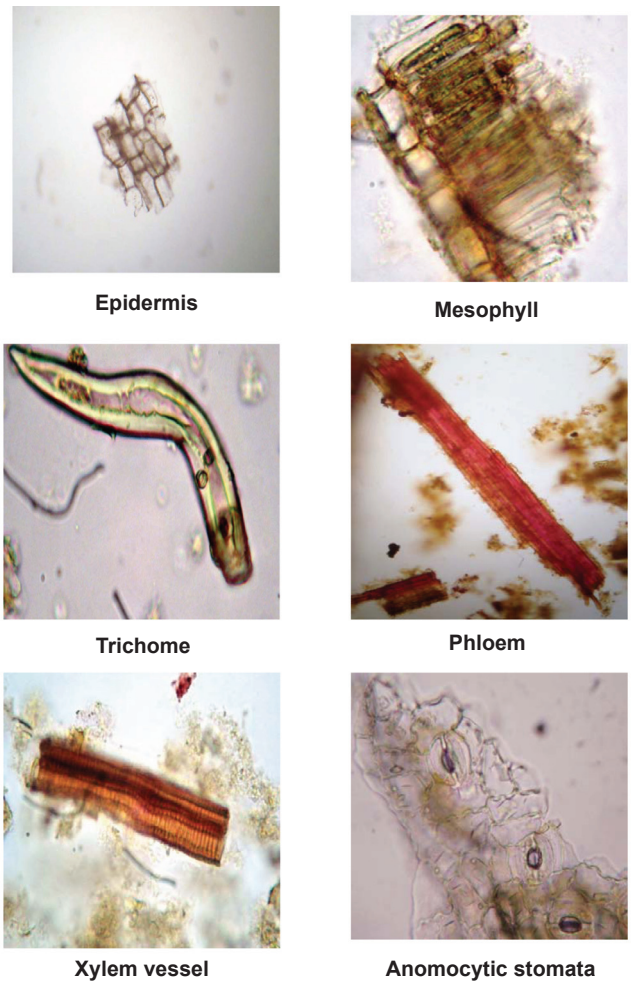


TABLE 1 : Physicochemical characterization of leaves of *P. longifolia*.

PARAMETERS	SAMPLE	VALUE
Loss on drying	crude powder	12 % (w/w)
Total ash	crude powder	5.8 % (w/w)
Acid insoluble ash	crude powder	0.4 % (w/w)
Water soluble ash	crude powder	2.5 % (w/w)
Petroleum ether soluble extractive	crude powder	4.60 % (w/w)
Alcohol soluble extractive	crude powder	20.11% (w/w)
Methanol soluble extractive	crude powder	20.16% (w/w)
Water soluble extractive	crude powder	15.25% (w/w)
pH	methanolic extract	5.51
Melting point	methanolic extract	90ºC
Boiling point	methanolic extract	93ºC

methanol (20.16%), followed by ethanol (20.11%) and water (15.25%). Lowest yield was in petroleum ether (4.60%) (Table 1). The variation in extractable matter in various solvents is suggestive of the fact the formation of the bioactive principle of the medicinal plant is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides, as also reported by Sharma et al.[14]

The solubility of the methanolic extract of *P. longifolia* was more in methanol followed by dimethylsulphoxide and less in acetone. All these parameters are useful for the compilation of a suitable monograph for it proper identification (Table 2).

CONCLUSION

The pharmacognostic studies and physicochemical properties of the *P. longifolia* leaf have been carried out for the first time. This could serve in the identification and preparation of a monograph of the plant. Pharmacognostic and physicochemical analysis of *P. longifolia* leaf is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf to establish as the drug.

TABLE 2 : Solubility of methanolic extract in different solvents.

SOLVENT		SOLUBILITY (MG/ML)
Non polar solvents	Petroleum ether	10
	Hexane	13
	Chloroform	6.2
	Ethyl acetate	9.9
Polar solvents	1,4, Dioxan	9.8
	Acetone	5
	Dimethylsulphoxide (DMSO)	23
	Methanol	25

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