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Pharmacognostical Evaluation of *Zanthoxylum nitidum* Root

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ABSTRACT

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), is a large shrub, and its root is used traditionally in North-East India for toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and pesticide. Present study has been undertaken to determine the necessary pharmacognostical standards for evaluating the crude plant material. Various investigations like organoleptic or morphological characters, microscopy, physico-chemical evaluations, phytochemical screening, TLC finger print profiling and fluorescence analysis were carried out and the salient qualitative and quantitative parameters have been reported. These studies provided referential information for identification and quality control of this crude drug.

KEY WORDS : Quality control, pharmacognostical, *Zanthoxylum nitidum*,

INTRODUCTION

Medicinal plants constitute an effective source of traditional and modern medicines. Standardization of plant products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential (1). Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. 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).

The genus *Zanthoxylum* L. belongs to Rutaceae family and is a large genus of aromatic prickly trees or shrubs distributed pan-tropically and 13 species of it are found in India (3). *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae) called *Tez-mui* in Assamese, is a morphologically variable plant species occurring in south-east Asian countries and in Australia (4). In India it grows as a large prickly shrub particularly in North-East India (Sikkim, Assam and Nagaland states). In India the plant is traditionally used for various medicinal purposes. The root is used in toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and pesticide. The fruit is used in the treatment of stomachache, cough, colic vomiting, diarrhoea, and paresis and as an aromatic, stimulant and pesticide. The small branches, seeds and stem bark are prescribed in fever, diarrhea and cholera (3, 5, 6). It has come to our notice that the rural people of Assam use the stems and its bark as chewing stick in treatment of toothache and gingivitis. No scientific standards or pharmacognostical data are available regarding the identity and quality control of the plant material. The present work therefore, attempts to report various necessary pharmacognostical parameters of *Z. nitidum* root.

MATERIALS AND METHODS

Plant Material

The fully matured entire plants of *Z. nitidum* were collected during the month of December 2006 from Dibrugarh district, Assam, India. The species was identified by Dr. S. J. Phukan, taxonomist, from Botanical Survey of India, Eastern Circle, Shillong, India, and a voucher specimen (No. DUPS-06-003) was deposited in Department of Pharmaceutical Sciences, Dibrugarh University for future reference. The roots were separated from the aerial parts, washed thoroughly with water. Then the roots were shade dried at temperature 21-24°C.

Reagents and Chemicals

All reagents and chemicals used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India. Brucine was obtained from Wilson Laboratories, Mumbai, India.

Organoleptic evaluation -

The freshly (just after collection) collected roots of the plant were spreaded on a clean dry plastic sheet

and investigated different organoleptic features by repeated observations using hand magnifying glass and ruler (where required) and recorded. Similarly the dried stem bark and root were also subjected to organoleptic evaluation.

Microscopic evaluation

The transverse sections (TS) of fresh root were obtained by usual techniques (7). Good sections were collected and placed on a grease free microscopic slide along with a drop of glycerin water (1:1). The sections were covered with clean cover slip and observed under the compound microscope. The TS was documented by photomicrography (8).

Physico-chemical evaluations

Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash, water soluble ash were determined as per the Indian Pharmacopoeia (9). Water and alcohol soluble extractives were estimated by hot extraction and cold maceration according to the method prescribed by WHO (2). All determinations were performed in triplicate and the results are presented as mean ± standard error of mean (SEM).

Phytochemical screening (10, 11)

The dried and powdered root was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents.

The dried and powdered root (150 g) was extracted successively with petroleum ether (40-60^{o}C), chloroform (59.5-60^{o}C), ethyl acetate (76.5-77.5^{o}C), and ethanol (90%) in a soxhlet extractor by continuous hot percolation. Finally the marc was macerated with chloroform water at room temperature. Each time before extracting with the next solvent of higher polarity the powdered drug (marc) was dried in a hot air oven below 50^{o}C for 10 minutes. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The concentrated extracts were evaporated about to dryness and the extracts obtained with each solvent were weighed. Their percentages were calculated in terms of initial air dried plant material. The colours of extracts were observed.

The successive extracts, as mentioned above, were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material.

Thin layer chromatographic studies (12) - Preparation of extracts (test solution): Approximately 2 g of dried coarsely powdered root was taken in a 100 ml glass beaker and moistened with little amount (sufficient to moisten) of 25% liq. NH_3 with occasional stirring for 20 mins. Then the beaker was kept on boiling hot water bath to dry the contents for a few minutes. The beaker was cooled at room temperature and added 10-15 ml of chloroform and extracted on boiling hot water bath for 10 mins. Then the extract (1-2 ml) was collected in clean stoppered glass test tube and used for spotting the chromatographic plates.

Reference solution: A minute quantity of brucine (reference alkaloid) was dissolved in minimum amount (around 1 ml) of chloroform and kept in clean stoppered glass test tube and used as reference.

Stationary phase: Silica gel G, particle size 10-40µ, applied as a thin layer on a clean glass plate support and activated just before use.

Mobile phase: The mobile phase chosen was,

Toluene: Ethyl acetate: Diethylamine = 70: 20: 10.

Development method: One dimensional ascending method by using standard protocol as per Indian Pharmacopoeia was followed (9). The root extract, prepared by above said method along with the reference alkaloid brucine solution, were chromatographed in the same plate.

Visualization: After development no visible spots were found. No spots were observed under short UV light (254 nm). However, two fluorescent spots were observed under long UV light (365 nm). Visualization was attempted by spraying with Dragendorff's reagent.

Documentation: After visualization by spraying with Dragendorff's reagent, dark and light orange brown spots were found. The R_f values of the spots were recorded carefully and the chromatogram was documented by graphical copying (13).

Fluorescence analysis (14, 15)

A small quantity of dried and finely powdered root was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded.

RESULTS

Organoleptic evaluation: The characters found were described below.

Fresh root: Branched taproot, buried up to 3-4 ft vertically.

Condition: Soft.

Shape: Elongated and cylindrical.

Dimensions: Varies, pieces are 2.5-4 cm in length and 2-18 mm in diameter.

Colour: Yellowish buff coloured outside, deep yellow coloured inside.

Odour: Aromatic and agreeable.

Taste: Bitter and aromatic.

The terminal portions of the roots have thin true rootlets.

Dried root:

Condition: Hard.

Shape: Same as fresh roots.

Dimensions: Same as fresh roots

Colour: Yellowish buff coloured outside, yellow coloured inside.

Odour: Same as fresh roots.

Taste: Same as fresh roots.

Fracture: Short.

Microscopic evaluation: The TS of root is shown in Fig. 1. Observations of the root transverse section showed that root is covered externally by a layer of yellowish brown cork cells. Periderm is multi-layered on the surface of the root. Cortex layer consists of parenchymatic cells. Phloem consists of irregular or rectangular cells. Cambium is 2-3 layered and distinguishable. Medullary rays are not distinguishable. Xylem consists of primary and secondary xylem and has trachea and tracheids. The pith containing parenchymatic cells is filled at the root centre.

Phytochemical screening: The results are shown in Table 1. The results demonstrated presence of true alkaloids, carbohydrates, flavonoids and amino acids in the root of *Z. nitidum.* The percent extractives in different solvents and the colours of the extracts are summarized in Table 2.

Physico-chemical evaluations: The values of all determinations were summarized in Table 3 & 4.Water soluble ash was found to be quite greater than acid insoluble ash value. The results showed greater extractive values (almost double) in hot extraction method. In both methods alcohol yielded higher extractives.

Thin layer chromatographic studies: The root extract yielded five orange-brown spots of different intensity, and the reference brucine showed one distinct spot. Two test spots were found to exhibit bright yellow fluorescence under long UV radiation (365 nm). These spots were also detected after visualization. The results are shown in Table 5. The chromatogram is shown in Fig. 2.

Fluorescence analysis: The results are summarized in Table 6.

Table 1. Results of phytochemical screenings of successive extracts of root of Z. nitidum

Constituents	Pet. Ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids	-	-	-	+	-
Purine alkaloids	-	-	-	-	-
Carbohydrates	-	-	-	+	+
Glycosides	-	-	-	-	-
Steroids	-	-	-	-	-
Flavonoids	-	-	-	+	-
Saponins	-	-	-	-	-
Fixed oils and fats	-	-	-	-	-
Tannins	-	-	-	-	-
Proteins and amino acids	-	-	-	-	+
Mucilage	-	-	-	-	-

+ = Present, – = Absent.

Table 2. Percent extractives and colours of successive extracts of Z. nitidum root

Solvent	% Extractives	Colours of extracts
Pet. Ether	1.289	Yellow
Chloroform	1.948	Brownish yellow
Ethyl acetate	4.117	Light yellow
Ethanol	16.057	Yellowish brown
Water	12.350	Dark brown

Table 3. Loss on drying (LOD) and ash values of powdered root of Z. nitidum

Parameters	Values of three replicates (%)	Mean ± SEM
Loss on drying	8.013	
	11.367	9.729 ± 0.9691
	9.808	
Ash values:	9.302	
1)Total ash	7.878	8.043 ± 0.6842
	6.949	
2)Acid insoluble ash	1.78	
	1.64	1.673 ± 0.0543
	1.60	
3)Water soluble ash	6.60	
	6.09	6.336 ± 0.1474
	6.32	

SEM = Standard Error of Mean.

Table 4. Extractive values of stem bark of Z. nitidum

Method of extraction	Values of three replicates (%)	Mean ± SEM
Cold maceration:	5.321	
1) Water soluble	4.691	4.954 ± 0.1889
	4.850	
2) Alcohol soluble	7.702	
	8.110	7.7616 ± 0.1862
	7.473	
Hot Extraction:	10.098	
1) Water soluble	11.334	10.6093 ± 0.3723
	10.396	
2) Alcohol soluble:	11.231	
	12.340	11.677 ± 0.3377
	11.462	

SEM = Standard Error of Mean.

Table 5. Observations of thin layer chromatographic studies of root of Z. nitidum

Extracts	Mobile phase	No. of spots	Rf values	hRf values	Intensity
			0.2222	22.22	+
Root	Toluene: Ethyl		0.4814	48.14	+
	acetate:	5	0.7333	73.33	++
	Diethylamine = 70:		0.8296	82.96	+++
	20: 10		0.9777	97.77	++
Brucine (Reference)	Do	1	0.3087	30.87	++

Visualizing reagent: Dragendorff's reagent. ; +++ = Most intense, ++ = Moderately intense, + = Least intense.

Table 6. Fluorescence analysis of powdered root of Z. nitidum

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder as such	Yellow	Light brown	Dark brown
Powder + 1M NaOH	Brownish yellow	Light brown	Dark brown
Powder + 1%Picric acid	Deep brownish yellow	Brownish yellow	Black
Powder + Acetic acid	Yellow	Brown	Dark brown
Powder + 1M HCl	Yellow	Brownish yellow	Brown
Powder + Dil. HNO_3	Yellow	Light brown	Dark brown
Powder + 5% Iodine	Brown	Dark brown	Black
Powder + 5% $FeCl_3$	Brownish yellow	Brown	Black

Contd..

Powder +HNO_3 + 25% NH_3	Light brown	Brown	Deep blackish brown
Powder + Methanol	Brownish yellow	Brown	Blackish brown
Powder +50 % HNO_3	Yellow	Brown	Dark brown
Powder + 1M H_2SO_4	Yellow	Light brown	Dark brown
Powder + Dil. NH_3	Brownish yellow	Light brown	Brown
Powder + Conc. HNO_3	Reddish brown	Dark brown	Black
Powder + 10% Pot. Dichromate soln	Deep yellow	Brownish yellow	Black
Powder + 25% Liq. NH_3	Fade light brown	Brown	Dark brown

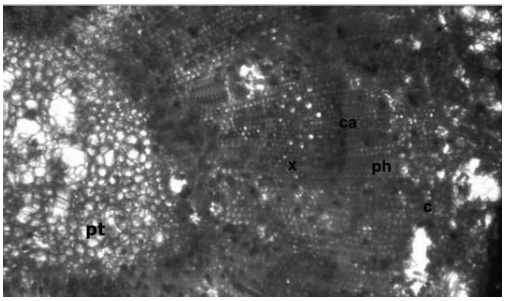

Figure 1. *The transverse section of the root, pt: pith, x: xylem, ca: cambium, ph: phloem, c: cortex, p: periderm.*

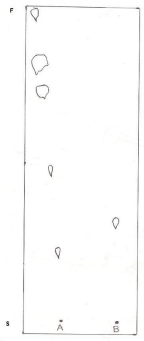

Figure 2. The chromatogram
A - Root extract of Z. nitidum, B- Brucine. S - Sample application point, F – Solvent front

DISCUSSION

The pharmacognostical 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. Organoleptic evaluation means conclusions drawn from studies resulted due to impressions on organs of senses i.e. it refers to evaluation of crude drugs by colour, odour, taste, size, shape and special features, like touch, texture, fracture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs (16). The organoleptic or macroscopic studies yielded important characteristics, such as the bitter and aromatic taste and aromatic and agreeable odour of the root; which are useful diagnostic characters. Similarly the microscopic features, e.g. presence of secondary thickenings, distinguishable cambium, indistinguishable rays etc. may be useful for this purpose.

The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. An excess of water in plant materials will encourage microbial growth, the presence of fungi or insects and deterioration following hydrolysis of constituents, thereby may cause loss of biological activity. Water content of plant materials should be minimized to prevent decomposition either due to chemical change or microbial spoilage. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water. The test for loss on drying determines both water and volatile matter (2, 16). 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. For determining ash, the powdered drug is incinerated so as to burn out all organic matter. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash.

The total ash method 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 (e.g. sand, soil etc) adhering to the plant surface. Total ash usually consists of carbonates, oxides, phosphates, silicates and silica.

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. It is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. This value is found higher for underground plant parts like root and rhizome. Water-soluble ash is the difference in weight between the total ash and the ignited residue after treatment (boiling) of the total ash with water. It is the water-soluble portion of the total ash (8, 16).

The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material. The values depend on the chemical nature, quantity, properties of constituents (solubility, polarity etc), the solvent employed, the type of plant part (leaf, bark, root etc) and the method of extraction employed. This parameter is employed for materials for which as yet no suitable chemical or biological assay exists (2, 9).

The results showed greater extractive values (almost double) in hot extraction, indicating the effect of elevated temperature on extraction. In all methods alcohol yielded higher extractives, as alcohol has unique feature of dissolving all polar and nearly all low polar constituents (17).

The chemical substances of plants that are responsible for biological activity are usually secondary metabolites. These are a multitude of compounds like alkaloids, glycosides, terpenoids, tannins etc. The plant material was subjected to preliminary phytochemical screening involving successive solvent extraction by different solvents in order of increasing polarity i.e. less polar (pet. ether) to more polar solvents (water) to obtain diverse polar and non polar phytoconstituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents (10,16). And it was found that true alkaloids and flavonoids are present in ethanol extract and carbohydrates and amino acids are in aqueous extract. Alkaloid content was found to be appreciable as compared with other constituents.

The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The colour of the extract sometimes can roughly indicate the physical and chemical features of constituents present.

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. As per phytochemical screening the stem bark of *Z. nitidum* contains mainly true alkaloids. Therefore, the powdered root was first basified with liquid ammonia to liberate the alkaloids as free bases, following extraction with chloroform, the solvent in which most of the free alkaloids are soluble. The solvent system employed was, Toluene: Ethyl acetate: Diethylamine = 70:20:10; as this was found to be the solvent system suitable for most of the alkaloids for preliminary study (12, 17). The standard alkaloid used along with was brucine with known R_f value of 0.253 with same solvent system to assess the accuracy of the study (12). The visualizing reagent employed was Dragendorff's reagent to effect visualization of all the resolved spots that were invisible visibly. The root extract yielded five orange-brown spots with different intensities, and brucine showed one distinct spot of R_f value 0.3087 which however, did not accurately comply with that of literature (mentioned above). In fact, it is very difficult to reproduce the experimental conditions of TLC and hence, the obtained R_f value differed to some extent from that of literature.

The test extract exhibited two bright yellow fluorescent spots (hRf values 73.33 and 97.77) under long UV radiation (365 nm). These spots were also observed after visualization with Dragendorff's reagent. Different alkaloids are known to exhibit fluorescence under UV light e.g. quinine, berberine etc (12). Hence, the root may contain certain fluorescent alkaloids. Fluorescence is very sensitive means of identifying small amounts of substances on chromatograms (13). This is quite distinctive feature in the TLC profile. The chromatographic profile may serve as a characteristic finger print for qualitative evaluation of root.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some plant materials show fluorescence in the visible range in daylight. The ultra violet light emitted from appropriate lamp produces fluorescence in many natural products (e.g. some alkaloids), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they can often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are evaluated qualitatively in this way and it is an important parameter of pharmacognostical evaluation (15, 18). Here the finely powdered root was subjected to various chemical reagents to let the phytochemical constituents react with those reagents, which can cause formation of compounds that would fluoresce in the visible day light or in the UV radiations employed in two wave lengths; short: 254 nm and long: 365 nm.

After present investigation it can be concluded that the pharmacognostical study of *Z. nitidum* root have furnished a set of qualitative and quantitative parameters that can serve as an important source of informations to ascertain the identity and to determine the quality and purity of the plant material in future studies.

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Evaluation of Pharmacognostic, Phytochemical and Antimicrobial Activity of *Euphorbia rothiana*

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ABSTRACT:
Antimicrobial activity of various parts of Euphorbia rothiana was studied using different solvent system against various gram positive, gram negative bacteria. Pharmacognostical evaluation including examinations of morphological and microscopical characterization was carried out. The phytochemical analysis reveals the presence of phenolic compound in various parts of the plant using alcoholic extract and flavonoid using aqueous extract. The antimicrobial activity of the plant may be due to the presence of phenolic and flavonoid constituents. Antimicrobial activity was compared with the standard antibiotic ampicillin.
KEYWORDS: *Alcoholic extract, Ampicillin, Antimicrobial, Flavonoid, Phenolic compound.*

INTRODUCTION

Euphorbia routhiana (family Euphorbiaceae) is an annual erect, glabrous, profusely branched sub shrub of one-meter height and distributed in India (Maharastra and Tamilnadu). In Indian system of medicine the plant is used as an antifungal (1), hypotensive agent and anthelmetic. Acne vulgaris is a disease which affects more than 90% of young people, which leads to permanent marking on the skin, disfiguring of the face. Propioni bacterium acnes are the most common gram-positive bacteria, acne is caused in parts from as infection, and it can be suppressed with topical and oral antibiotics. *P. acne* is highly susceptible to various beta lactum antibiotics and very resisting to penicillin G. Literature is available with respect to its phytochemical investigation and antifungal activity. This plant was known for its anti acne property; however the detail of antimicrobial evaluation has not been reported. This encouraged us to study the antibacterial activity of *euphorbia rothiana*.

MATERIALS AND METHODS

Materials

The plant *euphorbia rothiana* was collected from Velliangiri the foot hills of the Niligris in Tamilnadu and identified by Joint director, botanical survey of India, Coimbatore. The voucher specimen of the plant was deposited in the herbarium, KMCH College of Pharmacy, Coimbatore. The leaves, stems and roots of *euphorbia rothiana* were dried under sunlight and reduced to coarse powder.

The microorganisms used for antimicrobial study were *Kllebsiella aerogenosa (NCIM-2239), Escherichia coli (NCIM-2065), Proteous vulgaris (NCIM-2027), Pseudomonas aerogenosa (NCIM-2200), Bacillus subtilis (NCIM-2063), Staphylococcus albus (NCIM-2178), Staphylococcus epidermis (NCIM-2493) and Staphylococcus aureus (NCIM-2079).*

Extraction Process

Leaves, stems and roots of *euphorbia rothiana* (30gm) were sun dried and powdered. The powder was extracted separately by simple maceration process using macerator with occasional agitation. Powdered (leaves, stems and roots of *euphorbia rothiana*) were soaked with 100ml of solvent and allow to stand for 6 hr and during this period the drug swells then it is shifted to macerator and added 200ml of solvent and kept for 48hr. The active soluble principle was and dried under vacuum to get constant weight and stored in a desiccator until it is used (2).

Preliminary Phytochemical Studies

Preliminary Phytochemical analysis was done for alkaloids, glycosides, tannins, steroids, oil and wax, resins, carbohydrates, proteins and finally for flavonoid (3-4).

TLC Analysis

Phytochemical studies were confirmed by TLC analysis for the presence of flavonoids. Silica gel 60G absorbent was used for plate preparation. The spots were made with capillary tube, butanol-acetic acid-water (4:1:5) were used as solvent for alcoholic extract, where as Conc. HCl - acetic acid-water (3:30:10) were used as solvent for aqueous extracts. The spots were identified by ammonium vapour. Finally the R_f values were calculated (5-6).

Antimicrobial Study

Antimicrobial activity was studied by cup-plate method prescribed in Indian pharmacopoeia-1996. Nutrient agar medium in Petri plate was used as inoculum medium. After inoculation of gram positive and gram negative organism the plates were kept at room temperature. The holes of 5-8mm in diameter were bored in the medium by sterile borer maximum of 6 holes were made in the dish. Antimicrobial activity was studied and ampicillin (50microgram/ml) was used as standard. All extracts were used in the concentration of 100mg/ml. 0.1 ml of sample was loaded in to the well with the help of micropipette. DMSO was used to dissolve the extracts and standard. The applications of sample were done by micropipette and then incubated at 37^0C for 24 hr. The average zones of incubation were calculated (7). The antimicrobial activity of *Euphorbia rutiana* on *Escherichia coli* culture was shown in Figure 1.

RESULTS AND DISCUSSION

Euphorbia rutiana is an indigenous shrub. A tribal community in Coimbatore district traditionally used leaves and stems as antiacne medicine. Natural products can be developed as source of promising potent antiacne agent in order to replace the chemical and synthetic antibiotic treatment with many side effects. The present work focused on whole plant extraction, identification of chemical constituents by qualitative chemical analysis, TLC analysis and screening of antibacterial activity. The collected whole plant was identified and authenticated by botanical survey of India, Coimbatore. The morphological and microscopical character of plant was done. *Euphorbia rutiana* whole plant was extracted by maceration process by macerator with occasional agitation. Successive solvent extraction values in various organic solvents were observed. (Pet. Ether, chloroform, acetone, ethyl alcohol and water) and shown in *Table 1.*

The preliminary phytochemical studies were carried out for different constituent like alkaloids, glycosides, etc. The chemical test revealed the presence of phenolic compound in Leaf Alcoholic extract (LAL), Leaf Aqueous extract (LAQ), Stem Alcohol extract (SAL), Stem Aqueous extract (SAQ), Root Alcoholic extract (RAL), Root Aqueous extract (RAQ) and alkaloid in Leaf Chloroform extract (LCF) and Leaf Alcoholic extract (LAL).

Glycosides in Leaf Acetone extract (LAC), LAQ, SAQ, and RAQ extract. Tannins in LAL, SAL, and RAL extract. Steroids in Leaf Petroleum Ether extract (LPE), Stem Petroleum Ether extract (SPE), Root Petroleum Ether extract (RPE). Resins in LAQ, SAQ, and RAQ extract. Carbohydrates in Leaf Aqueous extract, Steam Aqueous extract and Root Aqueous extract. Flavanoids in LAQ, SAQ, and RAQ extract.

Table 1.*Extractive value of Euphorbia routhiana*

Solvent used	Average extractive value in %w/w on dry weight basis		
	Leaves	Stems	Root
Pet. Ether	2.33	0.4	0.6
Chloroform	3.28	0.1	0.85
Acetone	2.26	1.35	2.03
Ethyl alcohol	7.2	2.62	2.12
Water	5.65	8.72	5

Table 2. *TLC analysis of various alcoholic extracts*

Sample Extract	Distance Run By Solvent (cm)	Distance Run By Solute (cm)	Rf value
LAL	5.4	4.8	0.89
SAL	5.3	4.7	0.89
RAL	6.4	5.4	0.84

Table 3. *TLC analysis of various aqueous extracts*

Sample Extract	Distance Run By Solvent (cm)	Distance Run By Solute (cm)	Rf value
LAQ	5.4	5.2	0.96
SAQ	5.7	5.4	0.95
RAQ	5.1	4.1	0.8

Table 4. *Antimicrobial activity of various alcoholic and aqueous extracts*

Organism	Zone of Inhibition in mm (Diameter)						
	LAL	LAQ	SAL	SAQ	RAL	RAQ	Ampicillin(Std)
Klebsiella aerogenosa	20	16	17	18	21	16	22
Escherichia coli	21	15	20	19	19	15	24
Proteous vulgaris	23	16	15	19	18	15	22
Pseudomonas aerogenosa	20	18	18	18	15	14	26
Bacillus subtilis	22	21	17	18	14	17	24
Staphyococcus albus	23	19	18	17	17	17	26
Staphyococcus epodermis	20	21	17	19	16	18	24
Staphyococcus aureus	17	17	12	12	13	12	30

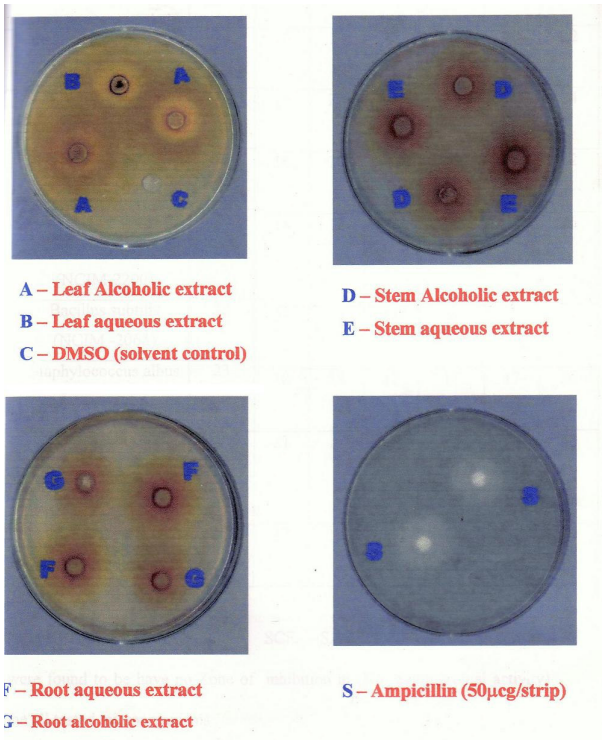

Figure 1 Antimicrobial activity of Euphorbia rutiana on Escherichia coli culture

Flavonoid is responsible for antimicrobial activity and was confirmed by TLC analysis. By the presence of three flavonoid in the Leaf Aqueous extract, two flavonoid in the Steam Aqueous extract and two flavonoid in Root Aqueous extract, three phenolic in Leaf Aqueous extract, two phenolic in Steam Alcohol extract and two phenolic in Root Alcohol extract. The results are tabulated in *Table 2 and Table 3*.

All the extracts of *Euphorbia rothiana* were subjected to microbial screening. The results indicates significant antibacterial activity observed by definite zone of inhibition produced by different zone of inhibition produced by LAL, LAQ, SAL, SAQ, RAL and RAQ extracts in the Petri dishes were inoculated with the cultures of different micro organisms, and these extracts were showed significant antimicrobial activity (*Table 4*). This confirmed the antimicrobial activity of the plant *Euphorbia routhiana* against gram-negative and gram-positive micro organism. Ampicillin was used as standard in the concentration of 50µg/ml and DMSO was used as solvent. The micro organism namely *Proteous vulgaris, Staphylococcus epidermis* causes inflammatic disease of sebaceous glands. The antimicrobial screening shows a remarkable inhibition of growth of these micro organisms (*Table 4*). The Phytochemical and TLC analysis reveals the presence of phenolic compounds in LAL, SAL, RAL extracts and flavanoids in LAQ, SAQ,RAQ extracts. So the antimicrobial activities of the plant *Euphorbia rothiana* may due to the presence of phenolic and flavanoidal constituents.

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PHCOG J: Research Article

Pharmacognostical studies on the bark of *Cassia fistula* Linn.

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ABSTRACT

The present communication deals with the macroscopical, microscopical & preliminary phytochemical studies on the bark of Cassia fistula Linn. Cassia fistula Linn is widely used in traditional medicine in many parts of India for the treatment of various diseases & ailments viz. hepatoprotective, antioxidant, antidiabetic etc. No reports are available on the pharmacognostic nature of the bark, hence, the present study was undertaken to investigate the same. All the parameters were studied according to the WHO & Pharmacopoeial guidelines. The qualitative chemical tests of the ethanolic extract revealed the presence of tannins, flavonoids, steroids, glycosides & carbohydrates.

KEYWORDS : *Cassia fistula; Pharmacognostic; Traditional medicine.*

INTRODUCTION

Cassia fistula Linn. also known as golden shower, Indian laburnum, belongs to the family Leguminoceae. *Cassia fistula* Linn is widely cultivated throughout India as an ornamental plant & deciduous tree. In traditional medicine, it is used in the treatement of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, & as antipyretic, analgesic & laxative (1-2). Herbal medicine is a triumph of popular therapeutic diversity. Almost in all the traditional medicine, the medicinal plants play the major role & constitute the backbone for the same. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety & efficacy (3). Keeping these facts into consideration, attempts are made to establish pharmacognostic standards of the plant bark.

Cassia fistula is a moderate sized deciduous tree, distributed throughout India. It is 8-15m to 24m in height, with greenish grey smooth bark when young & rough, dark brown when mature. Leaflets 8–12 pair, flowers yellow, long drooping racemes. Pod cylindrical & pulpy. Seeds light brown, hard & shiny (4-6). The present study investigates the macroscopical, microscopical & preliminary phytochemical nature of the bark of *Cassia fistula* Linn.

MATERIALS AND METHODS

The bark of the plant *Cassia fistula* Linn. was collected from the local area of Hubli (India). It was authenticated by Dr B.D.Huddar. Head, Department of Botany, H.S.K. Science Institute, Hubli (India). The bark was stored under the normal environmental condition. The macroscopical characters of the bark were studied as per the procedure given in WHO guidelines & Indian herbal Pharmacopoeia. Physico-chemical parameters such as extractive values, ash values, loss on drying were performed as per the official standard procedures (7-8). Microscopical investigations were made with transverse section & powder microscopy of the bark. Microtome section of the bark was taken for microscopic examination & the powder microscopy was performed according to the prescribed procedure (9-10).

For phytochemical screening, the bark was shade dried & powdered, 200 gm powdered bark was subjected for Soxhlet extraction with 90% alcohol. After complete extraction the extract was evaporated under reduced pressure & the percentage yield was determined. The total alcoholic extract was subjected for phytochemical screening (11). The chromatographic studies were performed using various solvent systems & finally benzene: ethyl acetate (1:9) was found to be suitable mobile phase for the proper separation of phytoconstituents. Anisaldehyde-sulphuric acid was used as spraying agent.

RESULT AND DISCUSSION

Macroscopically the bark was found to be dark brown to brown in color, odourless, single quilled with rough surface (Figure 1, Table1). The T.S. of the bark showed the presence of cork, cortex, medullary rays, phloem fiber, pericyclic fiber & sclerides (Figure 2). Powder microscopy shows the presence of thin & thick walled cork cells, calcium oxalate crystals, stone cells, lignified fibers & phloem fibers (Figure 3). The values of the physical constant like ash values, extractive values, loss on drying were determined (Table 2). The physical characteristics & percentage yield of the total alcoholic extract are given in (Table 3). Preliminary qualitative

phytochemical screening of the alcoholic extract revealed the presence tannins, flavonoids, glycosides,steroids & triterpinoids, carbohydrates and phenolic compounds.. Chromatographic studies of total alcoholic extract showed three distinct spots after spraying with detecting reagent anisaldehyde sulphuric acid with Rf values 0.531, 0.730 & 0.835.

CONCLUSION

Standardization of herbal drugs is very essential, as they are derived from heterogenous sources which can lead to variations. These variations can lead to erroneous results in various pharmacological and phytochemical studies. Since *Cassia fistula* bark is known for its various medicinal properties hence the present study may be useful to supplement information in respect to its identification, authentication & standardization, since no such data is available for the same.

Table 1 : Macroscopical Evaluation of Cassia fistula L bark.

Sr. No	Features	Observations
1	Colour	Inner surface - Pale brown Outer surface - Brown
2	Odour	Odourless
3	Taste	Bitter
4	Shape	Single curved, quilled
5	Fracture	Fibrous
6	Size	Length : 10 – 15 cm Bridth : 3 – 6 cm Thickness : 5 – 7 mm

Table 2: Extractive values, B. Loss on drying and C. Ash values of Cassia fistula L bark.

Sr. No.	Parameters	Determined values % w/w
A	Extractive values	
1	Alcohol soluble	23.10 ± 0.208
2	Water soluble	17.10 ± 0.321
B	Loss on drying	11.22 ± 0.151
C	Ash values	
1	Total ash	8.78 ± 0.197
2	Acid insoluble	1.98 ± 0.073
3	Water soluble	4.32 ± 0.060
4	Sulfated ash	12.98 ± 0.104

** Values are expressed as Mean ± SEM*

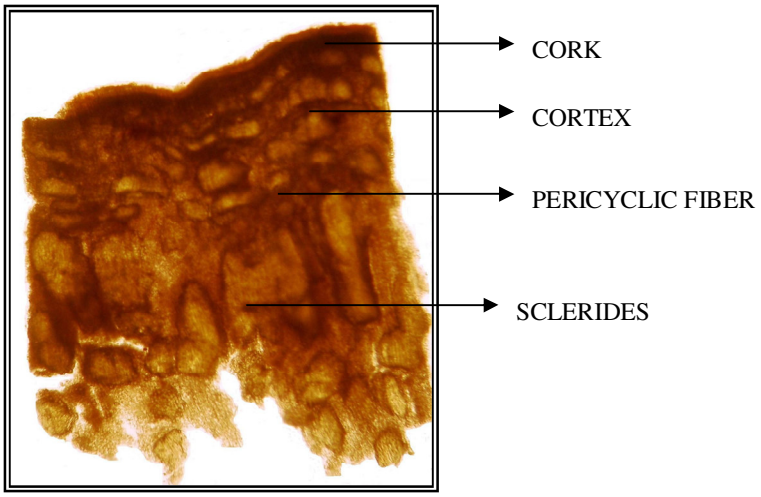
Table 3: Percentage yield and physical characteristics of alcoholic extracts of Cassia fistula L bark

% Yield in w/w	Colour	Odour	Nature
27.39	Dark brown	Characteristic	Powder

Figure 1 : Photograph of fresh bark.

Figure 2 : T.S. Cassia fistula bark(A,B)

A



B

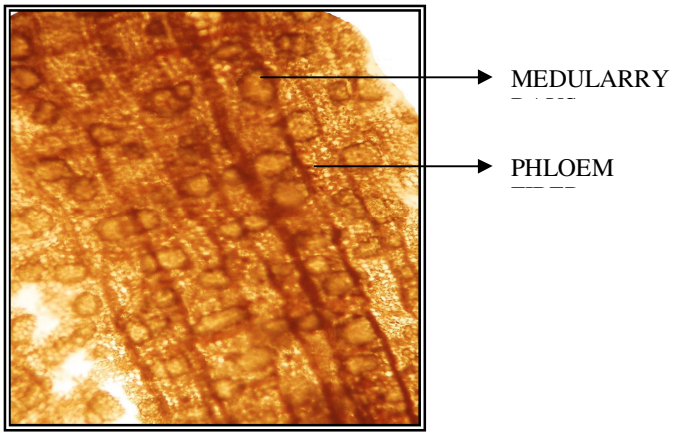
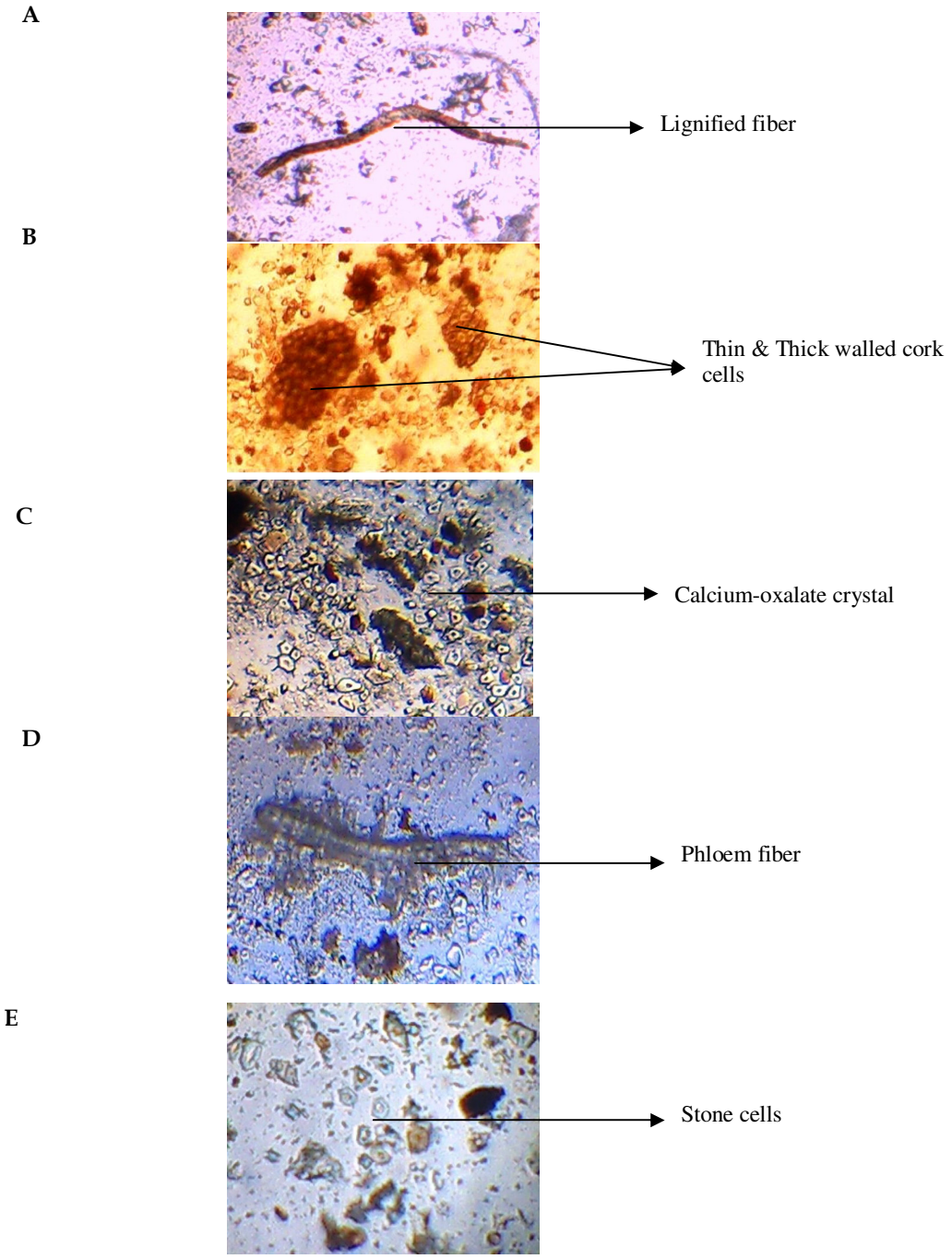


Figure 3: Photographs of powder characteristics of Cassia fistula L bark."(A-E)



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PHCOG J: Research Article

Pharmacognostic Evaluation and Phytochemical Studies on Leaves of *Albizia lebbeck* Benth.

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ABSTRACT
The leaves of Albizia lebbeck Benth. (Fabaceae) are reported to have great medicinal value. The present study deals with the macroscopical, microscopical, physiochemical and preliminary phytochemical investigation including qualitative chemical examination of Albizia lebbeck Benth. Some of the diagnostic features of the leaves are the presence of covering trichomes, fibers, epidermis, and collenchyma cells.
***KEYWORDS**: Albizia lebbeck, pharmacognostic, phytoconstituents*

INTRODUCTION

Albizia lebbeck Benth. (Fabaceae) known as Siris in Hindi has been advocated for various uses in Indian system of medicine. In ayurvedic literature the plant exhibited in treatment of allergic disorders, asthma, eczema and utricaria (1). Phytochemically, the leaves were found to contain cardiac glycosides, flavanoids (2), saponins, tannins (3). Barks of *Albizia lebbeck* Benth. have been established for anti-inflammatory activity (4). It has been reported to possess anti-allergic (5), antioxidant (6), antidiarrhoel (7), anticonvulsant (8) activities. In spite of its use in herbal formulation no systematic pharmacognostic studies have been reported and hence our efforts were devoted in this direction.

MATERIALS AND METHODS

The leaves of *Albizia lebbeck* Benth. were collected locally from Bhopal in August 2008 and authenticated by herbal specimen at NRI Institute of Pharmaceutical Sciences, Bhopal (voucher no. NIPS/08/001). The leaves were first dried in shade and than dried in an oven at 40-50°C for 1 hrs. The dried plant material was then subjected to size reduction to obtain coarse powder using grinding mill.

Pharmacognostical studies

Macroscopy

The plant was macroscopically examined for shape of leaves, apex, base, margin etc (9).

Microscopy

Microscopic studies were done by preparing a thin hand section of midrib and lamina region of *Albizia lebbeck* Benth. leaf. The section was stained by saffranin. Powder (# 60) of the dried leaf was used for the observation of powder microscopic characters (10).

Physicochemical parameters

Physicochemical parameters were determined as per Ayurvedic Pharmacopoeia of India. Moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value were determined (11).

Preliminary phytochemical studies

The powder of dried leaves was subjected to continuous soxhlet extraction with various organic solvent such as petroleum ether, benzene, chloroform, acetone and methanol respectively. After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out using chemical test and thin layer chromatography method by different detecting reagents (12).

RESULT AND DISCUSSION

The morphological studies revealed that it is compound opposite bipinnate leaf with oblong shape and mucoranate apex. Margin was entire and venation was reticulate. The transverse section of leaf of *Albizia lebbeck* Benth. revealed the presence of lower and upper epidermis, xylem and phloem, vascular bundle, mesophyll, covering trichome and collenchyma. The powder microscopy of the leaves revealed the presence of fibers, xylem, phloem, epidermal cell, stomata and covering trichome. The proximate analysis result shown that the moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value were 7.28 ± 0.36%, 6.33 ± 0.28%, 1.23

± 0.07%, 3.46 ± 0.21%, and 9.13 ± 0.38% respectively (Table 1). Successive solvent extractions were shown in percentage of yield along with physical appearance. The percentage yield values for petroleum ether, benzene, chloroform, acetone and methanol were 7.56%, 4.32%, 4.87%, 5.78%, and 3.66% respectively (Table 2). All extract were than subjected to study chemical nature of the drug (Table 3).

Table 1 : Evaluation of leaves of Albizia lebbeck Benth.

Parameters	Value obtained on dry weight basis (% w/w)*
Moisture content	7.28 ± 0.36
Total ash value	6.33 ± 0.28
Acid insoluble ash	1.23 ± 0.07
Alcohol soluble extract	3.46 ± 0.21
Water soluble extract	9.13 ± 0.38

* Average of three reading ± SEM

Table 2 : Successive solvent extraction of leaves of Albizia lebbeck Benth.

Solvent used	Color & consistency	Average extractive values on dry weight basis (% w/w)
Petroleum ether	Blackish green oily mass	7.56
Benzene	Green sticky mass	4.32
Chloroform	Dark green residue	4.87
Acetone	Brownish sticky mass	5.78
Methanol	Brown mass	3.66

Table 3 : Chemical examination of various extracts of leaves of Albizia lebbeck Benth.

Constituents	Extract				
	P	B	C	A	M
Alkaloids	-	-	-	+	+
Carbohydrates	-	-	+	+	+
Proteins & amino acids	+	+	-	+	-
Saponin	-	-	+	+	+
Fixed oil/ Fat	+	-	+	-	-
Gums/ mucilage	-	-	-	-	-
Flavonoids	-	-	-	+	+
Phenolic	-	-	+	-	+

P= Petroleum ether extract A= Acetone extract B= Benzene extract C= Chloroform extract M= Methanol extract

Table 4 : TLC screening of various crude drug extract of leaves of *Albizia lebbeck* Benth.

Solvent system used	Detection reagent	Observation	Inference	P	B	C	A	M
Ethyl acetate:Methanol: Water (75.5:13.5:10)	KOH	Red (Vis) Yellow	Anthraquinone Anthrone	-	-	-	-	-
	Vanillin sulphuric acid	Red/Yellow/Brown/ Blue-green	Bitter principle	-	-	-	-	-
	Dragendroffs reagent	Orange Red (Vis)	Alkaloid	-	-	-	+	+
	NP/PEG/ and UV	Yellow/Green/ Orange	Flavonoid	-	-	-	+	+
	VS reagent	Blue	Saponin	-	-	+	+	+
Tolune: Ethyl acetate (93:7)	VS reagent	Red/Yellow/Brown/ Blue-green	Essential oil	+	-	-	-	-
	HCl/acetic acid	Blue brown	Valepotriate	-	-	-	-	
	NH_3/KOH	Light blue brown	Coumarin	-	-	+	-	+

P= Petroleum ether extract A= Acetone extract B= Benzene extract C= Chloroform extract M= Methanol extract

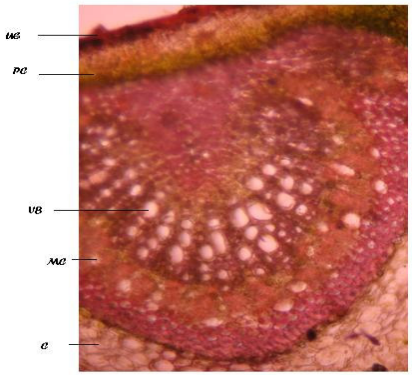
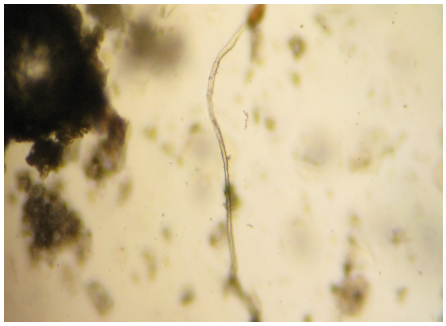
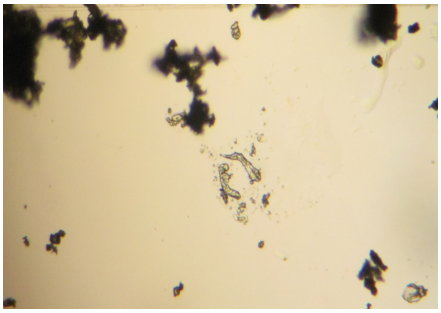


Figure 1 : T.S. of *Albizia lebbeck* Benth. leaf
UE:- Upper Epidermis, PC:-Palisade Cell, VB:-Vascular Bundles, MC:- Mesophyll Cell, C:- Collenchyma



(A)



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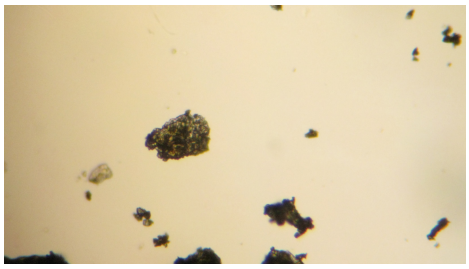

(C)

Figure 2 : Microscopy of powdered leaves of Albizia lebbeck Benth. (A) Fibers (B) Covering Trichomes (C) Epidermal cell with stomata

The preliminary phytochemical studies revealed that petroleum ether fraction contain fixed oil and amino acid, benzene fraction contain amino acid, chloroform fraction contain carbohydrate, saponin, flavanoids and phenolic, acetone fraction contain alkaloids, carbohydrate, amino acids saponins and flavanoids, while methanolic fraction contain alkaloids, carbohydrate, saponin, phenolic and flavanoids. The extract which showed presence of phytoconstituents of interest further subjected to TLC and result is shown in Table 4.

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PHCOG J: Research Article

Pharmacognostic and Phytochemical Investigation of stem bark of *Zanthoxylum rhetsa.*

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ABSTRACT
Bark of Zanthoxylum rhetsa commonly known as "Mullilam" belongs to the Rutaceae family and is widely distributed in India. Zanthoxylum rhetsa bark is a medicinal plant, traditionally used in diabetes, antispasmodic, diuretic, anti-inflammatory and in various ailments. No reports are available on the pharmacognostic work of the bark, hence, the present study was undertaken to investigate the same. All the parameters were studied according to the WHO & Pharmacopoeal guidelines. The qualitative chemical tests of the Pet ether, Chloroform, Methanol, Aqueous extracts revealed the presence of Alkaloids, tannins, flavonoids, steroids, glycosides & carbohydrates.
KEY WORDS: *Zanthoxylum rhetsa, Pharmacognostic standardization, physicochemical Analysis.*

INTRODUCTION

Herbal medicines are a wonderful and precious gift of the nature and have been playing a significant role in the prevention and treatment of various health ailments since the time immemorial, the major population of the south-eastern Asian countries relies heavily on the efficacy of herbal remedies. Some of the plant drugs promote positive health and maintain organic resistance against infections by re-establishing body equilibrium and conditioning the body tissues. Every herb is a complex mixture of biologically active components, some of them are synergistic, some antagonistic, some toxic and some inactive. Drugs used in the traditional system of medicine are in crude in their natural form which is safer to the human body. These have been a global interest in scientifically validating the therapeutic efficacy of the medicinal plants. Therefore, active interests in the pharmacognostic and phytochemistry of the plant drugs have been gaining momentum. scientists of modern era often ask for scientific validation of herbal remedies. So that herbals are standardized for active constituent. Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals.

Medicinal plant materials are characterized according sensory microscopic and macroscopic characteristics.Taking into consideration the variation in sources of crude drugs and their chemical nature, they are standardized by using different techniques of chief active constituent. Organoleptic evaluations can be done by means of organs of sense. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample. A number of different bases are used for morphological studies and natural variations in these characteristics plays an important role for preliminary evaluation of crude drugs.

A number of different bases are used for morphological studies and natural variations in these characteristics plays an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as for as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs. This study is an attempt to establish the standardization parameters for complete pharmacognostic evaluation of *Zanthoxylum rhetsa* bark (1).

Zanthoxylum rhetsa (Roxb) (Syn. *Zanthoxylum budrunga*, Fam. Rutaceae), locally known as ''*Mullilam*'' is a small or moderate sized tree with pale corky bark which is widely distributed throughout, India, Bangladesh Malaysia and other parts of South Asia (2-4).

MATERIALS AND METHODS:

Collection and authentication:

The bark of *Zanthoxylum rhetsa.* (Roxb) were collected from the local areas of Kumta, Karnataka, and authenticated by Dr. B.D. Huddar, Head, Department of Botany, Shri Kadasiddheshwar Arts College and H.S. Kotambari Science Institute, Vidyanagar, Hubli. A voucher specimen (07PG358, Vasudev Pai) has been deposited in the PG Pharmacognosy laboratory of the college for future reference.

The bark was stored under the normal environmental condition. The macroscopical characters of the bark were studied as per the procedure given in WHO guidelines and Indian Herbal Pharmacopoeia. Physico-chemical parameters such as extractive values, ash values, loss on drying were performed as per the official standard procedures.[5, 6] Microscopical investigations were made by microtome section and powder microscopy was performed according to the prescribed procedure.[7, 8]

Extraction:

The *Zanthoxylum rhetsa*. bark was shade dried at room temperature, pulverized, and 100g of coarse powder was successively extracted with petroleum ether (40-60), chloroform and methanol in a Soxhlet extractor. The extracts were concentrated in a rotary flash evaporator

and residues were dried in a desiccator over sodium sulfite.

Preliminary Phytochemical and Physicochemical analysis:

The extracts were subjected to preliminary phytochemical and physicochemical testing for the detection of major chemical group.

Isolation: [9]

The methanol extract was shown four spots. So an attempt were made to separate these spots by column chromatography using isocratic elution technique. The compound I and II was eluted with Ethyl acetate: Formic acid :Glacial acetic acid : Water (80:8:8:20). These compounds were further characterized by TLC, UV and IR analysis.

RESULTS AND DISCUSSION

The detailed and systematic pharmacognostical evaluation would give valuable information for the future studies. Macroscopically the bark was found to be Inner surface - Pale Brown. Outer surface –Woody, Ash coloured, odourless, bitter,shape is slightly curved, fracture is fibrous and thorny.(Figure 1,Table 1). The T.S. of the bark showed the presence of cork, cortex, medullary rays, phloem fiber, collenchyma, fibrous sclereids. (Figure 2). Powder microscopy shows the presence of thin and thick walled cork cells, cortex cells containing starch grains, calcium oxalate crystals, stone cells, fibrous sclerides, medullary rays crossing the crystal fiber and phloem fibers (Figure 3). The values of the physical constant like ash values, extractive values, loss on drying were determined (Table 2). Preliminary qualitative phytochemical screening of the extracts revealed the presence of various phytoconstituents (Table3). Compound I was shown positive test for flavonoids whereas compound II was shown positive test for alkaloids. The TLC, UV and IR data of the isolated compounds are as follows:

Compound-I:

TLC – Rf = 0.84, Ethyl acetate: Formic acid: Glacial acetic acid: Water, (80:8:8:20) UV light (Yellowish fluorescent spot)

UV spectra have shown one peak with λ_{max} at 342 nm.

IR spectra has shown wave numbers at, 3375.30 for OH Stretching, 2929.59-Alkyl stretching, 1705.24-C=O stretching. From the above data we can say that the isolated compound may be flavonoid which was confirmed by chemical test.

Compound-II:

TLC – Rf = 0.67, Ethyl acetate: Formic acid: Glacial acetic acid: Water, (80:8:8:20) UV light (Bluish fluorescent spot)

UV spectra have shown one peak with λ_{max} at 356 nm.

IR spectra has shown wave numbers at, 3402.18-O- H Stretching, 3268.29 –N-H Stretching, 1672.97– C= O Amide Stretching,1510.41- C=C, Aromaticity.

From the above data we can say that the isolated compound may be Alkaloid which was confirmed by chemical test.

Table 1: Macroscopical Evaluation of Zanthoxylum rhetsa bark.

Features	Observation
Color	Inner surface - Pale Brown. Outer surface –Woody, Ash coloured.
Odour	Odorless
Taste	Bitter
Shape	Slightly curved
Fracture	Fibrous and thorny.
Size	Length - 10-12 cm Bridth – 3-5 cm Thickness – 1 - 1.2cm

Table 2: A. Extractive values, B. Loss on drying and C. Ash values of Zanthoxylum rhetsa L bark.

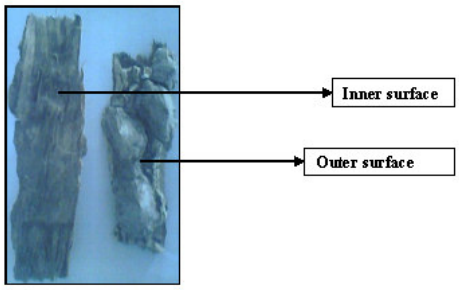
Parameter	Determined Value (% w/w)
Extractive values	
Alcohol soluble	8.00
Water soluble	12.69
Loss on drying	10.55
Ash Values	
Total ash	2.98
Acid insoluble ash	1.48
Water soluble ash	2.00
Sulfated ash	8.00

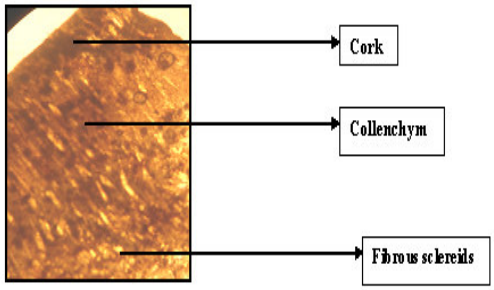
Table 3: Qualitative chemical analysis of various extracts of Zanthoxylum rhetsa L bark.

TEST	Tannin	Flavonoid	Glycoside	Steroid	Carbohy drates.	Phenol	Alkaloid
PE	--	--	--	+	--	--	--
CHCl3	--	+	--	+	--	--	+
MeOH	+	+	+	--	+	+	+
AQ	+	+	+	--	+	+	--

Keywords:
PE=Petroleum Ether $CHCl_3$= Chloroform MeOH = Methanol
AQ=Aqueous + = Present -- = Absent

Figure 1: Photograph of fresh bark of Zanthoxylum rhetsa bark





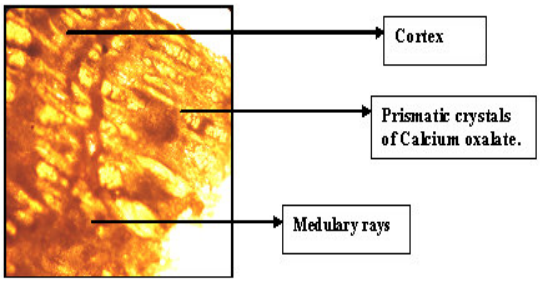


Figure 2: T.S. Zanthoxylum rhetsa bark (A) (B)

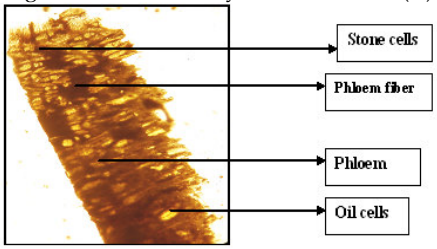
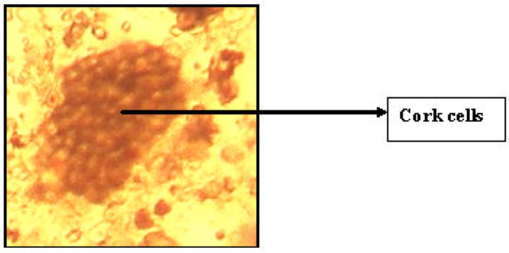
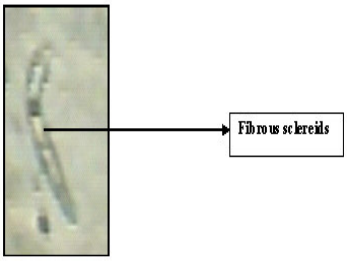
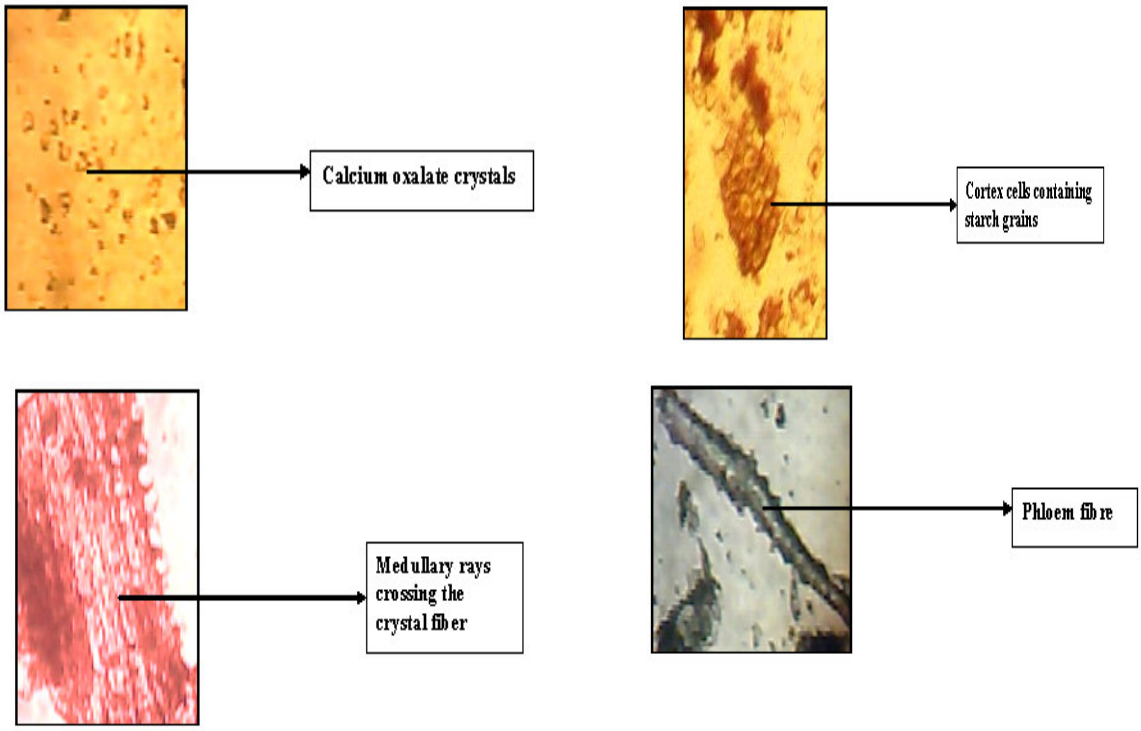


Figure 03: Powder microscopy of *Zanthoxylum rhetsa* bark.







CONCLUSION

Standardization of herbal drugs is very much essential, as they are derived from heterogenous sources which can lead to variations. These variations can lead to erroneous results in various pharmacological and phytochemical studies. Since *Zanthoxylum rhetsa* bark is known for its various medicinal properties hence the present study may be useful to supplement information in respect to its identification, authentication and standardization, since no such data is available for the same. From the TLC, UV and IR studies we can say that compound I may be flavonoid whereas compound II may be Alkaloid

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Anatomical studies on Balā - An Ayurvedic drug and its varieties

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ABSTRACT

The root and petiolar anatomy of Balā, an important Ayurvedic drug and its varieties (Sida spp.) has been studied in detail to identify the original drug from the adulterants. The diagnostic key for distinguishing the different varieties on the basis of the anatomical features of these drugs is also presented in detail.

INTRODUCTION

Balā is a reputed drug in Ayurveda is used for the treatment of rheumatism and it forms a chief ingredient of several important Ayurvedic preparations like *Ksirabala*, *Dhanvantaram*, *Balaristam*, *Rasnadhi kasayam*, *Asvagandhadi leham* etc. Root is the officinal part of the drug and is reported to be cool, sweet, demulcent, aphrodisiac and tonic. It produces strength, imparts beauty to the body and cures *vatarakta, raktapitta*, consumption, polyuria and ulcers. The drug is also useful in neurological disorders like hemiplegia, facial paralysis and sciatica, general debility, headache, opthalmia, dysuria, leucorrhoea, tuberculosis, diabetes, fever and uterine disorders (1).

In *Bhavaprakasa nighantu*, 4 varieties namely *balā*, *athibalā*, *nagabalā* and *mahabalā* are mentioned. The other two varieties are *rājabalā* mentioned in Rajanighantu and *bhūmibalā* in Ousadhinighantu are not in vogue or in practice. Of the four varieties, *balā* is the most widely used one. This has been equated with *Sida cordifolia* Linn. of Malvaceae (2 – 7). Ayurvedic formulary of India (8) has also accepted this and is widely used for source of *balā* in northern parts of India. But the traditional physicians of Kerala have adopted *Sida rhombifolia* ssp. *retusa* for this drug (9 - 11).

The other species of *Sida* ie. *Sida acuta*, *Sida rhombifolia* ssp. *rhombifolia* and *Sida cordata* are also adulterated or substituted with this drug. These species are common weeds found in abundance in many gardens and wastelands. The roots of these plants may simulate each other in dried condition and this may lead to either unintentional or intentional adulteration.

The present study puts forth a set of anatomical parameters of roots and petioles of some *Sida* species, which can be employed to distinguish the original drug as mentioned in the classical Ayurvedic drugs from the other adulterants. This study throws light on the need to properly identify the plant species to achieve standardization of Ayurvedic preparations which uses Balā as a key ingredient.

MATERIALS AND METHODS

Materials for the present study were collected from different places. They are as follows:

1. *Sida cordifolia, Sida acuta, Sida cordata* were collected from Cholayil Medicinal Plants Conservation Park (CMPCP), Velagapuram, Thiruvallur district, near Chennai and authenticated by regional floras (12 - 16).
2. † *Sida rhombifolia* ssp. *retusa* was collected from Kerala.
3. *Sida rhombifolia* ssp. *rhombifolia* from Kolathur, Chennai.
4. †† *Sida rhombifolia* ssp. *rhombifolia* var. *rhombifolia* from Korattur, Chennai and the material was referred and identified with the help of Auroville herbarium collections (Personal communication - Dr. Walter), Pondichery.

† Huang Hua Ren Shu (in Flora of China 12: 270-275, 2007) treated this species as *Sida alnifolia* var. *alnifolia* along with other 3 varieties under *Sida alnifolia* Linn.

†† *Sida rhombifolia* ssp. *rhombifolia* var. *rhombifolia* – This is very rare plant. Hence, this plant was not taken for root anatomy study with the objective of conserving the live plant. However the petiole anatomical study was carried out.

Fresh root and petiole samples of the above species were washed and fixed in FAA for 24 h and dehydrated, paraffin infiltrated and embedded in wax through customary techniques (17 - 18). Serial transections were obtained at 10-12µm thickness with rotary microtome and the sections were stained with Toluidine blue (19). Photomicrographs were taken with Nikon E400 microscope unit using 4X objective lens.

Observation

Anatomical descriptions of the root

1. *Sida cordifolia* Linn**.**

Macroscopic characters

Roots 5-15cm long and 0.75 –1cm thickness with few lateral roots of smaller size; tap root branched at the tip; outer surface is buff to grey-yellow; odourless; taste slightly bitter.

Microscopic characters (Fig. 2:1)

In transection of young root measuring about 4-5mm in diameter is roughly circular in outline with small fissures. Outermost zone consist of radial bands of rectangular, tangentially elongated, thin-walled cork cells about 4-5 rows. Secondary phloem composed of phloem fibres in wedge-shaped patches with thin walled parenchyma in

between. Phloem rays thin-walled tangentially elongated towards secondary cortex; a few rosette crystals of calcium oxalate found scattered in phloem parenchyma. The secondary xylem is very prominent forming dense solid cylinder occupying the major area of the root. Growth rings are fairly distinct and demarcated by broad zone. Vessels circular, wide, arranged in ring porous with pores solitary and in short radial multiples. It consists of prominent wide radial bands of rays, predominantly bi-seriate and fairly thick-walled fibres. Starch grains are abundant in xylem ray cells.

2. *Sida rhombifolia* Linn. ssp. *retusa* (Linn.) Borss. (Syn. - *Sida rhombifolia* var. *retusa* (Linn.) Mast.)

Macroscopic characters

Root consists about 5-7mm thick, slender, main tap root with small numerous rootlets, dark yellow-brown in colour. Surface is rough due to rootlets scar and peeled cork.

Microscopic characters (Fig. 2:2)

Cross section of root about 1-2mm in diameter shows circular in outline with prominent exfoliated cork layers. Cork consists of 4-7 rows of rectangular, tangentially elongated, thin-walled parenchymatous cells. Inner cortex consists of scattered phloem fibres in between the thin-walled parenchymatous. Secondary xylem consists of dense solid cylinder. Vessels predominantly solitary or less frequently short radial multiples, wide and polygonal shape in outline. Xylem fibres are thick-walled and wider lumen. Xylem rays are 1-2 seriate and starch grains absent.

3. *Sida rhombifolia* Linn. ssp. *rhombifolia*

Macroscopic

Root may occur as entire or cut pieces of varying lengths, 7-8mm in thickness, with wavy lateral roots comparatively thinner than main roots having numerous rootlets, brownish yellow, surface rough due to scars of small rootlets and lenticels; fracture, hard and splintery.

Microscopic (Fig. 2:4)

A thin root about 3-4mm shows circular in outline with exfoliated cork, consisting of 3-7 rows of narrow, rectangular, tangentially elongated, thin-walled parenchymatous cells. Secondary phloem composed of phloem fibres in wedge-shaped patches with thin walled parenchyma in between; phloem rays thin walled, tangentially elongated towards secondary cortex. A few rosette crystals of calcium oxalate found scattered in phloem parenchyma. Secondary xylem composed of vessels, fibres, parenchyma and rays. Vessels arranged in long radial multiples rows. Xylem rays 2-3 seriates, filled with starch grains.

4. *Sida acuta* Burm.f.

Macroscopic characters

Root consists of a short stout taproot system attaining a diameter of about 1cm and often long and flexuous with fairly thick lateral roots. The roots have a pale yellow colour; outer surface is fairly smooth except for the small filmy strips of exfoliating cork. The bark has thickness of about 1 to 11/2 mm and can be easily peeled off in fresh condition or by wetting.

Microscopic characters (Fig. 2:5)

A young root of 2-3mm thick shows circular in outline with exfoliated cork cells. It consisting of 5-6 rows of rectangular or tangentially elongated, thin walled cells followed by 2 to 3 layers of cork cambium towards inner side. Secondary cortex inner to cork cambium consists of 2 to 3 rows of tangentially elongated cells. Inner to the cortex, phloem elements, composed with thin-walled parenchyma alternate with phloem fibres. A few rosette crystals of calcium oxalate found scattered in phloem parenchyma. Secondary xylem is in dense solid cylinder occupying major part. Vessels are diffuse porous with pores solitary, wider, circular and/or in short radial multiples with abundant distribution. Xylem rays mostly bi-seriate with abundant starch.

5. *Sida cordata* (Burm.f.) Borss. (Syn. - *Sida veronicifolia* Lam.)

Macroscopic characters

Tap-root is very long, slender about 4-6mm in diameter having many small rootlets, yellowish brown in colour, outer surface is smooth.

Microscopic characters (Fig. 2:3)

About 3-4mm thick root shows circular outline with less frequent exfoliation of cork layers. Cork consisting about 4-5 rows of tangentially elongated, rectangular cells. The outermost cortex consist fairly distinct parenchymatous cells, which are loosely arranged with intercellular spaces. The inner cortex bounded internally by patches of phloem fibres in between the parenchymatous cells. Phloem rays broadly enter towards the cork. A few rosette crystals of calcium oxalate found scattered in phloem parenchyma. Secondary xylem is very prominent, dense solid cylinder. Vessels circular, wider and diffuse porous with pores solitary and in short radial multiples. Xylem rays prominent, 2-3 seriate with absence of starch grains.

Anatomical description of the Petiole

The petiolar anatomy has been studied based on the topographic arrangement of the vascular bundles.

1. *Sida cordifolia* Linn. (Fig. 3:1)

In transverse section of petiole measuring about 1mm diameter shows triangular shape in outline. A small groove is present on the adaxial side. Epidermis is single layer of compactly arranged barrel shaped cell and covered with abundant stellate trichomes. Hypodermis is represented by 3-4 layers of collenchymatous tissue occur just below the epidermis. The ground tissue made up of parenchymatous cells with distinct intercellular spaces. A few rosette type of calcium oxalate crystals found scattered in the ground tissue. Three vascular bundles present in the ground tissue and all are similar in size and shape and vascular system open type. They arranged as one dorsal (lower) and two laterals. Thus, the arrangement is expressed as 1 + 2.

2. *Sida rhombifolia* Linn. ssp. *retusa* (Linn.) Borss. (Fig. 3:2)

Petiole about 0.7mm in diameter and consist single layer of

epidermis with compactly arranged cubical cells and covered by abundant stellate trichomes. 2-3 layers of collenchymatous cell present just below the epidermis. Five vascular bundles present in the ground tissue and in different size and vascular system open type. They are arranged as one dorsal, two laterals and two ventrals and these are small in size compare to others. Thus, the arrangement is expressed as 1 + 2 + 2.

3. *Sida rhombifolia* Linn. ssp. *rhombifolia* (Fig. 3:3)

Petiole measuring about 1mm in diameter and shows single layer of epidermis with barrel shaped cells covered by stellate trichomes and less distribution. 1-2 layers of collenchymatous cells present just below the epidermis. A few rosette type of calcium oxalate crystals found scattered in the ground tissue. Four vascular bundles arranged as one dorsal, two laterals and one ventral. But these bundles are fused by thick-walled sclerenchymatous cells and form closed type of vascular system. Middle region of the vascular bundle made up of thin-walled parenchymatous cells. The arrangement of vascular bundles expressed as 1 + 2 + 1.

4. *Sida rhombifolia* Linn. ssp. *rhombifolia* var. *rhombifolia* (Fig. 3:4)

Petiole about 0.7mm in diameter and consist single layer of epidermis with compactly arranged cubical cells and covered by stellate trichomes and less distribution. 3-4 layers of collenchymatous cells present just below the epidermis. A few rosette type of calcium oxalate crystals found scattered in the ground tissue. Four vascular bundles arranged as one dorsal, two laterals and one ventral and open system. Dorsal and lateral bundles fused by sclerenchymatous cells and form girdle shape. The arrangement of vascular bundles expressed as 1 + 2 + 1.

5. *Sida acuta* Burm.f. (Fig. 3:5)

Cross section of petiole measuring about 0.5mm diameter shows wavy margin in outline with stellate trichomes. Hypodermis and ground tissue form few layers only. Four vascular bundles arranged as one dorsal, two laterals and one ventral and these bundles fused by thick-walled sclerenchymatous cells and form closed type as in *Sida rhombifolia ssp. rhombifolia*. Middle region of the vascular bundle made up of thick-walled sclerenchymatous cells. The arrangement of vascular bundles expressed as 1 + 2 + 1.

6. *Sida cordata* (Burm.f.) Borss. (Fig. 3:6)

In transverse section of petiole measuring about 0.8mm diameter shows triangular shape in outline. A small groove is present on the adaxial side. A few simple, unicellular elongate trichomes present in the adaxial groove. Well-developed hypodermis and ground tissues present. Vascular system open type and four vascular bundles are arranged as one dorsal, two laterals and one ventral and open. The arrangement of vascular bundles expressed as 1 + 2 + 1.

DISCUSSION

Balā is one of the most important drugs used in the various formulations of Ayurveda and other Indigenous medicines. Many species of *Sida* are used as balā and/ or its varieties. In North India, *Sida cordifolia* is used as balā. But in Kerala, *Sida rhombifolia* ssp. *retusa* have adopted. The other species of *Sida* may adulterate or substitute with the original balā. It is easy to distinguish these species when they bear flowers and fruits. However, the crude drug in the market is very difficult to identify (the original from the adulterant) due to lack of flowers and fruits. The anatomical features of root and petiole of these species are specific and these can be employed for the identification of drug of choice (original drug).

The following anatomical features are suggested to diagnose the root samples

1. *Sida cordifolia* Linn.

Growth rings are fairly distinct, vessels circular in outline, wider, arranged in ring porous with pores solitary and in short radial multiples. Xylem rays predominantly bi-seriate with abundant starch grains.

2. *Sida rhombifolia* Linn. ssp. *retusa* (Linn.) Borss.

Vessels polygonal in outline, wider, predominantly in solitary or very less frequent short radial multiples. Xylem fibres are thick-walled and wider lumen. Xylem rays are 1-2 seriate and starch grains absent.

3. *Sida rhombifolia* Linn. ssp. *rhombifolia*

Vessels circular in outline, moderately wide, arranged in long radial multiples. Xylem rays 2-3 seriates, filled with less starch grains.

4. *Sida acuta* Burm.f.

Vessels circular in outline, wider, arranged in diffuse porous with pores solitary, in short radial multiples with abundant distribution. Xylem rays mostly bi-seriate with abundant starch.

5. *Sida cordata* (Burm.f.) Borss.

Vessels circular in outline, wider, arranged in diffuse porous with pores solitary and in short radial multiples with less abundant compared to *Sida acuta*. Xylem rays prominent, 2-3 seriate with absence of starch grains.

The following petiolar anatomical features are suggested to diagnose the Sida spp.

1. *Sida cordifolia* Linn.

Triangular shape in outline. Vascular system open type and three vascular bundles similar in size and shape and arranged as 1 + 2.

2. *Sida rhombifolia* Linn. ssp. *retusa* (Linn.) Borss.

Vascular system open type and five vascular bundles different in size and arranged as one dorsal, two laterals and two ventrals and these are small in size compare to others and arrangement expressed as 1 + 2 + 2.

3. *Sida rhombifolia* Linn. ssp. *rhombifolia*

Vascular system closed type, four bundles arranged as one dorsal, two laterals and one ventral, these bundles fused by thick-walled sclerenchymatous cells. Middle region of the vascular bundle made up of thin-walled parenchymatous cells and arrangement expressed as 1 + 2 + 1.

4. *Sida rhombifolia* Linn. ssp. *rhombifolia* var. *rhombifolia*

Vascular system open type, four vascular bundles arranged as one dorsal, two laterals and one ventral. Dorsal and lateral bundles fused by sclerenchymatous cells and form girdle shape and arrangement expressed as 1 + 2 + 1.

5. *Sida acuta* Burm.f.

Vascular system closed type, four vascular bundles arranged as one dorsal, two laterals and one ventral and these bundles fused by thick-walled sclerenchymatous cells and middle region of the vascular bundle made up of thick-walled sclerenchymatous cells and arrangement expressed as 1 + 2 + 1.

6. *Sida cordata* (Burm.f.) Borss.

Vascular system open type, four vascular bundles arranged as one dorsal, two laterals and one ventral and the arrangement expressed as 1 + 2 + 1.

1.A key for identification based on root anatomical features of five *Sida* species.

1a. Growth rings present. Vessels ring porous .

Fig.1 - Morphology of *Sida spp.*

Sida cordifolia

Sida rhombifolia ssp.*retusa*

Sida rhombifolia ssp. *rhombifolia*

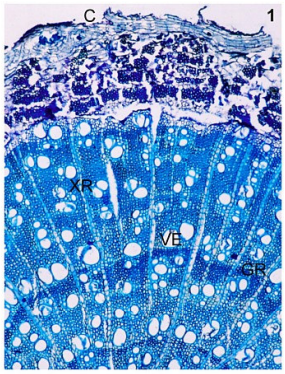
Sida rhombifolia ssp. *rhombifolia* var. *rhombifolia*

Sida acuta

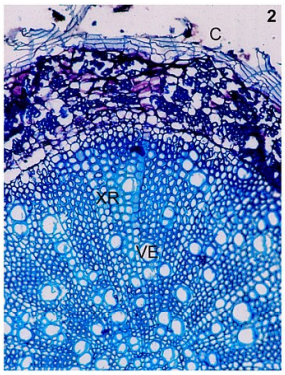
Sida cordata

Fig. 2 - Root Anatomy of *Sida spp.*

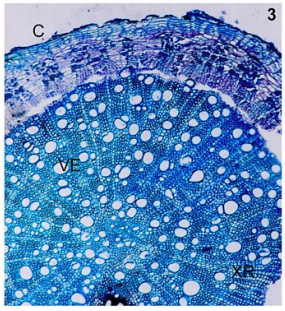
Sida cordifolia



Sida rhombifolia ssp. *retusa*



Sida cordata



Sida rhombifolia ssp. *rhombifolia*

Sida acuta

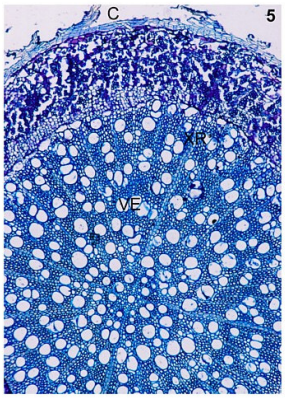
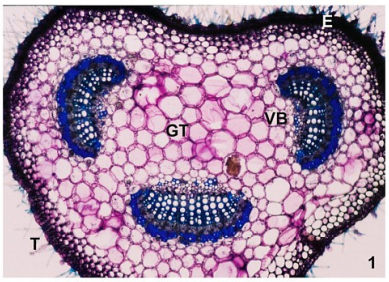
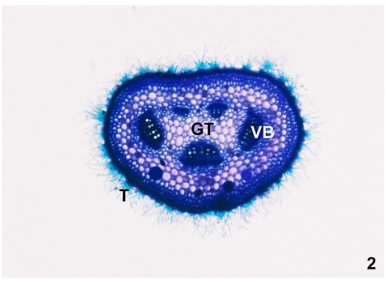


Fig. 3 - Petiolar Anatomy of *Sida spp.*

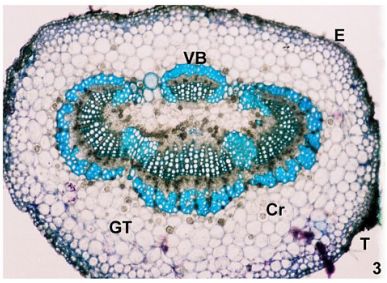
Sida cordifolia



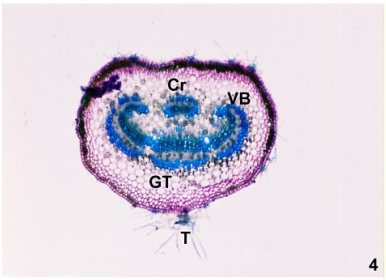
Sida rhombifolia ssp. *retusa*



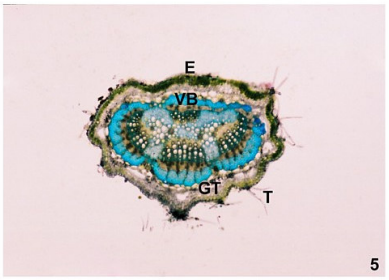
Sida rhombifolia ssp. *rhombifolia*



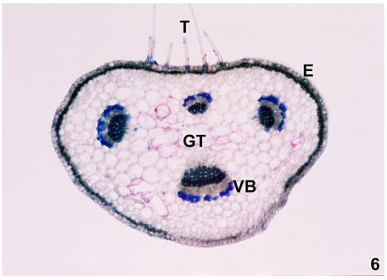
Sida rhombifolia ssp. *rhombifolia* var. *rhombifolia*



Sida acuta



Sida cordata



Sida cordifolia
1b. Growth rings absent. Vessels diffuse porous
2a. Vessels polygonal in outline and predominantly solitary .**Sida rhombifolia** ssp**. retusa**
2b. Vessels circular in outline and not as above
3a. Vessels arranged in long radial multiples
Sida rhombifolia ssp**. rhombifolia**
3b. Vessels not as above
4a. Starch grains absent in xylem rays, Vessels less abundant **Sida cordata**
4b. Starch grains present in xylem rays, Vessels abundant.

Sida acuta
2. A key identification based on petiolar anatomical features of six *Sida* species.
1a. Vascular system open type
2a. Dorsal and lateral vascular bundles fused
Sida rhombifolia ssp. **rhombifolia** var. **rhombifolia**
2b. Vascular bundles not as above
3a. Vascular bundles 5 nos
.**Sida rhombifolia** ssp. **retusa**
3b. Vascular bundles not as above
4a. Vascular bundles 4 nos

Sida cordata
4b. Vascular bundles 3 nos
Sida cordifolia
1b. Vascular system closed type
5a. Middle region of the vascular bundle made up of thin-walled parenchymatous cells
Sida rhombifolia ssp. **rhombifolia**
5b. Middle region of the vascular bundle made up of thick-walled sclerenchymatous cells
Sida acuta

ACKNOWLEDGEMENT

We sincerely thankful to Dr. V.P. Sidhan, Chairman, Mr. V.S. Pradeep, Managing Director and Ms. Jayadevi Pradeep, Directress R & D, Cholayil Private Limited for their constant support and encouragement.

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LEGEND

C – Cork, Cr – Crystals, E – Epidermis, GR – Growth ring, ,GT – Ground tissue, T – Trichomes, VB – Vascular bundle, VE – Vessel element, XR – Xylem rays

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PHCOG J: Research Article

Pharmacognostic Studies on the Leaves of *Anisomeles indica* Linn. (Labiatae)

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ABSTRACT
Pharmacognostical parameters for the leaves of Anisomeles indica Linn. (Labiatae) were studied with the aim of drawing the Pharmacognostical standards for this species. Macroscopical and microscopical characters, physio-chemical constants, quantitative microscopy parameters, extractive values with different solvents, fluorescence analysis of extracts, its reaction after treatment with chemical reagents under visible light and UV light at 254 nm and 366 nm. Preliminary phytochemical screening on the leaves Anisomeles indica was also studied. Caryophyllaceous stomata (in the upper and lower epidermis), glandular, non-glandular trichomes and starch grains were identified. The determination of these characters will help future researchers in their Phytochemical as well as Pharmacological analysis of this species.

KEY WORDS
Anisomeles indica Linn., Chemo-microscopy, Evaluation, Macroscopy, Micrometry, Pharmacognosy

INTRODUCTION

Anisomeles indica Linn. (Family: Labiatae) is one of the important medicinal plant found in satpuda vally, specifically in Toranmal (altitude 1800m), Maharashtra (India). It's an erect, camphor-scented, variable herb or woody under shrub, 1-2 m in height; stems acutely quadrangular, softly pubescent (1).

Anisomeles indica are used in folk medicine all over the world. It is popularly known as 'Jirnya' in northeastern part of India, where it receives widespread used as folk medicine, predominantly in the treatment of intestinal disorders and intermittent fever. *Anisomeles indica* have anti-microbial, astringent, carminative ethanolic extract (50%) of the herb showed hypothermic activity and when burnt acts as a mosquito repellant. The essential oil present in the herb is useful in uterine affections (2, 3).

This study is intended to establish, macroscopical, microscopical, chemo-microscopical, quantitative-leaf microscopical characters as well as quantitative evaluation of the powdered and fresh leaves of the plant to be used as diagnostic features in the identification, evaluation and monograph preparation of the plant (4, 5).

MATERIALS AND METHODS

Plant collection and identification

Whole plant of *Anisomeles indica* was collected from Toranmal forest (Satpuda Valley), Maharashtra, India in the month of Aug-Sep 2006. Plant sample was identified and authenticated by Dr. D.A.Patil taxonomist, SSVP'S Science College, Department of Botany, Dhule (MS), India. Voucher specimen of the plant material has been deposited at Institute level (RCPCOP/AI-06).

Chemicals and instruments

Compound microscope, simple microscope, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Photomicroscope provided with MOTIC IMAGES PLUS 2.0 software. Some crystals, starch grains and lignified cell slides were taken under projection microscope. Solvents viz. petroleum ether, chloroform, methanol, ethanol and reagents viz. phloroglucinol, glycerin, Hcl, Iodine and potassium hydroxide were procured from Loba Chemicals, Mumbai, India.

Macroscopical examinations

For morphological observations, fresh young leaves (approx. 3-7cm in length) were used. The macro-morphological features of the plant parts (leaves) were observed under magnifying lens and simple microscope (6).

Microscopical examinations

Fresh leaves of the plant were studied transversely and longitudinally, using surface preparations and sections. The different parts of leaf like lamina and midrib were studied according to the methods of Brain and Turner (7). For the microscopical studies, cross sections were prepared and stained as per the procedure of K. R. Khandelwal (8). Quantitative evaluations and quantitative-leaf microscopy were also carried out as outlined by Wallis T. E. (9, 10).

Chemo-microscopical examinations were also carried out, following thorough clearing of the powdered leaves with potassium hydroxide solution and a subsequent mounting with dilute glycerol on a microscope slide, and tested with various detecting reagents. Various ergastic substances/ chemical constituents were identified in accordance with (11).

Preliminary phytochemicals investigation

The investigation was carried out by using standard procedures (12).

Quantitative evaluations of the crude drug - Moisture content of the powdered leaves determined based on the

loss of drying method (13). The ash values (Total ash, acid-insoluble ash and water-soluble ash) were determined, to find out about the physiological state and level of extraneous matter. Total ash of the drug was subjected for testing different inorganic constituents (14, 15). Extractive values (ether, methanol, chloroform, alcohol and water) were determined according to the official methods prescribed in Ayurvedic pharmacopoeia (16). Fluorescence analysis of powdered leaf was done by standard method of Chase and Pratt (17).

RESULTS

Macroscopic examination (Figure 1)

The macro-morphological characteristic of the leaves of *Anisomeles indica* identified were acute apex, crenate margin, asymmetric base, reticulate venation and hairy to softly pubescent shape. Leaves surface thick, with dimension 3.8-10 x 5.5-6 cm. Color is green to yellowish green; taste is slightly astringent with characteristic odor. The plant shows covering trichomes more on the lower surface of the leaves.

Microscopic examination

Transverse section of leaf (Figure 2)

It is a dorsiventral leaf. Following tissues are present in lamina and midrib:

Lamina

The Upper epidermal cells are compactly arranged with no intercellular spaces except stomata. Epidermis is made up of single layer of spherical to polygonal cells with straight to slightly beaded anticlinal walls wavy in shape (Figure 2a). Mucilage is detected in epidermis. Mesophyll is differentiated into palisade and spongy parenchyma. Palisade formed from compactly arranged elongated, narrow columnar cells with beaded anticlinal walls. Triseriate layer has been continuous over the midrib region (Figure 2b). Palisade cells were filled with chlorophyll. Spongy parenchyma made up of parenchymatous cells with varying size and shape. Lower epidermis is made up of single layer of polygonal cells with straight to slightly beaded anticlinal walls. Chlorophyll is present in epidermal cells, numerous caryophyllaceous or diacytic stomata (Figure 2c), and multicellular clothing trichomes as well as glandular trichomes were observed on the both epidermis.

Midrib (Figure 2d)

Midrib present both surface with different degree of concavity. Dorsal surface is more convex than ventral surface. Lower palisade is replaced by patch of collenchymatous cells, which is about 37.5 µm in diameter. Collateral vascular bundle is prominent, occupying the central portion of the midrib. Xylem vessels are covered by xylem fibers. Xylem parenchyma is made up from semi-rectangular, lignified cells. The phloem is non-lignified and collenchymatous in nature. The micrometric analysis is tabulated in Table 1.

Powder characteristic

The leaf powder is greenish black in color with astringent taste. No greasy stain was observed when powder press between Whatmann's filter paper No. 40 indicates absence of fixed oil. But the strong aromatic odor of the filter paper is a mark of presence of essential oil. The behavior of powder with different chemical reagent is shown in Table 2. On microscopically examination, the powder showed

Trichomes - Two types of trichomes; one is non-glandular multicellular trichomes (Figure 3a) and another was glandular unicellular stalk with multicellular head type (Figure 3b). After addition of small quantity of water in powder it shows the following elements.

Xylem fibers (Figure 3c) - Scaliform with ladder like thickening.

Mesophylls – Fragments of leaf showing spongy parenchyma cells.

Starch grains are simple granules, spherical or irregularly ovoid with centric helium and less prominent striations. (Figure 3d). Large lignified cell entire or scattered forms (Figure 3e).

Quantitative Microscopical parameters of leaves

Pertaining to the stomatal index, stomatal number, vein-islet number and Vein-termination number data given in Table 3.

Chemo-microscopical examination

The counter idea about presence of phytoconstituents is obtained through this study like phenolic compound in palisade as indicated by brownish black stain on ferric chloride solution treatment (Table 4).

Preliminary phytochemicals investigation

Revealed the presence of primary and secondary metabolites as carbohydrates, mucilage, Tannins, Terpenoid, Glycosides, Alkaloids and Phytosteroids (Table 5)

Table 1: Micrometry of cellular elements

Cellular elements / cell content	Measurement (µ)
Upper epidermal cell	Length: 32.1 – 47.0 –60.5 ; Width: 20.5 – 29.0 – 38.4
Lower epidermal cell	Length: 13.9 – 23.4 – 29.6 ; Width: 6.60 – 14.9 – 21.7
Palisade cell	Length: 92.5 – 118 – 140 ; Width: 39.7 – 48.4 – 63.0
Upper collenchymatous cell	20.6 – 28.5 – 42.4
Lower collenchymatous cell	24.8 – 37.5 – 50.7
Parechymatous cell	55.1 – 79.3 – 98.6
Phloem cell	8.10 – 15.5 – 28.6
Xylem vessels	14.1 – 16.9 – 19.8

Table 2: Behavior of powdered leaves

Reagent	Color / ppt.	Constituent
Powder + Sulphuric Acid	Reddish	Steroids +ve
Powder + Aqs. Ferric chloride sol.	Blackish	Tannins* +ve
Powder + Iodine sol.	Blue	Starch +ve
Powder + Picric Acid sol.	Yellow color	Alkaloid* +ve
Powder + Aqs. Silver Nitrate sol.	No change	Protein +ve
Powder + Aqs. Potassium hydroxide sol.	No color change	Antraquinone Glycoside -ve
**may be present in powder drug; '+' presence; '	*' absence*	**may be present in powder drug; '+' presence; '*

Table 3: Quantitative parameters of leaves

Leaf Constants	Size (μ)
Diameter of stomata	Upper Surface: 35.0 - 37.0 - 40.0 ; Lower Surface: 34.0 - 36.0 - 38.0
Stomatal number	Upper Surface: 48.0 – 55.0 – 62.0 ; Lower Surface: 24.6 - 25.5 - 26.5
Stomatal index	Upper Surface: 56.0 – 62.0 – 68.0 ; Lower Surface: 21.9 - 22.9 - 24.1
Vein-islet number	11.5 sq.mm
Vein-termination number	21.9 sq.mm

Table 4: Chemo-microchemical tests of Anisomeles indica leaves

Reagent	Color	Test for	Histological zone
Phloroglucinol + Hcl	Pink	Lignin	Vascular bundle
Weak iodine solution	Blue	Starch	Mesophyll region
Ferric chloride solution	Brownish black	Phenolics	In palisade cell region
Libermann-Burchardlt reagent	Greenish	Steroids	Mesophyll region
Dragendorff's reagent	Orange	Alkaloids	Lamina
Million's reagent	Blue	Proteins	Vascular bundle

Table 5: Preliminary phytochemicals investigation

Phytoconstituent	Pet. ether	Chloroform	Methanol	Ethanol	Water
Steroids	+	+	+	+	-
Carbohydrates	-	-	-	-	+
Alkaloids	-	+	-	-	-
Glycosides	-	-	+	+	+
Reducing sugar	-	-	-	-	+
Phenolics	-	-	+	+	+
Tannins	-	-	+	-	-
Proteins	-	-	-	-	-
Amino acid	-	-	-	-	-
Mucilage	-	-	-	+	+

'+' presence; '-' absence

Table 6: Quantitative evaluations

Parameter	Values % (w/w)* ± SD
Total ash	11.87 ± 0.32
Acid insoluble ash	2.70 ± 0.10
Water soluble ash	6.17 ± 0.15
Sulphated ash	8.31 ± 0.10
Moisture content	5.37 ± 0.06
Extractives:	
Petroleum ether soluble	3.48 ± 0.24
Chloroform soluble	9.23 ± 0.15
Methanol soluble	13.63 ± 0.15
Ethanol soluble	11.20 ± 0.26
Water soluble	19.18 ± 1.01

* values expressed as mean of three readings; SD-Standard Deviation

Table 7: Qualitative analysis of elements present in total ash

Element	Inference
Aluminum	--
Calcium	++
Potassium	++
Magnesium	++
Sodium	++
Iron	--
Zinc	--
Antimony	--
Chlorides	--
Sulphate	--
Phosphates	++
Carbonates	--

'+ +' Presence of elements, '--' absence of elements

Table 8: Fluorescence analysis of extracts

Extract	Consistency	Color in Day light	Color in UV light	
			254nm	366nm
Petroleum ether	Sticky	Yellowish	Greenish yellow	Dark Brow
Chloroform	Sticky	Brownish	Dark Green	Dark Brown
Methanol	Sticky	Reddish	Green	Brownish
Ethanol	Solid	Blackish	Dark Green	Brownish
Aqueous	Solid	Blackish	Greenish Black	Brownish

***Figure 1: Anisomeles indica* Linn.**

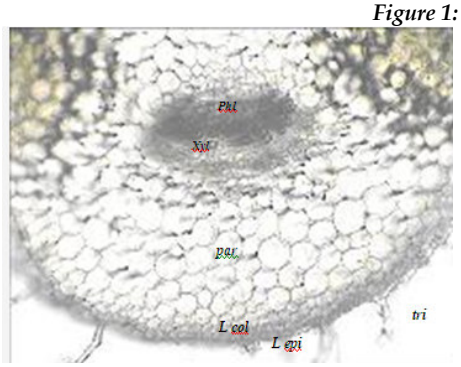

Figure 2: T. S. of midrib enlarged
[*Xyl*- Xylem; *Phl*- Phloem; *Par*- Parenchyma; *L col*- Lower collenchyma's; *L epi*- Lower epidermis; *tri*- Trichomes]

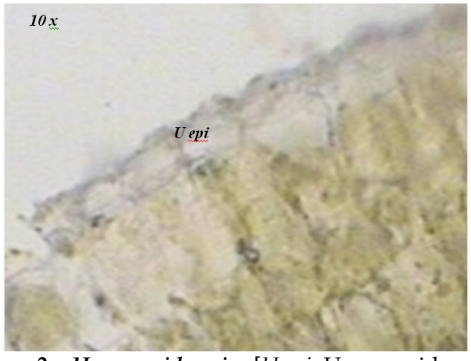

Figure 2a: Upper epidermis [*U epi*- Upper epidermis]

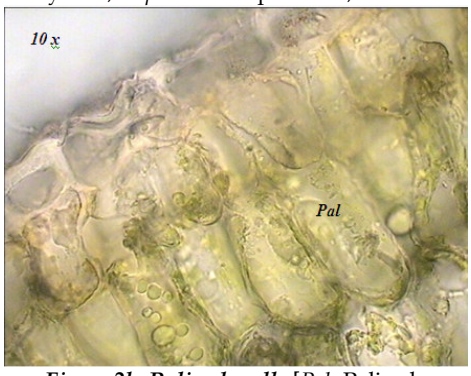

Figure 2b: Palisade cells [*Pal*- Palisade

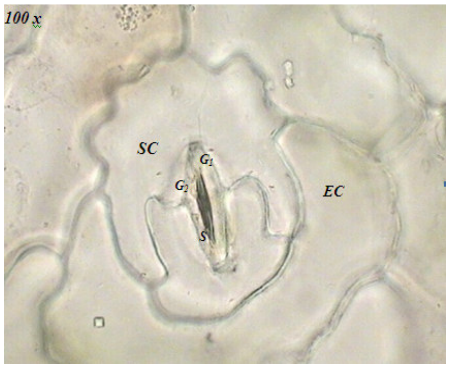

Figure 2c: Diacytic Stomata
[*S*- Stoma; $G_{1,2}$- Guard cells; *SC*- Subsidiary Cell; *EC*- Epithelial Cell]

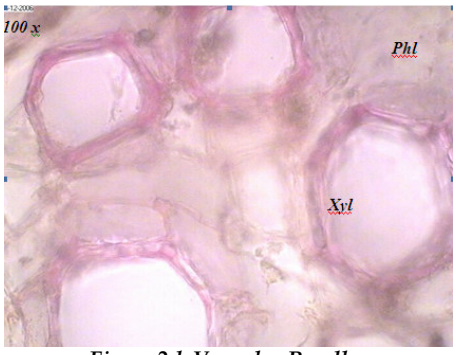

Figure 2d: Vascular Bundles
[*Xyl*- Xylem; *Phl*- Phloem]

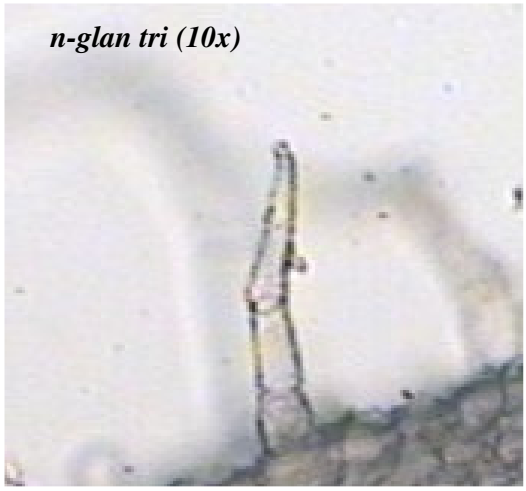

Figure 3b: Non-Glandular Trichome [*n-glan tri*]

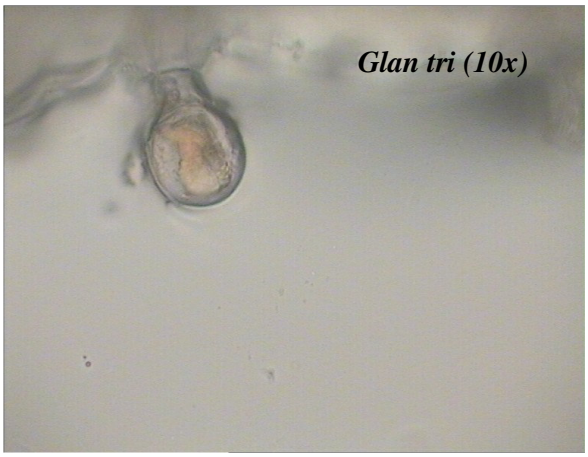

Figure 3a: Glandular Trichome [*Glan tri*]

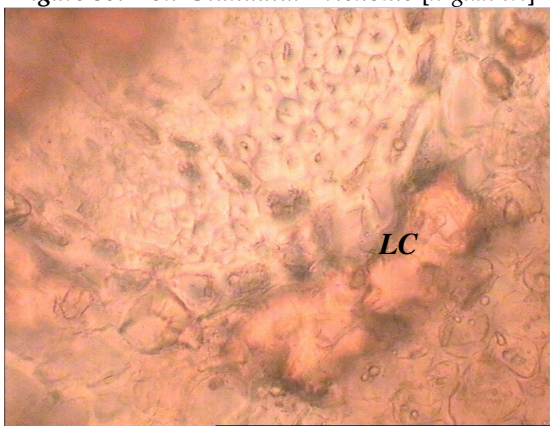

Figure 3e: Lignified cell [*LC*- Lignified Cell]

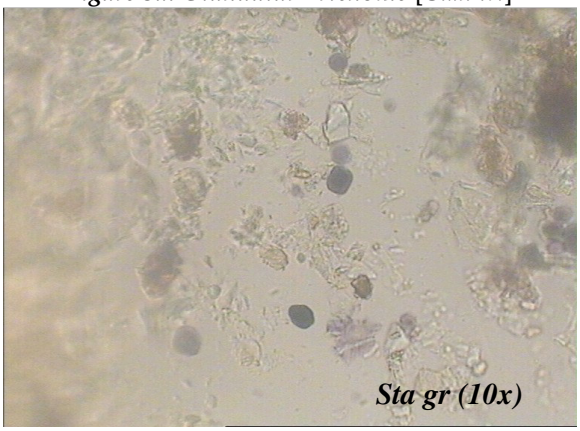

Figure 3d: Starch grains [*Sta gr*]

Quantitative evaluations of the crude drug

The moisture content (NMT 5.37 ± 0.06 % w/w) seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. Physical constant as ash value of the drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The results of the physical constants of the drug powder are given in Table 6. The qualitative analysis of ash indicated presence of calcium, Potassium, magnesium, sodium and phosphates (Table 7). The results of fluorescence analysis of the extracts presented in Table 8.

CONCLUSION

The exhausted literature survey conclude before the present work start there is no pharmacognostic study on record of this much valued traditional drug. Therefore present work was taken up with a view to lay down

standards which could be useful to find the authenticity of this traditional medicinal plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at Herbal industrial level in the coming days.

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PHCOG J: Research Article

Scientific Validation and Authentication of Genuine and Market samples of *Tinospora cordifolia*

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ABSTRACT
Guduchi (Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms) is one of the most versatile rejuvenate herbs. The Sanskrit name guduchi means the one, which protects the body. It is also called as amrta or nectar, as it is extremely useful in strengthening the immune system of the body and keeping the functions of its various organs in harmony. Therefore, in the present research work attempts have been made to provide a complete study on Guduchi Satva where its authentication and validation was worked out in a series of manner. In standardization, Tinospora cordifolia Genuine and market sample were screened for various pharmacognostic characterization, Phytochemical screening and antibacterial assay. Studies revealed that market sample was full of adulterations and cannot be use safe and effective drug. This identification was used to form various markers for standardization of guduchi satva. These characters can further be used in Ayurvedic Pharmacopoeia as Key markers. Hence these markers can scientifically used to check the adulteration and retain the quality of drug.
Key words: *Tinospora cordifolia, Adulteration, Validation, Markers*

INTRODUCTION

Guduchi (*Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms) is also known as *Amrita* in Ayurvedic System of medicine. *Amrita* means divine nectar is attributed to the drug in recognition of its capacity to impart youthfulness, vitality, and longevity to the patient (1).

Drug consists of the dried stem with bark intact. The plant is mainly known for its medicinal properties in Ayurvedic Medicine (2). Guduchi satva was used in ancient time as general tonic and now experimentally established as anticancer (3), antiulcer (4), antipyretic (5), antihepatitis (6), immunomodulatory (7), antioxidant (8), hypoglycemic (9), antineoplastic (10), cardiotonic, antibacterial, antimicrobial, antileishmanial, anti-inflammatory, antiarthritic, analgesic and diuretic (11) agent. A reverse phase HPLC method was also developed to quantitatively estimate the berberine content in the stem of *T. cordifolia* and *T. sinensis* (12). Though it is a multipurpose drug still its Ayurvedic authentication is so poorly studied that various parameters to identify the guduchi satva from genuine sample are still not available. Various drugs having sufficient clinical data are guggul, brahmi, ashwagandha, amlaki, guduchi, kutki, shatavari and shunthi (13) Pharmacopoeia of India (1996) (14) covers few botanical monographs like clove, guggul, opium, mentha etc. The Ayurvedic Pharmacopoeia of India gives monographs for 258 different Ayurvedic drugs. The standards mentioned are quite inadequate to build quality of the botanical materials (15). As increasing demand of the authentic drug lead to adulteration of various formulations is a common phenomenon. Even micropropagation of multipurpose drug - *Tinospora cordifolia* was also attempted (16). Therefore, in the present research work attempts have been made to complete study on Guduchi Satva where its authentication and validation was worked out in a series of manner. In standardization of Guduchi satva, authentic stem of *Tinospora cordifolia* and Market sample of Giloy Satva were compared and microscopic, tlc and bio- markers were generated to prove the authentication of the drug. These markers can be used to procure safe, efficacious and quality product of guduchi at quarantines.

MATERIALS AND METHODS

Plant Material

Collection and Identification

Authentic samples: *Tinospora cordifolia* (Genuine sample) was procured from wild region and authenticated from Herbarium, Department of Botany, Mahatma Gandhi Institute of Applied Sciences, JECRC Campus, Jaipur, India and authenticate as Voucher specimen No. AMP 102. Market samples of Guduchi Satva (Baidyanath ltd.) was procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of October, 2008.

Pharmacognostic Characterization:

Morphological Properties

Microscopical evaluation deals with the identification of various characters of tissues, cells and cell contents by microscope. Methods of preparing specimens of crude materials for microscopical studies vary depending on the part used like leaf, stem, root, bark, flower, fruit and also on the nature of the material i.e. entire, cut or powdered.

Preparation of sections: Drugs which are hard to cut, are boiled for 20 to 30 min in water. Cross section or transverse section with a razor blade was taken. Thin materials such as leaves, slender stems or flat seeds are placed in a potato slit and sections were taken with ordinary blade. The section was placed on a slide, clear with chloral hydrates, covered with a cover glass and observed under microscope.

Powder microscopy

Likewise, powder of the selected species and their adulterants were subjected to microscopic analysis and the structures of various evaluations were drawn.

For examination of powder characteristics, sufficient amount of powdered drug (fruit or leaves) in chloral hydrate on a slide was warmed over a low flame or on a hot-plate for a short time, covered it with a cover glass and observed under microscope (4, 10, 20 or 45 X). Disintegration of hard and woody tissues: The material was cut into pieces and few pieces transferred to a test tube containing 4 ml of nitric acid and heated to boiling. Later, powdered potassium chlorate was gently added, warmed and allowed to react leading to disintegration of the tissue. When completely bleached, pressure was applied with glass rod for complete disintegration of the tissues. The material was allowed to settle down, decant the liquid and the settled material washed repeatedly with water until the acidity is removed. The material was transferred on to a slide, a drop of glycerol added, covered with a cover glass and observed under microscope.

Microscopic test

Starch – For examining the presence of starch, the specimen was taken in I_2 wherein starch turns blue. Aleurone grains – For examining the presence of aleurone grains a specimen was prepared in I_2 and aleurone grains stained yellow. Fixed oil -For examining the presence of fixed oil, specimen was stained with sudan red resulting in the droplet of fixed oil to become pink coloured.

Phytochemical Screening Thin Layer Chromatography (17-18)

Extraction procedure

Preparation of guduchi satva as per the recommendation of API, India (9). For TLC profile of selected species each of the dried and powdered (100 gm.) test samples of Guduchi stem and Giloy satva were Soxhlet extracted successively in petroleum ether, benzene, chloroform, ethyl acetate and methanol for 6 h. These extracts were filtered, evaporated to dryness and weighed. Each extract (10 mg) was dissolved in 10 ml to make a concentration of 1mg/ml used for further studies.

TLC plates

Each extract was applied on silica gel G Thin Layer Chromatography[TLC] coated plates (Merck: 20x20 cm; with thickness 0.2-0.3mm) which were activated at 100°C for 30 minutes and brought to room temperature, just before use. Each extract of various species was applied 1cm above the edge of the chromatographic plates along with the reference compounds and developed in air-tight chamber already saturated with~200 ml of various solvent systems.

TLC spraying reagents

Various extracts of test samples were subjected to different solvent systems for identification of any significant biomolecules. After having used different solvent systems, on the basis of better resolution of spots for generating "Thin Layer Chromatography [TLC] fingerprints" for chemical libraries of the test drugs following solvent systems were used in the present study- hexane :acetone (3:1) for petroleum ether extracts, benzene :ethyl acetate (1:2) for benzene extracts, chloroform : methanol (1:2) for chloroform extracts, benzene: ethyl acetate (2:1) for ethyl acetate extracts, and chloroform :methanol (2:1) for methanol extracts.

TLC spraying reagents

During the work of present studies, different visualizing reagents i.e. 10% sulphuric acid {10 ml conc. sulphuric acid dissolved in 100ml absolute alcohol}, I_2 vapour (Saturated iodine chamber) and Drangendroff reagent were used.

Qualitative TLC

Thin glass plates were coated (0.2-0.3 mm) with silica gel G (30 g/60 ml distilled water) and dried at room temperature. The coated plates were activated in an oven at 100°C for 30 minutes and cooled. The plates were then placed in developing tanks having ~ 150 ml of an organic solvent mixture of pet ether: benzene (1:3). The lid of the developing tanks was sealed with vacuum grease. The plates were removed after making the solvent front and were air-dried. The dried plates were sprayed with Dragendroff reagent (8 g bismuth subnitrate dissolved in 25 ml 30% HNO_3 and further addition of 28 g KI and 1 ml of 6 N HCl) and alkaloid positive spot (R_f value) was calculated.

Antibacterial Assay

Sources of test organisms

Bacteria – Pure culture of all test organisms, namely

Escherichia coli (Gram negative), *Pseudomonas aeruginosa* (Gram negative) and *Bacillus subtilis* (Gram positive), *Staphylococcus aureus* (Gram positive), were obtained through the courtesy of Microbiology Lab, Mahatma Gandhi Institute of applied Science (MGiaS), Jaipur, which were maintained on Nutrient broth media.

Culture of test microbes

For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared using 20 gm Agar, 5 gm Peptone, 3 gm beef extract and 3 gm NaCl in one liter distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 minutes. Agar test plates were prepared by pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8 % NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 hours.

To prepare the test plates, in bacteria, 10-15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts

Crushed powder (50 g) of all the species were successively soxhlet extracted with petroleum ether (60°-80°C), Benzene, Chloroform, Ethyl acetate, Methanol and Distilled water. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness *in vitro* and re dissolved in respective solvents, out of which 80 mg/ 10 disc i.e. 8mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal assay

For both, bactericidal *in vitro* Disc diffusion method was adopted (19), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No.1 paper(6 mm in diameter), which were containing 8 mg of the text extracts, its control (of the respective solvent) and tetracycline as reference drugs separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The inhibition zones in each case were recorded and the activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample / Inhibition zone of standard).

RESULTS AND DISCUSSION

Microscopic Identification:

In microscopic studies, powdered drugs was studied for its starch grain at 10 and 40 x. resultant showed that *Tinospora cordifolia(genuine)* and Giloy satva (market sample) have different starch grains, simultaneously various other starch grains were studied for comparison. The structure of starch grain present in stem powder of *Tinospora cordifolia* are somewhat oval with concentric rings and hilum eccentric but it was surprising to see that slides of market sample resembled to the starch grain of maize powder (*Zea mays)* (Fig. 1 and 2).

Therefore it was proved that the market sample of guduchi satva is adulterated by powder of maize, a cheap adulterant and hence proved that microscopic character can safely be used in the screening and identification of *Tinospora cordifolia* in guduchi satva. Further, this identification character can be safely used in Ayurvedic Pharmacopoeia and check the quality of the drug and enhance its therapeutic potentials.

TLC Chromatograms as fingerprints

Thin Layer Chromatography has been regarded as a simple, rapid and inexpensive method for the separation, identification and semi-quantification of a wide variety of substances. An attempt was made to compare various extracts of *Tinospora cordifolia* and Giloy satva by running the chromo plates in different solvents so as to generate various fingerprints which can later be useful in its standardization.

***Tinospora cordifolia* (Genuine) TLC**

In *Tinospora cordifolia* petroleum ether extract in solvent system – petroleum ether: benzene (1:3) showed three spots on exposure to 10% sulphuric acid were seen at R_f value of 0.09 (purple), 0.21(light pink) and 0.34 (pink); benzene extract in solvent system – benzene : ethyl acetate (1:2) showed seven spots on exposure to 10% sulphuric acid were seen at R_f value of 0.06 (greenish brown), 0.14 (pinkish brown), 0.20 and 0.30 (dark pink), 0.40 (dark pinkish brown), 0.57 (pinkish green) and 0.99(dark green); chloroform extract in solvent system – chloroform: methanol (1:2) showed nine spots on exposure to 10% sulphuric acid were seen at R_f value of 0.04 (dark green), 0.11 (yellowish green), 0.22 (Light yellowish green), 0.36 (parrot green), 0.45 (light

parrot green), 0.59 (light green), 0.63 (very light green), 0.82 (light brownish green) and 0.91(dark brownish green); ethyl acetate extract in solvent system – benzene: ethyl acetate (2:1) showed two spots on exposure to 10% sulphuric acid were seen at R_f of 0.09 (very light yellow) & 0.93 (light yellow); methanol extract in solvent system – chloroform: methanol (2:1) showed four spots on exposure to dragendroff reagent were seen at R_f value of 0.09 (buff orange), 0.60 (light buff orange), 0.85 (buff orange) & 0.97 (dark buff orange) (Fig. 3).

Giloy satva (Market Sample)TLC

In Giloy satva petroleum ether extract in solvent system – petroleum ether: benzene (1:3); benzene extract in solvent system – benzene: ethyl acetate (1:2); ethyl acetate extract in solvent system – benzene: ethyl acetate (2:1); methanol extract in solvent system – chloroform: methanol (2:1) showed no spot on exposure to 10% sulphuric acid; chloroform extract in solvent system – chloroform : methanol (1:2) showed one spot on exposure to 10% sulphuric acid was seen at R_f value of 0.95(light yellow) (Fig. 4).

These results showed that the market sample not possessing bioactives that were present in authentic sample. Therefore, these TLC fingerprints of genuine sample can be safely used in future for generation of chemical libraries.

Antibacterial Efficacy

Various sequential extracts of Guduchi (*Tinospora cordifolia*) and Giloy satva (market sample) were screened for various test microbes and their inhibition zones and activity indexes were calculated.

Antibacterial efficacy of *Tinospora cordifolia*

Antibacterial activity of *Tinospora cordifolia* was performed against *Escherichia coli*, maximum efficacy was exhibited by distilled water extract (IZ – 24mm); *Bacillus subtilis*, maximum efficacy was exhibited by methanol extract (IZ – 11mm), mediocre efficacies were shown by pet ether, benzene, ethyl acetate and distilled water extracts having inhibition zone- 10mm, 9mm, 8mm & 7mm respectively; *Pseudomonas aeruginosa*, maximum efficacy was exhibited by methanol extract (IZ – 12mm); *Staphylococcus aureus*, maximum efficacy was exhibited by distilled water extract (IZ – 17 mm). While, mediocre efficacies were shown by pet ether, benzene, chloroform and ethyl acetate extracts having inhibition zone- 12mm, 9mm, 8mm & 12mm respectively (Fig. 5).

Antibacterial efficacy of Giloy satva market sample

Antibacterial activity of Giloy satva was performed against *Escherichia coli*, maximum efficacy was exhibited by distilled water extract (IZ – 22 mm) and no efficacies were shown by pet ether, benzene, chloroform and ethyl acetate; *Bacillus subtilis*, maximum efficacy was exhibited by methanol extract (IZ – 14 mm) and no efficacies were shown by pet ether, benzene, chloroform and ethyl acetate; *Pseudomonas aeruginosa*, maximum efficacy was exhibited by methanol extract (IZ – 11mm) and no efficacies were shown by pet ether, benzene, chloroform and ethyl acetate; *Staphylococcus aureus*, the maximum efficacy was exhibited by methanol and distilled water (IZ – 13 mm) and minimum efficacy was shown by pet ether, benzene, chloroform and ethyl acetate (Table 1).

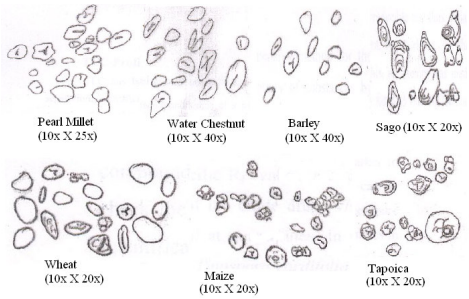

Figure 1: Structure of various types of starch grains

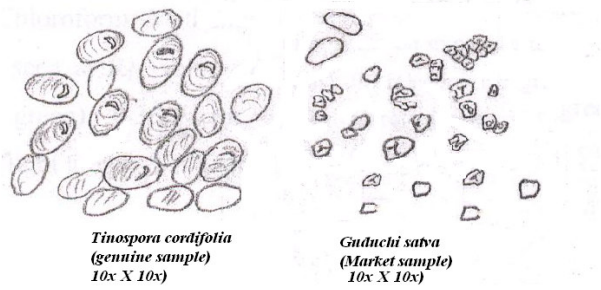

Figure 2: Structure of starch grain of Tinospora cordifolia genuine and market sample

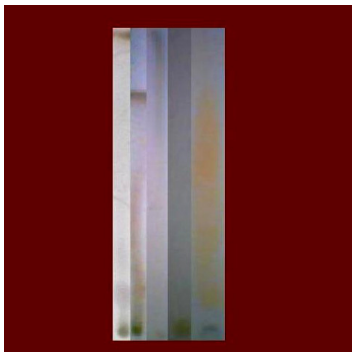

Figure 3: TLC of various successive extracts of Tinospora cordifolia genuine sample.

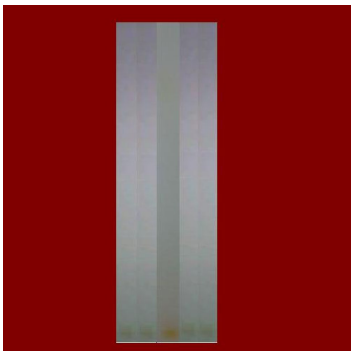

Figure 4: TLC of various successive extracts of Tinospora cordifolia market sample.

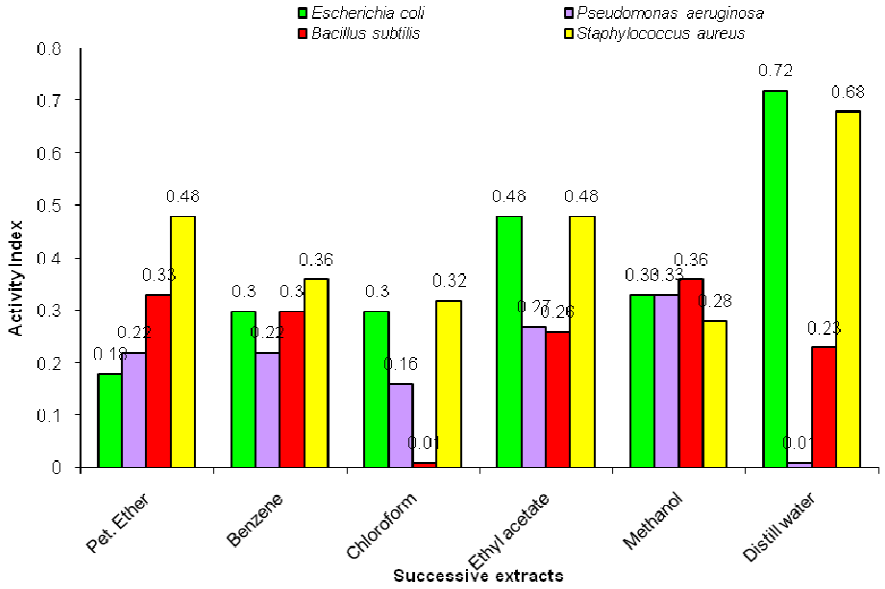

Figure 5: Various successive extracts of Tinospora cordifolia genuine sample exhibiting antibacterial activity in terms of activity index

Table 1: Various successive extracts of Tinospora cordifolia market sample exhibiting very less antibacterial activity in terms of activity index

	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
Pet. Ether	0.01	0.01	0.01	0.01
Benzene	0.01	0.01	0.01	0.01
Chloroform	0.01	0.01	0.01	0.01
Ethyl acetate	0.01	0.01	0.01	0.01
Methanol	0.24	0.34	0.43	0.54
Distill water	0.59	0.28	0.31	0.54

Standardization of ayurvedic drugs and plant materials is the need of the day. Several Pharmacopoeias containing monographs on plant materials describe only the physico-chemical parameters. Hence, modern methods describing the identification and quantification of active compounds in the plant material may be useful for proper standardization of herbs and their formulation. *Tinospora cordifolia* (Menispermaceae) is one such plant which is widely used in indigenous system of medicine. Among the complex mixture of biologically active compounds in the plant, microscopic identification, TLC, and bio efficacies can be used as an analytical characterization to determine the quality of plant material of different sources. It is very surprising that the simple tests which are not useful in identification sometimes become very beneficial for the characterization and validation of important Ayurvedic drugs. Hence, the Pharmacognostic markers are simply an authentic and easy tool for validation of Guduchi satva. The use of safe drug is the motto of our healthy being so

if we use the correct parameters for the identification of the drug it will be very useful for Ayurvedic drugs to not only enhance their quality and efficacy but also their therapeutic potentials as drugs.

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Evaluation and Identification of Meda and Mahameda to Common Substitutes and Adulterants In the Crude Drug Markets of India

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ABSTRACT –
Meda (Polygonatum verticiilatum (L.) Alt.) and Mahameda (Polygonatum cirrifolium (Wall.) Royle) are described as Astavargha group of drugs having antioxidant and anti-ageing effect in ancient texts of ayurveda but the botanical identity of Meda and Mahameda are controversial and different plant species are being used or sold in the name of Meda and Mahameda in different parts of India. In present paper efforts have been made to lay down pharmacopoeial characters and to identify botanical identity of market samples by comparing with genuine drugs.
KEYWORDS: *Macroscopy, Microscopy, Thin Layer Chromatography, Physico-chemical evaluation.*

INTRODUCTION

In Charak samhita *Meda* and *Mahameda* are components of *Jivaniyagana*[1] while 'Susruta' treated them under *Kakolyadigana* to promote longevity[2] but in modern period, these are considered under *Ashtavarga*[3] and are attributed to immunomodulators and tonics. These are widely used in fever; as restorative, galactogogue, blood purifier and spermatogenic[4].

In ayurveda rhizomes of *Polygonatum verticiilatum* (L.) Alt. (syn. *Convallaria verticillata* L., syn. *Evallaria verticillata* Necker) and *Polygonatum cirrifolium* (Wall.) Royle of family Liliaceae[5] are used as *Meda* and *Mahameda*. However it was observed that in different places, different plant species are being used as *Meda* and *Mahameda*. Hence there is an urgent need to evaluate the genuine drug and to identify which botanical species are being used / sold as substitute / adulterants[6].

In this context the detailed pharmacognostical study along with preliminary physicochemical study on roots and rhizomes of *P. verticillatum* and *P. cirrifolium* have been undertaken in order to establish salient diagnostic features of both the species and efforts have also been made to identify botanical equivalent of commercial samples procured from different crude drug markets of the Country.

MATERIAL AND METHODS

Fresh roots and rhizomes of botanically identified plants of *Polygonatum verticillatum* and *Polygonatum cirrifolium* were collected from medicinal plants garden of Regional Research Institute (Ayu.), Tarikhet and Indian Medicines Pharmaceutical Corporation Limited, Mohan (Almora). Roots and rhizomes were washed, cut into pieces and preserved in Formalo-acetyl-alcohol (FAA) and labeled PV5 and PC5 for pharmacognostical study and some were shade dried and coarse (20 to 30 #) powdered for qualitative tests and Physico-chemical study as per IP/API / WHO guidelines. The physicochemical parameters like total ash, acid insoluble ash, water and alcohol soluble extractives of all the samples were carried out according to IP procedures[7]. The thin layer chromatography of 90 % ethanolic extract of all samples were performed on pre-coated silica gel 60 F_{254} aluminum plates and the plates were developed in mobile phase Toluene: Ethyl acetate: Glacial acetic acid (5:6:4:1:0.3). The developed plates were observed under UV 254 nm and 366 nm and after derivatization in iodine vapours.

Market samples PV1 , PV2, PV3, PV4 and PC1, PC2, PC3, PC4 were collected in the name of *Meda* and *Mahameda* from Lucknow (Uttar Pradesh), Jaipur (Rajasthan), Mandi (Himachal Pradesh), New Delhi, respectively and compared Pharmacognostically as well as phytochemically with authentic drugs.

RESULTS

Pharmacognosy of *Polygonatum verticillatum* Alt.

Macroscopic Characters

The dried rhizomes branched irregularly, about 5 cm long and 1.5 cm thick, cylindrical – knotty, dorsiventrally flattened with blunt to tapering ends. The outer surface mostly dull reddish-brown to light grayish in colour, marked with numerous encircling leaf scars and show root scars and wiry adventitious roots. Hard in texture, fracture short and horny, internally deep reddish-brown with characteristic odour of burnt sugar and acrid in taste.

Microscopic characters

The T.S of adventitious roots (Figure 1a-b) show a single layered epidermis interrupted by unicellular hairs , the size of the hair varies between 45-355 µm followed by 12-16 layers of varying sizes (75-140-355 µm) at parenchymatous cortex. A few cortical cells contain bundles of raphides of calcium oxalate. The schizogenous mucilage canals lined by epithelium of 5-8 cells and varying in sizes (18-45-66 X 21-54-70) µm also present in the cortex. The endodermis distinct showing well developed thickening on the inner tangential and radial walls, a few thin walled passage cells are also observed.

The pericycle thin walled and encloses within it a polyarch stele.

The T.S. of rhizome (Figure 1c-d) shows 6-9 layers of cork cells (12-24-54 X 15-45-60) µm with lenticels at places. The cork followed by wide parenchymatous ground tissue, the outer region of which shows only large mucilaginous canals (90-120-240 µm). In the middle and central regions, a large number of vascular bundles present. The vascular bundles are mostly of amphivasal type but a few showing typical conjoint collateral arrangement and both type being enclosed by bundle sheath, mucilage canals also distributed in this region. The phloem consists of sieve-tubes, companion cells and phloem parenchyma and xylem mostly consists of tracheids, vessels and a few fibres. The vessels and tracheids greatly vary in shape and sizes mostly showing either peg like outgrowth or irregular lateral projections and scalarifolium thickening. However, few shows spiral thickening on their walls.

Pharmacognostical Studies of *Polygonatum cirrifolium* Royle

Macroscopic Characters

The dried rhizomes branched 3-5 cm long and 0.4-0.7 cm thick, somewhat conical, dorsiventrally flattened showing a distinct groove or furrow on one of the flattened side. few pieces show fused pear shaped or irregularly oblong rhizomes in pairs. Rough surface, wrinkled with indistinct leaf scars and root scars or wiry adventitious roots as in *P. verticillatum* observed. Grayish-brown in colour and show beak like tapering ends, fracture short exposing deep reddish brown waxy interior, taste sweetish becoming acrid afterwards and burnt sugar odour.

Microscopic Characters

The T.S of adventitious roots (Figure 2a) showed similar structure as in the roots of *P. verticillatum* except absence of the bundles of raphides of calcium oxalate in the cortical cells of size (12-30-45 X 9-21-39 µm) and the meta-xylem at the periphery of pith of pith cell size (5-12-16 X 9-18-21 µm).

The T.S. of rhizome (Figure 2b) showed 5-10 layers of cork cells (15-21-45 X 45-90-120 µm) with lenticels at places. Cork followed by wide zone of parenchymatous ground tissue, the outer region has large mucilage canals (124-165-180-210 µm) surrounded by 7-8 epithelial cells and bundles of raphides of calcium oxalate (27-50-90 X 42-70-115 µm). The middle and central region of ground tissue, however, exhibits a large number of scattered vascular bundles. The vascular bundles mostly of amphivasal type but few have conjoint, collateral arrangement as in *P. verticillatum* and both types being enclosed by a bundle sheath of thin walled cells. Mucilage canals also scattered in this region. The phloem consists of normal elements. The xylem shows conspicuous absence of xylem fibres. The vessels and tracheids have mostly reticulate thickening on their walls and are without any peg like out growth or irregular lateral projections as in *P. verticillatum*. However, an abrupt narrowing in the middle region observed.

Comparative Studies of Market samples

The detail macro and microscopical characters of all the market samples PV1 – PV4 & PC1-PC4 were studied and compared with both *Polygonatum* species. and it was observed that samples PV2 and PV4 shows more or less similar macro and microscopical details with that of *P. verticillatum* however some part of the stem pieces are found along with root and rhizomes of *P. verticillatum* in the market sample PV3, which are remain absent in other samples. Therefore, the sample PV3 is found to be adulterated one**.** The Physicochemical analysis (Table 1) and TLC of 90 % ethanolic extract (Figure 3) of powders of all the samples were carried out and the observations compared.

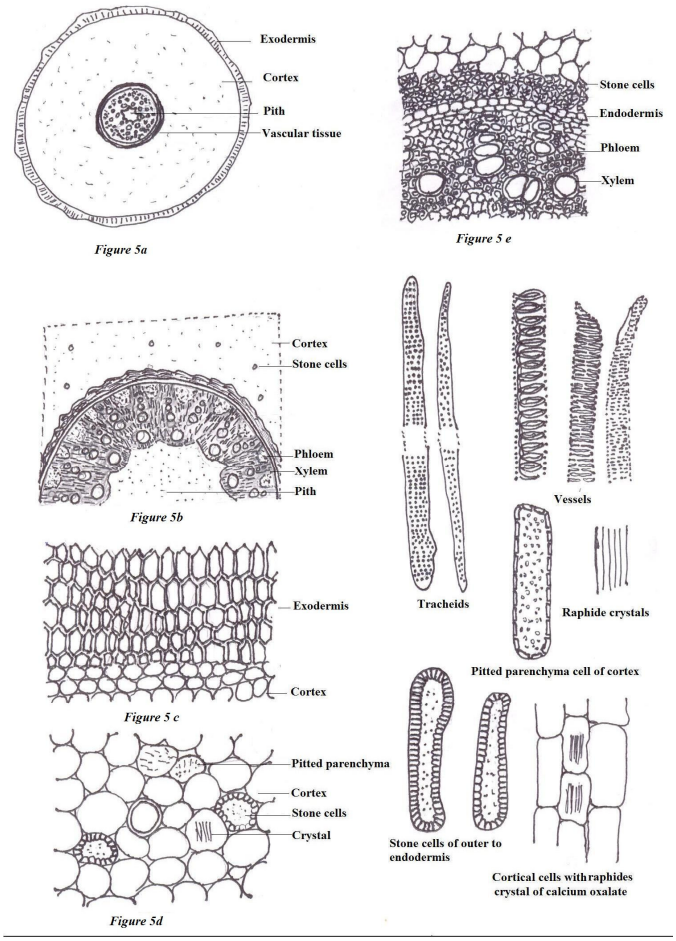
The pharmacognostic characters of PC1 and PC2 are identified same as *P. cirrifolium*. Sample PC4 resembles the pharmacognostic characters of *Asparagus racemosus* (Figure 5a-e).

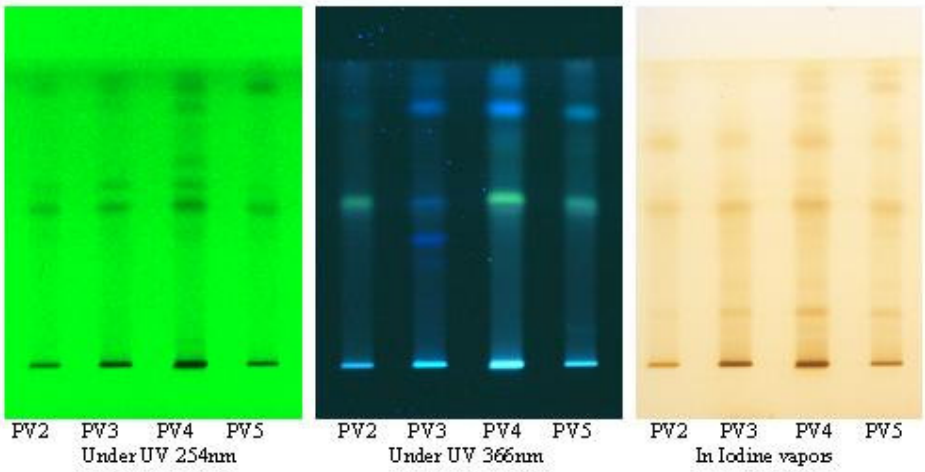
Table 1: Observations Physicochemical parameters of powdered samples of Meda

Parameters %		PV5 *Polygonatum verticillatum*	PV2	PV3	PV4
Total Ash (% w/w)		3.32	5.32	4.89	4.02
Acid insoluble ash (% w/w)		0.83	1.54	0.73	0.90
Water soluble ash (% w/w)		1.23	1.82	1.83	1.43
Water soluble extractive (% w/w)		49.51	43.44	38.08	48.25
Alcohol soluble extractive (% w/w)		33.13	29.54	25.05	35.54
TLC (Fig. 3)	Under UV 254 nm (R_f Values)	0.51,0.59, 0.93	0.51, 0.93	0.51, 0.59, 0.93	0.51, 0.59, 0.66, 0.84, 0.93
	Under UV 366 nm (R_f Values)	0.52, 0.82, 0.93	0.52, 0.80	0.40, 0.51, 0.82, O.92	0.40, 0.51, 0.82, O.92
	In Iodine vapors under white light (R_f Values)	0.18, 0.51, 0.73, 0.91, 0.94	0.18, 0.51, 0.72	0.18, 0.51, 0.72	0.12, 0.18, 0.51, 0.59, 0.72, 0.83, 0.91, 0.94

Table 2: Observations Physicochemical parameters of powdered samples of Maha-Meda

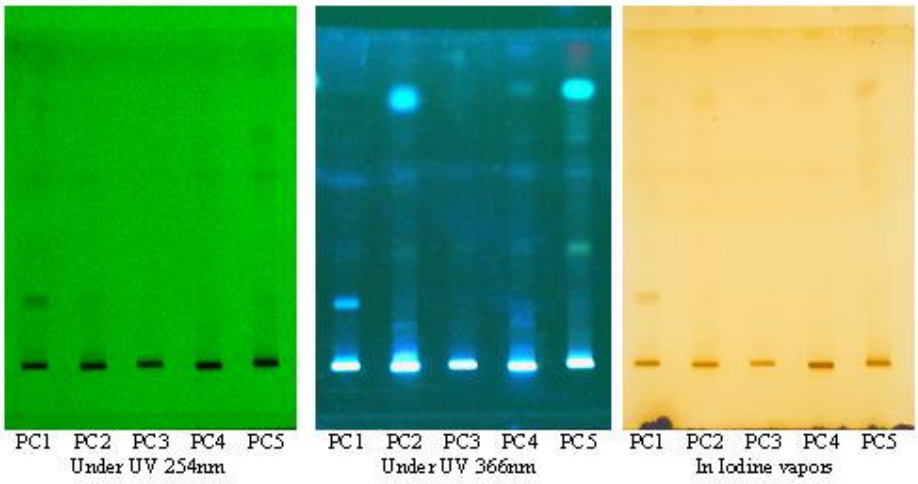
Parameters %		*Polygonatum cirrifolium*	PC1	PC2	PC3	PC4
Total Ash (% w/w)		2.9	6.54	3.5	6.52	5.0
Acid insoluble ash (% w/w)		0.78	2.78	1.52	1.34	0.5
Water soluble ash (% w/w)		1.12	2.12	1.90	2.83	2.60
Water soluble extractive (% w/w)		73.52	65.08	70.22	48.60	37.4
Alcohol soluble extractive (% w/w)		13.82	10.02	11.30	12.36	20.7
TLC (Fig.4)	Under UV 254 nm (R_f Values)	0.20, 0.55, 0.70	0.20, 0.55	0.20, 0.55	-	0.55
	Under UV 366 nm (R_f Values)	0.11, 0.20, 0.35, 0.55, 0.68, 0.80, 0.95	0.20,0.35, 0.55	0.11, 0.20 0.35, 0.55, 0.80	0.35, 0.55	0.11,0.35 0.55,0.80
	In Iodine vapors under white light (R_f Values)	0.20, 0.55, 0.70, 0.80	0.55,0.80	0.55, 0.80	0.55	0.55





Mobile Phase: Toluene: Ethyl acetate: Glacial acetic acid (5.6: 4.1 : 0.3)

Figure 3: TLC fingerprint of 90 % ethanolic extract of samples



Mobile Phase: Toluene: Ethyl acetate: Glacial acetic acid (5.3: 4.2 : 0.5)

Figure 4: TLC fingerprint of 90 % ethanolic extract of samples

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LEGENDS:

Samples of *Polygonatum verticillatum* from
PV2 :Jaipur (Rajasthan),PV3 : Mandi (Himachal Pradesh), PV4 : New Delhi, PV5 : Tarikhet (Uttarakhand)
Samples of *Polygonatum cirrifolium* from
PC1: Lucknow (Uttar pradesh) PC2 : Jaipur (Rajasthan),PC3 : Mandi (Himachal Pradesh), PC4 : New Delhi, PC5 : Tarikhet (Uttarakhand)

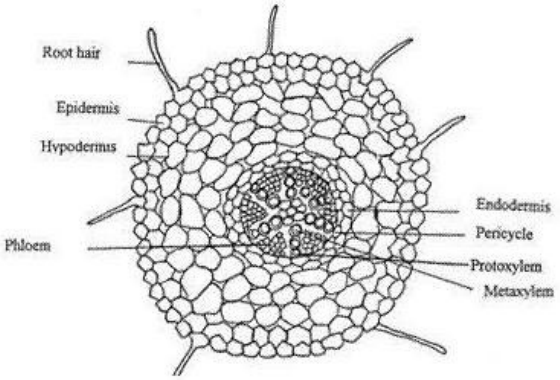


Figure 2a : T.S. of adventitious root of P. cirrifolium (diagramatic)

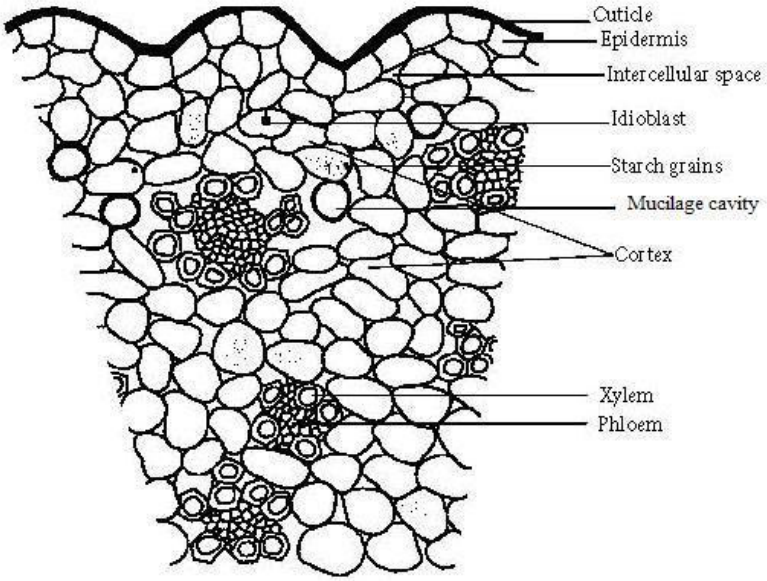
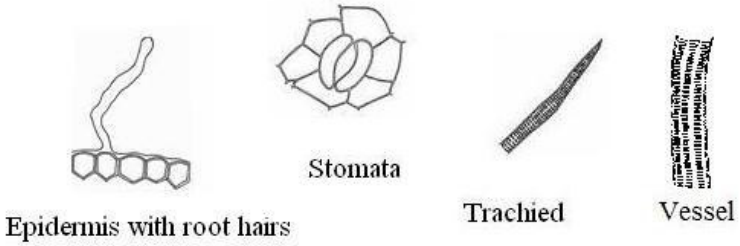


Figure 2b : T. S. of rhizome of P. cirrifolium (cellular details)



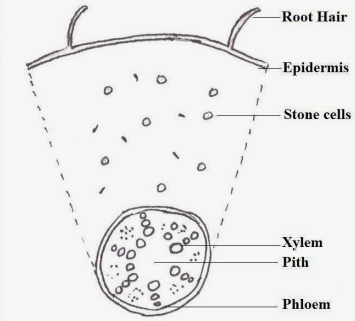


Figure 1a

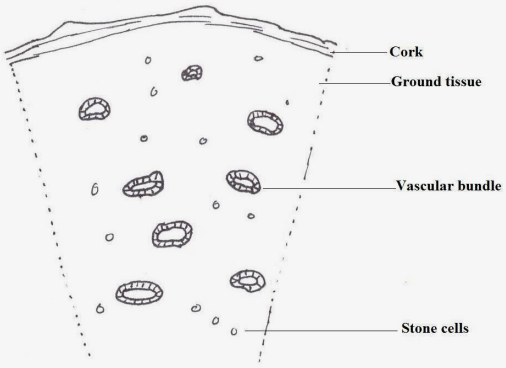


Figure 1c

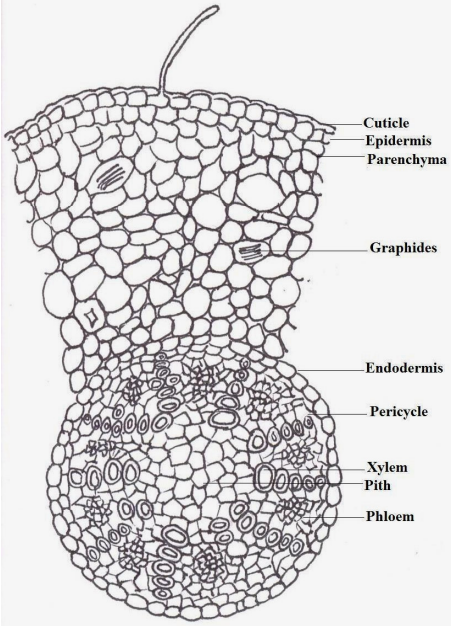
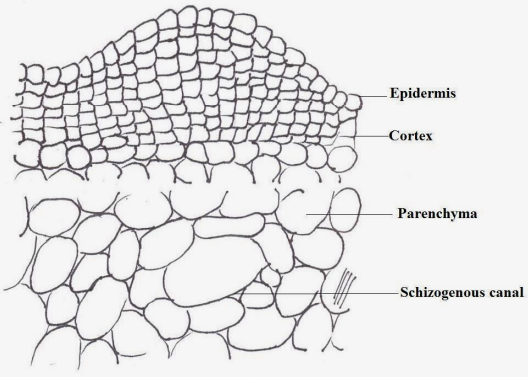
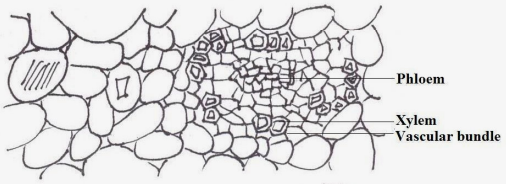
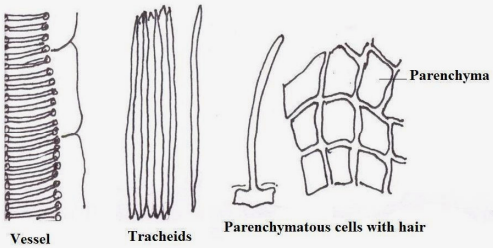


Figure 1b







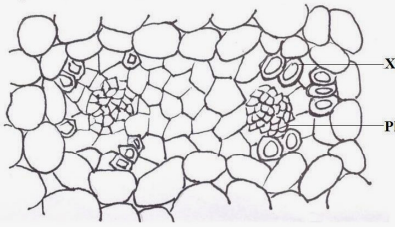


Figure 1d

Legends:

Figure 1a: T.S. of adventitous root of Polygonatum verticillatum Alt. (diagrammatic)

Figure 1b: T.S. of adventitous root of Polygonatum verticillatum Alt. showing cellular details

Figure 1c: T.S. of rhizome of Polygonatum verticillatum Alt. (diagrammatic)

Figure 1d: T.S. of rhizome of Polygonatum verticillatum Alt. cellular details

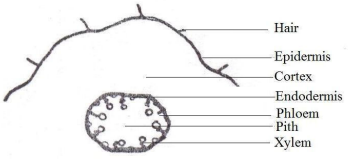


Figure 6a

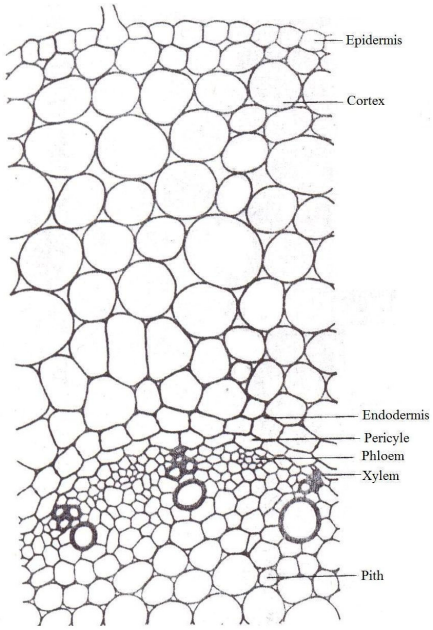


Figure 6b

Legends: Figure 6a: T.S. portion of Eulophia campestris Adventitous root (diagrammatic),
Figure 6b: T.S of adventitious root of E.campestris showing cellular details.

Market sample PC3 is identified as rhizomes of *Asparagus racemosus* admix with pieces of roots of *Eulophia campestris* Wall. The T.S. of adventitious roots (Figure 6a-b) of *E. campestris* show a single layered epidermis, some of the cells of which elongate to form unicellular hairs (380-450 – 570 µm).

DISCUSSION AND CONCLUSION

From on going studies it has been revealed that certain characters are common in both the species like in roots presence of wide cortex, endodermal cells showing thickening on radial and inner tangential walls except in thin walled passage cells and a polyarch stele which is only present in *P.verticillatum* similarly rhizomes show wide ground tissue in which a large number of mucilage canals and vascular bundles scattered in both the species. However, both the species can also be distinguished from each other for example in *P. verticillatum* 6-9 layers of cork cells, wide parenchymatous ground tissue, the outer region of which shows only large mucilaginous canals, large number of vascular bundles mostly being enclosed by bundle sheath, vessels and tracheids greatly vary in shape and sizes with either peg like outgrowth or irregular lateral projections and scalarifolium thickening present in rhizome. However number of cork cells 5-10 layers, large mucilage canals surrounded by 7-8 epithelial cells and bundles of raphides of calcium oxalate, number of scattered vascular bundles and absence of fibers in xylem observed in *P. cirrifolium.*

Like the anatomical structure of roots of *Asparagus racemosus* is quite characteristic and can be differentiated by having the cells of innermost layer are thick walled, lignified with simple pits, the bundles of raphides of calcium oxalate are present in cortex and the primary stele is polyarch from both the Polygonatum species

The observation of physico-chemical evaluation indicates that most of the drugs available in market are not genuine or some what adulterated which leads to deteriorate the quality and efficacy of drug.

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PHCOG J: Research Article

Phytochemical screening and antimicrobial efficacy of extracts from *Averrhoa bilimbi* (Oxalidaceace) fruits against human pathogenic bacteria

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ABSTRACT

Averrhoa bilimbi fruits have medicinal properties for the effective management of several human ailments. To enhance the pharmacological study for it traditional uses, the fruits were extracted with three different solvent with various polarities which is hexane, chloroform and methanol. All fractions were subjected to the phytochemical screening and antimicrobial activity against gram-positive and gram-negative bacteria using disc-diffusion methods. The preliminary phytochemical screening on the fruits extracts revealed the presence of flavonoids, saponins and triterpenoids but no alkaloids. The chloroform and methanol fruits extract were active on *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Saccharimyces cerevisiae, Straphylococcus aureus, Streptococcus agalactiae and Bacillus subtilis*. The results of our antibacterial study revealed that the fruits extract showed a good inhibitory activity against the tested pathogens compared with the standard antibiotics which is streptomycin. In conclusion, the *A. bilimbi* fruits posses a potential antibacterial activity that requires through this study.

Keywords: *Averrhoa bilimbi*, Oxalidaceace, antimicrobial activity, medicinal plants

INTRODUCTION

Averrhoa bilimbi (Oxalidaecea) is locally name as "belimbing buluh" among Malaysian (Morton, 1987). The *A. bilimbi* is native to the Moluccas (Indonesia) but is now cultivated in many other places in the tropics. *A. bilimbi* has sour taste but there's *A. bilimbi* with sweet taste found in Phillipines. *A. bilimbi* is a tropical tree, prefers seasonally humid climates and rainfall should be rather evenly distributed throughout most of the year but there should be a 2 till 3 month dry season. The tree makes slow growth in shady or semi shady situations. It should be in full sun. Its geographic distribution can divide to 2 parts, native and exotic. Only Indonesia and Malaysia are classified as native while others such as Argentina, Australia, Brazil, Colombia, Cuba, Ecuador, Guyana, India, Jamaica, Myanmar, Philippines, Puerto Rico, Singapore, Sri Lanka, Surinam, Tanzania, Thailand, Trinidad and Tobago, United States of America, and Venezuela are classified as exotic (Anon, 2007). *A. bilimbi* is generally regarded because it is too acidic for eating raw. As it contains large amount of juice about 40%, it is use to make cooling beverages. It also can be use to make jam or acid jelly other than act as preservative in food (Diliman, 1971).

In Malaysia, *A. bilimbi* fruits are used in various ways such as to treat skin disorders and fever. The leaves of *A. bilimbi* are used as a treatment for venereal disease and are taken as a medicine to relieve rectal inflammation (Anon, 2007). It seems to be effective against coughs and thrush. The Malay also belief leaf can cure diabetic. The root is use as tonic (Goh et al., 1995). In India, the leaf is applied on itches, pimples and treats gout (Latif, 2002). It also uses to relieve rectal inflammation. Flower infusion is useful in cough and thirst. The fruit are good remedy for scurvy and beneficial in diarrhea, hepatitis and in inflammatory condition (Goh et al., 1995). Other than that, in Indonesia, the local use *A. bilimbi* to treat goiter, cough, sore throat, and rheumatism. In Philippines, *A. bilimbi* is use to treat scabies and are applied on bites of poisonous creatures (Goh et al., 1995). There are few studies have been done previously on phytochemical screening and antimicrobial using *A. bilimbi* extracts. Therefore, this study was focused on the antimicrobial properties of the extract from the plant on some microorganism. Different approached had been use to enhance the research.

MATERIALS AND METHODS

PLANTS MATERIALS

Fresh fruits of *A. bilimbi* were collected from few villages near Gong Badak, Kuala Terengganu. Identifications were done by the science officer of Biological Department, Tuan Haji Razali.

BACTERIA CULTURES

Microorganisms tested in this study were *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Saccharimyces cerevisiae, Straphylococcus aureus, Streptococcus agalactiae and Bacillus subtilis*. All this pathogenic bacteria were obtained from a general hospital in Kuala Terengganu and were re-identified using API20E and API20NE system before the antimicrobial assays were conducted.

EXTRACTION

The fruits of *A. bilimbi* were air-dried for 48 h at the room temperature, chopped into small pieces and then soaked with three types of solvent with increasing polarity at room temperature. At early extraction, the sample was extracted with hexane for three days. The extract was filtered and the remaining was extracted again with chloroform for three days. The same step was repeated using methanol. The entire extract was concentrated to dryness using rotary evaporator under reduced pressure.

PHYTOCHEMICAL SCREENINGS

Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992). By this analysis, the presence of the several phytochemicals like alkaloids, flavonoids, saponins, steroids and terpenoids were tested.

ANTIBACTERIAL STUDY

The stock cultures were maintained on nutrient agar (NA) and subculture in nutrient broth (NB) for incubation at 37^{o}C overnight. The antibacterial activity was taken by considering the zone of growth and inhibition of the organisms by the test fractions. Streptomycin (10µg) was used as standards.

RESULTS

The phytochemical analysis of the fruit extract revealed the presence of flavanoid, saponin, triterpenoid and steroid. While alkaloids compound were not found in all crude extracts. Full results as presented in Table 1. From the presented table (Table 1), noted that flavanoid and saponin compounds were present in all crude. The test gave a negative result for determinations of alkaloids existence. This observation also indicated that only on chloroform extract shown a presence of triterpenoid compounds.

The results of antibacterial activities in terms of zone of inhibition (mm) were presented in Table 2 (24 h) and Table 3 (48 h). The antibacterial activities of *Averrhoa bilimbi* extracts were tested against seven bacterial strains. The results showed promising antibacterial activity against the bacteria tested. Among these, chloroform and methanol extracts were found to have a more potent inhibitory effect than hexane extracts. The observed activity may be due to the presence of potent phytoconstituents in the fruit extracts.

Generally, *Staphylococcus aureus* was observed to be the most susceptible organism t, while *Streptococcus agalactiae* was the least susceptible using chloroform extrac. The susceptibility of *Staphylococcus aureus* to the chloroform and methanol crude extracts of this species is an indication of the potential of the extract as a drug that can be used against this organism. Hexane extracts and *Saccharomyces cerevisiae* did not show any appreciable activity against the entire test. For *Bacillus subtilis*, at 24 hours, there are no inhibition zones for chloroform and methanol extracts. But after 48 hours they have inhibition zones that show positive results. It means that the rate to combat the *Bacillus subtilis* is slow.

DISCUSSION

The phytochemical compounds are known to play an important roles bioactivity of medicinal plants. Flavonoids exhibit as anti-inflammatory, anti-allergic, analgesic and antioxidant as well. Rather than that, flavonoids also exhibit a wide range of biological activities such as scanvenge for hydroxyl radicals. The presence of the saponin compound in this plant which is supported the usefulness of this plant in the managing inflammation. Other than fruits, the research also focused on the leaves, stems and barks of *Averrhoa bilimbi* extracts (using different extracts), for the presence of some phytochemical substances and pharmacognostic studies.

The acceptable range for control (streptomycin) is 17 – 24 mm by BSAC standard. All of the control is in acceptable range, so the results for this antimicrobial test should prove as significance results. The chloroform and methanol crude extracts showed the most significant zone of inhibition against all the bacteria. Inhibition zone were observed in almost all bacterial candidates except for the hexane crude extracts. The hexane crude extracts were not active against the bacteria compared to the chloroform and methanol crude extracts. It does not show any result in any tested bacterial.

Table -1: Preliminary screening of secondary metabolites from fruits extract.

Extract	Hex	$CHCl_3$	MeOH
Alkaloid Test	-	-	-
Flavanoid Test	+	+	+
Saponin Test	+	+	+
Triterpenoid Test	-	+	-
Steroid Test	-	-	-

\- : Negative result
\+ : Positive result

Table – 2: Antimicrobial activity of different extracts of A.bilimbi fruits against human pathogenic bacteria (zones of inhibition in mm) after 24 h

Sample	Fraction	Microorganism						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Averhoa bilimbi fruits	Hexane	-	-	-	-	-	-	-
	Chloroform	11	10	10	-	14	9	-
	Methanol	13	12	10	-	18	10	-
Streptomycin (control)		19	20	19	-	24	21	22

Table -3: Antimicrobial activity of different extracts of A.bilimbi fruits against human pathogenic bacteria (zones of inhibition in mm) after 48 h

Sample	Fraction	Microorganism						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Averhoa bilimbi fruits	Hexane	-	-	-	-	-	-	-
	Chloroform	10	10	10	-	12	9	10
	Methanol	13	11	10	-	18	10	12
Streptomycin (control)		20	20	18	-	13	22	22

1 : *Aeromonas hydrophila*
2 : *Escherichia coli*
3 : *Klebsiella pneumoniae*
4 : *Saccharimyces cerevisiae*
5 : *Straphylococcus. aureus*
6 : *Streptococcus agalactiae*
7 : *Bacillus subtilis*

CONCLUSION

In conclusion, the preliminary phytochemical screening of the fruits of *Averrhoa bilimbi* indicates the presence of secondary metabolities, having an essential role in medicine. Overall, this study indicates the antibacterial properties of *Averrhoa bilimbi* and provides some idea about phytochemical investigation on *Averrhoa bilimbi*. This study paves the way for further attention/research to identify the active compounds responsible for the plant biological activity.

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PHCOG J: Research Article

Pharmacognostic and Phytochemical Investigation of *Cordia dichotoma* Seeds

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***ABSTRACT**:*
Cordia dichotoma L. family Boraginaceae which is taken as food and the immature fruits are pickled and are also used as vegetable, has antidiabetic properties also. The plant is used for treatment for delaying effects of aging on skin. The current study is therefore carried out to provide requisite pharmacognostic detail about the plant. Pharmacognostic investigation of the anatomical section of the dried seeds as well as powder study was carried out to determine its morphological, anatomical and phytochemical diagnostic features. Physicochemical properties and qualitative phytochemical measures were established. The result of the study can be useful in setting some diagnostic indices for the identification and the preparation of the monograph of the plant.
***KEYWORDS**: Cordia dichotoma, Pharmacognostic investigation, Phytochemical screening*

INTRODUCTION

Diabetes is a multifactorial disease leading to several complications, and therefore demands a multiple therapeutic approach. Patients of diabetes either do not make enough insulin or their cells do not respond to insulin. In case of total lack of insulin, patients are given insulin injections. Whereas in case of those where cells do not respond to insulin many different drugs are developed taking into consideration possible disturbances in carbohydrate-metabolism. For example, to manage post-prandial hyper-glycaemia at digestive level, glucosidase inhibitors such as acarbose, miglitol and voglibose are used. These inhibit degradation of carbohydrates thereby reducing the glucose absorption by the cells. To enhance glucose uptake by peripheral cells biguanide such as metformine is used. Sulphonylureas like glibenclamide is insulinotropic and works as secretogogue for pancreatic cells. Although several therapies are in use for treatment, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc. Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy.

Medicinal plants are being looked upon once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated. Some of the herbs which has been investigated recently are *Artemisia pallens, Bombex ceiba, Cajanas cajan, Citrullus colocynthis, Eugenia jambolana, Ipomoea batatas, Tinospora cordifolia* etc. All these herbs have been found significant in treating diabetes. One such herb having the antidiabetic property is *Cordia dichotoma.*

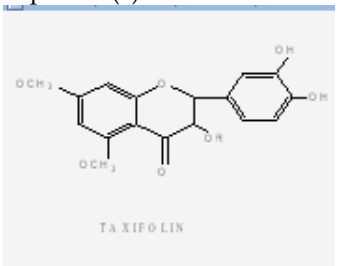
Cordia dichotoma L. family Boraginaceae is a medium sized tree with short crooked trunk; leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with a round and cordate base; flower white, small in lax terminal or axillary cyme; fruits drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part. The plant part used is bark, leaves and fruits (1).

It is also known by various other names; for example Bird Lime Tree (common name), Sebesten Plum, Indian cherry (English), Lasora, Lasura (Hindi) and Slesmatakah (Sanskrit) (2).

It is a tree of tropical and subtropical regions. It grows in the sub-Himalayan tract and outer ranges,

ascending up to about 1500 m elevation. It is found in a variety of forests ranging from dry deciduous forest of Rajasthan to the moist deciduous forest of Western Ghat and tidal forest of Myanmar. In Maharastra, it grows in moist monsoon forest also. It does not grow gregariously, but is found growing singly in moist shady ravines and valley. In areas with annual rainfall less than 500 mm, it thrives along streams or depressions where moisture is available.

It is taken as food. The immature fruits are pickled and are also used as vegetable. The rural people of coastal areas of Orissa eat the ripe fruits raw. The leaves contain 12-15% crude protein, 16-27% crude fibres, 42-53% nitrogen free extract, 2-3% ether extract, 13-17% total ash, 2-4% total calcium and about 0.3% phosphorus. The seed kernels of *C. dichotoma* contains high proportion of fatty oils and proteins (46 and 31% respectively) which has potential as cattle feed. The fruits also contain moisture 74, proteins 2, fats 2, crude fibres 2, total available carbohydrate 92 and ash 2 gm /100gm, Ca 55, P 275, Zn 2, Fe 6, Mn 2, Cr 0.2, Cu 1.6 mg/100 gm. Chromium is of therapeutic value in diabetes. The anti nutritive factor: phytic acid 355, phytate phosphate 100 and oxalic acid 250 mg/100gm are also reported from fruits. The mucilage of fruits has specific gravity 0.99, pH 4.5 and surface tension 64 dynes/cm (1). *Cordia dichotoma* seeds has disclosed the presence of α-amyrins, betulin, octacosanol, lupeol-3-rhamnoside, β-sitosterol, β-sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhamnoside and hesperitin-7- rhamnoside.The mucilage could sustain release of drug through tablet up to 11-12 hrs. The seeds contain alpha amyrins and taxifolin 35, dirhamnoside which show significant anti-inflammatory activity (71.4%, 67.8% respectively) by an oral dose of 1 gm/kg in albino rats (3). Crude extract of *C. dichotoma* suppresses larval hatching of Meloidogyne incognita. Seeds of the species are anti-inflammatory, 2 compounds alpha amyrins and 5-dirhamnoside have been isolated. The bark is medicinal and several chemicals have been identified including Allantoin, beta-sitosterol and 3', 5-dihydroxy-4'-methoxy flavanone-7-O-alpha-L-rhamnopyranoside. The seed kernel has medicinal properties (4).



α-AMYRIN

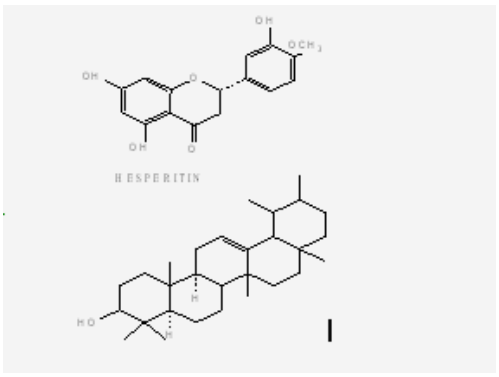


Fig 1: Cordia dichotoma- Chemical constituents

MATERIAL AND METHODS:

Collection:

The fruits of *Cordia dichotoma* were collected from the local market of Lucknow and were authenticated as *C. dichotoma* (Boraginaceae) by pharmacognostic evaluation and a voucher specimen was deposited at National Botanical Research Institute Lucknow, for future reference (voucher no. NBRI/CIF/Re./08/2008/32)

Morphological properties:

Organoleptic evaluation of drugs refers to the evaluation of drugs by colour, odour, size, shape, taste and special features including touch and texture etc. They are of primary importance before any further testing can be carried out. Organoleptic evaluations can be done by means of organs of sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity (5)

Anatomical properties:

Seeds were powdered and fine powder was taken up for microscopical evaluation using stains like phloroglucinol and HCl, sudan red III, ruthenium red, saffranin, iodine and acetic acid etc and was mounted with glycerine (6).

Physico-chemical Analysis (7)

Physico-chemical Analysis was performed using standard procedures (Table 1)

Extractive values (8)

Extractive values were determined by systematic solvent extraction using standard procedure (Table 2)

Successive extraction with various solvents:

The successive extraction was carried out with soxhlet using petroleum ether, chloroform and methanol and the extract was dried using rotary evaporator and percentage extractive value was determined (Table 3)

Preliminary Phytochemical Screening (9)

Extracts of pulverized Seeds of *Cordia dichotoma* with Petroleum ether, Chloroform and Methanol were subjected to qualitative chemical tests for the detection of major chemical groups (Table 4)

Chromatographic evaluation (10)

The Thin layer chromatography studies of various extract of *Cordia dichotoma* seeds confirmed the presence of triterpenoids, flavanoids and steroids and to ascertain the possible number of different constituents, the extracts were subjected to TLC by using the solvent systems specific for different class of compounds (Table 5-7).

Table 1: Physico-chemical Analysis of C. dichotoma seeds

ASH VALUES	PERCENTAGE (%) w/w
Total ash value	0.16-0.27
Acid insoluble ash	0.35-0.55
Water soluble ash	0.15-0.65
Moisture content	12

Table 2: Extractive values of C. dichotoma seeds

EXTRACTIVE VALUES	PERCENTAGE (%) w/w
Water soluble	0.52-1.04
Alcohol soluble	0.52-1.36

Table 3: Successive extraction with various solvents of C. dichotoma seeds

EXTRACTS	PERCENTAGE (%) w/w
Petroleum ether	0.16
Chloroform	0.18
Methanol	0.77

Table 4: Preliminary Phytochemical Screening of seeds of C. dichotoma

Extract	Alkaloid	Amino acid	Steroid/Tri-terpenoid	Tannin	Flavanoid	Glycoside
Methanol	-ve	-ve	+ve	-ve	+ve	+ve
Chloroform	+ve	-ve	+ve	-ve	+ve	-ve
Petroleum ether	+ve	-ve	+ve	-ve	-ve	-ve

Table 5: TLC profile of the extracts for steroidal substances

EXTRACT	SOLVENT SYSTEM	RATIO	NO. OF SPOTS	R_f VALUES
Petroleum ether	Chloroform : Dichloromethane :Benzene	2:2:6	4	0.44, 0.638, 0.77, 0.88

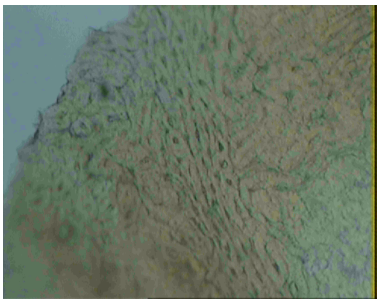
Table 6: TLC profile of the extracts for alkaloidal substances

EXTRACT	SOLVENT SYSTEM	RATIO	NO. OF SPOTS	R_f VALUES
Chloroform	Benzene: Acetone	2:1	7	0.11, 0.176, 0.27, 0.42, 0.576, 0.65 0.941

Table 7: TLC profile of the extracts for flavanoidal substances

EXTRACT	SOLVENT SYSTEM	RATIO	NO. OF SPOTS	R_f VALUES
Methanol	Chloroform: Methanol	90 : 10	4	0.154, 0.31, 0.871, 0.969

Fig 1 Cordia dichotoma seeds-Morphology



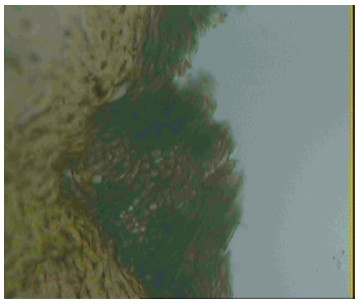
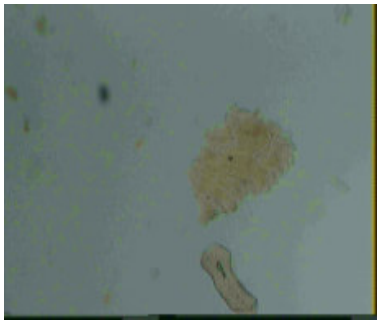


Fig 2 (a & b) T.S of Cordia dichotoma seeds (showing parts of the mesocarp and endosperm)



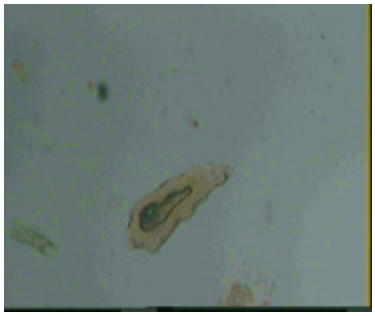
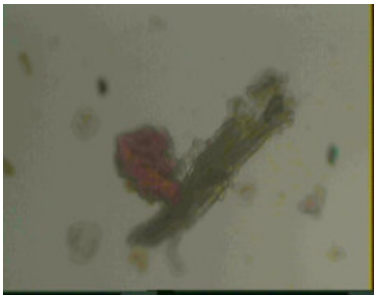


Fig 3 C. dichotoma seeds -powder microscopy (cluster of sclereid cells and single sclereid) (Lab star camera x 400)



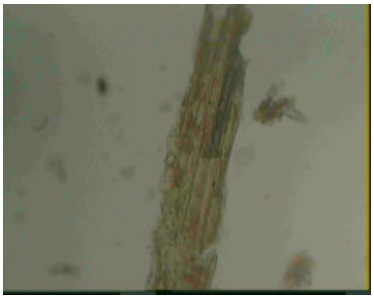


Fig 4 C. dichotoma seeds-powder microscopy (showing pigmented cells as well as xylem vessels and individual xylem vessel (x400)

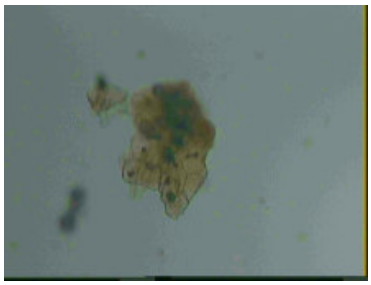


Fig 5 C. dichotoma seeds-powder (endospermal cells) (x400)

Fig 6: TLC of the chromatogram of Petroleum ether extract
Mobile phase: $CHCl_3$: Dichloromethane: Benzene 2:2:6
Visualization: anisaldehyde reagent 0.5 %

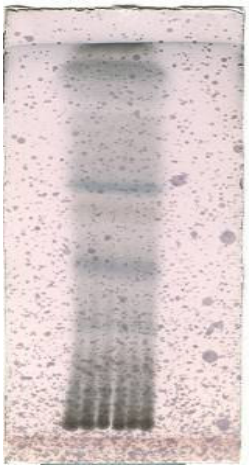


Fig 7: TLC of the chromatogram of Chloroform extract
Mobile phase: Benzene: Acetone 2:1
Visualization: anisaldehyde reagent 0.5 %

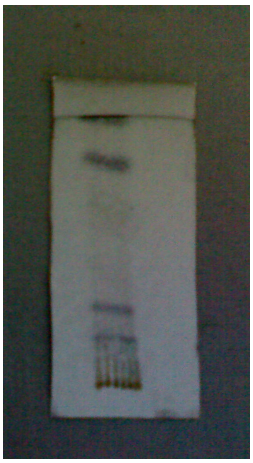


Fig 8: TLC of the chromatogram of methanol extract
Mobile phase: Chloroform: Methanol 9:1
Visualization: anisaldehyde reagent 0.5 %

RESULT AND DISCUSSION:

The seeds were light brown with characteristic odour and was tasteless. The seeds were near about 1 cm length and 1cm diameter. The shape was convex.The seeds on T.S showed normal histology of the seed with outer layers of seed coat and an inner endosperm which showed oily characters. The powdered drug showed endospermal cells. The abundant fragments of the endosperm composed of large oval or rectangular cells perforated with large pits which appeared circular or oval in surface veiw. They were filled with brown pigments occasionally. Vessels were found singly or in small groups. The walls were lignified and showed spiral or annular thickening. A group of sclereids from the central region of the bracts were observed. Individual cells varied in shape but they were usually oblongated, the ends were square or bluntly tapering or somewhat enlarged. The walls were strongly thickened and had scattered pits. The pigmented layer composed of single layer of thin walled cells, rectangular in surface view and slightly larger than the parenchyma of the inner layer, the cells were

filled with dark brown pigment. They were found associated to the inner layer of sclereid cells or the outer layer of endosperm. The fairly abundant starch granules mostly simple although the smaller granules tend to form tightly packed masses. Individual granules were spherical to polyhedral and had fairly distinct hilum. All these characters make the identification of the drug easy and also detect the degree of adulteration or substandard drug. The moisture content was 12 % which was not as high to facilitate bacterial growth. The other physico-chemical parameter which ascertain the quality and purity and also help in evaluating the crude drug, is the ash value and the acid insoluble value which was obtained to be not more than 0.12% w/w and 0.55% w/w respectively which indicated the presence of the total foreign inorganic matter. Preliminary phytochemical screening performed on the various extracts disclosed the presence of alkaloid and steroids/ triterpenoids in the petroleum ether extract, alkaloid and flavanoidal glycoside in chloroform extract and only flavanoidal glycoside in methanol extract. Therefore the TLC chromatography was performed on petroleum ether extract using solvent system specific for steroids i.e chloroform: dichloromethane: benzene (2:2:6) obtaining 4 spots with R_f values 0.44, 0.638, 0.77 and 0.88. The chloroform extract was subjected to solvent system specific for alkaloids i.e benzene: acetone (2:1) getting 7 spots having R_f values 0.11, 0.176, 0.27, 0.42, 0.576, 0.65, 0.941 revealing 7 constituents and similarly the methanolic extract was subjected to solvent system specific for flavanoidal glycosides i.e chloroform: methanol (9:1) with gave 4 spots of R_f values 0.154, 0.31, 0.871, 0.969 which may be of hesperitin and taxifolin glycoside.

Since *C. dichotoma* has multiple properties like wound healing, hepatoprotective, antihelminthic, anti-aging as well as antidiabetic property, it is important to standardize it for use as a drug. So the standard reports and the microscopic work can be used for compilation of a monograph for its correct identification.

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