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Research Article

PHARMACOGNOSTICAL AND ANATOMICAL STUDIES OF *LITSEA GLUTINOSA* (LOUR.) C.B. ROB AN IMPORTANT MEDICINAL PLANT IN CHITTOOR DISTRICT OF ANDHRA PRADESH

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ABSTRACT
The present study was aimed at pharmacognostical and anatomical studies of whole plant of *Litsea glutinosa* (*Lour.*) *C.B. Rob.* an important medicinal plant in Chittoor district of Andhra Pradesh. The pharmacognostical investigations carried out in terms of organoleptic, macroscopic, microscopic, and fluorescence analysis parameters. As there is no pharmacognostic and anatomical work recorded on this medicinally potent plant, the present work was undertaken. The parameters which are reported could be used for botanical identification of the drug in the crude form, for detecting low grade products and preparation of the monograph of *Litsea glutinosa* .

KEYWORDS: *Litsea glutinosa* , Pharmacognostical, Anatomical studies, Drug powder.

INTRODUCTION
Natural medicines are attracting renewed attention is encouraged from both practical and scientific viewpoints since their efficacy has been proven over the centuries. Plants offer novel bioactive compounds which added advantage of ethnobotanical observations, since many species are used in systems of natural and traditional medicine (Cox, 2000). Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances (Khare, 2007). The different tribal inhabitants of Chittoor district use different plant parts for procuring medicines for different ailments. The present study represents a prospective research on pharmacognostical evaluation of whole plant of *Litsea glutinosa* (*Lour.*) *C.B. Rob.*(Lauraceae) to explore the scientific information on pharmacognostical aspects . Every part of it is credited with its specific medicinal properties. Since no reports on systematic studies of whole plant present, an effort has been made to establish the pharmacognostical parameters (physicochemical), as well as anatomical study (histological characters, micrometric determinations) of *L. glutinosa* thereby facilitating authentication of the correct plant material.

MATERIALS AND METHODS
Collection and Authentication
The Plant specimen for the proposed study was collected in fruiting stage from wild source plant present in the Seshachalam hills of Chittoor district (Fig.1). The plant was identified and authentified with the help of local floras (Madhava Chetty *et al.,* 2010; Rangachari, 1991). Final identification was confirmed with reference to authentic specimen available in Madras Herbarium, Coimbatore (MH). A voucher specimen SVU/BOT/HB-LAU-1097 was deposited in the Botanical Herbarium, Sri Venkateswara University, Tirupati, Chittoor district for future reference.

Methods
Sample preparation: Samples of root, stem, bark, leaf were prepared in equal ratios by selecting each part, air-dried in shade, powdered and passed through a 70mm mesh sieve and stored in light-protected tight container. For the microscopical studies, transverse sections were prepared and stained. The powder microscopy was performed according to the methods of Kokate (2008) and Khandelwal (2002). Microscopic descriptions of tissue are supplemented with micrographs wherever necessary.

Fig.1 : *Litsea glutinosa* A. Tree habit B. Unriped fruits C. Riped fruits

Photomicrographs were taken using binocular photomicroscopic apparatus Canon Leitz microscope (24SLR camera integrated) of different magnifications in microscopic units. For normal observation bright field was used. For the study of crystals, lignified cells polarized light was employed. Magnifications of the figures are indicated by the scale-bars. For microscopic studies and macroscopic characterization methods adapted by Johansen DA, (1940) was considered**.** Anatomical Studies was referred from standard books such as Fahn (1982) and Easu (1964).

Physicochemical studies

The ash value (total ash, acid insoluble ash, water soluble ash) done according to Evan, W.C. and Trease, G.E. (2007). Extractive values (petroleum ether, chloroform and methanol) were determined according to the official methods of WHO guidelines (2002), Wallis TE (1953). Flouresence analysis was carried out as per Kokoski *et al.,* (1958**).**

RESULTS

Anatomical characterization of Leaf of *Litsea glutinosa*

The leaf consists of prominent midrib and lateral veins and uniformly thin lamina. The midrib, as seen in another view is biconvex, projecting equally on both adaxial and abaxial sides. The adaxial surface is slightly even while the adaxial side is undulate. The midrib is 550µm thick and 400µm wide on the upper end and 600µm wide on the abaxial side. The epidermal layer of the midrib is thin comprising small squarish thick walled cells. The ground tissue inner to the epidermis consists of two or three layers of circular parenchymatous cells, followed by a thick arc of crushed cells. On the adaxial part there occurs a horizontal leaved of thick walled cells. The vascular strand is wide occupying the entire midrib. It consists of collateral vascular tissues. There are about ten parallel lines of xylem elements, each line having 3-7 cells. On the lower and of the xylem strands occurs a thin horizontal band of phloem. Beneath the phloem zone, about five wide circular masses of sclerenchymatous elements (fibres) are situated. The lateral vein is 300 µm thick. The structure is similar to that of the midrib. It includes a thick and wide collateral vascular bundle with an abaxial horizontal pad of sclerenchyma (Fig 2.1)

Lamina

The lamina is 140 µm thick. The adaxial epidermis consists of narrow tabular cells with thick smooth cuticle. The cells are 15 µm thick. The abaxial epidermis is slightly thicker with wider rectangular cells and thick undulate cuticular layer. The mesophyll tissue consists of a thick zone of two layers of pillar-like palisade cells and abaxial spongy parenchyma cells of 4 or 5 layers of spherical or lobed cells. Some of the cells in the palisade zone are modified into wide circular or four angled cells which possess amorphous cell contents. These secretary idioblasts are more frequent, distributed randomly in the mesophyll tissue and are 20-40 µm wide. The lateral veinlets do not project beyond the surface level. They have a small collateral xylem, phloem elements surrounded by sclerenchymatous bundle sheath and adaxial and abaxial pillar like palisade zone is 70 µm.

Stomata and epidermal cells

The stomata are seen in the abaxial epidermis. They are predominantly paracytic type with semicircular subsidiary cells, one on either side of the guard cells in parallel position. The guard cells are elongated and narrowly elliptical. They are 20 µm long and 10 µm thick. The epidermal cells are wide with thin, much wavy anticlinal walls, so that the epidermal cells are amoeboid in outline (Fig. 2.2).

Venation pattern

The venation of the lamina is densely verticulate. The veins of the different orders are reduced in thickness successively. The veinlets are fairly thick and straight and forms well defined vein islet of polygonal outline. Vein-terminations are either short or slightly long, simple, less frequently forked which are restricted in distribution and are seen in only a limited number of vein-islets.

Crystal distribution

Calcium oxalate crystals are fairly abundant in the leaf, particularly along the veins. The crystals are predominantly prismatic type of rectangular shape (Fig. 5.1). The crystals are seen in ensheathing veins and are in a vertical orientation and parallel to the veins and are seen in the pith cells (Fig. 5.2). The pith crystal measures 12 µm long and 5 µm thick and are either cuboidal or rectangular (Fig. 5.3). Starch grains are sparsely seen in the pith cells.

Anatomical characterization of Stem of *Litsea glutinosa*

The stem is circular in outline with an even surface, nearly 3 mm thick. The stem consists of an epidermal layer of squarish cells with the heavy cuticle (Fig. 3.1). The sub-epidermal layer consists of semicircular cells with thick, lignified outer anticlinal walls. Inner to the lignified hypodermal layer has seen a narrow zone of 2-4 layers of periderms with 60µm thickness. The periderm is followed by the fairly wide parenchymatous cortex. The vascular cylinder is thick and hollow. It includes outer thick and continuous cylinder of phloem. The phloem elements in the outer part are crushed and collapsed into thick dark lines. A narrow inner zone of phloem elements are intact and consists of non-collapsed phloem. Total thickness of phloem is 100µm.

The Xylem cylinder comprises vessels and fibres. The xylem cylinder is 200µm thick. The vessels elliptical, they are wide and thin walled. They are either solitary or occur in long radial multiples. The vessels are 20-50µm in diameter. Xylem fibres are thick walled and lignified. Pith is wide and is occupied by wide central lysigneous cavity. The outer pith cells are thin walled, circular and compact (Fig. 3.2).

Anatomical characterization of Root of *Litsea glutinosa*

The root exhibits well developed secondary growth and periderm formation. It comprise a bark of secondary phloem (Fig. 4.1 & 4.2).

Periderm

It is superficial in position and consists of outer wide, fissued homogenous phellem and phelloderm (Fig. 4.1). The phellem cells are thin tabular in shape and occur in regular radial rows. The cell walls are thin and subersided. The phelloderm cells are fairly wide and have cell inclusions. The periderm is 200 µm thick. At certain loci, the phellogen originates at a deeper position within the cortex or even in the phloem zone. Thin deeply phellogen is bowl shaped and gets connected laterally with superficial original phellogen. The bowl shaped phellogen produces phellem and phelloderm, forming a bay of periderm. A thin portion of the periderm is called shell-bark. The shell-bark has an inner arc shaped boundary and a thick mass tissue within the arc.

Cortex

In between the periderm and secondary phloem the cortex is present as a narrow zone, where the cells are polyhedral, thin walled and compact. The cortex is gradually transformed into secondary phloem.

Secondary phloem

Secondary phloem is well defined comprising an outer portion of collapsed tissue, wide phloem rays and inner narrow region of intact non collapsed phloem. In the collapsed phloem, the phloem elements are

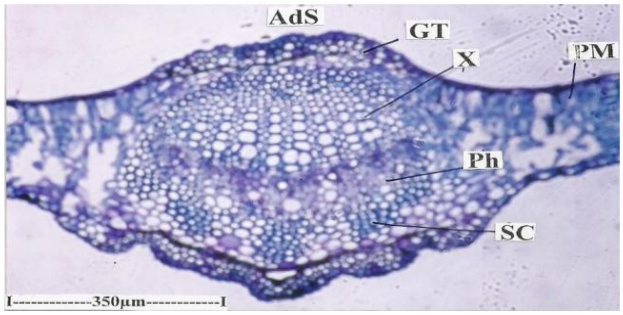


Fig.2.1 T.S of Leaf(Petiole –sector enlarged)

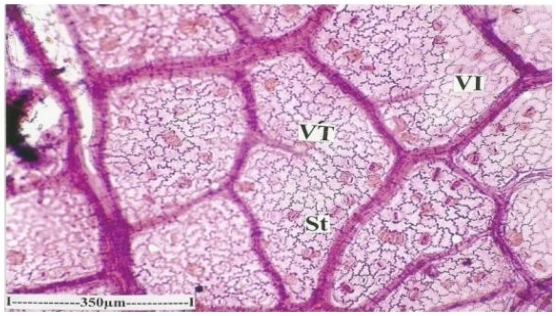
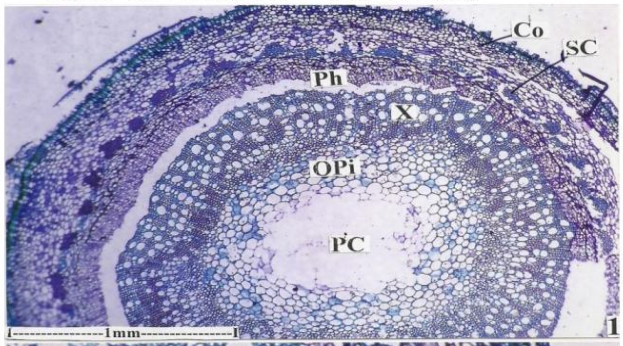


Fig.2.2 Anticlinal walls of epidermal cells and stomata



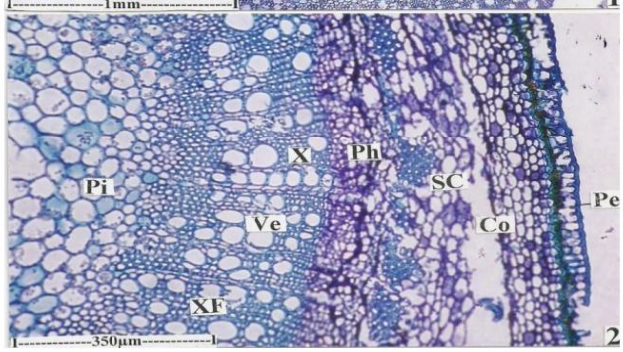
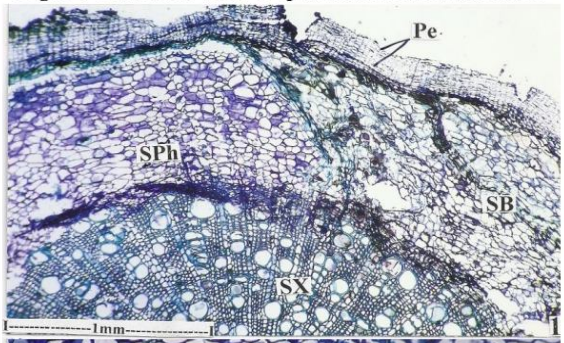


Fig.3.1 & 3.2 T.S. of stem & a sector enlarged



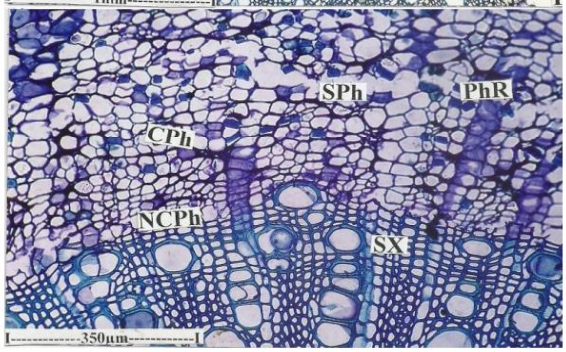


Fig.4.1 &4.2 T.S. of root bark and xylem

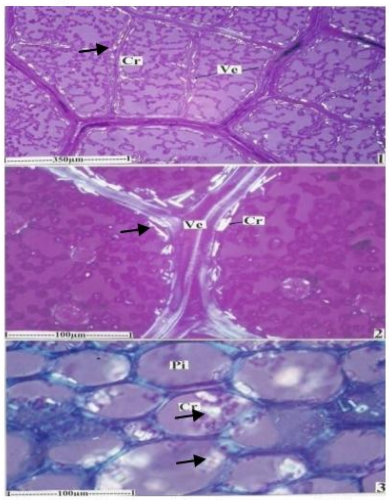


Fig.5.1,5.2 :Crystals along veins(Ve)
Fig.5.3 : Crystals (Cr) in the pith(Pi)

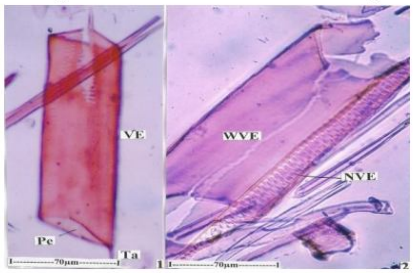


Fig.6.1: Wide vessel element (WVE)
Fig.6.2: Enlarged narrow vessel (NVE)

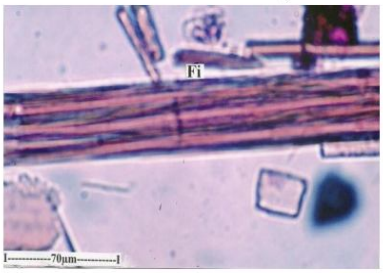


Fig.7: A bundle of fibres (Fi)

Fig.2.1 : Ads –Adaxial surface, **GT**-Ground tissue, **X**-Xylem**, Ph**-Phloem, **SC**-Sclerenchyma. **Fig.2.2 : VT**-Vein termination, **VI**-Vein islet, **St**-Stomata. **Fig.3.1 & 3.2 : Co**-Cortex, **OPi**- Outer Pith , **Pi**- Pith , **PC**- Central Pith, **Pe**- Periderm**, XF**-Xylem fibres . **Fig4.1 & 4.2 : SB**- Secondary Bark, **SPh**- SecondaryPhloem **, SX** – Secondary xylem**, PhR**- Phloem rays, **NCPh**-Non collapsed Phloem , **CPh**- collapsed Phloem, **Co**- Cortex, **Fig.5.1& 5.2 : Ve**- veins ;**Fig.6.1& 6.2 : VE**-vessel element,**Ta**-Tannin, **Pe**- Perforation,**WVE**- Wide vessel element , **NVE** - Enlarged narrow vessel**. Fig.7 :** Fibres **(Fi)**

crushed into dark streaks. In the non collapsed phloem, the sieve elements are angular, thick walled and are arranged in radial rows (Fig. 4.2).

Secondary xylem

Secondary xylem is a dense, solid, circular cylinder occupying the central core of the root. It consists of vessels, xylem fibres and rays (Fig. 4.2). Vessels are diffuse in distribution. They are angular, wide and thick walled, mostly solitary. The vessels are 40-80µm wide. Xylem rays are narrow and straight. Their walls are also thick and lignified. Xylem fibres have thick lignified walls with wide lumen.

POWDER MICROSCOPY

Fragments of lamina are common in the powder. These fragments exhibit veins-islet and vein terminations, epidermal cells and stomatal morphology. The vein-islets are wide and polygonal. The vein termination is simple and long or short and slightly wavy. The epidermal cells have thin wavy anticlinal walls. Stomata are seen in the peeling. They are anisocytic or actinocytic type.

Vessel elements

Wide, cylindrical vessel elements, as well as narrow vessel elements were frequently seen in the powder (Fig 6.1 & 6.2). The vessel elements have sharp narrow tails. Perforation plate is simple and oblique. Lateral wall pits are elliptic, multiseriate and dense. The vessel elements are 280µm long.

Fibres

Thick walled narrow fibres are abundant in the powder. And the wide thin walled fibres were also found. The terminal part of the fibre may be forked, forming two unequal lobes. The narrow fibres are 20µm thick and 560µm long, the wide fibres are 40 µm wide and 470 µm long (Fig 7).

Sclereids

Wide, rectangular or squarish sclereids are sparsely seen in the powder. They have thick walls and simple pits. The cells are 60 x 130 µm in size .

Periderm (Phellem)

Thick pieces of phellem cells of periderm are frequently seen in the powder. The cells are thick walled, rectangular or squarish and compact. The cells stain deeply. Their walls are thick and beaded.

PHARMACOGNOSY

Organoleptic studies

Powder characteristics of the drug showed brown colour, fine powder appearance with aromatic odour and slightly bitter taste.

Table 1 : Cell inclusions

Compounds	Stain / Reagents used	Results - Localisation
Starch	Potassium iodide	Not evident
Tannin	Ferric chloride	Found in the root cortex
Crystals	Polarized light	5 x 12 µm (present in the stem pith)
Lignin	Toulidine blue	Found in the fibres, sclereids & xylem elements
Mucilage	Toulidine blue	Mucilage cavity in the mesophyll tissue

Table 2 : Powder analysis of the drug

Powder treated with water	No-sticking
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Brown
Powder treated with 60% aqueous sulfuric acid	Brown
Powder pressed between filter paper for 24 hours	No oil stains

FLUOROSCENCE ANALYSIS

Experiments	Visible / Day light	UV light	
		254 nm	365 nm
Drug powder	Brown	Green	Pale brown
Drug powder + 1 N NaOH (aq.)	Brown	Green	Dark brown
Drug powder + 1 N NaOH (alc.)	Brown	Green	Dark brown
Drug powder + 1 N HCl	Brown	Pale green	Colourless
Drug powder + 50% H_2SO_4	Brown	Pale green	Pale brown
Drug powder + 50% HNO_3	Brown	Pale green	Colourless
Drug powder + Picric acid	Brown	Green	Yellow
Drug powder + Acetic acid	Brown	Green	Brown
Drug powder + Ferric chloride	Brown	Pale green	Brown
Drug powder + HNO_3 + NH_3	Brown	Green	Pale brown

Table 3.1 the drug powder analysis

Extract	Treatment	Observation
Ethanol	Daylight	Brown
	Short UV	Green
	Long UV	Brown
Water	Daylight	Brown
	Short UV	Green
	Long UV	Brown
Hexane	Daylight	Green
	Short UV	Brown
	Long UV	Green
Chloroform	Daylight	Brown
	Short UV	Green
	Long UV	Brown

Table 3.2 With Various extracts

Results for cell inclusions in coarse powder were mentioned in Table. 1 and the powder analysis filtrate colour, and residual nature was recorded in Table 2.

Table 4 : Ash value and its percentage

Methanol soluble extract	39.138(% w/w)
Ethanol soluble extract	35.68(% w/w)
Water soluble extract	48.90(% w/w)
Hexane soluble extract	2.82(ml)
Chloroform soluble extract	3.5176(% w/w)

Table 5 : Extractive values of the Drug powder

Ash value	**% (w/w)**
Total ash	11.44%
Water soluble ash	5.11%
Akalinity of water soluble ash	0.3 (ml)
Acid insoluble ash	9.17

Ash values of the drug results mentioned in Table 4. Extractive values of the drug showed a methanol soluble extract with 39.138 (% w/w), ethanol soluble extract with 35.68 (% w/w), Water soluble extract with 48.90 (% w/w), Hexane soluble extract 2.82 (ml), Chloroform soluble extract 3.5176(% w/w).

DISCUSSION

The traditional medicine require intensive and urgent investigation in the next few years from botanical, chemical, and biological perspective, particularly for the rapidly increasing diseases in the developing world. *L. glutinosa,* a traditionally, ethnobotanically important medicinal of Chittoor district of Andhra Pradesh. Ethnic studies and scientific reports on this traditionally used and clinically potential plant revealed that different parts of this plant are used in different ailments. According to the WHO, determining the macroscopic and microscopic characteristics are the first steps towards establishing the identity and the purity of such materials, and these steps should be carried out before any further tests are undertaken. The quantitative determination of physicochemical parameters is useful for setting standards for crude drugs. Biological activity of crude drug is mainly due to the active chemical constituents, and its properties. The constituent may be soluble in different polar, semi polar and non-polar solvents (Kokate, 2008) according to its chemical structure and chemical properties. It can work differently in different forms viz., ash form, fresh form and dried form. Ash content analyses indicate the degree of admixture of foreign inorganic matter either from the storage container or by intentional addition to disguise the appearance of the crude drug. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Methanol, ethanol and water showed highest extractive values, and both are able to extract most of phytoconstituents. The acid insoluble ash determines the acid insoluble material present in the drug materials. Flourescence analysis revealed noticeable colour with concentrated acids, which is an important character ascertain genuineness of the powdered drug. Present investigations were planned with an objective toestablish pharmacognostic standards of this plant, there by facilitating authentication of the correct plant material. These standardized parameters would be of immense help in authenticating. If any crude drug which claimed to be as *L. glutinosa* but whose

charecters significantly deviate from the character above would then be erejected as contaminated, adultered or downright fake as per results revealed by our study.

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