

PHARMACOGNOSTICAL STUDIES ON *LITSEA FLORIBUNDA* (BLUME) GAMBLE.

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ABSTRACT

Plant is man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization. In the last few decades there has been an exponential growth in the field of herbal medicine. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained. The selected plant *Litsea floribunda* (Blume) Gamble is a dioecious tree species which is endemic to India, and belongs to the family *Lauraceae*. It is used in the treatment of diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, edema, arthritis, pain. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. The preliminary organoleptic examination, and physicochemical constants like moisture content, ash values such as total ash, acid insoluble ash, water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value, was determined. The results of this study should provide a standard for identification and preparation of monograph of this drug.

Key words:-

Civilization, Gastroenteritis, *Litsea floribunda*, Pharmacognosy, Standardization

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times vaidyas used to treat patients on individual basics and prepared drugs according to the requirement of the patients. But the scene has been changed now, Herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters (Agarwal, 2005).

India, the richest floristic regions of the world, has got a source of plants and their products since antiquity. Man uses them as food and medicine as per his desires. Among the entire flora, estimated 2,500, 000 higher plant species on earth, only 35,000 to 70,000 species (less than 1%) have been used for medicinal purpose (Ponnu *et al.*, 2003).

The term pharmacognosy is derived from two Greek words *Pharmacon* meaning drug or medicine and *'ganosis* meaning knowledge. This term was first coined by C. A. Seydlor in his dissertation entitled '*Analecta pharmacognosia*' in 1895. Herbal medicines as the major remedy in traditional medical system have been used in medical practice for thousands of years and have made a great contribution to maintain human health (Emeka and Elizabeth, 2009). Pharmacognosy is a simple and reliable tool, by complete information of the crude drug can be obtained. There is a need for documentation of research work carried out on traditional medicines. Hence it becomes extremely important to make an effort towards standardization of the plant material to be used drugs. The process of standardization can be achieved by stepwise pharmacognostic studies (Dahanukar *et al.*, 2000).

Medicinal plants have played crucial roles in various traditional systems around the world, as well as in the discovery and development of pharmaceutical drugs (Taylor, 2013). In spite of the boom in pharmaceutical industry during the last century, the need for novel drugs is more pressing than ever due to either or both the adverse effects of the drugs or extensive evolution of drug resistance in the target pathogens (Cragg and Newman, 2013). A vast collection of research has indicated that many medicinal plants, which are relatively unexplored, offer promising sources for novel drugs (Brusotti *et al.*, 2014). Plant chemical are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are synthesized in all parts of the plant body, bark, leaves, stem, root, flowers, fruits, seeds etc. i.e any part of the plant body may contain active components (Solomon Charles *et al.*, 2013).

Litsea floribunda (Blume) Gamble leaves are used as one of the ingredients in the preparation of herbal shampoo, in Southern India (Girish *et al.*, 2014). In the health traditions the local inhabitants use *Litsea floribunda* to treat certain gastrointestinal and respiratory disorders. Till now, no data are available on the phytochemical profile, antioxidant and hepatoprotective potentials of the species. Hence the present investigation has been planned to study the Pharmacognostic characters of the whole plant of *Litsea floribunda* (Blume) Gamble.

MATERIALS AND METHODS

Collection of the plant materials:

Litsea floribunda (Blume) Gamble collected from Kothagiri. It is in Nilgiri District of Tamilnadu. It is the oldest and third largest hill station in Tamilnadu.



Fig. 1: **A** . *Litsea floribunda*
(Entire plant) **B**. A twig **C**. Buds **D**. Flowers **E**. Unripe fruits

Sample preparation:

Sample 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.

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).

RESULTS

Anatomical characterization of Leaf of *Litsea floribunda*

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 abaxial side is undulate. The midrib is 550 μ m thick and 400 μ m wide on the upper end and 600 μ m wide on the abaxial side. The epidermal layer of the midrib thin comprises 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 μ m 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 secretory 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 verticillate. 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. 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.

Anatomical characterization of Stem of *Litsea floribunda*

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 60 μ m 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 100 μ m. The Xylem cylinder comprises vessels and fibres. The xylem cylinder is 200 μ m thick. The vessels elliptical, they are wide and thin walled. They are either solitary or occur in long radial multiples. The vessels are 20-50 μ m in diameter. Xylem fibres are thick walled and lignified. Pith is wide and is occupied by wide central lysigenous cavity. The outer pith cells are thin walled, circular and compact (Fig. 3.2).

Anatomical characterization of Root of *Litsea floribunda*

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, fissured 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 subsided. The phelloderm cells are fairly wide and have cell inclusions. The periderm is 200 μ m 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 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)

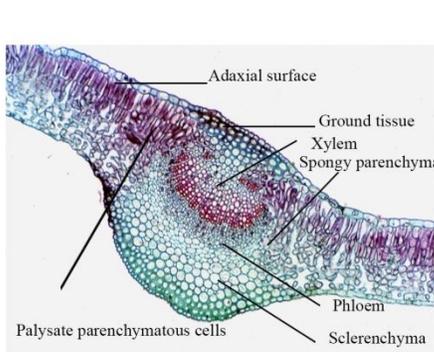


Fig. 2.1. T.S of leaf



Fig. 2.2. Actinal walls of epidermal cells and stomata

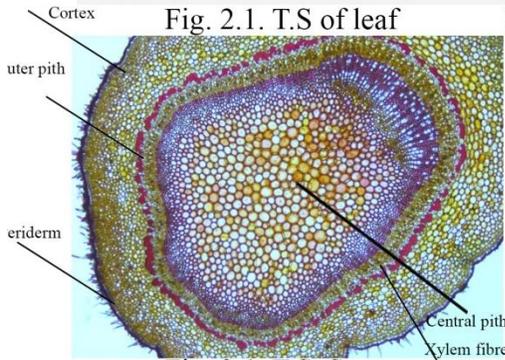


Fig. 3.1. T.S. Stem

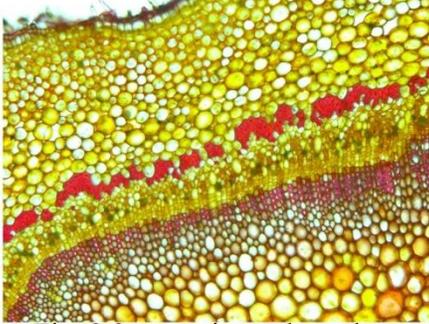


Fig. 3.2. a portion enlarged



Fig. 4.1. T.S. Root

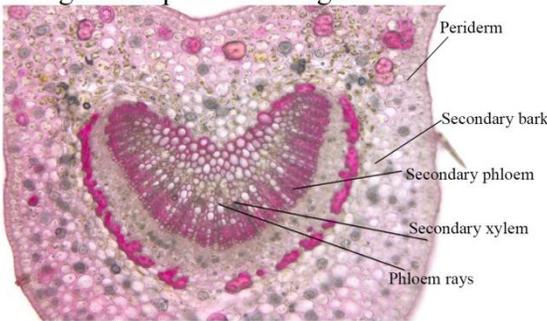


Fig. 4.2. a portion enlarged



Fig. 5.1. Secondary xylem

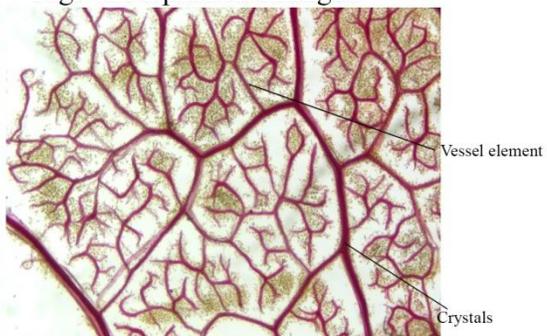


Fig. 5.2. Crystals along veins

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 5.1). Vessels are diffuse in distribution. They are angular, wide and thick walled, mostly solitary. The vessels are 40-80 μ m 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 5.2). 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 280 μ m 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 20 μ m thick and 560 μ m long, the wide fibres are 40 μ m wide and 470 μ m long .

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 μ m 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.

PHYSICOCHEMICAL ANALYSIS

Table -1
Physicochemical analysis of *Litsea floribunda* (Blume) Gamble.

S.No	Parameters	% Of Concentration
1.	Foreign matter	1.00
2.	Moisture	7.51
3.	Hexane	0.649
4.	Chloroform	1.771
5.	Ethyl acetate	0.800
6.	Ethanol	35.54
7.	Methanol	63.3
8.	Water	61.4

DISCUSSION

The traditional medicine requires 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. floribunda*. 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.

Conclusion

Medicinal value of *L. floribunda* in diseases i.e. ulcer, hepatotoxicity, wound healing etc is already reported. Still no research work is done to establish its Organoleptic, chemical and morphological evaluations. Therefore this research is a key step in this regard. This research is helpful for establishing the correct identification of a plant material. It will be a diagnostic tool for standardization and characterization of *L. floribunda*. It will also be helpful for other researchers to maintain the standards of this plant for their research projects.

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