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Anatomical studies on leaf and stem of *Gmelina asiatica* L.: An ethnomedicinal important plant

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Abstract
The present investigation is to provide information on the anatomical features of the leaves and stems of the plant *Gmelina asiatica*. Leaf and stem anatomy of *G. asiatica* was undertaken by rotary microtome and examined on photomicrographs. Anatomical characters such as echinate epidermal cells, glandular trichomes, anomocytic stomata, calcium oxalate crystals, periderm cylinder, phellem cells and vascular bundle of leaf and stem explains typical features of Verbenaceae. This study provides valuable information for reference and correct identification of the family Verbenaceae.

Keywords: *Gmelina asiatica*, plant anatomy, photomicrograph, identification

1. Introduction
Gmelina asiatica L. (Syn: *Gmelina parvifolia* Roxb.), is a large sized deciduous shrub about 4m to 6 m tall and much branched. Leaves are small petiolated and ovate. Leaves and young shoots are used for jaundice, rheumatism, syphilis, gonorrhea, burning sensation of eyes, fever, dysuria, wounds, dandruff, diabetes, hepatic diseases and also to reduce body heat [1-6]. Plant anatomy provides a novel perspective on the microscopic structure of plants. Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is a challenging task because of complex nature of chemical constituents. To ensure the quality of herbal products proper identification of the plant material is essential [7]. Anatomical characters help for identification, when the morphological features are indistinct [8]. Anatomical features of *G. asiatica* leaf and stem was undertaken with the aim to provide key diagnostic tools of identification.

2. Materials and Methods
2.1 Collection and Identification
Leaves of *G. asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamilnadu, India and identified using Gamble and Fisher [9].

2.2 Sectioning
For anatomical studies, the fresh samples were fixed in FAA (Formalin-5 mL+ Acetic acid-5 mL + 70% Ethyl alcohol-90 mL). The paraffin-embedded specimens were sectioned by rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure [10] and stained with Toluidine blue as per the method published by O'Brien *et al* [11]. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Sections were also stained with safranin and Fast-green and IKI (for Starch) wherever necessary.

2.3 Photomicrographs
Microscopic descriptions and photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For the study of crystals and lignified cells, polarized light was employed. Magnifications and anatomical features were reviewed [12].

3. Results
3.1 Anatomy of leaf
Gmelina asiatica leaf consists of thick flat convex midrib and thick lamina with glandular trichomes on the adaxial and semicircular on the abaxial sides. It is 340µm thick and 280µm wide. The adaxial part of the midrib consists of small, squarish thick walled epidermal cells and the abaxial semicircular part possesses conical echinate epidermal cells (Fig 1.1).

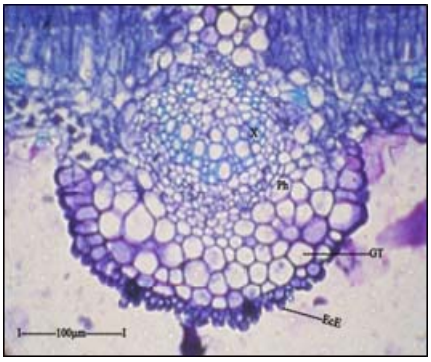

Fig 1.1: T.S. of leaf through midrib

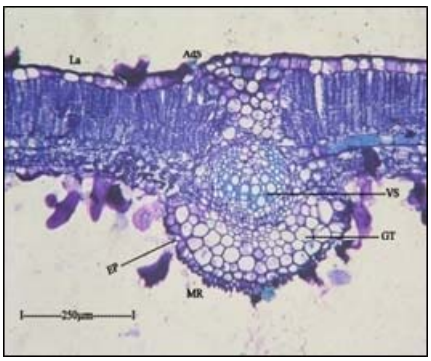

Fig 1.2: T.S. of midrib enlarged

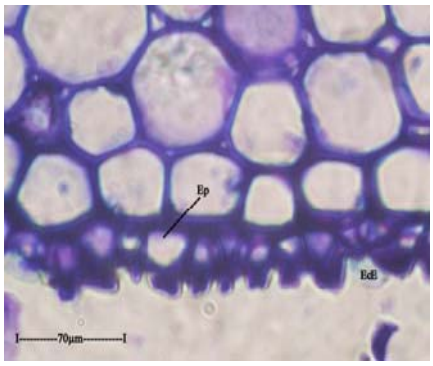

Fig 1.3: Echinate thick walled epidermis of the midrib enlarged

(AdS – Adaxial side, EcE – Echinate epidermis, Ep – Epidermis, GT – Ground tissue, MR – Midrib, Ph – Phloem, VS – Vascular strand, X – Xylem)

The echinate epidermal cells are about 10µm in thick and the ground tissue is 2 to 4 layered with angular and compact cells. The vascular strand is broadly top shaped and includes 6 to 7 rows of xylem elements with narrow, thick walled and fibrous. Phloem occurs as thin layer along the lower end of the xylem strand (Fig 1.2 and 1.3).

3.2 Lamina

The lamina is dorsiventral, hypostomatic and 90µm thick (Fig 1.4). It consists of thick, rectangular, adaxial epidermal layer of cells with prominent cuticle. The abaxial epidermis includes squarish, smaller cells with thin cuticle. The trichomes are small, lobed and with dense mucilaginous substances. The palisade mesophyll includes two horizontal rows of cylindrical cells with 3 to 4 layers of spherical and lobed spongy parenchyma cells in the lower part.

3.3 Leaf margin

The leaf margin is slightly bent below, thin and semi circular with 100µm thick. It consists of squarish, thick walled epidermal layer. The palisade and the spongy parenchyma cells are reduced in size (Fig 1.5).

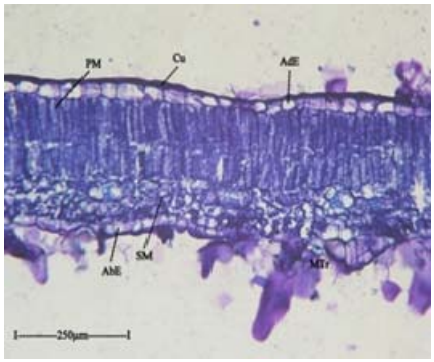

Fig 1.4: T.S. of Lamina

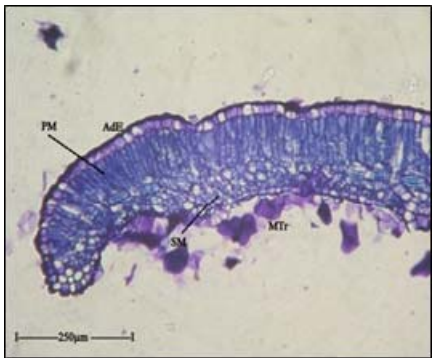

Fig 1.5: T.S. of Leaf Margin

Tetrad types of calcium oxalate crystals are seen in the mesophyll tissue. Each crystal is made up of 4 units forming a tetrad (Fig 1.6). The crystals are 10µm in diameter and sparsely distributed in the mesophyll.

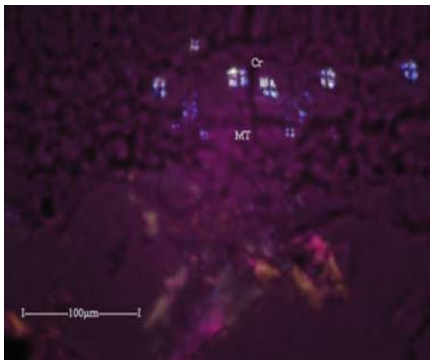

Fig 1.6: Tetrad type of calcium oxalate crystals in the mesophyll

(AbE – Abaxial epidermis, AdE – Adaxial epidermis, Cr – Crystal, Cu – Cuticle, LM – Leaf margin, MT – Mesophyll tissue, MTr – Mucilagenous Trichome, PM – Palisade mesophyll, SM – Spongy mesophyll)

3.4 Anatomy of stem

Thick and quadrangular stem of about 1.6 mm was studied. The stem shows thin less prominent epidermal cells with dark inclusions. Inner to the intact epidermal layer there is a thick periderm cylinder which is about 400µm thick with

homogenous, rectangular and thin phellem cells. Inner to the phellem zone the cortical cylinder consists of thick walled parenchymatous cells. The cortex is 4 to 5 layered. The inner boundary of the cortex includes large discontinuous masses of gelatinous fibres which encircle the vascular cylinder (Fig 2.1 and 2.2).

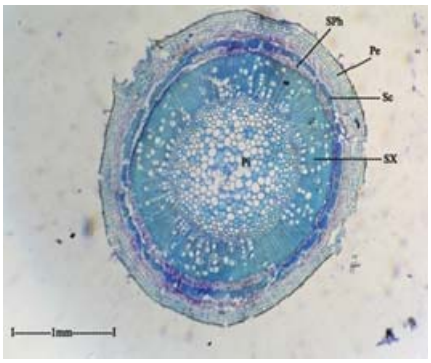

Fig 2.1: T.S. of stem entire view

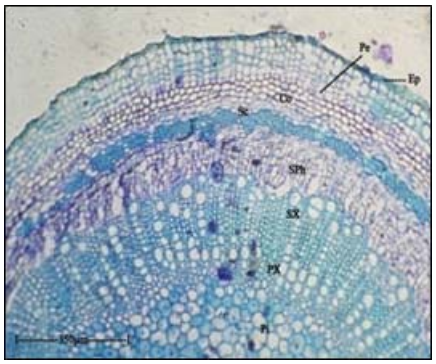

Fig 2.2: T.S. of stem a sector enlarged

The vascular cylinder is hollow and thick with outer continuous cylinder of radially arranged secondary phloem which measures about 110µm thick and includes sieve elements and parenchyma cells (Fig 2.3).

The secondary xylem consists of several radial chains of vessels and xylem fibres. The vessels are angular, wide thick walled and measures about 25µm in diameter (Fig 2.6). The xylem fibres are also thick walled with gelatinous layer (Fig 2.5). The inner boundary of the secondary xylem cylinder consists of several radial rows of primary xylem elements.

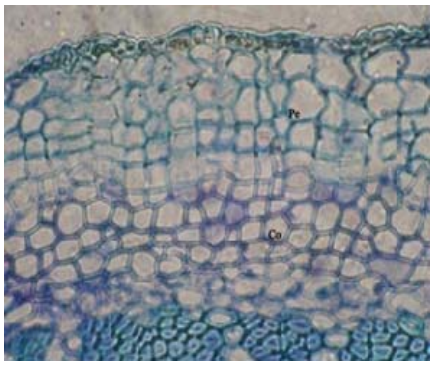

Fig 2.4: T.S. of stem showing periderm, cortex, gelatinous fibres and secondary phloem

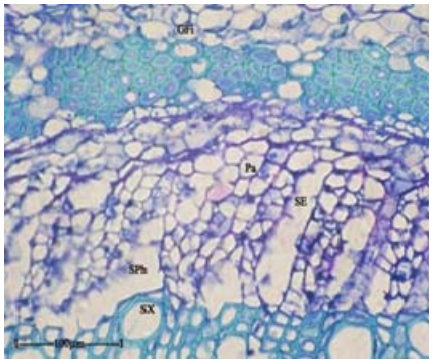

Fig 2.5: T.S. of secondary Xylem enlarged

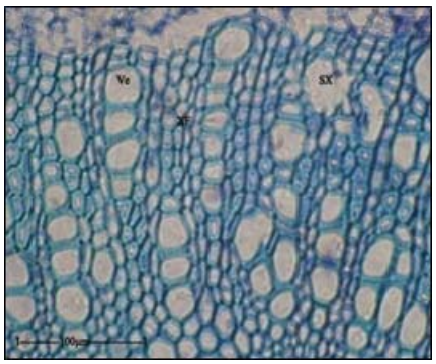

Fig 2.6: T.S. of stem showing secondary xylem, vessels and xylem fibres

(Co – Cortex, Ep – Epidermis, GFi – Gelatinous fibre, Pe – Periderm, SPh – Secondary phloem, SX – Secondary xylem, Ve – Vessel, XF – Xylem fibre, SE - Secondary epidermis, Pa - Parenchyma)

4. Discussion

4.1 Anatomy of leaf

Leaf anatomical features have been proved to be useful for species grouping and identification with great taxonomic significance [13-16]. The plant cuticle is a lipid layer of cutin intermeshed and coated with waxes that covers essentially all aerial organs and functions to restrict transpiration. By this mechanism, the cuticle is thought to play a critical role in plant drought tolerance through its ability to postpone the onset of cellular dehydration stress during drought [17-20]. During severe drought condition, nanoscale diffusion pathways are carried out by the crystalline wax of the cuticle membrane, and these crystalline waxes are thought to be a major determinant of cuticle permeability [19, 21-23]. Typical plant waxes consists of a homologous series of primary alcohols, aldehydes, alkanes, fatty acids, esters and sometimes cyclic compounds like triterpenoids and sterols [24]. But cuticular waxes are alkanes, alcohols, and aldehydes that show greater resistance to water diffusion [25]. The transverse section (T.S.) of *G. asatica* leaf showed thick walled epidermal cells but the abaxial semicircular part has prominently conical echinate thick walled epidermal cells covered by the cuticle layer (Fig 1.1). Echinate epidermal cells are observable and are considered to be a very specific feature of *G. asiatica* because it is densely covered with bristle like outgrowths which is helpful in taxonomical identification. The cuticle layer prevents water loss during hot environment [26].

The distribution and arrangement of trichomes on plant surfaces contribute to the control of transpiration [27] and the

phenolic compounds found in these structures afford the organ of protection against UV-B radiation, which results from exposure to sunlight [28]. The occurrence of glandular trichomes in the adaxial and abaxial side of epidermis is a distinctive feature of *G. asiatica* (Fig 1.1). Dassanayake, Walston and Dallwitz reported the characters of *G. asiatica* leaf which has round minute white-glands beneath the leaves which secrete tannins, terpenes and oils [29, 30]. Trichomes, particularly glandular trichomes secrete essential oils [31-35] which normally evaporates and are released under high temperature and low humidity; hence their preponderance on the abaxial surface is largely for protection [36]. The glandular trichomes also show ecological significance, being associated with the plant interaction with the environment, interfering efficiently against the attack of herbivores [37-40] and contribute to the control of transpiration and temperature. The function of trichomes affords protection against pathogens [27, 38].

G. asiatica possess anomocytic type of stomata. The epidermal cells surrounding the stomata were found only in the abaxial epidermis of the leaf (Fig 1.4). Similar types were reported by Inamdar [41] in selected members of Verbenaceae and it act as a preventive mechanism against photo-inhibition, since the adaxial surface is more exposed to solar radiation [42]. Tetrad type of calcium oxalate crystals appear in the mesophyll (Fig 1.6). Similar findings were observed by Acharya *et al*. [43] and Kannan *et al*. [44] on *G. asiatica* stem. The appearance, location, distribution and morphology of these crystals are a constant characteristic among the species, being important for taxonomic purposes, as its deposition is genetically controlled [45]. These crystals are a part of the plant's passive defense system and act in deterring herbivory [46, 47].

4.2 Anatomy of stem

Stems serve as a sink for several metabolites and as an important source of bioactive compounds. In the present investigation, the microscopic evaluation of transverse section shows prominent epidermal cells with dark inclusions, uniformly thick periderm and large discontinuous masses of gelatinous fibres which encircle the vascular cylinder. Inner to the phellem zone is thick parenchymatous cortical cylinder. Secondary xylem cylinder consists of several radial lines of primary xylem elements. The phloem tissue includes sieve elements and parenchyma cells (Fig 2.3). Rajesh *et al*. [48] noticed the replacement of epidermis by the periderm in the mature stem of *G. asiatica* which adds extra protection to the stem as it grows. The results of the present study reveal that the centripetal mode of formation of periderm from the epiderm and the variations observed in the anatomy of stem is similar to the reports of Kannan *et al*. [44] and Rajesh *et al*. [48]. The cracked cork cells with suberized wall, lenticels, patches of bundle cap fibres in the vascular cylinder of the present investigation is in agreement with the finding of Kannan *et al*. [44]. The suberized phellem is of great benefit during dry climate as it helps to seal off water loss. The thin layer of fibres found outside the vascular bundle exists to protect the outermost layer of phloem [49].

Secondary xylem consists of several radial chains of vessels and thick walled gelatinous layer of xylem fibres (Fig 2.4 and 2.5). Such characteristics are similar to the observations of Kannan *et al*. [44] and Rajesh *et al*. [48] in *G. asiatica*. Chew [49] noticed thick fibre band surrounding the innermost vascular bundles and the secondary growth proceeds, some of the parenchyma cells found within and outside this band become meristamatic and contribute to the formation of new vascular cambia and a new layer of fibres, providing them with extra strength and flexibility as the stem continuous to grow expand. The xylem vessels and xylem parenchyma are the main elements of secondary xylem that reflect ecological variation [50-53] and similar to the anatomical structure of the family Verbenaceae [32].

5. Conclusion

The present findings of the leaf and stem anatomy of *G. asiatica* exhibited echinate epidermis, glandular trichomes, anomocytic type of stomata, periderm cylinder, phellem cells and vascular bundles which are explaining the typical structure of Verbenaceae. This study provides valuable information in anatomical identification.

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