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).
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).

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.

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).

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.

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 [18, 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. asiatica* 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 axillary 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 abaxial 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,
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
meristematic 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.

6. References

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