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Research Article

Macro and microscopic evaluation of *Gmelina arborea* Roxb. – A botanical pharmacognostic approach for quality control of raw drug material

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Abstract

Gmelina arborea Roxb. of family Verbenaceae, is one of the highly valued medicinal plant used in numerous traditional medical formulations. It belongs to the 'Dasamoola' group of ayurvedic medicinal plants and hence widely exploited. Pharmacognostic techniques involving macroscopic, microscopic and also dry powder analysis serve as botanical methods which help in the correct identification of the crude drug. Leaf constants such as stomatal number, stomatal index, vein islet number, vein termination number also have been determined. Better quality control practices in nutraceutical and pharmaceutical industries demand the correct identification of the dried plants or powdered drug thereby detects and prevents the adulterations, if any. The challenge ahead of this investigation is to validate the therapeutic efficacy and safety of the plant following standard methodology.

Keywords: *Gmelina arborea*; raw drug; botanical pharmacognosy.

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Introduction

Gmelina arborea Roxb., a moderately small to large deciduous tree is a native to Indian subcontinent. It is called as 'Sriparni' in Sanskrit, 'Gambhar' in Hindi, 'Kumbil' in Malayalam and White teak in English. It belongs to the family Verbenaceae according to Bentham and Hooker's system of classification but Angiosperm phylogenetic system recognises it under the family Lamiaceae. The tree is used in various complementary and alternative systems of medicine like Ayurveda, Unani and Siddha. The root of *G. arborea* is one of the ingredients of 'Brihath panchmoola' of 'Dasamoola'. The root paste is applied externally on wounds, gout and abscesses and its decoction is given in cases of indigestion, fever and anasarca (1). Stem bark is used as an antidote to poisoning (2). Leaves are known to have carminative, purgative, diuretic and demulcent properties (3). *G. arborea* is one such plant used in folklore medicine practices in

the villages of India for abdominal tumors, piles, 'tridosha', urinary discharge and in post-delivery weakness (4). Owing to the increased demand for the drug, the plant parts are often substituted. Hence, the present study involves pharmacognostic studies of various parts of the plant through macroscopic and microscopic analyses.

Material and Methods

The studies were carried out in plant materials collected from Payattuvila, Thiruvananthapuram district, Kerala, India. The authenticity of the plant was confirmed with the Flora of the Presidency of Madras (5) by the Herbarium curator, Botany department, Kerala University and the voucher specimen (KUBH 10131) was deposited. The root, stem, petiole and leaf were subjected to pharmacognostic analyses. The external morphology of plant parts, and other structural peculiarities were studied. Anatomical characters of root, stem, leaf and petiole, stomatal and veinlet characters (6,7), palisade ratio and trichomes were included in the microscopic investigations. The leaf powder microscopy was also studied (8). The studies were performed using Leica compound microscope and photographed using LAS EZ software.

Results and Discussion

3.1 Macroscopic studies

The root has yellowish brown colour, cylindrical shape and rough surface owing to the presence of longitudinally running fissures (Fig. 1). The stem has almost cylindrical shape and has a solid pith at cut surface. Stem bark is light greyish brown coloured exfoliating as thin light coloured patches (Fig. 2). The branchlets are quadrangular, hairy and bear numerous white spots. The petiole is green, 0.2-0.5 cm in width and 10-15 cm in length. Leaves are broadly ovate having a basal width of about 15 cm and laminal length of 20-25 cm range and light green to dark green. The simple leaves show pinnate camptodromous venation, opposite decussate phyllotaxy and have hairy abaxial side. Similar venation patterns has been recorded in the six *G. arborea* clones in which the primary vein is moderate to stout and runs straight into lamina (9). The leaf margins are mostly entire but occasionally serrate (Fig. 3).

3.2 Microscopic studies

3.2.1 Transverse section analyses

3.2.1.1 Root: It consists of thin periderm, wide cortex, secondary phloem and central solid cylinder of secondary xylem. The periderm is superficial and it varies in thickness from 3 to 5 layers of phellem cells which are tubular in shape and suberized. Inner to the phellem is a 2 to 3 layered phellogen which is followed by a wide cortex, which includes parenchymatous cells. Occasional distribution of darkly stained highly thick walled stone cells could be seen in the outer cortical zone. Secondary phloem occurs inner to the cortical region but there is no clear cut border between cortex and secondary phloem. The secondary phloem zone consists of small dilated rays. The secondary xylem includes diffusely distributed wide circular thick walled vessels and xylem fibres. The vessels may be solitary or paired. The xylem rays are in thin straight radial lines (Fig. 4).

Fig. 1. *Gmelina arborea* root

Fig. 2. *Gmelina arborea* stem

Fig. 3. *Gmelina arborea* serrated and entire leaves

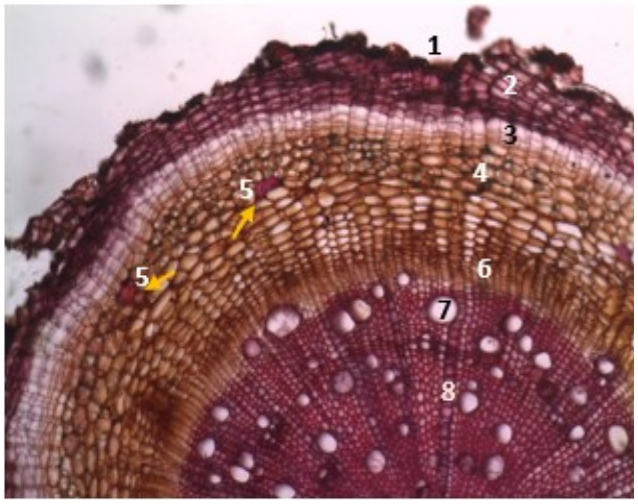

Fig. 4. T.S. of root - a portion enlarged 1-lenticel, 2-phellem, 3-phellogen, 4-phelloderm, 5- stone cells (darkly stained), 6-phloem, 7-xylem vessel, 8- uniseriate medullary ray

***3.2.1.2 Stem*:** The stem is circular in outline. It consists of a developing cork, wide cortex, hollow cylinder of vascular segments and wide pith. A crushed thin dark layer of epidermis could be observed. Cork consist of 3 to 4 layers of parenchymatous ray like cells. Beneath the cork cambium is 5 to 6 rows of collenchyma cells followed by a 3 to 5 layered chlorenchymatous cortex. The vascular segments have prominent, near-circular sclerenchymatous bundle cap, rectangular mass of phloem and radial xylem tissue. Xylem has radial chain of wide, circular, thick walled vessels, thick walled fibers and numerous xylem parenchyma towards the inner portion. Phloem has a mass of sieve elements and phloem parenchyma. An intrusion of thin chlorenchymatous cells could be observed in between the vascular segments as a uniseriate ray and consist of elongated narrow small chlorenchyma cells. The pith is wide and parenchymatous (Fig. 5).

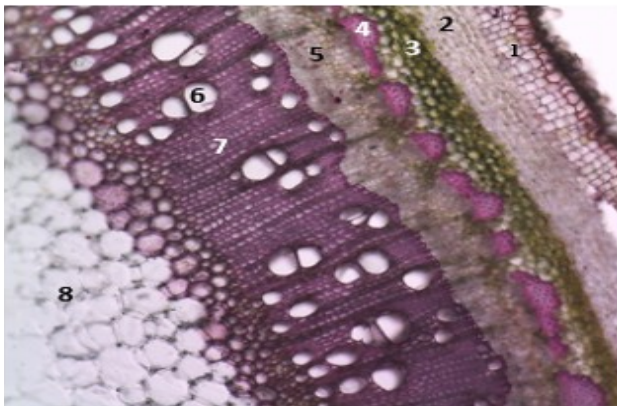

Fig. 5. T.S. of stem - a portion enlarged 1-cork, 2-collenchyma, 3-chlorenchyma, 4-bundle cap, 5-phloem, 6-xylem vessel, 7- vascular ray, 8-pith

***3.2.1.3 Leaf lamina*:** The leaf has smooth and even lamina and biconvex midrib. The midrib has a slight bulge on the adaxial side and semi-circular on the abaxial side. The adaxial epidermis is fairly thick with elliptical cells and prominent cuticle. The abaxial epidermis is with rectangular cells and thick cuticle. The lower epidermis also have characteristic uniseriate multicellular trichomes and numerous bleb-like structures. The collenchymatous hypodermis is wide in the adaxial and abaxial side but narrower at the sides. There is a single U-shaped vascular bundle which is collateral with several parallel lines of angular, thick walled xylem elements. The ground tissue beneath the vascular bundle is parenchymatous; the cells are circular and compact. The green mesophyll tissue is differentiated into adaxial band of single layer of longitudinally elongated, compact palisade cells and irregular abaxial spongy mesophyll tissue (Fig. 6).

Fig. 6. T.S. of leaf showing midrib 1-stomata, 2-epidermis, 3-trichome, 4-hypodermis, 4-xylem, 5-sclerenchymatous patch, 6-phloem, 7-xylem, 8-ground tissue, 9-palisade mesophyll, 10-spongy mesophyll

***3.2.1.4 Petiole*:** Petiole is circular in sectional view with a slight depression at the adaxial side. It consists of a thin epidermal layer of compactly arranged tangentially elongated cells and a heterogenous ground tissue in which the vascular tissue is embedded. The epidermis is characterised by the presence of a thick cuticle and numerous short trichomatous structures. The epidermis is followed by 5-6 layers of chollenchymatous cells and patches of chlorenchymatous cells mostly in the adaxo-lateral side. The major portion of ground tissue is parenchymatous which are small circular towards the outer zone of vascular strand and large angular in the central portion. The vascular strand assumes a broad arc shape owing to the presence of a subtending sclerenchymatous patch towards its abaxial side. It is collateral in nature with endarch xylem adjoining a small phloem patch towards the outer side, close to the sclerenchymatous zone. The arc is broken at the

adaxial side and two relatively small adaxial vascular bundles are seen, one on each side of the broken end (Fig. 7).

Fig. 7. T.S. of Petiole 1-epidermis, 2-chollenchyma, 3-chlorenchyma, 4- sclerenchymatous patch, 5-phloem, 6-xylem, 7- ground tissue

3.2.2 Leaf epidermal study

The epidermal cells are more clearly observed on the adaxial peel rather than on the abaxial surface as the abaxial side had numerous trichomes. The epidermal cells on both adaxial and abaxial surface are mostly irregular with thin anticlinal walls (Fig. 8A). Abaxial epidermis showed numerous multicellular uniseriate trichomes of 2-4 cells long (Fig. 8B and Fig. 9). A surface peel out analysis of abaxial epidermis further revealed the existence of anomocytic stomata surrounded by subsidiary cells which are not distinguishable from rest of the epidermal cells (Fig. 10). Stomatal distribution studies revealed the hypostomatous nature of the samples used in the present study. Existence of hypostomatous condition has been observed in two of the clones of *G. arborea* (9). However, there are reports of the presence of stomata on both surfaces indicating the occurrence of amphistomatous condition in the plant with comparatively more number of stomata on lower surface (10).

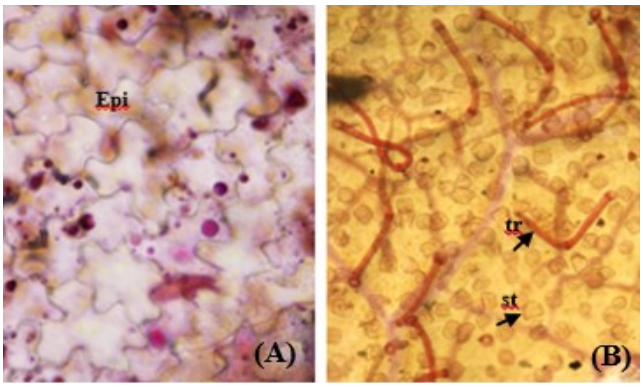

Fig. 8. (A) Adaxial epidermis- Epi and (B) Abaxial epidermal surface showing trichomes- tr and stomata-st.

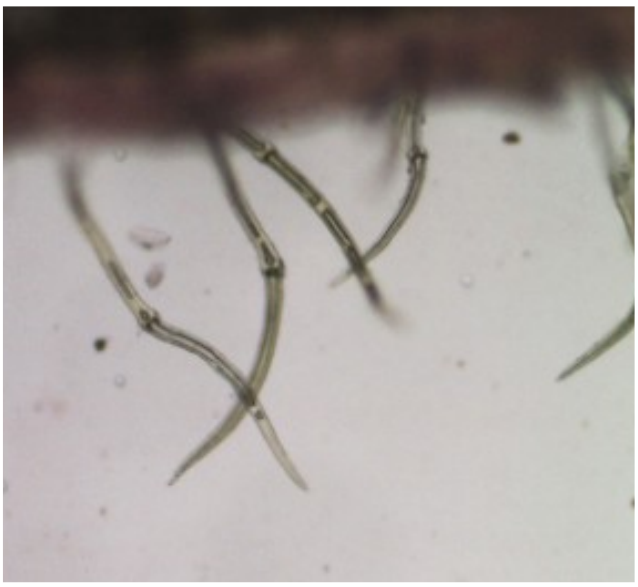

Fig. 9. *G. arborea* leaf trichomes

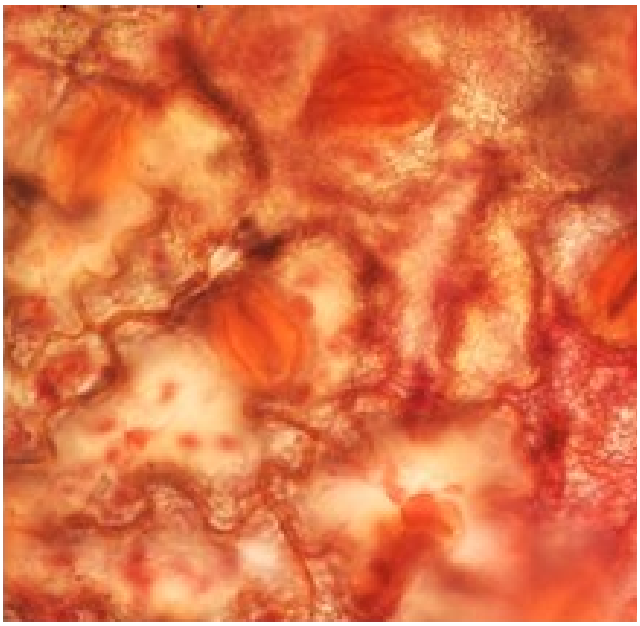

Fig. 10. Abaxial epidermal peel showing stomatal type (at 100X)

3.2.3 Quantitative microscopy

Table 1 shows recorded values of different leaf constants like stomatal number, index, palisade ratio, vein islet and termination numbers (Fig. 11).

3.2.4 Powder microscopy

The leaf powder of *G. arborea* is bright green. The microscopic analysis of the plant powder was characterised by a major portion of scattered broken parts of bleb-like indumentum observed in the abaxial part of leaves. Another diagnostic feature was the presence of uniseriate multicellular trichomes with nodal swelling. The trichomes varied in their size in

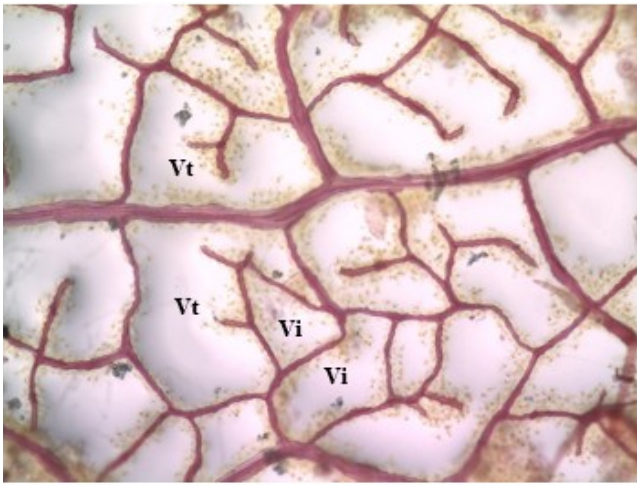

Fig. 11. Vein architecture of *G. arborea*; Vt- vein termination, Vi- vein islet

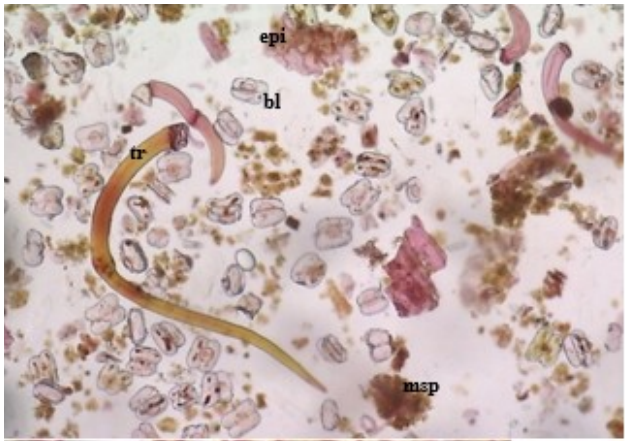

Fig. 12. Powder microscopy of the leaf; epi- epidermal tissue, tr- trichome, bl- broken part of bleb-like indumentum, msp- mesophyll tissue

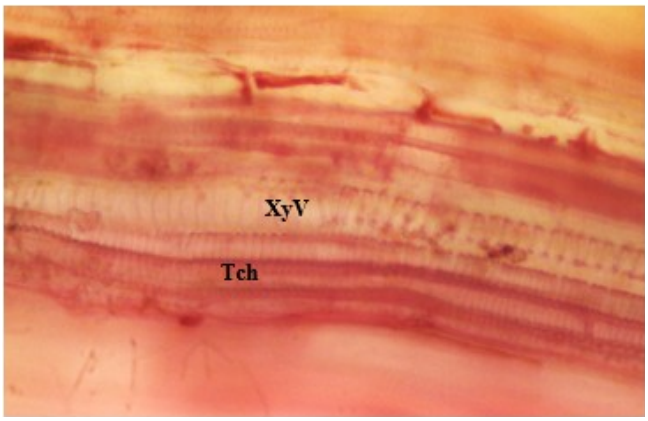

Fig. 13. Vascular elements showing xylem (XyV) and Tch-tracheid

terms of length. Rare appearance of unicellular trichomes were also noted. They also showed patches of epidermal tissue and vascular tissue. Light microscopy of the powder also revealed clumps of mesophyll tissue which were characteristically dull green to light brown colour owing to their chlorophyllous nature (Fig. 12). Closer examination of vascular tissue at higher magnification revealed xylem vessels with spiral thickenings and tracheids of varying length showing annular thickening (Fig. 13).

Table 1. Leaf constants of *G. arborea*

Parameter	Observed value (in 0.1mm^2 area)
Stomatal number	36 – 41
Stomatal index	~27.27
Palisade ratio	~5.25
Vein islet number	5– 8
Vein termination number	10– 12

Conclusion

The pharmacognostic studies serve as an ideal tool in determining the quality of the raw drug material as morphological and anatomical characters together could help in distinguishing the original drug plant from their adulterants. Pharmacognostic analysis of *G. arborea* showed many important features useful for the authentication of the medicinal plant. Presence of well-developed wood, lignified elements such as fibers and stone cells may indicate the woody and arboreus nature of the plant. Near-circular midrib with a slight bulge on the adaxial lamina were the important diagnostic feature of the plant. Numerous bleb-like indumentations on the abaxial epidermis is a unique feature of *G. arborea*. U-shaped single 100-150 µm size vascular bundles with parallel lines of angular thick walled xylem elements also assist the identification of the medicinal plant. Anomocytic type of stomata is another useful identification mark of the plant. High stomatal frequency and trichomatous leaf establishes the xerophytic character of *G. arborea* (8). Trichomes play a role in plant defence to phytophagous insects. Hairs or bristles were considered as specific diagnostic anatomical feature of plants (11). Various parameters like stomatal number, index, palisade ratio, vein islet and termination numbers serve as leaf constants in identification of the sample material. Further studies involving physicochemical pharmacognostic analyses needs to be carried out for substantiating the botanical pharmacognostic findings for more authentic identification of root and stem based crude drugs.

Author's contribution

SVR and VRM designed the objectives and plan of work. VRM carried out the work, analyzed the data and wrote the manuscript. SVR helped in data analysis and manuscript correction.

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Conflict of interests

The authors have declared that no competing interest exist.

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