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Research Article

Pharmacognostic Study and Establishment of Quality Parameters of Leaves of *Bombax insigne* Linn.

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ABSTRACT

Bombax insigne Linn. syn. *Salmalia insignis* (Family – Bombacaceae) is found commonly in India. No detailed pharmacognostic study or establishment of quality parameters has been done on any species of Bombax. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation. Chief characters of transverse section include primary and secondary vascular bundles surrounded by pericyclic fibres and a sheath of calcium oxalate cluster crystals. Chief characters of powder include xylem vessels having reticulate or annular thickening, pericyclic fibres, calcium oxalate prisms and clusters, and starch grains. Various physicochemical parameters were also established. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloids, flavonoids, phenolics, cardiac sterols, triterpenoids, saponins and carbohydrates. Such a study would serve as a useful tool in standardization of the leaf material, isolation of medicinally important phytoconstituents, performing pharmacological investigations and ensuring quality formulations in the future. It would also help in distinguishing the plant material of *Bombax insigne* from its related species, *Bombax ceiba*.

Keywords: Bombacaceae, Bombax, *Bombax ceiba*, *Salmalia insignis*, Shalmali.

INTRODUCTION

Bombax insigne syn. *Salmalia insignis* (Family – Bombacaceae) is also known as Shalmali (Sanskrit), Semul-tula (Bengali), Kalilavu (Malayalam), Kalilavu (Tamil), Didu (Andamans) and Shemlo (Gujarati)[1,2]. It is a tall deciduous tree found commonly in the plains of India, attaining a height up to 40m and a girth up to 6m, with straight buttressed trunk and wide spread branches. Flowers are numerous, near the ends of the branches, appearing before new leaves. Its trunk is devoid of prickles, a characteristic which differentiates it morphologically from its related species, *Bombax ceiba*[3]. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material of *B. insigne* and establishment of its quality parameters, including physicochemical and phytochemical evaluation. This thorough evaluation would be useful in standardization of the leaf material. It would also help in distinguishing *Bombax insigne* from *Bombax ceiba.*

MATERIALS AND METHODS

Collection and authentication of leaves

Leaves of *B. insigne* were collected from the herbal garden of R. K. College of Pharmacy, Rajkot in July, 2010. Herbarium was authenticated by Dr. A. N. Pandey, Department of Biosciences, Saurashtra University and deposited in R. K. College of Pharmacy (No. RKCP/COG/04/2010).

Figure 1: *Bombax insigne*

Pharmacognostic studies

Morphology of fresh leaves of *B. insigne* was studied. Photomicrography of unstained transverse sections and stained transverse sections (using phloroglucinol-HCl) of fresh leaves was performed. Leaf constants were established using camera lucida and stage micrometer[4] (Table 1). The leaves were dried under shade, powdered

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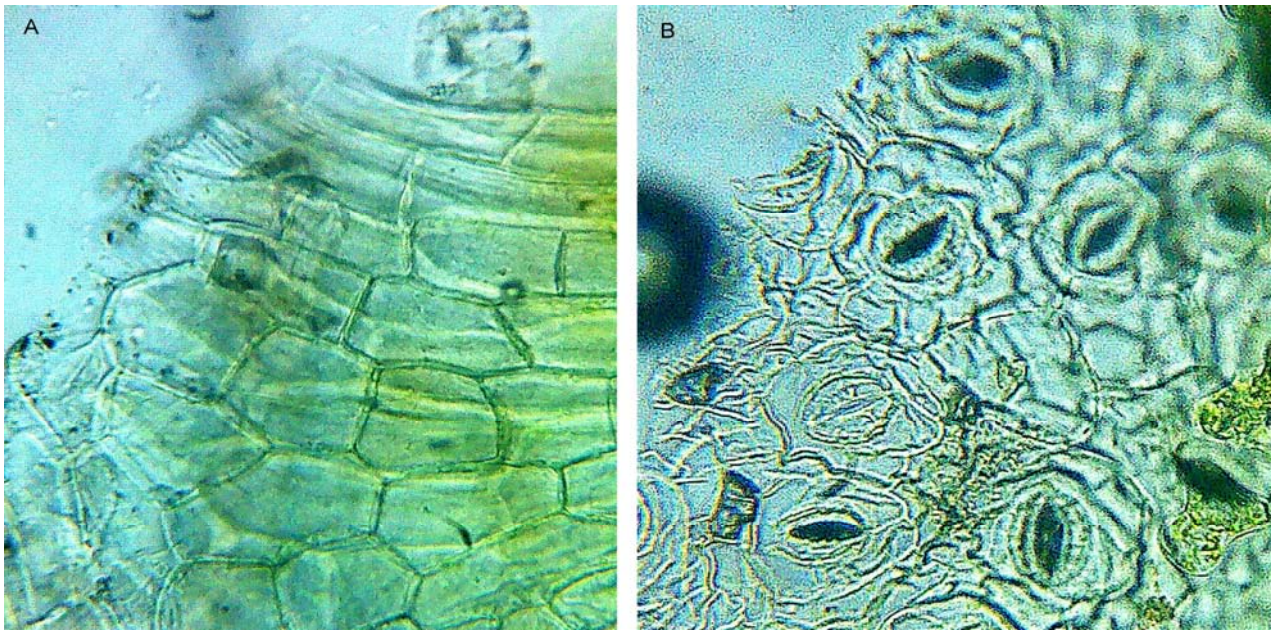

Figure 2: Surface preparation (X400)
A, Thick walled epidermal cells; B, Anomocytic stomata to 60#, stored in airtight containers and used for powder study, physico-chemical evaluation and phytochemical screening.

Physico-chemical evaluation
Various physico-chemical parameters like loss on drying, ash values (total ash, water soluble ash and acid-insoluble ash) and extractive values (water soluble and alcohol soluble extractives) and swelling index were established using the powdered drug[5] (Table 2).

Phytochemical study
The powder was extracted with 50ml each of water and ethanol at 60°C for two hours. Various phytoconstituents present in the leaves were detected by their respective chemical tests using the appropriate extracts [6-12] (Table 3).

Table 1: Quantitative microscopy

Leaf constant	**Mean value ± SD**
Stomatal Number	
Upper surface	3±1
Lower surface	6±1
Stomatal Index	
Upper surface	6.69±0.5
Lower surface	13.66±0.5
Palisade ratio	6 ± 1
Vein islet number	12±1
Vein termination number	7±1
Number of observations = 5, SD = Standard Deviation	

RESULTS
Macroscopy
Leaves are palmately compound, petiole 10-15cm, leaflets three to seven, 10-16 cm X 3-7 cm, leaflets sessile, exstipulate, lanceolate, apex cuspidate, margin entire, surface glabrous and pubescent, texture membranous, base symmetric, venation reticulate

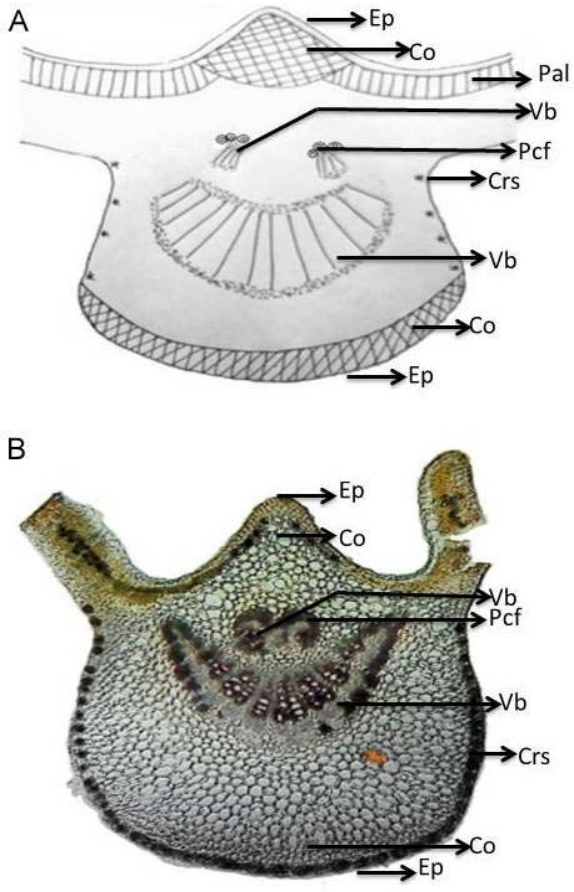

Figure 3: A - Diagrammatic T. S. of leaf. B - Detailed T. S. of leaf (X40) (Co, Collenchyma; Ep, Epidermis; Pal, Palisade; Vb, Vascular bundles; Crs, Calcium oxalate cluster crystal sheath; Pcf, Pericyclic fibers)

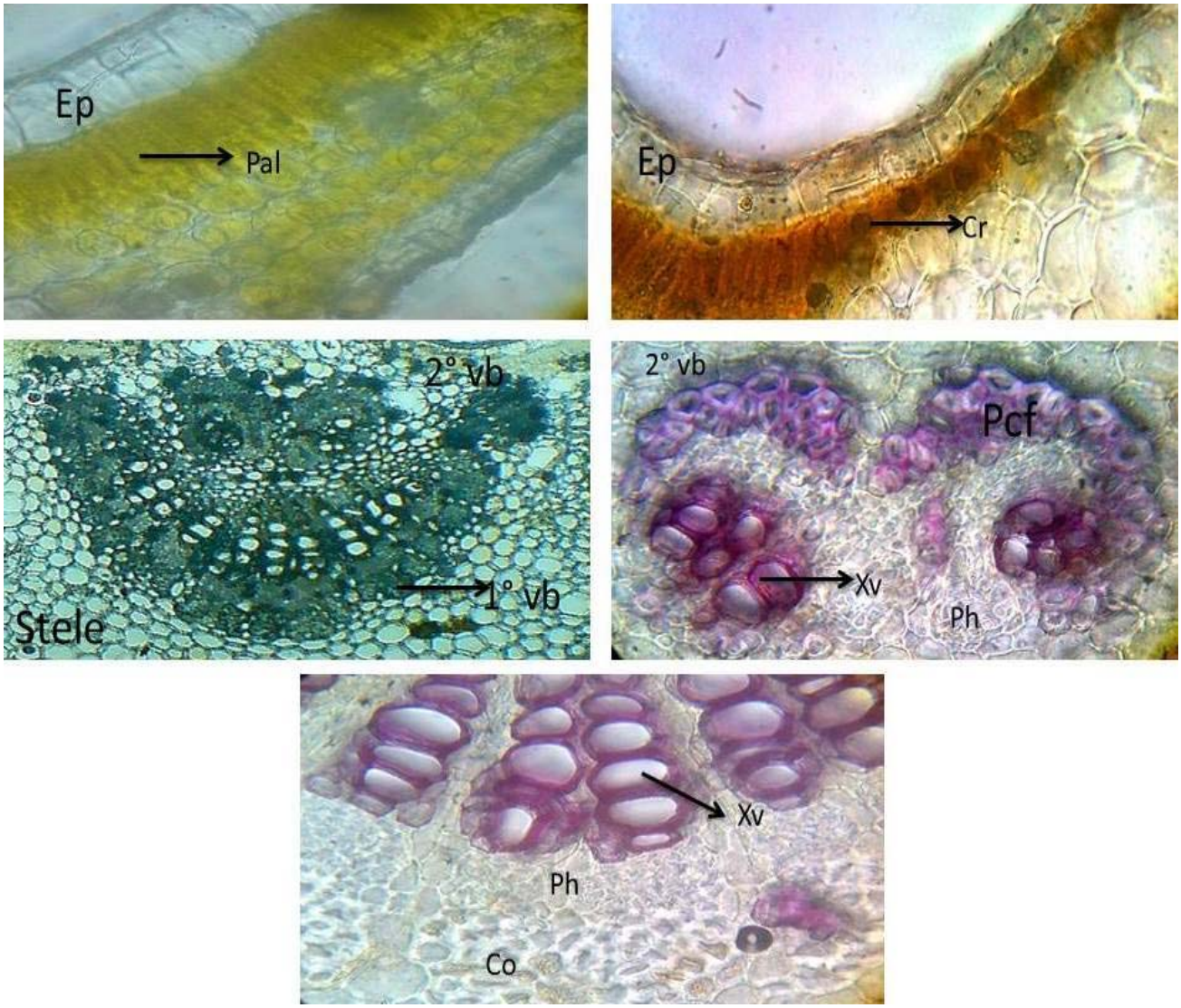

Figure 4: T. S. of leaf showing single enlarged portions (X400)
(Ep, Epidermis; Pal, Palisade cells; Xv, Xylem vessels; Ph, Phloem; Co, Collenchyma; Vb, Vascular bundles; Cr, Calcium oxalate cluster crystals; Pcf, Pericyclic fibers)

Table 2: Physicochemical evaluation

Parameter	**Mean ± SD**
Loss on drying	7.3 ± 0.2%w/w
Ash values	
Total ash	9.3 ± 0.2%w/w
Acid insoluble ash	1.7 ± 0.2%w/w
Water soluble ash	4.1 ± 0.2%w/w
Extractive values	
Water soluble extractive	23.2 ± 0.2%w/w
Alcohol soluble extractive	16.1 ± 0.2%w/w
Swelling index	16±0.7ml

Number of observations = 3, SD = Standard Deviation

(Figure 1). Phyllotaxy is sub-opposite. Color of upper surface is dark green and lower surface is light green. Leaves have a characteristic mucilaginous taste and odor.

Microscopy

Surface preparation shows the presence of thick walled epidermal cells and anomocytic stomata (Figure 2). Transverse section of the leaf shows a dorsiventral lamina, having a single layer of compact rectangular epidermal cells with a distinct cuticle. Beneath it is a single, compact layer of radially elongated palisade cells followed by loosely arranged spongy mesophyll rich in starch grains. The mesophyll covers 3/4th of the lamina. Mid-rib consists of a well-developed collenchyma below upper epidermis and above lower epidermis. A sheath of calcium oxalate cluster crystals is present above the lower epidermis in the lower collenchyma of the mid-rib. Ground tissue consists of loosely arranged polygonal parenchymatous cells having orange coloring matter. Vascular bundles are bicollateral. Primary vascular bundles showed 14 - 15 seriate xylem vessels whereas secondary vascular bundle showed 4 - 5 seriate xylem vessels. Bunch of pericyclic fibres are situated above the secondary vascular bundles, whereas they are discontinuous below the primary vascular bundles.

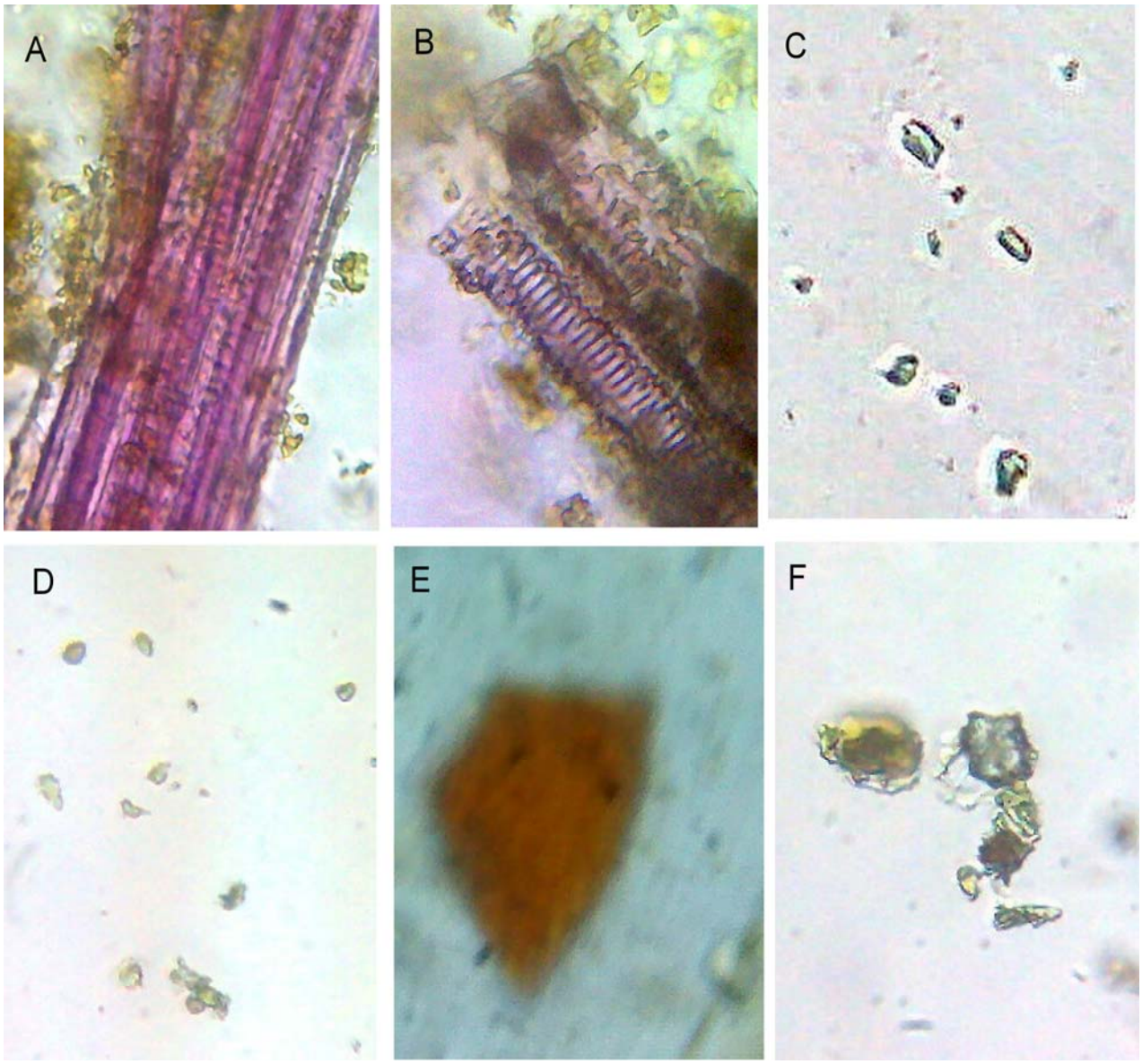

Figure 5: Powder study (X400)
A, Pericyclic fibers; B, Xylem vessels with reticulate thickening; C, Calcium oxalate prism crystals; D, Starch grains; E, Orange matter; F, Calcium oxalate cluster crystal

Starch grains and prisms of calcium oxalate are scattered throughout the ground tissue (Figure 3, 4).

Microscopy: Powder characteristics

Powder is dark green with characteristic odor and taste. Diagnostic microscopic features of the powder include xylem vessels with pitted, reticulate or annular thickening, bundles of pericyclic fibres, calcium oxalate prisms and clusters, starch grains and orange-colored matter (Figure 5).

DISCUSSION

The present work deals with the microscopic, physicochemical and phytochemical evaluation of the leaves of *Bombax insigne*. Main microscopic characters include anomocytic stomata, sheath of calcium oxalate cluster crystals present above the lower epidermis of the mid-rib and pericyclic fibres in groups above the secondary vascular bundles and discontinuous below the primary vascular bundles. Diagnostic characters of

Table 3: Phytochemical screening

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	+ve
	Hager's test	+ve
	Wagner's test	+ve
	Mayer's test	+ve
Flavonoids	Shinoda test	+ve
	Lead acetate test	+ve
Phenolics	Ferric chloride test	+ve
	Folin ciocalteu test	+ve
Sterols	Salkowski test	+ve
and triterpenoids	Libermann-Buchardt test	+ve
Cardiac	Legal test	+ve
glycosides	Baljet test	+ve
	Keller Killiani test	+ve
Saponin	Foam test	+ve
glycosides	Lead acetate test	+ve
Anthraquinone	Borntrager test	-ve
glycosides	Modified Borntrager test	-ve
Carbohydrates	Fehling's test	+ve
	Molisch test	+ve
Mucilage	Ruthenium red test	+ve

powder include bundle of pericyclic fibres, calcium oxalate clusters and prisms, starch grains and xylem vessels with reticulate or annular thickening. Various physicochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, flavonoids, phenolics, cardiac sterols, triterpenoids, saponins and carbohydrates, which may influence the pharmacological actions of the plant. Such a detailed study would be decisive in performing standardization of the leaf material, preparation of its monograph, isolation of phytoconstituents, performing further pre-clinical and clinical investigations and manufacturing of its formulations and differentiating it from its closely related species, *Bombax ceiba*.

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