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Pharmacognostic Studies on Leaves of Salmalia Malabarica

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Abstract:
Salmalia malabarica, commonly called Silk Cotton Tree is a deciduous tree popularly used in tribal and folklore medicine. Almost all parts of the tree are medicinally used and forms an important ingredient of different Ayurvedic formulations. The leaves, bark, seeds and the gum exudates are reported to contain various bioactive phytochemicals. Despite the reported medicinal uses of various parts of silk cotton tree, no systematic pharmacognostic evaluation has been reported on the leaves of *Salmaliamalabarica*.The current research,which reports the pharmacognostic characteristics of leaves of *Salmaliamalabarica*,for the first time, is an attempt towards sustainable use of this tree.The transverse section of the leaf lamina reveals a thick midrib and mesophyll tissue covering most of the laminal area. There is cuticle on adaxial side followed by 2-4 layers of epidermis. The palisade layer shows tubular cells with chloroplasts and has spongy tissue to the abaxial side. The 1 -2 layered epidermis on abaxial surface shows anisocytic stomata. Vascular bundles are bicollatoral and the mesophyll cells show starch grains. These anatomical features are discernable in leaf powder when examined under the microscope. The proximate analysis of the leaf powder and its extractive values in various solvents are provided. The phytochemical fingerprint of the leaf powderhas been developed using HPTLC, and can be used as an effective quality control tool. The leaf anatomy is similar to that reported in *Bombax insigne* but the stomatal anatomy is distinctive. Both microscopic evaluation and phytochemical evaluation will help distinguish the leaf powders of these two closely related trees.

Keywords: *Salmalia malabarica*, phytochemicals, HPTLC, Pharmacognosy

Introduction:

Salmaliamalabarica, commonly calledSilk Cotton Tree is an important tree which is reported to be used in the Indian traditional systems of medicine.It isalso known as Indian Red Kapok tree, Semal, Shimul, Shalmali etc.In tribal medicineand folklore medicine almost all parts of the plant are used medicinally and thus the tree has ethnobotanic significance [1].Plants of family Bombacaceae are already reported to possess a variety of phytochemical compounds present in their different parts [2].*Salmaliamalabarica*Schott. &Endl, syn *Bombax ceiba Linn.*is widely distributed in deciduous forests throughout the drierand arid regions of India, including the Andaman Islands.It is seen upto an altitude of 1500 meters and is also planted as an avenue tree. It is a deciduous tree, which can grow up to 40 meters tall and at least 6 meters in diameter. The bark of*Salmaliamalabarica*is 1.8 to 2.5 cm thick, pale

ash to grey in appearance, and is rough and it cracksas it matures. The trunk bears numerous conical spines when young, butthese get eroded as the trunk gets older.Leaves are compoundand palmate with about 6-7 leaflets radiating from the tip of petiole. The flowers are large, bright red in colour and bloom in January. The fruits develop as green pods that become brown when they mature. The pods bear numerous oily seeds covered in silk cotton. The silky cotton is used as fillings for soft pillows and is highly priced[3,4].

Phytochemical constituents:

Leaves contain shamimin and mangiferin. The seeds of *Salmaliamalabarica*contain palmitic acid, stearic acid, gallic acid, tannic acid, lupeol. Lupeol is also reported from root, bark and leaves. The stem as well as bark contains lupeol, β-sitosterol, shamimin, flavonoids, glycoside, sterol and terpenoids. The gum exudate (mocharas) from the trunk contains tannic acid, gallic acid and a mixture of L-arabinose, D-glactose and D-galacturonic acid. The flowers contain carbohydrates (fructose, glucose, galactose, sucrose, lactose, arabinose), amino acids (lysine, arginine, alanine, glutamic acid, leucine) and β-sitosterol, hentraicontane, hentriacontanaol, quercetin, kaempferol, bombasin, and bombalin[5].

Therapeutic properties:

In Ayurveda, *Salmaliamalabarica* is identified with properties like stimulant, astringent, hemostatic, aphrodisiac, diuretic, antidiarrheal, cardiotonic, emetic, demulcent, antidysenteric, and antipyretic[6, 5].The roots and pods are used for the treatment of low vitality, debility, sexual weakness, leprosy and as a body coolant. The leaves are reported to be good for strangury and skin eruptions. They possess antibacterial, antioxidant, hypotensive and antipyretic activities. Seeds are used to cure chickenpox, smallpox, catarrhal affections, chronic cystitis, genitourinary diseases and is also used as an abortifacient. The stem bark is reported to be useful in dysentery, bleeding disorders, acne vulgaris. The gum exudate from the trunk have astringent, demulcent, styptic properties and is used against diarrhea, dysentery, hemoptysis, bleeding piles, menorrhagia, infertility and spermatorrhea. Flowers are used as diuretic, laxative, and have astringent, anti-diabetic, hepatoprotective properties. They are also useful in the treatment of menorrhagia [7, 8, 9, 10].

Despite the reported medicinal uses of various parts of silk cotton tree, no systematic pharmacognostic evaluation has been reported on the leaves of *Salmaliamalabarica*. There arepublished reports available on the pharmacognostic evaluation of root and bark of *Salmaliamalabarica* [3, 4]whereasleaves of *Salmaliamalabarica*have not been rereported so far. There is, however, a report on the pharmacognostic studies on the leaves of *Bombax insigne* syn. *Salmalia insignis*, which is a species related to *Salmaliamalabarica*[11, 12]. The current research was therefore undertaken to record the pharmacognostic characteristics of leaves of *Salmaliamalabarica* as an attempt towards sustainable use of this tree for medicinal purposes by encouraging the use of leaves instead of bark and roots.

Materials and Method

Collection of leaves and its processing:

Collection of plant material requires consideration to be given to factors like, the time and month of collection, the phenological stage of the plant, the geographic location, etc. [13]. Fresh leaves of *Salmaliamalabarica* were collected fromvarious geographical regions of India like- Nerul (Navi Mumbai), Karnala, Mumbai - Pune expressway, Ratnagiri, Medicinal Plant Garden (Maharaja Sayajirao

University of Baroda), Varanasi. A specimen of a branch with leaves of *Salmaliamalabarica* was mounted on the herbarium sheets (30 X 42 cm) and labelled with detailed information[14]. The herbarium was then authenticated from Agharkar Research Institute, Pune and the certificate of authentication was obtained.

The leaves after collection were cleaned of exogenous material and were shade dried for two days. The partially dried leaves were then dried in oven at 45°C (± 2°C) for 4 days. The dried leaves were pulverized using a motorized pulverizer and sieved through BSS mesh No 85. The powder was stored in neatly labeled PearlPet® containers and stored at room temperature away from direct sunlight in a rodent- free, fire and water-proof area. Thus, prepared leaf powder waseventually used for pharmacognostic evaluation.

To study leaf anatomy, transverse sections of fresh leaves were stained with dilute saffranine, mounted in pure glycerol and sealed with paraffin wax to prepare semi-permanent slides. For observation of lamina for stomatal morphology, 5 mm pieces from the middle portion between the lamina and midrib of a mature leafwere taken. They were boiled with 60% nitric acid in a test tube placed in a water bath. Within few minutes of boiling, the epidermis separated out. With the help of a brush, the separated epidermis was placed in a watch glass with water. The washed epidermis was placed on a slide along with 1-2 drops of glycerin. A fine brush was moved to straighten the epidermis and wasthen covered with a cover glass. These preparations were then photographed under a compound microscope using 10X and 40X objectives and 10X eye piece to obtain 100X and 400X magnifications respectively.

Microscopic and Phytochemical evaluation:

Instruments used for analysis :

The HPTLC system used for the analysis was a CAMAG system equipped with Linomat V Automatic Sample Spotter (CAMAG, Muttenz, Switzerland) and CAMAG TLC Scanner IV with win CATS planar chromatography manager software version 1.4.7. The TLC plates used were pre-coated with silica gel 60 F_{254} (Merck, Germany) of 0.2 mm thickness. All chemicals used were of analytical grade. Leica Microscope (DM750) with image analyzer software (LAS EZ Imaging Software) was used for light microscopic studies.

Microscopic studies:

To study microscope characteristics of the leaf powder particles, maceration technique was used. 1.0 g of leaf powder was taken in a beaker, 10 % nitric acid with a pinch of $K_2Cr_2O_7$ (potassium dichromate), wasadded as a clearing agent. This mixture was boiled at 225°C for 5 minutes on a hot plate. The residue was washed thoroughly with water, several times,to remove every trace of nitric acid. Before each repeated washing, the residue was allowed to settle down and was collected by decantation). The final washed residue was collected in a Petri plate andstained with 1% aqueous safranine for 30 minutes. The residue particles were picked up using a brush (Camlin no. 2), just enough to cover the tip of the brush. These were placed on a slide, mounted in glycerol using coverglass and sealed with paraffin wax. The slides with evenly distributed particles were selected for microscopic evaluation of the leaf powder

Proximate and Phytochemical Analysis:

Proximate analysis of the leaf powder was carried out to evaluate the quality parameters for the crude raw material. The preliminary qualitative phytochemical analysis of leaf powder of *Salmaliamalabarica* was carried out in order to check the presence of various classes of phytochemicals such as alkaloids, flavonoids, terpenoids, phenolics, saponins etc.The plant powder (3.0 g) was extracted with ethanol (30

mL) and water (30.0 mL), vortexed for a minute and sonicated for 20 min. The mixture was filtered through Whatman filter paper (No. 1). The filtrates were used to perform the preliminary phytochemical tests.

Extractive values of the leaf powder were determined using solvents of varying polarities. 500mg of leaf powder was mixed with 100 mL of each solvent. The mixture was vortexed on a vertical shaker overnight. The mixture was centrifuged, and the residue was separated by filtration. The filtrate was evaporated to dryness in a water bath. The residue was accurately weighed to obtain the percent yield[15,16].

Ethanolic extract of the leaf powder was subjected to HPTLC separation of its phytoconstituents. The HPTLC was carried out using an optimized mobile phase that gave good number of bands which were well separated. The Rf values for each band was calculated and recorded.

Observations:

The transverse section of leaf (Figure 1) reveals a thick midrib, lateral veins with a single layer of the thin dorsiventral compact lamina. 3/4tharea of the lamina is covered with mesophyll tissue. The leaf lamina hascuticle on the adaxial surface, andrectangular epidermis, which is 2-4 layered followed by single layer of hypodermis. The photosynthetic palisade cellsform the next layer beneath it. The palisade cells are tubularwith prominent chloroplasts. The palisade layer is followed by a layer of spongy tissue which is loosely arranged. The spongy tissue layer is followed by the epidermis that forms the abaxial surface. This epidermis is thick walled 1-2 layered and anisocytic stomata is seen on the surface.The stomata show, two kidney shaped guard cells with three subsidiary cells of which one is small and the remaining two are larger (Figure 2 – A & B). Iinterspacedwithin the spongy tissue are bicollateral vascular bundles of the veinlets (Figure 3–C&D). Starch grains are seen in the mesophyll cells.

The midrib shows prominent vascular bundleswith xylem, phloem and cambium (Figure 3–E&F).The vascular bundles are bicollateral and arranged in an arc in the central region of the midrib. There are 4-5 seriate xylem vessels (Figure 3 - G) in secondary vascular bundles at the adaxial side while there are 14-15 in the primary vascular bundles at the abaxial side. Pericyclic fibers are discontinuous beneath the primary vascular bundles and arranged in a bunch above the secondary vascular bundles. Midrib is made up of robust collenchyma cells, which are situated directly below the layers of upper epidermis and hypodermis and just above the lower epidermis. Thin walled Parenchymatous cells line the region below the Collenchyma at the adaxial side. The central region of the midrib shows thick walled Sclerenchymatous cells.An envelope of crystals of calcium oxalate (Figure 3–H&I) is observed above the lower epidermis in the midrib region. The crystals in clusters.

Figure 1 :Transverse Section of leaf of *Salmaliamalabarica*(Photo college - X100)

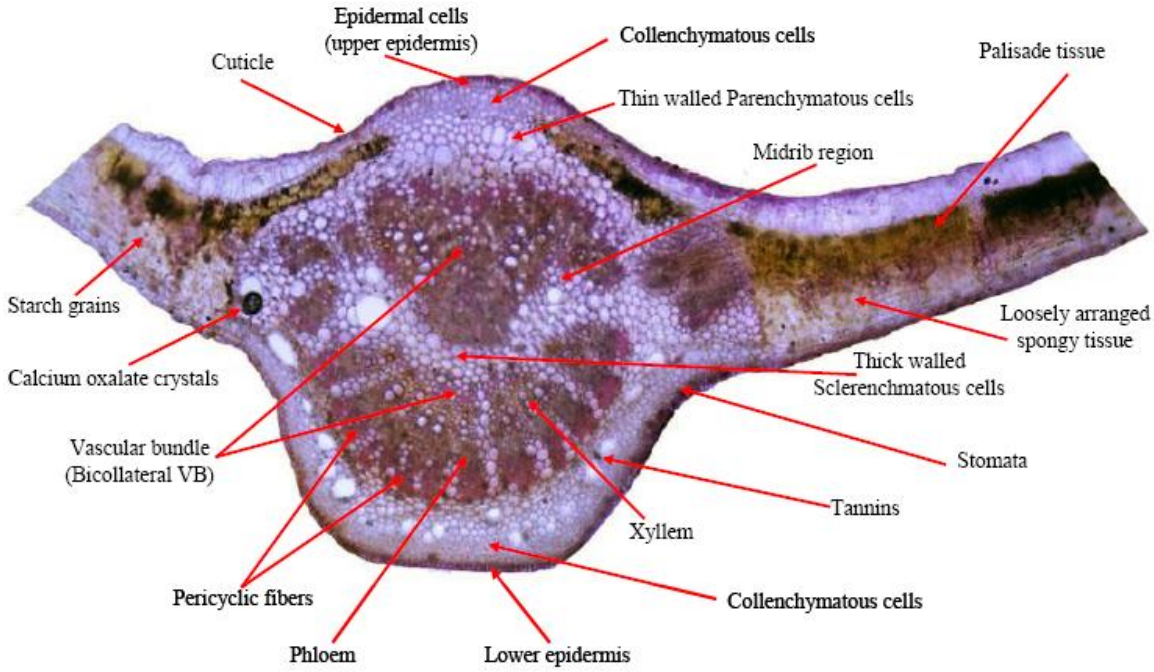


Figure 2: Stomata as seen in the lamina peel under microscope (A. 100 X, B. Digitally enhanced)

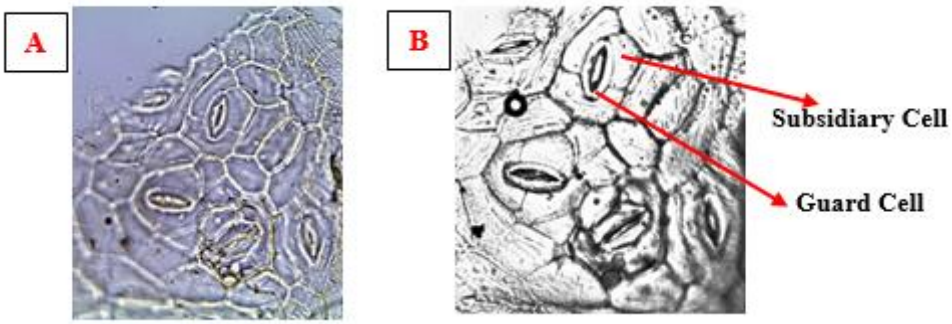
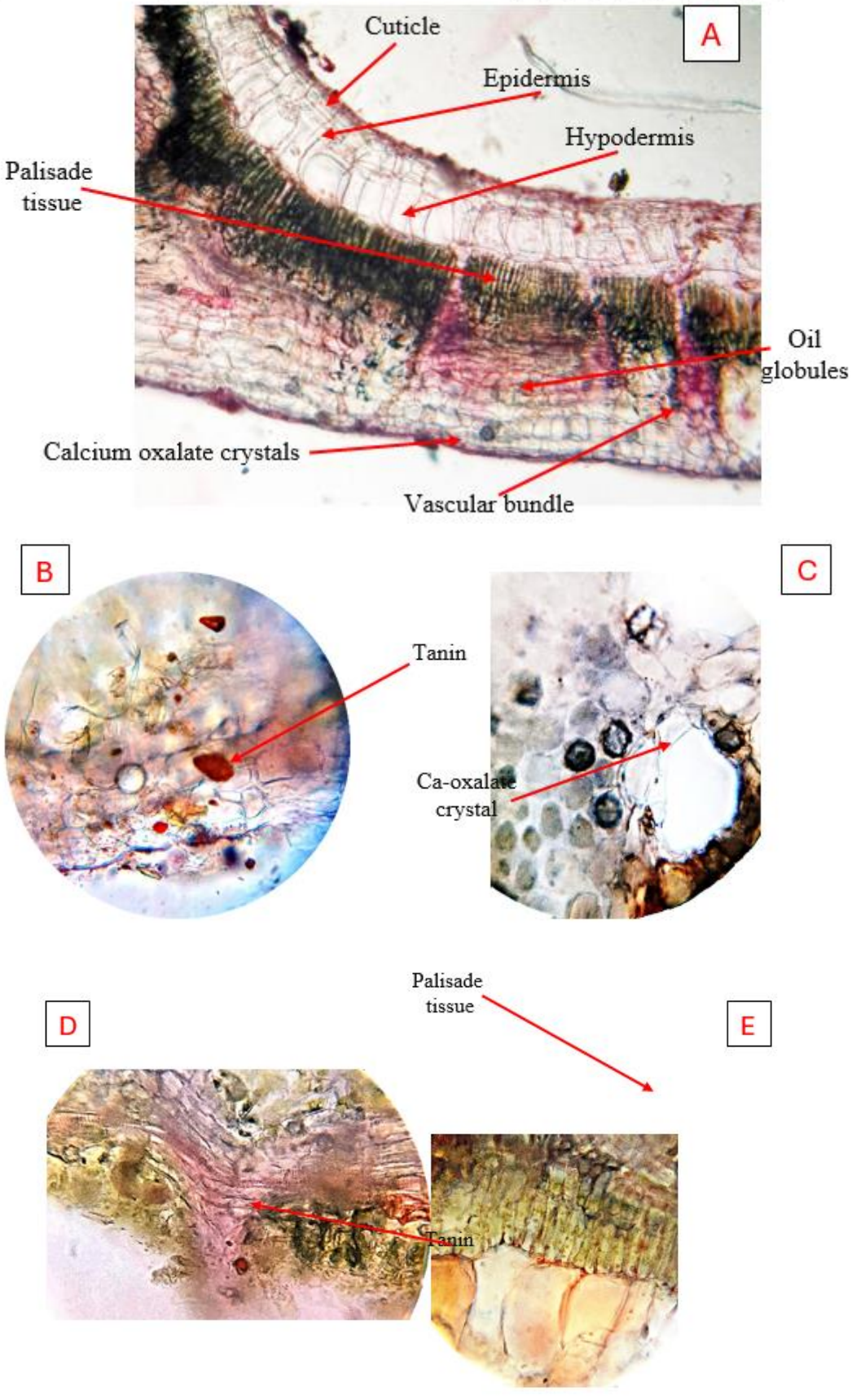
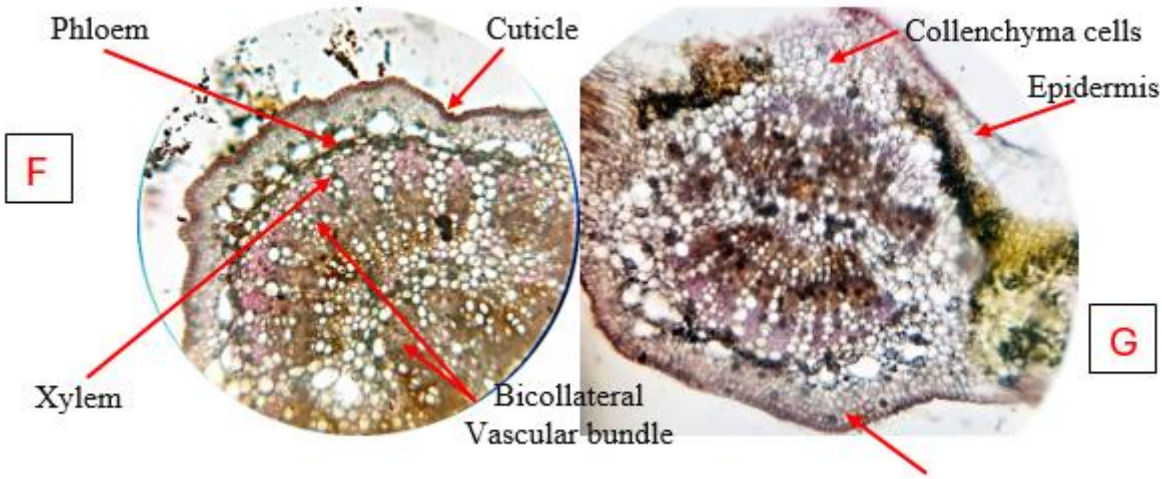
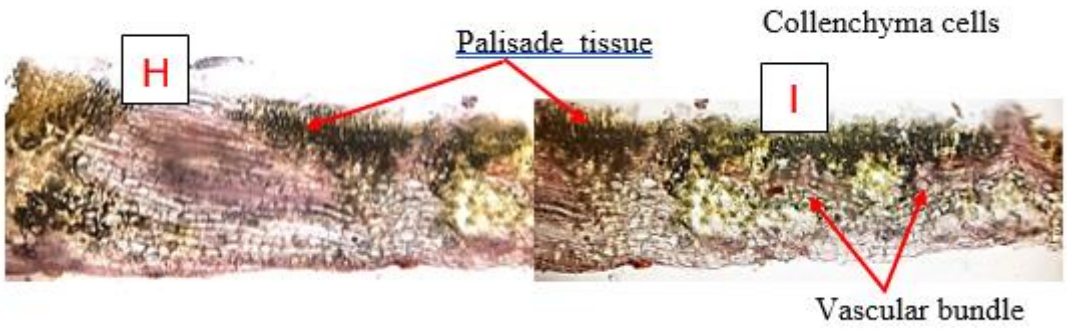
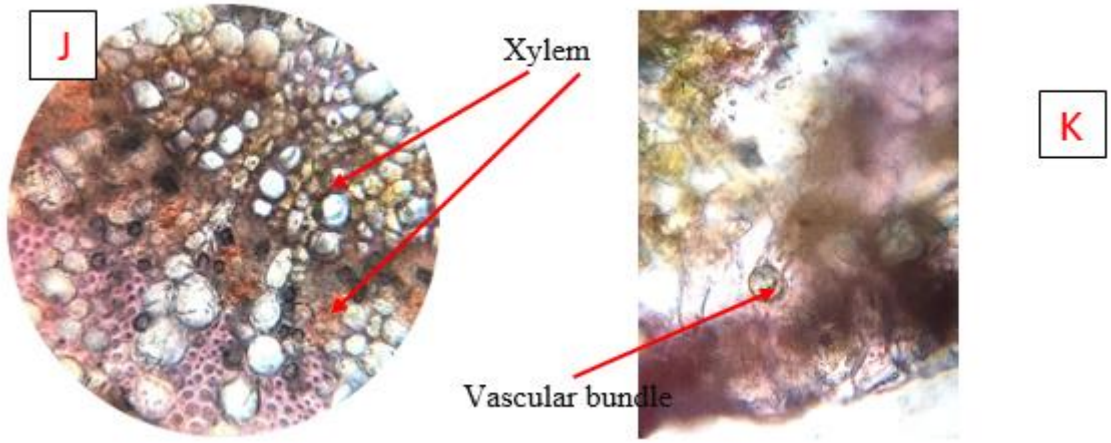


Figure 3: Lamina part of *Salmaliamalabarica* leaf (A,B,C,D,E,F,G,H,I,J,K,L and M)




Phloem
Cuticle
F
Xylem
Bicollateral
Vascular bundle
Collenchyma cells
Epidermis
G


H
Palisade tissue
Collenchyma cells
I
Vascular bundle


J
Xylem
Vascular bundle
K

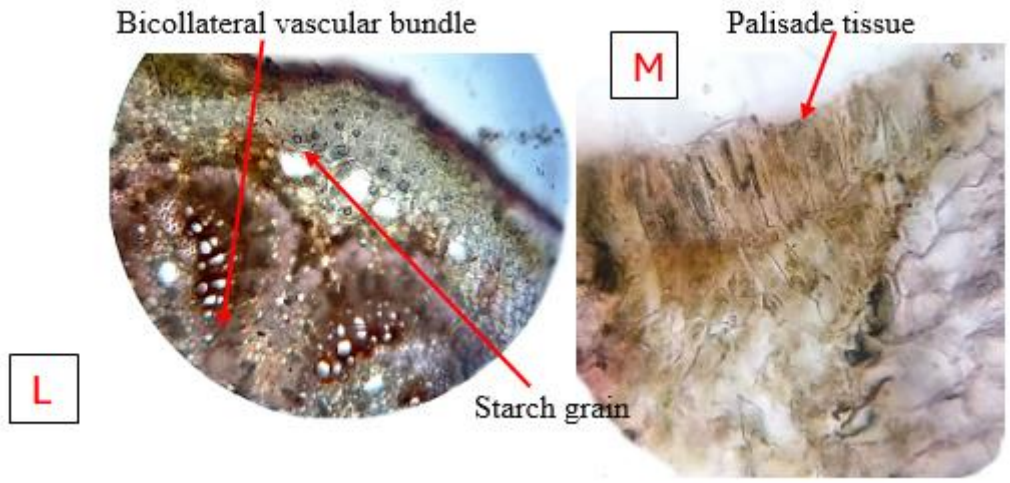
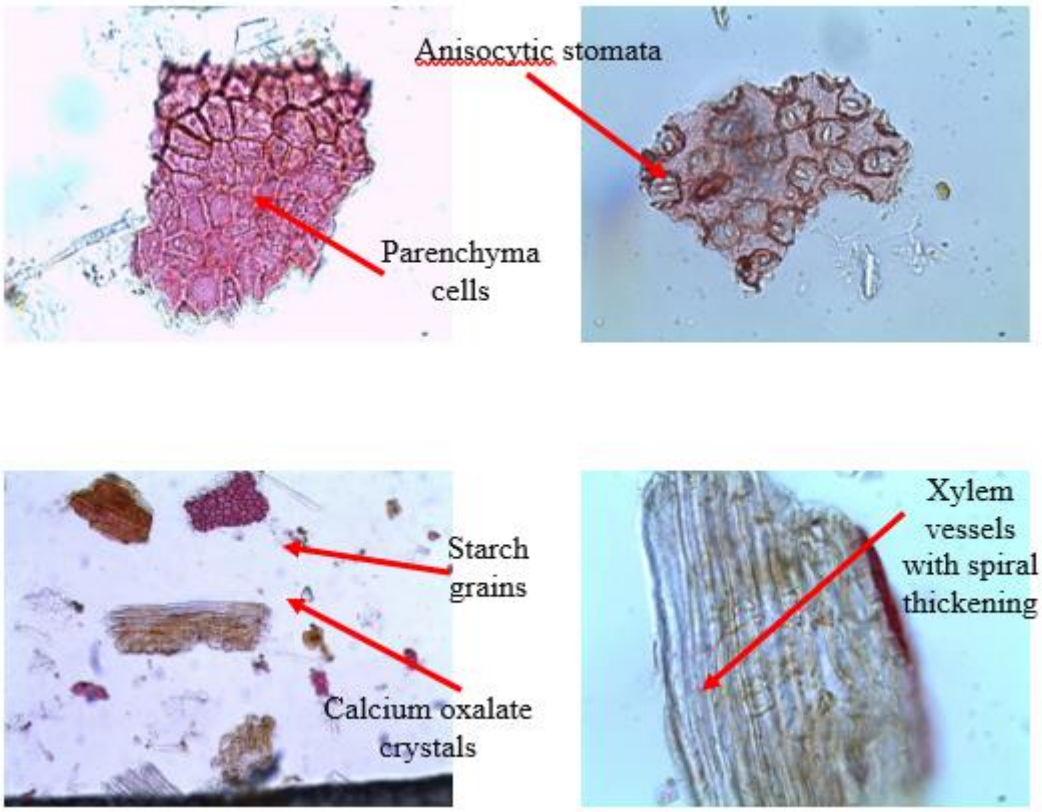
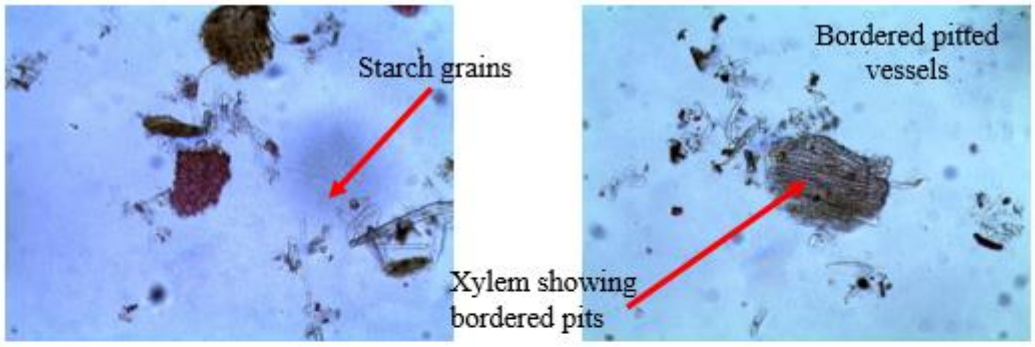
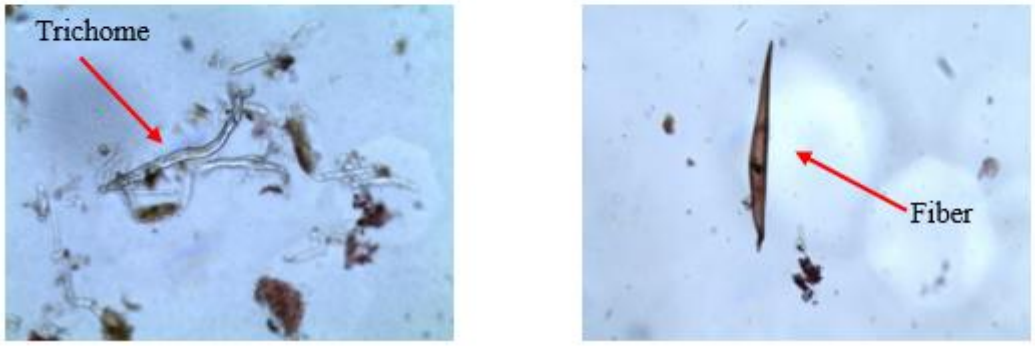
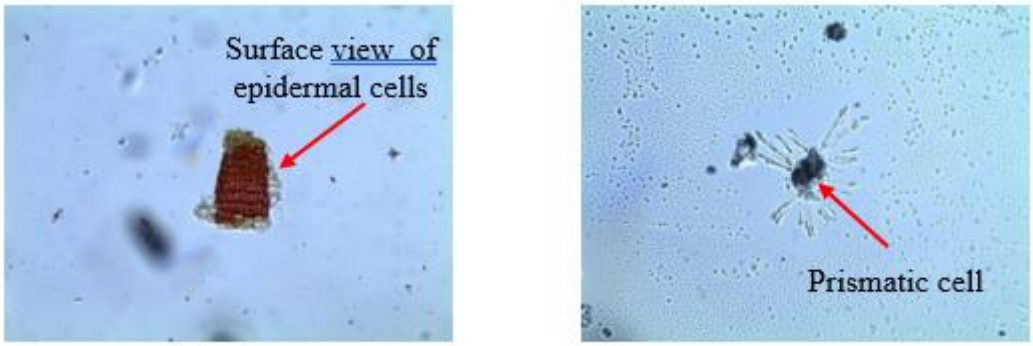


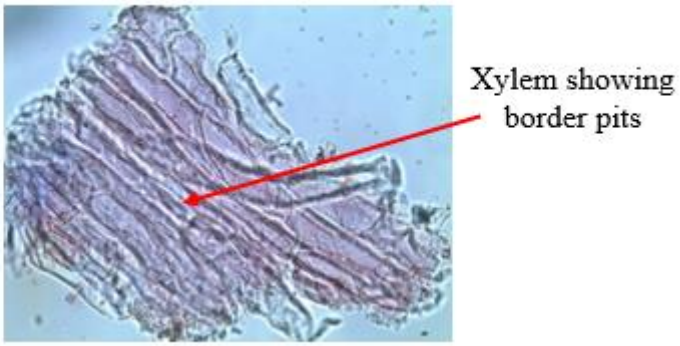
Figure 4 : Microscopic characters of *Salmaliamalabarica* leaf Powder (400X)




Starch grains
Bordered pitted vessels
Xylem showing bordered pits


Trichome
Fiber


Surface view of epidermal cells
Prismatic cell


Xylem showing border pits

2-3 layered Epidermis

Xylem with spiral thickening

Characteristics of leaf powderof *Salmaliamalabarica*:

*Salmaliamalabarica*leaf powder shows the presence of tissue fragments that could be correlated with the features seen in the transverse section of the leaf. The powder shows few parenchyma cells, (Figure 4 - A)fragments of xylem (Figure 4 – B & C)showing spiral thickening and few anomocytic stomata (Figure 4 - D).Fragments of palisade cells and calcium oxalate crystals (Figure 4 - E)are also present. Fragments of pericyclic fibers (Figure 4 - F), starch grains (Figure 4 - G), oil globules and trichome (Figure 4 - H)could also be observed. The predominant particles in the leaf powder, that could be easily discernable and identified included parenchyma cells, xylem showing spiral thickenings, fragments of palisade cells.Stomata are specific to the particular plant and act as diagnostic characters, in the identification of plant powders. In case of leaf, surface preparations give the details of type of stomata, trichomes and Ca-oxalate crystals.

Proximate Analysis of the leaf

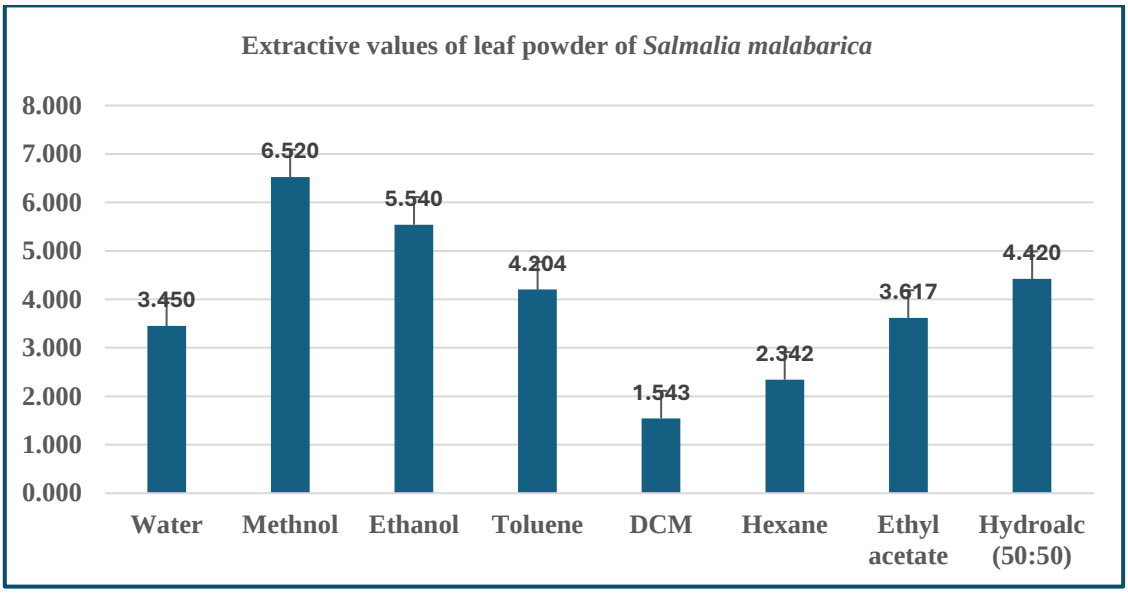
The raw material of leaf of *Salmaliamalabarica*was analyzed for its proximate parameters. The results are listed in Table No. 1

Table 1: Proximate Parameters of *Salmaliamalabarica*leaves

Parameter	Observed value (%)
Foreign Matter	0.212 ± 0.004
Total Ash	6.726 ± 0.077
Acid Insoluble Ash	0.488 ±0.009
Water Soluble Ash	4.293 ±0.075
Loss on Drying	9.371 ± 1.030
Moisture Content	8.162 ±0.121

These values can be used by the industry for evaluating the quality of crude raw material of the leaves. The leaf powder was extracted with different solvents of varying polarities. The extractive yields in percentage are shown in Figure 5.The maximum extraction is obtained with methanol followed by hydro-ethanol.[17].

Figure 5 :Extractive values of leaf powder of *Salmaliamalabarica*



The methanolic extract of the leaves was subjected to evaluation of its phytochemical fractions. The observations are listed in Table no.2.

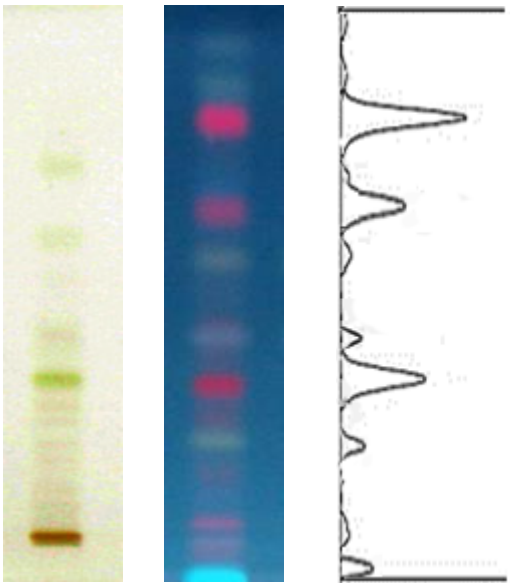
Table 2 : Phytochemical Evaluation of leaf extracts of *Salmaliamalabarica*

Parameter	Observed Value (%)
Fats and Waxes(Neutral Extract)	15.560± 0.016
Terpenoids & Phenolics(Moderately Polar Extract)	32.100±0.008
Quaternary alkaloids and N-oxides (polar extract)	116.900±0.013
Alkaloids(basic extract)	13.640±0.012
Crude Fibers	4.232±0.004

The methanolic extract of the leaf is rich in phenolics and alkaloids which is indicative of its medicinal value. The methanolic extract was further evaluated for its phytochemical profile using HPTLC. A phytochemical fingerprint that was generated with HPTLC is shown in Figure No. 6.

Figure 6 : Phytochemical fingerprint of ethanolic extract of leaves of *Salmaliamalabarica*before and after derivatization at 366nm

Band No.	R_f
1	0.257
2	0.300
3	0.400
4	0.542
5	0.642
6	0.714
7	0.814
8	0.914



Thephytochemical fingerprint of leaves of *Salmaliamalabarica*showed 8 distinct bands.The maximum intensity is seen for the band at R_f 0.814.The fingerprint showed well resolved bands and can be a quality evaluation test for the leaf raw material of *Salmaliamalabarica*.

Discussion:

Salmaliamalabarica is a medicinal plant with its significance in human health recorded in Ayurveda, traditional knowledge and folklore. Though various parts of the tree are used in therapeutic practices, there is no systematic study reported on the Pharmacognostic characteristics of the leaves of *Salmaliamalabarica*. There are reports of the Pharmacognostic studies of its bark [4] and histochemical studies on its leaves[18]. Use of leaves would be a more sustainable utilization of the tree as compared to bark and roots. The current study is a systematic evaluation of the Pharmacognostic properties of the leaves of *Salmaliamalabarica*. The transverse section of *Salmaliamalabarica*isdistinctive from that of *Bombax insigne*in the arrangement of vascular bundles in the midrib. Though the number of primary and secondary vascular bundles could be similar, the vascular bundles are more peripheral in arrangement as compared to those in *Bombax insigne*. The ridge on the adaxial side of the midrib is more prominent in *Bombax insigne*as compared to *Salmaliamalabarica*. The stomata in *Bombax insigne* are anomoctytic while in *Salmaliamalabarica* they are anisocytic. Calcium oxalate crystals and starch grains are more prominent in *Salmaliamalabarica* as compared to *Bombax insigne* [11]. The characteristic differences in the anatomy of the leaf are not clearly seen in the characteristic particles seen under the microscope of the leaf powder. It would therefore be very difficult to differentiate the leaf powder of *Salmaliamalabarica* from the leaf powder of *Bombax insigne*. Phytochemical fingerprints would be a possible differentiating test. The HPTLC fingerprint of leaf powder of *Salmaliamalabarica*sshows bands with maximum intensity at R_f 0.814.

The current study reveals similarities in the leaf anatomy *Salmaliamalabarica* and *Bombax insigne*. Since *Salmaliamalabarica* is reported to be medicinally very useful in traditional practices of medicine, it is important to ensure that authentic material of *Salmaliamalabarica* is used in medicinal formulations.

The microscopic evaluation of lamina peel very clearly indicates that *Salmaliamalabarica* and *Bombax insigne* differ in their stomatal anatomy. This brings out the significance of microscopy in authentication of medicinal plant raw materials. Additionally, phytochemical evaluations of two plantsshould be carried out with sensitive analytical techniques so that the raw materials of both these plants could be used authentically. It is also important to note that *Bombax insigne* is not an accepted substitute for *Salmaliamalabarica*.

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