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Microscopic and Physicochemical Evaluation of *Lagerstroemia lanceolata* Wall Leaves

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Original Research Article

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Abstract: The aim of the present study was to perform the microscopic evaluation of *Lagerstroemia lanceolata* Wall. Leaves Fresh *L. lanceolata* Wall. Leaf was studied macroscopically and microscopically. Preliminary phytochemical investigation of the plant specimen was done along with other WHO parameters for the standardization of drug. The Scanning Electron Microscopy (SEM) of leaf and powder of the drug was done. The detailed microscopy revealed the presence of uniseriate unicellular covering trichomes, anomocytic stomata, calcium oxalate crystals, palisade cells, xylem vessels. The SEM revealed the nature of stomata, trichomes, epidermis, collenchyma and cuticle layer. The Physiochemical parameters such as ash values, loss on drying, extractive values, fluorescence powder analysis were also determined. The microscopic, SEM and physicochemical analysis of the *L. lanceolata* leaf is useful in standardization for quality, purity and sample identification.

Keywords: *L. lanceolata*, Scanning Electron Microscopy (SEM), physicochemical analysis.

INTRODUCTION

Lagerstroemia lanceolata Wall. (Lythraceae) is a moderate to large deciduous tree, sometimes attaining 30 metres in height and 2.4 to 3.0 metres in girth with a clean cylindrical bole of 12 to 15 metres. It is found from Bombay to Kerala and in the hills of Deccan Peninsula upto an altitude of 1,200 metres. Bark is smooth, greenish or yellowish white, exfoliating in papery strips; leaves elliptic – lanceolate or broadly ovate, 6.2 to 10.0 cm x 1.8 to 5.0 cm, coriaceous, glabrous, shining above, usually white or greyish blue; flowers small, white, in large panicles; capsules ellipsoid; seeds winged[1].

The wood is most commonly used for building construction, bridges, ships and boats. The leaves are used as green manure in arecanut gardens. Tannin is present in leaves[1].

Authenticity, purity and assay are important attributes for assuring the quality and standardization of herbal drugs. Hence, in this work we report an attempt of standardization of *L. lanceolata* leaf by performing Pharmacognostic evaluation, microscopic evaluation and scanning electronic microscopy.

MATERIALS AND METHODS

Chemicals

Phloroglucinol, glycerine, hydrochloric acid, chloral hydrate, potassium hydroxide and all other chemicals used in the study were of analytical grade.

Plant material

L. lanceolata leaves were collected from Maharashtra Forest Department, Tansa Wildlife Sanctuary, Tansa WLS, Shahapur.

Macroscopic and microscopic evaluation

The macroscopy and microscopy of plant were studied according to the method of Brain *et al.* [2]. Transverse sections and ground powders were observed under a microscope to determine the anatomical and histological characteristics [3,4].

SEM of leaves and powder

Fresh leaf samples on both sides observed by using FEI (Field Emission Ion)-Quantum 200 SEM with microscope Control Software., LFD – Large Field Detector, Light source is electron beam by tungsten filament. During SEM study, some parameters were adjusted such as high voltage range in between 200 V and 30 kV, Magnification range variable use in between 30× and 100,000×, Pressure Range is between 10 Pa

and 130 Pa (Low vacuum); 65 Pa is generally used. All parameters mentioned were adjusted depending on type surface of the leaves of the plant [10].

Physicochemical analysis

Physicochemical parameters such as ash value, extractive value, moisture content were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plant materials [5-7]. Fluorescence powder analysis was also carried out as per the standard procedure [9].

RESULTS AND DISCUSSION

Macroscopic characteristics

Lagerstroemia lanceolata Wall. (Lythraceae) is a moderate to large deciduous tree (Fig 1a) and the leaves are simple with an opposite leaf arrangement. The shape of the leaf is lanceolate, elliptic. It has an acute apex, cuneate base and entire leaf margin (Fig 1b).

Fig- 1a: *L. lanceolata* tree

Fig-1b: *L. lanceolata* leaf

Microscopic characteristics

Leaves are dorsiventral. Cells of upper epidermis are polygonal with no stomata, while lower epidermis possesses many anomocytic stomata. Two layers of palisade cells are present below the upper epidermis. Mesophyll consists of 6-8 layers of spongy parenchymatous cells some of them containing calcium oxalate rosette and with intracellular spaces. Leaf bears uniseriate unicellular covering trichomes on lower epidermis. The midrib region shows collenchymatous cells beneath both epidermal layers. The central region is occupied by an arc of radiate xylem and narrow phloem and pericyclic fibers associated with calcium oxalate prisms (Fig.2).

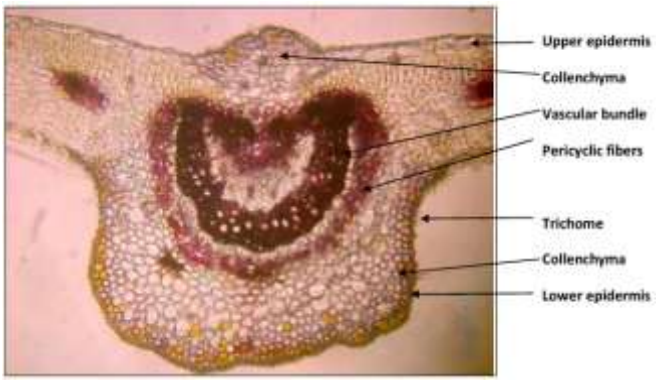

Fig-2: T.S. of leaf passing through midrib

Powder characteristics

The powder microscopy shows the presence of epidermis with underlying palisade cells, epidermis with unicellular covering trichomes, Calcium oxalate prisms associated with fibers, calcium oxalate rosette in mesophyll cells and spiral and reticulate vessels (Fig. 3a). The presence of upper epidermis in surface view beneath which palisade cells are seen (3b). Prism shaped calcium oxalate crystals are observed (Fig. 3c). Fragment showing spiral and reticulate vessels are seen (Fig. 3d). Few unicellular covering trichomes present on lower epidermis (Fig. 3e). Lower epidermis showing trichomes and stomata (Fig.3f).

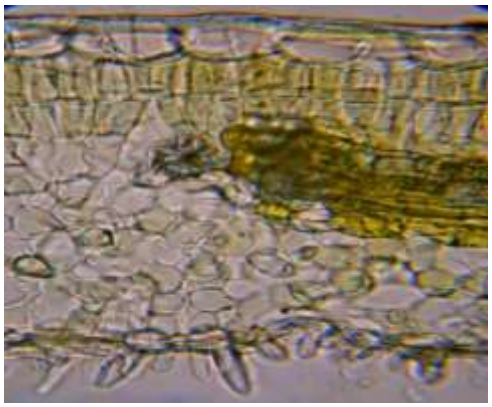

Fig-3a: Part of lamina

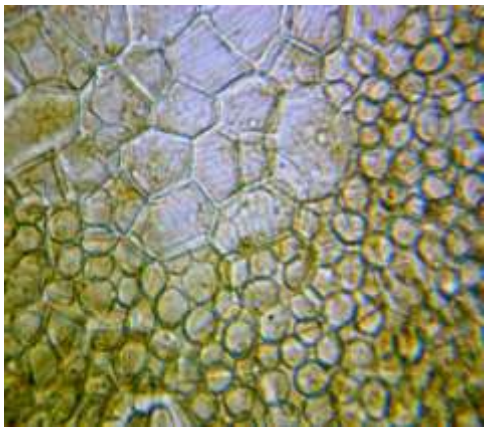

Fig-3b: Upper epidermis in surface with underlying palisade cells

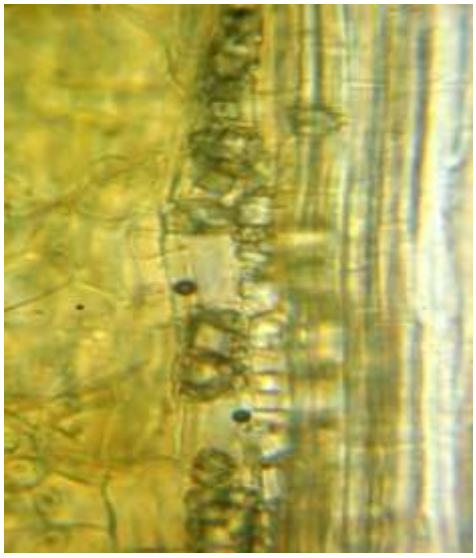

Fig-3c: Calcium oxalate prisms in midrib region

Fig-3 d: Fragment showing spiral and reticulate vessels

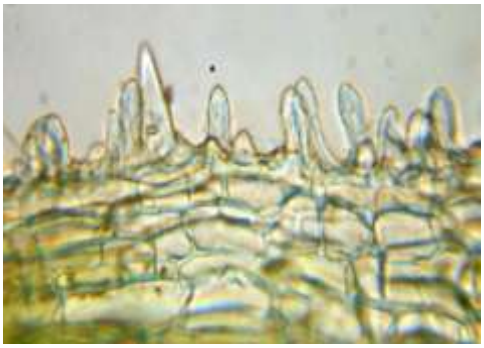

Fig-3e: Lower epidermis showing trichomes

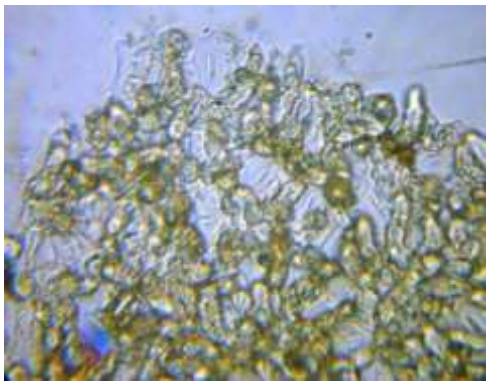

Fig-3f: Lower epidermis showing trichomes and stomata

SEM Analysis

SEM analysis of leaves shows spongy parenchyma on upper leaf surface with excessive cuticle on the lower leaf surface (Fig. 4a and 4b), venation pattern of leaf, and further helps for the identification of plant species (Fig. 4c and 4d). Anomocytic type of stomata was observed predominantly on lower epidermal surface having an average diameter of 23.96 µm (Fig. 4e and 4f). Uniseriate unicellular covering trichomes were observed on lower epidermal surfaces. Trichomes on lower surface had an average length of 41.215 µm and width of 11.033 µm (Fig. 4g and 4h). Epidermis layer and collenchyma cells are clearly seen (Fig 4i) along with the reticulate xylem vessels (Fig 4j).

So, the important diagnostic features of leaf of *L. lanceolata* W shows anomocytic stomata on lower epidermis with uniseriate unicellular covering trichomes. The specific observed characteristics such as types of stomata and trichomes were predominantly observed on lower epidermal surface of leaves. This is an important characteristic in identification, authentication and standardization of botanicals.

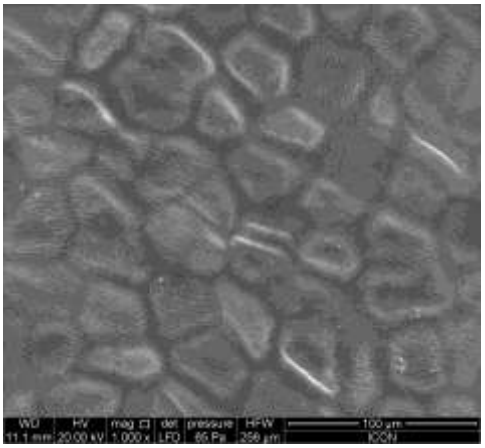

Fig-4a: Epidermis on Upper leaf surface

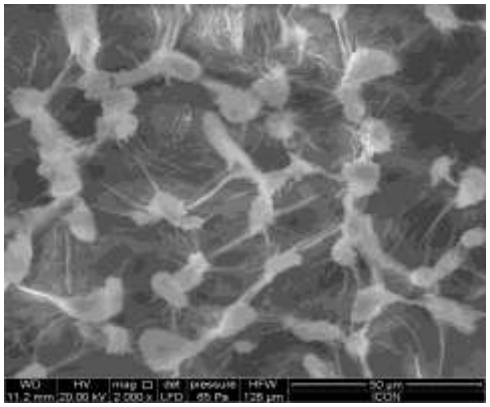

Fig-4b: Cuticle on lower leaf surface

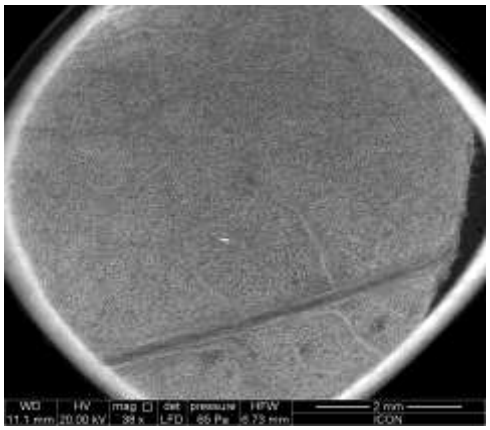

Fig-4c: Veination pattern(Lower leaf)

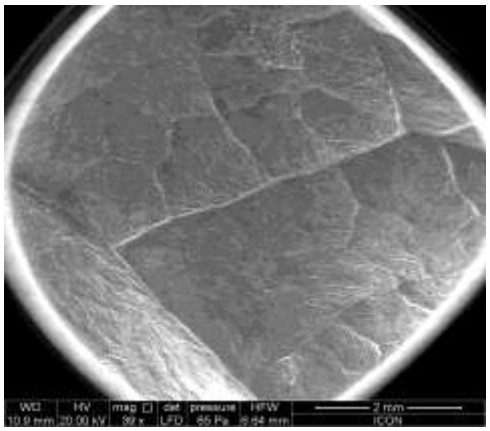

Fig-4d: Veination pattern (Upper leaf)

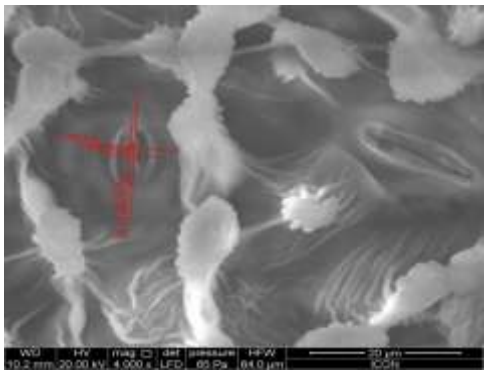

Fig-4e: Anomocytic stomata on Lower epidermis

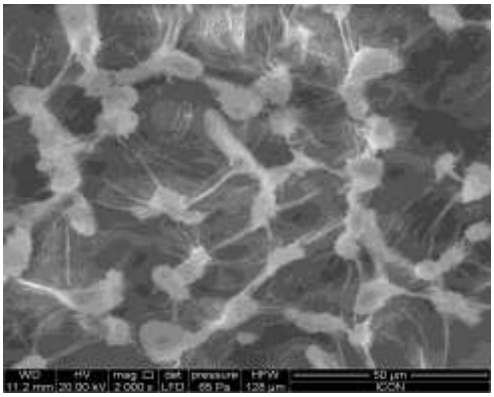

Fig-4f: Anomocytic stomata

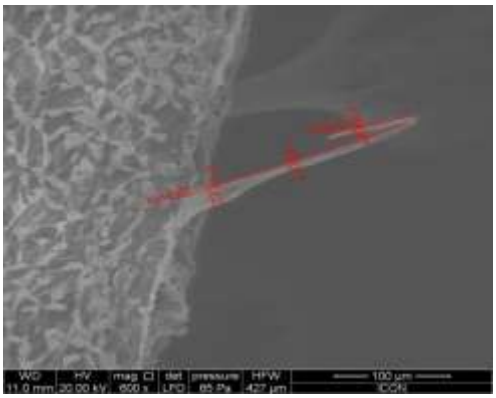

Fig-4g: Unicellular Covering trichome on the lower epiderm

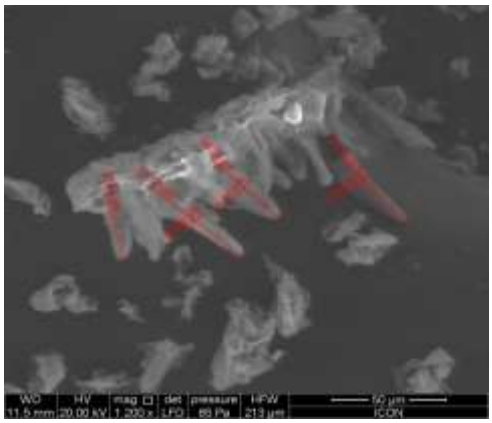

Fig-4h: Covering Trichomes

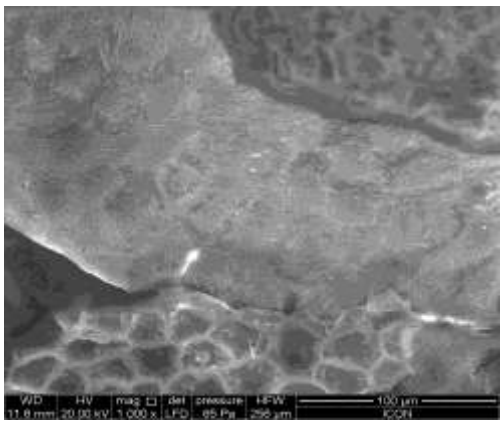

Fig-4i: Epidermis with collenchyma cells

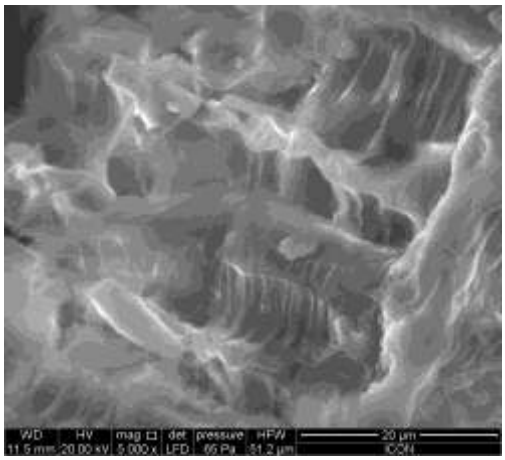

Fig-4j: Reticulate xylem vessels

Table-1: Physicohemical analysis

Sr. No.	Parameter	Value (%w/w)
1.	Total ash value	7.75
2.	Acid Insoluble ash value	5
3.	Water soluble ash value	1
4.	Loss on Drying	7.45
5.	Alcohol soluble Extractive	6.2
6.	Water soluble Extractive	0.92

Table-2: Fluorescence powder analysis

Sr. No.	Powdered drug + Chemicals/ Solvents	UV 254 nm (Short)	Visible/Day light	UV 365 nm (Long)
1.	Powdered drug as such	Green	Pale green	Dark green
2.	Powder + Methanol	Faint green	Green	Brown
3.	Powder +1% glacial acetic acid	Faint green	Faint green	Black
4.	Powder + 10% NaOH	Light green	Faint green	Black
5.	Powder + dil. NH_3	Light green	Yellow green	Faint black
6.	Powder + Conc. HNO_3	Muddy green	Yellow brown	Black
7.	Powder + dil.NH_3 + Conc.HNO_3	Bright green	Yellow	Black
8.	Powder + 1M H_2SO_4	Bright green	Faint brown	Brown
9.	Powder + 1M HCl	Light green	Green	Black
10.	Powder + 10% $FeCl_3$	Bright green	Yellow	Brown
11.	Powder + Acetone + Methanol	Bright green	Faint green	Reddish orange
12.	Powder +10% Iodine	Dark green	Yellow brown	Brown

Today sophisticated modern research tools for evaluation of the plant drugs are available but microscopic method is still one of the simplest and cheapest methods to start for establishing the correct identity of the source materials [8]. The specific morphological and histological characters of the leaf will help in identifying the crude drug. The microscopic studies reveal the presence of anomocytic stomata and uniseriate unicellular covering trichomes on lower epidermis respectively which is one of the important diagnostic parameter to identify the crude drug. Estimation of ash value is one of the significant parameter for the detection of impurities, adulteration and determination of authenticity, quality and purity of the respective crude drug. Ash value gives an idea about the content of earthy matter or inorganic composition and other impurities present along with the drug. In the present study the total ash value indicates the presence of carbonates, phosphates, silicates and silica. Extractive values are primarily useful for the determination of exhausted or adulterated drugs [8]. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [8]. The water soluble extractive value indicates the presence of sugar, acids and inorganic compounds and the alcohol soluble extractive value indicates the presence of polar constituents like phenols, steroids, glycosides and flavonoids. Determination of moisture content indicates the medicinal use of the drug. The higher or lower percentage of moisture indicates the presence of wet or dry climate which may favour the growth of microorganisms and inturn will result in deterioration of the crude drug. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

CONCLUSION

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Microscopic study and physicochemical standards can be useful to substantiate and authenticate the drug.

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