DOI: 10.55522/jmpas.V13I1.5928 **ISSN NO. 2320 – 7418**

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Comparative morpho-anatomical studies of *Albizia lebbeck* (L.) Benth and *in vitro* micro propagated plantlets

Md. Nazneen Bobby*[1], Michael Helan Soundra Rani[2], Edward Gnanaraj Wesely[3], M. Rajasekara Pandian[4]

[1]Department of Biotechnology, Vignan's Foundation for Science Technology and Research, Andra Pradesh, India.
[2]Department of Biotechnology, Sri Ramakrishna College of Arts & Science, Coimbatore, India.
[3]Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.
[4]Department of Zoology, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

Corresponding author: Md. Nazneen Bobby, ✉ slh41025@gmail.com, **Orcid Id**: https://orcid.org/0000-0002-1857-9001
Department of Biotechnology, Vignan's Foundation for Science Technology and Research, (Deemed to be University) Andra Pradesh, India

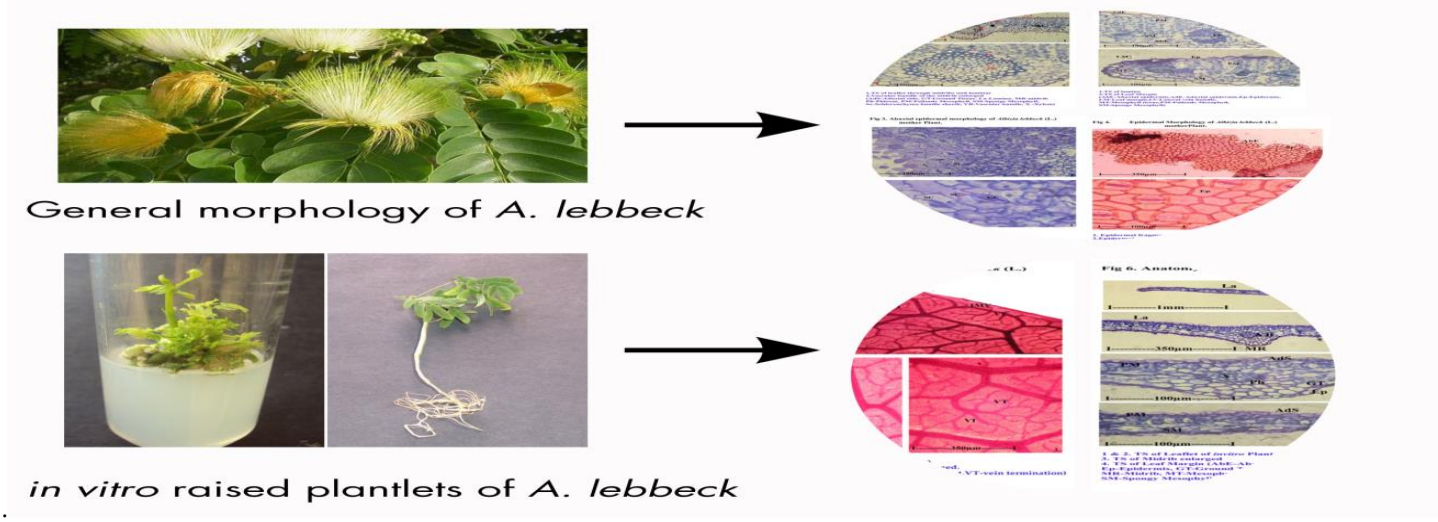
Received - 22-11-2023**, Revised** - 14-01-2024, **Accepted** - 18-02-2024 **(DD-MM-YYYY)**

Refer This Article

Md. Nazneen Bobby, Michael Helan Soundra Rani, Edward Gnanaraj Wesely, M Rajasekara Pandian, 2024. Comparative morpho anatomical studies of *Albizia lebbeck* (L.) Benth and *in-vitro* Micro propagated plantlets. Journal of medical pharmaceutical and allied sciences, V 13 - I 1, Pages- 6401 – 6407. Doi: https://doi.org/10.55522/jmpas.V13I1.5928.

ABSTRACT

The current investigation was characterizing the pharmacognostical screening of the mother plant *Albizia lebbeck* and its *in vitro* propagated plantlets. The macroscopic analysis showed the presence of various parts of the plants and the anatomical studies revealed the presence of paracytic stomata of equal subsidiary cells; dendroid vein terminations; Thick–walled epidermal cells and parenchymatous ground tissue; cuticular projections with striations in the epidermal cells of a leaf; smaller vascular bundles with lateral veins; thick-walled bundle sheath fibers with thick vascular strands; irregularly lobed sclereids and tannin cells in the upper epidermis of lamina; druse idioblasts in the mesophyll of the leaf; accessory vascular bundles in the petiole were observed in the mother plant, which was compared with the *in-vitro* micro propagated plantlet with the presence of thick and wide midrib with lamina; Thick leaf margin with thin Phloem; short xylem; vascular showed reduced in size; Reduce palisade tissue; Thick and compact parenchyma; Squamish and papillate epidermal cells were observed in epidermal and stromata region. The current studies contribute as a standard monograph for the identification and evaluation of plants.



Keywords: *Albizia lebbeck* (L.) Benth, Anatomy, Pharmacognosy, *in-vitro* propagated plantlet, Comparative studies, Venation pattern

INTRODUCTION

Plants are important resources for medicine, and therefore their pharmacognostical, pharmacological, and safety parameters need to be assessed. As a source of medicine, they have always been at the forefront almost in all cultures of civilizations which are rich in resources of traditional medicines which also help in the manufacture of herbal drugs. For more than ten decades, medicinal plants have been used to treat health disorders. In recent days, the significance of pharmacognosy has been felt extensively. Unlike taxonomic identification, pharmacognostic study includes various parameters, which help in the identification of dried and powdered plant materials. Pharmacognostic approaches ensure plant identity and provide standardization parameters that will be helpful in the prevention of adulteration [1]. Plant anatomy is vital in plant science research [2] and its developmental studies lead us to a better understanding of their cell functions. Studies of growth, differentiation, and organogenesis are the prime developmental investigations [3]. It deals with morphology, cells, and tissue development. It also helps in finding its physiology, morphogenesis, taxonomy, ecology, genetics, evolution, reproduction, etc. Systemic anatomy mainly focuses on vegetative organs. Unerring both macro scopical and micro scopical studies of the medicinal plants and also for the safety and quality standards help to authenticate crude drug materials accurately [4]. Macro scopical identifications are very difficult in drugs extricated from leaves, barks, roots, and rhizomes instead of micro scopical characterization and it is used for different purposes. These studies help in diagnostic features in stratifying anatomical characters between similar species to circumvent adulteration [5]. Apart from physiognomic characteristics, flowering parts can also be distinguished using taxonomic inferences in different groups of flowering plants [6 - 9]. Morpho-anatomical characters play a vital role in crude drug standardization [10]. This was directly affected by fluctuations in the environmental conditions to which plants are exposed during developmental stages [11]. The lack of literature regarding the anatomical characterization of micromorphological characters plays a key role in authenticating and classifying any plant as phylogenetic studies which require malleable data. Structures of cells and tissues may involve complex regulatory mechanisms. It is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction, breeding, and some others.

Albizia lebbeck (L.) Benth (Shirisha) is widely distributed in India, with is scale of 800-900m in the Himalayas and also located in Andamans. It has greenish-black rough bark that carries longitudinal and transverse fissures on the outer surface and whitish fine longitudinal stations on the inner surface with yellowish white sap, and dark brown heartwood with white shades on it. Leaves arranged with 8-18 bipinnate leaflets, and greenish-yellow stalked flowers. Season of flowering and fruiting begins from April to June with 6-10 yellowish-brown Pods, available till May-July. *A. lebbeck* ranks first place in Teak (*Tectonagrandis* Linn.) and Sala (Shorearobusta Gaertn) [12]. The present investigation proposed the anatomical description of *Albizia lebbeck* and its *in vitro* micro propagated plantlets.

MATERIALS AND METHODS

A. lebbeck Benth validated voucher specimens were placed in the St. Xavier's College Herbarium (XCH 00000) for future use after the specimens were obtained from the natural habitats of Rasipuram, Namakkal district, Tamil Nadu, India. Together with the petiole's length, measurements were made of the leaves length, breadth, and area. Safranin was used to color fine leaf pieces that were transversely sliced through the midrib, and glycerin was used to mount them. Similarly, leaves were collected from an immaculate site on campus. Replica leaves were collected from plants growing in a uniform form, with comparable DBH. The epidermal layer, stomatal, veins, and mesophyll cells with ocular scale were quantitatively measured under a microscope. All the chemicals were procured from GK Life Sciences, Guntur, Andra Pradesh, India. *in-vitro* raised leaflet produced from the maximum number of shootlets regeneration (13.9 ± 0.73) was observed from MS medium supplemented with 0.5 mg/L of TDZ in combination with 0.25 mg/L of IAA and *in vivo* mother. Macroscopically, the plant reveals the morphology of leaves, apex, base, margin, etc [13]. Histochemical studies were carried out with the petiole, midrib, and lamina region of the leaf [14]. Phloroglucinol and conc. HCl is used to stain the sections. Furthermore, shade-dried (#60) was carried out by Powder microscopy (15). Carl Zeiss binocular microscope attached to the camera was used to take the Photomicrographs [15].

RESULTS AND DISCUSSION

The plant is a majestic deciduous tree height of 18–30 m tall with a 50 cm to 1 m in diameter trunk. The bi-pinnate leaves are 7.5–15 cm long, with 1- 4 pairs of pinnae, with each 6 –18 leaflets. It has a thin leaflet with coriaceous glabrous above, pubescent along the veins, base oblique, margins entire nerves prominent.

Microscopic Structure of Leaf

The leaf is dorsi ventral with a plano convex midrib. The adaxial side of the midrib is flat and the abaxial side hemi- spherical, 250µm thick midrib and 200 µm wide. It consists of small spindle-shaped, fairly thick-walled epidermal cells and 3 or 4 leaves of compact parenchymatous ground tissue. It contains a palisade layer like a horizontally transcurrent along the adaxial part of the midrib, with a single large top-shaped collateral vascular strand. It consists of about 10 parallel rows of xylem elements with sclerenchyma layers in

between the xylem elements are angular and thick-walled. The metaxylem elements are 20 µm in diameter. Phloem occurs in 6 or more discrete masses beneath the xylem strand. The entire vascular bundle is covered by 2 or 3 layers of thick-walled bundle sheath fibers, 150 µm thick vascular strands (Fig1).

Lamina

It is 150 µm thick and has thick epidermal layers with thin-walled cells and smooth prominent cuticles. The outer walls were slightly projecting into short papilla. Stomata occur only on the abaxial epidermis. The mesophyll tissue consists of a thick zone of palisade tissue, comprising 50-70 µm thick, 4 or 5 layers of short cylindrical cells. The lower part of the mesophyll includes 3 or 4 layers of parenchyma cells which are lobed and spongy. In the lower part of the mesophyll cells, there are smaller vascular bundles with lateral veins. The bundled veins have a small clump of vascular elements and thick sclerenchyma bundle sheath fibers (Fig 2). The leaf margin is semicircular and blunt 100 µm thick along with Squamish and thick-walled epidermal cells. The cuticle is echinate. The mesophyll tissue is undifferentiated and includes large compact parenchyma cells.

Epidermal cells and stomata

The epidermis was studied from para-dermal sections which show the surface view of the cells. These are thin, wavy anticlinal walls; the cells are lobed and amoeboid in appearance. The stomata are densely distributed on the abaxial epidermis. It has elliptical guard cells associated with closed stomatal aperture measuring 10×20 µm in size and also contains paracytic stomata with 2 subsidiary cells which are collateral to guard cells (Fig 3 & 4).

Venation pattern

The veins are densely reticulate. Both main veins and veinlets are thick. There is a less distinct vein – islets. They are variable in outline and terminations are well defined. Veins are fairly thick and most of them are branched once or twice, some of them are profusely branched forming a dendroid outline (Fig 5).

Powder microscopic results

The leaf powder exhibits fragment epidermal peelings. Especially the abaxial epidermal fragments exhibit hexagonal or polygonal epidermal cells. The stomata are well preserved. They are 25×40 µm in size. They have narrow slit-like aperture. There are two parallel subsidiary cells with paracytic stomata.

***In-vitro* leaf**

Leaflets of *in vitro* sample are wide around 1.6 mm and 100 µm thick midrib, with lamina 40 µm thick (Fig 6 & 7). The vascular strand is reduced in size. It is 50 µm thick. They have only three or four short rows of xylem elements that are less than 10 µm in diameter. Phloem elements are also reduced to a thin, reso prominent layer. The vascular bundle lacks the sclerenchymatous bundle sheath.

The leaf margin is 50 µm thick. It is blunt and semicircular epidermal cells and two layers of compact mesophyll tissue.

Epidermal cells and stomata

It maintains highly lobed epidermal cells because of thin wavy anti-clinal walls. The stomata have circular guard cells that are 15 µm in diameter. They have a fairly wide elliptic aperture. The cells of the veins are thin-walled, they have elongated rectangular cells. Nuclei are well preserved in the cells. Some of the cells have lignified walls which appear bright under polarized light. The midrib is convex on the abaxial side and planoconvex on the adaxial side. The epidermal cells are Squamish, thick-walled, and papillate. The ground tissue of the midrib includes compact parenchyma cells with a thick-walled angular shape. The palisade tissue is reduced to a single layer of vertical rectangular cells across the adaxial side of the vascular strand.

The anatomical study of various medicinally important plants plays a vital role in pharmacognosy to avert adulteration and also to investigate certain criteria for authenticity and for raw drugs to estimate the quality [16]. Previous reports include a thorough investigation [17] proposed the morphological studies in *B. repanda*, *Vitex leucoxylon*, and *Vitex negundo* (Verbenaceae) plant parts and their anatomical studies. Barks of *Albizzia* were characterized by irregularly shaped, thicker-walled stone cells and cortical tissues bounded internally accurately. Studies also report the similarity between the wood of *Albizia gummifera* and *Albizia zygia* used for multiple purposes [18-19].

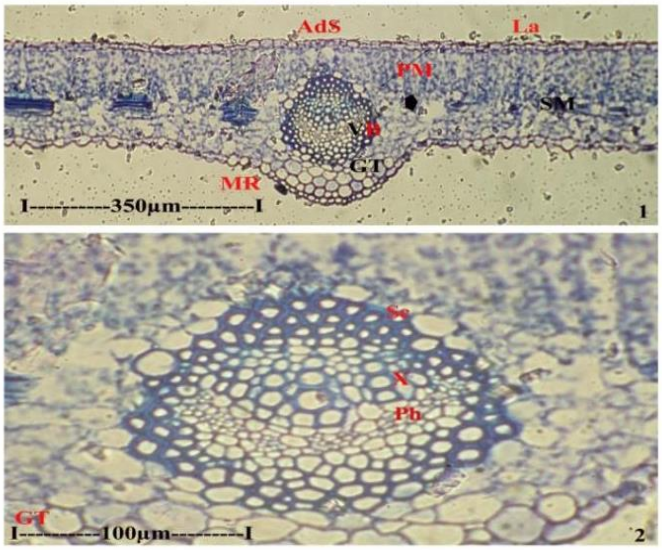
CONCLUSION

The present experiment was conducted to study the histological variations that occurred between the mother and micro-propagated plants of *A. lebbeck*. The thin sections of the leaf were used for the study. The leaf possesses dorsi ventral and planoconvex midrib. The adaxial side of the midrib is flat and the abaxial side is hemi spherical with 250µm thick midrib and 200 µm wide. Also contains ten parallel rows of angular and thick-walled xylem elements with sclerenchyma layers in between. The Meta xylem are measured at 20 µm in diameter. Phloem occurs in 6 or more discrete masses beneath the xylem strand. The entire vascular bundle is covered by 2 or 3 layers of thick-walled bundle sheath fibers. It holds 150 µm thick vascular strands, and 150 µm thick lamina. The epidermis was studied from para-dermal sections which show the surface view of the cells with thin epidermal cells, wavy anticlinal walls; lobed cells with amoeboid in appearance. The stomata are densely distributed on the abaxial epidermis. Guard cells are 10×20 µm in size. The stomata are paracytic type. The veins are densely reticulate.

The leaflets of the *in vitro* sample are 1.6 mm wide the thickness of the midrib is 100 µm, lamina is 40 µm. The vascular

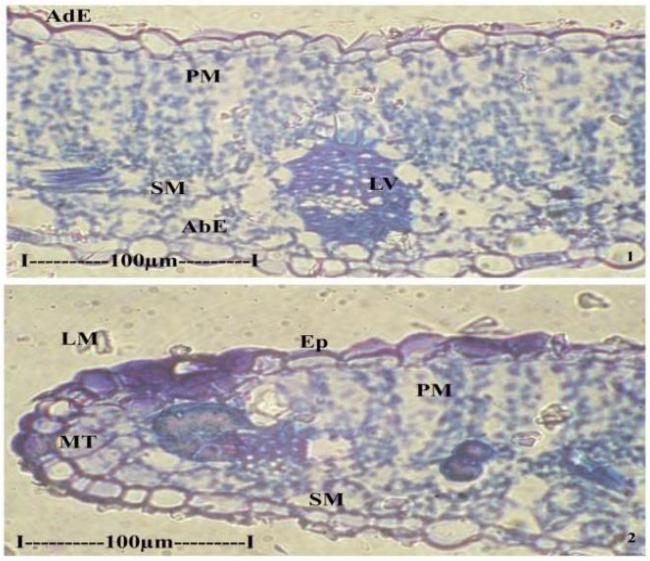
strand is reduced in size. It is 50 µm thick. They have only three or four short rows of xylem elements that are less than 10 µm in diameter. The leaf margin is 50 µm thick. The stomata have circular guard cells which are 15 µm in diameter.

Fig 1. Anatomical details and leaflet morphology of *A. lebbeck* (L.) mother Plant.



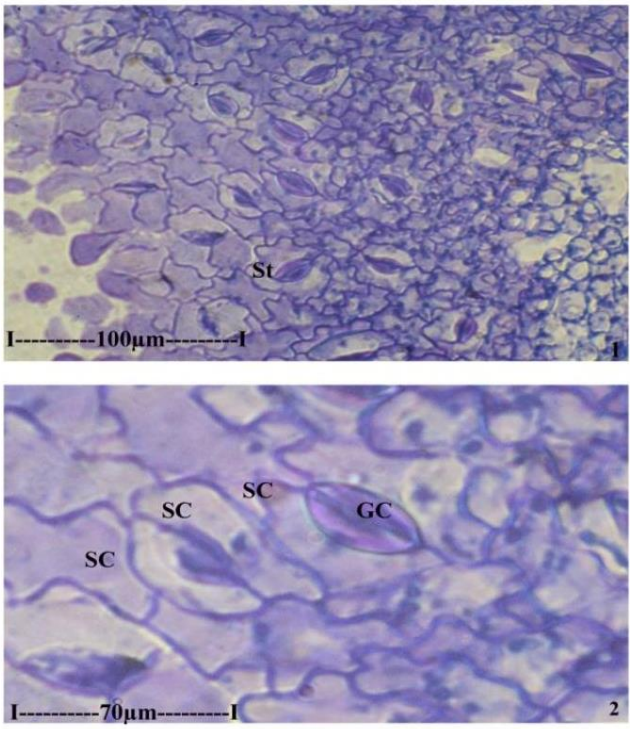
1.TS of leaflet through midribs and laminar
2.Vascular bundle of the midrib enlarged
(AdS-Adaxial side, GT-Ground Tissue, La-Lamina, MR-midrib Ph-Phloem, PM-Palisade Mesophyll, SM-Sponge Mesophyll, Sc-Schlerenchyma bundle sheeth, VB-Vascular bundle, X -Xylem)

Fig 2. T.S of lamina and leaf margin of *Albizia lebbeck* (L.) mother plant.



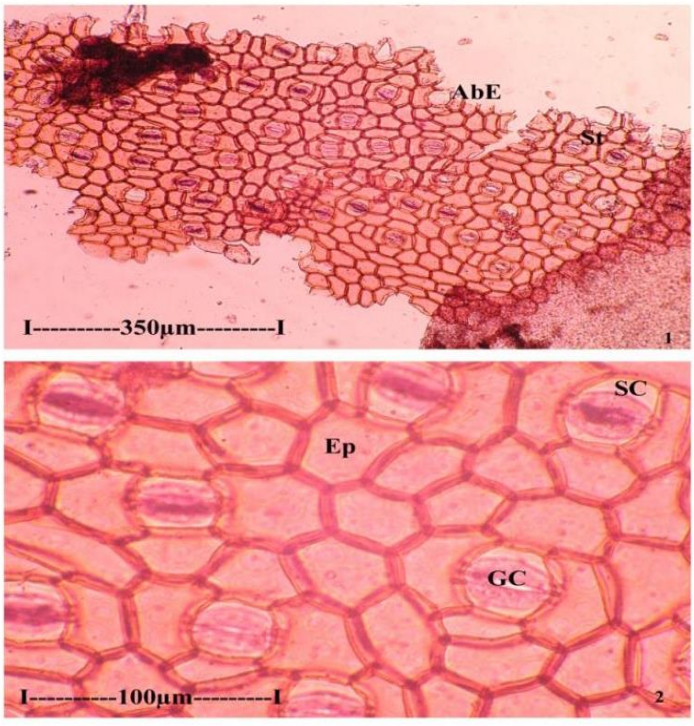
1.TS of lamina
2.TS of Leaf Margin
(AbE-Abaxial epidermis,AdE-Adaxial epidermis,Ep-Epidermis, LM-Leaf margin,LV-Lateral vein bundle, MT-Mesophyll tissue,PM-Palisade Mesophyll, SM-Sponge Mesophyll)

Fig 3. Abaxial epidermal morphology of *Albizia lebbeck* (L.) mother Plant.



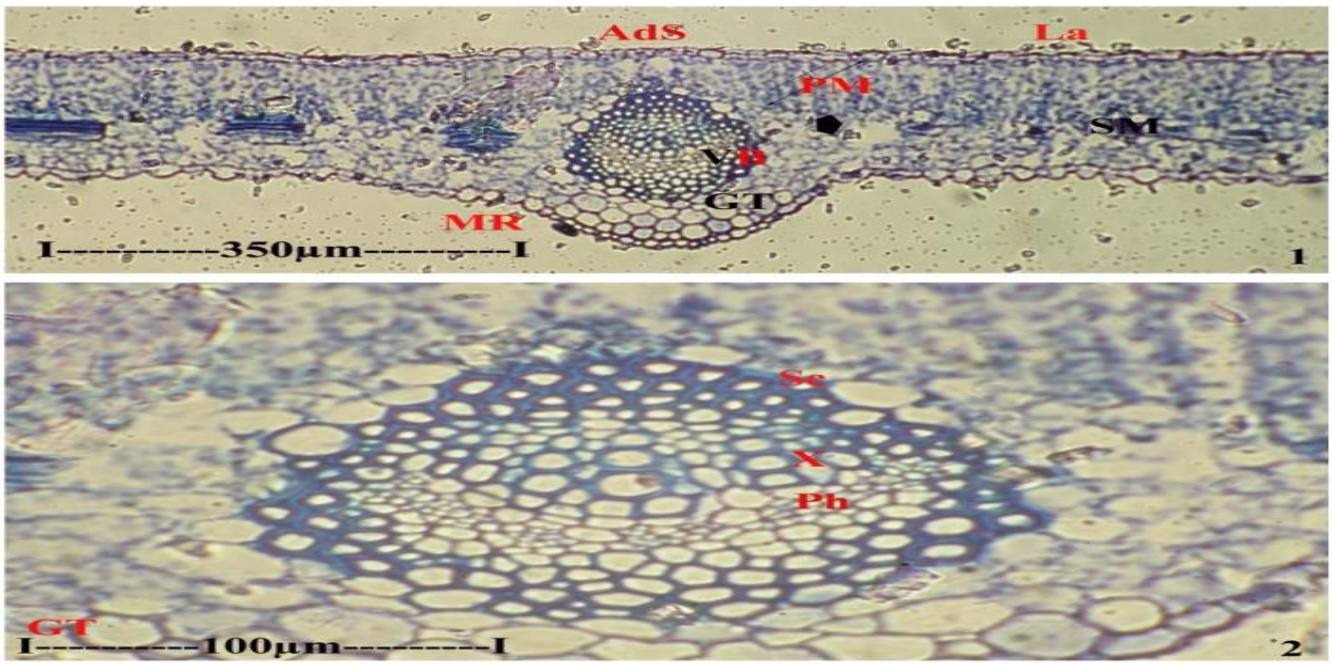
1.Paradermal section of the Abaxial epidermis
2.Paracytic Stomata enlarged
(GC-Ground cell,Sc-Subsidiary cell,St-Stomata)

Fig 4. Epidermal Morphology of *Albizia lebbeck* (L.) motherPlant.



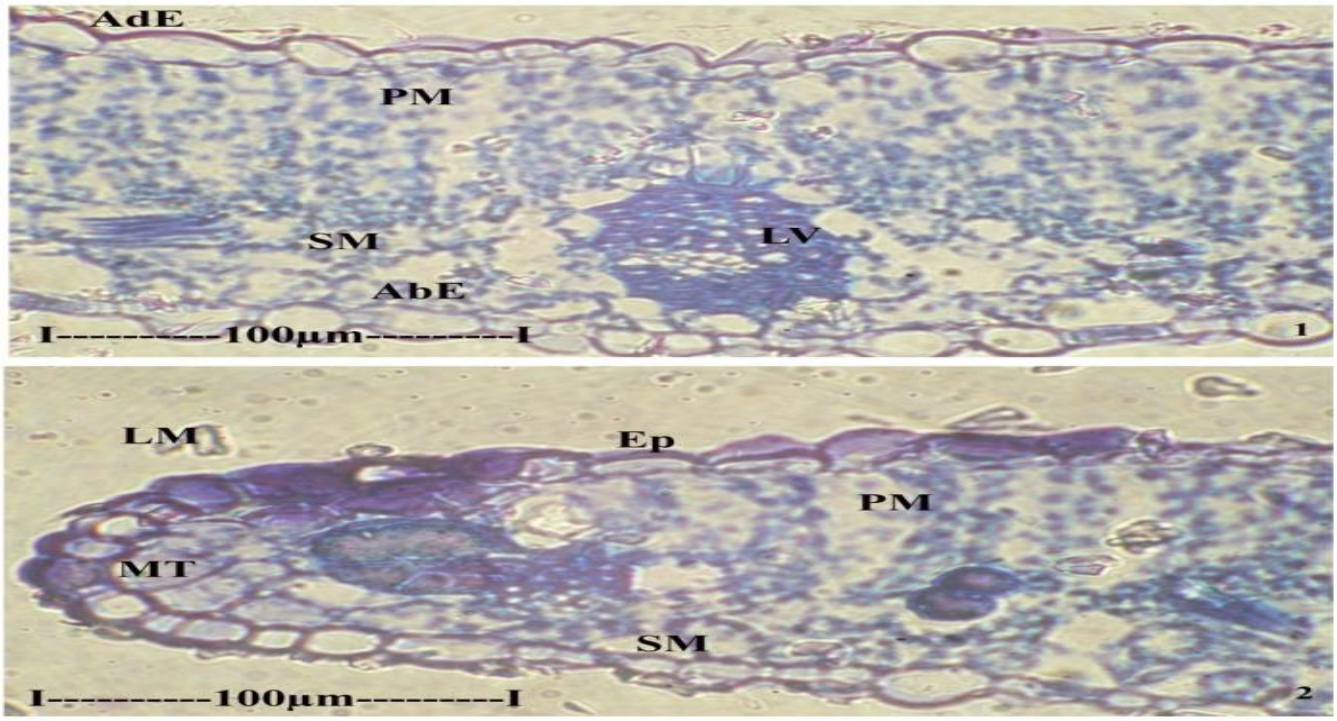
1. Epidermal fragment with leaf powder.
2.Epidermal cells and stomata enlarged
(AbE-Abaxial epidermis, Ep-Epidermal cells,Gc-Guard cells, SC-Subcidiary cells,St-Stomata cells)

Figure 1: Anatomical details and leaflets morphology of *A. lebbeck* (L.) mother plant.



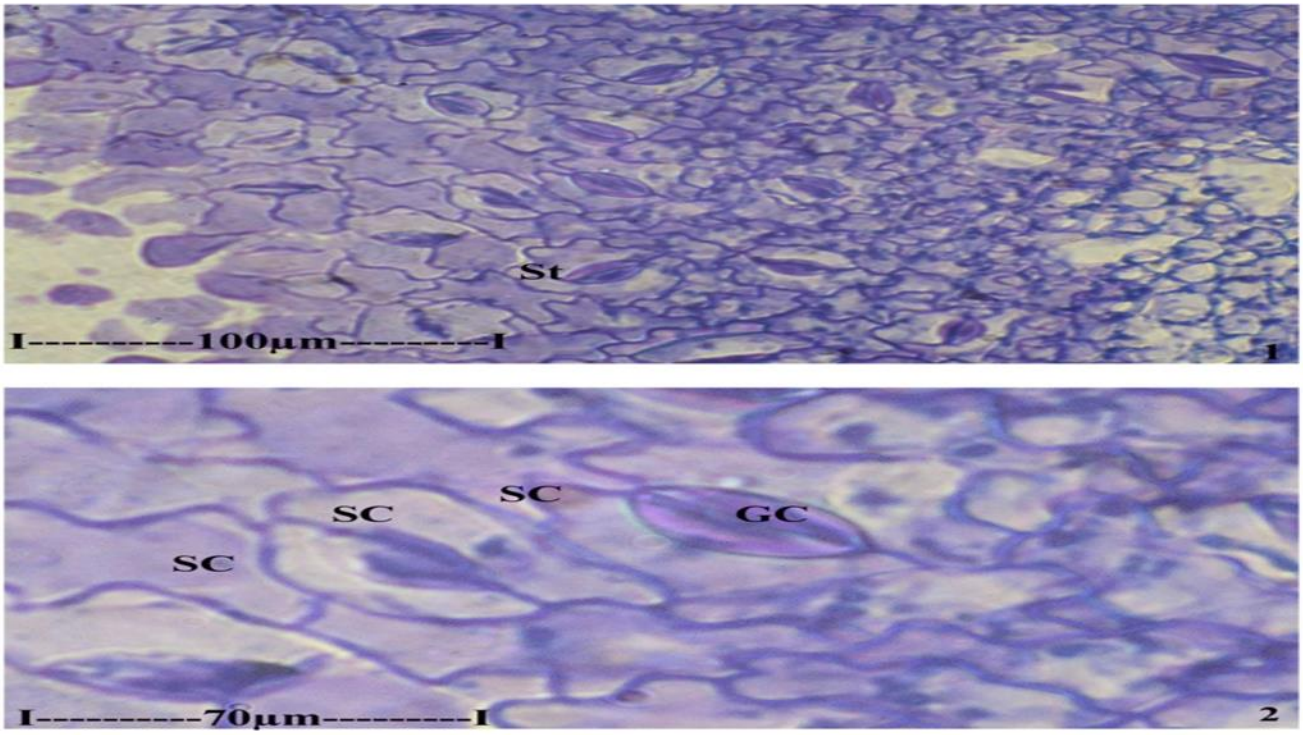
1.TS of leaflet through midribs and laminar
2.Vascular bundle of the midrib enlarged
(AdS-Adaxial side, GT-Ground Tissue, La-Lamina, MR-midrib
Ph-Phloem, PM-Palisade Mesophyll, SM-Sponge Mesophyll,
Sc-Schlerenchyma bundle sheeth, VB-Vascular bundle, X -Xylem)

Figure 2: T.S of lamina and leaf margin of *A. lebbeck* (L.) mother plant.



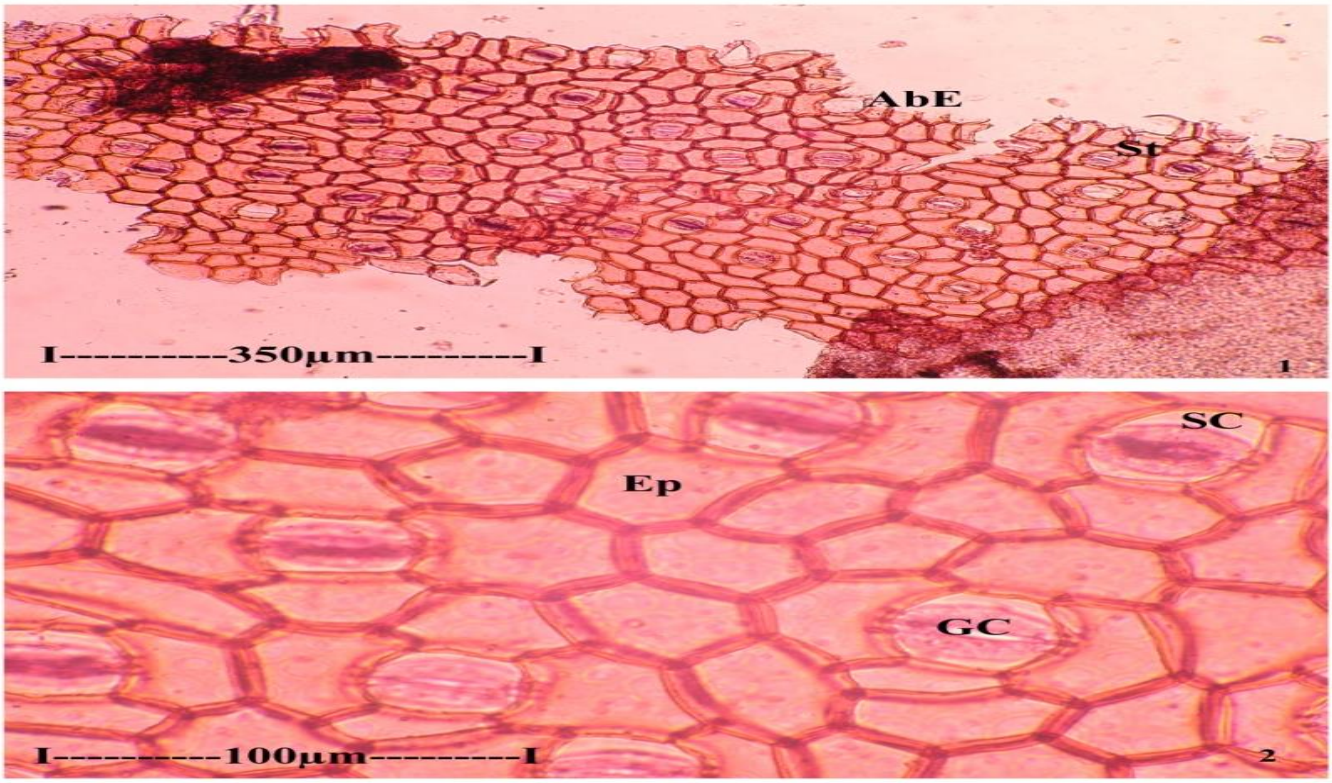
1.TS of lamina
2.TS of Leaf Margin
(AbE-Abaxial epidermis,AdE-Adaxial epidermis,Ep-Epidermis,
LM-Leaf margin,LV-Lateral vein bundle,
MT-Mesophyll tissue,PM-Palisade Mesophyll,
SM-Sponge Mesophyll)

Figure 3: Abaxial epidermal morphology of *A. lebbeck* (L.) mother plant.



1.Paradermal section of the Abaxial epidermis
2.Paracytic Stomata enlarged
(GC-Ground cell,Sc-Subsidiary cell,St-Stomata)

Figure 4: Epidermal Morphology of *A. lebbeck* (L.) mother plant.



1. Epidermal fragment with leaf powder.
2.Epidermal cells and stomata enlarged
(AbE-Abaxial epidermis, Ep-Epidermal cells,Gc-Guard cells,
SC-Subcidiary cells,St-Stomata cells)

REFERENCES

1. Sumitra C, 2014. Importance of pharmacognostic study of medicinal plants: An overview. Journal of Pharmacognosy and Phytochemistry 2(5), Pages - 69-73. 2(5) jan_2014/28.1.

2. Presena J, Pragasam A, 2016. Evaluation of anatomical features of *Adenia wightiana* Engl. of Passifloraceae for proper identification of the species. International Journal of Plant, Animal and Environmental Sciences 6(2), Pages - 132-138.

3. Maiti R, Satya P, Rajkumar D, 2012.Crop Plant Anatomy. Wallingford, UK: CABI, Experimental Agriculture, 49(2), Pages - 320-320 http://dx.doi.org/10.1017/S0014479712001469.

4. Cutler DF, 1978. Applied Plant Anatomy. 1st edition Longman LTD, London. https://www.biblio.com/9780582441286.

5. Edeoga HO, Omosun G, Osuagwu GGE, et al, 2007. Microscopic anatomy and histochemistry of stem and root of some Mimosa species. (Leguminosae- Mimosoideae). Asian Journal of Plant Sciences 6(4), Pages - 688-691. https://dx.doi.org/10.3923/ajps.2007.688.691.

6. Pant DD, Mehra B, 1961. Nodal anatomy of *B. diffusa* L. Phytomorphol 11, Pages - 384-405.

7. Guimeraes AC, Kuster RM, Amaral AF, et al, 2007. Histological study of the leaf and stem of the Amazonian medicinal Mistletoe Cladocoleamicrantha (Loranthaceae). International Journal of Botany 3(2), Pages - 218-221. https://dx.doi.org/10.3923/ijb.2007.218.221.

8. Kaplan A, Hasanoglu A, Ince IA, 2007. Morphological, anatomical, and palynological properties of some Turkish *Veronica* species (Scrophulariaceae). International Journal of Botany 3(1), Pages - 23-32. Doi: 10.3923/ijb.2007.23.32.

9. Keshavarzi M, Zare G, 2006. Anatomical study of Salicornieae Dumort. (Chenopodiaceae Vent.) Native to Iran. Int. Journal of Botany. 2(3), Pages - 278-285. Doi: 10.3923/ijb.2006.278.285.

10. Agarwal S, Kumar VR, Kumar A, 2013. Pharmacognostical Characterization of *Ocimum* spp. Journal of Pharmacognosy and Phytochemistry 2(1), Pages - 219-224.

11. Guimeraes AC, Kuster RM, Amaral AF, et al, 2007. Histological study of the leaf and stem of the Amazonian medicinal Mistletoe Cladocoleamicrantha (Loranthaceae). International Journal of Botany 3(2), Pages - 218-221. https://dx.doi.org/10.3923/ijb.2007.218.221.

12. Rajni R, Vandana T, Negi KS, 2016. A comparative study of morphological and anatomical structures of four *Ocimum* species in Uttarakhand, India. Journal of Drug Delivery and Therapeutics 6(6), Pages - 1-6. http://dx.doi.org/ 10.22270/jddt.v6i6.1322.

13. Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products 2006. 1st ed. Vol. I: A, New Delhi, CSIR. 126.

14. Khandelwal KR, 2006. Practical Pharmacognosy Techniques and Experiments Nirali Prakashana, Pune, 157-160.

15. Wallis TE, 1985. Textbook of Pharmacognosy. 15th ed. London, Churchill Publication; 572-82. https://doi.org/ 10.1002 /jps.2600600146.

16. Banerjee G, Mukherjee A, 2001. Pharmacognostic studies on *Portula caoleracea* L. I. Leaf. In (Eds) J. K. Maheshwari and A. P. Jain Recent researches in plant anatomy and morphology, Scientific Publishers (India), Jodhpur, pp. 69-78.https://doi.org/10.1590/S0102-695X2008000400005.

17. Edeoga HO, 1991. Comparative morphology of the leaf epidermis in *Costus lucanusianus* (Costaceae) complex and its systematic importance. Nigerian Annual National Science 1, Pages - 25-30.

18. Idu M, Olorunfemi DI, Omonhinmin AC, 2000. Systematic value of stomata in some Nigerian hardwood species of Fabaceae. Plant Biosystem. 134(1), Pages - 53-60 https://doi.org/10.1080/11263500012331350345.

19. Silvy M, John Britto S, Sinjumol T, 2014. Anatomical Studies on *Vitex leucoxylon* and *Vitex negundo* (Verbenaceae). International Journal of Research & Review 1(3), Pages - 7-10.