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MICROSCOPICAL EVALUATION OF LEAF OF BALANITES AEGYPTIACA

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ABSTRACT

Microscopical investigation was carried out on leaves of *Balanites Aegyptiaca* (L.) Delile belonging to family Zygophyllaceae, also known as 'Desert date' in English. Literature survey revealed that not much work has been done on this plant, especially on leaves. So we have taken its detailed microscopical studies to prove its appropriate identification. Microscopical study provide information that the leaf exhibits dorsiventaral midrib and smooth, even and thick lamina. The lamina is 350 µm thick. It is isolateral. The marginal part of the lamina is bluntly conical measuring 200 µm thick. Calcium oxlate crystals are abundant in the mesophyll tissue. The crystals are druses and rosette types. The veins form well defined vein-islets of polygonal outline. The vein terminations are well developed and thick, short and straight. At the end of the vein termination occurs a cluster of squarish, thick walled wide sclereids. The leaf is amphistomatic, However, the stomata on the abaxial side are more in frequency than on the adaxial side. The abaxial epidermal cells are small, polygonal and fairly thick walled. The anticlinal walls are straight. The trichomes are unicellular, unbranched and thick walled. Crystal bodies are wide spread in the powder. The crystals are exclusively druses, which are spherical bodies comprising many pointed triangular small crystal units. The above studies provide useful information in regard to its correct identity, evaluation and help to differentiate from the closely related other species of *Balanites Aegyptiaca (L.) Delile*.

KEYWORDS: *Balanites Aegyptiaca (L.) Delile*, Microscopical, Pharmacognostic, Powder Microscopy.

INTRODUCTION

The leaves of *Balanites Aegyptiaca (L.) Delile* belonging to family Zygophyllaceae was selected for project, on the basis of ethnobotanical information which reveals its uses against diabetes by the people.[1]

Balanites aegyptiaca (L.) Delile, also known as 'desert date' in English. This tree is native to much of Africa and parts of the Middle East. In India, It is particularly found in drier parts of Rajasthan, Madhya Pradesh, Gujarat and Deccan. This is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia. The tree can grow up to 10 meters in height with spiny branches, compound leaves and greenish yellow flowers, double root system and pale brown date-like fruits.[2]

It is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture.[3] Literature has revealed antifeedent, molluscicide, antidiabetic, contraceptive activities and antihelminthic in various *Balanites aegyptiaca* (L.) Delile extracts. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidysentric properties.[4,5]

Literature survey revealed that not much work has been done on this plant, especially on leaves. So we felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed pharmacognostical, and phytochemical and pharmacological studies to prove its appropriate identification and rationalize its use as drug of therapeutic importance.[6]

Pharmacognostical studies mainly include study of morphological characters, microscopical characters and powder microscopy. Microscopy method is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentification of the crude drugs, study of powdered drugs visualizing calcium oxalate crystals.[7]

Microscopy method allows more detailed examination of a drug it can be used to identifying the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered form.[7]

MATERIALS AND METHODS

Plant Collection and Identification

Plant material

The proposed study of the plant leaves of *Balanites aegyptiaca* (L.) Delile were collected from uncultivated fields in and around the Village Maroth of Nagaur District, Rajasthan, India during july-august 2011. The Plant was identified from "Department of Botany, University of Rajasthan, Jaipur and confirmed by compared with the help of herbarium maintained at the Department of Botany, University of Rajasthan, Jaipur. The voucher specimen (RVBL21073) was deposited and preserved in Herbarium Department of Botany, University of Rajasthan, Jaipur for further reference.

Taxonomical Identification

The species for the proposed study was identified and authentified as *Balanites aegyptiaca* (L.) Delile (Certificate no. PARC/2013/2064.) by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai.

MICROSCOPICAL STUDIES

Collection of specimen

Most care was taken to select the fresh leaf of healthy plant *Balanites Aegyptiaca* (L.) Delile. The required sample of fresh leaf with petiole was collected from the plant and fixed in FAA (Farmalin-5ml + Acetic acid -5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.[8]

Sectioning

The paraffin embedded specimens were section with the help of rotary Microtone. The thickness of the sections was 10-12 µm. De-waxing of the sections was done and the sections were stained with Toluidine blue.[9] Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some phytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and I_2-KI (for Starch). Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.[10]

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.[11]

POWDER MICROSCOPY

First of all the leaves were washed with water and dried it in sunlight for one hour and then it was dried in shade. By the help of wood grinder the dried leaves were powdered and were passed through the sieve no. 60 for powder microscopy. The leaf powder is boiled with chloral hydrate for 5-10 minutes, and then stained with phloroglucinol, Toludiene and observed for the microscopic features under high power (40 x).[8]

RESULTS

Fig. No. 1: An exomorphic feature of *Balanites Aegyptiaca* (L.) Delile fresh leaf.

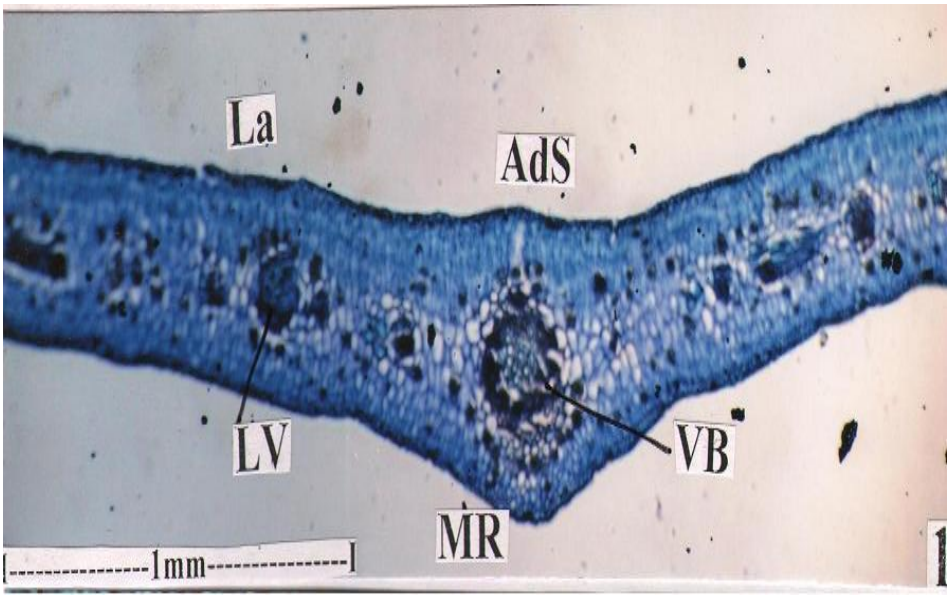

Fig. No. 2: T.S. of leaf of *Balanites aegyptiaca (L.) Delile* through midrib.

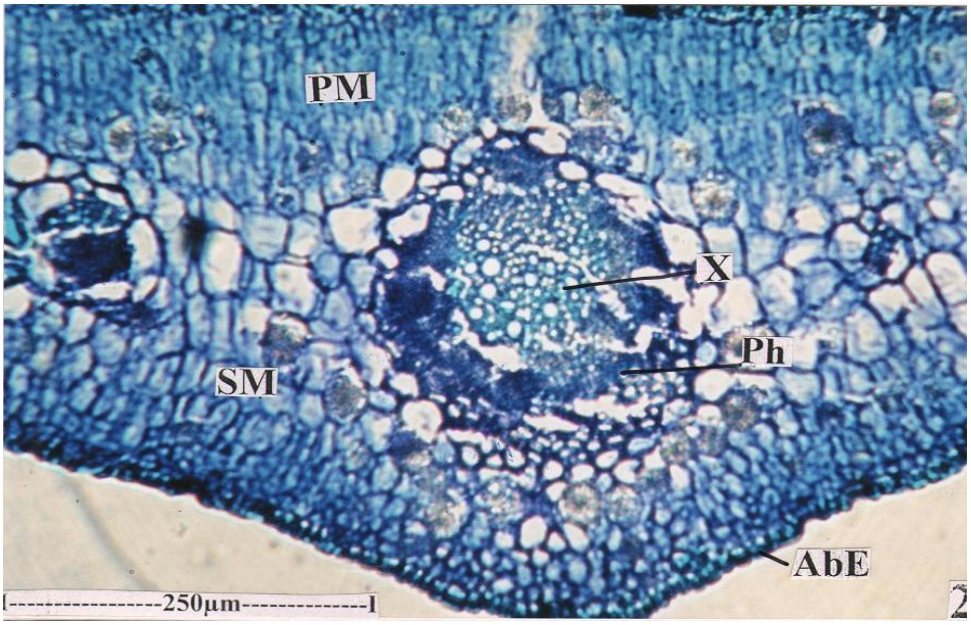

Fig. No.3: T.S. of midrib enlarged.

(AbE- Abaxial Epidermis, AdS- Adaxial Side, La- Lamina, LV- Lateral Vein, MR- Midrib, Ph- Phloem, PM- Palisade Mesophyll, SM- Spongy Mesophyll, VB- Vascular bundle, X- Xylem).

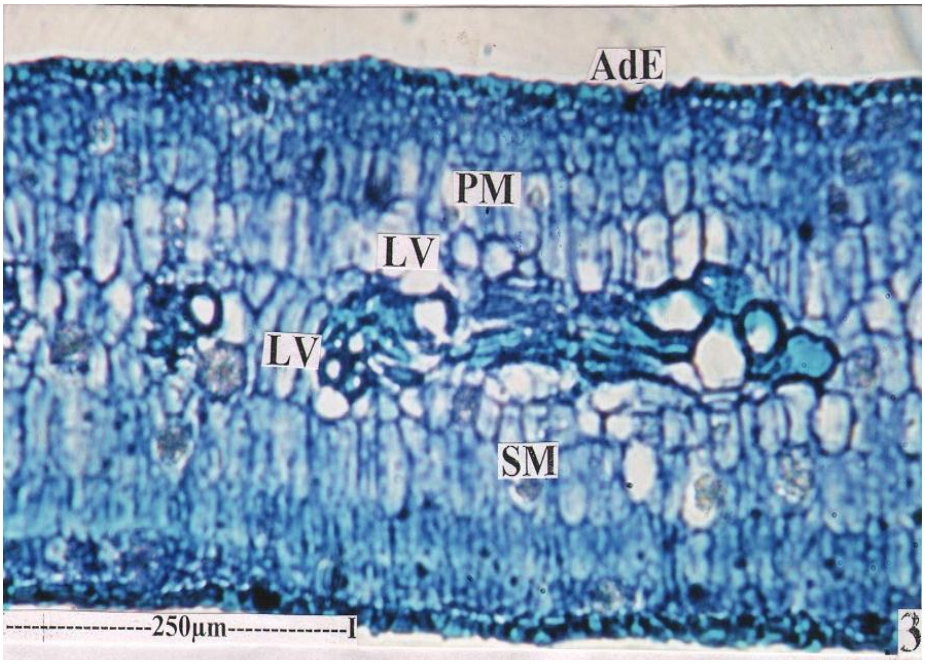

Fig. No. 4: T.S. of lamina.

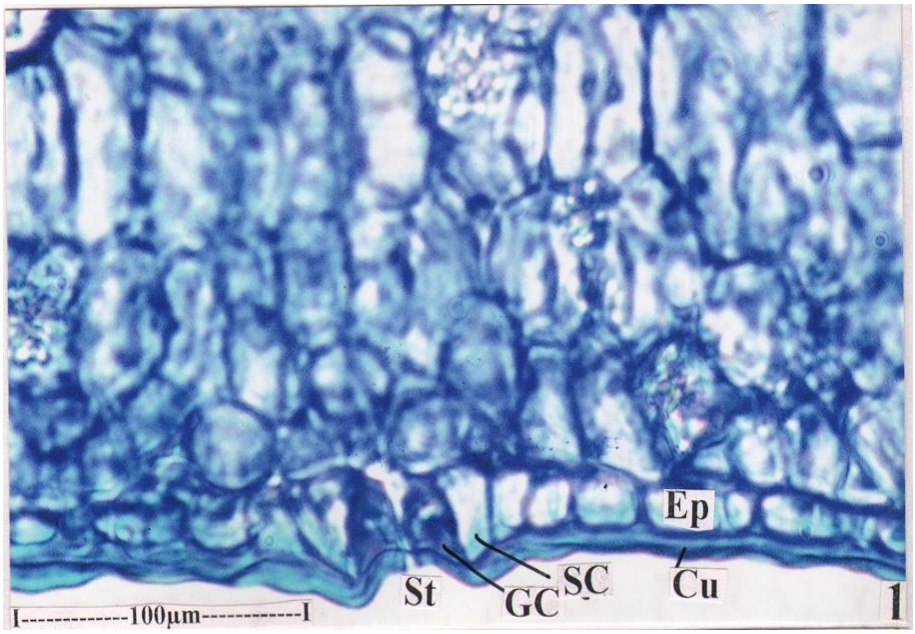

Fig. No. 5: T.S. of leaf showing sunken stomata.

(AdE- Adaxial Epidermis, La- Lamina, LV- Lateral Vein, PM- Palisade Mesophyll, SM- Spongy Mesophyll, VB- Vascular bundle, X- Xylem).

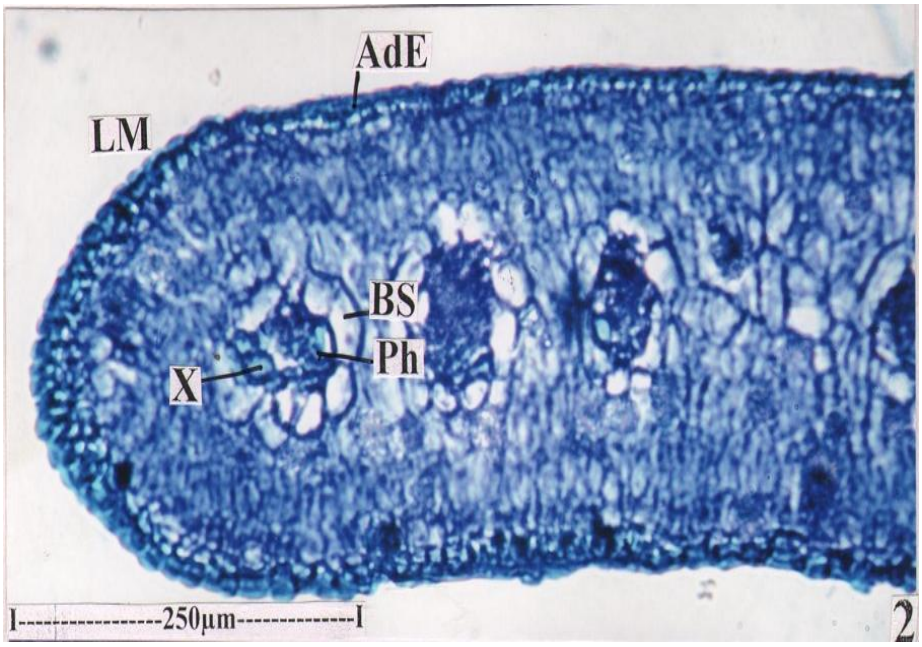

Fig.No. 6: T.S. of marginal part of lamina.

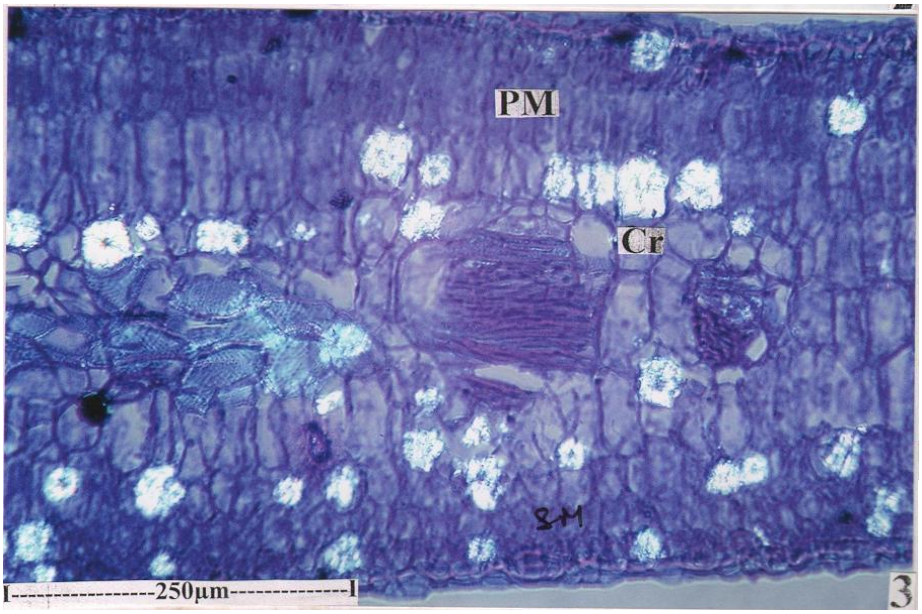

Fig. No. 7: T.S. of leaf showing cristal distribution (Under polarized light.)

(AdE- Adaxial Epidermis, BS- Bundle Sheath, Cr- Crystals, Cu- Cuticle, Ep- Epidermis, Gc- Guard cells, LM- Leaf margin, Ph- Phloem, PM- Palisade Mesophyll, Sc- Subsidiary cell, St- Stomata, X- Xylem.).

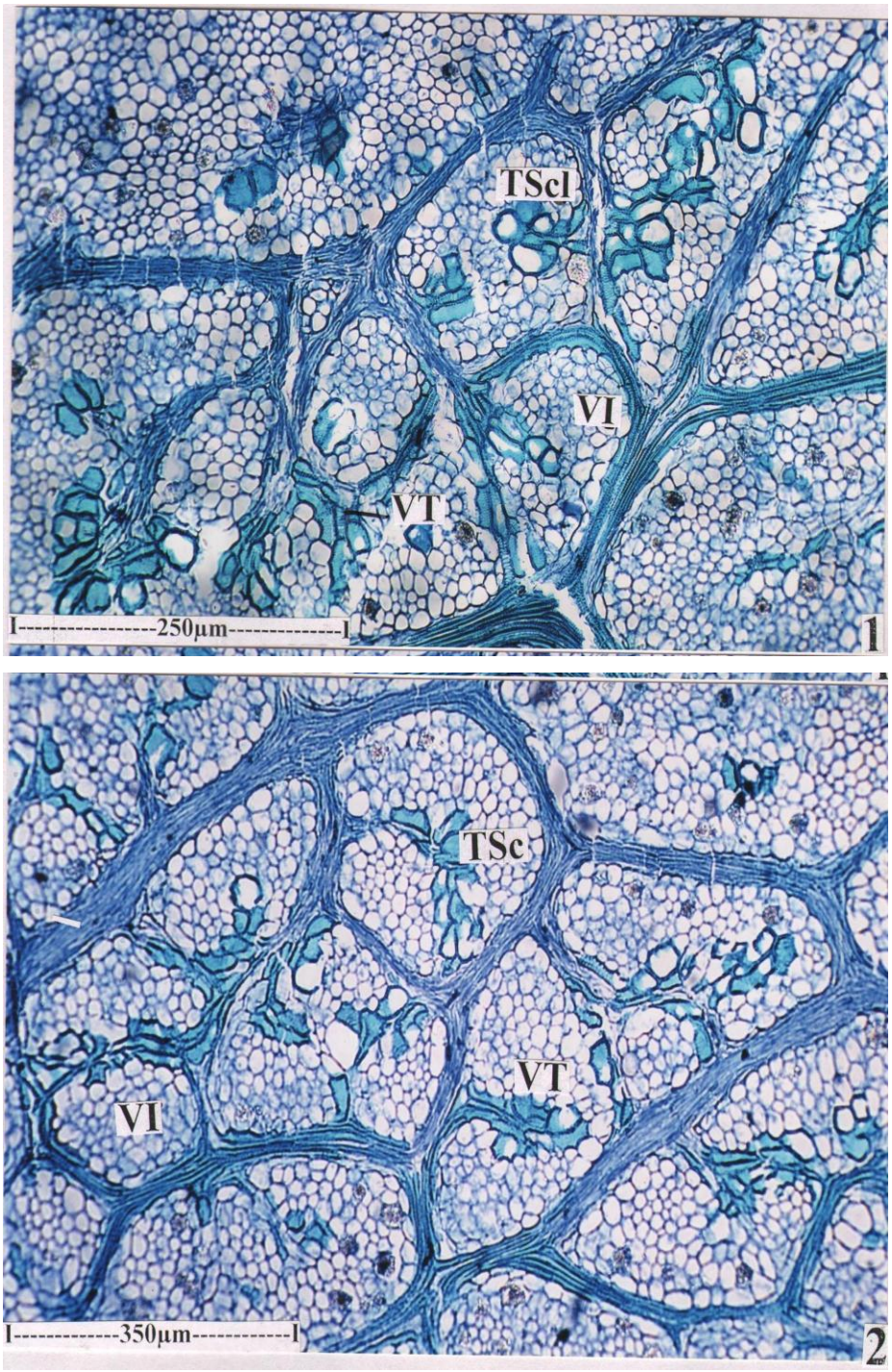

Fig. No. 8: Paradermal section of the lamina showing vein islets, vein termination and cubical clusters of terminal sclereids.

(TScl- terminal Sclereids, VI-Vein Islet, VT- Vein Termination).

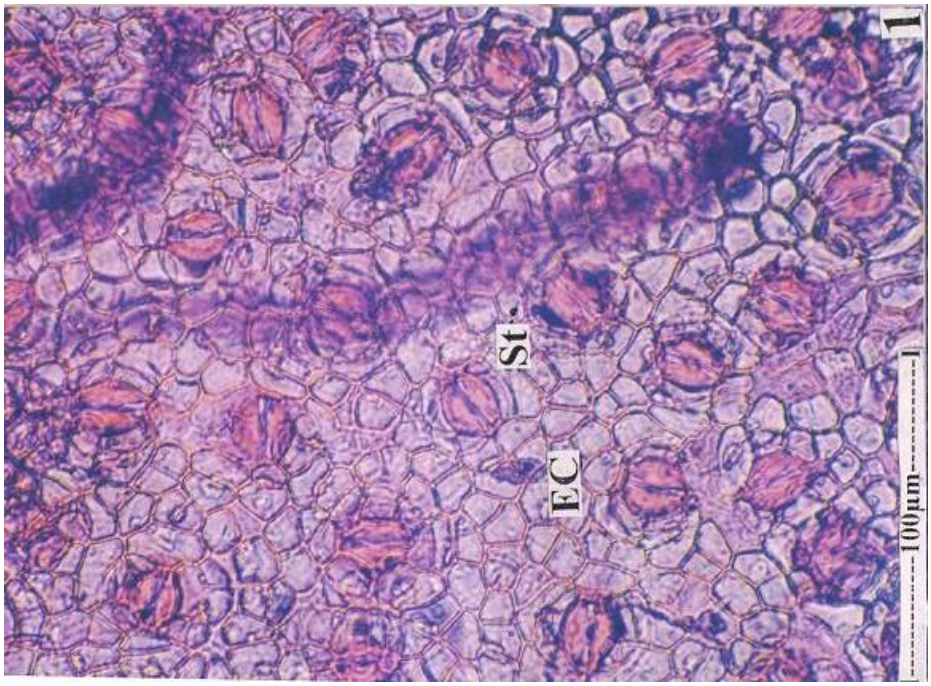

Fig. no. 9: Abaxial epidermal peeling of the leaf showing stomata in surface view.

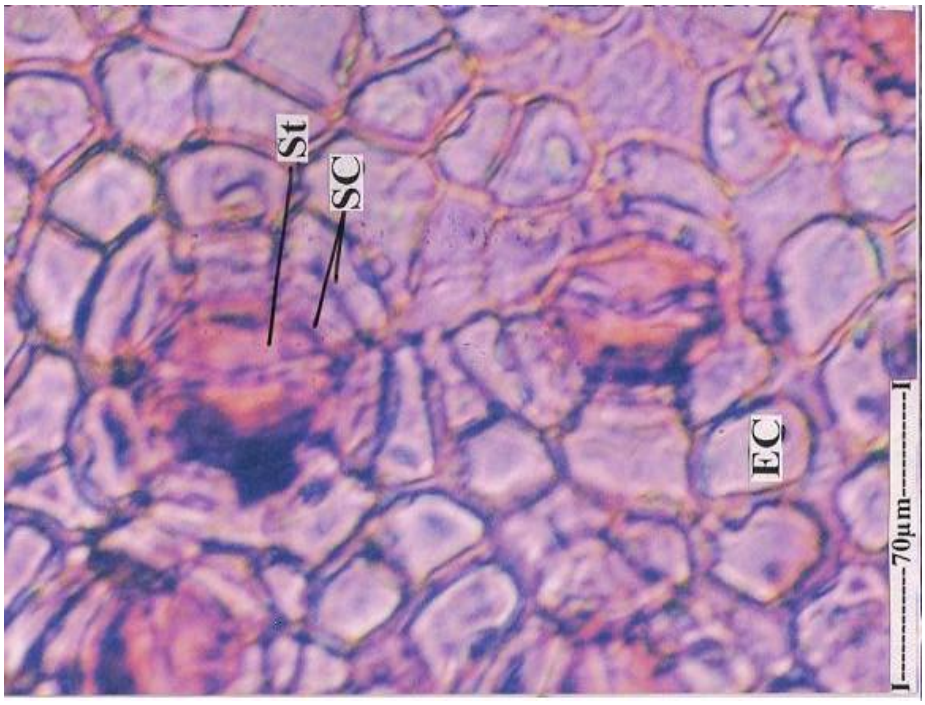

Fig. no.10: Cyclocytic stomata enlarged.

(AW- Anticlinal Wall, EC- Epidermal Cell, Sc- Subsidiary cell, St- Stomata.)

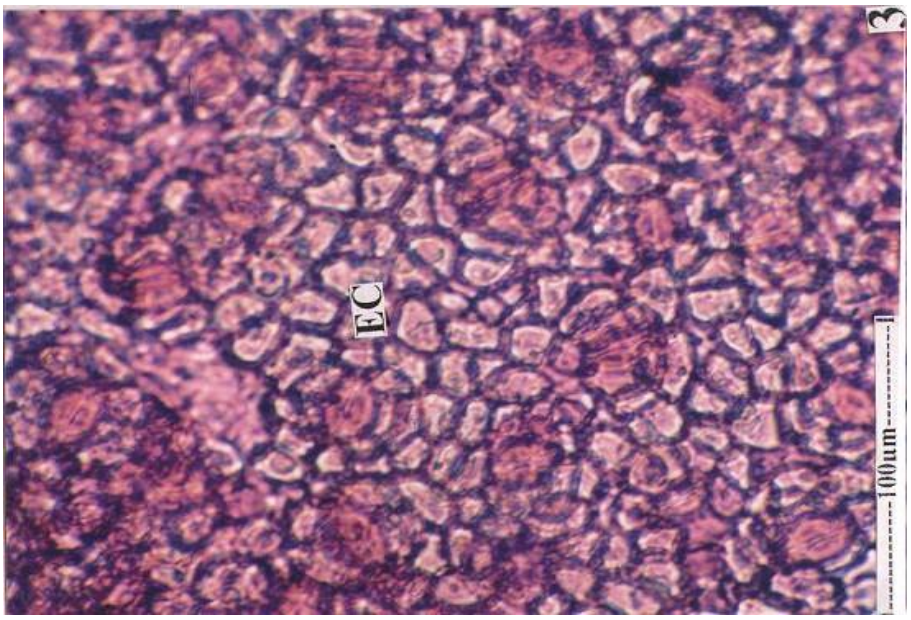

Fig.no. 11: Adaxial epidermis in surface view.

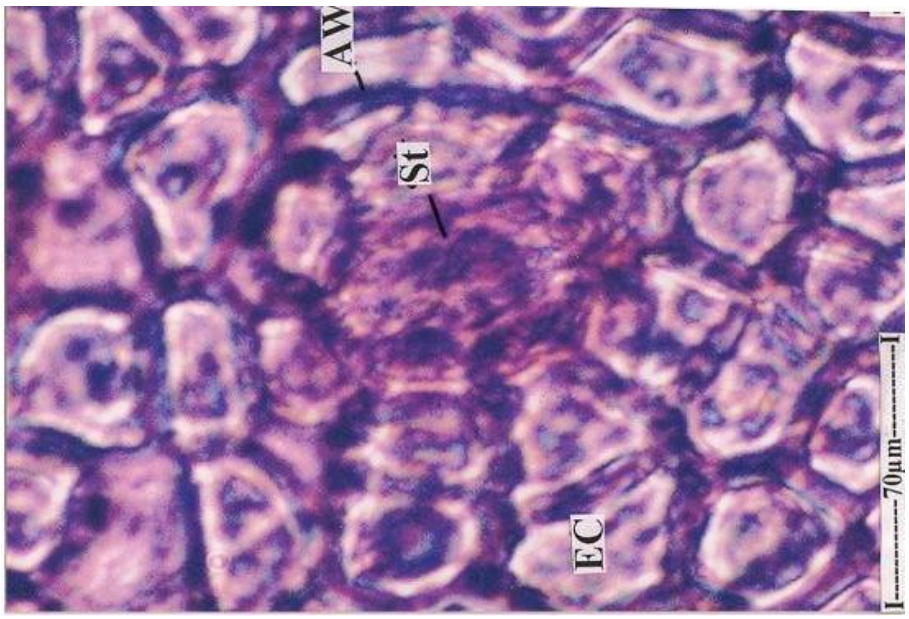

Fig. no.12: A stoma of the abaxial epidermis.

(AW- Anticlinal Wall, EC- Epidermal Cell, Sc- Subsidiary cell, St- Stomata.)

Powder Microscopy

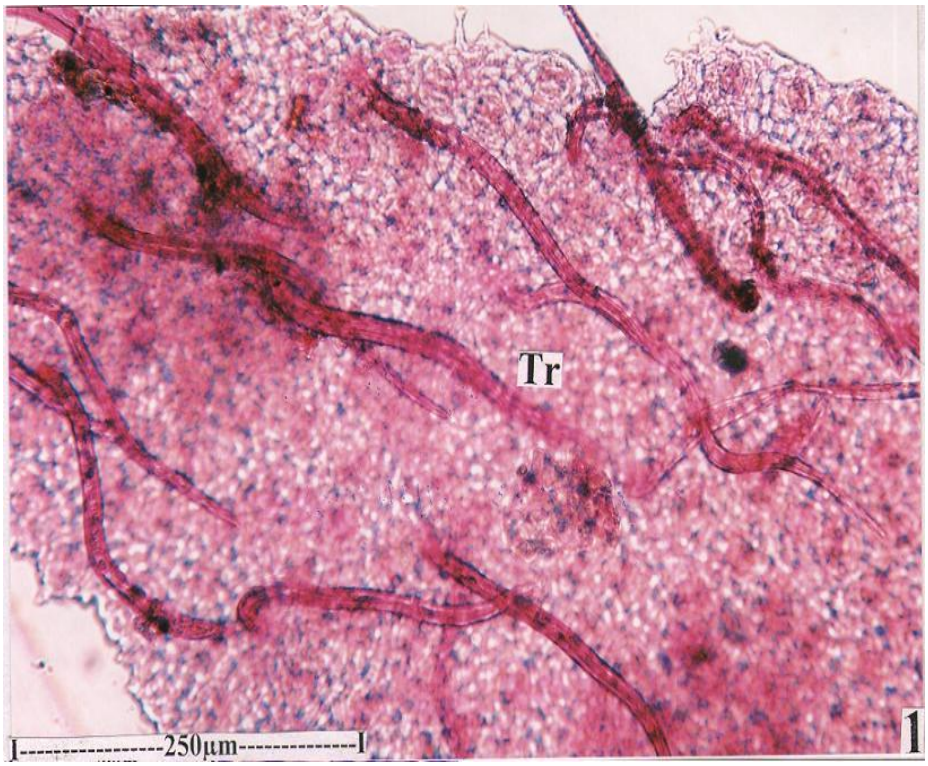

Fig.no. 13: Epidermal non-glandular trichomes attached on the lamina.

Fig. no. 14: A single trichome enlarged.

(CI- Cell Inclusion, Tr- Trichome)

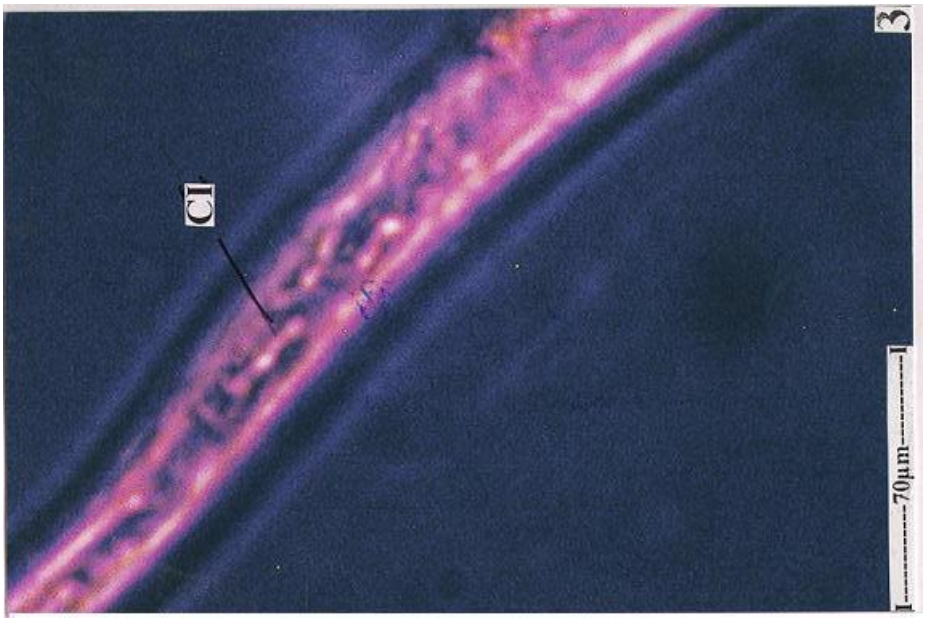

Fig. no. 15: Lumen of the trichome having cell inclusions.

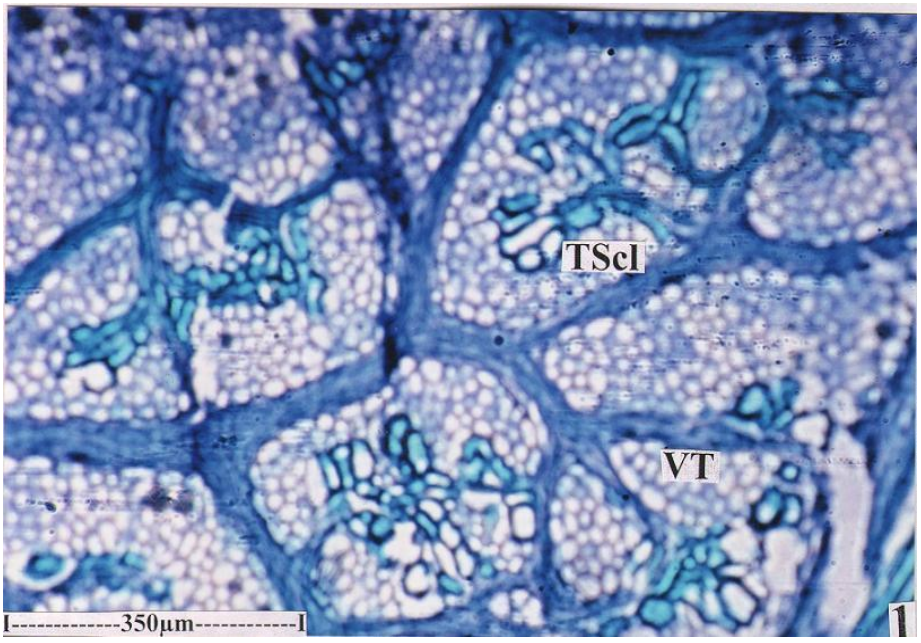

Fig. no. 16: Lamina showing vein terminations and terminal sclereids.

(CI- Cell Inclusion, Tr- Trichome, Dr- Druses, TScl- Terminal Sclereids)

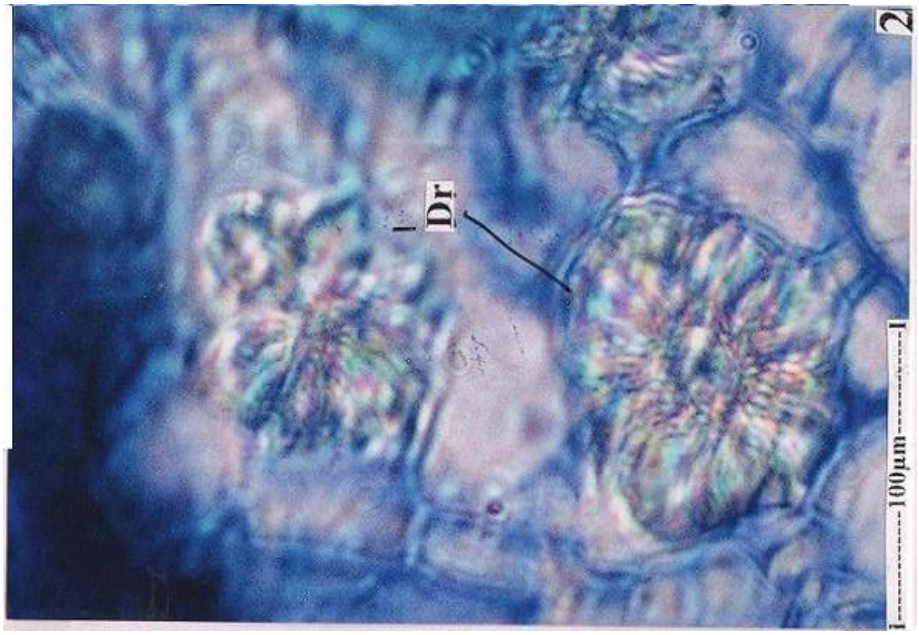

Fig. no.17: Calcium oxalate druses under bright field light.

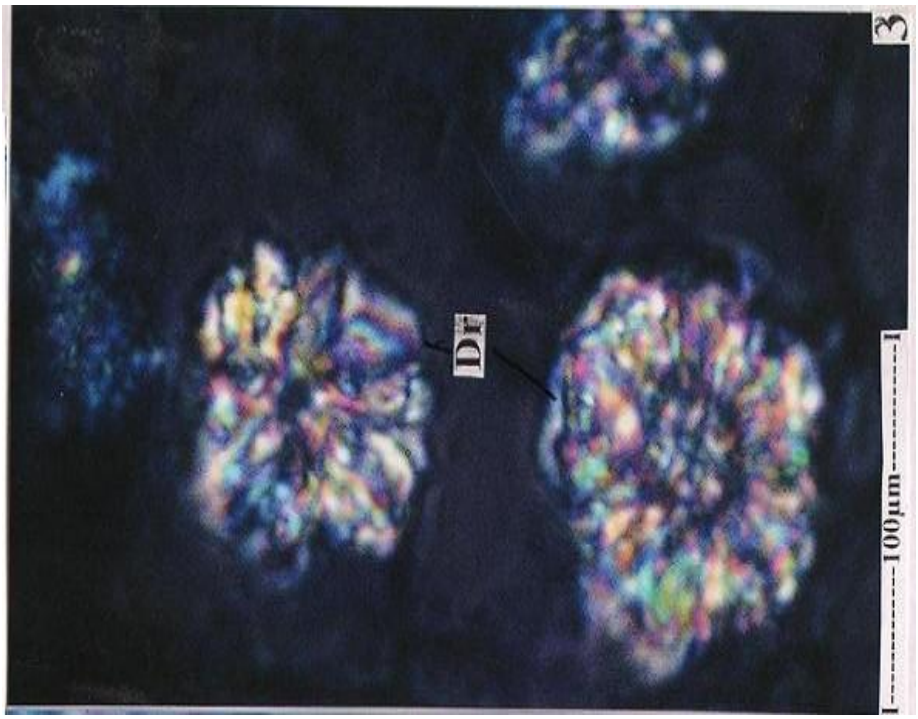

Fig. no. 18: Calcium oxalate druses under bright field light (Under polarized light)

(Dr- Druses, TScl- Terminal Sclereids)

DISCUSSIONS

In transactional view, the leaf exhibits dorsiventaral midrib and smooth, even and thick lamina. The midrib is more or less flat on the adaxial side and slightly conical on the abaxial side (Fig. no. 2). The midrib is 500µm thick. It consists of thin epidermal layers of small

thick walled cells (Fig. 3). The cuticles were prominent and uneven. The vascular bundle of the midrib is single, more or less circular, prominent and collateral**.** The vascular bundle consists of wide cluster of narrow thick walled xylem elements and thin is of phloem located on the lower end of the xylem strand. The vascular strand is surrounded by a thin layer of sclerenchyma sheath (Fig. 3). There are also one or two layers of large hyaline parenchyma cells around the vascular straind. The vascular bundle is 200 µm in transverse plane. The vascular bundles of the lateral veins are circular and they do not project beyond the level of the surface of the lamina.

Lamina (Fig. 4)

The lamina is 350 µm thick. It is isolateral**,** ie; The adaxial and abaxial sides are not well differentiated. The epidermal cells on both sides are large and thick walled with thick cuticle. The mesophyll tissue is consists of adaxial zone of two to three layers of vertically elongated compact palisade cells. Similar type of palisade cells are also seen on the abaxial part, the abaxial palisade cells are in two layers. The median part of the lamina consists of two or three layers of spherical, less compact spongy parenchyma cells. The stomata occur at the level of epidermis, the guard cells have short cubicular ledges (Fig. 5). The epidermis including cutical is 20 µm thick.

Leaf margin (Fig. 6).

The marginal part of the lamina is bluntly conical measuring 200 µm thick. The structure of the margin is not much different from the remaining part of the lamina. The marginal portion has thick walled epidermal cells with prominent cuticle. The mesophyll includes adaxial and abaxial zones of palisade cells and median portion of spherical cells and prominent, circular collateral vascular bundles with bundle sheeth parenchyma cells (Fig. 6).

Crystal distribution (Fig. 7)

Calcium oxlate crystals are abundant in the mesophyll tissue. The crystals are druses and rosette types. They are sypherical bodies with spiny surface. The crystal bodies are located inside the unmodified ordinary cells of the mesophyll. The crystals are 30 µm in diameter.

Venation pattern of the lamina (Fig. 8)

The lamina was cleaned and view in surface view to study the venation system. The venation is reticulate, the vein are thick and prominent. The veins form well defined vein-islets of polygonal outline. The vein boundaries of the islets are straight and thick. The vein

terminations are well developed and thick, short and straight. At the end of the vein termination occurs a cluster of squarish, thick walled wide sclereids. They are called terminals sclereids. The sclereids in a terminal vary from a few to many (Fig. 8.1).

Epidermal cells and stomata (Fig. 9,10,11,12).

Epidermal cells and stomata were studied from the paradermal sections of the lamina. The leaf is amphistomatic, ie; the stomata occure on both side of the lamina. However, the stomata on the abaxial side are more in frequency (Fig. 9) than on the adaxial side (Fig. 11). The abaxial epidermal cells are small, polygonal and fairly thick walled. The anticlinal walls are straight (Fig. 10). the stomata are cyclocytic type. Each stoma is surrounded by two to three circles of subsidiary cells; each circle consists of about seven narrow rectangular cells. The guard cells are 15×20 µm in size.

The adaxial epidermal cells are thick and straight walled. These are many simple pits on anticlinal walls of the cells (Fig. 10, 11). The adaxial stomata are also cyclocytic type.

Powder Microscopy

Fragments of lamina were observed under the microscope.

Epidermal trichomes (Fig. 13,14,15)

Druse non glandular epidermal trichomes are seen the abaxial surface of the lamina. The trichomes are unicellular, unbranched and thick walled. The trichomesare pointed at the cell inclusions (Fig. 14, 15). The trichomes are up to 250µm long and 15µm thick.

Veins and terminal sclereids (fig. 16)

Veins with vein islets and Veins terminations are also seen on the leaf fragments. The vein-islets are many sided, and have thick, straight vein boundaries. The vein terminations have characteristic cluster of squarish sclereids with wide lumen and fairly thick walls (fig. 16).

Crystal bodies are wide spread in the powder. The crystals are exclusively druses, which are spherical bodies comprising many pointed triangular small crystal units (fig. 17,18).

CONCLUSION

The leaves of *Balanites aegyptiaca*(L.) Delile belonging to family Zygophyllaceae was selected for the project, on the source of ethnobotanical information and Literature survey which revealed that not much work has been done on this plant, especially on leaves. So we

have taken its detailed microscopical studies to prove its appropriate identification. The above studies provide helpful information in regard to its correct valuation and identity and give a hand to distinguish from the closely related other range of Balanites aegyptiaca (L.) Delile. The above studies also provide proper future identification of the plant and serves as a standard monograph for identification and evaluation of plant. The determination of these characters will help future researchers in phytochemical as well as a pharmacological analysis of this species.

CONFLICT OF INTEREST: We declare that we have no Conflict of interest.

ACKNOWLEDGEMENT

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