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Microscopic Evaluation of Leaf of *Balanites Aegyptiaca* (L.) Delile: A Review Article

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ABSTRACT:

Introduction: Leaves of *Balanites aegyptiaca* (L.) Delile are commonly used for diabetes. Literature survey revealed that not much work has been done on this plant, especially on leaves. So detailed microscopical study was done for its identification. **Methods**: Transverse section and powder microscopy was carried out on leaves of *Balanitesaegyptiaca* (L.) Delile.

Result: Microscopical study showed dorsiventaral midrib and smooth, even and thick lamina. The lamina was isolateral and 350 μ m thick. The marginal part of the lamina was bluntly conical measuring 200 μ m thick. Calcium oxalate crystals were abundant in the mesophyll tissue. The crystals were druses and rosette types. The veins formed well defined vein-islets of polygonal outline. The vein termination occurred a cluster of squarish, thick walled wide sclereids. The leaf was amphistomatic, However, the stomata on the abaxial side were more in frequency than on the adaxial side. The abaxial epidermal cells were small, polygonal and fairly thick walled. The anticlinal walls were straight. The trichomes were unicellular, unbranched and thick walled. Crystal bodies were wide spread in the powder. The crystals were exclusively druses, which were spherical bodies comprising many pointed triangular small crystal units.

Analysis and Discussion: The present research provided useful information in regard to its correct identity, evaluation and helped to differentiate from the closely related other species of *Balanites aegyptiaca* (L.) Delile. Hence, it furnished the data for future standardization of the drug.

Keywords: *Balanites aegyptiaca* (L.) Delile, Microscopy, Pharmacognostical, Powder Microscopy.

INTRODUCTION

The leaves of *Balanites aegyptiaca* (L.) Delile belonging to family Zygophyllaceae were selected for the study, 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 antifeedant, molluscicide, antidiabetic, contraceptive activities and anthelmintic in various *Balanites aegyptiaca* (L.) Delile extracts. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidysentery properties.^{4,5}

Literature survey revealed that not much work has been done on this plant, especially on leaves. So, it was worthwhile to validate the folk claim for its therapeutic activity scientifically. Its detailed pharmacognostical, and phytochemical and pharmacological studies were done 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 f crude drugs which is applicable at various levels such as the authentication of the crude drugs, study of powdered drugs, etc.⁷

Microscopy method allows more detailed examination of a drug which can be used to identify 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 of Plant material (Fig 1: Leaf of *Ingudi* with Stem) The plant leaves of *Balanites aegyptiaca* (L.) Delile were collected from uncultivated fields in and around the Village Renwal of Jaipur District, Rajasthan, India during July-August 2021. The freshly collected leaves were identified and authenticated by CSIR - National Institute of science Communication and Information Resources, New Delhi-110012 (NISCAIR), vide Authentication number: -NISCAIR/RHMD/Consult/2021/3836-37-1. Microscopical Studies

Utmost 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 58-60°C) until TBAsolution attained super saturation. The specimens were cast into paraffin blocks.8Microscopic 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 scalebars.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, Tolu diene and observed for the microscopic features under high power (40 x).

OBSERVATIONS AND RESULTS

In transverse section, the leaf exhibits dorsi-ventral 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. The midrib is 500µm thick. It consists of thin epidermal layers of small thick-walled cells. 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. There are also one or two layers of large hyaline parenchyma cells around the vascular strand. The vascular bundle is 200 um 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. (Fig 2)

Lamina

The lamina is $350 \,\mu\text{m}$ thick. It is iso-lateral, i.e.; 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 consists of adaxial zone of two to three layers of vertically elongated compact palisade cells. Similar type of palisade cells is also seen on the abaxial part, the abaxial palisade cells are in two layers. The median part of the lamina consists of two or threelayers of spherical, less compact spongy parenchyma cells. The stomata occur at the level of epidermis, the guard cells have short cubicular ledges. The epidermis including cuticle is $20 \,\mu m$ thick.

Leaf margin

The marginal part of the lamina is bluntly conical measuring 200 μ m 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 sheath parenchyma cells.

Crystal distribution

Calcium oxalate crystals are abundant in the mesophyll tissue. The crystals are druses and rosette types. They are spherical 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

The lamina was cleaned and viewed in surface view to study the venation system. The venation is reticulate, the veins 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 squares, thick walled wide sclereids. They are called terminal sclereids. The sclereids vary from a few to many.

Epidermal cells and stomata

Epidermal cells and stomata were studied from the paradermal sections of the lamina. The leaf is amphistomatic, i.e.; the stomata occur on both side of the lamina. 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 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 $15 \times 20 \ \mu m$ in size.

The adaxial epidermal cells are thick and straight walled. These are many simple pits on anticlinal walls of the cells. The adaxial stomata are also cyclocytic type.

Epidermal trichomes

Druse non glandular epidermal trichomes are seen the abaxial surface of the lamina. The trichomes are unicellular, unbranched and thick walled. The trichomes are pointed at the cell inclusions. The trichomes are up to $250\mu m$ long and $15\mu m$ thick. (Fig. 3)

Veins and terminal sclereids

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. 3).

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. 3).

ANALYSIS AND DISCUSSION

The leaves of *Balanites aegyptiaca* (L.) Delile belonging to family Zygophyllaceae were selected for the study, 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 detailed microscopical study was carried out to prove its appropriate identification. The present research provided helpful information in regard to its correct evaluation and identitification and gave a base to distinguish from the closely related other varieties of *Balanites aegyptiaca* (L.) Delile.

CONCLUSION

The current study provided proper future identification of the plant and will serve 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.

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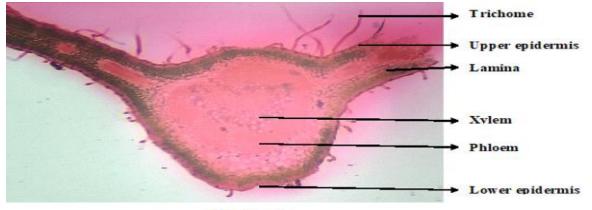
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Fig 1: Leaf of Ingudi with Stem



T.S. of Leaf

Fig 2: T. S. of Leaf

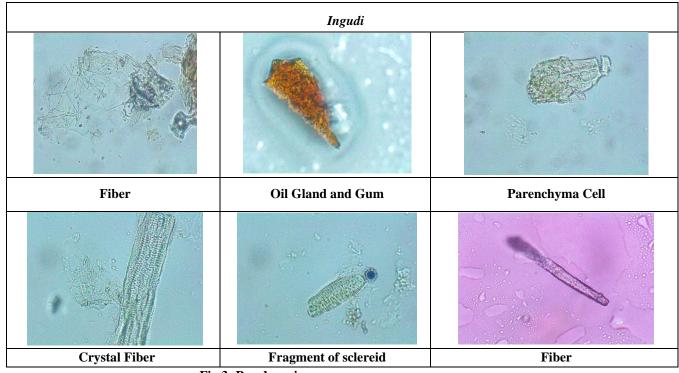


Fig 3: Powder microscopy