ejpmr, 2024, 11(7), 695-704

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INVESTIGATION OF PHARMACOGNOSTICAL PROFILE OF *Delonix regia (Bojer ex hook)* RAF LEAVES

Mercy Jenifa A.[1]* and Mahendran R[2]

[1]Master of Pharmacy, Department of Pharmacy Practice, School of Pharmacy, PRIST Deemed to be University, Thanjavur -613405.

[2]Master of Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625020.

***Corresponding Author: Mercy Jenifa A.**

Master of Pharmacy, Department of Pharmacy Practice, School of Pharmacy, PRIST Deemed to be University, Thanjavur - 613405. **Email ID:** mercyjenifa111@gmail.com

Article Received on 28/05/2024 **Article Revised on 18/06/2024** **Article Accepted on 08/07/2024**

ABSTRACT

Herbal medicine has become very popular in developing countries, with growing awareness of its safety, efficacy, and quality. This study aimed to investigate the pharmacognostical profile of *Delonix regia* (bojer ex hook) leaves. We evaluated pharmacognosy parameters, including macroscopical, organoleptic, and microscopical characteristics. Macroscopical characteristics examined were size, margin, and shape. Organoleptic characteristics included colour, odour, and taste. Microscopical characteristics involved the transverse section, quantitative microscopy, and powder microscopy.

KEYWORDS: *Delonix regia*, Pharmacognosy, macroscopy, microscopy.

INTRODUCTION

Pharmacognostical studies include parameters which help in identifying adulteration. Pharmacognostical studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. At present this study deals with pharmacognostical evaluation of leaves of *Delonix regia* (Caesalpiniaceae) anatomical studies (Transverse section, powder analysis and quantitative microscopy) were done to establish the salient diagnostic characters by using standard methods. These study help in the accurate identification and authentication of medicinal plant and their parts, ensuring the correct species and quality of the plant material used. Belonging to the Caesalpiniaceae family, *Delonix regia* is a prominent ornamental tree. Within the *Delonix* genus, there are two notable species*: Delonix regia* Rafin and *Delonix elata*. *Delonix regia* is particularly renowned for its vibrant blossoms. Each flower consists of five petals, with four of them displaying a uniform color, while the fifth petal stands out with distinctive white streaks.

MATERIALS AND METHODS

Collection and Authentication of Plants

The Plant *Delonix regia* (Bojer ex Hook) Raf. was collected from Madurai, Tamil Nadu, India, on December 2023 and the leaf was botanically identified and authenticated by **Dr. D. Stephen, M.Sc., Ph.D.,** Assistant Professor in Botany, The American College, Madurai, Tamilnadu. The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical Studies

Fresh leaves were subjected to pharmacognostical studies includes organoleptic and morphological studies.

Macroscopical Studies

A. Organoleptic Studies

Colour, odour, taste, shape was identified by sensory characters and displayed in Table.

B. Morphological studies

Leaves were studied separately for its morphological characters like venation, apex, base, margin, length and width. The results were displayed in Table 1.

Microscopical Studies

Fresh leaves are investigated for the microscopical parameters which includes, Transverse section and Quantitative microscopy which covers

A. Stomatal number
B. Stomatal index
C. Vein islet number and vein termination number
D. Palisade ratio
E. Powder microscopy

Transverse Section

Sample (*Delonix regia* (Bojer ex Hook.) Raf leaves) was preserved in fixative FAA (Formalin-5 ml + Acetic acid – 5 ml + 70% Ethyl alcohol – 90 ml) for more than 48hr. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% safranin and 0.5% Astra blue. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam Erc5s digital camera under bright field light.

Quantitative Microscopy

Determination of Leaf Constants (Kokate 2002)

Fresh leaves were taken for the determination of vein islet and vein termination number, epidermal number, stomatal index, stomatal number, and palisade ratio.

Stomatal Number

The Stomatal number may be defined as the average number of stomata per sq.mm area of epidermis of the leaf.

Procedure

Small pieces of upper and lower epidermal peelings of the leaves were mounted in a slide and then by using the camera lucida and stage micrometre 1mm. sq was drawn on a paper. Then the stage micrometre was replaced by the preparation slide and stomata were observed and marked in that unit area. The number of stomata present in unit area was calculated and recorded.

Stomatal Index

It is the percentage, which the numbers of the stomata from the total number of epidermal cells, each stoma being counted as 1 cell.

Stomatal index = S/S+E x 100a

S = Number of stomata per unit area
E = Number of epidermal cells in the same unit area.

Procedure

The same procedure adopted for the determination of stomatal number was followedand the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the standard formula and recorded.

Determination of Vein Islets Number and Vein Termination Number

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per sq.mm area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq.mm area of the photosynthetic tissue.

Procedure

Small pieces of fresh leaves were cut on the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide. With the help of a stage micrometre, camera lucida and microscope, 1 mm square was drawn on the paper. Then the stage micrometre was replaced by the sample slides and the veins were traced over the square. The vein islets and vein terminations were counted in the square and recorded.

Palisade Ratio

A piece of the leaf was boiled in chloral hydrate and was placed under microscope. Camera lucida and drawing board were arranged and the outline of four cells of the epidermis was traced using 4 mm objective. Then, palisade layer is focused down and sufficient cells for covering the tracing of the epidermal cells were traced off. The outline of those palisade cells which were intersected by the epidermal walls was completed. The palisade cells under the four epidermal cells were counted and recorded. The results were displayed in Table and Figure.

Preparation of Leaf Powder

The collected leaves were thoroughly washed with the cold water and the water is drained completely, then the leaves are allowed to dried under shade until it becomes moisture free and it was finely powdered and passed through the Sieve no: 40. The sieved powder was collected and stored properly in a well closed air tight container which is used for the further works.

Powder Microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

RESULTS AND DISCUSSION

Pharmacognostical studies include parameters which help in identifying adulteration. Pharmacognostical studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. At present this study deals with pharmacognostical evaluation of leaves of *Delonix regia* (Caesalpiniaceae) anatomical studies (Transverse section, powder analysis and quantitative microscopy) were done to establish the salient diagnostic characters by using standard methods.

Figure 1: Leaves *of Delonix regia.*

MACROSCOPICAL FEATURES

Fresh leaves of *Delonix regia* was subjected to organoleptic and macroscopical studies and the result was presented in Table 1.

ORGANOLEPTIC EVALUATION

Colour: Green colour
Odour: Characteristic odour
Taste: Characteristic taste.

Table 1: Macroscopical studies of *Delonix regia* (Bojer ex Hook).

S.NO	MORPHOLOGICAL CHARACTERS	OBSERVATION
1	Type	Bi-pinnately compound leaf
2	Arrangement	Alternate
3	Size	0.9 to 1.3 cm long and 0.5 to 0.7 cm wide
4	Shape	oblong or oval
5	Apex	Rounded
6	Base	8 to 20 pairs of pinnae, 12 to 30 pairs leaflet
7	Margin	Entire
8	Surface	Smooth with papery texture

Figure 2: *Macroscopy of Delonix regia* (Dorsal view).

Figure 3: *Macroscopy of Delonix regia* (Ventral view).

Fresh leaves are green coloured, large, alternate, bi-pinnately compound with 8 to 20 pairs of pinnae, 12 to 30 pairs leaflet, leaflets oblong or oval, apex rounded, margin entire, measuring 0.9 to 1.3 cm long and 0.5 to 0.7 cm wide; odour and taste is characteristic.

Microscopic Evaluation

Rachis

- T.S of rachis is oval shaped with a notch on upper surface formed by projection of either side.
- Epidermis is single layered and formed of circular to oval shaped small cells covered by moderately thick cuticle, few epidermal cells bears simple covering trichomes
- 1 to 2 layers of collenchymatous hypodermis is present followed by 4 to 5 layers of cortex formed of thick walled parenchyma cells of which some are pitted.
- Colored cell contents and cluster crystals are present.
- Major portion of the section is occupied by large concentric and closed vascular bundle.
- Bundle is surrounded by 4 to 5 layers of thick pericyclic Fibers.
- Few prismatic crystals are scattered in pericycle; phloem is formed of thin walled group of cells.
- 3 to 4 layers of phloem parenchyma can be seen after pericycle; xylem consists of vessels, Fibers and parenchyma.
- Small wedge shaped parenchymatous pith is present at the center.

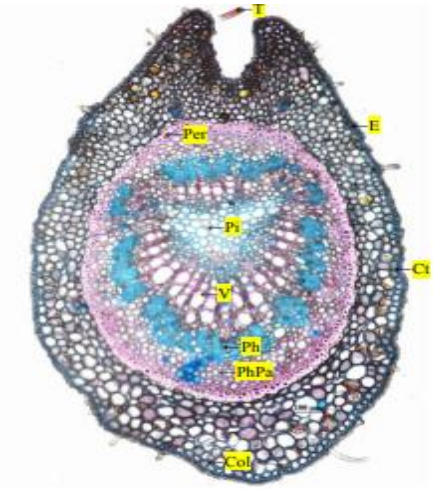

Figure 4: ***TS of Delonix regia petiole.***

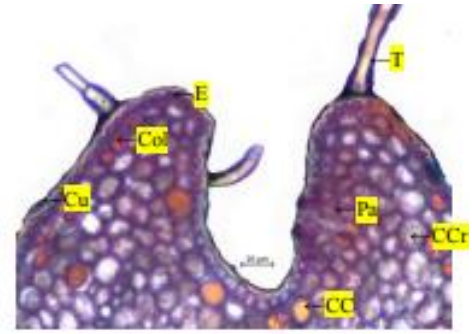

Figure 5(a): Rachis-Upper region enlarged.

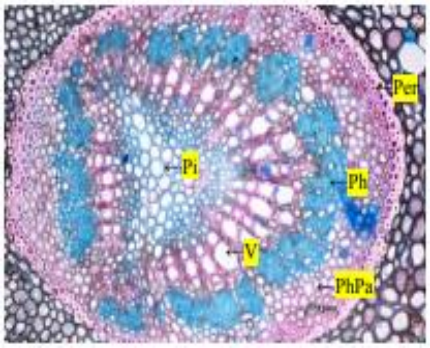

Figure 5(b): Rachis-Vascular bundle.

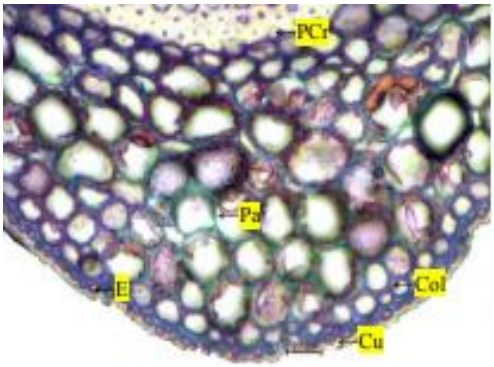

Figure 5c: Rachis-Enlarged view of lower region.

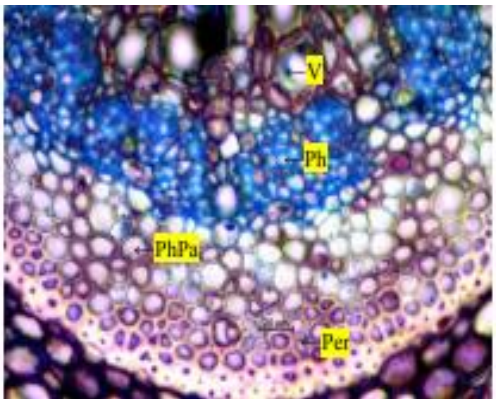

Figure 5D: Rachis-Enlarged view of lower region.

Figure 5: TS of Rachis.

Lower region lower region

CC - cell contents;**CCr** - cluster crystal; **Col** - collenchyma; **Cu** - cuticle; **Ct** - cortex; **E** - epidermis; **Pa** - parenchyma; **PCr** - prismatic crystal; **Per** - pericycle; **Ph** - phloem; **PhPa** - phloem parenchyma**; Pi** - pith; **T** - trichome; **V** - vessel;**Xy**– xylem.

Leaf

T.S of leaf shows convex shaped lower surface and flat upper surface with lateral laminar extensions.

Midrib

T.S of midrib shows single layered epidermis formed of oval shaped cells covered by cuticle and few of them bearing covering trichomes; beneath epidermis is parenchymatous ground tissue embedded with conjoint, collateral and closed vascular bundle; phloem is arranged towards lower side and formed of thin-walled group of cells; xylem is found facing upper side and made up of vessels, Fibers and parenchyma.

Lamina

T.S of lamina is dorsiventral; shows upper and lower epidermis covered by cuticle and bears simple covering trichomes and few glandular trichomes; epidermis is single layered and formed of oval to somewhat elongated cells; mesophyll tissue is differentiated into upper single layered compactly arranged elongated columnar palisade cells and lower loosely arranged spongy parenchymal cells with intercellular spaces; abundant chlorophylls are present in mesophyll tissue; veins can be seen traversing through the mesophyll cells.

Figure 6: T.S of Leaf.

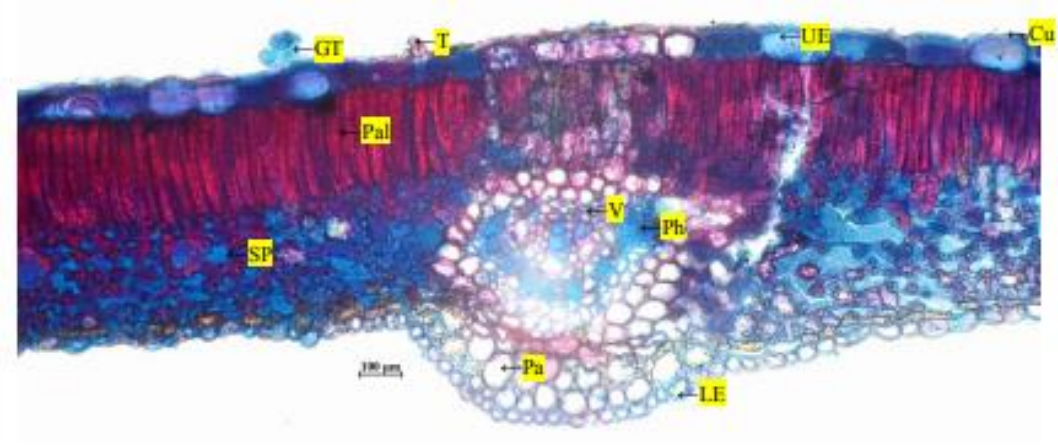

Figure 7: T.S of Leaf passing through Midrib.

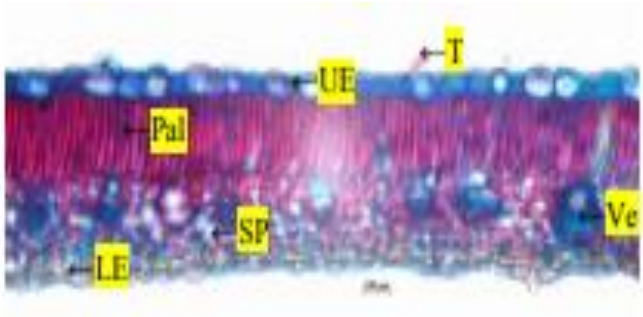

Figure 8: T.S of Lamina.

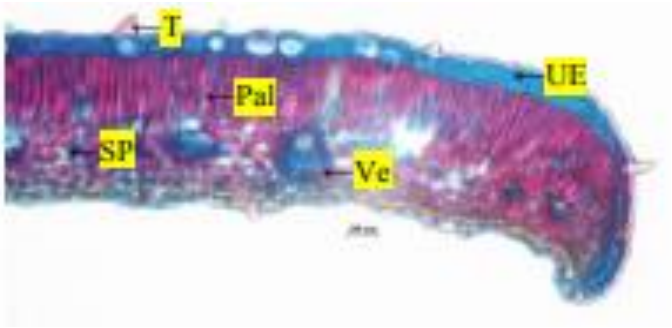

Figure 9: T.S of Leaf Margin.

Cu - cuticle; **GT** - glandular trichome; **LE** - lower epidermis; **Pa** - parenchyma; **Pal** - palisade cells; **Ph** - phloem; **SP** - spongy parenchyma; **T** - trichome; **UE** - upper epidermis**; V** - vessel; **VB** - vascular bundle; **Ve** – vein.

QUANTITATIVE MICROSCOPY

The fresh leaves of *Delonix regia* (Bojer ex hook) was treated with 0.1% saturated chloral hydrate solution and slides were prepared. Vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined. The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded.

Table 2: Quantitative microscopy of *Delonix regia* leaf.

PARAMETERS	UPPER EPIDERMIS (Cell /mm^2)	LOWER EPIDERMIS (Cell /mm^2)
Epidermal number	390-420	400-440
Stomatal number	-	50-60
Stomatal index	-	11-12
Palisade ratio	10-15	
Vein islets number	30-35	
Vein termination number	12-20	

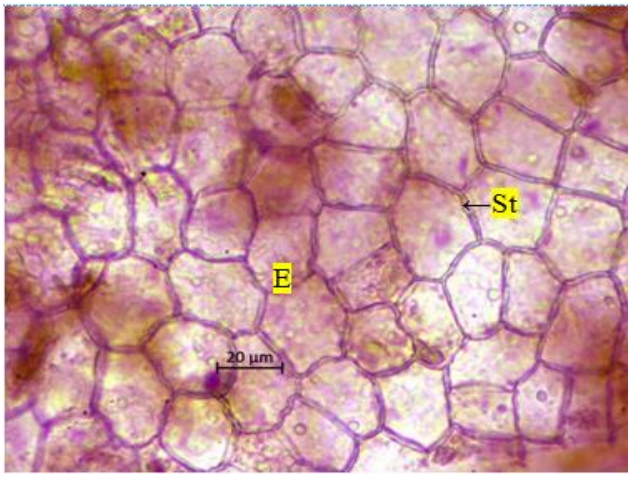

Figure 10(a): Upper epidermis.

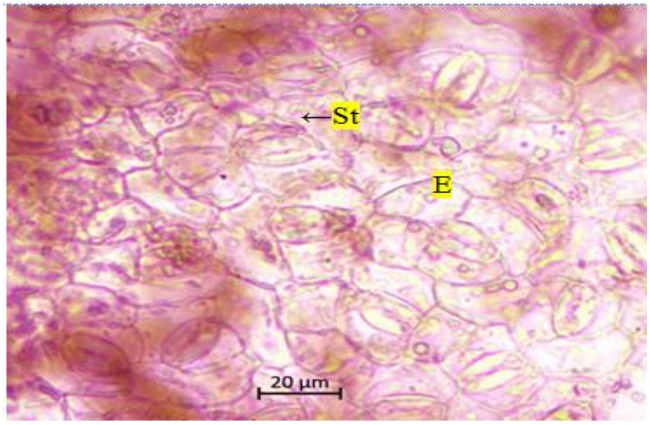

Figure 10(b): Lower epidermis.

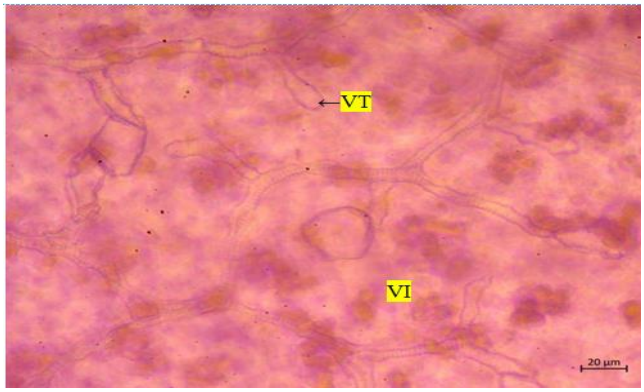

Figure 10(c): Vein islets and terminations.

Figure 10: Quantitative microscopy of *Delonix regia* leaf.

E - Epidermis; **St** - Stomata; **VI** - Vein Islet; **VT** - Vein Termination.

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded (Table 2). The leaf is hypostomatic with anisocytic stomata onlower surface of epidermis. The quantitative constants were determined for the leaves of this plant and these parameters were considered as significant one especially for the evaluation of this plant.

POWDER MICROSCOPY

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light.

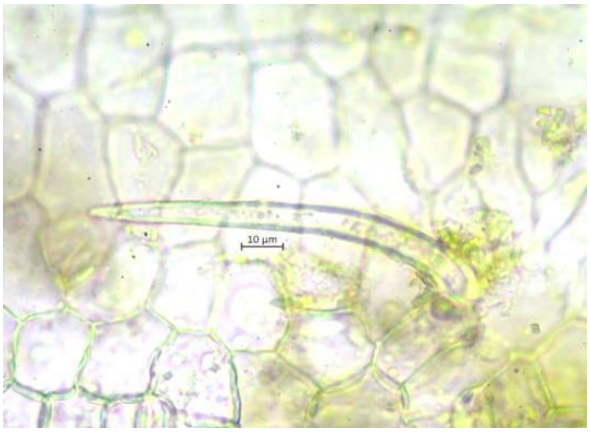

Figure 11(a): Simple covering trichome.

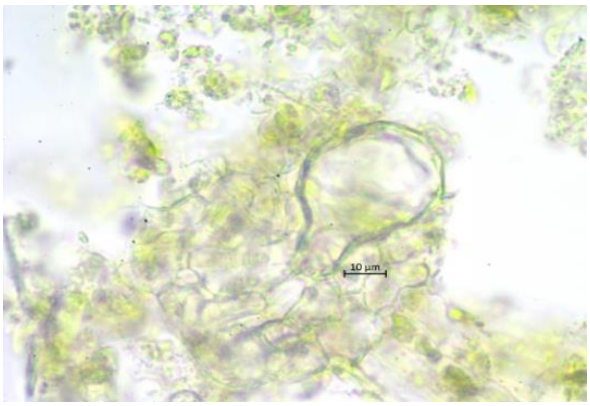

Figure 11(b): Glandular trichome.

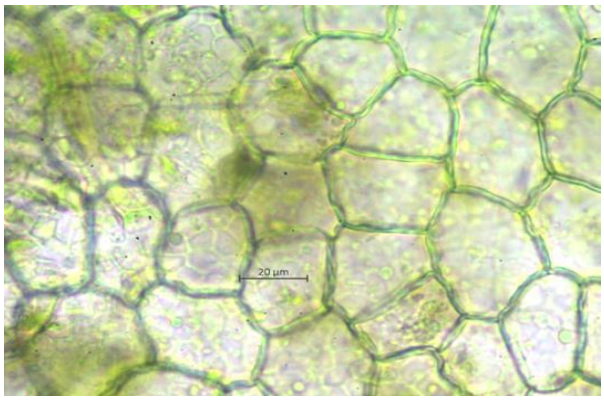

Figure 11(c): Upper epidermis.

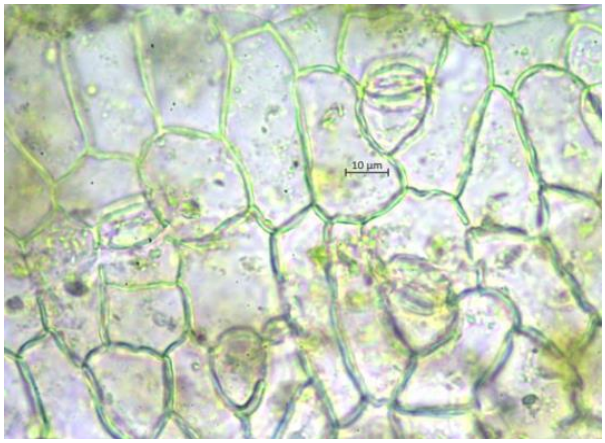

Figure 11(d): Lower epidermis- anisocytic stomata.

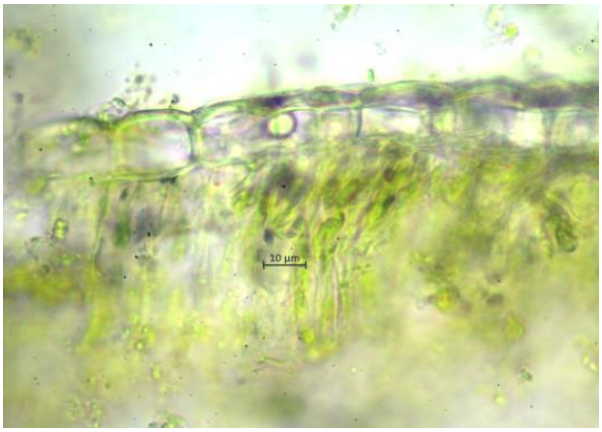

Figure 11(e): Palisade in sectional view.

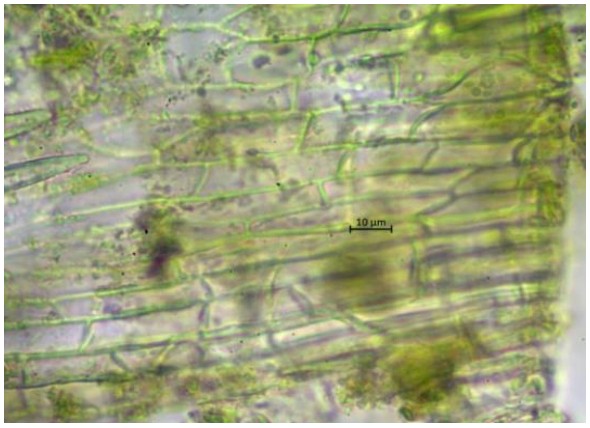

Figure 11(f): Parenchyma cells in midrib.

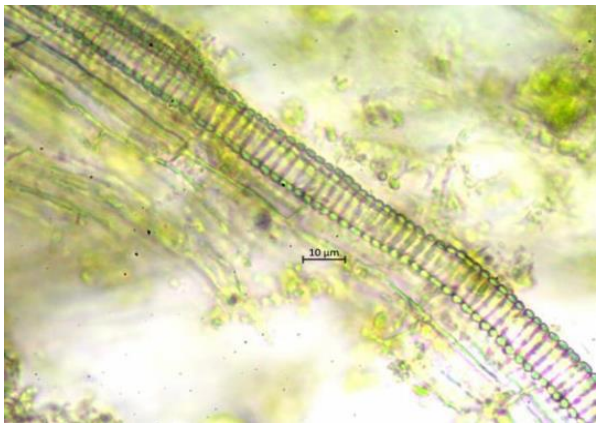

Figure 11(g): Annular vessel.

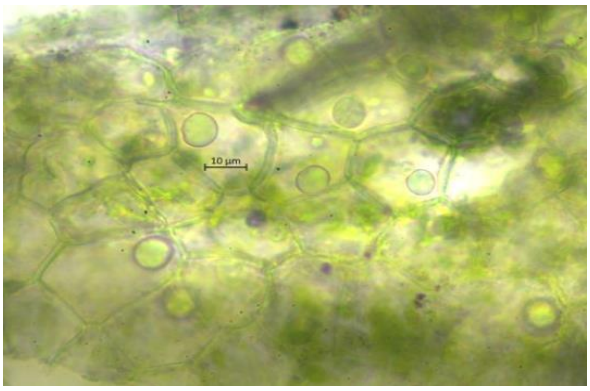

Figure 11(h): Oil globules.

The macroscopic examination revealed leaves are green colour, characteristic odour and taste. Transverse section of showed the presence of petiole, rachis, leaf, midrib, lamina.

Quantitative microscopy of *Delonix regia* was carried out. The reports revealed that the number of epidermal cells in the upper and lower epidermis were 390 – 420 cell /mm^2 and 400– 440 cell/ mm^2. Stomatal number in the lower epidermis is 50 – 60 cell / mm^2 and the stomatal index was found to be 11-12 cell /mm^2 in the lower epidermis. Palisade ratio present in the lamina region is about 10-35 cell / mm^2, vein islets number was found to be 30-35 and vein termination number was found to be 12-20.

The powder is dark green coloured with characteristic odour and taste; shows the characters like simple unicellular covering trichomes, glandular trichomes, upper epidermis in surface view, lower epidermis with anisocytic stomata, sectional view of palisade cells, parenchyma cells of midrib, vessels with annular thickening, oil globules, cells with contents, prismatic and cluster crystals.

CONCLUSION

Pharmacognostic studies provide the basis for quality control measures, detecting adulteration and ensuring the purity and potency of herbal drug. By documenting the characteristics and uses of medicinal plants, pharmacognostic studies support the conservation of biodiversity and sustainable use of plant resources. Overall pharmacognostical studies play a fundamental role in development, quality assurance and safe use of herbal medicines.

REFERENCES

1. Wallis TE. Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs. Third Edition. Boston: Little, Brown and Company, 1965.
2. Fahn A. Plant anatomy. Third Edition Pergamon Press, Oxford, 1980.
3. Brindha S, Divya KG, Remya A, Sunil Kumar KN, Sathiya Rajeswaran P. Foliar Macro-micro-morphology of *Capparis zeylanica* L. J Ayu Med Sci., 2017; 2(2): 174-8. DOI: 10.5530/jams.2017.2.9 (ISSN 2456–4990).
4. Chopra R N, Nayar S L, Chopra I C. Glossary of Indian Medicinal Plants CSIR, New Delhi, 1956; 92.
5. Sumitra Singh, Sonia Naresh Kumar. A Review: Introduction to Genus *Delonix* World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(6)
6. Kokate C K, Purohit A P, Gokhale S B. Analytical Pharmacognosy. 23rd edition. Pune. Nirali Prakashnan, 2003.