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Original Article

Integrated Standardization of *Pergularia daemia* (Forssk.) Chiov. Using Pharmacognostic and Molecular Techniques

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ABSTRACT

Background: This research paper aims to establish comprehensive pharmacognostical standards and DNA barcode markers for *Pergularia daemia* (Forssk.) Chiov., a medicinally important plant in traditional medicine systems. Pharmacognostical work reveals it's identification, make more easier for formulating traditional system. **Materials and Methods:** The research involved macroscopic, microscopic, powder microscopic, and quantitative microscopic analyses along with histochemical evaluations to develop quality control parameters. DNA barcoding using rbcL as a marker was performed to authenticate the species at a molecular level. **Results:** The microscopic studies revealed diagnostic features including rosette crystals, multicellular trichomes, latex content, paracytic stomata, and distinct vascular structures. The DNA barcode for the species was successfully generated and submitted to GenBank (Accession numbers: PQ740391 and PQ740392). The quantitative parameters (stomatal index 25-28, palisade ratio 14-16, macroscopic/microscopic features (rosette crystals, multicellular trichomes, paracytic stomata) and seed characteristics (dorsiventrally flattened, 850-1100 µm thick: trichomes 150-350 µm) while histochemistry reveals alkaloids, phenolic, mucilage, oils, lignin and starch mostly in vascular/epidermal tissues palisade ratio 14-16), macroscopic/microscopic features (rosette crystals, multicellular trichome, paracytic stomata) and seed characteristic (dorsiventrally flattened 850-1100 µm thick; trichome 150-350 µm). DNA barcoding via rbcL market established molecular identity and pharmacognosy for strong authentication. **Conclusion:** This standardization study provides a comprehensive set of parameters for the authentication of *P. daemia*, which can be used to ensure the quality and purity of this medicinal plant in herbal formulations.

Keywords: DNA barcoding, Microscopy, *Pergularia daemia*, Pharmacognosy, rbcL, Standardization.

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INTRODUCTION

Medicinal plants have been the cornerstone of traditional healthcare systems worldwide. However, the authenticity and standardization of these medicinal plants remain a challenge due to adulteration, substitution, and misidentification. *Pergularia daemia* (Forssk.) Chiov. (Family Apocynaceae) is a twining perennial herb widely distributed in tropical and subtropical regions, particularly in Asia and Africa (Bhaskar *et al*., 2009).

P. daemia, commonly known as "Uttaravaruni" in Sanskrit, "Veliparuthi" in Tamil, and "Utranajutuka" in Hindi, has been extensively utilized in various traditional medicine systems including Ayurveda, Siddha, and folk medicine for centuries.

The genus *Pergularia* belongs to the subfamily Asclepiadoideae of the family Apocynaceae, which is known for its latex-producing species with diverse medicinal applications (Vere *et al*., 2015).

The plant possesses a remarkable repertoire of pharmacological activities, making it a valuable resource in traditional healing practices. Ethnomedicinal surveys have documented its use in treating a wide range of ailments. The leaves are traditionally used as an emetic, expectorant, and antipyretic. The plant extract is employed for treating bronchitis, asthma, and amenorrhea (Bhusari *et al*., 2018). In certain communities, it is used as an anthelmintic, laxative, antipyretic, and employed in the treatment of infantile diarrhea and malarial intermittent fevers.

Modern pharmacological studies have validated many of these traditional uses. Extracts of *P. daemia* have been scientifically proven to possess anti-inflammatory, antipyretic, antioxidant, analgesic, hepatoprotective, anticancer, antibacterial, antifungal, and antifertility activities. The plant's anti-diabetic potential has been demonstrated in various animal models, supporting its use in traditional medicine for managing diabetes (Johnson *et al*., 2015).

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Phytochemical investigations have revealed that *P. daemia* contains a rich array of bioactive compounds including alkaloids, flavonoids, steroids, terpenoids, saponins, and cardenolides. Specific compounds like Pergularin, Tylophorine, Tylophorinine, and Pergularinine have been isolated, which contribute to its medicinal properties (Karthishwaran *et al*., 2010). The presence of various cardiotonic steroids, including calotropin, calactin, and uzarigenin, further enhances its therapeutic potential (Khandelwal *et al*., 2008).

The plant's aerial parts have shown significant antioxidant activity attributed to the presence of phenolic compounds, which help neutralize free radicals and reduce oxidative stress (Narayana *et al*., 2019). The hepatoprotective activity of *P. daemia* has been validated through studies on carbon tetrachloride-induced hepatotoxicity in rats, where treatment with plant extracts significantly reduced liver damage markers

Despite its significant medicinal value, comprehensive Pharmacognostical standards for *P. daemia* are still inadequate, hampering quality control during its utilization in herbal formulations (Nikam *et al*., 2012). Pharmacognostical standardization, which includes macroscopic, microscopic, and phytochemical evaluation, is essential for establishing the identity, purity, and quality of medicinal plants (Sathish *et al*., 2010).

The increasing demand for herbal medicines has raised concerns about the quality, safety, and efficacy of plant materials used in traditional formulations (Sridhar *et al*., 2014). This is particularly pertinent for *P. daemia*, which is often collected from wild sources and may be confused with morphologically similar species. Furthermore, the potency of herbal medicines largely depends on the quality of the plant material used, which is influenced by various factors including harvesting time, geographical location, processing methods, and storage conditions (Kumar *et al*., 2008).

Furthermore, molecular authentication using DNA barcoding has emerged as a powerful tool for species identification, complementing traditional Pharmacognostical approaches (Suresh Kumar *et al.,* 2008). DNA barcoding utilizes short genetic markers in an organism's DNA to identify it as belonging to a particular species, providing a reliable method for authentication regardless of the plant part used or its physical condition. This technique has gained importance in the quality control of medicinal plants, especially for species that are morphologically similar or processed into forms where anatomical features are no longer distinguishable (Suresh *et al*., 2008).

This study aims to establish comprehensive Pharmacognostical standards and generate DNA barcode markers for *P. daemia* to facilitate its proper identification and quality control (Techen *et al*., 2014). The integration of traditional Pharmacognostical methods with modern DNA barcoding would provide robust parameters for the authentication of this medicinally important plant, thereby ensuring the safety and efficacy of herbal formulations containing *P. daemia*.

MATERIALS AND METHODS

Plant Material

The whole plant of *Pergularia daemia* (Forssk.) Chiov. was collected and authenticated. The sample was assigned the code 849.10052401 for DNA barcoding analysis and 849.P10052401D for Pharmacognostical evaluation.

Macroscopic Analysis

External features of the sample were documented using a Nikon D-5600 Digital camera following standard procedures (SOP No. PCOG-004-SOP).

Microscopic Analysis

Transverse Section Studies

The sample was preserved in FAA (Formalin-Acetic acid-Alcohol) fixative for more than 48 hours. Thin transverse sections were prepared using a sharp blade, stained with toluidine blue, and photographed using an Axiolab 5 trinocular microscope attached with a Zeiss Axiocam208 color digital camera under bright field light.

Powder Microscopy

A small amount of powdered sample was cleared with chloral hydrate, mounted in 50% glycerol, and observed using a Nikon ECLIPSE E200 trinocular microscope attached to a Zeiss ERc5s digital camera

Quantitative Microscopy

Leaf fragments (5 × 5 mm) were cleared with chloral hydrate, mounted in glycerin, and examined under a microscope equipped with a camera lucida. Parameters including stomatal number, stomatal index, vein islet number, vein termination number, and palisade ratio were determined (Wallis *et al*., 1965).

Histochemical Analysis

Plant sections were subjected to various histochemical tests following standard procedures (SOP No. PCOG-008-SOP) to detect the presence of crystals, fats, oils, resins, starch, tannins, mucilage, lignin, suberin/cutin, and alkaloids (Wahi *et al*., 2002).

DNA Barcoding

Genomic DNA Isolation

Approximately 100 mg of plant tissue was ground with liquid nitrogen, mixed with preheated CTAB extraction buffer containing β-mercaptoethanol, and incubated at 65°C. After centrifugation, the supernatant was processed with chloroform alcohol (24:1) mixture, followed by precipitation with ice-cold

isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and suspended in $T_{10}E_1$ buffer (SOP No. PCOG-009).

DNA Quality and Quantity Assessment

The quality and concentration of genomic DNA were assessed by 1% agarose gel electrophoresis and visualization under a UV-transilluminator (Biorad, GelDoc Go, USA). The quantity was further checked using a Nanodrop spectrophotometer (Thermoscientific, Nanodrop One, USA).

PCR Amplification

The rbcL DNA barcode marker was used for PCR amplification. The reaction was carried out in a thermocycler (Applied Biosystem, Veriti™, USA), and the amplified products were visualized on a 1% agarose gel.

Sequence Analysis and Submission

The nucleotide sequences in FASTA format were obtained using Finch TV from the chromatogram and analyzed using the BLAST algorithm of NCBI to identify matching sequences. The confirmed sequences were submitted to GenBank with necessary details and converted to barcodes using BioRad barcode generator software (Verma *et al.*, 2008).

RESULTS

The comparative table comparing the phytochemical constituents of *Pergularia daemia* with similar medicinal plants in the Apocynaceae family, which were tabulated in Table 1.

Macroscopic Characteristics

Pergularia daemia is a twining pubescent or tomentose undershrub with opposite cordate leaves. The roots are thick and brownish in color. The stem is cream to white. The leaves are green, turning pale yellow on maturity, measuring 4 to 7 cm in length and 3 to 6 cm in width, with an entire margin and acute tip. The midrib is prominent on the ventral side (Figure 1). The plant has a characteristic odor and taste.

Microscopic Characteristics

Root

The Transverse Section (TS) of the root is nearly circular in outline with numerous small ridges and furrows. The epidermis is replaced by multicellular cork tissue composed of thick-walled cells. The cortex is broad and composed of 4 to 5 layers of parenchyma cells. The vascular region shows well-developed secondary phloem as a complete ring encircling the inner mass of secondary xylem. A discontinuous pericycle comprising thick-walled cells surrounds the vascular cylinder. Numerous starch grains are present in the cortical and phloem regions. The pith is obscure (Figure 2).

Stem

The TS of stem is circular in outline with a single-layered epidermis covered with distinct cuticle and uniseriate hairs. The cortex is divided into an outer cortex composed of collenchyma cells followed by an inner cortex made up of parenchyma cells. Isolated patches of bast fibers are scattered in the inner cortex. The vascular region consists of an outer ring of secondary phloem encircling the secondary xylem. Medullary rays traverse through the xylem elements. Laticifers are confined to the medullary rays and pith cells (Figure 3).

Quantitative Microscopy

Leaf

Quantitative microscopic analysis revealed that the leaf is hypostomatic with paracytic stomata on the lower epidermis. The epidermal number ranged from 110-120/mm² in the upper epidermis and 120-130/mm² in the lower epidermis. The stomatal number was 40-50/mm² with a stomatal index of 25-28 on the lower epidermis (Verma *et al.*, 2008). The palisade ratio was 14-16, vein islet number 14-18, and vein termination number 8-10 (Table 2 and Figure 4).

Figure 1: *Pergularia daemia*, thewhole plant.

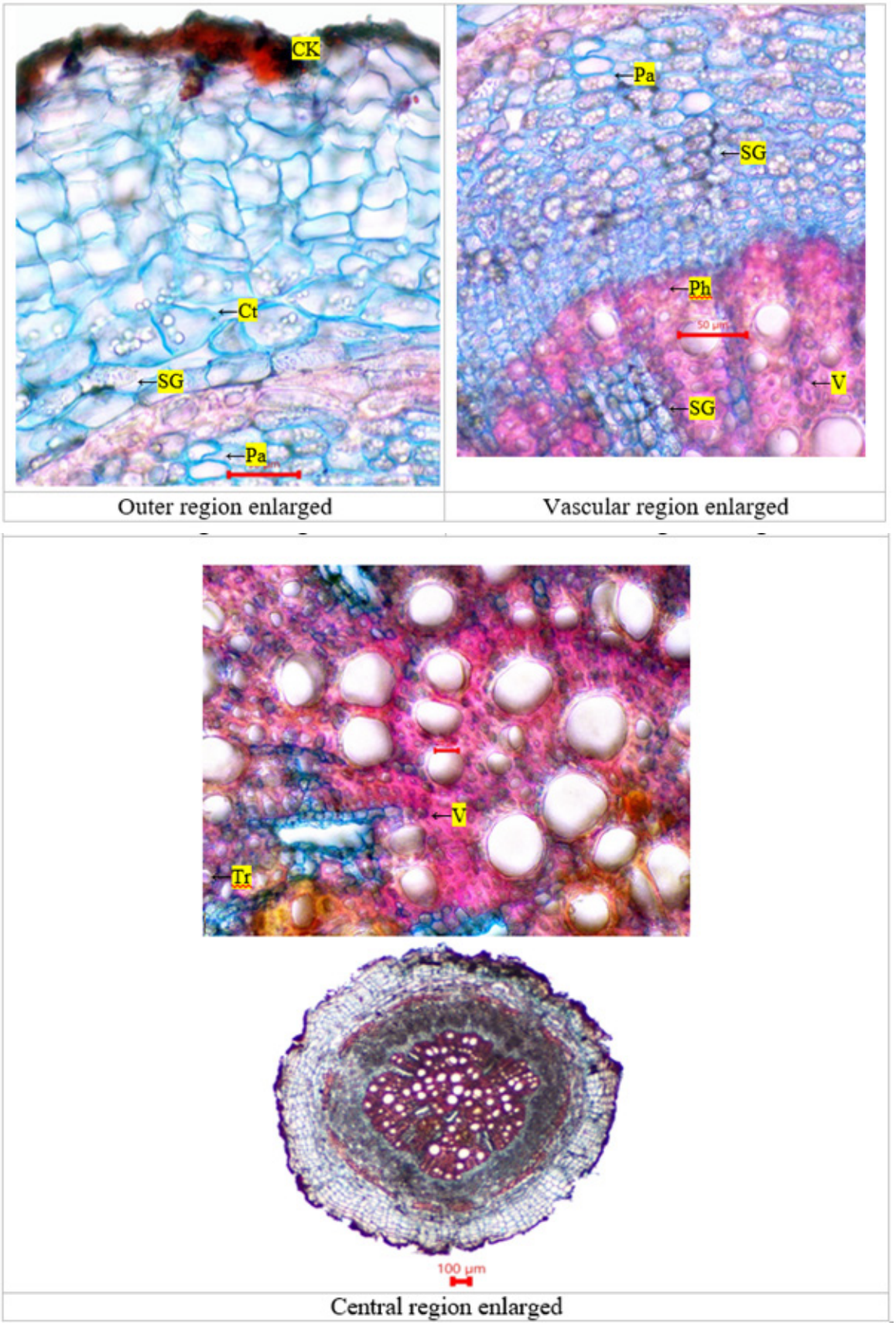


Figure 2: TS of *Pergularia daemia*-root. Ck - cork; Cot - cortex; Pa - parenchyma; Per - pericycle; Ph - phloem; SG - starch grain; Tr -tracheid; V - vessel; Xy – xylem.

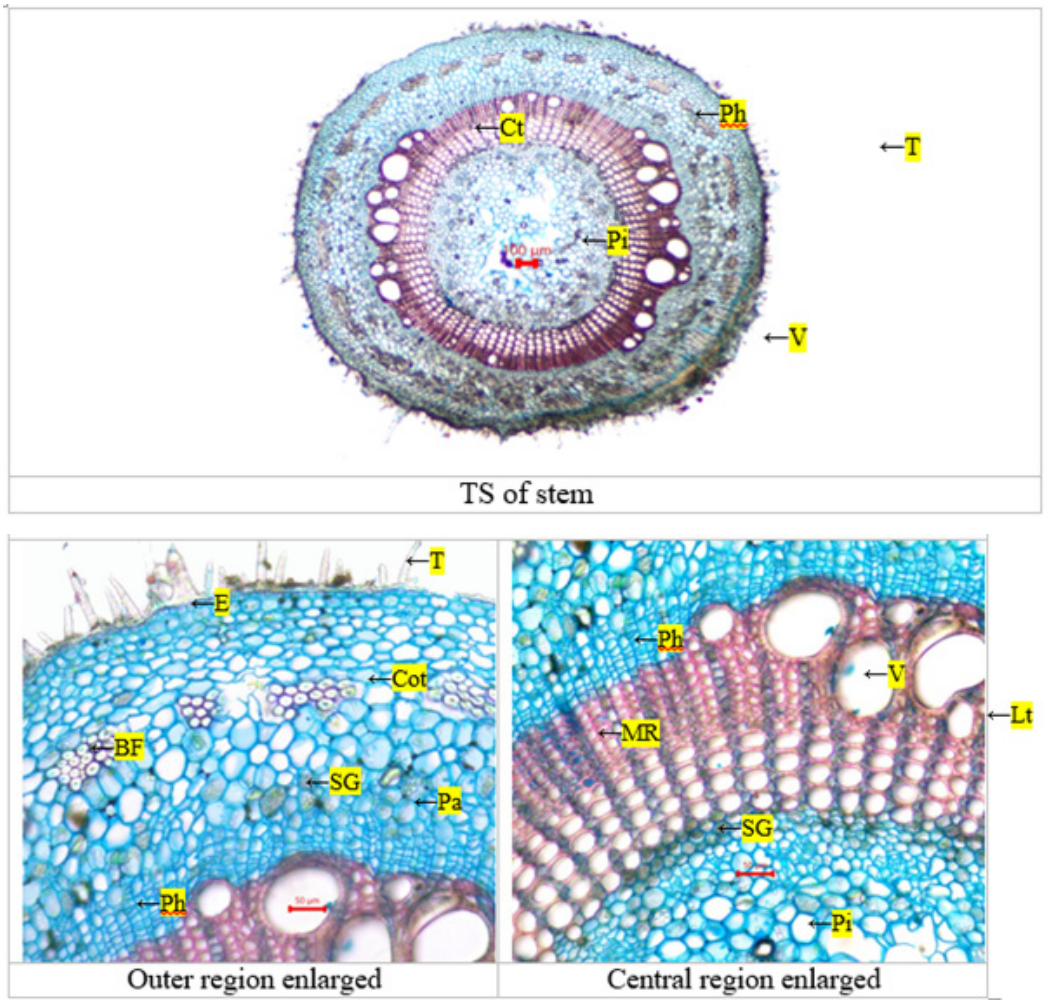


Figure 3: TS of *Pergularia daemia* – stem. BF - bast fibres; Cot - cortex; Cu - cuticle; E - epidermis; Lt - latex; MR - medullar ray; Pa - parenchyma; Ph - phloem; Pi - pith; SG - starch grain; V - vessel; Xy – xylem.

Table 1: Comparative Phytochemical Constituents Table.

Phytochemical Class	*P. daemia*	Comparator Plant 1	Comparator Plant 2	Significance
Alkaloids	Present	Trace	Moderate	Potential medicinal activity
Flavonoids	Moderate	Low	High	Antioxidant potential
Steroids	Present	Low	Moderate	Pharmacological importance
Terpenoids	Present	High	Low	Therapeutic diversity
Cardenolides	Present	Moderate	Low	Cardiotonic properties

Flower

Peduncle

The transverse section of the peduncle exhibits an oval outline. The epidermis is uniseriate, composed of small, thin-walled parenchymatous cells covered by a prominent cuticle. A high density of uni- to multicellular trichomes is recorded. Beneath the epidermis, a two-layered collenchymatous hypodermis is observed. The cortex consists of 8-10 compact layers of parenchymatous cells. The vascular system forms a continuous ring, with phloem encircling the xylem. The xylem is endarch, with protoxylem directed towards the center and metaxylem towards the periphery. A parenchymatous pith occupies the central region (Figure 5).

Sepal

In transverse view, the sepal is crescent-shaped. The outer epidermis is composed of compact, bulbous cells, while the lower epidermis bears abundant trichomes. The mesophyll comprises 6-8 layers of rounded cells. Four to five collateral vascular bundles are distributed in the upper mesophyll region. These bundles are relatively uniform in size (Figures 6a-6c).

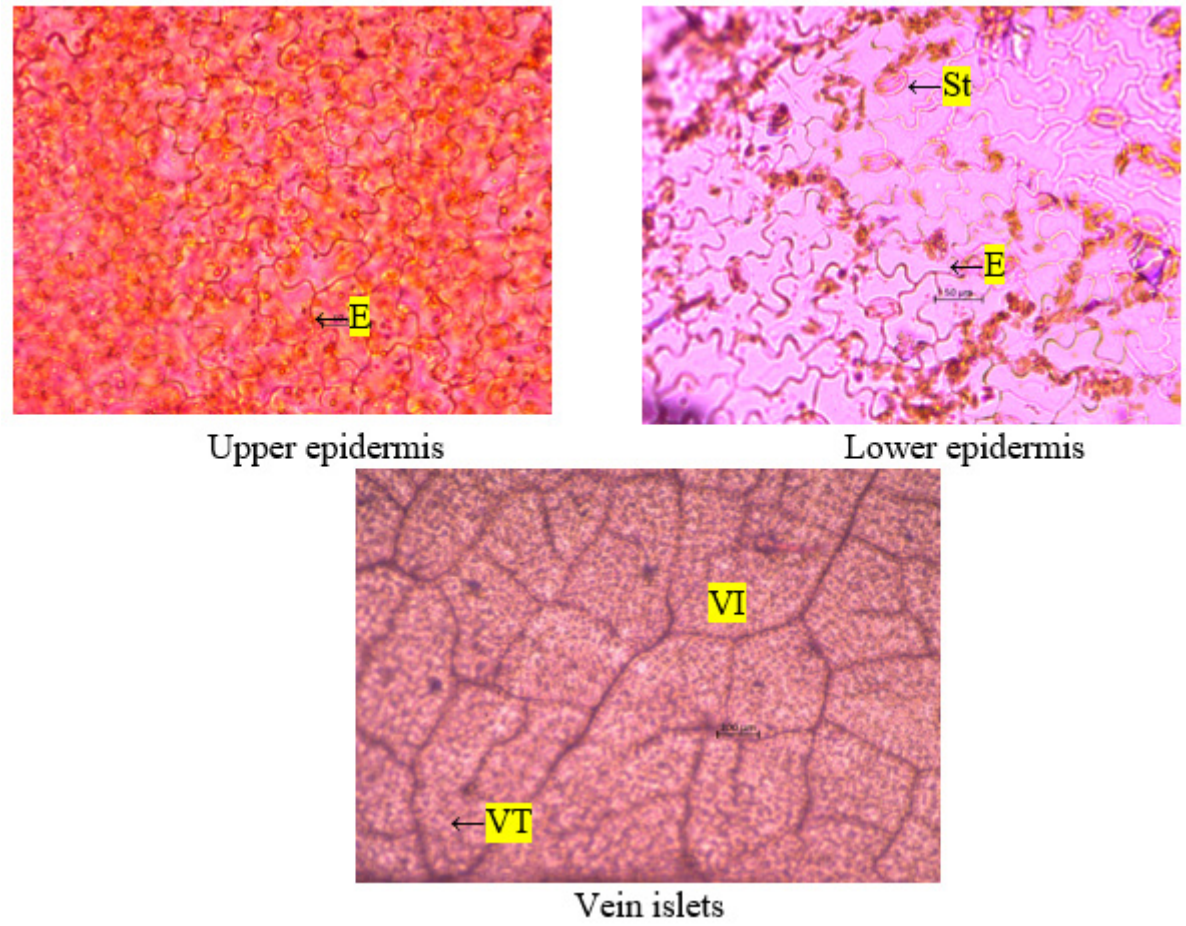


Figure 4: Quantitative microscopy of *Pergularia daemia* – leaf. E - epidermis; St - stomata; VI - vein islet; VT - vein termination.

Table 2: Quantitative microscopy of *Pergularia daemia* leaf.

Parameters	Upper epidermis (/ mm²)	Lower epidermis (/ mm²)
Epidermal number	110 - 120	120 - 130
Stomatal number	-	40 - 50
Stomatal index	-	25 - 28
Palisade ratio	14 - 16	
Vein islet	14 - 18	
Vein termination	08 - 10	

Petal

The transverse section of the petal resembles that of the sepal in shape. The outer epidermis is formed of small, closely packed bulbous cells. Both unicellular and multicellular covering trichomes, along with occasional glandular trichomes, are present on the upper epidermis. The mesophyll region is comparatively loose, consisting of 6-8 layers of thin-walled parenchyma. Two to four collateral vascular bundles are embedded in the mesophyll, smaller in size than those of the sepal (Figures 6d-6f).

Gynostegium

The transverse section of the gynostegium reveals a double corona arrangement. The outer corona forms a ring with five interstaminal lobes, originating at or just above the mouth of the corolla tube. The inner corona consists of five staminal lobes that are adnate to the staminal column approximately halfway along the anther wings. These lobes are semi-sagittate in shape, with a free, tapering apex arching over the column head, and a short basal projection.

The pollinaria are well developed, comprising pendant, subsessile pollinia with translucent margins. The ovary is apocarpous and bicarpellary, with two superior ovaries exhibiting marginal placentation. The ovary wall is comparatively thick, consisting of 4-5 concentric layers of parenchymatous cells. The style and stigma are fused into a prominent stigma head, which is organized into an outer epidermis, a compact parenchymatous ground tissue, and a central vascular bundle (Figure 7).

Quantitative Microscopic results of Fruit

Pericarp

The transverse section of the mature fruit is oval in outline, with distinct surface ridges. The pericarp is differentiated into

three regions: epicarp, mesocarp, and endocarp. The outermost epidermis is uniseriate, composed of compactly arranged parenchymatous cells covered by a well-defined cuticle. Numerous covering trichomes are present. This zone is stratified into two regions. The outer mesocarp consists of one to two layers of compact parenchyma cells interspersed with groups of sclereids. The inner mesocarp is made up of comparatively larger, thin-walled parenchymatous cells. Several conjoint, collateral vascular bundles traverse the mesocarp. A well-developed band of sclerenchymatous fibres, 5-7 layers thick, is clearly distinguishable beneath the mesocarp (Rabe *et al.*, 1997). An elongated placenta arises from one end of the pericarp, extending towards the locular cavity (Figure 8).

Quantitative Microscopic results of Seed

The transverse section of the seed appears broadly V-shaped with distinctly flattened arms. Testa, the outer epidermis consists of compactly arranged parenchymatous cells, externally bearing abundant trichomes. Beneath this, a single layer of columnar cells is present, with cell length progressively increasing toward the arms. The mesotesta comprises small, rounded parenchymatous cells. The endotesta is reduced to 3-4 crushed layers of parenchyma, with the outermost stratum rich in prismatic crystals. A distinct pigment layer occurs just below, contributing to the seed coat coloration. The endosperm is elongated and closely adheres to the central cotyledons (Barceló *et al.*, 2001). Cotyledons occupy the core of the seed and are surrounded by the nutritive endosperm tissue (Figure 9).

Powder Microscopy

Whole Plant

The powder is dull green with a characteristic odor and taste. Microscopic examination revealed the presence of cork cells, multicellular trichomes, parenchyma cells, epidermis with paracytic stomata, mesophyll cells, thin-walled fibers, thick-walled fiber bundles, pitted and spiral vessels, latex content, and numerous rosette crystals (Figure 10).

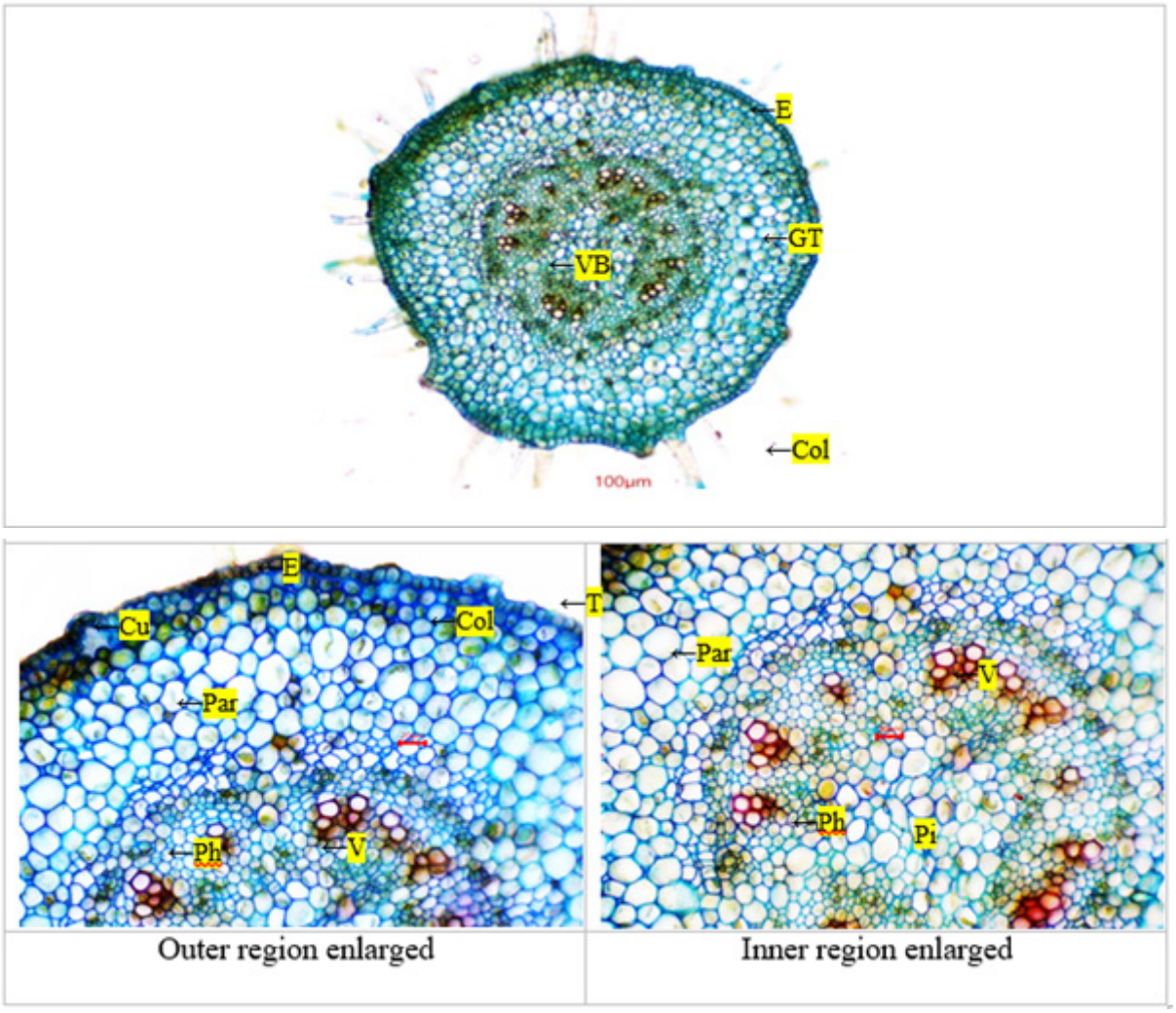


Figure 5: TS of *Pergularia daemia* peduncle. Col - collenchyma; Cu - cuticle; E - epidermis; GT - ground tissue;Pa - parenchyma; Ph - phloem; Pi -pith; T - trichome; V - vessel; VB - vascular bundle.

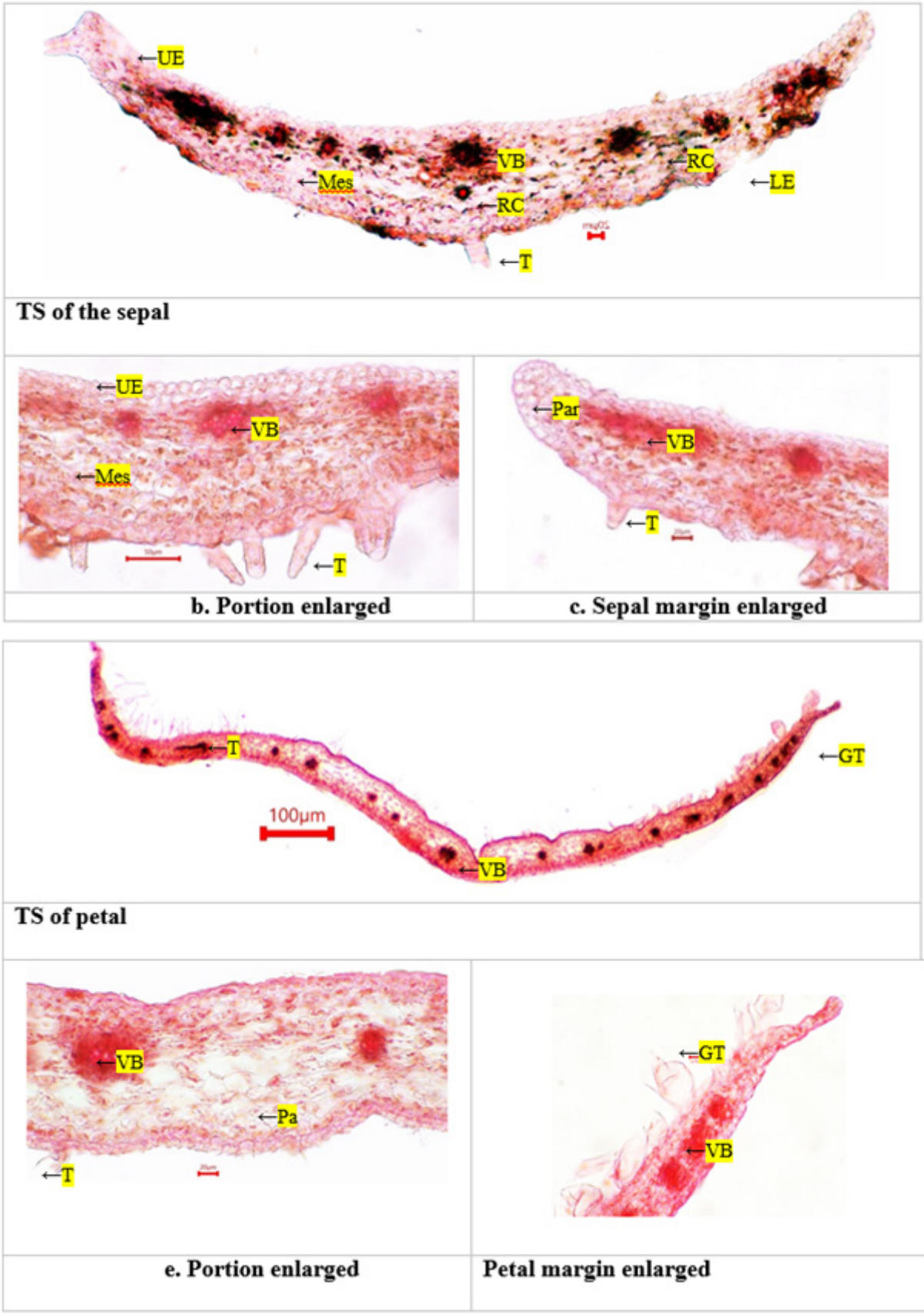


Figure 6: TS of *Pergularia daemia* sepal and petal. Col - collenchyma; Cu - cuticle; E - epidermis; GT - ground tissue; Pa - parenchyma; Ph - phloem; Pi -pith; T - trichome; V - vessel; VB - vascular bundle.

Flower Powder

The powdered drug was light brown in color, possessing a distinct characteristic odor and bitter taste. Microscopic observation revealed the presence of Long, unicellular, warty trichomes, Fragments of calyx and corolla epidermis, Parenchymatous cell clusters, Isolated xylem vessels, and Numerous pollen grains with well-preserved exine structure (Figure 11).

Fruit Powder

The fruit powder also appeared light brown, with a characteristic odor and slightly bitter taste. Microscopic examination showed Fragments of the pericarp bearing abundant trichome, Mesocarpic parenchyma cells, Pieces of cotyledonary tissue, Xylem vessels, and Numerous prismatic crystals of calcium oxalate. These elements collectively confirm the fruit identity and provide diagnostic characters for pharmacognostic evaluation (Figure 12).

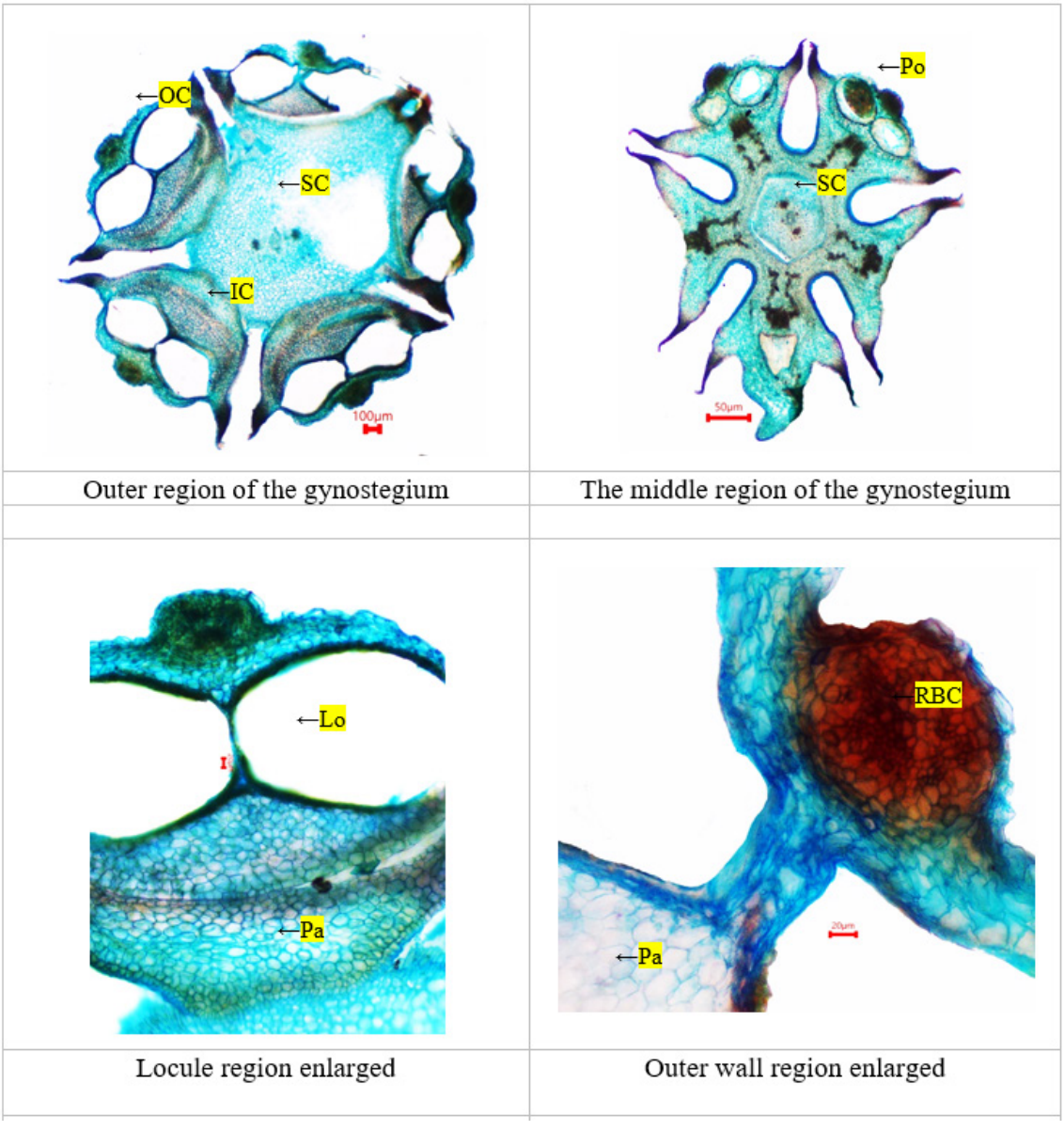
Histochemistry

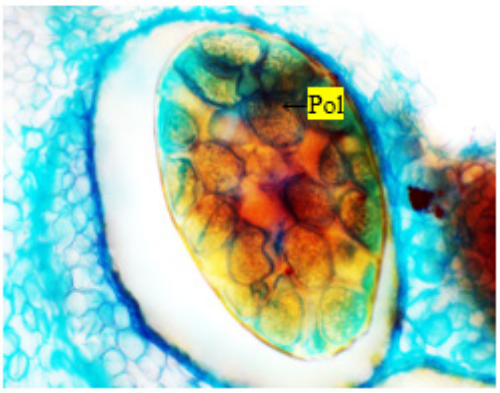
Whole Plant

Histochemical tests revealed the presence of cutin on the walls of the epidermis and trichomes, and phenolic depositions in the cortical region. Alkaloids were present in a few cells of the cortex. Lignin deposition was observed in the vascular region along the walls of xylem vessels. Abundant starch grains and a few oil droplets were present in the corticalregion. Mucilage depositions were observed at the base of trichomes (Figure 13).

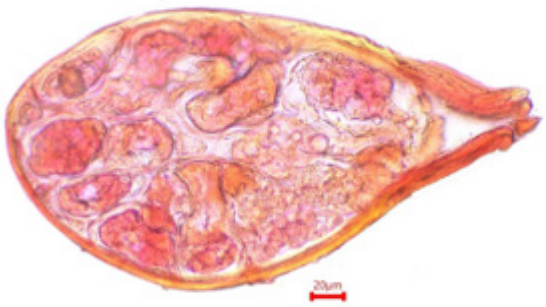
Histochemistry results of Flower

Histochemical screening of the flower and fruit tissues revealed the deposition of multiple classes of biomolecules (Figure 14). A continuous cutinized layer was observed on the epidermal cells,

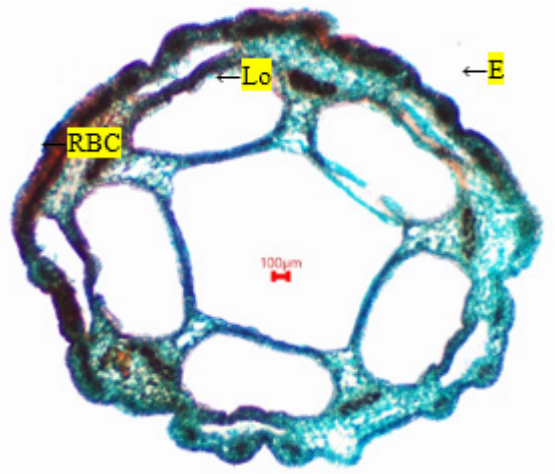




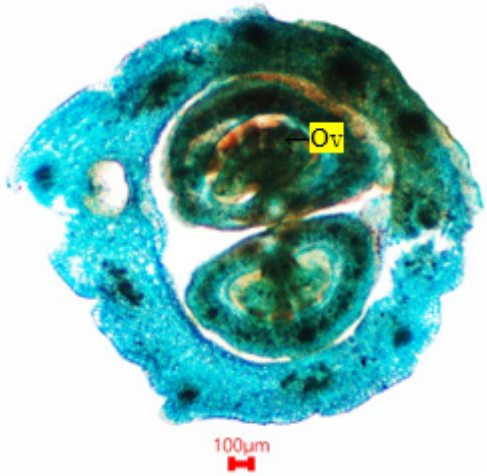
Developing pollinia



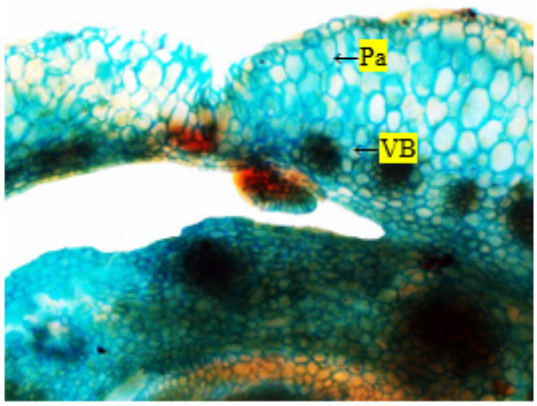
Pollinia



Lower region of the gynostegium



Middle part of the gynostegeium



Connecting walls of locules enlarged

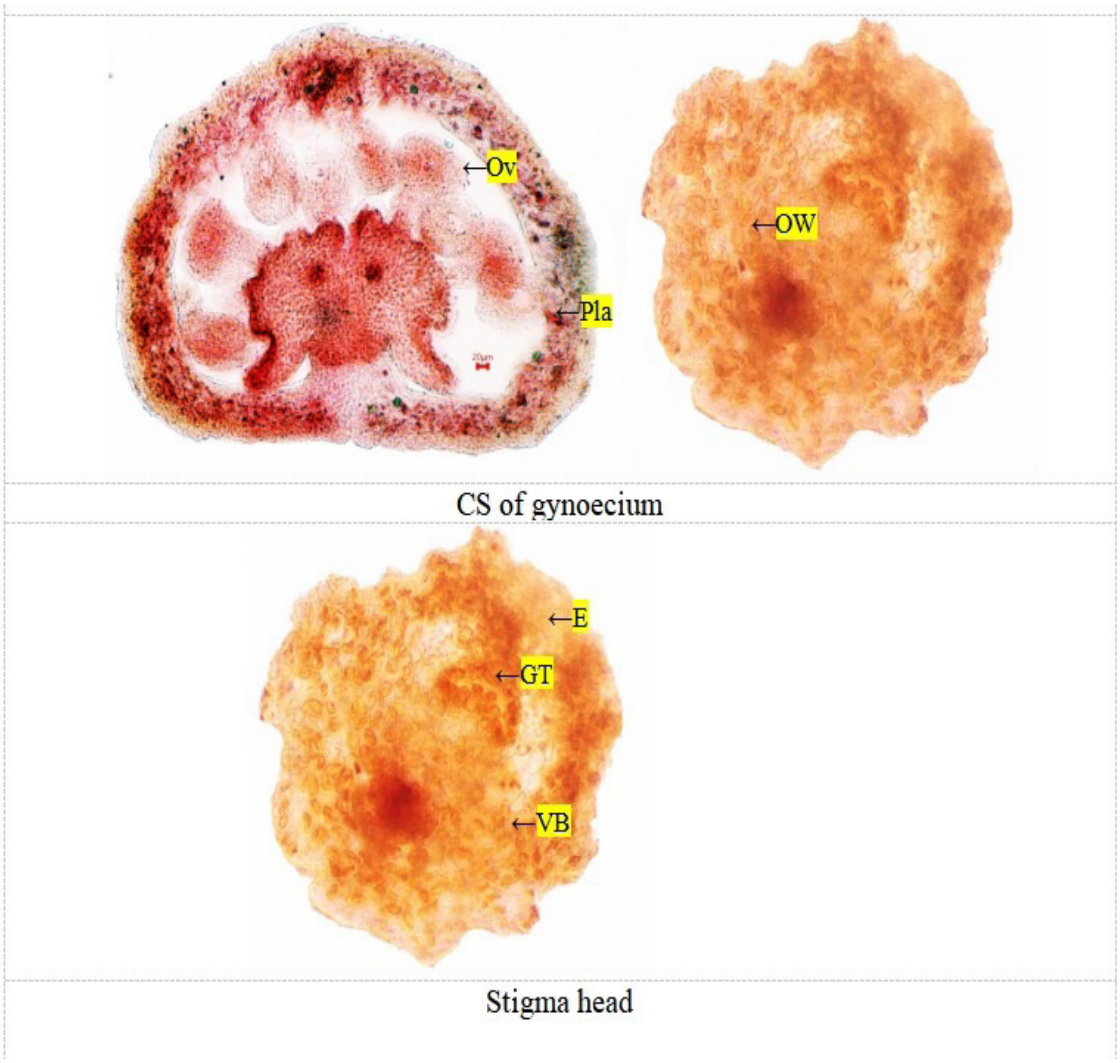


Figure 7: TS of *Pergularia daemia* gynostegium. GT -ground tissue; E - epidermis; IC - inner corona; Lo - locule; OC - outer corona; Ov - ovule; OW - ovary wall; Pa -parenchyma;Po - pollinia; RBC - reddish brown content; SC - staminal column; VB - vascular bundle.

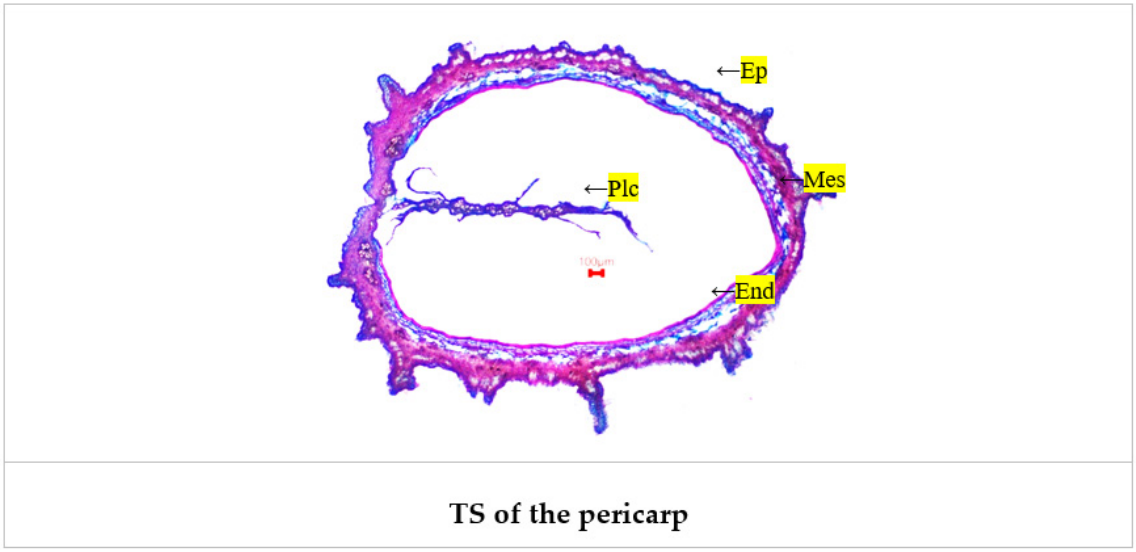
indicating protective adaptation. Thickness of cutinized walls measured. Distinct mucilage deposition was localized within trichomes. Ground tissue cells stained positively for alkaloids, with intensity strongest in the cortical zone. Strong lignin deposition was detected in the secondary walls of xylem vessels, confirmed by phloroglucinol-HCl reaction. Phenolic compounds were concentrated in parenchymatous ground tissue adjacent to the vascular bundles. Numerous starch grains were recorded in the ground tissue.

Histochemistry results of Fruit

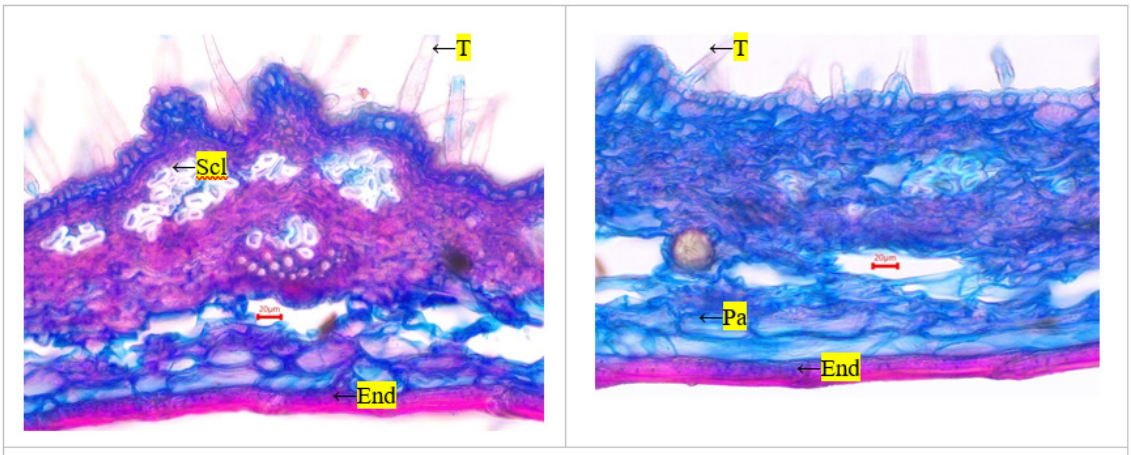
Histochemical staining of the fruit pericarp revealed distinct patterns of secondary metabolite deposition (Figure 15). Strong cutinization was evident in both the outer and inner epidermal cells. Mucilage accumulation was localized in the ground tissue cells adjacent to the vascular bundles. Resin droplets, alkaloid inclusions, and starch grains were scattered irregularly within the ground tissue. Intense lignin deposition was recorded in the innermost layer of the pericarp and in the walls of xylem elements, confirmed by phloroglucinol-HCl staining. Mesocarp cells exhibited marked phenolic deposition. These findings indicate that the pericarp functions as both a protective barrier (cutin, lignin) and a storage site for secondary metabolites (alkaloids, phenolics, starch), thereby contributing to fruit defense and seed development.

Histochemistry results of the seed

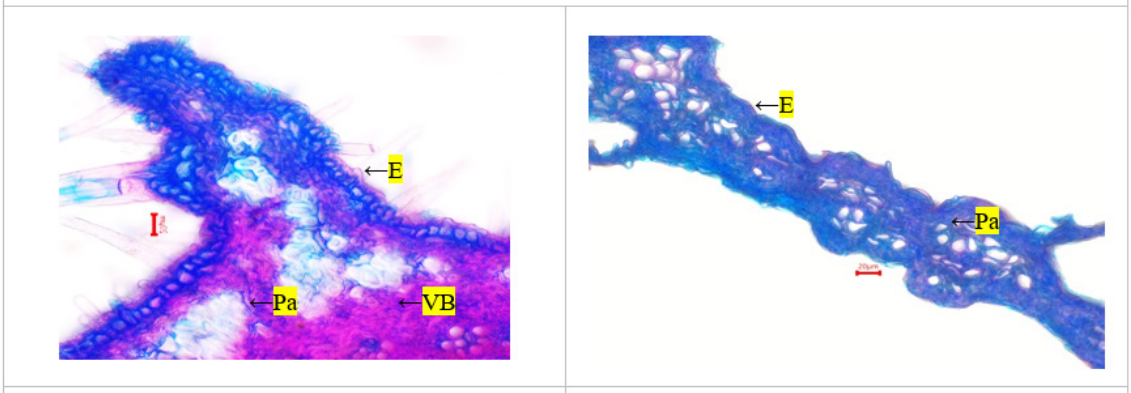
Histochemical analysis of the seed revealed distinct localization of metabolites and storage inclusions (Figure 16). A uniform layer of cutin was evident in the epidermal cells of the testa. Both phenolic compounds and alkaloids were strongly detected in the epidermal layer of the testa. Abundant oil-containing cells were observed in the endosperm region. Numerous prismatic crystals were concentrated in the outer cotyledonary region. These results demonstrate that the seed serves as a major storage site for lipids,



TS of the pericarp



Portion enlarged



Ridge portion enlarged | Placenta

Figure 8: TS of *Pergularia daemia* Pericarp. E - epidermis; Ep - epicarp; End-endocarp;Mes - mesocarp; Pa - parenchyma; PCr - prismatic crystal; Plc - placenta; Scl - sclereids; T -trichome; Te - testa; VB - vascular bundle.

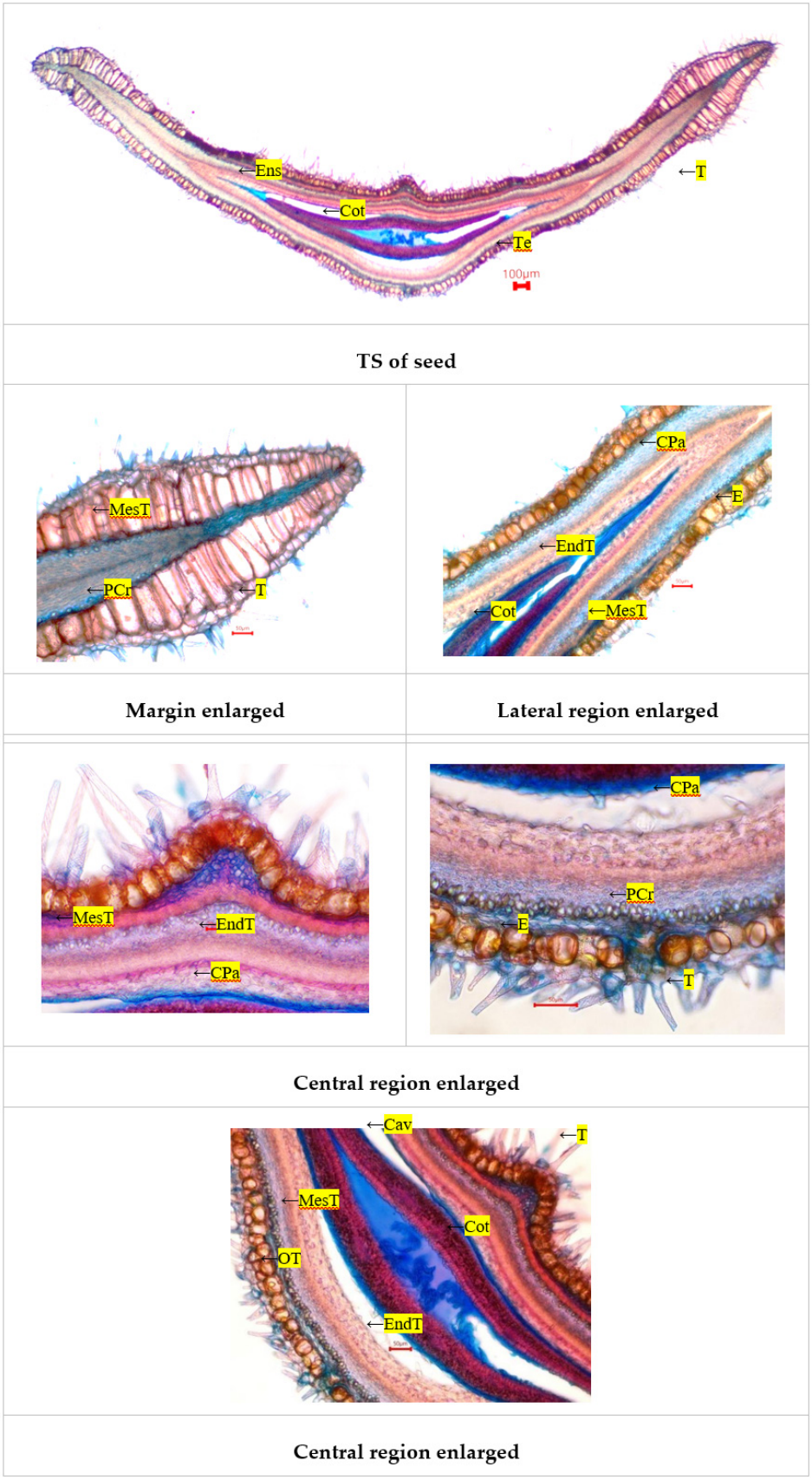


Figure 9: TS of *Pergularia daemia* seed. Cav - cavity; Cot - cotyledon; CPa- crushed parenchyma; E - epidermis; EndT-endotesta; Ens - endosperm; Mest- mesotesta;OT - outer testa; PCr - prismatic crystal; T -trichome; Te - testa; VB - vascular bundle.

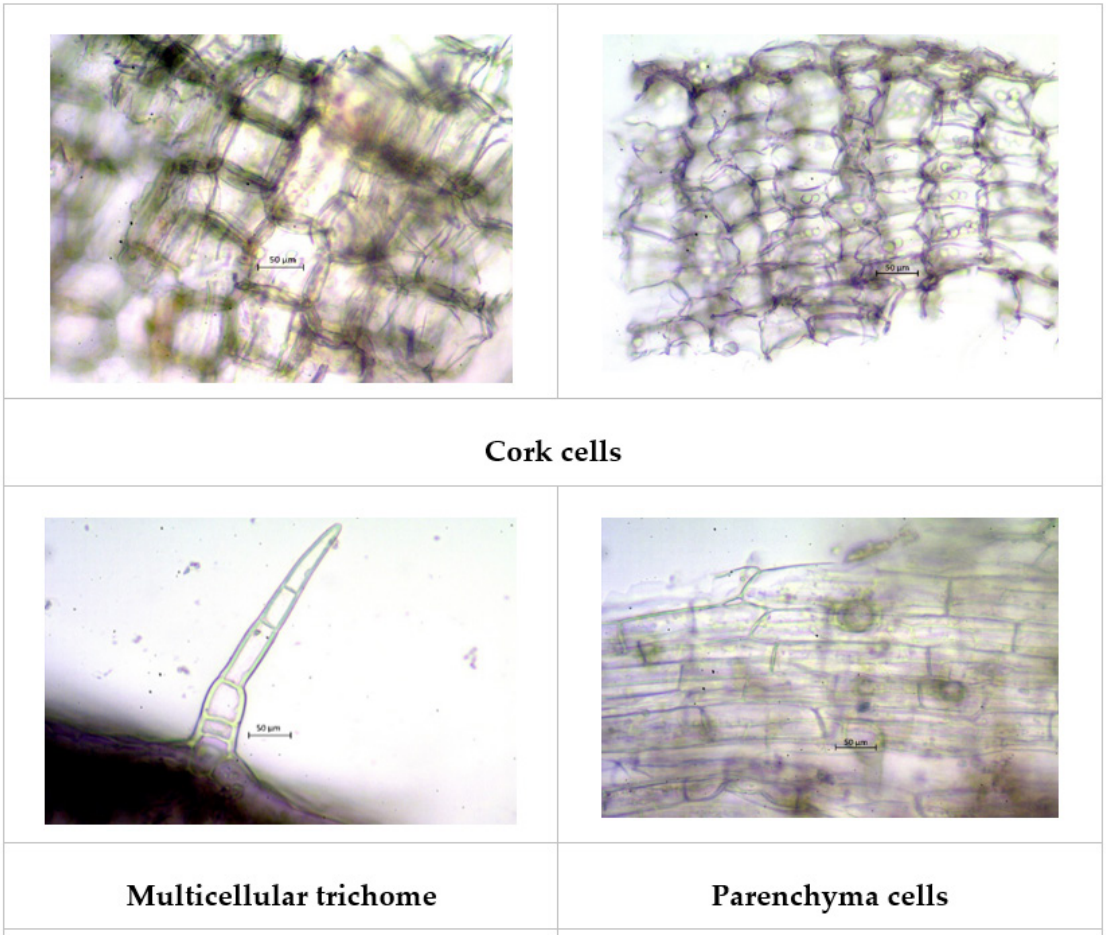
phenolics, alkaloids, and crystals, which collectively support defensive, nutritive, and protective roles.

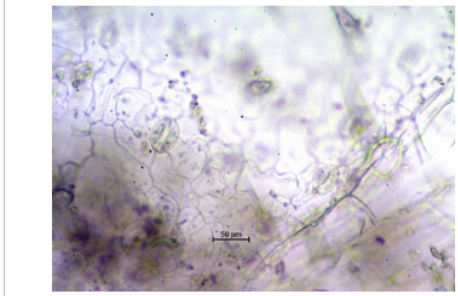
DNA Barcoding

Genomic DNA was successfully isolated from the authenticated sample with a concentration of 127.4 ng/μL and $A_{260}/_{280}$ ratio of 2.13, indicating good quality. PCR amplification using the rbcL marker yielded a clear band on agarose gel electrophoresis. The sequences obtained were submitted to GenBank with accession numbers PQ740391 (SCRIPCOGPD54) and PQ740392 (SCRIPCOGPD55). The barcode was successfully generated using BioRad barcode generator software.

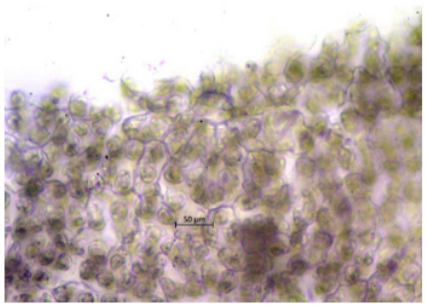
DISCUSSION

This study provides a comprehensive Pharmacognostical standardization and DNA barcoding of *Pergularia daemia* (Forssk.) Chiov., contributing valuable data for its authentication and quality control. The macroscopic and microscopic characteristics identified in this study serve as diagnostic tools for the identification of this medicinal Plant. Microscopically, the plant displays hallmark features of Apocynaceae, such as rosette calcium oxalate crystals, multicellular uniseriate trichomes (150-350 μm long, with prominent basal cells), abundant latex ducts, paracytic stomata, and well-defined vascular bundles (Nikam *et al.*, 2012). These observations align seamlessly with familial diagnostic criteria reported in standard pharmacognostic literature. The microscopic features, including the presence of rosette crystals, multicellular trichomes, latex content, paracytic stomata, and distinct vascular structures, are consistent with the characteristics of the Apocynaceae family to which *P. daemia* belongs. The quantitative microscopy data, particularly the stomatal index (25-28) and palisade ratio (14-16), can serve as reliable parameters for quality control. The dorsiventrally flattended seed shown distinct testa, endosperm with embryo, thickness ranges from 850-1100 μm. The trichome 150-350 μm with basal cells, Mesocarp of seeds had 15-20 layers with 30-60 μm. The innerlayer of endocarp composed of 2-3 layers of parenchymatous layer with cell size 24-30 μm. The powder character shown polygonal thick walled uniserate trichome, which also authenfied the *Pergularia daemia* (Forssk.) It reveals the trichomes, mesocarp, parenchyma, cotyledon tissue, xylem, prismatic Ca-oxalate crystals collectively authenticating the Pharmacognostical parameters too. The histochemical studies revealed the presence of various phytochemical constituents, including alkaloids, phenolic compounds, mucilage, and oils, which correlate with the reported medicinal properties of *P. daemia*. A wide range of phytochemicals is revealed by histochemical

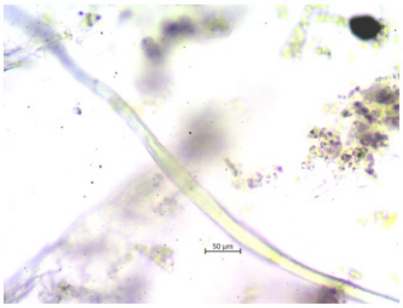




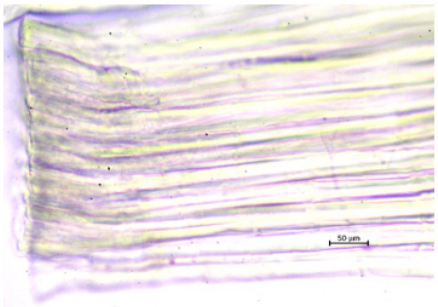
Epidermis with paracytic stomata



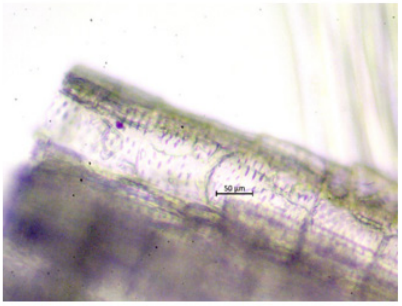
Mesophyll cells



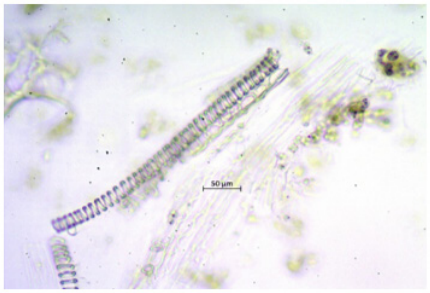
Fibre



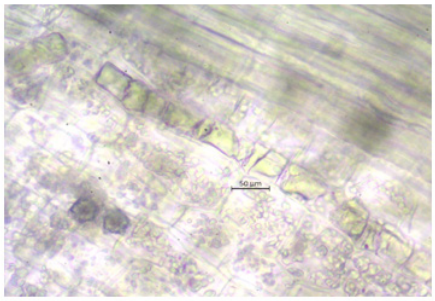
Fibre bundle



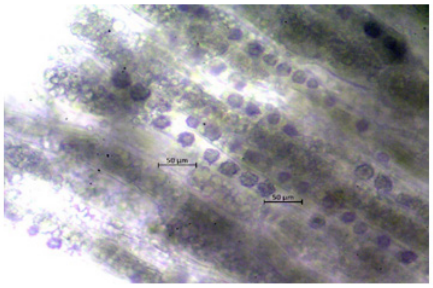
Pitted vessel



Spiral vessel

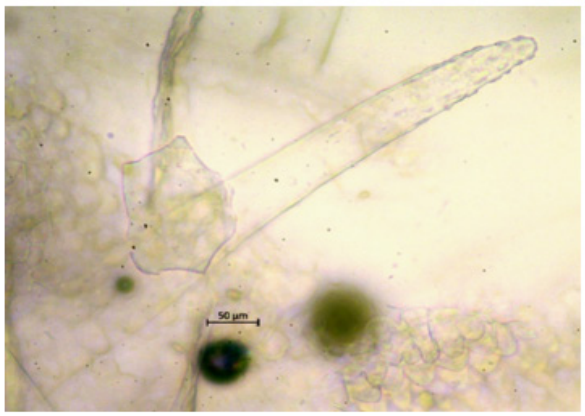


Latex content

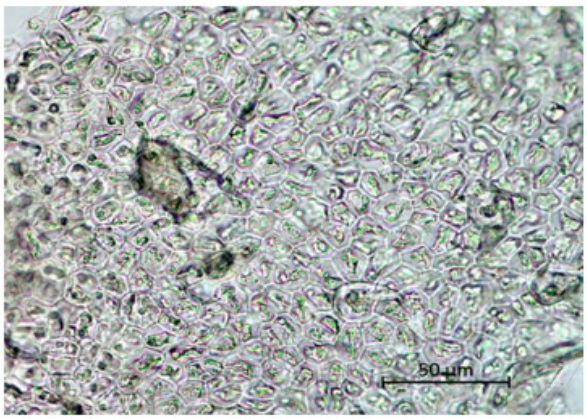


Rosette crystals

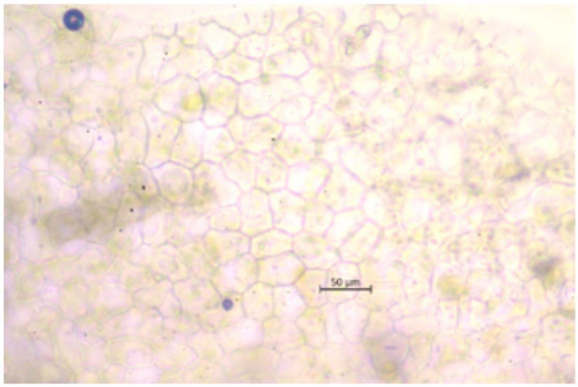
Figure 10: Powder microscopy *Pergularia daemia* - whole plant.



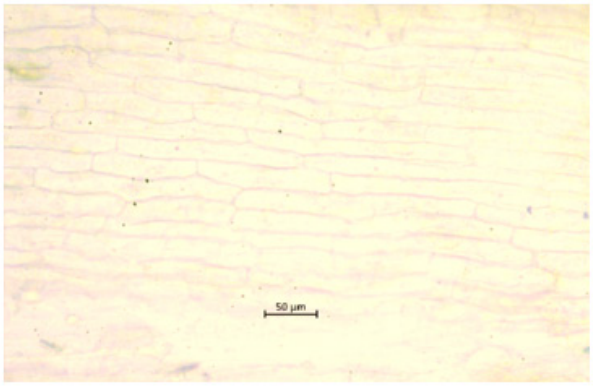
Trichome



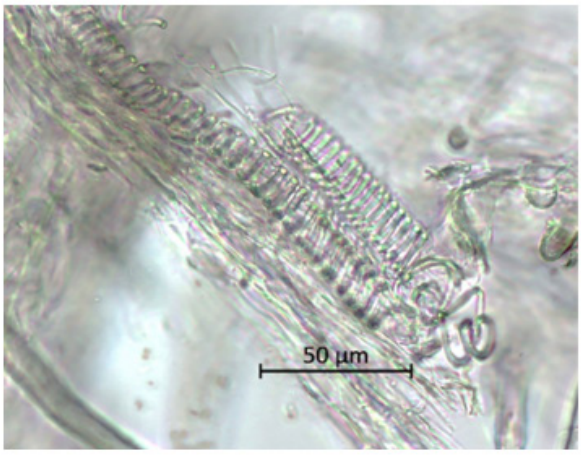
Peduncle epidermis



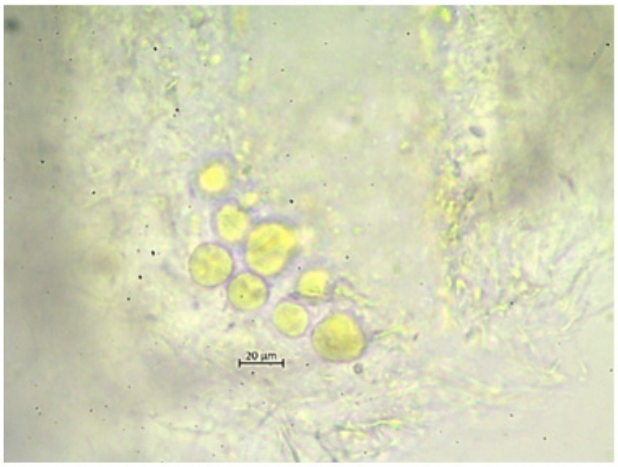
Corolla epidermis



Parenchyma cells

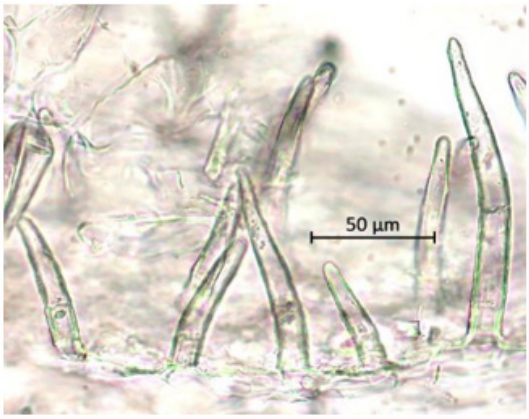


Vessel

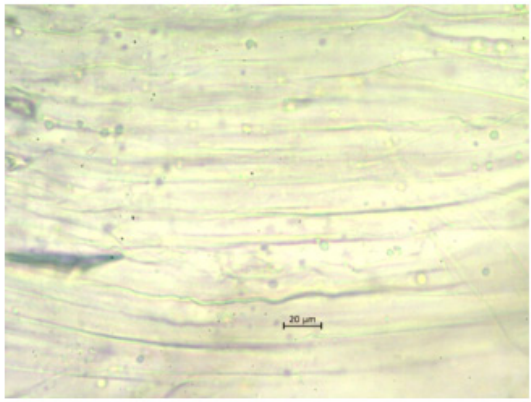


Pollen grains

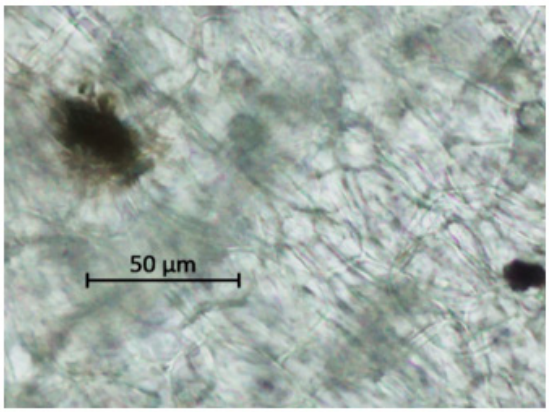
Figure 11: Powder microscopy of *Pergularia daemia* flower.



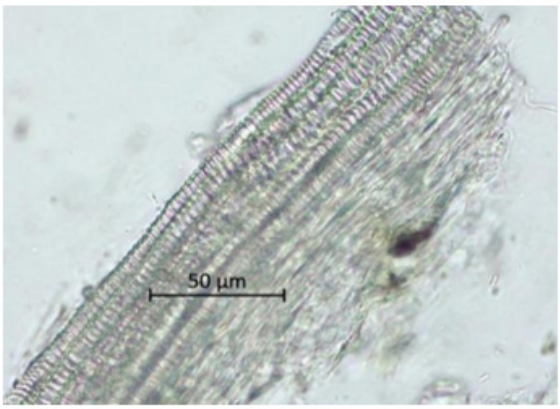
Pericarp with trichomes



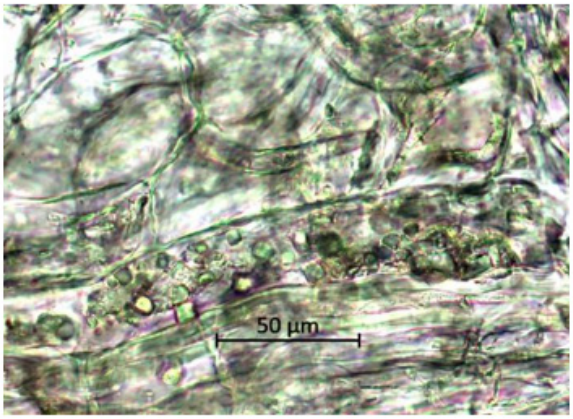
Parenchyma cells of mesocarp



Cotyledon cells

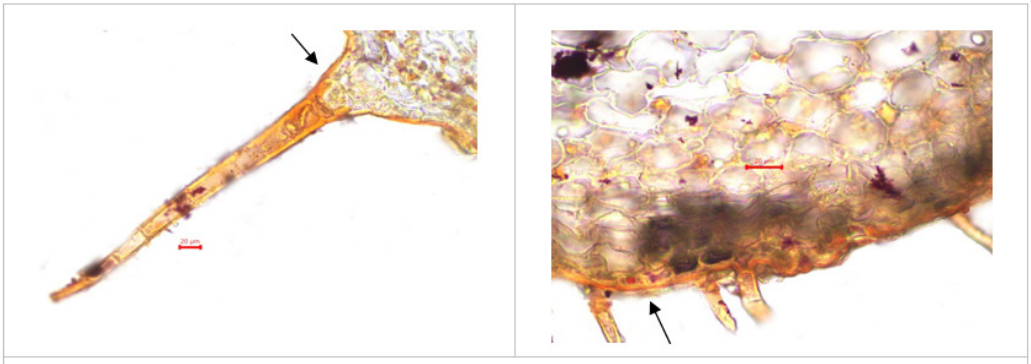


Spiral vessel

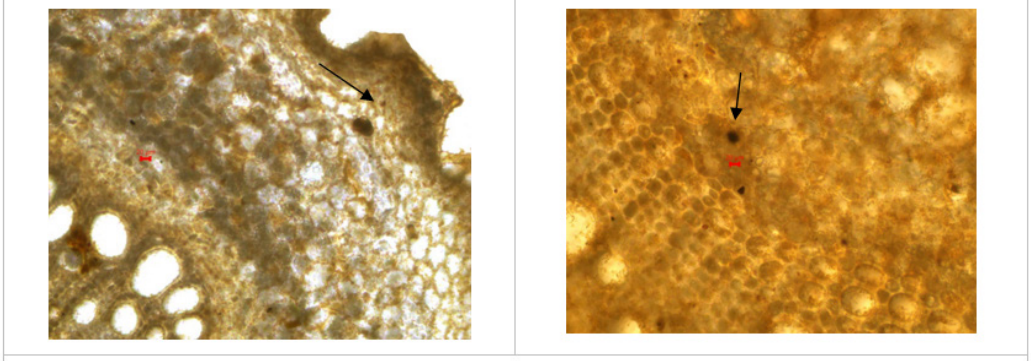


Prismatic crystal

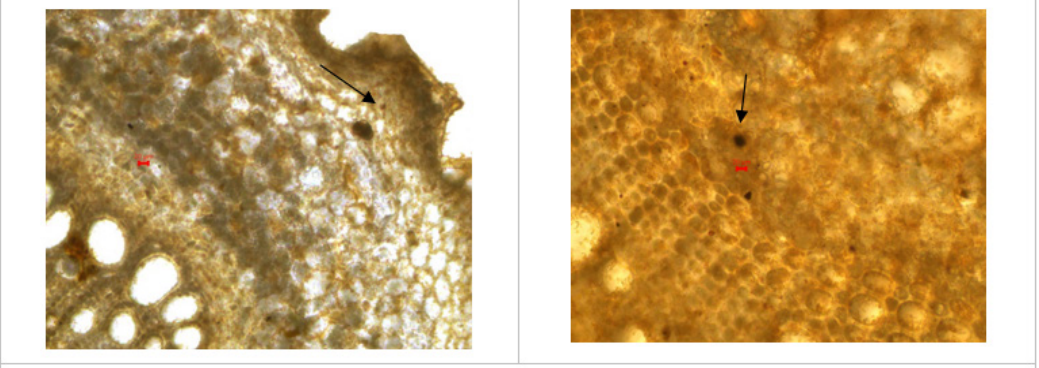
Figure 12: Powder microscopy of *Pergularia daemia* fruit.



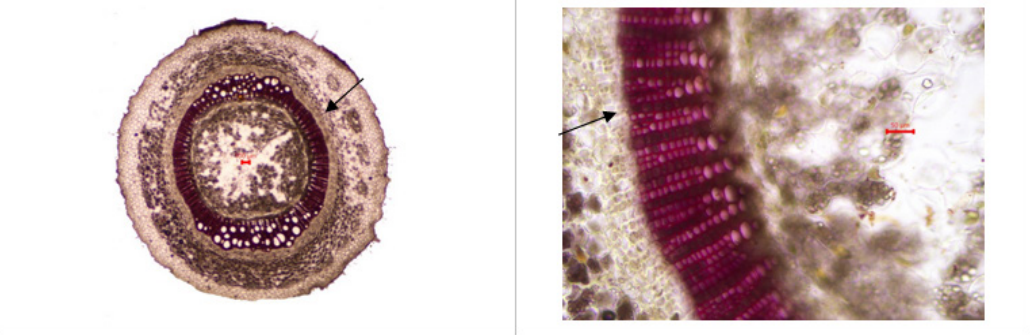
Cutin deposition



Phenolic compounds



Phenolic compounds



Lignin

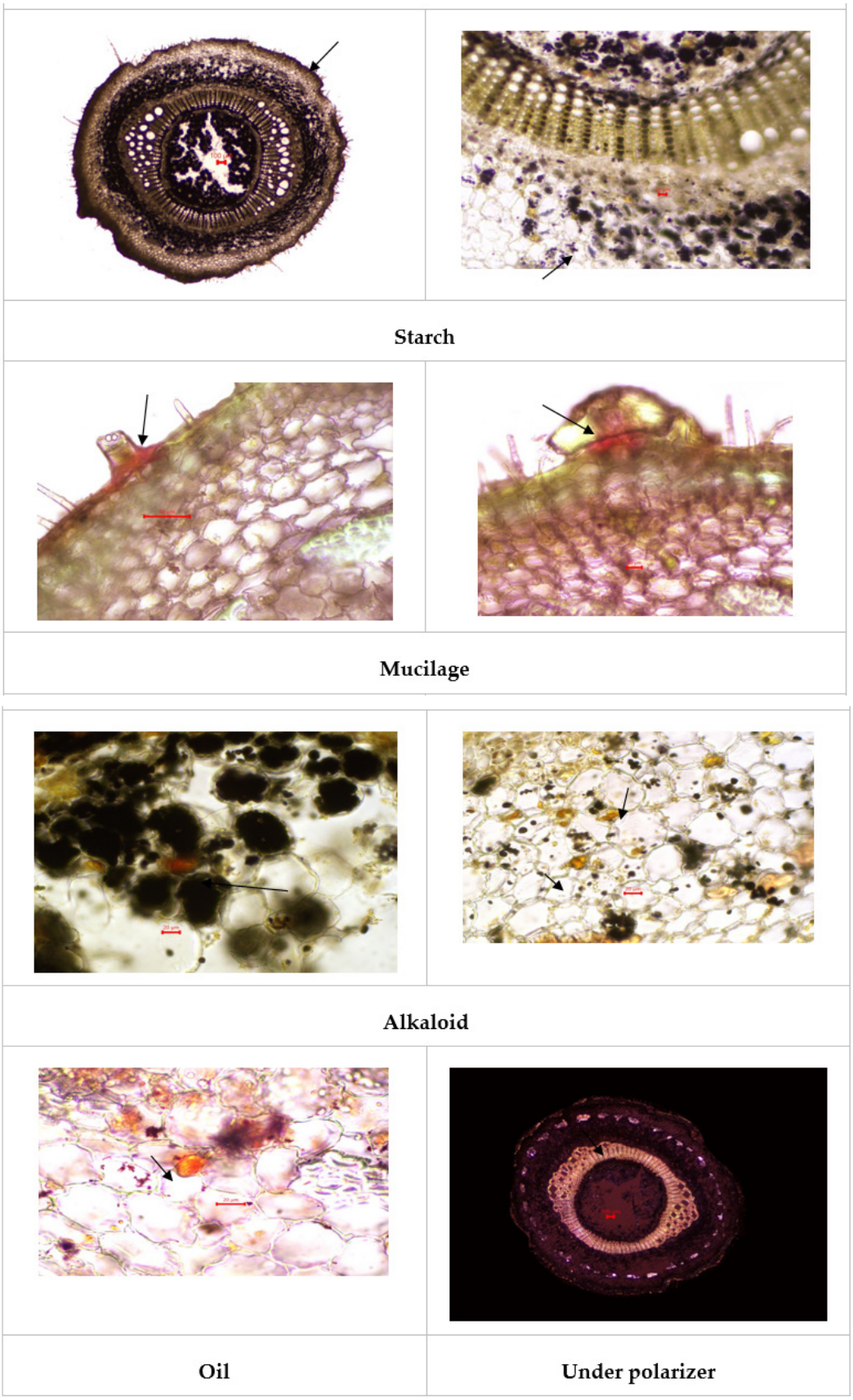


Figure 13: Histochemistry of *Pergularia daemia* – stem.

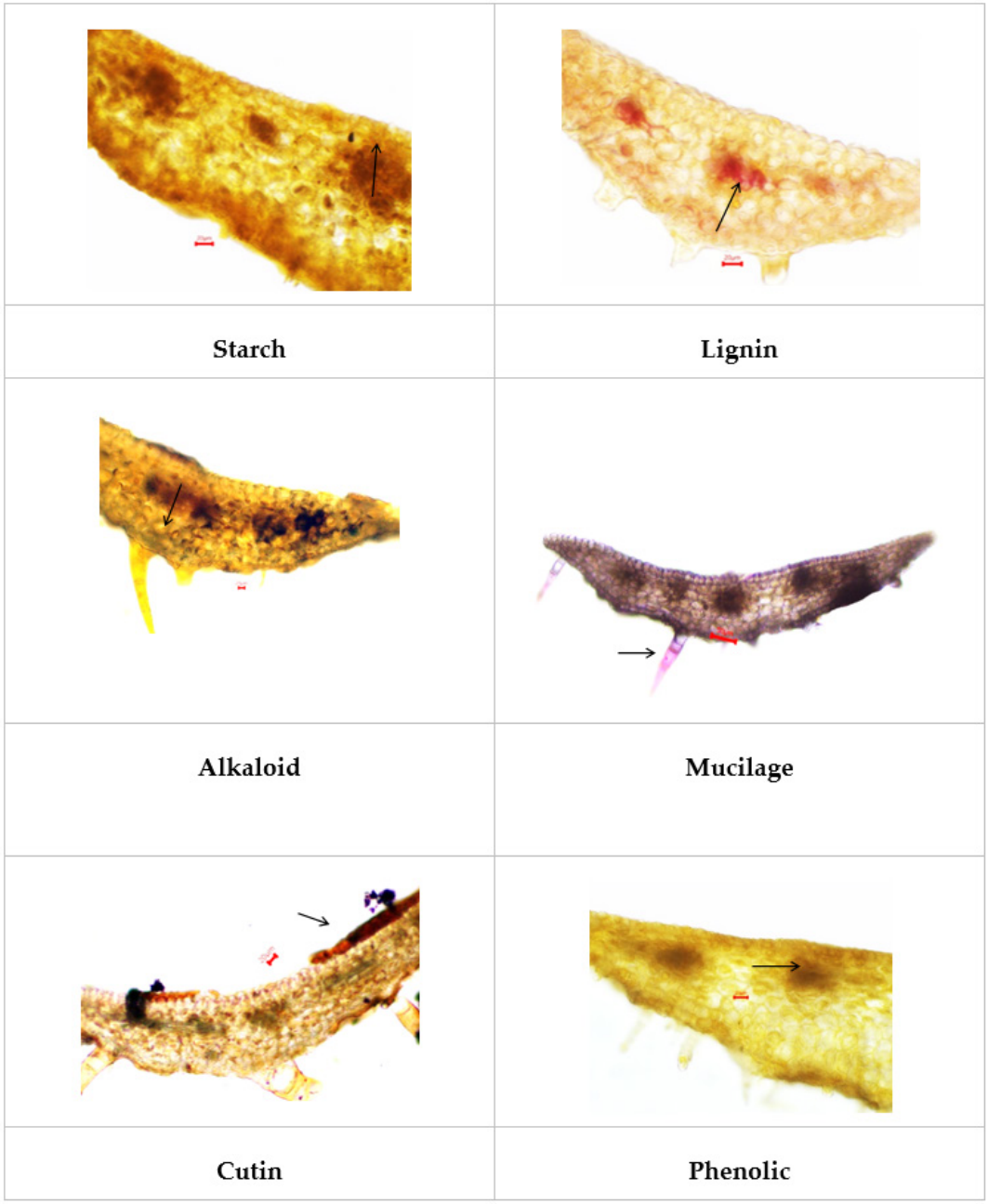
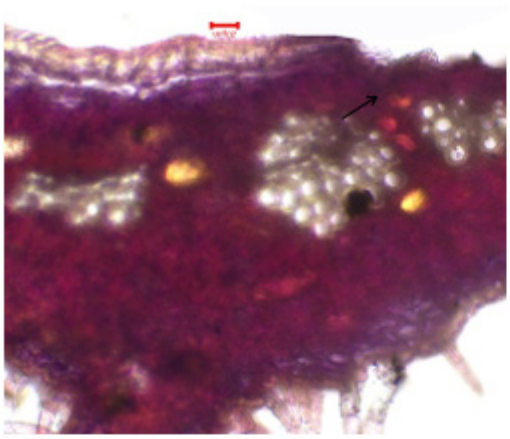
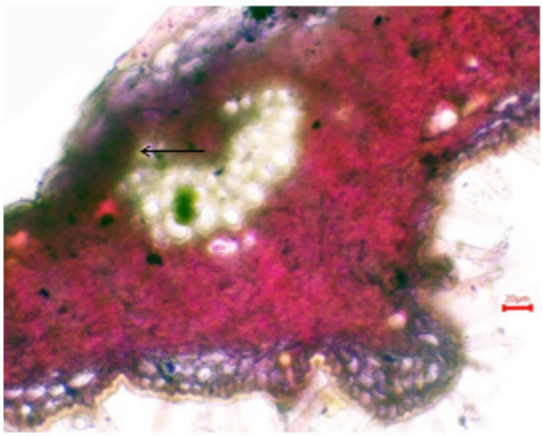


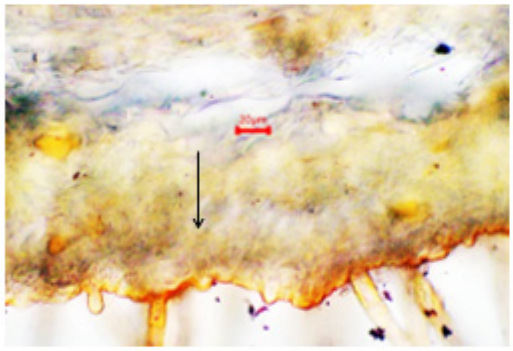
Figure 14: Histochemistry of *Pergularia daemia* flower.

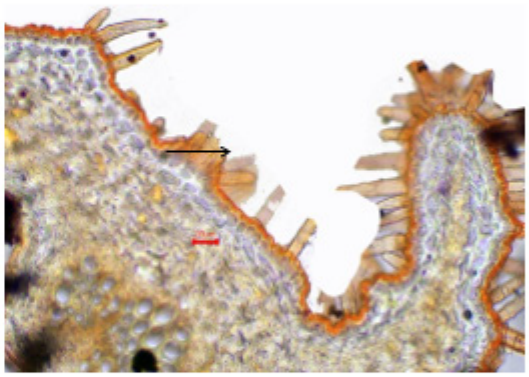
profiling, supporting*P. daemia's* potential as a treatment for conditions like inflammation, respiratory conditions, and reproductive health in conventional systems like Ayurveda. Mucilage is abundant in the epidermal and sub-epidermal layers, alkaloids stain strongly in secretory structures, phenolics and lignin support vascular tissues, starch builds up in parenchymatous cells for energy storage, and oils and resins predominate outer tissues along with cutin (Rabe *et al.*, 1997). The presence of these phytoconstituents supports its traditional use in various therapeutic applications (Harborne *et al.*, 2012). The phenolic compounds, lignin and starch prominately present in vascular tissue indicate reinforcement and observed as storage of parenchymatous cell. The histochemistry of mucilage is vast at epidermal and sub- epidermal, while resin concentrated at secretory structure. Comparatively cutin and resin dominate present at outer tissus. *Pergularia daemia* histochemical profiling (Forssk. Chiov. reveals spatiotopically coordinated phytoconstituents: cutin/resins engirdle peridermal tissues, mucilage predominates in epidermo-subepidermal mucocytes, starch accumulates in amyloplast-filled parenchyma, phenolics/ lignin strengthen vascular sclerenchyma, and alkaloids localize in laticifers. According to Ayurvedic Ethnopharmacognosy. Quantitative Histometry supports therapeutic pharmacophores: Mucilaginous reserves provide anti-inflammatory hydration, while resinous idioblasts support antimicrobial efficacy (Prakash *et al.*, 2015). The molecular identity also confirmed



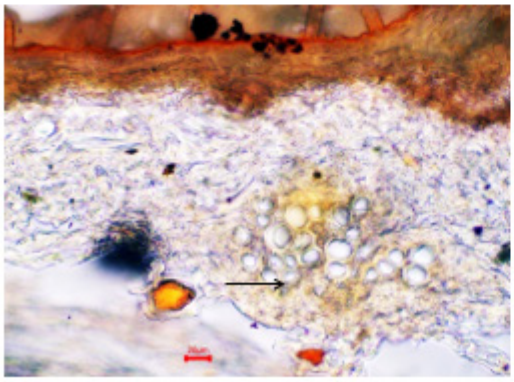


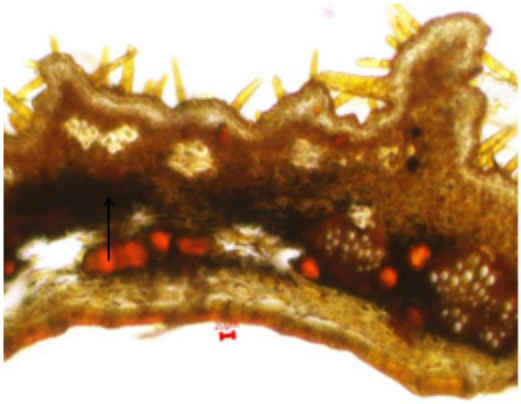
Mucilage





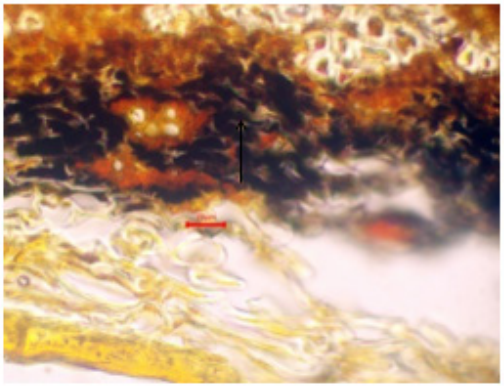
Cutin

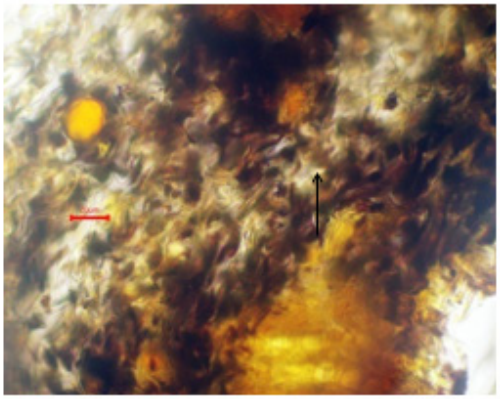




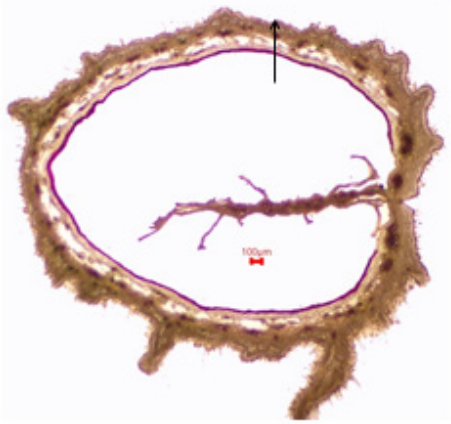
Resin

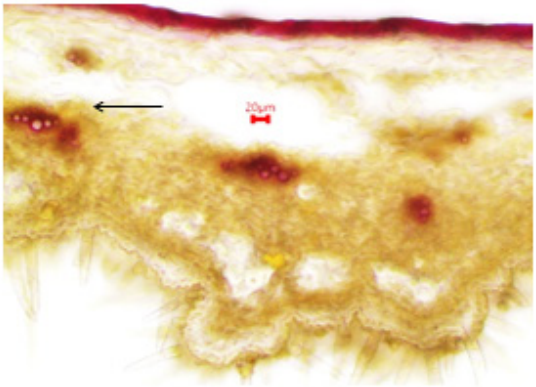
Alkaloid



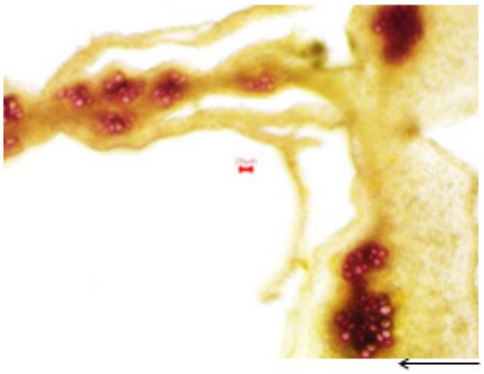


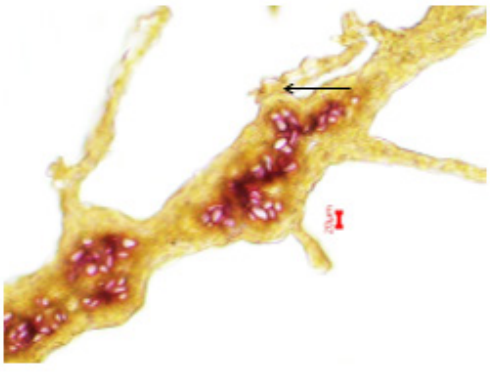
Starch





Lignin compound





Lignin compound

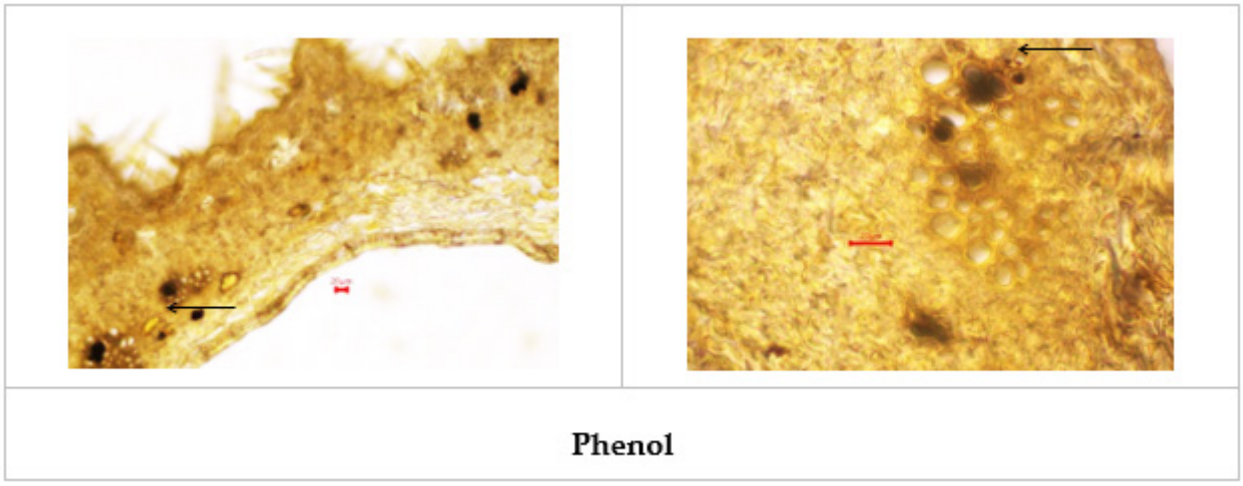
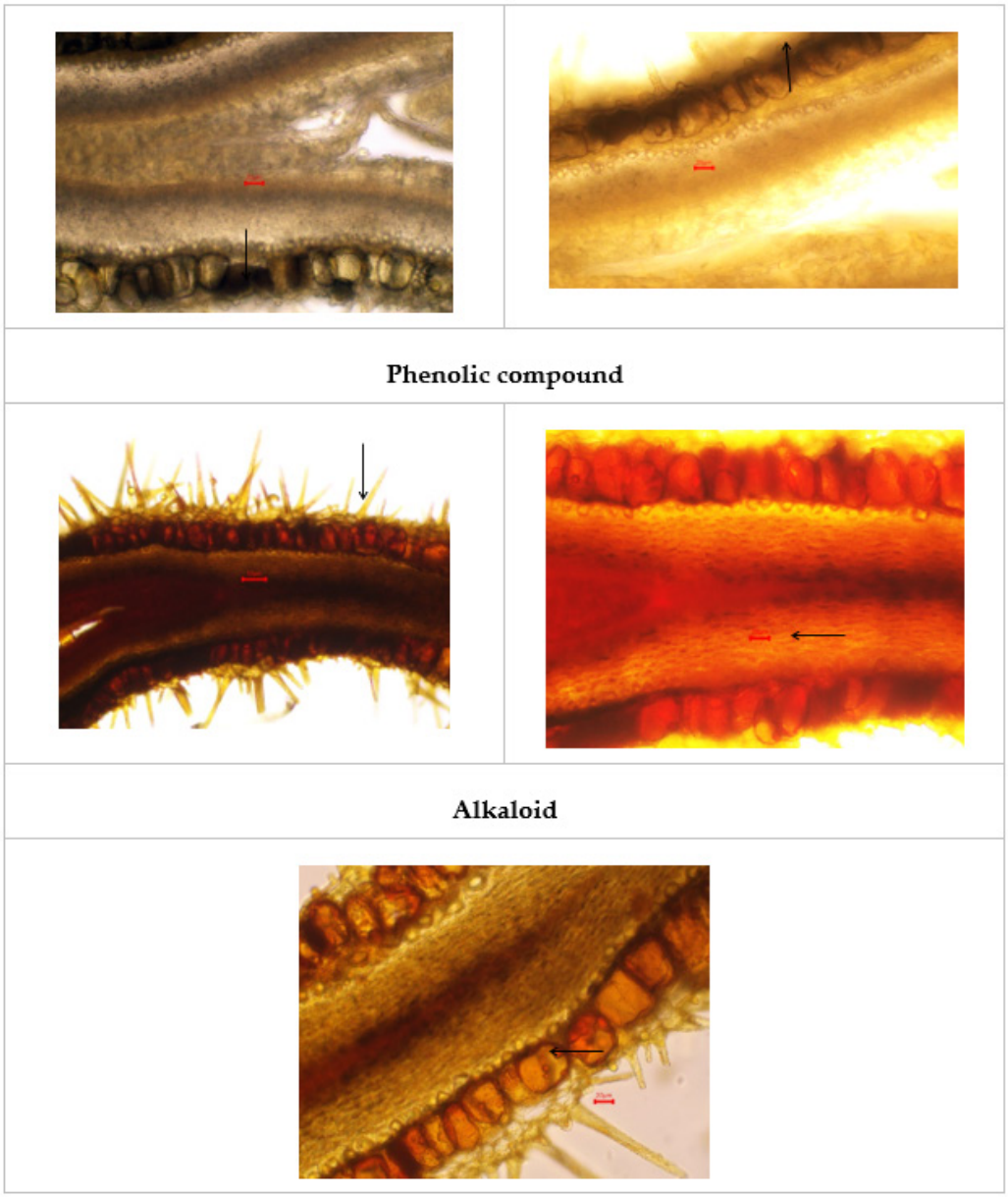


Figure 15: Histochemistry of *Pergularia daemia* pericarp.



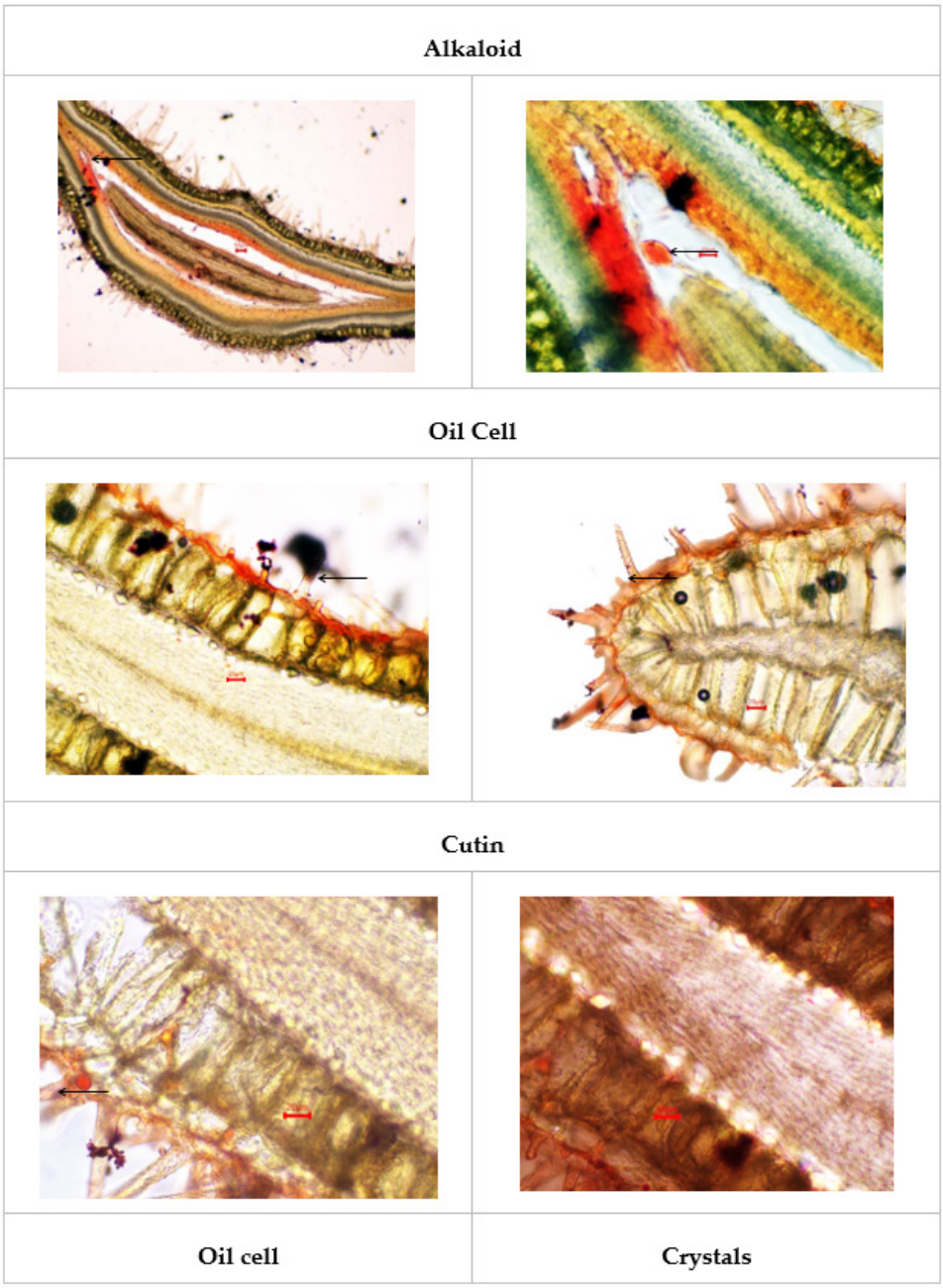


Figure 16: Histochemistry of *Pergularia daemia* seed.

by DNA barcoding using the rbcL chloroplast gene marker, which complements these traditional techniques. These results collectively establish *P. daemia* as a standard herbal supplement. DNA barcoding using the rbcL marker successfully established the molecular identity of *P. daemia*. The generated DNA barcode provides a reliable tool for authentication at the molecular level, complementing the traditional Pharmacognostical approach. This integrated approach enhances the reliability of the identification process, addressing the challenges of adulteration and substitution in herbal medicine. Here is the radar chart for the quantitative microscopic parameters of *Pergularia daemia* (Figure 17). The DNA barcoding of *Pergularia daemia* (Forssk) using rbcL and pharmacognostical standardization is a paradigm. The Chiov. provide clear authentication paradigms that outline diagnostic macromorphology, histoarchitecture (paracytic stomata; stomatal index 25-28, palisade ratio 14-16), and histochemical phytoconstituent cartography (alkaloids, phenolics, mucilage, resins) The rbcL amplicons exhibit 99-100% conspecificity with *Pergularia daemia* vouchers, supporting molecular authentication. They were deposited in GenBank under accessions PQ740391 (SCRIPCOGPD54) and PQ740392 (SCRIPCOGPD55). It confirms the *Pergularia daemia* identity with 99-100% homology, synergizing Pharmacognostical standardization against adulteration.

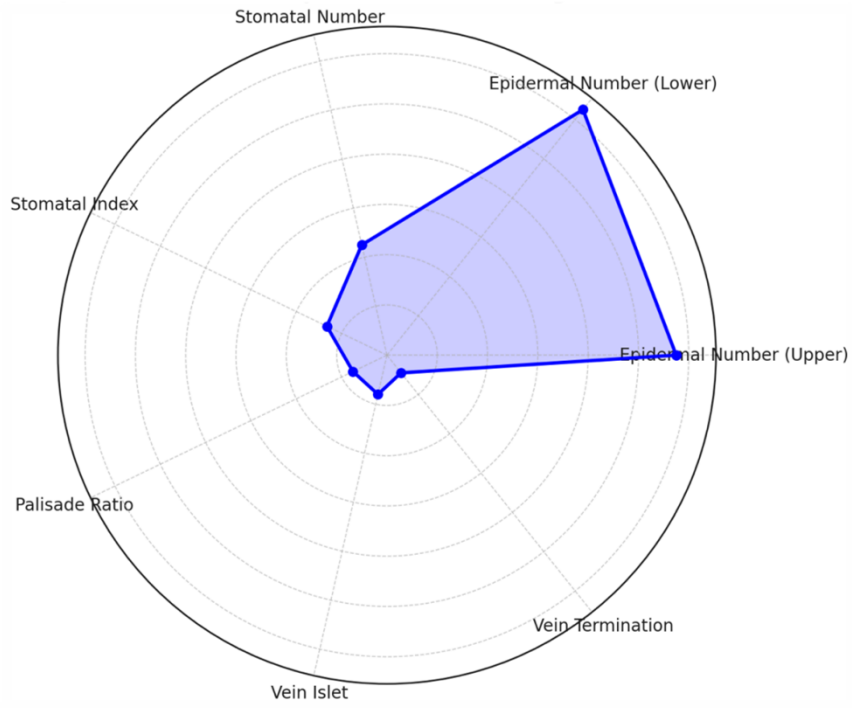


Figure 17: Quantitative microscopic parameters of *Pergularia daemia*.

CONCLUSION

This study establishes comprehensive Pharmacognostical standards and DNA barcode markers for *Pergularia daemia* (Forssk.) Chiov. The integrated approach of traditional Pharmacognostical methods and modern DNA barcoding provides robust parameters for the authentication of this medicinally important plant. These standardization parameters can serve as valuable tools for ensuring the quality and purity of *P. daemia* used in herbal formulations, contributing to the safety and efficacy of traditional medicine.

ACKNOWLEDGEMENT

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ABBREVIATIONS

CTAB: Cetyl Trimethyl Ammonium Bromide buffer; **Ultraviolet light:** Commonly 302-312 nm; **FASTA:** FAST-All in bioinformatics.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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