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Original Article

Phytotomy and Microchemistry of *Peperomia tetraphylla* G. (Forst.) Hook. & Arn.: A Lead to Accurate Identification

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ABSTRACT

Background: Our study aims to explore the anatomical characters of *Peperomia tetraphylla*, commonly called four-leaved peperomia and belonging to the family *Piperaceae*. **Objectives:** The investigation was undertaken to address the dearth of existing reports on the anatomical and histochemical aspects of *Peperomia tetraphylla*. **Materials and Methods:** Plant materials were collected, identified and preserved in Formalin-Aceto-Alcohol (FAA). The preserved specimens were sectioned using a rotatory microtome and stained with Toluidine blue, safranin and astra blue. Powder Microscopic studies and histochemical analysis were done using standard procedure. **Results:** *Peperomia tetraphylla* is a succulent herb with spike inflorescence of minute, perianthless flowers. The stem outline was undulate with cutinized single layered epidermis having glandular trichomes. Scattered collateral vascular bundles were present in the parenchymatous ground tissue. The section of the petiole shows a distinct epidermis with glandular trichomes. Vascular bundles are arranged in crescent shaped manner in ground tissue. Histochemical analysis shown the presence of alkaloids, tannins, starch, cutin, lignin, resin and crystals in various zone of leaf and stem of *Peperomia tetraphylla*. **Conclusion:** These characteristics are essential since it portrays the physical appearance of the plants which helps to regulate the genuineness of the drug.

Keywords: Histochemistry, Microscopic, *Peperomia tetraphylla*, Piperaceae, Powder microscopy.

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INTRODUCTION

Piperales signifies one of the major orders of Basal angiosperms with around 4300 species and includes the family Piperaceae. Piperaceae is characterized by five genera *Verhuellia, Zippelia, Manekia, Piper* and *Peperomia*, with all genera commonly distributed in tropical and sub-tropical regions.[1-4] *Peperomia* and *Piper* are the richest genera in this family with nearly 1500-1700 and 2000 species, respectively.[5,6] Piper commonly exists as herbs, shrubs, trees and lianas whereas Peperomia is predominantly parasites, epiphytes, succulents and geophytes.

Peperomia Ruiz and Pavon (*Piperaceae*) is generally distributed in tropical and subtropic areas with particularly high diversity in the Neotropics, Southern Asia, Africa, Australia, and New Zealand.[7,8] They are among the largest genera of palaeo herb angiosperms and one of numerous synonyms. There are 3249 plant name records and 1564 accepted species.[9,10]

Peperomia tetraphylla G. (Forst.) Hook. Arn. is a succulent tufted herb growing epiphytically on tree-trunks, widely distributed in natural and undisturbed areas in the Neotropics, Africa, Pacific Islands, New Zealand, Asia, and Australia.[11] In India, this plant is found in Mahendragiri, Western Ghats, in all districts from the Coimbatore Hills and Nilgiris southwards, usually above 4000 feet msl.[12]

Generally, Piperaceae is characterized by the presence of aromatic compounds like terpenoid essential oils and other odorous volatile substances.[13] These substances are found in fruits, seeds and leaves inside glandular pockets and trichomes, and confer the intense flavour and the characteristic aromatic fragrance in most species of the genus[14] Previous phytochemical studies on species of Piperaceae led to the discovery of flavonoids,[15] secolignans,[16] tetrahydrofuran lignans[17] and polyketides.[18]

The genus *Peperomia* has been proved to be a high source of lignans,[19] amides,[20] polyketides,[21] terpenoids,[22] phenols,[23] chromones,[24] quinones[25] and flavones.[26] These bioactive compounds contribute to the immense pharmacological properties of *Peperomia tetraphylla*. The plants were mainly used to treat kidney disorders[27] and also showed the anti-urolithiatic activity.[28] The plants also possess cytotoxic activity[29] and the essential oil showed antidepressant activity.[30] It is often used in treatment of digestion, relieving cough, bone fracture, traumatic bleeding, scabies, swelling, infantile malnutrition, uterine prolapse and tuberculosis.[31]

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Herbal drugs play a significant role in health care programs especially in developing countries.[32] Unfortunately, the usage of medicinal plants in these countries is based primarily on empirical knowledge and many of the plants have not been scientifically assessed for their safety and efficiency.[33] Standardization is the basic requirement for the plant material to be used as medicine. Simple pharmacognostic procedures were used in the standardization of plant material include its morphological, anatomical and biochemical characteristics. These studies assist in recognizing and authenticating the plant material. Accurate identification and quality assurance of the preliminary materials is a crucial prerequisite to ensure reproducible quality of herbal drug which will contribute to its safety and efficacy.[34]

Despite its medicinal properties, pharmacognostic standardization of *Peperomia tetraphylla* is currently lacking. Therefore, this contribution aims to study the anatomy and microchemistry of vegetative organs of *Peperomia tetraphylla* to establish salient features to assist pharmacobotanical studies.

MATERIALS AND METHODS

Collection

Fresh plant *P. tetraphylla* was collected from Banagudi forest, Kotagiri, Nilgiri district, Tamil Nadu, India. It was identified and authenticated by BSI, Coimbatore, Voucher specimen No: BSI/SRC/5/23/2023-24/Tech-509. The herbarium was deposited at the PG & Research Department of Botany, Vellalar College for Women, Erode, Tamil Nadu, India. The fresh sample was preserved in fixative FAA for more than 48 hr and the aerial parts were also dried and stored for future applications.

Morphological and anatomical studies were performed on fresh and fixed material. The nomenclature to describe the morphological characters was based on (Mathew,1983).[35] Cross-sectional and paradermal sections were made using a rotary microtome and the sections were stained with Toluidine blue (stem, leaf and spike), 0.8% safranin and 0.5% astra blue (fruit).[36] Transverse sections were snapped using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Organoleptic features like aroma, taste, color, size, shape etc., of the aerial parts were also examined.

Sections of fresh material were subjected to histochemical tests: Wagner's reagent[37] for the detection of alkaloids; 10% ferric chloride solution[38] to highlight phenolic compounds; Lugol's reagent for starch localization; dilute sulphuric acid (5-10%), to localize calcium oxalate crystals ruthenium red solution[39] to evidence mucilage or polysaccharides; Sudan IV solution to locate cutinized walls and other lipid materialsand acdified fluoroglucinol for lignin identification. The diaphanization of the leaves followed the technique of Fuchs[40] and the description of the general pattern of venation used the criteria proposed by Hickey.[41]

RESULTS

Macroscopic Evaluation

Stem is reddish green in color, succulent, much branched herb, round, smooth and glabrous measuring 20 cm long and 0.4 to 0.6 cm in diameter, fracture fibrous, no characteristic odor in stem. Leaves are succulent, nerves obscure, green colored on upper surface and light green in lower side, in whorls of 4, ovate to rhomboid, petiolate with characteristic smell and pungent taste. Spikes are terminal, up to 1 to 2.5 cm long, pubescent; bracts orbicular, peltate, 3mm, margin elevated. flowers are tiny and deeply sunken into axis, greenish-yellow, black color fruits occur in the spike (Figure 1).

Organoleptic Evaluation

Organoleptic investigation of the aerial parts of *Peperomia tetraphylla* revealed that the colour was pale green, with acrid taste and characteristic smell (Table 1).

Microscopic Evaluation

T.S. of Stem

The outline was undulate in *P. tetraphylla*, (Plate 1: A). The epidermis was distinct, single layered comprising of pearl shaped cells and heavily cutinized. Epidermis often bears unicellular trichomes. The ground tissue is parenchymatous with intercellular spaces of varying dimensions. Starch grains, cluster crystals and mucilage cells were observed in the ground tissue (Plate 1: B). Scattered vascular bundles are noted in the ground tissue which is considered as an anomaly. The vascular system was collateral.

T.S. of Petiole

The sectional view of the petiole is nearly heart shaped, multi-layered with oval or polygonal epidermal cells with thick cuticle and few short bicellular trichomes. (Plate 1: C). Just below the epidermis, six to seven layers of collenchyma cells are present. Ground tissue is made up of parenchyma cells with small intercellular space. Tanniferous cells, starch grains and prismatic crystals were scattered in the parenchyma. Three vascular bundles are arranged in a crescent shape and enclosed by parenchymatous pericycle. In general, vascular bundles are collateral with xylem fibres. (Plate 1: D).

T.S. of Leaf

The leaf shows dorsiventral surface, the midrib has wide adaxial convex and abaxial wavy surface having ridges and furrows with laminar extensions. The epidermis along prominent ridges at the adaxial is smooth and has rectangular cells and is compactly arranged. Cells along the abaxial part are circular, wide, single layered with thin cuticle, bicellular trichomes and many paracytic

Table 1: Organoleptic investigation of the aerial parts of *Peperomia tetraphylla*.

Sl. No.	Characters	Observation		
		Stem	Leaf	Spike
1.	Condition	Fresh	Fresh	Fresh
2.	Color	Reddish green	Green coloured on upper surface and light green in lower side	Greenish-Yellow
3.	Size	8 to 10 cm long and 0.4 to 0.6 cm in diameter	0.9 to 1.3 cm long and 0.7 to 0.9 cm wide	1 to 2.5 cm
4.	Shape	Round	Obovate to Orbicular	Terminal Spike
5.	Texture	Smooth	Glabrous	Smooth

Table 2: Quantitative microscopy of *P.tetraphylla*.

Sl. No.	Parameters	Upper Epidermis (/ mm^2)	Lower Epidermis (/ mm^2)
1	Epidermal number	190 - 200	260 - 275
2	Stomatal number	-	15 - 20
3	Stomatal index	-	5 - 7
4	Palisade ratio	25 - 48	
5	Vein islets	1 - 2	
6	Vein terminations	1 - 2	

stomata. Hence the leaf is hypostomatic (Plate 1: E). Below the adaxial epidermis is a wide hydrophilic hypodermal region made up of multi-layered oval-shaped parenchymatous cells with few cystoliths. A very small collateral vascular bundle is embedded at the central wavy ridge region in abaxial surface. Vascular bundle is formed of normal phloem and xylem cells. In lamina portion the mesophyll tissue is not differentiated and confined to the wavy abaxial part. It consists of oval or dome shaped and loosely arranged spongy parenchyma cells (Plate 1: F) with starch grains, prismatic crystals, few cluster crystals and mucilage cells.

Venation

The major lateral veins and vein lets are thin. The vein-islets are wide, polygonal in outline and have distinct vein boundaries. The vein-terminations are mostly repeatedly branched forming dendroid outline within the islets (Plate 1: G).

T.S. of Spike

Sectional view of spike shows an irregularly circular outline with several arm-like out growths (Plate 1: H). It consists of continuous epidermis consisting of thin-walled cutinized oval cells often bearing short simple bicellular trichomes. Cortical zone is large forming a continuous ring. The outer zone is made up of five to six layers of chlorenchyma cells, followed by parenchymatous cells. Plenty of starch grains and few cystoliths are scattered in the arm region of the cortex. Vascular strands are present in central portion of cortical tissue and fruits are noticeable in the furrow region of the arms (Plate 1: I).

T.S. of Fruit and Seed

Transverse section of the fruit has a circular outline with a short lateral projection measuring 50µm in thickness (Plate 1: J). Pericarp is formed of outer single layered epicarp followed by parenchymatous thick mesocarp and thin membranous endocarp. Inner to the pericarp seed is present enclosed within a thick-walled testa layer, followed by wide endosperm region with polygonal cells enriched with aleurone grains. Embryo is present in the centre comprising of minute, polygonal cells. (Plate 1: K).

Powder Microscopy

Powder microscopy of the aerial plant parts displayed the presence of fragments of spiral vessel elements (Plate 2: A and B), mucilage cells (Plate 2: C) parenchyma cells (Plate 2: D), collenchyma cells (Plate 2: E), cells with reddish brown content (Plate 2: F and G), trichome 20µm in thickness (Plate 2: H, I, J), Paracytic stomata (Plate 2: K), Palisade layer (Plate 2: L), cluster and prismatic crystals (Plate 2: M and N) oil drops and cystolith (Plate 2: O and P) (Table 2).

Histochemical Analysis

Histochemical analysis is crucial for identifying the several bioactive compounds found in plant cells, through a colour shift produced by certain reagents or dyes reacting with particular metabolites. It has been carried out to detect various phytochemicals groups localized in different tissue zones of the plant parts. The results revealed the presence of alkaloids, tannins, starch, cutin, lignin, resin, cystolith, prismatic crystals

Table 3: Histochemical characterization of *P. tetraphylla*.

Sl. No.	Bioactive compounds	Reagents	Coloration	Tissue Location	
				Stem	Leaf
1.	Alkaloids	Wagner's Reagent	Yellow to Reddish Brown	Epidermis Cortex	Epidermis, Hypodermis and Mesophyll Tissue
2.	Tannin	10% Ferric Chloride	Bluish Black	Cortical Parenchyma	Epidermis, Hypodermis and Midrib Region
3.	Starch	Lugols reagent	Blue Colour	Ground Tissue	Midrib (Very few cells)
4.	Crystals	Dil.H_2SO_4	Formation of air bubbles	Ground Tissue (Cluster Crystal)	Mesophyll Tissue (Prismatic Crystal)
5.	Mucilage	Ruthenium Red	Pink to Red	Ground Tissue	Mesophyll Cells and Lower portion of leaf
6.	Cutin	Sudan Red IV	Pink	Epidermis	Upper and Lower Epidermis
7.	Lignin	Drop of fluroglucinol and 50% HCL	Blue to Black	Xylem	-
8.	Oil and Resin	Sudan - IV	Orange, Red or Pink- Fatty Oil; Red- Resin	Cortex	Hypodermis and Mesophyll Tissue

and mucilage in various tissue zones of the stem and leaf parts of *Peperomia tetraphylla* which is represented in the (Table 3 and Plates 3 and 4). This test highlights that epidermis, hypodermal cells, vascular bundles cortical zone and mesophyll tissue are the main sites for the synthesis or storage of different phytochemical groups.

DISCUSSION

Morphological and anatomical analysis plays a pivotal role in classifying medicinal plants. Also, microscopic examination of raw materials enables to detect the undesirable impurities. Top of Form

In our study, the morphological features of *Peperomia tetraphylla* showed resemblances with certain species of Peperomia, which aligns with the findings of Yu-Chen and Yang (2020),[42] who suggested a morphological similarity within the genus.

Stem

In our investigation, we noted that the stem displayed an undulating single-layered epidermis characterized by a thick cuticle and frequently adorned with unicellular trichomes. This follows the findings of Metcalfe and Chalk (1957)[43] who stated that the stem outline in *Peperomia* species is commonly entire, rounded, or undulate. Likewise, Suwanphakdee *et al.* (2024)[44] identified a similar undulating epidermis in species such as *P. heptaphylla, P. masuthoniana*, and *P. portulacoides* but with a thinner cuticle. However, they noted differences, reporting a grooved epidermis in *P. heptaphylla* and *P. portulacoides*, and a ridged epidermis in *P. laevifolia* and *P. pellucida.*

Variations in the trichomes form and number of cells can be used to recognize the plants, including in the powder form (Sonibare MA *et al.*, 2012)[45] Trichomes are commonly noticed in the epidermal region of *P. tetraphylla*, consistent with the earlier observation reported by Metcalfe and Chalk (1957).[43] In agreement with our findings, Suwanphakdee *et al.* (2024)[44] noted the prevalence of unicellular trichomes in eight species of *Peperomia*, namely *P. cochinensis, P. masuthoniana, P. moulmeiniana, P. multisurcula, P. nakaharae, P. portulacoides, P. ranongensis*, and *P. tetraphylla*. In contrast, they observed the presence of multicellular trichomes in *P. cavaleriei, P. dindygulensis, P. heptaphylla, P. kotana, P. masuthoniana*, and *P. ranongensis* while glandular trichomes were identified in *P. dindygulensis, P. heptaphylla, P. masuthoniana, P. moulmeiniana, P. multisurcula, P. portulacoides* and *P. ranongensis.*

The scattered vascular bundles in the ground tissue of *Peperomia tetraphylla* resemble those found in monocotyledonous plants, as previously reported by Solereder (1908),[46] Metcalfe and Chalk (1957),[43] and Setijati (1967).[47] This unusual characteristic of the stem supports the classification of *Peperomia* as an anomalous dicotyledonous type (de Bary 1884, Solereder 1908, Balfour 1958).[46,48,49] However, the vascular bundles form rings and mucilage canals in the pith in *Peperomia dindygulensis*, in a similar arrangement to the stem of *Piper* (Suwanphakdee 2012, Nugroho *et al.* 2019).[50,51]

Petiole

The petiole exhibited a cordate outline with a distinct multi-layered cutinized epidermis in our study. This feature was also described in the studies of Suwanphakdee *et al.*, (2024)[44] where *Peperomia* petioles have been described as nearly rounded, semi-circular or heart-shaped, sometimes presenting one or two ridges or being convex with two ridges. Moreover, Hawaiian species described by Setijati (1967),[47] were also noted for their grooved and winged petioles. Suwanphakdee *et al.*, (2024)[44] has documented various

trichome types, including unicellular, bicellular, tricellular, multicellular and glandular in a few species of *Peperomia* but our study primarily observed bicellular trichomes on the petiole's epidermis.

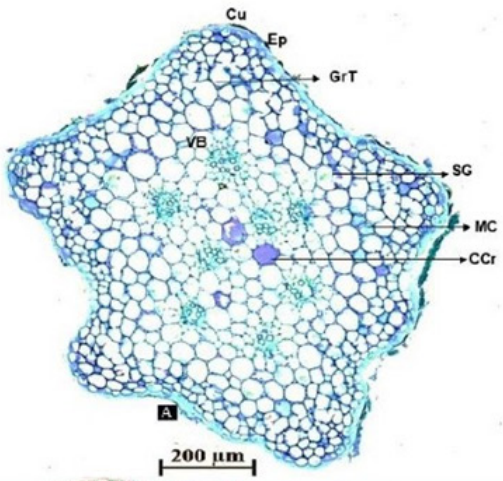
The present findings reported the occurrence of collenchyma, parenchymatous ground tissue with tanniferous cells, starch grains and prismatic crystals. This was on par with the findings of Suwanphakdee *et al.* (2024).[44] Setijati (1967)[47] also reported prismatic and druse crystals in the Hawaiian species of *Peperomia*.

Three collateral vascular bundles are arranged in crescent shape in the current study. This aligns with the findings reported by Tihurua *et al.* (2011).[52] However, scattered vascular bundles were reported in the Hawaiian species (Setijati 1967)[47] whereas Metcalfe and Chalk (1957)[43] recorded types that form approximate circles, or have an outer ring and inner scattered bundles and sometimes scattered bundles surrounded by 2 or 3 rings. Similarly, Wanke *et al.* (2006)[8] noticed that the vascular bundle is always of collateral type with xylem fibres. The number of vascular bundles varied from 3-12 (Suwanphakdee *et al.* 2024).[44]

Leaf

Succulent thick and fleshy leaves are very common in *Peperomia* as reported by Metcalfe and Chalk 1957).[43] In the present study, the leaf shows dorsiventral surface, the midrib having a wide adaxial convex and abaxial wavy surface having ridges and furrows with laminar extensions. The epidermis has compactly arranged rectangular cells with thin cuticle and bicellular trichomes and paracytic stomata. Similarly, Suwanphakdee *et*

Figure 1: A vegetative habit of *Peperomia tetraphylla*.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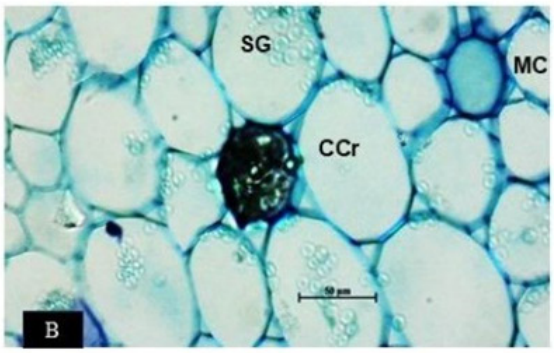
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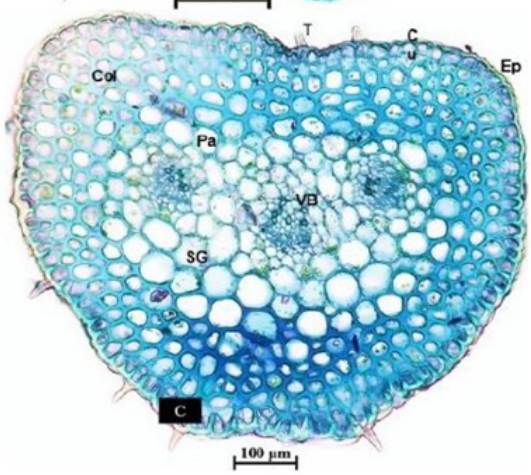
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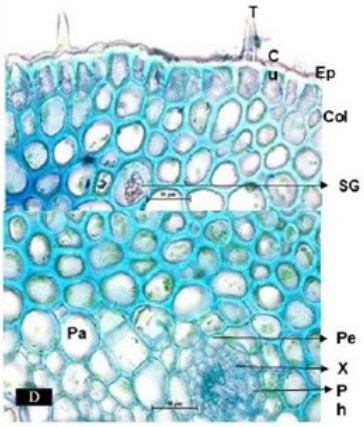
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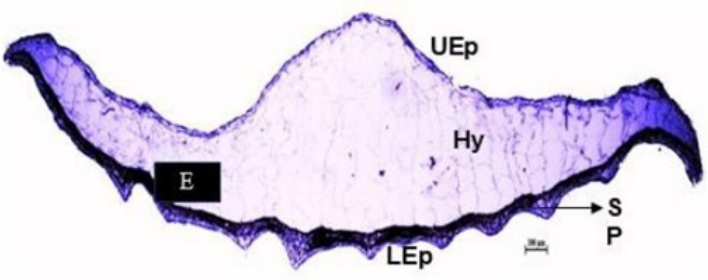
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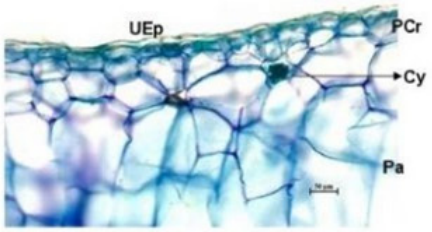
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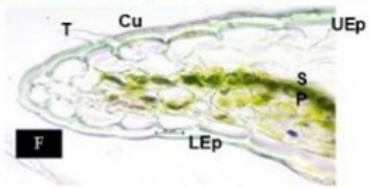
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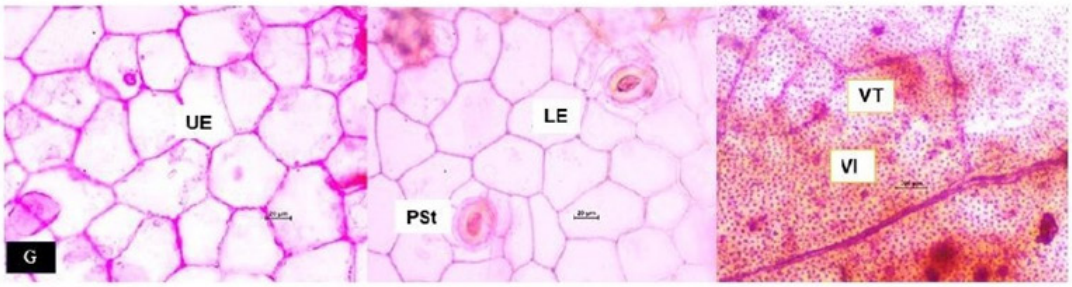
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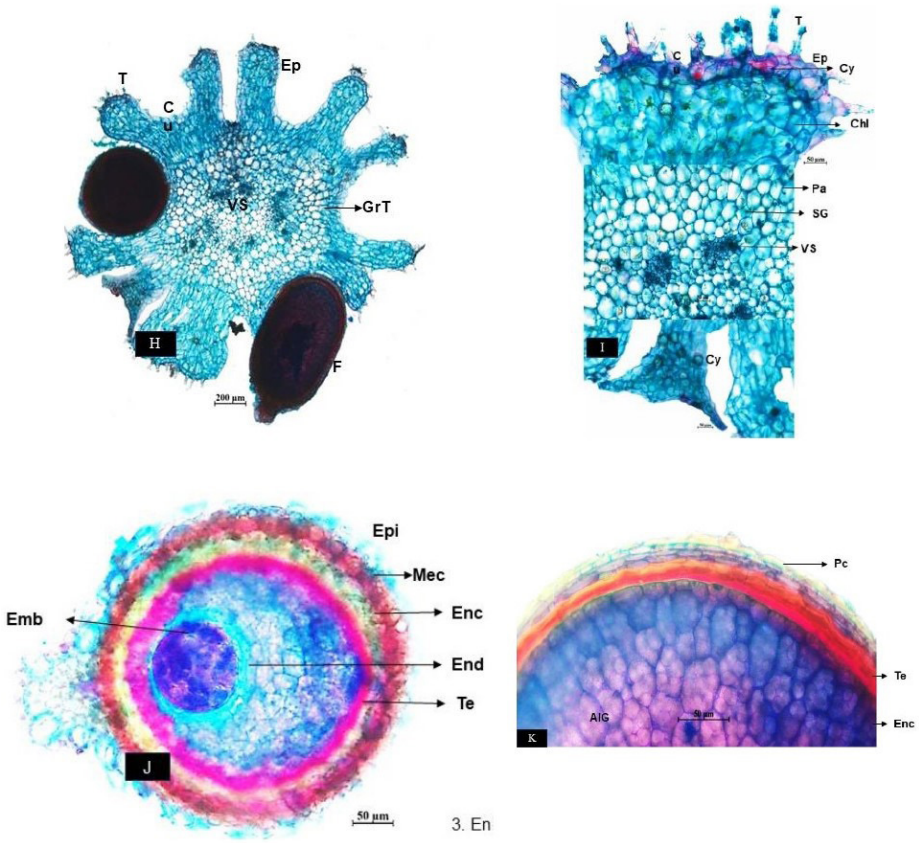

Plate 1: A- T.S of *P. tetraphylla* stem, B- cluster crystal, C- T. S of *P. tetraphylla* petiole, D- Enlarged portion of petiole, E- T.S of *P. tetraphylla* leaf, F- Enlarged portion of leaf, G- Venation, H- T.S of *P. tetraphylla* Spike, I- enlarged portion of Spike, J- T. of *P. tetraphylla* fruit, Enlarged view of Endosperm and Testa, Cu: Cuticle, Ep: Epidermis, GrT: Ground Tissue, VB: Vascular Bundle, SG: Starch Grain, CCr: Cluster Crystal, MC: Mucilage Cell, T: Trichome, Col: Collenchyma, Pa: Parenchyma, Ph: Phloem, X: Xylem, Pe: Pericycle, UEp: Upper Epidermis, SP: Spongy Parenchyma, PCr: Prismatic Crystal, Cy: Cystolith, LEp: Lower Epidermis,VI: Vein Islet, VT: Vein Termination, PSt: Paracytic Stomata, Chl: Chlorenchyma, VS: Vascular Strand, F: Fruit, Emb: Embryo, Enc: Endocarp, End: Endosperm, Epi: Epicarp, Mec: Mesocarp, Te: Testa, AlG: Aleurone Grains, Pc: Pericarp.

al. (2024)[44] reported thin or thick cuticle; single or multiple epidermis with 2–3 layers, oval or polygonal shaped cells in the species of *Peperomia* they studied. Also, Suwanphakdee *et al*. (2024)[44] reported hypostomatic leaves with a variety of stomatal types, including paracytic, anisocytic, cyclocytic, tetracytic, and staurocytic in all the species they studied indicating the existence of diverse stomatal structures across the genus. Our findings align with this, as we observed that the leaves of *P. tetraphylla* are hypostomatic with paracytic stomata. Similarly, Pant and Barnerji, (1965) and Setijati (1967),[53,47] stated that hypostomatic leaves are common in *Peperomia.* In contrast, Yuncker (1933)[54] reported the presence of stomata on the adaxial surface.

In *Peperomia tetraphylla* hypodermal region is of multi-layered oval-shaped parenchymatous cells with few cystoliths. Additionally, Solereder (1908)[46] also stated that the hypodermis of *Peperomia* species has been widely studied, showing significant variability in the number of hypodermal layers across different species with some considerable thickness. In the present findings, a very small collateral vascular bundle is embedded at the central wavy ridge region in abaxial surface. Normally, the midvein is embedded in the lamina and has 1–3 bundles with xylem fibres as reported by Suwanphakdee *et al*. (2024)[44] In the present findings the mesophyll tissue is not differentiated and consisted of oval, loosely arranged spongy parenchyma cells with starch grains, prismatic crystals, few cluster crystals and mucilage cells. As like to that of Hawaiian species by Yuncker and Gray (1934),[55] *Peperomia tetraphylla* neither showed palisade layer in the mesophyll region but spongy mesophyll with prismatic crystals were noticed. On the contrary, a few south East Asian species reported by Suwanphakdee *et al*., (2024)[44] possessed palisade and spongy mesophyll differentiation. Presence of prismatic crystals in leaf spongy mesophyll was analogous to reports of Suwanphakdee *et al*., (2024)[44] but it was not reported in Hawaiian species (Setijati 1967).[47]

Spike, Fruit and Seed

Peperomia tetraphylla spike has an outer continuous epidermis with thin cuticle-bearing bicellular trichomes. Analogous to our

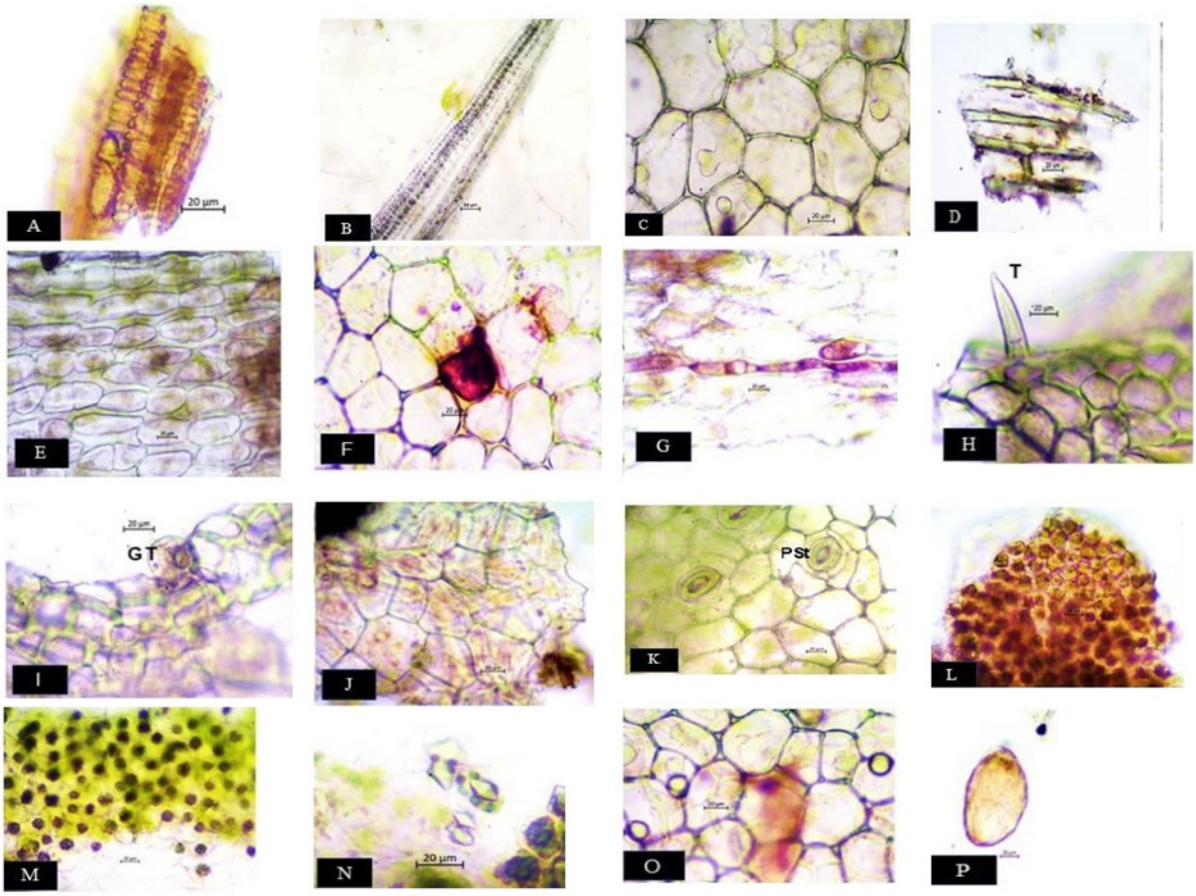

Plate 2: Powder microscopic studies describe the characters of *Peperomia tetraphylla*; A and B- Stem showing Spiral vessel in surface view, C-Leaf Cells with mucilage content, D- Parenchyma cells, E- Collenchyma cells, F and G- Cells with reddish brown content, H, I, J- Trichomes, K- paracytic stomata, L- Palisade layer, M and N- Cluster Crystals and Prismatic Crystals, O and P- Oil drops and Cystolith.

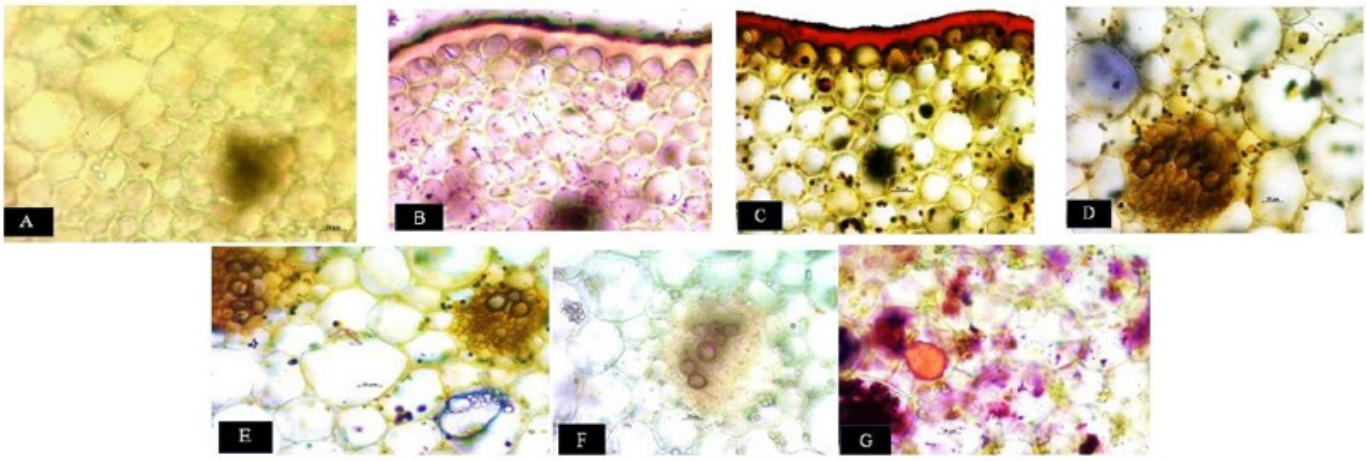

Plate 3: Histochemical Analysis of T.S. Stem of *Peperomia tetraphylla* shows the A- Tannin in cortical parenchyma, B- Cutin on Epidermis, C and D- Alkaloid in Epidermis and cortical parenchyma, E- Starch Grains in Cortical Parenchyma, F- Lignin in xylem, G- Resin in Cortex

findings, *Piper longum* displayed an epidermis with barrel-shaped cells with thick cuticle and the parenchyma-cells containing chloroplast, starch grains and vascular strands in center *P. tetraphylla* fruit had many resemblances to the *Piper trichostachyon* including the presence of pericarp, testa endosperm with embryo, and aleurone grains. (Updhaya, 2016).[56] With relevance to our study (Dahanayake *et al.*, 2019)[57] stated that fruits of *Piper longum, Piper nigrum* showed the existence of a wide mesocarp composed of parenchymatous cells.

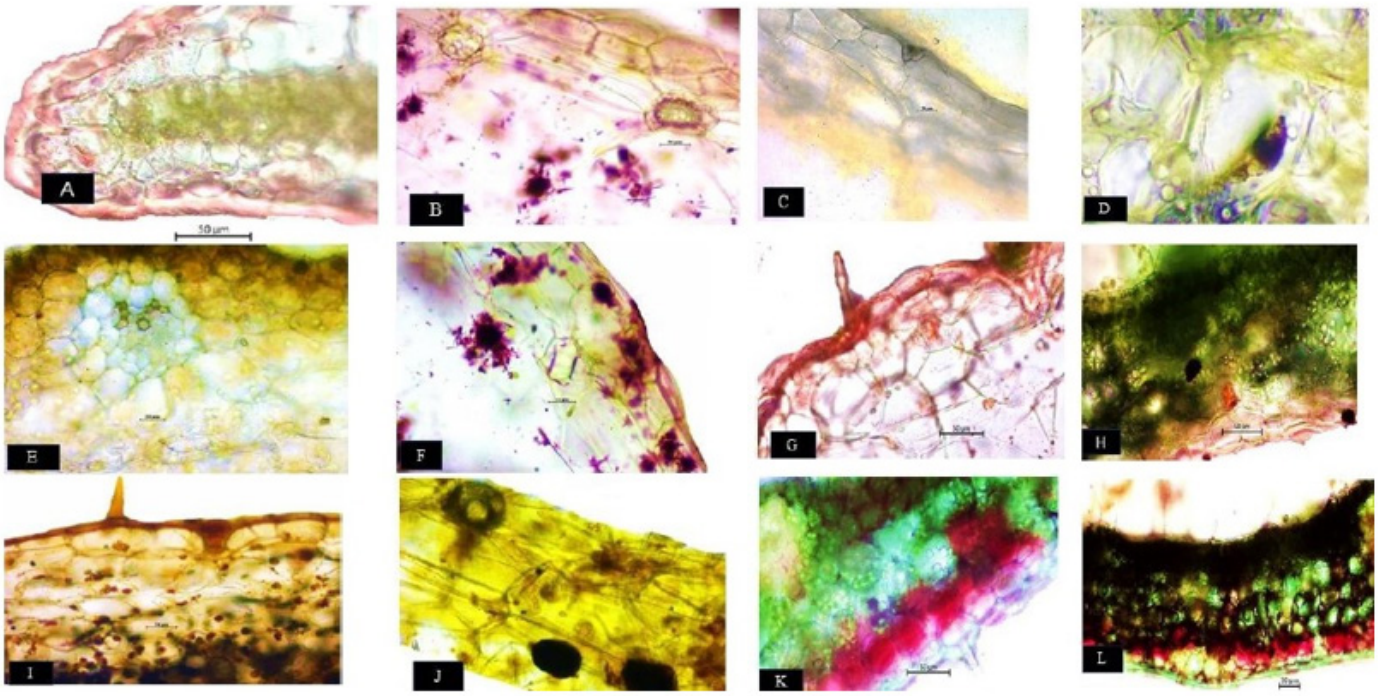

Plate 4: Histochemical Analysis of T.S. *Peperomia tetraphylla* leaf displays the presence of bioactive compounds are A- Cutin on upper and lower epidermis, B- Cystolith, C, D, E- Tannin in epidermis, hypodermal cells and midrib, F- prismatic crystals, G and H- resin in hypodermis and mesophyll tissue, I and J- Alkaloids in epidermis, mesophyll tissue and hypodermal cells, K and L- Mucilage in lower portion of leaf and mesophyll cells.

Powder Microscopy

The observation of the cellular level is most important for the authentication of powdered drugs to prevent contaminants, due to the loss of physical characteristics (Periyanayakam, 2012).[58] In *P. tetraphylla* spiral vessel, starch grains, oil drops, paracytic stomata, cystoliths and reddish-brown content were seen. Akin to this (Pulak majunder, 2012)[59] reported the oil drops and vessel fibres in *Peperomia pellucida* and cyclocytic stomata, glandular trichome in *Piper betle* (Periyanayakam, 2012).[58]

Histochemical Analysis

Histochemical methods are useful in the *in situ* localization of various metabolites, and aid in identifying their respective sites of production or action. In the present study, the distribution and localization of various phytochemicals were investigated, providing insights for identification and authentication. Alkaloids, naturally occurring organic compounds known for their therapeutic properties were detected in the epidermis and cortex regions of the stem and the epidermis, hypodermis as well as in mesophyll tissues of leaf as Yellow to Reddish Brown with Wagner's reagent. Similarly, Corti *et al.*, (2021)[60] documented the occurrence of alkaloids in the leaf tissues of *Peperomia* species, particularly in the upper hypodermis and mesophyll tissue of leaves. Tannins are known by black coloration in epidermis, hypodermis and mesophyll tissues of leaf and Cortical Parenchyma of stem using 10% ferric chloride. Meanwhile, El Babili *et al.*, (2021)[61] also observed the presence of tannin in cortical parenchyma and leaf midrib of *Peperomia obtusifolia*. In contrast, tannin was reported to be absent in the stem of *Peperomia aduncum* by Arroyo *et al.*, (2022).[62]

The current research displayed the presence of fine blue coloration (starch) in the ground tissue of the stem and a few cells of the leaf midrib. Consistently, Arroyo *et al.*, (2022)[62] recognized starch grains in the cortical parenchyma of *Piper aduncum* stem. While, da Silva *et al.*, (2014)[63] found starch in mesophyll tissue of *Peperomia pellucida*. In present study, Cutin was observed as a pink colour in the epidermis of both the leaf and stem, while in *Peperomia obtusifolia* it was reported only in the leaf midrib. Lignin was noticed exclusively in the xylem region of stem to the same fashion as in *Piper aduncum* and *Peperomia tetraphylla* species. Mucilage cells was noted in the ground tissue of stem as well as in the mesophyll and lower epidermis of the leaf in present study. Also, Silva *et al.*, (2017)[64] stated that mucilage cells were found in the ground parenchyma and mesophyll cells of *Piper callosum* leaves. However, in contrast, species such as *Piper malgassicum, Piper guineense* and *Piper tsarasotrae*, did not exhibit mucilage cells. (Ilodibia *et al.* 2016; Palchetti *et al.* 2018).[65,66] As in *P. callosum,* oil and resins were observed in stem cortex along with hypodermis and mesophyll tissue of leaf. In *Peperomia tetraphylla* cluster crystals in stem and prismatic crystals in mesophyll tissue were noted. In *P. malgassicum* crystals were reported in stem hypodermis and leaves.

In conclusion, our outcomes shows that *Peperomia tetraphylla* produces secondary metabolites with defense actions, particularly in the leaves. By tradition, the vegetative parts of *Peperomia*

species have been depended on for species identification, as the reproductive parts tend to be simple, uniform and lacking distinct characteristics for differentiation Suwanphakdee *et al.* (2024).[44] Therefore, it may be concluded that *Peperomia tetraphylla* reveals notable morphological diversity, particularly in stem, petiole, and trichome characteristics, which is crucial for accurate species identification and classification within the genus.

CONCLUSION

In conclusion, our study aimed to establish standardized protocols for the precise identification and quality control of *Peperomia tetraphylla*. The diverse morphological characteristics observed, including traits of the stem, leaf, petiole, spike and fruit are anticipated to serve as crucial parameters for validating and ensuring the quality of this medicinal plant in future applications. Furthermore, histochemical analysis provides a significant understanding of the distribution and localization of various phytochemicals and enhances the reliability of species identification and quality control assessment. Ultimately this comprehensive report on micro-morphological and histochemical data provides a fundamental understanding of *Peperomia tetraphylla* in therapeutic applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

%: Percentage; **MSL:** Mean Sea Level; **µm:** Milli micron; **cm:** Centimeter; **FAA:** Formalin acetic acid; **hr:** Hour; **mm:** Millimeter.

SUMMARY

The study is to scrutinize the botanical characters of *Peperomia tetraphylla*, a small herb from the *Piperaceae* family through macroscopic, microscopic and histochemical analysis. The study plant exhibits unique features such as four number of leaves in a whorl, much branched herbs, terminal spike and characteristic flower and fruit structure. Through anatomical study of stem, expressed the presence of scattered vascular bundle which is considered anamoly. The transverse section of Petiole is nearly heart shaped with three vascular bundles arranged in a crescent shape. The leaf is hypostomatic with collateral vascular bundle embedded in central wavy ridge region in abaxial surface. Sectional view of spike shows irregular circular outline with many arm-like outgrowth and fruits are present in the furrow region of the arms. Fruits consists of pericarp, Testa, endosperm with embryo. In endosperm aleurone grains are seen. In histochemical analysis, the alkaloids and tannins are found majorly in both stem and leaf cells. Hence, the study underscores the critical visions for the characterization of this medical plant, while it also facilitates the validation of its purity, safety and healing efficiency in herbal formulation derived from *Peperomia tetraphylla*.

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