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LEAF ANATOMY OF MEDICINALLY VALUABLE *Melastoma malabathricum* L. IN PENINSULAR MALAYSIA

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ABSTRACT
Melastoma malabathricum, commonly known as senduduk, is a member of the Melastomataceae family. It is well-known for its therapeutic and medicinal properties, deeply rooted in local traditional knowledge and supported by scientific evidence. Often considered a robust plant and even labeled as a weed, *M. malabathricum* can be easily spotted along roadsides. However, despite its potential value, recent publications have provided limited information about its leaf anatomy. This study aimed to improve species identification by thoroughly examining the leaf anatomical characteristics of *M. malabathricum*. Leaf samples were collected from 10 different locations in Peninsular Malaysia and subjected to laboratory procedures involving sectioning and staining. The prepared permanent slides were then observed under a light microscope. A total of 35 leaf anatomical characteristics, including 10 types of trichomes, were successfully identified. Important characteristics such as the petiole, midrib, marginal outline, paracytic stomata, and vascular bundle types were systematically documented. Notable discoveries included the presence of hypodermal layers, collenchyma cells, druses, mucilage cells, and medullary phloem, all of which are distinctive anatomical traits of *M. malabathricum*. During the leaf anatomical analysis, it became evident that the majority of examined *M. malabathricum* samples displayed substantial similarities with minor variations in the combination of trichome types on the leaf surface. These variations might be influenced by ecological and geological factors. Nonetheless, it is essential to emphasize that the types of trichomes and other anatomical characteristics remained consistent within each sample. Consequently, these findings offer valuable insights into the systematic classification of *M. malabathricum* through the documentation of leaf anatomy characters.
Keywords: Leaf anatomy, *Melastoma*, *Melastoma malabathricum*, Medicinal herbs

ABSTRAK
Melastoma malabathricum, biasanya dikenali sebagai senduduk, merupakan famili Melastomataceae. Ia terkenal dengan sifat terapeutik dan perubatannya, secara mendalam berteraskan pengetahuan tradisional tempatan dan disokong oleh bukti saintifik. *Melastoma malabathricum* sering ditemui sepanjang tepi jalan dan tergolong dalam kategori rumpai yang tersebar dengan luas. Walaupun tumbuhan ini mempunyai potensi dalam perubatan, namun penerbitan berkaitan maklumat anatomi daunnya adalah terhad. Kajian ini bertujuan untuk menambah baik pengecaman spesies dengan memeriksa dengan teliti ciri-ciri anatomi daun *M. malabathricum*. Sampel daun telah dikumpulkan dari 10 lokasi berbeza di Semenanjung Malaysia dan seterusnya terlibat dengan kerja pemotongan dan pewarnaan di makmal. Kemudian penyediaan slaid kekal dijalankan untuk tujuan pemerhatian di bawah mikroskop cahaya. Sejumlah 35 ciri anatomi daun, termasuk 10 jenis trikom telah berjaya dikenalpasti. Ciri-ciri penting seperti bentuk luaran petiol dan midrib, tepi daun, stomata jenis parasitik, dan jenis berkas vaskular secara sistematik didokumenkan. Penemuan ketara termasuk kehadiran lapisan hipodermal, kolenkima sel, drus, sel musilaj, dan floem medulari, telah dikenalpasti sebagai ciri tersendiri *M. malabathricum*. Kesemua sampel *M. malabathricum* yang dikaji menunjukkan persamaan yang ketara dengan variasi kecil dalam gabungan jenis trikom pada permukaan daun. Variasi ini mungkin dipengaruhi oleh faktor ekologi dan geologi. Walau bagaimanapun, jenis trikom dan ciri anatomi lain kekal konsisten dalam setiap sampel. Justeru, kajian ini dapat membuktikan bahawa dokumentasi ciri anatomi daun dapat membantu pengelasan sistematik *M. malabathricum*.
Kata kunci: Anatomi daun, *Melastoma*, *Melastoma malabathricum*, Tumbuhan ubatan

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INTRODUCTION

The therapeutic properties of *Melastoma* are well-known and have been established via numerous scientific studies, most notably on *M. malabathricum*. Older people utilized the leaves, shoots, barks, seeds, and roots of *M. malabathricum* ethnobotanically to treat cuts and wounds, toothache, diarrhea, dysentery, hemorrhoids, and stomachache (Burkill, 1966). Scientists have identified bioactive compounds with pharmacological activities such as antinociceptive, antiulcerogenic, anti-inflammatory, anticoagulant, antidiarrheal, cytotoxic, antioxidant and anthelmintic by isolating several flavonoids from this plant (Isnaini et, al 2019; Zheng et al., 2021). The extracts of *M. malabathricum* leaves and flowers exhibit promising anticancer activity against human breast cancer cell lines (Edianto et al., 2020).

With the significant usage of *M. malabathricum* leaves as a raw material for product development, it is essential to determine their characteristics, especially when they are in ground form. Therefore, the anatomical characteristic of the leaves needs to be the focus. Anatomical approaches are valuable for the food industry and herbal research and innovation. This alludes to the difficulties encountered in identifying powdered plants. It is critical to choose the correct species when developing a plant-based product. Errors in identifying the incorrect species may have a detrimental effect on the entire process, rendering the research and development insignificant.

However, studies on the leaf anatomy of *M. malabathricum* are scarce in the primary literature. According to Noorma Wati (2015), some *Melastoma* species possess paracytic stomata and non-glandular trichomes on the leaf's surface. However, their study solely investigated micromorphology using Scanning Electron Microscopy (SEM). A comprehensive anatomical study of *M. decemfidum* (white *Melastoma*) was previously conducted, as reported by Khatijah and Noraini (2007). Their research focused on various aspects of leaf anatomy, specifically targeting stomata, trichomes, leaf venation in laminar and margin regions, as well as the ground and vascular tissues in the midrib and petiole. To date, this is the only such leaf anatomical study published in the literature, highlighting the need for further anatomical investigations into this species. Morphologically, the only distinguishing feature between *M. malabathricum* and *M. decemfidum* is the color of their flowers (inflorescence) (Figure 1). Without flowers, distinguishing between them is challenging. Moreover, there are significant differences in chemical compounds and bioactivities between *M. malabathricum* (purple) and *M. decemfidum* (white) Zheng et al., 2021). Therefore, it is imperative to conduct an extensive study involving a larger number of samples to gain a more comprehensive understanding of the anatomical characteristics of *M. malabathricum* in Peninsular Malaysia.

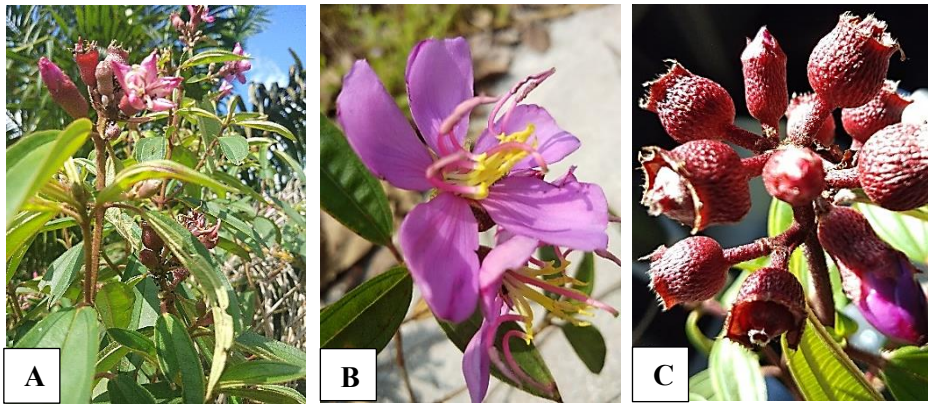

Figure 1: *Melastoma malabathricum* (A) Leaves, (B) Inflorescences and (C) Fruits

MATERIALS AND METHODS

Plant Materials

Mature leaf specimens of *M. malabathricum* were collected from 10 localities in Peninsular Malaysia, covering the north, south, east, and west regions of Peninsular Malaysia. Three fresh leaf sample replicates were prepared for each locality, and the voucher specimens were deposited in the Herbarium of the International Islamic University Malaysia (IIUM).

Leaves Anatomy Observation

Fresh leaves samples were fixed in 3:1 AA solutions (70% Alcohol: 30% Acetic Acid). The middle part of the petiole was sectioned using a microtome, stained with Safranin and Alcian Blue, dehydrated using a series of alcohol, and mounted on slides using Euparal before being observed under the light microscope. Anatomical images were captured using a video (3CCD) camera attached to a Leitz Diaplan microscope using cell^B software. Suitable modifications in terms of fixation and embedding followed the method by Johansen (1940) and Sass (1940).

RESULTS

Leaf anatomical characterization of M. malabathricum.

Lamina cuticle layer: one thin layer, presence at the adaxial surface. **Epidermal cells**: adaxial and abaxial, both ± rectangular, cells twice wide as high with ratio 2:1. **Hypodermal cells**: a single layer underneath adaxial epidermis (Figure 2 B). **Chlorenchyma cells**: palisade cells presence in one layer equal to 1/2 of leaf thickness, each cell ca. 5 - 7 times higher than wide. Spongy mesophyll cells in ca. 3 – 5 layers, without intercellular spaces. **Vascular bundles**: **primary vascular bundles**; prominent, concave with V-shaped on adaxial and convex-shaped on abaxial epidermis, U-shaped bicollateral vascular bundles (Figure 2 C), **secondary vascular bundles;** prominent, flat on adaxial and V-shaped on abaxial, simple vascular bundles (Figure 2 D). **Sclerenchyma cells:** a cluster of cells present underneath epidermis adaxial surface of secondary vascular bundles figure. **Collenchyma cells**: presence with ca. 4 – 5 layers underneath epidermis abaxial surface, parallel with vascular bundles. **Cell inclusions and mucilage cells** mucilaginous idioblast and druses (Figure 2 B). **Trichomes**: setose (short, blunt), strigose setose (short, pointed end) and capitate glandular trichome (unicellular- uniseriate) (Figure 2 E and F). **Leaf marginal**: rounded with U-shaped outline, recurved 30º towards abaxial

epidermis, the size decreasing gradually towards the edge of margin (Figure 2 A).

Petiole outline: adaxial surface (ad) flat and abaxial surface (ab) U-shaped. **Vascular tissue**: main vascular bundle (opened type with V-shaped continuous ring of vascular bundles, bicollateral); two clusters medullary phloem presence in the main vascular bundle; additional vascular bundles on adaxial (opened system with six continuous rings of vascular bundles) (Figure 3 A). **Cell inclusions**: the presence of druses in parenchyma cortex, vascular bundles, and epidermis cells (Figure 3 A). **Mucilage cells/canal**: presence in parenchyma cortex. Outer parenchyma: ca. 5 – 7 layers of parenchyma cortex. Collenchyma cells: ca. 2 – 4 layer of cells presence underneath the epidermis. **Trichomes:** setose trichome (short, pointed); setose trichome (long, pointed), scales trichome (wide), scales trichome (long, narrow) and scales trichome (fused) (Figure 3 B, C, D & E).

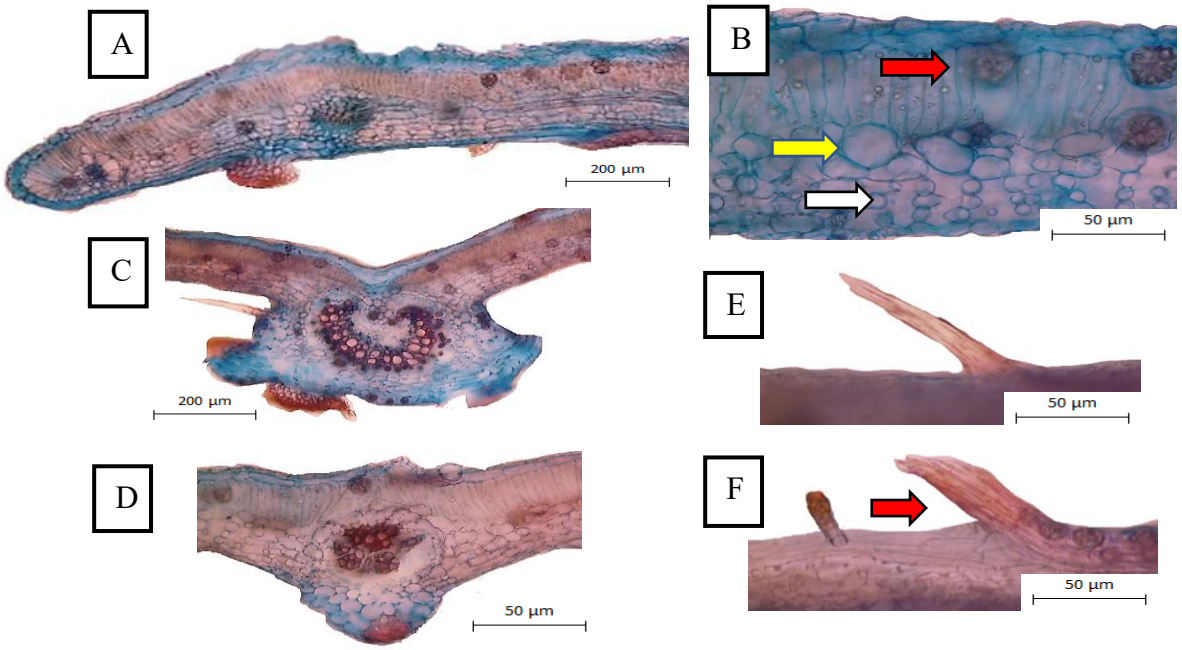

Figure 2: **Lamina** A) Marginal leaf, B) Cross-section of lamina: mucilaginous idioblast (white arrow), hypodermis layer (red arrow) and druses (yellow arrow), C) Primary vascular bundle, E) Secondary vascular bundle, G) Strigose setose trichome (short, pointed end), H) setose (short, blunt) (red arrow) and capitate glandular trichome (unicellular- uniseriate). Scales: A & C) 200 µm. B, D, E & F) 50 µm.

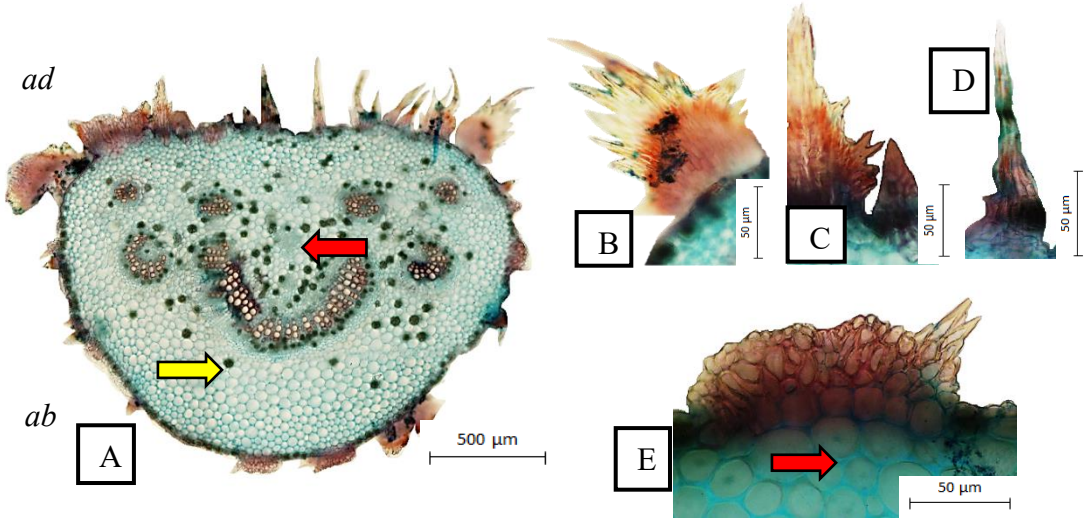

Figure 3: **Petiole** A) Overall view of petiole cross-section; medullary phloem (red arrow); druses on parenchyma cortex (yellow arrow), B) Scales trichome (long, wide), C) Setose trichome (short, pointed end) and scales trichome (long, narrow), D) setose trichome (long, pointed), E) Scales trichome (fused) and collenchyma cells (red arrow). Scale: A) 500 µm. B, C, D & E) 50 µm.

Midrib outline: adaxial surface (*ad*) flat channel formed by vertical attachment of lamina and abaxial surface (*ab*) rounded shaped (Figure 4 A). **Vascular tissue**: Main vascular bundle (opened system with continuous ring of vascular bundles, crescentic bicollateral); one cluster of phloem medullary presence in main vascular bundles; a vascular bundle medullary in adaxial. **Cell inclusions**: the presence of druses in parenchyma cortex, vascular bundles and epidermis cells. **Mucilage cells/canal**: Presence of mucilage cells in vascular bundle and parenchyma cortex. (Figure 4 B). **Outer parenchyma**: ca. 3 – 5 layers of parenchyma. **Collenchyma cells**: ca. 1 - 2 layers under epidermis layer (Figure 4 B). **Trichomes**: Setose trichome (short, pointed end), setose trichome (long, pointed end), simple multiseriate trichome (short, pointed end) and scales trichome (partially fused) (Figure 4 A).

Anticlinal wall of abaxial epidermis: Straight to wavy. Anticlinal wall of adaxial epidermis: Straight to wavy**. Stomata**: Hypostomatic (present on abaxial only), densely scattered, paracytic (Figure 5 A). **Trichome on abaxial surface**: Setose trichome (long, pointed end) and simple multiseriate trichome (long, pointed end). **Trichome on adaxial surface**: Strigose setose trichome (long, pointed end) (white arrow) and setose trichome (short, pointed end) **Main venation**: Majority open, minority closed, veinlets ending branched and swollen tracheid. **Marginal venation**: Incomplete (Figure 5 B).

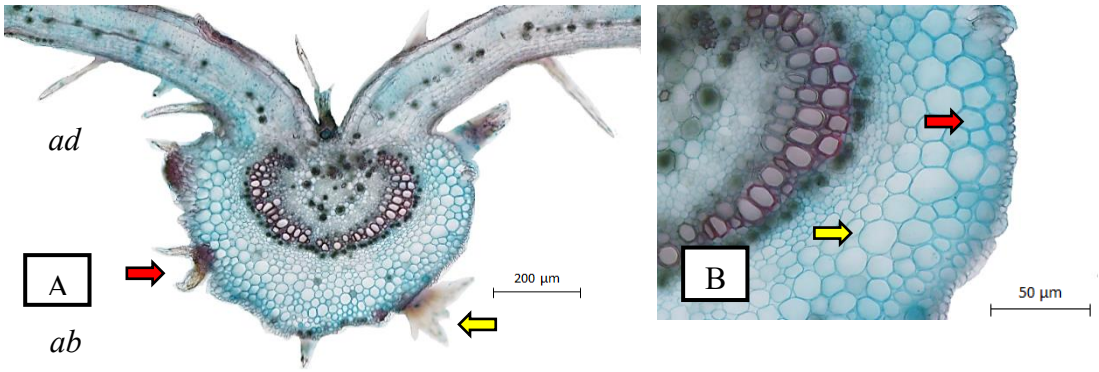

Figure 4: **Midrib** A) Overall view of midrib cross-section; setose trichome (short) and scales (short, wide) scales trichome (partially fused), B) collenchyma cells (red arrow); mucilage cells (yellow arrow). Scale: A: 200 µm; B: 50 µm.

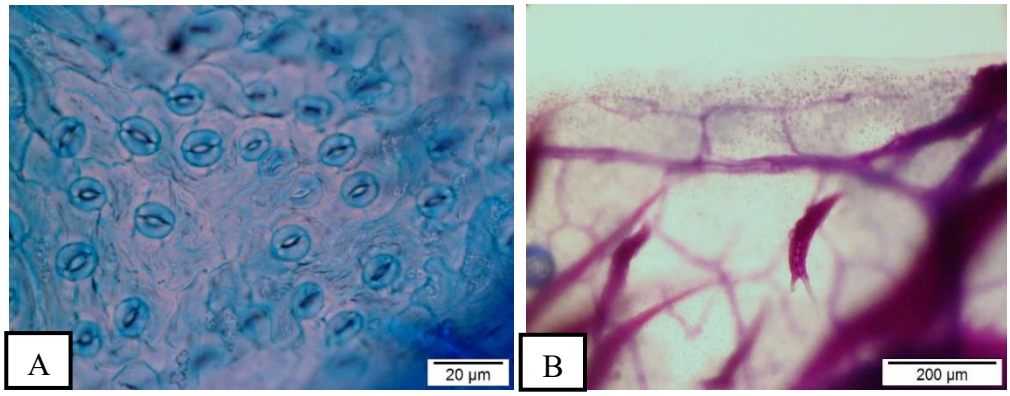

Figure 5: A) Paracytic type of stomata, B) Incomplete marginal venation

DISCUSSION

The utilization of cross-sectional features such as leaf, petiole, midrib, and marginal outline has been a well-established method for demarcating plant species (Metcalfe & Chalk., 1979); (McKown et al., 2010); (Tan & Buot., 2020). Results showed the midrib cross-sectional displayed a concave channel on the adaxial surface, resulting from the vertical attachment of the lamina, and a U-shaped profile on the abaxial surface in all species studied. Conversely, the petiole exhibited a flat adaxial surface and a U-shaped abaxial surface. Notably, the petiole's cross-sectional outline serves as a stable and environment-independent feature, making it a valuable tool for taxonomic identification (Metcalfe & Chalk., 1979); (McKown et al., 2010). Regarding the leaf margin, it featured a pointed end with a U-shaped cross-sectional outline, with a gradual size reduction towards the margin's edge. Additionally, the margin displayed a recurved orientation of approximately 10º to 20º towards the abaxial epidermis.

The examination of *M. malabathricum* revealed specific vascular characteristics in both the midrib and petiole. In the midrib, a primary vascular bundle was identified, which exhibited an open system with a continuous ring of vascular bundles, forming a crescentic bicollateral arrangement. Furthermore, a single cluster of medullary phloem was observed within the main vascular bundle,

and an additional vascular bundle was noted medullary on the adaxial side. A principal vascular bundle was observed in the petiole, featuring an open type with a V-shaped continuous ring of vascular bundles, arranged bicollaterally. Interestingly, two clusters of medullary phloem were present within the main vascular bundle, and additional vascular bundles were identified on the adaxial side, following an open system with six continuous rings of vascular bundles. These vascular patterns in *M. malabathricum* are consistent with those observed in several other genera within the Melastomataceae family, including *Lavoisiera*, *Microlicia*, and *Trembleya*, where bicollateral vascular bundles are a characteristic feature in both petioles and midribs, as reported by Mentink and Bass (1992) and Silva et al. (2018).

The presence of mucilaginous idioblast cells and druses in the petiole and midrib was observed in all samples studied. The mucilaginous cells and druses were found to be dispersed within the parenchyma cortex and vascular bundles. Additionally, druses were also identified within the setose strigose and scales trichomes. A previous study by Watanabe et al. (2018) provided compelling evidence for the existence of specialized mucilage cells containing a high content of polysaccharides in the roots of *M. malabathricum.* Interestingly, it was noted that *M. malabathricum* exclusively exhibited a single type of crystal in the form of druses. These crystal shapes and their specific locations within a taxon are frequently distinctive and can serve as valuable taxonomic characteristics, as previously discussed by Gómez-Espinoza et al, (2021).

The characterization of *Melastoma* trichomes was substantiated by prior research conducted by Noorma Wati et al. (2015), which revealed an even greater diversity in trichome characteristics within the *Melastoma* genus. Specifically, this study disclosed that *M. malabathricum* exhibited both non-glandular and glandular trichomes. Notably, the identification of glandular trichomes in *M. malabathricum* contradicts earlier findings. The findings of this study demonstrated that all examined samples possessed trichomes on either the abaxial or adaxial leaf surfaces. These trichomes were identified as strigose setose trichomes (short with pointed ends), setose trichomes (short with blunt ends), and capitate glandular trichomes (unicellular-uniseriate). It is noteworthy that strigose setose trichomes (non-glandular) predominated on both leaf surfaces; however, multiple types of trichomes were present on each surface. In addition to leaf surfaces, trichome variations were also observed on the midrib and petiole, including setose trichomes (short with pointed ends), setose trichomes (long with pointed ends), simple multiseriate trichomes (short with pointed ends), scales trichomes (short and wide), partially fused scales trichomes, short setose trichomes, and fused scales trichomes. The consistent presence of trichomes in specific plant species underscores the utility of this characteristic in taxonomic delineation, as previously discussed by Weryszko-Chmielewska and Chernetskyy (2006) and Siti-Maisarah (2020).

Based on the findings, *M. malabathricum* exhibits collenchyma cells in its petiole, midrib, and lamina. Specifically, these collenchyma cells take the form of peripheral collenchyma due to the presence of collenchyma layers beneath the epidermal surface (Filartiga et al., 2023). Apart from collenchyma, hypodermis cells were also identified in the lamina of all samples. These findings align with prior research on other genera within the Melastomataceae family, which has shown occasional occurrences of hypodermal (Mentink, H., & Baas, P., 1992). However, it's worth noting that hypodermal cells are typically associated with xerophytic plants and are recognized as specialized cells located on the inner

side, adjacent to the epidermal cells (Cutler et al., 2008). They serve as a water storage tissue, contributing to water conservation mechanisms (Nurnida., 2012). Research by Nurul-Aini et al. (2018) also reported the importance of hypodermal cells in Acanthaceae in differentiating species.

Most recently, research has demonstrated that stomatal characteristics carry taxonomic significance in various plant families, including Asparagaceae (Klimeš et al., 2022), Euphorbiaceae (Rayos & Hadsall, 2018) and Scrophulariaceae (Ullah et al., 2021). In the context of the present anatomical study, it was observed that all examined samples displayed hypostomatic stomata with a dense distribution of stomata on the abaxial surface. The stomatal type observed was paracytic which is consistent with results reported by Khadijah and Noraini (2007) and Noorma Wati (2015). The stomata were relatively small in size, with an average width of 6 µm and a height of 9 µm.

It is worth noting that Cutler et al. (1978) have emphasized that leaf epidermal characteristics can serve as diagnostic features in plant identification, particularly due to variations in the patterns of anticlinal and periclinal walls of the epidermal cells. Following a thorough examination, it was determined that both leaf surfaces exhibited straight to wavy anticlinal walls, a finding corroborated by Noorma Wati (2015). Regarding leaf venation, major open and swollen tracheids were observed, and the marginal venation was found to be incomplete. Lamina venation is recognized as a crucial element in providing mechanical support and facilitating the transport system in plants. It is distinctive among species and genera due to variations in the shape and size of leaves (Rolland., 2008).

CONCLUSION

A total of 35 leaf anatomical characters and 10 types of trichomes were successfully identified. During the leaf anatomical examination, it was observed that the majority of *M. malabathricum* samples exhibited similarities with minor variations in the combination of trichome types on the leaf surface, possibly influenced by ecological and geological factors. These leaf anatomical features of *M. malabathricum* can serve as valuable supporting characteristics for species identification, particularly in instances when flowers and fruits are not available for reference.

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