

Comparative Study on Leaf Anatomy in Selected *Garcinia* Species in Peninsular Malaysia

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ABSTRACT

A comparative study of leaf anatomy was attempted on *Garcinia* species in Peninsular Malaysia to identify anatomical features useful in species identification and classification. The species are *Garcinia mangostana* var. *mangostana*, *Garcinia mangostana* var. *malaccensis*, and *Garcinia celebica*. Leaves were collected from two different regions: Kuantan, Pahang and Kepong, Kuala Lumpur. The leaf anatomical study was done using the methods of leaf peeling, leaf venation, leaf cross-section, and scanning electron microscopy. The assessment of the leaf anatomy found that these three *Garcinia* species showed similarities in anatomical features, including the presence of paracytic stomata on the abaxial surface, a straight to wavy anticlinal wall of both adaxial and abaxial surfaces, a thick cuticle wax layer, the presence of druses, mucilage canal, petiole vascular bundle, the presence of collenchyma cells in the midrib, and also the presence of sclerenchyma cells in midrib and petiole. Meanwhile, the notable anatomical variation observed in this study included three types of midrib vascular bundles: the outline of the leaf margin, the presence of tanniferous idioblast, leaf marginal, and laminal venation as six types of epicuticular waxes present on epidermal surface. Overall, this study highlighted the anatomical features that are taxonomically valuable, which could be used to identify selected *Garcinia* species in Malaysia.

ARTICLE INFO

Article history:

Received: 11 November 2022

Accepted: 23 February 2023

Published: 16 May 2023

DOI: <https://doi.org/10.47836/pjtas.46.2.18>

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Keywords: *Garcinia*, *Garcinia celebica*, *Garcinia mangostana* var. *malaccensis*, *Garcinia mangostana* var. *mangostana*, leaf anatomy, taxonomy

INTRODUCTION

Plant systematics is established in the fundamental elements of evolution theory, and it purposely reveals and reconstructs phylogeny among species, genera, and families (Simpson, 2020). Plant anatomy serves as a tool that aids in plant systematics in terms of identification and classification based on specific characteristics. However, more than anatomical features are needed to implement the established classification, as it exclusively provides extra details to external morphological features (Metcalf & Chalk, 1950). It only can be used as secondary evidence to the initial classification based on morphological features. Given the significance of anatomy as a tool for systematics and taxonomy, acquiring anatomical data is essential to overcome classification and phylogeny challenges. Due to inadequate morphological details, plant anatomy sometimes delivers primary evidence for classification at the species or subspecies level (Maiti et al., 2012). Both morphological and anatomical data can obtain more accuracy in plant classification.

The characteristics of the leaf epidermis provide important information for identifying plant species within various plant families (Adedeji et al., 2007), such as trichomes, stomata types, stomata pore, stomata index, and subsidiary cells (Ullah et al., 2018). These micromorphological features assist species delimitation (Stefano et al., 2008). Several *Garcinia* species reported the absence of trichomes (Gupta et al., 2018; Priya et al., 2018). Some

features in the petiole cross sections, including vascular pattern, the appearance of sclerenchyma cells, and the presence of medullary vascular bundles and trichomes types, are helpful in species identification, especially in Dipterocarpaceae (Noraini et al., 2016). The structure, composition, and tissue arrangement in the leaf midrib provide excellent additional sources for plant delimitation (da Silva et al., 2015; Dalvi et al., 2014; Mantovani et al., 2009). Besides, the utilisation of plant anatomy is widespread for plant physiology and ecology (Sokoloff et al., 2021). Previous studies have incorporated the correlation of anatomies and environmental conditions, such as water stress on *Garcinia brasiliensis* (de Souza et al., 2013) and *Garcinia madruno* (Abreu et al., 2017), as well as *in vitro* gamma irradiation on *Garcinia mangostana* var. *mangostana* calli in combating photosynthesis deficiency (Qosim et al., 2011).

Garcinia is a notably large genus from Clusiaceae and subfamily Clusioideae Burnett (Stevens, 2007). Approximately 240 *Garcinia* species were discovered across Asia and Africa, predominantly in Southeast Asia, and 49 *Garcinia* species were distributed in Peninsular Malaysia (Nazre et al., 2007). The species range from small to large trees or shrubs in tropical forests (Nazre et al., 2018). Moreover, *Garcinia* can be distinguished through gummy latex and hard timber. It is also mentioned that *G. mangostana* var. *mangostana* (mangosteen), labelled the “Queen of Fruit”, is a well-known cultivated species in Southeast

Asia. Some of *Garcinia* species such as *G. mangostana* var. *mangostana* and *Garcinia mangostana* var. *malaccensis* can adapt better to tropical climates and organic rich soils (Lim, 2012a, 2012b). Meanwhile, the coastal nature of *Garcinia celebica* tolerates salty conditions well, especially in sandy, rocky, and acid clay soils or areas with heavy rainfall and drought environments (Lim, 2012c).

For *Garcinia* species, the classification and phylogeny have been well constructed based on the morphological and genetic features from previous studies over the decades. The morphological features are widely documented by including the most important parts: inflorescences, stamens, and fruits in *Garcinia*. The recognition of *Garcinia* species is also possible through vegetative features such as the leaf, particularly in *G. mangostana* var. *mangostana* (Nazre et al., 2018). However, Nazre et al. (2018) also stated that leaf features must be carefully treated for species isolation due to the convergence of vegetative features, as they inaccurately showed upper-level relationships in all instances. Besides, the anatomical studies of *Garcinia* still need to be widely utilised, and only a few pieces of data were documented recently. Additional material, such as leaf anatomy, can provide more evidence and justification for species identification in *Garcinia*. Thus, this study aims to identify the leaf anatomical features of the selected *Garcinia* species in Malaysia, which are *G. mangostana* var. *mangostana*, *G. mangostana* var. *malaccensis*, and *G. celebica*.

MATERIALS AND METHODS

Plant Collection and Preparation

The study was conducted on three selected *Garcinia* species, namely *G. mangostana* var. *mangostana*, *G. mangostana* var. *malaccensis*, and *G. celebica*. The leaves samples were collected from Glasshouse Nursery Complex at International Islamic University Malaysia (IIUM), Kuantan Campus, Pahang and Forest Research Institute Malaysia (FRIM), Kepong, Kuala Lumpur. The leaves samples were then compressed and dried in the oven for approximately two weeks. The voucher specimens were preserved in the IIUM Herbarium for future reference and analysis.

Leaves Peeling

The fresh leaves samples at the epidermal surface of the abaxial and adaxial were scraped off using a sharp blade until the desired epidermis could be reached. The epidermal peels were placed on the glass slide and stained with safranin solution (System, Malaysia) for 5 min. The features of the epidermal surface were viewed under a light microscope (Leica ICC50 HD, Germany).

Leaves Venation

The fresh leaves samples in a petri dish were submerged in a basic fuchsin solution (Bendosen, Malaysia) as a clearing agent. The petri dish was placed in the oven at 60°C until the leaves samples were fully cleared. The cleared leaf sample was placed on a

glass slide, and the features of the veins were viewed under a light microscope (Leica ICC50 HD, Germany).

Leaves Cross Section

The fresh leaves parts (margin, petiole, midrib, and lamina) were sliced in a range of thickness (35-40 μm) using a sliding microtome. The sliced leaves samples were stained in safranin (System, Malaysia) and Alcian blue (Sigma, USA) for approximately 5 min for each staining. The stained leaves samples were dehydrated using alcohol (ethanol) solutions at different concentrations (50, 70, 95, and 100%). The method, including slicing, staining, and dehydration, were modified following Johansen's (1940) and Sass's (1958) recommendations. The samples were placed on a slide and mounted in Euparal. Anatomical images were captured using the LAS EZ software (version 3.0.0) attached to a microscope (Leica ICC50 HD, Germany). The anatomical features were described properly.

Scanning Electron Microscopy

The selected leaf samples were collected from the dried herbarium samples. The lamina part from the samples was excised in 1 cm^2 measurements, attached to aluminium stubs, and mounted on a mounting holder. The mounted samples were sputter-coated with a film layer of gold. The sample's notable features, such as waxes and stomata structure, were observed under a scanning electron microscope Zeiss Model EVO 50 (Germany).

RESULTS AND DISCUSSION

The findings of this study showed that all three *Garcinia* species shared eight common anatomical features, which are the presence of paracytic stomata on the abaxial surface (Figure 1A), a straight to wavy anticlinal wall of both adaxial and abaxial surfaces (Figure 1B), a thick cuticle wax layer (Figure 1C), the presence of druses (Figure 1D), mucilage canal (Figure 1E), petiole vascular bundle (Figure 1F), the presence of collenchyma cells in midrib (Figure 1G), as well as the presence of sclerenchyma cells in midrib and petiole (Figure 1H).

This study also reported the notable variations that can be used to differentiate all three *Garcinia* species studied. First, three types of midrib vascular bundles were observed in the three *Garcinia* species in this study (Table 1). The midrib of the vascular bundle consists of a main vascular bundle (closed system with a non-continuous ring of the vascular bundle and O-shaped) and a medullary vascular bundle (opened system with a continuous ring of the vascular bundle and U-shaped), which can be found in both *G. mangostana* var. *mangostana* (Figure 2A) and *G. celebica* (Figure 2B). These two species only varied in the number of additional vascular bundles (opened system with continuous rings of vascular bundles). Meanwhile, the medullary vascular bundle was not observed in *G. mangostana* var. *malaccensis* (Figure 2C). Another variation is the outline of the leaf margin (Table 2). *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* showed a 20° curved downward pattern toward the abaxial

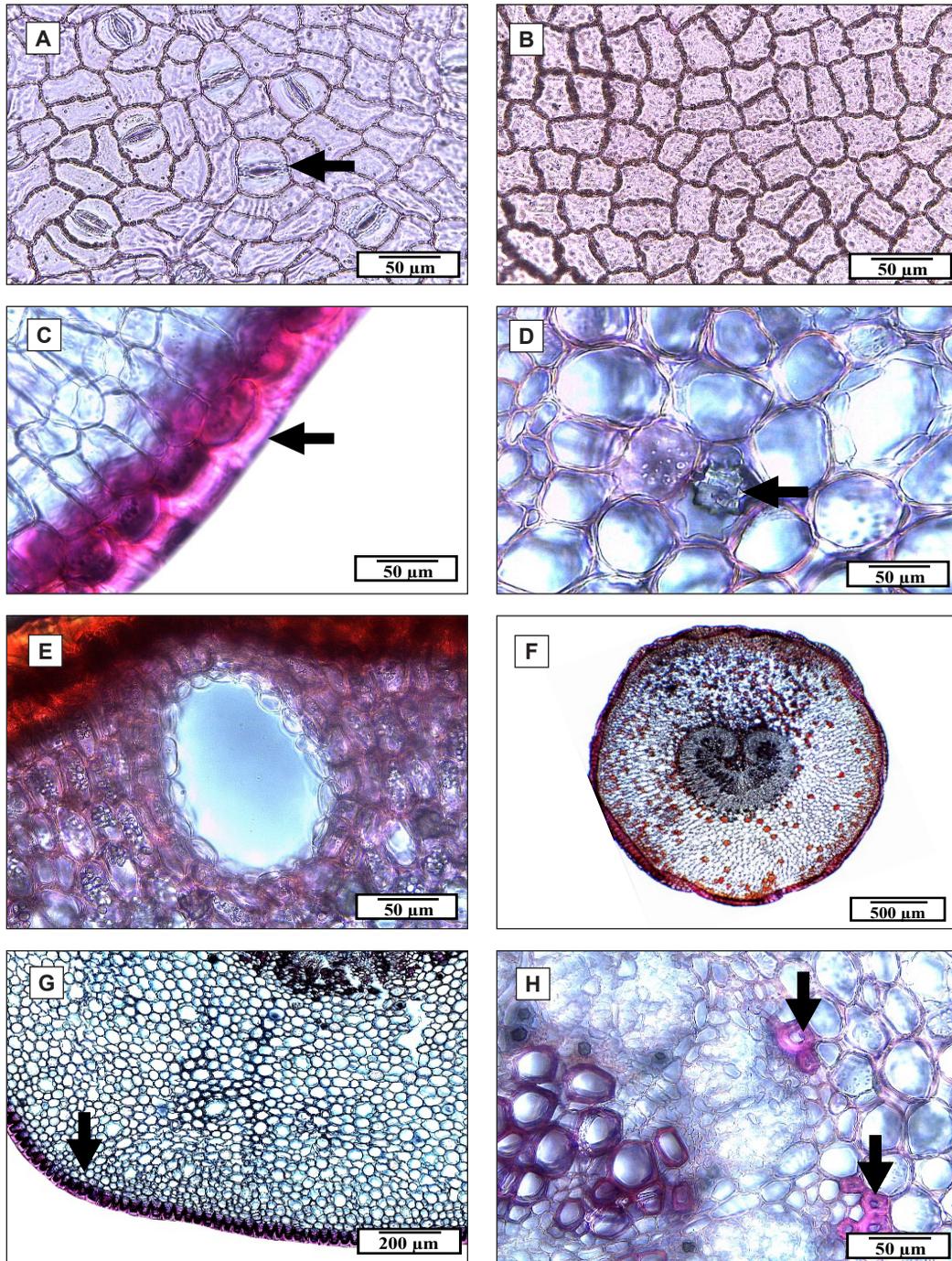


Figure 1. Common features of leaf anatomy and micromorphology in *Garcinia*. (A) Presence of paracytic stomata (black arrow), (B) Straight to the wavy anticlinal wall of adaxial and abaxial surfaces, (C) Presence of thick cuticular wax (black arrow), (D) Presence of druses (black arrow), (E) Presence of mucilage canal, (F) Petiole vascular bundle, (G) Presence of collenchyma cells in midrib (black arrow), and (H) Presence of sclerenchyma cells in midrib and petiole (black arrow)

Table 1
The identification and classification of midrib vascular bundles

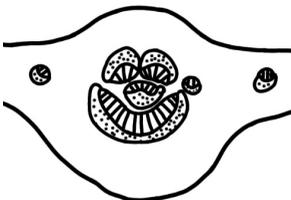
Type	Description	Species	Illustration
1	Closed system (non-continuous ring of the vascular bundle) with O-shaped, one medullary vascular bundle (opened system with a continuous ring of the vascular bundle) with U-shaped, five additional vascular bundles (opened system with continuous rings of vascular bundles)	<i>Garcinia mangostana</i> var. <i>mangostana</i>	
2	Closed system (non-continuous ring of the vascular bundle) with O-shaped, four additional vascular bundles (opened system with continuous rings of vascular bundles), no presence of medullary vascular bundle	<i>Garcinia mangostana</i> var. <i>malaccensis</i>	
3	Closed system (non-continuous ring of the vascular bundle) with O-shaped, one medullary vascular bundle (opened system with a continuous ring of the vascular bundle) with U-shaped, three additional vascular bundles (opened system with continuous rings of vascular bundles)	<i>Garcinia celebica</i>	

Table 2
The classification and description of the outline of the leaf margin

Type	Description	Species	Illustration
1	Tapered with a blunt end, 20° curved downward towards the abaxial epidermis. The size decreases gradually toward the edge of the margin	<i>Garcinia mangostana</i> var. <i>mangostana</i> <i>Garcinia mangostana</i> var. <i>malaccensis</i>	
2	Tapered with a blunt end, straight. The size decreases gradually toward the edge of the margin	<i>Garcinia celebica</i>	

epidermis (Figure 2D), while *G. celebica* showed a straight pattern (Figure 2E).

Next is the presence of tanniferous idioblast (Figure 2F), which can be found only in *G. celebica* and *G. mangostana* var. *malaccensis*. Furthermore, complete leaf

marginal venation (Figure 2G) was observed in *G. mangostana* var. *mangostana*, while incomplete venation (Figure 2H) was found in *G. mangostana* var. *malaccensis* and *G. celebica*. For lamina venation, minority opened, and majority closed venation with

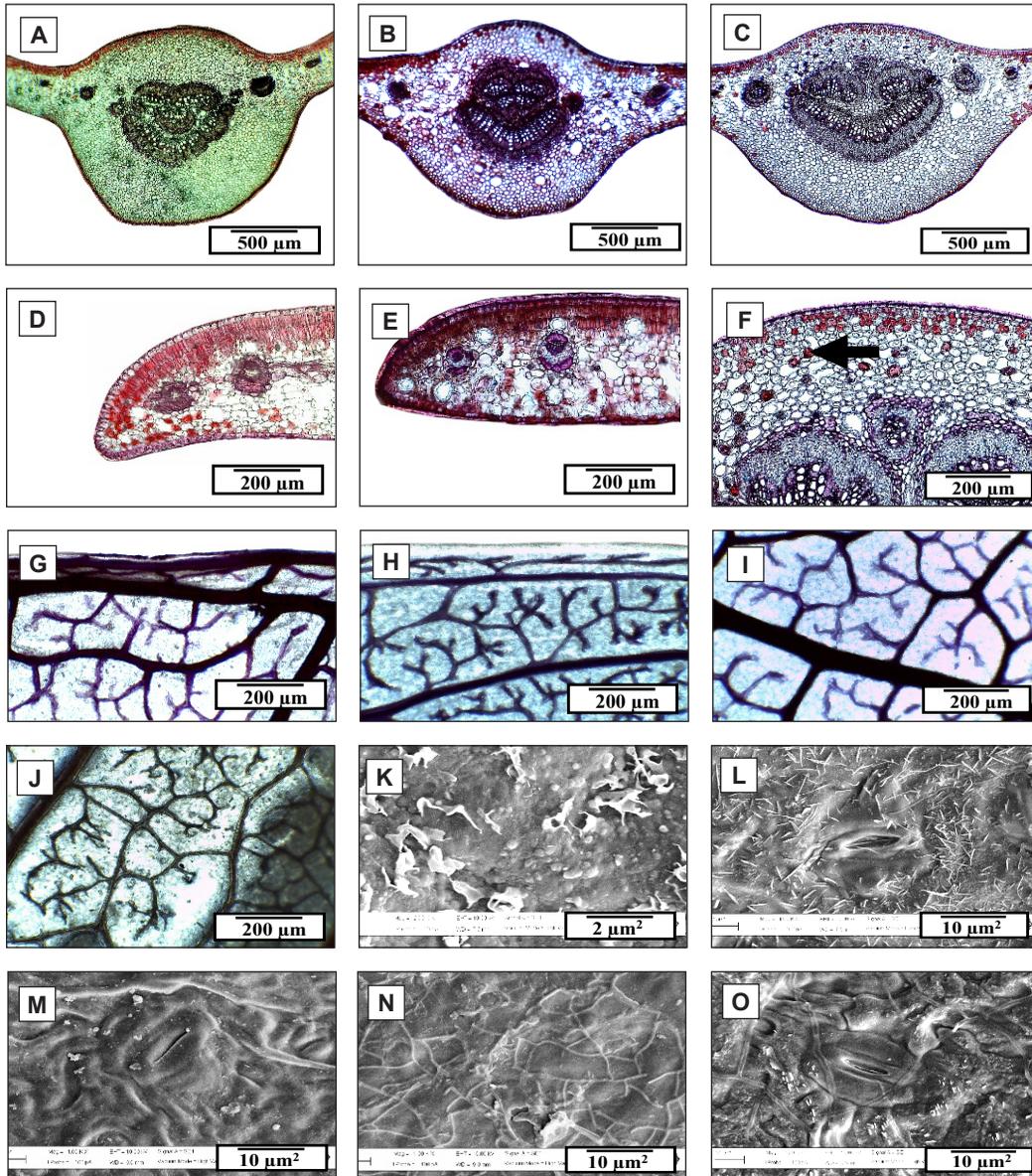


Figure 2. Variation characteristics of leaf anatomy and micromorphology in *Garcinia*. (A) Midrib of *Garcinia mangostana* var. *mangostana*, (B) Midrib of *Garcinia celebica*, (C) Midrib of *G. mangostana* var. *malaccensis*, (D) Marginal leaf outline of *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis*, (E) Marginal leaf outline of *G. celebica*, (F) Presence of tanniferous idioblast (black arrow) in *G. mangostana* var. *malaccensis* and *G. celebica*, (G) Complete marginal venation in *G. mangostana* var. *mangostana*, (H) Incomplete marginal venation in *G. mangostana* var. *malaccensis* and *G. celebica*, (I) Lamina venation with minority opened and majority closed with swollen tracheid in *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis*, (J) Lamina venation with minority closed and majority opened with swollen tracheid in *G. celebica*, (K) Irregular platelets cuticular wax on leaf abaxial of *G. mangostana* var. *mangostana*, (L) needle-like cuticular wax on abaxial part of *G. mangostana* var. *mangostana*, (M) Smooth layer cuticular wax on abaxial part of *G. mangostana* var. *malaccensis*, (N) Crust and granules cuticular wax on leaf adaxial of *G. mangostana* var. *malaccensis*, and (O) flakes cuticular wax on leaf abaxial of *G. celebica*

swollen tracheid (Figure 2I) was reported in *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis*, while minority closed and majority opened with swollen tracheid was seen in *G. celebica* (Figure 2J). Lastly, six types of epicuticular waxes presented on both epidermal surfaces were reported in all *Garcinia* species studied, which are irregular platelets (Figure 2K), needle-like wax (Figure 2L), smooth layer (Figure 2M), granules (Figure 2N), crust (Figure 2N), and flakes (Figure 2O). The variation in terms of epicuticular wax in *Garcinia* species in this study was summarised in Table 3.

Watson (1962) stated that the stomata arrangement and distribution are taxonomically essential above the species level. The stomatal distribution on the leaf surface and the type of stomata were similar among all *Garcinia* species in this study. All *Garcinia* species showed hypostomatous stomata, which means the stomata are distributed on the leaf's lower surface (abaxial part). The distribution of stomata is affected by the surrounding environment, especially in mesophytic habitats. In adapted aerial habitats, plants with hypostomatous stomata usually reduce water loss (Stebbins & Khush, 1961). The type of stomata is significant in determining numerous ranks,

establishing the linkage among distinct taxa, and clarifying taxonomic challenges (Baranova, 1992). Stomata are determined into various shapes through the guard's position and neighbouring cells' position. Van Cotthem (1970) listed several types of stomata, such as anomocytic, anisocytic, paracytic, diacytic, actinocytic, cyclocytic, and tetracytic. Paracytic stomata are identified when the stomata are enclosed by two subsidiaries, which are arranged side by side to the stomatal pore and the guard cells. This study found that all *Garcinia* species shared similar paracytic types of stomata. The paracytic pattern observed in *G. mangostana* var. *mangostana* complements the finding from the previous research done by Priya and Hari (2019). Hypostomatous and paracytic stomata were also reported on *Garcinia brasilliensis* (Cardoso et al., 2013), *Garcinia cambogia* (Sreelakshmi et al., 2014), *Garcinia zhangpuensis* (Wang et al., 2018), *Garcinia dulcis* (Begum, 2020), and *Garcinia atroviridis* (Susilowati et al., 2022). These findings supported the evidence that these two features of stomata are common in the *Garcinia* genus (Pathirana & Herat, 2004) and the Clusiaceae family (Metcalf & Chalk, 1950).

Cutler et al. (1978) highlighted that plant species could be identified through

Table 3
The identification and classification of epicuticular wax

Plant species	Types of cuticular wax	
	Adaxial	Abaxial
<i>Garcinia mangostana</i> var. <i>mangostana</i>	Irregular platelets, granules	Smooth layer, needle-like wax
<i>Garcinia mangostana</i> var. <i>malaccensis</i>	Crust, granules	Smooth layer, granules
<i>Garcinia celebica</i>	Crust, granules	Crust, flakes, granules

leaf epidermis characterisation. Shah et al. (2018) mentioned that the structure and pattern of anticlinal walls are believed to be a form of environmental adaptation, given that xerophytes plant located in a dry area typically have straight walls, while mesophytic species located in average to hot areas typically have sinuous walls (Gifford & Foster, 1989). The findings of this study showed that all *Garcinia* species exhibited straight-to-wavy anticlinal walls on the abaxial and adaxial epidermis. According to Pathirana and Herat (2004), all *Garcinia* taxa for the adaxial and abaxial epidermis consist of rectangular to square cells. A similar pattern was also observed in *G. zhangpuensis* (Wang et al., 2018), *Garcinia indica*, and *Garcinia livingstonei* (Priya et al., 2018). The types of epidermal cells which are straight, undulated, and sinuate observed in the *Tomovita* species can be utilised in the phylogenetic study of morphological features and determination of linkages across species (Gahagen, 2015). In *Garcinia* cases, epidermal cells of *G. dulcis* (Begum, 2020) and *G. madruno* (Abreu, 2017) showed sinuosity, while *G. cambogia* exhibited undulating to irregular cells (Sreelakshmi et al., 2014). An epidermal anticlinal wall pattern can be a suitable anatomical feature for species delimitation in *Garcinia*.

Furthermore, all *Garcinia* species in this study exhibited a thick cuticular wax in the leaf midrib, petiole, lamina, and margin. Metcalfe and Chalk (1950) mentioned that cuticle thickness is essential to characterise *Garcinia* species. Data

collected on the Sri Lanka *Garcinia* species included dorsiventral leaves and a thick cuticle (Pathirana & Herat, 2004). Other *Garcinia* species, such as *G. livingstonei* and *G. indica*, also showed thick cuticles on the leaf midrib, petiole, and lamina (Priya et al., 2018; Priya & Hari, 2019), except for *G. zhangpuensis*, which has a thin cuticle on the leaf abaxial part (Wang et al., 2018). This study proposed a thick cuticle wax to serve as a delimiting feature in the examined *Garcinia* species. The presence of cuticles attests to the interaction between plants and the environment. A previous study by Abreu et al. (2017) found thick cuticle wax on *G. madruno* under flooded conditions. They pointed out that this condition led to low water potential (Medri et al., 2011); thus, thick wax offered more protection to *G. madruno* (Abreu et al., 2017).

This study observed abundant mucilage canals or secretory cavities in all parts of the leaf cross-section. Mucilage canals were widely distributed surrounding the tissues of the vascular bundle, parenchyma, spongy mesophyll, and palisade mesophyll. This finding was justified by the report of Metcalfe and Chalk (1950). They also mentioned that the distribution of the secretory canal is important for characterising the *Garcinia* species. As Nnamani and Nwosu (2012) mentioned, the mucilage canal is used in closed and distinct related families since it possesses taxonomic value (Vieira et al., 2001). The taxonomic value of secretory cavities is demonstrated in numerous genera of the angiosperm families (Metcalfe & Chalk, 1957). The appearance of mucilage

canals was reported in *G. brasiliensis* (Cardoso et al., 2013) and *Garcinia kola* (Nnamani & Nwosu, 2012). Meanwhile, the presence of this feature was restricted to *G. dulcis* (Begum, 2020).

The presence of calcium oxalate crystal, commonly in a woody plant (Wu & Huang, 1997), justified the abundance of druses observed in all *Garcinia* species studied. This study found that the druses (Figure 1D) exhibited compact and spherical aggregates of angular crystal appearance. Beck (2010) explained that druses are commonly found in plants beside raphides, which appear as needle-like crystals. The presence of druses was observed in every tissue of the leaf cross-section. However, it was restricted to the leaf margin of *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis*. Druses could be used as a diagnostic character to identify *Garcinia* species, as observed in *Garcinia morella*, *Garcinia spicata* (Pathirana & Herat, 2004), *G. madruno* (Abreu et al., 2017), *G. indica* (Priya & Hari, 2019), *Garcinia dioica* (Wulansari et al., 2020), and *G. dulcis* (Begum, 2020). All of these *Garcinia* species exhibited a similar appearance of druses. Oxalate removal from the metabolic process, calcium storage, light regulation, and physical protection are all related to the occurrence of crystals (Franceschi & Nakata, 2005).

Moreover, a similar pattern of the vascular bundle in the petiole (Figure 1F) was observed in all *Garcinia* species in this study. Pathirana and Herat (2004) described the pattern of the petiole vascular bundle as consisting of a single arc-pattern collateral

strand, open on the adaxial part with incurved distal ends that almost connect to form a complete tube. This pattern was also comparable according to the study reported by Priya and Hari (2019) on *G. mangostana* var. *mangostana*, where the vascular bundle consists of an incurved vascular strand with a narrow gap. A similar pattern of a vascular bundle in petiole was also found in other *Garcinia* species, which are *Garcinia talbotii* (Palkar et al., 2017) and *G. indica* (Priya et al., 2018). Some *Garcinia* species showed an incurved pattern but with a large gap on the adaxial part of the vascular bundle, such as *G. livingstonei* (Priya et al., 2018) and *G. spicata* (Palkar et al., 2017). The pattern of the vascular bundle is utilised to delimit the genus taxa in Clusiaceae (D'Arcy & Keating, 1979). Delimitation is crucial for allocating individuals to the same species or different species. In the Dipterocarpaceae family, the petiole vascular bundle is taxonomically essential to identify species and categorise different genera (Noraini et al., 2016).

The presence of collenchyma cells in the midrib is necessary for support against the strong wind (Amri et al., 2019) by facilitating bending to avoid vascular damage (Leroux, 2012). This statement could validate the presence of collenchyma cells in *G. celebica*, where the plants are commonly found in coastal areas (Nazre et al., 2018). In this study, about 2 to 3 layers of collenchyma cells were concentrated underneath the adaxial and abaxial epidermis in the midrib of all *Garcinia* species. This anatomical feature

could be used as a promising identification tool. Ibrahim et al. (2016) demonstrated the taxonomic significance of the number of collenchyma layers on two species, *Datura innoxia* and *Datura stramonium*. In the family Euphorbiaceae, the distribution of collenchyma is significantly important as the continuous rings of collenchyma were observed in the petiole of three *Acalypha* species (Tadavi & Badhane, 2014). The morphology of collenchyma was considered for a taxonomical study on Cucurbitaceae (Abbas et al., 2022).

Sclerenchyma cells include numerous cell types with thick secondary walls that will lignify in maturity (Dickison, 2000). In this study, sclerenchyma cells were scattered as smaller patches encompassing vascular bundles in the petiole and midrib of all *Garcinia* species. However, these cells were absent in the midrib of *G. celebica* only. These patches of sclerenchyma cells were observed in *Garcinia pedunculata* (Gupta et al., 2018) and most Euphorbiaceae species studied (Tadavi & Bhadane, 2014). According to Noraini et al. (2016), the appearance of sclerenchyma cells was a notable feature in the petiole vascular bundle. Besides, the selected *Parashorea* species can be identified by the appearance of sclerenchyma cells surrounding the main and additional vascular bundles in the lamina (Noraini & Cutler, 2009). Sclerenchyma layers were included as a diagnostic feature of taxonomy value in Cucurbitaceae (Abbas et al., 2022). Noor-Syaheera et al. (2015) stated that both sclerenchyma and collenchyma cells act

as structural support but vary in elasticity (Coyle, 2004). Sclerenchyma cells play a role in ecological adaptation under seasonal water shortage, soil nutrient deficits, leaf protection against herbivores, or leaf carbon uptake improvement, as highlighted by Edwards et al. (2000).

The previous study by Pathirana and Herat (2004) delivered six main groups of midrib vascular bundles that could be utilised to distinguish the *Garcinia* taxa in Sri Lanka. They also described the pattern of the midrib vascular bundle in *G. mangostana* var. *mangostana*, which consisted of two adaxial and abaxial vascular arcs opposing each other with a middle vascular plate, each individually encircled by a bundle sheath. A similar pattern was observed in *G. mangostana* var. *mangostana*, as seen in Table 1. Table 1 shows three different types of midrib vascular bundles that could be used to separate the *Garcinia* taxa individually. The presence of a medullary vascular bundle and the number of additional vascular bundles were two key points used to distinguish these *Garcinia* species studied. Priya et al. (2018) also described three different shapes of the midrib's vascular zone, including the appearance of cowry, owl, and cordate in *G. mangostana* var. *mangostana*, *G. livingstonei*, and *G. indica*, respectively. Noor-Syaheera et al. (2015) reported multiple types of midrib vascular bundles (opened system) in selected taxa in Acanthaceae by including the shape of the vascular bundle (O-shaped, V-shaped, and U-shaped) and the number of separated vascular bundles. Numerous studies have

documented the systematic importance of the pattern of vascular bundles, including those on *Parashorea* (Noraini & Cutler, 2009) and the family Rhizophoraceae (Nurnida, 2012). Thus, it is justifiable that this feature is taxonomically significant.

Furthermore, this study suggested that the outline of the leaf margin could be used for species delimitation, as seen in Table 2. This study revealed two different patterns of a marginal outline: curved downward and straight. Similarly, these two patterns were also reported in certain species under the family Anacardiaceae (Norfaizal & Latiff, 2013) and selected *Mangifera* species (Tipmontiane et al., 2018). Noraini et al. (2012) revealed that two shapes (rounded and pointed) of the marginal outline act as diagnostic features in the selected *Johannesteijsmannia* species (Arecaceae). The comparative study based on the marginal leaf outline is not widely reported in *Garcinia*. A similar case happened in Orchidaceae because the marginal leaf outline is unrecognisable for anatomical comparison compared to midrib and petiole (Raffi et al., 2019). Raffi et al. (2019) also proposed that the marginal leaf outline should provide preliminary evidence for the characterisation and delimitation of *Vanilla* species.

The current study also showed that tanniferous idioblasts were scattered within the parenchyma tissue of the petiole and midrib except in *G. mangostana* var. *mangostana*. However, the absence of tanniferous idioblast in *G. mangostana* var. *mangostana* was contrary to evidence

provided by Pathirana and Herat (2004), where these phenolic idioblasts were highly distributed in this respected species. Regardless, this feature could isolate *G. mangostana* var. *mangostana* from other species in this study. The abundance of tanned cells in the family Clusiaceae was considered by Metcalfe and Chalk (1957) as one of the diagnostic keys besides secretory canals. The distribution pattern of tanned cells in selected species, particularly *G. kola*, may be taxonomically useful by considering the intensity of the cells (Nnamani & Nwosu, 2012). The occurrence of tanniferous idioblast was one of the taxonomically valuable features in the petiole of the *Microcos* genus in the family Malvaceae (Nurul-Aini et al., 2013). This feature may be applicable for identifying and classifying species. Tanniferous idioblast is also linked with protection against stress (da Silva Lobato et al., 2020).

Leaf venation is linked with plant evolution and is taxonomically essential for plant systematics (Hickey, 1973). The architectural features of leaf venation are intensively studied due to their significance for systematic classification (Zetter, 1984), for instance, leaf venation patterns. This study identified two types of marginal venation, complete and incomplete, that can be potentially used to distinguish *Garcinia* species in this study. Complete marginal venation (Figure 2G) is visualised as higher vein orders attached to a vein that runs just beyond the margin, while incomplete venation (Figure 2H) is viewed as freely ending veinlets straight alongside

the margin (Badron et al., 2014). Similar types were also described in the *Ficus* genus (Badron et al., 2014) and the *Carallia* genus (Nurshahidah et al., 2011). Nurul-Aini et al. (2010) revealed that the two distinctive features: incomplete and complete in leaf venation, were significant for distinguishing the selected taxa, namely *Grewia* and *Microcos* (Grewioideae), respectively. This study also reported two different lamina venations with swollen tracheids in the *Garcinia* species studied; hence, these features can be important for classifying species. Leaf venation is considered an underutilised feature, but it has been used for species delimitation in Combretaceae by revealing intra- and intergeneric variation among all the species studied (Akinsulireta et al., 2020). Akinsulireta et al. (2020) also considered the areolar (lamina) venation and the number of veinlets endings useful to characterise the species. Incomplete areolar venation with swollen veinlet ending and closed areolar venation was reported for the *Carallia* species (Nurshahidah et al., 2011). Besides, the system of leaf venation mainly works on structural stability as well as water and solute transportation (Roth-Nebelsick et al., 2001).

Epicuticular waxes are also taxonomically significant for species identification and characterisation, as confirmed by Barthlott (1998). Several attempts have been made to utilise epicuticular wax as taxonomy characteristics to isolate the group of species within a genus or family (Maffei, 1996; Mimura et al., 1998). The irregular granules, flakes, smooth layer, crust, and

needle-like epicuticular wax exhibited by each *Garcinia* species, as seen in Table 3, could distinguish these three species. The variation of epicuticular waxes in terms of type, density, and distribution found in the selected *Ficus* species could contribute as extra material for taxonomy (Araújo et al., 2014). The granules type of epicuticular waxes in the *Ficus* species (Araújo et al., 2014) was similar to the *Garcinia* species in this study. The interspecific variation among the selected *Hypericum* species can be seen through abundant waxes, listed as granules, flakes, crust, layer, road, and filament. These features can also aid *Hypericum*'s taxonomy and evolutionary study (Perrone et al., 2013). Perrone et al. (2013) also stated that epicuticular waxes prevent water loss by improving water binding, lessening solar ray interference, and limiting mechanical impairment caused by fungi and insects (Eglinton & Hamilton, 1967).

CONCLUSION

The leaf anatomical features in this study are essential for identifying and classifying the *Garcinia* genus. This study's description of anatomical features might be valuable for plant systematics by providing additional evidence for classification. The findings of this study provide some common features, which are hypostomatic and paracytic stomata, anticlinal walls pattern, thick cuticle wax layer, druses, mucilage canal, petiole vascular bundle, collenchyma cells, and sclerenchyma cells in all three *Garcinia* species studied. This study proposed that these common features could be used for

species delimitation among the *Garcinia* genus. On the other hand, a variation of features was also detected, such as three types of midrib vascular bundles, marginal leaf outline, tanniferous idioblast, leaf venation, and six types of epicuticular waxes on epidermal surfaces. These features possess taxonomical value to distinguish *Garcinia* species. Overall, the findings of this study significantly supported the establishment of species identification and classification among species and genera.

ACKNOWLEDGEMENTS

The authors greatly appreciated all organisations involved in the Department of Plant Science, Kulliyyah of Science, Kulliyyah of Medicine for scanning electron microscopy (SEM) service, the International Islamic University of Malaysia, Kuantan, Pahang, for their guidance and advice. Our deepest gratitude is devoted to the Ministry of Higher Education and FRGS/1/2019/STG03/UIAM/02/2 (Project ID: 13507) for the financial support during this research.

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