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Taxonomic Significance of Leaf Micromorphology in Selected *Garcinia* from Peninsular Malaysia

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ABSTRACT
Garcinia is a notably large genus from Clusiaceae. This genus is approximately discovered across Asia and Africa, predominantly in Southeast Asia. The recent taxonomic revision revealed several changes in the species classification within the *Garcinia* taxa. Additionally, few data on the anatomical characteristics of *Garcinia* species in Malaysia have been documented recently. This study aims to identify the leaf micromorphological characters using scanning electron microscopy (SEM) on *Garcinia mangostana* var. *mangostana, G. mangostana* var. *malaccensis* and *G. celebica* in Peninsular Malaysia. The result of this study found several important characteristics in terms of stomatal size, stomatal shape as well as abaxial and adaxial cuticular sculpturing. In conclusion, this study proposed that the anatomical characteristics in these selected *Garcinia* species might have a significant taxonomic value that can be applied to species identification and classification.
Keywords: *Garcinia, Garcinia celebica, Garcinia mangostana* var. *malaccensis, Garcinia mangostana* var. *mangostana,* leaf micromorphology

ABSTRAK
Garcinia merupakan genus yang besar dari famili Clusiaceae. Genus ini boleh ditemui di Asia dan Afrika, terutamanya di Asia Tenggara. Semakan taksonomi terkini mendedahkan beberapa perubahan dalam klasifikasi spesies di dalam takson Garcinia. Beberapa data mengenai ciri anatomi spesies Garcinia di Malaysia juga telah didokumenkan. Kajian ini bertujuan untuk mengenal pasti karakter mikromorfologi daun dengan menggunakan mikroskop elektron imbasan (SEM) pada Garcinia mangostana var. mangostana, G. mangostana var. malaccensis dan G. celebica di Semenanjung Malaysia. Hasil kajian ini mendapati beberapa ciri penting dari segi saiz stomata, bentuk stomata serta ukiran kutikula abaksial dan adaksial. Kesimpulannya, kajian ini mencadangkan bahawa ciri-ciri anatomi dalam spesies Garcinia terpilih ini mungkin mempunyai nilai taksonomi yang ketara yang boleh digunakan untuk pengenalpastian dan pengelasan spesies.
Kata Kunci: *Garcinia, Garcinia celebica, Garcinia mangostana* var. *malaccensis, Garcinia mangostana* var. *mangostana,* mikromorfologi daun

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INTRODUCTION

Plant anatomy studies the microscopic structure of cells and tissues, which are smaller than those that are easily visible to the naked eye. It emphasizes high-throughput screening and characterization of plant anatomy in a field-developed plant. Plant anatomy also aids in plant systematics by providing details of certain characteristics for plant identification and classification. However, anatomical characters alone cannot fully implement the established classification as it exclusively provides extra information on external morphological characteristics [1]. It can only be used as secondary evidence to support the initial classification made using morphological characters. In addition, anatomical data provide more accuracy and it tackles classification and phylogeny challenges for plant systematics.

Two hundred and forty species from the notably large genus, *Garcinia* were roughly discovered across Asia and Africa, predominantly in Southeast Asia and 49 *Garcinia* species were distributed in Peninsular Malaysia [2]. *Garcinia* is known from small to large trees or shrubs in tropical forests [3] with the presence of gummy latex and hard timber. Earlier research studies have shown that the classification of *Garcinia* in plant systematics is well established by implying the morphological and genetic characteristics over the years. However, a recent taxonomic review has revealed several changes within the *Garcinia* taxa. For example, *Garcinia malaccensis* (wild mangosteen) is limited to a variety of *G. mangostana* (mangosteen) due to similar morphology, particularly fruit and comparative molecular sequence data [3-4]. Recently, these 2 species are recorded as *Garcinia mangostana* var. *malaccensis* and *Garcinia mangostana* var. *mangostana*. Both are distinguished through fruit and flower morphological characters [3]. Taxonomic confusion has occurred on seashore mangosteen, namely *Garcinia hombroniana*, which was recently named *Garcinia celebica* due to misinterpretation in nomenclature and variable characters used for species delimitation [3]. The confusion is presumably caused by insufficient material studied or the unavailability of samples for morphological variation [5].

Besides, there is still limited reports of the anatomical studies on *Garcinia* in Malaysia. The additional material such as leaf anatomy perhaps can provide more evidence and justification for species identification in *Garcinia.* Leaf characters could be utilized for identifying *Garcinia* species [6], and this idea is supported by the pattern of glandular lines on the leaves [7]. Thus, this research aims to identify the leaf anatomical characters of the selected *Garcinia* species in Malaysia which are *G. mangostana* var. *mangostana, G. mangostana* var. *malaccensis* and *G. celebica* by using the scanning electron microscopy (SEM) technique.

MATERIAL & METHODS

The study was conducted on 3 selected *Garcinia* species, namely *G. mangostana* var. *mangostana, G. mangostana* var. *malaccensis* and *G. celebica*. The leaf samples were collected from Glasshouse Nursery Complex, IIUM Kuantan Campus and Forest Research Institute Malaysia (FRIM), Kepong. The leaf samples were then compressed and dried in the oven for approximately 2 weeks. The voucher specimens were kept and preserved in International Islamic University Malaysia Herbarium for future reference and analysis. The selected samples for scanning electron microscopy (SEM) were collected from the dried sample of the herbarium (Figure 1). The lamina part from the sample was excised in 1 cm^2 measurement, attached to aluminium stubs and mounted on a mounting holder. The mounted samples were sputter-coated with a film layer of gold. The notable features such as stomata structure and leaf

cuticular sculpturing in the sample were observed under the scanning electron microscope (SEM) Zeiss Model EVO 50.

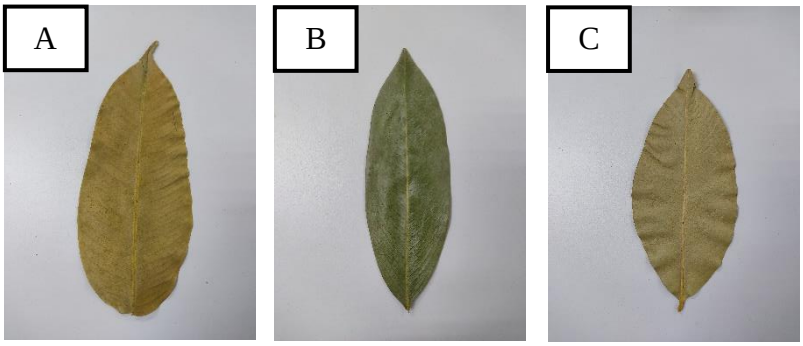

Figure 1: Leaf herbarium sample: (A) *G. mangostana* var. *mangostana*, (B) *G. mangostana* var. *malaccensis* and (C) *G. celebica*

RESULTS AND DISCUSSIONS

***G. mangostana* var. *mangostana*.**

Stomata: Epidermal and subsidiary cells cannot be distinguished, the cuticular rim is clear and raised, stomata elliptical in shape, size of stomata on abaxial surface: width (44.81 µm - 50.65 µm), length (35.78 µm - 40.16 µm). (Figure 2A). **Adaxial cuticular sculpturing:** Anticlinal wall and periclinal wall cannot be distinguished (Figure 2B). **Abaxial cuticular sculpturing:** Slightly distinguishable, periclinal walls raised into ridges and anticlinal walls sunken with striae present on some part of abaxial surface (Figure 2C).

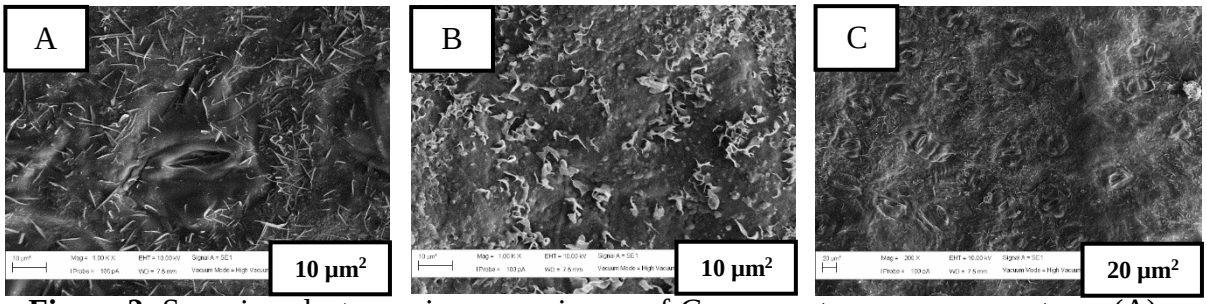

Figure 2: Scanning electron miscrocopy image of *G. mangostana* var. *mangostana*: (A) Stomata, (B) Adaxial leaf surface, and (C) Abaxial leaf surface. Scale: (A) 10 µm^2, (B) 10 µm^2, (C) 20 µm^2

G. mangostana* var. *malaccensis.

Stomata: Epidermal and subsidiary cells cannot be distinguished, the cuticular rim is clear and raised, stomata elliptical in shape, size of stomata on abaxial surface: width (38.98 µm - 40.08 µm), length (31.68 µm - 38.22 µm). (Figure 3A). **Adaxial cuticular sculpturing:** Anticlinal walls and periclinal wall cannot be distinguished (Figure 3B). **Abaxial cuticular sculpturing:** Distinguishable, periclinal walls raised into ridges and anticlinal walls sunken with striae present on some part of abaxial surface (Figure 3C).

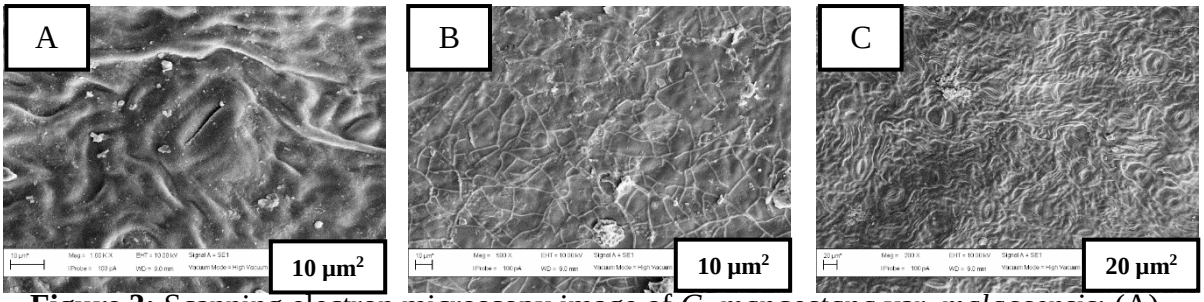

Figure 3: Scanning electron microscopy image of *G. mangostana* var. *malaccensis*: (A) Stomata, (B) Adaxial leaf surface, and (C) Abaxial leaf surface. Scale: (A) 10 µm^2, (B) 10 µm^2, (C) 20 µm^2

Garcinia celebica.

Stomata: Epidermal and subsidiary cells can be distinguished, the cuticular rim is clear and raised, stomata enclosed by a pair of subsidiary cells, stomata elliptical in shape, size of stomata on abaxial surface: width (35.64 µm - 47.34 µm), length (33.41 µm - 38.37 µm). (Figure 4A). **Adaxial cuticular sculpturing:** Anticlinal wall and periclinal wall are slightly distinguishable, periclinal walls raised into ridges and anticlinal walls sunken (Figure 4B). **Abaxial cuticular sculpturing:** Anticlinal wall and periclinal wall are slightly distinguishable periclinal walls raised into ridges and anticlinal walls sunken with striae present on some part of abaxial surface (Figure 4C).

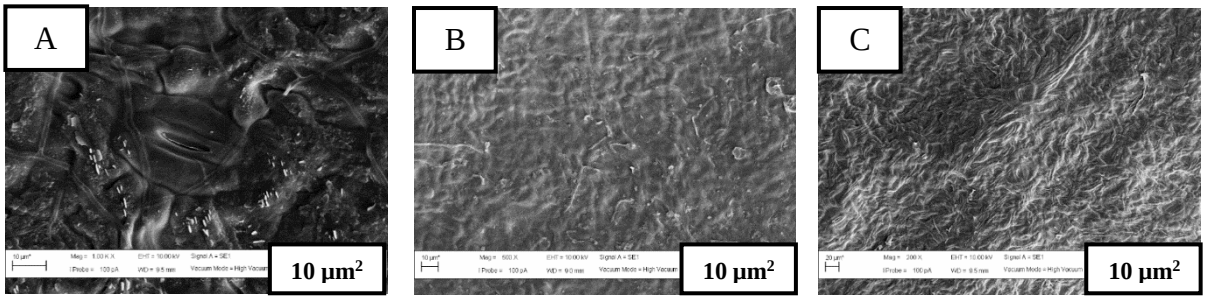

Figure 4: Scanning electron microscopy image of *G. celebica*: (A) Stomata, (B) Adaxial leaf surface, and (C) Abaxial leaf surface. Scale: (A) 10 µm^2, (B) 10 µm^2, (C) 20 µm^2

Taxonomy and phylogenetic studies have greatly utilized anatomical studies on leaf epidermal surfaces as the characters deliver beneficial material [8-10]. A study was done on leaf micromorphological characteristics as it showed the significant taxonomic value and provide valuable data to the currently available morphological characters used for identifying species. This study found leaf character, particularly the presence of stomata and leaf cuticular sculpturing might be useful in *Garcinia* species identification. The details of the stomata and leaf cuticular sculpturing were summarized in Table 1 and Table 2 respectively.

Stomata are essential components in phylogenetics studies as the characters are associated with the theory of plant origin and evolution as well as classification studies [11]. Stomata links with the physiological functions of plants, including photosynthesis, respiration, and transpiration [12] by working as the main channel for the exchanges of water and gas [13] between plants and environments and also nutrient absorption and digestion [14]. The presence of stomata is highly sparse in leaves compared to stems and roots Based on Table 1, both *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* showed similar observation by the nonvisible difference between neighbouring epidermal cells and subsidiary cells. This will indicate a close relationship shared by these two species as they closely resembled each other based on the morphology and molecular studies [4]. However, the position of subsidiary cells

was not obvious enough to determine the type of stomata. Hence, the other method such as leaf peeling and observation under light electron microscopy can be helpful for clear stomatal observation. Meanwhile; the difference between epidermal cells and subsidiary cells in *G. celebica* is well observed in this study. Two subsidiary cells are positioned parallel to the guard cells and the stomata are deeply entrenched. indicating that *G. celebica* exhibited paracytic type of stomata. Paracytic stomata are common in *Garcinia* genus [15] and Clusiaceae family [1]. A previous study found the highest occurrence of paracytic stomata (91.46%) was observed in plant species, particularly *Garcinia atroviridis* followed by anomocytic (6.02%), anisocytic (1.20%), and diacytic (1.20%) in 83 trees species studied in green open space located in Universitas Sumatera Utara (USU) campus [16]. Other *Garcinia* species, *Garcinia pedunculata, Garcinia lanceifolia, Garcinia morella* and *Garcinia xanthochymus* exhibited a similar paracytic stomatal type [17].

All 3 *Garcinia* species in this study showed an elliptical stomatal shape. A recently discovered *Garcinia* species, namely *Garcinia zhangpuensis* also revealed similar elliptical or rounded shapes of stomata [18]. Wang et al. (2018) [18] also concluded that this new *Garcinia* species thrived in humid, tropical or subtropical regions. Moreover, *G. mangostana* var. *mangostana* recorded the highest maximum value of width and length compared to the other two *Garcinia* species (Table 1). The widest stomata (37.62 µm) of *G. mangostana* var. *mangostana* was also reported in the previous study [16]. An important character such as stomata is purposely identified to define and classify species within the *Garcinia* taxa. These characteristics of stomata in this study can be used as diagnostic characters in plant systematics.

Table 1: The characteristics of leaf stomata in *Garcinia* species studied

Species	**Type**	**Shape**	**Width (Min – Max)**	**Length (Min – Max)**
G. mangostana var. *mangostana*	Subsidiary cells undistinguishable	Elliptical	44.81 µm - 50.65 µm	35.78 µm - 40.16 µm
G. mangostana var. *malaccensis*	Subsidiary cells undistinguishable	Elliptical	38.98 µm - 40.08 µm	31.68 µm - 38.22 µm
G. celebica	Paracytic	Elliptical	35.64 µm - 47.34 µm	33.41 µm - 38.37 µm

The finding of this study found several variations in terms of adaxial and abaxial sculpturing as summarized in Table 2. The top leaf surface (adaxial part) of both *G. mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* appeared to be smooth and no striation occurred except for *G. celebica*. On the other hand, the lower leaf surface (abaxial part) of all *Garcinia* species studied was certainly covered by cuticular striation. The structure of the anticlinal wall and periclinal wall were well visible with the aid of SEM. These two walls have different structures and shapes that had been described by the previous studies in another genus and species. Kim et al. (1999) [19] revealed that subgenus *Strobus* exhibited a fine granular surface of the periclinal wall and thin with tapering and rough apex of the anticlinal wall while subgenus *Pinus* possessed a granular periclinal wall and thick with rounded and smooth apex of the anticlinal wall. In Lecythidaceae family, the species showed psilate, striate and rarely granulate cuticular sculpturing, but these characteristics cannot be utilized for species identification [20]. Thammarong et al. (2014) [20] also concluded that there is a limitation in the taxonomic value of the leaf

epidermal characters in Lecythidaceae. Oladele (1983) [21] revealed that two main features of cuticular sculpture from the abaxial surface which are the macrorelief of the anticlinal and the microrelief of the periclinal provided no intermediary in hybrid *Cupressocyparis leylandii* where this species only showed smooth and small, fairly uniform and widely spaced granules. Another *Garcinia* species, *G. zhangpuensis* showed a straight anticlinal wall and smooth periclinal wall [18].

Numerous character traits of the cuticular membrane sometimes have less taxonomic value [22]. Typically, these mostly deal with the size and distribution of micropapillae, reticulations, and striations as observed under SEM [23]. Darók et al. (2000) [23] also stated that anticlinal undulations of leaf epidermal cells typically have significant taxonomic value and frequently characterise taxa at the species and genus levels as well as delimit subgroup and groups [24]. For the lowest taxonomic levels, the exterior periclinal wall`s curvature can be a valuable diagnostic characteristic. However, the limited variety of leaf micromorphology either as flat or convex epidermal cells are found all over the plant kingdom, resulting in the minimal systematic impact to be identified in these characters [23]. The systematic importance of cuticular sculptures for species delimitations is fairly limited, although they may be excellent diagnostic characters [24]. To add, the use of these leaf characters may or may not accurately represent the relationship among *Garcinia* species studied as the leaf cuticular documentation is severely limited. The use of leaf characters in isolation should be treated with caution due to the convergence of leaf characters which means no accuracy in indicating higher-level relationships in all instances [3]. Nazre et al. [3] also suggested that the identification of species can be done by employing single 'spot' characters or with the combination of characters of the leaves.

Table 2: The characteristics of leaf cuticular sculpturing in *Garcinia* species studied

Species	Adaxial cuticular sculpturing	Abaxial cuticular sculpturing
G. mangostana var. *mangostana*	Not distinguishable	Slightly distinguishable
G. mangostana var. *malaccensis*	Not distinguishable	Distinguishable
G. celebica	Slightly distinguishable	Slightly distinguishable

Countless lists of the taxonomically most useful characters have been created in a taxon, such as the one by Hickey and Wolfe (1975) [25] regarding leaf structure. Barthlott (1981) [26] believes that their substantial structural diversity makes them best ideal for classification between the levels of family and species. The application of leaf-surface characters has thus substantially supported the taxonomy of plants. The ability of a plant to create different phenotypes under different ecological conditions is indicated by the relationship between anatomy and environmental surrounding. For instance, most tropical plants have smooth leaf surfaces, whereas the majority of herbaceous plants feature sculptured surfaces [26]. The pattern of leaf sculpturing may link with the physical properties of the plant in terms of air absorption, water resistance and also thermal and electrical permeability of the leaf epidermal layer [27]. Airflow and light interception may influence the change in temperature caused by leaf microtopography in three-dimensional [28]. The morphology of the leaf surface is

also associated with the ability to carry out efficient photosynthesis. The removal of pollutants and dust from the plant surface is influenced by the texture of the leaf epidermis (29-30). This is because the attached particles prevent effective light absorption and any remaining water layer on leaf surfaces is likely to impede gas exchanges during photosynthetic respiration [27]

CONCLUSION

The result of this study revealed that the important characteristics which are stomata and leaf cuticular sculpturing can be valuable taxonomic tools to aid and support the evidence for species identification and classification. The common characteristics and variations observed, especially leaf stomata and cuticular sculpturing on both adaxial and abaxial surfaces were detected through scanning electron microscopy (SEM) in all *Garcinia* species in this study. The characteristics of cuticular sculpturing alone may or may not possess taxonomic significance but it certainly aids in regulating plant physical properties. Based on these comparisons, these anatomical characters, considered together, might be relevant for the accurate species identification and classification within the *Garcinia* taxa.

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