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Research

Taxonomic Value of Leaf Anatomical Characteristics in *Lepisanthes* (Roxb.) Leenh. (Sapindaceae) of Peninsular Malaysia

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ABSTRACT

Taxonomic value of leaf anatomical characteristics in *Lepisanthes* (Roxb.) Leenh. (Sapindaceae) of Peninsular Malaysia. Leaf anatomical study was carried out on five species of *Lepisanthes* (Roxb.) Leenh. (Sapindaceae), namely *L. amoena, L. fruticosa, L. rubiginosa, L. senegalensis,* and *L. tetraphylla*. The studies involved a cross-section method using sliding microtome on petioles, lamina, midribs, and leaf margins, leaf clearing method and leaf epidermis mechanical scrapping, and light microscope observation. The objective was to observe the taxonomic value of the leaf anatomical features. The results showed that there are three common generic features, nine features whose variations can be used for species differentiation, and three diagnostic features that can be used for species identification. The common generic features consist of the presence of a layer of collenchyma cells in the petioles and the presence of cells or mucilaginous canals in the midribs. The anatomical features with variations consist of features of outer shape and pattern of petiolar vascular tissue, outer shape and vascular tissue of midribs, leaf margin shape, presence of brachysclereid cells, and leaf venation type. The results showed that the anatomical features of leaves in *Lepisanthes* have taxonomic value, especially in species differentiation and recognition.

Key words: Leaf anatomical characteristics, *Lepisanthes*, Sapindaceae, Peninsular Malaysia

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INTRODUCTION

Sapindaceae is one of the major flowering plant families in the world. To date, it is estimated that there are 141 genera and 1,900 species that have been identified (van Welzen, 1999; World Flora, 2021; Puccio, 2024). According to Boonsuk and Chantaranothai (2016), the distribution of Sapindaceae species includes Tropical Africa, Madagascar, Sri Lanka, India, China (Hainan) Malesia, extending to New Guinea and as far north as Australia. This family is widely distributed and can mostly be found in tropical and subtropical regions with some specific genera being found in the subtemperate. A total of thirty-one (31) accepted species classified under the genus Lepisanthes, and five species that can be found in Peninsular Malaysia namely *L. amoena* (Hassk.) Leenh., *L. fruticosa* (Roxb.) Leenh., *L. rubiginosa* (Roxb.) Leenh., *L. tetraphylla* (Poir.) Leenh. and *L. senegalensis* (Vahl.) Radlk. (Turner, 1995; POWO, 2024). Species such as *L. fruticosa* and *L. rubiginosa* can be found planted in backyards, and small gardens while some species such as *L. amoena, L. tetraphylla,* and *L. senegalensis* still grow wild in their original habitats (Mohd Norfaizal & Masrom, 2014). This genus has been classified in the tribe Lepisanthae, subfamily Sapindoideae, and has a total number of 24 species, mostly in tropical Africa, Madagascar, southern Asia, Sri Lanka, China, Malesia and extending to eastern Australia (van Welzen, 1999). This genus is also usually found as a plant

under the canopy in primary and secondary forests, river banks, or coastal areas up to 1200 m above sea level. Species from this genus also have similar morphological characteristics and are sometimes difficult to determine if they are not accompanied by their fruits and flowers in the field. Therefore, the purpose of this study is to see the similarities, differences, and variations in leaf anatomy that can be used as additional data to help identify species in *Lepisanthes*.

MATERIALS AND METHODS

The study samples included a combination of fresh leaf specimens and herbarium samples obtained from the Herbarium of the Malaysian Agricultural Research and Development Institute (MDI), Selangor, the Herbarium of the Malaysian Forestry Research Institute (KEP), Selangor, and the herbarium, Universiti Kebangsaan Malaysia (UKMB). A complete list of species and information is listed in Table 1. A few taxa were studied using herbarium specimens and the dry leaves were boiled for 15 min. Boiled leaf samples were preserved in fixing solution A: A (Alcohol 70%; Acetic acid 30%) in a ratio of 1:3, and the same preservation method was also applied to the fresh specimens used for the leaf anatomical methods, part of petioles, midribs, leaf lamina and marginal were sectioned in a range of thickness (20 µm–40 µm) using sliding microtomes. The epidermal peels were conducted manually by scrapping off the underside of leaf surfaces using a razor blade. The leaf clearing involved the immersion of leaf lamina and margin into the Basic Fuchsin solution. All the anatomical procedures which also involved the staining and dehydration process followed the suitable modification methods by Johansen (1940) and Saas (1958).

Table 1. *Lepisanthes* studied specimens

Species	Specimens and their localities
L. amoena	Pahang, Jerantut, Sungai Pahang, Burkill, FRI 118693, 7.12.1924 (KEP); W.P. Kuala Lumpur, Taman Awam Kuala Lumpur, Ahmad, FRI 118695, 7.7.1919 (KEP); Selangor, Ladang Kepong, Abdul Hamid FRI 118648, 1.3.1934 (KEP); W.P. Kuala Lumpur, Taman Awam Kuala Lumpur, Jaamal FRI 118696, 5.4.1926 (KEP); Pahang, Kuala Lipis, Kg. Awah, Mohd Norfaizal & Masrom, MDI 2513, 8.4.2011 (MDI); Perak, Tanjung Malim, Shohimee, MDI 4405, 17.7.1984 (MDI); Selangor, MARDI Serdang, Mohd Norfaizal, MNFG 7676, 21.7.2015 (MDI)
L. fruticosa	W.P. Kuala Lumpur, Hutan Simpan Bukit Nanas, Abdul Rahman FRI 118690, 26.2.1918 (KEP); Johor, Gunung Panti, Everett FRI 8684, 21.1.1970 (KEP); Johor, Gunung Panti, Kochummen FRI 115591, 5.3.1980 (KEP); W.P. Kuala Lumpur, Jalan Nakhoda Yusoff, Kochummen FRI 119270, 15.2.1981 (KEP); Terengganu, Kuala Berang, Mohd Norfaizal, MNFG 2323, 18.5.2015 (MDI); Johor, Pontian, Mohd Norfaizal, MNFG 2324, 20.6.2015 (MDI); Selangor, MARDI Serdang, Mohd Norfaizal, MNFG 2328, 19.7.2015 (MDI)
L. rubiginosa	Johor, Pulau Aur, Henderson FRI 115558, 28.4.1927 (KEP); Kedah, Yan, Gunung Jerai, Saw FRI 85678, 29.3.2011 (KEP); Perak, Hutan Simpan Piah, Mohd Isa & Jaamal FRI 11554, 15.2.1935 (KEP); W.P. Kuala Lumpur, Jalan Belengkok, Hamid FRI 11556, 20.8.1919 (KEP); Terengganu, Kuala Berang, Mohd Norfaizal, MNFG 2310, 18.5.2015 (MDI); Selangor, Serdang, Mohd Norfaizal, MNFG 1963, 9.5.2014 (MDI); Selangor, Seri Kembangan, Jalan Puchong, Mohd Norfaizal, MNFG 1964, 9.5.2014 (MDI)
L. senegalensis	Negeri Sembilan, Bahagian 8, Gunung Angsi, Loh Hong Shing FRI 17357, 21.2.1971 (KEP); Perak, Hutan Simpan Gunung Bubu, Chan FRI 17595, 21.3.1971 (KEP); Pahang, Kampung Aur, Suppiah FRI 14769, 18.2.1971 (KEP); Negeri Sembilan, Hutan Simpan Pasoh, Mat Asri FRI 21664, 2.3.1976 (KEP); Johor, Pontian, Benut, Kamaruddin Salleh MDI 6175, 10.9.1988 (MDI)
L. tetraphylla	Negeri Sembilan, Jelebu, Kg. Pantai, La Frankie FRI 6006, NIL (KEP); Terengganu, Ulu Terengganu, Kuala Petang, Sungai Trenggun, Cockburn FRI 8427, 3.6.1968 (KEP); Terengganu, Bukit Bauk, Bahagian 20B, Chelliah FRI 104370, 19.6.1967 (KEP); Hutan Simpan Gunung Lesong, Yap FRI 15491, 24.8.1928 (KEP); Perak, Manong, Bukit Bubu, Anthony FRI 3914, 7.5.2005 (KEP); Terengganu, Tasik Kenyir, Pulau Tekak Besar, Mohd Norfaizal, & Masrom, MDI 7457, 8.7.2012 (MDI); Terengganu, Tasik Kenyir, Pulau Tekak Besar, Mohd Norfaizal, & Masrom, MDI 7458, 9.7.2012 (MDI)

RESULTS

The results showed the presence of several common characteristics of the genus and consistent variations in leaf anatomy. Common features shared by all five species of this study can be used as genus characteristics within the family. In general, general anatomical characteristics of the genus *Lepisanthes* are the presence of a layer of collenchyma cells in the petiole and the presence of mucilage cells or vessels in the petiole.

The genus *Lepisanthes* shows a high variation in the external shape of the petiole for each species studied. There are four outline shapes of petioles have been identified in this study. *Lepisanthes amoena* shows the outline shape of the petiole - adaxial surface: convex and unevenly indented with a small lobe projection on the left adaxial surface; abaxial surface: convex and indented shape *L. fruticosa* shows the external shape of the petiole - adaxial surface: convex, with lobe-shaped projections on the left and right of the petiole; abaxial surface: 3/4 circle shape, *L. rubiginosa* and *L. senegalensis* show petiole outline shape - adaxial surface: ½ circle; abaxial surface: ½ circle; whole round while *L. tetraphylla*

shows the exterior of the petiole - adaxial surface: almost flat and has a lobe-like projection towards the left side of the petiole; abaxial surface: ¾ oval shape) (Figure 1) .A previous study by Olowokudejo (1987) on several species and genera in Cruciferae has also identified differences in terms of external petiole shape that can be used in differentiating the studied species. Therefore, it clearly shows the variation in the outer shape of the petiole gives significant value in the identification of species in the genus *Lepisanthes*.

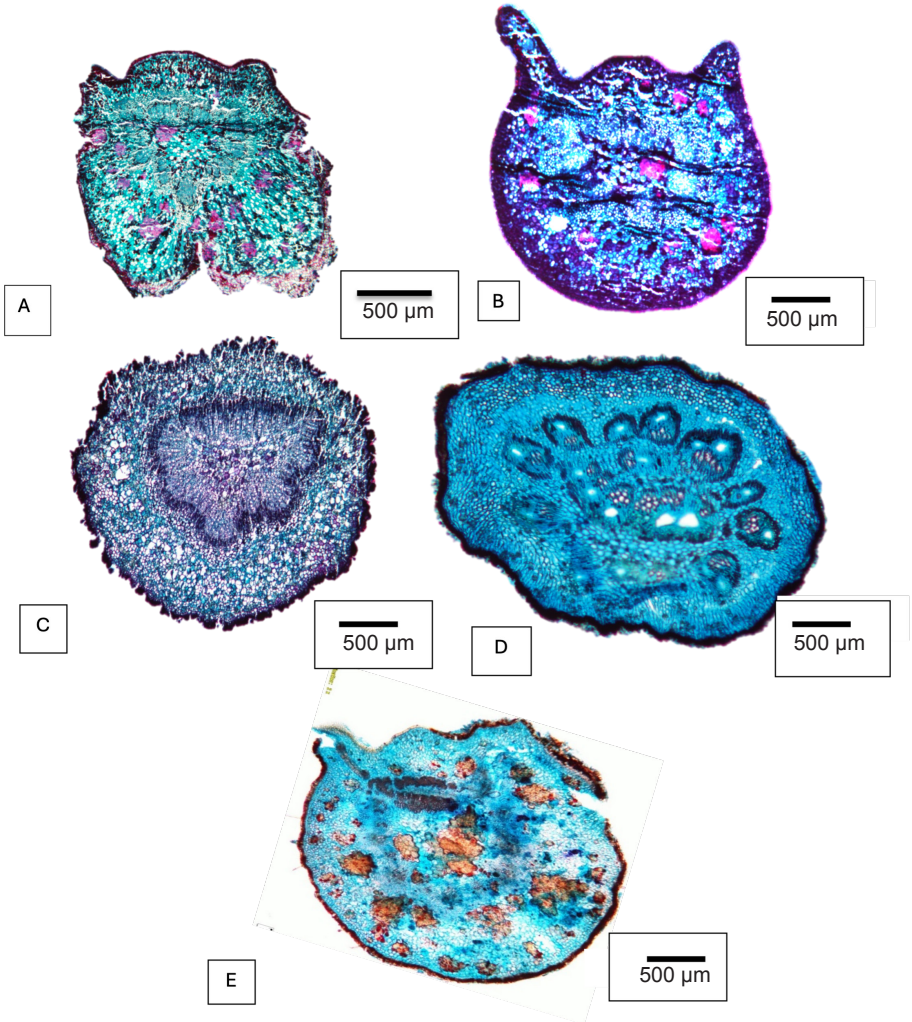

Fig. 1. Transverse section of *Lepisanthes petiole*: A) *Lepisanthes amoena*, (B) *L. fruticosa*, (C) *L. rubiginosa*, (D) *L. senegalensis*, (E) *L. tetraphylla*. Scale: A-E: 500 µm.

Analysis of the midrib outline shape in *Lepisanthes* shows variation in its outline shape characteristics. The difference in the shape of the midrib can be referred to as an additional feature of species identification in *Grewia* (Che Nurul-Aini, 2011). The results of this study have shown four midrib outline shapes have been identified, which are *L. amoena* and *L. fruticosa* with the adaxial surface: large hump in the middle; abaxial surface: concave –V shape, *L. rubiginosa* - adaxial surface: hump; abaxial surface: arc shape, *L. senegalensis* with adaxial surface: slightly humped inverted V-shape; abaxial surface: V-shape and *L. tetraphylla* - adaxial surface: slightly humped; abaxial surface: 3/4 circle shape (Figure 2). The observation of the outer shape of the midrib of *Lepisanthes* does not show high variation and is not very suitable to be used as a species identification feature because several species show the same outline shape of the midrib, but is still useful for the differentiation of the species studied above.

A study on the Dipterocarpaceae family shows that the variation in the pattern of the complex vascular bundles of petioles can be used to distinguish species and even genera in this family (Metcalfe & Chalk, 1950; Ashton, 1982; Noraini & Cutler, 2009). The results of this study found that the pattern of vascular tissue for *Lepisanthes* consists of a closed system with main vascular tissue: consisting of several separate vascular bundles arranged discontinuously and irregularly with an O-shape which

is observed in *L. amoena, L. fruticosa,* and *L. tetraphylla* meanwhile closed system for main vascular tissue: consisting of a ring of vascular bundles arranged continuously in an O-shape was observed in *L. rubiginosa* and closed system, accessory vascular tissue: composed of several separate vascular bundles arranged discontinuously –O shape; accessory vascular bundles: 5-6 medullary vascular bundles present were observed in *L. senegalensis* (Figure 1). The O-shaped closed-system vascular bundles in *L. senegalensis* and *L. tetraphylla* is interrupting or discontinuous, whilst the others have continuous O-shaped closed-system vascular bundles. Therefore, the results of this study can help in the identification of the species of *Lepisanthes*, especially for the identification of the species *L. rubiginosa* and *L. senegalensis*.

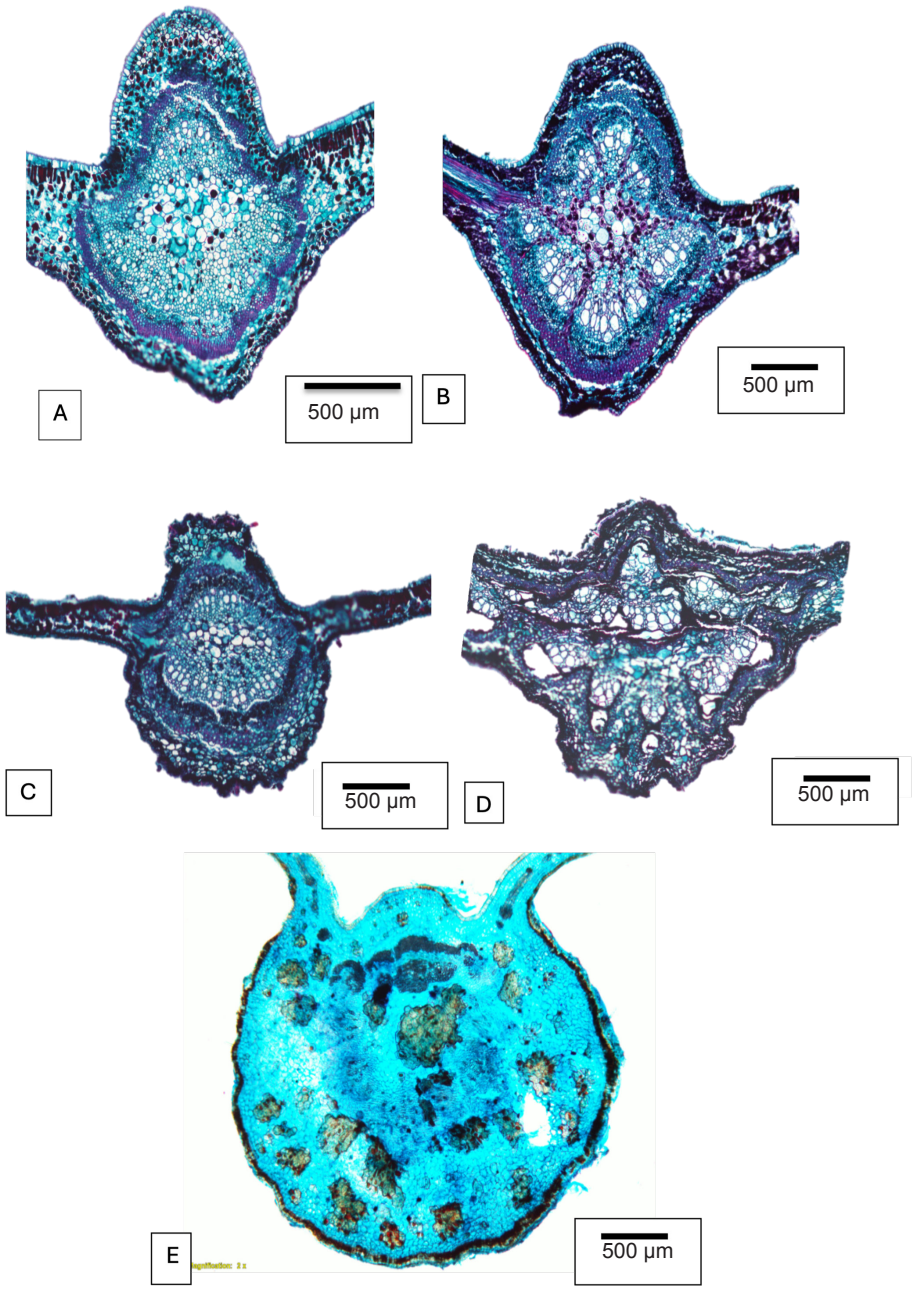

Fig. 2. Transverse section of Lepisanthes midrib: A) L. amoena, (B) L. fruticosa, (C) *L. rubiginosa*, (D) *L. senegalensis*, (E) *L. tetraphylla*. Scale: A-E: 500 µm.

Leandro *et al*. (2015) showed that the variation of midrib vascular tissue pattern can be used as one of the characteristics to distinguish 16 sympatric species in the subfamily Bambusoideae found in Brazil. The results of this study show that the vascular tissue pattern of *Lepisanthes* midrib consists of open system - main vascular tissue: with several separate vascular bundles that are present discontinuously and irregularly -0; medullary vascular bundles: a medullary vascular bundle is present observed in *L.*

fruticosa, L. tetraphylla and *L. senegalensis*. Closed system -main vascular tissue: consisting of one continuous vascular bundle on the adaxial side and one ring of continuous arcuate vascular bundles on the abaxial side observed in *L. rubiginosa* and closed system for main vascular tissue: composed of main vascular tissue and adaxial vascular tissue forming discontinuous O-shaped rings was observed in *L. amoena* (Figure 2). Therefore, the analysis of the diversity of midrib vascular tissue patterns for *Lepisanthes* is suitable to be used as a species-level identification feature, especially to distinguish *L. amoena* from other *Lepisanthes* species. Three external leaf margin shapes have been identified in the study species - rounded, curved 20° towards the abaxial surface *in L. amoena* and *L. rubiginosa*, tapered, curved 30° towards the abaxial surface observed in *L. fruticosa* and *L. senegalensis* while tapering-acute, straight was recorded in *L. tetraphylla*. This study also showed that no trichomes were present at the tip of the leaf margin in all the species studied (Figure 3).

Leaf venation pattern is also useful for plant identification. For *Lepisanthes*, variation can be observed in marginal venation, *L. amoena* shows complete leaf margins while other species show incomplete leaf margins (Table 2). In addition, *L. amoena* and *L. fruticosa* showed swollen tracheids when compared to *L. tetraphylla, L. senegalensis,* and *L. rubiginosa* which showed non-swollen tracheids. Therefore, the characteristics of the leaf arrangement can be used to distinguish *L. amoena* and *L. fruticosa* from other *Lepisanthes* species (Figure 4).

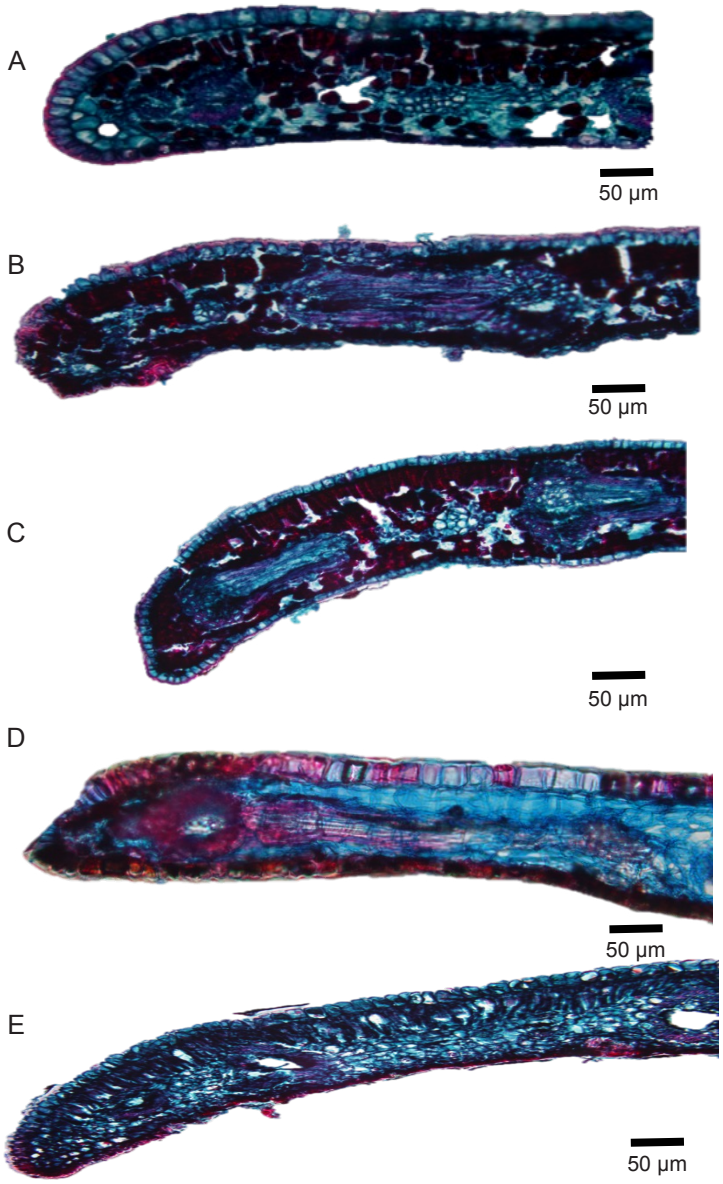

Fig. 3. Transverse section of *Lepisanthes* leaf margin: A) *L. amoena*, (B) *L. fruticosa,* (C) *L. rubiginosa*, (D) *L. senegalensis*, (E) *L. tetraphylla.* Scale: A-E: 50µm.

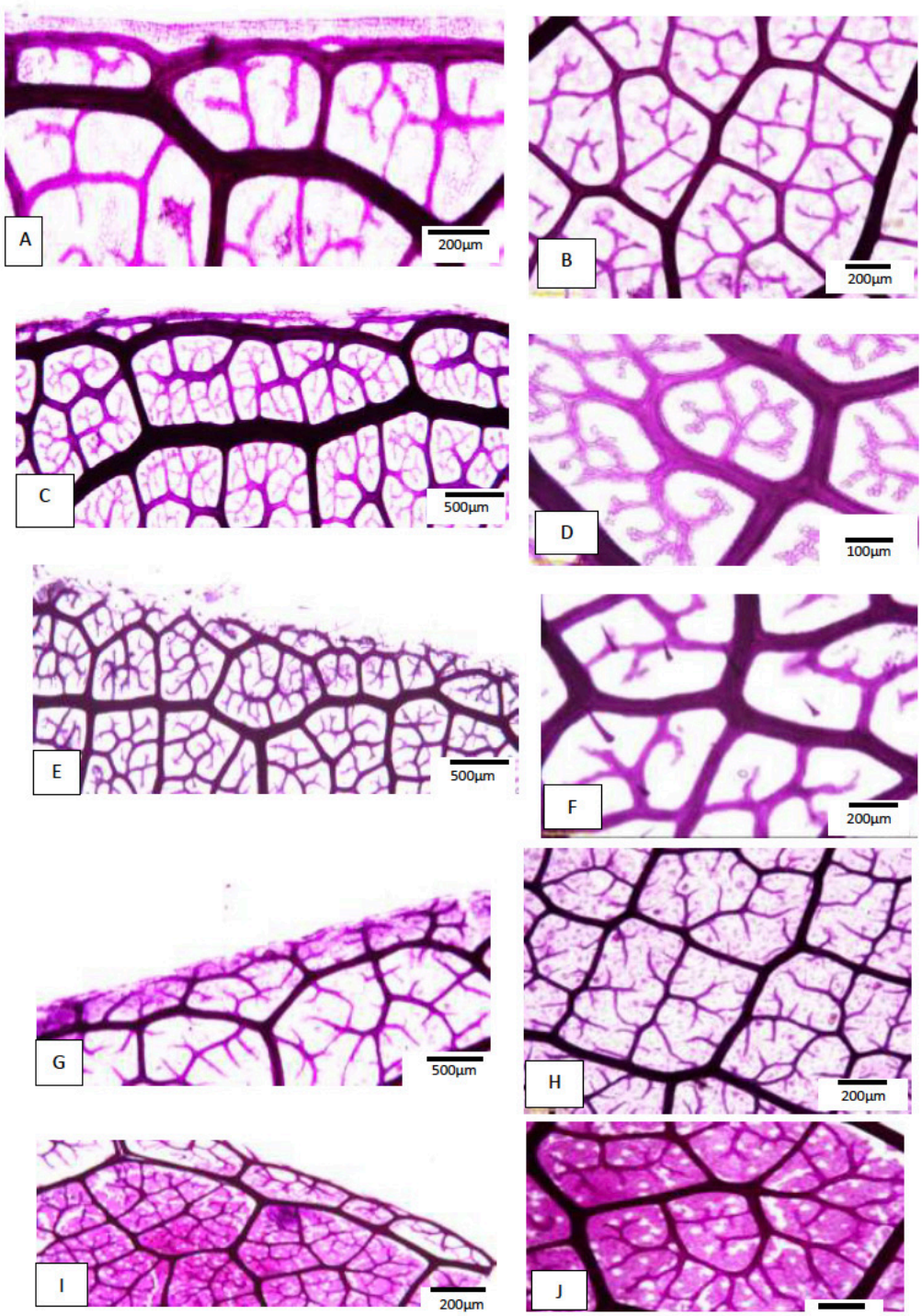

Fig. 4. Venation pattern variation in *Lepisanthes*: A-B) *L. amoena*, (C-D) *L. fruticosa*, (E-F) *L. rubiginosa*, (G-H) *L. senegalensis*, (I-J) *L. tetraphylla*. Scale:: A, B, H, I J: 200µm; C,E, G: 500µm; D: 100µm.

Table 2. Venation pattern variation in *Lepisanthes*

Species	Marginal Venation	Tracheid features
Lepisanthes amoena	Complete	Swollen tracheid; with branched end
L. fruticosa	Incomplete	Swollen tracheid
L. rubiginosa	Incomplete	Normal tracheid; with simple trichome
L. senegalensis	Incomplete	Normal tracheid
L. tetraphylla	Incomplete	Normal tracheid

DISCUSSION

The variation of leaf anatomical characteristics in *Lepisanthes* can be observed through several characteristics such as the outline shape and pattern of vascular tissue in the petiole, outline shape

and pattern of vascular tissue in the midrib, outline shape of the leaf margin, the pattern of the anticlinal wall of leaf epidermis cells, the presence of brachysclereid cells and type of leaf arrangement. A study conducted on *Parashorea* (Dipterocarpaceae) shows that the outline shape of the petiole can be used for the classification of this genus if combined with the characteristics of the vascular bundle pattern of the petiole (Noraini & Cutler, 2009).

In general, the collenchyma cell layer is present in the petiole and midrib in all five *Lepisanthes* species studied. Collenchyma cells are either sphere, isodiametric, or elongated cells that can be found in the stems, petioles, leaf bones, and young parts of the woody structure of plants, usually thick-walled due to the presence of cellulose, hemicellulose, and pectin and its' function as mechanical support for plant structures (Fahn 1974, 1979). Its function is of being as a mechanical support for plants, collenchyma cells also have significant value in plant taxonomy (Noraini *et al*. 2014). For example, in the Apiaceae family, the characteristics of the collenchyma layer present on the petiole can be used in species identification (Metcalfe & Chalk, 1979). Metcalfe and Chalk (1979) showed that the collenchyma cell layers are present in most taxa found in temperate regions, especially for species that climb using tendrils from the genera of *Atalaya, Melicoca,* and *Talisia*. The number of collenchyma cell layers present is different according to the genus or species and this feature has value for taxonomic studies in *Lepisanthes* showing the presence of four to five layers of collenchyma cells in the petiole and midrib, while *L. amoena* showed four to six layers of collenchyma cells in the petiole. Mucilaginous cells contain natural compounds, consisting of a mixture of polysaccharides and can be found in most organs of higher plants, and have a high correlation with the environmental response in which a species is found (Beck, 2005). Mucilage material can be found in the rhizome, root, leaf, and endosperm of seeds, where the mucilage on the leaf is secreted if the leaf is cut or injured (Clarke *et al*., 1979; Franz, 1979; Bass & Gregory, 1985; Zimmermann *et al*., 2001). This mucilage secretion is usually released through cell structures and mucilage canals that can be seen through plant parts and cross sections (Noraini & Cutler, 2009). The presence of mucilage cells and vessels in the epidermis is a common feature for the Order Sapindales (Metcalfe & Chalk, 1963) and can be used as a diagnostic feature for some species. The results of this study show that mucilage cells and vessels are present either in the petiole, lamina, and midrib of the *Lepisanthes* species studied, specifically in the parenchyma, pith, and palisade mesophyll.

Vascular tissue patterns in higher plants play an important role, especially in identification analysis at the family level (Akinnubi *et al*., 2015). Khatijah *et al*. (1996) through a study on the genus *Mallotus* (Euphobiaceae) stated that the O-shaped continuous ring type of vascular tissue is a general characteristic of the genus. The presence of discontinuous rings or circles of vascular tissue is a common feature for the majority of Sapindaceae taxa (Metcalfe & Chalk, 1963). The results of this study show the presence of ring-type vascular tissue, either continuous or discontinuous in all the species studied. The importance of the pattern of vascular tissue has been proven by Akinnubi *et al*. (2015) and Nor-Ashila *et al*. (2016) who analyzed the variation of vascular tissue patterns in the Asteraceae and *Marantodes* (Primulaceae) and found that the variation of vascular tissue arrangement has high taxonomic value in distinguishing species.

The presence of brachysclereid cells is unique for each species in which brachysclereid cells can be observed in the parenchyma part of the cortex or pith, or both parts (Metcalfe & Chalk, 1979). An early study conducted by Metcalfe and Chalk (1979) recorded the presence of brachysclereid cell layers in some Sapindaceae species. The results of this study have shown that brachysclereid cells are present in selected *Lepisanthes* species studied namely *L. amoena, L. fruticosa,* and *L. tetraphylla*, and their distribution characteristics in the parenchyma part of the cortex can be used to distinguish the three species above, with *L. tetraphylla* showing highest distribution of brachysclereid cells which is > 70% relatively when compared to the other two species (Figure 5). Therefore, the characteristic observation of the presence of brachysclereid cells has a high value for the identification of species in the Sapindaceae.

UPGMA phenetic numerical analysis using Kovach MVSP 3.1 software was constructed based on leaf anatomical characteristics obtained from this study. A phenogram through the UPGMA method assists the classification hierarchy from the diagram between the studied species (Figure 6). The results of the analysis show that there is significance in terms of group division. Two main groups formed; the first group is the *L. senegalensis* with a similarity index of 0.69 or 65%. This is because there is a high variation observed in the analysis of cross-sections of midrib. Petioles, epidermal characteristics, and leaf venation pattern of this species. The second group consists of *L. tetraphylla, L. fruticosa, L. rubiginosa,* and *L. amoena* with a similarity index between 0.69 and 69%. *Lepisanthes tetraphylla, L. fruticosa, L. rubiginosa,* and *L. amoena* have the closest relationship based on the characteristics of

the venation, anticlinal walls, and stomata features. This phenogram clearly shows that leaf anatomical features can be used to classify related species while also showing that these features are also useful for species identification. The diagnostic features of this species in *Lepisanthes* are shown in Table 3.

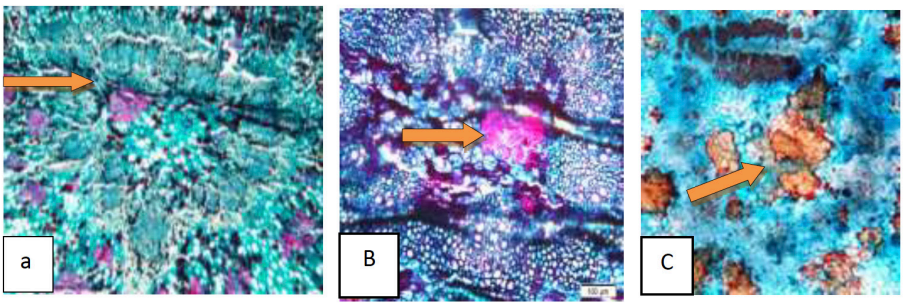

Fig. 5. Brachysclereid cells distribution in *Lepisanthes*. A) *L. amoena*), B) *L. fruticosa* and C) *L. tetraphylla*.

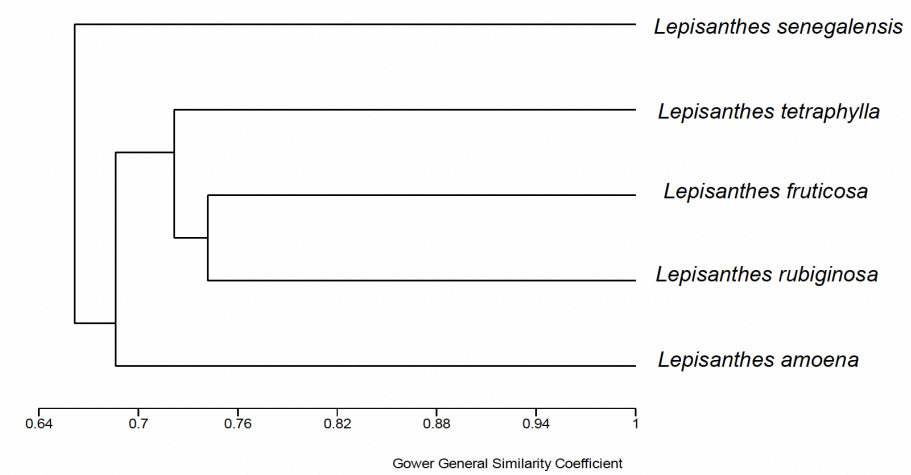

Fig. 6. UPGMA analysis on five Lepisanthes (Sapindaceae) of Peninsular Malaysia.

Table 3. Diagnostic characteristics of *Lepisanthes* (Sapindaceae)

Species	Diagnostic characters
	Petiole outer
Lepisanthes amoena	Outline shape 13
Lepisanthes fruticosa	Outline shape 15
Lepisanthes tetraphylla	Outline shape 1
	Midrib outer shape
Lepisanthes tetraphylla	Outline shapes 5 dan 6
Lepisanthes fruticosa	Outline shape 6
	Collenchyma cells
Lepisanthes amoena	4-6 layers of collenchyma cells

CONCLUSION

The results of the study show that there are three common characteristics, and nine variation characteristics that can be used for species differentiation while three diagnostic characteristics can be used for species identification using the leaf anatomy feature of *Lepisanthes*. The outline shape of the petioles and leaf midribs as well as the characteristics of the collenchyma cell layers can be used as a diagnostic feature for some species in *Lepisanthes*, with a combination of some other anatomical features the species identification can be done with certainty. The results of the study clearly show that the anatomical characteristics of leaves have taxonomic value, especially in differentiation and identification at the species level. Further studies on other genera in the same subfamily and family need to be conducted to analyze the taxonomic value of the anatomical and micromorphological characteristics for classification at the genus, subfamily, or family level.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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