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Research Article

Taxonomic Significance of Petiole Anatomical Characteristics of Selected *Hoya* R.Br. (Apocynaceae) Species in Malaysia

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ABSTRACT

This study aims to identify the common characteristics, variations and diagnostics features of petiole anatomy that can be used to differentiate, identify and classify ten *Hoya* species. This anatomical study involves incision with a sliding microtome, staining with Safranin and Alcian green, hydration with a series of alcohols, slide preparation, drying process, and observation under a light microscope. The results of the study show that all species share five common characteristics: the presence of cuticles, the number of petiole epidermal cells, the presence of latex cells and ducts, and the presence of hypodermis layers. A total of nine anatomical variations of the petiole were observed, namely the outline shape of the petiole, the pattern of vascular tissue, the type of additional vascular bundles, the presence and pattern of sclerenchyma cells, the presence of sclereid and collenchyma cells, the presence and type of crystals, the presence of starch nodules, the presence and type of trichomes. Diagnostic features of the petiole anatomy of some study species can also be identified. The dichotomous key for identifying the studied *Hoya* species was constructed using the petiole anatomical features obtained from the study. In conclusion, the anatomical characteristics of *Hoya* leaves have taxonomic value and can provide added value for the differentiation and identification of *Hoya species* in Peninsular Malaysia.

Key words: Botany, systematic anatomy, taxonomy, microscopy, species identification

INTRODUCTION

Genus *Hoya* R.Br. is the largest genus of Apocynaceae from the tribe Marsdenieae subfamily Asclepiadoideae (Rodda *et al.* 2020). Its main specialty is its waxy, beautiful, durable, fragrant and frequent flowering season. Due to these qualities, it is popular as an ornamental plant and sold commercially at a high price (Rodda *et al.* 2020) (Figure 1). Despite having a high demand, *Hoya* breeding efforts are still limited, and this prompts suppliers to obtain it from rural residents who make *Hoya* an economic commodity and source of income (Rahayu 2019). Additionally, *Hoya* also holds potential medicinal and pharmaceutical value (Burkill 2002). In India, *Hoya* treats stomach aches (Ambasta 1990); in Bangladesh, *H. diversifolia* treats eczema, dermatitis, acne, and pain relief (Mollik *et al.* 2010). Sarkar *et al.* (2022) proved that *H. parasitica* leaf extract has analgesic, antidiarrheal and antipyretic activity. The *H. carnosa* species can absorb pollutants in the air and improve air quality by absorbing pollutants such as benzene, toluene, and xylene (Yang *et al.* 2009).

Hoya was published by Robert Brown in 1810 and named after botanist Thomas Hoy (Quattrocchi 2012). There are nine synonymous names for *Hoya*, namely *Absolmsia* Kuntze, *Centrostemma* Decne., *Clemensiella* Schltr., *Cyrtoceras* Benn., *Cystidianthus* Hassk., *Eriostemma* (Schltr.) Kloppenb. & Gilding, *Phyostelma* Wight, *Plocostemma* Blume and *Triplosperma* G. Don. (World Flora Online 2022). According to Kloppenburg (1990, 2004) and Rodda *et al.* (2020), *Hoya* has been divided into several sections based on morphological characteristics of corolla, corona, and pollinarium. Still, until now, no complete infrageneric system has been published.

According to World Flora Online (2022), there are 489 *Hoya species* published worldwide, and the number constantly increases with the discovery of new species. The geographical distribution *of Hoya* is concentrated in the tropical and subtropical regions of Asia and Australia, where 350-450 species have been found (Lamb & Rodda 2016). The natural distribution *of Hoya* is from India in the west to Samoa and the Fiji Islands in the east, from Southern Japan and China in the north to Northeast Australia (Rahayu *et al.* 2018).

In Peninsular Malaysia, *Hoya* was reviewed for the first time by King and Gamble (1908), who listed 23 species, and the latest review recorded 36 species in Peninsular Malaysia (Rodda & Zakaria 2020). The habitat for *Hoya* is along the river and under the forest canopy (Rahayu *et al*. 2010) or at the edge of the forest, which is relatively protected. A previous anatomical study by Melo-de-Pinna *et al*. (2016) recorded that *H. retusa* has terete leaves and collateral vascular bundles with xylem towards the adaxial epidermis and phloem towards the abaxial epidermis. Hafiz *et al.* (2013) conducted a study on 10 Indonesian *Hoya* species namely as *H. diversifolia, H. latifolia, H. dolichosparte, H. bilobata, H. lacunosa, H. verticillata, H. purpureofusca,*

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H. kuhlii, H. oblanceolata, and *H. micrantha*. The anatomical features found are the hypostomatic stoma, the cuticle layer on the abaxial and adaxial leaf epidermis and the hypodermis cell layer. Salas *et al.* (2018) conducted a study on *H. incrassata* and *H. soligamiana* from the Philippines. Both species have waxes on the abaxial and adaxial epidermis, arcuate midrib vascular tissue and amphicribal vascular bundles. In contrast, *H. incrassata* differs from *H. soligamiana* with a sunken cyclocytic stoma rather than an actinocytic stoma.

Plant identification and classification are usually done using morphological features because these characters are easy to observe, but accurate identification can only be done if they have flowers, fruits, and leaves (Noraini *et al*. 2019; Cutler *et al.* 2008). Anatomical characteristics have been used as additional identification features, especially for incomplete specimens without fruits and flowers (Baltazar & Buot 2019; Jumawan & Buot 2016). These characteristics help a lot in solving the problem of plant species identification. An accurate description of anatomical and morphological characteristics is very important to verify the purity of raw materials in the plant-based medicine industry to ensure the safety and quality of the medicine.

Microscopy methods can also be used to detect contaminated materials found in food products, especially for products in powder form. Besides that, anatomical studies are also important in forensic science to help identify criminal evidence found on suspects, victims, or at crime scenes (Noraini *et al.* 2019). Therefore, based on the importance of *Hoya*, the data on its anatomical characteristics are very important to ensure correct identification. This study was conducted to identify general anatomical features, variations and diagnostics that can be used to differentiate and identify the studied *Hoya species.*

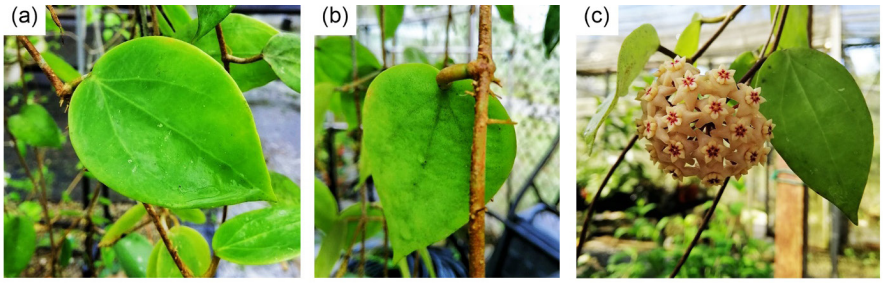

Fig. 1. *H. limoniaca.* (a) Adaxial epidermal surface, (b) Abaxial leaf, (c) Flower.

MATERIALS AND METHODS

Material

Hoya species from Peninsular Malaysia were selected for this study. The complete list of species studied is shown in Table 1.

Table 1. List of *Hoya* species studied

No.	Species and specimen code	Locality	Date
1.	*H. archboldiana* C. Norman H23	Bangi Botanical Garden, FST, UKM	28.12.2020
2.	*H. bhutanica* Grierson & DG Long H24	Bangi Botanical Garden, FST, UKM	28.12.2020
3.	*H. glabra* Schltr. H26	Bangi Botanical Garden, FST, UKM	28.12.2020
4.	*H. halconensis* Kloppenb. H25	Bangi Botanical Garden, FST, UKM	28.12.2020
5.	*H. hanhiae* VT Pham & Aver. H12	Bangi Botanical Garden, FST, UKM	28.12.2020
6.	*H. limoniaca* S. Moore H9	Bangi Botanical Garden, FST, UKM	28.12.2020
7.	*H. mindorensis* Schltr. H27	Bangi Botanical Garden, FST, UKM	28.12.2020
8.	*H. multiflora* Blume H8	Bangi Botanical Garden, FST, UKM	28.12.2020
9.	*H. paziae* Kloppenb. H49	Bangi Botanical Garden, FST, UKM	05.01.2021
10.	*H. pubifera* Elmer H7	Bangi Botanical Garden, FST, UKM	28.12.2020

This study involves collecting and preparing study voucher specimens, using the petiole cross-section method, comparative analysis, and constructing dichotomous keys for species identification. Fresh specimens were obtained from the *Hoya species* collection at the Bangi Botanical Garden Nursery, Universiti Kebangsaan Malaysia. Specimens were compressed, dried in an oven at 50ºC for two weeks, stitched on herbarium cards, labeled, documented, and stored at the Universiti Kebangsaan Malaysia (UKMB) Herbarium. Anatomical studies involve the incision method with a sliding microtome based on Noraini *et al*. (2022). The leaf specimens were stored pre-preserved in AA fixing solution (acetic acid: alcohol 70%) with a ratio of 1:3 for 48 hours. A sliding microtome incision method was performed to obtain petiole cross-sections. The staining process was done using Safranin and Alcian Blue and dehydration with a series of alcohol solutions of 20%, 50%, 70% and 100%. Sample slices were mounted on glass slides using Euparal mount, covered with glass inserts, dried in an oven at 50ºC for two weeks, and observed under an Olympus microscope (Olympus BX43) connected to an Olympus DP 72 digital camera. Images were captured using Analysis Docu software 5.0 by Olympus Soft Imaging Solutions, observed at magnification x4, x10, x20, x40 and x60 and saved in Tagged Image File Format (TIFF) and processed with Adobe Photoshop. Description of anatomical features according to the method of Noraini *et al.* (2019). A comparative study of the anatomical characteristics of the leaves was carried out. The dichotomy key constructed for this study is a *bracketed type* to facilitate reference to the anatomical features of the study species.

RESULTS AND DISCUSSION

General characteristics of petiole anatomy of *Hoya species* study

Common features can be used to support the clustering of species in the same genus or family. The study revealed several

key characteristics. Five common characteristics of leaf anatomy for the genus *Hoya* can be identified: the presence of cuticle, the number of epidermal cell layers, the presence of latex cells and vessels, additional vascular bundles and the presence of the hypodermis layer (Figure 2). The cuticle is a translucent layer on the outer surface that reduces water loss and provides mechanical support to the plant (Cutler *et al.* 2008). The thickness of the cuticle is closely related to water availability for plants, where the cuticle will be thicker in species that live in dry and hot habitats. The findings of this study indicate that the comparatively thick cuticle on the epidermis of the petiole contributes to *Hoya's* resistance to extreme weather, same as to the results observed in *H. mirabilis*, which also possesses a thick outer wall cuticle (Kidyoo 2012). In the previous research, Weissflog *et al.* (2010) also reported the presence of a thick layer of wax on the cuticle of *H. carnosa*. A thick cuticle is present in the epiphytic *Hoya species,* and a thin one is in the non-epiphytic *Hoya* species (Rayos & Hadsall 2022). An epidermal layer was found in all *Hoya species* studied, matching the findings of Duarte and Larrosa (2011) and El-Gendy *et al.* (2019). The shape of the epidermal cells in the studied *Hoya* species is rectangular to square and this feature is similar to the findings of Kidyoo (2012) on *H. mirabilis.* Secretory cells or idioblast cells are cell structures that contain specific secretory substances such as tannin, mucilage, oil, resin or latex (Noraini *et al.* 2019). Secretory cells can be identified by their characteristic absorption of different colors and cell contents and are larger in size than the surrounding parenchymal cells. The results of the study showed that latex cells and vessels and the hypodermis layer were observed on the petioles of all species studied (Figure 2). The results of this study are similar to those of previous studies that reported the presence of cells and latex ducts on the leaves of other Apocynaceae species (da Silva *et al*. 2019; Gama *et al*. 2017).

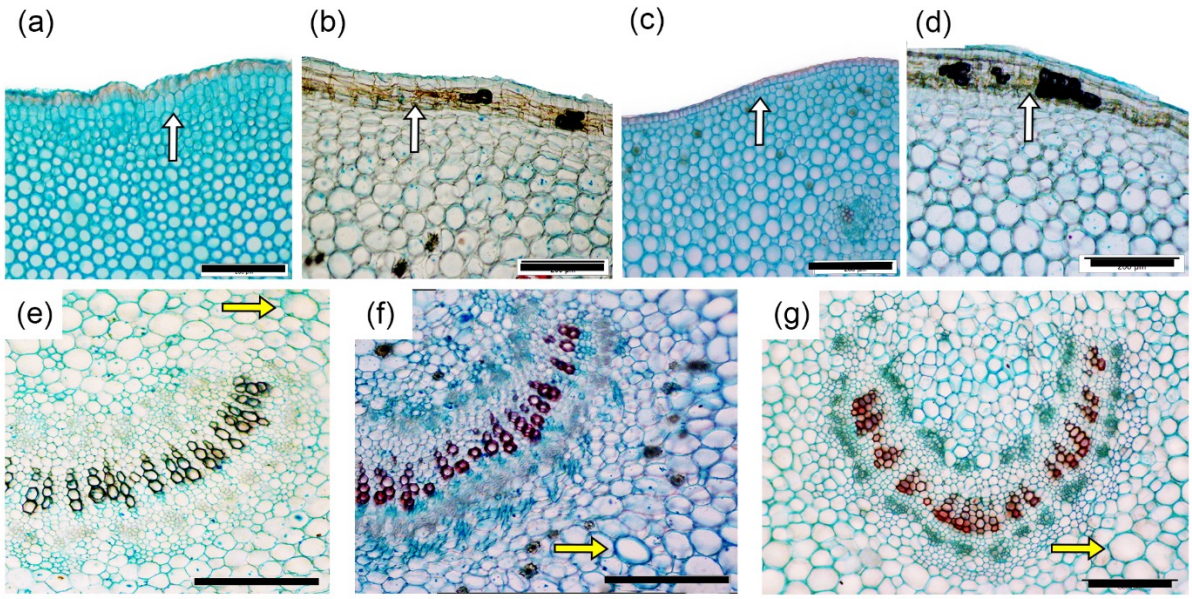

Fig. 2. Hypodermis layer (white arrow), (a) *H. archboldiana,* (b) *H. bhutanica,* (c) *H. halconensis,* (d) *H. mindorensis.* Cells and latex ducts (yellow arrows): (e) *H. multiflora*, (f) *H. hanhiae,* (g) *H. pubifera.* Scale bar: 200 µm.

Variation of petiole anatomical features

Variations in petiole anatomical features can be observed in outline shape, vascular tissue pattern, type of additional vascular bundles, presence and pattern of sclerenchyma cells, presence of sclereid and collenchyma cells, presence and type of crystals, presence of starch nodules, presence and type of trichomes. Table 2 and Figure 3 show the variations in petiole anatomical characteristics that can be used to construct key identification of each species.

Number of hypodermis cell layers

Hypodermis cells are specialized cells located beneath the epidermal layer, distinguished by their thick cell walls and containing sparse chloroplasts (Cutler *et al.* 2008). These cells originate from cortical cells, which are parenchyma cells located in the cortex area. The characteristics of hypodermis cells, especially shape, cell wall thickness, number, and continuity have taxonomic value as supporting characteristics for identifying certain taxa (Martins *et al.* 2012).

According to Metcalfe and Chalk (1979), the number of hypodermis cell layers in Apocynaceae species can vary, with some species having just one layer while others have more. These layers are found on both the adaxial and abaxial parts of the leaf. Although the hypodermis cell layer is present in all *Hoya species*, the number varies. One layer in *H. halconensis, H. limoniaca, H. multiflora, H. paziae* and *H. pubifera,* 1-2 layers in *H. archboldiana,* whereas two layers in *H. bhutanica, H. glabra, H. hanhiae* and *H. mindorensis*. This finding shows that this feature can be used for *Hoya species* differentiation.

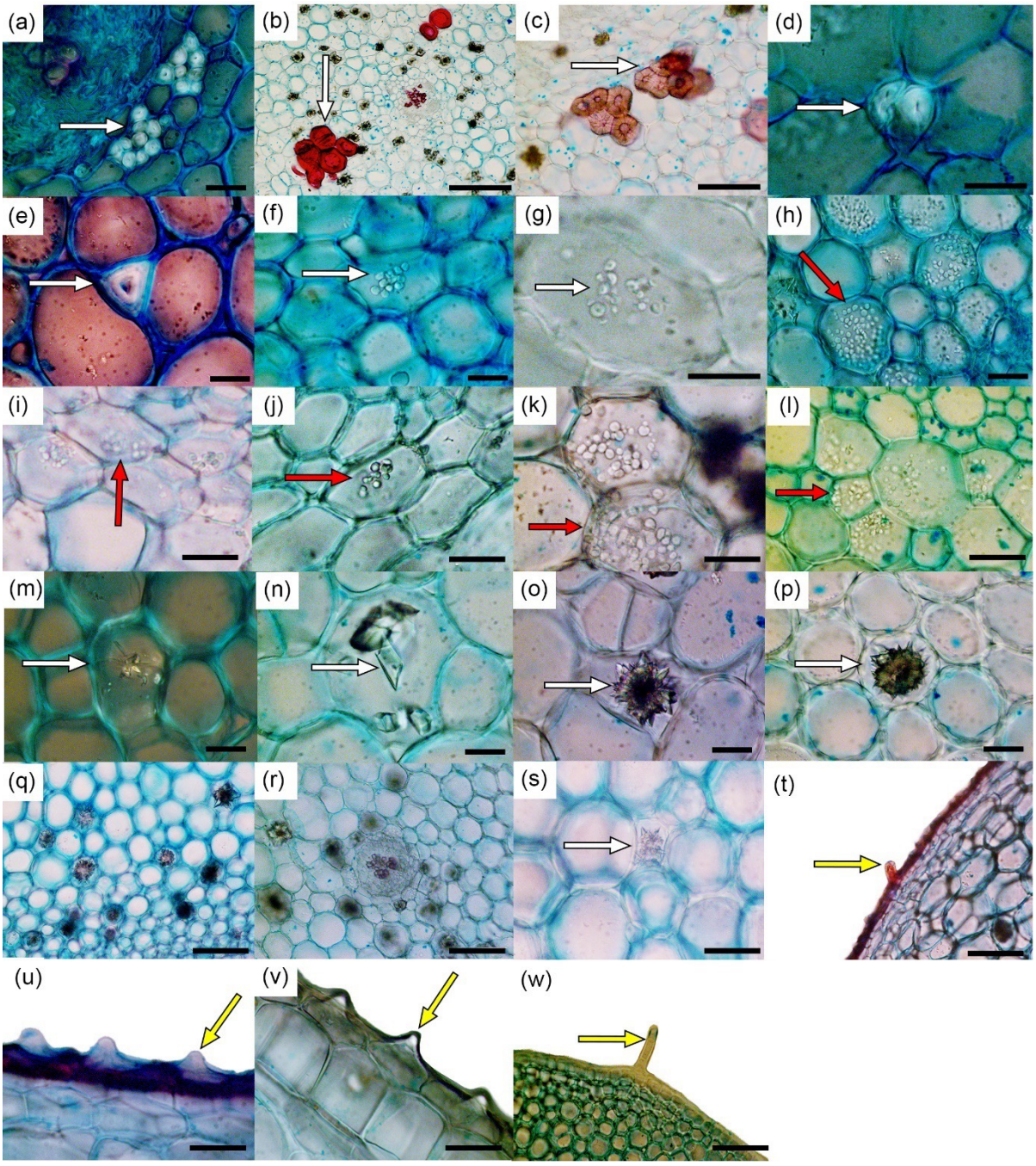

Fig. 3. Sclerenchyma and brachysclereid cells: (a) *H. archboldiana* , (b) *H. bhutanica,* (c) *H. glabra,* (d) *H. halconensis,* (e) *H. pubifera.* Starch nodules: (f) *H. archboldiana,* (g) *H. glabra,* (h) *H. halconensis,* (i) *H. hanhiae,* (j) *H. limoniaca,* (k) *H. mindorensis,* (l) *H. multiflora.* Drus and single crystals: (m) *H. archboldiana* , (n) *H. bhutanica,* (o) *H. bhutanica,* (p) *H. glabra,* (q) *H. halconensis,* (r) *H. mindorensis,* (s) *H. pubifera,* Trichomes: (t) *H. hanhiae* (simple trichome), (u) *H. hanhiae* (papillae trichome), (v) *H. limoniaca* (papillae trichome), (w) *H. multiflora* (multicellular simple trichome). Scale: (b) = 200 µm; (c,i,q,r,t&w) = 100 µm; (m,s) = 50 µm; (a,d,e,f,g,h,j,k, l,n,o,p,u&v) = 20 µm.

The outline shape of the petiole

A study by Noraini *et al.* (2017) on *Hopea species* (Dipterocarpaceae) demonstrated that variations in the outline shape of petioles can serve as a useful method for identifying and classifying species within the genus. The results of this study show that there are five variations in the outline shape of the petiole. Table 3 shows the classification and description of the outline shape of the petiole obtained from the study.

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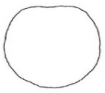
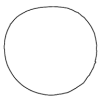
Table 2. *Hoya* petiole anatomical variation

Features	*H. archboldiana*	*H. bhutanica*	*H. glabrous*	*H. halconensis*	*H. hanhiae*	*H. limoniaca*	*H. mindorensis*	*H. multiflora*	*H. paziae*	*H. pubifera*
The outline shape of the petiole	Adaxial: nearly flat Abaxial: ¾ circle	Adaxial: slightly convex Abaxial: ¾ circle	Adaxial and abaxial ½ circle	Adaxial: concave Abaxial: ¾ circle	Adaxial: slightly convex Abaxial: ¾ circle	Adaxial: slightly convex Abaxial: ¾ circle	Adaxial and abaxial ½ circle	Adaxial: slightly concave Abaxial: U-shaped	Adaxial: concave Abaxial: ¾ circle	Adaxial and abaxial ½ circle
Pattern of vascular tissue on the petiole	Open, bilateral, discontinuous, arcuate system	Open, bilateral, discontinuous, arcuate system	Open system, bilateral, discontinuous, U-shaped	Open system, bilateral, discontinuous, U-shaped	Open, bilateral, discontinuous, arcuate system	Open, bilateral, discontinuous, arcuate system	Open system, bilateral, discontinuous, U-shaped	Open, bilateral, discontinuous, arcuate system	Open, bilateral, discontinuous, arcuate system	Open system, bilateral, discontinuous, U-shaped
Additional vascular bundles	Amphicribal	Collateral	Collateral	Collateral	Amphicribal	Collateral	Amphicribal	Amphicribal	Amphicribal	Amphicribal
Fibrous cells	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present
Sclereid cells	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Collenchyma cells	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent
Crystal type	Druses	Single and druse	Druses	Druses	Druses	Druses	Druses	Druses	Druses	Druses
Starch nodules	Present	Absent	Present	Present	Present	Present	Present	Present	Present	Absent
Types of trichomes	Absent	Absent	Absent	Absent	Lithops-type papillae and simple unicellular (short, blunt end)	Cone-type papillae and peltate glands (multicellular terminals)	Absent	Multicellular short (short, blunt end)	Absent	Absent

Table 3. Classification and description of the outline shape of petioles

Outline shape	Description of the outline shape of petioles and species	Illustrations
Shape-1	Adaxial surface almost flat; abaxial surface ¾ circle *H. archboldiana*	
Shape-2	Adaxial surface slightly convex, abaxial surface ¾ circle; The overall shape is nearly round *H. bhutanica, H. hanhiae* and *H. limoniaca*	
Shape-3	Adaxial and abaxial surfaces ½ circle; overall round shape *H. glabra, H. mindorensis* and *H. pubifera*	
Shape-4	Adaxial surface concave, abaxial surface ¾ round; overall shape like an apple *H. halconensis* and *H. paziae*	
Shape-5	Adaxial surface slightly concave; U-shaped abaxial surface *H. multiflora*	

Vascular tissue pattern

The study's findings revealed four distinct patterns of petiole vascular tissues (Table 4). The type and system of vascular tissue are the same, i.e. bilateral type and open system, but there are variations in the pattern. Six study species have non-continuous arc-shaped vascular tissue, namely *H. archboldiana, H. bhutanica, H. hanhiae, H. limoniaca, H. multiflora* and *H. paziae*. Discontinuous U-shaped vascular tissue was observed in *H. glabra, H. halconensis, H. mindorensis* and *H. pubifera*.

Vascular tissue pattern

Sclerenchyma cells are supporting cells with thick, lignin secondary walls and do not contain protoplasts when mature. Sclerenchyma cells allow plant tissues or organs to deal with stress caused by stretching, bending and weight. Sclerenchyma cells can be found singly or in groups in plant tissues such as basal tissue, vascular tissue, and under the epidermal cell layer (Noraini *et al.* 2019). There are two types of sclerenchyma cells which are fibrous cells which are elongated cells with a spindle-like shape and have a small lumen in the middle and sclereid cells are short cells with a thick secondary wall and lignin and have several simple pits.

The results of the study show that sclerenchyma cells are present on the petiole of five species studied with six classification patterns (Table 5). Table 6 shows the variation of the sclerenchyma cell position pattern on the petiole of the *Hoya* species studied. Diagnostic features were observed in *H. archboldiana* with Pattern 6, a cluster of sclerenchyma cells outside the phloem of additional vascular bundles. Pattern 5 is a diagnostic feature for *H. halconensis*, which is sclerenchyma cells present singly outside the phloem of additional vascular bundles. Diagnostic features are also present in *H. glabra* with Pattern 3 and Pattern 4, which are sclerenchyma cells present singly and in clusters outside the phloem of the main vascular tissue. Therefore, it is evident that the presence and position pattern of sclerenchyma cells on the petiole can be used as a feature to distinguish *Hoya species*.

The presence of brachysclereid cells

Sclereid cells have a variety of unique shapes based on their function and brachysclereid cells or stone cells are one of the types of sclereid cells commonly found in plants (Evert 2006). Brachysclereid cells are usually isodiametric and commonly found on leaves, stems and fruits (Noraini *et al*. 2019). The results of the study found that brachysclereid cells were only present in *H. bhutanica* and *H. glabra*. The results of the study by Abeysinghe and Scharaschkin (2019) on *Cinnamomum* (Lauraceae) recorded the presence of brachysclereid cells on the petiole has a high taxonomic value and can be used as a feature to distinguish species. This is proven in this study where the presence of brachysclereid cells is a useful feature for differentiating and identifying *Hoya species*.

The presence of collenchyma cells

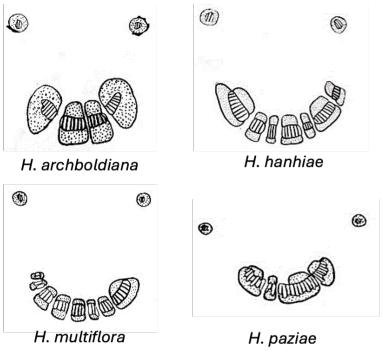
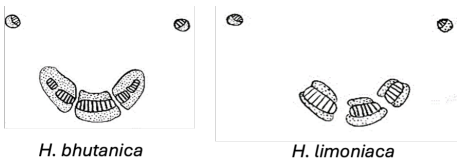
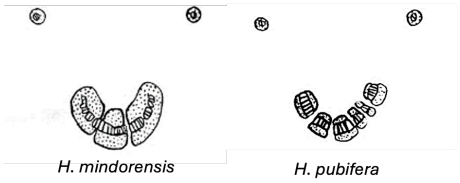
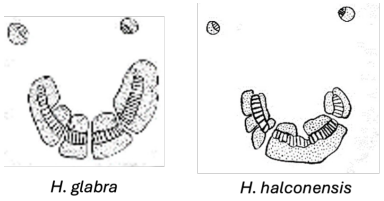
Collenchyma cells are support cells by thickening the cell wall during and after the cell elongation process occurs with uneven and non-lignin wall thickening and contains active protoplasts that can continue meristematic activity when necessary. The study's results revealed that only two species, *H. halconensis* and *H. paziae*, exhibited a layer of collenchyma cells beneath the hypodermis layer.

The presence and type of crystals

Calcium oxalate crystals or crystals are ergastic substances commonly found in plant cells and can be present singly or in clusters in various forms. Calcium oxalate crystals are the end product of plant cell metabolism. The presence of crystals can be

used as supporting data in plant taxonomy, especially in identification at the species, genus or family level (Noraini *et al.* 2019). Cutler *et al.* (2008) have also stated that the presence of crystals can be used as additional data for the identification of certain species. From the findings, druses were observed to be present on the petiole cross-section of all the study species in the form of druses except *H. bhutanica* which exhibited both druses and single crystals.

Table 4. Variation of petiole vascular tissue pattern

Pattern	Description of petiole vascular tissue pattern	Illustrations
Pattern 1	Main vascular tissue: bilateral type, open system with discontinuous vascular tissue arranged in an arc shape. Additional vascular tissue: two simple vascular bundles of amphicribal type.	
Pattern 2	Main vascular tissue: bilateral type, open system with discontinuous vascular tissue arranged in an arc shape. Additional vascular tissue: two simple vascular bundles of the collateral type.	
Pattern 3	Primary vascular tissue: bilateral type, open system with discontinuous vascular tissue arranged in a U-shape. Additional vascular tissue: two simple vascular bundles of amphicribal type.	
Pattern 4	Primary vascular tissue: bilateral type, open system with discontinuous vascular tissue arranged in a U-shape. Additional vascular tissue: two simple vascular bundles of the collateral type.	

Indication: phloem xylem sclerenchyma cell

The presence of starch nodules

Starch nodules are long-chain molecules with symmetrical spacing and have crystal-like characteristics, under a microscope with polarized light, starch nodules appear bright and have a dark cross band called the Maltese Cross (Noraini *et al.* 2019). Mercader *et al.* (2018) conducted an anatomical study to identify the shape of starch nodules in two species of Apocynaceae namely *Ceropegia rendalii* and *Fockea angustifolia***.** The study identified several shapes of starch nodules including pear-like, round, trapeziform, hemispherical and flat-like. The diversity of starch nodule shapes and sizes allows starch nodule characteristics to be used for plant species identification. For example, the results of this study show that starch nodules can be used to distinguish *H. bhutanica* and *H. pubifera* compared to other *Hoya* species.

Presence and type of trichomes

Trichomes are projections from the outer wall of epidermal cells that exist in various forms and have serve multiple functions such as reducing water loss, protecting plants from high temperatures, radiation and ultraviolet rays, as well as acting as a defense against herbivore attacks (Noraini *et al.* 2019). Trichomes can be classified into glandular and non-glandular categories (Cutler *et al.* 2008). In glandular trichomes, there is a specific structure at the end such as a head or terminal containing certain metabolites, while non-glandular trichomes do not have a head (Huchelmann *et al.* 2017). The study's results documented the identification of three types of trichomes: glandular, simple and papilla trichomes. Detailed descriptions of their morphology are provided in Table 7. The types of trichomes identified in *Hoya* are unicellular simple trichomes (short, blunt end), multicellular simple trichomes (short, blunt end), peltate glandular trichomes (multicellular terminal) and papillae either cone-shaped, dome or lithops. Metcalfe and Chalk (1979) also recorded the presence of papillae, glandular and non-glandular trichomes in the Apocynaceae family. Pirolla-Souza *et al.* (2019) also recorded the presence of dome and cone-type papillae in two study species from the genus *Rhabdadenia* (Apocynaceae) which can be used to distinguish species.

Table 7. Presence and type of trichomes

Species	Type of trichomes
H. bhutanica	Cone-type papillae, Lithops-type papillae
H. hanhiae	Lithops-type papillae, unicellular simple trichomes (short, blunt tip)
H. limoniaca	Cone-type papillae, peltate gland trichomes (multicellular terminals)
H. mindorensis	Dome type papillae
H. multiflora	Multicellular simple trichomes (short, blunt ends)

Anatomical diagnostic features of *Hoya species petiole* study

Among the studied *Hoya* species, diagnostic features were identified in only one species, enabling its direct identification. Table 8 lists the eight diagnostic features successfully identified in the *Hoya species* studied.

Table 8. Diagnostic features of the *Hoya species* studied

No.	Species	Diagnostic features
1.	*H. archboldiana*	Outline shape of petiole, shape-1 The position of sclerenchyma cells on the petiole Pattern 6
2.	*H. bhutanica*	A solitary crystal is present on the petiole
3.	*H. glabra*	The position of sclerenchyma cells on the petiole, Pattern 3 and Pattern 4
4.	*H. halconensis*	The position of sclerenchyma cells on the petiole, Pattern 5
5.	*H. hanhiae*	Simple unicellular trichomes (short, blunt ends)
6.	*H. multiflora*	The outline shape of the petiole, shape-5 Multicellular simple trichomes (short, blunt end)
7.	*H. paziae*	Collenchyma cells are present on petioles and leaf bones

***Hoya* species identification dichotomous key study using petiole anatomical features**

The construction of this dichotomous key demonstrates that the petiole anatomical features hold significant have a high taxonomic value. These features can be used for the differentiation and identification of the studied *Hoya* species. However, this constructed key is only subject to the studied species and more species need to be added to obtain a complete identification dichotomous key for the genus *Hoya*. Adding other anatomical features will also further strengthen the construction of dichotomous keys for identifying and differentiating *Hoya* species.

1. Additional vascular bundles on collateral-type petioles..... 2
1. Additional vascular bundles on petioles of bilateral type, amphicribal..... 5

2. Brachysclereid cells present on the petiole…..... 3
2. Brachysclereid cells absent on petiole 4

3. Outline shape of the petiole: slightly convex adaxially; abaxial: ¾ circle, vascular tissue pattern :open system; bilateral; discontinuous; arc shape, solitary crystals and druses present..... *H. bhutanica*
3. Outline shape of the petiole; adaxial and abaxial ½ circle, pattern of vascular tissue open system; bilateral; discontinuous; U-shaped, only druses present…….....*H. glabra*

4. Outline shape of the petiole: a slightly convex adaxially; abaxial ¾ circle, open system vascular tissue pattern; bilateral; discontinuous; arc shape, cone-type papillae and peltate glands (multicellular terminals) trichomes ……..... *H. limoniaca*
4. Outline shape of the petiole: a concave adaxially; abaxial: ¾ circle, open system vascular tissue pattern; bilateral; not casual; U-shaped, no trichomes ..... *H. halconensis*

5. Main vascular tissue of the arch-shaped petiole 6
5. Main vascular tissue of U-shaped petiole..... 7

6. Collenchyma cells are present on the petiole. 8
6. Collenchyma cells are absent on the petiole. 9

7. Starch nodules and sclerenchyma cells present on the petiole..... *H. mindorensis*
7. Starch nodules and sclerenchyma cells are absent on the petiole. ……..... *H. pubifera*

8. Outline shape of the petiole: a slightly concave adaxially; abaxial U-shaped, multicellular simple trichomes (short, blunt tip) ..... *H. multiflora*
8. Outline shape of petiole adaxially: concave; abaxial ¾ circle, no trichomes..... *H. paziae*

9. Outline shape of the petiole: slightly convex adaxially; abaxial ¾ circle, trichome papillae lithops type and simple unicellular (short, blunt end), no sclerenchyma cells..... *H. hanhiae*
9. Outline shape of the petiole: adaxially almost flat; abaxial ¾ circle, no trichomes, clusters of sclerenchyma cells outside the phloem additional vascular bundles ..... *H. archboldiana*

CONCLUSION

The study findings on the anatomical characteristics of selected *Hoya's* species' leaves can provide added value for identification and differentiation at the species level, especially for sterile specimens lacking field information. This research contributes to a more complete and comprehensive dataset on the anatomical features of *Hoya* leaves, enhancing taxonomy efforts, thus enabling a better classification of *Hoya* species in Peninsular Malaysia.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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