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Taxonomic Significance of Leaf Epidermis, Lamina and Venation of Selected *Hoya* R.Br. Species in Peninsular Malaysia

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ABSTRACT

This study focused on five species of Hoya, namely *H. mcgregorii* Schltr, *H. ischnopus* Schltr., *H. pubicalyx* Merr., *H. flavescens* Schltr and *H. micrantha* Hook.f. The objective of this study was to list common, variations and diagnostics characteristics of the anatomical and micromorphological characteristics of the veins, lamina and epidermis of the *Hoya* species studied and to construct a dichotomous key for species identification based on the characteristics obtained. Methods applied in this anatomical study involved sample preservation with AA solution, sliding microtome sections, leaf epidermal sections, leaf clearing and observation under a light microscope. The study of leaf micromorphological characteristics involved critical drying point, gold plating and observation under a scanning electron microscope. The results of this study showed that there are similarities and differences between the studied species. Common features observed in this study are the presence of latex, the layer and size of epidermal cells, the anticlinal wall pattern of epidermal cells and the type of wax on the leaf surface. The variation characteristics observed were leaf margins and lamina, stomatal index, type of stomata and trichomes that present in species studied. Several diagnostic features were observed in certain species. A dichotomous key for species identification has been constructed from the leaf anatomy and micromorphology characteristics found in this study, which then has taxonomic value.

1. Introduction

In Peninsular Malaysia, there are more than 1200 species of medicinal plants that have the potential to be commercialized including *Hoya* R.Br. (Apocynaceae: Asclepiadoideae) [13]. *Hoya* has also gained attention as an ornamental plant and has become one of the most famous plants in Europe and is now becoming famous in Malaysia and Singapore [23]. As many as 300 *Hoya* species have been found in tropical and subtropical regions of Asia, Oceania and the Pacific islands [19-21]. However, this number will always increase according to new studies and discoveries.

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This climbing plant can grow up to 18 meters high and is famous as an ornamental plant because its flowers have a variety of interesting colours and a unique smell [12]. *Hoya* flower colours include white, pink, yellow, blackish purple, green and orange [18]. The demand for this genus is increasing because many hybrids produce interesting and unique flowers. The diversity and uniqueness of *Hoya*'s scent have the potential to be commercialized as a new fragrance [25]. The anatomical and micromorphological characteristics of a species have been proven to be used to produce herbal products [4,6,7,26]. Research on *Hoya* is still meagre even though *Hoya* has medicinal values that can potentially be used as herbal medicine. This study was conducted because there has not yet been a comprehensive anatomical and micromorphological study done on the five selected *Hoya* species in this study.

2. Methodology

2.1 Materials

A total of five species from the *Hoya* genus were selected for this study, namely *H. mcgregorii, H. ischnopus, H. pubicalyx, H. flavescens* and *H. micrantha*. Specimens were obtained from the Bangi Botanical Garden Nursery, Rumah Tumbuhan Complex, UKM Bangi, Selangor.

2.2 Methods

The research method was carried out according to [16,17]. Leaf specimens were preserved in AA fixing solution (30 % Acetic Acid: 70 % Alcohol) with a ratio of 1:3, for 48 hours. Slice method using a sliding microtome (Reichert) for the leaf lamina and margin. A disposable knife was used for slices at a thickness of 20-30 µm, the slices were transferred to a petri dish, the clarification process was done with Clorox bleach, staining using Safranin and Alcian green solutions, and dehydration involved a series of alcohols. For epidermal sections, specimens were soaked with Jeffrey's solution until clear, stained with HCL, dehydrated, dried at 60°C and observed under a light microscope (Olympus BX43) connected to a camera (Olympus DP72) and (Canon EOS 700D), using Analysis DoCu software and EOS Utility 2 software. Micromorphological studies involve fresh leaf samples being cleaned, dehydrating process, cleaning using Phosphate Buffer Solution, and dehydrating in an acetone series, at 99 % acetone concentration, the sample is placed in a specimen container and inserted into a critical dryer (Critical Dryer, Model RF-10) for 30 minutes, the plating process involves the sample being mounted on a stab using double-sided adhesive and then transferred into a gold plating (Sputter coater, Model SC 500). The abaxial and adaxial epidermal surfaces of the leaves were observed using an electron scan (Philips XL Series XL 30) at 200x, 500x, 1500x and 3000x magnification. All features that have taxonomic value were used for the construction of dichotomous keys for the identification of *Hoya* species study.

3. Results

3.1 Common Characters

The presence of crystals is a common feature of most dicotyledonous plants [6] especially woody plants [28]. Crystals are present in palisade cells and spongy mesophyll cells but are more often found in epidermal cells and mesophyll cells in various shapes such as diamonds, cubes, rods, aciculars and druses [16]. The results of the study found that drusen-type crystals were present in all five species studied, especially in the epidermal cells of the leaf lamina. The presence of crystals can be used as a taxonomic feature that can distinguish the genus [9]. Crystals are also associated with protection for

plants and are important for removing oxalate from the plant's metabolic system and helping in the photosynthesis process of plants in the shade [1,2].

Latex cells or laticifiers also known as plant rubber are usually present in certain families such as Euphorbiaceae, Moraceae, Apocynaceae and Musaceae [16]. In this study, the latex cells present are non-joint latex. The presence of latex cells is a general feature of the Apocynaceae family [5]. Latex cells have a secondary metabolism and act as a defense mechanism for plants from being eaten by herbivorous animals [3,7]. There is a study that found that latex from the Apocynaceae has medicinal value such as anti-inflammatory, analgesic and able to treat wounds [29].

3.2 Variations in the Leaf Anatomical and Micromorphological Characteristics
3.2.1 Venation

The vascular system is important for the transport process of water, solutes, hormones and carbohydrates through xylem and phloem. The tissue also functions as a mechanical defense mechanism [3,21]. In plant taxonomy, veins play a very important role as a distinguishing and diagnostic feature of a plant species. Leaf thickness can adapt to environmental changes, but leaf veins remain genetically fixed [21]. The leaf margins of *H. ischnopus* and *H. flavescens* are incomplete, *H. pubicalyx* and *H. micrantha* are looped, while *H. mcgregorii* is complete. As for the leaf lamina veins arrangement, *H. mcgregorii, H. ischnopus* and *H. pubicalyx* have open veinlets, while *H. flavescens* and *H. micrantha* have closed veinlets. This feature definitely has taxonomic value for the differentiation of the studied *Hoya* species.

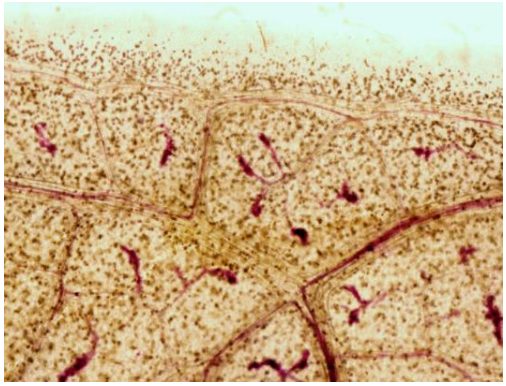

Fig. 1. The venation of the leaf margin is complete and the areolar is open veinlets *H. mcgregorii*

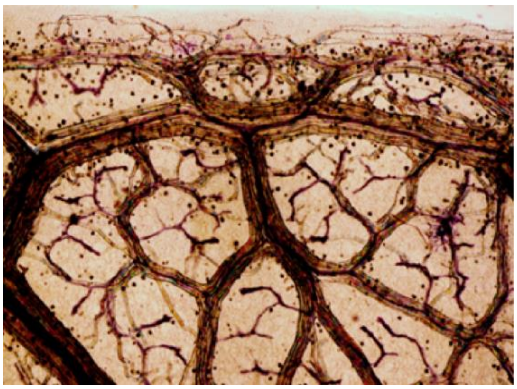

Fig. 2. The venation of the leaf margin is incomplete and the areolar is open veinlets in *H. ischnopus*

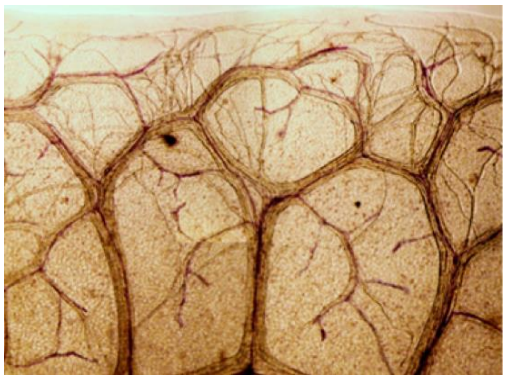

Fig. 3. The venation of leaf margin is looped and the areolar is open veinlets *H. pubicalyx*

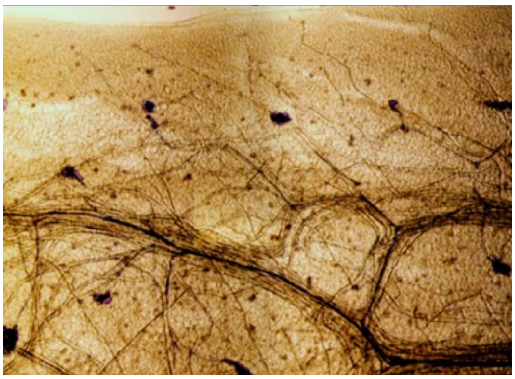

Fig. 4. The venation of leaf margin is incomplete and closed veinlets in *H. flavescens*

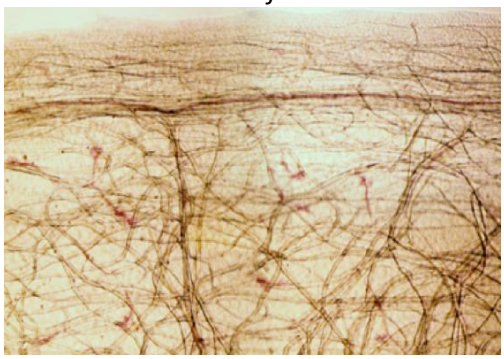

Fig. 5. The venation of leaf margin is looped and the areolar is closed *H. micrantha*

3.2.2 Index stomata

The lamina surface has been preferred in taxonomic studies because the leaf surface has stable and constant characteristics [22,24]. On the surface of the lamina, there are also stomata, trichomes, wax, cuticle decoration, pericline and anticline walls, stomata are the features with the highest taxonomic value [16]. Stomatal index values for example are not influenced by the habitat environment in which the tree is located [27]. A study by [14] showed that the value of the stomatal index is influenced by the density of stomata and the number of epidermal cells. High epidermal cells and low stomatal density will show low stomatal index values, on the other hand low epidermal cells and high stomatal density will show high stomatal index values. There are also studies that say stomatal index values can be influenced by various environmental factors [1,2]. Nevertheless, stomatal density has been considered an important taxonomic trait although it is often linked to the environment [24]. The stomatal index is calculated using the following Eq. (1),

$$SI = \frac{S}{S+E} \times 100 \quad (1)$$

Where, SI = Stomatal Index, S = number of stomata per unit area (mm^2) and E = number of epidermal cells per unit area (mm^2) [16].

The results of this study found that the stomatal index for all species is different. *H. micrantha* recorded the highest stomatal index value of 16.53 % while *H. ischnopus* recorded the lowest

stomatal index value of 4.18 %. Stomatal index values for *H. mcgregorii* could not be calculated due to the high density of trichomes on the abaxial surface, covering the stomata. *H. pubicalyx* and *H. flavescens* recorded values of 14.58 and 11.54 % respectively. Overall, the stoma index for all species studied is in the low range of 4.18 to 16.53 %. This may be due to the distribution of species around Southeast Asia such as Malaysia, Indonesia and the Philippines which have hot weather, less stomata will reduce the rate of water loss.

3.2.3 Presence and type of Trichomes

Diverse trichome features such as shape, distribution, type and pattern are the most easily observed features and have been successfully used for additional classification of genus and species as well as interspecies hybrids within certain plant families. The presence of papillae as a diagnostic feature has been reported by [10,15,16]. Some species of *Hoya* such as *H. caudata* and *H. verticillata* are also recorded as having papillae and there is also a species that has simple unicellular trichomes namely *H. coronaria* [11].

The results of this study found simple multicellular type trichomes in *H. ischnopus* and *H. micrantha*, with echinate ornamentation in *H. micantha*. On the abaxial surface of the leaf, all species have trichomes except *H. pubicalyx*. The abaxial surface of the leaf epidermis of *H. mcgregorii* (Figure 6) and *H. flavescens* (Figure 8) is filled with papillae trichomes. Lithops-type papillae, which are dome-shaped papillae, are present on the abaxial epidermal surface cells of *H. ischnopus* (Figure 7). Both species *H. flavescens* (Figure 8) and *H. micrantha* (Figure 9) have multicellular glandular trichomes on the abaxial surface of the leaf epidermis.

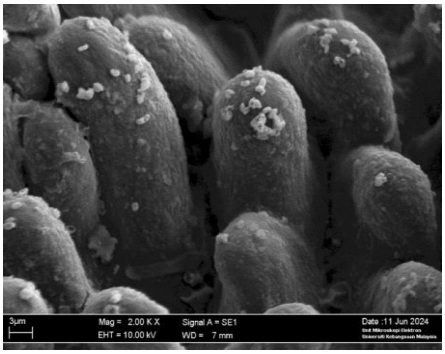

Fig. 6. Papillae trichomes- *H. mcgregorii*

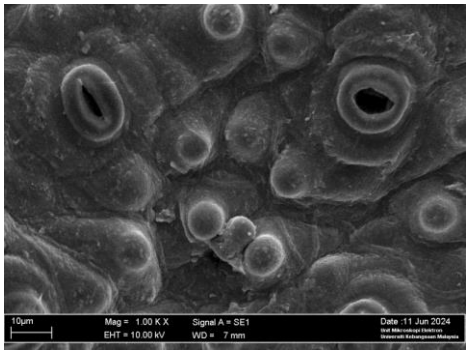

Fig. 7. Papillae lithops trichomes - *H. ischnopus*

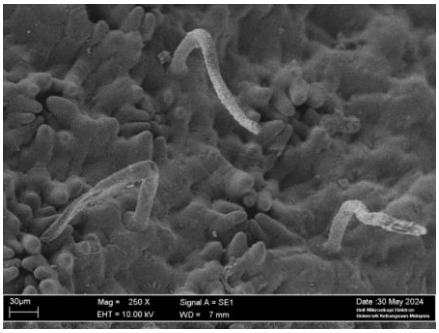

Fig. 8. Simple multicellular and papillae trichomes - *H. flavescens*

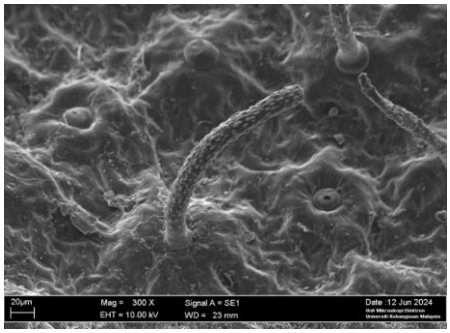

Fig. 9. Simple multicellular with echinate ornamentation trichomes- *H. micrantha*

3.3 Dichotomous Key

Based on the combination of anatomical and leaf micromorphological characteristics obtained from the study, a dichotomous key for the identification of the study species is constructed.

1. The venation of leaf margin is incomplete and looped, the outline shape of the leaf margin is pointed and tapering, have stomatal index value……..2
1. The venation of leaf margin is complete, the external shape of the edge of the leaf is rounded, no stomatal index value……..*H. mcgregorii*

2. Areolar venation is closed and unbranched, stomata present hypostomically ……..3
2. Areolar venation is open and branched, stomata present amphistomically.....*H. ischnopus*

3. The anticlinal walls on the adaxial and abaxial epidermal surfaces are straight to curved, stomatal index value more than 14.58% or less than 14.58%.....4
3. The anticlinal walls on the adaxial epidermal surface is straight and on the abaxial epidermal surface is straight to curved, stomatal index value at 14.58 %.....*H. pubicalyx*

4. Multicellular simple trichomes with echinate ornamentation, and papillae on the abaxial epidermal surface, stomatal index value of 11.54 %.....*H. flavescens*
4. Simple multicellular trichomes with echinate ornamentation only on the abaxial epidermal surface, stomatal index value of 16.53 %.....*H. micrantha*

4. Conclusion

The results of the study have proven that the anatomical and micromorphological features of the leaves have taxonomic value for differentiation and identification of *Hoya* species through the construction of a dichotomous key. This leaf anatomy study on *Hoya* species in Peninsular Malaysia has contributed in adding information to the *Hoya* species anatomical database in Malaysia. The information obtained can be used for reference by the horticultural sector as well as the medicine and fragrance industry based on Hoya species in Malaysia.

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