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Leaf Anatomy of *Morinda citrifolia* L. in Pahang, Malaysia, and its Taxonomic Significance

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ABSTRACT

Leaf anatomical study has been conducted on *Morinda citrifolia* L. from family Rubiaceae in Kuantan, Pahang. The aim of this study was to identify and list characteristics of leaf anatomy that can be used to identify and classification of species and genus in the Rubiaceae family. Methods used in this study were cross section using sliding microtome, epidermis peeling and leaf clearing and observation under a light microscope. Results from this study showed few characteristics can be used in identification in species studied. The result of this study showed that the presence of collenchyma cells, sclerenchyma cells, cell inclusions such as raphide at the petiole and midrib and hypostomatic stomata can be useful as an additional data to identify species studied. In conclusion, leaf anatomy characteristics can be used as an additional characteristic in the identification and classification of selected Rubiaceae species.

Keywords: Leaf anatomy, *Morinda citrifolia*

ABSTRAK

Kajian anatomi daun telah dijalankan ke atas *Morinda citrifolia* daripada famili Rubiaceae di Kuantan, Pahang. Objektif kajian dijalankan ialah untuk mengenalpasti dan menyenaraikan ciri anatomi yang boleh digunakan untuk pembezaan, pengecaman dan pengkelasan spesies dan genus bagi famili Rubiaceae. Kajian ciri anatomi melibatkan kaedah hirisan mikrotom gelongsor (ciri anatomi petiol, lamina, tulang dan tepi daun), kaedah siatan epidermis (ciri anatomi epidermis daun), kaedah penjernihan (ciri anatomi peruratan) dan serta pemerhatian di bawah mikroskop cahaya. Hasil kajian menunjukkan beberapa ciri anatomi yang boleh digunakan untuk pengecaman spesies kajian. Hasil kajian menunjukkan kehadiran sel kolenkima, sel sklerenkima, hablur seperti rafid yang mana sangat berguna sebagai data tambahan untuk mengenalpasti spesies kajian. Kesimpulannya, ciri anatomi daun boleh digunakan sebagai ciri tambahan dalam pengecaman dan pengkelasan bagi spesies Rubiaceae.

Kata kunci: Anatomi daun, *Morinda citrifolia*

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INTRODUCTION

According to Koehbach and Gruber (2015), Rubiaceae is the fourth largest family among angiosperm plants with approximately comprise of 13 000 species within 650 genera. Almost 4% of all flowering plants in this world belong to the Rubiaceae family, which puts this families as one of the largest family among the eudicotyledons. These species can represent in many forms such as trees, shrubs, annual or perennial herbs and climbers. Rubiaceae species can adapt to a wide range of environment conditions. Hence, this is the reason why the Rubiaceae species can easily found in almost any type of habitat around the world. This includes arid to desert environments, humid rain forests, subarctic cold climate, and tropical hot climate regions. Usually, in temperate regions, this family can be found as herbaceous species, while woody in tropical regions.

Morinda is classified as one of the genera under the Rubiaceae family. As stated by Desai et al. (2011), 7 out of 80 species of *Morinda* are originated from India. *Morinda citrifolia* is known as bush or small tropical evergreen tree of 3-10 m tall. Leaf of *Morinda citrifolia* consist of abundant wide elliptical leaves with 5-17 cm length and 10-40 cm width while the flowers are small tubular white flowers are grouped together and inserted on the peduncle. The corolla is greenish white. The fruit has 3-10 cm length and 3-6 cm width is oval and fleshy with an embossed appearance. It is slightly wrinkly, semi-translucent and ranges in colour from green to yellow and to almost white at maturity. It is covered with small reddish-brown buds containing the seeds. The fruit can grow up to 12 cm or more. The seeds have triangular shaped and reddish brown (Rethinam & Sivaraman, 2007).

Compared with the other species, *Morinda citrifolia* is the most popular and prominent species, especially in the medicinal field over the years. According to Singh and Sharma (2019), a variety of phytochemical compounds can be found in *Morinda* species like iridoids, flavonoids, flavonoid glycosides, anthraquinones, coumarins, lignanas, non-iosides, phenolics, and triterpenoids. Furthermore, genus *Morinda* is widely used for the treatment of cancer, diabetes, inflammation, and bacterial and viral infections. According to Algenstaedt *et al.* (2018), the daily intake of noni fruit juice has the positive effect in regulating elevated blood sugar levels and some of the pathological parameters in patients who suffered from Type 2. In addition to that, noni fruit also showed the potential to prevent the progression of inflammatory diseases, such as inflammatory bowel disease, as it has phytochemical properties (Sousa et al. 2017).

Complete samples data is necessary for the identification and classification of the plants in taxonomy studies. Otherwise, lack of sample data will cause the incomplete and unsuccessful identification of plants. One of the crucial parts in plant identification is anatomical part. Hence, the research on anatomical part for this family species is very useful because lack of research has been done before. Other than that, the study of anatomical structures can assist the taxonomist and botanist by giving extra information to identify and classify the plants especially the plants that have almost same characters in terms of morphology.

MATERIALS AND METHOD

Five replicates of *Morinda citrifolia* were collected from Bandar Indera Mahkota, Kuantan, Pahang. The plant samples collected were placed on newspapers and arranged neatly and properly. Then, the plant samples wrapped with newspapers were pressed by using presser and dried in an oven that set up with 55°C for two weeks duration. The dried samples were then mounted, labeled, and placed in a herbarium room for future reference. The

plant specimens collected will be cut to three sections which are apex, middle and base, which connected to petiole. These specimens were preserved in sample bottles containing acetic acid (AA) solution. This fixative solution is made from the mixture of 70% ethanol and 30% acetic acid in ratio 1: 3 [7]. The fresh leaves were fixed in 3:1 AA solutions (70% ethanol: 30% acetic acid). Its petiole and midribs were sectioned transversely in a range of thickness (15–30 μm) by using a sliding microtome, LEICA SM2010R.

The knife microtome was lubricated before use by applying 50% alcohol with a soft brush. The sections were stained with diluted Safranin and Alcian blue. The sections were dehydrated by a series of 50%, 70%, 95%, and 100% ethanol solutions and later were mounted on microscope slides using Euparal [8]. The images were captured using a three-CCD (3CCD) camera attached to a microscope (Leitz Diaplan; United Kingdom) and an imaging software (Cell^B). For the leaf epidermal peel method, the adaxial and abaxial epidermis of the fresh leaves were scraped by using a sharp blade. After getting a small, thin, and clear surface, the leaf sample was immersed in Jeffrey solution for a few minutes and was stained with Safranin. The leaf sample was placed on a slide, covered with a cover slip and observed under the light microscope. Then, for the leaf venation process, the leaf specimen was cleared by using Basic Fuchsin solution (10% Basic Fuchsin and 10% KOH) in an oven with temperature at 60°C for 1 to 2 days depending on the leaf thickness. The cleared leaf specimen was then proceeded with dehydration process in alcohol series (50%, 70%, 95% and 100%), cleared in xylene and mounted properly on the slide.

RESULTS AND DISCUSSION

Cross Section of Petiole (Figure 1A & 1B)
Epidermal cell: One layer cell with (1:1) ratio of height:width. **Vascular tissue:** Opened vascular system with continuous rings together with two additional vascular bundles located at the above right and left of the main vascular bundle near each wing. S**clerenchyma cell:** Present. **Parenchyma cell:** 13-14 layers of parenchyma cells. **Collenchyma cell:** 8-9 layers of collenchyma cells under the epidermis of abaxial and adaxial. **Idioblast tannin:** Absent. **Mucilage cell:** Present at the parenchyma cells. **Crystal oxalate:** Present (raphide). **Trichome:** Absent.

Cross Section of Midrib (Figure 1C)
Epidermal cell: One layer cell with (1:1) ratio of height:width. **Vascular tissue:** Opened vascular system with continuous rings together with two additional vascular bundles located at the above right and left of the main vascular bundle. **Sclerenchyma cell:** Absent. **Parenchyma cell:** 12-13 layers of parenchyma cells. **Collenchyma cell:** 6-7 layers of collenchyma cells under the epidermis of abaxial and adaxial. **Idioblast tannin:** Absent. **Mucilage cell:** Present at the parenchyma cortex. **Crystal oxalate:** Present (Raphide). **Trichome:** Absent.

Leaf Epidermis (Figure 1E & 1F)
Anticlinal wall of adaxial epidermis: Straight to wavy. **Anticlinal wall of abaxial epidermis:** Straight to wavy. **Stomata:** Hypostomatic; only present on abaxial epidermis. Type; paracytic. Stomata size; abaxial: W=17.43 μm, H=28.66 μm (width: min= 14.36 μm, max=20.50 μm; height: min=22.92 μm, max=34.39 μm). **Trichome:** Absent.

Leaf Venation (Figure 1G &1H)
Main venation: Majority open and minority close. **Marginal venation:** Complete. **Tracheid ending:** Swollen.

The outcome of this study reported the significance of leaf anatomical characteristics in the identification of *Morinda citrifolia*. Collenchyma cells can be identified based on few morphological

characteristics such as their axially elongated cells, cell wall thickening and living protoplasts inside the cells. The shape of collenchyma cells is varied from prosenchymatic, isodiametric and prismatic cells to long, fibre-like cells with tapering ends (Leroux, 2012). Based on cross section of petiole and midrib of species studied which is *Morinda citrifolia* have portrayed the presence of collenchyma cells beneath the epidermis layer. The same result was obtained by Roonyamarai et al. (2011) and Mownika et al. (2020), where they found collenchyma cells in the petiole and midrib of *Morinda citrifolia* and *Morinda elliptica*. *Morinda citrifolia* has recorded 7-9 layer of collenchyma cells.

Previous study by Nurul-Aini et al. (2013) identified the vascular bundles arrangement in midrib can be used to differentiate between two species in genus *Grewia* which is *G. laevigata* and *G. polygama*. The pattern of vascular bundles that have been identified in the petiole and midrib for *Morinda citrifolia* is the main vascular bundle (opened system with continuous ring). Two additional vascular bundles are located on the above left and right sides of main vascular bundle near each wing. Ghazalli et al. (2021) also reported the taxonomic importance of midrib and petiole vascular bundle in species identification of *Mitragyna speciosa* with supported by previous research on Rubiaceae family by Metcalfe and Chalk (1950).

In plants, epidermal cells are the cells of the outermost layer that act as protective mechanism against biotic and abiotic agents that enter the cells and control the essential exchange of gas, water, and the nutrients between cell and environment (Javelle et al. 2011). Usually, epidermal cells are uniseriate which is only consists of one layer of cell only. The ratio of height and width of epidermal cells can determine the variation between plant species. Results showed the ratio of height and width of epidermal cells for this species is 1:1 (height:width). The patterns of anticlinal walls are varying in each plant which it can be either straight, sinuolate, sinuous or sinuate (Ao, 2006). Other than that, as stated by Siti-Maisarah et al. (2020), the pattern of anticlinal walls also can be varied between straight to wavy. The anticlinal walls in abaxial and adaxial epidermis of *Morinda citrifolia* showed pattern which is straight to wavy due to some of the epidermal cells are irregular in shape. Meanwhile, Mownika et al.)2020) reported the anticlinal wall for *Morinda citrifolia* was thick and straight.

In cross section of petiole and midrib, mucilage cell can easily distinguish between other cells as it has distinct appearance which is large. According to Lloyd (1999), mucilage cell is distributed thoroughly in the parenchyma, in both medullary and cortical. Number of mucilage cells and where they distributed are based on the plant species. In the mucilage cell, there are present chloroplasts, starch grains and a large cluster of calcium oxalate crystals inside it. Then, based on the study by Lyshede (1977), mucilage cell also contains cellulose. The mucilage cell can either consist of only a thin layer of primary wall that is strong enough to prevent the mucilage content from leaking out into intercellular spaces or be composed of primary wall and the suberized layer. In all plant species studied, they showed the presence of mucilage cells in the parenchyma cortex. This finding was supported based on a study by Vieira et al. (2001) where they have listed that Rubiaceae family has cavities with mucilaginous contents.

As stated by Perveen et al. (2007), there are five types of stomata which are anisocytic, cyclocytic, diacytic, parallelocytic and paracytic. Firstly, in anisocytic type, the guard cells are surrounded by three irregulars sized of subsidiary cells. Next, two or more subsidiary cells create one or two narrow rings around the guard cells in the cyclocytic type of stomata. Later, a diacytic

stomata type is formed when one or more pairs of subsidiary cells enclose the stomata while a parallelocytic stomata is developed when there is presence of three or more C-shaped subsidiary cells. Lastly, stomata with paracytic type arises when guard cells are enclosed with two subsidiary cells and the longitudinal axis are parallel to the guard cells and stomata pore. According to the result of this study, paracytic stomata were recorded in *Morinda citrifolia*. This study supported previous research by Shekhawat & Manokari (2017), showing that the stomata were paracytic where both subsidiary cells were placed parallel to long axis of guard cells in *Morinda citrifolia*. Besides, stomata in this species were recognized as hypostomatic where it only presents on abaxial epidermis while absent on the adaxial epidermis surface.

Leaf venation can be an element for the identification and classification in plant species as there are some characters or patterns that can be observed in leaf venation such as veinlets, ultimate marginal venation, areolar venation and areolation shape (2016). Results of this study showed the type of main venation is majority open and minority close while complete type of ultimate marginal venation can be observed in species studied. Crystal formation in idioblasts is commonly related with membranes, chambers or inclusions found within the vacuoles. Crystal oxalates play crucial roles in tissue calcium regulation, protection from herbivory and metal detoxification [2003). Based on this study, *Morinda longifolia* showed the presence of raphide type of crystal oxalate in petiole.

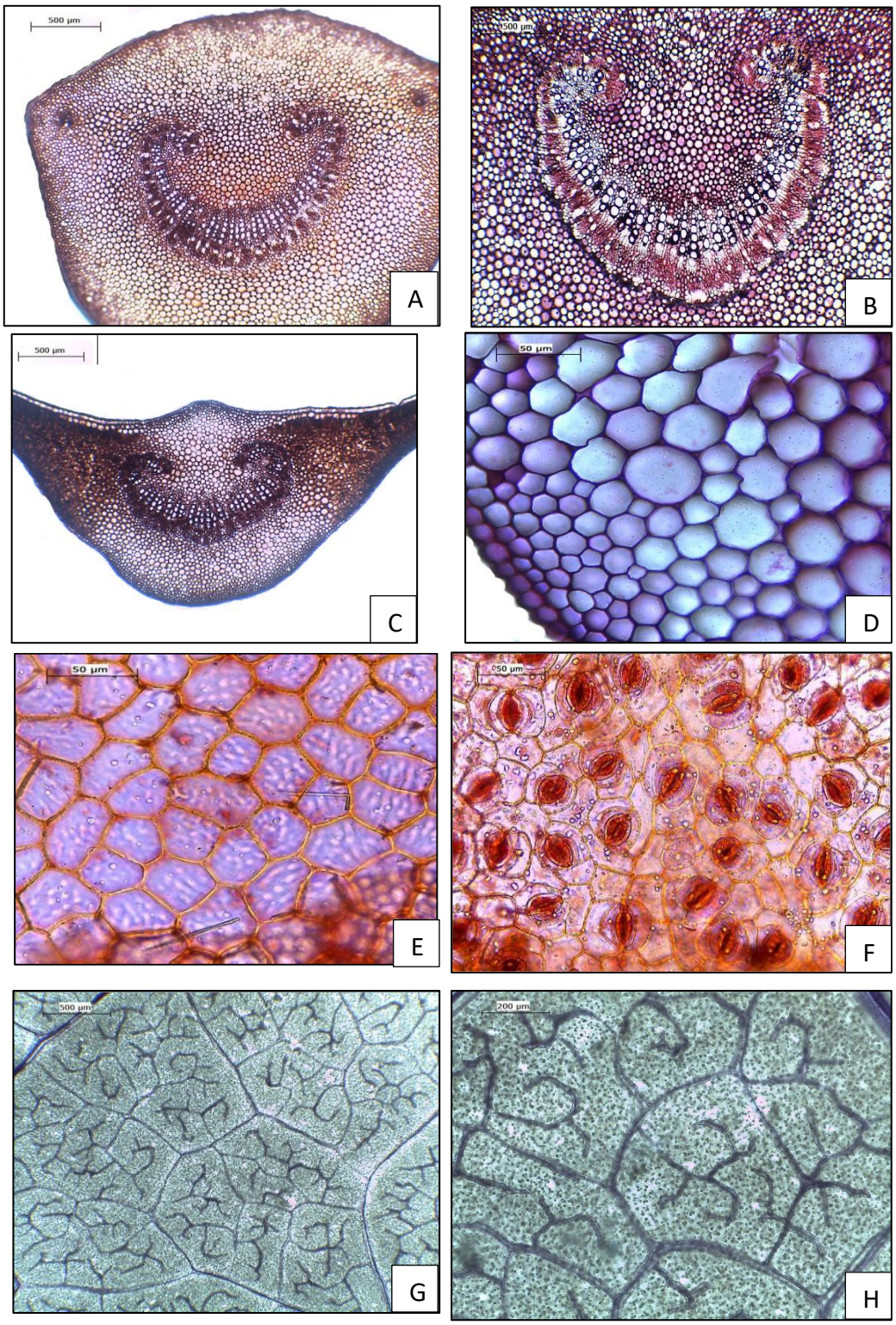

Figure 1: *Morinda citrifolia*. A&B) Cross Section of Petiole. C) Cross Section of Midrib. D) Mucilage cells. E) Adaxial Anticlinal Wall. F) Abaxial Anticlinal Wall. G&H) Leaf venation.
Scale: A,B,C,G) 500 µm. H) 200 µm. D-F) 50 µm.

CONCLUSION

The result of this study showed that the leaf anatomical characteristics can be used as an additional data to identify plant species. This research described many characteristics of the studied species that can be used to identify *Morinda longifolia* which are the presence of raphide, stomata types, anticlinal wall patterns, epidermal cell size, and vascular bundle patterns in petiole and midrib.

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