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RESEARCH ARTICLE

LEAF ANATOMY AND MICROMORPHOLOGY OF *STROBILANTHES CRISPA* (BLUME) (PECAH BELING) FROM KUANTAN, PAHANG, MALAYSIA

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ABSTRACT

The anatomical studies of selected plant species of Acanthaceae in Kuantan, Pahang are scarce. Thus, the aim of this study is to identify the leaf anatomical and micromorphological characteristics of *S. crispa* which can give additional information for identification of species. In addition, not only for taxonomy purpose, but also useful for ethnobotany perception on using as medicinal purpose because nowadays people are more searching on traditional plants as the alternative for drugs medicines, especially *S. crispa*. Methods used in this study were sectioning using sliding microtome, epidermal peeling and leaf micromorphology method by using scanning electron microscope (SEM). Findings from this study showed that the presence of collenchyma, sclerenchyma and mucilage cells in either petiole or midrib of the leaves. Meanwhile, the anatomical characteristics observed include petiole and midrib outlines, patterns of petiole and midrib vascular bundles, presence of cystolith cells and presence and types of trichomes. As a conclusion, leaf anatomy and micromorphology characteristics are useful for identification of species studied.

KEYWORDS

Acanthaceae, Strobilanthes crispa, leaf anatomy and micromorphology, ethnobotany

1. INTRODUCTION

Acanthaceae, is known as one of 24 families in the new order (Lamiales) of flowering plants, containing around 346 genera and nearly 4,300 species distributed mostly in tropical and subtropical regions of the world. The range of habitats extends from swamps and bays to extremely dry situations, but most of these plants are found in moist and shady habitat. The largest genus in this family is *Justicia* which consist of 600 species while the small genus *Avicennia* contains at least eight species of ecologically important mangroves (Petruzzello, 2013). Strobilanthes Blume is the second largest genus in the Acanthaceae. There are approximately 400 species in the world that belong to the genus (Rekhajohn et al., 2017). *S. crispa* is the plant that has higher mineral content and vitamin. It is herbaceous plant that can grow up to1 m tall. The foliage is oblong-lanceolate blade, rather obtuse and shallowly crenate crispate and the leaves have rough surface, covered with short hairs. The leaves are darker in color at the upper surface of the leaves and less rough as compared to underside. The stem is near the branch tips and distinctly 4-sided. The immature bark is purplish and will turning brown when mature. The flowers of this plant are short, dense and have panicle spikes. The flowers are yellow in colour (Nurraihana and Hanoon, 2013). The fruit is spindle in shape and measure about 11 cm long.

S. crispa is traditionally used for antidiabetic, antilytic, laxative, anticancer and as a diuretic agent (Ghasemzadeh et al., 2015; Rhun et al., 2017). This plant has many cystoliths of calcium carbonate which is one of the main characteristics to differentiate the family of Acanthaceae with other families. The high content of this calcium carbonate makes this boiled water of this plant become mildly alkaline and functioning in ease of urination. A study reported that orang asli in Kampung Bawong, Perak of West Malaysia chewed and swallowed the fresh leaves of this plant to enhance the immune system. A survey of the Malay herbal medicine in the Gemencheh settlement, Negeri Sembilan state, Malaysia, revealed the application of *S. crispa* is to treat kidney stones by placed the heated leaves on the hips of the men who suffer from it (Nurraihana and Hanoon, 2013). The growing interest in *S. crispa* as medicinal plants that has been used by local peoples therefore can be a good starting point, especially for the pharmaceutical industry. Therefore, a comprehensive study on *S. crispa* is very significant to ensure the correct identification of the species. Hence, the aim of this study is to identify the leaf anatomy and micromorphological characteristics of species studied in Kuantan, Pahang, Malaysia that later can be used as additional data in the taxonomic classification.

2. MATERIALS AND METHOD

Fresh samples leave of *S. crispa* were collected at Glasshouse and Nursery Complex, International Islamic University Malaysia, Kuantan, Pahang. Three replicated were used throughout this research. Voucher specimens were deposited at Herbarium laboratory in IIUM Kuantan. Part of petioles, midribs, leaf lamina and marginal were sectioned in a various range of thickness (15-40 µm) using sliding microtomes and stained using Safranin and Methylene blue. The specimens obtained from the sliding microtome were undergone a dehydration process in a series of alcohol (50 %, 70%, 95% and 100%) and mounted in Euparal. All slides were covered with coverslips and kept in the oven for two weeks at about 55°C for drying purposes. Anatomical images were captured using a video (3CCD) camera attached to a Leitz Diaplan microscope using Cell^B software. Suitable modifications in terms of fixation and embedding followed the method (Johansen, 1940; Saas, 1958). For the micromorphology study, the scanning electron microscopy (SEM) method was applied. The specimens were taken from a dried sample of the herbarium in which 1cm^2 lamina portions of leaf samples was cut and mounted on a mounting holder. The specimens were then coated with gold by using a sputter-coated machine. The observation of micromorphological characteristics was done under Scanning Electron Microscope Zeiss Model Evo 50.

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3. Results and Discussion

The characteristics of leaf anatomy and micromorphology for *S. crispa* are summarized as below:

3.1 Cross Section of Petiole (Figure 1A)

Epidermal cell: One layer with ratio of height:width (1:1). Vascular tissue: Opened system with continuous rings of vascular bundle with two additional vascular bundle located at the above right and left of the main vascular bundle near each wing. Sclerenchyma cell: clusters of sclerenchyma cells below the upper epidermis of petiole. Parenchyma cell: 6-8 layers of parenchyma cells. Collenchyma: Present at the epidermis of adaxial and abaxial. Mucilage cell: Present. Cystolith: Present. Trichome: Simple multicellular trichomes with pointed ends (echinate ornamentation) and and simple unicellular trichome with blunted end.

3.2 Cross Section of Midrib (Figure 1B)

Epidermal cell: One layer with ratio of height:width (1:1). Vascular tissue: Opened system with continuous rings of vascular bundle. Sclerenchyma cell: Densely scattered around the vascular bundle. Parenchyma cell: 5-6 layers of parenchyma cells. Collenchyma: Present at the epidermis of adaxial and abaxial. Mucilage cell: Present at the parenchyma cortex. Cystolith: Present at the epidermis of the cell and parenchyma cortex. Trichome: Nil.

3.3 Cross Section of Leaf Margin (Figure 1C)

Outline: slightly rounded, 30-45º recurved downwards to the abaxial side. Trichome: Nil.

3.4 Cross Section of Leaf Lamina (Figure 1D)

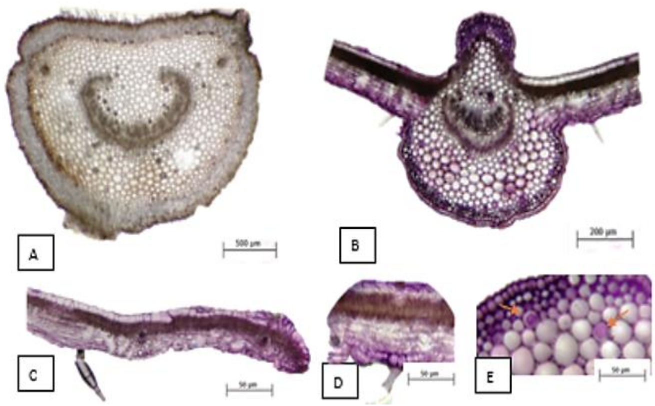
Cuticular layer: relatively thin. Adaxial epidermis: single layer with height: width ratio – 1:1. Abaxial epidermis: single layer with height: width ratio – 1:1. Chlorenchyma cells: mesophyll palisade: one= layer filling 1/3 part of the height of leaf lamina. Spongy mesophyll: 5-6 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircling the vascular bundle. Trichome: Simple multicellular trichome with pointed end (echinate ornamentation).

3.5 Peeling

Anticlinal wall of adaxial epidermis: Straight to wavy (Figure 1). Anticlinal wall of abaxial epidermis: Straight to wavy. Stomata: Amphistomatic; present on abaxial and adaxial, densely scattered on abaxial part. Homostomatic (dicytic). Stomata size; adaxial: W= 3.19 µm, H= 13.85 µm (width: max= 3.19 µm; height: max= 13.85 µm) stomata size; abaxial: W= 3.99 µm, H= 11.73 µm, (width: min= 3.72 µm, max= 4.34 µm; height: min= 11.34 µm, max= 12.15µm). Trichome: Simple multicellular trichome with tip end and glandular trichomes present at adaxial (Figure 1K) and abaxial part. Cystolith: Present

3.6 Micromorphology

Wax: Film-like layer, crust and granules found on adaxial and abaxial surfaces. Adaxial cuticular sculpturing: Anticlinal wall and periclinal wall are slightly distinguishable. Abaxial cuticular sculpturing: Anticlinal wall and periclinal wall cannot be distinguishable. Stomata: Oblong shaped stomata, homostomatic, stoma enclosed by a pair of subsidiary cells. Trichome: Peltate trichome found on adaxial surface. Simple multicellular trichome (long with blunt end, echinate ornamentation) and glandular peltate trichome are found on abaxial surface.



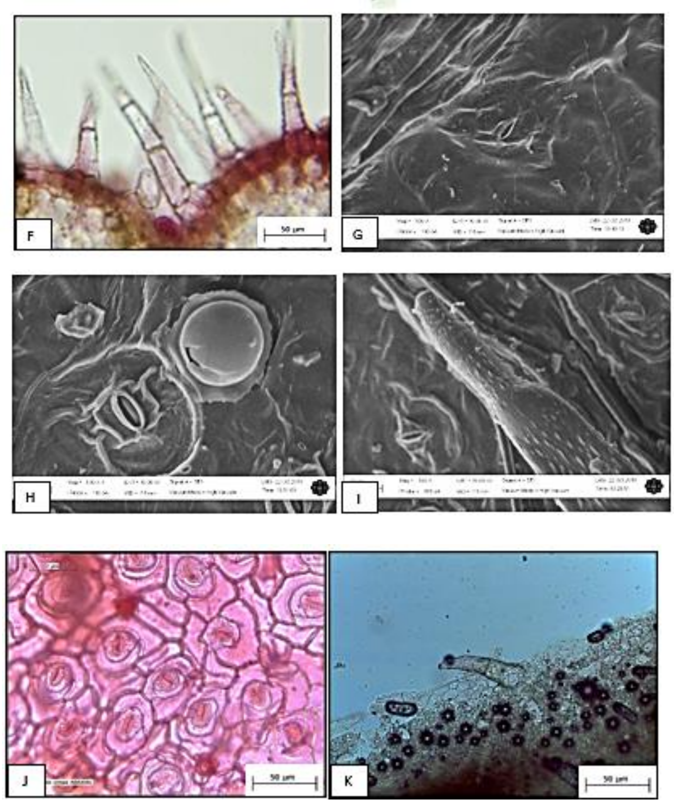


Figure 1: *Strobilanthes crispa*: A) Petiole cross section. B) Midrib cross section. C-D) Lamina and margin cross section. E) Cystolith. F) Simple multicellular trichome. G) Adaxial surface. H) Abaxial surface. I) Simple multicellular trichome (long with pointed end, echinate ornamentation) found on abaxial surface. J) Anticlinal wall. K) Simple multicellular trichome.

Scale: A) 500 µm. B) 200 µm. C-F) 50 µm. G-K) 50 µm.

The result of this study reported the significance of leaf anatomical and micromorphological characteristics in identification of *S. crispa*

3.7 Cystolith

In the midrib of *S. crispa*, the cystolith is in single and rounded shape. The presence of cystolith cells is very useful in Acanthaceae families in identification and classification of genus and species (Amri et al., 2018). The variation of cystolith cells also can be a diagnostic value for the species identification because the shape and size of the cystolith is different among species especially in Acanthacaceae species.

3.8 Pattern of Vascular Bundle

The finding from this study showed the variation in pattern of vascular bundle in petiole and midrib of the species studied. Previous study showed that petiole vascular bundle has taxonomic value for identification of certain species and can be used for classification of different genera in family Dipterocarpaceae. The pattern of vascular bundles that have been successfully identified in petiole for *S. crispa* which is opened system, continuous rings of vascular bundle and have two additional vascular bundles located above right and left wings of the main vascular bundle. Other than that, this study also observed the the midrib vascular bundle arrangements in the species studied which is opened system with continuous rings of vascular bundle. Candolle's early research in his first comprehensive study of petiole anatomy explained several fundamental concepts of vascular bundles. He distinguished two types of vascular systems which are 'opened system' and 'closed system' (Amri et al., 2018).

3.9 Sclerenchyma Cells

Sclerenchyma cells have thickened lignified walls, which make them strong and waterproof to protect the plant. The species that have been studied composed of sclerenchyma cells. The cells are classified into support system types and conducting forms. The support system of sclerenchyma tissue reduces wilting, but it is costing energy for the plant to create. The sclerenchyma is mature along with the surrounding tissues and provides more permanent support than collenchyma by maintaining the established morphology of the plant (Lopez and Barclay, 2017). There are two types of sclerenchyma cells cell walls which are primary and

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secondary walls. There are also two main types of sclerenchyma cells which are fibers and sclereids (Armando and Elhadi, 2019). All plant cells include Acanthaceae family initially have only a primary wall made mainly of cellulose. As sclerenchyma matures, a secondary wall with a high amount of lignin is deposited inside the primary wall. The secondary wall is very thick and highly lignified than the primary wall and gives a great rigidity and hardness to the cell and tissue. They also function in primarily in mechanical support and water conduction. The sclerenchyma cell is dead at maturity as the protoplast usually dies during development (Thomas et al., 2009).

3.10 Trichomes

Finding of this study showed that the type of trichomes can be useful character to the species of *S. crispa* as it has simple simple unicellular trichome with blunted end, simple multicellular trichomes with pointed ends (echinate ornamentation) and also glandular peltate trichome that are found in adaxial and abaxial surface of the species. According to a study, the types of trichomes include ribbon, simple and glandular trichomes on various vegetative plant organs in *Vitis* representatives (Yao et al., 2016). These trichomes can play various roles with variable morphological, mechanical and phytochemical characteristics in plant physiology and ecology. It is a specialized structure that function in stress resistance, including excessive light or temperature, and insect and pathogen defence and also decrease water loss (Yao et al., 2016). According to Amirul Aiman, glandular trichomes secrete essential oils to protect the plant's aerial sections from herbivores and diseases (Amirul et al., 2017). The secretion procedures of peltate trichomes differ as well. The secretory product of peltate trichomes is held in a huge subcuticular cavity, which will only rupture if external variables like high temperatures, low air humidity, or animal hostility are present.

3.11 Types of Stomata

Stomata are the minute pores that found in epidermis of the plants. The stomata are always surrounded by guard cells which they are bean shaped epidermal cells. The stomata are found in all parts of the plant except for the root. There are epidermal cells that bordering the guard cells and called as accessory cells or subsidiary cells. The guard cells contain chloroplast in it and they are alive. There are a few types of stomata which are anomocytic, anisocytic, paracytic, diacytic, actinocytic, cyclocytic and tetracytic. The type that had been found in *S. crispa* is diacytic. In this type, the stoma remains surrounded by a pair of subsidiary or accessory cells and whose common wall is at right angles to the guard cells (Harika, 2019). The main function of stomata is it used for the exchange of gases in between the plant and atmosphere. Each stoma opens in a substomatal chamber or respiratory cavity as to facilitate this function. The other function is evaporation of water which also takes place through stomata (Harika, 2019).

3.12 Types of Waxes

Other than that, waxes can be defined as the esters of long-chain acids and long-chain primary alcohols. In plant surface lipids, it is usually called as epicuticular waxes that are composed of a large mixture of different chemical compound (Wilhelm et al., 1997). One of the types is film which very thin coverings, usually only a few nm thick, expressive the obligate outermost border of the cuticle, hardly visible in the SEM and not showing cracks after drying. Layers and crust are a continuous covering that form fissures which granule is irregular in shape, mostly isodiametric, often rounded crystalloids, which may sometimes be hollow. These three types of waxes are found in *S. crispa* studied.

4. Conclusion

The result of this study showed that it is possible to use leaf anatomy and micromorphology to identify plant species in the Acanthaceae family. This research also described few characteristics of the species studied that can be used to identify the *S. crispa*. *S. crispa* was found to have cystolith that is in single and rounded shape, simple multicellular cell that is found in adaxial and abaxial surface of the leaves and diacytic type of stomata. The characteristics are cystolith presence, stomata and trichomes types, anticlinal wall patterns, type of epicuticular wax structures, cuticular ornamentation, epidermal cell size, and vascular bundle patterns in petiole and midrib.

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