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LEAF ANATOMY OF THE MEDICINAL PLANT *Sphagneticola trilobata* (L.) PRUSKI

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Abstract: Leaf anatomical and micromorphological studies were conducted on *Sphagneticola trilobata* in the Asteraceae family. Since there has been no taxonomic study of *S. trilobata* in Malaysia, this study aims to determine and investigate the leaf anatomical and micromorphological characteristics of the species that can be used in its identification and for supportive data in its classification. The methods used in this study include the preparation of transverse sections of the petiole, midrib, lamina and margin using a sliding microtome, as well as epidermal peels, and they were observed under a light microscope. The leaf micromorphological study was conducted using a scanning electron microscope. The findings demonstrated characteristics of the leaf anatomy and micromorphology of *S. trilobata* that could be used in the identification and classification of the species, such as having arc-shaped separated vascular bundles, anomocytic stomata, flakes, crusts and granules of waxes, as well as simple multicellular (long, pointed end, with echinate ornamentation), capitate (multicellular, short stalk, unicellular head) and peltate glandular trichomes. In conclusion, the study revealed that the anatomical and micromorphological characteristics of *S. trilobata* have a taxonomic value that can be useful in terms of identification, differentiation, and classification at the species level.

Keywords: Leaf anatomy; leaf micromorphology; Asteraceae, *Sphagneticola trilobata*.

Introduction

The word "Aster" means "star" in Greek, and it is used to refer to plants with a star-like inflorescence. The Asteraceae family is commonly known to have medicinal value (Koc *et al.,* 2014). There are approximately 25,000 species in this family, including 950 to 1500 genera based on the review by Ekeke and Ogazie (2020) from research by De Souza *et al.* (2018).

Sphagneticola is one of the genera in the Asteraceae family under the order Asterales, which is the subfamily of Asteroideae and a tribe of Heliantheae. *S. trilobata* was native to tropical America and was later spread to Brazil around the Caribbean area. Now, it has been commonly spread to other parts of the world, regardless of the elevation. Even though records are limited, it is believed that *S. trilobata* existed in West Africa based on a study by the Centre for Agriculture and Bioscience International (2021).

S. trilobata is known as the Singapore daisy (English) or *bunga serunai* (Malaysia) and it is recognised as a perennial plant and can grow up to 70 cm. Based on Flora and Fauna Web (2020), above the ground, the species forms dense crust coverings. The stems are rounded with roots on the nodes, and the stolons can grow up to 2 m horizontally. The leaves are simple and oval-shaped, and fleshy-looking, with a thick and smooth surface on the adaxial. They are faded green on the abaxial surface, have a toothed or irregularly serrated leaf margin, are palmately lobed, with an acute apex and a cuneate base. A matured leaf has a glossy surface. The estimated size of one leaf is 20.25 cm^2 to 45 cm^2.

It has an opposite leaf arrangement, and the venation of the leaf is pinnate. The flowers are bisexual, are yellow or golden in colour, form a radial symmetry, and are not influenced by the environment. The inflorescence has a capitulum and flowers throughout the year. The mature achenes are brown and produce simple fruits (Flora & Fauna Web, 2020).

Traditionally, *S. trilobata* is used to treat hepatitis, indigestion and infection. It is anti-hepatotoxic and is used to prevent liver damage. It also exhibits antibacterial properties against *Bacillus subtilis*. It has also been used to clean the placenta following birth (CAB International, 2021). A pharmacology study showed that the hydroalcoholic dried extract of *S. trilobata* has a significant anti-inflammatory effect on the ears of mice, along with a reduction of plasma extravasation and migration of leukocyte activity (Fucina *et al.,* 2016).

Nurul-Aini (2011) stated that the importance of plant anatomy cannot be denied as it provides additional data for the identification and classification of a species. Therefore, this study intends to identify the anatomical and micromorphological characteristics of *S. trilobata* so that the data can be used in the taxonomical study of the species.

Materials and Methods

Three to five replicates of *S. trilobata* were collected from Bukit Pelindung, Kuantan, Pahang. The specimens, with the voucher number ARSZ04, were deposited at the International Islamic University Malaysia (IIUM) Herbarium after being verified by botanist Assistant Professor Dr. Rozilawati Shahari. Fresh leave samples were fixed in a 3:1 AA Solutions (70% alcohol: 30% acetic acid) (Johansen, 1940 as cited in Amirul-Aiman *et al.,* 2017). The petioles and midribs were sectioned into various thicknesses (15-30µm). A soft brush was soaked in 50% alcohol and was applied on the knife of the microtome. Safranin and alcian blue were used to stain the sample slices. Later, the process was continued with dehydration by soaking the slices with a series of alcohol solutions (50%, 70%, 95% and 100%) and finally mounted with Euparal (Cutler *et al.,* 2007). The images were captured using a three-CCD (3CCD) camera attached to a microscope (Leitz Diaplan, United Kingdom) and were processed using the Cell^B software.

For the leaf epidermis, specimens were placed in a basin of freshwater to avoid drying. A sharp blade was used to carefully scrape the abaxial and adaxial surfaces. After getting a small, thin, and clear surface, the leaf samples were immersed in Jeffrey's solution for a few minutes and later stained with safranin. Then, the leaf peels were placed on a slide and observed under a light microscope. For the scanning electron microscope (SEM) method, small pieces of leaves of 1.0 cm^2 were cut from the leaf laminas and were affixed to aluminium stubs with double-sided adhesive tapes. The specimens were then coated with a thin film of gold. The specimens were examined under an SEM (EVO®50, Carl Zeiss AG) as done by Cutler *et al.* (2007).

Results and Discussion

The characteristics of the leaf anatomy and micromorphology of *S. trilobata* are summarised as follows:

Petiole

Adaxial outline: Concave. Abaxial outline: convex. Vascular tissue: Separated vascular bundle arranged in an arc-shaped. Mucilage canal: Present at the parenchyma cortex. Mucilage cell: Present at the parenchyma cortex. Parenchyma cells: Six to ten layers. Collenchyma cells: Two to three layers under the adaxial and abaxial of the epidermis. Sclerenchyma cell: Cluster of sclerenchyma cells present at the vascular bundle. Trichome: Capitate glandular trichomes (multicellular stalk, unicellular head) (Figure 1A).

Midrib

Adaxial outline: Concave. Abaxial outline: Arc-shaped. Vascular tissue: Open system, continuous ring of vascular bundle, with two additional vascular bundles. Mucilage canal: present at the parenchyma cortex. Mucilage cell: Present at the parenchyma cortex. Parenchyma cells: seven to nine layers. Collenchyma cells: Two to three layers under the adaxial and abaxial of the epidermis. Sclerenchyma cell: Cluster of sclerenchyma cells present at the vascular bundle. Trichome: Simple multicellular trichomes (long, pointed end, echinate ornamentation) (Figure 1B).

Lamina

Cuticular layer: Relatively thick. Adaxial epidermis: Single layer with height-width ratio of 1:1. Abaxial epidermis: Single layer with height-width ratio of 1:1. Chlorenchyma cells: Mesophyll palisade cells, two layers filling half of the height of the leaf lamina. Spongy mesophyll: Six to seven layers. Vascular bundles: Simple vascular bundles. Parenchyma cells: Single layer encircling each vascular bundle. Trichome: Capitate glandular trichomes (multicellular stalk, unicellular head) (Figure 1C).

Margin

Outline: Straight outline with rounded blunt end (Figure 1D).

Leaf Peeling

Adaxial epidermis anticlinal wall: Straight to wavy. Stomata type: Anomocytic. Stomata size range: W × H = (21.00 µm – 23.04 µm) × (30.64 µm – 37.99 µm). Type of trichome: Simple multicellular trichomes (long, pointed end, echinate ornamentation). Abaxial epidermis anticlinal wall: Straight to wavy. Stomata type: Anomocytic. Stomata size range: W × H = (12.73 µm – 19.74 µm) × (29.45 µm – 33.78 µm). Trichome: Capitate glandular trichomes (multicellular stalk, unicellular head) (Figure 1G - H).

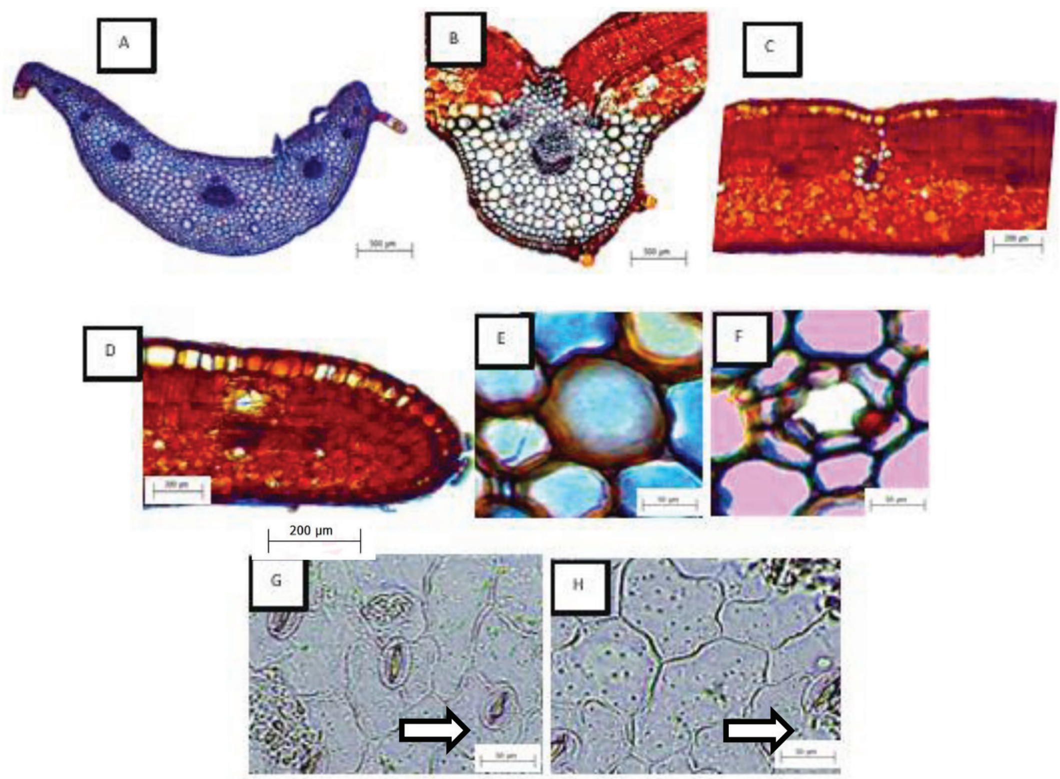

Figure 1: The cross section and leaf epidermis of *S. trilobata.* A) The petiole, B) midrib, C) lamina, D) margin, E) mucilage cell, F) mucilage canal, G) anomocytic stomata, and H) straight to wavy anticlinal wall. Scales: A-B) 500 µm, C-D) 200 µm, and E-H) 50 µm

Leaf Epidermis

Epicuticular waxes: Both adaxial and abaxial surfaces have flakes, crusts and granules of waxes. Adaxial cuticular sculpturing: The epidermal cell outline is slightly distinguishable, the anticlinal walls are raised into ridges and the periclinal walls sink. Abaxial cuticular sculpturing: The epidermal cell outline is slightly distinguishable, the anticlinal walls are raised into ridges and the periclinal walls sink. Stomata: Amphistomatic, the guard cell pair is elliptical in shape. Trichome: Simple multicellular trichomes (long, pointed end, echinate ornamentation) at both surfaces, capitate glandular trichomes (multicellular stalk, unicellular head) and peltate glandular trichomes at the epidermis of the abaxial surface (Figure 2N - P).

Vascular Bundle Pattern

There were many significant anatomical and micromorphological characteristics in the additional data that identify the species (Nurul-Aini *et al*., 2018). A previous study by Hare (1942), as cited by Talip *et al.* (2017), reported that the characteristics of vascular bundles have a significant value in terms of taxonomical data. Metcalf (1944) stated that there are open-system (U-shaped) vascular bundles and closed-system (O-shaped) vascular bundles. He also emphasised the significance of the shape and pattern of the vascular in identifying plants. Liese (1980) also reported that the whole structure of the anatomy of any cross-sectional part can be determined by the vascular bundle, including the shape, number, arrangement and size of the vascular. The vascular bundle can be

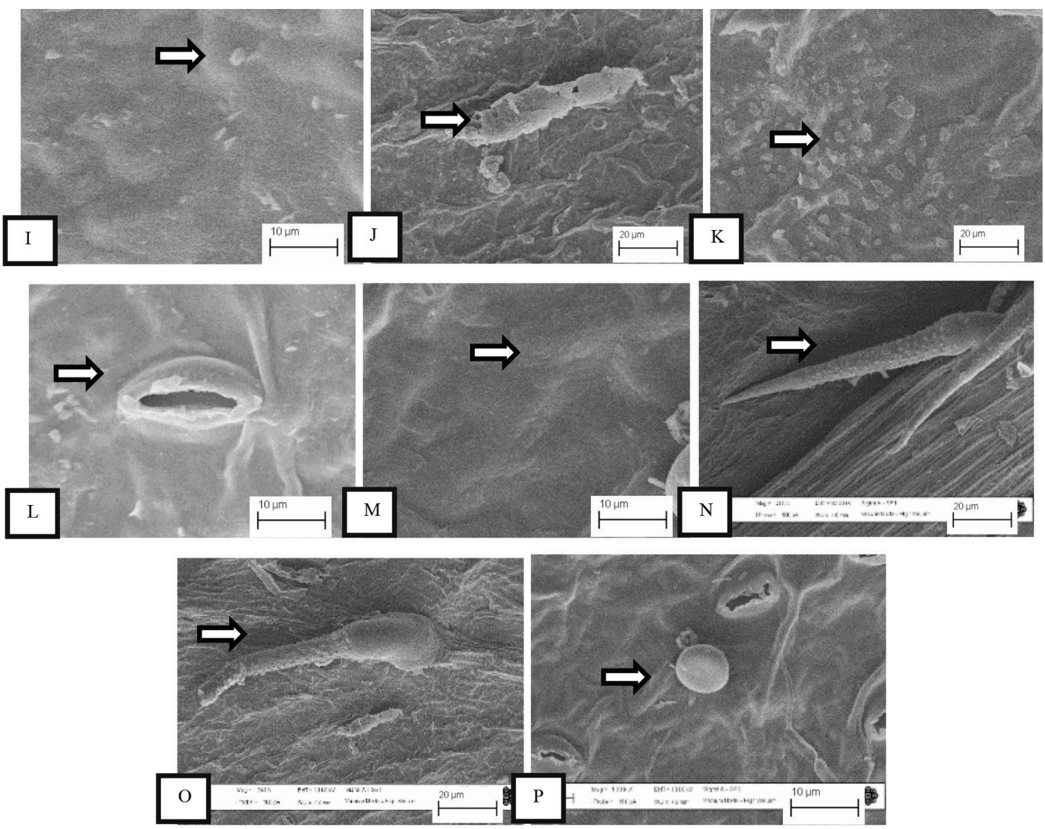

Figure 2: The micromorphology of *S. trilobata*. I) The granules, J) crusts, K) flakes, L) elliptical stomata, M) anticlinal and periclinal wall, N) simple multicellular trichomes (long, pointed end, echinate ornamentation), O) capitate glandular trichomes (multicellular stalk, unicellular head), and P) peltate glandular trichomes Scales: I) 10 µm, J-K) 20 µm, L-M) 10 µm, N-O) 20 µm and P) 10 µm

distinguished by its characteristics, compared with the parenchyma cells.

Silva *et al*. (2012) stated that the leaves of *S. trilobata* have a secretory canal on the parenchyma cortex, and they are linked to the vascular bundles to form a network between tissues. Vieira *et al*. (2008), as cited by Silva *et al*. (2012), reported that another species from the Asteraceae family, such as *Ageratum fastigiatum*, also has the characteristic of the secretory canal being linked to the vascular bundle. In this study, it was found that *S. trilobata* has open-system vascular bundles on the petioles and midribs, with a continuous ring of vascular bundle and additional vascular bundles as shown in Figures 1A and 1B.

Ekeke and Mensah (2015), in their study, focused on the anatomical characteristics of the midribs of 17 species (Asteraceae family) in Nigeria. One of the characteristics studied was the vascular bundles. The number of vascular bundles in the midribs varied from one to eight. The shapes observed were arced, oval, U-shaped and V-shaped. The arrangement of the vascular bundle, meanwhile, is significant in identifying a species, which helps in the classification of a species in the taxonomical study. A study by Ozcan *et al.* (2015) on the *Cirsium* genus (Asteraceae family) in Turkey revealed that the anatomical characteristics of the vascular bundles can be used to classify plants into their taxa. They not only observed the number of vascular bundles in the midribs, but they also observed the length and width of the vascular bundles to obtain more specific data.

Budel *et al.* (2018) showed that the Asteraceae family always has characteristics that can be added from one place to another. They observed and studied the differences between six Brazilian species of *Baccharis*. They found that the midribs have vascular bundles that were circular and open arced. The shapes of midrib, such as biconcave, concave-convex, flat-concave and slightly concave-convex, would add significant differences between the species. Among the six species of *Baccharis*, the vascular bundle was clearly embedded in the parenchyma cortex.

Two years later, Ekeke and Ogazie (2020) studied approximately 30 plants in the Asteraceae family. They found that there were five types of vascular bundles in the distal and basal portions. Among the types of vascular bundles were three major vascular bundles with an open separate arc in *S. trilobata* and two accessary arc vascular bundles in *Vernonia cinera*. The outline of the adaxial petiole for the basal and distal portions of *S. trilobata* was V-shaped. Comparing the discovery of Ekeke and Ogazie (2020) with the present study, *S. trilobata* had separated vascular bundles arranged in an arc, but with one major vascular bundle and six extra vascular bundles. In addition, the adaxial outline of the petiole of *S. trilobata* was concave, as seen in the study by Ekeke and Ogazie (2020). In addition, this study on *S. trilobata* showed that the vascular bundles on the petiole and midrib are open-system vascular bundles, with a continuous ring of vascular bundle with additional vascular bundles.

Hulley *et al.* (2010) reported that *Pteronia incana* (Asteraceae) contained similar anatomical characteristics with *Pteronia divaricate* in terms of the presence of vascular bundles in the leaf sections. Besides that, there was a solitary secretory canal observed below the midrib and vascular bundles that secreted oil. According to Karthika and Manivannam (2018), the midrib of *Wedelia trilobata* has more than one vascular bundle. *W. trilobata* is the old name of *S. trilobata*, but it is still used as the common name for *S. trilobata*.

The Thickness of the Parenchyma Layer

Another anatomical characteristic is the thickness of the parenchyma layer. According to Evert (2006), there are three types of fundamental tissue systems (or known as ground systems), namely parenchyma, collenchyma and sclerenchyma cells. The most common one are parenchyma cells, which are still undifferentiated. Meristematic cells are similar to young parenchyma cells. Parenchyma cells may change functions or combine with other cells. Due to their functions that can be differentiated, parenchyma cells are responsible for regeneration, wound healing, and the formation of roots and shoots. In addition, parenchyma cells function as water reservoirs. Parenchyma cells may be thick or thin according to the plant's growth.

Four *Aldama* species (Heliantheae tribe, Asteraceae family) in Brazil were observed by Bombo *et al.* (2017). The species showed different parenchyma cell characteristics. Instead of measuring the parenchyma, the study found that canals were present at the ground parenchyma of the midribs of all the *Aldama* species. Secretory cavities were also found in the parenchymatic pith in the roots. According to a study by Prasanth and Lakshmana (2018), the root of *W. trilobata* observed in India revealed that the hypodermal layer consisted of parenchymatous cells and a few unicellular spaces for ease of gaseous exchange and water movement. Meanwhile, the powder root of *W. trilobata* also showed parenchymatous cells, lignified spiral vessels and cork cells. The presence of anatomical characteristics can be used to avoid misidentification of the plant since the benefits of *W. trilobata* can be accessed by powder materials besides avoiding an unauthorised person wrongly claiming that another powder plant is the original powder of *W. trilobata*.

The research conducted by Ekeke and Ogazie (2020) measured the thickness of parenchyma cells in the distal and basal portions of the petiole of *S. trilobata*. On average, the measurements of the adaxial surface in the distal portion varied from 153 µm to 164 µm and the abaxial surface varied from 173 µm to 180 µm. Meanwhile, the measurements of the basal portion of the adaxial surface varied from 31 µm to 35 µm and the abaxial surface ranged from 53 µm to 60 µm. In comparison, the parenchyma layers of *S. trilobata* were seven to nine layers, which varied between 147.90 µm and 171.35 µm for the adaxial layer and between 330.23 µm and 396.89 µm for the abaxial layer.

A study by Milan *et al.* (2006) examined the parenchyma cells of selected select number of species of Asteraceae, which were *Mikania glomerata* Spreng, *Porophyllum ruderale* Cass and *Vernonia condensata* Baker. At the leaf base, the parenchyma layers were adjacent to the collenchyma cells and the parenchyma layers contained idioblasts, which can later form druses for protection. The leaf mesophyll also consisted of parenchyma tissues.

A study by Jesus *et al.* (2016) on parenchyma cells found that there is a direct relationship between the size of parenchyma cells and aluminium toxicity in *Helianthus annuus* (Heliantheae tribe, Asteraceae family). Plants that were exposed to aluminium had a bigger and thicker size of parenchyma cells compared with plants that were not exposed to aluminium toxicity. Hence, it can be concluded that the bigger parenchyma cells can be a guide to other researchers on aluminium toxicity.

Bezerra *et al.* (2018) studied *Verbesina macrophylla* (Cass.) F. S. Blake (Asteraceae family) in Brazil and found that its oil was produced from the plastids and parenchyma cells and secreted into the secretory ducts. It is believed that the secretion involved in chemical defence and sesquiterpenes were detected as much as 55.1% in the plant. The plant is also commonly used for medicinal purposes since it is native in Brazil. Based on the authors' observation, the vascular bundles are enclosed with large parenchyma cells.

The Thickness of Collenchyma Cells

O'Neill (2010) stated that the thickness of collenchyma cells helps the plants to withstand strong wind. Matured collenchyma cells are less elastic and more rigid compared with the young ones (Fahn, 1967). Beck (2010) stated that the collenchyma and sclerenchyma cells function as support for the plants, especially to the margin of the leaf near the vascular bundles. Collenchyma cells consist of hemicellulose, cellulose, water and pectin. Normally, the collenchyma cells can be found around the vascular bundle of leaf stems and petiole, which are adjacent to the cortex.

Bezerra *et al.* (2018) reported that *V. macrophylla* (Asteraceae family) in Brazil having an angular collenchyma in the midrib is a significant characteristic in identifying the plant since it has a good essential oil and sesquiterpenes, which would not be misidentified with other plants. The type of collenchyma cells can be used as one of the diagnostic characteristics of a species. Ajao and Moteetee (2017) reviewed the species of *Tithonia diversifolia* (Hemsl) A. Gray. (Asteraceae family, Heliantheae tribe), and the collenchyma of the stem was angular-tangential. This characteristic helps the plant withstand bad weather.

Khammas *et al.* (2019) compared the anatomical characteristics between three *Centaurea* species (Asteraceae family) in Iraq and observed the various thickness of the collenchyma in the stem. The location of the collenchyma and chlorenchyma alternated, with the largest vascular bundle facing the collenchyma and the smallest facing the chlorenchyma.

Wahua and Nwuzi (2021) examined the morphology and anatomy of *Ageratum conyzoides* Linn (Asteraceae family) in Nigeria. They found that the hypodermis in the midrib, petiole and stem consist of collenchyma, general cortex and pith. The collenchyma has two to three layers, and it supports the plant against the wind.

Ramachandran and Radhakrishnan (2020) studied nine plants in the *Acmella* genus (Asteraceae family) in India and they observed that there were significant differences among the nine species, which was their various number of collenchyma layers. The thickest collenchyma layers can provide the plant with the strongest protection against strong wind. This characteristic may help the plant stay still and not break. In this study, it was found that there are two to three layers of collenchyma cells on the petiole and the midrib of *S. trilobata* at the adaxial and abaxial of the surfaces.

Ibrahim *et al.* (2016) showed the importance of the number of layers of collenchyma cells between two planst of the same genus, *Datura* (Solanaceae family). The adaxial and abaxial layers of the collenchyma cells of *D. innoxia* were –four to five, and –two to three layers, respectively. Meanwhile, the collenchyma numbers in the adaxial and abaxial surfaces of *D. stramonium* are –five to eight layers and –three to four abaxial layers. *Datura* are known as toxic plants, and they are used for medicinal purposes and its use must be highly supervised by a qualified practitioner. If not carefully observed, the consumption of the species may cause blurred vision, fever, amnesia and coma (Krenzelok, 2010).

Sclerenchyma Cells

According to Dickison (2000), sclerenchyma cells consist of a compilation of cells that are lignified after maturing and do not maintain the living protoplast in their mature stage. Sclerenchyma cells are also one of the important elements in plant tissues as they provide mechanical support. They contain lignin and cellulose. When sclerenchyma cells mature, they may die. Sclerenchyma cells consist of fibres and sclereids. Examples of fibres that have been commercialised are rattan and cotton (Glimn-lacy & Kaufman, 1984). This study found that sclerenchyma cells were present in the vascular bundles of the petioles and midribs of S. *trilobata*.

According to Rivera *et al.* (2019), Asteraceae plants are found in xerophytic scrubs in Mexico City. They observed 61 species and 13 tribes of the Asteraceae family to identify the plants at the genus and species levels. One of the tribes, Heliantheae, was also examined. Sclerenchyma cells were present among the *Aldama* and *Lagascea* genera. Sclerenchyma cells could be used as significant data when comparing plants in the Heliantheae tribes.

Che Amri *et al.* (2019) found that the characteristics of sclerenchyma cells were similar in the different species in coastal areas. Since the selected species were found in coastal areas (Balok and Separ beaches), the presence of sclerenchyma cells is important in protecting the plants against strong winds in the coastal region. Since sclerenchyma cells are close to each other, they may provide support during turbulent weather. Thus, the characteristic of the sclerenchyma is considered important and this is supported by Ramachandran and Radhakrishnan (2020). They observed nine species of *Acmella* (Asteraceae family) in India and agreed that the sclerenchyma has a taxonomic value. It is present between the vascular bundles, and adaxial and abaxial epidermises.

Makbul *et al.* (2011) examined 18 species in the Asteraceae family. They found that latex and secretory channels, sclerenchyma and collenchyma cells were found to be important in examining a plant. The position of the vascular bundle, secretory cells in the cortex and the phloem and xylem sclerenchyma characteristics can facilitate the identification process, even though some species are similar to one another.

A study by Batista and De Souza (2017) filled the gap of the weed of the Asteraceae family. All the species have uniserite or pluriseriate sclerenchymas. All the Heliantheae species among the Asteraceae family have phytomelanin, a mesocarp with three layers and middle mesocarp. All the characteristics from the study can be used as diagnostic characteristics for the Asteraceae species. *S. trilobata* examined in this study consists of clusters of sclerenchyma cells around the vascular bundle as extra support to the leaf.

Mucilage Cells

Beck (2010) stated that the mucilage cell is a cell that contains gums or mucilages or any related carbohydrate materials that swell in water. The plant *Hydrangea paniculata* has mucilage cells in the secondary phloem. The cells contain a package of raphides and needle-like crystals composed of calcium oxylate. The epithelial cells secrete different substances into the cavities, such as mucilage or oils in the Hypericaceae family. The secretory cavities are found in the leaves of all genera and appear macroscopically as translucent dots or opaque dots. They also appeared as gums or resins in conifers, such as in the Anacardiaceae family, or are also known as a layer of secretory cells that restricts the resulting cavity.

Gregory and Baas (1989) mentioned that ruthenium red can be used in the histochemical analysis of mucilage cells. The trichomes on the mucilage cells are a significant feature for the species (Filartiga *et al.,* 2016). A summary by Dickison (2000) stated that mucilage cells have lignified walls, which helps in the storage of water in the plant. The water stored may avoid the plant from dehydrating, leading to no issues in terms of water stress. The mucilage cell is a parenchymatous cell that undergoes specialisation and contains mucilage, normally round and bigger. However, sometimes it is difficult to differentiate between the mucilage and oil cells just by looking at their appearances.

However, Dickison (2000) also mentioned that there are differences between the oil cells and mucilage cells. The site of oil accumulation is between the cupule wall and plasma membrane, while the mucilage accumulation is between the cell wall and plasma membrane. The main organelles in the oil and secretion cells are plastids and Golgi vesicles, respectively. The chemistry compositions in the oil cells are flavonoid aglycones, fats and terpenes, and the mucilage contains polysaccharides. Besides the leaf, mucilage cells are also present in the plant seed when comparing between the Asteraceae species (Plachno *et al.,* 2020). According to Plachno *et al.* (2020), there are two types of mucilage in the plant seeds. First, mucilage cells that secrete mucilage to form a mucilage drop, andsecond, mucilage cells that compile mucilages in a cell.

A study by Mariani *et al.* (1988) found that mucilage cells are modified epidermal cells. In the genus *Salix*, mucilage cells are seen when the buds opened, and the leaflet cells became enlarged. However, mucilage cells are already in the zone of the upper leaf, but they are differentiated in the basal zone. The number of mucilage cells increased as the leaf ages. The mucilage could be present in the adaxial, abaxial or both of the epidermises. It could be an individual cell or in a grouping, and the cells may become broad. By using a light microscope, the mucilage cells can be identified as having thick tangential walls and are larger than the parenchyma cells.

Liesenfeld *et al.* (2019), in their study, connected the functions of mucilage cells and plants in sand fields. Selected Asteraceae plants in Brazil were observed, and they found that *Aspilia montevidensis* (Heliantheae tribe) has mucilage on the epidermis, which shows that mucilage cells help plants survive with minimal water in a dry environment. Noraini *et al.* (2019) reported that the mucilage cell is commonly found in the Sterculiaceae family in the genus *Sterculia*. The mucilage cell is bigger than the parenchyma cell, and it is convex shaped and has mucus. In this study, it can be related to the leaves of *S. trilobata*, which look fleshy due to the mucilage cells that hold water in the leaf and prevent dehydration.

Mucilage Canals

Another family that contains mucilage canals or cavities is Lycopodiaceae, discovered by Hegelmaier (1872), as cited in Bruce (1976), which are three species, namely *Lycopodium inundatum*, *L. alpecuroides* and *L. annotinum*. There was a canal in the rhizome of *L. inundatum* and he agreed that it was schizogenous. Another study by Brebner (1895) defined the mucilage canal as schizogenous intercellular spaces surrounded by living secretory epithelium.

The mucilage canal is found in the fronds of *Angiopteris evecta*, *Marattia alata* and *Marattia cicutaefolia*. In the family Marattiaceae, the mucilage canal characteristics are more attractive than others. Brebner (1895) affirmed that there was no mucilage canal in the root of *A. evecta*, but it consists of tannin sacs that form a canal.

Kasahara (1985) revealed that mucilage canal formations occurred in two species, namely *Laminaria yezoensis* and *Laminaria angustata* (Laminariaceae family) through the secretory cells generated from the periclinal epidermal cell division. After that, the process is followed by the collapse and reorganisation of cells to form the mucilage canals. Some of the mucilage canals may appear in the lamina as a by-product of secondary growth during transition. The existence of a mucilage canal in the lamina depends on the season, the age of the plant collected and the area in the lamina that was observed.

Che Amri *et al.* (2019) reported that the mucilage canals in *Canophyllum inophyllum* are present in the parenchyma cortex. Thus, the mucilage canals and cells may provide a taxonomic value in identifying the species. According to Metcalfe and Chalk (1950), as cited in Wilkinson (1992) too, the Araliaceae has secretory canals in the petioles of 34 genera that have been investigated.

Noraini *et al*. (2019) found that the mucilage canal is commonly found in a basic tissue, such as the petiole. Sometimes, the mucilage canal is surrounded by epithelial cells. The mucilage canal could be used in a systematic study of a species. They are also found on the parenchyma cells of the petiole and midrib of *S. trilobata*. The mucilage canal can be a diagnostic characteristic for *S. trilobata* since this characteristic is commonly found in the Asteraceae family.

Leaf Margin

Wightman *et al.* (2018) examined several *Saxifraga* species and they found that the leaf margin of one of the species, *S. scardica*, was thick and partially transparent when compared with *S. cochlearis*. By using SEM, it was observed that *S. cochlearis* has steep-sided margins and *S. scardica* has tapered margin. The margins of *S. cochlearis* consist of tightly packed cells for the palisade tissue layer, but the margins of *S. scardica* show that the palisade layer cells were extended and rounded inside the tapered margins. The pointed margin of *S. cochlearis* was built from the layer of the epidermal cell, and the tapered margin of *S. scardica* was built from the thick-walled cylindrical cells.

Noraini *et al.* (2019) stated that the leaf margin of a plant can be observed from the last vascular bundle in the lamina until the edge of the leaf. The leaf margin could be straight, straight-to-downward or blunt-upward. The specimen must be placed carefully on the slide to avoid errors in the observation of the shape of the leaf margin. The fresh sample can be viewed in the field to observe the leaf margin.

Mason and Donovan (2015) described the morphology of the leaf margin as full, teeth/dentate, large teeth or reduced teeth. Using the cross-sectional method, the anatomy of the leaf margin can either be straight, curved-downward or any condition viewed by any researcher. Trichomes were not present at the end of the leaf margin of *S. trilobata*. This species has an epidermal cell surrounding the leaf margin as protection. The outline of the leaf margin is straight-outline with a rounded blunt end. This characteristic can be used to identify the species.

A recent study by Lusa *et al.* (2018) on Lycnophorinae (Asteraceae family) in Brazil showed that the leaf margin is different among the species with two major differences, in which some of the species have flat margins and some have revolute margins.

Dickison (2000) stated that the structure of the leaves was strongly affected by the environment, which can be seen in the morphology and anatomy of the leaf. Historically, the anatomy and morphology of the leaf can be used as an indication of the conditions of an environment. For leaves that have an entire margin and apical drip on the tip, they are usually found in lowland tropical rain forests. Leaves in xeric climate must be different from leaves that gets enough rainfall.

Stomata

The stomata can be used as the key differences among the different genera as seen in the study by Ghimire *et al.* (2014). They found that the genus *Austrotaxus* (Taxaceae family) had similarities in the morphology of subsidiary cells of the *Taxus* genus in terms of having a band or a ring surrounding the stomata. The characteristics, such as the morphology of stomata and mesophyll layers, can be used to differentiate the tribe of plants, and they can be used as additional data in the classification and identification of species. The types of anticlinal walls for both epidermises were straight to wavy when each epidermal cell was observed.

A group of researchers from Turkey investigated the leaf anatomy of 26 plants from the *Cirsium* genus (Asteraceae). Ozcan *et al.* (2015) observed the characteristics of the stomata and proposed the study as a pioneer study of *Cirsium*. The number of stomata is important in examining the stomata using the quantitative method. Asteraceae is commonly known for having anisocytic-anomocytic stomata. Based on the observation, plants that grow in dry conditions and are exposed to full sunlight have bigger stomata.

Bano *et al.* (2015) investigated the different characteristics of stomata among the Asteraceae species in the Western Himalayas. Some species have stomata with an irregular shape, and some species have polygonal stomata. Most species have anomocytic stomata and a few species has anisocytic stomata. The adaxial epidermis has fewer stomata compared with the abaxial epidermis. However, the size of stomata at the abaxial epidermis is bigger than at the adaxial epidermis. The study that focused on stomata characteristics may help in adding taxonomic value at the genus and species levels.

A study by Budel *et al.* (2018) supported the study by Bano *et al.* (2015) on the connection between the identification of a species and the characteristics of the stomata. Among the six Brazilian species of *Baccharis* (Asteraceae family), they observed that each of them have different types and occurrence of stomata. For example, *B. punctulate* is hypostomatic (the stomata are present only at the abaxial epidermis) and *B. pauciflosculosa* is amphistomatic (the stomata are present at both epidermises). The distribution of stomata in *B. punctulata* is the highest among the *Baccharis* plants.

Several authors have considered the characteristics of the *Centaurea* genus (Asteraceae) in Turkey (Citak & Dural, 2018). Several characteristics that can be observed are the measurement, type, and shape of stomata, and the type of anticlinal wall. They examined 21 species and found that the stomata are amphistomatic as they are present on both the adaxial and abaxial epidermises. The type of stomata is anomocytic. These characteristics can be observed using a light microscope and they show that the stomata are key in classifying the plants into their taxonomy.

The type of stomata in *S. trilobata* is anomocytic, which means that there are no subsidiary cells around the stomata. The shape of the stomata is elliptical as reported in this study on *S. trilobata*. Usually, anomocytic stomata can be found in Myrtaceae, Ebenaceae, Malvaceae, Sapotaceae and Rhizophoraceae families. Subsidiary cells re different from epidermal cells that cover the leaf surface. Different types of stomata have a taxonomic value that identifies the plant at the genus and species levels. Other types of stomata are diacytic, paracytic, anisocytic, cyclocytic andtetracytic (Noraini *et al.,* 2019).

Hussain *et al.* (2019) found various types of stomata in 13 species of *Artemisia* (Asteraceae family) plants in Pakistan. Anomocytic, diacytic, anomotetracytic, and anisocytic stomata are present due to the way the subsidiary cells are arranged around the stomata. The shapes of the stomata among the species could be irregular, polygonal and elongated. These diagnostic properties give an added value in the taxonomic traits to solve the clash of taxonomy in the *Artemisia* genus.

Epicuticular Wax

Epicuticular wax can be used as a taxonomic value to identify a species. Turunen and Huttunen (1990) shared the results of the importance of epicuticular wax in a species. Any air pollutant or other factors that directly react to epicuticular wax may cause a negative impact on the outer structures of the wax. Wax is normally present at the outermost layer of the leaf surface (Beck, 2010). In some plants, the wax can be seen using a highly magnified microscope, revealing scale-like platelets. If observed with the naked eye, the leaf appears to be smooth. The epidermis of the leaf has the same specialised elements as the stem, such as trichomes, surface sculpturing, stomata and epicuticular wax. The physical appearance of epicuticular wax can be in the crust surface, flakes or a filament and granules.

Nurul-Aini *et al.* (2014) found different types of epicuticular wax, such as film-like flakes, crust of wax, verrucate, granular coating and stellate-like layers, on the adaxial, abaxial or both of the surfaces in eight species of Acanthaceae. For example, verrucate wax layers are present on the adaxial surface of *Acanthus montanus*, and stellate-like layers are present on the abaxial surface of *Acanthus ilicifolius*. These observations show that the species have various types of epicuticular wax, with high diversity in terms of micromorphology.

The observations in this study found three types of epicuticular wax in *S. trilobata*, which are flake, crust and granules, which can be seen using an SEM. With the naked eye, the wax cannot be seen clearly and the leaves appear to have a smooth and shining surface. The structure is slightly distinguishable and the anticlinal walls are raised into ridges.

Fabbri *et al.* (1986) studied strawberry leaves (*Fragaria ananassa*) in the field and they were compared with in-vitro samples (control) using an SEM and light microscopy. The leaves under the in-vitro culture were smaller in size and have poor palisade cells. Significant wax deposited was focused on the adaxial and abaxial epidermises. After removal of the plant from the in-vitro culture, the crystalline epicuticular wax increased significantly and covered most of the persistent leaves and new leaves. This feature can help plants from losing water and survive in the field. This observatiopn can be used as additional data to compare the control strawberry and the wild strawberries.

A study by Ferraro and Scremin-Dias (2018) found the importance of epicuticular wax in seasonally dry environments in the South American Chaco for the Asteraceae species. The species that were studied were *Pterocaulon purpurascens* Malme, *Wedelia trichostephia*

DC., and *Pectis gardneri* Baker. Even though the species are not from the same genus, they are from the same subfamily (Asteroideae) and live in the dry conditions. The presence of waxes on the ridges of the stomata increases the water flow and prohibits water from flowing through the cuticle. This feature helps in terms of water deficit in dry weather. The stomatal ridges look bigger due to the presence of the epicuticular wax.

Bezerra *et al.* (2018) studied *V. macrophylla* in Brazil and reported the characteristics of the plant's epicuticular wax. They washed the leaves using chloroform for one minute and observed the leaves under an SEM. The epicuticular wax on the adaxial epidermis is smooth without any ornamentation. The characteristic is similar to *M. glomerata* Spreng (Asteraceae family).

Chen *et al.* (2021) conducted a study using a different method with the same objective, which is to examine the epicuticular wax on the flower of three plants in the *Dubyaea* genus (Asteraceae family) in China. They found that the three species have well-developed epicuticular wax on the cypsela surface. The characteristic of epicuticular wax and the combination of other characteristics between the three species can prevent misidentification of the species as the waxes are similarly found in new flowers.

Liesenfeld *et al.* (2019) focused on identifying and evaluating the epicuticular waxes on the Asteraceae species in the sand fields of Brazil. In a xeric environment, plants must adapt to the surroundings to ensure their survival. Due to the waxes that help them store water, the plants have epicuticular waxes in the forms of granules, crusts and aggregate plaques on the leaf surfaces. Other characteristics that could aid in the plants' survival in sandy conditions and avoid water loss are thickened cuticles and the presence of trichomes.

Trichomes

A recent study by Panchal and Patel (2017) showed that most plants have various defence mechanisms against insects or animals. The first two layers are the trichomes and glands. As a protection, some plants increase the density of trichomes in younger leaves. The term "trichomes" came from the Greek word "trachoma", which means "hair". Usually, most plants have hair-like epidermal structures. If the hair grew at the aerial part, it is known as trichome.However, if the hair grew at the root, it is known as root hair. Trichomes have various functions, and the shape, structure and size of the trichomes depend on their functions, whether to protect, absorb, secrete or support. The cell wall of trichomes is made of cellulose. The sharp and rigid hair can prevent small animals and insects from crawling onto the leaf or stem.

Panchal and Patel (2017) emphasised that the shape of trichomes can be used as diagnostic characteristics and have taxonomic value for plant identification. Trichomes can be divided into unicellular or multicellular, and glandular or non-glandular. Normally, non-glandular trichomes exist in angiosperms, but some gymnosperms and bryophytes may also have them. Some species have glandular and multicellular trichomes, and some species may have non-glandular but unicellular or multicellular trichomes. Star-shaped trichomes are also found in certain species. Trichomes may be present at the stem, petiole, leaf and pedicel.

Glimn-Lacy and Kaufman (1984) stated that trichomes may contain one or multiple hair cells and some of them may be glandular trichomes. Glandular trichomse may excrete terpenes, such as essential oil, rubber or saponin, or crystals such as salt. If trichomes are present in the petal, they may produce fragrance for the flower.

The leaf primordia of *H. annuus* were previously studied by Aschenbrenner *et al.* (2014), specifically the trichomes of the Heliantheae tribe. They found four types of trichomes, which are capitate glandular, glandular, linear glandular and non-glandular. From the authors' observation, the linear glandular, capitate glandular and non-glandular trichomes could be viewed clearly after 72 hours of germination. This showed that trichomes are easily grown on the species.

Redonda-Martínez *et al.* (2016) expanded the term "non-glandular trichome" to unicellular and multicellular trichomes, and unicellular could be elaborated to papillae, conical, and filiform. The specific term for trichome could lead the species into a more accurate dichotomous key and, later, the taxonomic traits since the trichome is the most valuable characteristic for taxonomic study. The authors studied 12 species of Leiboldiinae (Asteraceae family) in Mexico, specifically the leaves, phyllaries, florets, and cypselae. Since trichomes are easily grown on the surface of vegetative and reproductive parts, this would make the identification of a species smooth. The adaxial epidermis is a common location to find trichome rather than the abaxial epidermis.

The glandular trichomes founded by Muravnik *et al.* (2016) in the species of *Tussilago farfara* (Asteraceae family) showed that trichomes could produce chemical properties, such as phenols and terpenoids. The majority of glandular trichomes would accumulate secondary substances in the model. One of the trichomes is capitate glandular trichome, whicbh has an oblong head and long stalk. The time or season of collecting samples may also influence the occurrence of trichomes in the species. The authors compared the ultrastructural trichomes for the sample collected in May, which was more visible than the samples collected in April.

Hayat *et al.* (2009) conducted a study on trichomes in *Artemisia* (Asteraceae family). They found that this genus has glandular and non-glandular trichomes. A study by Choi and Kim (2013) on *Mentha spicata*, *Mentha suaveolens*, and *Mentha piperita* var. (Lamiaceae family) also observed various glandular and non-glandular trichomes. Most of the capitate glandular trichomes were present on the abaxial surfaces. Compared with the peltate glandular trichomes, there were no peltate trichomes on the adaxial and abaxial surfaces. Huttunen *et al.* (2010) stated that one of the functions of trichomes is similar to the epicuticular wax, in which trichomes protect plants by minimising exposure to solar radiation, thus reducing the heat load.

The characteristics of the trichomes can be used as one of the diagnostic characteristics in some of the studied taxa and can help resolve taxonomic problems in the species identification (Noor-Syaheera *et al.,* 2020). The six taxa studied in *Melastoma* were separated by their differences in trichome characteristics, suggesting that they are distinct from one another, while the common characteristics revealed a relationship among the *Melastoma* species.

This study found various type of trichomes in *S. trilobata*, which are simple multicellular (long, pointed-end, echinate ornamentation), capitate glandular (multicellular, short stalk, unicellular head) and peltate glandular trichomes that are mostly found on the abaxial of the leaf. The different types of trichomes have clear taxonomic value in identifying the species of a plant, especially if the species are quite similar to each other. This is supported by Nurul-Aini *et al.* (2018) who stated that the trichome characteristics can be useful in the identification of the *Staurogyne* species in Acanthaceae.

Conclusion

S. trilobata is well known in traditional and modern science for having medicinal value, and a systematic study of the anatomical and micromorphological characteristics is important to identify the species. This study showed the pattern of the vascular bundle, such as separated vascular bundles arranged in an arc shape; the thickness of the parenchyma layer; the thickness of the collenchyma layer; the sclerenchyma cells; the mucilage cells; the mucilage canals; the leaf margin; stomata; and, the epicuticular waxes, such as flakes, crusts and granules of the plant. The multicellular and peltate glandular trichomes can be used as additional data to identify *S. trilobata* in the Asteraceae family.

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