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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1525-1530. **OPEN ACCESS**

Anatomical Delineation of *Syzygium myrtifolium* Walp.

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Genus Syzygium is the largest genus within the family Myrtaceae found in the tropical and subtropical regions of the world. Members of the genus are known for their medicinal value. Popularly used for the treatment of diabetes. Family Myrtaceae are known vexing with replating, nomenclatural proactive, and problematic systematic classification. *Syzygium* is regularly misidentified in ecological inventories which caused serious problems for biodiversity management. The study aimed to evaluate the anatomical features of *Syzygium myrtifolium.* Macroscopic and microscopic analysis was utilized. The anatomical features revealed an open system of the vascular bundle at the midrib and a closed system of the vascular bundle at the petiole. Diagnostic characters from morphoanatomy would serve as markers for the identification of the species. The above information will serve as a baseline for feature research in natural development.

Keywords: Genus, Myrtefolium, Myrtacea.

INTRODUCTION

Myrtaceae is a known family with more than 100 genera and more than 3000 known species evenly distributed in the tropics and subtropics region of the world. Family Myrtaceae are known vexing with replating, nomenclatural proactive and problematic systematic classification. The largest genus of the family is Syzygium which is made of up about 1000 to 1200 species all over the world. Most members of the genus are trees from Southeast Asia, China, and Australia. Members of the genus are classified as horticulturally important plants with medicinal potential. They appeared to be tolerant trees. *Syzygium* consist of densely foliaceous and large evergreen plants with thick bark and grayish-brown wood utilized in the woody industry (Alerico et al. 2016).

Wood from *Syzygium* is durable, close grained and whitish (Soh & Parnell, 2011). They have leathery leaves, obovate elliptic or oblong ovate shapes with six to twelve centimeters (Retamales et al. 2015; Soh & Parnell, 2011). There is wide variation in shape, presence of primary, secondary and tertiary vein, shining, smooth and the tips of the leaves are less acuminate and broad (Retamales et al. 2015).

From the branchlets below the leaves, panicles arise of which are four to six centimeters in length and being terminal or axillary (Hussin et al. 1992; Retamales et al. 2015). All over the globe especially in Asia particularly, Malaysia and Indonesia plants from this genus are used to treat diverse ailments ranging from diabetes, high blood pressures, postpartum, cough, fever, vaginal infection, respiratory ailments, gastrointestinal, cancer, microbial diseases . It has also been reported that some members of the group are utilised in the treatment of oral infection through the usage of juvenile leaves (Gamage et al. 2003). Some members of the genus have also been reported the usage of their bark for the treatments of infection in skin, wounds and ulcer.

Inflammation has also been reportedly cured by the genus through the decoction of the bark (Gamage et al. 2003). The Anatomy of plants is concerns with structures and the way they developed inside and outside the cells of plant tissue. It is the building block and foundation of taxonomy, genetics, evolution and physiology of the plant.

The intent of anatomy and morphological studies of plant tissue is mainly for taxonomic discrimination (Kantachot et al. 2007; Pacheco-Silva & Donato, 2016). For authentication and standardization of modern drugs, comprehensive macroscopically and microscopic studies of medicinal plants must be documented in other to have safety and quality in modern medicine (Abdulrahman et al. 2018). Micromorphological and anatomical characters have proven to be taxonomic relevant for delineating and discrimination of Syzygium family. The evolutionary basis and the taxonomic classification challenges have been broadly reviewed and discussed by many authors(Abdulrahman et al. 2018). Nevertheless only a few species have been studied. The present study will provide a detailed description of anatomical description of the *Syzygium Myteforlium*. Therefore, the study will add knowledge to the taxonomic information and for utilisation in natural product development

MATERIALS AND METHODS

Matured fresh samples of *Syzygium myrtifolium* were collected from the wild in Terengganu, Peninsular Malaysia.

4.2.2 Morphological Study

Parts of the plant were subjected to studies on taxonomical and morphological characteristics reported by (Abdulrahman et al. 2018). The following features including leaf length, leaf width, leaf attachments, leaf organization, leaf color, leaf odour, lamina shape, lamina symmetry, petiole length, the position of the petiole attachments, vein type, presence of tertiary vein, vein spacing, flowers type, and bark color were examined. The selection of the leaves was based on (Abdulrahman et al. 2018).

4.2.3 Micromorphology

Leaf micromorphology were determined with scanning electron microscope (JEOL6360LA) (SEM) through the cross section of 4-6 µm and immediately fixed in a solution of glutaraldehyde and sodium cacodylate buffer at 2.5% and 0.1M respectively for 24 hours at room temperature using gas oven dryer (SHEL LAB). Buffer of sodium cacodylate 0.1M at pH 7.2 was used in washing the oven dried sample three times at 10min interval at room temperature, osmium tetroxides in sodium cacodylate buffer (pH 7.2) at 1% and 0.1M was used for post fixation for two hours at room temperature. Another series of washing was carried out three times at 10 min submersion interval using 0.1 M sodium cacodylate buffer (pH 7.2). Dehydration was carried out using series of ethanol submersion 35, 50, 60, 70, 80, 90 and 100% at 10 min intervals, respectively. Samples were air dried using Hexamethyldisilazane (HMDS) at room temperature for 48 hours and immediately mount on a stub iron mounted with double sided carbon and coat with gold using with the aid of auto fine coater (Amponsah, Mensah, Otoo, Mensah, & Jonathan, 2014; Haron, Anuar, & Veeramohan, 2015; Wosch et al. 2015).

4.2.4 Microscopic Study

Light Microscope (Leica) was used to determine the anatomical features on transversal sections of petiole, midrib, and lamina, of fresh leaves, that were obtained by a manual microtome. The plant specimens were bleached with commercial sodium hypochlorite (clorox) for 3 to 24 hours, dehydrated with a series of ethanol as shown in Table 1 and 2, in order to observe the stomata, as well as the features from the adaxial and abaxial surfaces (Abdulrahman et al. 2018).

Table 1: Decolourization and Staining Process

S/N	Solutions	Time	Description
1	Sodium hydrochloride	30 minutes	Time varies and needs to wait until it decolorizes sometimes up to one hour depend on the plant part
2	Distilled water	5 minutes	To remove excess bleach from the plant part
3	Fast green	30 minutes	To stain the vascular bundle sometimes it varies depending on the plant part
4	Distilled water	1-2 minutes	To remove excess color
5	Safranin	1-5 minutes	To stain other cells

Note: S/N= Serial Number

Table 2 : Dehydration Process

S/N	Solutions	Time	Description
1	50 % Ethanol	2 minutes	Petri dish was covered
2	70 % Ethanol	2 minutes	Concentrated hydrochloric acid, a drop was added to the solution and shake, gently to remove excess colors
3	95% Ethanol	2 minutes	Petri dish was covered
4	100% Ethanol	2 minutes	Petri dish was covered

Note: S/N= Serial Number

The abaxial and adaxial leaf surface were also polish with nail polish and masked with celotape and directly transfer to glass slide to observe stomata (Abdulrahman et al. 2018; Luković et al. 2009). The slides were mounted in glycerine and covered with a cover slip. The structures were analyzed and photomicrographed in an optical microscope with a digital camera attached (Leica Malaysia).

RESULTS

Leaf Morphology

Leave morphology of the of *Syzygium mytifolium* were study morphologically. The leaf length was found to be 2 to 18 (cm) . The leaf shape is elliptic a situation whereby the widest area is found at the middle of the leaf with an approximation of 1 to 3 (cm). The leaf base angle is obtuse, both the cultivar has acute apex shape, the lamina symmetry the species was found to be symmetrical. Petiolar insertion, petiole size, vein type, presence of vein type and vein spacing.

Lamina transverse section

The leaf lamina transverse section revealed the presence of five to seven layers of collenchyma mesophyll filling $^{2}/_{3}$ part leaf lamina at adaxial part. Oil glands was seen at adaxial part lamina transverse section (Fig. 1).One layer palisade mesophyll present at abaxial part of the lamina transverse section filling $^{1}/_{3}$ part of the lamina at the abaxial part.Small intracellular space at both the adaxial and abaxial lamina transverse section (Fig. 1). The arch lamina transverse section was seen to be maintained with collenchyma cells with triangle shape.

Figure 1: Laminar Transverse section of *Syzygium Myteforlium*

Vascular Tissue at Midrib

The vascular system at the midrib cross section were found open system of vascular tissue, $^{3}/_{4}$ or circular shaped with an opening at the adaxial side (Fig. 2).Schlenchyma ensheathing the vascular tissue (Fig. 2). Layers of parenchyma cells were seen all over the midrib (Fig. 2) Occupied $^{3}/_{4}$ of the adaxial part of the midrib. Two to three layers of collenchyma cells is seen at the abaxial part of the midrib. Oil glands was absent in both the adaxial and abaxial part of the midrib (Fig. 2).

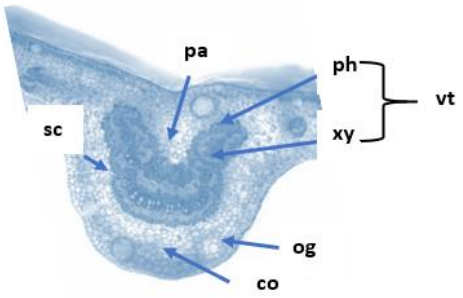

Figure 2: Vascular Tissue at the Midrib Transverse section of *Syzygium Myteforlium:* Co-collenchyma, og- oil gland, pa-parenchyma pith, ph- phloem, xy- xylem, vt-vascular tissue, sc-sclerenchyma cells

Vascular Tissue at Petiole

The petiole cross section were found to be closed system vascular tissue (Fig. 3). Flat surface is seen in the adaxial part of the vascular tissue with protruding arms and circular shape at the abaxial part of the vascular tissue (Fig. 3). Parenchyma pits was seen at the centre of the vascular tissue (Fig. 3). Sclerenchyma cells ensheathing the vascular tissue. At the abaxial part of the petiole transverse section, layers of collenchyma cells were seen (Fig. 3). $^{3}/_{4}$ of the petiole was occupied by parenchyma cells. No oil

glands was seen all over the petiole transverse section (Fig. 3).

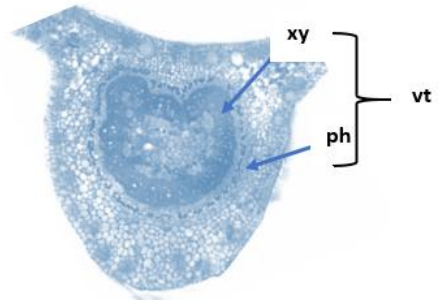

Figure 3: Vascular Tissue at the Petiole Transverse section of *Syzygium Myteforlium*: ph- phloem, xy- xylem, vt-vascular tissue

Stomata and Epiticular Wax

The stomata appeared raised above the epidermis (Fig. 4). Anisocytic stomata; the guard cells are surrounded by three cells that are not radially arranged. The subsidiary cells are mostly unequal in size, and one of the three is smaller than the other two cells. The subsidiary cells usually have the same staining properties as the surrounding epidermal cells. Stomata confined to abaxial surface; hypostomatic stomata. The average stomatal length was 7-24 and width 3 -12 µm.

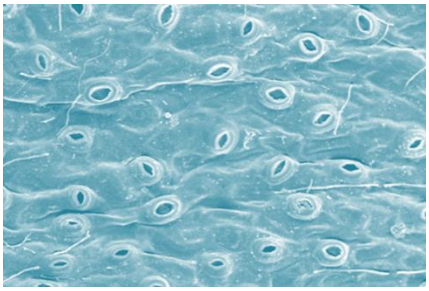

Figure 4: Stomata and Epiticular Wax of *Syzygium Myteforlium*

DISCUSSION

The taxonomic importance of the leaf epidermal layer has been highlighted to be a very important taxonomic character to be utilised for authentication identification of plant species (Zoric et al. 2009; Haron et al. 2015; Lin & Tan, 2015; Liu et al. 2015;Retamales et al. 2015). Retamales & Scharaschkin (2015) established anatomical features of the leaves of Myrtaceae provide the ecological features and taxonomic differentiation of the family. The examination of the lamina transverse section of *Syzygium Myteforlium* revealed a greater variation with other species of the genus Syzygium. *Syzygium Myteforlium* was found to have a regular shape of epidermis, compact, layers of dense collenchyma cells, and absence of spongy mesophyll layer. The following feature was previously reported by Retamales et al. (2015) in the comparative study of *Chilean Myrtaceae.* Soh & Parnell, (2011) in the same way reported similar characteristics on the Comparative leaf anatomy and phylogeny of *Syzygium.* Kantachot et al. (2007) used similar features for delimiting Thai *Syzygium*.Similarly, Abdulrahman et al. (2018) on the taxonomic discrimination of *Syzygium polyanthum* cultivars.

Vascular tissue arrangement has been previously reported to be sufficient enough to discriminate or delimited plant species into the various groups (Hussin et al. 1992; Zoric et al. 2009; Retamales et al. 2015). Much scientific research delimited plant species with the arrangement of the vascular tissue at the midrib (Hussin et al. 1992; Luković et al. 2009; Retamales et al. 2015). The vascular tissue at the midrib cross section of *Syzygium Myteforlium* has clearly shown the anatomical difference having no layers of palisade mesophyll at both adaxial and abaxial part. The vascular tissue at the midrib cross section was ensheathing by schlenchyma cells. Collenchyma cells were seen at the abaxial part of the midrib cross section. Previous research has also reported this kind of feature from the midrib of Myrtaceae (Donato & Morretes, 2013). The study agrees with Retamales et al. (2015) where they document similar features in *Syzygium floribundum* and *Myrceugenia rufa* respectively. Oil gland was seen at both the adaxial and abaxial part of the midrib. The presence of the oil secretion gland is a clear indication of the production of essential oil, responsible for the aromatic odour and sweetness of the plant. This character is a very important feature that would be utilised in authentication and taxonomic identification of Myrtaceae (Donato & Morretes, 2013; Retamales et al. 2015). Noraini et al. (2016) have reported the utilisation nature, structure and position of the vascular system in the petiole as one of the important anatomical character diagnoses of plants. Vascular system arrangement in the petiole is the most valuable character for the classification of plants using anatomy (Hussin et al. 1992; Zoric et al. 2009; Retamales et al. 2014). The vascular system at the petiole is character for taxonomic identification from generic to the family level. The systematic value of the taxonomic importance of the petiole depends on the individual plants (Haron et al. 2015; Noraini et al. 2016; Norfaizal, Noraini, Latif,

Masrom, & Salmaniza, 2018). Many previous studies on the petiole anatomy have proved the character has systematic significance in many plant families (Luković et al. 2009; Retamales et al. 2015). Noraini et al. (2016) stated that the petiole vascular patterns can be used in the taxonomic distinction of certain taxa. The taxonomic importance of the vascular system at the petiole transverse section varies with individual species ranging from generic to species level (Retamales et al. 2015). Variation in the vascular system in combination petiole outline is very useful in plant taxonomic classification. According to Noraini et al. (2016), petiole anatomy has diagnostic taxonomical value for species identification and later distinguished petiole vascular system as opened and 'closed system, respectively. The vascular system at the petiole transverse section of *Syzygium Myteforlium* was found to be circle or oval shape vascular tissue, flat at the adaxial surface and complete circle at the abaxial surface, sclerenchyma ensheathing the vascular tissue, follows by seven to eight layers collenchyma cells and no oil gland was seen all over the part. The over roll petiole transverse section is a circle shape at the abaxial surface and U shaped at the adaxial surface. The vascular tissue at the petiole has provided supportive information. Noraini et al. (2016) taxonomically identified *Hopea* species with aid of petiole anatomical features. Similarly by Donato and de Morretes (2013).

According to Hussin et al. (1992), the morphology of stomata has long been looked upon as one of the useful taxonomic criteria. Stomata occurrence and types are also very useful in species recognition. Cuticular features are good parameters for plant taxonomic identification (Yang et al. 2012). The stomata appeared raised above the epidermis. Anisocytic stomata; the guard cells are surrounded by three cells that are not radially arranged. The subsidiary cells are mostly unequal in size, and one of the three is smaller than the other two cells. Similar to studies carried out on genus Eugenia in Peninsular Malaysia three types of stomata are observed by Hussin et al. (1992): anomocytic, paracytic and anisocytic stomata. Haron & Moore (1996) show the anomocytic and paracytic types are the most abundant type in the family Myrtaceae. Previous studies carried out in the genus *Syzygium* by Retamales et al. (2014) reported a similar type of stomata found in the present study. Soh & Parnell, (2011) established four stomatal types anisocytic, anomocytic, cyclo-staurocytic and paracytic that occur exclusively or in combinations are found in the genus *Syzygium*. The hypostomatic nature of *S. Myteforlium* coincides with Haron et al. (2015) where they report species *Melastoma* as hypostomatic. While Luković et al. (2009) in their studies of leaf epidermal characteristics *Trifolium* species where they report the presence of stomata at both the abaxial and adaxial surface. Norfaizal et al. (2018) reported four different types of stomata from the stomatal studies of *Sapindaceae* Species in Malaysia. Abdulrahman et al. (2018) on studies of Myrtaceae family finds out the cell walls of the stomata vary in members of the same family or even same species and further conclude the features can be fully utilized for taxonomic classification of plants up to species level. Therefore, the following studies found anatomical characters to be of paramount importance for taxonomic identification. The following observation was previously reported by Guimaraes et al. (2015) and Haron et al. (2015). The result was also in agreement with Wosch et al. (2015) on their studies on taxa *Passiflora* whereby different features were observed on the same leaf of a particular plant. Donato and de Morretes (2013) reported similar features on *Plinia edulis* a member of Myrtaceae. Pacheco-Silva and Donato (2016) reported the appearance of special features like autofluorescence that have not been previously reported. Amponsah et al. (2014) reported from evaluating the pharmacognostic qualities of the whole plant part of *Hilleria latifolia* (Lam.) H. Walt species concluded that the identification of similar structures found in this study would be a very good parameter for the evaluation of drug standards from plant material.

CONCLUSION

The anatomical features were found to be variable as regards the type of stomata, the shape of vascular tissue located in the midrib petiole transverse section. The similarities and discrimination documented from both morphological, micromorphology, and anatomical features have added taxonomic information to the genus *Syzygium.* It will also be a guide to overcome adulteration of the said species.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The author wish to acknowledge the support

of Universiti Sultan Zainal Abidin Malaysia, Kaduna State University, Nigeria and Tishk International University, Erbil Iraq.

AUTHOR CONTRIBUTIONS

I designed and performleed the experiments and also wrote the manuscript.

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