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RESEARCH ARTICLE

LEAF ANATOMY AND MICROMORPHOLOGY OF SELECTED EUPHORBACEAE SPECIES IN KUANTAN, PAHANG, MALAYSIA

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ABSTRACT

Leaf anatomy and micromorphology study was carried out on three selected taxa in Euphorbiaceae. The three chosen taxa were *Jatropha curcas* Linn., *Euphorbia milii* Des Moul. and *Euphorbia hirta* Linn. The methods involved procedures such as sectioning using sliding microtome, epidermal peeling and leaf micromorphology method using scanning electron microscope (SEM). The aim of this study is to identify the common, variation and diagnostic characteristics of leaf anatomy and micromorphology of selected species collected from Kuantan area which can give additional information for identification and classification of species. Findings in this study have shown that there were two common characters present in all the species studied which include the presence of mucilage cells and cuticular sculpturing of abaxial and adaxial epidermis. Meanwhile, several variable characters have been recorded in this study which can be useful in species differentiation such as vascular bundles arrangement in the midrib, pattern of anticlinal wall, types of stomata, waxes and trichomes. The diagnostic characteristics identified in this study are sinuous anticlinal wall pattern and anomocytic type of stomata that can only be found in *E.hirta* while crust of wax type and absence of trichomes can only be found in *E.milii*. The study reveals that leaf anatomy and micromorphology characteristics have taxonomic values and can be used as an additional data for identification and classification of species in Euphorbiaceae.

KEYWORDS

Euphorbia milii, leaf micromorphology, sculpturing, anomocytic, taxonomic.

1. INTRODUCTION

According to a study, anatomy is the science of the structure of the bodies of humans, animals and plants derived from the Greek stems *ana-* and *tomy-*, meaning "repeated cutting" (Lopez and Barclay, 2017). Plant anatomy is closely related to plant physiology, which is the study of essential processes taking place in plants. In addition, plant anatomy has given rise to the independent science of cytology, which is the study of the cell, a rapidly developing discipline that plays a major role in understanding the essential processes in general (Esau, 1961). Moreover, stated that the term anatomy is generally used for plants analysis through light microscope or electron microscope with high capacity of identification (Yigit, 2016). The Euphorbiaceae family is one of the largest families of angiosperms made up of over 300 genera and 8000 species, with plants ranging from massive woody trees to climbing lianas to simple weeds growing prostrate to the ground.

The members of this family are cosmopolitan in nature, constituting both old and new world plants (Bijekar and Gayatri, 2014). Many research obtained only focus on the morphological parts of the family and not much information can be found on the leaf anatomy of Euphorbiaceae. Thus, this research is aimed to study the anatomical and micromorphology of Euphorbiaceae in three different species which are *J. curcas*, *E. milii* and *E. hirta* collected in Pahang area. These species are selected mainly due to their significant values in medicinal that can be useful in pharmacological research.

A study stated, the species of Euphorbiaceae has been used and practised as herbal medication by the rural Malaysian Malays for a long time (Norhanom and Yadav, 1995). Taxonomic studies require a complete samples data to identify and classify the plants. This is because incomplete data may lead to the conflicting and unsuccessful of plants identification and classification. Anatomical studies also known as one part of taxonomic data which is one of the important tools that necessary in plants identification and classification. However, there are lack of research has been conducted in anatomy for this plant species. Hence, researches on anatomical part of plants are crucial especially for the taxonomist to gain extra information to identify and classify the plant species.

2. MATERIALS AND METHODS

Three selected plant species from Euphorbiaceae family which consist of two genera, *Jatropha* and *Euphorbia*, were collected in Kuantan, Pahang, Malaysia. These three selected plant species are *J. curcas*, *E. milii* and *E. hirta*. Specimens obtained from the field sampling were dried, curated, identified, and deposited at the Herbarium of International Islamic University of Malaysia, Kuantan, Pahang. Fresh leaf samples were fixed in 3:1 AA solutions (70% alcohol: 30% acetic acid). The methods of this study involved cross-section using sliding microtome, epidermal peeling and scanning electron microscope. Part of petioles and midribs was sectioned in a range of thickness (30–40 µm) using sliding microtomes and stained with Safranin and Alcian blue. The epidermal peels were prepared

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using Jeffrey's solution and stained with Safranin. Slides were mounted in Euparal after dehydration process.

The leaf clearing method was prepared using Basic Fuchsin's solution. Slides were then mounted in Canada balsam after dehydration process. All slides were dried in oven at 60°C for 1 week. Anatomical images were captured using LEICA ICC50 HD camera. For scanning electron microscope, small pieces of leaves were cut from the same area of the leaf lamina, affixed to aluminium stubs with double sided adhesive tape and then sputter-coated with a thin film of gold to improve the electrical conducting properties of the leaf surface. The critical point drying and gold coating was done as follows; samples were dried in a critical point drier and mounted on aluminium stubs. Then, samples were gold-coated using a sputter coater and examined under a scanning electron microscope Zeiss Model EVO 50.

3. RESULTS AND DISCUSSION



The study of three selected species in Euphorbiaceae family in Kuantan has shown some common and variation of leaf anatomical and micromorphological characteristics. Common characteristics that have been reported among the species were significant as supporting data for the identification and classification between the genus and species in family Euphorbiaceae. In addition, the variations in the characteristics of leaf anatomy and micromorphology of selected species studied help in the identification and differentiation of the species in Euphorbiaceae. The results of this analysis showed the common features in leaf anatomy and micromorphology found in the selected Euphorbiaceae species. A similar phenotype shared by the species may be used to facilitate the authentication and identification of Euphorbiaceae plant species studied.

The common characteristics found in this study are the presence of mucilage cells and the presence of cuticular sculpturing in both abaxial and adaxial epidermis surface. Some researchers in their study revealed that plant mucilages of certain families have been used widely in a food production, pharmaceutical and cosmetics industries such as in Malvaceae family (Vignesh and Nair, 2018). The characteristics of physical and chemical attribution of mucilages are noteworthy parameters in differentiating the Malvaceae from other plant species. Results obtained from this study have shown the presence of mucilage cells in the parenchyma cortex in both petioles and midribs of the species in Euphorbiaceae (Figure 1D). Nevertheless, the size of the mucilage cells are varied depending on the organisms studied. Noraini and Cutler stated that the cuticular sculpturing may be used as a criterion for the level of diagnostic species that offers some systematic knowledge on the plant species studied (Noraini and Cutler, 2009).

The results in this research study has identified that the cuticular sculpturing on the epidermal cell of *J. curcas*, *E. milii* and *E. hirta* can be distinguished as the anticlinal wall slightly raised into ridges. All the selected species have shown similarities in the sculpturing of cuticle of both adaxial and abaxial epidermis surfaces (Figure 2 E-H). Therefore, the similar criteria for these species studied can also be used for identification and recognition of species in the Euphorbiaceae family. This study also showed that there are several variations characteristics that can be used to differentiate between the species studied based on leaf anatomy and micromorphology. The characteristics recorded are the pattern of anticlinal walls on the abaxial and adaxial epidermis, types of stomata, types of waxes and types of trichomes. Some researchers reported that the classification of the type of stomata is based on the configuration of the subsidiary cells and the developmental patterns (Norfaizal et al., 2018).

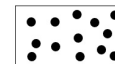
The stomata surface distribution patterns based on their orientation and dispersion are also found to be stable and can be categorized as taxonomically stable. From the obtained results, the stomata type recorded in this research denote that the *Euphorbia* species mostly composed of amphistomatic that present in both abaxial and adaxial epidermal surfaces of the leaf. Meanwhile, the stomata type in *J. curcas* is hypostomatic which it only presents at the abaxial parts of the epidermal surface. This study supports where the stomata types are greatly used in observing on the classification and identification of plant species (Essiett et al., 2012; Rai, 2012). Apart from the vascular bundles found in the petiole, this research has also reported two other vascular bundle structures in the midrib. A group researchers proclaimed, the characteristics of the midrib vascular bundles that can be used as a method for distinguishing between species or genera are the vascular bundles form and arrangement of the main vascular bundles (Nurul-Syahirah et al., 2016).

Moreover, in the research conducted the presence of collateral midrib vascular bundles and characterize of the abaxial surface in genus *Jatropha* has outlined essential traits in distinguishing the species (Jie et al., 2014). Table 1 revealed the details and illustrations of the vascular bundle's arrangement found in the midrib of the selected species. Findings in this study has shown the variations in vascular bundles arrangement in midrib can be used to distinguish taxa in Euphorbiaceae (Figure 1A-C). Previous research by also reported the significance of vascular bundle arrangements can be useful as an additional data to identify species in Acanthaceae (Nurul-Aini et al., 2018). A research has proven that the pattern of anticlinal walls have some taxonomic significant in classifying and differentiating the genus in Euphorbiaceae from other species studied (Devi et al., 2013). Previous research reported by has recorded the anticlinal wall patterns of adaxial and abaxial epidermis in *Euphorbia* and *Jatropha* species respectively (Essiett et al., 2012; Soyewo et al., 2015).

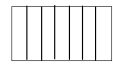
| Table 1: Classification and description of vascular bundles in midrib | | |
|---|---|---|
| Structures | Details | Illustration |
| Type 1 | Main vascular bundle (opened system with continuous rings of vascular bundle) |  |
| Type 2 | Main vascular bundle (opened system with continuous ring of vascular bundle); additional vascular bundles (opened system with five continuous ring of vascular bundles) |  |

Indication:

Phloem



Xylem



The anticlinal wall patterns are varying from sinuous, wavy, slightly curly and straight patterns. In this research, two types of anticlinal walls of abaxial and adaxial epidermal surfaces have been listed which are the straight to wavy and sinuous. The anticlinal wall of the *E. hirta* leaf showed a differentiation which revealed the sinuous pattern of both abaxial (Figure 1H) and adaxial epidermis as compared to *J. curcas* and *E. milii* which showed straight to wavy pattern (Figure 1F and 1G). Hence, this anticlinal wall of abaxial and adaxial epidermis can be used to differentiate and classify various genus in Euphorbiaceae. The result from this research also reported that the stomata types include the paracytic which referred to stoma surrounded by two subsidiary cells, anisocytic which stoma surrounded by three subsidiary cells and anomocytic which lack of subsidiary cells. The stomata in both genus, *Jatropha* and *Euphorbia* comprised of heterostomatic which having paracytic and anisocytic types of stomata in a species (Figure 1 E-H). However, *E. hirta* is only having a single type of stomata which is the anomocytic type.

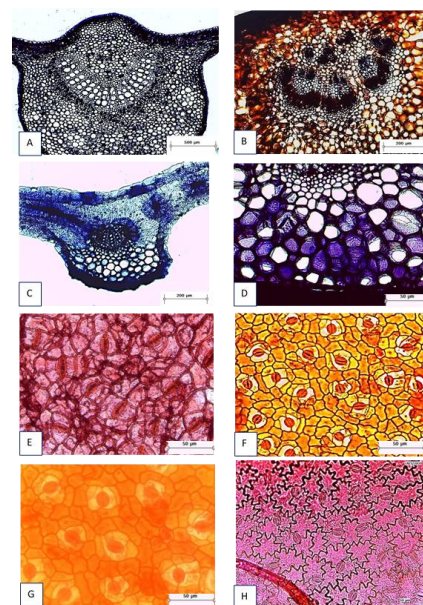


Figure 1: A) Vascular bundles of midrib of *J. curcas*; B) Vascular bundles of midrib of *E. milii*; C) Vascular bundles of midrib of *E. hirta*; D) Mucilage cells; E-H) Distribution of stomata on the epidermal cells. Scale; A) 500um, B&C) 200um, D-H) 50um

Therefore, these stomatal types can be used in determining the classification of the species in Euphorbiaceae. According to a study, the scanning electron microscopic (SEM) studies on Euphorbiaceae have identified various forms of epicuticular wax coats that used for differentiation and classification of species studied (Raju and Rao, 1977). This research has found four types of waxes present on abaxial and adaxial epidermis in all species studied. The types of waxes are film layer, granular coatings, verrucate and crust of wax layers. Through this analysis, leaf wax is considered as taxonomic criteria or markers for the separation and identification of certain plants, often at the level of the genus and species. In previous study reported that variation in foliar trichome characters was able to address the taxonomic conflicts of selected taxa in Acanthaceae (Nurul-Aini et al., 2014).

Hence, the micromorphology characters of trichomes are widely used as one of the features in classification and identification of the plants. The results achieved in this research showed a significant value in distinguishing the genus and species of Euphorbiaceae. The trichomes present at abaxial and adaxial epidermis of genus *Jatropha* is peltate glandular trichomes (Figure 2B). Then, simple multicellular trichomes (Figure 2A) and peltate trichomes are found on the adaxial epidermis while simple multicellular trichomes are investigated on the abaxial epidermis of *E. hirta*. However, there are no trichomes recorded on the *E. milii* in this research as contended by which denote the absent of trichomes in *Euphorbia* species including *E. microphylla*, *E. pulcherrima*, *E. tirucalli*, *E. milii*, *E. neriifolia* and *E. rothiana* (Inamdar and Gangadhara, 1977). Thus, the differentiation of trichome characters showed in this research can be used in classification and identification of Euphorbiaceae species.

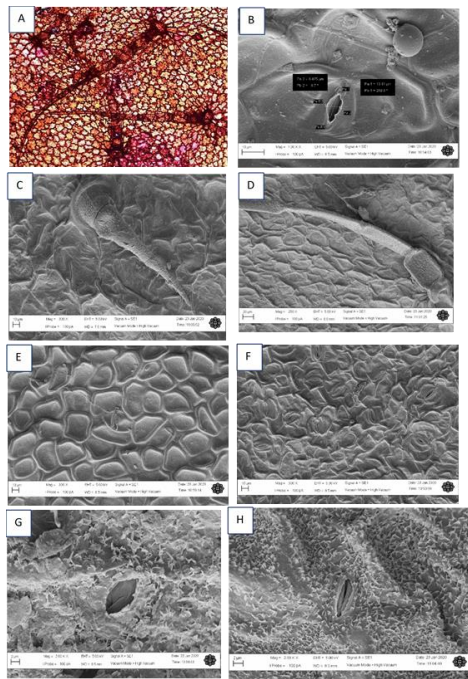


Figure 2: A-D) Type of trichomes. E-H) Type of anticlinal walls in species studied. Scale: A) 200 μ m. J-F) 10 μ m. G-H) 2 μ m.

4. CONCLUSION

The results from this research showed that anatomical and micromorphological characteristics of the leaf can be used in identifying and classifying plant species of the family Euphorbiaceae. There are two specific characteristics that can be described among all the species studied. The presence of mucilage cell and the cuticular morphology of the abaxial and adaxial epidermis were found to be identical in three distinct species. This research also described some differences in the characteristics of the species being studied that could be used to distinguish between them. The characteristics are the pattern of anticlinal walls on the abaxial and adaxial epidermis, types of stomata, types of waxes and types of trichomes. In conclusion, the study of leaf anatomy and micromorphology has helped in better understanding of the ways in observing and differentiating various species that has been used in the classification and identification of plants.

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