TAXONOMIC SIGNIFICANCE OF FOLIAR EPIDERMIS IN FOUR *Euphorbia* SPECIES IN JOS, PLATEAU STATE, NIGERIA

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Abstract. An investigation was carried out on four species of *Euphorbia* Linn. in Jos North LGA, Plateau State Nigeria to assess the anatomical characters that can serve as taxonomic markers. The species investigated include *E. cotinifolia* L., *E. hirta* L., *E. milii* Des Moul. and *E. thymifolia* L. The leaf epidermal characters studied were the types of stomata, trichomes, cell shapes amd patterns. The stomatal distribution varied within the studied species and can be used to differentiate the studied species into two groups, *E. cotinifolia* with hypostomatic leaves can conveniently be distinguished from *E. hirta*, *E. milii* and *E. thymifolia* with amphistomatic leaves. The trichome morphology can also be used to delimit the studied species into two groups, *E. cotinifolia* and *E. milii* with no trichome on the surfaces can be separated from *E. hirta* and *E. thymifolia* with multiellular uniseriate trichomes on both surfaces. The studied *Euphorbia* species displayed a number of characters of taxonomic importance such as the presence of both the anomocytic and anisocyctic stomata types on the abaxial and adaxial surfaces of *E. cotinifolia* and *E. hirta* respectively, different epidermal cell shapes and anticlinal wall patterns that can increase our understanding of the species.

Keywords: *Euphorbia*, epidermal cell, trichomes, stomata, epidermis.

INTRODUCTION

The genus *Euphorbia* consists of over 2000 species and with the exception to the polar region, it is present in almost every part of the world [22]. It is one of the largest and most diverse genera in the plant kingdom [21]. The species are annual or perennial herbs, woody shrubs or trees with a caustic poisonous milky sap (latex).

The milky latex produced by the *Euphorbia* species had been used both as medicine and as poisons. The sap of *Euphorbia cotinifolia* had been used in folk medicine as both an emetic and cathartic substance. Fishermen have been known to add the sap in water bodies when fishing to stun the fish and force them to float on the top. It was also historically used as a poison for arrowheads by the natives of Curacao [5]. The sap can also cause irritation if it comes into contact with human skin or eyes, and if ingested, the sap can cause severe damage to internal organs [12].

Euphorbia hirta is claimed to have a curative effect on dengue patients as being backed up by testimonies, hence becoming one of the most popular folk medicine in the Philippines. However, there is no evidence to support this claim despite its wide spread use. *Euphorbia hirta* possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties [9]. The aqueous extract of *Euphorbia hirta* has also been reported to inhibit aflatoxin contamination in rice, wheat, maize and mustard crops [19]. The dried leaves and seeds of *Euphorbia thymifolia* are used in India and Indonesia to treat diarrhea and dysentery in children. It is also useful in cases of flatulence and constipation [24]. The plant is also used by Indians to treat chronic cough, asthma, rheumatism, toothache and bronchitis [7]. *Euphorbia milii, Euphorbia pulcherrima* and *Euphorbia cotinifolia* are popularly utilized for ornamental beautification of landscape.

The importance of epidermal features in taxonomy and phylogeny of flowering plants is widely known. The use of data generated from leaf epidermal surfaces in resolving the taxonomy of taxa has gained much recognition for a long time [20]. The contributions of leaf surface structure to improve the identification of indigenous flora of Nigeria are evident from a number of works [2, 3, 8, 13, 14]. Some anatomical features are diagnostic and are commonly used in routine identification, rather than being confined to use in problems of phylogeny or classification, or in the identification of fragments of plants [20].

The present study described the leaf epidermal morphology in some *Euphorbia* species with the aim of providing useful characters with a view to enhancing our knowledge of their classification, delineation and identification.

MATERIALS AND METHODS

Sources of Materials

The fresh leaves of four species *Euphorbia* (*E.cotinifolia*, *E. hirta*, *E. milii* and *E. thymifolia*) were collected from the Bauchi road campus of the University of Jos, Plateau state Nigeria (9.9503^0N, 8.8892^0E). Specifically, *Euphorbia cotinifolia* was collected around law Faculty, *Euphorbia hirta* and *Euphorbia milii* were collected around the faculty of Pharmaceutical Sciences while *Euphorbia thymifolia* was collected around the Multipurpose Auditorium (MPA) on the same campus. The species were identified at the Federal College of Forestry herbarium, authenticated and deposited at the herbarium of the Department of Pharmacognosy, University of Jos.

Preparation of Leaf Epidermal Surfaces

The preparation of leaf samples for permanent slides to enhance epidermal morphology follows the method of Wilkinson [26] with slight modifications to accommodate the peculiarity of the plant specimens. Leaves collected from plants of equal age and from the mid region of the stems were soaked in 70% sodium Hypochlorite for three to five hours in order to remove the colouring pigments and surface debris followed by washing in several changes of water to remove excess reagents. Using fine grade camel hair brush, epidermal peels were carefully removed from the leaf sample surfaces. The slides of both the adaxial and abaxial surfaces of the leaves were prepared, labelled, viewed for micromorphological characters with the digital Olympus BX 51 light microscope and photomicrographs were captured on the computer. For statistical analysis, ten (10) epidermal cells and ten (10) stomata were chosen at random from each species and measured using a micrometer. For each quantitative character, the range, mean, standard deviation and standard error were determined for each species.

The stomata Index was determined according to Metcalfe and Chalk [11] using the formula:

$$\frac{S}{E+S} \cdot 100 = SI$$

where:

S - Number of stomata per unit area

E - Number of epidermal cells in the same area.

RESULTS

The results obtained from the study of the leaf epidermal features of the *Euphorbia* species in were shown in Figures 1A – 1L as photomicrographs and tabulated in Tables 1-2.

The leaf epidermal cells are polyhedral, polygonal but more often irregular. The anticlinal wall patten was mostly straight to slightly curved on the adaxial surfaces but wavy on the abaxial surfaces. The smallest epidermal cell size on the abaxial surface ranging from 27.20-34.00µm long and 13.60-20.40µm wide was observed in *Euphorbia cotinifolia* while on the adaxial surface it ranges from 27.20-37.40µm long and 17.00-23.80µm wide. The largest epidermal cell size was observed in *E. thymifolia* ranging from 47.60-54.40µm long and 23.80-40.80µm wide on the abaxial surface while on the adaxial surface it ranges from 44.20-61.20µm long and 27.20-34.00µm wide in *E. hirta* .

Two patterns of stomata occurrence with respect to leaf surface were observed. It was amphisomatic in all the species studied except for *E. cotinifolia* which was hypostomatic having stomata only on the abaxial surface. Similarly, the orientation of the subsidiary cells in relation to the guard cells in each stomatal complex revealed three discrete types. These are anisocytic stomata type with three cells which may be of unequal size enclosing the guard cells, anomocytic with the epidermal cells around the guard cells not being distinguishable from other epidermal cells and paracytic stomata accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and the guard cells. The most common types of stomata observed in the studied species are anisocytic and anomocytic. In some cases, more than one stomata type was observed on the abaxial surface of the same species. The anisocytic and anomocytic types were observed in all the species studied except in *E. milii* with paracytic stomata on both surfaces distinguishing it from other species. The stomata index varied on both adaxial and abaxial surfaces of the species studied.

The lowest stomata index was found on the abaxial surface of *Euphorbia milii* (4.79%) and the highest stomata index was found on the adaxial surface of *Euphorbia hirta* (14.77%). Mean stomata length is in the range of 18.02 and 20.40µm in *E. thymifolia* and *E. milii* respectively on the adaxial surface. On the abaxial surface, the mean stomata length ranges from 15.64 and 25.84µm in *E. hirta* and *E. milii* respectively. The mean stomata width ranges from 9.52 and 16.66µm in *E. hirta* and *E. milii* respectively.

The shape of the subsidiary cells in the species studied varied from unequal and angular. The mean guard cell width is in the range of 4.42 and 5.95µm in *E. hirta* and *E. thymifolia* respectively on the adaxial surface. On the abaxial surface, the mean guard cell width ranges from 4.08 and 7.65µm in *E. hirta* and *E. milii* respectively.

Long multicellular uniseriate trichomes with pointed apices were present on both surfaces of *E. hirta* and *E. thymifolia*. The longest trichome was found on the abaxial surface of *E. thymifolia* and the shortest trichome was found on the adaxial surface of *E. hirta*. The number of cells found on each trichome in *E. thymifolia* ranges from 4 to 11 while the number of cells on the trichomes in *E. hirta* ranges from 3-6. Trichomes were not found on the surfaces of *E. milii* and *E. cotinifolia*.

DISCUSSION

This study showed a number of important micro morphological characters on the leaf surfaces of *Euphorbia* species. Although, new characters were observed that were not previously recorded, generally, there is an agreement in the results obtained from this present study and previous studies.

Foliar anatomy is one of the most noteworthy taxonomic characters from the systematic point of view and the characters are attributes of potential taxonomic significance both at genus and species level found on the leaves of plant [2]. Anatomical characters of the vegetative organs in flowering plants like epidermal cell shape, anticlinal wall pattern, stomata type, trichome types have been employed with great success in resolving taxonomic problems. They are also very helpful in the identification of fragments of plant materials and of herbarium samples not accompanied by flowers and fruits [1, 10, 18].

Table 1. Qualitative foliar micro morphological characters of the studied species

Species	Epidermal cell shape		Anticlinal wall pattern		Stomata type		Trichome type	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
E. cotinifolia	Undulate	Irregular	Wavy	Wavy	-	Anisocytic/ Anomocytic	-	-
E. hirta	Polyhedral	Undulate	Straight/ slightly curved	Wavy	Anisocytic	Anisocytic/ Anomocytic	Multicellular uniserriate	Multicellular uniserriate
E. milii	Polygonal	Irregular/ Undulate	Straight/ slightly curved	Straight/ Curved	Paracytic	Paracytic	-	-
E. thymifolia	Polygonal	Undulate	Straight/ slightly curved	Wavy	Anisocytic	Anisocytic	Multicellular uniserriate	Multicellular uniserriate

Table 2. Quantitative foliar micro morphological characters of the studied species, min (mean ± SE) max

Species		Stomata measurement		Epidermal measurement		Trichome measurement	Guard cell measurement width (at widest point) (µm)	Stomata index (%)
		Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)		
E. cotinifolia	Ad	-	-	27.20 (31.79 ± 0.87) 37.40	17.00 (19.89 ± 0.80) 23.80	-	-	-
	Ab	20.40 (22.44 ± 0.49) 23.80	15.30 (16.49 ± 0.26) 17.00	27.20 (31.79 ± 0.88) 34.00	13.60 (17.17 ± 0.82) 20.40	-	5.10 (5.74 ± 0.33) 6.80	9.82
E. hirta	Ad	17.00 (19.21 ± 0.51)20.40	10.20 (12.92 ± 0.46) 13.60	44.20 (49.64 ± 1.99) 61.20	27.20 (29.24 ± 0.91) 34.00	139.40 (255.68 ± 27.53) 455.60	3.40 (4.42 ± 0.28) 5.10	14.77
	Ab	13.60 (15.64 ± 0.49) 17.00	8.50 (9.52 ± 0.28) 10.20	34.00 (41.82 ± 1.53) 51.00	23.80 (27.37 ± 0.82) 30.60	251.60 (457.30 ± 45.57) 690.20	3.40 (4.08 ± 0.28) 5.10	8.05
E. milii	Ad	17.00 (20.40 ± 0.72) 23.80	10.20 (10.88 ± 0.35)13.60	30.60 (38.76 ± 1.70) 47.60	17.00 (22.15 ± 1.16) 27.20	-	3.40 (5.44 ± 0.42) 6.80	9.09
	Ab	23.80 (25.84 ± 0.49) 27.20	15.30 (16.66 ± 0.22) 17.00	37.40 (43.12 ± 1.44) 51.00	20.40 (24.99 ± 0.72) 27.20	-	6.80 (7.65 ± 0.53) 10.20	4.79
E. thymifolia	Ad	17.00 (18.02 ± 0.52) 20.40	10.20 (12.07 ± 0.47) 13.60	34.00 (45.90 ± 1.99) 57.80	20.40 (25.84 ± 1.04) 30.60	408.00 (857.14 ±92.68)1213.80	5.10 (5.95 ± 0.28) 6.80	14.48
	Ab	20.40 (22.44 ± 0.49) 23.80	11.90 (12.76 ± 0.28) 13.60	47.60 (51.68 ± 0.99) 54.40	23.80 (30.60 ± 1.97) 40.80	190.40 (364.48 ± 46.69) 557.60	5.10 (6.12 ± 0.28) 6.80	10.84

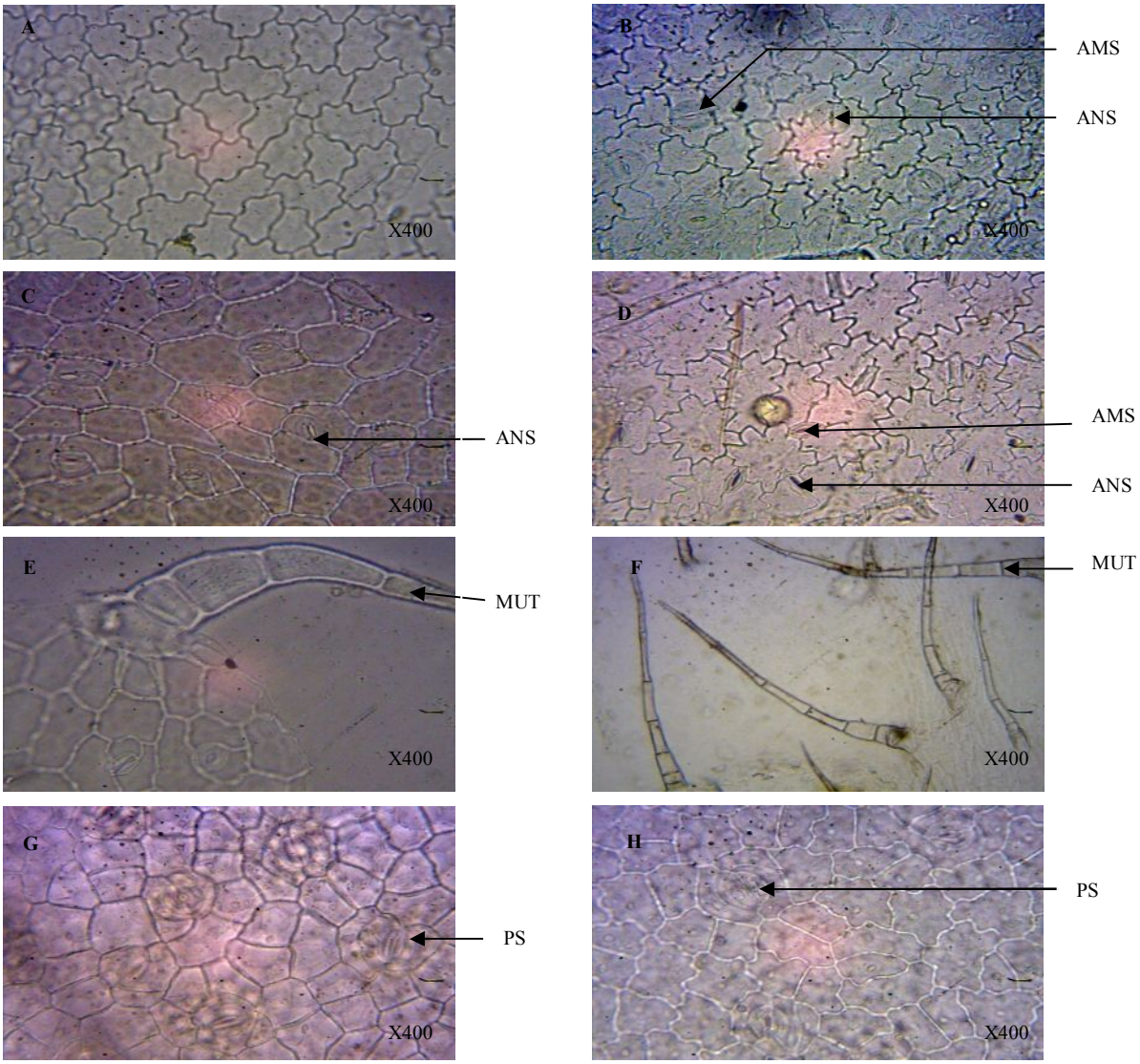

Figure 1. Photomicrographs of leaf surfaces of the studied *Euphorbia* species, where: **A -** Adaxial surface of *E.cotinifolia* showing undulate epidermal cells; **B -** Abaxial surface of *E. continifolia* showing anomocytic (AMS), anisocytic (ANS) stomata and irregular epidermal cells; **C -** Adaxial surface of *E. hirta* showing anisocytic stomata (ANS) and polyhedral epidermal cells; **D -** Abaxial surface of *E. hirta* showing anomocytic (AMS), anisocytic (ANS) stomata and wavy epidermal cells; **E -** Adaxial surface of *E. hirta* showing multicellular uniseriate trichome (MUT); **F -** Abaxial surface of *E. hirta* showing multicellular uniseriate trichome (MUT); **G -** Adaxial surface of *E. milii* showing paracytic stomata (PS) and polygonal epidermal cells; **H -** Abaxial surface of *E. milli* showing paracytic stomata (PS) and undulate epidermal cells.

The epidermal cell shape and anticlinal wall pattern varied within the studied species and thus are useful for delimitation of species. Based on the epidermal cell shape and anticlinal wall pattern, the studied species can conveniently be divided into three groups. *Euphorbia cotinifolia* which has undulate and irregular cell shape on the adaxial and abaxial surfaces respectively and wavy anticlinal cells on both surfaces can be separated easily from other species studied. Also, *Euphorbia hirta* and *Euphorbia thymifolia*, both with polygonal and undulate cell shapes on their adaxial and abaxial surfaces respectively, and straight or slightly curved cell walls on their adaxial surfaces with wavy cell walls on their abaxial surfaces are distinctly different from other species. Similarly, *Euphorbia milii* with different epidermal cell shape and anticlinal wall pattern with polygonal and irregular cell shape on adaxial and abaxial surfaces respectively and straight to slightly curved anticlinal walls on both surfaces can be separated from other species.

The occurrence of irregular, undulate, polygonal and polyhedral epidermal cell shape is in agreement with the works of differnt authors [1, 6, 17] who reported the presence of irregular, polygonal or variously elongated epidermal cells in the *Euphorbia* species studied.

The importance of stomatal complex in taxonomy especially in plant identification had been emphasized by Stace [20]. The stomata complex types recorded in this study are paracytic, anisocytic and anomocytic. This diversity of stomata was also reported by [6] in the *Euphorbia* species studied. Also the occurrence of more than one stomata type on the same leaf surface of a species as seen in *Euphorbia hirta* and *Euphorbia cotinifolia* having the anomocytic and anisocytic stomata types on their abaxial surfaces can serve as a

useful identification character. This is in agreement with some authors [2, 6, 15, 16] who also observed the occurrence of more than one stomata type on a leaf surface.

Furthermore, the cuticular examination revealed the presence of stomata on both the abaxial and adaxial surfaces of the species studied with higher frequency on the adaxial surfaces except in *Euphorbia cotinifolia* with no stomata on the adaxial surface. This in agreement with the findings of [6, 17] who observed stomatal complexes more on the adaxial surfaces in the species studied. Based on the stomata type, the *Euphorbia* species studied can be separated into three groups there by delimiting the species. *Euphorbia cotinifolia* and *Euphorbia hirta* having a combination of both the anisocytic and anomocytic stomata types, *Euphorbia milii* having only paracytic stomata type and *Euphorbia thymifolia* with anisocytic stomata type only.

The presence or absence, type, size as well as the distribution of trichomes on the epidermal surface can be used in the infrageneric classification of a genus and thus serve as classification tools [4, 23]. Furthermore, Metcalfe and Chalk [10] suggested that the types of epidermal trichomes can frequently delimit species, genera or families in plant and also hold that the trichome frequency and size are environmentally controlled, while Stace [20] believes that hairs are constant in a species when present and showed a constant range of form and distribution useful in diagnosis.

In this study, the presence or absence of trichomes can be used in characterizing the species. The presence of multicellular uniseriate trichomes on both surfaces of *Euphorbia hirta* and *Euphorbia thymifolia* made them distinctly different from *Euphorbia cotinifolia* and *Euphorbia milii* that lack trichomes. This is in agreement with Silva [17] who reported multicellular uniseriate trichomes in *Euphorbia hirta* and *Euphorbia thymifolia*. Furthermore, the sizes of trichomes on the *Euphorbia* species vary with *Euphorbia thymifolia* having the longest trichome on the adaxial surface and *Euphorbia hirta* having the shortest trichome on the adaxial surface.

The data recorded from the leaf epidermal study provided further evidence for identification and delimitation of *Euphorbia* species. The diagnostic characters used for species delimitation and identification are stomata type, stomata size, epidermal shape and size, stomata index, presence or absence and type of trichome.

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