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## Taxonomic Significance of Leaf Epidermal Attributes of some Herbaceous Species of *Euphorbia* L. (Euphorbiaceae) in Nigeria

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**ABSTRACT:** Foliar epidermis of six species of *Euphorbia* Linn. in Nigeria was studied with the aim of ascertaining reliable characters for easy identification, delimitation and indicating relationships among species. Light microscopy was employed to evaluate leaf surface features such as shape of epidermal cell, stomata and trichome and their distribution frequency. Epidermal shapes are variable i.e. polygonal, irregular and undulate. Stomatal complex types include anomocytic, anisocytic, paracytic, diacytic were encountered. Diacytic stomatal complex was observed only in *E. heterophylla* and *E. thymifolia*; anomocytic, anisocytic, paracytic and diacytic in *E. heterophylla*; *Euphorbia hirta* has anomocytic, anisocytic and paracytic stomata while *E. graminea* has paracytic, anomocytic and anisocytic stomatal complexes. All the six examined species are amphistomatic. Trichomes are widespread in the representative species except for *E. hyssopifolia* which has no trichome. Observed foliar trichomes were non-glandular, unicellular and multicellular with swollen base in the examined species. The distinguishing shape and size of epidermal cell, anticlinal cell wall pattern, guard cell size, stomata type and trichome type varied among species. Similarities in leaf anatomical structures indicate interspecies relationships and this justify their grouping in the same genus. A dichotomous bracketed key to the *Euphorbia* species is provided.

**Keywords:** *Euphorbia*, Foliar epidermis, Delimitation, Identification

### Introduction

*Euphorbia* Linn., a cosmopolitan genus in its distribution, an annual or perennial herbs, shrubs and trees which is represented by 2000 species. They occur as prostrate, decumbent, or erect, monoecious or dioecious, succulent and non-succulent (Dutta, 1974; Olorode, 2012). The genus belongs to the family Euphorbiaceae, tribe Euphorbieae and subtribe Euphorbiinae and is rated as third largest genus of angiosperms after *Astragalus* Linn. (Fabaceae) and *Psychotria* Linn. (Rubiaceae) (Govaerts et al., 2000). According to Hutchinson and Dalziel (1958); Aigbokan and Ektu (2012), thirty-one (31) species of the genus are found in Nigeria. Some of the species are edible and can be used for ornamental, economical and medicinal purposes (Etukudo, 2003; Spano et al., 2012). An important and rather unusual feature of this genus is its simultaneous possession of three-carbon

compound (C<sub>3</sub>), four-carbon compound (C<sub>4</sub>) and Crassulacean acid metabolism (CAM) photosynthetic systems. This feature makes *Euphorbia* adaptable to warm, mesic, semi-arid and arid conditions at varying altitudes (Sage *et al.*, 2011).

The taxonomic value of foliar epidermis is one of the most noteworthy micromorphological characters explored in taxonomic studies of different families (Faboyede, 2015; Oladipo *et al.*, 2015; Ashidi *et al.*, 2017; Karakish *et al.*, 2020; Ishak *et al.*, 2021; Damaiyani *et al.*, 2022; Abid *et al.*, 2023). The exploration of leaf epidermal micromorphology included shape of leaf epidermal cells, stomata types and types of trichomes (glandular and non glandular) on adaxial and abaxial surface both quantitatively and qualitatively (Albert and Sharma, 2013; Bahadur *et al.*, 2022; Taib, 2023). Epidermal glands are also important features in taxonomic studies as they originate from epidermal cells (Celka *et al.*, 2006).

*Euphorbia* species have several medicinal uses; it is used in the treatment of skin diseases, gonorrhoea, intestinal parasites, cough, dysentery, and warts (Singia and Pathak, 1990). In Nigeria, *E. hirta* extracts are used in the treatment of pimples, boils, wounds, rashes and can also stop bleeding. Root decoction also helps to increase milk flow in nursing mothers. Plant extracts are used in treating asthma, respiratory tract inflammatory, cancer, gastrointestinal disorders, conjunctivitis etc. (Etukudo, 2003). In India, plant extract is used to treat worm infestations in children and for gonorrhoea, jaundice, and tumours (Kirtikar and Basu, 1991) while root decoction is also used to cure snake bite. *Euphorbia graminea* Jacq. is used in the treatment of skin infections, ulcers, cancers, tumour warts (Betancur-Galvis *et al.*, 2002). The decoction leaf of *E. heterophylla* is used as a purgative, source of diet in southern Nigeria and sometimes cultivated as an ornamental plant. In the northern Malaya, decoction of roots and bark has been used to treat fever and malaria (Etukudo, 2003).

The taxonomy of the genus was re-evaluated using leaf anatomical characters to unravel the complex inter-relationships existing among the six selected species. The six selected species, from existing observations and records are distinct and distinguishable from other members of the genus *Euphorbia* because of their generally weedy nature. However, they fall into two “natural” groups on the bases of their general sizes, physiognomy, longevity, ecology and geographic amplitude. *Euphorbia heterophylla* Linn. and *Euphorbia graminea* Jacq. on one hand and *Euphorbia hyssopifolia* Linn., *Euphorbia hirta* Linn., *Euphorbia prostrata* Ait. and *Euphorbia thymifolia* Linn. on the other hand. The extant bases of identities, differences and relationships among the selected species are their habit and gross exomorphological characteristics. These bases are not exhaustive and, consequently, are not satisfactory for comprehensive characterization of the selected species. It is this problem that the study sets out to address through the evaluation of leaf epidermal characters of these six species.

## Materials and methods

**Plant collection:** Freshly collected specimens (of *E. graminea*, *E. heterophylla*, *E. hirta*, *E. prostrata*, *E. hyssopifolia* and *E. thymifolia*) from the field (e.g. Ogun, Osun, Oyo States etc.) and specimen from different herbaria (such as Forestry Research Institute of Nigeria (FRI) and the specimens were deposited both at FRI, Elikaf Herbarium of Olabisi Onabanjo University, Ago-Iwoye, Ogun State and Obafemi Awolowo University, Ile-Ife herbarium (IFE)) were employed for the study. The fresh specimens were collected from different parts of Nigeria (Fig. 1). The fresh specimens were pressed with the aid of plant press. Identification and authentication was done appropriately at the above-mentioned herbaria.

**Leaf peeling:** Mature leaves were cut into manageable pieces and macerated in concentrated nitric acid until the surfaces became swollen under the hot sunlight. The pieces of leaves with swollen surfaces were transferred to water in petri-dish. Gentle use of fine tip forceps was employed to separate the adaxial and abaxial epidermises from the mesophyll layers. The peels were preserved in 50% ethanol (Akinloye *et al.*, 2005). Epidermal cell shape, anticlinal cell wall pattern, stomata type and trichome type were studied. The trichome frequency, stomatal and trichome indices were calculated using the following formula below:

$$(i) \text{ Trichome frequency} = \frac{\text{Number of trichome per unit area of leaf surface}}{\text{Number of stomata + number of epidermal cells per leaf unit area}} \times 100$$

$$(ii) \text{ Stomatal index} = \frac{\text{No. of stomata per unit area of leaf}}{\text{No. of stomata + number of epidermal cells per leaf unit area}} \times 100$$

$$(iii) \text{ Trichome index} = \frac{\text{No. of trichome per unit area of leaf}}{\text{No. of trichome + number of epidermal cells per leaf unit area}} \times 100$$

**Staining:** The epidermal peels, cleared leaves and wood macerates of the species of *Euphorbia* studied were rinsed for three minutes in Safranin -O-. They were rinsed twice in water and mounted in 25% glycerol on glass slides cover slip with the edge of cover slip ringed with nail varnish to seal the edges of the cover slips and prevent dehydration.

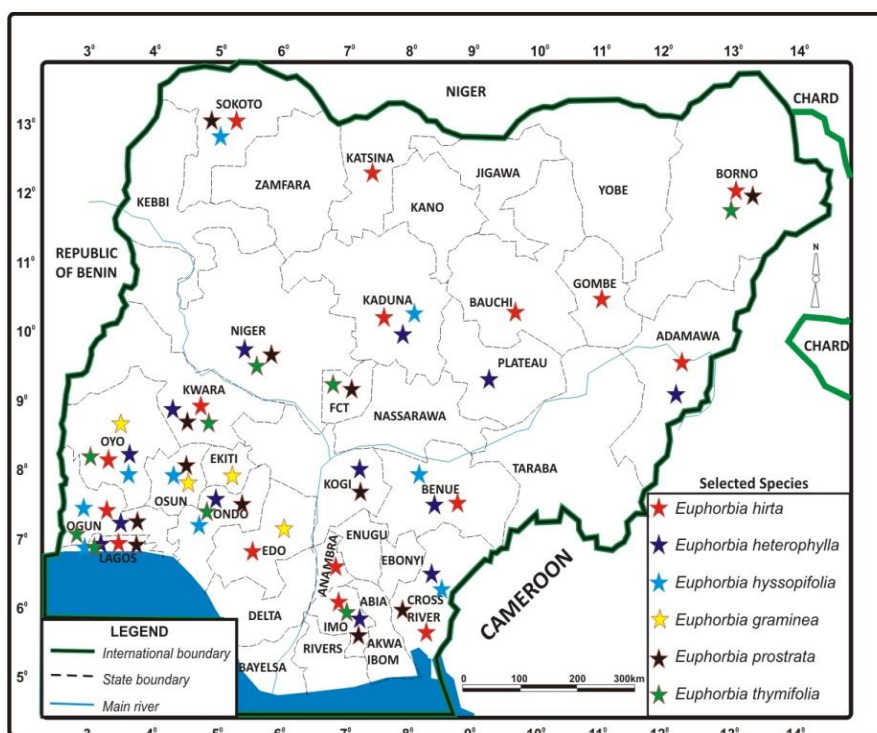


Fig. 1: Distribution map of *Euphorbia* species in Nigeria (Sanusi et al., 2021)

**Microscopy of anatomical characters:** Appropriate microscopic observations were carried on the mounted specimens at lower magnification (x400 magnification). Photomicrographs of the slides were made using an Acuu-scope trinocular microscope (ACUU-scope 3001 LED Trinocular microscope with 3.2 MP CMOS digital camera). Tissues and cells identification and description of wood samples of the *Euphorbia* species was done according to IAWA hardwood features list, definition, and illustration (IAWA, 1989). Tissues and cell identification and description of leaf were done according to Dickison (2000).

**Statistical analysis:** All measurements were made using ocular micrometer at x400 magnification. For each abaxial and adaxial surface, twenty-five readings of each characteristic were recorded. The measurement was taken to the nearest micrometers. Counts were taken of the epidermal, stomatal and trichome frequencies. Corresponding mean values of each quantitative character along with standard errors were obtained for each character. The values generated were then input into microsoft excel spreadsheet and raw data were coded to allow analysis using SPSS 15.0 analysis sheet.

## Results

***Euphorbia graminea* Jacq.:** **Adaxial Surface:** Epidermal cells are irregular with wavy to undulating anticlinal walls (Table 1). They vary in size, shape, and arrangement. Epidermal cell is  $62.60 \pm 12.50 \times 36.20 \pm 7.60$   $\mu\text{m}$  and epidermal cell frequency is  $203.5 \pm 35.8$   $\mu\text{m}$  (Table 3). The species are amphistomatic with anomocytic, paracytic and anisocytic stomata on the adaxial side. Stomatal shape is circular or elliptic, subsidiary cell size is  $60.8 \pm 10.7 \times 37.8 \pm 7.3$   $\mu\text{m}$ ; guard cell  $125.9 \pm 2.2 \times 7.8 \pm 1.6$   $\mu\text{m}$ , stomatal frequency is  $10.4 \pm 3.2$   $\mu\text{m}$ , stomatal index is  $5.29 \pm 2.07\%$ . Trichomes are multicellular and glandular  $737.2 \pm 138.1 \times 50.8 \pm 8.9$   $\mu\text{m}$ , its frequency is  $39.6 \pm 16.9$ /field, and its index is  $20.5 \pm 10.0\%$  (Table 3).

**Abaxial Surface:** Epidermal cells are irregular with wavy or undulating anticlinal wall (Table 2). They vary in size, shape, and arrangement. Epidermal cell size is  $64.1 \pm 13.9 \times 30.4 \pm 7.4$   $\mu\text{m}$ , its frequency is  $193.9 \pm 23.1$ /per field. Lamina amphistomatic, mostly anisocytic, also anomocytic, circular or elliptic in shape restricted to the non-venous regions. Subsidiary cell is  $51.5 \pm 12.45 \times 33.7 \pm 5.60$   $\mu\text{m}$ ; guard cell is  $24.4 \pm 2.80 \times 5.8 \pm 1.20$   $\mu\text{m}$ ; stomatal frequency  $29.4 \pm 8.10$  /field and its index is  $15.2 \pm 3.80\%$  (Table 4).

**Table 1:** Qualitative features of the foliar adaxial epidermal morphology of six studied species of *Euphorbia*

Species Feature	<i>E. graminea</i>	<i>E. heterophylla</i>	<i>E. hyssopifolia</i>	<i>E. hirta</i>	<i>E. prostrata</i>	<i>E. thymifolia</i>
Epidermal shape	Irregular, rectangular or polygonal	Rectangular, polygonal	Irregular, rectangular at costal region	Rectangular, polygonal	Rectangular, polygonal	Rectangular, polygonal
Anticlinal cell wall	Undulating, straight	Straight	Undulating straight at the coastal region	Straight to curve	Straight	Straight
Stomata type	Anomocytic, anisocytic and paracytic	Anomocytic, anisocytic, paracytic and branchparacytic	Anisocytic and anomocytic	Anisocytic, anomocytic and paracytic	Anomocytic, anisocytic, paracytic and branchparacytic	Anomocytic, anisocytic, paracytic and branchparacytic
Stomata shape	Oval and angular	Circular oval	Oval and angular	Circular and oval	Circular, oval and angular	Oval, circular and angular
Trichome type	Multicellular	Muti- and unicellular	Absent	Multicellular	Multicellular	Multicellular
Radiated epidermal cells around the trichome base	Absent	Present	Absent	Present (11-14)	Present (7-8)	Present (7-8)

**Table 2:** Qualitative features of the foliar abaxial epidermal morphology of six studied species of *Euphorbia*

Species Feature	<i>E. graminea</i>	<i>E. heterophylla</i>	<i>E. hyssopifolia</i>	<i>E. hirta</i>	<i>E. prostrate</i>	<i>E. thymifolia</i>
Epidermal cell shape	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Anticlinal cell wall	Undulating	Undulating	Undulating	Undulating	Undulating	Undulating
Stomatal type	Anomocytic, anisocytic and paracytic	Anomocytic, anisocytic, paracytic and branchparacytic	Anisocytic and anomocytic	Anisocytic, anomocytic and paracytic	Anomocytic, anisocytic, paracytic and branchparacytic	Anomocytic, anisocytic, paracytic and branchparacytic
Stomata shape	Oval and angular	Circular and oval	Oval and angular	Circular and oval	Circular, oval and angular	Oval, circular and angular
Trichome type	Multicellular	Multi- and unicellular	Absent	Multicellular	Multicellular	Multicellular
Radiated epidermal cells around the trichome base	Absent	Present	Absent	Present (11-14)	Present (7-8)	Present (7-8)

***Euphorbia heterophylla* Linn:** Adaxial surface: Epidermal cells are more or less isodiametric with straight anticlinal walls (Table 1). They vary in size, shape and arrangement. Epidermal cell is  $29.40 \pm 1.17 \times 27.5 \pm 0.61$   $\mu\text{m}$ , its frequency  $752.50 \pm 5.24/\text{field}$ . Stomata present, anomocytic, anisocytic, paracytic, diacytic, stomata frequency  $96.8 \pm 2.73/\text{field}$ , stomata index  $11.32 \pm 0.36\%$ , subsidiary cell is  $22.10 \pm 0.93 \times 15.6 \pm 0.51$   $\mu\text{m}$ ; guard cell is  $15.5 \pm 0.48 \times 6.20 \pm 0.33$   $\mu\text{m}$  (Table 3). Trichome present on the venous and non-venous regions. Trichomes are nonglandular, multicellular, its base is often circular or oval. Trichome size is  $80.40 \pm 6.42 \times 30.40 \pm 1.29$   $\mu\text{m}$  its frequency is  $90.4 \pm 4.10/\text{field}$  and its index is  $10.64 \pm 0.55\%$  (Table 3).

Abaxial surface: Epidermal cell is  $29.4 \pm 1.17 \times 17.5 \pm 0.61$   $\mu\text{m}$ , its frequency is  $629.4 \pm 9.21/\text{field}$  (Table 2). Stomata present, brachparacytic, anomocytic and anisocytic types of stomata, restricted in the non-venous regions, its frequency is  $102.7 \pm 4.10/\text{field}$ , and its index is  $16.30 \pm 0.66\%$ , subsidiary cell size is  $67.1 \pm 3.93 \times 38.2 \pm 2.57$   $\mu\text{m}$ . Guard cell is oval, its size is  $30.6 \pm 2.54 \times 10.6 \pm 2.26$   $\mu\text{m}$  (Table 4). Trichomes unicellular and multicellular, non glandular, present on both venous and non-venous regions, trichome is  $82.5 \pm 2.00 \times 38.2 \pm 0.67$   $\mu\text{m}$  its frequency is  $15.4 \pm 15.9/\text{field}$  and its index  $38.85 \pm 5.74\%$  (Table 4) with circular base.

***Euphorbia hyssopifolia* Linn:** Adaxial surface: Epidermal cells are irregular with undulating walls (Table 1). They vary in size, shape and arrangement, its size is  $48.2 \pm 1.18 \times 21.8 \pm 0.74$   $\mu\text{m}$ , and  $201.9 \pm 4.06$  frequency/field (Table 3). Stomata are present, anisocytic and anomocytic; with frequency  $32.8 \pm 1.94/\text{field}$ , its index is  $16.46 \pm 1.04\%$ . Subsidiary cell size is  $50.2 \pm 3.32 \times 36.00 \pm 3.94$   $\mu\text{m}$ , guard cell size is  $16.50 \pm 0.56 \times 5.20 \pm 0.25$   $\mu\text{m}$  (Table 3), circular, oval and cylindrical. Trichome is absent.

Abaxial Surface: Epidermal cells are irregular polygonal, rectangular with undulating anticlinal walls (Table 2). They vary in size, shape, and arrangement. Epidermal cell size is  $59.00 \pm 1.98 \times 43.7 \pm 1.56$   $\mu\text{m}$ , its frequency is  $322.5 \pm 11.12/\text{field}$  (Table 4). Stomata anomocytic and anisocytic with frequency  $43.9 \pm 2.34/\text{field}$ , its index is

14.09±0.98 %. Subsidiary cell size is 30.3±1.97×19.3±1.04 µm, guard cell is 16.9±0.46×5.5±0.25 µm (Table 4), which are round, elliptic and cylindrical. Trichomes are absent.

***Euphorbia hirta* Linn.** *Adaxial surface:* Epidermal cells are rectangular, polygonal to irregular, anticlinal walls straight or arch or curve (Table 1). The size, shape and arrangement are variable. Epidermal cell is 37.30±2.46×21.80±0.74 µm, its frequency is 344.50±4.61/field. Stomata are paracytic and anisocytic, shape often oval or circular, stomata frequency is 63.63±2.33/field, its index is 18.53±0.72 %, subsidiary cell size is 32.90±2.03×32.2±10.10 µm, guard cell occasionally circular sometimes oval, guard cell size is 16.7±0.35×5.7±0.23 µm (Table 3). Trichomes are multicellular with circular glandular-like base, surrounded by radiated epidermal cells which range from 9 - 25 cells often rectangular or polygonal. Trichome size is 49.5±33.40×42±2.31 µm with frequency 213±29.30/field, its index is 62.73±9.00 % (Table 3).

*Adaxial surface:* Epidermal cells are irregular with undulating anticlinal walls (Table 2). They vary in size, shape and arrangement. Epidermal cell is 40.24±1.53×23.84±1.35 µm, its frequency 278.10±13.70/field (Table 4). Stomata anisocytic and anomocytic, restricted to non-venous regions, stomata frequency 69.20±2.18/field and its index is 32.68±7.90 %. Subsidiary size is 27.30±1.10×16.2±0.86 µm, guard cell cylindrical, rectangular, size is 15.6±0.46×5.4±0.19 µm (Table 4). Trichomes are multicellular with swollen base surrounded by radiated epidermal cells ranging from 7 - 12 cells often irregular in shape. Trichome size is 212±22.3×46.7±2.73 µm, its frequency is 58.7±11.8/field, with index 58.7±8.44 % (Table 4). The trichome base is irregular, polygonal, oval to circular.

***Euphorbia prostrata* Aiton.** *Adaxial surface:* Epidermal cells are rectangular to polygonal; with straight anticlinal walls (Table 1) of various sizes, shapes, and arrangement. Epidermal cells size is 64.5±2.80×33.10±2.60 µm, its frequency is 312.10±3.30/field. Stomata are anomocytic, anisocytic and paracytic, its frequency is 60.00±1.53/field, and index is 19.25±0.48 %. Subsidiary cell size is 10.70±0.27×3.10±0.22 µm, guard cells are elliptic, round, and angular. Trichomes are multicellular, non-glandular with circular base, trichome size is 15.10±0.55×8.50±0.58 µm; and its frequency is 8.30±0.53/field (Table 3).

*Abaxial surface:* Epidermal cells are rectangular to polygonal with straight to arch anticlinal walls (Table 2). The size, shape and arrangement vary considerably, its size is 64.50±2.80×33.10±2.60 µm, with frequency 312.10±3.30/field. The stomata in the non-venous regions are anomocytic, anisocytic and paracytic, its frequency is 60±1.53/field and the stomata index is 19.25±0.48. Subsidiary cell size is 57.60±3.70×30.50±2.18 µm, guard cell size is 10.70±0.27×3.10±0.22 µm (Table 4), guard cell shapes are elliptic to round. Trichomes are multicellular non-glandular on the non-venous and leaf margin. Trichomes are often with circular base.

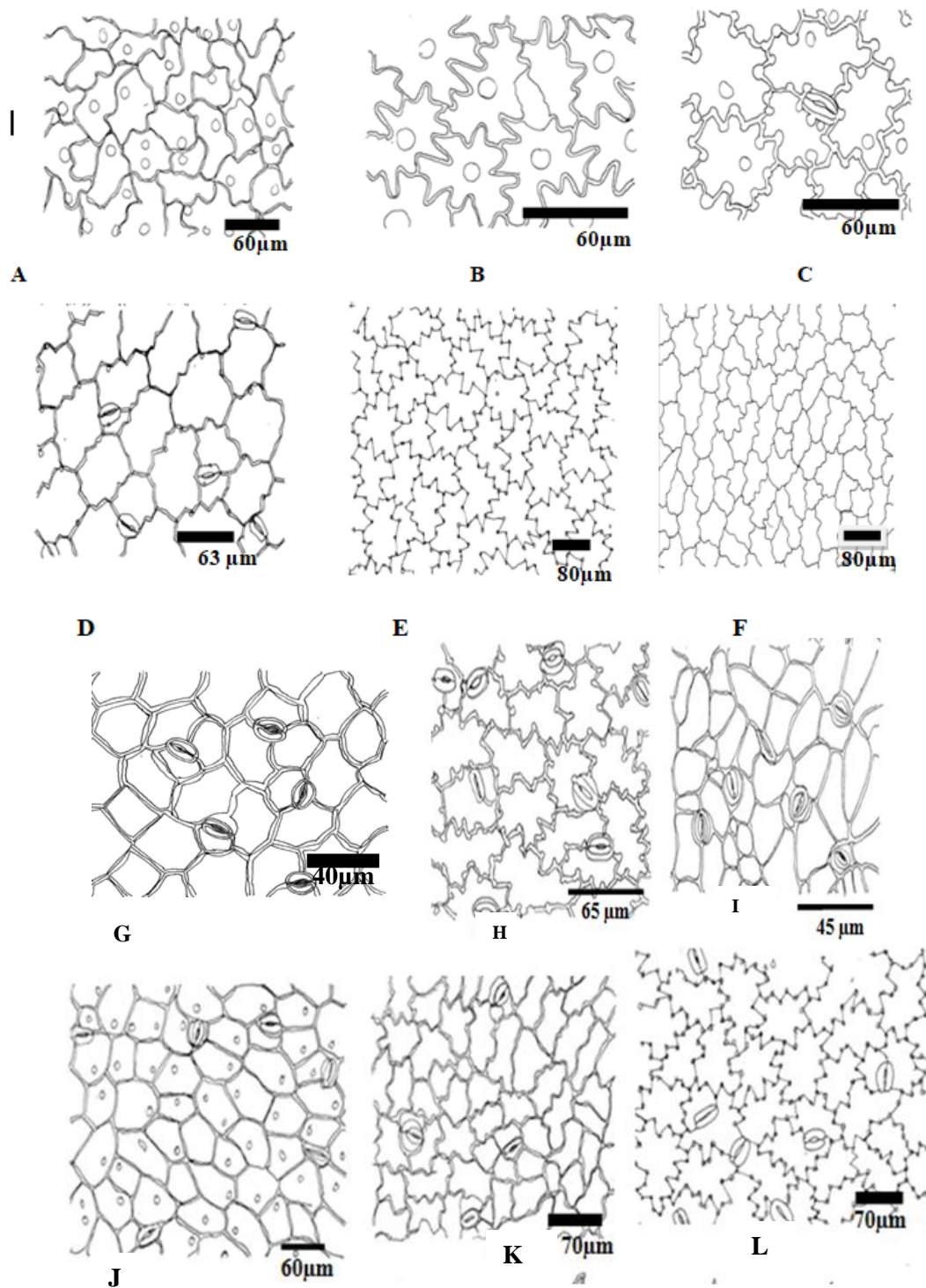
***Euphorbia thymifolia* Linn.** *Adaxial surface:* Epidermal cells are rectangular to polygonal more or less isodiametric with straight anticlinal walls (Table 1) of different sizes, shapes and arrangements. Epidermal cell size is 46.40±1.52µm×28.20±1.04 µm with frequency 232.60±6.96/field (Table 3). Stomata are anisocytic, anomocytic, paracytic and diacytic, restricted to non-venous regions, its frequency is 45.10±1.77/field and stomata index is 19.89±1.02. Subsidiary cell size is 37.70±1.99×17.30±0.96 µm. Guard cell may be oval circular, polygonal or cylindrical in shape, size 18.30±0.49×6.00±0.25 µm. Trichomes are non-glandular, multicellular with radiated trichome ranging from 7-9 cells; its base is usually circular or oval; trichome size is 499.00±34.70×26.00±1.41 µm with frequency 170.00±11.50/field and its index is 74.57±5.27% (Table 3).

*Abaxial surface:* Epidermal cells irregular with undulating to sinuous anticlinal walls (Table 1) its size is 32.50±1.79×18.10±1.14 µm, with frequency 463.00±6.47/field. Stomata are anisocytic, anomocytic, anisocytic, paracytic and diacytic restricted to non-venous regions, its frequency is 107.00±3.71/field while its index is 23.00±0.93. Subsidiary cell size is 31.80±1.54×16.00±0.90 µm; guard cell oval, circular, rectangular, or polygonal, its size is 20.10±0.91×7.20±0.26 µm; trichomes are non-glandular, multicellular with radiated trichome base (Table 2 and 4).

*Artificial key for the identification of Euphorbia species using foliar epidermal characters.*

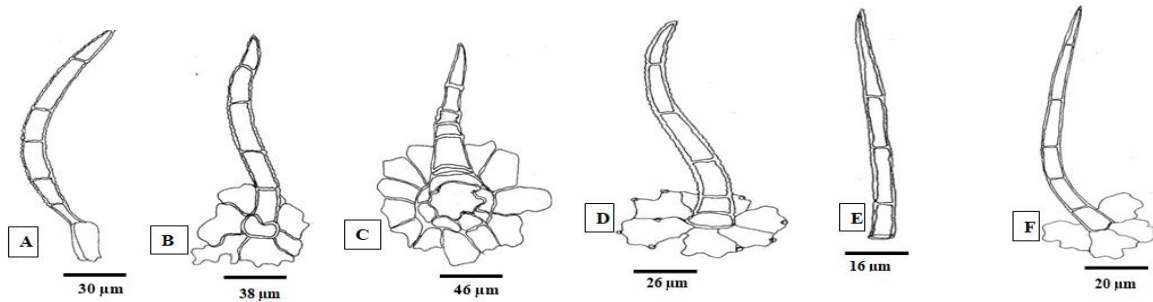
- 1a. Epidermal cell rectangular to polygonal with straight anticlinal walls ..... (2)
- 1b. Epidermal cell irregular with undulating anticlinal walls ..... (3)
- 2a. Epidermal cell size 46.40±1.52×28.20±1.04 µm.  
epidermal frequency 463.00±6.47 µm/field.  
subsidiary cell size 31.80±1.54×16.00±0.90 µm ..... *E. thymifolia*
- 2b. Epidermal cell size 64.50±2.80×33.10±2.60 µm with frequency 312.10±30 µm/field.  
subsidiary cell size 57.60±3.70×30.50±2.18 µm ..... *E. prostrata*
- 3a. Trichomes present, stomata are anomocytic, anisocytic, paracytic and branchyparacytic .....(4)
- 3b. Trichomes absent; stomatal are anomocytic and anisocytic ..... *E. hysopifolia*.
- 4a. Trichomes glandular; papillae absent ..... *E. hirta*
- 4b. Trichomes non-glandular, papillae present ..... (5)
- 5a. Papillae conspicuous; stomata elevated above the epidermal surface; stomatal index 16.30±0.66 %,  
trichome index 38.85±5.74 % .....*E. heterophylla*.
- 5b. Papillae non conspicuous; stomata levelled with the epidermal surface.  
stomata index 15.17 % and trichome index 18.90±1.18 % ..... *E. graminea*



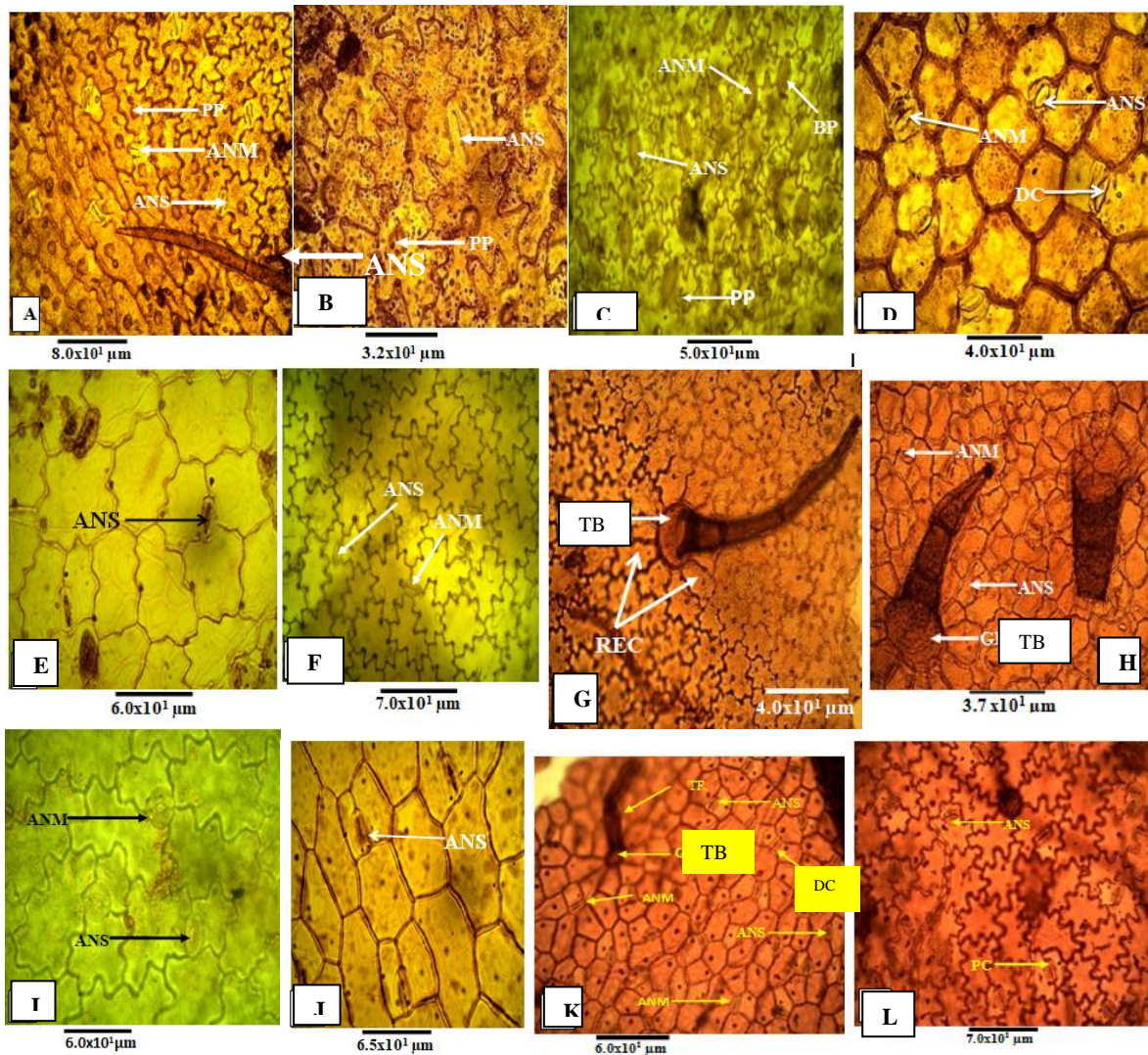


**Fig. 2:** Lamina in surface view of *Euphorbia* species

Abaxial epidermis of *E. graminea*; B. and C. Adaxial epidermises of *E. graminea*; D. and E. Abaxial epidermis of *E. hyssopifolia*; F. Adaxial epidermises of *E. hyssopifolia*; G. Adaxial epidermis of *E. heterophylla*; H. Abaxial epidermis of *E. prostrata*; I. Adaxial epidermis of *E. prostrata*; J. Adaxial epidermis of *E. thymifolia*; K. Abaxial epidermis of *E. thymifolia*; L. Adaxial epidermis of *E. hirta*



**Fig. 3:** Trichomes of *Euphorbia* species E. *graminea*; B. *E. heterophylla*; C. *E. hirta*; D. *E. thymifolia*; E and F. *E. prostrata*



**Plate 1:** Leaf Anatomy of *Euphorbia* species. A and B. Abaxial and Adaxial epidermises of *E. graminea* respectively; C and D. Abaxial and Adaxial epidermises of *E. heterophylla* respectively; E and F. Abaxial Adaxial epidermises of *E. hyssopifolia* respectively; G and H. Abaxial and Adaxial epidermises of *E. hirta* respectively; I and J. Abaxial and Adaxial epidermises of *E. prostrata* respectively; K and L. Abaxial and Adaxial epidermises of *E. thymifolia* respectively. ANS – Anisocytic stomata; ANM – Anomocytic; DC – Diacytic; REC – Radiated epidermal cells; TB – Trichome base; MTR – Multicellular trichome; PP – Pappilae; TR – Trichome

**Table 3:** Quantitative parameters of the foliar adaxial epidermal morphology of six studied species of *Euphorbia*

Species	Epidermal Cell		Subsidiary cell		Guard Cell		Trichome		Frequencies			Indices	
	Length	Width	Length	Width	Length	Width	Length	Width	EF	TF	SF	SI	TI
<i>E. graminea</i>	62.60±2.56	36.24±1.56	60.80±2.19	37.80±1.5	25.90±0.45	7.80±0.33	737.00±28.20	50.80 ±1.82	203.50±7.31	39.60±3.44	10.40±0.66	5.30±0.42	20.48±2.64
<i>E. hirta</i>	37.30±2.46	21.80±0.74	32.90±2.03	32.20±10.1	16.70±0.35	5.70±0.23	495.00±33.40	42.00±33.4	344.50±4.61	213.00±29.3	63.60±2.33	18.53±0.72	62.73±9.00
<i>E. hyssopifolia</i>	59.00±1.98	43.70±1.56	30.30±1.97	19.30±1.04	16.90±0.46	5.50±0.25	-	-	322.50±11.12	-	43.90±2.34	14.09±0.98	-
<i>E. heterophylla</i>	29.40±101.7	27.50±0.61	22.10±0.93	15.60±0.51	15.50±0.40	6.20±0.33	80.40±6.43	0.40±1.29	752.50±5.24	90.40±4.10	96.80±2.34	11.32±0.36	10.69±0.55
<i>E. prostrata</i>	36.60±1.48	17.50±0.80	34.00±0.98	17.40±0.85	17.00±0.48	5.60±0.36	15.10±0.55	8.50±0.58	655.30±36.23	83.00±0.53	134.30±4.25	23.57±2.46	4.20±0.16
<i>E. thymifolia</i>	32.50±1.79	18.10±1.14	31.80±1.54	16.00±0.9	20.10±0.91	7.20±0.26	545.00±32.5	25.20±1.02	463.00±6.47	168.00±8.04	107.00±3.371	23.30±0.93	36.60±1.98

Means with the same letters along the same column are not significantly different at P≤0.05; number of measurements = 25

**Keys:** EF – Epidermal Frequency; TF – Trichome Frequency; SF – Trichome Frequency; SI – Stomata Index; TI – Trichome Index

**Table 4:** Quantitative parameters of the foliar abaxial epidermal morphology of six studied species of *Euphorbia*

Species	Epidermal Cell		Subsidiary cell		Guard Cell		Trichome		Frequencies			Indices	
	Length	Width	Length	Width	Length	Width	Length	Width	EF	TF	SF	SI	TI
<i>E. graminea</i>	64.1±2.84	30.4±1.51	51.5±2.54	33.7±1.15	24.4±0.56	5.8±0.24	64.6±2.46	58.2±2.22	93.9±4.72	36.00±20	29.4±1.66	15.17±0.78	18.90±1.18
<i>E. hirta</i>	40.2±1.53	23.8±1.35	27.3±1.10	16.20±0.86	15.6±0.46	5.4±0.1	21.2±2	46.7±2	278.1±13.7	144.00±10.6	69.2±2.18	32.68±	58.7
<i>E. hyssopifolia</i>	48.20±1.18	36.4±1.03	50.2±3	36±3.94	16.5±	5.2±0.2	-	-	201.9±	-	32.8±1.94	16.46±	-
<i>E. heterophylla</i>	69.4±3.17	37.5±0.61	67.1±3.93	38.7±2.57	30.6±2.57	10.6±2.26	82.5±2.65	38.2±0.67	629.4±9.21	154.00±159	102.7±4.10	16.30±0.66	38.85±5.74
<i>E. prostrata</i>	64.5±2.80	33.1±2.6	57.6±3.70	30.5±2.18	10.7±0.27	3.1±0.22	17.1±7.62	16.3±0.36	312.1±3.30	104.00±0.66	60.00±1.53	19.25±0.48	3.33±0.20
<i>E. thymifolia</i>	46.4±1.52	28.2±1.04	37.7±1.99	17.3±0.96	18.3±0.49	6±0.25	49.9±34.7	26.00±0.1	232.6±6.96	170.00±11.5	45.1±11.77	19.89±1.02	74.57±5.2

Means with the same letters along the same column are not significantly different at P≤0.05; number of measurements = 25

**Keys:** EF – Epidermal Frequency; TF – Trichome Frequency; SF – Trichome Frequency; SI – Stomata Index; TI – Trichome Index



## Discussion

Foliar epidermal characters play an increasingly important role in determining the relationship between different taxa in different taxonomic categories. Leaf anatomical data are useful in solving several problems of plant systematics (Albert and Sharma, 2013). The comprehensive work done by Roy (2008) on the anatomy of plants revealed that flowering plant exhibits a great variation in their foliar anatomical characters. The leaf is one of the plant organs that show great variation in types, forms and arrangement. The anatomical survey of the leaf in this present study has provided supplementary evidence and is of great taxonomic importance in the delimitation of the species studied.

The leaf epidermal characters studied in this work correspond largely to those of Oladipo *et al.* (2015); Ishak *et al.* (2021); Chapeta *et al.* (2023). They used leaf epidermal characters in characterizing some species in genus *Euphorbia*. However, few salient characters (such as guard cell size, epidermal, trichome and stomatal frequencies, trichome and stomatal indices) that were not put forward by these authors and which may enhance the taxonomy of these species in Nigeria have been established in this study.

The species of *Euphorbia* examined here show remarkable interspecific relationships. Generally, stomata occur on both lower and upper surfaces of the leaves i.e. (amphistomatic leaves) and this corroborates the findings of Metcalfe and Chalk (1989); and Zokia (2011). Stomata complex types in the categories of anomocytic, anisocytic, paracytic, diacytic were encountered. Diacytic stomatal complex was observed only in *E. heterophylla* and *E. thymifolia*.

It is clear from the present findings that stomata of more than one type have been observed on the same leaf surface. For instance, in *E. heterophylla*, four types of stomatal complexes (i.e., anomocytic, anisocytic, paracytic and diacytic) were encountered on one leaf surface (abaxial surface). *Euphorbia hirta* has anomocytic, anisocytic and paracytic stomata while *E. graminea* has paracytic, anomocytic and anisocytic stomatal complexes. The predominance of anomocytic and anisocytic stomata found in this study corroborates the findings of Kakkar and Paliwal (1974); Zokia (2011). Anomocytic stomata was also reported in *E. hirta* by Aworinde (2009) and Essiet *et al.* (2012).

Stomatal frequencies vary in the studied species. Of the six species selected for the present study, on the upper epidermis, the highest stomatal frequency was observed in *E. prostrata* and the lowest stomatal frequency was in *E. graminea*. On the lower epidermis, the highest stomatal frequency was recorded in *E. heterophylla* and the lowest in *E. graminea*. According to Stace (1981), the frequency of stomata has been found helpful in solving taxonomic problems, although, many controversies have arisen that stomatal frequency is less reliable feature in taxonomy in that stomatal frequency varies considerably with the age of the plant (Esau, 1977; Raju and Rao, 1977). The stomatal index has also proven to be helpful in species delimitation (Olatunji, 1983; Ashidi, 2017). On the adaxial epidermal surface, the stomatal index is low in *E. graminea* ( $5.30 \pm 0.42 \mu\text{m}$ ) and *E. heterophylla* ( $11.32 \pm 0.36 \mu\text{m}$ ) and considerably high in *E. prostrata* ( $23.57 \pm 2.46 \mu\text{m}$ ) and *E. thymifolia* ( $23.30 \pm 0.43 \mu\text{m}$ ). On the abaxial, it is high in *E. hirta* ( $32.68 \pm 7.90 \mu\text{m}$ ) and low in *E. graminea* ( $15.17 \pm 0.78 \mu\text{m}$ ).

The shape of stomata is of little importance in genus *Euphorbia*. Therefore, it cannot be solely used in differentiating species. The shape ranges from circular, oval and rectangular to polygonal. In *E. thymifolia*, *E. hirta* and *E. graminea*, there are three to four different types of stomatal shapes on a leaf surface whereas in *E. hyssopifolia*, the shape is largely oval. On the abaxial surface, the stomatal shape is oval in *E. heterophylla* while on the adaxial surface, it is circular and oval. In *E. prostrata*, the shape is largely circular. The guard cell size is diagnostic value and can be used to distinguish the species. On the lower epidermis, the highest guard cell size ( $30.60 \pm 2.54 \times 10.60 \pm 2.26 \mu\text{m}$ ) was found in *E. heterophylla* while the lowest guard cell size ( $10.70 \pm 0.27 \times 3.10 \pm 0.22 \mu\text{m}$ ) was observed in *E. prostrata*. On the adaxial surface, *E. graminea* has the largest guard cell size ( $25.90 \pm 0.45 \times 7.80 \pm 0.33 \mu\text{m}$ ) while *E. heterophylla* has the smallest guard cell size ( $15.50 \pm 0.48 \times 6.20 \pm 0.33 \mu\text{m}$ ).

The epidermal cell size and frequency also show some variations which are useful in separating the species. Ashidi *et al.* (2017) employed epidermal size and frequency to separate ten species of *Milletia* L. in the family Fabaceae. Oladipo (2012) also utilized these characters to delimit some selected species of *Jatropha* L. *E. heterophylla* has the largest epidermal cell on the lower epidermis while *E. thymifolia* has the smallest size on the upper epidermis, the largest epidermal cell size was shown in *E. graminea*, and the smallest value was recorded in *E. heterophylla*.

Variation in epidermal cell frequency was also observed with the highest epidermal cell frequency in *E. heterophylla* and the lowest in *E. graminea* on the abaxial surface while on the adaxial surface, the highest value of epidermal cell frequency was recorded in *E. heterophylla* and the lowest value in *E. graminea*. Apparently, it is observed from the present studies that epidermal frequency is inversely proportional to epidermal cell size, when epidermal cells are small, the epidermal frequency is high and when cells are large, the frequency is low.

Trichomes are widespread in the studied *Euphorbia* species except *E. hyssopifolia*. Observed foliar trichomes were non-glandular, unicellular and multicellular in the examined species. Trichomes were observed on both adaxial and abaxial surfaces of the leaves. The common trichome is the non-glandular type found in *E.*

*heterophylla*, *E. hirta*, *E. prostrata* and *E. thymifolia*. Trichomes with swollen bases are infrequent, it displays an interesting character for taxonomic distinction which is only observed in *E. hirta*. This observation is consistent with the earlier report on the presence of swollen base trichome on the leaf epidermis of *E. hirta* (Essiet, 2012; Zokia, 2011).

*E. heterophylla* has both multicellular and unicellular trichomes with pointed or curved apices. In *E. hirta*, *E. heterophylla*, *E. prostrata* and *E. thymifolia*, the trichomes show typical arrangement of radiated epidermal cells around the trichome base which gives radiated-epidermal-trichome appearance. In *E. hirta*, the epidermal cells are arranged around the base vary from 11 to 14 cells, in *E. thymifolia* and *E. prostrata*, 7-8 cells while they are 5-6 cells in *E. heterophylla*.

The distribution of trichomes on the leaf epidermis can be used as diagnostic characteristics as these are observed on the venous and non-venous regions. In *E. heterophylla*, trichomes are abundant on the leaf veins than on the non-venous regions. The leaf epidermis of *E. prostrata* and *E. thymifolia* are similar but can be differentiated by the distribution of trichomes on the venous and non-venous regions. In *E. prostrata*, trichomes are also distributed on the leaf margins but not in *E. thymifolia*. It is obvious from the present findings that trichome size, trichome frequency and trichome index are of considerable relevance in species differentiation. On the lower epidermis, the highest trichome frequency ( $170.00 \pm 11.50$ /field) appeared in *E. thymifolia* and the lowest ( $104.00 \pm 0.66$ /field) in *E. prostrata* while on the upper epidermis, the highest trichome frequency ( $213.00 \pm 29.30$  /field) was found in *E. hirta* and lowest value ( $8.30 \pm 0.53$ /field) was recorded in *E. prostrata*. The trichome indices were also calculated for the six studied species and variations were observed among species with minimum value ( $33.30 \pm 0.20$ ) in *E. prostrate* and maximum value in *E. thymifolia* ( $74.57 \pm 5.20$ ) per field on the lower epidermal surface whereas on the upper epidermal surface, the highest trichome index ( $62.73 \pm 9.00$ ) was in *E. hirta* and lowest in *E. prostrata* ( $14.20 \pm 0.16$ ).

On the adaxial surface, the longest and widest trichomes were noticed in *E. graminea* and the shortest in *E. prostrata* whereas on the abaxial surface, the largest trichomes were found in *E. graminea* and the smallest in *E. heterophylla*. The presence of papillae in some of the species studies is also of diagnostic importance. Papillae were encountered in three species i.e., *E. heterophylla*, *E. graminea* and *E. thymifolia*. They are of various shapes and sizes, in *E. heterophylla*, the papillae are very conspicuous occupying more than half of the epidermal cell, followed by *E. graminea* whereas in *E. thymifolia*, the papillae are very small in size.

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