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(RESEARCH ARTICLE)

Foliar epidermal anatomy and its taxonomic implications within the family Euphorbiaceae in the Niger Delta Region of Nigeria

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Abstract

Micro-morphological investigations of the foliar epidermal anatomy of seven species of the family Euphorbiaceae: *Acalypha hispidia* Burm. f., *A. wilkesiana* Mull. Arg, *Euphorbia heterophylla* L., *E. hirta* L., *Jatropha curcas* L., *J. gossypifolia* L. and *Manihot esculenta* Crantz was carried out to assess the systematic relevance of epidermal features, stomata and trichome diversity within the family using light microscope and camera. Members of this family contain many important medicinal herbs and there has been increasing misidentification of species in this family. Leaf micro-morphological characteristics are commonly used in the identification and authentication of plants. Results showed that the stomatal index varied from species to species. Epidermal cell shapes were found to be irregular to polygonal on both surfaces of the species. The epidermal cells possess straight to undulating anticlinal walls but in some few cases exhibited wavy to straight anticlinal walls. The presence of papillae was observed to be a unique character of *E. heterophylla* among the species studied. The stomatal types observed were Anisocytic, Anomocytic, Diacytic, tetracytic, paracytic, and actinocytic. The stomatal complex was mainly amphistomatic except for *A. wilkesiana* that was hypostomatic. The hypostomatic character of A. wilkesiana as reported in this paper is the first of such report as previous report found was amphistomatic. Non-glandular, multiseriate uniseriate and in some cases branched trichome types were observed only on both surfaces of *A. wilkesiana* and *E. hirta* but absent on all other studied species. This study revealed detailed descriptive micromorphological structures which could serve as a source of information and reference for the taxonomic description, identification and delineation of the species studied.

Keywords: *Acalypha; Euphorbia*; Euphorbiaceae; *Jatropha; Manihot*; Micro- morphology

1. Introduction

The family Euphorbiaceae which is classified under the group of plants referred to as Angiosperm is one of the largest families of the plant kingdom [1]. The family contains over 800 species organized into 300 genera which may be woody trees, shrubs, climbing lianas or weeds [2]. The family is characterized by the production of milky sap, possession of unisexual flowers, superior and trilocular ovary, axile placentation with a collateral ovule, pendulous and ventral raphe, usually carunculate [2].

In Nigeria, species of Euphorbiaceae are well distributed across free and forest reserved areas. Nigerians make use of a number of these species of Euphorbiaceae for various purposes in both crude and processed forms. The use of these species in Nigeria can be classified into food and fodder, landscape and beautification, superstitious use, medicine, hedges, timber, and others [3].

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Micro-morphological characteristics of leaves have played important roles in plant taxonomy, especially at the generic and specific levels. Plant morphologists and systematists have resolved lots of taxonomic conflicts in different groups of plants using studies in this field [4]. Most taxonomic studies are based on the studies of the leaf epidermis and the foliar epidermal structure has been one of the most important taxonomic characters in biosystematics. Epidermal cells, stomata, and trichomes, their sizes, lengths, distribution, orientation, and frequency are of significant importance in taxonomy as well as phylogeny [5].

Most plants have epidermal cells containing hairs which are functionally called glandular or nonglandular trichomes. Glandular trichomes, which are specialized secretory structures, vary in size, form, location, and function in different plant species and have been reported to possess higher levels of secondary metabolites than those produced from other plant organs [6, 7]. Secretory structures such as stomata, glandular trichomes, and nonglandular trichomes are of scientific interest because of their functional characteristics and economic significance in the production of phytochemicals.

Despite the numerous ways members of family have been put into use, much research has not been done on the use of micro-morphological features for the purpose of identification, delineation and classification of the various taxa in this family as a whole. Previous works basically delved into medicinal potentials of members of the family [8], the developmental anatomy [9], propagation, growth and physiology [10, 11, 12].

Also, a crucial subject of discussion among Plant taxonomists for years now is the problem of accurate plant identification and classification: a challenge which abounds among species of family Euphorbiaceae too [1, 13, 14]. For years now, a lot of plants are classified based on their flora and fruits which are considered as external morphological features whose production are seasonal, hence limiting their availability for study and proper identification [15, 16]. Currently, there are quest for some novel protocols for easy and better identification of plant at any time or season of the year [15, 16]. Some method suggested includes: the use of cytological, anatomical, molecular and epidermal systematics from the leaves, roots and stems which are not time bound [15]. It is based on this foregoing reason that this study was carried out to investigate the detailed micromorphological features of the leaves of the selected species for taxonomic identification, delineation and validation of the species.

2. Material and methods

2.1. Sample Collection and Herbarium Specimen Preparation

Fresh matured leaf materials for the micro morphological studies were collected from the wild and cultivated field and voucher specimens were prepared using standard method for plant taxonomy and deposited at the Rivers State University Herbarium. The middle vein, secondary veins, leaf teeth, and leaf apex were selected for investigations, and both the adaxial and abaxial leaf surfaces were examined.

2.2. Micro-morphological Study

The upper (adaxial) and lower (abaxial) epidermal surfaces were peeled with blades and in some cases depending on the genus involved, they were soaked in concentrated nitric acid or trioxonitrate (v) acid, rinsed in distilled water and then stained in 1% aqueous safranin solution and mounted in glycerin. Thereafter, the cover slips were placed over the peels and then sealed with nail varnish to prevent dehydration [17, 18]. The slides were observed using a light microscope and pictures were taken with the use of a XSZ-N107 Microscope with (MA88-900) camera. The leaf epidermal characteristics were determined based on the methods of Metcalfe and Chalk [19].

2.3. Stomatal Index

The stomatal index was determined using the formula: $\text{Stomatal Index (SI)} = \frac{S}{E+S} \times \frac{100}{1}$

Where S = number of stomata per unit area; and E = number of epidermal cells in the same area.

3. Results

3.1. Foliar Micromorphology

3.1.1. Epidermal cells

For adaxial (AD) and abaxial (AB) leaf surfaces, the epidermal cell patterns were described separately. The Adaxial epidermal cells were irregular to polygonal in shape in *A. hispida, A. wilkesiana* and *M. esculenta*; rectangular, irregular and polygonal in *E. heterophylla*; Polygonal in *E. hirta* and *J. curcas* and irregular in *J. gossipifolia* (Figure 1 A, C, G, I, J, M, O, and Q). On the abaxial surface, the epidermal cells were also irregular to polygonal in shape in *A. hispida, A. wilkesiana* and *M. esculenta,* but irregular in *E. heterophylla, E. hirta* and *J. gossipifolia* and polygonal in *J. curcas* (Table 1 & Figure 1: B, E, F, H, K, L, N, P, and R)

The anticlinal cell walls on the adaxial surfaces of the species were wavy in *A. hispida,* undulating in *A. wilkesiana, E. hirta, J. gossipifolia* and *M. esculenta*; straight to wavy in *E. heterophylla* and straight to undulating in *J. curcas* (Plate 1: A, C, G, I, J, M, O, and Q). On the abaxial surfaces, the walls were undulating in *A. hispida*, *A. wilkesiana* and *J. gossipifolia*; wavy in *E. heterophylla* and *E. hirta*; straight to undulating in *J. curcas* and *M. esculenta* (Table 1 & Figure 1: B, E, F, H, K, L, N, P, and R).

3.2. Stomatal Complex

Amphistomatic leaves were found in *A. hispida*, *E. heterophylla, E. hirta, J. curcas, J. gossipifolia* and *M. esculenta* but hypostomatic in *A. wilkesiana* (Figure 1: A, B, D – R). Six types of stomatal complexes were recognized—actinocytic, anisocytic, anomocytic, diacytic, tetracytic, and paracytic. The most common type of stomatal complex in the taxa investigated was paracytic. However, two to five types of stomata occurred on the same surface. An anisocytic and paracytic stomatal complex was observed in *A. hispida,* only paracytic in *A. wilkesiana,* anisocytic, tetracytic, actinocytic and paracytic complex in *E. heterophylla,* paracytic, diacytic, anisocytic, tetracytic and anomocytic in *E. hirta;* anomocytic, tetracytic, anisocytic and paracytic in *J. curcas and J. gossipifolia;* anomocytic and paracytic in *M. esculenta* ((Table 1 & Figure 1: A– R).

The stomatal index (SI) of all amphistomatic taxa was higher on the AB surface than on the AD surface. On the AD surface, the highest SI was observed in *M. esculenta* (48.4), and the lowest SI was observed in *J. curcas* (2.25). On the AB surface, the highest SI was recorded in *E. hirta* (78.43), and the lowest SI was recorded in *J. gossipifolia* (25.49).

3.3. Trichomes

The leaf epidermis of *A. wilkesiana* and *E. hirta* had multicellular uniseriate non-glandular trichomes on both the adaxial and abaxial surfaces, including branched multicellular uniseriate non-glandular trichomes in *A. wilkesiana,* while the epidermis of all other studied species were glabrous except *J. gossipifolia* where there were trichomes on the abaxial surface (Table 1 & Figure 1: D, F, J, L and P).

3.4. Papillae

Papillae, a small rounded protuberance was found to be present on both surface of only *E. heterophylla* but was found to be absent on all other studied species. This character (papillae) is peculiar to *Euphorbia heterophylla* among the species studied (Figure 1: G & H).

Light microscopy (LM) micrographs of adaxial (AD) and Abaxial (AB) leaf surfaces in Euphorbiaceae species studied. (A) AD of *Acalypha hispida* with paracytic stomata, irregular to polygonal epidermal cells with wavy anticlinal walls (B) AB of *Acalypha hispida* with anisocytic and paracytic stomata, irregular to polygonal epidermal cells with undulating anticlinal walls. (C) AD of *Acalypha wilkesiana* lacking stomata, having irregular to polygonal epidermal cells with undulating anticlinal walls. (D) AD of *Acalypha wilkesiana* with multicellular uniseriate non-glandular trichomes (arrow). (E) AB of *Acalypha wilkesiana* with anisocytic and actinocytic stomata, irregular to polygonal epidermal cells with undulating anticlinal walls. (F) AB of *Acalypha wilkesiana* with multicellular uniseriate non-glandular branched trichomes (arrow). (G) AD of *Euphorbia heterophylla* showing papillae with anisocytic and tetracytic stomata, irregular to polygonal epidermal cells with straight to wavy anticlinal walls. (H) AB of *Euphorbia heterophylla* showing papilla with actinocytic and paracytic stomata, irregular epidermal cells with wavy anticlinal walls. (I) AD of *Euphorbia hirta* with paracytic and diacytic stomata, polygonal epidermal cells with undulating anticlinal walls and multicellular uniseriate non-glandular trichomes (arrow). (J) AB of *Euphorbia hirta* with anisocytic and tetracytic stomata, irregular epidermal cells with wavy anticlinal walls. (K) AB of *Euphorbia hirta* with multicellular uniseriate non-glandular trichomes (arrow). (L) AD of *Jatropha curcas* with anomocytic and tetracytic stomata, polygonal epidermal cells with straight to undulating anticlinal walls. (M) AB of *Jatropha curcas* with anisocytic and paracytic stomata, irregular epidermal cells with straight to undulating anticlinal walls. (N) AD of *Jatropha gossipifolia* with anomocytic and tetracytic stomata, irregular epidermal cells with undulating anticlinal walls. (O) AB of *Jatropha gossipifolia* with multicellular uniseriate non-glandular trichomes (arrow). (P) AD of *Manihot esculenta* with anomocytic stomata,

polygonal to irregular epidermal cells with undulating anticlinal walls. (Q) AB of *Manihot esculenta* with paracytic stomata, irregular to polygonal epidermal cells with straight to undulating anticlinal walls. Scale bars = 20 µm.

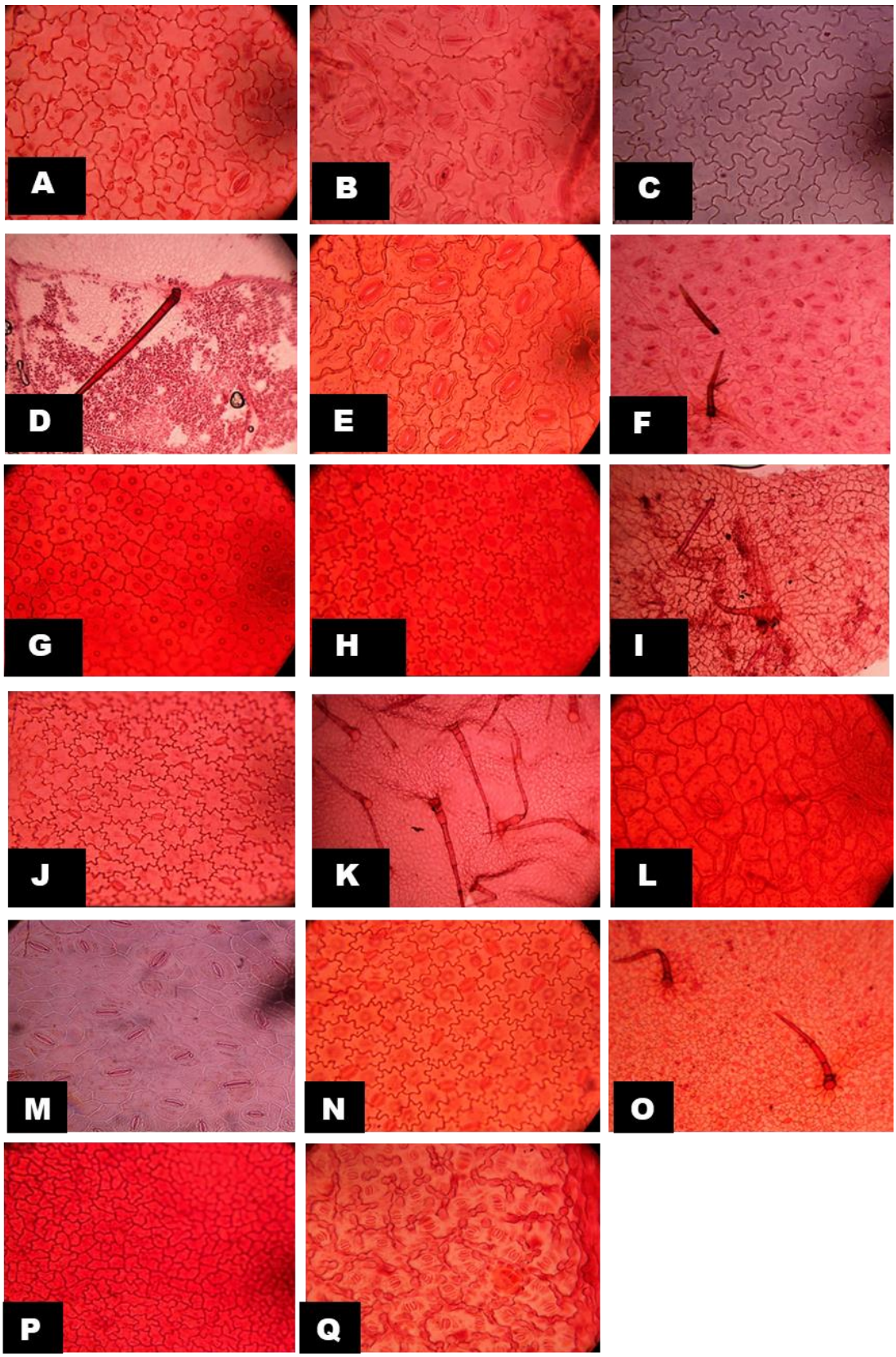

Figure 1 Light microscopy (LM) micrographs of adaxial (AD) and Abaxial (AB) leaf surfaces in Euphorbiaceae species studied.

Table 1 Micro morphological Characters of Species of Euphorbiaceae Studied

S/N	Character	*A. hispida*	*A. wilkesiana*	*E. heterophylla*	*E. hirta*	*J. curcas*	*J. gossipifolia*	*M. esculenta*
1	Stomatal index (%) (AD)	2.27	4.43	23.68	25.00	2.25	5.41	48.4 (1.3)
	(AB)	32.61	37.5	55.17	78.43	57.89	25.49	67.5
2	Epidermal Cell shape (AD)	Irregular to polygonal	Irregular to polygonal	Rectangular, Irregular to Polygonal	Polygonal	Polygonal	Irregular	Irregular to polygonal
	(AB)	Irregular to polygonal	Irregular to polygonal	Irregular	Irregular	Polygonal	Irregular	Polygonal to Irregular
3	Stomatal type (AD)	Paracytic	Absent	Tetracytic, Anisocytic	Paracytic, Diacytic	Anomocytic, Tetracytic	Anomocytic, Tetracytic	Anomocytic
	(AB)	Anisocytic and Paracytic	Anisocytic, Actinocytic and Paracytic	Actinocytic, Paracytic	Anisocytic, Tetracytic, Anomocytic and Paracytic	Anisocytic, Paracytic	Anisocytic, Paracytic	Paracytic
4	Trichomes (AD)	Absent	Present	Absent	Present	Absent	Absent	Absent
	(AB)	Absent	Present	Absent	Present	Absent	Present	Absent
5	Papillae (AD)	Absent	Absent	Present	Absent	Absent	Absent	Absent
	(AB)	Absent	Absent	Present	Absent	Absent	Absent	Absent
6	Anticlinal cell wall (AD)	Wavy	Undulating	Straight to wavy	Undulating	Straight to Undulating	Undulating	Undulating
	(AB)	Undulating	Undulating	Wavy	Wavy	Straight to Undulating	Undulating	Straight to Undulating
7	Stomatal distribution	Amphistomatic	Hypostomatic	Amphistomatic	Amphistomatic	Amphistomatic	Amphistomatic	Amphistomatic

AD: Adaxial Surface; AB: Abaxial Surface

4. Discussion

This study provides comprehensive micro-morphological information for the seven taxa studied. The micro-morphological characters basically coincide with the descriptions of other Euphorbiaceae species previously studied by [20].

Epidermal cell shape was found to be irregular to polygonal on both surfaces of studied species. This finding is supported by the work of [21] who also reported that the irregular cell shapes were observed to inter mixed with polygonal cells in species of Euphorbiaceae. The polygonal and irregular epidermal cells usually have straight to undulating anticlinal walls but in some few cases they have wavy to straight anticlinal walls.

The stomatal index from the studied species varied from species to species. The stomatal index for adaxial surfaces varies from 1.3 for *M. esculentus* which was the lowest to 25.00 for *E. hirta* which was the highest. For the abaxial surfaces, *E. hirta* had 78.43 with the highest stomatal index while *J. gossipifolia* had 25.49 with the lowest stomatal index. The results showed that the stomatal distribution of the epidermal cells of the studied plant species of family Euphorbiaceae were mainly Amphistomatic. This is in agreement with the report of [22], while only *A. wilkesian* was hypostomatic in its stomatal distribution as the stomata was only present in the abaxial surfaces of the epidermal cell.

The stomatal type patterns in the studied species represented a useful diagnostic characteristic. Variations in types, distribution, and arrangement of stomata are characters which are taxonomically important at the generic level of classification [5]. Stomata are microscopic openings on plant leaves surfaces that allow for the easy passage of water vapour, carbon dioxide, and oxygen. Different stomatal types were observed and recorded for the species of family Euphorbiaceae studied. These are: Anisocytic with three cells which may be of unequal size enclosing the guard cells, Anomocytic: having epidermal cells around the guard cells which cannot be differentiated from the other epidermal cells, Diacytic having stomata that are enclosed by one or more pairs of subsidiary cells with walls that are at right angle to the guard cells. Other stomatal types observed are tetracytic, paracytic, and actinocytic stomatal type. In some cases, more than one stomatal type may be found present on the same surface of the same species, e.g Anisocytic, tetracytic, anomocytic and paracytic found on the abaxial surface of *E. hirta.* It was also observed that the stomatal types found on the adaxial surface of one species may differ from those on the abaxial surface of the same species, e.g Anomocytic for adaxial surface and Paracytic for abaxial surface for *M. esculenta*. This observation is also in conformity with the report of [22].

Trichomes were also observed only on both surface of *A. wilkesiana* and *E. hirta* but absent on all other studied species. The presence, absence and density of trichomes in plant species are affected by different ecological factors. Also, trichomes types in plants are very useful for the delimitation and identification of such plants even in the present study [23].

Scientifically, interest in leaf trichomes is based on their functions such as lowering of the temperature of leaves, increase in its tolerance to freezing, phytochemical storage, protection of the leaves inner tissues from UV radiations from sun and against insect herbivores/airborne fungi, retention of water [24], and a very important feature in delimitation of species in taxonomic studies [5]. The multicellular uniseriate non-glandular trichomes on both the adaxial and abaxial surfaces of *A. wilkesiana* and *E. hirta*, and only on the abaxial surface of *J. gossipifolia* may serve the function of mechanical protection against insect herbivores that use the leaves as a nest. This study supports previous study by [25] who stated that there is a positive correlation of herbivory with increased trichome density. Trichomes are also known to function as temperature buffer in reducing the rate of water lost during transpiration. The multicellular uniseriate non-glandular trichomes observed in this study could serve as a source of taxonomic information in identifying this species from the rest of the species/genera.

Papillae, a small rounded protuberance was found to be present on both surface of only *E. heterophylla* but was found to be absent on all other studied species. This character (papillae) is peculiar to *Euphorbia heterophylla* among the species studied. This is in agreement with the report of [26].

5. Conclusion

The plant species studied are widely used in alternative medicine; therefore, any information which could be useful in identifying them is of utmost importance. The epidermal line of evidence is of great significance in plant taxonomic studies as it presents characters such as shape of epidermal cells, nature of anticlinal cell walls stomatal types, index, distribution, presence and absence of papillae, trichomes etc. This study has presented detailed information of the

micromorphological structures of the species studied. These characters could be useful for identification, delineation and authentication of this species. It could also serve as a database for future references of the taxa and are of great relevance in the field of systematics and taxonomy.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors (Onisodumeya Elemchukwu James, Blessing Okpakiri Green, Mercy Gospel Ajuru and Victoria Wilson) declare that there are no competing interests on this work.

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