



COMPARATIVE EPIDERMAL MORPHOLOGY AND PETIOLE ANATOMICAL STUDY OF *ACALYPHA HISPIDA* BURM. F AND *ACALYPHA WILKESSIANA* MUELL. ARG VARIETIES (EUPHORBIACEAE).

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ABSTRACT

Epidermal morphology and petiole anatomy of some members of the genus *Acalypha*: *Acalypha hispida* Burm. *Acalypha wilkesiana* Mull. Arg and *Acalypha wilkesiana* var. Java White in Nigeria were investigated to establish and document taxonomic data that could help in the proper identification, delineation and classification of the taxa. Leaf epidermal peels and transverse sections of the petiole of each species were made (with the aid of a Riechert sliding microtome). Staining and counter staining were done using Safranin O and Alcian blue. The epidermal surfaces and cross sections of petioles were observed with the aid of light microscope. Quantitative and qualitative characters observed were documented. Results revealed close similarity among the taxa in the shape of epidermal cells, stomata types and trichome morphology. Wavy anticlinal wall is classificatory of *A. hispida* and *A. wilkesiana* var. Java white while epidermal cells with undulating anticlinal wall are diagnostic of *A. wilkesiana*. Parallelocytic stomata are classificatory of *A. wilkesiana* and *A. hispida* while staurocytic stomata is common to *A. wilkesiana* and its variety. Absence of medullary bundles separates *A. wilkesiana* var. Java White from other taxa. The anatomical characters observed can be used in the identification, delimitation and classification of these species.

Keywords: *Acalypha*, delimitation, foliar anatomy, taxonomy, stomata

INTRODUCTION

The genus *Acalypha* belongs to the family Euphorbiaceae and sub-family Acalyphoideae. The genus includes ca. 500 species mostly in tropical and subtropical regions of the world and 13 species reported in the flora of West Tropical Africa (Hutchinson and Dalziel, 1958).

Most are herbs but some especially in the tropics are also shrubs and trees while some are succulent and resemble cacti. The diversity of the genus can be summarized from Webster's (1994) classification which reported 20 tribes, 116 genera, and 2000 species found

throughout the world but are especially abundant in the tropics.

The species of *Acalypha* are very important in the traditional medicinal practices of most tribes in Africa and Asia as observed by Sofowora (1982) and Duraipandiyani *et al.* (2006). In Nigeria for instance, *Acalypha wilkesiana* (otherwise called fire dragon and copper leaf) is important in the treatment of headache and cold. Also, cold extracts of the leaves is used to bath babies with skin infection (Adesina *et al.*, 2000). The leaf extract is reported to be active against Gram positive bacteria while the extracts of seed have Immunomodulating properties that work against some tumors (Bussing *et al.* 1999).

Similarly, Ogundaini (2005) reported *A. wilkesiana* to have antimicrobial properties. Onocha *et al.* (2011) reported that different parts of *A. hispida* have been used in the treatment of debilitating diseases like leprosy, Gonorrhoea, Asthma, pulmonary problems and kidney ailments. However in spite of the numerous medicinal importances of members of the family Euphorbiaceae, the diversity within the family (and by implication the genus *Acalypha*) has been a major problem to the taxonomy of the taxa (Craig, 1978). The description and taxonomy of the genus *Acalypha* have been attempted at different times by various authors (Qin *et al.*, 2004 and Kadiri *et al.* 2009). Some of these studies were largely focused on the epidermal morphology. In this study, both epidermal morphology and petiole anatomy are employed and this is with a view to deepening the depth of information on available foliar characters that may be useful in the identification of the species studied. Therefore, the objective of this study is to describe the foliar epidermal morphology and petiole anatomical structure of two species of *Acalypha* and a variety with a view to providing useful data that would give further insight into proper identification and classification of the studied taxa.

MATERIALS AND METHODS

The epidermal peels of both the adaxial and abaxial surfaces of the leaves of the each plant were made. The leaf materials were placed on a clean glass slide and soaked with concentrated trioxonitrate (v) acid (HNO₃). The glass slides were covered and allowed to stand for between 3 and 5 hours. The epidermal surfaces were separated from the macerated leaf materials and transferred into Petri dishes containing water for cleaning the surfaces. Staining was done with safranin O for about five minutes before mounting in glycerine.

$$\text{Stomata index (S.I)} = \frac{S}{S + E} \times 100$$

Where,

S= Number of stomata per unit area.

E= Number of ordinary epidermal cells in the same area.

For the study of the petiole anatomy of the species, transverse sections of the petiole of each of the species were obtained from the proximal, median and distal regions using a microtome. The sections were stained with 10 % aqueous solution of Safranin O and toluidine blue, the stained sections were then thoroughly rinsed in 2-3 changes of water to remove excess stains; they were then mounted on a clean slide with 25 % glycerol solution. The specimens were observed using the light microscopy under different power of the objective lenses.

Photomicrographs of each slide were made using Olympus microscope with attached Amscope digital camera. Tissue and cell identification and description followed Fahn (1997). All microscope measurements were made with ocular and stage micrometers.

RESULTS

Detailed epidermal morphology and petiole anatomy of the taxa studied is presented below. Summary of qualitative and quantitative epidermal and petiole anatomical characters are presented in Tables 1 and 2.

Acalypha hispida (Plate 1A-M)

Epidermal cells are irregular to polygonal, anticlinal walls are wavy on the adaxial lamina surface (Plate 1A-E); size ranges from 4.00 - 6.75 μm long and 1.25-4.00 μm broad, costal cells are rectangular, size variable; 7.25-13.00 μm long and 1.78-4.00 μm broad. Stomata anisocytic, Laterocytic, and Parallelocytic (Plate 1B), guard cells are mostly elliptic, mean stomata index 2.53 %. Indumentum-multicellular, uniseriate, hooked, non-glandular trichomes present, the mean length and breadth up to 53.88 μm and 11.37 μm respectively; druses were present. However, on the abaxial surface (Plate 1F-I), epidermal cells are irregular with highly undulating anticlinal walls, costal cells are variable in size, 4.00 - 8.12 μm long and 1.52 - 2.55 μm broad. Anomocytic stomata were present in addition to the types present on the adaxial lamina surface. Mean stomata index on the abaxial surface is 48.07 %. Indumentum the same as for the adaxial lamina surface, mean length and width are 34.00 μm and

7.92 μm respectively. Prismatic crystals and druses are found

Petiole Anatomy (Plate 1J-M)

Petiole outline is oval in proximal section (Plate 1J) with thin cuticle but reniform in median and distal section (Plate 1K-M) but grooved on the adaxial in all regions. Epidermis is uniseriate having multicellular, non-glandular trichomes. Collenchyma angular, 4-6 layers in proximal, 4-8 and 6-9 in median and distal section respectively. Parenchyma polyhedral, 6-9 layers in proximal, 5-7 in median and 4-7 in distal. Vascular bundle collateral, forming an arc shape of about 8-9 bundles in proximal, 8 in median and 7 in distal. Medullary bundle present between two vascular bundle in distal region (Plate 1L), pith consists of thin walled polyhedral parenchyma. Druses are randomly distributed in the cortex and pith.

Acalypha wilkesiana var. *Java White* (Plate 2A-G)

The adaxial epidermal cells are irregular with wavy anticlinal walls; size variable, ranging from 3.50-5.25 μm long and 1.50-2.75 μm broad (Plate 2A-B). Costal cells are rectangular, size varies from 3.50-6.00 μm long to 0.75-1.50 μm broad. Stomata absent. Trichomes: multicellular, uniseriate, non-glandular (Plate 2A); mean length and width up to 24.25 μm and 11.29 μm . Druses and prismatic crystals are present. On the abaxial surface (Plate 2C-D), epidermal cells are irregular to polygonal with highly undulating anticlinal walls, cell size ranges from 4.25-7.75 μm long and 1.50-3.00 μm broad. Costal cells are rectangular, 4.75-10.00 μm long and 1.50-3.50 μm broad, Stomata present, largely laterocytic, paracytic, anisocytic and staurocytic (Plate 2D), guard cells are oval, mean stomata index is 35.74%. Multicellular, hooked, non-glandular trichomes present (Plate 2D). Mean length and width of the trichomes about 40.85 μm and 12.50 μm respectively. Druses are present.

Petiole Anatomy (Plate 2 E-G)

Petiole outline generally circular to oval in all regions, with shallow groove on distal section (Plate 2G). Epidermis is uniseriate, having numerous multicellular, uniseriate, non glandular trichomes. Angular

collenchyma is present in all regions, 7-10 layers in proximal, 4-6 and 5-9 layers in median and distal. Parenchyma thin walled and polyhedral, 5-8 layers in proximal, 6-9 in median and 5-7 layers in distal. Vascular bundles are collateral, arranged in ring in all regions, consisting 9 bundles in proximal, 8 in median and about 7-8 bundles in distal. Pith relatively wide and parenchymatous. Druses are present in all regions.

Acalypha wilkesiana Muell Arg (Plates 3A-K)

Epidermal cells on the adaxial surface (Plate 3A-E) are irregular to polygonal with anticlinal walls slightly undulating; cell size vary, ranging from 2.75-4.25 μm long and 1.25-2.25 μm wide. Costal cells present, predominantly rectangular; 6.25-10.75 μm long and 2.50-4.00 μm broad. Stomata types observed include anisocytic (Plate 3E), Staurocytic (Plate 3D) and Parallelocytic (Plates 3B and E). Guard cells are mostly elliptical and mean stomata index up to 20.30%. Trichomes present, uniseriate, non-glandular, mean length and width about 56.75 μm and 10.55 μm respectively. Crystal sand and druses are present. However, on the abaxial surface, epidermal cells are similar to those on the adaxial surface but with highly undulating anticlinal walls. Cells vary in sizes, ranging from 3.25-6.00 μm long and 0.75-2.75 μm broad. Costal cells are rectangular; 8.00-11.75 μm long and 1.75-3.50 μm broad. Stomata laterocytic and paracytic (Plate 3F). Trichomes absent. Druses are present.

Petiole Anatomy (Plates 3I-K)

Petiole outline is oval in proximal and median with thin cuticle though oval with thick cuticle and deep groove in distal region; epidermis is uniseriate consisting non-glandular, uniseriate, multicellular trichomes. Collenchyma angular, 4-6 layers in proximal, 4-7 in median and 5-9 layers in distal. Parenchyma polyhedral, 5-8 layers in proximal, 6-8 in median and 4-6 layers in distal, Vascular bundle collateral, 8 in proximal and median region, 8-9 bundles in distal, forming an arc. Pith consists of polyhedral parenchyma; medullary bundles present in distal region (Plates 3J and K). Druses present in cortex and pith.

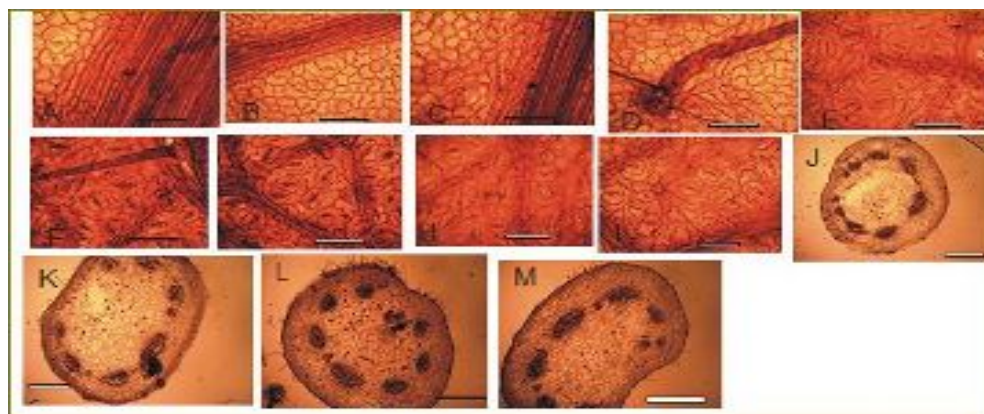


Plate 1(A-M): Epidermal and petiole anatomical features of *A. hispida*

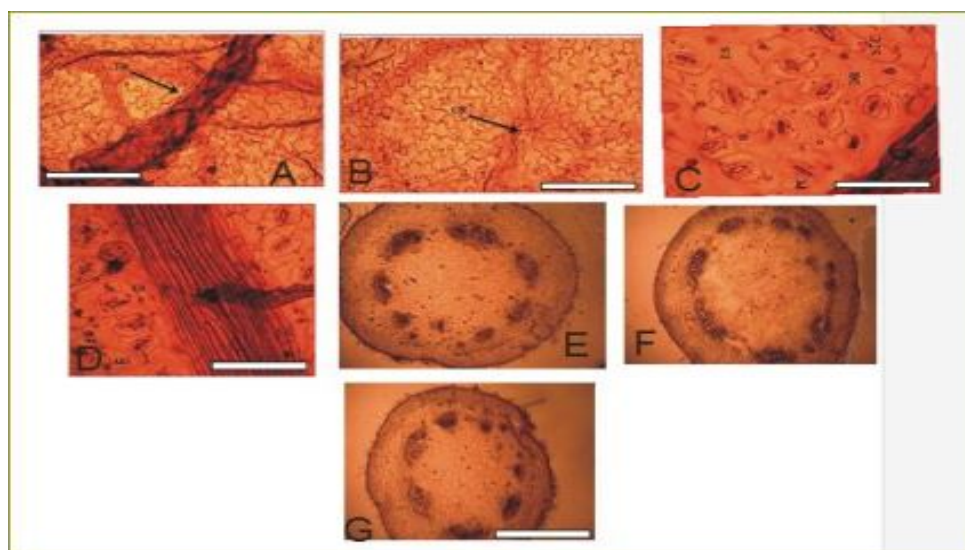


Plate 2 (A-G): Epidermal and petiole anatomy of *A. wilkessiana* var. java White

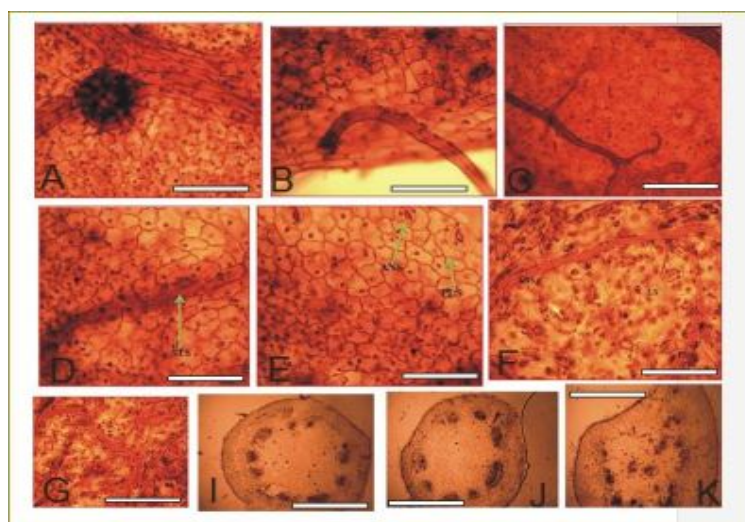


Plate 3 (A-K): epidermal morphology and petiole anatomy of *A. wilkessiana* Muell Arg.

Table 1: Qualitative and Quantitative foliar epidermal characters of *Acalypha* species

Species	<i>Acalypha hispida</i>		<i>Acalypha wilkesiana</i> var. Java White		<i>Acalypha wilkesiana</i>	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Surfaces	Surface	Surface	Surface	Surface	Surface	Surface
Anticlinal walls	Wavy	Highly undulating	Wavy	Highly undulating	Slightly undulating	Highly undulating
Epidermal cell size(μm)	4.00-6.75	4.00-8.12	3.50-5.25	4.25-7.75	2.75-4.25	3.25-6.00
Mean Stomata Index (%)	2.53	48.07	Absent	35.74	20.30	18.38
Trichome types	mung	mung	mung	mung	mung	Absent
Costal cells	Rectangular and oblong		Rectangular		Rectangular	
Crystals	Present		Present		Present	
Druses	Present		Present		Present	
Epidermal cells	Irregular and polygonal		Irregular and polygonal		Irregular and polygonal	
Guard cells	Elliptical		Oval		Oval	
Stomata types	Anomocytic, Anisocytic Laterocytic, Paracytic, and Parallelocytic		Anisocytic, Laterocytic Paracytic and Staurocytic only on Abaxial Surface		Anisocytic, Laterocytic, Paracytic, Parallelocytic, Staurocytic	

Legend: MUNG- Multicellular Uniseriate Non glandular

Table 2: Qualitative anatomical characters of petiole of *Acalypha* species

Species	<i>Acalypha wilkesiana</i> var.								
	<i>Acalypha hispida</i>			Java White			<i>Acalypha wilkesiana</i>		
Character	Pr	Md	Ds	Pr	Md	Ds	Pr	Md	Ds
Parenchyma shape		Polyhedral			Polyhedral			Polyhedral	
Petiole Outline		Oval and reniform			Oval			Oval	
Tannins		Absent			Absent			Absent	
Trichomes type		Multicellular, uniseriate			Multicellular, uniseriate			Multicellular, uniseriate	
Vascular bundle		Collateral			Collateral			Collateral	
Collenchyma		Angular			Angular			Angular	
Druses		Present			Present			Present	
Groove		Present			Present			Present	
Medullary bundle		Present			Absent			Present	

Legend: Pr- Proximal, Md- Median Ds- Distal

DISCUSSION

Epidermal morphology

The result of this study as presented in Tables 1 and 2 and illustrated in Figures 1-3, outlined the foliar epidermal and petiole anatomical characters which are important in differentiating the species studied. The level of affinity among the species of *Acalypha* studied was revealed through data derived from qualitative and quantitative foliar epidermal and petiole anatomical characters of the taxa. General similarity was observed between *A. hispida* and *A. willkessiana* var. Java

White in the nature of anticlinal walls. On the adaxial lamina surface of *A. hispida* and *A. willkessiana* var. Java White, it is wavy but slightly undulating in *A. willkessiana*. This is in line with the report of Kadiri *et al.* (2009). They reported curved and undulate anticlinal walls for *A. hispida*. However, on the abaxial surface, anticlinal wall is highly undulating in all taxa. The highly undulating anticlinal wall on the abaxial surface of *A. hispida* contradicts the observation of Essiet and Etukudo (2012) that documented simple undulating anticlinal walls. The

considerable overlap among the taxa in the size of epidermal cells limits its value in separating the taxa. The mean length of the epidermal cell is highest on the abaxial surface of *A. hispida* while the lowest is observed on the adaxial surface of *A. wilkessiana* (Table 1). This corroborates the observation of Essiet and Etukudo (2012) and could be used in demarcating the two species. In all species, epidermal cells were largely irregular to polygonal with oval guard cells except in *A. hispida* with elliptic guard cells

Considerable variations exist in stomata type and distribution in the taxa studied. Generally, anisocytic, laterocytic, paracytic stomata were common in all species. These appear to be generic features and is in line with the report of Metcalfe and Chalk (1950). Staurocytic stomata are peculiar to *A. wilkessiana* and its variety, *A. wilkessiana* var. Java White. However, *A. wilkessiana* and its variety, *A. wilkessiana* var. Java White can further be separated based on the presence of the Staurocytic stomata on the abaxial surface in the variety but on adaxial in *A. wilkessiana*. Parallelocytic stomata is common to *A. hispida* and *A. wilkessiana*. Morphological affinities between *A. hispida* and *A. wilkessiana* have been previously reported (Soladoye 2008). Anomocytic stomata is diagnostic of *A. hispida*. The variations in stomatal index observed in this study can be reasonably employed in demarcating the species. On the adaxial surface, the stomatal index is highest in *A. wilkessiana* while on the abaxial surface, it is highest in *A. hispida* and lowest in *A. wilkessiana* (Table 1).

Trichomes in the three species are generally multicellular, uniseriate and non-glandular except in *A. wilkessiana* lacking trichomes on the abaxial surface (Table 1). This differs from the result of Essiet and Etukudo (2012) that indicated the presence of trichome on the abaxial surface of *A. wilkessiana*. The pubescence observed in the abaxial surface of *A. wilkessiana* makes it a unique diagnostic character which could be used to separate the taxon from the rest. However, the quantitative variation in trichome length and width is in line with the report of

Essiet and Etukudo (2012) that reported the highest rate for trichome length and width in *A. wilkessiana* var. and the lowest in *A. hispida*.

Petiole Anatomy

Srinaul and Thammathaaworn (2008) observed that petiole anatomy offers considerable anatomical variations that could be utilized in separating taxa. Variations have been observed in the petiole anatomy of several species of the family Euphorbiaceae with respect to the vasculature, number of hypodermal layers, resolution of vasculature in different number of arcs and vascular bundles, association of sclerenchyma with the vascular tissue, occurrence of sacs, occurrence of crystals and tannins (Thakur and Patil, 2011). These features can be easily employed in taxonomic distinction at lower ranks.

In this study, crescentiform outline in the proximal region is diagnostic in *A. hispida* (Plates 1L and M). Deep groove and thick cuticle in *A. wilkessiana* separates it from the other two taxa having shallow grooves and thin cuticle. However, medullary bundle in *A. wilkessiana* var. separates it from the rest of the taxa

Generally, characters like uniseriate epidermis, collateral vascular bundle, angular collenchyma in all sections and in all species are generic features.

CONCLUSION

Though similarities abound in both the epidermal morphology and petiole anatomy of the taxa studied, variations that are diagnostic and which can be utilized in separating the species exist in the foliar anatomy

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