

## MORPHOLOGICAL AND LEAF EPIDERMAL FEATURES OF SOME *PHYLLANTHUS* SPECIES IN JOS, NIGERIA

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### ABSTRACT

A research was carried out on five species of *Phyllanthus* L. in Jos North L.G.A., Plateau State, to study the leaf morphological and anatomical characters that can serve as taxonomical markers. The species examined were *P. discoideus* (Baill.) Mull-Arg, *P. muellerianus* (O. Ktze) Excel, *P. niruri* L., *P. nivosus* W. Bull, *P. reticulatus* Poir. The macro morphological characters studied were leaf type, leaf arrangement, leaf apex, leaf base, leaf margin, leaf length, leaf width, petiole length and leaf area, while the anatomical characters studied were the stomata, trichomes, epidermal cell shape and pattern. The morphology of the leaf base serves as an important feature for delimitation of the species, thus separating the species into two groups. *Phyllanthus discoideus* and *Phyllanthus reticulatus* with cuneate leaf bases can be distinguished from *Phyllanthus muellerianus*, *Phyllanthus niruri* and *Phyllanthus nivosus* with rounded leaf bases. All the studied species are hypostomatic and the stomata type varied from paracytic, anisocytic to anomocytic. No trichome was observed on all the species studied. The *Phyllanthus* species studied also displayed different epidermal cell shapes, anticlinal wall patterns and other characters of taxonomic significance that would aid their identification and delimitation.

**KEY WORDS:** characters, epidermis, *Phyllanthus*, stomata, trichomes.

### INTRODUCTION

The genus *Phyllanthus* is the largest genus in the family Phyllanthaceae with 750-1200 species which are widely distributed throughout the tropical and subtropical regions of the world (Kathriarachi *et al.*, 2005). Members of this family are mostly trees, shrubs and a few herbs (Hoffmann, 2007). The larger genera are *Phyllanthus* (1270 spp), *Cleistanthus* (140 spp), *Antidesma* (100 spp), *Aporosa* (90 spp), *Uapaca* (60 spp), *Baccaurea* (50 spp) and *Bridelia* (50 spp) (Kanchana *et al.*, 2008).

*Phyllanthus* has a remarkable diversity of growth or life forms. The plants are nearly all annual and perennial herbs, shrubs and trees. A few are climbers, pachycaulous succulents and one species, *Phyllanthus fluitans* is floating aquatic. The genus also has a wide variety of floral morphologies and has one of the widest ranges of pollen types (Hoffmann *et al.*, 2006). Despite their diversity, almost all *Phyllanthus* species express a specific type of growth called phyllanthoid branching in which the vertical stems bear deciduous, floriferous (flower-bearing), plagiotropic (horizontal or oblique) stems. The leaves on the main vertical axes are reduced to scales called

cataphylls, while leaves on the other axes develop normally. Some species have flattened leaf-like stems called cladodes (Webster, 1994). *Phyllanthus* are monoecious or dioecious. Leaves are simple, alternate, spiral or distichous; leaf margin is almost always entire and petiole is nearly always present often with pulvinus at its base. Flowers are unisexual and actinomorphic in form, while the fruit is a schizocarp, drupe or berry. Seeds are trigonous, smooth and usually not elongate while latex, thorns and other armament are very rare (Webster, 1994).

The genus is generally well known for its biological active compounds such as flavanoids, glycosides, terpenoids, vitamins, minerals and antibodies which have definite physiological action on the human body (Uka *et al.*, 2014). Experimental preparation of the leaves, bark or fruit have shown potential efficacy against laboratory model of diseases such as diabetes, cancer, anaemia, jaundice, inflammation and age-related renal diseases (Puri, 2002). Apart from medicinal values, the plant serve as food, fodder and ornaments (Nahar *et al.*, 2011). Despite the medicinal and non-medicinal value of this plant, a number of *Phyllanthus* species are recognized as weed of minor importance in both arable and non-arable lands (Edeoga & Eriata, 2001).

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 (Stace, 1989; Ianovici *et al.*, 2011). The contributions of leaf surface structure to improve the identification of indigenous flora of Nigeria are evident from a number of works including Jayeola *et al.* (2001), Ogundipe & Pereira-Sheteolu (2006), Ayodele & Olowokudejo (2006) and Aworinde *et al.* (2009). 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 (Stace, 1989; Ianovici *et al.*, 2009).

*Phyllanthus* L. is regarded as a taxonomically challenging group (Kathriarachchi *et al.*, 2006), hence the present study described the leaf epidermal morphology in some *Phyllanthus* species with the aim of providing useful taxonomic data with a view to enhancing our knowledge of their classification and identification.

## MATERIALS AND METHODS

Plant materials used for this investigation were collected in Jos North LGA of Plateau State. The species were identified and authenticated in the herbarium of University of Jos, Plateau State. The species studied were *Phyllanthus nivosus*, *P. reticulates*, *P. niruri*, *P. muellerianus* and *P. discoideus*.

Matured leaves were measured for each species at comparative position. Macro characters measured include leaf length and width (taken at a widest part of the leaf) and petiole. Qualitative characters such as leaf shape, base, arrangement, margin, surface and apex were also noted. Micro characters such as size of the epidermal cell

(at the widest point), size of the guard cell (at the widest point), length and width of stomata and stomata index were also measured.

Epidermal preparation was by obtaining sizeable portion (5mm<sup>2</sup>-1cm<sup>2</sup>) of the mature leaves from the transverse section of each leaf soaked in concentration solution of trioxonitrate (v) acid (HNO<sub>3</sub>) for a period of about 18-24 hours. The appearance of air bubbles on the surface of the leaf fragments indicated their suitability for separation. They were then transferred into water in a Petri dish from where the upper and lower epidermises were carefully peeled. Selected specimens were stained in Safranin. Stained specimens cleared in ethanol to remove excess stain were mounted on slides with glycerin.

For statistical analysis, six (6) epidermal cells and six (6) stomata were chosen at random from each species. Length and width of stomata were measured at X400 magnification using ocular micro-meter. The measurements were later converted to microns using a pre-calibrated stage micrometer.

The stomata index (SI) was obtained by expressing the number of stomata per unit area as a percentage of the total number of cells in the same unit area using Metcalfe and chalk (1979).

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

where, SI represents the stomata index, S, number of stomata per unit area and E, number of ordinary epidermal cells in the same unit area.

## RESULTS AND DISCUSSIONS

### Macromorphological Characters

The leaves of all the species studied are simple with alternate leaf arrangement and entire margin. The leaf shape is elliptical in *P. muellerianus* *P. niruri* *P. reticulatus*, ovate in *P. nivosus* and oblanceolate in *P. discoideus*. The leaf surfaces were glabrous while the leaf apices varied from acute, obtuse and rounded. *P. discoideus* and *P. reticulatus* have cuneate leaf bases while *P. muellerianus*, *P. niruri* and *P. nivosus* possess rounded leaf bases (Table 1). There was variation in the sizes of the leaves examined with *P. niruri* having the smallest size while the largest size was found in *P. discoideus*. *P. discoideus* had the longest petiole length with mean number of 0.79cm while *P. niruri* had the shortest with mean number of 0.09cm (Table 2).

### Micromorphological Characters

The stomata distribution showed hypostomatic leaves (stomata occurred only on the abaxial surface) in all the species studied. The stomata types recorded were paracytic, tetracytic, anisocytic and anomocytic. The stomata number varied from three in *P. muellerianus* to eleven in *P. reticulatus* while the stomata index varied from 7.41 in *P. discoideus* to 14.62 in *P. muellerianus*. The mean length of stomata varied between 6.80μm in *P. reticulatus* to 11.60μm in *P. discoideus* while the mean width of

stomata varied from 7.48µm in *P. reticulatus* to 15.35µm in *P. muellerianus* (Table 3). The shapes of epidermal cells on both the adaxial and abaxial surfaces are polygonal and mostly irregular in all the taxa. The anticlinal walls are curved, slightly straight and straight in all the taxa except *P. niruri* with wavy anticlinal patterns on both adaxial and abaxial surfaces. The irregular cells usually have curved to slightly straight anticlinal walls and rarely wavy, while the polygonal cells usually have straight anticlinal walls. The number of cells on the adaxial surface is more than those on the abaxial surface of the studied species except in *P. reticulatus* with higher number of cells on the abaxial surface. *P. muellerianus* and *P. reticulatus* had the highest number of cells on the adaxial and abaxial surfaces respectively while *P. reticulatus* and *P. nivosus* had lowest number of cells on the adaxial and abaxial surfaces. The smallest epidermal cell size on the adaxial surface ranging from 20.40- 40.80 µm long and 17.00- 44.20 µm wide was observed in *P. muellerianus* while on the abaxial surface, it ranges from 13.60-37.40 µm long and 20.40- 34.00 µm wide in *P. nivosus*. Further, the largest epidermal cell size on the adaxial surface ranging from 27.70- 54.40 µm long and 30.60- 54.40 µm wide was observed in *P. niruri* while on the abaxial surface, it ranges from 23.80 µm long and 27.20- 54.40 µm wide in *P. niruri*.

**TABLE 1. Qualitative leaf macro morphological characters of the *Phyllanthus* species**

Species	Leaf type	Leaf arrangement	Leaf shape	Leaf apex	Leaf margin	Leaf base	Glabrous/pubescent
<i>P. discoideus</i>	Simple	Alternate	Oblanceolate	Acute	Entire	Cuneate	Glabrous
<i>P. muellerianus</i>	Simple	Alternate	Elliptical	Acute	Entire	Rounded	Glabrous
<i>P. niruri</i>	Simple	Alternate	Elliptical	Obtuse	Entire	Rounded	Glabrous
<i>P. nivosus</i>	Simple	Alternate	Ovate	Rounded	Entire	Rounded	Glabrous
<i>P. reticulatus</i>	Simple	Alternate	Elliptical	Acute	Entire	Cuneate	Glabrous

**TABLE 2. Quantitative leaf macro morphological characters of the *Phyllanthus* species**

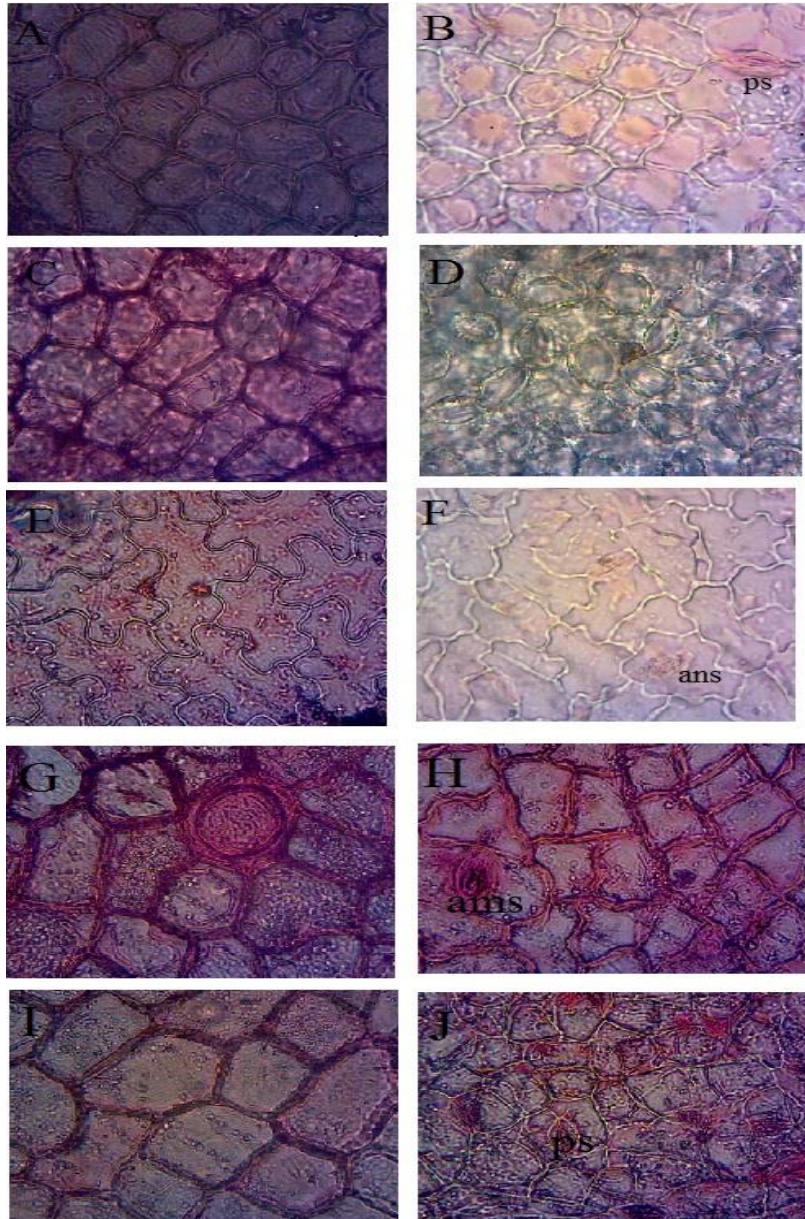
Species	Leaf Length (cm)		Leaf Width (cm)		Petiole Length (cm)		Leaf area (cm <sup>2</sup> )	
	min	(mean ± SE) max	min	min (mean ± SE) max	min	(mean ± SE) max	min	(mean ± SE) max
<i>P. discoideus</i>	6.30	(7.72 ± 0.40) 9.00	2.60	(3.18 ± 0.14) 3.60	0.70	(0.79 ± 0.40) 0.90	16.38	(24.89 ± 1.02) 32.40
<i>P. muellerianus</i>	4.60	(5.74 ± 0.55) 8.30	2.60	(3.18 ± 0.19) 3.90	0.30	(0.34 ± 0.02) 0.40	12.48	(18.81 ± 1.32) 32.37
<i>P. niruri</i>	1.00	(1.33 ± 0.09) 1.60	0.45	(0.59 ± 0.05) 0.80	0.09	(0.09 ± 0.003) 0.10	0.45	(0.81 ± 0.05) 1.28
<i>P. nivosus</i>	3.40	(5.08 ± 0.58) 6.90	2.10	(3.40 ± 0.59) 4.50	0.20	(0.36 ± 0.04) 0.50	7.14	(18.59 ± 1.77) 31.05
<i>P. reticulatus</i>	3.40	(4.06 ± 0.22) 4.80	1.80	(2.30 ± 0.16) 2.90	0.50	(0.52 ± 0.02) 0.60	6.30	(9.54 ± 0.52) 13.90

**TABLE 3. Qualitative foliar micro morphological characters of the studied species**

Species	Epidermal cell shape		Anticlinal wall pattern		Stomata type		Trichome type	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
<i>Phyllanthus discoideus</i>	Polygonal	Irregular	Straight	Slightly curved	-	Paracytic	-	-
<i>Phyllanthus muellerianus</i>	Polygonal	Circular	Straight	Curved	-	Tetracytic	-	-
<i>Phyllanthus niruri</i>	Irregular	Irregular	Wavy	Wavy	-	Anisocytic	-	-
<i>Phyllanthus nivosus</i>	Polygonal	Polygonal	Straight	straight/slightly curved	-	Anomocytic	-	-
<i>Phyllanthus reticulatus</i>	Polygonal	Irregular/undulate	Straight	Curved	-	Paracytic	-	-

**TABLE 4. Quantitative anatomical characters of the studied *Phyllanthus* species, min (mean ± SE) max**

Species		Epidermal measurement		Stomata measurement		Guard cell measurement. Width (at widest point (µm))	Stomata index (%)
		Length (µm)	Width (µm)	Length (µm)	Width (µm)		
<i>Phyllanthus discoideus</i>	Adaxial	20.40 (27.77 ± 0.91)34.00	27.20 (40.23 ± 1.71)54.40	-	-	-	-
	Abaxial	20.40 (33.43 ± 1.64)47.60	23.80 (28.33 ± 0.93)37.40	11.90 (11.60 ± 0.31)13.60	10.20 (14.73 ± 0.64)20.40	3.40 (4.11 ± 0.22)6.80	7.41
<i>Phyllanthus muellerianus</i>	Adaxial	20.40 (31.17 ± 1.44)40.80	17.00 (33.43 ± 1.77)44.20	-	-	-	-
	Abaxial	17.00 (30.60 ± 1.32)47.60	17.00 (26.07 ± 1.39)34.00	8.50 (10.48 ± 0.31)13.60	11.90 (15.35 ± 0.55)20.40	3.97 (5.12 ± 0.18)6.80	14.62
<i>Phyllanthus niruri</i>	Adaxial	27.20 (37.40 ± 1.76)54.40	30.60 (43.63 ± 1.73)54.40	-	-	-	-
	Abaxial	23.80 (35.70 ± 1.51)47.60	27.20 (41.37 ± 1.86)54.40	10.20 (11.60 ± 0.4)15.30	10.20 (12.47 ± 0.51)17.00	3.40 (4.16 ± 0.17)3.67	12.00
<i>Phyllanthus nivosus</i>	Adaxial	23.80 (33.90 ± 1.63)47.60	30.60 (36.27 ± 0.85)44.00	-	-	-	-
	Abaxial	13.60 (24.93 ± 1.60)37.40	20.40 (27.80 ± 0.91)34.00	10.20 (12.75 ± 0.47)7.00	10.20 (14.73 ± 0.75)20.40	3.40 (4.91 ± 0.25)6.50	9.30
<i>Phyllanthus reticulatus</i>	Adaxial	23.80 (32.30 ± 1.16)34.80	27.20 (35.70 ± 0.65)44.20	-	-	-	-
	Abaxial	20.40 (26.63 ± 1.20)37.40	20.40 (28.90 ± 1.16) 37.40	6.80 (9.24 ± 10.25)10.20	6.80 (7.48 ± 0.31) 8.50	2.27 (2.49 ± 0.04)2.33	9.42



**FIG.1.:** Photomicrographs of the studied species. A-B: Adaxial and abaxial surfaces of *P. discoideus*, C-D: Adaxial and abaxial surfaces of *P. muellerianus*, E-F: Adaxial and abaxial surfaces of *P. niruri*, G-H: Adaxial and abaxial surfaces of *P. nivosus*, I-J: Adaxial and abaxial surfaces of *P. reticulatus*. Ps-paracytic stoma, ans- anisocytic stoma, ams-anomocytic stoma. Mg X400

This study showed a number of important macro and micro morphological characters on the leaf surfaces of five *Phyllanthus species*. Generally, there is an agreement in the results obtained from this present study and previous studies although some few characters that were previously recorded were not observed in this study.

The *Phyllanthus* species studied possess glabrous simple and alternate leaves, entire margin, with leaf apices varying from acute, sub-acute, obtuse to rounded while the leaf bases varied from cuneate to rounded. Similarly, the leaf shapes varied from elliptical, oblanceolate to ovate. This agrees with the findings of Aziagba *et al.* (2014) and Daniel *et al.* (2014).

Foliar epidermal anatomy is one of the most noteworthy taxonomic characters from the systematic point of view and also the taxonomic studies of a number of families are made on the basis of leaf epidermis (Aworinde *et al.*, 2014). The epidermal characters such as stomata type, size and distribution, epidermal cell shape, anticlinal wall pattern as well as trichome type and distribution are useful tools used in the identification and classification of many angiosperms (Mbagwu & Edeoga, 2006).

The epidermal cell shape and anticlinal wall pattern varied within the studied species thus can serve as distinguishing factors. Based on the epidermal cell shape and anticlinal wall pattern, the studied species can be divided into four groups. *Phyllanthus discoideus* and *Phyllanthus nivosus* with polygonal and irregular cell shapes on their adaxial and abaxial surfaces respectively, and straight cell walls on their adaxial surfaces with wavy to slightly curve cell walls on their abaxial surfaces distinctly differ from other species. Also, *Phyllanthus muellerianus* had polygonal and circular cell shape on the adaxial and abaxial surface respectively with straight and curve cell wall on the adaxial and abaxial surfaces respectively thus, can easily be separated from the other species studied. Similarly, *Phyllanthus niruri* with irregular cell shapes and wavy cell walls on both the adaxial and abaxial surfaces can also be distinguished from the other species. However, *Phyllanthus reticulatus* with polygonal and irregular to undulate cell shapes on the adaxial and abaxial surfaces respectively, with straight and slightly curve anticlinal walls on the adaxial and abaxial surfaces respectively can also be separated from other species.

The occurrence of polygonal, irregular, circular and undulate epidermal cell shapes are in agreement with the works of Sayyada *et al.* (2006), Aziagba *et al.* (2014) and Daniel *et al.* (2014) who reported the presence of varying epidermal cell shapes including polygonal, irregular sinous or variously elongated epidermal cells in the *Phyllanthus* species studied.

The epidermal cell sizes varied significantly among the studied *Phyllanthus* species. However, Ogundipe *et al.* (2009) reported that there is always an overlap in the epidermal cell size and therefore cannot be used to distinguish between species but can be used to separate genera, thus justifies the inclusion of the species in the same genus.

The stomata types recorded in this study are anisocytic, anomocytic, paracytic and tetracytic. Aziagba *et al.* (2014) also reported the diversity of stomata in the *Phyllanthus* species studied. Similarly, the occurrence of only one type of stomata on the leaf surfaces of all the *Phyllanthus* species studied can serve as a useful identification character. This is in agreement with the findings of Uka (2012), Daniel *et al.* (2014) and Aziagba *et al.* (2014) who also observed the occurrence of only one type of stomata on the leaf surface of the *Phyllanthus* species they studied.

Furthermore, the epidermal examination revealed the presence of stomata only on the abaxial surface of all the *Phyllanthus* species studied. This could be due to adaptation to water loss which is in agreement with Metcalfe & Chalk (1979). The occurrence of stomata only on the lower surfaces is in agreement with Claudia *et al.* (2004), Aziagba *et al.* (2014) and Daniel *et al.* (2014) who reported hypostomatic leaves in a number of the *Phyllanthus* species they studied. On the contrary, Uka (2012) reported *Phyllanthus niruri* as amphistomatic with anisocytic stomata observed on both the adaxial and abaxial surfaces of the species. Also, Inamdar and Gangadhara (1978) reported *Phyllanthus nivosus* and *Phyllanthus reticulatus* as amphistomatic (having stomata on both the adaxial and abaxial surfaces) with higher frequency observed on the abaxial surfaces of the two species.

Metcalf & Chalk (1979) hold that the trichome frequency and size are environmentally controlled while Stace (1984) 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, no trichome was observed in all the *Phyllanthus* species studied. This is in agreement with the work of Sayyada *et al.* (2006), Uka (2012) and Aziagba *et al.* (2014). On the contrary, Aswatha *et al.* (2009) reported scarcely distributed multicellular uniseriate trichomes on the lower surface of *Phyllanthus reticulatus*.

### CONCLUSIONS

The information from this study has provided evidence that could help to resolve the taxonomic problem of the studied *Phyllanthus* species from Jos North L.G.A., Plateau State. Also, the information would help to confirm the identity of the studied *Phyllanthus* species as well as support the observation of earlier researchers that micro morphological characters and other epidermal characters could be employed for species delimitations. The leaf morphological characters used for the identification of *Phyllanthus* species were leaf type, leaf arrangement, leaf shape, leaf margin, leaf base, leaf apex and leaf size. The diagnostic characters used for the species identification and delimitation in the anatomical study were epidermal cell shape and size, stomata type, stomata size, stomata index as well as presence or absence of trichome.



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