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Leaf epidermal studies of four species of *Chlorophytum* Ker- Gawl in Nigeria

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ABSTRACT: Studies of the foliar epidermal morphology in four species of *Chlorophytum*; *C. orchidastrum, C. bichetii*, *C. stenopetalum* and *C. macrophyllum* revealed the presence of stomata on both sides of the leaves (amphistomatic stomata distribution). In *C. orchidastrum*, few stomata are present on the adaxial surfaces. Stomata type has no diagnostic importance as all the species studied have tetracytic stomata. Stomata index (<10%) on the adaxial surface in *C. orchidastrum* easily distinguished the species from others. In *C. bichetii* and *C. macrophyllum* stomata index (<50%) was recorded while stomata index (>50%) was recorded for *C. stenopetalum* on the adaxial surface. Stomata index on the abaxial surface also shows that fewer stomata occur in *C. macrophyllum* compared with *C. stenopetalum.* The studies also revealed smooth leaf margin for *C. orchidastrum.* Papillea out growth were observed on the leaf margin of *C. stenopetalum* and *C. macrophyllum,* Papillea projections were however more pronounced in *C. bichetii*. Leaf epidermal character is hereby indicated as an important tool in delimiting species in the genus *Chlorophytum*.

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Chlorophytum Ker-Gawl is a rhizomatous geophtypes in the family Asperagaceace, sub-family Agavoideae of monocotyledonous perennial flowering plant order Asparagales (Angiosperm Phylogeny Group IV, 2016). The genus (*Chlorophytum*) is well known for its economic, ornamental and medicinal values (Tarafder, 1983; Wolverton *et al.,* 1984). However, little of its medicinal importance is known in Nigeria. *Chlorophytum* species are characterized by having linear, oblong or lanceolate leaves, lamina narrowed into clasping petioles and arranged alternately in a rosette. Scapes are naked usually shorter than leaves. Bracts are linear or lanceolate, coloured and persistent. Fruits are deeply lobed, with compressed seed. According to Adsul *et al*. (2014) species in the genus have similar sized flowers and leaf pattern hence the use of these features for delineation of species is unreliable. Attention is therefore directed toward the use of other anatomical features with proven reliability.

Kothari & Shah (1975) opined that studies of stomata can have great taxonomic significance for the delimitation of different levels of taxa. Metcalfe and Chalk (1979) observed that Stomatal Index is independent of the environment, size or portion of the leaf surface and size of the intervening epidermal cell and also highly constant for any given species. Oladele (*personal communication*) also remarked that stomata index (SI) is an indication of the proportion of spread of stomata on a leaf surface. It is independent of the changes in epidermal cell size brought about by environmental factors e.g. light and water. Hence, it is a reliable taxonomic character. Baderinwa and Morakinyo (2012) explored epidermal characters in distinguishing three species of *Corchorus*. According to Carpenter and Smith (1975), variations in stomata frequencies have taxonomic importance at a generic level. Patil and Patil (1987) investigated stomatal distribution, frequency, index, and size in the leaves of 11 species and varieties of *Chlorophytum* L. and showed that these characters were significant at the subgenus level. In this study, it is the intention of the authors to document folia epidermal morphology, in four members of the genus *Chlorophytum* and their importance in delimitation of species boundary within the genus.

MATERIALS AND METHODS

Plant materials used for this study were collected from different locations in Nigeria, between November, 2014 and December, 2015 and raised under the same environmental condition in the Plant Biology

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Department of University of Ilorin. The four species of *Chlorophytum* under investigation are; *Chlorophytum orchidastrum* Lindl, is an ornamental species of *Chlorophytum* found in Nigeria (Omokanye, 2015). It is popularly referred to as "Fire flash' because of its pinkish leaf petioles. The plant has false stem surrounded by leaf sheath. Leaves are obvolanceolate or lanceolate about 33cm long and 6 cm broad. Inflorescence may be up to18cm long usually shorter than the leaves and taper towards the tip (Plate 1a).

Chlorophytum bichetii (karra) Backer. is another ornamental species of *Chlorophytum*, the species goes by the names *Chlorophytum laxum*, Bichetii grass, False Lily Turf, Siam lily, Wheat Plant, Saint Bernard Lily e.t.c. It leaves are variegated, linear and very gradually narrowed to the base. Leaves are about 23cm long and 3cm broad. Inflorescence is slender, sometimes as long as the leaves. Root tubers are found in the proximal region (Plate 1b). The species is used in Nigeria for ornamental beautification as household plants (Omokanye, 2015).

Chlorophytum stenopetalum Bak is a tuberous herb with rosette of leaves close to the ground. Leaves are linear-lanceolate from base to apex up to 40cm long, 4cm broad with wavy leave margin. Inflorescence is about one fifth as long as leaf, bearing congested fruit (Plate 1c). One to three inflorescences grow simultaneously from the centre at the base of the leaf rosette, with the prime one at the middle. Roots are long, bearing tubers at the median position. The root tubers are said to be used for curing cancer.

Chlorophtum macrophyllum (A. Rich.) Aschers**;** is a herb with rosette of leaves inclined to the horizontal ground. Leaves are lanceolate or oblanceolate about 60cm long and 8cm broad attenuated toward the base and broaden toward the point of insertion. The leaf margins are straight. It has longer peduncle about 15.3cm long bearing congested fruits in the upper 1/3. Roots are long bearing tubers at the median part.

Table 1. Botanical name, area of collection and voucher number of *Chlorophytum* species investigated.

Botanical name	Area of collection	Voucher number
Chlorophytum orchidastrum Lindl.	Unibadan, Ibadan	UIL 001/1139
Chlorophytum bichetti Karra Backer	Olokemeji FR, Abeokuta	UIL 001/1141
C. stenopetalum Bak	Shika dam, Zaria	UIL 001/1142
Chlorophtum macrophyllum (A. Rich.) Aschers	Shika dam, Zaria	UIL 001/1143

Methods: Leaf epidermal morphology was studied using matured leaves of each of the taxa. The studies include both the leaf blade and the leaf margin. Epidermal strips from the leaf for each of the two studies were obtained based on the method described by Essietti *et al*. (2012) as modified for the plant materials use. About 5 mm^2-1 cm^2 leaf portions were obtained from the standard median portion of the leaves. Epidermal peels of both abaxial and adaxial surfaces for the two studies were made by soaking the leaf portion in concentrated HNO_3 in a Petri dish for a period of about 6-12 hrs with the surface to study facing down. They were later transferred into water in a Petri dish with a pair of forceps. Care was taken to ensure that the previous leaf orientation was maintained.

To strip off thin slices of epidermis, the specimens were held downwards from one end, and then the epidermis above the desired surface was scraped-of carefully with a sharp razor blade. The loose cells were washed away from the epidermal peels with the aid of soft camel hair brush and water until the desired epidermis below was reached. The epidermal peels were lifted on to a clean glass slide, stained in 1% aqueous solution of Safranin for 4-8 minutes and mounted in 10% glycerol.

The slides were labeled appropriately and examined under the light microscope. Stomata count was done at the field of view of x40, while photographs of the micro-morphological features were also taken at a magnification of x40 using digital camera optics.

Guard cell area was calculated by multiplying their length and width by Francós constant which is 0. 7854. The stomatal index was determined according using the formula:

$$SI = \frac{S}{S + E} x100$$

Where SI = stomatal Index; S= Number of Stomata per square milimeter E= Number of ordinary Epidermal cells per square millimeter

RESULTS AND DISCUSSION

The four species examined in the present study are listed in Table 1. Voucher specimens have been preserved in the Herbarium of University of Ilorin.

LEAF EPIDERMIS: Leaf blade Epidermal Cells: The four species studied were observed to have sandwich pattern of arrangement of rows of cells in which stomata are distributed and the parallel rows of cells devoid of stomata (Plate 3).

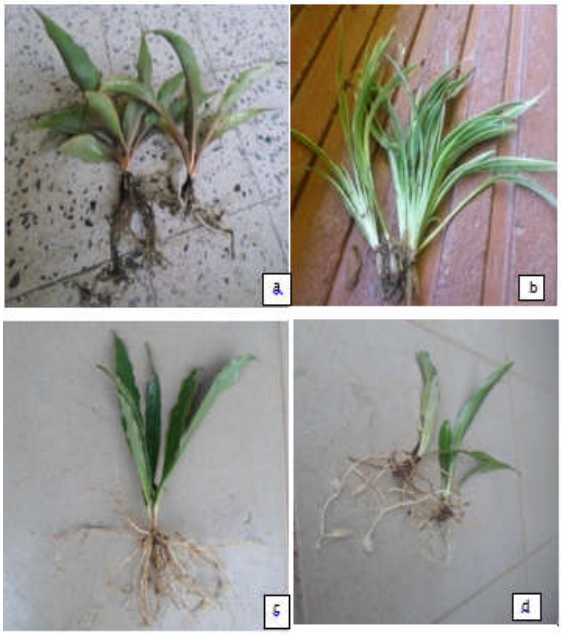

Plate 1: Some representative sample of each of the species of *Chlorophytum* investigated.
a, C. orchidastrum b, *C. bichetii,* c, *C. stenopetalum* and d, *C. macrophyllum*

The thickness of these two regions varies from one species to another as well as the surface viewed. Also folia epidermal cells on both surfaces are rectangular shaped and anticlinal wall patterns are straight to curve on both surfaces in all the species studied. Subsidiary cells are isodiametic on the abaxial surface, but rectangular on the adaxial surface in the four species.

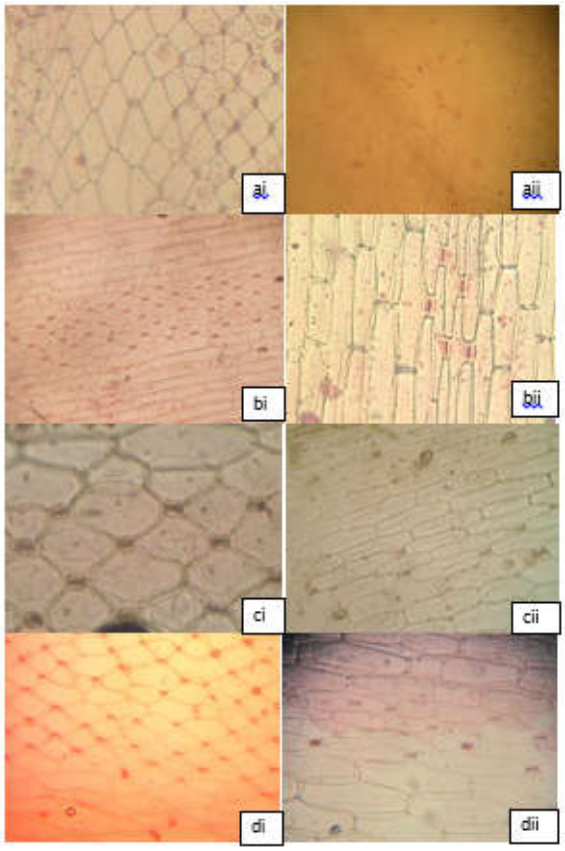

Plate 2: Photomicrographs of folia epidermis of *Chlorophytum* plant studied. ai, bi, ci *and* di show tetracytic stomata on the abaxial surface while aii, bii, cii *and* dii show tetracytic stomata on the adaxial surface of *C. orchidastrum C. bichetii, C. stenopetalum* and *C. macrophyllum* respectively.

Table 2: Epidermal characteristics of the four species of *Chlorophytum* studied

TAXA	Leaf Surface	Cell wall types	Stomata complex type	Shape of epidermal cell	Shape of subsidiary cell	Stomata distribution
C. orchidastrum	Abaxial	Straight to curve	Tetracytic	Hexagonal	Isodiametric	Amphistomatic
	Adaxial	Straight to curve	Tetracytic	Hexagonal	Polygonal	
C. bichetii	Abaxial	Straight	Tetracytic	Rectangular	Isodiametric	Amphistomatic
	Adaxial	Straight	Tetracytic	Rectangular	Rectangular	
C. stenopetalum	Abaxial	Straight to curve	Tetracytic	Rectangular	Isodiametric	Amphistomatic
	adaxial	Straight to curve	Tetracytic	Rectangular	Rectangular	
C .macrophyllum	Abaxial	Straight to curve	Tetracytic	irregular	Isodiametric	Amphistomatic
	Adaxial	Straight	Tetracytic	Rectangular	Rectangular	

Table 3A: Data on the Epidermal characteristics of the four species studied (mean value in µm ± standard deviation)

Taxa	Guard cell area		Epidermal cells/unit area
	Abaxial	Adaxial	Abaxial
C, orchidastrum	274.84(468.50)701.82	377.90(530.24)706.73	19(41.6)63
C. bichetii	157.05(247.94)539.86	353.36(527.79)701.82	143(169.4)190
C.stenopetalum	441.70(644.49)809.76	490.78(591.49)829.78	7(12.6)27
C.macrophyllum	176.47(336.48)736.23	309.30(673.16)834.23	6(30.8)54

Table 3B: Data on the Epidermal characteristics of the four species studied (mean value in µm ± standard deviation)

Taxa	Stomata frequency		Stomata index		
	Adaxial	Abaxial	Adaxial	abaxial	adaxial
C, orchidastrum	248(234.2)356	162(188.7)221	1(16.6)29	81.9	4.87
C. bichetii	252(323.8)483	121(133.4)162	35(41.0)51	88.71	47.13
C.stenopetalum	107(129.2)163	158(180.8)217	27(58.6)103	93.48	68.79
C.macrophyllum	71(104.4)129	120(157.2)183	26(30.8)36	60.09	21.97

Stomata complex type and Stomata distributions: Generally stomata occur on both surfaces of the leaf (amphistomatic leaves) in all the species investigated, however fewer stomata exist on the adaxial surface (that is hypoamphistomatic condition). Stomata are present in between the vein, while the vein region is devoid of stomata, except in *C. orchidastrum,* where the few stomata present on the adaxial surface were found on the vein. The stomata complex type in all the species is tetracytic (Plate 2) and (Table 2). It is characterised by four subsidiary cells sorrounding the stomata apperatus. The subsidiary cells are joined end to end with stomata apparatus found at the adjoining point, forming netlike structure. Epidermal cells run parallel to this region as shown in Plate 6. The stomata number varies from 1 in *C. orchidastrum* to 35 in *C.bichetii* on the adaxial surface, with stomata index ranging from 4.87 in to 31.2 in *C. stenopetalum.* On the abaxial the stomata number varies from 120 in *C. macrophyllum* to 221 in *C. orchidastrum* with stomata index ranging between 44.02 in *C.bichetii* to 93.48 *C. stenopetalum.*

Guard cell area appears to be wider on the adaxial surface than those on the abaxial surface. The guard cell area of 809.76 µm in *C.stenopetalum* on the abaxial surface is higher than others, while *C.bichetii* with guard cell area of 157.05 µm is the least. Also on the adaxial surface, guard cell area of 829.78µm in *C.stenopetalum* is higher than all others. Least guard cell area of 309.30µm is however seen in *C.macrophylum*. Number of epidermal cells on the abaxial surface range between 6 cells per unit area in *C. macrophyllum* to 190 cells/unit area in *C. bichetii.* Also on the adaxial surface highest number of epidermal cells/unit area was obervered in *C. bichetii* (483 cells /unit area) and lowest number of epidermal cell/unit area was observed in *C. macrophyllum* (71 cells per unit area).

Leaf Epidermis: Leaf margin: Based on the epidermal morphological features observed on the leaf margin, the species can be separated into two distinct groups. The first group composed of *C. orchidastrum* having smooth leaf margin (Plate 3a). In the second group, the leaf margin have minute papillae out growth (plate 3c and d). Members of this group are *C. bichetii, C. stenopetalum,* and *C. macrophyllum*. The papillae appear to be more prominent in *C. bichetii* (plate 3b) than the others.

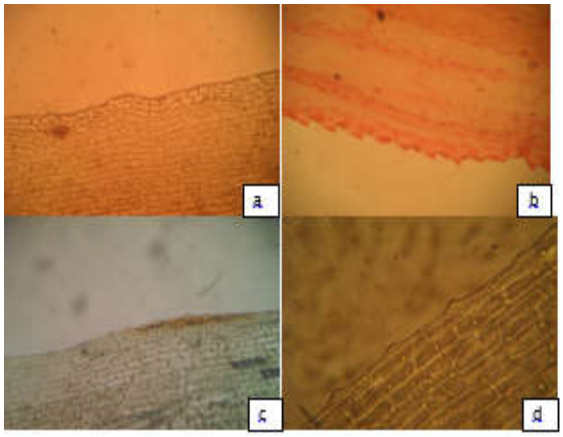

Plate 3: Photomicrographs of folia epidermis (leaf margin) of four species of *Chlorophytum* studied. a, shows smooth leaf margin of *C. orchidastrum* b, profound seration of leaf margin of *C. bichetii* c and d show minute serration in *C. stenopetalum* and *C. macrophyllum* respectively.

Taxonomic confusion in the genus *Chlorophytum* had been noticed over the ages and attributed to a number of factors. Hooker (1892) reported that the species of *Chlorophytum* are most difficult to circumscribe based on variability in its morphological features: leaves and the length and robustness of the scape and racemes. These features according to Hanid (1974) are influenced by environmental factors. In spite of the fact that morphological features are modified by the environmental factors, the results obtained above showed a level of diversity in the epidermal morphology which could be useful in taxonomic delimitation. Based on features of the leaf margin, *C. orchidastrum* with smooth leaf margin can be conveniently separated from the others. Though papillae outgrowths were observed on the leaf margin in the other three, it is arching and more prominent in *C. bichetii*.

Patil and Patil (1987) reported that, most species of *Chlorophytum* from Asian country which were investigated (with the exception *C. tuberosum*), have hypostomatic stomata distribution, with stomata present in between the vein on the abaxial surface and the adaxial surface is devoid of stomata. They also observed that the vein line is devoid of stomata.

However, the Nigerian species studied indicates the presence of stomata on both the abaxial and adaxial surfaces in the four species studied. On the adaxial surface however, the stomata were fewer in number, and in *C. orchidastrum* are located underneat the vein. It should be noted that, similar observation was recorded for *C. tuberosum* Patil and Patil (1987). This finding thus emphasis some basic relationship between the African and Asian species of the genus *Chlorophytum*.

According to Stace (1965), the size of the epidermal cells cannot be assumed to be a reliable taxonomic feature. This is due to variability in the epidermal cell size, which may be correlated with the age of the leaf, genetic variation, and the environment. However, Olowokudejo (1993) observed that characteristics of epidermis, including cell size and periclinal cell walls, had taxonomic significance in identification of species of the genus Euphorbiaceae. The shape of epidermal cells is mainly rectangular on the adaxial surface in all the species investigated except in *C. holstii,* which has hexagonal cell shaped. The subsidiary cells are mainly isodimetric on the abaxial surface in all the species studied. On the adaxial surface however, *C. orchidastrum* also stand out having hexagonal shaped subsidiary cells.

Studies of stomata can have great taxonomic significance for the delimitation of different levels of taxa (Kothari & Shah, 1975). In the present study stomata type has no diagnostic importance as all the species have tetracytic stomata. Stomatal Index is independent of the environment, size or portion of the leaf surface and size of the intervening epidermal cell (Metcalfe and Chalk, 1979) and also highly constant for any given species. Stomata index (<10%) on the adaxial surface in *C. orchidastrum* easily distinguishes the species from others. In *C.bichetii* and *C.macrophyllum* stomata index (<50%) was recorded on the same surface. Stomata index (>50%) in *C. stenopetalum* separate it from *C.macrophyllum* (the two are morphologically similar).

Stomata index on the abaxial surface also indicates that fewer stomata occur in *C. macrophyllum* as compared with *C. stenopetalum.* These may in part account for the glaborous texture of the leaves in the former, and scabrid texture of the later, as high number of stomata predisposes the plant to greater water loss. The papillae out growth observed on the leaf margin supports the closseness of between *C. stenopetalum* and *C. macrophyllum.* The papillae out growth is more pronounced in *C. bichetii* and completely absent in *C. orchidastrum.*

Conclusion: The study indicates the importance of leaf epidermal characters in delimiting species in the genus *Chlorophytum*. The species show variations as far as the following characters are concerned, these include; presence of papilla on the leave margin, stomata index and shape of the subsidiary and the other epidermal cells. Leaf epidermal character is hereby indicated as an important tool in delimiting species in the genus *Chlorophytum* and should be used for this purpose.

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