Leaf Epidermal Characteristics of Some *Alternanthera* Species in Nigeria

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Abstract. The leaf epidermis of some species of *Alternanthera* such as *A. bettzickiana, A. sessilis, A. pungens* and *A. brasiliana* have been characterized using light microscope and following standard procedures. To the authors' knowledge, the epidermal features of *A. bettzickiana* and *A. brasiliana* are being documented and reported for the first time. The shapes of epidermal cells ranged from polygonal to hexagonal shapes in both leaf surfaces of all the species examined except in both leaf epidermis of *A. brasiliana* and lower leaf epidermis of *A. bettzickiana* whose cells were irregular in shape. On the other hand, the walls of the epidermis in the species examined were slightly straight in both epidermal surfaces except in the lower epidermis of *A. bettzickiana* and upper epidermis of *A. brasiliana* which were zig-zag and sinuous respectively. There were gross absence of trichomes in both leaf epidermis of *A. bettzickiana* and *A. pungens* whereas scanty and short trichomes were observed on both epidermis of *A. sessilis* and *A. brasiliana*. In terms of stomata, *A. brasiliana* and *A. pungens* had the largest sizes (µm) with 57.01 ± 6.87 x 39.21 ± 6.93 (length x width) and 48.13 ± 4.06 x 37.34 ± 3.07 (length x width) in their lower and upper epidermis respectively. On the whole, *A. sessilis* had the smallest size of stomata with length and width of 31.55 ± 3.15 and 21.90 ± 6.47 in the upper epidermis. It was concluded that the results obtained in the present study suggest close relationship among the species. However, these need to be complemented with results from other sources of taxonomic inferences in order to adjudge possible delimitation and regrouping of these species where necessary.

Key words: Leaf epidermis, cells, stomata, micrometer, *Alternanthera*

Introduction

The genus *Alternanthera* (Amaranthaceae) includes approximately 80 species, native to tropical and subtropical regions of Australia and South America (Gupta *et al*., 2012). *Alternanthera sessilis, Alternanthera pungens, Alternanthera brasiliana, Alternanthera bettzickiana* and *Alternanthera nodiflora* are the most common five species in this genus in Nigeria.

Alternanthera sessilis (Linn.) DC. is a semi-prostrate annual herb with erect shoots up to 50cm high that reproduces from seed. The stem is round, more or less woody, slightly hairy and roots at the nodes. It has opposite leaves that are broadly lanceolate, about 2-8 cm long and 1-2 cm wide, and smooth and minutely toothed at the margins. The inflorescence consists of small sessile flowers clustered in axillary spikes. The flowers are white and have segments that are acute, not spinuous, and are about 2-2.5 mm long. The fruits are indehiscent, 1-seeded utricles. It is a weed of shady, damp soils in cultivated and waste areas. A pan-tropical weed found everywhere including hydromorphic rice fields in West Africa. On the other hand, *Alternanthera pungens* H. B. & K. (= *A. repens* [Linn.] Link.) is a prickly, creeping perennial herb, 50-60 cm high that reproduces by seed and vegetatively through stem cuttings. The stem has a woody rootstock, is prostrate, rooting at the nodes, pubescent and sometimes reddish in colour. The leaves are opposite, broadly oval, have entire margins and vary in size up to 4 cm long and 2 cm wide. The inflorescence occurs in dense axillary clusters about 1 cm long. The flowers are small, silvery-white, with sharply-tipped perianth and bracts. The fruit is a one-seeded indehiscent capsule. The seed is small and shaped like a maize seed. It is a common weed of waste areas, field crops, lawns, roadsides and open areas (Akobundu and Agyakwa,

1998). Similarly, *Alternanthera bettzickiana* (Regel) G. Nicholson is a herbaceous perennial plant, often with variegated leaves, growing 20-80cm tall. The plant is sometimes harvested from the wild for its edible leaves. It is widely grown as an ornamental in tropical and subtropical gardens, being valued especially for the colorfully variegated leaves. Furthermore, *Alternanthera brasiliana* (L.) Kuntze is an erect, herbaceous plant that can grow up to 3 meters tall, but is usually less than 1 meter in cultivation. The plant is sometimes harvested from the wild for local use as a food and medicine. It is often grown as an ornamental (Ken, 2014).

The morphological characters of angiosperms have been extensively used both for producing classification and for diagnostic purposes. These characters are indispensable as they play important roles in plant taxonomy. The morphological characters do not undermine the importance of other characters like anatomical and palynological which have been helpful in their own ways; the greatest advantage of the morphological characters is that they can be easily observed in the field (Singh and Jain, 1991). There are a large number of anatomical characters of taxonomic importance pointed out by Metcalfe and Chalk (1950) and Van Cotthem (1970). Some of these important anatomical characters are: trichomes, stomata and epidermis. Trichomes have been employed very frequently for taxonomic comparisons because of their diversity, their universal presence in the angiosperms and their simple means of preparation. For the epidermis, shape of the cells, thickness and characteristics of their walls, nature of the sculpturing on their walls as seen in surface view and inclusions of epidermal cells provide useful taxonomic criteria (Nwankwo and Ayodele, 2017). According to Das and Mukherjee (1997) mature morphological features are the conventional criteria used to establish taxonomic status. Similarly, Edeoga and Eboka (2000) observed that the use of leaf epidermal morphology in systematics is becoming popular just as the use of other markers like DNA sequence, chemical compositions, histochemical attributes and presence/absence of several groups of secondary metabolites. To this end, curious researchers over the years have made several scientific reports about the features in the epidermis of leaves of both dicots and monocots with respect to their usefulness and reliability as diagnostic characters in plant identification and delimitation of taxa. Consequently, the reports of Edeoga and Eboka (2000), Jayeola and Thorpe (2000), Edeoga and Otoide (2001), Nwachukwu and Mbagwu (2007), Aworinde *et al.* (2013), Kemka-Evans (2014), Gupta *et al*. (2012) and Nwankwo and Ayodele (2017) are pertinent references.

Edeoga and Eboka (2000) studied the morphology of the leaf epidermis of some *Dissotis* species and reported the presence of paracytic stomatal type on the abaxial and adaxial surfaces of all the taxa except in *Dissotis rotundifolia* where the stomata were anomocytic and variations in epidermal cells architecture ranging from pentagonal to polygonal in all the species except in *Dissotis rotundifolia* where they were sinuous and irregular. They recommended the usefulness of their observations in the systematics of the genus. Similarly, Jayeola and Thorpe (2000) studied the *Calyptrochilum* with the aid of microscope. They reported that *Calyptrochilum chrystyanum* was characterized by a network of horizontal grooves, dome shaped micropapillae and a mass of soft wax, while *C. emarginatum* was distinguished by the presence of densely overlapping conical and globular types of micropapillae. They concluded that sculpturing features in the genus were characteristic and therefore of utility in identifying even sterile specimens and fragments. In the same vein, Edeoga and Otoide (2001) reported the characteristics of leaf epidermal morphology of *Amaranthus dubius, A. hybridus, A. lividus, A. spinosus, A. tricolor and A. viridis* that irregularly shaped epidermal cells were observed in *A. dubius* while rectangular or polygonal epidermal cell shapes, paracytic stomata and calcium oxalate crystals were observed in *A. tricolor* and *A. lividus.* These authors were the first to report and document the leaf epidermal characteristics of *A. dubius, A. lividus* and *A. tricolor*. Furthermore, Nwachukwu and Mbagwu (2007) reported variation in shape of epidermal cells-mostly rectangular and sinuous in *Indigofera pulchra, I. senegalensis* and *I. stenophylla* while

it varies from pentagonal in *I. paniculata, I. prieureana and I. terminalis* though *hexagonal* in *I. hirsuta* and *I. tinctoria.* Similarly, Aworinde *et al.* (2013) compared the leaf architecture of some *Vernonia* species in south western Nigeria. He reported the occurrence of wavy anticlinal wall with polygonal cell shape in *V. ambigua* and straight anticlinal wall with irregular cell shape in *V. amygdalina* and *V. cinerea*. Paracytic stomatal types were also reported about the leaves of the genus. He concluded that the features were sufficient enough to delimit them from one another. Kemka-Evans *et al.* (2014) studied the leaf epidermis of three species of *Vernonia* and reported the occurrence of contiguous stomata and sinuous anticlinal walls on the abaxial leaf surface of non-bitter variety of *V. amygdalina* and on both leaf surfaces of *V. cinerea.* Also reported were irregular T- Shaped trichomes on the leaf surfaces of *V. amygdalina* and the cuticular striations on the adaxial surface of the same taxa. They concluded that the features were of taxonomic importance in determining the relationship among the species. Furthermore, Gupta *et al*. (2017) reported the presence of anomocytic and diacytic stomatal types of uniseriate and biseriate layers of epidermis on the upper and lower leaf surfaces of *Alternanthera sessilis* respectively. Whereas, uniseriate layer of epidermis occurred in both epidermis of *A. pungens* with only anomocytic stomatal type. They concluded that the leaf anatomical features observed on the species would be useful in confirming the authenticity of raw drugs resulting from leaves of the two species. Similarly, Nwankwo and Ayodele (2017) studied the taxonomy of the genus *Indigofera* Lin in Nigeria and reported anisocytic and paracytic stomatal types common in all the species. Also reported were irregular epidermal cell shapes on both surfaces of all the species except in *I. capitata, I. nummulariifolia* and *I. oblongifolia* in which they are polygonal. And that anticlinal wall patterns were curved, sinuate and undulate in all the species. They concluded that the stomatal type and epidermal cell shapes are significant in the taxonomy of the genus *Indigofera.*

Methods

Collection of Plant Samples

Matured fresh leaves of *Alternanthera sessilis, A. pungens, A. brasiliana, and A. bettzickiana* were collected from Ado-Ekiti and Ikere towns of Ekiti State, Nigeria. The specimens were then taken to the Herbarium of Ekiti State University, Ado- Ekiti for authentication.

Preparation of Slides

The epidermal peels of each leaf sample were obtained using the methods of Ogundipe and Kadiri (2013) with slight modifications. The leaves were placed, with the outer surface facing downward, on a flat surface and flooded with 8% sodium hypochlorite solution (NaOCl). An area of about 1cm square was removed from a central / standard position, always midway between the base and the apex of the leaves. The peels were mounted temporarily on slides. 10 slides (each of lower and upper epidermis) were prepared per population.

Data Collection

The slides were examined under the light microscope using x10 and x40 objectives. Data were collected from 10 microscopic fields selected at random from each slide. The lengths and widths of stomatal pores and guard cells were measured using ocular micrometer. Data were collected from 50 stomata per leaf epidermis. This was done in 10 replications. Line drawings of the epidermal tissues using camera lucida are shown in figures as illustrations. The data obtained were subjected to relevant statistics using mean and standard deviation.

Measurement of Stomatal Index (SI):

The Stomatal Index was measured following Metcalfe and Chalk (1988):

$$SI = \frac{S}{E+S} \times \frac{100}{1}$$

Where, S denotes the number of stomata per unit area and E the number of epidermal cells of the same area

Results and Discussion

Table1. Characteristics of the epidermis of the *Alternanthera* leaves

		Species			
S/n	**Parameters**	***A. bettzickiana***	***A. sessilis***	***A. pungens***	***A. brasiliana***
1	Shape of Epidermal Cells	**U:** Polygonal – Hexagonal. **L:** Irregular	**U:** Polygonal – Hexagonal **L:** Polygonal - Hexagonal	**U:** Polygonal – Hexagonal **L:** Polygonal - Hexagonal	**U:** Irregular **L:** Irregular
2	**N**ature of Epidermal Cell Wall	**U:** Slightly straight **L:** Zig-zag	**U:** Slightly straight **L:** Slightly straight	**U:** Slightly straight **L:** Slightly straight	**U:** Sinuous **L:** Slightly straight
3	Type of Trichomes	**U**: Absent **L**: Absent	**U:** Scanty, short and uniseriate **L:** Scant, short, Septate and uniseriate	**U:** Absent **L:** Absent	**U:** Very, short, scanty and uniseriate **L:** Septate, very short, scanty and multicellular
4	Type of Stomata	**U:** Anomocytic **L:** Paracytic and Diacytic	**U:** Diacytic **L:** Diacytic	**U:** Anisocytic **L:** Anisocytic	**U:** Anomocytic **L:** Diacytic
5	Stomata Index (%)	**U:** 21.10 **L:** 31.10	**U:** 24.04 **L:** 35.61	**U:** 22.04 **L:** 38.12	**U:** 14.50 **L:** 32.00
6	Stomatal Length (µm)	**U:** 41.03 ± 3.12 **L:** 35.98 ± 4.01	**U:** 31.55 ± 3.15 **L:** 38.57 ± 9.11	**U:** 48.13 ± 4.06 **L:** 46.15 ± 5.51	**U:** 36.16 ± 3.86 **L:** 57.01 ± 6.87
7	Stomatal Width **(**µm**)**	**U:** 27.09 ± 3.53 **L:** 24.92 ± 2.60	**U:** 21.90 ± 6.47 **L:** 25.18 ± 3.44	**U:** 37.34 ± 3.07 **L:** 33.92 ± 2.94	**U:** 24.59 ± 2.59 **L:** 39.21 ± 6.93

The results of the present study have been summarized in Table 1 and illustrated in Fig. 1(a-h).

Trichomes were absent at the upper and lower leaf epidermis of both *Alternanthera bettzickiana* and *A. pungens* but present at both the upper and lower epidermis of *A. sessilis* and *A. brasiliana.* The trichomes were short, scanty and uniseriate at the upper and lower epidermis of *A. sessilis.* The trichomes were septate at the lower epidermis of *A. sessilis* and *A. brasiliana*. In the same vein, very short, scanty and uniseriate trichomes were observed at the upper epidermis of *A. brasiliana.* Although the trichomes were very short and scanty, they were also multicellular at the lower epidermis of *A. brasiliana.* The shapes of the epidermal cells in all the species studied ranged from polygonal to hexagonal shapes except in the upper and lower epidermis of *A. brasiliana* and the lower epidermis of *A. bettzickiana* were they were irregular in shape. Furthermore, the anticlinal and periclinal walls of the epidermal cells were slightly straight at both the upper and lower epidermis of all the species except in the upper and lower surfaces of *A. brasiliana* and the lower epidermis of *A. bettzickiana* where they were sinuous (Fig. 1g-h and b). In addition, the cell walls in all the species examined were thick.

The leaves of all the taxa were amphistomatic with numerous stomata. Diacytic and Anomocytic stomatal types were present at the upper and lower leaf epidermis of *A. sessilis* and *A pungens* respectively. Conversely, at the upper leaf epidermis of both *A. bettzickiana* and

A. brasiliana were anomocytic stomatal type while at their lower epidermis were paracytic and diacytic stomatal types. Stomatal density was highest at the lower epidermis and lowest at the upper epidermis in all the taxa investigated. Hence, stomatal indexes (%) of 21.10, 24.04, 22.01 and 15.50 were deduced for the upper leaf epidermis of all the taxa whereas, 31.10, 35.61, 38.12 and 32.00 were deduced as stomatal indexes of the lower leaf epidermis of all the taxa investigated.

The mean length (µm) of stomata were 41.03 ± 3.12, 31.55 ± 3.15, 48.13 ± 4.06 and 36.16 ± 3.86 for the upper epidermis of *A. bettzickiana, A. sessilis, A. pungens* and *A. brasiliana* respectively. At the lower epidermis, on the other hand, the length of stomata were 35.98 ± 4.01, 38.57 ± 9.11, 46.15 ± 5.51 and 57.01 ± 6.87 for each of the species respectively. The mean width (µm) of the stomata at the upper leaf epidermis were 27.09 ± 3.53, 21.90 ± 6.47, 37.34 ± 3.07 and 24.59 ± 2.59 for *A. bettzickiana, A. sessilis, A. pungens* and *A. brasiliana* respectively while, the mean width (µm) of the stomata at the lower leaf epidermis of the species were 24.92 ± 2.60, 25.18 ± 3.44, 33.92 ± 2.94 and 39.21 ± 6.93 respectively.

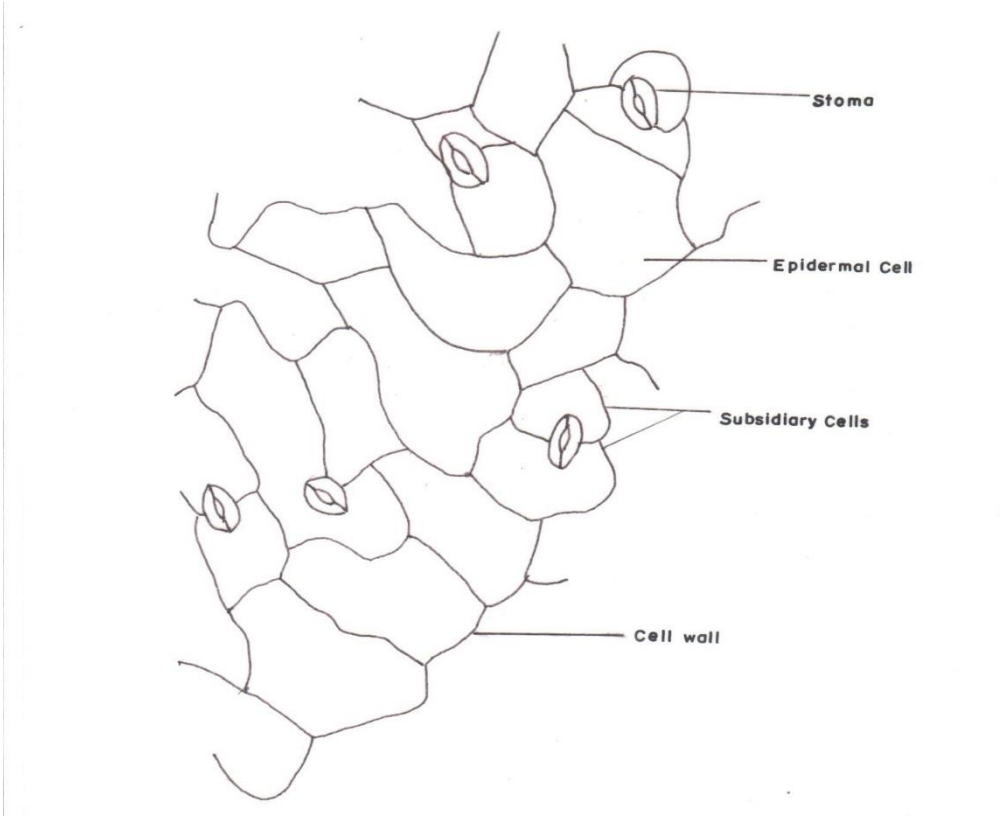

Figure 1(a). Upper epidermis of leaf of *Alternanthera bettzickiana* x 100

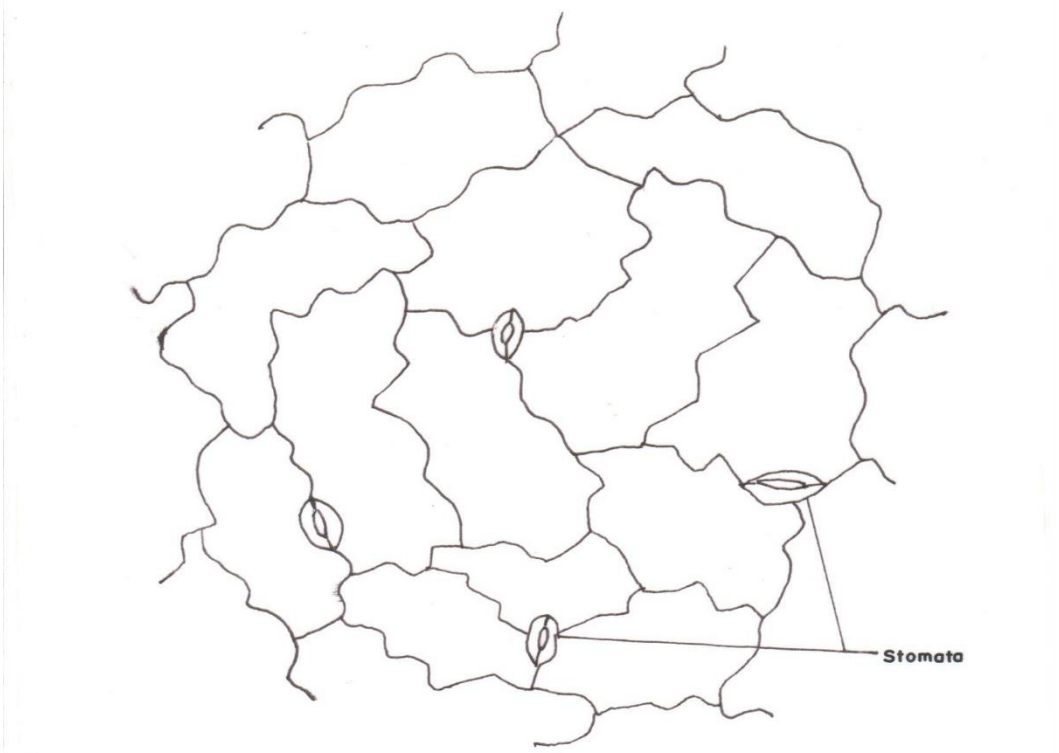

Figure 1(b). Lower epidermis of leaf of *Alternanthera bettzickiana* x 100

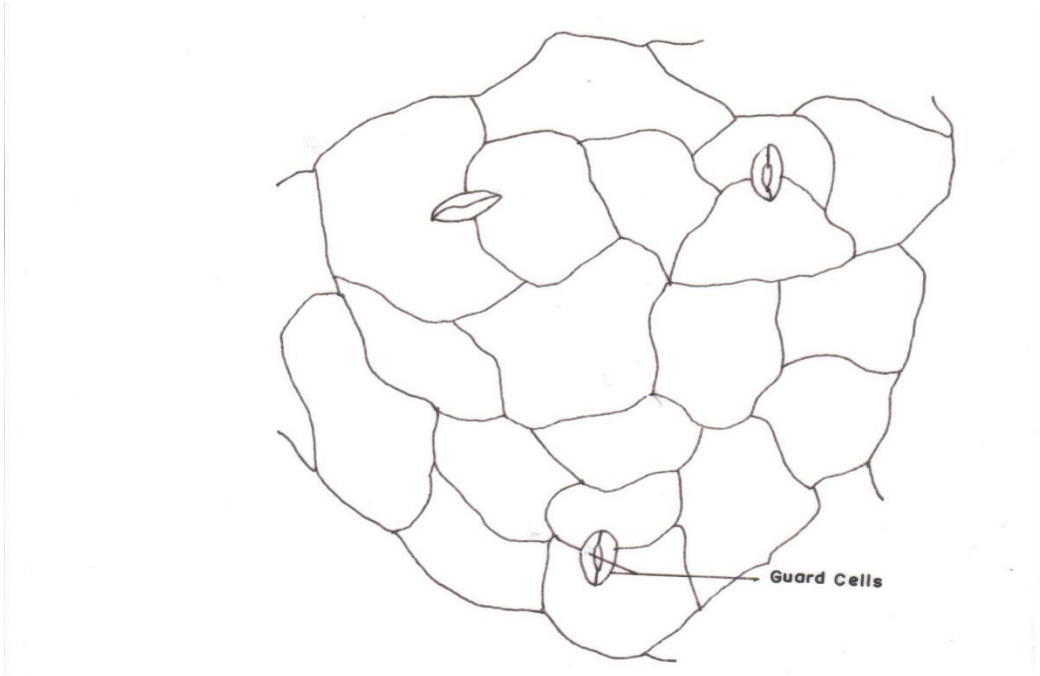

Figure 1(c). Upper epidermis of leaf of *Alternanthera sessilis* x 100

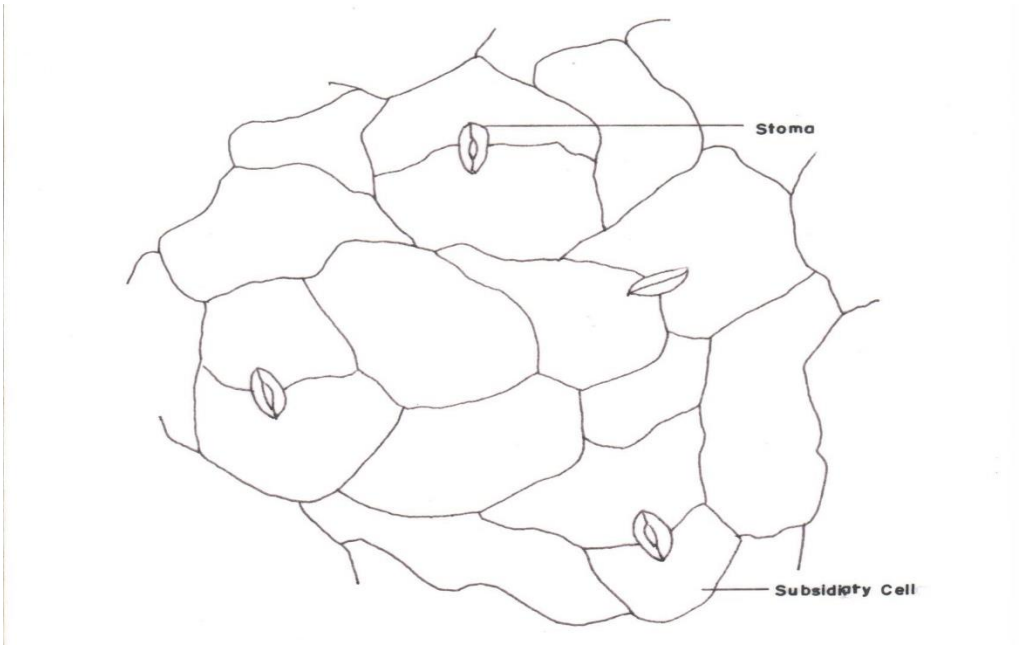

Figure 1(d). Lower epidermis of leaf of *Alternanthera sessilis* x 100

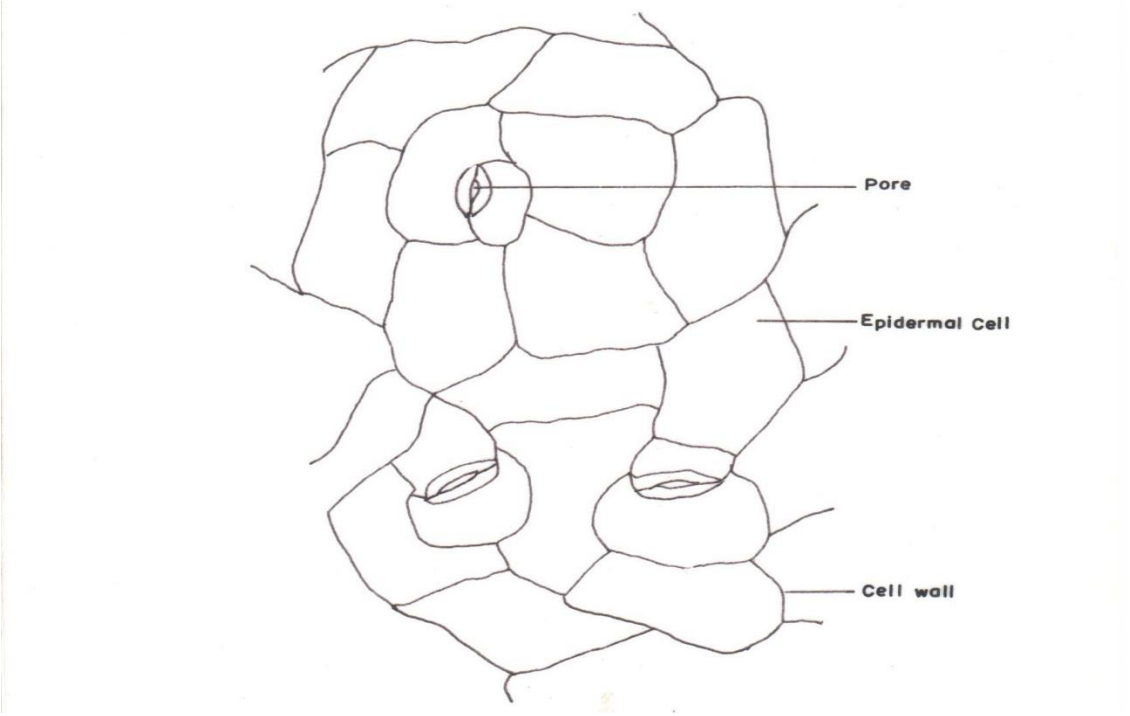

Figure 1(e). Upper epidermis of leaf of *Alternanthera pungens* x 100

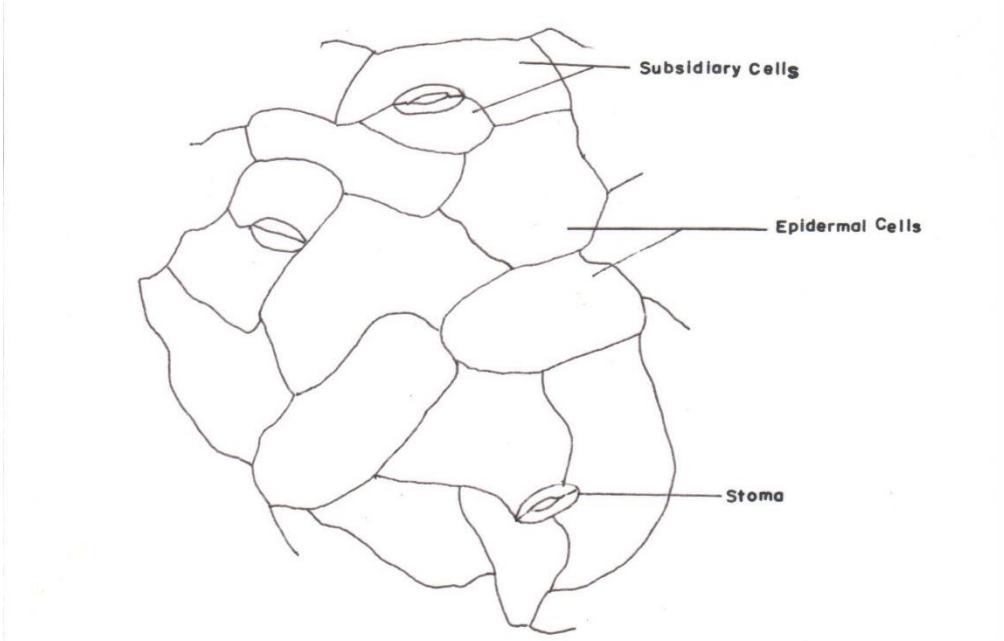

Figure 1(f). Lower epidermis of leaf of *Alternanthera pungens* x 100

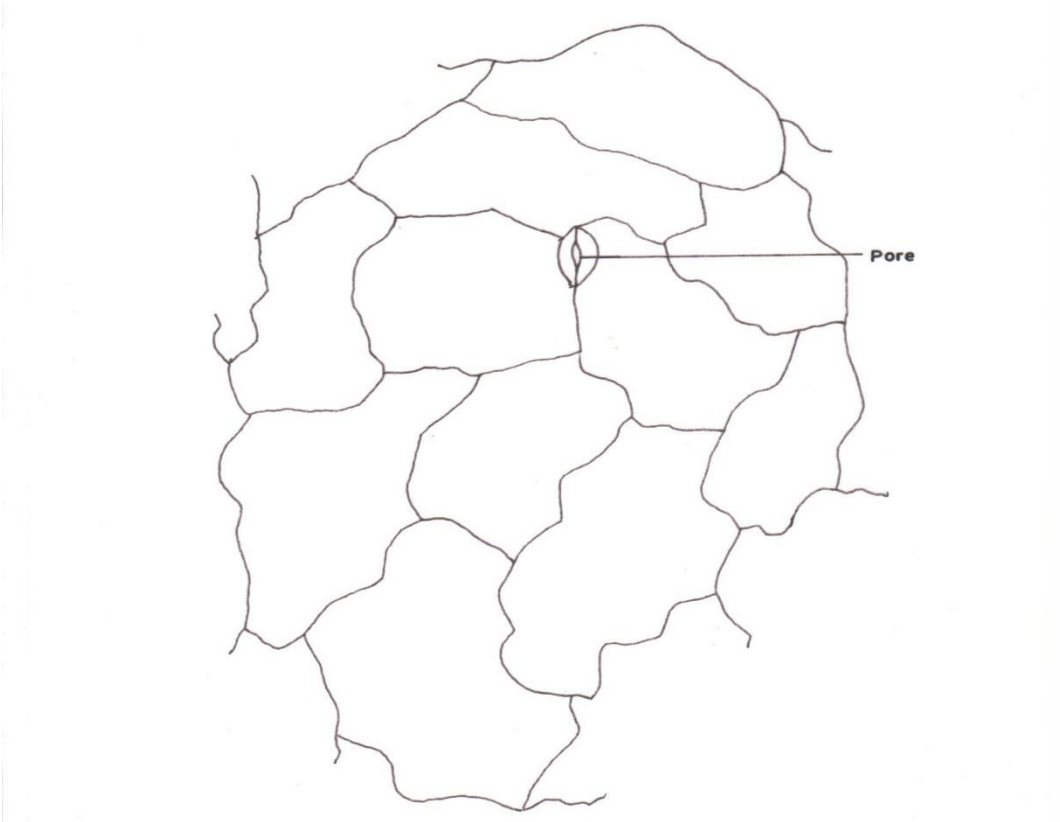

Figure 1(g). Upper epidermis of leaf of *Alternanthera brasiliana* x 100

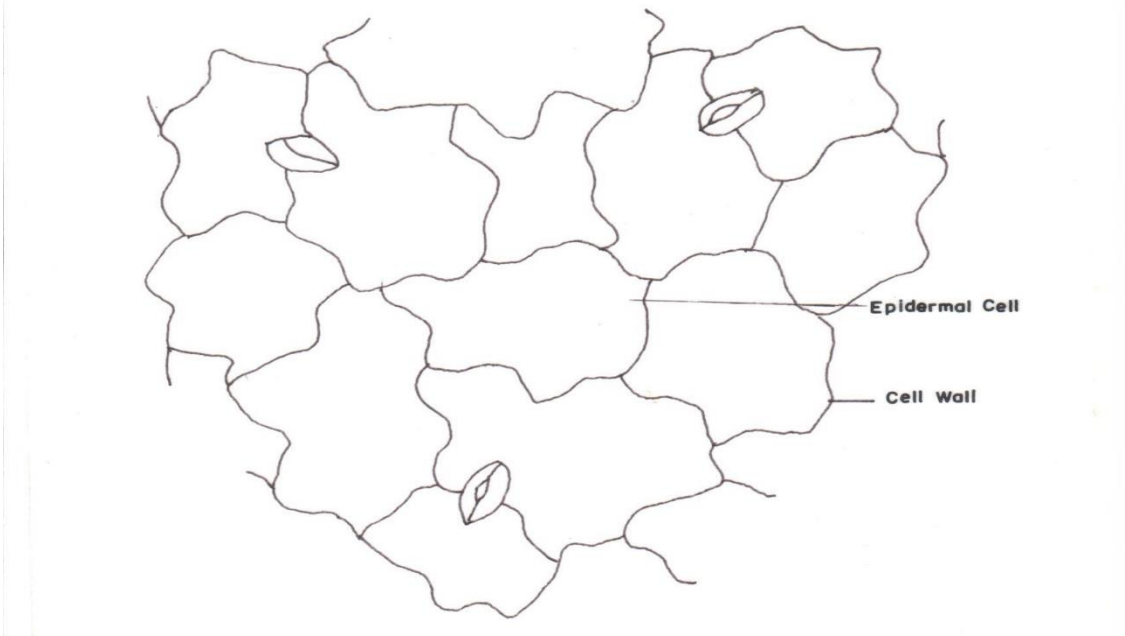

Figure 1(h). Lower epidermis of leaf of *Alternanthera brasiliana* x 100

The observed leaf epidermal features of the four *Alternanthera* species investigated are useful in identifying them when presented in sterile or fragmentary condition and in establishing possible relationship among the different species. This assertion is in line with the previous ones made by Jayeola and Thorpe (2000), Edeoga and Otoide (2001) and Nwankwo and Ayodele (2017).

The presence of short, scanty and uniseriate trichomes in both leaf surfaces of *A. sessilis* and *A. brasiliana* is a diagnostic taxonomic tool useful in distinguishing them from *A. bettzickiana* and *A. pungens* where trichomes were grossly absent on both the upper and lower epidermal surfaces. This assertion is in line with the previous ones made by Metcalfe and Chalk (1950) and Singh and Jain (1991) on the reliability of trichome type and distribution pattern in delimitation of taxa.

The amphistomatic nature of the epidermis as observed in all the species investigated agrees with the earlier reports of Gupta *et al.* (2012) on *Alternanthera sessilis* and *A. pungens.* To the authors' knowledge, no report has been documented on the leaf epidermal features of *A. brasiliana* and *A. bettzickiana.* Both the upper and lower leaf epidermal surfaces of *A. sessilis* showed diacytic stomatal type and on the basis of this, the upper and lower leaf epidermis is the same for this species. This observation correlates with that of Gupta *et al.* (2012) on *A. sessilis.* This is a distinguishing factor that separate *A. sessilis* from the rest of the taxa investigated. Similarly, the occurrence of anisocytic stomatal type in both the upper and lower leaf epidermis of *A. pungens* is a distinguishing taxonomic anatomical character separating the taxon from the rest of the species in the present study.

The irregular nature of epidermal cells shape at both upper and lower leaf epidermis of *A. brasiliana* is characteristic and diagnostic in separating the taxon from the rest of the members investigated whose epidermal cells shape ranged from hexagonal to polygonal in both upper and lower epidermis of their leaves except in the lower epidermis of *A. bettzickiana* where the cell shape was irregular as well.

On the other hand, the sinuous and heavily sinuous architectural patterns of the anticlinal and periclinal walls of the epidermal cells in the upper and lower epidermis of *A. brasiliana* is a reliable source of taxonomic inference useful in delimiting the species from the rest of the taxa investigated whose anticlinal and periclinal walls were slightly straight in both upper and lower leaf epidermis. Strange in this context, is the zig-zag architectural pattern of the cell walls

in the lower epidermis of *A. bettzickiana.* This is a taxonomic tool in identifying the species when in sterile or fragmentary condition.

The entire species possessed large size of stomata. The longest and fattest among the taxa was *A. brasiliana* having mean length and mean width of stomata of 57.01 ± 6.87 and 39.21 ± 6.93 at the lower leaf epidermis. This nature is eco-physiologically advantageous to this species as it would be able to manufacture enough food at the shortest rate and compete successfully in adverse conditions. Conversely, the shortest and smallest of the stomata in the taxa was found on the upper leaf epidermis of A. sessilis having mean length and width of 31.55 ± 3.15 and 21.90 ± 6.47 respectively.

Information on the internal features of the leaf epidermis of *Alternanthera* species is very scanty and not properly documented. To the authors' knowledge only Gupta *et al.* (2013) had recently studied the anatomy of *A. sessilis* and *A. pungens*. Consequently, it suffices to assert heretofore, that the present report on the leaf epidermis of *Alternanthera bettzickiana* and *A. brasiliana* is being documented for the first time.

Conclusion

Although there is hitherto no discrepancy about the taxonomic status of the *Alternanthera* species covered in the present study, the results obtained are informative though, rather not sufficient to recommend and adjudge delimitation and regrouping of the species, they suggest relationship among these species within the same genus, *Alternanthera*. On this note, it is highly recommended that the results of the present study be complemented with those from other sources of taxonomic inferences such as Palynology, Cytology, Taximetrics, Histochemistry, DNA fingerprinting and Barcoding and Phytochemistry. This will go a long way to scrutinize the taxonomic position of each of the species that make up the genus, *Alternanthera.*

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