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Research article

LEAF EPIDERMAL STUDIES IN THE SPECIES OF STACHYTARPHETA FOUND IN AWKA, NIGERIA AND ITS TAXONOMIC IMPLICATIONS

Chisom F Iroka[1], Clement U Okeke[2] and Nkumah C Okereke[3]

[1&2]Department of Botany, Nnamdi Azikiwe University, P. M. B 5025 Awka, Anambra State Nigeria.
[3]Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State
Corresponding Author: Iroka, Finian Chisom (harlyz14@yahoo.com) +234 8067 588 025.

ABSTRACT: The foliar epidermal and stomata study of the *Stachytarpheta* species present in Awka, south eastern Nigeria was carried out using standard anatomical procedure. The genus is a family of Verbenaceae and has three species in it which are present in the region. They include *Stachytarpheta jamaicensis*, *Stachytarpheta cayannensis* and *Stachytarpheta angustifolia*. The study showed that epidermal cell shape, stomatal type and stomata shape both on adaxial and abaxial surfaces of the three plants were almost similar with little variation in *S. cayannensis*. The analysis of variance of stomata length, stomata size and epidermal cell size showed no significant difference between *Stachytarpheta* species ($p>0.05$) but thickness of upper epidermis, lower epidermis, stomata width and stomata index showed a significant difference between *Stachytarpheta* species ($p<0.05$). Finally, most of these characters studied were important taxonomic tools in the delimitation of the three species. Duncan multiple range test however, revealed the species relationship. Therefore, this study showed more affinity between *S. jamaicensis* and *S. angustifolia*, hence, delimiting *S. cayannensis*.

Key words: *Stachytarpheta, Jamaicensis, Cayannensis, Angustifolia*, Stomata, Epidermal.

INTRODUCTION

Verbenaceae is predominantly a tropical family exhibiting a wide range of growth habit and inhabiting diverse habitats (Lillyamma and Shah, 1987). The family has about 98 genera and 3,000 species (Idu et al., 2009). They are low shrubs, herbs or trees. Flowers are in spikes. The genus *Stachytarpheta* Vahl. Belongs to the family Verbenaceae and is represented in West Africa and Nigeria by three species namely: *Stachytarpheta cayannensis* (Rich.) Vahl; *S. angustifolia* (Mill.) Vahl and *S. jamaicensis* (L.) Vahl (Hutchinson and Daziel, 1963). They are economic plants and may be grown as ornamentals (Gill, 1988). Members of family Verbenaceae are popular in traditional medicine. Moreover, all the *Stachyrapheta* species have been used ethnomedically as anti-diabetic, arbotifacient, emmenagogue, sedative, antihypertensive, anti-asthmatic and anti-fever (Schwontkowski, 1993). *Stachytarpheta* is an erect and branched half-woody plant, with stem slightly angled. The leaves are elliptic to oblong-ovate and 2 to 10cm long. The leaf tips ere pointed with toothed margins. The leaf base is decurrent on the petiole. The spikes are terminal, rather slender, 10-30cm long, 3-4mm thick, green and continuous. The calyx is small, oblique and 4-toothed. The corolla is deep-blue or blue-purple, 1cm long. The fruit is enclosed in the calyx and oppressed to and somewhat sunk in the rachis which is smooth, oblong and about 4mm long (Idu et al., 2009). Plant anatomy has been found to be very essential in plant taxonomy. The purpose is to develop a system of classifying plants in a way that all the differences and similarities are set out in ordered manner (Olorode, 1984). In spite of the fact that vegetative and floral characters are markedly modified in relation to the habitat and pollination mechanisms, the preceding observations and the summaries of character variation indicated that the taxonomic application of the diversity of epidermal morphology in plants cannot be over emphasized. The decision to choice epidermal characters to carryout studies in plants was informed by earlier declaration that these characters represented genetic variations and have been used to solve taxonomic problems in certain plant groups by Taxonomists (Oladele, 1990; Adegbite, 1995; Ogukunle and Oladele, 1997; Ogunkunle and Oladele, 2008). Now-a-days virtually every anatomical aspect of plants has been studied by Taxonomists and the quality of information accumulated is enormous. Particularly valuable taxonomic evidence has been obtained from the study of pollen, wood, leaf, epidermis, cuticle, trichomes and stomata. Some of these anatomical features are so diagnostic that they are now commonly used in routine identification, rather than being confined to a use in problems of phylogeny or classification or in the identification of fragments of plants. These variations in the epidermis on the other hand, have been attributed to the functional multiplicity of the dermal tissue.

The variation in the epidermal anatomy has also been studied in family Boraginaceae. Major and diverse uses of stomata have been made in plant classification. Metcalfe and Chalk (1950); and Dilcher (1974) have emphasized the systematic application of stomata types while Shah and Gopal (1969) considered the stomata as a weak point in taxonomic classification. But they felt that like other characters they can be advantageously used for that purpose. Pant and Khara (1969) reported that the stomata type is not constant for a taxonomic category of any rank. The reason for this is the diversity of stomata types in different taxa. In view of these considerations in family Boraginaceae, it was decided to undertake a survey of frequency, distribution pattern, types and percentages of open and close stomata. This family was however, chosen because it was found more or less in all types of climates and at all altitudes. Moreover, Altaf et al. (2003) found that despite the enormous diversity in the stomata type in Boraginaceae (with more than one type occurring even on the same surface lying side by side) the anomocytic was the dominant type of stomata. Dissimilarities in the stomata types in different species of same genera and even on the same surface of one leaf e.g. *Cordia gharaf, C.myxa* and *C. obliqua* have anomocytic and anisocytic as the common types while diacytic and actinocytic types occur in *Cordia myxa.* Paracytic and amphiparactic types were present only in *C.obliqua* while *C.gharaf* had brachyparacytic type, which is absent in *Cordial myxa* and *C.obliqua.* Such types of diversity occurred in species of other genera such as *Heliotropium, Cynoglossum, Paracarysum, Eritrichium, Myosotis, Nonnea* and *Onosma*. Similarities of stomata types encountered in species of different genera of the same tribe and even belonging to different tribes' e.g. anomocytic and anisocytic types of stomata occurred in all the tribes. *Cordia myxa* and *Amebia grandiflora* belong to different tribes but have equal frequency of paracytic types on the lower epidermis (Altaf et al., 2003). More so, Altaf et al. (2003) observed that all the species of the family Boraginaceae they studied had hypoamphistomatic leaves. The epidermal cells in the mature epidermis of all the members were irregularly arranged and were either elongated in various directions or were isodiametric in shape. The walls of epidermal cells were straight sinuous, arched or papillose. In all the leaves, the epidermal cells on the veins or near the veins had either straight or slightly wavy all more cell walls. Most of the observed species had more cell wall undulations on the abaxial than adaxial ones. Apart from the stomatal types, a great diversity was observed in the size of stomata. Two fold differences were found in the width of guard cells ranging from 7.0 (*Eritrichium fruticosum*) to 17.4F (*Paracrysum perpeurum*). Similar variations were observed in the length of guard cells ranging from 20.00f (*Cynoglossum tomentosa*) to 34.4f (*Paracarysum perpeurum*). Differences in the stomatal pore size were also observed between the species. However, all these parameters were not consistent and exhibited variations from one locality to another. Results indicated that epidermal characters do not exhibit a uniform pattern and hence have very little significance as a taxonomic character within the family Boraginaceae. Altaf et al. (2003) in their studies showed that the species studied belonging to different tribes show similar value of stomatal indices as in *Cordia gharaf* of the tribe Cordieae. However, due to the complex taxonomic status of *Stachytarpheta* species, this research is aimed at the following

- To study the epidermal features of these plants so as to know their various attributes.
- To delimit the species by knowing and comparing these characters in order to establish a relationship.

MATERIALS AND METHODS USED IN THE STUDY

Collection and Identification of Plants

Samples of *S.cayannensis* and *S.jamaicensis* were collected from Nnamdi Azikiwe University Premises. *S.angustifolia* was collected from Adabebe village in Amawbia Community; Awka South L. G. A. Samples of *S. cayannensis, S. angustifolia* and *S. jamaicensis* collected were properly and authenticated by Prof. J.C Okafor and vouchers deposited at the Herbarium, Department of Botany, Nnamdi Azikiwe University, Awka.

Anatomical Analysis

Anatomy follows the methods of Kadiri et al. (2007); Kadiri and Ayodele (2010) and Ajayi et al. (2011) with some modification. Terminologies of stomatal complex type used are according to Metcalfe and chalk (1950; 1979).

Leaf epidermis was examined after Nitric acid treatment which involved cutting $4cm^2$ of the standard median part of the leaf lamina near the midrib following the methods of Olowokudejo (1993); Ogundipe and Wujeck (2004); Ogundipe and Kadiri (2013) with some modifications. Dried leaves were boiled in water for thirty minutes and subsequently soaked in concentrated Trioxonitrate (V) acid (HNO_3) in capped specimen bottles for about 8 – 24 hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into petri dishes containing water for cleansing. Tissue debris cleared off the epidermis with fine hair brush and washed in several changes of water. They were passed through a series of ethanol ranging from 50% - 100% drops of different grades of ethanol to dehydrate the cells. The preparation, were later stained with Safranin O in 50% alcohol for 5minutes before mounting in glycerine on glass slides. The epidermal layers were mounted on glass slides with the adaxial and abaxial surfaces facing up, covered with cover slips and edges of cover-slip sealed with nail varnish to prevent dehydration.

All preparations were observed with an Olympus microscope and photographs were taken with a digitized camera (Nikkon). Stomata size was calculated by multiplying the length and width of the stomata. Stomata index is the percentage proportion of the number of stomata to the other epidermal cells present on a leaf portion.
The stomata index in expressed by the formula

$$S.I = \frac{S \times 100\%}{E + S}$$

Where, S.I = Stomata Index
S = Number of stomata per unit area.
E = Number of ordinary epidermal cell plus the subsidiary cells in the same unit area.

RESULTS

Table 2 shows the various foliar epidermal features of *stachytarpheta* species. The table indicates that the thickness of upper epidermis (µm) was highest on the abaxial surface of *S. angustifolia* (64.00±5.657) and lowest on adaxial surface of *S. jamaicensis* (39.50±2.121); the analysis of variance shows a significant difference in the thickness upper epidermis between *Stachytarpheta* species ($p<0.05$) but not between epidermal surface ($p>0.05$). Thickness of lower epidermis (µm) was highest on the abaxial surface of *S. angustifolia* (36.75±1.061) and lowest on the adaxial surface of *S. jamaicensis* (15.25±1.061); the analysis of variance shows a significant difference in the thickness of lower epidermis between *Stachytarpheta* species ($p<0.05$) but not between epidermal surface ($p>0.05$). More so, stomata length (µm) was highest on the adaxial surface of *S. cayannensis* (44.00±6.364) and lowest on adaxial surface of *S. jamaicensis* (41.25±2.475); the analysis of variance shows no significant difference in the stomata length between *Stachytarpheta* species ($p<0.05$) and between epidermal surface ($p<0.05$).

Table 1: Summary of the qualitative foliar epidermal characters

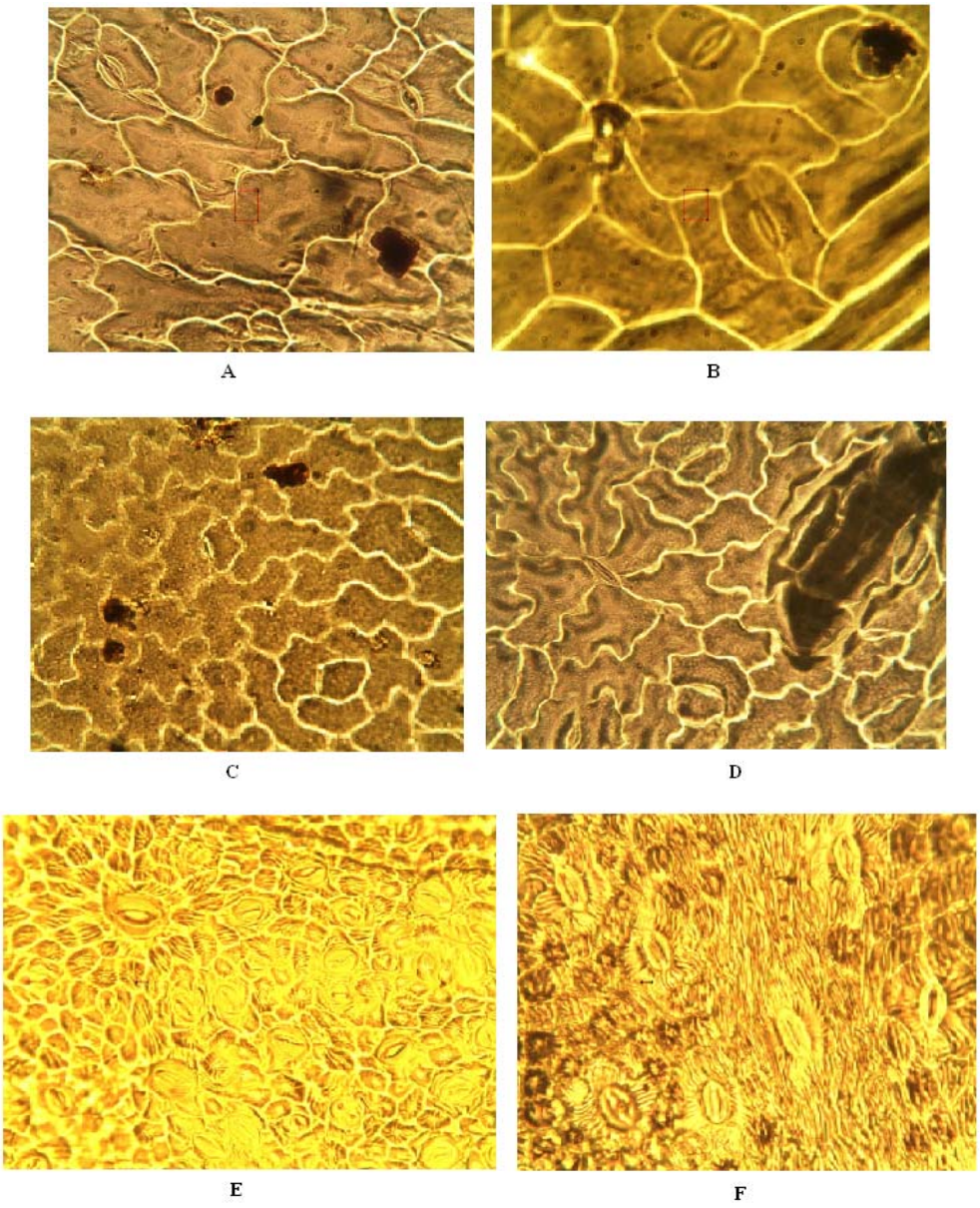
Species	Epidermal Cell shape		Stomatal type		Stomatal shape	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
S. angustifolia	Polygonal and Irregularly arranged	Polygonal and Irregularly arranged	Diacytic Anisocytic Anomocytic	Diacytic Anisocytic Anomocytic	Elliptic	Elliptic
S. cayannensis	Polygonal and regularly arranged	Polygonal and regularly arranged	Diacytic Anisocytic Anomocytic	Diacytic Anisocytic Anomocytic	Circular to Elliptic	Circular to Elliptic
S. jamaicensis	Polygonal and Irregularly arranged	Polygonal and Irregularly arranged	Diacytic Anisocytic Anomocytic	Diacytic Anisocytic Anomocytic	Elliptic	Elliptic

Table 2: Quantitative Epidermal Features of the *Stachytarpheta* species

Treatment	Thickness of Upper epidermis	Thickness of lower epidermis	Stomata length (µm)	Stomata Width (µm)	Stomata size (μm^2)	Stomata index (%)	Epidermal cell size (µm)
S. angustifolia (Adaxial)	62.00 ± 5.657^{b}	34.01 ± 5.657^{b}	42.49 ± 3.550^{a}	33.54 ± 2.164^{b}	764.7 ± 288.2^{a}	14.61 ± 5.508^{a}	140.0 ± 63.6^{a}
S. cayannensis (Adaxial)	40.25 ± 3.182^{a}	19.25 ± 1.061^{a}	44.00 ± 6.364^{a}	27.80 ± 1.697^{a}	480.4 ± 240.5^{a}	20.07 ± 5.141^{ac}	95.3 ± 49.1^{a}
S. jamaicensis (Adaxial)	39.50 ± 2.121^{a}	15.25 ± 1.061^{a}	41.25 ± 2.475^{a}	29.00 ± 0.707^{a}	493.8 ± 273.8^{a}	18.26 ± 4.999^{ab}	96.5 ± 51.3^{a}
S. angustifolia (Abaxial)	64.00 ± 5.657^{b}	36.75 ± 1.061^{b}	43.81 ± 4.674^{a}	35.80 ± 0.424^{b}	677.8 ± 384.8^{a}	34.33 ± 5.756^{c}	150.6 ± 91.1^{a}
S. cayannensis (Abaxial)	42.50 ± 3.536^{a}	17.00 ± 1.414^{a}	42.50 ± 3.536^{a}	28.50 ± 0.707^{a}	479.4 ± 242.0^{a}	33.22 ± 6.760^{bc}	81.3 ± 47.7^{a}
S. jamaicensis (Abaxial)	42.50 ± 3.536^{a}	17.25 ± 1.061^{a}	42.75 ± 3.889^{a}	29.00 ± 0.707^{a}	498.4 ± 267.4^{a}	31.64 ± 7.488^{bc}	81.3 ± 47.7^{a}
p-value							
Epidermal surface	Ns	Ns	ns	ns	ns	**	ns
Stachytarpheta species	**	**	ns	**	ns	ns	ns

Results are in Mean ± STD
*Columns with the same superscript (or sharing a common letter) are not significantly different

Stomata width (µm) was also highest on the abaxial surface of *S. angustifolia* (35.80±0.424) and lowest on the adaxial surface of *S. cayannensis* (27.80±1.697); the analysis of variance shows a significant difference in the stomata width between *Stachytarpheta* species ($p<0.05$) but not between epidermal surface ($p>0.05$). Stomata size (μm^2) as well was highest on the adaxial surfaces of *S. angustifolia* (764.7±288.2) and lowest on the abaxial surface of *S. cayannensis* (479.4±242.0); the analysis of variance shows no significant difference in the stomata size between *Stachytarpheta* species ($p<0.05$) and between epidermal surface ($p<0.05$). On the other hand, stomata index (%) was highest on the abaxial surface of *S. angustifolia* (34.33±5.756) and lowest on the adaxial of *S. angustifolia* (14.61±5.508); the analysis of variance shows a significant difference in the stomata index between epidermal surface ($p<0.05$) but not between *Stachytarpheta* species ($p>0.05$). Epidermal cell size (µm) was highest on the abaxial surface of *S. angustifolia* (150.6±91.1) and lowest on the abaxial surface of *S. cayannensis* and *S. jamaicensis* (81.3±47.7); the analysis of variance however, shows no significant difference in the epidermal cell size between *Stachytarpheta* species ($p<0.05$) and between epidermal surface ($p<0.05$).



A. Abaxial surface of leaf of *S.jamaicensis*; B. Adaxial surface of leaf of *S.jamaicensis*;
C. Abaxial surface of leaf of *S.cayannensis*; D. Adaxial surface of leaf of *S.cayannensis*;
E. Abaxial surface of leaf of *S.angustifolia*; F. Adaxial surface of leaf of *S.angustifolia*

Figure-1: Stomata on Epidermal Surfaces Of Leaf Of Stachytarpheta Species

DISCUSSION

Stomata in the genus *Stachytarpheta* are largely diacytic occasionally anisocytic and anomocytic, this however supports the findings of Adedeji (2012). Besides, stomata type cannot be used as a taxonomic tool in delimiting any species in the genus. Dilcher (1974) stated that stomata are ecologically less significant variable characters. Metcalfe and Chalk (1950) in their work on the Verbenaceae family reported that stomata on the adaxial surface of a few species of the genus *Stachytrapheta* are in a group arrangement. Stomata abnormalities have been reported by Inamdar (1969) in the Verbenaceae family where *S. jamaicensis* and *S. cayannensis* had aborted guard cells and contiguous stomata (stomata occurring in group) in them respectively.

Studies by Shaheen et al. (2010) delimited *Althaea* and *Alcea*, the two closely related genera in the family Malvaceae using anatomical sizes of the epidermal cells. But in this study however, cell size of the epidermal cells is of little or no taxonomic significance and may not be too helpful in delimiting the three species of *Stachytarpheta* studied as indicated by the specie grouping from the Duncan Multiple Range Test (Table II). The foliar epidermis is one of the most significant taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families of leaf epidermis have been evidential (Bhatia, 1984; Adedeji, 2004). Also, the rigorous and critical anatomical study of fewer morphological characters in the context of molecular phylogenies is fruitful to integrate the strength of morphological data with those of sequence data (Hayat et al., 2009).

Leaves are amphistomatic in all three *Stachytarpheta* species studied. Epidermal cell shape on adaxial surface was generally polygonal, regularly arranged in *S. cayannensis*, irregularly arranged in the other two *Stachytarpheta* species. This delimits *S. cayannensis* from the other two species of *Stachytarpheta*. On the abaxial epidermal surface however, the epidermal cell shape was polygonal, irregularly arranged but also regularly arranged in *S. cayannensis* and these agrees with the reports of Adedeji (2012).

From the study, stomata index was found to be higher on the abaxial surface of the three species than on the adaxial, this therefore agrees with the reports from Adedeji (2012) and this however, seems to be a protective mechanism against photo-inhibition, since the adaxial surface is more exposed to solar radiations most leaves are in horizontal position (Smith et al., 1998). Stomata shape and size are important taxonomic characters (Thai and Rajput, 2009). Stace (1965) stated that stomata size may vary on the same leaf, but this does not prevent it from being used as a taxonomic character in delimiting different species within a genus. Somata index and width are also important taxonomic quantitative characters in the genus *Stachytarpheta* as shown in the study. In addition stomata shape is largely circular to elliptic in *S. cayannensis* but mostly elliptic in *S. jamaicensis* and *S. angustifolia*.

On the other hand, stomatal frequency varies from one leaf to the other and these were observed among the species in the family of many plants studied (Oyeleke et al., 2004). Homogeneous nature of stomata complex type on leaves of the species of Cucurbitaceae was responsible for 100% occurrence of stomata complex types (SCTs), where a single SCT was present on a leaf surface. Stomata density (SD) according to Esau (1977) was found to vary from one plant to another. Thus, all species of Cucurbitaceae studied possessed more SD in the abaxial surface than in the adaxial surface (Abdulrahaman and Oladele, 2003). This pattern is in corroboration with Oyeleke et al. (2004); Abdulrahaman (2009); Abdulrahaman and Oladele (2009) and Sa'adu et al. (2009). More so, stomata index (S.I) and stomata size (S.S) were higher (ranging from 22.73 – 26.91%) and larger in the abaxial surface than in the adaxial surface in most species of Cucurbitaceae where stomata occurred on both surfaces of leaves. According to Esau (1977) stomata index varied on different parts of the leaves or on different leaves of same plants. While Davis and Heywood (1963) considered stomata size to be too variable as diagnostic feature, other researchers like Wilkinson (1971) had contrary view. He indicated that stomata size showed, a much wider range in some taxa than in others, as it may sometimes be useful as diagnostic character when dealing with taxa in which size ranges are restricted.

However, the above leaf epidermal features could be said to be taxonomically significant because of discontinuities that occurred within and between genera and within family. The leaf epidermal features observed in all the fourteen species of Cucurbitaceae were enough taxonomic characters which could be implored to support hitherto external morphological characters used to classify plants in this family. In addition, based on epidermal features, some members of the family Cucurbitaceae can readily be distinguished from one another. Such epidermal features as in stomata were thus useful tools for diagnostic and taxonomic works. The approach can however, be put to use in herbal medicine and forensic science where identification and authentication of plant specimens are essential (Abdulrahaman and Oladele, 2003; Iroka et al., 2015).

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