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Leaf Epidermal Features in 14 Species of Vernonia.

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INTRODUCTION

The leaf is one of the most important organs of the plant. Studies of the leaf epidermal anatomy are available for a number of African plants (Kadiri et al., 2011; Kadiri and Olowokudejo, 2010). The foliar epidermis offers useful taxonomic characters and many taxonomic decisions have been made based on them. Not only this, the characters have facilitated proper understanding the relationship of taxa. Given that these characters are under the control of genes (Davies and Heywood, 1963), leaf epidermis has offered solutions to unravel problems of genetic diversity of some taxa. The epidermis possesses a number of important diagnostic characters that offer valuable clues to identification (Munir et al., 2011). Kadiri and Olowokudejo (2010) who studied the West African species of Ludwigia (Onagraceae) reported that there are good taxonomic accounts of the diagnostic features of the genus based on exomorphological features (Hutchinson and Dalziel, 1958). Similarly, distinguishing features were obtained from the cuticle, genetics and stem anatomy and cellular inclusions in leaf. The use of leaf epidermal features in systematic botany, though older than molecular studies, is now becoming popular just like the use of other makers like
DNA sequence and chemical compositions (Mbagwu and Edeoga, 2006; Mbagwu et al., 2007). However, it must be noted that multiple levels of evidence strengthen taxonomic practice.

Plant classification has been a subject of discussion among taxonomists over the years. Plants are classified and re-classified as soon as new evidence arise, and this has been a continuous exercise. Most plants are classified based on external morphological structures such as flowers and fruits. There is no substitute for floral morphology in plant taxonomy. When these structures are not available on plants because they are seasonal in production, other means of classification may be involved; one of which is anatomical study, especially of leaves (Davies and Heywood, 1963; Soyombo, 2012). When herbs are dried and folded, identification and authentication become rather difficult. An approach to overcome the problem of identification is the use of anatomical features of the plant materials, especially, the plant epidermis. Most leaves show dorsiventral anatomy: the upper (adaxial) and lower (abaxial) surfaces which have somewhat different anatomy and may serve different functions (Hardie, 2009). This can also be used as the taxonomic character in delimiting plants. Some characters or features on the epidermis which are useful taxonomically include epidermal cells, subsidiary cells, stomata and trichomes. These features have been used previously to resolve some taxonomic problems or to contribute to the ever-increasing taxonomic database in some genera and even families of plants (Kadiri et al., 2011; Munir et al., 2011).

Studies on the leaf epidermal anatomy of Vernonia species are too old (Oladele, 1990; Narayana, 1979). According to Oladele (1990), among the species found in Nigeria, Vernonia amygdalina and Vernonia cinerea formed an interesting pair to study, because one (V. amygdalina) is treated as a crop and the other (V. cinerea) as a weed. Narayana, (1979) cited Goodspeed (1954) who found that the trichome complement is of phyletic significance because the type of trichomes points to species origins and their relationships. According to Narayana, (1979), the hypothesis of Gleason (1923) forms a basis for comparative study of trichome patterns and schemes of phyletic groupings. Trichomes of Vernonia are very variable in their structure, development and organographic distribution (Narayana, 1979). Metcalfe and Chalk (1950) have detailed the types of trichomes in Vernonia. The report of Metcalfe and Chalk (1950; 1979) was based on fewer species of the genus and a general family account which provides scanty anatomical data. In view of this, obviously the endomorphological characteristics of the genus in Nigeria are poorly known and the present investigation was carried out so as to document those characters of systematic importance and present comparative information on the species.

**MATeRIALS AND METHODS**

**Plant Materials:**

The foliar epidermal characteristics of 14 Vernonia species collected from field trips within the five agro-ecological zones of Nigeria were used for the study.

**Methodology:**

Studies were carried out by means of light microscopy following the methods of Kadiri and Olowokudejo (2008) with slight modification. Leaf epidermal preparations involved cutting one to five centimeters square portions from the standard median portion of the leaf lamina near the mid-rib and then hydrated by boiling in water for twenty to thirty minutes. The leaf pieces were later soaked in concentrated trioxonitrate (v) acid (HNO₃) in capped specimen bottles for about eight to twenty-four hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles, and the epidermis was transferred into Petri dishes containing water for cleansing and then, epidermis were separated with forceps and mounting needles. Tissue debris was cleared off the epidermises with the fine hair brush and washed in several changes of water. Drops of different grades of Ethanol: 50%, 70%, 75% up to 100% were added in turn to harden the
Preparations were later stained with Safranin O in 50% alcohol for about five minutes before mounting in glycerine on a glass slide. The epidermis were mounted on a glass slide with upper surfaces facing up and then covered with cover-slips and ringed with nail varnish to prevent dehydration.

Slides were examined with light microscopes with x10 and x40. All measurements in the light microscope (LM) were made using a micrometer with ×40 objective. From each species, 20 cells and stomata were randomly selected for measurement. Stomata and trichome indices were calculated using the formulae of Stace (1965):

\[ \text{Stomata index} = \frac{\text{Number of stomata per unit area}}{\text{Number of cells per unit area}} \]

\[ \text{Trichome index} = \frac{\text{Number of trichomes per unit area}}{\text{Number of cells per unit area}} \]

**Analysis of Data from Leaf Epidermal Anatomy**

The statistical analysis of the leaf epidermal data was done using Microsoft Excel software. The statistical calculations included mean, standard deviation, standard error and stomata index. The calculations were carried out on both the adaxial and abaxial layers. Stomata index was calculated following the method of Stace (1965).

**RESULTS AND DISCUSSION**

**Results from Leaf Epidermal Study:**

The result from the studies on leaf epidermal anatomy is presented in Tables 1-3 as well as Plates 1-7 and these data sources were used to construct an artificial indented dichotomous key for *Vernonia* species in Nigeria as presented below: the plates.

### Table 1: Quantitative Epidermal Characters of *Vernonia* species on Adaxial Layer

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Thickness (µm)</th>
<th>Number</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Stomata Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. amygdalina</em></td>
<td>90 (103.10 ± 1.80)</td>
<td>7 (8.85 ± 0.36)</td>
<td>4 (5.20 ± 0.20)</td>
<td>1 (1.50 ± 0.50)</td>
<td>2 (4.85 ± 0.34)</td>
<td>4 (5.65 ± 0.17)</td>
<td>1 (2.00 ± 0.08)</td>
<td>19.0 (4.5 ± 1.5)</td>
</tr>
<tr>
<td><em>V. caloana</em></td>
<td>51 (60.60 ± 1.35)</td>
<td>7 (11.40 ± 0.53)</td>
<td>5 (8.15 ± 0.32)</td>
<td>1 (1.00 ± 0.00)</td>
<td>1 (4.90 ± 0.74)</td>
<td>3 (4.60 ± 0.21)</td>
<td>1 (1.25 ± 0.09)</td>
<td>1.56 (7.25 ± 0.96)</td>
</tr>
<tr>
<td><em>V. camporum</em></td>
<td>113 (144.11 ± 3.69)</td>
<td>12 (13.60 ± 0.68)</td>
<td>6 (8.10 ± 0.20)</td>
<td>2 (2.00 ± 0.00)</td>
<td>8 (9.65 ± 0.31)</td>
<td>7 (9.40 ± 0.35)</td>
<td>2 (7.25 ± 0.10)</td>
<td>4.9 (6.4 ± 0.9)</td>
</tr>
<tr>
<td><em>V. cinerea</em></td>
<td>15 (39.30 ± 4.10)</td>
<td>6 (8.90 ± 0.32)</td>
<td>5 (7.70 ± 0.48)</td>
<td>2 (2.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. glabra</em></td>
<td>39 (49.25 ± 1.50)</td>
<td>14 (18.75 ± 0.57)</td>
<td>10 (11.30 ± 0.33)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. glabra var.</em> occidentalis*</td>
<td>44 (59.80 ± 1.73)</td>
<td>10 (12.05 ± 0.51)</td>
<td>4 (8.65 ± 0.50)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. guineense</em></td>
<td>88 (120.95 ± 5.54)</td>
<td>10 (12.90 ± 0.40)</td>
<td>6 (9.70 ± 0.39)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. migeodii</em></td>
<td>38 (41.95 ± 0.66)</td>
<td>18 (24.15 ± 0.75)</td>
<td>11 (13.35 ± 0.36)</td>
<td>9 (11.15 ± 0.35)</td>
<td>9 (11.13 ± 0.35)</td>
<td>8 (8.95 ± 0.15)</td>
<td>2 (2.80 ± 0.09)</td>
<td>16.4 (21.0 ± 2.9)</td>
</tr>
<tr>
<td><em>V. myrianthza</em></td>
<td>70 (74.80 ± 0.91)</td>
<td>8 (11.20 ± 0.50)</td>
<td>4 (6.80 ± 0.36)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. nestor</em></td>
<td>76 (92.05 ± 1.97)</td>
<td>6 (10.75 ± 0.60)</td>
<td>4 (6.25 ± 0.29)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. oocphala</em></td>
<td>54 (61.35 ± 1.47)</td>
<td>13 (15.65 ± 0.43)</td>
<td>9 (10.95 ± 0.27)</td>
<td>1 (1.13 ± 0.07)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. purpurea</em></td>
<td>33 (39.65 ± 0.78)</td>
<td>12 (17.03 ± 0.69)</td>
<td>8 (10.40 ± 0.27)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. smithiana</em></td>
<td>90 (123.85 ± 3.69)</td>
<td>7 (11.45 ± 0.79)</td>
<td>6 (9.20 ± 0.48)</td>
<td>1 (1.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. tenoreana</em></td>
<td>47 (52.50 ± 0.95)</td>
<td>23 (24.30 ± 0.19)</td>
<td>10 (13.00 ± 0.44)</td>
<td>2 (2.00 ± 0.00)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Species with different letter superscripts (a–h) differ significantly from each other at 5% level of significance.
Table 2: Quantitative Epidermal Characters of Vernonia species on Abaxial Layer

<table>
<thead>
<tr>
<th>Species</th>
<th>Number(µm)</th>
<th>Length (µm)</th>
<th>Width(µm)</th>
<th>Thickness (µm)</th>
<th>Number (µm)</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Stomata Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. amygdalina</td>
<td>94 (107.55 ± 0.37)</td>
<td>105 ± 2.37</td>
<td>5 (6.10 ± 0.19)</td>
<td>2 (4.25 ± 0.00)</td>
<td>1 (1.00 ± 0.94)</td>
<td>12 (19.20 ± 0.26)</td>
<td>7 (5.30 ± 0.12)</td>
<td>1 (1.90 ± 0.34)</td>
</tr>
<tr>
<td>V. calvoana</td>
<td>100 (117.60 ± 0.58)</td>
<td>147 ± 3.12</td>
<td>8 (10.70 ± 0.37)</td>
<td>4 (6.70 ± 0.00)</td>
<td>1 (1.00 ± 0.45)</td>
<td>5 (7.60 ± 0.11)</td>
<td>5 (4.00 ± 0.11)</td>
<td>1 (1.20 ± 0.38)</td>
</tr>
<tr>
<td>V. camporum</td>
<td>56 (62.50 ± 0.21)</td>
<td>76 ± 1.36</td>
<td>10 (11.05 ± 0.11)</td>
<td>5 (5.35 ± 0.00)</td>
<td>2 (2.00 ± 1.00)</td>
<td>20 (28.55 ± 0.96)</td>
<td>6 (6.90 ± 0.26)</td>
<td>2 (2.00 ± 0.08)</td>
</tr>
<tr>
<td>V. cinerea</td>
<td>13 (56.50 ± 12c)</td>
<td>12a</td>
<td>6 (8.45 ± 0.37)</td>
<td>5 (9.10 ± 0.45)</td>
<td>1 (1.00 ± 0.25)</td>
<td>1 (1.20 ± 0.23)</td>
<td>3.0 (5.00 ± 1.05)</td>
<td>0.5 (1.00 ± 0.10)</td>
</tr>
<tr>
<td>V. glabra</td>
<td>43 (62.90 ± 0.25)</td>
<td>75 ± 1.51</td>
<td>5 (6.45 ± 0.34)</td>
<td>5 (6.45 ± 0.34)</td>
<td>1 (1.00 ± 0.63)</td>
<td>7 (13.35 ± 0.25)</td>
<td>6 (8.20 ± 0.11)</td>
<td>2 (2.35 ± 0.11)</td>
</tr>
<tr>
<td>V. guineense</td>
<td>95 (105.75 ± 0.56)</td>
<td>130 ± 1.97</td>
<td>8 (11.55 ± 0.60)</td>
<td>5 (9.10 ± 0.83)</td>
<td>1 (1.00 ± 1.52)</td>
<td>1 (1.00 ± 0.70)</td>
<td>0 (0.20 ± 0.20)</td>
<td>0 (3.84 ± 5.58)</td>
</tr>
<tr>
<td>V. migeodii</td>
<td>41 (51.85 ± 0.36)</td>
<td>63 ± 1.18</td>
<td>18 (20.25 ± 0.32)</td>
<td>10 (10.90 ± 0.32)</td>
<td>2 (3.00 ± 0.11)</td>
<td>11 (13.60 ± 0.37)</td>
<td>8 (8.95 ± 0.15)</td>
<td>2 (2.80 ± 0.09)</td>
</tr>
<tr>
<td>V. myriantha</td>
<td>54 (70.30 ± 1.92)</td>
<td>84 ± 1.20</td>
<td>8 (12.30 ± 0.46)</td>
<td>5 (7.60 ± 0.46)</td>
<td>1 (1.00 ± 0.46)</td>
<td>2 (5.40 ± 0.46)</td>
<td>3.0 (5.70 ± 0.29)</td>
<td>1 (0.20 ± 0.27)</td>
</tr>
<tr>
<td>V. nestor</td>
<td>92 (108.25 ± 0.50)</td>
<td>125 ± 2.37</td>
<td>5 (8.00 ± 0.23)</td>
<td>2 (4.30 ± 0.00)</td>
<td>1 (1.00 ± 0.89)</td>
<td>10 (18.75 ± 0.16)</td>
<td>3 (4.10 ± 0.16)</td>
<td>2 (2.10 ± 0.07)</td>
</tr>
<tr>
<td>V. ooechala</td>
<td>60 (78.40 ± 0.69)</td>
<td>108 ± 3.37</td>
<td>6 (9.10 ± 0.30)</td>
<td>4 (5.65 ± 0.30)</td>
<td>1 (1.00 ± 0.00)</td>
<td>12 (17.75 ± 0.00)</td>
<td>6 (6.00 ± 0.00)</td>
<td>2 (2.50 ± 0.12)</td>
</tr>
<tr>
<td>V. purpurea</td>
<td>43 (51.45 ± 0.99)</td>
<td>62 ± 2.54</td>
<td>9 (16.00 ± 0.24)</td>
<td>6 (9.60 ± 0.33)</td>
<td>1 (0.80 ± 0.25)</td>
<td>4 (9.65 ± 0.45)</td>
<td>5 (7.50 ± 0.25)</td>
<td>2 (2.70 ± 0.13)</td>
</tr>
<tr>
<td>V. smithiana</td>
<td>185 (193.55 ± 0.42)</td>
<td>210 ± 1.31</td>
<td>14 (21.75 ± 0.31)</td>
<td>5 (7.35 ± 0.31)</td>
<td>1 (1.00 ± 0.00)</td>
<td>1 (1.00 ± 0.00)</td>
<td>4.0 (5.60 ± 0.31)</td>
<td>1.0 (1.40 ± 0.11)</td>
</tr>
<tr>
<td>V. tenoreana</td>
<td>31 (51.25 ± 0.50)</td>
<td>73 ± 2.54</td>
<td>8 (11.50 ± 0.33)</td>
<td>5 (6.90 ± 0.33)</td>
<td>1 (1.00 ± 0.00)</td>
<td>8 (11.80 ± 0.08)</td>
<td>5 (6.15 ± 0.21)</td>
<td>2 (2.60 ± 0.17)</td>
</tr>
</tbody>
</table>

Species with different letter superscripts (a – h) differ significantly from each other at 5% level of significance.
The presence of stomata was variable. Only *Vernonia amygdalina, Vernonia calvoana, Vernonia camporum*, *V. glabra var. occidentalis* and *Vernonia migedii* were amphibistomatic whereas four others were hypostomatic namely: *Vernonia glabra, Vernonia glabra var. occidentalis, Vernonia guineense* and *Vernonia tenorea*. All other taxa...
studied possessed stomata at either the adaxial or abaxial surfaces. Stomata, when present, was always anomocytic (stoma lacks morphologically differentiated subsidiary cells in both the adaxial and abaxial surfaces of all the species studied) while epidermal cell shape was mostly polygonal with a few that were sinuous. Anticlinal wall patterns were mostly straight with a few that were undulate/curved or wavy/undulate. The taxonomic value of the leaf epidermal characters is well documented (Jayeola et al., 2001; Adedeji and Illoh 2004; Adedeji 2004; Munir et al., 2011). Adedeji and Jewoola (2008) noted that the epidermal cells of *V. cinerea* and *V. amygdalina* are slightly irregular to polygonal with wavy or undulating anticlinal walls on the adaxial surface and sinuous anticlinal walls on the abaxial surface. They also observed that the leaf surfaces were amphistomatic. The occurrence of anomocytic stomata on all the taxa studied suggests that the species are related (Kemka-Evans et al., 2014). Okoli (1987) found contiguous stomata and cuticular striations to be of the useful diagnostic feature on the leaf epidermis of *Telfairiaoccidentalis* Hook F. Mbagwu and Edeoga (2006) constantly reaffirmed the point that epidermal and cuticular traits of plants could serve as vital tools exploitable in the systematics of the present day angiosperms. Also, different shapes of epidermal cells, the type of arrangement of stomata, size and shape of trichomes and number of vascular bundles are all vital in systematic botany (Nwachukwu and Mbagwu, 2006). Munir et al., (2011) reported that the foliar epidermis is one of the fundamental taxonomic characters from biosystematics point of view and taxonomic studies of a number of plant families are made on the basis of leaf epidermis anatomy. Leaf epidermal features like the shape of epidermal cells, stomata and trichomes are useful anatomical tools. Length and width of epidermal cells is a useful aid in distinguishing varieties (Kadiri et al., 2005 / 2006). Edeoga and Osawe (1996) used the leaf epidermal morphology of some members of *Costus, Senna* and *Boerhaavia* species to establish possible relationships among the different species they investigated.

Although stomata appeared on both the upper and lower surfaces but they were more on the lower leaf epidermis except for those species which were hypostomatic. This is probably an adaptation to water loss. Metcalfe and Chalk (1960) observed the same in some dicotyledonous plants and Mbagwu and Edeoga (2006) also noticed same in *Vigna* species as well as in *Solanum* species (Mbagwu et al., 2007). Several workers have established that leaf anatomical characters are of considerable diagnostic value and may also be of assistance in elucidating taxonomic relationships (Kadiri and Olowokudejo 2010; Mbagwu et al., 2007; Stace, 1965). Metcalfe and Chalk (1950; 1979) reported that the anatomical structure of the family Compositae (which includes the genus *Vernonia*), shows considerable diversity in correlation with these habit differences whilst ecological specializations also occur. Leaves are generally dorsiventral but sometimes exhibiting ecologically specialized forms. The epidermis is not uncommon including groups of silicified cells. Rosettes of silicified cells sometimes surround the bases of the hairs. Vascular bundles of the veins are frequently provided with a distinct parenchyma. Stomata are generally ranunculaceous. An artificial indented dichotomous key constructed from leaf epidermal data obtained from this study was presented earlier which was similar to that obtained by an earlier worker for some *Vernonia* species (Soyombo, 2012).and on other species (Kadiri et al., 2011; Kadiri and Olowokudejo, 2010). Most of the keys constructed by earlier workers cited here were on other species and did not deal with *Vernonia* specifically, while the key presented by Soyombo (2012) dealt with only eight (8) species of *Vernonia*. On the other hand, the key for *Vernonia* presented in this study have considered 14 species of *Vernonia* used in the study making it the first product with the highest number of species of *Vernonia* from Nigeria, and hence giving it a unique status.

**Conclusion:** The leaf epidermal features of 14 *Vernonia* species evaluated here shows that they are diagnostic and can be used for interspecific delineation among the species.
Plate 1 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. amygdalina* and *V. calvoana* (Mag. X40) Scale bar ( ) is 50µm.
Plate 2 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. camporum* and *V. cinerea* (Mag. X40) Scale bar ( ) is 50µm.
Leaf Epidermal Features in 14 Species of *Vernonia*.

Plate 3 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. glabra* and *V. glabra* var. *occidentalis* (Mag. X40) Scale bar ( ) is 50µm.
Plate 4 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. guineensis* and *V. migeodii* (Mag. X40) Scale bar is ( ) is 50µm.
Leaf Epidermal Features in 14 Species of *Vernonia*.

Plate 5 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. guineensis* and *V. migeoidii* (Mag. X40) Scale bar ( ) is 50µm.
Plate 6 (i-iv): Photomicrographs of the epidermal surfaces of Vernonia (Adaxial and Abaxial layers) of *V. oocephala* and *V. purpurea* (Mag. X40) Scale bar is 50 µm.
Leaf Epidermal Features in 14 Species of *Vernonia*.

Plate 7 (i-iv): Photomicrographs of the epidermal surfaces of *Vernonia* (Adaxial and Abaxial layers) of *V. smithiana* and *V. tenoreana* (Mag. X40) Scale bar ( ) is 50 µm.
REFERENCES


