The Taxonomic Significance of Certain Anatomical Variation in Four Genera of Asteraceae

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ABSTRACT
Epidermal structures of four genera of Asteraceae from Akwa – Ibom State are described. The mature stomata types were diacytic, anisocytic, staurocytic, anomocytic and brachyparacytic stomata. The anisocytic stomata were the commonest. Abnormalities observed here include unopened stomatal pore, stomata with one guard cell, aborted stomata, vertical and parallel contiguous stomata and one subsidiary cell shared by two stomata variously oriented. Diacytic stomata were distributed on both surfaces of B. pilosa but absent in A. africana, E. prostrata and E. floribundus. Parallel contiguous stomata were found only in the adaxial surface of E. floribundus and vertical contiguous stomata were also found only in the abaxial surfaces of E. prostrata and B. pilosa. Leaves are amphistomatic in B. pilosa and A. africana, but hypoamphistomatic in E. prostrata and E. floribundus. Glandular trichomes occurred in the abaxial surface of B. pilosa. Crystal druses are found on the abaxial surface of E. floribundus but absent in other species surfaces studied. Other systematically useful characters are shapes of the epidermal anticlinal walls, stomata index and guard cell area can also be used for distinguishing the species.

Keywords: Asteraceae, Taxonomy, Anatomy, Leaf epidermis, Trichome, Stomata.

INTRODUCTION
The family Asteraceae is a very large cosmopolitan family consisting of about 23,000 currently accepted species, spread across 16,200 genera and 12 subfamilies and also the largest group of flowering plants [36]. The family is highly advanced, easily recognised and with worldwide distribution in the tropical and subtropical region of South Africa, West Africa and North America [4]. Asteraceae is characterized by having simple leaves, pinnately lobed some species alternate or opposite leaves in some genera; they have capitulum or head, an inferior, unilocular ovary with one ovule, and with few exceptions fused anthers surrounding the style. The flowers are mainly yellow while some are white in colour. The flowers are of two basic types: those with tubular actinomorphic corollas and those with strap-shaped or radiate zygomorphic corolla [30]. Some species in the family Asteraceae have achene structure which is called the cypsela. Cypsela is often used to determined plant relationship at the genus and species level [25].

In Nigeria, the indigenous people have utilized number of species in the Asteraceae family for various purposes; In Uganda and in Mexico, the leaves are used as an invigorating or stimulant substitute for tea; while in the Philippines the flowers are used in the preparation of a kind of wine. The flowers are a good source of nectar for honeybees, and also serves important weed of pasture as well as herbicides [17]. In Ecliptaprostrata, the plant is commonly used in hair oil all over India for health black long hair. It is reported that to improve hair growth and colour growth and colour [31]. The fresh juice of the leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. Tender leaves and young shoots are cooked and used as vegetables. The plant Aspilia africana amongst other weed was reported to be a source of protein although the quantity is not sufficient for both human and livestock demands [40]. Aspilia africana leaves is widely used in ethno-medicinal practice in Africa for its used effectively to arrest bleeding from fresh wound, growth inhibited microbial of known wound contaminants and accelerated wounds healing process [11]. Aspilia africana is thought to be used as herbal medicine by
some chimpanzees and the potential of the leaves of the haemorrhage plant is used in traditional medicine in treatment of rheumatic pains, bees and scorpion strings and in removal of opacities and foreign bodies from eyes [28]. In Mbaise and most Igbo speaking parts of Nigeria, the leaf is used in preventing conception suggesting potential contraceptive and anti-fertility properties [29]. Bidens pilosa can be used in treatment of various ailments such as coughs, laryngitis, headache, conjunctivitis, rheumatism, infections, and digestive and stomach disorders including peptic ulcers, hepatitis, diabetes, malaria, and inflammation [37]. Eclipta prostrata is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarhral jaundice and for skin diseases [32]. Erigeron floribundus: The fresh leaves from Erigeron floribundus play an important role in the folk medicine of West Africa and are used against infectious diseases (dental pain, diarrhoea, gastroenteritis, athlete’s foot, influenza) [12]. Taxonomic significance of foliar epidermis in some members of the family Asteraceae in Nigeria has been investigated [7].

MATERIALS AND METHODS

The fresh leaves of four species of Asteraceae (Bidens pilosa, Erigeron floribundus, Eclipta prostrata, and Aspilia africana) were collected on October, 2012 from a bush in Uyo local Government area of Akwa-Ibom State. The plants were authenticated by Dr. (Mrs.) U. A. Essiett, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo.

The fresh plant materials of Bidens pilosa, Erigeron floribunda, Eclipta prostrata, and Aspilia africana collected in the field were immediately taken to the laboratory for the purpose of anatomical studies; small portions were obtained from the standard median part of the leaves of mature and well expanded leaves. Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade taken from a standard median portion of the leaves on a clean glass slab, with the surfaces to be studied facing down. The specimens were irrigated with water holding it downward from one end, and then the epidermis above the desired surface was scrapped-off carefully with sharp razor blade. The loose cells was 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 strained in 1% aqueous solution of Safranin O for 4-8 minutes, rinsed carefully in water to remove excess strain and mounted in 10% glycerol.

Guard cells area was calculated by Francos constant method (Guard cells area = Length x Width x 0.7854). The stomata index was determined according to Metcalfe and Chalk [26] using the formula:

\[
\text{Stomatal Index (SI)} = \frac{S \times 100}{E + S}
\]

Where: 
- \(S\) = number of stomata per unit area
- \(E\) = number of epidermal cells in the same area.

All measurements were made with the aid of an ocular micrometer and finally converted by the ocular constant with respect to the power under which they were taken and images were computerized digitally with a Motic image plus version 2.0ml mounted on Zeiss Light Microscope.

RESULTS

While the salient features of the epidermal morphology of four genera of Asteraceae studied are summarized in Table 1 and 2, the most important characters are described in some details below:

Aspilia africana (Pers.) C. D. Adams
Epidermal cells on both adaxial and abaxial surfaces were irregular to polygonal in shape and size with undulating anticlinal cell walls. The largest epidermal cells are found on the abaxial surface (48x17µm) while adaxial surface showed the smallest cells (38x17µm) (Table 1).

**Stomata**
The distribution of stomata in the investigated taxa is amphistomatic (stomata abundant on both adaxial and abaxial surfaces). The mature stomatal types found on both surfaces are anisocytic, anomocytic, brachyparacytic and staurocytic stomata. The anisocytic stomata were abundant and evenly distributed on both surfaces. The smallest stomatal size is found on the adaxial surface (25x17µm) while the largest were found on abaxial surface (26x15µm) has shown in Table 1. Stomatal index varied, the highest stomatal index was found on the abaxial surface (30%) and the lowest stomatal index was found on the abaxial surface (14%). Guard cell area on adaxial surface off(13µm) is longer than those on the abaxial surface (11µm) as shown in table 1.

**Abnormal Stomata**
Abnormal stomata observed, are unopened stomatal pore (Plate 1B and I), stomata sharing one subsidiary cell (Plate 1C and J), stomata with one guard cell (Plate 1E) were found on both surfaces.

**Hairs**
The morphology of trichomes including their shape, size and frequency which are of characteristics different in species and hence of great interest. The Presence of unicellular non glandular trichomes straight to curve with tips found on both surfaces (Plate 1F and H). Trichomes were scanty on both surfaces. The length of trichomes varied, on the adaxial surface is (186x81µm) while that of abaxial is (232x73µm) (Table 2).

**Bidens pilosa Linn.**
Epidermal cells on the abaxial surface of *B.pilosa* are mostly irregular to polygonal while on the adaxial surface are irregular to rectangular. The largest epidermal cells were found on the abaxial surface (63x18µm) while adaxial surface showed the smallest cells (59x20µm) (Table 1). Anticlinal cell walls of both surface of *Bidens pilosa* are highly undulating.

**Stomata**
The distribution of stomata in the investigated taxa is amphistomatic (stomata abundant on the abaxial and adaxial surfaces). Stomata are randomly distributed and their axes are oriented in different direction. The mature stomatal types present were diacytic, anisocytic, staurocytic and anomocytic stomata found on both surfaces of *B. pilosa* while brachyparacytic stomata are present only on the abaxial surface (Plate 2L). The largest stomatal size was found on the adaxial surface (33x21µm) while smallest stomatal size was found on the abaxial surface (28x18µm). Stomatal index varied, the highest stomatal was found on the abaxial surface (46%) while the lowest stomatal index was found on the adaxial surface (34%). The most abundant type of stomata present on both surfaces are anisocytic stomata and guard cells area on the adaxial surface (14µm) is longer than those of the abaxial surface (9µm) Table 1.

**Abnormal Stomata**
Various abnormal stomata were observed, on both surfaces includes one subsidiary cell shared and unopened stomatal pore (Plate 2A), one guard cell (Plate 2C) and aborted guard cell (Plate 2F) found on adaxial surface while on the abaxial surface abnormal stomata found are two stomata sharing one subsidiary cell (Plate 2I), unopened stomatal pore (Plate 2K), vertical contiguous stomata (Plate 2M) and aborted guard cell (Plate 2O).

**Hairs**
The morphology of trichomes including their shape, size and frequency which are of characteristics interest. Trichomes types present are glandular and non glandular trichomes. On the adaxial surface uniseriate non glandular trichome (Plate 2E) are present while on the abaxial, glandular and uniseriate trichomes on the vein cells are also present (Plate2G and H). Trichomes are scanty on the abaxial and adaxial surfaces. The length of trichomes varied, on the abaxial surface is (219x76µm) and are larger than those on adaxial surface (184x79µm) (Table 2).

**Eclipta prostrata Linn.**
Epidermal cells of both surfaces are irregular to polygonal. The largest epidermal cells are found on the abaxial surface (55x18µm) while adaxial surface showed the smallest cells (39x17µm) (Table 1). Anticlinal cell walls on adaxial surface are straight to slightly undulating and undulating on abaxial surface.

**Stomata**
The distribution of stomata on the investigated taxa is hypo- amphistomatic (stomata abundant on abaxial surface and scantly on adaxial surface). The mature stomatal types found on the abaxial surface are mostly anisocytic and staurocytic (Plate 3A-B), and on adaxial surface anisocytic, staurocytic (Plate 3H) and brachyparacytic stomata (Plate 3L) are present. The most abundant types of stomata are
anisocytic stomata found on both surfaces. The smallest stomatal size is found on the adaxial surface (36x12µm) while the largest were found on abaxial surface (21x17µm). Stomatal index varied, and the highest stomata index was found on the abaxial surface (37%) and the lower stomatal index was found on the adaxial surface (26%). Guard cell area on adaxial surface of (12µm) is longer than that of the abaxial surface (10µm) has shown in Table 1.

**Abnormal Stomata**

Various abnormal stomata were observed on both surfaces include stomata sharing one subsidiary cell (Plate 3B and J), vertical contiguous stomata (Plate 3C), aborted guard cell (Plate 3D and I), unopened stomatal pore (Plate 3E) and one guard cell (Plate 3F and K) were found on the abaxial and adaxial surfaces.

**Hairs**

The morphology of trichomes including their shape, size and frequency which are of characteristics interest. Trichomes types present are uniseriate non glandular trichomes found on both surfaces (Plate 3G and M). Trichomes on the abaxial surface (213x64µm) are larger than the adaxial surface (177x62µm) Table 2. Trichomes are abundant on the abaxial surface and scanty on the adaxial surface.

**Erigeron floribundus** (Kunth) Sch. Bip

Epidermal cells were slightly rectangular to polygonal on adaxial surface and irregular to polygonal on abaxial surface. The largest epidermal cells are found on the adaxial surface (68x35µm) while abaxial surface shows the smallest cells (55x21µm) (Table 1). Anticlinal cell walls of both surfaces are straight to slightly undulating.

**Stomata**

The distribution of stomata in the investigated taxa is hypoamphistomatic (stomata abundant on the abaxial surface and scanty on adaxial surface). The mature stomatal types found on the both surfaces are anisosytic, anomocytic, brachyparacytic and staurocytic stomata. Anisosytic stomata are abundant and evenly distributed on both surfaces and their axes are oriented in different directions. The smallest stomatal size is found on the adaxial surface (29x20µm) while the largest were found on abaxial surface (30x20µm). Stomatal index varied, the highest stomatal index was found on the abaxial surface (32%) and the lower stomatal index was found on the adaxial surface (15%). Guard cell area on adaxial surface (17µm) is longer than that on the abaxial surface (14µm) as shown in Table 1.

**Abnormal Stomata**

Various abnormal stomata were observed on both surfaces, these include parallel contiguous stomata shared (Plate 4C) found on the adaxial surface while on abaxial surface one subsidiary cell shared are present (Plate 4G).

**Hairs**

The morphology of trichomes including their shape, size and frequency which are of characteristics interest. Trichomes types present are uniseriate non glandular trichomes on both surfaces. Trichomes are abundant on both surfaces. Trichomes are long and wide, adaxial surface (176x82µm) while abaxial surface (242x59µm) (Table 2). Crystal druses were present on the abaxial surface (Plate 4E) but absent on the adaxial surface.

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**TABLE 1: EPIDERMAL FEATURES OF THE LEAVES OF FOUR GENERA OF ASTERACEAE STUDIED.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Stomatal size(µm)</th>
<th>Epidermal cells size (µm)</th>
<th>Guard cells Area (µm)³</th>
<th>Stomata index (%)</th>
<th>Stomata distribution</th>
<th>Epidermal cell wall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bidens pilosa</strong></td>
<td>33x21</td>
<td>28x18</td>
<td>59x20</td>
<td>14</td>
<td>9</td>
<td>34</td>
</tr>
<tr>
<td><strong>Aspilia africana</strong></td>
<td>25x17</td>
<td>26x15</td>
<td>38x17</td>
<td>13</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td><strong>Eclipta prostrata</strong></td>
<td>21x17</td>
<td>36x12</td>
<td>39x17</td>
<td>12</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td><strong>Erigeron floribundus</strong></td>
<td>29x20</td>
<td>30x20</td>
<td>68x35</td>
<td>17</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>
TABLE 2: TRICHOMES CHARACTERISTICS OF THE LEAVES OF FOUR GENERA OF ASTERACEAE STUDIES. TRICHOMES MEASUREMENT (µm)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Glandular trichomes</th>
<th>Non-glandular trichomes</th>
<th>Moniliform trichomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad</td>
<td>Ab</td>
<td>Ad</td>
</tr>
<tr>
<td>Bidens pilosa</td>
<td>-</td>
<td>147x74</td>
<td>184x79</td>
</tr>
<tr>
<td>Aspilia africana</td>
<td>-</td>
<td>-</td>
<td>186x81</td>
</tr>
<tr>
<td>Eclipta prostrata</td>
<td>-</td>
<td>-</td>
<td>177x62</td>
</tr>
<tr>
<td>Erigeron floribundus</td>
<td>-</td>
<td>-</td>
<td>176x82</td>
</tr>
</tbody>
</table>

KEYS: Ad - Adaxial surface (upper), Ab - Abaxial surface (lower)
Plate 1G: B.S.P, Brachyparacytic, (A.S) Anisocytic and A.S.O.S, anomocytic of *A. africana* (lower surface) x400

Plate 1J: S.S, Two Staurocytic stomata sharing one subsidiary cell of *A. africana* (lower surface) x400

Plate 1I: U.S.P, Unopened stomatal pore of *A. africana* (lower surface) x400

Plate 1H: U.N.T, Unicellular trichome of *A. africana* (lower surface) x400

Plate 2A: A.S, Anisocytic, (S.S) Staurocytic, one subsidiary cell shared and U.S.P, Unopened stomatal pore of *B. pilosa* (upper surface) x400

Plate 2B: D.S, Diacytic, (A.S) Anisocytic, and (U.S.P) Unopened stomatal pore of *B. pilosa* (upper surface) x400
Plate 2C: O.G.C, One guard cell and 2 stomata sharing one subsidiary cell of *B. pilosa* (upper surface) x400

Plate 2D: D.S Diacytic and (A.S) Anisocytic stomata of *B. pilosa* (upper surface) x400

Plate 2E: U.S.T, Uniseriate trichome of *B. pilosa* (upper surface) x400

Plate 2F: A.G.C; Aborted guard cell of *B. pilosa* (upper surface) x400

Plate 2G: U.S.P, Uniseriate trichome of *B. pilosa* (Lower surface) x100

Plate 2H: G.T, Glandular trichome on vein cell of *B. pilosa* (Lower surface) x100

Plate 2I: A.S, Anisocytic and two stomata sharing subsidiary cell of *B. pilosa* (Lower surface) x400

Plate 2J: D.S, Diacytic stomata of *B. pilosa* (Lower surface) x400
Plate 2K: U.S.P, Unopened stomatal pore of *B. pilosa* (Lower surface) x400

Plate 2L: B.P.S, Brachyparacytic stomata of *B. pilosa* (Lower surface) x400

Plate 2M: V.C.S, Vertical contiguous stomata of *B. pilosa* (Lower surface) x400

Plate 2N: A.S.O.S, Anomocytic stomata of *B. pilosa* (Lower surface) x400

Plate 2O: A.G.C, Aborted guard cell of *B. pilosa* (Lower surface) x400

Plate 3C: V.C.S, Vertical contiguous stomata of *E. prostrata* (Lower surface) x400

Plate 3D: A.G.C, Aborted guard cell and (A.S.O.S) Anomocytic stomata of *E. prostrata* (Lower surface) x400
Plate 3E: U.S.P, Unopened stomatal pore of *E. prostrata* (Lower surface) x400

Plate 3F: O.G.C, One guard cell of *E. prostrata* (Lower surface) x400

Plate 3G: U.S.T, Uniseriate trichome of *E. prostrata* (Lower surface) x400

Plate 3H: S.S, Staurocytic and (A.S) Anisocytic of *E. prostrata* (Upper surface) x400

Plate 3I: A.G.C, Aborted guard cell of *E. prostrata* (Upper surface) x400
Plate 3J: One subsidiary cell sharing two (S.S) staurocytic stomata of *E. prostrata* (Upper surface) x400

Plate 3K: O.G.C, One guard cell of *E. prostrata* (Upper surface) x400

Plate 3L: B.P.S, Brachyparacytic stomata of *E. prostrata* (Upper surface) x400

Plate 3M: U.S.T, Uniserate trichome of *E. prostrata* (Upper surface) x100

Plate 4A: A.S.O.S, Anomocytic stomata and (AS) Anisocytic stomata of *E. floribundus* (Upper surface) x400

Plate 4B: S.S, Staurocytic stomata of *E. floribundus* (Upper surface) x400

Plate 4C: P.C.S, Parallel contiguous stomata of *E. floribundus* (Upper surface) x400

Plate 4D: U.S.T, Uniseriate trichome of *E. floribundus* (Upper surface) x100
DISCUSSION

Taxonomic relevance of vegetative anatomy in taxa delimitation recognition has been reported by Tomblinson [39, 35, 23]. The current study of the epidermal characters of the four genera is quite significant because they distinctly separate the taxa under study. The functions of epidermis are water regulation, protection against sunlight and defence to other organisms [24].

The present investigation described the epidermal structure of four genera of Asteraceae. Epidermal cells varied in shape and size from irregular to polygonal with straight to slightly undulating anticlinal cell walls in some genera. The anticlinal cell walls are highly undulating on *A. africana* and *B. pilosa* and on abaxial surface of *E. prostrata* and straight to slightly undulate onadaxial of *E. prostrata* and on both surfaces of *E. floribundus*. The epidermal cell size varies significantly and can fairly use for the separation the species in this group. The largest epidermal cells observed in *E. floribundus* (68x35µm) while *A. africana* shows the smallest cells (38x17µm) (Table 1). The stomata distributions on the investigated taxa were amphistomatic in *B. pilosa* and *A. africana* but hyp amphistomatic in *E. floribundus* and *E. prostrata*. The presence and combination of different types of stomata on the surfaces of leaves can be useful in the classification and delimitation. The findings in the four genus of Asteraceae studied exhibited this; it is possible for most species to have more than three types of stomata. This has been shown by Patel and Inamdar [45] on some Polemoniales. Adeleji and Jewoola [3] on the variation in the structure and development of foliar stomata in the family Asteraceae. The presence of various types of stomata in the taxa is of taxonomic interest in these studies, the presence of diacytic stomata in both surfaces of *B. pilosa* and absent of brachyparacytic stomata in both surface of *E. floribundus* distinguishes them from other species, also brachyparacytic was found on both surfaces of *A. africana*, abaxial surface of *B. pilosa* and on adaxial surface of *E. prostrata*. The presence of anisocytic stomata in both surfaces of *B. Pilosa, E. prostrata, A. africana* and *E. floribundus* shows their relationship in the family Asteraceae. The highest stomatal size is observed on the abaxial surface (36x12µm) of *E. prostrata* also adaxial surface recorded the smallest (21x17µm).

Other important characters presence is abnormal stomata observed in all the species surfaces studied. These include vertical contiguous stomata found on the abaxial surfaces of *E. prostrata* and *B. pilosa* while
parallel contiguous stomata are present only on the adaxial surface of E. floribundus and one subsidiary cell shared by two stomata are present in all the species surfaces studied except on the adaxial surface of E. floribundus. Aborted guard cells were found on both surfaces of B. pilosa and E. prostrata but absent in A. africana and E. floribundus. Unopened stomatal pore are present on both surfaces of B. Pilosa and A. africana and on abaxial surface of E. prostrata but absent on both surface E. floribundus which can be used in separation of the species. The importance of abnormalities in leaves has been the result of environmental factor as confirmed by Carr and Carr [9].

Stomatal Index is independent of the environment size or portion of the leaf surface size of the leavesepidermal cell [27] and it is a reliable factor for identification.

The variation in stomatal index observed in these studies can be reasonable employed in delimiting the genera in Asteraceae. Stomatal index is highly constant for any given species and the value is more uniform upon the abaxial than the adaxial surface. The role of stomatal index in systematic work to separate species has also been reported by Abdulrahaman and Oladele [1, 7]. The highest stomatal index was found on the abaxial surface of B. pilosa (46%) while the smallest stomatal index is found on the adaxial surface of A. africana (14%).

The guard cell area on adaxial surface of E. floribundus (17µm) are longer than those on the abaxial surface of E. prostrata (9µm) and Stomatal index can also be useful for Identification of the studied taxa. Trichomes are of different shapes and sizes in Asteraceae and are of great diagnostic interest. Uniseriate non-glandular hairs are present in both surfaces of the four genera studied and also glandular trichome are found only on abaxial surfaces of B. pilosa. The long unicellular hairs in them serve to reduce the rate of transpiration in the plants. The importance of trichomes in taxonomy has been highlighted by Stace [34, 20, 18, 19]. Corsi [10] suggested that the primary function of stinging trichomes might be the regulation between the plant and environment.

At the specific level, studies of trichomes have been found to be of value by many workers [4, 15]. Many researchers have found the presence or absence and types of trichomes on the epidermal surface as classification tools [13, 43, 44].

According to Metcalfe and Chalk [42] the presence of particular types of epidermal trichomes can frequently delimit species, genera or families in plant. Isawummi [21] in his study on the genus Jatropha that the difference in trichomes types is more useful taxonomically in discrimination of species into sections than any of the other epidermal characters. In these studies, trichomes occurred on both surfaces of B. pilosa, E. prostrata, A. africana and E. floribundus. Although quantitatively the variation in trichome length observed in this study can be reasonable employed in delimiting the species.

The importance of crystals in taxonomy as diagnostic tools was emphasized by Terwelle [38, 16] went further to mention that their mode of distribution is equally important in taxonomy. The presence of different types of crystals of calcium carbonate and their distribution in the leaf epidermal are therefore of interest. Crystal druses were present only on the abaxial surface of E. floribundus among the species studied.

In conclusion, foliar epidermal anatomy with special reference to stomata, trichomes, crystals in the studied genera, many serve as a valuable supportive taxonomic tool.

REFERENCES


Essiett and Archibong


*Citation of this article*