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Leaf Epidermal Structures and Stomata Ontogeny in Some Members of the Family Cucurbitaceae

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Authors' contributions

This work was carried out in collaboration between both authors. Author SMJ designed the study, and wrote the first draft of the manuscript and managed the literature searches. Author BHJ managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this research work is to study the leaf epidermal structures and stomata ontogeny of some members of the family Cucurbitaceae.
Study Design: Research Article.
Place and Duration of Study: Department of Biological Science, Bayero University Kano (BUK), between November 2012 and February, 2013.
Methodology: The leaves of *Citrullus lanatus*, *Cucumis sativus* and *Cucurbita pepo* were collected from Imawa Village of Kura Local Government Kano state with a global positioning of N 11º 48' 14.6' ' E 008º 48' 45.4". Epidermal peels of both abaxial (lower) and adaxial (upper) surfaces were made by placing the leaf on a clean glass slab with the surfaces to be studied facing downward. The specimens were irrigated with water holding it downward from one end, the epidermis above the desired surfaces was scrapped off carefully with a sharp razor blade, loosen cells were washed

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away from the epidermal peel with the aid of soft camel hairbrush and water until the desired epidermis below was reached. The epidermal peels were mounted in glass slide stained with aqueous solution of safranin for 4-8 minutes, then rinsed carefully in water to remove excess stain, a drop of 50% paraffin was added and examined using light power microscope at (x40) objective magnification. For the study of stomata types, epidermal peel was made using mature leaves and for stomata ontogeny fresh immature leaves were used. Measurement of stomata was made with the aid of an ocular micrometer and stage micrometer. The data obtained of lower surface was subjected to analysis of variance (ANOVA) at 5% level of significance to determine the least significant difference (LSD).
Results: *Stomata Types:* In *Citrullus lanatus* mature stomata are anomocytic, In *Cucumis sativus* the stomata are anisocytic and present on abaxial (lower) surface only and in *Cucurbita pepo* are anomocytic stomata.
Epidermal Cells and Trichomes: In *Citrullus lanatus* epidermal cells on lower and upper surface is irregular in shape, unicellular non glandular epidermal hair (trichome) were present in upper surface, *Cucumis sativus* the epidermal cells were polygonal or irregular in shape, unicellular non-glandular trichomes were present in upper and lower surface.
Stomatal Ontogeny: In all the species studied mesogenous stomata development was observed
Conclusion: The species can be distinguished by the type of stomata and variation in stomatal index, there were similarities based on stomata development between the three species.

Keywords: Abaxial surface; adaxial surface; epidermal cell; epidermal peel; epidermal structure; systematics; taxonomy.

1. INTRODUCTION

Plant classification has been a subject of discussion among plant taxonomists and systematists over the years, plants are classified and reclassified as soon as new evidences arise and this is going to be a continuous exercise over some years to come [1]. Most plants are classified based on external morphological structures such as flowers and fruits [2]. These structures are not always available on plants because they are seasonal in production. Due to this reason, other means of classification need to be involved; one of which is anatomical studies especially of leaves [3].

The epidermis is the outer layer of cells covering the leaf [4]. It has several functions: Protecting the plants against water loss by transpiration, regulation of gaseous exchange, secretion of metabolic compounds and absorption of water [5]. Most leaves show dorsiventral anatomy: The upper (adaxial) and lower (abaxial) surfaces have somewhat different construction and may serve different functions [6]. It can also be used as taxonomic character delimiting plants, some characters or features on the epidermis which are useful taxonomically include several differentiated cell types: Epidermal cells, stomata, subsidiary cells, guard cells and epidermal hairs (i.e., trichomes) [5]. These features have been used previously to resolve some taxonomic problems or to contribute to ever increasing taxonomic data base in some genera and even families of plants [7-9]. Continuity and/or discontinuity of the characters can be used to show relationships and differences between plant taxa within a genus or between families [1].

Stomata in dicotyledon may originate by division resulting in an oblique wall in an epidermal cell, the smaller cell resulting from this division functions as the guard cell mother cell, in many monocotyledons the guard cell mother cell is formed by an asymmetric division of an epidermal cell, the process is similar to the formation of some trichoblast in the root epidermis [10]. These complexes are generated by a series of cell divisions that are stereotypical yet dynamic, during organogenesis, a protodermal cell faces a decision to undergo proliferative division to form pavement cells or undergo asymmetric division to initiate stomatal lineages [5]. A subset of protodermal cells, called meristemoid mother cells (MMC), divides asymmetrically to generate two daughter cells with distinct sizes and fates, the larger cell adopts pavement-cell identity where the smaller, triangular cell is called a meristemoid, which possesses a transient, stem-cell like property and continues a few rounds of asymmetric division [11]. In each asymmetric division of a meristemoid, the smaller daughter cell renews its property as a meristemoid, while amplifying the number of surrounding cells. The meristemoid

then differentiates into a round, guard mother cell (GMC), which divides symmetrically once to give rise to a pair of guard cells [12]. The larger daughter cells produced by the amplifying asymmetric divisions of the meristemoids are termed stomatal-lineage ground cells [13]. The SLGCs resemble the pavement cells. The SLGC may divide asymmetrically to give rise to a secondary, satellite meristemoid, which differentiates into a secondary satellite stomatal complex [14]. The SLGCs of a secondary stomatal complex may divide asymmetrically to generate a tertiary meristemoid, and so on [12]. Three categories of stomatal development are known in angiosperm termed mesogenous and perigenous and a third mesoperigenous where some adjacent cells were derived and some were not derived from guard cell initials, these three main categories are further subdivided into ten categories and more recent authors have increased this total considerably [2]. It must be emphasized that there is no strict correlation between mature stomatal type and the mode of development [6]. In particular, the presence of subsidiary cells does not necessarily indicate a mesogenous development (and their absence a perigenous one) [1]. A study on stomatal development and application of these descriptive terms which are now equally applied to all vascular plants often elicits more useful taxonomic character than the use of mature characteristics alone, especially when distinctive subsidiary cells develop at a late stage in a perigenous fashion or when a mesogenous development does not give rise to a distinguishable subsidiary cells [15]. However, development studies do not give extra data for example; the anomocytic and anisocytic stomata of *Streptocarpus* develop perigenously and mesogenously respectively [16].

In most species the number and arrangement of the subsidiary cells around the stomata are relatively constant; various classifications of stomata according to these arrangements have been drawn up, and are sometimes useful to taxonomist [17,10].

Four types of stomatal complex that occur especially in certain families of dicotyledons, and to which he gave family names rather more descriptive names to these types as follows:

Anomocytic; Stoma surrounded by a limited number of cells that are indistinguishable in size, shape, or form from those of the remainder of the epidermis (irregular-celled) type.

Anisocytic; Stoma surrounded by three cells of which one is distinctly smaller or larger than the other two (unequal-celled) types.

Paracytic; Stoma accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells (parallel-celled) type.

Diacytic; Stoma enclosed by a pair of subsidiary cells whose common wall is at right angles to the guard cells the (cross-celled) type.

The leaves of family Cucurbitaceae have amphistomatic or hypostomatic and anomocytic type of stomata [18,19]. However study shows that the stomata type in Cucurbitaceae is anisocytic, paracytic and diacytic [20]. The type of stomata in *Curcurbitaceae* is anomocytic, anisocytic and paracytic, and the leaves are amphistomatic or hypostomatic [20-22,15].

1.1 Aim

The aim of this work is to show some anatomical characters of the family Cucurbitaceae.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *Citrullus lanatus*, *Cucumis sativus* and *Cucurbita pepo* were collected from Imawa Village along Zaria road, Kura Local Government of Kano state. The place has a global positioning system of N 11º 48' 14.6'' E 008º 48' 45.4''

2.2 Epidermal Peel

Epidermal peels of both abaxial (lower) and adaxial (upper) surfaces were made by placing the leaf on a clean glass slab with the surfaces to be studied facing downward, the specimens were irrigated with water holding it downwards from one end, the epidermis above the desired surfaces was scrapped off carefully with a sharp razor blade loose cells were washed away from the epidermal peel with the aid of soft camel hairbrush and water until the desired epidermis below was reached, the epidermal peels were mounted in glass slide stained with aqueous solution of safranin for 4-8 minutes, then rinsed carefully in water to remove excess stain, a drop of 50% paraffin was added and examine using light power microscope at (x40) objective magnification [23].

Fig. 1.0. Photograph showing seedlings of watermelon

Fig. 1.1. Photograph showing cucumber seedlings

Fig. 1.2. Photograph showing pumpkin seedlings

2.3 Study of Stomata Types and Measurement

For the study of stomata types the epidermal peel was made using mature leaves, for stomata ontogeny a fresh immature leaves was used to study stomata ontogeny in the member of the family Cucurbitaceae, stomata measurement were made with the aid of an ocular micrometer and stage micrometer, at first stage the ocular micrometer was placed carefully in the ocular lens, the stage micrometer was then placed and focused, the point where the ocular micrometer and stage micrometer calibration met was taken and arrived at:

Ocular micrometer/stage micrometer = 20/60 = 0.3 mm.

All the measurements were later done using ocular micrometer after which each value was multiplied using above scale [24,11]. The number of stomata per field and epidermal cells per field was counted and the stomatal index was determined Using the formula: S / E+S x 100 = Stomatal Index (S.I) [19]

Where

S - Number of stomata per unit area
E - Number of epidermal cells in the same area.

2.4 Statistical Analysis

The data obtained of lower surface was subjected to analysis of variance (ANOVA) at 5% level of significance to determine the least significant difference (LSD) between the three members of the family Cucurbitaceae. In upper surface t-test was used to determine whether there was significant difference between the two species.

3. RESULTS

Three Members of the Family Cucurbitaceae were studied and the following result was obtained:

3.1 Type of Stomata

In *Citrullus lanatus* mature stomata are anomocytic, the stomata type of stomata in lower surface of *Citrullus lanatus* is shown in plate 3.0.

In *Cucumis sativus* the stomata are anisocytic and present in abaxial (lower) surface only, the anisocytic stomata in lower surface of *Cucumis sativus* is shown in plate 3.1.

Stomata in *Cucurbita pepo* are anomocytic, the anomocytic stomata on lower surface of *Cucurbita pepo* is shown in plate 3.2.

3.2 Epidermal Cells and Trichomes

In *Citrullus lanatus,* epidermal cells on lower and upper surface are irregular in shape, and unicellular non glandular epidermal hair (trichome) are present in upper surface as shown in plate 3.3.

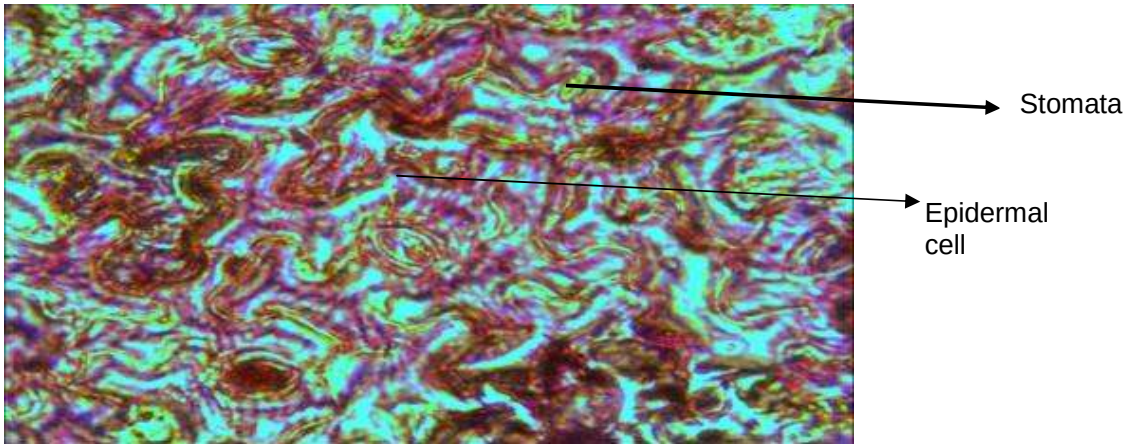

Plate 3.0. Photomicrograph showing anomocytic type of stomata in lower surface of *Citrullus lanatus* (x100)

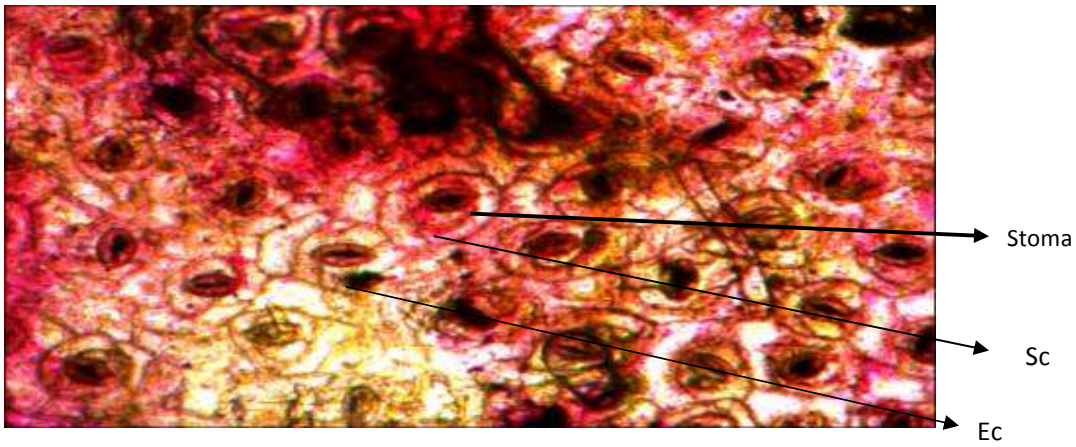

Plate 3.1. Photomicrograph showing anisocytic stomata lower surface in *Cucumis sativus* (x100)

Sc= subsidiary cell, Ec= epidermal cell

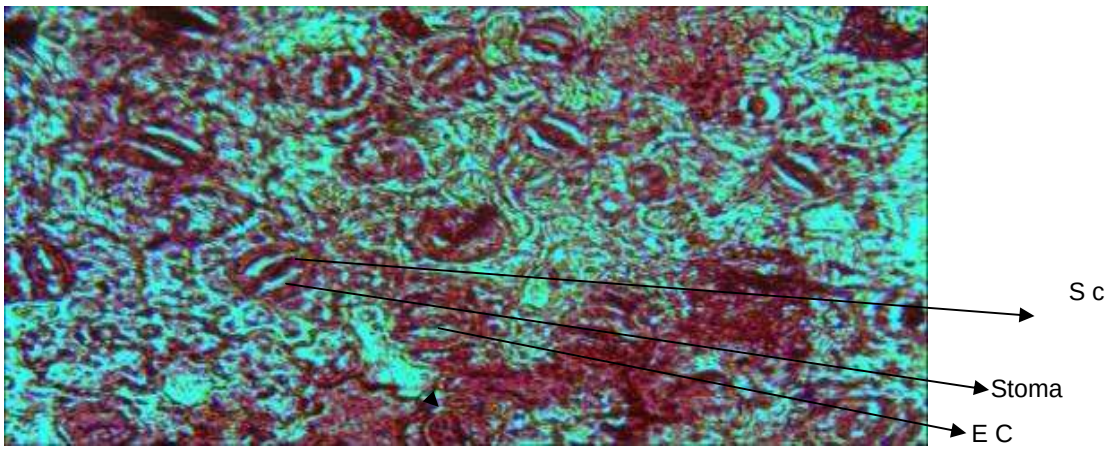

Plate 3.2. Photomicrograph showing anomocytic type of stomata in lower surface of *Cucurbita pepo* (x100)

Sc= Subsidiary cell, Ec= Epidermal cell

Cucumis sativus the epidermal cells are polygonal or irregular in shape; unicellular non-glandular trichomes are present in upper and lower surface. Trichome in the upper surface of the epidermal peel is shown in plate 3.4.

3.3 Stomatal Ontogeny

In all the species studied mesogenous stomata development were observed, the stomata develop from a meristemoid which function directly as a guard mother cell the stomata is formed by an asymmetrical division of proterdermal cells and other two division resulted in the partitioning of the precursor into the guard cell mother cell after the formation of the guard cells the growth adjustment make the subsidiary cells appear as part of stomata complex. The mesogenous stomata development is shown in plate 3.5.

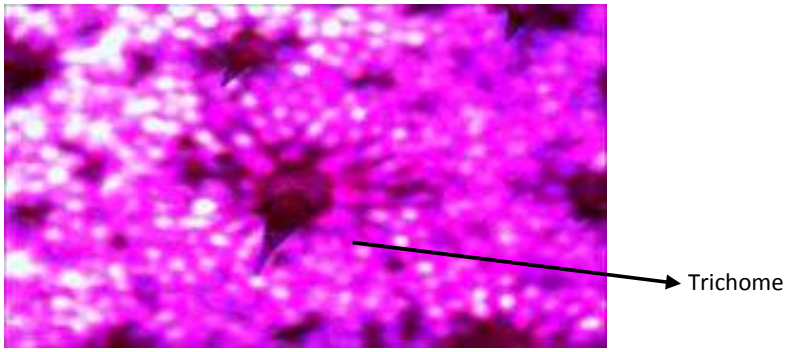

Plate 3.3. Photomicrograph showing unicellular uniseriate trichome in *Citrullus lanatus* upper surface (x100)

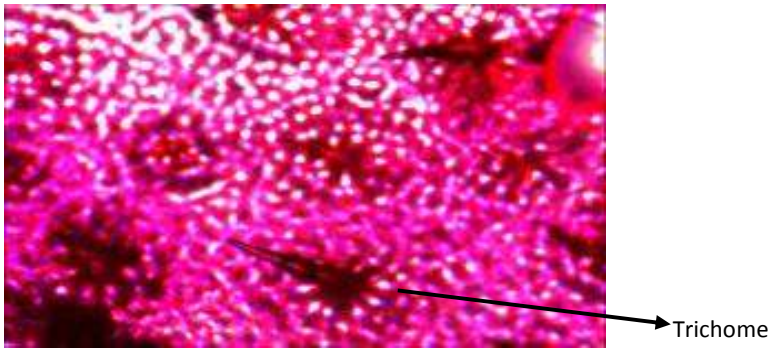

Plate 3.4 Photomicrograph showing unicellular uniseriate trichome in upper surface of *Cucumis sativus* (x100)

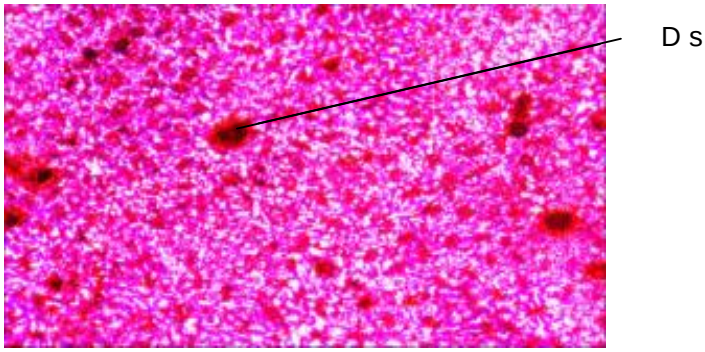

Plate 3.5. Microphotograph showing developing stomata in *Citrullus lanatus* (x100)
Key: DS= Developing stomata, for the above micrographs; On the scale of ×100 magnification; 1 mm=1000 µm

3.4 Stomata Measurement

The mean difference between stomata measurement and the least significance difference were compared at 5% level of significance to determine the least significant difference among the three species studied and the following results were obtained:

3.4.1 Lower surface

The various stomata measurement of the lower surface of the three species is shown in the Table 3.0.

3.4.2 Upper surface

For the two means of upper surface between *Citrullus lanatus and Cucurbita pepo*, t-test were used to test the differences between the two at 5% t- distribution as shown in Table 3.1.

4. DISCUSSION

4.1 Stomata

Leaf epidermal structures of the three members of the family Cucurbitaceae were studied based on the occurrence of stomata, the species can be categorized into two; those in which stomata occur on both abaxial and adaxial surface, *Citrullus lanatus* (water melon) and *Cucurbita pepo* (pumpkin). And those in which the stomata occur on only adaxial surface that is *Cucumis sativus* (cucumber). The leaves whose stomata occur on both surfaces are termed amphistomatic and those whose stomata occur on abaxial surface only are termed hypostomatic leaves. In *Citrullus lanatus* and *Cucurbita pepo* the stomata are anomocytic in which the surrounding cells are indefinite in number and do not differ from the other epidermal cells, the stomata are more numerous on abaxial surface than on adaxial surface [20,25,1,26]. In *Cucumis sativus* the stomata are anisocytic that is three subsidiary cells surround the stomata, one cell being considerably smaller or larger than the other two, the stomata occur on abaxial surface only, the leaf of *Cucumis sativus* is therefore said to be hypostomatic leaf. Similar observations were made [24,25,19]. Stomata index are higher on abaxial surfaces than on adaxial surfaces [20,27].

4.2 Epidermal Cells and Trichome

The epidermal cells are polygonal or irregular in shape in the three species studied. Trichomes are another taxonomic significance of the species studied. Unicellular non glandular, uniseriate trichome was found on both abaxial and adaxial surfaces of *Cucumis sativus* and occurs on upper surface in *Citrullus lanatus*. In *Cucubiata pepo* no trichome was observed,

Table 4.0. Mean stomata measurement in some members of Cucurbitaceae abaxial at (lower) surface of three species (x400) mm

S_no.	Species	Type of stomata	Length of stomata	Breadth of stomata	Pore length	Pore breadth	No. of stomata per field	No. of epidermal cell per field	Stomta index
1	*Citrullus lanatus*	Anomocytic	3.27	2.16	2.22	1.05	22.00	50.30	30.32
2	*Cucumis sativus*	Anisocytic	3.12	2.10	2.28	1.02	21.10	52.20	28.59
3	*Cucurbita pepo*	Anomocytic	3.24	2.13	2.28	1.08	23.20	50.10	34.14
	LSD 5%		1.837 Ns	0.522 Ns	0.244 Ns	0.142 Ns	7.095 Ns	6.896 Ns	2.120 S

Key: Ns = not significant, s = significant

Table 4.1. Mean of stomata measurement in some members of Cucurbitaceae at adaxial (upper) surface of two species (x400) mm

S/N	Species	Type of stomata	Length of stomata	Breadth of stomata	Pore length	Pore breadth	No. of stomata per field	No. of epidermal cells per field	Stomata index
1	*Citrullus lanatus*	Anomocytic	3.13	2.13	2.16	1.02	14.00	50.30	23.03
2	*Cucurbita pepo*	Anomocytic	3.18	2.06	2.06	1.05	13.50	49.30	21.47
	Difference of means		*0.23*	*1.03*	*1.17*	*0.58*	*0.26*	*0.16*	*0.93, table value at 5% = 2.265*

Similar observation was made and many researchers have found the presence or absence and types of trichomes on the epidermal surfaces as classification tool [20,5,23,28].

4.3 Stomata Ontogeny

In all the species studied, mesogenous stomata development was observed. Stomata originate by a division resulting in an oblique wall in epidermal cell; stomata develop from a merestimoid which function directly as a guard mother cell. it is formed by asymmetrical division of protodermal cells, the first division occurs when a neutral protodermal cell becomes a meristemoid mother cell defining characteristics of the meristemoid mother cell in the asymmetrical division of the cell into one daughter cell called ameristemoid and another larger daughter cell [26]. The small meristemoid cell has two options; it may remain in the same state or undergo a second cell transition to guard mother cell which divides asymmetrically just like its mother to generate one small daughter cell. The small meristemoid daughter cell undergoes a second division to a guard mother cell. The guard meristemoid mother cell undergoes a simultaneous third cell division to become a mature guard cell, the mature guard cell does not divide, it exhibits a characteristic shape which allows it to carry out its highly specialized role in gas exchange [25,28].

4.4 Stomata Measurement

The mean of lower surface measurement was compared at 5% level of significance. Significant difference was found in stomata index among the three species studied. The variations in stomatal index observed in this study can be reasonably employed in Separation of the species [5,19,29,15], systematic relevance has been documented [24]. No significant difference was found in terms of length and breadth of stomata, pore length and breadth of the stomata. For upper surface, t-test was used to test the differences between *Citrullus lanatus and Cucurbita pepo*. After comparing the means of the two species, no significant difference was found as the table value is higher than the calculated value in the two species.

5. CONCLUSION

Based on leaf epidermal features the species studied can be distinguished from one another by stomata types. The data from this study has provided evidences for delimiting of the species to some extent based on stomata type and index. The presence of various stomata is of taxonomic interest in this study which can be used to distinguish the species; two of which are amphistomatic and another is hypostomatic. Based on stomata ontogeny, the species showed one type of development which is mesogenous. Similarities were observed based on development of stomata [25].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oyeleke MO, Abdulrahaman AA, Oladele FA. Stomatal anatomy and transpiration rate in some afforestation tree species. NISEB J. 2004;4:83-90.
2. Olorode O. Taxonomy of West African flowering plants. Longman Publishing Company, New York. 1984;25-32.
3. Davis PA, Heywood VH. Principle of angiosperm taxonomy. Oliver and Boyd, Edinburgh. 1963;210-230.
4. Dutta AC. Botany for degree students (26th Ed.) Oxford University Press, London. 2009;155,565-566.
5. Adedeji O, Jewoola DA. Importance of leaf epidermal characters in the asteraceae family. Notulae Botanicae. Horti, Agrobotani cluj-Napoca. 2008;36(2):7-16.
6. Hardie J. Autum leaves seen through herbivour eye. Proc. Royal Soc. Biology. Sci. 2009;276:121-127.
7. Saheed SF, Illoh HC. A taxonomic study of some species in *Cassiinae* (Leguminosae) using leaf epidermal characters. Not. Bot. hort. Agrobot. 2010;38:21-27.
8. Van Wyk AE, Robbertse PJ, Kok PDF. The genus *Eugenia L*. (Myrtaceae) in Southern Africa: The structure and taxonomic value of stomata. Botanical Journal Linnean Society. 1982;84:41-56.
9. Vishnu Swarup. Vegetable science and technology in India. Kalyani Publishers. New Delhi, India. 2006;448-483 .
10. Cuttler EG. Plant Anatomy part 1 cells and tissues contemporary biology. Published by Great Britain Williams cloves and sons limited, London. 1978;95-127
11. Essiett UA, Bala DN, Agbakali JA. Leaf epidermal studies in three Nigerian species

of aspilia (*Heliantheae* – Asteraceae) and two hybrids. Nigerian Journal of Botany. 2010;8:25-33.
12. Nadeau JA, Sack FD. Stomatal development in Arabidopsis. In Arabidopsis. Oxford University press. 2002;55-62.
13. Shpak ED, Mc Abee JM, Pillitteri LJ, Torii KU. Science. 2005;309:290-293-3.
14. Pant DD. Stomata ontogeny and other homologous structures. Clarendon press Oxford. 1965;11-24.
15. Stace CA. Cuticular studies as an aid to plant taxonomy. Bull Br. Mus. Bot. 1965;4: 1-78.
16. Clive AS. Plant taxonomy and biosystematics. Cambridge University Press. 1991;81-82.
17. Abdulrahaman AA, Oladele FA. Stomatal complex types and epidermal cells in *Jatropha species* L. (Euphorbiaceae). Nigerian Journal of Pure Applied Science. 2009;23:2160-2163.
18. Jeffrey C. Notes on Cucurbitaceae, including a proposed new classification of the family. Kew Bull. 1962;15:337-371.
19. Metcalfe CR, Chalk L. Anatomy of the dicotyledons, systematic anatomy of the leaf and stem. 1st. Ed. Clarendon Press Oxford. 1950;1:82-84.
20. Abdulrahaman AA, Oyedotun RA, Oladele FA. Diagnostic significance of leaf epidermal features in the family Cucurbitaceae. Insight Botany. 2011;1: 22-27.
DOI: 10.5567/BOTANY-IK.2011.22.27.
21. Hutchinson J, Dalziel JM. Flora of West Tropical Africa ed. 2. London. 1954; 1(1):243.
22. Kolb D, Muller M. Light, conventional and environmental scanning electron microscopy of the trichomes of *Cucurbita pepo* ssp. *pepo* var. *styriaca* and histochemistry of glandular secretory products. Annals of Botany. 2004;94: 515-526.
23. Essiett UA, Akpabio E. Aniesua & Silas; 2012. International Journal of Biotechnsol and Allied Science. 2009;4(1):424-432.
24. Abdulrahaman AA, Oladele FA. Stomatal complex types in some Nigerian species of *Ocimum* hyptis and tinnea. Nigerian Journal of Pure Applied Science. 2003;23: 2160-2163.
25. Gills LS, Karatela YY. Epidermal structure and stomatal ontogeny in some Nigerian Cucurbitaceae. Published by: Botanischer Garten and Botanisches Museum, Berlin-Dahlem. 1982;303-310.
Available:http://www.jstor.org/stable/3995942
26. Saadu RO, Abdulrahaman AA, Oladele FA. Stomatal complex types and humidification potential of some root tuber species. African Journal of Plant Science. 2009;3:107-112.
27. Watson l. The taxonomic significance of certain anatomical observation on *ericaceae.* New Phytologist. 1964;63: 274-280.
28. Inamdar JA, Gangadhara M. Structure, ontogeny, classification and organographic distribution of trichomes in some Cucurbitaceae. Feddes Repertorium. 2008;86:307-320.
29. Olatunji OA. Practical manual for plant anatomy. Obafemi Awolowo University, Ile-ife, Nigeria (Manuscript). 1983;14-19.

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