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Biosystematic studies in the Loganiaceae (series 1): Foliar trichome morphology of Tree Species of *Anthocleista* Afzel Found in Parts of the Niger Delta, Nigeria

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ABSTRACT

Comparative systematic study of foliar epidermal trichome morphology of species of *Anthocleista* found in Nigeria was carried out in parts of Niger Delta area of the country. The area covered includes parts of Rivers, Bayelsa, Akwa-Ibom and Cross River States. The present study brings out the trichome morphology in leaf epidermal surfaces in four species belonging to the genus *Anthocleista* of the Loganiaceae. The micromorphological observations reveal some interesting features with reference to the type, and frequency or abundance of trichomes on foliar surfaces of the species studied. Though there could be similarity in terms of type and morphology in both surfaces of the species, *A. liebrechtsiana* showed a lesser abundance of trichome on both surface. *Anthocleista djalonesis* has in addition to other types, conical, falcate and stellate trichomes. Based on the variations in the above feature 10 types of trichomes among the four groups of species of *Anthocleista* are recognized. These include: cylindrical unbranched, 3- armed, 5- armed, dendritic, scale, 2- armed, conical, falcate, stellate and simple unbranched trichomes. The adaptive variation and taxonomic significance of the trichome epidermal features observed in this study further highlight the usefulness of micro anatomical structures in taxonomic delimitation. This therefore confirms earlier suggestions on the use of this epidermal structure including trichomes in plant classification.

Keywords: Niger Delta Nigeria, *Anthocleista djalonesis*, *Anthocleista liebrechtsiana*, *Anthocleista nobilis*, and *Anthocleista vogelii*.

INTRODUCTION

Trichome type has received less attention than fruit morphology and seed anatomy as a potentially informative character for delimiting tribes in plant family [1]. They were among the first anatomical features of plants to be recognized by early plant scientists and they have played a key role in plant taxonomy [2]. Trichomes are epidermal appendages of diverse form, structure and functions [3, 4] or are hairs or bustles [5]. They are seen as epidermal protuberances that have distinguishing height / width ratio [6, 7] and are generally of two types: simple or non-glandular and glandular secreting types [8, 9]. Morphology of both types as found on different plants varies greatly. They are represented by protective supporting and glandular hairs, by scales, by various papillae and by the

absorbing hairs of the roots. They may occur on all parts of a plant either by persisting throughout the life of an organ or by been ephemeral. Simple trichomes are present on aerial surfaces of most angiosperms and on some gymnosperms and bryophytes. In angiosperms trichomes may occur on leaves, petals, stems, petioles, peduncles, seed coats and roots depending on the species [8, 10]. Species completely devoid of trichomes on all parts of the plant are very few among the angiosperms. The cell walls of trichomes are commonly of cellulose and are covered with a cuticle. They may be lignified and sometimes impregnated with silica or calcium carbonate [11]. Their content are varied in relation to function, with the most complex been those of the glandular cells. Chloroplasts are often present, though may be small and not persisting [12]. From the epidermal surface a trichome is initiated as a protuberance which elongates into various divisions and develops into a multicellular structure [12, 13]. The epidermal trichomes usually develop early in relation to the growth of organ [3, 9, 7]. They are the first cell to differentiate from the epidermis in developing leaf primordial [14, 15, 16, 17, 18].

Trichomes may show wide variations within families and smaller plant groups and even in the same plant. Thus, there is sometimes considerable uniformity in trichomes within a plant group [19]. Trichomes have been successfully used in the classification of genera and even of species in certain families and in the recognition of interspecific hybrids [20, 21, 19].

The usefulness of epidermal studies including those in trichomes (epidermal hairs) in taxonomic delimitation of taxa had been reported [19, 22, 23]. It is equally significant to delimit taxa with micro morphological features in addition [24]. The value of leaf micromorphology in the taxonomic delimitation of *Emilia* spp [25] and *Euphorbia* spp. [26] has been elucidated. Metcalfe and Chalk [19] note that trichomes length; size and density are more variable in response to varied environmental conditions than the types.

The presence of a particular type of trichomes can frequently delimit species, genera or even whole families [19]. Stace [24] reported that trichome morphology provides the most important epidermal features in the taxonomy of the family Combretaceae. He reported a peculiar hair type (Combretaceous hair) in the family. This helped to confirm his previous notion that trichome as a taxonomic character is important from the specific to the family level in many plant groups. The report was similar to that of Dehgan [27] who employed the type of hair to demarcate different subgenera and sections of the genus *Jatropha* L.

Indeed, epidermal features have been severally used in taxonomic classification of such angiosperm genera like *Boerhavia* L. [28], *Terminalia* L. [29]. Osuji [30] reported the type and nature of trichomes in *Cucumeropsis mannii* while the elucidation of the epidermal micromorphology of *Cucurbita* L. species in Nigeria was carried out by Agbagwa and Ndukwu [31]. Similar trichome morphology and characterization have also been reported and utilized in taxa elucidation by Metcalfe and Chalk, [32]; Oladele, [33]; Edeoga and Osawe, [34]; Ogundipe and Akinrinlade, [35]; Agbagwa and Ndukwu, [31]; Ndukwu and Agbagwa, [25].

Various types of trichomes have been found among members of the Loganiaceae, which has also been noted as one of the plant family with numerous types of trichomes [32]. From vast literature available, no known systematic studies of trichomes morphology and anatomy have been carried out on Nigerian tree species of *Anthocleista*. Thus the present work shall exhaustively examine the presence, type and distribution of trichomes in this taxon.

MATERIALS AND METHODS

Description of the study area.

Geographically, the Niger Delta is the Southern segment of Nigeria, created by myriads of Islands segmented by lagoons and channels, which empty into the Bight of Benin in the East Atlantic Ocean. The Delta is supplied with water by the Rivers Niger and Benue. These Rivers (now joined) break up at Abor into the Rivers Nun and Forcados (including their tributaries). The portion of the Niger Delta traversed by the River Nun is the present Bayelsa State, while the present Delta State is traversed by the River Forcados. The Nun River breaks out into many channels and creeks such as *Santa Barbara*, *St. Nicholas*, *Brass*, *Nun*, *Sangana*, *Fishtown*, *Kohlama*, *Middleton*, *Digatoru*, *Pernnington*, *Dodo* and *Ramos* Rivers, which empty into the Atlantic Ocean [36].

The Niger Delta has also been seen from the ecological perspective as that portion of the Southern Nigeria stemming from a Northern apex situated at Aboh, bounded in the East and West by the Imo River and the Benin River respectively and on the South by the Atlantic Ocean [37]. It is the Africa's largest Delta covering some 70,000

square kilometers (Km²), of which one third of the area is made up of wetland [38]. The Niger Delta area includes Rivers, Bayelsa, Akwa Ibom, Cross River, Delta, and Edo States. Others included are Abia, Imo and Ondo States. However, the areas covered in this study include parts of Rivers, Bayelsa, Akwa-Ibom and Cross River States in designated local government areas (LGA), (Fig.1). The sampling areas covered include:

- i. Rivers State: Comprising areas such as Opu-oko town Nyokhana in Khana LGA, Sakpewa in Tai LGA, Choba in Obio/Akpor, Isiodu in Emuoha LGA, and Omuoko axis in Aluu town, Ikwerre LGA.
- ii. Bayelsa State: Comprising the Yenagoa axis of the forest lying on both side of the road by the gate way to Edipie/Yenagoa.
- iii. Akwa Ibom State comprising parts of Ikot Osuete town in Oruk Anam LGA along the Bori/Ikot Abasi high way.
- iv. Cross River State, comprising parts of Akai Effa-Idundu and Atimbo forest in Calabar municipal.

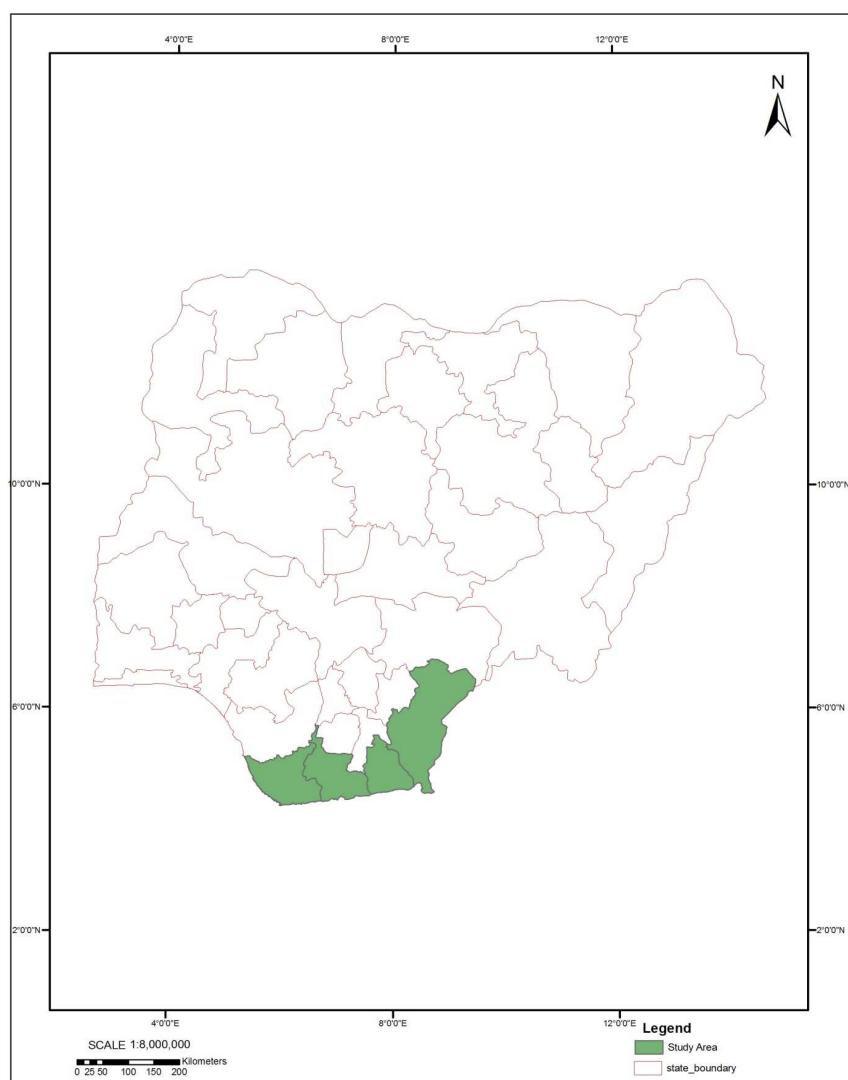


Fig. 1: Map of Nigeria showing sampling / study areas

Species Description

The genus *Anthocleista* Afzel *ex. Br.* is a medium sized Tropical African tree composed usually of small trees or scrambling shrubs with soft white wood with a few herbs like *Spigelia* [39, 40]. It belongs to the order Loganiales

[41]. The family Loganiaceae contains about thirty (30) genera and over 600 species [42]. About 50 species in the genus *Anthocleista* are endemic to Tropical Africa, Madagascar and Mascaree Island. Of the 50 species six (6) species are known to be found and of economic importance in various parts of Nigeria [40]. Of the six species in Nigeria, phytogeographical study has revealed four species of common occurrence in parts of Niger Delta [43], most of which are generally mesophytic in habitat adaptation and occur in some parts of tropical rainforest zone. Despite the level of disparity in certain ecological habitats, the species are perennial shrubby trees with marked preference for tropical climate [43]. The delimitation of the family is a much debated taxonomic issue [39]. While Hutchinson [41] has placed *Loganiaceae* with six other families: *Potaliaceae*, *Buddlejaceae*, *Antoniaceae*, *Spigeliaceae*, *Strychnaceae* and *Oleaceae* in the order Loganiales, Takhtajan [44] and Backlund *et al.*, [42] placed *Loganiaceae* with eight other families in the order Gentianales. This has heightened the need for a micromorphological study especially of valuable tree species of *Anthocleista* in the area. The current study is part of the effort at documenting the epidermal trichomes in species of *Anthocleista* in the study area. The information obtained is expected to aid as assessment of the taxonomic value of these features as well as their functions in taxonomic delimitation in this genus.

Epidermal Studies: Trichomes

Fresh materials of the leaves collected from the field were directly preserved into the fixative FAA (Formalin, Acetic and Alcohol in the ratio of 1:1:18 mls respectively). The trichomes on the adaxial and abaxial leaf surfaces were examined. The epidermis of the leaf organ was cleared, by placing the sample in 5% sodium hypochlorite (bleach) for 24 hours. The cleared epidermal layers obtained were then washed in several changes of distilled water, lightly stained with 1% Safranin and viewed under the photomicroscope. The morphology of the trichomes was described following Metcalfe and Chalk [32]. Photographs and drawings by free hand were made from good slides on temporary mounts.

RESULTS

Trichome Morphology

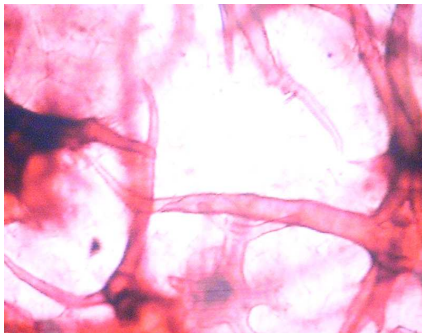
Different types of trichomes occur among the species studied. A summary of the trichomes is presented in Table 1. Variations and similarities in trichome distribution on both epidermal surfaces are recorded among the species found in parts of Niger Delta studied.

Table 1: Summary of Trichome Morphology in *Anthocleista* spp.

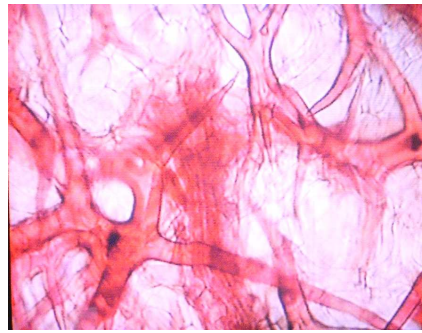
Species	Adaxial	Abaxial
<i>A. nobilis</i> G. Don	Cylindrical unbranch trichomes, 3-armed, 5-armed, dendritic branched and scale trichomes.	Dendritic branched trichomes and scale trichome.
<i>A. liebrechtsiana</i> De Wild and Th. Dur	Scale and dendritic branched trichomes	Scale and dendritic branched trichomes
<i>A. djalonesis</i> A. Chev	Scale trichomes, dendritic trichomes 2-armed forked trichomes.	2-armed forked trichome, conical, scale, falcate, dendritic and stellate trichomes.
<i>A. vogelii</i> Planch	Scale dendritic branched trichomes	Scale, dendritic and simple unbranched trichomes

Anthocleista nobilis has five different trichomes. These include: the cylindrical (unbranched trichomes), 3-armed trichomes, 5-armed trichomes, dendritic branched trichomes and scale trichomes on its adaxial surface. While the abaxial surface has only two morphologically different types namely the dendritic branched trichomes and scale trichome (Plates. 1a -d), (Figs. 2a-e & 2¹a-d).

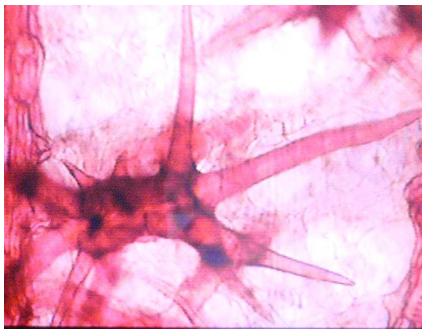
A x10, Adaxial surface



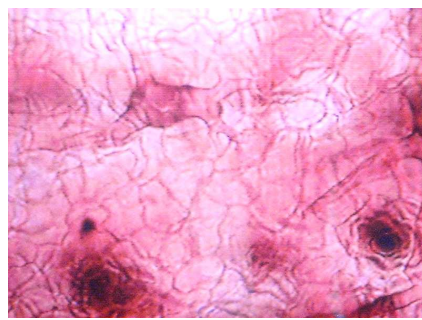
B x10, Adaxial surface



C x10, Abaxial surface



D x10, Abaxial surface



Plates. 1A – D: showing branched trichomes of various morphological forms on the adaxial and abaxial surfaces of *A. nobilis*

A-B = Dendritic branched trichome on the adaxial surface

C = Dendritic branched trichome on the abaxial surface

D = Scale trichome on the abaxial surface

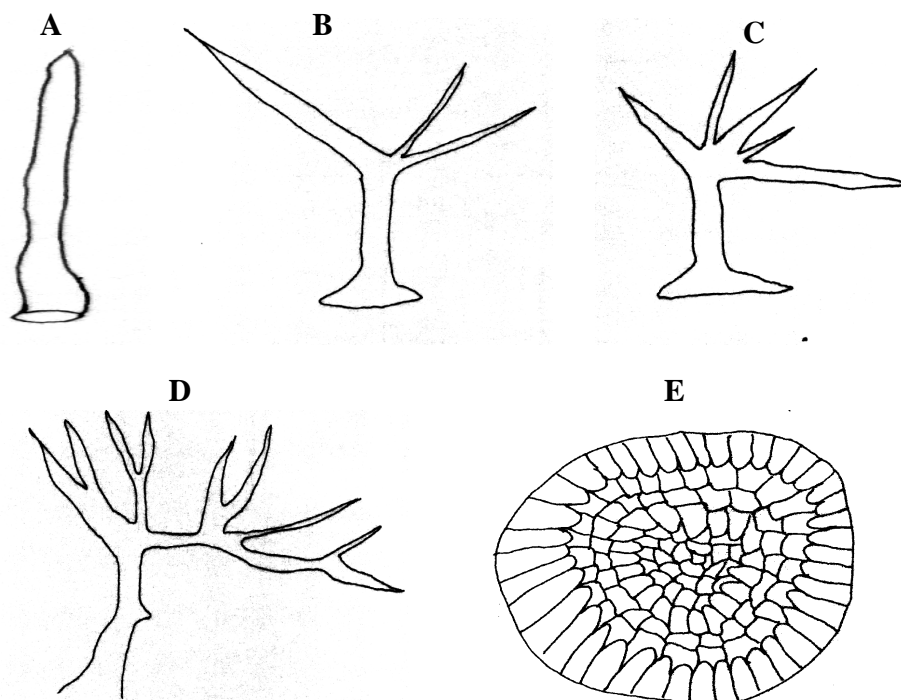


Fig. 2. Adaxial surface of microscopic cartograph of trichomes in *A. nobilis*
 A – Cylindrical (unbranched) trichomes, B – Three - armed trichomes
 C – Five armed trichomes, D – dendritic branched trichomes, E – Scale trichomes

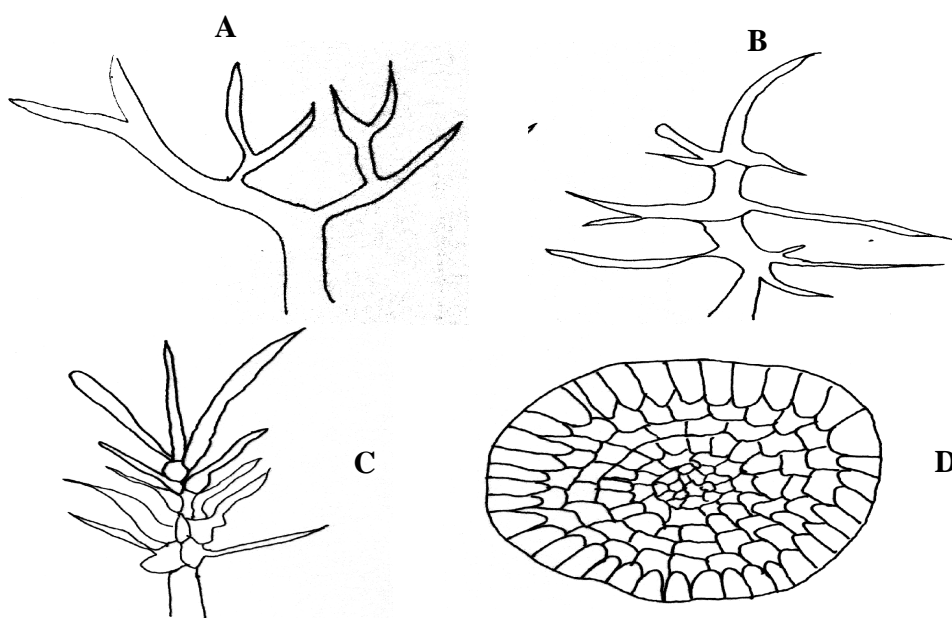
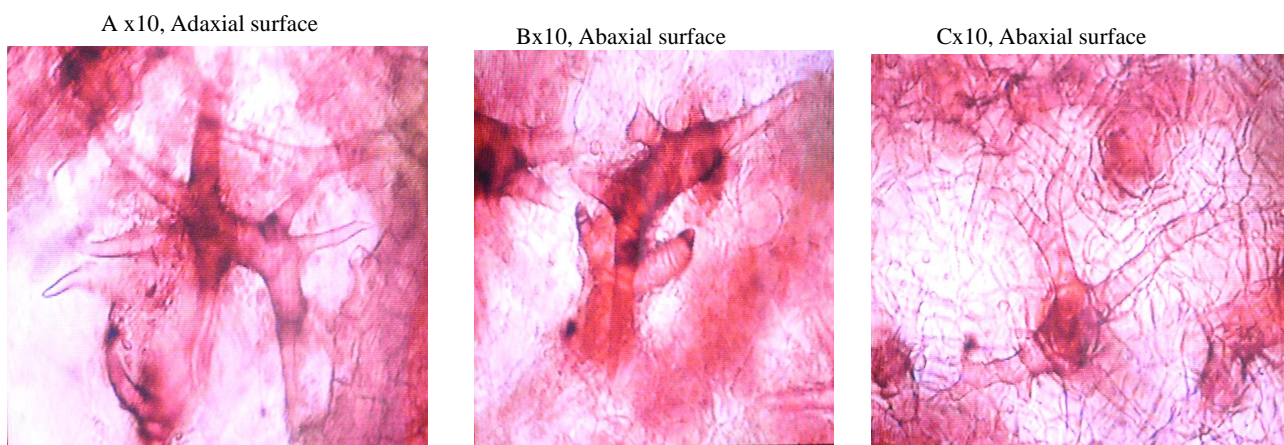


Fig.2¹ Abaxial surface of microscopic cartograph of trichomes in *A. nobilis*.
 A-C – dendritic branched trichomes, D- scale trichomes

Anthocleista liebrechtsiana has scale trichome and dendritic branched trichomes of diverse morphology at the adaxial and abaxial surfaces (Plates.2 a-c), (Figs. 3a-c & 3¹a-c).



Plates. 2 A-C: showing dendritic branched trichomes on the adaxial and abaxial surfaces of *A. liebrechtsiana*

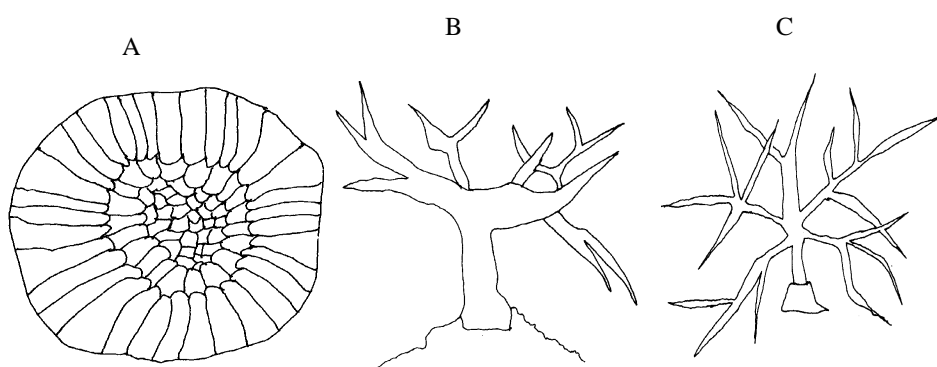


Fig. 3: Adaxial surface of microscopic cartograph of trichomes in *A.liebrechtsiana*
A- scale trichomes, B, C – dendritic branched trichomes.

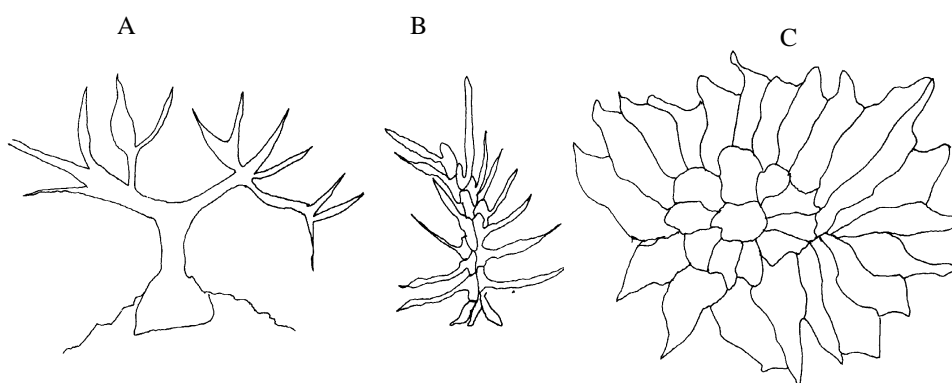


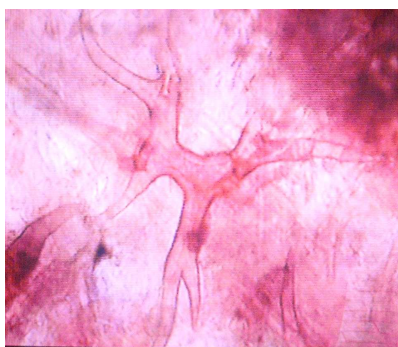
Fig. 3¹: Abaxial surface of microscopic cartograph of trichomes in *A. liebrechtsiana*
A-B –dendritic branched trichomes, C – scale trichomes

Anthocleista djalonesis is also characterized by scale trichomes of diverse morphology, dendritic trichome and 2 - armed trichomes at the adaxial surface. Similarly, 2-armed, conical scale, falcate, dendritic and stellate trichomes are located on the abaxial (Plates. 3a- g), (Figs. 4a-j & 4¹a-k).

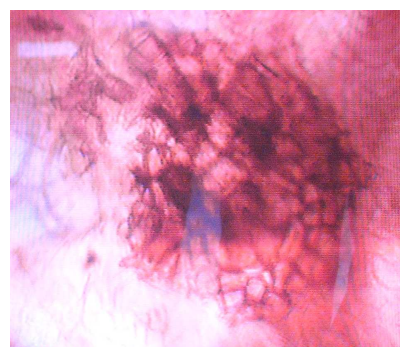
A x10, Adaxial surface



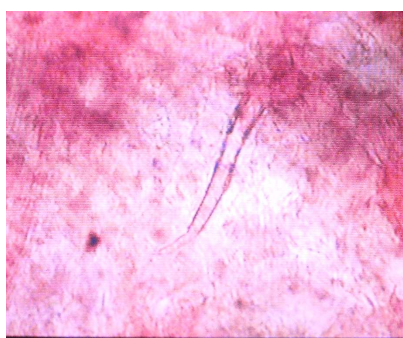
B x10, Adaxial surface



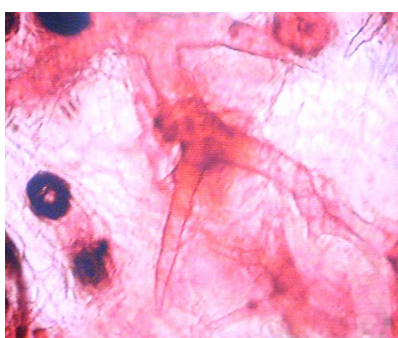
C x10, Adaxial surface



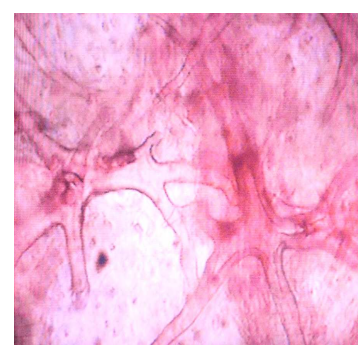
D x10, Abaxial surface



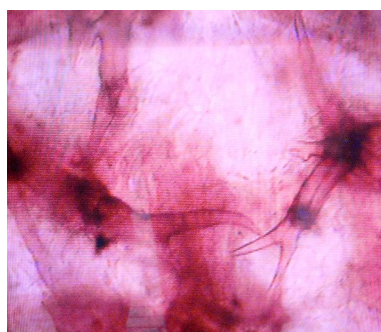
E x10, Abaxial surface



F x10, Abaxial surface



G x10, Abaxial surface



Plates. 3A-C; Showing trichomes of various morphological forms on the adaxial surfaces of *A. djalonesis*

A - B = Dendritic branched trichomes on the adaxial surface
C – Scale trichome on the adaxial surface

Plates. 3D-G; Showing trichomes of various morphological forms on the abaxial surfaces of *A. djalonesis*

D = Simple unbranched trichome on the abaxial surface
E & F = Branched dendritic trichomes on the abaxial surface
G = Two-armed Forked trichome on the abaxial surface

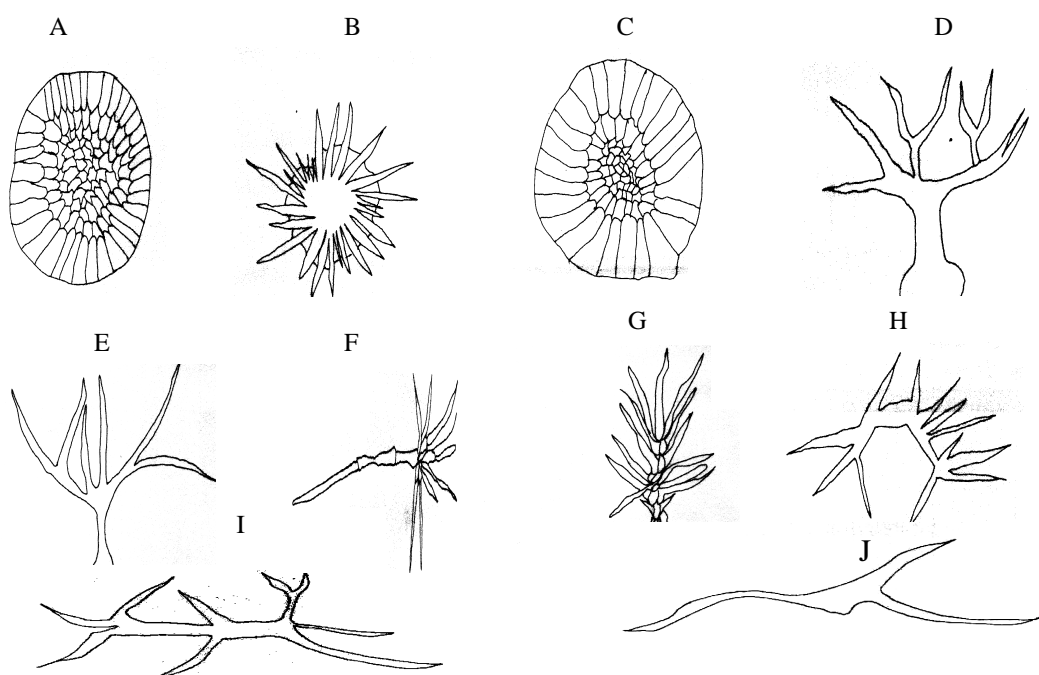


Fig.4: microscopic cartograph of trichomes on the adaxial surface of *A. djalonesis*
 A-C – scale trichomes, D-I –dendritic branched trichomes, J- two-armed forked trichomes

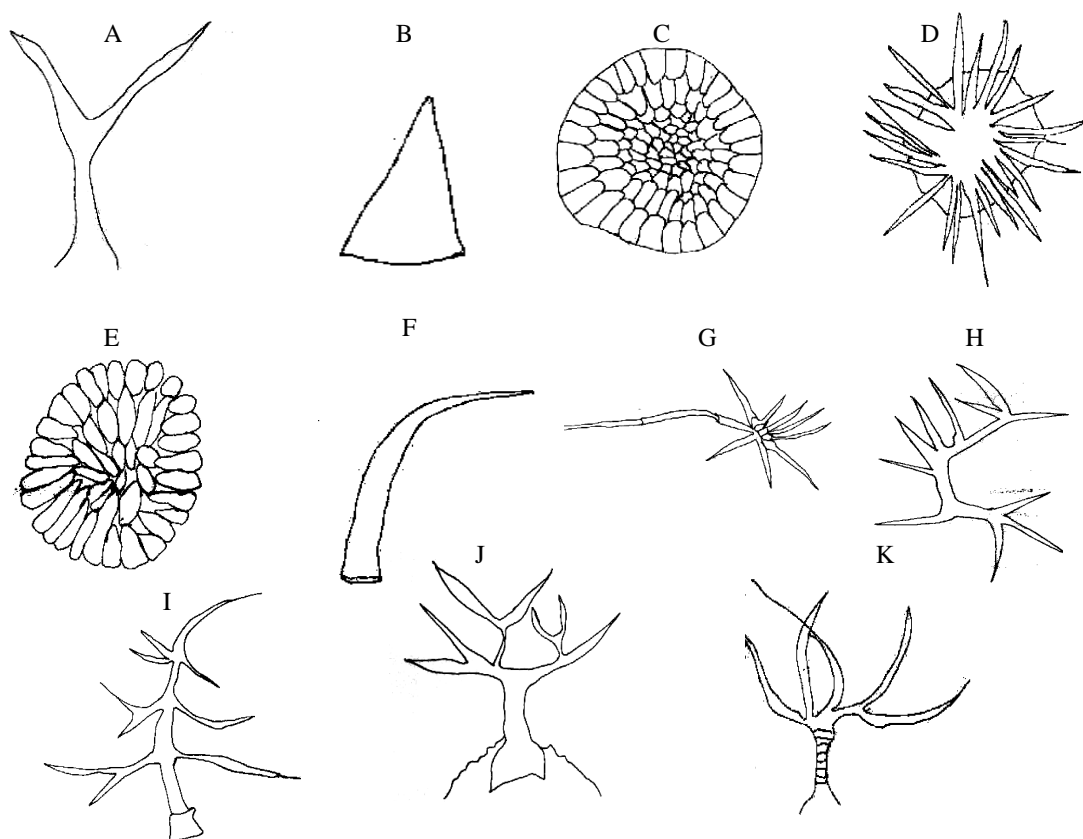
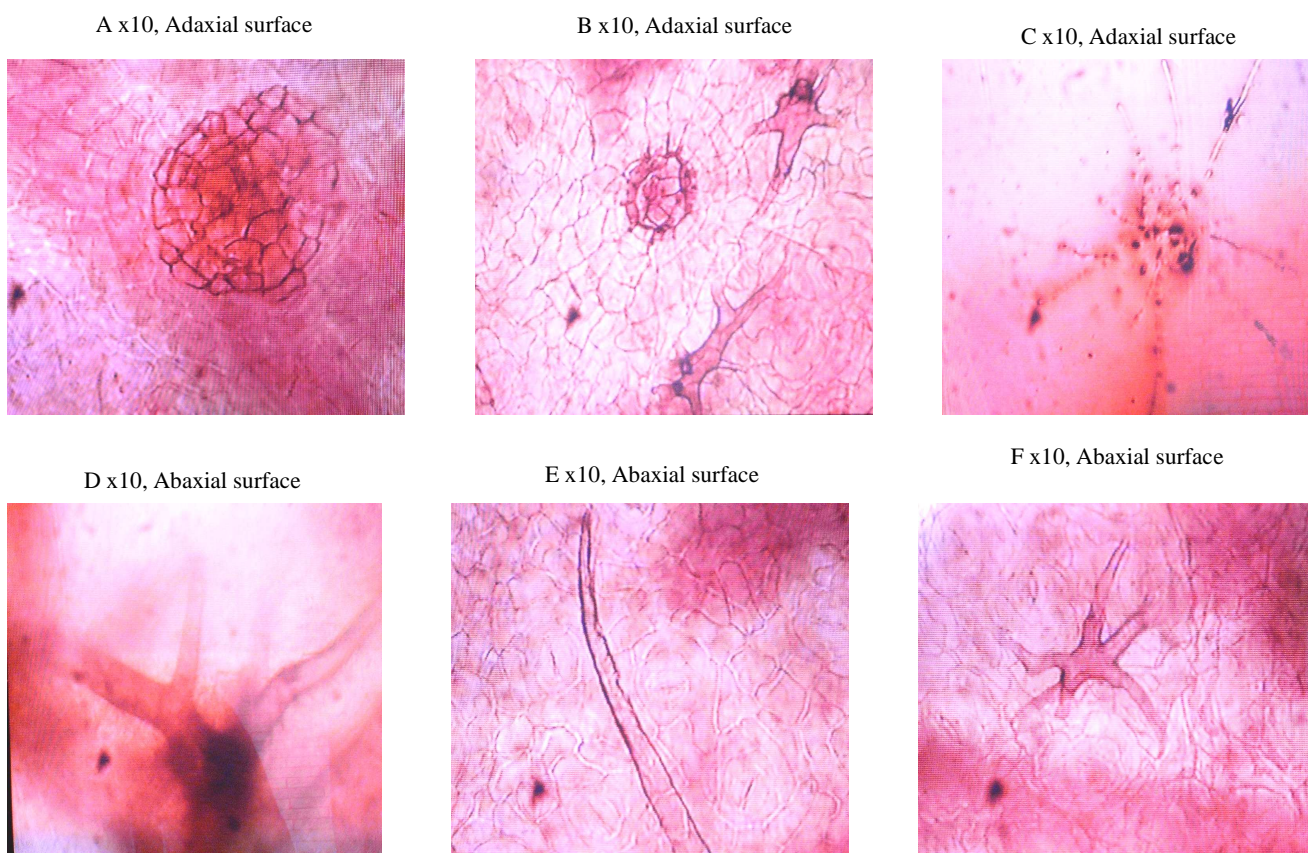


Fig.4': microscopic cartograph of trichomes on the abaxial surface of *A.djalonesis*
 A- Forked two - armed trichome, B- conical simple trichome, C-E – scale trichomes
 F- Falcate trichome, G-J –dendritic branched trichomes, K- stellate trichome.

Anthocleista vogelii is characterized by scale trichomes of diverse morphology, dendritic trichomes in both adaxial and abaxial surfaces in addition to a simple unbranched trichome in the abaxial surface (Plates. 4a - f), (Figs. 5a-c).



Plates 4A-F: Showing trichomes of various morphological forms on the adaxial and abaxial surfaces of *A.vogelii*

A, B&C – Scale trichomes
B, D &F – Branched dendritic trichomes
E – Simple unbranched trichome

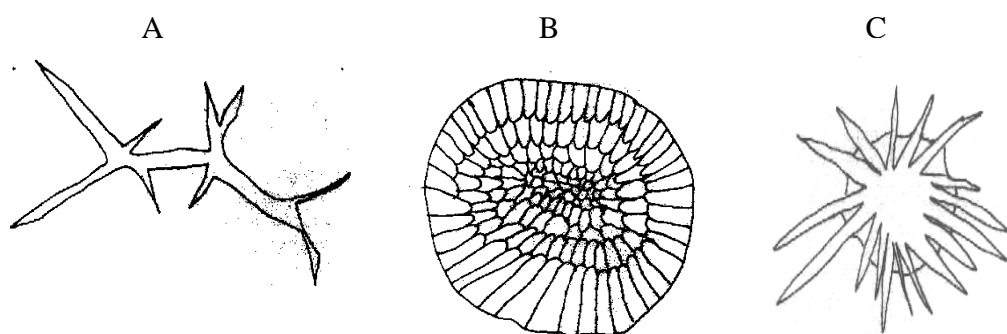


Fig. 5 Cartograph microscopic observation of trichomes on the surface of *A.vogelii*

A – Dendritic branched trichome on the adaxial
B – Scale trichome on the adaxial
C – Scale trichome on the abaxial

DISCUSSION

The survey on the foliar surface of the *Anthocleista* species studied revealed 10 types of trichomes: simple unbranched, dendritic though common to all but of various morphology in terms of shapes, 3 – armed trichomes, 5 – armed trichomes, scale trichomes, 2 – armed trichomes, conical, falcate, and stellate . Trichomes have long been of considerable importance in comparative systematic investigation of angiosperm. They are frequently present, easily observable, and have often been found to have variation patterns which correlate with other features of the taxa under investigation [32].

The use of particular types of trichomes in the studies of different groups of taxa has also been highlighted. Metcalfe and Chalk [19] have drawn attention to various types and their occurrence in particular families within the dicotyledons. In the Poaceae Metcalfe [45] has shown the value of a careful study of minute trichomes as well as large ones for numerous genera of this family. Tomlinson [46] described the types within the Commelinales and Zingiberales and draw attention to their value in delimiting various groups. He also noted the diagnostic value of the trichomes based on the definitive studies in the Zingiberaceae. Cowan [47], in a study of trichomes in the genus *Rhododendron*, illustrated and described the numerous and often elaborate types of trichomes within this genus and showed their value in delimiting subgenera and species. Heintzelmann and Howard [20], working with the Icacinaceae, have shown the value of determining types of trichomes, both relative to the number of types within a genus and their distribution on the plant. Carolin [48] has surveyed the trichomes found within the Goodeniaceae and questioned the taxonomic groupings made by earlier works.

At the specific level studies of trichomes have been found to be of value by many workers [49]. The taxonomic significance of the trichome complement or range of trichomes on the entire plant has been emphasized by Carlquist [50, 51, 52, 53] for members of the Compositae (Asteraceae) especially by clearly illustrating the evolution of particular types of trichomes through comparative studies of development within and between taxa of the tribe Madiinae. Trichome variation has been used to delimit both genera and species in Brassicaceae [54, 55, 56, 57] and for members of the Asteraceae [25].

Various types of trichomes have been found among members of the Loganiaceae, which has also been noted as one of the plant family with numerous types of trichomes [32]. The result of the microscopic observations has shown differences in the type, morphology and frequency and abundance of these trichomes on foliar surfaces of the species studied. Though there could be similarity in terms of types of trichomes and morphology in both adaxial and abaxial surfaces of the species, *A. liebrechtsiana* has shown a lesser abundance of trichomes on both surfaces while *A. nobilis* has a lesser abundance of trichomes on the abaxial surface. *Anthocleista djalonesis* has in addition to the other types, conical, falcate and stellate trichomes. Several other workers have used leaf epidermal study as a taxonomic tool for species delimitation [58, 59, 60, 61, 62]. Therefore, this confirms the earlier suggestions and use of these foliar structures in plant classification [27, 63].

CONCLUSION

Though the various types of trichomes have been documented on the Loganiaceae by some other investigations, the present study will enhance the already existing information. Thus, have elucidated the differences in the type, morphology, frequency and abundance of these trichomes on foliar surfaces of the species studied. This will further contribute to the usefulness of micro-epidermal structures in the taxonomic delimitation of species in plant classification.

REFERENCES

- [1] Prantl, K. Cruciferae. In: A. Engler, K. Prantl (Eds.) *Die Natürlichen Pflanzenfamilien* 145 206 Wilhelm Engelmann, Leipzig, Germany, **1891**
- [2] Behnke, H.D. Plant trichome structure and ultrastructure: general terminology, taxonomic applications and aspect of trichome bacterial interaction in leaf tips of *Dioscorea*. In: Rodrigues E., Healey P.L., Mehta .I. (Eds.) *Biology and Chemistry of Plant Trichomes*. (Pp.1-21) New York, Plenum Press, **1984**.
- [3] Esau, K. *Plant Anatomy* (1st edition), John Wiley, New York, Chapman and Hall, London, **1958**.
- [4] Esau, K. *Plant Anatomy* (1st Ed.) John Wiley, London, **1965**.

- [5] Lawrence, G.H.M. *Taxonomy of Vascular Plants* 1st ed. Macmillan, New York, **1951**.
- [6] Kim, H.J., Triplett, B.A. *Plant Physiol.* **2001**, 127: 1361–1366.
- [7] Wang, E., Hall J.T, Wagner G.J. Transgenic *Nicotiana tabacum* L. with enhanced trichome exudate cembratrieneols has reduced aphid infestation in the Molecular Breeding, **2003**.
- [8] Fahn, A., Structure and function of secretory cells. In: Hallahon, D. and Gray, J. (Eds.) *Advances in Botanical Research*, 31:37-75, Incorporating Advances in Plant Pathology, Plant Trichomes, Academic Press., **2000**.
- [9] Werker, E., *Advances in Botanical Research*, **2000**, 31: 1-35.
- [10] Kellogg, E. A. *Trends in Plant Science*, **2001**, 6: 550-552.
- [11] Beyrich, H. *Flora*, **1943**, 36:313-324.
- [12] Netolitzky, F. Die Plarzenhaare In: K. Linsbauer *Handbuch der Pflanzenanatomie*. B and 4 Lief 29. **1932**.
- [13] Copper, D.C. *Amer. Jour Bot.*, **1932**, 19:423 - 428
- [14] Alvarez-Buylla E. R, Liljegren S. J, Pelazz S, Gold S. E, Burgeff C, Ditta G.S, Vergara-Silva F, Yanofsky M. F. *Plant Cell*, **2000**, 24: 457- 466.
- [15] Schwab B, Folkers U, Ilgenfritz H, Hulskamp M. *Philosophical Transactions of the Royal Society of London*. **2000**, 355: 879-883.
- [16] Szymanski D.B. *Journal of Plant Growth Regulation*. **2001**, 20: 131-140.
- [17] Schmittger A, Hulskamp M. *Philosophical Transactions of the Royal Society of London*. **2002**, 357: 823-826.
- [18] Wang, S., Wang J-W, Yu N, Li C-H, Luo B, Gou J-Y, Wang L-J, Chen X-Y. Control of plant trichome development by a cotton fiber MYB gene. *Plant Cell*. **2004**, 16:2323-2334.
- [19] Metcalfe, C.R. Chalk, L. *Anatomy of Dicotyledons* Vol. 172. Clarendon Press, Oxford. **1950**.
- [20] Heintzelmann C.E. Jr. Howard, R.A. *Amer. Jour. Bot.* **1948**, 35:42-52
- [21] Hoff, A. *Deut.Bot.Gesell Ber.* **1950**, 63:31-35.
- [22] Stace, C.A. *J. Linn. Soc. Lond. Bot.* **1965**, 59: 229-252.
- [23] Cantino P.O. *Journ. of the Arnold Arboretum*. **1990**, 71: 323-370.
- [24] Stace, C.A. *Plant taxonomy and Biosystematics: Contemporary Biology*. Edward Arnold, London, **1980**.
- [25] Ndukwa, B. C.; Agbagwa, I.O. *Glo. J. pure and Applied Sciences*. **2006**, 12 (2): 183-187.
- [26] Ndukwa, B.C.; Okoli, B.E. *GJPA*, **2007**, 13(4): 493 – 496.
- [27] Dehgan, B. *Bot. J. of Linn. Soc.* **1980**, 80: 257-778.
- [28] Edeoga, H.O.; Ikem, C.I. *Morphology J. Econ. Taxon Bot. Addl. Ser.* **2001**, 19:197-205.
- [29] Obute, G.C.; Ademosu, A.H. *Journal of Applied Sciences and Environment Management*. **2001**, 5 (2): 31-34
- [30] Osuji, J. O. Taxonomic studies on *Cucumeropsis manni* Naud. B.Sc. Project, Dept. of Botany University of Port Harcourt. **1988**.
- [31] Agbagwa, I.O.; Ndukwa, B.C. *JASEM*. **2001**, 5 (2): 59-64.
- [32] Metcalfe, C.R.; Chalk, L. *Anatomy of the Dicotyledons*. 2nd ed. vol. 1 Systematic anatomy of the leaf and stem, with a brief history of the subject Clarendon Press Oxford. **1979**.
- [33] Oladele, F.A. *Nigerian Journal of Botany*. **1990**, 3:71-77.
- [34] Edeoga, H.O.; Osawe, P.I. *Acta Phytotax. Geobot.* **1996**, 47(1): 41-46
- [35] Ogundipe, O.T.; Akinrinlade, O.O. *Phytomorphology*. **1998**, 48(4): 325-333.
- [36] Alagoa, I. O. *The Land and People of River State, Central Niger Delta*. Onyema Research Publication, Port Harcourt, Rivers State Nigeria. **1999**.
- [37] Fubara, D. M. J.; Teme, S. C.; Mgbeke, T.; Gobo, A.E.T. Abam, T.K. S. *Master Plan Design of Flood and Erosion Control Measures in the Niger Delta* IFERT Technical Report NO.1. **1988**.
- [38] Afolabi, D. *The Nigerian Mangrove ecosystem*. Third Regional Workshop of the Gulf Guinea Large Marine Ecosystem (GOGLME), Lagos Nigeria. **1998**.
- [39] Leeuwenberg, A.J.M. *Acta Botanica Neerlandica*. **1961**, 10: 460 - 463.
- [40] Keay, R.W.J. *Trees of Nigeria*. Clarendon Press Oxford. 476 pp. **1989**.
- [41] Hutchinson, J. *Key to the Families of Flowering Plants of the World*. Clarendon Press, London. 828 pp. **1967**.
- [42] Backlund, M.; Oxelman, B. Bremer, B. *American Journal of Botany*. **2000**, 87(7): 1029 – 1043.
- [43] Edwin-Wosu, N.L. Studies on species of *Anthocleista Afzel ex. R. BR.* (Loganiaceae) in parts of Niger Delta of Nigeria. M. Sc. Thesis, University of Port Harcourt. 2010.
- [44] Takhtajan, A. *Diversity and Classification of Flowering Plants*. New York, Columbia University Press. USA. **1997**.
- [45] Metcalfe, C.R. *Anatomy of the Monocotyledons*. I. Graminea. Clarendon Press, Oxford. **1960**.
- [46] Tomlinson, P.B. *Anatomy of the Monocotyledons*. II Commelinales. Zingiberales, Clarendon Press, Oxford. **1969**.

-
- [47] Cowan, J.M. *The Rhododendron leaf, a study of the epidermal appendages*. Oliver and Boyd, Edinburgh. **1950**.
- [48] Carolin R. C. *Proc Linn. N.S.W.* **1971**, 96:8-22.
- [49] Faust, W.Z. Jones, S.B. Jr. *Rhodora*, **1973**, 75: 517 – 528.
- [50] Carlquist, S. *American Journal of Botany*.**1958**, 45: 675-682.
- [51] Carlquist, S. *American Journal of Botany*, **1959a**, 46: 70-80.
- [52] Carlquist, S. *American Journal of Botany*, **1959b**, 46: 70-80.
- [53] Carlquist, S. *Comparative Plant Anatomy. A Guide to Taxonomic and Evolutionary Application of Anatomical Data in Angiosperms*. Holt, Rinehart and Winston, N.Y. **1961**.
- [54] Al-Shehbooz, I.A. *Journal of the Arnold Arboretum*.**1990**, 71: 221 – 250.
- [55] Al-Shehbooz, I.A. *Novon*. **1994a**, 4:191 – 196.
- [56] Aneev, M.E. Genus *Alyssum* in Bulgarian Flora. In: S.I. Kozhukharov; B.A. Kuznov (Eds.) *Evolution of flowering plants and florogenesis, 2. Asteraceae, Brassicaceae, Poaceae, Cyperaceae*. 85 – 117 Bulgarian Academy of Sciences, Sofia, Bulgaria. **1991**.
- [57] Mulligan, G.A. *Rhodora*, **1995**, 97: 109 – 163.
- [58] Isawumi, M.A. *Feddes Rapertorium*, **1989**, 100: 335 355.
- [59] AbdulRahaman, A. A. Oladele, F.A. *Nigerian Journal of Botany*. **2003**, 16: 144 – 150.
- [60] Adedeji, O. *Botanica Lithuania*, **2004**, 10(2): 121 – 133.
- [61] Folorunso, A.E.; Olorode, O. *Research Journal of Botany*, **2006**, (3):118 – 124.
- [62] Folorunso, A.E.; Olorode, O. *Research Journal of Botany*. **2008**, 3(1):118 – 124.
- [63] Olowokudejo, J.D. *Phytomorph*. **1990**, 40 (3 & 4): 407- 422.