Global Journal of Science Frontier Research: C
Biological Science
Volume 15 Issue 1 Version 1.0 Year 2015
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Systematic Values of Foliar Anatomical Features in some Members of Nigerian Clusiaceae

By Nnamani, C. V. & Nwosu, M. O.
Ebonyi State University Abakaliki, Nigeria

Abstract- Foliar epidermal features of four Nigerian species of the family Clusiaceae were studied. This was with a view to exploiting their systematic and taxonomic values to aid their taxonomy. Representatives of the four species were obtained from various parts in Southern Nigeria and passed through standard treatments to make permanent anatomical slides for the study by light microscopy (LM). Micrographic evidences of distinguishing and affinity taxonomic features were recorded. Variations in stomata, epidermal and guide cells attributes were obvious and they could be used as systematic evidence to taxonomically delineate these taxa even at generic levels. Data accruing from this study could help to resolve the taxonomic problems in this family and confirm their identity.

Keywords: *epidermal features, trichome; anatomical, clusiaceae, nigeria.*

GJSFR-C Classification : *FOR Code: 069999*

Strictly as per the compliance and regulations of :

Systematic Values of Foliar Anatomical Features in some Members of Nigerian Clusiaceae

Nnamani, C. V. [α] & Nwosu, M. O. [σ]

Abstract- Foliar epidermal features of four Nigerian species of the family Clusiaceae were studied. This was with a view to exploiting their systematic and taxonomic values to aid their taxonomy. Representatives of the four species were obtained from various parts in Southern Nigeria and passed through standard treatments to make permanent anatomical slides for the study by light microscopy (LM). Micrographic evidences of distinguishing and affinity taxonomic features were recorded. Variations in stomata, epidermal and guide cells attributes were obvious and they could be used as systematic evidence to taxonomically delineate these taxa even at generic levels. Data accruing from this study could help to resolve the taxonomic problems in this family and confirm their identity.

Keywords: epidermal features, trichome; anatomical, clusiaceae, nigeria.

I. Introduction

The Clusiaceae formerly recognized as Guttiferae includes herbs, shrubs, trees with sap resinous and oil glands present in most species. Leaves are opposite or whorled, rarely alternate and exstipulate. Flowers are usually unisexual, sometimes bisexual on the same plant and functionally polygamodioecious, actinomorphic with sepals of 2-10 or more (Keay 1954). Petals range from 4-12 usually imbricate, subvalvate or contorted. Stamens are few or numerous, hypogenous, distinct and variously united. The anther is 2-celled and dehiscing longitudinally with 1 pistil and superior ovary (Robson, 1961).

The family is distributed mainly in the temperate and tropical regions of the world (Keay *et al*., 1964; Kokwaro, 1976). Robson, (1961) and Matig *et al.* (2007) opined that, they occur commonly in humid lowland rainforest or gallery of West and Central Africa sub regions, extending from Congo to Sierra Leone, Madagascar and the Mascarene Islands. The major centers of diversity and species richness of this family in Nigeria are mainly in the Southern and Southeastern parts of the country (Keay, 1989).

In Nigeria, the family is traditionally represented by 16 species distributed in 5 genera, consisting of *Symphonia* L., *Allanblackia* Oliv., *Pentadesma* Sabine., *Mammea* L. and *Garcinia* L. (Keay, 1954; Gill, 1988). However, the reclassification by Keay (1989), to include other genera such as *Vismia* Vand., *Harungana* Lam *ex* Poir. and *Endodesmia* Benth makes them vulnerable to much debate.

Gustafsson *et al*. (2002) started the phylogeny reconstruction of the Clusiaceae using the chloroplast gene rbcL. The analysis provided support for the monophyly of three clades viz., Kielmeyeroideae, Clusioideae, and Hypericoideae + Podostemaceae, except for *Clusiella*, that they traditionally placed in Clusioideae, when it initially appeared in Kielmeyeroideae. Sharma *et al* 2013) noted that the Clusiaceae, even with the removal of the Hypericaceae (from the traditional Guttiferae Juss.), remains a heterogeneous agglomeration partly due to their taxonomic works which are confined to restricted geographical areas and unclear mode of reproduction Malaysia (Whitmore, 1973) and tropical Africa (Robson, 1961; Bamps *et al*., 1978). In the same line Nnamani and Nwosu, 2013a & b) used pollen morphology and distribution of secretory canals respectively to verify their taxonomic status.

The use of data generated from leaf epidermal studies in resolving the taxonomic problems in plants have gained much recognition for a very long time (Aworinde *et al,* 2009). They reiterated that the epidermal and cuticular traits of plants could serve as vital tools exploitable in the systematics of the present day angiosperms. Leaf epidermal features in systematic botany is now popular just like the use of other markers like DNA sequencing and chemical compositions in providing valuable data for taxonomic affinity (Edeoga and Ikem, 2001; Mbagwu and Edeoga, 2006). It is the second most important character after cytology for solving taxonomic problems (Folorunso and Olaniyan, 2009).

Foliar epidermal features particularly the stomata have proved very useful in the delineation of doubtful families (Yasmin *et al.,* 2009). The major aim of this work is to present a more precise characterization of the foliar epidermal features using light microscopy to identify the taxonomic potential in some members of Nigerian Clusiaceae. The main objectives were to 1) determine the various types of epidermal features and 2) utilize the data occurring from the stomata to aid the classification of Nigerian Clusiaceae.

Author α: Department of Applied Biology Ebonyi State University Abakaliki, Nigeria. e-mail: drnnamanikate@gmail.com
Author σ: Department of Botany, University of Nigeria, Nsukka, Nigeria.

II. Materials and Methods

a) Study Location

This study was conducted in Southeastern Nigeria that covers an area of 95,488 sq km. It lies between Latitudes 4° and 7° North of the equator and Longitudes 3° and 15° East of the Greenwich Meridian (Iloeje, (1999) Ofomata, (1975). These regions comprise of Abia, Anambra, Ebonyi, Enugu and Imo States of Nigeria.

b) Selection of Taxa

Plants used for this study were from four genera out of the eight genera recorded in Nigeria by Keay (1989). Representative species examined were, *Harungana madagascariensis, Garcinia kola Allanblackia floribunda* and *Pentadesmia butyracea.* This decision was based on the availability of samples, considering the fact that *Garcinia kola, Allanblackia floribunda* and *Pentadesma butyracea* are listed as "threatened to near endangered species" in (Cheek, 2004; Isichei, 2005).

c) Leaf Epidermal Structures

Leaves for epidermal studies were collected fresh from samples growing in the field. To avoid and minimize spatial heterogeneity effects on foliar epidermal characters, sample materials for slide preparations were taken from the same internodes of each branch. The method used is the Impression Technique, where a thin layer of colourless nail vanishes was spread over the leaf surfaces and allowed to dry for 1 hour. Peels were made from both adaxial and abaxial surfaces of each leaf with the aid of a transparent adhesive cello tape on these regions. These strips were mounted on glass slides.

d) Microscopy

Both qualitative and quantitative micro morphological foliar characters were observed using Olympus CH Trinocular Microscope (LM), fitted with 650 IS Cannon Digital Camera. Four slides were prepared for each of these samples. The epidermal features studied include: Nature of the epidermal cells and cell wall, types of trichome (if present or absent), distribution and types of stomata, stomata frequency, length and breadth of stomata. Other dimensions of stomata considered were, length and breadth of guard cells, shape of stomata, size of the stoma, pore length and breadth, nature of subsidiary cells and stomata index (SI) Metcalfe and Chalk (1957) which was given as:

$$SI = \frac{S}{S + E} \times 100$$

Where: S = the number of stomata per field of view,

E = the corresponding number of epidermal cells.

e) Statistical Analysis

Data obtained from quantitative assessments were synthesized and presented in tables and figures. These values were tabulated with the species description citing mean standard errors only for all the morphological features. Statistical analyses on anatomical features were based on 20 measurements of each feature per slide by the four slides per sample. Values derived were subjected to statistical analysis using the General Linear Model (GLM) procedure in Statistical Analysis System (SAS), SAS Institute (2000), version 9. Means of those traits which show significant differences between taxa were separated by Least Significant Difference (LSD) tests at P = 0.05.

III. Results

a) Epidermis

Epidermal cell walls were irregular in outline with a conspicuous presence of pipillose cells on the abaxial surface partially obscuring the epidermal cells in *H. madagascariensis*, irregular for *G. kola*, straight for both *A. floribunda* and *P. butyracea* (Plate 1A-D).

b) Trichome

There were the presence unicellular stellate hairs of about 189.02 ± 5.7 μm long which have leaf-like structures attached to the body of the epidermis in *H. madagascariensis*, while in *G. kola trichome were* unicellular non-glandular of about 109.2 ± 1.07μm long, and an elongated multicellular trichome with branched tips of about 197.89 ± 4.09 long in *A. floribunda* (Plate1E-G). However, there was complete absence of trichome on both surfaces of *P. butyracea* Qualitative evaluation of stomata are strictly hypostomatic mainly paracytic (Rubiacous type) surrounded by four to six subsidiary cells. Their shapes were oblate spheroidal for *H. madagascariensis*, oblate for *G. kola* and prolate for *A. floribunda* and *P. butyracea* (Table 1).

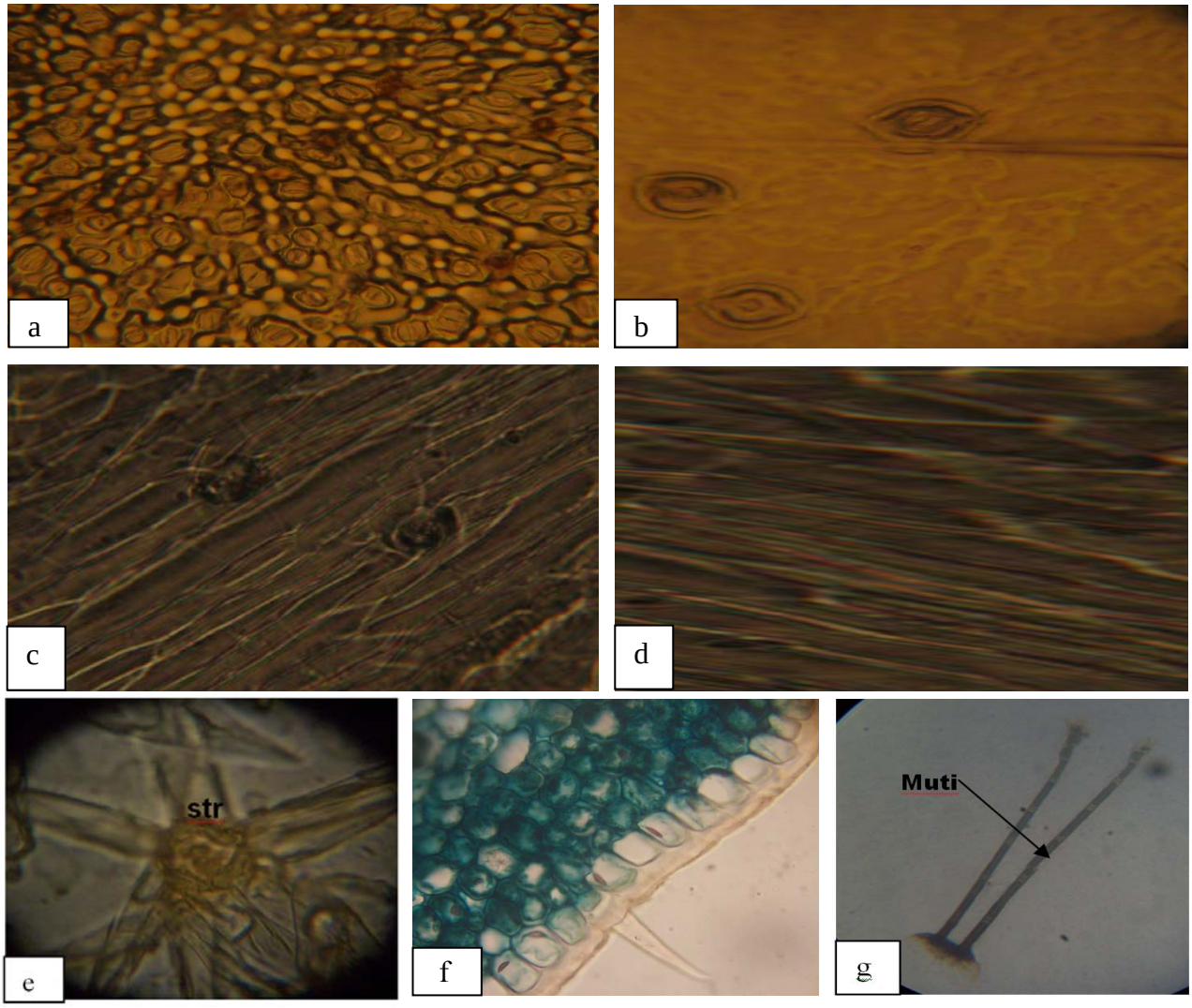

Plate 1: Foliar epidermal features of, a= H. madagascariensis, pc-papillose cell , Abaxial surface devoid of stomata. b.= A. floribunda, c= G. kola, d= P. butyracea icw- irregular cell wall, e= str- stellate trichome with crystal base in H. madagascariensis, f= unicellular trichome in G.kola, g= multicellular trichome in A. floribunda

Table 1 : Qualitative Values of Stomata Features of the Four Species of Nigerian Clusiaceae

Species	Stomata			Trichome	Nature of Epidermal Wall	
	Distribution	Type	Shape	Nature	Abaxial	Adaxial
H madagascariensis	Hypostomatic	Paracytic	Oblate Spheroidal	Stellate	Irregular	Irregular
G. kola	Hypostomatic	Paracytic	Oblate	Uniseriate	Irregular	Irregular
A floribunda	Hypostomatic	Paracytic	Prolate	Multicultural	Straight	Straight
P. butyracea	Hypostomatic	Paracytic	Prolate	Glabrous	Straight	Straight

c) Stomata Features

Stomatal features vary significantly in *H. madagascariensis, G. kola, A. floribunda* and *P. butyracea* in most of the features, but they were statistically the same in *A. floribunda* and *P. butyracea. H. madagascariensis* and *G. kola* varied in all their features except in their stomata breadth (Table 2). Statistical analysis of some of the attributes of stomata showed that, there are very high significant differences ($p < 0.05\%$) between stomata. lengths, pore lengths and breadths, number of stomata per field of view, guard cell lengths and breadths among the four species

with LSD of 0.1, 0.13, 0.08, 0.15, 3.21, 0.11 and 0.04, respectively (Table 2).

Table 2 : ANOVA on Stomata Features of the Four Species (mean standard error in μm x 400)

Species	STB	STL	PL	PB	NSPV	GCL	GCB
H. madagascariesis	0.41c	1.31b	0.77b	0.11b	132.25a	0.83c	0.11c
G. kola	1.81a	1.40b	1.15a	0.37a	32.85b	1.1b	0.25a
A floribunda	1.26b	1.95a	1.15a	0.12b	18.5c	1.29a	0.21b
P. butyracea	1.23b	1.86a	1.15a	0.12b	17.95c	1.26a	0.21b
LSD	0.1	0.13	0.08	0.15	3.21	0.11	0.04

Note: Means followed by the lower case letters were not significantly different, but were significantly different from mean of a different case letters.

Legend: STB - somatal breadth, STL- stomata length, PL - pore length, PB - pore breadth, NSPV- number of stomata per field of view , GCL- guard cell length, GCB- guard cell breadth

d) Stomata Density, Number of Epidermal Cell and Stamata Index

Stomata densities per a field of view varied in the four species from 133.1 ± 19.0, 32.6 ± 4.10, 17.00 ± 1.50 and 17.00 ± 1.50 in *H. madagascariensis, G. kola, A. floribunda* and *P. butyracea,* respectively (Fig 1).

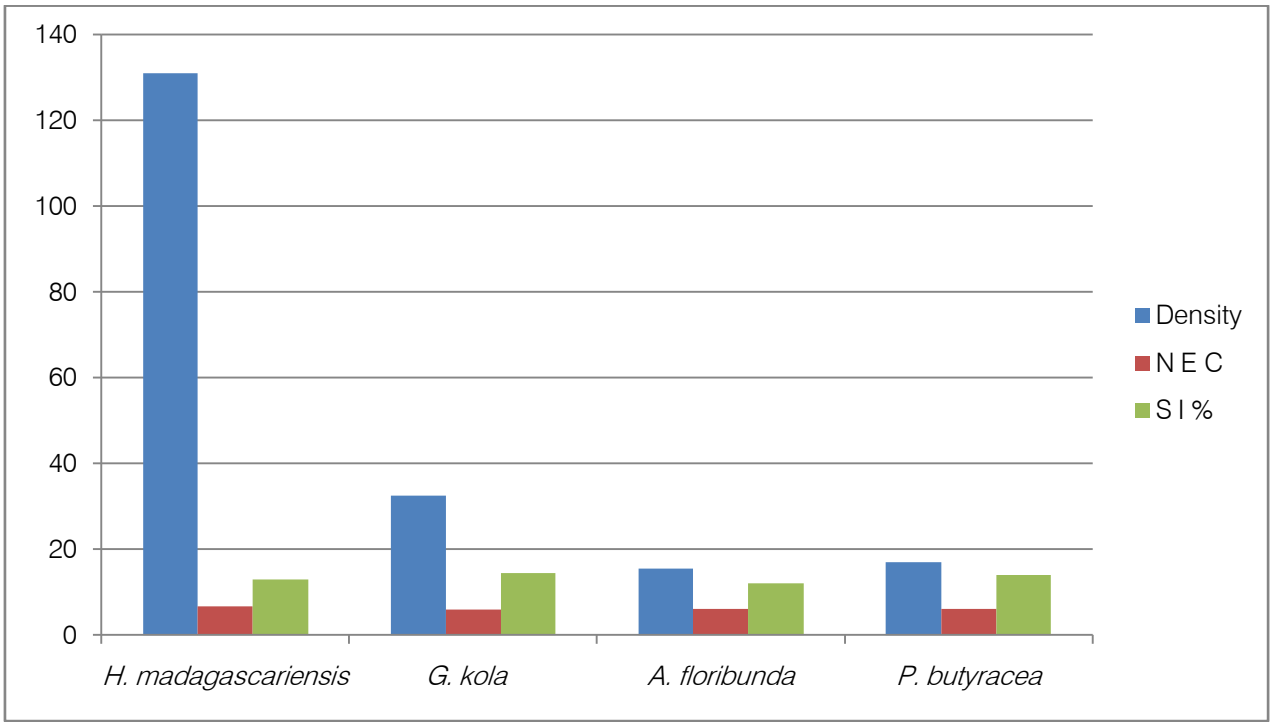

Figure 1 : Stomata Density, No of epidermal cell and Stomata Index (μm, x 400). Where NEC=No of epidermal cell, SI= Stomata Index

IV. Discussion

a) Foliar Epidermal Features

Foliar epidermal features from the four species showed that, the shape of the epidermal cell walls were highly variable in outline among these species studied. Cell walls were irregular with palpilose cells on the abaxial surface in *H. madagascariensi*, irregular in *G.kola,* but straight in *A. floribunda and P. butyracea.* These findings are in line with the findings of Ahmad *et al.,* (2009), who reported that epidermal cell shapes and anticlinal cell wall patterns are highly variable from taxon to taxon and even within the same taxon. Stace (1965) stressed that curves or irregular walls of plant epidermal cells are more of mesomorphic character and that environmental conditions such as temperature and

relative humidity play significant roles in determining the patterns of these cell walls. He pointed out that irregular walls are features of open vegetation while straight walls are associated with forest environments.

This present work is in conformity to some extent with the above reports, as *H. madagascariensis* and *G. kola* with irregular cell walls are found in and around open environment, while *A. floribunda* and *P. butyracea* with their straight cell walls are associated with forest environments, where the relative humidity and temperature are more of constant.

Bearing the above opinions in mind, the number of epidermal cells and the presence of palpilose cells in *H. madagascariensis* seem to have more taxonomic value. These tend to suggest the uniqueness of this taxon from others. The above diagnostic features could serve in the delimitation of the genus *Harungana* from the rest of the genera in the Clusiaceae. These findings are in line with the report by Metcalfe and Chalk (1957) who observed some of these features in some exotic members of the Hypericaceae where they placed *H. madagascariensis*.

b) Trichome

Three basic types of trichome were encountered in this study; stellate types in *H. madagascariensis* (Plate 1e), unicellular type in *G. kola* (plate 1f) and multicellular elongate type with branched tips in *A. floribunda* (Plate 1g). The presences of these diverse types made it difficult to infer their taxonomic value. Moreover, their taxonomic value in this work is greatly limited by the complete absence of trichome in *P. butyracea*. The above findings were in accordance with the reports of Pandey (2004), who stated that a whole family may be recognized by the occurrence of one or more types or by the presence or complete absence of one distinctive type of trichome or the other in members of the same family.

Although the significance of these hairs as revealed in this study is not yet fully understood because of the above facts, Metcalfe and Chalk (1957) held that trichome frequency, size and types were environmentally controlled. They reiterated that it was therefore possible that each species responds to its environment in a specific way, by modifying the basic plan of certain features to improve its adaptation to such environment.

c) Stomata

The relevance of stomata in the taxonomy of angiosperms has severally been emphasized by (Edeoga and Ikem, 2001; Edeoga and Ogbobor, 2001). These authors mostly considered epidermal features at the species and genus levels, thus making it worthwhile to investigate its usefulness at the generic levels within this Nigerian Clusiaceae. A look at (Table 2) shows that *A. floribunda* and *P. butyracea* are the same in all their stomata features. This could be an indication of their close phylogenetic relatedness.

However, the size and density of stomata have been reported by several authors to be correlated in the sense that small stomata often have a high density while large stomata are associated with lower density (Dessine *et al.,* 2005). They further stated that small stomata are particularly present in plants with microphyllous leaves often having thick cuticle and/or are densely hairy. Similarly, Abdul rahaman and Oladele (2012) reported that the rate of transpiration affects stomata type and size, according to them, leaf having higher rate of transpiration are with larger stomata sizes than those having lower rate of transpiration. The above observation was taxonomically relevant in the classification of these taxa. This present study is in conformity in some respect with the above reports, wherein *H. madagascariesis* that had the highest stomata density of 131.1± 19.0 was characteristically in domentous and had smaller stomata as against others with lower densities.

V. Conclusion

Conclusively, data from this study could help to resolve the taxonomic problems in this family, confirm the identity and supports the observation of earlier worker that micro morphological characters and other epidermal features could be employed for species delimitations.

References Références Referencias

1. Ahmad, K. M. A., Khan, M., Ahmad, M., Zafar, M., A. and Ahmad, F. (2009). Taxonomic Diversity of Stomata in Dicot Flora of a District Tank (N.W.F.P) in Pakistan. *African Journal of Biotechnology, 8*:1046–1055.
2. Aworinde, D.O., Nwoye, D.U., Jayeola, A. A., Olagoke, A.O. and Ogundele, A. A. (2009). Taxonomic Significance of Foliar Epidermis in some Members of Euphorbiaceae Family in Nigeria. *Res. J. Bot., 4*: 17-28.
3. Bamps, P., N. Robson & B. Verdcourt. 1978. Guttiferae. In R. M. Polhill, ed., *Flora of tropical East Africa.* Crown Agents for Oversea Governments, London.
4. Cheek, M. (2004). *Garcinia kola*. *2008 IUCN Red List of Threatened Species*. IUCN 2008.
5. Dessein, S., Harwood, R., Smmets, E. and Robbrecht, E. (2005). Pollen of the Spermacoce (Rubiaceae) Species from the Northern Territory of Australian: Morphology and Taxonomic Significance. *Australia Systematic Botany, 18*: 367-382.
6. Edeoga, H. O and Ikem, C. I. (2001). Comparative Morphology of the Leaf Epidermis in Three Species

of *Beorhavia* L. (Nyctaginaceae). *Journal of Economic Taxonomy and Botany. 19:* 197- 205.
7. Edeoga, H. O and Ogboabor, N. O. (2001). Epidermal Studies Features of some Nigerian Species of *Aneilema* R. Br (Commelinaceae). *Journal of Economic and Taxonomy and Botany. 19:* 117-124.
8. Folorunso, A. E. and Olaniyan, O. F. (2009). Comparative Foliar Epidermal Studies in *Andropogon gayanus* (Kunth) and *Andropogon tectorum* (Schum & Thonns) in Nigeria. *Thaiszia – Journal of Botany*, 19: 27-35.
9. Gill, L. S. (1988). *Taxonomy of Flowering Plants*. Africana- FEP Publishers Limited, Nigeria. 338, pp.
10. General Linear Model (GLM) procedure in Statistical Analysis System (SAS), SAS Institute (2000), version 9.
11. Gustafsson, Mats H. G. (2002), "Phylogeny of Clusiaceae Based on *rbc*L sequences", *International Journal of Plant Sciences 163*: 1045, doi:10.1086/342461, http://www.jstor.org/stable/3080291
12. Iloeje, N. P. (1999). *A New Geography of West Africa*. Longman Group Ltd. 201, pp.
13. Isichei, A. O. (2005). The Role of Plant Resources in Nigerias Economic Recovery Agenda. *Nigerian Journal of Botany 18*: 1-22.
14. Keay, R. W. J., Onochie, C. F. A. and Stanfield, D. P. (1964). *Nigerian Trees*. Federal Department of Forest Research. Oxford Science Publications, New York. 465, pp.
15. Keay, R. W. J. (1989). *Trees of Nigeria*. Clarendon Press Oxford University Press, New York. 476 pp.
16. Kokwaro, J. O. (1976). *Medicinal Plants of East Africa.* 2. Kenya Literature Bureau; Nairobi, Kenya;
17. Metcalfe, C. R. and Chalk, L. (1957). *Anatomy of the Dicotyledons,* Vol 1, Oxford University Clarendon Press, London. 726 pp. ___ (1965). *Anatomy of the Dicotyledons*. Vol. 2. Clarendon Press, Oxford, London. pp. 726-1460.
18. Matig, A. L., Aoudji, A. K. N. and Linsoussi, A. (2007).*Gkola* Seed Dormancy- Breaking *Applied Ecology and Environmental Research 5(1*): 63-71.
19. Nnamani, C. V. and Nwosu, M. O (2012a). Pollen Morphology of Some Members of the Nigerian Clusiaceae and Its Taxonomic Significance. *Journal of Pharmacy and Biological Sciences 3 (3),* 14-19.
20. Nnamani, C. V. and Nwosu, M. O (2012b) Taxonomic Significance of the Occurrence and Distribution of Secretory Canals and Tanned Cells in Tissues of some Members of the Nigerian Clusiaceae. *Journal of Biology, Agriculture and Healthcare, 2 (10),* 100-105.
21. Pandey, B. P. (2004). *Plant Anatomy*. Chand and Company Ltd, India. 498, pp.
22. Robson, N. K. B., 1961. Guttiferae. In A. W. Exel and H. Will, eds., *Flora Zambesiaca*. Vol. 1: 371-375. Crown Agents for Overseas Governments and Administrations, London.
23. Sharma, P.H.B, Handique, P.J. and Devi, H. S. (2013). A Historical and Taxonomic Overview of *Garcinia* L. and its reproductive ecology, *Folia malaysiana Vol. 14 (1)* 63-76.
24. Stace, C. A. (1965). Cuticular Studies as an Aid to Plant Taxonomy. *Bulletin of the British Muesum (Natural History) Botany 4:* 1-78.
25. Whitmore, T. C. (1973). *Garcinia* L. In T. C. Whitmore, ed., *Tree Flora of Malaya:A Manual for Foresters*: 196-225. Longman, London.
26. Yasmin, G., M.A. Khan, N. Shaheen, M.Q. Hayat, Z. Ullah and S. Munsif, 2009. Systematic value of foliar anatomical features in *Polygonum* L. species, family polygonaceae from Pakistan. *Int. J. Agric. Biol.*, 11: 731–736.