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COMPARATIVE ECOLOGICAL AND FUNCTIONAL ANATOMY OF THE FOLIAR VARIABLES OF SOME TREE SPECIES IN THE SOUTHERN NIGERIA

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ABSTRACT

Classification of plants has suffered a number of setbacks because of the inexplicit analysis of plant characters used in the delimitation of taxa. The earlier plant classification relied only on the morphological characters without putting anatomy and ecological influences into consideration. This work aimed at utilizing anatomical traits to assess the compatibility or suitability of the nine taxa in the families where they belong. Comparative foliar anatomy of nine tree genera *Antiaris toxicaria* Lesch. (Moraceae), *Ficus exasperata* Vahl. (Moraceae), *Milicia excelsa* Welw. (Moraceae), *Dacryodes klaineana* Lam. (Burseraceae) *Canarium schweinfurthii* Engl. (Burseraceae), *Dacryodes edulis* Lam. (Burseraceae), *Erythrophleum suaveolens* Taub. (Fabaceae), *Pelthophorum pterocarpum* De Cand. (Fabaceae) and *Pterocarpus soyauxii* Taub. (Fabaceae), from various locations in the Southern Nigeria, was studied using five samples of each species randomly collected from their natural regions of provenance. Three genera were selected from each of the three families Moraceae, Burseraceae and Fabaceae. Leaf samples were sectioned with a Reichert (Heidelberg) sledge microtome and conventional clearing methods were followed and finally observed under light Olympus (CHBS) model XSZ-107BN-Japan Biological Microscope on which a Motican camera was attached. Comparison of anatomical parameters showed the hypostomatic leaf types. Variations occurred in Moraceae, Burseraceae and Fabaceae in their epidermal cell. Stomatal types varied. Non-septate trichomes were present in the two taxa of Moraceae but absent in one. In Burseracea, trichomes were present in *C. schweinfurthii* but absent in the other two taxa. It occurred in only one species in Fabaceae. In the transverse sections of the leaves, different vascular bundle arrangements were observed. *E. suaveolens* had the highest stomatal number while *P. pterocarpum* had the least. *C. schweinfurthii* had the highest number of epidermal cells while *F. exasperata* had the least. *P. pterocarpum* had the highest aperture sizes while *D. klaineana* had the least. Some ecological factors influenced some parameters. The data obtained from the morphological and anatomical parameters were subjected to one way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) to compare their means and for multiple separations of means respectively ($P \leq 0.05$).

Keywords: Anatomy, Ecology, Foliar, Variables and Tree genera

INTRODUCTION

Leaves play vital roles in the productivity of plants in a forest ecosystem through photosynthesis, gas exchange and stomatal transpiration. Leaf surfaces act as boundary between atmosphere and internal leaf tissues. The types and sizes of leaves vary greatly among taxa, used in the taxonomic determination of the species along with their anatomical features. Leaf anatomy provides various characters of taxonomic importance as has been rightly stated by **[1]**, that the leaf is perhaps anatomically the most varied organ of angiosperms and its anatomical variations often concur closely with generic and specific and occasionally familial lines. In a study carried out by **[2]**, he investigated ten species of *Crucus* which showed that palisade cell height and spongy cell width are among the parameters to distinguish species. On the other hand, few studies discussed the role of the anatomical traits in the adaptation of the species to different habitats. **[3]**, carried out a study on *Solanum nigrum* collected from different habitats within the same ecological zone in Europe and it showed variability in terms of stomata number, number of hairs, thickness of lamina, palisade and spongy tissue, as well as the size of mesophyll cells of the species collected from different localities. The earliest taxonomic practices were based only on morphological parameters. However, In no case can we depend on external characters alone in generic and specific distinctions but if it is taken hand-in-hand with the anatomy, it is frequently observed that the two supplement one another in a most agreeable manner. In other words, a truly scientific classification can moreover be based only on a complete knowledge of all the morphological and anatomical features of the plant **[4]**. The internal structures of the plants have added a lot in understanding the relationships between the taxa. The organization of the vascular system in stem and leaf, petiole and nodal anatomy, leaf vasculature and architecture and epidermal studies are considered important characters in phylogeny and taxonomy. Some articles on trichomes, stomata, cuticles and leaf architecture have been useful in helping to establish relationships **[5, 6, 7]**.

MATERIALS AND METHODS

Collection of study samples

Replicates of five (5) samples of leaves of each of the nine Genera namely: *Antiaris toxicaria, Ficus exasperata, Milicia excelsa, Dacryodes klaineana, Canarium schweinfurthii, Dacryodes edulis, Erythrophleum suaveolens, Pelthophorum pterocarpum and Pterocarpus soyauxii,* were collected from five different locations in each of the three ecological zones in the Southern Nigeria as shown in the **Table 1**.

The leaf samples were put in polythene bags to keep their cells and tissues in the active cellular state from the point of collection to the Plant Anatomy Laboratory where they were fixed in Formalin Acetic Alcohol, (FAA) separately in a well labeled glass jars. This solution contained 90 ml of 70% ethanol, 5 ml of glacial acetic acid and 5 ml of formaldehyde. This preserved the leaves and their cell contents for a long period as well as softening them for easy sectioning with the microtome **[8]**. Five (5) mature leaves from the terminal axes of twigs of the nine woody genera were collected. Foliar epidermis of the adaxial (upper) and abaxial (lower) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in commercial bleach "Hypo" containing 3.5% sodium hypochlorite for 18 hrs. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip. The slides were viewed with a model XSZ-107BN-Japan, Biological Microscope at x40, x100 and x400 magnifications and photomicrographs were taken with a Moticam Camera 2.0.

The following parameters were observed and assessed:

1. **Epidermal cells:** the type and number of epidermal cells were counted and recorded.
2. **Stomata type:** the stomatal types were observed and recorded following the terminologies of Miguel (2011).
3. Stomata size (length and width): the stomata length and width were measured using Moticam micro-scope software in a total of 10 fields of view for each sample.
4. Stomatal density: the stomatal density was determined as the number of stomata per square millimetre.

Table 1: The locations, ecological zones, coordinate annual rainfall and average temperature

Locations	Ecological zones	Coordinate	Annual rainfall	Average temperature	Authors citation
Nsukka, Enugu State	**Derived Savannah**	**6.86°N, 7.39°E**	**1051 mm**	**27.3 °C**	**Chinago, (2015)**
Awka, Anambra State	**Southern Guinea Savannah**	**6.22°N, 7.08°E**	**1828 mm**	**26.5 °C**	**Okereke *et al.*, (2016)**
Obio/Akpo, Rivers State	**Fresh Water Swamp Forest**	**4.87°N, 7.01°E**	**2708 mm**	**25.8 °C**	**Adoki, (2012)**

RESULTS

FOLIAR STUDIES: Observations on the leaves of all the species showed the hypostomatic type, that is, stomata appearing only on one side of the leaf.

Epidermal cell

In Moraceae, the epidermal cell types in *A. toxicaria* are irregularly shaped with wavy anticlinal cell walls on both adaxial and abaxial surfaces **(Plates 1a and d)** while in *F. exasperata* and *M. excelsa* they were polygonal with straight cell walls on the upper surface and irregularly shaped with wavy cell walls on the abaxial surface **(Plates 1 b, c, e and f)**. In Burseraceae, the epidermal cells in *D. klaineana* is irregularly shaped with wavy anticlinal cell wall on both surfaces (Plate 2a and d) while *C. schweinfurthii* and *D. edulis* have the same polygonal shape with straight cell wall on both surfaces **(Plates 2 b, c, e and f)**. The epidermal cells in *E. suaveolens* and *P. soyauxii* of Fabaceae are irregularly shaped with wavy anticlinal cell wall on both surfaces **(Plates 3a, c, d and f)** while *P. pterocarpum* had polygonal shape with straight cell wall on both surfaces **(Plates 3 b and e)**.

Stomatal type

The stomatal types in *A. toxicaria* and *M. excelsa* were anomocytic – (irregular celled), the stomata remained surrounded by limited number of subsidiary cells which were quite alike the remaining epidermal cells **(Plates 1d and f)**. Presence of paracytic type of stomata (stomata surrounded by two subsidiary cells which were parallel to the longitudinal axis of the pore and guard cells) was observed in *F. exasperata* **(Plate 1e)**. In Burseraceae, *C. schweinfurthii* and *D. edulis* had anomocytic type **(Plates 2e and f)** while *D. klaineana* had tetracytic type **(Plate 2d).** *E. suaveolens* and *P. soyauxii* of Fabaceae had paracytic type **(Plates 3 d and f)** while *P. Pterocarpum* had anomocytic type **(Plate 3e)**.

Stomata sizes

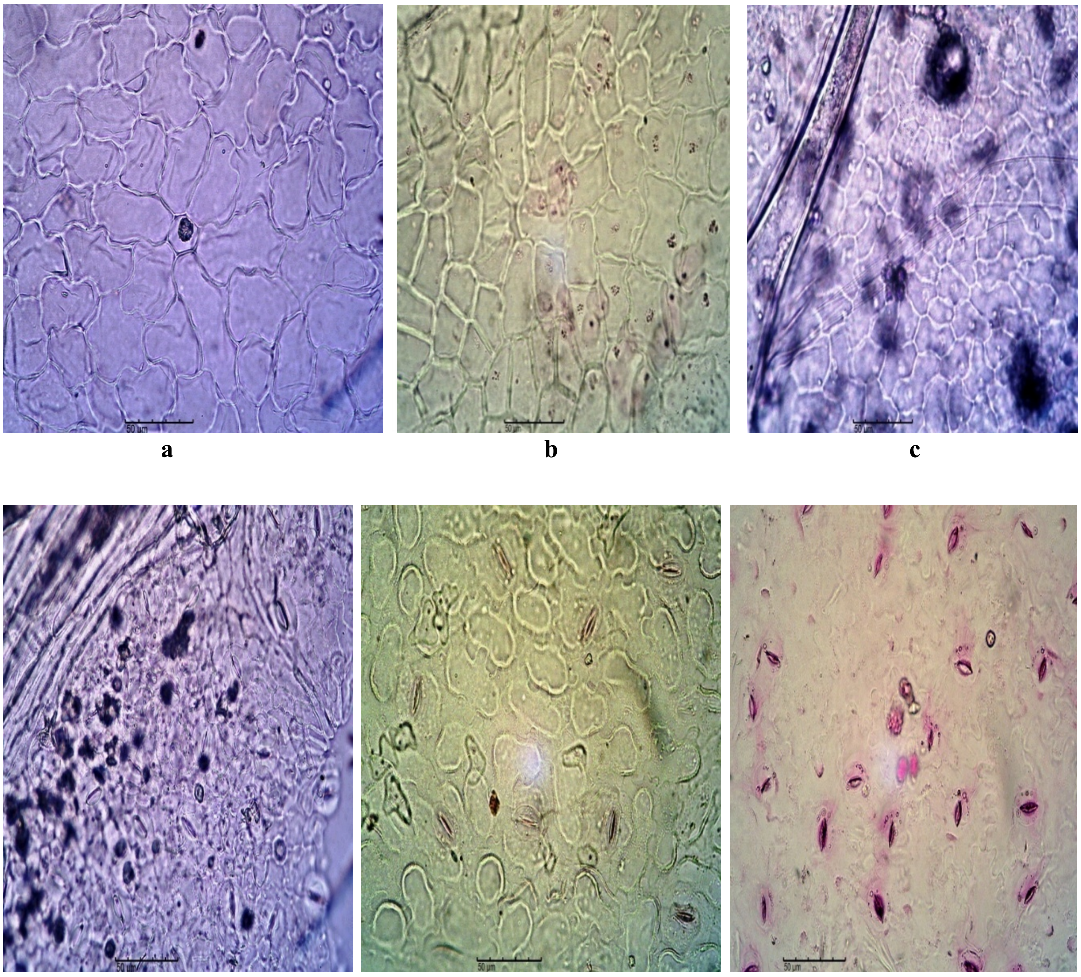
Comparison of the stomatal sizes of the nine species showed that they all differed significantly ($P \leq 0.05$) across families and across ecological zone, increasing from Southern Guinea Savannah through the Derived Savannah to the Fresh Water Swamp Forest. Across families, the stomata sizes decreased in this order; *P. pterocarpum, F. exasperata, A. toxicaria, C. schweinfurthii, D. edulis, M. excels, P. soyauxii* and *E. suaveolens* **(Table 4)**.

Trichomes

There was the presence of unicellular and unbranched trichomes in all the species in Moraceae **(Plates 1)**. In Burseraceae, there were no trichomes in *D. klaineana* and *D. edulis* but in *C. schweinfurthii* there were unicellular and unbranched trichomes **(Plate 2)**. *E. suaveolens* and *P. soyauxii* possessed no trichomes while *P. pterocarpum* possessed trichome **(Plate 3)**.

Vascular bundle arrangement

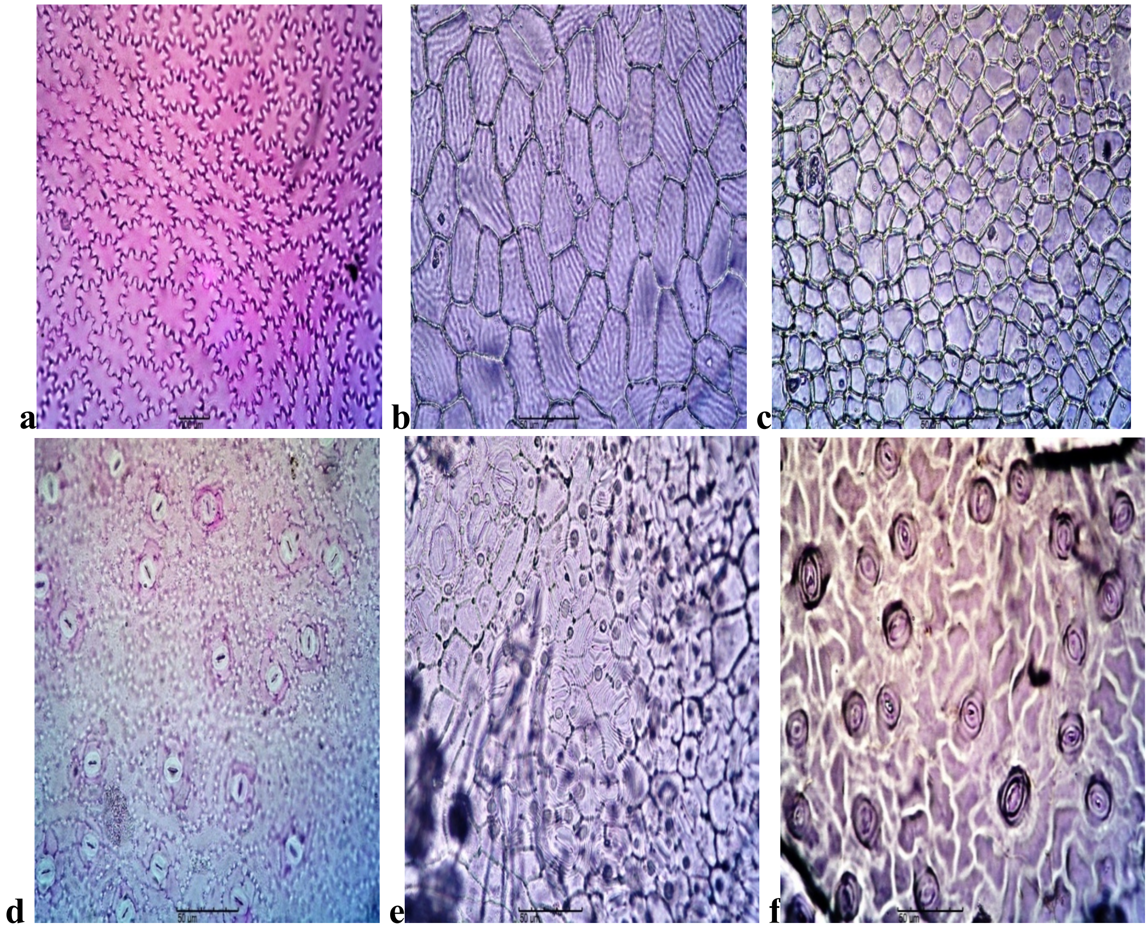
The transverse sections of the leaves of all the genera in Moraceae were 'O'-shaped, *D. klaineana* had inverted triangular shape, *C. schweinfurtii* had 'O' shape while *D. edulis* had polygonal shape. In Fabaceae, *E. suaveolens* had 'O' shape while *P. soyauxii* had triangular shape. They were all said to be conjoint, where the vascular tissues were arranged in bundles. The arrangement was said to be amphicribral and collateral.



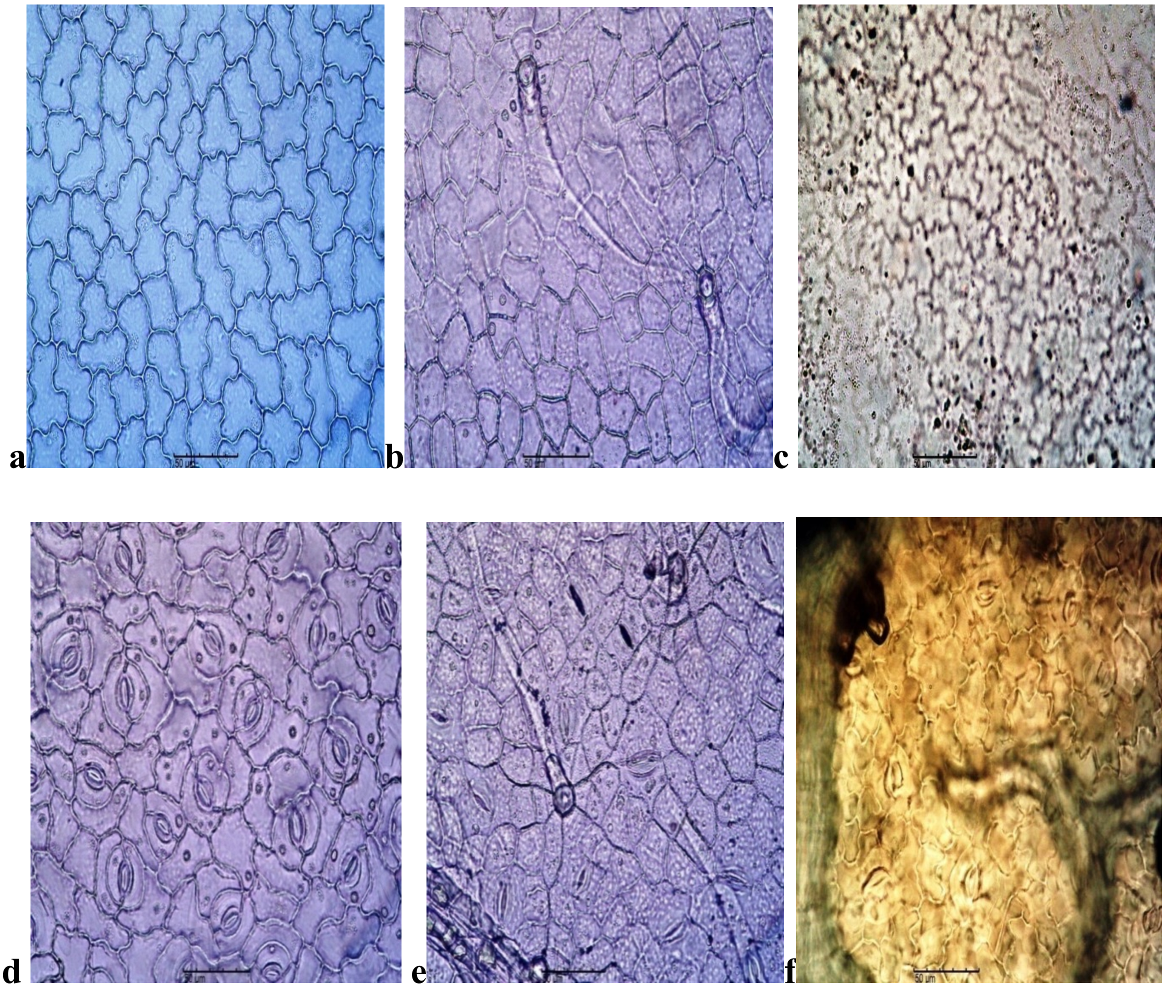
a b c

d e f

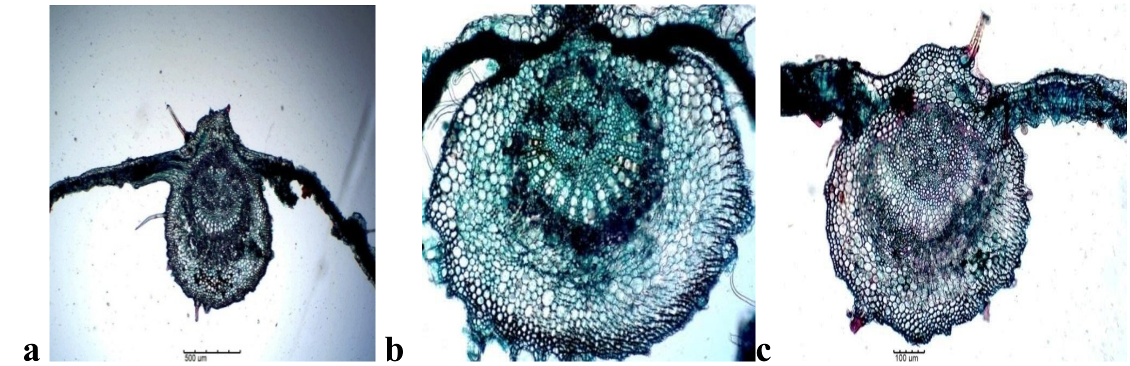
Plates1(a-f): Comparison of the features of the leaves of *a- adaxial surface of A. toxicaria leaf* showing irregular and wavy epidermal cells; b - adaxial surface of the leaf of *Ficus exasperata* showing epidermal cells with polygonal and straight cell walls; c - adaxial surface of the leaf of *Milicia excelsa* leaf showing polygonal epidermal cells with straight cell wall; d - the abaxial surface of *A. toxicaria leaf* showing some stomata, stomata aperture, guard cells and irregular wavy epidermal cell; e - abaxial surface of the leaf of *F. exasperata* showing irregularly shaped with wavy epidermal cell wall; d - abaxial surface *of M. excelsa* showing irregularly shaped and wavy epidermal cell wall. EC=epidermal cell, ECW=epidermal cell wall, SA= stomatal aperture, GC= guard cell



Plates 2(a-f): Comparison of the abaxial features of the leaves, a - *D. klaineana* showing epidermal cell with straight cell walls and crystals of calcium oxalate; b - Adaxial surface of the leaf of *Canarium schweinfurthii* showing polygonal with straight cell walls; c - Adaxial surface of the leaf of *Dacryodes edulis* showing epidermal cell with straight cell walls and crystals of calcium oxalate; d- abaxial surface of the leaf of *D. klaineana* showing stomatal apparatus, irregularly shaped epidermal cells with cyclocitic type of stomata; trichomes were absent; e- the abaxial surface of *Canarium schweinfurthii* showing polygonal shaped with straight cell walls, stomata, trochomes and crystals of calcium oxalate; f- the abaxial surface of *Dacryodes edulis* showing subsidiary cell, irregularly shaped epidermal cell wall and stomatal apparatus

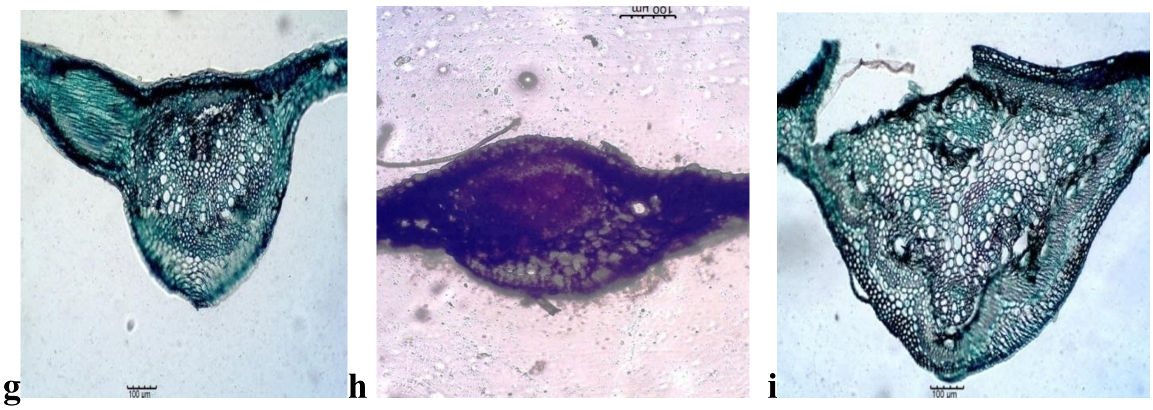


Plates 3 a: Adaxial surface of the leaf of *Erythrophleum suaveolens* showing irregularly shaped and wavy anticlinal epidermal cell walls; b - Adaxial surface of the leaf of *Peltophorum pterocarpum* showing polygonal with straight epidermal cell wall and the presence of crystal of calcium oxalate; c - Adaxial surface of the leaf of *Pterocarpus soyauxii* showing irregularly shaped with wavy anticlinal epidermal cell wall; d- abaxial surface of *Erythrophleum suaveolens* showing paracitic and anomocytic type of stomata, irregularly shaped epidermal cell, stomatal apparatus and crystals of calcium oxalate; e- abaxial surface of leaf of *Peltophorum pterocarpum* showing unicellular and non septate trichomes, stomata and polygonal with straight epidermal cell walls; f- abaxial surface of *Pterocarpus soyauxii* showing the stomata apparatus with irregularly and wavy anticlinal epidermal cell wall



A . toxicaria **(O- shaped)** ***F. exasperata*** **(O- shaped)** ***M. excelsa*** **(O-shaped)**

D. klaineana **(inverted triangle)** ***C. schweinfurthii*** **(O- shaped)** ***D. edulis*** **(O- shaped)**



E. suaveolens **(O- shaped)** ***P. ptericarpum*** **(O- shaped)** ***P. soyauxii*** **(inverted triangle)**

Plates 4 (a-i) T.Ss of the leaves of all the genera showing the types of vascular bundles

Table 2: Comparison of the stomata number within and across families and across locations ($p \leq 0.05$)

Families	Species	Nsukka	Awka	Obia/Akpo
Moraceae	*A. toxicaria*	$30.24\pm0.58^{c,1}$	$29.13 \pm 0.58^{b,2}$	$23.70 \pm 0.54^{b,3}$
	F. exasperata	$24.19\pm1.15^{d,1}$	$15.09 \pm 0.01^{h,2}$	$9.67 \pm 0.57^{g,3}$
	M. excelsa	$30.49\pm0.58^{bc,1}$	$26.32 \pm 0.01^{d,2}$	$20.67 \pm 0.58^{c,3}$
Burseraceae	*D. klaineana*	30.24 ± 0.57^{c1}	$27.59 \pm 0.58^{c,2}$	$22.33 \pm 0.58^{c,3}$
	C. schweinfurthii	$41.23\pm0.58^{a,1}$	$38.93 \pm 0.01^{a,2}$	$31.67 \pm 0.58^{a,3}$
	D. edulis	$30.10\pm0.57^{c,1}$	$24.71 \pm 0.57^{e,2}$	$21.33 \pm 0.01^{c,3}$
Fabaceae	*E. suaveolens*	$32.45\pm0.58^{b,1}$	$25.13 \pm 0.58^{e,2}$	$18.67 \pm 0.01^{d,3}$
	P. pterocarpum	$14.68\pm0.01^{e,1}$	$21.61 \pm 0.58^{f,2}$	$14.33 \pm 0.58^{f,3}$
	P. soyauxii	$24.13\pm0.58^{g,1}$	$20.19 \pm 0.58^{g,2}$	$15.67 \pm 0.57^{e,3}$

Means with letter superscripts showed that there were significant differences across genera and figure superscripts showed that there were significant differences across location in a decreasing order from Nsk to Obio/Akpo ($P\leq0.05$)

Table 3: Comparison of Epidermal cell numbers within and across families and across locations ($P \leq 0.05$)

Families	Species	Nsukka	Awka	Obia/Akpo
Moraceae	*A. toxicaria*	$136.22 \pm 0.58^{b,3}$	$139.26 \pm 0.57^{b,2}$	$140.75 \pm 0.57^{b,1}$
	F. exasperata	$55.86 \pm 0.57^{i,3}$	$56.89 \pm 0.57^{i,2}$	$58.53 \pm 0.34^{i,1}$
	M. excelsa	$121.57 \pm 0.58^{e,3}$	$121.94 \pm 0.57^{e,2}$	$123.35 \pm 0.58^{e,1}$
Burseraceae	*D. klaineana*	$131.37 \pm 0.58^{c,3}$	$131.72 \pm 1.14^{c,2}$	$134.62 \pm 0.57^{c,1}$
	C. schweinfurthii	$186.27 \pm 0.58^{a,3}$	$186.49 \pm 0.01^{a,2}$	$189.22 \pm 0.58^{a,1}$
	D. edulis	$123.49 \pm 0.57^{d,3}$	$125.69 \pm 0.01^{d,2}$	$126.44 \pm 1.16^{d,1}$
Fabaceae	*E. suaveolens*	$105.80 \pm 0.01^{f,3}$	$108.80 \pm 0.58^{f,2}$	$109.97 \pm 0.02^{f,1}$
	P. pterocarpum	$83.31 \pm 0.58^{h,3}$	$84.38 \pm 0.01^{h,2}$	$84.87 \pm 0.58^{h,1}$
	P. soyauxii	*$93.16 \pm 0.01^{g,1}$*	*$92.38 \pm 0.01^{g,2}$*	*$90.10 \pm 0.58^{g,3}$*

Means with letter superscripts showed that there were significant differences across species and figure superscripts showed that there were significant differences across location in a decreasing order from Nsk to Obio/Akpo ($P \leq 0.05$). The italised figures behaved in a reverse manner from the other species

Table 4: Comparison of the Stomata Sizes (µm) within and across families and across locations ($P \leq 0.05$)

Families	Genera	Nsukka	Awka	Obia/Akpo
Moraceae	*A. toxicaria*	$424.04 \pm 0.57^{c,3}$	$436.35 \pm 0.57^{c,2}$	$440.76 \pm 1.74^{c,1}$
	F. exasperata	$428.03 \pm 0.58^{b,3}$	$445.79 \pm 0.58^{a,2}$	$451.58 \pm 0.57^{a,1}$
	M. excelsa	$332.80 \pm 0.57^{f,3}$	$337.03 \pm 0.57^{f,2}$	$340.85 \pm 0.58^{f,1}$
Burseraceae	*D. klaineana*	$170.70 \pm 0.01^{i,3}$	$173.40 \pm 1.72^{i,2}$	$176.40 \pm 0.58^{i,1}$
	C. schweinfurthii	$375.90 \pm 0.58^{d,3}$	$382.09 \pm 0.01^{d,2}$	$390.09 \pm 0.01^{d,1}$
	D. edulis	$359.79 \pm 0.01^{e,3}$	$361.37 \pm 0.58^{e,2}$	$365.13 \pm 0.58^{e,1}$
Fabaceae	*E. suaveolens*	$241.18 \pm 0.58^{h,3}$	$248.02 \pm 0.58^{h,2}$	$252.08 \pm 0.59^{h,1}$
	P. pterocarpum	$430.69 \pm 0.01^{a,3}$	$438.03 \pm 0.58^{b,2}$	$442.38 \pm 0.58^{b,1}$
	P. soyauxii	$248.27 \pm 0.58^{g,3}$	$252.40 \pm 0.57^{g,2}$	$440.76 \pm 1.74^{c,1}$

Means with letter superscripts showed that there were significant differences across species and figure superscripts showed that there were significant differences across location in a decreasing order from Nsk to Obio/Akpo ($P\leq0.05$)

DISCUSSION

The foliar types in all the nine genera that were tall trees by habit were hypostomatic which were in consonance with the works of **[9, 10]** who stated that the leaves of most of the tall trees were characterized by the hypostomatic foliar types. The variations that occurred in their epidermal cells showed that not all the species within each family were supposed to be grouped together**. [11]** stated that variations in the leaf epidermal characters have been experimentally shown to be gene-dependent as they are only slightly influenced by environmental conditions. Their proven genetic stability and high structural diversity have been the basis for their use in identification and classification of many groups. Taxonomically therefore, as can be seen in **Plates 1, 2 and 3**, if classification was to be based on the epidermal cell structures, *F. exasperata* and *M. excelsa* of Moraceae; *C. schweinfurthii* and *D. edulis* of Burseraceae; *P. pterocarpum* of Fabaceae were more closely related in their epidermal cells and should be grouped together while *A. toxicaria* of Moraceae; *D. klaineana* of Burseraceae and *E. suaveolens* of Fabaceae are more closely related.

The stomata types in all the species that were dicotyledons, were randomly distributed which was in line with the work of **[12]**, where he observed that in a number of species, particularly the monocotyledonous plants, stomata and pavement cells are arranged in a regular pattern of alternating cell files whereas in dicotyledonous plants, stomata patterning appeared to be relatively random. This therefore follows that if classification was to be made based on the stomata type, *F. exasperata* of Moraceae and *E. suaveolens* and *P. suyauxii* of Fabaceae would be grouped together.

There were differences in their stomata sizes in an increasing order from Nsukka to Obio/Akpo through Awka but they were not significant ($P \leq 0.05$). This was in accordance with the works of **[13, 14]** who stated that water deficit leads to a decrease in stomata size and an increase in stomata density. This also followed the work of **[15]** who stated that stomata sizes respond to water status - that the stomata size decreased with a reduction in the water availability. Functionally, the work done by **[16, 17]** showed that stomata size is more effective in water transpiration and gaseous exchange than stomata frequency for carbon fixation. Therefore, the rate of carbon fixation in all these genera will increase from Nsukka through Awka to

Obio/Akpo since the stomata sizes increased the same way and while *P. pterocarpum* fixes the highest carbon, *D. edulis* will fix the least.

The presence of non-glandular trichome in Moraceae was in conformity to **[9]** who stated that both non-glandular and glandular trichomes and ranunculaceous stomata occurred in the four families he worked on which included Ulmaceae, Ulticaceae, Moraceae and Cannabaceae. Functionally, the overall role of these trichomes appeared to have a protective function by covering the layer of epidermis in response to environmental changes. This distribution pattern might also be regarded as an adaptation associated with the environmental pattern and habitats of specific plants **[17]**. Although, studies of the effect of plant trichomes upon loss of water have not produced uniform results, it is believed that these trichomes insulate the mesophyll cells from excessive heat. In addition, they might also indirectly influence the water economy of the leaves or stem at early stages of development through temperature regulation **[16]**.

It can therefore, be said that *A. toxicaria, F. exasperata, M. excelsa, C. schweinfurthii* and *P. pterocarpum* will be more protected or more resistant to extreme temperature and/or sunlight than the rest of the genera. This can also be ecologically applicable.

The transverse sections of the leaves of all the genera in Moraceae were 'O'-shaped, in Burseraceae, *D. klaineana* had inverted triangular shape, *C. schweinfurtii* had 'O'-shape while *D. edulis* had polygonal shape. In Fabaceae, *E. suaveolens* had 'O'- shape while *P. soyauxii* had triangular shape. All these were said to be conjoint, amphicribral and collateral where the vascular tissues were arranged in bundles, a situation where xylem is located towards the pith while phloem is located towards the epidermis. These types of vascular bundles are characteristics of the angiosperm leaves **[18]**. This was a further confirmation that all the genera studied were angiosperms.

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