

## ORIGINAL RESEARCH ARTICLE

Comparative Foliar Epidermal Morphology of the Genus *Terminalia* L. in Nigeria<sup>1,4</sup>Nodza, G. I.\*, <sup>2</sup>Adeniran, S.A., <sup>1,4</sup>Igbari, A.O., <sup>3</sup>Hammajoda, S. A., and <sup>1,4</sup>Onuminya, T.O<sup>1</sup>Molecular Systematics Laboratory, Department of Botany, University of Lagos, Akoka, Lagos, Nigeria<sup>2</sup>Department of Plant Biology, University of Ilorin, Ilorin Nigeria<sup>3</sup>Department of Biological Sciences, Taraba State University, Jalingo, Nigeria<sup>4</sup>TETFUND Centre of Excellence in Biodiversity Conservation and Ecosystem Management (TCEBCEM), Univ. of Lagos, Lagos, Nigeria

## ABSTRACT

Foliar epidermal features play an important role in clarifying the taxonomy and systematics of flowering plants. This study investigates leaf epidermal morphology of the eight *Terminalia* species represented in Nigeria using light microscopy to resolve besetting taxonomic issues. Leaf sections were macerated, cleaned, mounted, stained, and sealed on slides for microscopic examination. Stomatal index and other measurements were then recorded. The findings revealed variability in stomatal and other epidermal features among the species, although some characteristic features remained consistent. Epidermal cells generally exhibit a regular and sinuous shape, with the exception of *Terminalia schimperiana*, which displays isodiametric cells on both surfaces. The anticlinal cell walls are either straight or undulating; however, they are curved on the adaxial surface of *Terminalia macroptera*. All the species studied exhibit hypostomatic and anomocytic stomata except for *Terminalia macroptera*, which has amphistomatic leaves. Multangulate trichomes were recorded in *Terminalia avicennioides*, and falcate trichomes in *Terminalia schimperiana*. The stomatal indices varied significantly, ranging from 28.75% in *Terminalia laxiflora* to 5.95% in *Terminalia macroptera*. Moreover, the examined species showed significant variations in stomatal type, anticlinal wall type, epidermal cell type, and trichome presence. However, these taxonomic features have been recognized as diagnostic and highly significant characteristics for resolving taxonomic issues and aiding the accurate identification of these species for effective conservation and monitoring. An artificial taxonomic key was developed based on foliar epidermal characteristics to accurately delineate and identify the Nigerian *Terminalia* spp. Therefore, further anatomical research utilizing a scanning electron microscope is recommended to strengthen the systematics of *Terminalia* in Nigeria.

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## INTRODUCTION

*Terminalia* L. is the second-largest genus of the Combretaceae family after the genus *Combretum* (Wahua and Iheaka, 2017; Zhang *et al.*, 2019). This genus consists of 200-250 species widely distributed throughout tropical and subtropical regions and is well-established in every habitat (Waman, 2015). *Terminalia* species range from shrubs to large deciduous forest trees, occupying a wide range of habitats, from savannas and forests to woodlands. *Terminalia* trees are mostly large trees reaching heights up to 80 m tall and are cosmopolitan in distribution (Aliyu *et al.*, 2018; Cock, 2015). Common characteristic features of this genus are nectar glands at the base of the lamina or petiole, flowers borne on spicate inflorescences, and flattened or winged fruits (Nithaniyal and Parani, 2016). In the flora of West Tropical Africa (Hutchinson and Dalziel, 1954; Keay, 1989), the genus is represented by 11 species, with eight species occurring in

Nigeria (Uzoechina, 1978; Keay, 1989). Many *Terminalia* species have multiple uses in traditional medicine preparations, such as timber, as raw materials in the pharmaceutical and leather processing industries (Burkhill, 2004, Cock, 2015). Many species are used for their antibacterial, antifungal, antiprotozoal, antiviral, antidiarrhoeal, analgesic, antimalarial, antioxidant, antidiabetic, anti-inflammatory, laxative, astringent, purgative, anticancer, and wound healing activities (Burkhill, 2004, Cock, 2015.). In Nigeria, wood from certain *Terminalia* species is employed in producing commercial charcoal and in manufacturing tools, such as mortar and hoe handles (Nodza *et al.*, 2013). However, these species' combined use and overexploitation jeopardize the continued existence of many *Terminalia* species in the wild. For example, *Terminalia ivorensis* A. Chev has been classified by the International Union of

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Conservation of Nature (IUCN) as Vulnerable (VU) (Hawthorne, 1998). While *T. superba* Engl. & Diels have been reported to be greatly decimated and face extinction within their natural habitat (Onyekwelu and Stimm 2004; Onana, 2011). Therefore, accurate identification is imperative for effective conservation and monitoring of species (Nithaniyal and Parani, 2016). Traditionally, the identification of *Terminalia* species has relied heavily on floral morphological traits. However, this method has proven problematic because of the synonymy among species, which results from the overlap of morphological characters and high variations between individuals (Deshmukh *et al.* 2009; Maurin *et al.* 2010; Waman 2015; Nithaniyal and Parani 2016; Akinsulire *et al.* 2018). Similarly, *Terminalia* species exhibit significant variations in morphology and karyotype (Mishra *et al.*, 2017), which seldom complicates their identification. A typical example is the pair of *Terminalia ivorensis* A. Chev., and *Terminalia glaucescens* (Uzoechina 1978). To resolve this issue, the foliar epidermal method can play a crucial role in the accurate species-level identification of *Terminalia*, even when specimens are sterile or fragmented (Paul and Chowdhury 2021). This is because variations within a species, genus, or family are often reflected in anatomical traits, as documented by several authors (Waman, 2015; Akinsulire *et al.*, 2018; Kadiri *et al.*, 2019; Onuminya *et al.*, 2020; Damaiyani *et al.*, 2022). Recently, Akinsulire *et al.* (2018) conducted a leaf anatomical characterization on five of the eight *Terminalia* species found in Nigeria: *T. superba*, *T. ivorensis*, *T. catappa*, *T. mantaly*, and *T. avicennioides*. The current study surpasses their studies by providing anatomical characteristics for the eight species of *Terminalia* represented in Nigeria, thus filling the missing knowledge gap in the literature on *Terminalia* species in Nigeria.

## MATERIALS AND METHODS

### Specimen collection and identification of plant specimens

A total of eight plant species of *Terminalia* were collected from different areas of Nigeria, and voucher specimens were subsequently prepared. The collected plant samples were taken to the herbarium and identified with the help of available literature and the Flora of Nigeria (Hutchison and Dalziel, 1927–1954; Keay, 1989). The specimens were also compared with herbarium samples obtained from the Lagos University Herbarium (LUH), Holmgren *et al.* (1990) of the Department of Botany at the University of Lagos, Nigeria. The prepared voucher specimens were deposited in LUH along with their georeference data (Table 1). The correct names of the plants were checked and verified using Plants of the World Online (POWO 2023: <https://powo.science.kew.org> ).

### Epidermal preparation

An anatomical study of the leaf epidermal surface was conducted by excising a 1-5 cm<sup>2</sup> section from the standard

median portion of the leaf lamina near the midrib. The sample was soaked in concentrated nitric acid (HNO<sub>3</sub>) for approximately 2-6 hours to macerate the mesophyll and bleach the leaf tissue. Tissue disintegration was indicated by the formation of bubbles, allowing for the separation of the epidermal layers. These layers were then transferred to Petri dishes containing water for cleaning and further separation using forceps. This method followed the protocol established by Kadiri *et al.* (2013). The tissue debris was carefully removed from the epidermis with a clean camel hair brush and rinsed in water. The epidermal layers were mounted on glass slides with the upper surfaces facing upward. A drop of 70% ethanol was applied for a few minutes to harden and dehydrate the cells. The preparations were subsequently stained with safranin for approximately 2 minutes before glycerine was added. The prepared epidermal layers were covered with coverslips and sealed with nail varnish to make the slides more permanent. These slides were labeled appropriately and examined under a light microscope. All measurements were calibrated using a micrometer eyepiece with 40x objectives. The stomatal index was calculated using the formula reported by Stace (1965).

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

Where SI = Stomatal index

S = Number of stomata per unit area

E = Number of epidermal cells

## RESULTS AND DISCUSSION

Numerous taxonomists have relied on leaf epidermis for systematic studies and species classification across various plant families and groups (Bhatia, 1984; Stace, 1984; Jones, 1986; Illo, 1995; Adedeji, 2004; Kadiri and Olowokudejo, 2019). Research has demonstrated that leaf epidermis can serve as a valuable tool for delineating the genus *Terminalia*, as Stace (1965) and Tilney (2002) established. Our results on the characteristics of the adaxial and abaxial foliar epidermis were evaluated and are presented in Tables 2 and 3 and Figures 2 and while Figure 1 present the Stomatal Index for all the 8 species. This study identified significant variations in the configuration of the foliar epidermal anatomy, which can be utilized as an additional taxonomic tool for identifying and differentiating *Terminalia* species (Figure 3). The findings revealed distinct differences in the size and shape of epidermal cells, stomata, and trichomes. Epidermal cells were irregular in *T. avicennioides*, *T. laxiflora*, and *T. macroptera* on both surfaces and irregular on both the adaxial and abaxial sides of *T. catappa* and *T. superba*. This result is consistent with the findings of Akinsulire *et al.* (2020) regarding leaf and petiole microanatomical diversity in various Nigerian species of Combretum. The remaining species exhibited either regular or sinuous epidermal cells on either surface, with the exception of *T.*

*schimperiana*, which displayed isodiametric cells on both abaxial and adaxial surfaces. However, the recorded

length of the epidermal cells was greater on the adaxial surface than on the abaxial surface.

Table 1: Species studied

S/N	Species	Coll. ID	GPS	Elevation(m)	Location
1	<i>Terminalia laxiflora</i> Engl	LUH 3395	N 06° 31.139°, E003° 24.033°	1900	CH, Taraba state
2	<i>Terminalia superba</i> Engl.& Diels	LUH 5087	N06° 30.666°, E003° 23.799°	100	ONP, Edo state
3	<i>Terminalia schimperiana</i>	LUH 1961	N06° 31.032°, E003° 23.976°	500	GGNP, Taraba State
4	<i>Terminalia macroptera</i> Guill & Perr	LUH 4217	N06° 30.803°, E003° 23.642°	80	KNP Kaduna state
5	<i>Terminalia catappa</i> Linn	LUH 8924	N06° 31.116°, E003° 24.043°	50	UNILAG, Lagos
6	<i>Terminalia ivorensis</i> A. Chev	LUH 1983	N06° 31.078°, E003° 24.052°	100	IJO, Ogun
7	<i>Terminalia avicennioides</i> Guill. & Perr.	LUH 802	N06° 30.987°, E003° 23.925°	80	KLNP, Kainji
8	<i>Terminalia mantaly</i> H. Perrier	LUH 8925	N06° 30.610°, E003° 23.708°	50	UNILAG, Lagos

Plant collection, herbarium deposition voucher number, altitude, locality, and district/province

**Keywords:** CH= *Chabal Hendu*, ONP= *Okomu National Park*, GGNP= *Gashaka Gumi National Park*, KNP= *Kamuku National Park*, UNILAG= *University of Lagos*, IJO= *Ijebu-Ode*, KLNP= *Kainji Lake National Park*

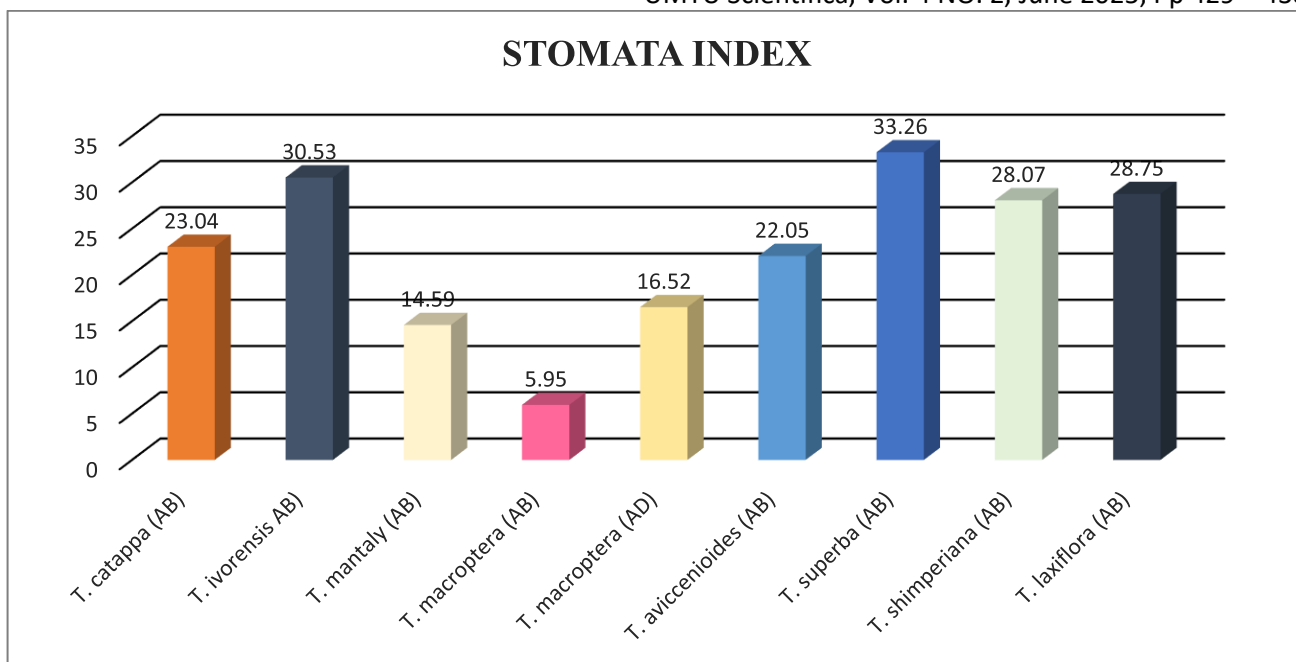


Figure 1: Bar graph of Stomatal Index in the eight *Terminalia* species

The stomatal index (SI) values revealed considerable interspecific variation in stomatal density, which is an important diagnostic trait. *T. shimperiana* exhibited the highest stomatal index (33.26%), followed by *T. ivorensis* (30.53%) and *T. laxiflora* (28.75%), indicating a higher density of stomata relative to epidermal cells on the abaxial surface. On the other hand, *T. macroptera* demonstrated the lowest stomatal index (5.95%) on its adaxial surface, suggesting a pronounced hypostomatic adaptation to reduce water loss. Species such as *T. catappa* (23.04%), *T. superba* (22.05%), *T. avicennioides* (16.52%), and *T. mantaly* (14.59%) exhibited moderate stomatal indices. These variations in stomatal index correlate with physiological adaptations to their respective environments and provide valuable insights into species differentiation within *Terminalia*.

We observed significant variations in the number of epidermal cells among different *Terminalia* species. *T. laxiflora*, *T. shimperiana*, *T. superba*, *T. avicennioides*, *T. macroptera*, and *T. ivorensis* exhibited a higher number of epidermal cells on their adaxial surfaces (see Tables 2 & 3). Most species of the genus *Terminalia* exhibit a hypostomatic anatomical structure, which is consistent with previous research (Chimezie and Agbagwa, 2015; Ramassamy, 1998; Wahua and Iheaka, 2017). However, the eight species that we examined predominantly displayed anomocytic stomata. All the leaves were hypostomatic, except for *T. macroptera*, which exhibited amphistomatic stomata. The stomatal index was useful in

distinguishing species. On the abaxial surface, *T. superba* had the highest stomatal index at 33.25%, whereas *T. mantaly* had the lowest index at 5.95%. *T. catappa* had the highest average mean number of stomata at approximately 24.9%, whereas the adaxial surface of *T. macroptera* had the least. Waman (2015) also documented hypostomatic leaves in their study on leaf epidermal characteristics. In the present study, all species exhibited hypostatic traits; however, *T. macroptera* displayed anomocytic stomata on both surfaces. This finding is consistent with those of Tilney (2002) and Akinsulire et al. (2020), who also reported numerous anomocytic stomata in the African *Terminalia*. The stomatal distribution was primarily observed on most species' abaxial surface, except for *T. macroptera*, which had stomata on both surfaces. Associated epidermal characteristics, such as trichomes, were also found to be valuable for species identification. Trichomes were only present in *T. avicennioides* and *T. shimperiana*. Multiangular trichomes were observed on the abaxial surface, whereas falcate trichomes were found on the adaxial surface of *T. avicennioides* and *T. shimperiana*. In addition, cell inclusions were visible on the adaxial surface of *T. ivorensis*. The anatomical characteristics of the leaves of *Terminalia* species are consistent and distinguishable among species, making them valuable for identifying morphologically related species. To facilitate identification, we developed a dichotomous identification key for the eight *Terminalia* species. Consequently, the findings of this study support the use of anatomical and morphological features for the delimitation and identification of *Terminalia* species in Nigeria, thereby addressing a gap in the literature.

**Key to *Terminalia* species in Nigeria based on anatomical characteristics**

- 1a) Epidermal cell sinous/isodiametric, cell number less than 83.8 at the adaxial surface -----2
- 1b) Epidermal cell shape regular, cell number more than 83.8 at the adaxial surface ----- 4
- 2a) Stomata index up to 33.26%, anticlinal wall pattern curve on the abaxial plant-----*T. superba*
- 2b) Stomata index less than 33.26%, anticlinal wall pattern straight/undulate-----3
- 3a) Epidermal cell at abaxial surface: Isodiametric, Stomata present: *T. shimperiana*

- 3b: Epidermal cell at the abaxial surface sinuous, Stomata absent-----*T. catappa*
- 4a Mean epidermal number at the abaxial surface up to 105.3 -----*T. mantaly*
- 4b Mean epidermal number at an abaxial depth less than 105.3 -----5
- 5a Stomata amphistomate, wall pattern curved on both surfaces-----*T. macroptera*
- 5b Stomata hypostomate, wall pattern straight to undulate on both surfaces-----7
- 6a Stomata index up to 28.75%, cell shape sinous on the abaxial surface----- *T. laxiflora*
- 6b Stomata index more than 28.75%, cell shape regular on the abaxial -*T. ivorensis*

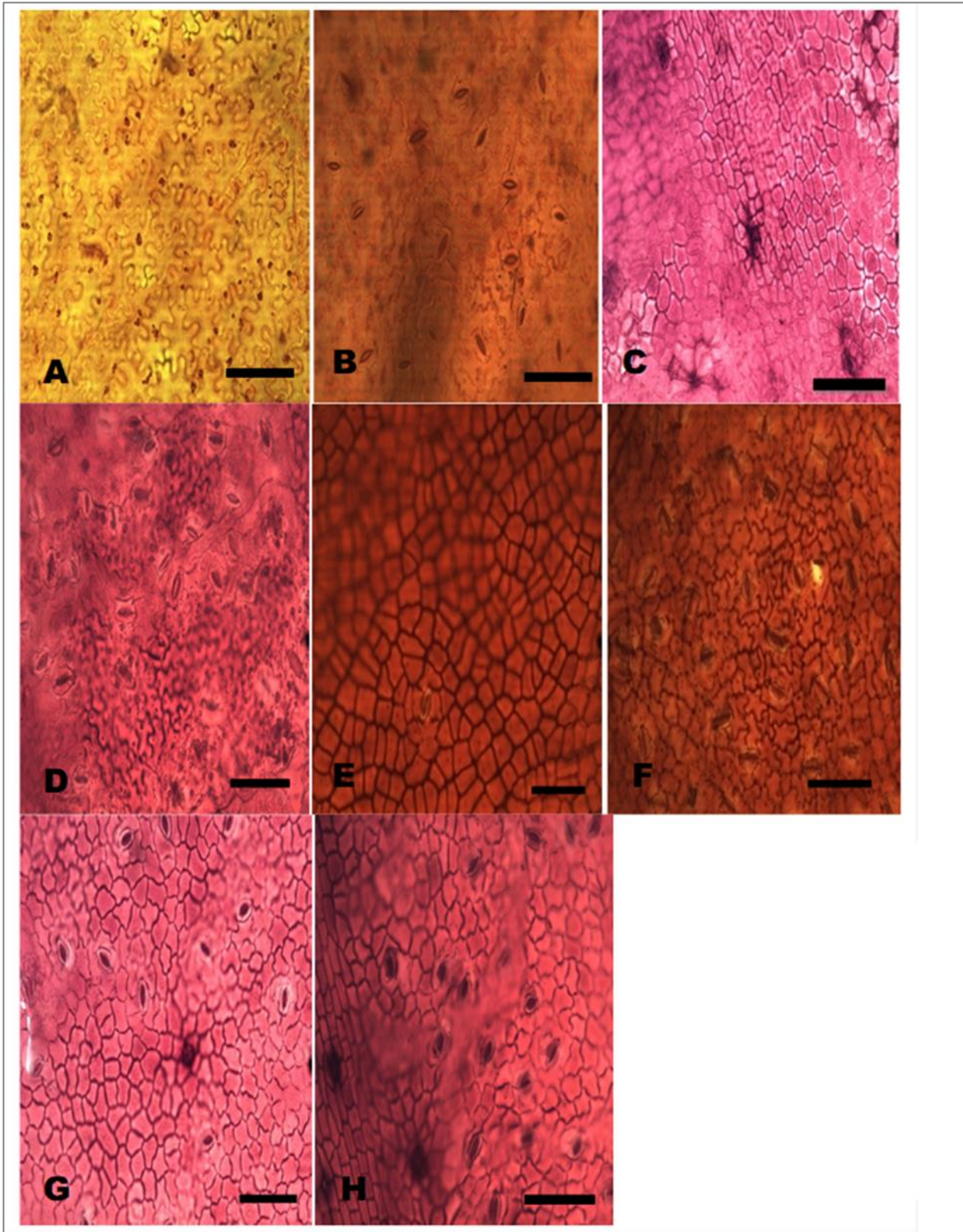


Figure 2: Leaf epidermal from Nigeria species of Terminalia: adaxial on the left, abaxial on the right, A,B = Terminalia catappa, C,D = T. ivorensis, E,F = T. mantaly, G,H T. macroptera. Scale bars = 50 µm

Table 2: Comparative epidermal characteristics of 8 Terminalia species

Species		Epidermal Number	Epidermal Length	Epidermal Width	Stomatal Number	Trichomes Number	Stomata Index
<i>T. catappa</i>	Adaxial	45(75.1±5.11)95	40.5(51.75±0.05)72	31.5(39.6±0.15)49.5	14(24.9±2.73)42		23.04
	Abaxial	70(83.3±3.54)105	27(43.65±0.08)63	13.5(21.15±0.05)36			
<i>T. inorensis</i>	Adaxial	65(83.9±4.62)105	31.5(45±0.08)67.5	13.5(26.1±0.07)49.5	10(17.1±1.47)24		30.53
	Abaxial	30(38.6±2.61)55	22.5(35.1±0.06)54	13.5(20.7±0.03)27			
<i>T. mantaly</i>	Adaxial	70(86.6±2.99)100	22.5(35.1±0.06)49.5	18(27.45±0.05)40.9	10(18.6±2.21)30		14.59
	Abaxial	79(105.3±5.38)140	18(35.1±0.06)49.5	9(15.3±0.04)27			
<i>T. macroptera</i>	Adaxial	80(89.5±1.86)100	29.3(34.2±0.04)40.5	13.5(21.6±0.04)031.5	3(5.7±0.72)10		5.95
	Abaxial	60(78.7±2.83)90	22.5(35.55±0.05)45	13.5(24.75±0.05)36	10(15.7±1.54)25		16.52
<i>T. anicenioides</i>	Adaxial	82(97.4±3.25)105	22.5(33.3±0.06)49.5	13.5(23.4±0.04)29.25	7(10.7±0.72)15	1(2.13±0.25)3	22.05
	Abaxial	22(38.4±2.52)50	13.5(30.15±0.08)54	18(25.65±0.04)31.5		1(2.29±0.33)5	
<i>T. superba</i>	Adaxial	30(48.2±3.69)68	27(45.45±0.13)67.5	13.5(23.4±0.05)33.75	10(17.1±1.26)23		33.26
	Abaxial	27(34.5±1.85)45	31.5(49.5±0.12)90	13.5(20.25±0.04)27			
<i>T. shimperiana</i>	Adaxial	15(32.4±4.52)50	22.5(32.85±0.06)45	13.5(21.6±0.04)31.5	5(10.8±1.07)15	2(4±0.75)10	28.07
	Abaxial	16(29.3±3.60)50	27(34.2±0.04)45	13.5(25.2±0.05)36		2(5.8±0.96)11	
<i>T. laxiflora</i>	Adaxial	66(85.1±3.33)94	18(33.75±0.08)54	13.5(26.1±0.05)33.75	10(21.9±2.07)30		28.75
	Abaxial	40(57±3.41)75	13.5(29.25±0.06)45	13.5(21.15±0.04)29.2			

Min(mean ±S.E. )Max

**Table 3: Variations in qualitative microscopic characters of species studied**

Species	Adaxial Epidermal Cell Shape	Abaxial Epidermal Cell Shape	Stomata type	Adaxial Anticlinal Wall	Abaxial Anticlinal Wall	Trichome type
<i>Terminalia catappa</i>	Sinuous	Sinuous	Anomocytic	Undulate	Undulate	
<i>Terminalia inorensis</i>	Regular	Sinuous	Anomocytic	Undulate	Undulate	
<i>Terminalia macroptera</i>	Regular	Regular	Anomocytic Anomocytic	Curved	Curved	
<i>Terminalia avicenioides</i>	Regular	Regular	Anomocytic Anomocytic	Straight	Straight	Multangulate Falcate
<i>Terminalia laxiflora</i>	Regular	Regular	Anomocytic	Straight	Straight	
<i>Terminalia shimperiana</i>	Isodiametric	Isodiametric	Anomocytic	Straight	Straight	Falcate
<i>Terminalia superba</i>	Sinuous	Sinuous	Anomocytic	Straight	Curved	
<i>Terminalia mantaly</i>	Regular	Sinuous	Anomocytic	Straight	Curved	

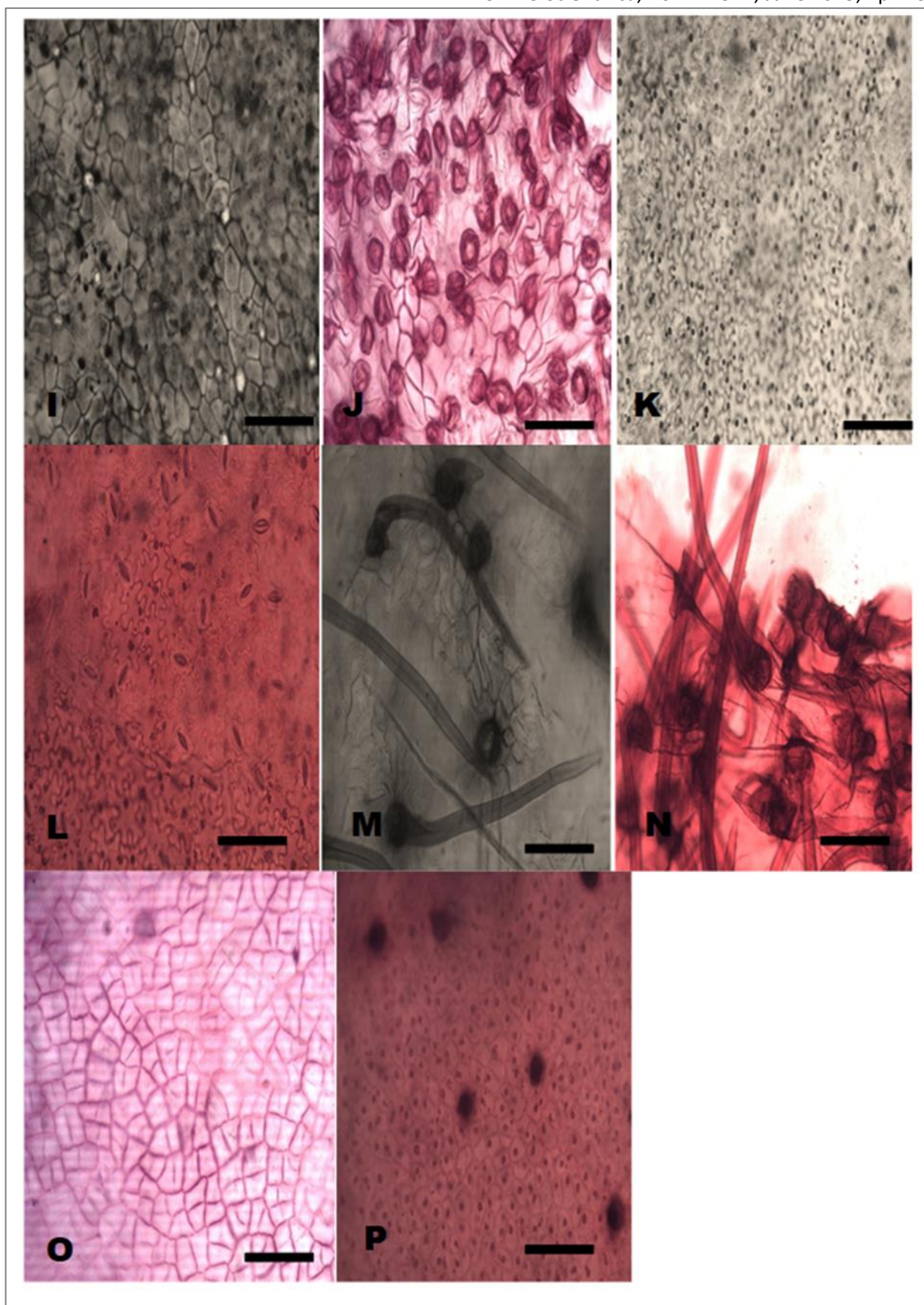


Figure 3: Leaf epidermal from Nigeria species of *Terminalia*: adaxial on the left, abaxial on the right, I, J = *Terminalia avicennioides*, K, L *T. schimperiana*, M, N = *T. laxiflora*, O, P = *T. macroptera*. Scale bars = 50  $\mu$ m

### CONCLUSION

The current study on the leaf anatomy of *Terminalia* provides valuable insights for identifying various species.

<https://scientifica.umyu.edu.ng/>

These findings highlight the taxonomic utility of foliar epidermal traits in resolving species-level identification within the genus *Terminalia*. The stomatal type, anticlinal wall type, epidermal cell type, and trichomes have been

identified as diagnostic features that are highly significant for addressing taxonomic disputes, thereby expanding the scope of taxonomic knowledge. Further research that integrates scanning electron microscopy and molecular methods is recommended to validate and refine these findings for broader conservation and ecological studies.

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## CONFLICTING OF INTEREST

The authors have declared that no conflicts of interest exist.

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## INFORMED CONSENT STATEMENT

Not applicable.

## DATA AVAILABILITY

All data generated or analysed during this study are included in this article

## AUTHORS' CONTRIBUTIONS

**NG** designed the study and wrote the original draft and the final manuscript. Samples were collected from the field during which the study was conducted. **ASA** conducted the research and contributed to the discussion. **IAO** assisted with study design, data analysis, and interpretation. **HAB** and **OTO** contributed to interpretation and logistics. All authors read and approved the final draft.

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