



# Comparative foliar epidermal morphology of the *Entandrophragma* species (*Meliaceae*) in Nigerian ethnomedicine

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Received: December 21, 2024 | Accepted: March 07, 2025 | Published: June 13, 2025

## Abstract

A comparative leaf epidermal study of four species of the genus *Entandrophragma* used in Nigerian ethnomedicine was conducted by light microscopy. The aim was to explore their taxonomic significance and provide further pharmacopoeial information for delimitation and on the relationship among these species. The results have shown diagnostic characters, including wavy anticlinal cell walls with trichomes on the adaxial surface in *E. angolense*, straight to curved anticlinal cell walls on the adaxial surface in *E. candollei*, cyclocytic stomata in *E. cylindricum*, and striations on the abaxial surface in *E. utile*. All species were hypostomatic, with anomocytic stomata.

## Key words

*Entandrophragma*, ethnomedicine, foliar epidermis, Nigeria, stomata, taxonomy

## Citation

Osiyemi, O.A., Adeniji, K.A., Oyebola, T.O. & Ugbogu, O.A. 2025. Comparative foliar epidermal morphology of *Entandrophragma* species (*Meliaceae*) in Nigerian ethnomedicine. – *Phytologia Balcanica*, 31: 65-74. -- ISSN 1310-7771 (print), 1314-0027 (online).

## Introduction

Use of medicinal plants is a centuries-old practice among Nigerians (Iyiola & Wahab 2024). Nigerian forest floras are one of the most accessible forest resources for disease control and treatment. However, misuse of herbal medicines or products starts with wrong plant identification. Different plant species from the same genus are particularly difficult to identify due to their similar features, even during flowering or fruiting, and especially when sterile (Bashyal & Robertis 2023).

The genus *Entandrophragma* consists of many medicinal timber species without obvious visual differences (Yakusu & al. 2021) frequently mentioned in Nigerian ethnomedicine. *Entandrophragma* belongs to the family *Meliaceae* (the Mahogany family). It includes ten species

of deciduous dicotyledonous trees (African Plant Database version 4.0.0) endemic to tropical Africa (Koenen & al. 2015). Four of those species [*Entandrophragma angolense* Welw. C. DC., *Entandrophragma candollei* Harms. *Entandrophragma cylindricum* (Sprague) Sprague and *Entandrophragma utile* (Dawe & Sprague) Sprague] are native to Nigeria (Hutchinson & Dalziel 1958).

The *Entandrophragma* species in Africa are economically useful, with a wide-ranging pharmacological and ecological potential (Burkill 1997; Happi & al. 2018; Yakusu & al. 2021). *Entandrophragma angolense* has been extensively used in the ethnomedical treatment of various diseases such as peptic ulcer, inflammations and malaria (Kamkumo & al. 2020; Zingue & al. 2021; Youn & al. 2022). Its antioxidant and anti-inflammatory effect have been also reported (Olaniyi & al. 2017).

*Entandrophragma utile* is used in Nigerian ethnomedicine to treat peptic ulcers, stomach ache, eye inflammation, rheumatism, otitis, and kidney pain (Bickii & al. 2007; John & al. 2012). The extract of *E. utile* bark has displayed anti-inflammatory, fungicidal and antioxidant effect (Mujuni 2008; Usman & al. 2018; Kola-Mustapha & al. 2023). The stem bark of *Entandrophragma candollei* is used for the treatment of abdominal pain and malaria (Kueete & Efferth 2011). Decoction of the fresh stem bark of *E. cylindricum* is applied for the treatment of dysentery and rheumatism (Noumi & Yomi 2001; Kouam & al. 2012). Antidiabetic and antioxidant effect of the extract of *E. cylindricum* leaves has also been reported (Oladoja & al. 2021). *Entandrophragma* species are among the most important timbers harvested in Africa (Hall & al. 2003). Consequently, those species have been subject to severe logging in many African countries since 1970 and are now registered as Vulnerable in the IUCN Red List (Hawthorne 1998). They are traded under various names (Sipo Mahogany and African Cedar) in Nigeria. *Entandrophragma cylindricum* is a major source of African hardwood, and as such it is exploited heavily (Monthe & al. 2017).

Foliar epidermis is one of the most outstanding taxonomic characters from a biosystematic viewpoint. A technique based on the metabolomic profiling for automated timber species identification shows that *E. angolense* is mostly confused with *E. candollei* due to the

great similarity of their chemical fingerprints (Deklerck & al. 2019). Delimitation complexities of the species in a number of plant families and genera are solved on the basis of leaf epidermal characters (Adeniji & Ariwaodo 2012; Oliveira & al. 2018; Ullah & al. 2018; Gul & al. 2019; Cardiel & al. 2020). The role of anatomical data in traditional taxonomy has been long recognized since the variations within the species, genera or a family are usually reflected in the anatomical features. Leaf epidermal anatomical features such as stomata, trichomes and other characters are useful as taxonomic tools. Although the studies conducted into general morphology and wood anatomy of *Entandrophragma* species have proved valuable for their delimitation, identification criteria would be still incomplete without foliar epidermal morphology (Kadiri & al. 2005). Measurements of the leaf epidermal cell length and width are regarded as useful in distinguishing varieties with similar flowering date in perennial grasses (Wilkins & Sabanci 1990). Anatomical features are of particular value to scientists who need to identify small scraps of plant material. Thus, the current study presents the foliar epidermal characters of *E. cylindricum*, *E. utile*, *E. angolense*, and *E. candollei*, in order to complement the existing identification criteria upon which the recognition and delimitation of these species have been based. The study will also assist prevention of adulteration of these plants when used for subsequent drug development and identification for timber harvesting.

**Table 1.** List of the taxa studied.

Taxa	Name of collector(s)	Date of collection	Herbarium number	Distribution in Nigeria
<i>E. angolense</i>	K.A. Adeniji & O.A. Osiyemi	06.12.2023	FHI 1142065	Ibadan State
	Otulana Jubril & Shitta Oyinkansola	21.02.2017	FHI 110819	Osun State
	R.W.J. Keay	14.04.1958	FHI 37653	Niger State
<i>E. cylindricum</i>	O.A. Osiyemi & K.A. Adeniji	06.12.2023	FHI 1142066	Ibadan State
	Adebusoye & Ubom	16.11.1963	FHI 45901	Osun State
	R.W.J. Keay	19.10.1946	FHI 21554	Ondo State
<i>E. candollei</i>	J.A. Emwiogbon	23.04.1966	FHI 58476	Benin State
	S.N. Iheuwa	07.09.1952	FHI 32356	Ondo State
	J.D. Kennedy	10.07.1931	FHI 9134	Ogun State
<i>E. utile</i>	C.F.A. Onochie	Aug. 1960	FHI 50859	Oyo State
	A.P.D. Jones	20.08.1946	FHI 20706	Ondo State
	C.F. Symington	12.04.1943	FHI 3393	Ondo State

## Material and methods

### Leaf epidermal morphology

Fresh and herbarium specimens of the *Entandrophragma* species were used in the leaf epidermal study. The fresh specimens were obtained in December 2023 from the Forestry Research Institute of Nigeria, Ibadan, and the herbarium samples from the collections of the Forest Herbarium Ibadan (FHI). The fresh *Entandrophragma* species were validated and their voucher specimens deposited in FHI. Epidermal preparations were obtained from fresh and herbarium leave specimens using the technique of Ayodele & Olowokudejo (2006). Each leaf specimen was immersed in concentrated nitric acid (HNO<sub>3</sub>) in covered Petri dishes for 3-4 hours. Bubbles observed on the soaked specimen implied tissue disintegration. Subsequently, the leaf specimen was removed and placed in another Petri dish containing distilled water, while the abaxial and adaxial epidermises were gently separated with forceps. The mesophyll tissue of the leaf was cautiously cleared with a camel hair brush. The separated epidermises were rinsed in distilled water several times and placed in 50% alcohol for one or two minutes for hardening. Then the isolated epidermal layer was mounted on a clear glass microscopic slide, stained with safranin-O for five minutes and rinsed with distilled water to remove excess stain. The stained epidermis was mounted in glycerin on a microscopic slide and ringed with nail varnish to prevent dehydration and seal the cover slips to the slides. All slides were labeled accordingly and examined under an Olympus light microscope. Photomicrographs  $\times 40$  were taken using a cx31 Olympus Biological microscope, fitted with Olympus E-330 digital SLR m camera via E 330-ADU 1.2 microscope adapter.

### Statistical analysis

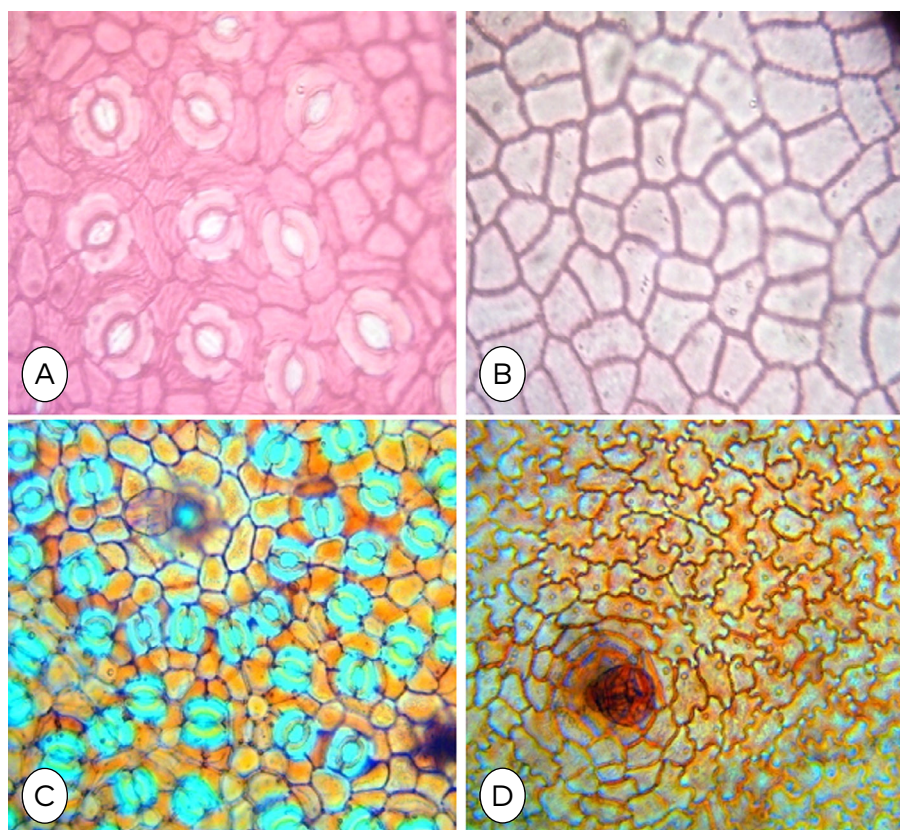
The micromorphological character measurements were expressed as mean values  $\pm$  standard error (S.E.M.). The means for all microscope parameters were calculated on the basis of occurrence of each character in a total of 10 fields of view.

## Results

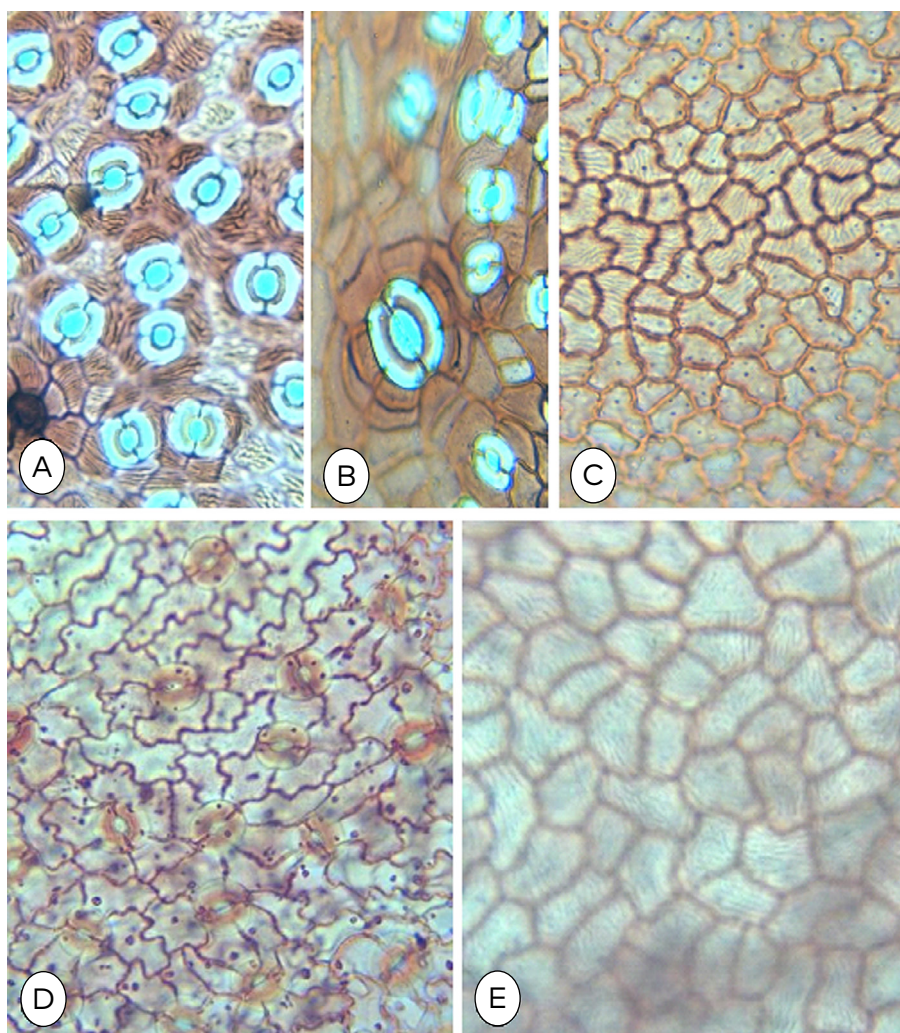
The synopses of the leaf epidermal features of the four investigated *Entandrophragma* species (*E. cylindricum*, *E. angolense*, *E. utile*, and *E. candollei*) are presented in Tables 2 and 3 and Figs 1–6. Striations have been observed on the abaxial surface in *E. cylindricum* and *E. utile* (Table 2). Glandular and non-glandular trichomes have been found on the abaxial and adaxial surfaces of *E. angolense* and *E. utile* (Figs 3–4 and Table 2). On the abaxial surface of *E. cylindricum* and *E. candollei* there have been also glandular and non-glandular trichomes, while there were no trichomes on their adaxial surface (Figs 5–6 and Table 2). Other diagnostic characters found have been polygonal and irregular epidermal cells, straight, curved, wavy and undulate anticlinal walls, and starch grain cell inclusions (Table 2). *Entandrophragma angolense* had the largest epidermal cell size of  $27.52 \times 20.80 \mu\text{m}$ . The smallest epidermal cell size of  $19.52 \times 11.20 \mu\text{m}$  was found in *E. candollei* (Table 3). *Entandrophragma angolense* had the largest stoma size of  $25.6 \times 20.16 \mu\text{m}$ . *E. utile* had the smallest stoma size of  $20.48 \times 16.64 \mu\text{m}$  (Table 3). The leaves were hypostomatic in all studied species (Figs 1–4 and Table 2). Anomocytic stomata have been the constant generic feature of all species, while the cyclocytic stomata type was found in *E. cylindricum*, in addition to the anomocytic stomata (Fig. 2B).

**Table 2.** Qualitative features of the epidermal morphology of *Entandrophragma* species.

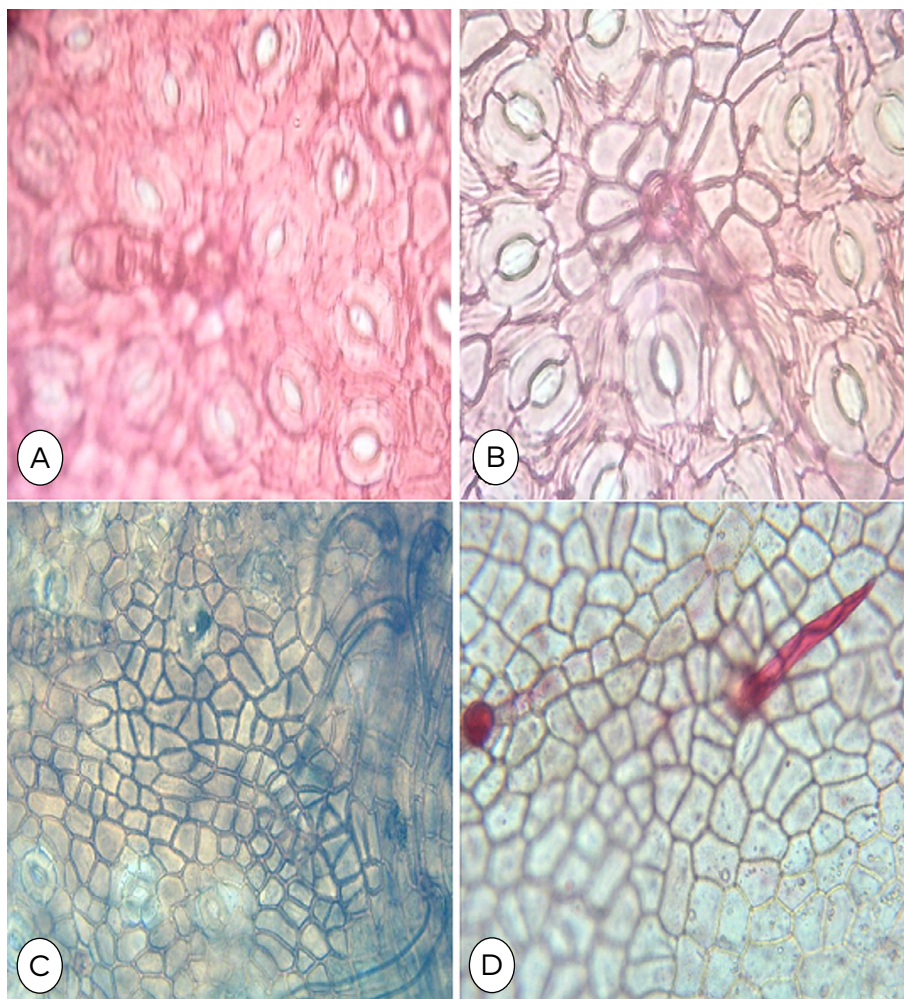
Taxa	Leaf surface	Stomatal type	Epidermal cell shape	Anticlinal wall pattern	Cell inclusion	Trichome type	Striations
<i>E. angolense</i>	abaxial	anomocytic	polygonal	straight/curve	absent	non glandular and glandular	absent
	adaxial	absent	irregular	wavy	absent	non glandular and glandular	absent
<i>E. cylindricum</i>	abaxial	anomocytic/cyclocytic	polygonal	straight/curve	starch grain	non glandular and glandular	present
	adaxial	absent	irregular	straight/curve or undulate	starch grain	absent	absent
<i>E. candollei</i>	abaxial	anomocytic	irregular	undulate	absent	non glandular and glandular	absent
	adaxial	absent	polygonal	straight/curve	absent	absent	absent
<i>E. utile</i>	abaxial	anomocytic	polygonal	straight/curve	absent	non glandular and glandular	present
	adaxial	absent	polygonal	straight/curve	absent	non glandular	absent



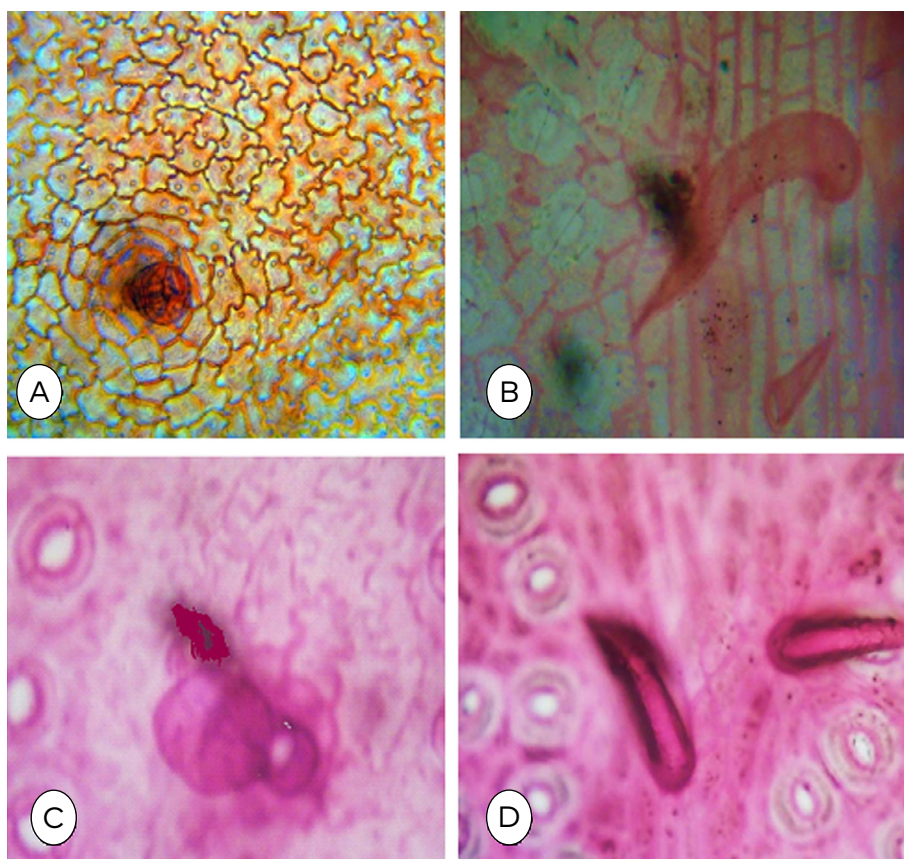
**Fig. 1.** Photomicrographs (A–D,  $\times 40$ ) of the leaf surfaces of genus *Entandrophragma*: **A.** Abaxial surface of *E. utile* with anomocytic stomata (St) and polygonal cell shape with straight/curved anticlinal walls (Aw); **B.** Adaxial surface of *E. utile* with polygonal cell shape and straight/curved anticlinal walls; **C.** Abaxial surface of *E. angolense* with anomocytic stomata and polygonal cell shape with straight/curved anticlinal walls; **D.** Adaxial surface of *E. angolense* with irregular cell shape and wavy anticlinal walls.



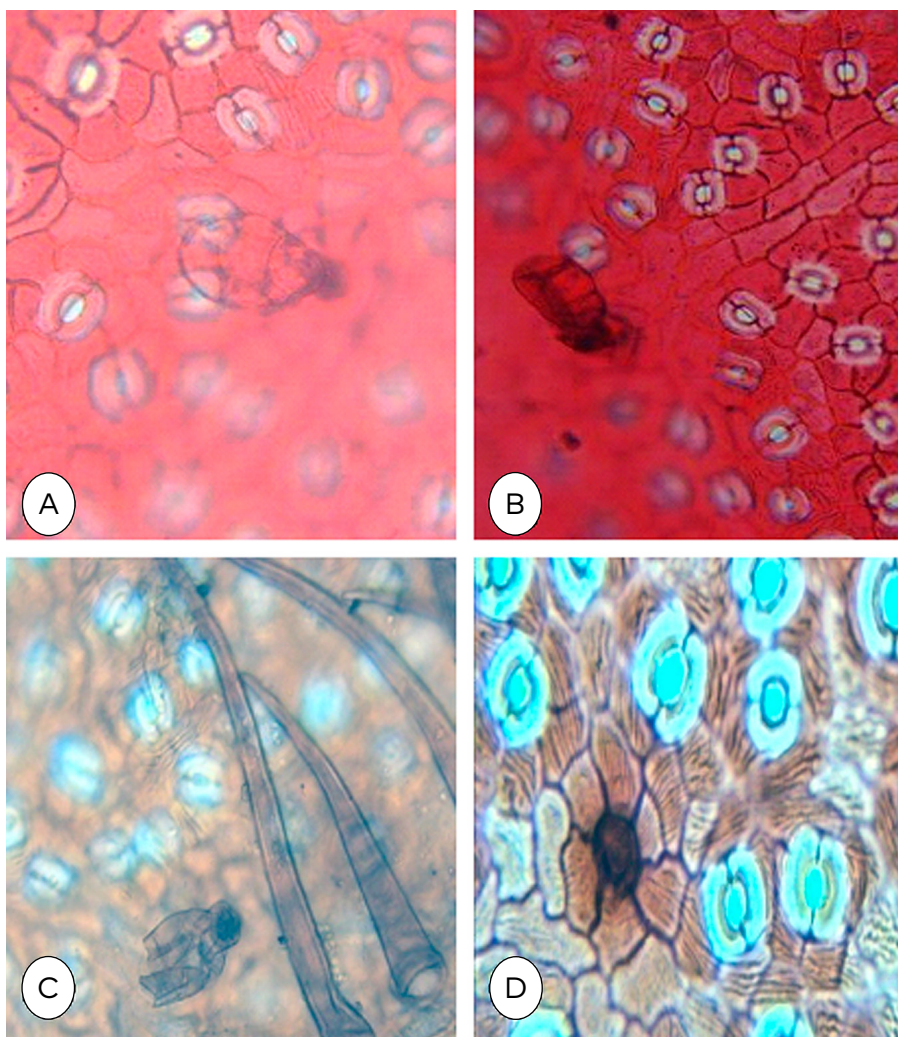
**Fig. 2.** Photomicrographs (A–E,  $\times 40$ ) of the leaf surfaces of genus *Entandrophragma*: **A.** Abaxial surface of *Entandrophragma cylindricum* with anomocytic stomata and striations; **B.** Abaxial surface of *E. cylindricum* with cyclocytic stomata and polygonal cells with straight/curved anticlinal walls; **C.** Adaxial surface of *E. cylindricum* with irregular cell shape and undulate anticlinal wall; **D.** Abaxial surface of *E. candollei* with anomocytic stomata and sinuous anticlinal walls; **E.** Adaxial surface of *E. candollei* with polygonal cell shape and straight /curved anticlinal walls.



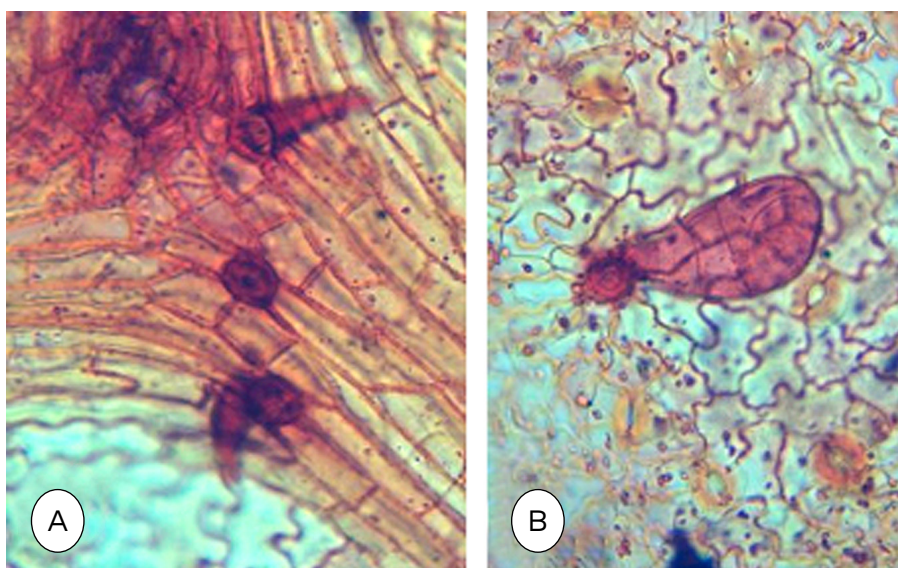
**Fig. 3.** Photomicrographs (A-D, x40) of the leaf surfaces of *Entandrophragma utile*: **A.** Glandular trichomes with unicellular head and stalk on the abaxial surface; **B.** Unicellular linear non-glandular trichomes on the abaxial surface; **C.** Unicellular curved non-glandular trichomes on the abaxial surface; **D.** Unicellular linear non-glandular trichomes on the adaxial surface.



**Fig. 4.** Photomicrographs (A-D, x40) of the leaf surfaces of *Entandrophragma angolense*: **A.** Glandular trichomes with unicellular head and unicellular stalk on the adaxial surface; **B.** Unicellular spine-like and curved non-glandular trichomes on the adaxial surface; **C.** Glandular trichomes with unicellular head and stalk on the abaxial surface; **D.** Unicellular non-glandular trichomes on the abaxial surface.



**Fig. 5.** Photomicrographs (A–D,  $\times 40$ ) of the leaf surfaces of *Entandrophragma cylindricum*: **A.** Glandular trichomes with unicellular head on the abaxial surface; **B.** Glandular trichomes with multicellular head on the abaxial surface; **C.** Unicellular non-glandular spine-like and long trichomes on the abaxial surface; **D.** Trichome base with striations on the abaxial surface.



**Fig. 6.** Photomicrographs (A–B,  $\times 40$ ) of the leaf surfaces of *Entandrophragma candollei*: **A.** Unicellular non-glandular spine-like trichomes on the abaxial surface; **B.** Glandular trichomes with unicellular stalk and multicellular head on the abaxial surface.

**Table 3.** Quantitative features in the epidermal morphology of *Entandrophragma* species.

Taxa	Leaf surface	Cell length (µm)	Cell width (µm)	Stomata length (µm)	Stomata width (µm)
<i>E. angolense</i>	abaxial	19.2 – 28.8 24.31 ± 0.97	9.6 – 16.0 13.76 ± 0.68	22.4 – 28.8 25.6 ± 0.67	19.2 – 22.4 20.16 ± 0.48
	adaxial	22.4 – 28.8 27.52 ± 0.70	12.8 – 25.6 20.80 ± 1.28	absent	absent
<i>E. cylindricum</i>	abaxial	22.4 – 28.8 24.32 ± 0.70	12.8 – 22.4 16.32 ± 1.00	19.2 – 25.6 22.40 ± 0.67	12.8 – 16.0 14.08 ± 0.52
	adaxial	19.2 – 25.6 22.08 ± 0.88	9.6 – 12.8 11.20 ± 0.52	absent	absent
<i>E. candollei</i>	abaxial	16.0 – 22.4 19.52 ± 0.74	19.2 – 28.8 11.20 ± 0.53	19.2 – 25.6 23.40 ± 0.92	16.0 – 22.4 18.24 ± 0.68
	adaxial	19.2 – 25.6 21.76 ± 0.64	16.0 – 19.2 16.96 ± 0.48	absent	absent
<i>E. utile</i>	abaxial	16.0 – 22.4 19.84 ± 0.92	12.8 – 19.2 14.40 ± 0.71	19.2 – 22.4 20.48 ± 0.52	16.0 – 19.2 16.64 ± 0.42
	adaxial	19.2 – 25.6 22.40 ± 0.67	9.6 – 16.0 13.76 ± 0.68	absent	absent

## Discussion

All examined *Entandrophragma* species had common foliar epidermal features, but some of those were distinguishing and useful for the species delimitation. According to Wilkinson (1979), taxonomic importance of the resemblance of stomata apparatus in a full-grown leaf regularly provided a consistent diagnostic character, especially when ontogeny of the stomata was unidentified or different. In the present study, *E. cylindricum* has stood out by having both cyclocytic and anomocytic stomata types.

Omokanye & al. (2020) has observed that the epidermal cell shape had taxonomic significance in identification of species in the genus *Chlorophytum* ker Gawl. The epidermal cell shape on the abaxial surfaces of all investigated species was polygonal, except in *E. candollei*, where it was irregular (Figs 1A, C, 2A, D), thus delimiting *E. candollei*. There have been polygonal epidermal cells on the adaxial surfaces of *E. utile* and *E. candollei* (Figs 1B, 2E). However, *E. angolense* and *E. cylindricum* also manifested irregular epidermal cells (Figs 1D, 2C). The anticlinal wall was straight/curved on the abaxial surfaces of all examined species in the current study, except in *E. candollei*, where it was undulating and that character distinguished *E. candollei* from the other three species of *Entandrophragma*. On the adaxial surfaces, the anticlinal walls were straight to curved in *E. utile* and *E. candollei*, but sinuous and undulate in *E. angolense* and *E. cylindricum*, respectively (Figs 1D, 2C). Findings in the present study supported the earlier research connected with the use of variations of the anticlinal cell walls in identi-

fication of some *Melastoma* species (Siti-Maisarah & al. 2020). Striations were present in *E. utile* and *E. cylindricum* (Figs 1A, 2A). That character delimited them from *E. angolense* and *E. candollei*. The presence of striations has proven to be a reliable diagnostic character of the epidermis and has been used to support the taxonomic studies into *Oleaceae*, *Euphorbiaceae* and *Phyllanthaceae* families (Bahadur & al. 2022; Akhtar & al. 2024; Batool & al. 2024). Calcium oxalate was observed on the adaxial surface of *E. angolense* and the abaxial surface of *E. candollei*, and that character has delimited them from *E. cylindricum* and *E. utile*. Calcium oxalate has been found a valuable character contributing to taxonomy of *Conamomum vietnamense* N.S. Ly & T.S. Hoang (Nhi & al. 2023). Calcium oxalate has also been reported as a botanical marker that can help differentiate *Baccharis articulata* (Lam.) Pers. from other species (Raeski & al. 2023). The trichome types and distribution in the studied *Entandrophragma* species are presented in Figs 3–6 and summarized in Table 2. Two types of trichomes have been identified: glandular and non-glandular. All studied species possess both glandular and non-glandular trichomes on their abaxial surfaces. Only *E. angolense* had the two trichome types on its adaxial surface, and only non-glandular trichomes were present on the adaxial surface of *E. utile*. Trichomes were absent on the adaxial surface of *E. cylindricum* and *E. candollei* (Figs 3–6 and Table 2) and thus, the presence and type of trichomes on the adaxial side of *E. angolense* and *E. utile* delimited them from the other investigated species. The great morphological diversity of trichomes - type, shape and size - has allowed

botanists to use them for biosystematic purposes in many plant families (Carvalho & al. 2017; Arabameri & al. 2020; Santos & al. 2020; Hussin & al. 2024). Trichome morphology has helped the identification of species within the alpine flora of Hindukush (Abdullah & al. 2024); the poisonous plants (Abid & al. 2023) of the megadiverse genus *Croton* L. (Pinto-Silva & al. 2023) and the *Kaunia* species (Viera Barreto & al. 2024).

## Conclusion

The comparative foliar epidermal study of *E. cylindricum*, *E. angolense*, *E. utile*, and *E. candollei* has displayed some diagnostic characters which can be used to delimit these species. These characters would permit a more accurate and consistent identification of *E. cylindricum*, *E. angolense*, *E. utile*, and *E. candollei*, even if they were collected sterile or in fragmentary form. The present study has provided in particular further useful pharmacopoeial information for delimitation and relationship among

these species. It has also offered a more solid basis for relevance of the foliar epidermal research in resolving perplexity in taxonomic studies and identifying adulteration in plant-based drugs. Based on the observed diagnostic features, an artificial indented dichotomous key is presented below, aimed at delimiting the four investigated *Entandrophragma* species.

### An artificial indented dichotomous key to four *Entandrophragma* species in Nigeria

- 1 Presence of striations on the epidermal cell ..... 2
  - 2 Stomata cyclocytic on the abaxial surface ...  
..... *E. cylindricum*
  - 2\* Stomata anomocytic on the abaxial surface ...  
..... *E. utile*
- 1\* Absence of striations on the epidermal cell ..... 3
  - 3 Wavy anticlinal cell walls and presence of trichomes on the adaxial surface ..... *E. angolense*
  - 3\* Straight to curved anticlinal cell walls and absence of trichomes on the adaxial surface...  
..... *E. candollei*

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