MACRO- AND MICRO-MORPHOLOGICAL ANALYSES OF *Entandrophragma angolense* (Welw.) C.D.C. and *Entandrophragma utile* (Dawe & Sprague) Sprague

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ABSTRACT

Entandrophragma utile and *Entandrophragma angolense* are timber species with significant economic and ecological importance in the forestry sector. Understanding their morphological differences at both macro and micro levels is crucial for their identification, management and conservation. This study conducted a comparative analysis of the macro- and micro-morphological characteristics of *Entandrophragma utile* and *E. angolense*. Samples of *E. angolense* and *E. utile* were collected from Forestry Research Institute of Nigeria (FRIN), Oyo State, and Aponmu Forest Reserve, Ondo State, Nigeria, respectively. The bark texture, leaf structure, and fruit/seed shapes were determined following standard methods. The leaf epidermal cells of *E. utile* were wavy-patterned, while those of *E. angolense* were polygonal-shaped. The number of stomata of *E. utile* (42.60±1.70) was significantly higher than *E. angolense* (22.80±1.40), while epidermal cell length was 32.40±6.40 µm and 63.96±5.80 µm, respectively. The number of trichomes was higher in *E. angolense* (7.20±0.30) than *E. utile* (1.80±0.30). The micro-morphology of leaves differed between *E utile* and *E. angolense*. Hence, the cellular structure (abaxial and adaxial epidermal cell layers) and anatomy (mid-rib and bark features) of the two species were uniquely different. These are essential findings for their ethnobotanical use, identification, management, and conservation strategies.

Keywords: Micro-anatomical features, Leaf epidermis, Adaxial layer, Abaxial layer

INTRODUCTION

Entandrophragma angolense and *E. utile* are medicinal plants traditionally used for the treatment of various ailments. However, the species are often misidentified, due to their morphological similarities. Evaluation of their anatomical and morphological differences could aid their differentiation and use in ethnomedicine.

Morphological studies provide key information important for species identification, forest management, and conservation (Lang *et al*., 2010; Amonum *et al*., 2019; Rust and Stoinski, 2024). Macro-morphology is the study of visible structures such as leaves, bark, and tree architecture, while micro-morphology is the examination of microscopic details like cell structure and wood anatomy, for characterisation of the intrinsic qualities and typologies of plant species (Vignesh and Sumitha, 2020; Ruffinatto *et al*., 2023).

Although *Entandrophragma angolense* and *E. utile* are in the same genus, the two species may exhibit distinct morphological differences due to environmental conditions and genetic factors (CABI International, 2022a; 2022b; Quadroni *et al*., 2023). The assessment of these differences could enhance species identification and classification, while also providing crucial information for ecological research, conservation and wood utilisation.

This study compared the macro- and micro-morphological attributes of *E. angolense* and *E. utile*, using field and laboratory examinations. A clear understanding of the features of the two species could facilitate better ethnobotanical use, taxonomic characterisation and phytomedicinal potentials.

MATERIALS AND METHODS

Sample Collection

Samples of *Entandrophragma utile* were collected from Aponmu Forest Reserve, Akure, Ondo State, while *E. angolense* were obtained from the Forestry Research Institute of Nigeria, Oyo State, Nigeria. The mature trees were identified using morphological markers and taxonomic keys. Following standardised protocols, bark, leaves, fruits, and seeds were excised using sterilised tools. The samples were placed in labelled bags, which were sealed to prevent degradation. Tree heights were measured, and signs of disease or stress were recorded. Photographic documentation was done before samples were transported to the laboratory.

Identification and Authentication

The trees were identified using field guides, taxonomic keys, and expert consultation. Samples were authenticated at the Forest Herbarium Ibadan at FRIN, with voucher numbers FHI-113968 and FHI-113969 allocated to *E. utile* and *E. angolense*, respectively.

Macro- and Micro-morphology Analyses

The bark was assessed for characteristics such as texture, colour and pattern. The shape, size and colour of leaves were evaluated. Fruits and seeds were assessed for distinctive features, including size and shape. Observations were documented and supplemented with photographic evidence.

The scanning electron microscope was used for the micro-morphological characterisation of *E. utile* and *E. angolense* following the method of Harun *et al.* (2020).

The bark samples were cut into uniform cubes, with each side measuring 2 cm. Leaf samples were carefully soaked in paraffin wax to facilitate thin sectioning. The leaf samples were mounted on a microtome sliding machine and sectioned to approximately 20 μm thickness for microscopic analysis. Leaf sections were stained with Safranin to highlight specific cellular structures. After staining, the sections underwent a dehydration process, which involved passing the wood sections through an ascending series of ethanol baths with concentrations increasing sequentially from 30%, 50%, 70% to 90%. The cleared sections were mounted on microscopic slides using Canada balsam as the mounting medium. The slides were examined under an Olympus light microscope (CH series), and micrographs of the sections were captured using a digital camera. These micrographs provided detailed illustrations of the cellular structure and composition of the samples.

RESULTS AND DISCUSSION

The leaves of *E. angolense* were usually dark green, with nine leaflets arranged alternately, which are oblong to obovate in shape, 8-12 cm long, with smooth edges and pointed tips. The bark was greyish-brown on the exterior and whitish-yellow in the inner layers, with deep vertical fissures (2-4 mm depth) that create a rough texture (Figure 2). The species produces pendulous capsules (12-15 cm) containing 40-60 light brown, winged seeds (3-4 cm in length) with feathery appendages adapted for wind dispersal (Figure 3).

Entandrophragma utile has distinct characteristics that contrast with *E. angolense*, featuring pinnately compound leaves with five oppositely arranged leaflets that were oblong shaped (length: width ratio 3.5-4:1) and 10-14 cm in length (Figure 1), displaying dark green colouration with pronounced acuminate tips (10-20° angle). The bark presents a uniform reddish-brown colouration with shallow fissures (0.5–1.5 mm deep), resulting in a notably smoother texture compared to its congener (Figure 2). While maintaining a similar capsular fruit morphology, *E. utile* produces slightly larger fruits (14-18 cm) containing 60-80 seeds that have more developed wings (4-5 cm length), reflecting an enhanced adaptation for wind dispersal. *Entandrophragma angolense* and *E. utile* have several physical traits in common, such as compound leaves with smooth edges, capsular fruits that have winged seeds, and pointed leaf tips.

The macro-morphological traits provide crucial taxonomic markers for correct identification and classification of the species (James *et al*., 2020; Ullah *et al.,* 2022). Accurate species identification is vital for conservation efforts, particularly in regions where these trees are exploited for timber (Bisong and Buckley, 2014). The differences in the number of leaflets and the bark features (deep cracks in *E. angolense* versus shallow ones in *E. utile*) are helpful in distinguishing the two species. The presence of winged seeds in both species indicates an evolutionary strategy for wind dispersal (anemochory).

The distinct morphological features of *E. angolense* and *E. utile* reflect specialised adaptations to their ecological niches, with *E. angolense* exhibiting greyish-brown, deeply fissured bark that enhances water retention and fire resistance in forest-savanna ecotones, while *E. utile* displays smoother reddish-brown bark that is better suited to humid forest conditions, where it reduces fungal colonisation (Liukko and Elfowsson, 1999; Benedict *et al.,* 2014; Loram-Lourenço *et al.,* 2022). The differences in leaf morphology correspond with their light environments, as *E. angolense* heteroblastic elliptic-oblong leaflets optimise light capture in variable transitional zones, whereas the oblong leaflets of *E. utile* help to maximise photosynthesis in stable understory conditions. These structural variations are supported by phytochemical differences, with *E. angolense* producing more suberin and phenolics for drought resistance while, *E. utile* contains higher terpenoid and alkaloid concentrations for pathogen defence (Bazargani *et al.,* 2021; Liao *et al.,* 2023; Abbasi *et al.,* 2024). These adaptive specialisations have direct implications for conservation strategies, particularly in predicting climate change responses and guiding species-specific reforestation efforts, as documented in recent ecological and phytochemical studies (Bazargani *et al*., 2021; Loram-Lourenço *et al*., 2022).

The micro-morphological characterisation of the epidermal layers in *Entandrophragma utile* and *E. angolense* offers insightful information about leaf architecture and potential adaptive strategies, with both species possessing stomata and trichomes in their abaxial epidermal layers (Table 1, Figure 5).

Entandrophragma utile *Entandrophragma angolense*

Figure 1. Leaves of *Entandrophragma angolense* and *Entandrophragma utile*

Entandrophragma utile *Entandrophragma angolense*

Figure 2. Stem bark of *Entandrophragma angolense* and *Entandrophragma utile*

Figure 3. Winged seeds of *Entandrophragma angolense* and *Entandrophragma utile*

Entandrophragma utile *Entandrophragma angolense*

Figure 4. Fruit pods of *Entandrophragma angolense* and *Entandrophragma utile*

The epidermal cells in *E. angolense* leaves were predominantly polygonal, suggesting a structural adaptation that might influence light interception and water retention. In *E. utile*, the cells had a wavy shape, which suggests they handle bending and gas exchange differently. There were significant differences in the adaxial epidermal layers, with *E. angolense* exhibiting polygonal epidermal cells without trichomes, while *E. utile* had wavy epidermal cells and trichomes. This highlights the adaptive feature of each species to herbivory and environmental challenges (Wang *et al*., 2021).

The midribs of leaves in both species were corrugated on the abaxial surface and flat on the adaxial surface. However, the leaf blade in *Entandrophragma utile* formed a more obtuse angle with the abaxial surface, whereas the angle was acute in *E. angolense*. On the adaxial surface, *E. utile* exhibited an acute to obtuse apex, while *E. angolense* displayed a flat orientation.

Vascular bundles in both species were D-shaped, but the pits and vessels were wider in *E. utile* than in *E. angolense* (Figure 5, Table 1). The flat adaxial surfaces are likely to enhance the optimisation of light absorption and photosynthetic efficiency, while the convex and corrugated abaxial surfaces, could provide mechanical support and improve gas exchange efficiency (Migacz *et al.,* 2018; Ornellas *et al.,* 2019).

The bark of both species was predominantly composed of cork (phellem) and phloem (secondary phloem) regions (Figure 6). The differences in the cork and phloem regions have significant ecological and physiological implications. The thinner and compact cork layer of *E. angolense* indicates a higher mechanical resilience and better protection against external damage, pests, and pathogens. However, this compactness might compromise its ability to store water and gases, affecting its overall drought and fire resistance. On the other hand, the thicker and more loosely arranged cork layer of *E. utile* may offer better insulation and water retention. These aid survival in areas prone to fire and drought.

The secondary phloem in *E. utile* had many sieve tube elements and companion cells, which suggest improved movement of nutrients, rapid growth and better recovery from injuries or environmental stress. In contrast, the higher proportion of fibre cells in the phloem region of *E. angolense* underscores a trade-off between mechanical support and nutrient transport efficiency (Crespo-Martínez *et al*., 2019). The increased mechanical strength provided by these fibres might be advantageous for supporting tall structures and resisting physical damage, yet it may limit the species ability to quickly transport nutrients, possibly affecting growth rate and recovery (Clair *et al*., 2018).

Quantitative analysis of the abaxial epidermal characteristics of *Entandrophragma utile* and *E. angolense* revealed significant and non-significant differences across variables (Table 2). The stomatal length (22.17±1.8 µm) and width (14.06±1.4 µm) of *E. utile* were not significantly different from those of *E. angolense* (21.45±2.2 µm and 15.20±0.61 µm, respectively). The stomatal index was not significantly different, but the number of stomata of *E. angolense* (22.80±1.4) differed from *E. utile* (42.60±1.7). Although, *E. utile* had a lower number of epidermal cells (113.80±9.4) compared to *E. angolense* (133.80±14.6), the difference was not statistically significant ($t = -1.146$, $p = 0.285$ ns). The epidermal cell length of *E. utile* (32.40±6.4 µm) was significantly lower than that of *E. angolense* (63.96±5.8 µm). Similarly, a significant difference was observed for the epidermal cell width of *E. utile* (18.00±2.9 µm) and *E. angolense* (32.88±2.9 µm). The number of trichomes in *E. utile* leaves was significantly lower (1.80±0.3) than *E. angolense* (7.20±0.3). The guard cell area was 79.54±8.4 µm² and 84.89±7.9 µm² for *E. utile* and *E. angolense*, respectively. The trichome length for *E. utile* (30.60±1.9 µm) was shorter than that of *E. angolense* (57.20±4.4 µm).

Table 1. **Midrib anatomical features of *Entandrophragma utile* and *Entandrophragma angolense***

Species	Midrib	Vascular Bundles	Trichomes	Adaxial	Abaxial
E. angolense	D-shaped	D-shaped	Absent	Flat	Convex and corrugated
E. utile	D-shaped	D-shaped	Present	Flat	Convex and corrugated

Entandrophragma angolense *Entandrophragma utile*

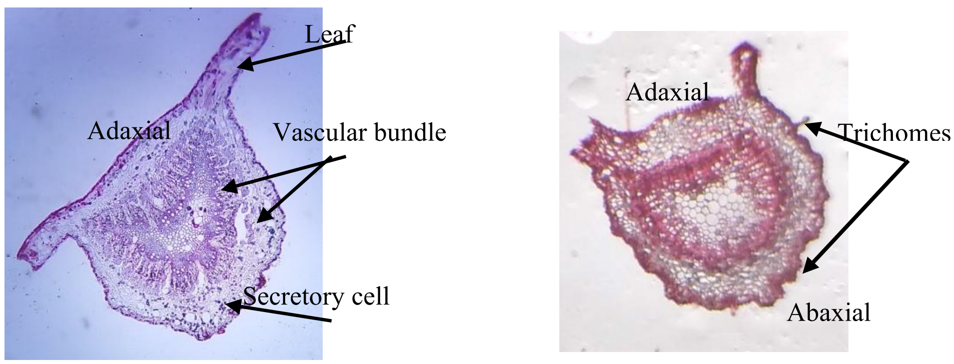

Figure 5. Abaxial and adaxial anatomical features of *Entandrophragma angolense* and *Entandrophragma utile*

Entandrophragma angolense *Entandrophragma utile*

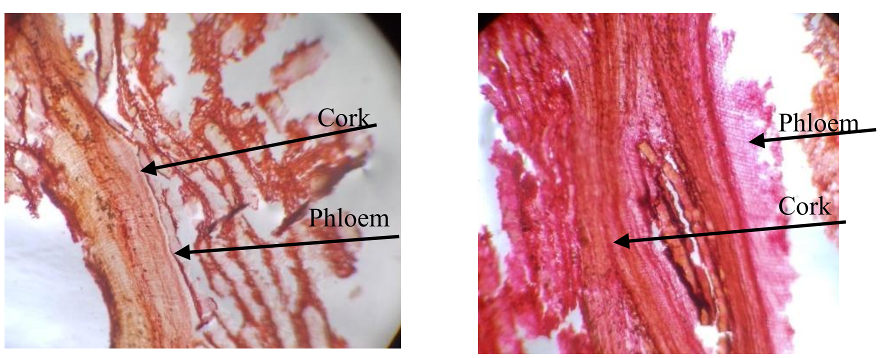

Figure 6. Anatomical features of the bark of *Entandrophragma angolense* and *Entandrophragma utile*

Table 2. Quantitative abaxial epidermal characteristics of *Entandrophragma utile* and *Entandrophragma angolense*

Epidermal variables	Tree species	Mean±SD	T-value	p-value
SL (µm)	*Entandrophragma utile*	22.17±1.80	0.245	0.812^{ns}
	Entandrophragma angolense	21.45±2.20		
SW (µm)	*Entandrophragma utile*	14.06±1.40	-0.71	0.498^{ns}
	Entandrophragma angolense	15.20±0.61		
SI (%)	*Entandrophragma utile*	6.80±2.20	-0.667	0.524^{ns}
	Entandrophragma angolense	10.85±5.60		
NS	*Entandrophragma utile*	42.60±1.70	8.683	0.000*
	Entandrophragma angolense	22.80±1.40		
NEC	*Entandrophragma utile*	113.80±9.40	-1.146	0.285^{ns}
	Entandrophragma angolense	133.80±14.60		
ECL (µm)	*Entandrophragma utile*	32.40±6.40	-3.616	0.007*
	Entandrophragma angolense	63.96±5.80		
ECW (µm)	*Entandrophragma utile*	18.00±2.90	-3.553	0.007*
	Entandrophragma angolense	32.88±2.90		
NWS	*Entandrophragma utile*		-	-
	Entandrophragma angolense	4.40±0.50		
NT	*Entandrophragma utile*	1.80±0.30	-10.205	0.000*
	Entandrophragma angolense	7.20±0.30		
GCA (µm^2)	*Entandrophragma utile*	79.54±8.40	-0.457	0.660^{ns}
	Entandrophragma angolense	84.89±7.90		
TL	*Entandrophragma utile*	30.60±1.90	-5.441	0.001^{ns}
	Entandrophragma angolense	57.20±4.40		
PS(µm^2)	*Entandrophragma utile*	70.84±12.30	0.424	0.682^{ns}
	Entandrophragma angolense	64.26±9.30		

Keys: NS = number of stomata; NEC= number of epidermal cells; NWS = number of wall sides; ECL = epidermal cell length; ECW= epidermal cell width; GCA = guard cell area; SL= stomatal length; SW= stomatal width; SI = stomatal index; NT = number of trichome; PS= pore size; TL = trichome length. * = significant at 5% probability level; ns= not significant 5% probability level

The stomatal features were similar for both species. However, a notable distinction was observed in the number of stomata (NS), with *E. utile* having significantly higher stomata. This higher stomatal density may imply higher rates of transpiration and CO_2 uptake. Hence, the species might be better adapted to environments with high light intensity and water availability (Miyazawa *et al.*, 2006; Xuan *et al.,* 2011; Hasanuzzaman *et al.*, 2013; Bertolino *et al.*, 2019).

Entandrophragma angolense had larger epidermal cells, implying tougher leaf texture, and this could help the plant deal with challenges like drought and extreme temperatures, as well as impact its interactions with pests and diseases (Wang *et al.*, 2021; Watts and Kariyat*,* 2021; Li *et* *al.,* 2023). A significant difference was noted in the number of trichomes (NT), with *E. angolense* having a higher count compared to *E. utile*, indicating a divergent adaptation strategy, with *E. angolense* potentially offering better protection against herbivory and possibly influencing microclimatic conditions around the plant surface (Valkama *et al.,* 2004; Curvers *et al.,* 2010; Lu *et al.,* 2021; Oviedo-Pereira *et al.,* 2022; Wang *et al.,* 2021; Li *et al.,* 2023).

These findings can inform targeted breeding programs and conservation strategies. For instance, species with higher stomatal densities like *E. utile* might be prioritised in reforestation efforts in areas where high photosynthetic capacity is desirable, whereas *E. angolense,* with its robust epidermal structure, might be better suited for environments where physical leaf durability is important (Miyazawa *et al.,* 2006; Xuan *et al.,* 2011). Furthermore, understanding these epidermal characteristics also contributes to the broader knowledge of plant physiological ecology and evolutionary biology, offering insights into how different species within the same genus can adapt to varied environmental conditions through subtle morphological changes (Watts and Kariyat, 2021).

The adaxial epidermal layers of *E. angolense* and *E. utile* showed notable differences. *Entandrophragma angolense* displayed polygonal epidermal cells without trichomes, while *E. utile* had wavy epidermal cells with trichomes (Figure 8). A comparative analysis of epidermal characteristics (Table 3) revealed significant differences between the two species. *Entandrophragma utile* had a lower number of epidermal cells (138.20±3.60) compared to *E. angolense* (167.60±8.60) (p=0.014). Additionally, the epidermal cells of *E. utile* were significantly smaller in length (25.44±5.6 µm) and width (15.74±3.3 µm) than *E. angolense* (56.72±5.1 µm and 28.94±3.4 µm, respectively). The number of wall sides was only recorded for *E. angolense* (6.0±0.6), with no differences in number of trichomess.

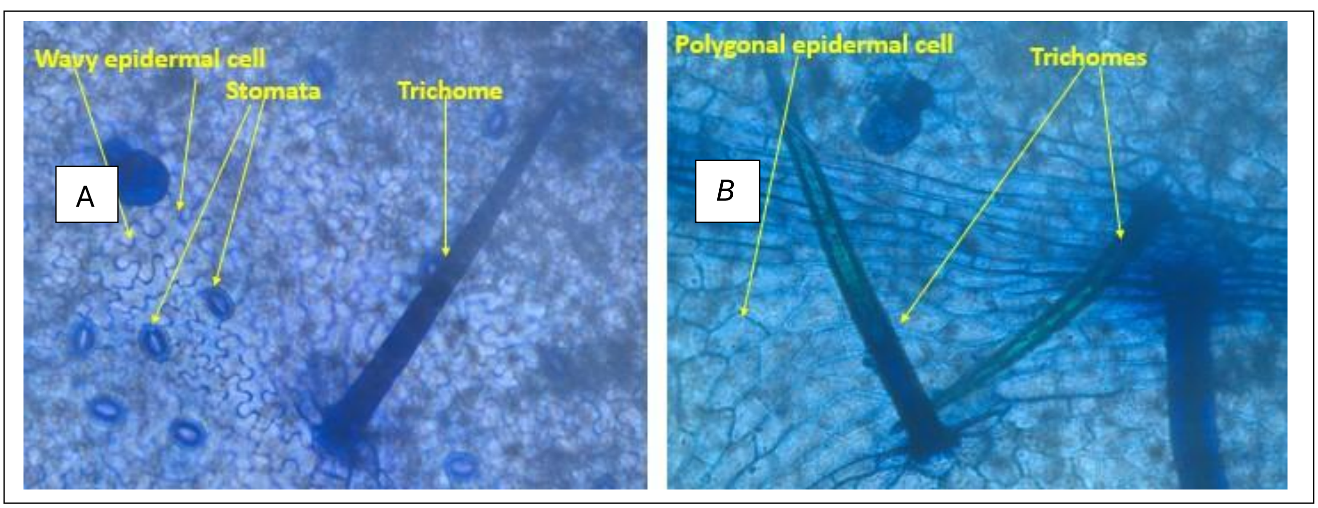

Figure 7. Abaxial epidermal layer (mgx40) of *Entandrophragma utile* (A) and *Entandrophragma angolense* (B)

Table 3. Quantitative adaxial epidermal characteristics of *Entandrophragma utile* and *Entandrophragma angolense*

Epidermal variables	Tree species	Mean±SD	T-value	p-value
NEC	*Entandrophragma utile*	138.20±3.6	-3.148	0.014*
	Entandrophragma angolense	167.60±8.6		
ECL (μm)	*Entandrophragma utile*	25.44±5.6	-4.096	0.003*
	Entandrophragma angolense	56.72±5.1		
ECW (μm)	*Entandrophragma utile*	15.74±3.3	-2.752	0.025*
	Entandrophragma angolense	28.94±3.4		
NWS	*Entandrophragma utile*	.		
	Entandrophragma angolense	6.0±0.6		
NT	*Entandrophragma utile*	2.60±0.2	10.614	
	Entandrophragma angolense			
TL (μm)	*Entandrophragma utile*	33.20±2.0	16.317	
	Entandrophragma angolense			

Key: NEC=number of epidermal cells, NWS=number of wall sides, ECL=epidermal cell length, ECW=epidermal cell width, NT = Number of trichome, TL = trichome length. *= Significant at 5% probability level, ns= not significant 5% probability level.

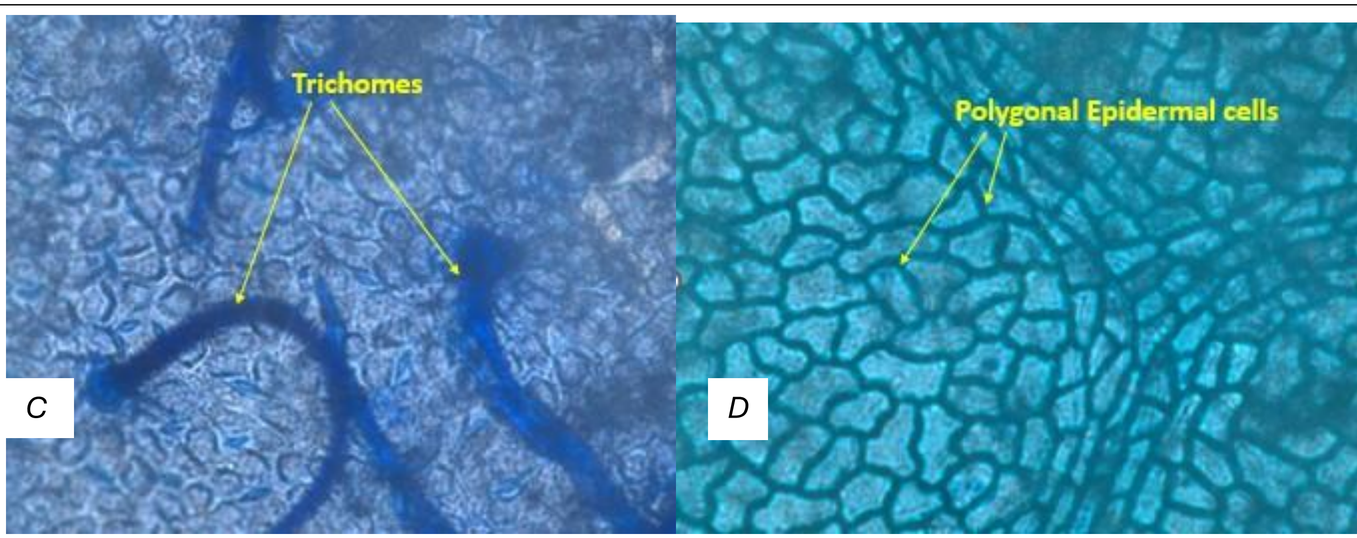

Figure 8. Adaxial epidermal layer (mgx40) of *Entandrophragma utile* (A) and *Entandrophragma angolense* (B)

CONCLUSION

This study identified the distinct macro- and micro-morphological differences between *Entandrophragma utile* and *E. angolense*, providing critical insights for species identification and ecological adaptation. Bark anatomical analyses revealed that *E. utile* prioritizes nutrient transport through a well-developed phloem, while *E. angolense* emphasizes structural integrity with a fibre rich phloem, aligning with their respective humid forest and forest-savanna ecotone habitats. These findings not only resolve long-standing identification challenges but also offer a functional basis for conservation strategies, silvicultural practices and climate-resilient reforestation efforts.

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