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SIGNIFICANCE OF LEAF (MIDRIB AND LAMINA) CHARACTERS IN THE IDENTIFICATION OF *TERMINALIA* L. (COMBRETACEAE)

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ABSTRACT

Leaf (midrib and lamina) anatomical characters were investigated to enhance the identification of Terminalia L. The central portions of the mature leaves were fixed in formaldehyde, glacial acetic acid, and ethanol (FAA) for 12 hrs. They were dehydrated in alcohol series, hand-sectioned, stained with safranin and alcian blue, mounted on a slide, viewed, and photographed with Optika B-1000 FL LED fitted with digital camera. Our findings showed the absence of rib trace in T. ivorensis, presence of secretory ducts in T. catappa, open vascular cylinder with incurved ends in T. mantaly, and medullary phloem in T. avicenniodes. These characters were key to the delimitation of the Terminalia species.

Keywords: Anatomy, Midrib, *Terminalia*, Rib trace, Secretory ducts

INTRODUCTION

Terminalia L. is a member of the family Combretaceae with over 200 species of trees and shrubs (Stace, 2002). Members of this genus are pantropical and occur in the tropical areas of America, Africa, Asia, the Pacific Islands, and subtropical regions of Australia (Nahed *et al*., 2020). This genus has 54 species occurring across eastern, western, and southern Africa (Smith *et al*., 2004), 10 species in West Africa (Hutchinson and Dalziel, 1954), and nine species including the representative species of the genus *T. catappa* L., *T. superba* Engl. & Diels., *T. ivorensis* A. Chev., *T. mantaly* H. Perr., and *T. avicennioides* Guill. & Perr. in Nigeria (Keay, 1989). Members of this genus such as *T. superba*, and *T. ivorensis* are known to be among the popular timbers in Nigeria (Jayeola *et al.*, 2009; Awe *et al.*, 2019) and *T. acuminata* and *T. ivorensis* (Kayode *et al*., 2019).

The anatomical, morphological, and karyotype attributes of members of *Terminalia* differ greatly and as useful in their classification (Stace, 2008; Ohri, 1996). Also, the morphological features of the leaf, bark, and fruit are significantly different in the genus and could help in the delimitation of the taxa (Wickens, 1973; Fyhrquist, 2007). Noraini & Cutler (2009), reported that the fragment of plant materials from members of this genus could be identified using anatomical features. At the seedling stage members of this genus have similar morphological features which make it difficult to easily differentiate them but several studies including the anatomy of Combretaceae were discussed (Verhoeven, 1969; Verhoeven & Vander- Schüff, 1973, 1974). Moreover, Metcalfe & Chalk (1979) described the anatomical features of the family with some specifications on genus *Terminalia* and emphasized the importance of the arrangement of sclerenchymatous cells surrounding the vascular bundles. The study of

leaf epidermis of *Terminalia* has been documented by Stace (2008), Nawani & Kulshreshtha (1982), Narasimha Rao & Ramayya (1984), and Ekeke & Agbagwa (2015).

Medical, cultural, religious, and social importance has been recorded throughout the genus (Schmidt *et al*., 2002; Smith *et al*., 2004; Satardekar & Deodhar, 2010; Sharma & Mukundan, 2014; Rathinamoorthy & Thilagavathi, 2014). In Africa and Asia, traditional remedy from *Terminalia* species has been utilized (Lawes et al., 2004; Steenkamp et al., 2004; Moshi & Mbwambo, 2005). Also, some *Terminalia* species are sources of raw materials for some pharmaceuticals and cosmetics preparations (Dalziel & Hutchinson, 1937; Irvine, 1961). Derivatives of *Terminalia* species are used in the remedy of many diseases such as diabetics, eczema, tuberculosis, leprosy, candidiasis, dermatitis, gonorrhea, malaria, scurfy affection, kidney and liver disorders (Batawila *et al.*, 2005; Masoko and Eloff, 2005; Fyhrquist, 2007; Kamtchouing *et al.*, 2006; Gupta, 2012). Various colours of dyes utilized in many industrial productions are obtained from bark, root, leaf, and fruit extracts and utilized for many industrial productions (Dalziel and Hutchinson, 1937; Errington and Chisumpa, 1987). In Egypt, biological studies on the leaves revealed numerous activities *in vivo* and *in vitro* studies; including antioxidant, anti-inflammatory, anti-diabetic, anti-ulcer, anti-hyperlipidemic, anti-microbial anti-cancer, anti-parasitic, hepatoprotective, and cardioprotective activities (Fahmy *et al.*, 2015).

In recent past years, the leaf anatomical characters in the genus *Terminalia* have been studied by many authors (Tilney, 2002; Ingle and Dhabe, 2011, 2015; Akinsulire *et al*., 2018) for the identification of the relationships between its species. The nine cultivated species in Egypt have been studied (Hamdy *et al.*, 2007; Hamdy, 2010; Fahmy *et al.*, 2015). Notwithstanding, Nahed *et al.* (2020) reported taxonomic conflict and difficulty in identification in some species of *Terminalia* due to morphological similarities, especially at the seedling stage. Among other plant families, midrib outline and anatomy have been used in their identification (Mantovani & Pereira 2005; Bačić *et al.*, 1992; Woltz *et al.*, 1987; Mantovani *et al*., 2009; Dalvi *et al.*, 2014; da Silva *et al.*, 2015). All the authors concluded that the midrib outline is a useful character for taxonomy. We therefore aimed at investigating the use of anatomical features leaf (lamina and midrib) in the identification of some members of *Terminalia* in Nigeria.

MATERIALS AND METHODS

Specimens for anatomical analysis were obtained fresh from matured plants growing at the University of Port Harcourt, properly identified and authenticated by the curator in the Department of Plant Science and Biotechnology Herbarium, and fixed in FAA (1 part of 40% formaldehyde, 1 part of glacial acetic acid and 18 parts of 70% ethanol) for 12 hrs. They were transferred to 50% and 70% ethanol (each for 2 hours) and finally kept in absolute ethanol at room temperature until required. When required the fruit stalk and tendril were collected from the absolute ethanol and hand-sectioned using sharp razor blades (Okoli and Ndukwu, 1992). Thin sections were stained in 1% Safranin red for two minutes, counter-stained with Alcian blue, mounted on a slide, viewed, and Good sections were mounted on slides, viewed, and photographed with Optika B-1000 FL LED fitted with a digital camera.

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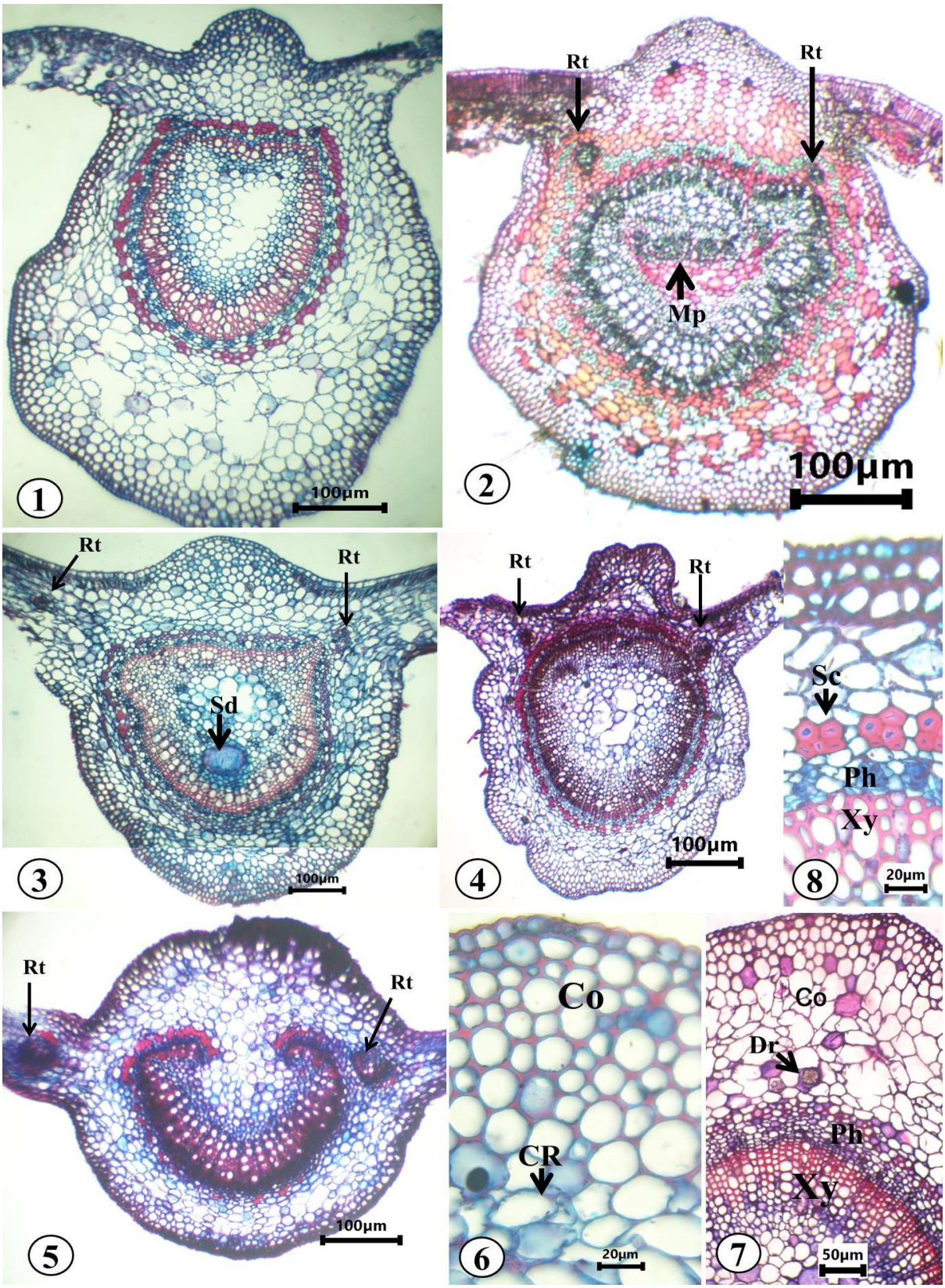
RESULTS AND DISCUSSION

Generally, the epidermal surfaces of the species were hairy and partly glabrous in *T. ivorensis* and the vascular bundles in the midribs were surrounded by patches of sclerenchymatous fibre or sclereids. The abaxial outline is relatively smooth in *T. ivorensis* (Fig. 1), *T. avicennoides* (Fig. 2), and *T. catappa* (Fig. 3); rough or undulating in *T. superba* (Fig. 4), but smooth in *T. mantaly* (Fig. 5). There are comparative differences in their midrib and lamina anatomical features which could be used to delimit them especially when they are not flowering or fruiting. Generally, there are crushed parenchyma cells in the abaxial midrib cortex of the *Terminalia* species studied (Fig. 6) except in *T. mantaly*. Druse crystals are located in the parenchymatous cortex close to the phloem cells (Fig. 7) in all the species studied but are more abundant in *T. superba.* Also, the adaxial cuticle outline of all the species is elevated with varying heights and shapes.

The vascular bundles formed closed cylinders in *T. superba*, *T. mantaly, T. avicenniodes, T. ivorensis,* and *T. catapa* but formed an open semi-circle with strongly incurved ends in *T. mantaly.* This character made *T. mantaly* distinct from other members of *Terminalia* studied. Also, all the species investigated have rib traces on both sides of the adaxial portion of their vascular cylinder except *T. ivorensis.* This character further delimits *T. ivorensis* from all these species. Among the species with closed vascular cylinders, the adaxial portion of the cylinder in *T. ivorensis* and *T. catappa* is flat however; *T. catappa* (Fig. 3) has rib traces on both sides of the vascular cylinder including the secretory duct in the central abaxial part of the vascular cylinder. These features are lacking in *T. ivorensis* (Fig. 1).



Figures 1 – 8: Midrib transverse sections of *Terminalia* species; (1) *T. ivorensis*, (2) *T. avicenniodes*, (3) *T. catapa*, (4) *T. superba*, (5) *T. mantaly*, (6) *T. catapa* showing cortex and crushed parenchyma, (7) *T. superba* showing druse crystal in cortex parenchyma, (8) *T. ivorensis* showing patches of sclerieds, Co – cortex, Dr – druse, Tr – rib trace, CR – crushed parenchyma, Sd – secretory duct, Mp – medullary phloem, and Sc – scleried.

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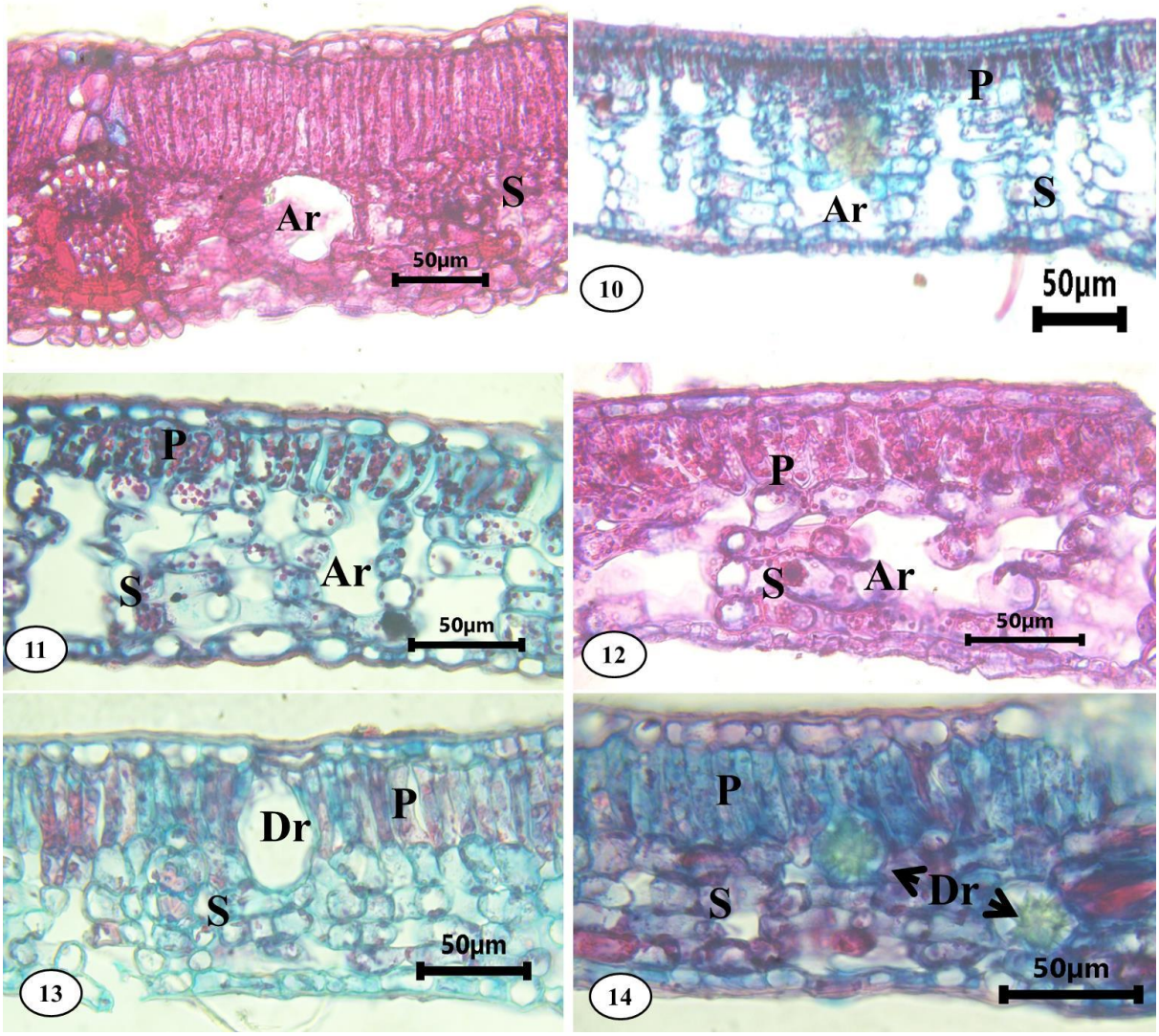
Furthermore, the adaxial portion of the vascular cylinder in *T. avicenniodes* (Fig. 2) and *T. superba* (Fig. 4) have convex shape but *T. avicenniodes* has medullary phloem which distinguishes it from *T. superba*. Previous research works have shown that the midrib outlines and anatomy could be of taxonomic value in some plant species, genera, and families. Mantovani and Pereira (2005) and Mantovani *et al.* (2009) demonstrated that the midrib outline of *Anthurium* (section *Urospadix*; sub-section *Flavescentiviridia*) (Araceae) varies greatly among them and is relevant in their identification. Also, Bačić *et al.* (1992) compared the midribs of three species of *Arbutus* (Ericaceae) while Woltz *et al.* (1987) described the midrib outline prominence or concavity for 112 of the 184 species of Podocarpineae. These authors observed that the midrib anatomy and outline is a useful features in the taxonomy of these species. In the same way, we observed variations in the midrib anatomical characters of the *Terminalia* species studied which are dependable and diagnostic in this genus. The findings of our study, conform with the previous documentation (Tilney, 2002; Ingle & Dhabe, 2011, 2015; Akinsulire *et al*., 2018; da Silva, 2015; Hamdy *et al.*, 2007; Hamdy, 2010; Fahmy *et al.*, 2015).

The lamina of all the specimens studied had the vascular bundles of the small veins embedded in the mesophyll. Among the species investigated, only *T. avicenniodes* had 1-2 layers of palisade parenchyma and an undulating adaxial surface (Fig. 9). The spongy mesophyll cells are loosely packed with large air/intercellular spaces in *T. avicenniodes* (Fig. 9), *T. catappa* (Fig. 10), *T. ivorensis* (Fig. 11), and *T. superba* (Fig. 12), but closely packed in *T. mantaly* with druse crystals in the palisade and spongy mesophylls (Figs. 13 and 14). Though Ekeke *et al.* (2014) reported that the size and distribution of druse crystals in the leaf lamina of *Combretum* Loelf., could be used to differentiate the genus, druse crystals were observed only *T. mantaly*. This character in combination with other leaf anatomical features could be diagnostic in *Terminalia.* The presence of druses and solitary crystals in the palisade and spongy mesophyll cells of angiosperms have been commonly reported (Dickison 2000). The ratio of the palisade cells to the leaf thickness and the leaf thickness in the species studied showed slight variation among them.



Figures 9 – 14: Lamina transverse sections of *Terminalia* species; (9) *T. superba*, (10) *T. mantaly*, (11) *T. catapa*, (12) *T. ivorensis*, (13) *T. avicenniodes*, (14) Arrows show druse crystals in mesophyll, P – palisade parenchyma, S – spongy parenchyma, Ar – intercellular air space, Dr – position of druse crystal.

The range (mean±standard deviation) of the ratio of the palisade cells to the leaf thickness is *T. avicenniodes*, 2.44 – 3.12(2.75±0.22); *T. catappa*, 4.52 – 5.71(5.03±0.46); *T. ivorensi*, 4.53 – 5.77(4.91±0.43); *T. mantaly*, 2.75 – 3.60(3.08±0.28); and *T. superba*, 3.31 – 4.73(3.99±0.44). Also, the average thickness of the leaves include *T. avicenniodes* (140µm), *T. catappa* (110µm), *T. ivorensi* (160µm), *T. mantaly* (96µm), and *T. superba* (130µm). From our findings, we observed that this character alone cannot be used to identify the species but in combination with other characters. Similar observation was made by Noraini and Cutler (2009) who reported a palisade-leaf thickness ratio of ⅓ to ½ in *Parashorea* Kurz. and noted that it was not diagnostic in the genus.

The results of our study revealed some interesting characteristics which could be of taxonomic and diagnostic values in this genus. The absence of rib trace in *T. ivorensis*, presence of secretory ducts in *T. catappa*, open vascular cylinder with incurved ends in *T. mantaly*, and medullary phloem and undulating adaxial leaf surface in *T.*

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avicenniodes. This showed that the leaf midrib characters could be used to identify *Terminalia* species.

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