Anatomical Studies on the Genus *Terminalia* L. (Combretaceae) in Egypt, I- Leaf Structure

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**Introduction**

*Terminalia* L. is a pantropical genus growing across the tropical areas of Africa, Asia, America and subtropical regions of Australia and Pacific Islands, considered to be the second-largest genus of the family Combretaceae comprising more than 200 species of trees and shrubs (Stace, 2002). The 54 species of *Terminalia* are distributed across eastern, western and southern Africa (Lebrun & Stork, 1991; Smith et al., 2004). Taxonomic treatment of *Terminalia* has long been recognized by many workers (Clarke, 1878; Engler & Diels, 1900; Exell, 1954, 1978; Griffiths, 1959; Al-Mayah, 1983; Pedley, 1990).

The species of this genus differ greatly in anatomy, morphology and karyotype features (Stace, 1965; Ohri, 1996). According to Wickens (1973) and Fyhrquist (2007), the morphological characters of the bark, leaf and fruit are remarkably different throughout the genus and could be a significant guide for the identification of taxa. In case of incomplete materials, the anatomical features could be useful (Noraini & Cutler, 2009).

Several studies including the anatomy of Combretaceae were discussed (Heiden, 1893a, b; Solereder, 1908; Verhoeven, 1969; Verhoeven & Vander- Schifff, 1973, 1974). Moreover, Metcalfe & Chalk (1950) described the anatomical features of the family with some specification on genus *Terminalia* and emphasized the importance of the arrangement of sclerenchymatic cells surrounding the vascular bundles.

*Corresponding author email: ashsoliman@sci.cu.edu.eg*  
Received 24/11/2019; Accepted 2/5/2020  
DOI: 10.21608/ejbo.2020.20019.1398  
Edited by: Prof. Fco Martin Dr. Huerta-Martinez, University of Guadalajara, Mexico.  
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As well, the study of leaf epidermis of *Terminalia* has been documented by Stace (1965), Nawani & Kulshreshtha (1982) and Narasimha Rao & Ramayya (1984).

The leaves in *Terminalia* are simple, alternate, subopposite or spirally rarely opposite arranged, single along the shoots or clustered at the end of branches with brochidodromous venation, exstipulate, margin slightly crenulate, glabrous or pubescent, often minutely verrucose with 2 or more glands at or near the base of the blade or at the top of the petiole.

Medical, cultural, religious and social importance has been recorded throughout the genus (Irvine, 1961; Srivastava et al., 1992; Lemmens et al., 1995; 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 (Lawn et al., 2004; Steenkamp et al., 2004; Moshi & Mbambo, 2005). As well pharmaceuticals and cosmetics preparations utilized *Terminalia* species as sources of raw materials (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 disorder (Batawila et al., 2005; Masoko & Elof, 2005; Fyhrquist, 2007; Kametchouing et al., 2006; Gupta, 2012). Various colors of dyes utilized for many industrial productions were obtained from bark, root, leaf and fruit extracts and utilized for many industrial productions (Dalziel & Hutchinson, 1937; Errington & Chisumpa, 1987). Recently, in Egypt, biological studies on the leaves revealed numerous activities in vivo and in vitro studies; included antioxidant, anti-inflammatory, anti-diabetic, anti-ulcer, anti-hyperlipidemic, anti-microbial anti-cancer, anti-parasitic, hepatoprotective and cardioprotective activities (Fahmy et al., 2015).

In the recent past years, the leaf anatomical characters in the genus *Terminalia* (lamina and petiole) have been studied by many authors (Tilney, 2002; Ingle & Dhabe, 2011, 2015; Akinsulire et al., 2018) for the identification of the relationships between its species.

In Egypt, *Terminalia* is represented by nine cultivated species (Sickenberger, 1901; Muschler 1912; Hamdy et al., 2007; Hamdy, 2010; Fahmy et al., 2015).

Due to the taxonomic conflict of the genus *Terminalia* and the difficulty in the identification of some of its species morphologically. Moreover, there is no comprehensive coverage of genus *Terminalia* in Egypt that distinguishes between different species anatomically, despite of its medicinal importance. Therefore, this research therefore set out to define the leaf (lamina and petiole) anatomical characters of the nine species of the genus *Terminalia* in Egypt as bases for the authentication and the discrimination among species.

**Material and Methods**

Ten fresh samples for each species were collected from different botanical gardens in Cairo and Giza Governorates, compared with authenticated materials kept in Cairo University Herbarium (CAI), identified (Table 1) then fixed in Formalin, Glacial Acetic acid (FAA) then 70% ethyl alcohol. The transverse sections of leaves and petioles were cut using Reichert Sliding Microtome at a thickness of 8-10 microns. These sections were stained in Safranin for 3-5min, rinsed thoroughly in water to remove excess stain and counterstained in Light green solution for 3-5 min. The sections were again washed with water and treated in a series of ethanol dilution 50%, 70%, 80%, 90% and 100% to enhance the dehydration process. After dehydration, sections were transferred into absolute xylene to remove any remaining trace of water and ethanol. These made sections clearer and prevented the cloudiness of the slides. Sections were therefore mounted in Canada balsam on a clean glass slides for light microscopy.

Sections were investigated with light microscope; Olympus BX51. Photomicrographs were taken using photomicroscope; Olympus DP12 in the Central Laboratory, Geology Department, Faculty of Science, Cairo University, Egypt.

In this work, anatomical characters were the basis to separate the taxa, including petiole and leaf outline, epidermal feature, vascular pattern, the occurrence of cortical and pericycle fibers, presence/absence of crystals and trichomes in addition to the shape of main vascular bundle and number of accessory vascular bundles.
TABLE 1. The localities of collected specimens, geographic coordinates and the date of collection of the investigated *Terminalia* species, kept at Cairo University Herbarium (CAI).

<table>
<thead>
<tr>
<th>N</th>
<th>Species</th>
<th>Locality</th>
<th>Geographic coordinates</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>T. arjuna</em> (Roxb. ex DC.)</td>
<td>Giza: Zoological garden</td>
<td>30° 01’24.7” N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wight &amp; Arn.</td>
<td></td>
<td>31° 12’40.6” E</td>
<td>7 Nov. 2016</td>
</tr>
<tr>
<td>2</td>
<td><em>T. bellirica</em> (Gaertn.) Roxb.</td>
<td>Giza: Zoological garden</td>
<td>30° 01’27.4” N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31° 12’52.7” E</td>
<td>7 Nov. 2016</td>
</tr>
<tr>
<td>3</td>
<td><em>T. bentzoe</em> (L.) L. f.</td>
<td>Giza: Zoological garden</td>
<td>30° 01’34.0” N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°12’46.0” E</td>
<td>7 Nov. 2016</td>
</tr>
<tr>
<td>4</td>
<td><em>T. brownii</em> Fresen.</td>
<td>Giza: Mazhar botanical garden</td>
<td>30° 03’ N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°08’ E</td>
<td>10 Dec. 2016</td>
</tr>
<tr>
<td>5</td>
<td><em>T. catappa</em> L.</td>
<td>Giza: Orman botanical garden</td>
<td>30° 01’ N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°12’ E</td>
<td>7 Nov. 2016</td>
</tr>
<tr>
<td>6</td>
<td><em>T. laxiflora</em> Engl. &amp; Diels</td>
<td>Cairo: El Zohriya garden</td>
<td>30° 02’ N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°13’ E</td>
<td>16 Nov. 2016</td>
</tr>
<tr>
<td>7</td>
<td><em>T. mantaly</em> H. Perrier</td>
<td>Giza: Mazhar botanical garden</td>
<td>30° 03’ N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°08’ E</td>
<td>10 Dec. 2016</td>
</tr>
<tr>
<td>8</td>
<td><em>T. muelleri</em> Benth.</td>
<td>Giza: Zoological garden</td>
<td>30° 01’35.2” N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31°12’52.4” E</td>
<td>7 Nov. 2016</td>
</tr>
<tr>
<td>9</td>
<td><em>T. myriocarpa</em> Van Heurck</td>
<td>Giza Zoological garden</td>
<td>30° 01’34.8” N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and Mull. Arg.</td>
<td></td>
<td>31°12’46.0” E</td>
<td>7 Nov. 2016</td>
</tr>
</tbody>
</table>

Terminology of the anatomical characters of leaf and petiole was described according to Metcalfe & Chalk (1950), Tilney (2002) and Akinsulire et al. (2018).

In order to conduct which species are similar or dissimilar to each other, we use the clustering analysis of Multi-Variate Statistical Package version 3.13g. The matrix of the 9 studied species with the investigated 24 anatomical characters is subjected to the clustering method (UPGMA) with the average similarity distance. All categorical characters are transformed into binary characters (0, 1) in order to allow the use of cluster analysis techniques. This is followed by generalization of an artificial key depending on the anatomical characters investigated.

**Results**

*Terminalia petiole in transverse section*

The outline was more or less spherical, triangular or cave-shaped. Epidermis covered with very thin to rarely thick cuticle and simple, unicellular combretaceous trichomes, cells of epidermis square to more or less papillate. Hypodermis, if present, single layered formed of circular to polygonal cells. Cortex differentiated into outer and inner regions; outer collenchymatous and parenchymatous and inner collenchymatous (the collenchyma varies between angular and lamellar) throughout. Vascular system is either amphiphloic siphonostele, with outer and inner phloem continuous (Plate 1; F) or ectophloic siphonostele, with outer phloem only (Plate 2; L); lysigenous ducts 0-5 present in inner phloem, either solitary located centrally or in groups extending along the xylem length, sclerenchyma scares present. Accessory vascular bundles 2 to 3 similar or dissimilar. Calcium oxalate crystals (druses) rare to abundant occurring in cortex, phloem, rarely in bundle sheath (Plates 1, 2, 3).

*Egypt. J. Bot.*** 60, No.3 (2020)
Plate 1. Transverse section of petiole in *Terminalia* species; A & B: *T. arjuna*, C & D: *T. bellerica*, E & F: *T. bentzoe*

[AVB= Accessory vascular bundle, EP= Epidermis, COL= Collenchyma, LD= Lysigenous duct, MVB= Main vascular bundle, PAR= Parenchyma, PI= Pith, PF= Pericyclic Fibers, TR= Trichome].

_Egypt. J. Bot. 60_, No. 3 (2020)

[AVB= Accessory vascular bundle, LD= Lysigenous duct, MVB= Main vascular bundle]
Plate 3. Transverse section of petiole in *Terminalia* species; M & N: *T. mantaly*, O & P: *T. muelleri*, Q & R: *T. myriocarpa* [AVB = Accessory vascular bundle, COL = Collenchyma, DC = Druses, IPH = Inner phloem, LD = Lysigenous duct, MVB = Main vascular bundle, OPH = Outer phloem, PAR = Parenchyma, PF = Pericycle fibers, XY = Xylem].

_Egypt. J. Bot._ 60, No. 3 (2020)
Terminalia leaf in transverse section

Leaf blade dorsiventral, hairs present of combretaceous type. Epidermis covered with very thin to medium cuticle layer on both surfaces, 1-layered with square to periclinal elongated cells (Plate 4: C); outer periclinal cell walls flattened on the upper surface and flattened to slightly convex on the lower surface or often ridged, usually strongly convex often appearing somewhat papillae over larger vascular bundles, rarely mucilaginous. Upper epidermal cells usually conspicuously larger than lower ones but occasionally similar, outer periclinal cell walls especially on adaxial surface often conspicuously cutinized but minute striate on the surface, inner periclinal and anticlinal cell walls always slightly cutinized. Mesophyll usually differentiated into palisade and spongy parenchyma; with palisade cells usually markedly elongated, 1-layered, small tubular extensions interconnecting cells frequently very conspicuous; spongy parenchyma, 3-6 layered, cells oval polygonal and irregular, in T. laxiflora, the mesophyll was formed of only 3 rows of palisade layer. Vascular bundles collateral in small veins or bicollateral in large veins, sclerenchyma nearly always associated with phloem only but rarely with phloem and xylem, surrounded by a bundle sheath formed of parenchyma cells (Plate 5: J).

Druses crystals always present, in varying numbers and sizes, usually very large in idioblasts, palisade parenchyma, spongy parenchyma, and at the junction of palisade and spongy parenchyma. Midrib area variously raised or flattened. Cuticle was very thin to medium thick. Epidermis 1-layered usually followed by a single hypodermal layer (Plate 5: K); cells square to anticlinal elongated with the outer periclinal cell walls mostly slightly convex on the upper surface but usually strongly convex often appearing almost papillae on periclinal cell walls. Midrib formed of one or two type of cells, often forming a continuous collenchymatous layer usually more than one cell thick adjacent to adaxial epidermis tissue followed by 1-6 thin wall parenchymatous layers. Central vascular bundle was conjoint; open bicollateral endarch vascular bundle of different shape and size (Plate 6: P). Sclerenchyma fibers combined with external phloem or external and internal phloem. Patches of fibers interrupted with tanniniferous cells in complete or incomplete bands. Mucilage ducts if present, usually one or up to three towards the inside of the inner xylem. Druses crystals frequently present in collenchyma, parenchyma and phloem accessory vascular bundle, if present, one or two equal or unequal situated on either side towards the epidermis, conjoint, concentric, closed and collateral type (Plates 4, 5, 6).

Table 2 shows the 24 investigated anatomical characters of the leaf and petiole of Terminalia species in Egypt. These characters are distinguishable and used to construct the following cluster.

The goal of cluster analysis is to group a collection of objects in such a way that characters in the same group are more similar to one another than characters in other groups. The hierarchical structure of UPGMA with the average similarity distance (Fig. 1) helps in the generalization of the artificial key adopted below.

Fig. 1. The cluster analysis of the nine Terminalia species based on the investigated anatomical characters (Table 2).

*Egypt. J. Bot.* **60**, No. 3 (2020)
## TABLE 2. The anatomical characters of the nine investigated species of *Terminalia* in Egypt.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>T. arjuna</em></th>
<th><em>T. bellirica</em></th>
<th><em>T. bentzoe</em></th>
<th><em>T. brownii</em></th>
<th><em>T. catappa</em></th>
<th><em>T. laxiflora</em></th>
<th><em>T. mantaly</em></th>
<th><em>T. muelleri</em></th>
<th><em>T. myriocarpa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Petiole outline: Ovate (1), triangular (2), cat face (3), cave (4)</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Petiole hypodermis: Present (1), absent (2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Petiole ground tissue ratio - Collenchyma/Parenchyma: 1:1 (1), 1:2 (2), 1:3 (3), 1:4 (4), 2:3(5)</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Petiole lenticels &amp; Trichomes: Present (1), absent (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Petiole main vascular bundle shape: Heart-shaped (1), closed triangular (2), open triangular (3), crescent-shaped (4)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Petiole bundle sheath sclerenchyma cells: Continuous ring (1), patches (2)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Petiole accessory vascular bundles distribution: One on each side (1), two on each side (2), one in one side &amp; two on other side (3)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Petiole accessory vascular bundle type: Primitive (1), open collateral (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Petiole lysigenous ducts: Present (1), absent (2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Petiole lysigenous ducts number: Zero (1), two (2), more than two (3)</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Petiole tannins: Present (1), absent (2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Petiole crystals: Few (1), abundant (2)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Leaf mesophyll ratio-palisade/spongy: 1:2 (1), 1:3 (2), 1:4 (3)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Leaf midrib region: One type of cells (1), two types of cells (2)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leaf main vascular bundle shape: Triangular (1), open mouth (2), cat face (3), crescent (4), open heart (5)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leaf main vascular bundle size: Small (1), medium (2), large (3)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Leaf accessory vascular bundle: Present (1), absent (2)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leaf number of accessory vascular bundles in each side: Zero (1), two (2), more than two (3)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leaf vascular bundle of small veins: Present (1), absent (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leaf crystals: Few (1), abundant (2)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leaf shape of upper epidermis: Rectangular (1), papillose (2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td>Leaf length of palisade in mesophyll: &lt;50μm (1), &gt;50μm (2)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Leaf length of Spongy in mesophyll: &lt; 70μm (1), 70 &gt;μm (2)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leaf bundle sheath shape: Patches (1), continuous ring (2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Artificial key based on the petiole and leaf anatomical characteristics

I- Petiole shape in TS cave-shaped......................1
   1a- Main vascular bundle in leaf open heart-shaped .................................................................T. brownii
   1b- Main vascular bundle in leaf crescent-shaped...........................................T. mantaly

II- Petiole shape in TS cat face..............T. bellerica

III- Petiole shape in TS triangular......................2
   2a- Main vascular cylinder in petiole, open triangular (amphiphloic siphonostele). T. bentzoe
   2b- Main vascular cylinder in petiole, closed triangular or heart-shaped (ectophloic siphonostele) ................................................3
   3a- Bundle sheath sclerenchyma cells in petiole form a continuous ring .........................T. muelleri
   3b- Bundle sheath sclerenchyma cells in petiole form separate patches....................................T. catappa
   4a- main vascular bundle shape cat face..................T. catappa
   4b- Leaf main vascular bundle shape open mouth ............................................................T. laxiflora

IV- Petiole shape in TS ovate..........................5
   5a- Leaf vascular bundle in small veins and petiole hypodermis are present...............T. arjuna
   5b- Leaf vascular bundle in small veins and petiole hypodermis are absent ..........T. myriocarpa

Discussion

Many researchers in various plant taxa used leaf anatomy for taxonomic purposes (Illo, 1995; Adedeji, 2004; Adedeji & Illoh, 2004; Okeke et al., 2015; Rodriguez et al., 2016). Among the most anatomical features, the leaf and petiole anatomy provide diversity of characters which could be used for taxonomic purposes. In addition, the leaf the most diverse organ of angiosperms, and their anatomical variation frequently correlate closely with generic and common, often phylogenetic lines (Carquist, 1961).

Anatomical characters, along with other characters, have been considered important in providing additional features; they are typically of taxonomic significance in plant identification and classification. Howard (1979) and Khatijah et al. (1992) have reported that anatomical characteristics often indicate relationship between species and often classify the position of the species, and the data obtained from anatomy can be used extensively for taxonomic and phylogenetic elucidation (Illoh & Inyang, 1998).

Many anatomical characters of leaf and petiole can be investigated as taxonomic tools in plant identification and differentiation between the taxa. The epidermal cells in the investigated species are arranged irregularly that are polygonal, isodiometric or elongated in various directions. The anticlinal epidermal walls are sinuous, undulating, straight or arched, characteristic as cited by Ekeke & Agbagwa (2015) for T. catappa. The presence of a thin cuticle helps protection against multiple stress factors including dehydration (Gonzalez, 2002). The epidermal characteristics have been utilized for taxonomic specification of taxa at the genus and family levels (Van Cothiem, 1970; Paliwal et al., 1980; Naidu & Shah, 1981; Das, 2002; Agbagwa & Okoli, 2005 & 2006; Amaral & Mello-Silva, 2008). Particularly, Ramassamy & Kannabiran (1994) used the epidermal characteristics as stomata and trichome types for taxonomic considerations of some Terminalia species in India. There is significant diversity observed in the epidermal area among the investigated species. Therefore, the epidermal characters were utilized for differentiation between species.

The structure of the petiole is significant for the discrimination of taxa and has considerable taxonomic importance in many genera, once it is less affected by environmental changes (Metcalfe & Chalk, 1950); the shape and type of vascular cylinder in the petiole were remarkable characters (Ayensu, 1968) as the morphologic separation is hard with some species (Leme & Scremin-Dias, 2014). Particularly, Akinsulire et al. (2018) utilized the shape of the vascular cylinder and the arrangement of the accessory vascular bundles of the petiole as diagnostic characters for the separation between five species of Terminalia in southwest Nigeria. He placed T. mantaly and T. catappa in one group based on their quantitative leaf anatomical characters.

The vascular cylinder in petiole in the genus Terminalia is siphonostele. Most of the higher vascular plants display centered steles with a pith. The pith remains surrounded by xylem, which in
turn is surrounded by phloem on the peripheral side (ectophloic siphonosteole) or both in and out (amphiphloic siphonosteole). Those steles are called siphonosteoles. Ectophloic siphonosteole has a continuous cylinder of the vascular tissues in its primitive form. The advanced form consists of a bundling network.

The secretory structure including ducts can also be used as diagnostic parameters for the separation between the species (Milan et al., 2006) and identification of the genera (Castro et al., 1997).

Amongst the studied species of Terminalia, anatomical characters of leaf and petiole seem to be of high taxonomic value in plant identification. Twenty four anatomical characters that have significant anatomical diversity have been investigated; in leaf, type of mesophyll, length of palisade tissue, number and size of the main and accessory vascular bundles, presence or absence of vascular bundles in small veins as well lysigenous ducts and crystals, while in petiole: the type and shape of the vascular cylinder, the number and distribution of the accessory vascular bundles, types of bundle sheath, number of lysigenous ducts, etc. All these characters help to differentiate the nine Terminalia species and help to create an artificial key.

The main vascular cylinder in the petiole has various types and shapes; we recognize crescent shape with amphiphloic siphonosteole in T. brownii and T. mantaly, while the other seven species viz: T. arjuna, T. muelleri, T. bellerica, T. bentzoe, T. catappa, T. myriocarpa and T. laxiflora varies between heart or triangular shape and amphiphloic or ectophloic siphonosteole.

The main vascular bundle in the leaf was of diagnostic characters, it takes an open-heart shape in T. brownii (Plate 2; G & H) and a crescent shape in T. mantaly (Plate 3; M & N). Akinsulire et al. (2018) described the vascular bundle of T. mantaly as deeply crescent form or lunar-shaped. Seema et al. (2015) described the vascular bundle of the mature leaves of T. catappa as an arc-shaped.

Moreover, the distribution of accessory vascular bundles in the petiole of Terminalia play a great role as a diagnostic character, T. bellerica (Plate 1; C & D) has two vascular bundles on each side of the petiole while the other 8 species have only one vascular bundle on each side. Presence, absence and number of lysigenous ducts in Terminalia petiole differentiate between the nine investigated species. Lysigenous ducts was absent in T. bellerica (Plate 1; C & D), T. bentzoe (Plate 1; E & F), T. brownii (Plate 2; G & H) and T. mantaly (Plate 3; M & N) while present with various number in T. arjuna (Plate 1; A & B), T. muelleri (Plate 3; O & P), T. catappa (Plate 2; I & J), T. myriocarpa (Plate 3; Q & R) and T. laxiflora (Plate 2; K & L). It was two ducts in T. muelleri (Plate 3; O & P) and T. catappa (Plate 2; I & J), three ducts in T. arjuna (Plate 1; A & B) and T. laxiflora (Plate 2; K & L) and numerous ducts in T. myriocarpa (Plate 3; Q & R).

The bundle sheath that surrounds the main vascular bundle in the petiole of Terminalia consists of sclerenchyma cells that can take different shapes. In both, T. catappa (Plate 2; I & J), and T. laxiflora (Plate 2; K & L), the bundle sheath was in a continuous ring while in the others in separate patches.

T. catappa (Plate 2; I & J / Plate 5; I & J) and T. laxiflora (Plate 2; K & L / Plate 5; K & L) are more or less similar to each other in the leaf and petiole anatomical characters, but we can differentiate between them by the shape of the main vascular bundle in the leaf which takes a cat face shape in T. catappa (Plate 5; I & J) and an open mouth shape in T. laxiflora (Plate 5; K & L). In addition, trichomes and lenticels in petiole was absent in T. laxiflora but present in T. catappa.

The petiole outline of Terminalia species shows a great variation. We can recognize a triangular shape in T. muelleri (Plate 3; O & P), T. bentzoe (Plate 1; E & F), T. catappa (Plate 2; I & J) and T. laxiflora (Plate 2; K & L); but an ovate shape in T. arjuna (Plate 1; A & B), T. brownii (Plate 2; G & H) and T. myriocarpa (Plate 3; Q & R).

T. arjuna (Plate 1; A & B / Plate 4; A & B) and T. myriocarpa (Plate 3; Q & R / Plate 6; Q & R) are more or less similar in their leaf and petiole anatomical characters, however, we can differentiate between them by the presence of vascular bundle in the small veins in leaf and the hypoderms in petiole in T. arjuna and their absence in T. myriocarpa.
Conclusion

The anatomical characteristics of the petiole and leaf described in this study are quite significant in the generic delimitation and differentiation of the studied members within genus *Terminalia*.

Conflict of interests: The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Authors contribution: N.W., R.H. and H.M. conceived of the presented idea. N.W. and R.H. developed the theory. R.H. and H.M. performed the measurements and processed the experimental data. A.S. performed the computations and verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

Ethical approval: Not applicable.

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*Egypt. J. Bot.* 60, No. 3 (2020)


Egypt J. Bot. 60, No.3 (2020)


ANATOMICAL STUDIES ON THE GENUS TERMINALIA L. (COMBRETACEAE) IN EGYPT

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