RESEARCH ARTICLE

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Cladistic analysis of some taxa in Malvaceae s.l. “Core Malvales” based on anatomical characteristics

ABSTRACT:
Malvaceae s.l. “Core Malvales” in their broad circumscription include members of four traditional families; Malvaceae s.s., Bombacaceae, Sterculiaceae and Tiliaceae. The aim of the present study is to evaluate the use of stem, petiole and lamina anatomical characters in analyzing the phylogenetic relationships among 39 taxa from Malvaceae s.l. in addition to one representative of Elaeocarpaceae as an outgroup using the parsimony analysis upgraded in WinClada and TNT cladistics programs. The anatomy in cross section of stem, petiole and lamina of the 40 studied taxa were examined by light microscope and photos were taken. Certain anatomical synapomorphic diagnostic characters has great taxonomic values which in turn were used for analyzing the phylogenetic relationships between the studied taxa. Such as petiole outline, vascular system, cuticle thickness, diverse shapes of secondary phloem fibers, presence and types of crystals and trichomes, gossypol gland, resin ducts, tanniferous cells, star-shaped idioblasts, vessels size and distribution, anomalous xylem structure and extrafloral nectaries. The present results of the phylogenetic analysis based on 44 anatomical characters and 111-character states strongly support the monophyly of family Malvaceae s.s. while, the other three families are para- or polyphyletic. It shows, also the graduation of the taxa under study in relation to the outgroup; from Tiliaceae and Sterculiaceae are directly attached to the tree (ancestor) while taxa from Bombacaceae and Malvaceae are far away from the root of the tree with little deviation. This indicates that Tiliaceae is the most primitive family followed by Sterculiaceae, Bombacaceae ending recently by the most advanced family Malvaceae. The present results agree to some extent with APG IV classification in transferring Elaeocarpaceae to order Oxalidales instead of Malvales also agree with other worker in transferring Gossypium and Lagunaria in family Sterculiaceae while transferring Dombeya to family Tiliaceae. For more accurate assignment of Malvaceae s.l. still more studies on many taxa are needed.

KEY WORDS:
Anatomy, Bombacaceae, Cladistics, Elaeocarpaceae, Malvaceae, Phylogeny, Sterculiaceae, Tiliaceae.

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INTRODUCTION:
The Malvaceae sensu APG II (2003) corresponds to the four traditional families; Malvaceae, Bombacaceae, Sterculiaceae and Tiliaceae. They form a natural grouping – the “Core Malvales”. In the distant past, most genera of Elaeocarpaceae were classified within Tiliaceae (Engler, 1890) but now are not considered for membership of Malvaceae sensu APG. From the previous studies on Core Malvales “Malvaceae s.l.”, it was clear that the delimitation of the four families is problematic (Cronquist, 1981; Edlin, 1935). Certain genera have been transferred between these families (Kelman, 1991;
Nilsson and Robyns, 1986; Robyns et al., 1977).

Most of the systematists knowing the close relationship between these four families and placing them within a single order “Malvales”, only Hutchinson (1973) differed placing the three families (Tiliaceae, Sterculiaceae and Bombacaceae) in order “Tiliales” while placing Malvaceae in a separate order “Malvales” (Table 1).

Table 1. Systematic position of Malvaceae according to different systems of classifications.

<table>
<thead>
<tr>
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<td>Rosopsida</td>
<td>Magnoliopsida</td>
<td>Rosopsida</td>
<td>Magnoliopsida</td>
<td>Eurosids I</td>
<td>Eurosids II</td>
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<td>Magnoliidae</td>
<td>Rosopsida</td>
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<td>Family</td>
<td>Malvaceae Juss.</td>
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<td></td>
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</table>

Family Malvaceae s.l. “Core Malvales” is a large group, containing about 2330 species of trees and herbs and many tropical to temperate species. It has long been known to be of great economic importance, for the cotton as well as its vegetables, horticultural members and for medicinal applications (Thorne, 1992).

In modern systems of classification, the sequence of four families from the most primitive to the most advanced are as follows: Tiliaceae to Sterculiaceae then Bombacaceae to Malvaceae (Cronquist, 1981 & 1988; Takhtajan, 1980 & 1987). While Warming (1895) and Rao (1952) have considered the Sterculiaceae to be the most primitive.

Several authors treated Sterculiaceae as a separate family (Bentham and Hooker, 1862; Schumann, 1890; Edlin, 1935; Melchior, 1964; Cronquist, 1988; Dahlgren, 1989; Takhtajan, 1997). Within the order Malvales, Judd and Manchster (1997), Bayer et al. (1999), Bayer and Kubitzki (2003), and APG II (2003) merged Sterculiaceae, Tiliaceae, Bombacaceae with Malvaceae and subdivided the enlarged family Malvaceae into nine subfamilies; Bombacoideae, Brownlowioideae, Byttnerioideae, Dombeyoideae, Grewioideae, Heliceroideae, Malvoideae, Sterculioideae and Tilioideae, based on molecular, morphological and biogeographical data.

There are many contradictory hypotheses about the phylogenetic relationships between the families within Core Malvales. Some traditional view suggests that, these four families constitute a monophyletic group. However, it also suggests that only the Malvaceae are monophyletic. Bombacaceae usually have been allied with Malvaceae (Bentham and Hooker, 1862; Cronquist, 1988; Rendle, 1925; Thorne, 1992). Although Hutchinson (1973) considered this family closely related to Tiliaceae and Sterculiaceae.

The main objective of this study was to clarify the phylogenetic relationships as possible between the four families “Core Malvales” through cladistic analysis based on anatomical characteristics.

MATERIAL AND METHODS:

In the present study, 39 selected species belonging to 24 genera of family Malvaceae s.l. “Core Malvales” (i.e. Tiliaceae, Sterculiaceae, Bombacaceae, Malvaceae) along with one species representative to family Elaeocarpaceae as outgroup (mostly related to Core Malvales as a sister group) were collected from different localities in Egypt. A list of species studied with their localities and data of collection is given in (Table 2).

Table 2. List of the studied taxa, their location and date of collection.

<table>
<thead>
<tr>
<th>Families s.s.</th>
<th>No.</th>
<th>Taxa</th>
<th>Localities</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvaceae</td>
<td>1</td>
<td>Abelmoschus esculentus (L.) Moench</td>
<td>1, 13</td>
<td>June, September, 2014, 2015</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Abutilon hirtum (Lam.) Sweet</td>
<td>1, 9</td>
<td>March, February, 2014</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Hibiscus acutifolia Welw. ex Hiern</td>
<td>1</td>
<td>November, 2015</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Hibiscus mutabilis L.</td>
<td>1</td>
<td>December, November, 2014, 2015</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Hibiscus pedunculatus L.</td>
<td>1</td>
<td>November, 2015</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Hibiscus rosa-sinensis L.</td>
<td>9</td>
<td>May, February, 2014</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>*Hibiscus sabdariffa L.</td>
<td>1, 13</td>
<td>June, October, 2014, 2015</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Hibiscus tiliaeus L.</td>
<td>1, 3</td>
<td>November, April, 2015</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Lagunaria patersonii (Andrews) G.Don</td>
<td>1</td>
<td>June, 2015</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>*Malva parviflora L.</td>
<td>9, 14</td>
<td>February, March, 2014</td>
</tr>
</tbody>
</table>
The specimens identified according to Täckholm (1974), Boulos (2000) for wild species and Bailey and Bailey (1976) and Kubitzki (2003) for cultivated species. The collected species were prepared as herbarium specimens and deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University.


To study the anatomical characteristics, cross sections of the stem, petiole and lamina were made using hand microtome at 13-16µm., then double stained with safranine and light green, mounted in Canada Balsam according to Johanson (1940) method. The sections were examined using OPTIKA B-150 light microscope and the photos were taken using Nikon coolpix p600 (60x) Digital Camera.

The specimens identified according to Täckholm (1974), Boulos (2000) for wild species and Bailey and Bailey (1976) and Kubitzki (2003) for cultivated species. The collected species were prepared as herbarium specimens and deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University.

Most parsimonious tree were estimated using WinClada, the shortest tree was condensed and summarized by a strict consensus tree. The data were conducted by using heuristic searches and tree bisection recognition (TBR) branch swapping algorithm. Some other characters were studied but finally excluded from the analysis because of the high instance of polymorphism.

Characters variable with taxa were coded as polymorphic ($), and no attempts to determine the plesiomorphic (ancestral) state within the genus were made, characters with inapplicable states were coded using the reductive coding strategy as recommended by Strong and Lipscomb, (1999). Multistate characters were treated as unordered to avoid false statements on character polarity. Nearly all characters that we selected were readily divisible into discrete states, thus avoiding arbitrary decisions relating to state delimitation (Stevens, 1991).

The character states changes were optimized on the cladogram by making ACCTRAN test (ACCELERATES THE TRANSFORMATION OF CHARACTERS ON TREE) (Farris optimization). Reliability of clades was assessed by bootstrapping (using 1000 replicates addition) (Felsenstein, 1985). Bootstrapping was used to estimate statistic support for individual nodes. Numbers of < 50% of bootstrapping values mean uniform support (Berry and Gaseuel, 1996).

RESULTS AND DISCUSSION:

The obtained anatomical characteristics of the studied taxa were summarized as follows:

I. **Stem characteristics**: (Tables 3 & 4 and Plates I - IX)

![Fig. 1. Thin in Malvaviscus arboreus var. penduliflorus (X = 40).](image1)

![Fig. 2. Thick in Pseudobombax ellipticum (X = 40).](image2)

![Fig. 3. Very thick in Pachira aquatica (X = 40).](image3)

Plate I. Cuticle thickness

![Plate II. Types of crystals](image4)

![Fig. 1. Solitary in Elaeocarpus angustifolius (X = 40).](image5)

![Fig. 2. Druses in Abelmoschus esculentus (X = 40).](image6)

![Plate III. Secretory glands and ducts](image7)

![Fig. 1. Secretory glands (gossypol) of Gossypium barbadense (X = 40).](image8)

![Fig. 2. Resin duct of Pterygota alata (X = 40).](image9)

![Fig. 3. Gum duct of Gossypium barbadense (X = 40).](image10)

![Fig. 4. Extra-floral nectaries of Pachira aquatica (X = 40).](image11)

![Fig. 5. Mucilagenous cavity of Abelmoschus esculentus (X = 40).](image12)
Fig. 1. Unicellular unbranched in *Hibiscus pedunculatus* (X = 40).

Fig. 2. Unicellular branched in *Hibiscus pedunculatus* (X = 40).

Fig. 3. Multicellular unbranched in *Firmiana simplex* (X = 40).

Fig. 4. Multicellular glandular in *Pterospermum acerifolium* (X = 40).

Fig. 5. Stellate in *Hibiscus mutabilis* (X = 40).

Fig. 6. Capitate in *Hibiscus mutabilis* (X = 40).

Fig. 7. Peltate in *Lagunaria patersonii* (X = 40).

Fig. 8. T-shaped in *Thespesia populnea* (X = 40).

Plate IV. Types of trichomes

Fig. 1. Two types (angular collenchyma, and polyhedral parenchyma with mucilagenous cavities) in *Brachychiton australis* (X = 40).

Fig. 2. Three types (chlorenchyma, angular collenchyma, and polyhedral parenchyma with mucilagenous cavities) in *Malva parviflora* (X = 40).

Plate V. Types of cortical cells

Fig. 1. Circular in *Hibiscus mutabilis* (X = 4).

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Fig. 2. Ovate in *Lagunaria patersonii* (X = 4).

Fig. 3. Concave elliptic in *Firmiana simplex* (X = 4).

Fig. 4. Angled in *Pachira aquatica* (X = 4).

Fig. 5. Irregular in *Brachychiton populneus* (X = 4).

Plate VI. Types of stem outline

Fig. 1. Hollow and homogenous in *Corchorus olitorius* (X = 4).

Fig. 2. Solid and heterogenous in *Elaeocarpus angustifolius* (X = 4).

Plate VII. Types of pith

Fig. 1. Large in *Ceiba pentandra* (X = 10).

Fig. 2. Medium in *Grewia occidentalis* (X = 10).

Fig. 3. Moderately small in *Abelmoschus esculentus* (X = 10).

Fig. 4. Very small in *Alcea rosea* (X = 10).

Plate VIII. Different vessel size

Fig. 1. Solitary in *Sterculia foetida* (X = 10).
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**A. The epidermis:**
1. Cell arrangement: either radially arranged in 22 taxa or tangentially elongated in the remainder 18 taxa.
2. Shape of epidermal cells: normal in 17 taxa and papillose in eight taxa while of two types in 15 taxa.
3. Cuticle thickness: mostly between thin in 19 taxa to thick in ten taxa while is very thick in 11 taxa.
4. Trichomes: present in 30 taxa while absent in the remainder ten taxa.
5. Types of trichomes: each species has more than one type of trichomes. It was varied from unicellular, unbranched or branched, nonglandular - stellate - peltate - T-shaped - multicellular unbranched, nonglandular - multicellular, glandular capitate or globose.
6. Mucilaginous cavity: present in all the studied 39 taxa except for the outgroup (Elaeocarpus).
7. Types of crystals: solitary only in Elaeocarpus, while druses in all Malvaceae s.s. species beside Pseudobombax and mixed (druses and solitary together) or having polymorphism in the remainder taxa.
8. Shape of stem in transverse section: circular in 29 taxa, ovate in Elaeocarpus, Hibiscus rosa-sinensis, Lagunaria, Dombeya wallichii, Pterygota, angled in Gossypium, Hibiscus tiliaeus, Pachira, while irregular in Brachychiton australis, Brachychiton populneus, and elliptic in Firmiana.
10. Lenticels: present in 11 taxa and absent in the remainder 29 studied taxa.

**B. The cortex:**
1. Gossypol: present in Gossypium and Thespesia and absent in all the other studied taxa.
2. Gum: present in 28 taxa and absent in the remainder 12 taxa.
4. Resin ducts: present in 23 taxa and absent in the remainder 17 taxa.
5. Types of cortex: composed of two types of cells in 15 taxa while of three types of cells (mostly chlorenchyma, collenchyma; either angular or lacunar and polyhedral parenchyma) in the remainder 25 taxa.

**C. The vascular system of the stem:**
1. Secondary phloem fibers: present in 32 taxa while absent in eight taxa only.
2. Types of secondary phloem fibers: either scattered in eight taxa, patches in 14 taxa or strands in Hibiscus rosa-sinensis, and Sterculia foetida while polymorphic in eight taxa.
3. Phloem ducts: present in 18 taxa while absent in the remainder 22 taxa.
4. Cambium: one type of cells in Abutilon, Alcea rosea, Hibiscus acetosella, and Malva while of two types of cells in the remainder 36 taxa.
5. Shape of xylem vessel: angled in 23 taxa while rounded in the remainder 17 taxa.
6. Vessel size: large in 13 taxa (mostly Bombacaceae and Sterculiaceae), medium in ten taxa, moderately small in 11 taxa, while very small in the remainder six taxa.
7. Distribution of vessels: either solitary in Sterculia foetida, cluster of 2-3 in nine taxa, or radial multiples of four or more in 20 taxa, while polymorphic in the remainder ten taxa.
8. Tylosis: present in 11 taxa while absent in 29 taxa.
9. Arrangement of axial wood parenchyma: paratracheal in 11 taxa
or apotracheal in 13 taxa while polymorphic in the remainder 16 taxa.

10. Anomalous structure of vessels: present in *Brachychiton rupestris* only while absent in all the remainder taxa.

11. Rays: either uniseriate in *Grewia occidentalis*, multiseriate in 32 taxa, while polymorphic (uni-, bi-, and multiseriate) in *Hibiscus sabdariffa*, *Firmiana*, *Grewia asiatica*, and absent in four remainder taxa.

12. Tile cells: present in 18 taxa while absent in 22 taxa.

D. The pith:

1. Pith section: solid in all the studied taxa except in *Brachychiton rupestris*, and *Corchorus* is hollow.

2. Pith type: homogenous in all the studied taxa except the *Elaeocarpus* show heterogenous pith of angular collenchyma and polyhedral parenchyma.

II. Petiole characteristics: (Tables 3 & 4 and Plates X - XI)

1. Tanniferous cells: present in 22 taxa while absent in the remainder 18 taxa.

2. Vascular system:
   - Most of the Malvaceae taxa either separated vascular bundles or separated but closely related except in *Gossypium* shows complete vascular cylinder. While in *Hibiscus tiliaceus*, *Lagunaria* shows incomplete vascular cylinder.
   - In Bombacaceae, most of them show complete vascular cylinder except *Pseudobombax*, incomplete vascular cylinder.
   - Most of Sterculiaceae taxa vary from complete – incomplete vascular cylinder or separated vascular cylinder, only *Dombeya* spp. show Malvalean characters, that having separated vascular bundles.
   - Tiliaceae vary from separated vascular bundles in *Corchorus* to vascular cylinder in *Grewia*, either complete or in complete.

3. Medullary bundles: present in ten taxa and absent in the remainder 30 taxa.

4. Types of medullary bundles: amphivasal in *Ceiba pentandra*, *Brachychiton acerifolius*, *Sterculia foetida* or amphicerebral in *Pterygota*, *Grewia asiatica* or collateral in *Pachira* and polymorphic in *Elaeocarpus*, *Hibiscus tiliaceus*, *Lagunaria*, *Bombax ceiba*.

5. Accessory strands: only *Elaeocarpus* (out group) having these bundles while absent in all the studied taxa.

III. Lamina characteristics: (Tables 3 & 4 and Plates XII- XV)

- **Fig. 1.** Elongated in *Hibiscus acetosella* (X = 40).
- **Fig. 2.** Cubic in *Malva parviflora* (X = 40).
- **Plate X. Shapes of palisade cells**
- **Fig. 1.** L.s. of tile cells of *Grewia asiatica* (X = 10).
- **Fig. 2.** L.s. of tile cells of *Grewia asiatica* (X = 40).
- **Fig. 3.** Star-shaped idioblasts and bulliform cells in the wing of lamina of *Abelmoschus esculentus* (X = 40).
Plate XI. Tile cells, star-shape idioblasts, and tanniferous cells

Fig. 4. Tanniferous cells in *Hibiscus sabdariffa* (X = 40).

Plate XII. Different shapes of midrib transverse section

Fig. 1. Complete in *Pachira aquatica* (X = 10).

Fig. 2. Arc-shaped in *Alcea rosea* (X = 10).

Fig. 3. Incomplete in *Elaeocarpus angustifolius* (X = 10).

Plate XIII. Different shapes of midrib vascular cylinder

A. Wings:
1. Bulliform cells in adaxial surface: present in 30 taxa while absent in the remainder 10 taxa.
2. Spongy tissue: present in 28 taxa and absent in 12 taxa.
3. Shape of palisade cells: cubic in eight taxa while elongated in the remainder 32 taxa.
4. Star-shaped idioblasts: most common in all Malvaceae taxa except *Gossypium, Lagunaria, Pavonia,* and *Thespesia.* Also, absent in all studied taxa in other families and in the out group.
5. Shape of lamina in cross section: ribbon in *Brachychiton populneus, Brachychiton rupestris;* half-circle in four taxa; oval in *Pterygota* while biconvex in the remainder 33 taxa.

B. Midrib:
1. Midrib center: hollow in *Pachira* while solid in the remainder 39 studied taxa.
2. Vascular cylinder shape: complete vascular cylinder in *Pachira* only, arc-shaped in 24 taxa while incomplete vascular cylinder in the remainder 15 taxa.
3. Medullary bundles: present in 23 taxa and absent in the remainder 17 taxa.
4. Types of medullary bundles: amphivasal in six taxa, amphicerebral in ten taxa, collateral in *Brachychiton australis* while polymorphic in the remainder six taxa.

**C. Extrafloral nectaries:** present in 16 taxa while absent in the remainder 24 taxa.

Anatomical features are more conservative than morphological features, therefore, useful for taxonomic study. Anatomy along with morphology always treated as backbone of plant taxonomy and systematists elucidated the phylogeny and evolution from these criteria (Endress *et al.*, 2000). Also, anatomy is providing evidence concerning the interrelationships of larger groups such as families or helping to establish the real affinities of genera of uncertain taxonomic status (Metcalfe and Chalk, 1950). This opinion obviously clear in our results.

Table 3. Anatomical characters and character states with their codes used in cladistic analysis of Core Malvales

<table>
<thead>
<tr>
<th>Characters</th>
<th>Character states and their codes</th>
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<tbody>
<tr>
<td>Cell arrangement</td>
<td>Radial (0) Tangential (1)</td>
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<tr>
<td>Shape of cells</td>
<td>Normal (0) Papillose (1)</td>
</tr>
<tr>
<td>Cuticle thickness</td>
<td>Thin (0) Thick (1) Very thick (2)</td>
</tr>
<tr>
<td>Trichomes</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Types of trichomes</td>
<td>Unicellular unbranched non-glandular (0) Unicellular branched non-glandular (1) Stellate (2) Peltate (3) T-shaped (4) Multicellular unbranched non-glandular (5) Multicellular capitate glandular (6) Multicellular globose glandular (7)</td>
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<td>Mucilagenous cavities</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Types of crystals</td>
<td>Solitary (0) Druses (1)</td>
</tr>
<tr>
<td>Shape of transverse section</td>
<td>Circular (0) Oval (1) Elliptic (2) Angled (3) Irregular (4)</td>
</tr>
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<td>Periderm</td>
<td>Absent (0) Present (1)</td>
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<td>Lenticels</td>
<td>Absent (0) Present (1)</td>
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<td>Secretery glands (gossypol)</td>
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<td>Gum</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Stone cells</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Resin ducts</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Types of cortex</td>
<td>Two types (0) Three types (1)</td>
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<td>Secondary phloem fibers</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Type of secondary phloem fibers</td>
<td>Scattered (0) Patches (1) Strands (2)</td>
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<td>Phloem ducts</td>
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<td>Shape of xylem vessel</td>
<td>Angled (0) Rounded (1)</td>
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<td>Vessel size</td>
<td>Large (0) Medium (1) Moderately small (2) Very small (3)</td>
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<tr>
<td>Distribution of vessels</td>
<td>Solitary (0) Cluster of 2-3 (1) Radial multiples of 4 or more (2)</td>
</tr>
<tr>
<td>Tylosis</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Arrangement of axial wood parenchyma</td>
<td>Paratracheal (0) Apotracheal (1)</td>
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<td>Anomalous structure of vessel parenchyma</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Rays</td>
<td>Uniseriate (0) Biseriate (1) Multiseriate (2)</td>
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<tr>
<td>Tile cells</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Pith section</td>
<td>Hollow (0) Solid (1)</td>
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<tr>
<td>Type of pith</td>
<td>Homogenous (0) Heterogenous (1)</td>
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<tr>
<td>Tanniferous cells</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Separated vascular cylinders (0) Complete vascular cylinder (1) Incomplete vascular cylinder (2) Separated but closely related vascular bundles (3) Separated vascular bundles (4)</td>
</tr>
<tr>
<td>Medullary bundles</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Type of medullary bundles</td>
<td>Amphivasal (0) Amphicerebral (1) Collateral (2)</td>
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<tr>
<td>Accessory strands</td>
<td>Absent (0) Present (1)</td>
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<tr>
<td>Bulliform cells in adaxial surface</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Spongy tissue</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Shape of palisade cell</td>
<td>Cubic (0) Elongated (1)</td>
</tr>
<tr>
<td>Star-shaped idioblasts</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Shape of cross section</td>
<td>Flattened-ribbed (0) Half circle (1) Biconvex (2) Oval (3)</td>
</tr>
<tr>
<td>Midrib center</td>
<td>Hollow (0) Solid (1)</td>
</tr>
<tr>
<td>Vascular cylinder</td>
<td>Complete vascular cylinder (0) Arc-shaped (1) Incomplete vascular cylinder (2)</td>
</tr>
<tr>
<td>Medullary bundles</td>
<td>Absent (0) Present (1)</td>
</tr>
<tr>
<td>Type of medullary bundles</td>
<td>Amphivasal (0) Amphicerebral (1) Collateral (2)</td>
</tr>
<tr>
<td>Extra-floral nectaries</td>
<td>Absent (0) Present (1)</td>
</tr>
</tbody>
</table>
Phylogenetic Analysis Based on Anatomical Characters:

For cladistic analysis to the obtained anatomical data (44 characters and 111-character states) (Table 3 & Fig. 1), by using (TNT) programme, the equally weighted parsimony analysis yield ten anatomical trees (cladograms). To choose the most parsimonious tree we make strict consensus tree which have a shorter length (L = 234), consistency index (CI = 0.28) and retention index (RI = 0.64), while homoplasy index (HI = 0.72). Only three nodes are collapsed in this tree; two of them are in one clade containing family Sterculiaceae and Bombacaceae while the last one in clade Malvaceae (Figs 1 & 2).

<table>
<thead>
<tr>
<th>No.</th>
<th>Taxa</th>
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<tbody>
<tr>
<td>6</td>
<td>Elaeocarpus angustifolius (Out group)</td>
</tr>
<tr>
<td>1</td>
<td>Abellmosus esculentus</td>
</tr>
<tr>
<td>2</td>
<td>Abellion hirtum</td>
</tr>
<tr>
<td>3</td>
<td>Alca rosea</td>
</tr>
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<td>4</td>
<td>Gossypium barbadense</td>
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<td>5</td>
<td>Hibiscus acetosella</td>
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<td>6</td>
<td>Hibiscus mutabilis</td>
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<td>7</td>
<td>Hibiscus pedunculatus</td>
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<td>8</td>
<td>Hibiscus rosa-sinensis</td>
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<td>9</td>
<td>Hibiscus sabdariffa</td>
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<td>Lagunaria patersoni</td>
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<td>Malva parviflora</td>
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<td>15</td>
<td>Malvaviscus arboreus var. drummondii</td>
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<tr>
<td>16</td>
<td>Malvaviscus arboreus var. penduliferus</td>
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<td>Pavonia spinex</td>
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<td>Thespesia populnea</td>
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<td>Bombax ceiba</td>
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<td>Pseudobombax ellipticum</td>
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<td>Brachychiton acerifolium</td>
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<td>Brachychiton rupestris</td>
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<td>Dombeya burgessiae</td>
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<td>Dombeya tiliace</td>
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<td>Dombeya wallichii</td>
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<td>Guazuma ulmifolia</td>
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<td>Ptygea alata</td>
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<td>Sterculia foetida</td>
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<td>37</td>
<td>Corchorus olitorius</td>
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<tr>
<td>38</td>
<td>Grewia asiatica</td>
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<tr>
<td>39</td>
<td>Grewia occultalis</td>
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</tbody>
</table>

Table 4. Character values for Core Malvaceae taxa used in cladistics analysis (Data Matrix)
Fig. 1. Strict consensus tree (cladogram) based on 44 anatomical characters and 111 character states of the studied taxa of Core Malvales. Numbers above the branches represent character number, and those below the branch indicates number of character states, black circle shows homologous character, while white rectangular shows homoplasy character.
Ibrahim et al., Cladistic analysis of some taxa in Malvaceae s.l. “Core Malvales” based on anatomical characteristics

From resulted cladogram (Figs 1 & 3) we have a root common ancestor which is the out group (Elaeocarpus angustifolius), rooted in a separated clade and weak supported by ovate stem cross section, heterogenous pith consisting of angular collenchyma and polyhedral parenchyma, presence of medullary bundles and accessory strands in petiole, presence of solitary crystals, incomplete vascular cylinder in lamina, absence of mucilaginous cavities, trichomes, gossypol, resin ducts, secondary phloem fibre, tanniferous cells, star-shaped idioblasts; while the four families under study are connected in a large clade sharing the following characters: tangential epidermal cell arrangement, presence of mucilaginous cavities, druses crystals, and resin ducts, rounded xylem vessels, presence of tile cells, and bulliform cells at the lamina.
In this respect Cronquist (1988) noted that Elaeocarpaceae stand apart from the rest of the order Malvales, but even so the relationship is so close that they have often been included in Tiliaceae. He considered Elaeocarpaceae to be the most “archaic” family in the order due to the absence of all the diagnostic characters shared by the Core Malvalean families. Our results in agreement with this finding and support the view of APG IV (2016) in transferring Elaeocarpaceae to order Oxalidales.

We can see the graduation of the studied taxa among familial level in relation to the out group (root of the tree). We can clearly see that taxa belonging to families (Tiliaceae and Sterculiaceae) except some subclades are directly attached to the root of the tree (consider as primitive ancestor) while most taxa of Bombacaceae and all taxa of Malvaceae (except Gossypium and Lagunaria)
are found far away from the root of the tree. This indicates that Tiliaceae is the most primitive family among our studied families followed by Sterculiaceae, then Bombacaceae and ending with the most recent (advanced) family Malvaceae.

From the obtained cladogram (Fig. 1) five large clades and few small subclades supported evolutionary trend have been identified within the studied taxa of Malvaceae s.l.

As regards to family Tiliaceae we have two small separated clades; the first clade includes Corchorus olitorius in a separated clade attached with other family and strongly supported by characters of thick cuticle and separated petiolar vascular bundles while separated by hollow pith and absence of medullary bundles in the lamina, absence of bulliform cells.

The second clade includes two Grewia spp. (Grewioideae) strongly supported by uniseriate rays. Grewia asiatica separated by radially arranged epidermal cells and presence of petiolar medullary bundles. While Grewia occidentalis was separated by complete petiolar vascular cylinder, cubic palisade cells and amphivasal medullary bundles in lamina. So, it is clear that clade of Grewia is monophyletic while Corchorus clade is paraphyletic in relation to them.

Our results agree with the finding of Nurul-Aini et al. (2013) on some species of Malvaceae (Grewioideae) whom shown that, some common petiolar anatomical characters viz. petiole vasculature and outline, types of trichomes and crystals are useful and of taxonomic value in species delimitation.

In relation to family Sterculiaceae, the resulted cladogram revealed the presence of three large clades (Dombeyaspp. clade), (Brachychiton spp. and Sterculia clade), (Brachychiton spp. and Pterospermum clade), and three taxa (Guazuma, Firmiana, Pterygota) in three separated small subclades.

In case of the first large three Dombeya spp. clade (Dombeyoideae): it is strongly supported by presence of gum, spongy tissue, and cubic palisade cells. Our results cleared that tribe Dombeyaeae from Sterculiaceae is nested together within Tiliaceae. This indicates that Dombeya is more closely related to core Dombeyoidae than core Sterculioidae. Dombeya tiliae supported by absence of resin ducts, presence of tanniferous cells, Dombeya burgessiae supported by presence of trichomes while Dombeya wallichii supported by ovate stem, cortex of three types of cells, and elongated palisade cells. These taxa of Dombeya moved between the two families; Sterculiaceae and Tiliaceae.

Guazuma: appeared in separated small subclade and weakly supported by cubic palisade cells and presence of lamina medullary bundles, while connected to large clade with other family and supported by stellate trichomes, cortex of three types of cells, presence of phloem ducts, xylem vessels clusters of 2-3 and presence of extrafloral nectaries.

Firmiana: appeared in separated small subclade and strongly supported by absence of gum and resin ducts, cortex of two types of cells, absence of phloem ducts and spongy tissue. While linked with the clades of Bombacoideae and Sterculioideae) through radially arranged epidermal cells, patches of secondary phloem fibre, large xylem vessels, and incomplete vascular cylinder in lamina.

Pterygota: also connected to the same clade and strongly supported by ovate stem, absence of extrafloral nectaries and separated alone by presence of periderm, gum and phloem ducts, complete vascular cylinder of the petiole and oval midrib shape.

The second large clade which includes three taxa; Brachychiton discolor separated alone and strongly supported by circular stem and presence of bulliform cells while B. acerifolius connected with Sterculia foetida by presence of trichomes, stone cells, spongy tissue and cubic palisade cells while Sterculia supported by tangentially elongated epidermal cells, presence of periderm, lenticels and solitary xylem vessels distribution, presence of secondary phloem fibre strands.

The third large clade of Sterculiaceae nested with family Bombacaceae and supported by these characters: multicellular unbranched non-glandular trichomes, medium sized and angled xylem vessels, presence of tile cells, absence of petiole medullary bundles, half-circle midrib shape. The clade includes: Pterospermum sp. separated in one subclade and strongly supported by presence of multicellular glandular trichomes, and periderm, absence of gum and bulliform cells, presence of medium size vessels and extrafloral nectaries.

The second subclade includes Brachychiton spp. and weakly supported by absence of tile cells, flatted ribbon-shaped midrib cross section, amphiclereral medullary bundles in midrib. Brachychiton rupestris separated by having anomalous structure in xylem and hollow pith. The two other Brachychiton australis and B. populneus connected by irregular stem cross section and separated vascular cylinder in the petiole. While Brachychiton australis separated than B. populneus by having stone cells, spongy tissue, biconvex shape of lamina and with collateral medullary bundles.

We must note that the last two subclades of Brachychiton are poorly resolved.
due to the weak support of boot strapping. Brizicky (1966) suggested that family Sterculiaceae is the most primitive between Elaeocarpaceae, Tiliaceae, Bombacaceae and Malvaceae. Also, Hooker (1874) regarded that tribe Sterculieae as the most primitive while Malvatheca is the most advanced tribe. Our results cleared that clade Dombeyoideae is the most primitive than Sterculioideae, so supported their placement to family Tiliaceae.

When delimiting the four traditional families the sequence of progression from the primitive Tiliaceae through the intermediate Sterculiaceae and Bombacaceae to the most advanced Malvaceae (Cronquist, 1981 & 1988; Takhtajan, 1987 & 1997; Dahlgren, 1989; Thorne, 1992; Judd et al., 1994; La Duke and Doebly, 1995; Judd and Manchester, 1997). While Warming (1895) and Rao (1952) deviated by considering the Sterculiaceae to be the basal group. The present results agree with the first opinion with small deviation that (Grewioideae + Dombeyoideae) are the basal group and more primitive. Also, our results indicate that Sterculiaceae is paraphyletic or polyphyletic.

Baum et al. (1998 & 2004) and Alversen et al. (1999) revealed that nine well supported evolutionary lineages have been identified within Malvaceae s.l., one of which was named (Malvatheca). This lineage consists of the two well supported large clades; clade Malvoideae (which includes Malvaceae s.s.) and clade Bombacoideae (corresponding to the taxa of Bombacaceae). From our results and in case of family Bombacaceae, we can see that all taxa collected in one clade (Bombacoideae) except Pachira is separated in subclade with malvaceous and sterculiean taxa. It is strongly supported by absence of trichomes, angled stem cross section, presence of tile cells, collateral medullar bundles in the petiole, presence of bulliform cells, hollow midrib, complete vascular cylinder and amphivasal medullary bundles in the lamina.

The other clade including Bombacaceae (Bombacoideae) within Sterculiaceae supported by absence of trichomes, cortex of two types of cells, rounded xylem vessels. It includes Pseudobombax, Bombax and 2 species of Ceiba. Only Pseudobombax from this group separated in small subclade alone by having spongy tissue and absence of tanniferous cells. It is connected to some Sterculiaceae taxa by angled medium size xylem vessels, absence of petiolar medullary bundles and half-circle midrib. So, the results obviously cleared that tribe Bombacaceae is nested within Sterculiaceae as the sister to it. The rest Bombacaceae taxa (Bombax and two Ceiba sp.) are nested in one suboclade supported by thin cuticle, cortex of three types of cells, presence of tylosis and tile cells.

Ceiba speciosa separated alone by having tangential elongated epidermal cells, absence of petiolar medullary bundles while Bombax and Ceiba pentandra are joined by presence of tanniferous cells and spongy tissue. Bombax can be separated by presence of trichomes and stone cells, while Ceiba pentandra characterized by presence of gum, extrafloral nectaries and angled xylem vessels. The results cleared that family Bombacaceae is paraphyletic.

Finally, with respect to family Malvaceae s.s. all taxa grouped in one large clade (Malvoideae) and strongly supported by presence of separated but closely related vascular bundles in the petiole. Only two taxa are separated and nested in other family (Sterculiaceae) clade, they are Gossypium and Lagunaria, sharing by papillose epidermal cells, T-shaped trichomes, absence of tile cells, elliptic stem and absence of bulliform cells.

Gossypium can be separated by having capitate and globose trichomes, angled stem cross section, presence of gossypol gland, rounded xylem vessels, moderately small, parastracheal parenchyma and complete petiolar vascular cylinder.

Presence of gossypol consider synapomorphy character for distinctive tribe Gossypiaceae which has been considered transitional between Bombacaceae and Malvaceae (Cronquist, 1981). The present results support this opinion. While Lagunaria can be separated by having moderately small vessels, cubic palisade cells and half-circle lamina.

In the clade Malvoideae: Thespesia separated alone in clade and weakly supported by radial epidermal cells, presence of gossypol gland, cortex of two types of cells, absence of tanniferous cells, separated vascular bundles in the petiole, while the rest of taxa among Malvaceae remain in one clade and supported by moderately small vessels, radial multiples of 4 or more vessel distribution and absence of lamina medullary bundles.

We see Abelmoschus separated by branched trichomes, absence of resin ducts, tile cells and presence of tylosis. There are two large subclades; one including five Hibiscus species (H. sabdariffa, H. schizopetalus, H. syriacus, H. rosa-sinensis, H. tiliaceus) sharing in the absence of phloem ducts. The other large sub-clade includes the remaining studied taxa among Malvaceae sharing in absence of tanniferous cells.

In the first subclade, Hibiscus sabdariffa can be separated by absence of secondary phloem fibre and uniseriate rays. Hibiscus syriacus separated by absence of tanniferous cells. Hibiscus rosa-sinensis separated by presence of secondary phloem fibre strands while H. tiliaceus having angled stem cross

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section and rounded xylem vessels, incomplete petiolar vascular cylinder, absence of bulliform cells, presence of petiolar medullary bundles and half-circle lamina cross section.

The second large subclade, *Hibiscus mutabilis* is separated by presence of lamina medullary bundles, *H. acetosella* by absence of phloem fibre and one type of cambial cells and *H. pedunculatus* was separated by thin cuticle, absence of gum and secondary phloem fibre patches.

There are another small subclades includes (*Pavonia*, two varieties of *Malvaviscus*, *Abutilon*, *Alcea*, and *Malva*), weakly supported by small vessel size, absence of tylosis, paratracheal parenchyma, and absence of extrafloral nectaries. *Pavonia* and two *Malvaviscus* varieties are included together by radially epidermal cells, angled xylem vessels, presence of tile cells. *Pavonia* can be separated by thick cuticle, absence of gum and star-shaped idioblasts, presence of stone cells, resin ducts and tanniferous cells. While *Malvaviscus* subclade is supported by secondary phloem fibre patches.

Finally, we can see (*Abutilon*, *Alcea*, and *Malva*) included in one subclade and weakly supported by one type of cambial cells, cluster of 2-3 xylem vessels, and absence of bulliform cells. *Abutilon* can be separated by absence of gum, and radial multiples of 4 or more xylem vessels, *Alcea*, by thin cuticle, and presence of bulliform cells while *Malva* by absence of secondary phloem fibres, cubic palisade cells and arc-shape lamina vascular cylinder.

From cladistic results, we can see that family Malvaceae is homogenous and mainly monophyletic except *Gossypium* and *Lagunaria*, in comparison with the other families under study, and these results agree with the other advanced previous results. While *Tiliaceae*, *Sterculiaceae* and *Bombacaceae* are paraphyletic or polyphyletic. This result in accordance with Judd and Manchester (1997), Alverson *et al.* (1998), Bayer (1999), and Bayer *et al.* (1999).

In conclusion, the present study cleared that, Malvaceae is redefined to refer to the most recent and advanced common ancestor of plants considered to be *Tiliaceae*, *Sterculiaceae*, *Bombacaceae* and *Malvaceae*. Our cladogram based on the anatomical characteristics shows, the root common ancestor which is the out group (*Elaeocarpus angustifolius*), we can see the graduation of the studied taxa among familial level in relation to the out group (root of the tree); from *Tiliaceae* and *Sterculiaceae* families are directly attached to the root of the tree (primitive ancestor), while taxa from *Bombacaceae* and *Malvaceae* are far away from the root of the tree. This indicates that, *Tiliaceae* is the most primitive family among the other studied families. Then, we have *Sterculiaceae* followed by *Bombacaceae*, ending recently by the most advanced family *Malvaceae*.

The present results showed also that most of the anatomical characters which may be synapomorphic and have some taxonomic value for taxa delimitation are; cuticle thickness, diverse shapes of secondary phloem fibre in the stem, presence and types of crystals, trichomes, petiolar anatomical vascular system, presence of gossypol gland, resin ducts, tanniferous, idioblast and tile cells, vessel size and distribution, anomalous xylem structure and extrafloral nectaries.

The present study present additional criteria for the phylogenetic relationship between the four traditional families and for more accurate assignment of Malvaceae s.l., still more studies on a large number of taxa are needed. Also there are still some relationships between some taxa need to be clarified.

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