Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Macro- and microscopical characterization of the stem and flowers of *Malvaviscus arboreus* Cav. (Malvaceae)

Omnia H. Abdelhafez[1], John R. Fahim[2*], Usama R. Abdelmohsen[1,2], Samar Y. Desoukey[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, New Minia 61111, Egypt

[2]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt

Received: May 27, 2020; revised: August 28, 2020; accepted: August 31, 2020

Abstract

Malvaviscus arboreus Cav. (Sleeping Hibiscus) is a species of flowering plants belonging to family Malvaceae with a natural distribution throughout Central and South America. However, this perennial, medium-sized shrub has been also widely cultivated in several other tropical and subtropical areas of the globe. Sleeping Hibiscus is characterized by an erect, cylindrical, and glabrous stem with monopodial branching, reaching up to 0.5–1 m in height and 0.2–1 cm in diameter. The stem carries simple, alternate, oval or ovate to broadly cordate leaves and red solitary flowers. Flowers of Sleeping Hibiscus are tube-shaped with totally wrapped petals that only slightly open at the top, carried on hairy, pendulous green pedicels. Besides their ornamental value, *M. arboreus* plants enjoy multiple folk, medicinal, and culinary applications worldwide owing to their wide range of bioactive secondary metabolites, principally phenolics. Hence, in continuance of our interest in this medicinal plant, which included formerly the comprehensive botanical description of its leaves, along with the phytochemical and biological characterization of its leaf and stem extracts, this paper presents the macro- and microscopical characters of *M. arboreus* stems and flowers. Such botanical data collectively draw a helpful image regarding the identification and authentication of this plant species.

Key words

Botanical study, Flower, Malvaceae, Malvaviscus, Stem.

1. Introduction

Malvaviscus arboreus Cav. (syn. *Malvaviscus mollis* (Aiton) DC. or *Hibiscus malvaviscus* L.) is a perennial flowering shrub within the plant family Malvaceae [1, 2]. It grows naturally throughout the southern parts of USA, the Mexican forests, and Latin America [1, 3]. This plant has multiple common names, encompassing Wax mallow, Drummond Wax Mallow, Turk's cap, and Sleeping Hibiscus [3, 4]. Generally, the freely-branching shrubs of *M. arboreus* can grow up to 1 m in height, carrying simple ovate to cordate leaves with alternate phyllotaxis and brilliant red flowers that usually appear for most of the year, adding an attractive appearance to the plant [1, 5]. The showy blossoms of *M. arboreus* are particularly noted for their both pendulous stalks and the tightly wrapped red corollas that usually remain closed, giving rise to the common name "Sleeping Hibiscus" of the plant [1, 6]. The flowers also show a central column formed of the pistil and the fused numerous stamens; this staminal tube protrudes conspicuously beyond the petals. Outwardly, these flowers are surrounded by a small green calyx and an additional hairy epicalyx [7]. From a botanical point of view, it is worth mentioning that *Malvaviscus* plants are generally related to those of the genus *Hibiscus*; however, they are typically distinguished by their ten, rather than five, capitate stigmas covering a central style, together with the production of schizocarpic fruits consisting of five separate parts [4]. Besides the ornamental and medicinal reputation of *Malvaviscus* plants [6, 8–13], their flowers, fruits, and leaves are also added to salads, jellies, and herbal preparations [1, 13]. Phytochemically, *M. arboreus* plants have been shown to contain a range of phenolic metabolites, e.g. flavonoids, anthocyanins, and phenolic acids, in addition to fatty acids, sterols, and triterpenes [6, 8, 10, 13–15]. Our previous investigation of *M. arboreus* has deliberated the botanical characteristics of the leaves, the phytochemical composition of the aerial parts, in addition to the hepatoprotective, antioxidant, and anti-infective properties of their different extracts [5, 6, 13], whereas the current study delineates the macro- and microscopical features of the stems and flowers.

2. Experimental

2.1. Plant material

The flowering plants of *M. arboreus* were obtained from the Campus of Minia University and authenticated by Prof. Mahmoud A. Hassan, Horticulture Department, Faculty of Agriculture, Minia University. A specimen with the number Mn-Ph-Cog-027 was deposited in the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Minia University.

2.2. Plant sample preparation

Fresh stem and flower samples were kept in a mixture of ethanol–glycerine–water (1:1:1), whereas other samples were air-dried and ground to a powder form suitable for

**** Correspondence: John R. Fahim***

Tel.: *+2 01227454025****; Fax:*** *+20 862369075*

Email Address: *john.michael@mu.edu.eg*

microscopical investigation. Different plant sections were stained with safranin, light green, iodine solution, as well as phloroglucinol and conc. HCl [16]. A microscope with Leica® camera (Germany) and a 10-megapixels digital camera (Samsung, South Korea) were used for the microscopical studies.cology Centre (AUMC) under the voucher number 6837.

3. Results and discussion

3.1. Macroscopical characters of the stem

The stem of M. arboreus (**Figure 1**) is erect, cylindrical, and glabrous with monopodial branching, measuring 0.5–1 m in length and 0.2–1 cm in diameter, having internodes of 0.5–1 cm. The main stem and branches are mostly woody and brown in colour, whereas the branchlets are green and softer; carrying alternately arranged oval or ovate to broadly cordate leaves. The fresh stem is flexible, while the dry one breaks with a fibrous fracture. The stem is generally odourless and slightly mucilaginous.

Figure 1: Stems of Malvaviscus arboreus Cav. (x 0.5)

3.2. Microscopical characters of the stem

3.2.1. The upper part of the stem

The transverse section (T.S.) in the upper part is approximately circular in outline and shows an outer epidermis, followed by the cortex region, which consists of few rows of collenchyma and a wider zone of parenchyma cells. A continuous ring of vascular tissues traversed by medullary rays and central wide parenchymatous pith are also observed (**Figure 2**).

3.2.1.1. Epidermis

It is represented by a single row of rectangular cells with thin cuticle (**Figure 2**).

3.2.1.2. Cortex

It consists of 2–3 rows of small, rounded, and thick-walled collenchyma cells without intercellular spaces, followed by 3–5 rows of large, oval, and thin-walled parenchyma cells having narrow intercellular spaces. The endodermis is parenchymatous and indistinct (**Figure 2).**

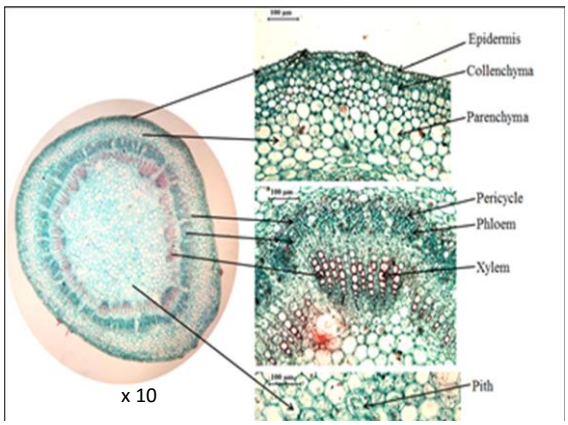


Figure 2: A detailed T.S in the upper part of the stem (x 100).

3.2.1.3. Vascular bundles

The pericycle is parenchymatous with a few intermittent groups of lignified fibres. Pericyclic fibres are long, having wide lumina and tapering ends, as shown in the powder of the stem (Figure 5). Numerous scattered clusters of calcium oxalate are also observed in the pericyclic region (**Figure 2**). The phloem is comprised of thin cellulosic elements, including sieve tubes and companion cells, with associated phloem parenchyma; some of the latter contain clusters of calcium oxalate (**Figure 2**). The cambium is represented by several rows of tangentially arranged meristematic cells. The xylary region contains numerous lignified vessels, tracheids, wood fibres, and wood parenchyma. Xylem vessels are of spiral thickening (**Figure 5**). Wood parenchyma are polygonal with pitted walls. Medullary rays are uni- or biseriate traversing the xylem (**Figure 2**).

3.2.1.4. Pith

The pith is wide and consists of rounded, thin-walled parenchyma cells containing clusters of calcium oxalate (**Figure 2**).

3.2.2. The lower part of the stem

The transverse section of the lower part of the stem is circular in outline, showing an outer layer of dark brown cork cells surrounding the cortex, followed by the vascular tissue, which consists of pericycle, phloem, and a wide zone of xylem extending towards the center. Central narrow parenchymatous pith also appears (**Figure 3**).

3.2.2.1. Cork

The cork is formed of one or two layers of brown, tangentially elongated, rectangular cells with thick walls (Figure 3). In surface view, they appear as polygonal, isodiametric to slightly elongated cells (**Figure 5**).

3.2.2.2. Secondary cortex

It consists of 4–5 rows of parenchyma cells with thin walls and small intercellular spaces; some of which contain clusters of calcium oxalate (Figure 3).

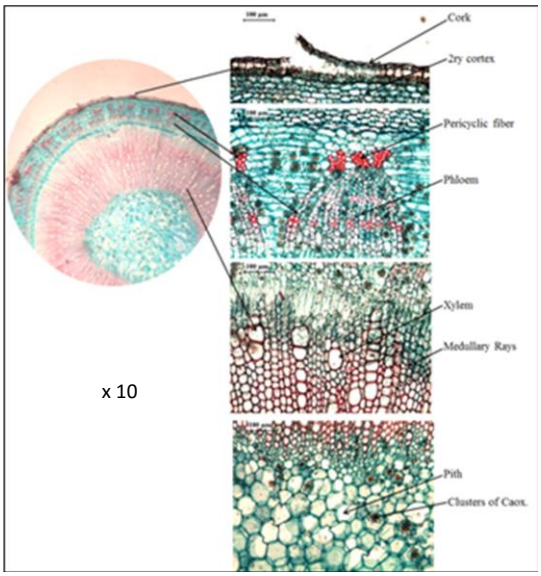


Figure 3: A detailed T.S in the lower part of the stem (x 100).

3.2.2.2. Secondary cortex

It consists of 4–5 rows of parenchyma cells with thin walls and small intercellular spaces; some of which contain clusters of calcium oxalate **(Figure 3)**.

3.2.2.3. Vascular bundles

The pericycle consists of groups of lignified fibres separated by parenchyma cells. The fibres are thick, lignified with wide lumina and acute apices, as shown in the powder **(Figure 5)**. The phloem contains soft cellulosic phloem parenchyma, sieve tube, and companion cells, accompanied with groups of lignified phloem fibres. Several clusters of calcium oxalate are also found in the phloem region. The cambium is represented by a continuous ring of tangentially elongated and radially arranged cells arranged in several rows **(Figure 3)**. The xylary tissue consists of lignified vessels, tracheids, and tracheidal vessels, along with wood parenchyma and wood fibres. Xylem vessels are lignified with spiral thickenings **(Figure 5)**. Wood parenchyma are polygonal cells showing thick, pitted, and lignified walls. Wood fibres have lignified walls, wide lumina, and acute ends. Tracheids are simple pitted with lignified walls. Medullary rays are uni-, bi-, or multiseriate with elongated cells and thick pitted, lignified walls **(Figures 3 & 4)**.

3.2.2.4. Pith

The pith consists of large, rounded parenchyma cells containing clusters of calcium oxalate (**Figure 3**).

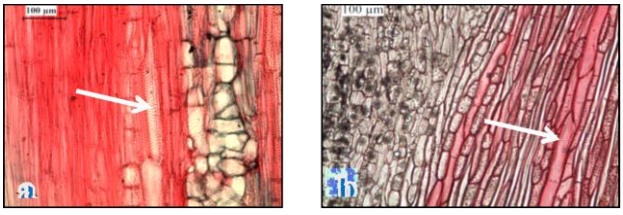


Figure 4: A longitudinal cut in the lower part of the stem showing tracheids (a) and medullary rays (b) with lignified walls (x 100).

3.2.3. Powder of the stem:

The powder of the stem is dark green and odourless, with a slightly mucilaginous taste, showing the following elements **(Figure 5)**:

A- Fragments of cork cells.
B- Lignified wood fibres
C- Fragments of spiral xylem vessels.
D- Fragments of wood parenchyma with thick pitted walls
E- Fragments of medullary rays with pitted walls.
F- Lignified pericyclic fibres
G- Scattered calcium oxalate clusters.

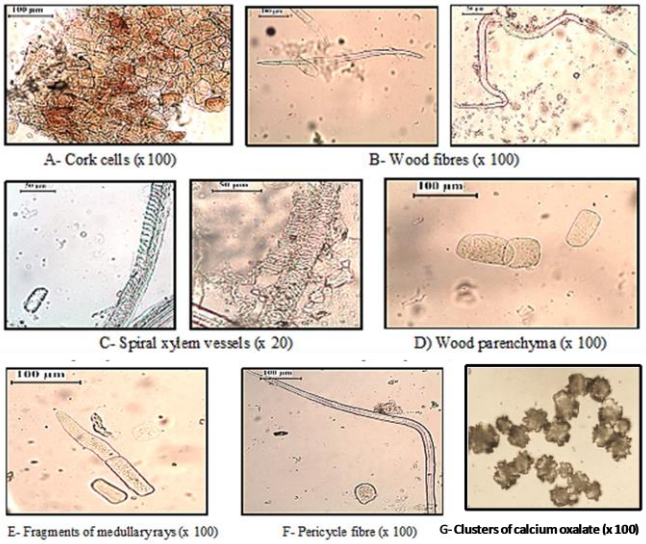


Figure 5: Elements of the powdered stem.

3.3. Macromorphology of the flower

Flowers of *M. arboreus* **(Figure 6)** are solitary, axillary, and tubular in shape, measuring 5–6 cm in length and 0.5–1.5 cm in width. They are scarlet red in colour, having a faint odour and a mucilaginous taste. The flowers are pendulous, slightly expanding at the top, and are carried on the plant with hairy green pedicels, which are 3–15 mm long. They are hermaphrodite, pentamerous, actinomorphic, and hypogenous. The flower consists of a hairy epicalyx of 6–8 green spatulate bracts, a campanulate calyx of five united green sepals, a corolla of five united and twisted red petals, an androecium with numerous stamens; The staminal column is about 5–7 cm in length, exceeding the corolla tube, and a gynoecium of a superior pentalocular pentacarpellary ovary, a branched style, and ten capitate stigmas. The floral formula is represented by ⊕ Epik 6-8, K(5), C(5), A(∞), G(5). The floral diagram is depicted in **(Figure 7)**.

Figure 6: Flowers of Malvaviscus arboreus Cav. (x 0.5)

Figure 7: The floral diagram.

3.4. Micromorphology of the flower

3.4.1. The epicalyx

A transverse section in the epicalyx consists of upper and lower epidermises enclosing a wide parenchymatous cortex and numerous vascular bundles. Both epidermises consist of elongated rectangular cells covered with thin cuticle. The cortex region comprises several rows of almost rounded thin-walled parenchyma cells with numerous clusters of calcium oxalate. The vascular tissue is formed of several collateral vascular bundles with lignified spiral xylem vessels towards the upper epidermis and phloem towards the lower one **(Figure 8).**

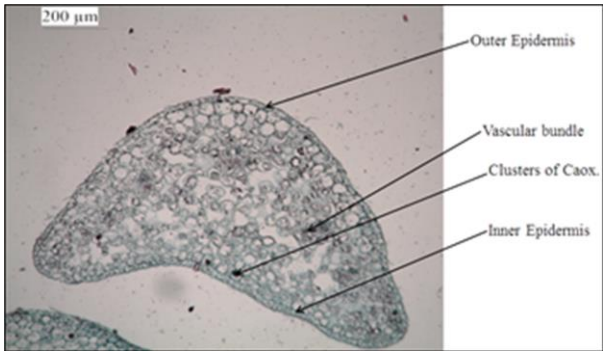


Figure 8: A T.S. in the epicalyx (x 100).

3.4.2. The calyx

A transverse section in the calyx shows an upper glabrous epidermis and a lower hairy one enclosing a wide parenchymatous cortex and several vascular bundles. The upper (outer) epidermis is represented by one row of rectangular cells with thin cuticle. The cortex contains several layers of rounded or slightly oval parenchyma cells containing several clusters of calcium oxalate. The vascular tissue is formed of several collateral vascular bundles with xylem vessels towards the outer epidermis and phloem towards the inner one. The lower (inner) epidermis is similar to the upper one except for carrying numerous non-glandular uniseriate multicellular hairs (Figure 9).

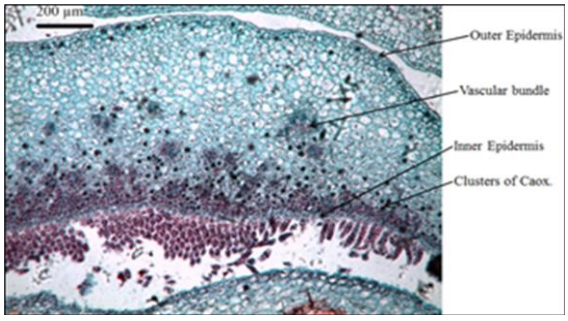


Figure 9: A T.S. in the calyx (x 40).

3.4.3. The corolla

A transverse section in the petals shows upper and lower epidermises and a wide parenchymatous cortex, followed by small scattered vascular bundles. The upper (outer) and lower (inner) epidermises are formed of rectangular cells with thin cuticle, while in surface view; they appear as polygonal cells with wavy anticlinal walls **(Figure 19)**. Trichomes and stomata are both absent. The cortex is formed of polygonal, thin-walled parenchyma with small intercellular spaces. Several clusters of calcium oxalate are scattered in the cortical region. The vascular tissue is composed of small collateral vascular bundles with lignified spiral xylem vessels towards the upper epidermis and phloem towards the lower one **(Figure 10)**.

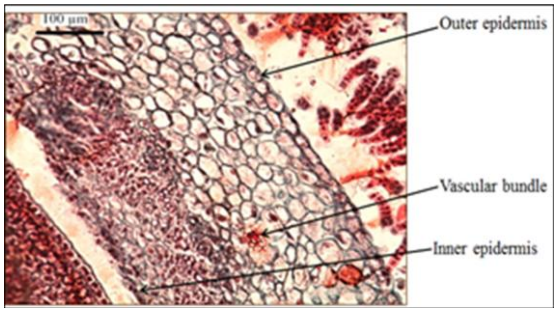


Figure 10: A T.S. in the corolla (x 100).

3.4.4. The androecium

3.4.4.1. The filament

The epidermal cells of the filament appear in surface view as polygonal, axially elongated cells with straight anticlinal walls **(Figure 11)**. Both stomata and trichomes are completely absent.

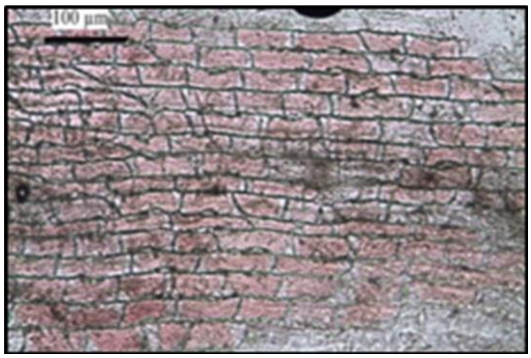


Figure 11: Surface preparation of the filament (x 100).

3.4.4.2. The anther

A transverse section in the anther shows two anther lobes attached together by a parenchymatous connective tissue. The two anther lobes contain numerous spherical spiny pollen grains. The anther wall is formed of an epidermis followed by a fibrous layer and remaining of the tapetum (Figure 12). The fibrous layer of anther consists of polygonal axially elongated cells with thick lignified walls showing bar-like thickening **(Figure 13)**.

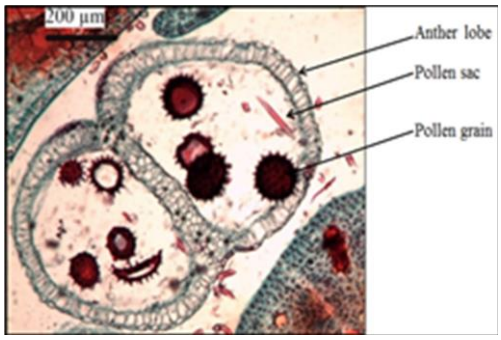


Figure 12: The anther (x 40).

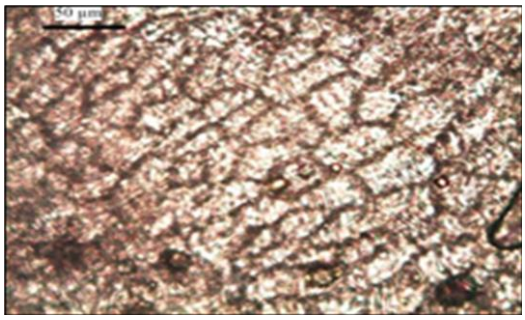


Figure 13: Fibrous layer of anther (x 200).

3.4.4.3. Pollen grains

They are spherical and brown in colour showing a spiny exine **(Figure 14)**.

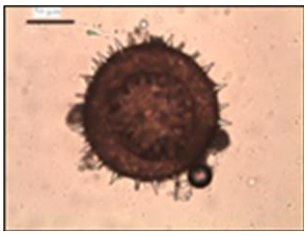


Figure 14: A pollen grain (x 200).

3.4.5. The gynoecium

3.4.5.1. The ovary

A transverse section in the ovary is circular in outline showing an epidermis enclosing five united carpels with five locules; each contains one or two ovules. The ovary shows outer and inner epidermises with a parenchymatous mesophyll in between **(Figure 15)**.

3.4.5.2. The style

The epidermal cells of the style appear in surface view as polygonal, axially elongated cells with straight anticlinal walls covered with thin cuticle **(Figure 16)**.

3.4.5.3. The stigma

The epidermis of stigma consists of polygonal papillosed cells with straight anticlinal walls **(Figure 17)**.

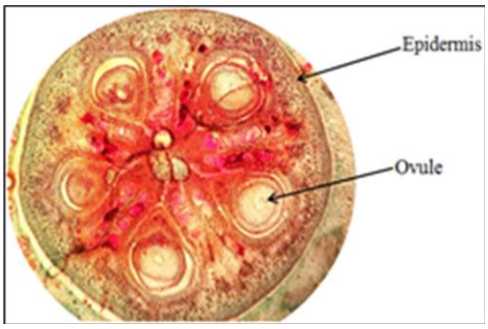


Figure 15: A T.S. in the ovary (x 40).

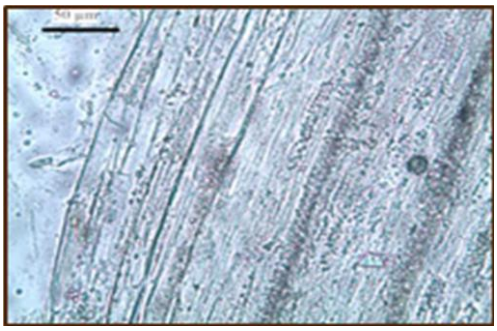


Figure 16: Surface preparation in the style (x 200).

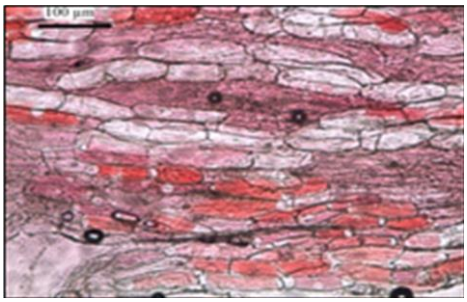


Figure 17: Surface preparation in the stigma (x 100).

3.4.6. The pedicel

A transverse section in the pedicel shows a slightly hairy epidermis, followed by a wide cortex, collateral vascular bundles, and wide parenchymatous pith (**Figure 18**).

3.4.6.1. The epidermis

It is represented by one row of rectangular cells covered with thin cuticle carrying numerous non-glandular stellate hairs with 3–6 unicellular arms and thin cuticle **(Figure 18)**.

3.4.6.2. The cortex

It is formed of one row of small rounded collenchyma with no intercellular spaces followed by several rows of large parenchyma cells with thin cellulosic walls and wide intercellular spaces. Clusters of calcium oxalate are scattered in the cortex. The endodermis is parenchymatous and indistinguishable. The pericycle is formed of small rounded thin-walled parenchymatous cells with no pericyclic fibres. Clusters of calcium oxalate are also scattered in the pericyclic

region. The vascular tissue contains small collateral vascular bundles. The phloem consists of thin-walled sieve tubes, companion cells, and associated phloem parenchyma. Some cells phloem parenchyma also contain clusters of calcium oxalate. The xylem is formed of lignified xylem vessels and wood parenchyma. The pith consists of large parenchyma cells with thin walls and wide intercellular spaces **(Figure 18)**.

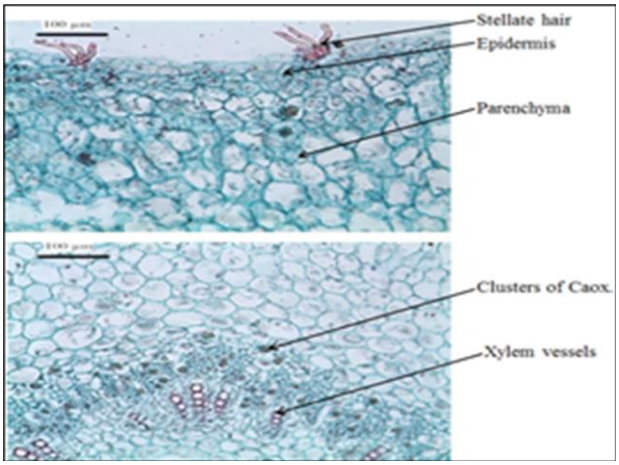


Figure 18: A T.S in the pedicel (x 100).

3.4.7. Powder of the flower

The powered flowers are dark red in color, odourless, with a mucilaginous taste. Microscopically, it is characterized by the following elements **(Figure 19):**

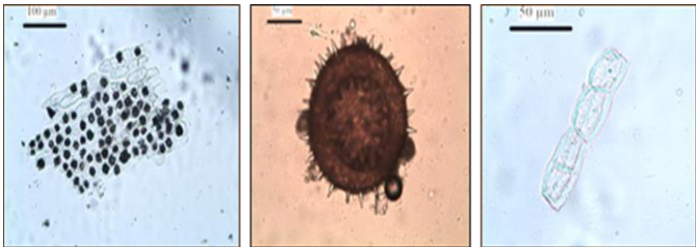
- Scattered clusters of calcium oxalate
- Fragments of the outer and inner epidermises of corolla
- Stellate hairs.
- Spherical spiny pollen grains
- Fragments of cortex parenchyma.
- Fragments of spiral xylem vessels
- Fragments of wood fibers.

3.5. Microscopical measurements

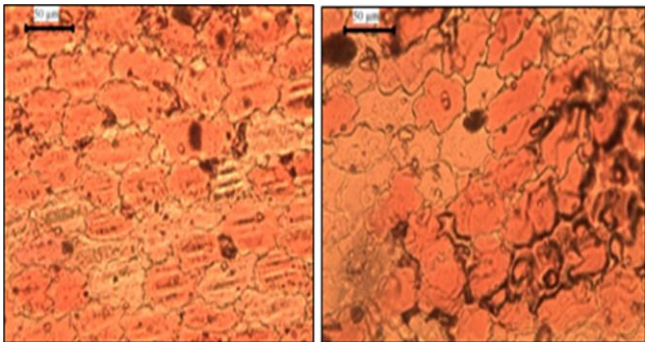
Microscopical measurements of various stem and flower tissues of M. arboreus are summarized in **Table 1**.

4. Conclusion

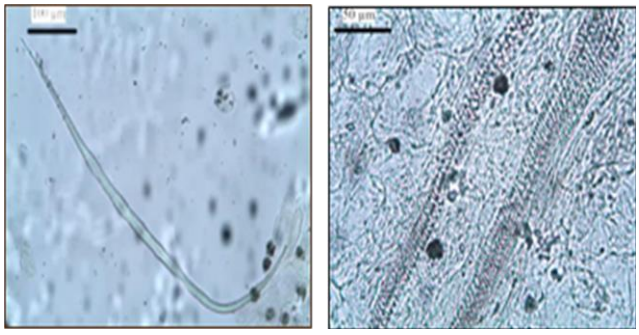
The detailed botanical features of M. arboreus stem and flowers were deliberated herein for the first time. Tissues of the lower part of the stem exhibited prominent secondary thickening in comparison with those of the upper one, resulting in narrowing of the pith region. Various stem tissues of M. arboreus are generally rich in calcium oxalate clusters, with both the non-glandular and glandular hair types are totally absent. Flowers of M. arboreus, on the other hand, are generally hermaphrodite, actinomorphic, and hypogenous, characterized by wrapped red corollas, pendulous stalks, and hairy epicalycies. Microscopically, the spiny spherical pollen grains, cluster crystals of calcium oxalate, and stellate hairs are the most diagnostic elements of the powdered flowers. These data represents an important authentication tool for M. arboreus among different Malvaceae species, particularly the most related Hibiscus plants.



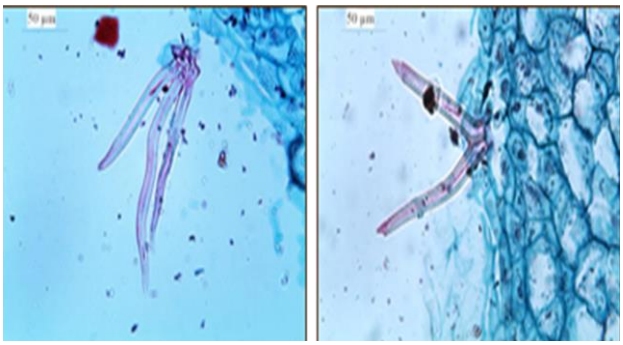
Clusters of Ca Ox. (x 200 | A pollen grain (x 200 | Cortex Parenchyma (x 200



Fragments of outer epidermis of corolla (x 200) | Fragments of inner epidermis of corolla (x 200)



(Wood fibres (x 100 | (Spiral xylem vessels (x 200



Stellate hair (x 200)

Figure 19: Elements of the powdered flower.

Table 1: Microscopical measurements of M. arboreus stem and flower tissues in micron.

Item	Length	Width	Height	Diameter
A- Stem:				
Epidermal cells	14-18-22	8-12-16	18-23-27	–
Collenchyma	–	–	–	9-18-27
Parenchyma of the cortex	–	–	–	36-50-64
Wood parenchyma	38-41-45	18-19-20	–	–
Xylem vessels	–	–	–	33-45-56
Tracheids	90-115-140	10-13-26	–	–
Medullary rays	40-55-70	15-20-25	–	–
Cork cells	19-25-31	22-28-33	11-17-22	–
Parenchyma of the secondary cortex	–	22-34-45	11-17-22	–
Parenchyma of the pith	–	–	–	33-61-89
Calcium oxalate clusters	–	–	–	11-22-33
B- Flower:				
Epicalyx				
Inner epidermis	25-41-57	12-17-22	10-13-16	–
Outer epidermis	50-72-95	35-44-54	13-15-18	–
Calyx				
Inner epidermis	22-36-72	10-20-30	10-13-15	–
Outer epidermis	43-66.5-133	20-30-40	13-15-18	–
Wood fiber	385-460-535	15-18-21	–	–
Calcium oxalate clusters	–	–	–	17-24-30
Corolla				
Outer epidermis	45-62.5-125	44-59-73	10-15-20	–
Inner epidermis	47-64-82	22-32-42	25-30-35	–
Parenchyma	–	–	–	15-33-50
Calcium oxalate clusters	–	–	–	8-11-15
Androecium				
Epidermis of filament	14-18-22	36-50-64	–	–
Fibrous layer of anther	11-15-18	18-36-54	–	–
Pollen grains	–	–	–	107-112-117
Gynoecium				
Epidermis of style	75-100-125	13-19-25	–	–
Epidermis of stigma	12-20-27	67-94-120	–	–
Pedicel				
Epidermis	10-20-30	10-16-22	15-18-20	–
Collenchyma	–	–	–	13-23-33
Parenchyma	–	–	–	33-45-56
Calcium oxalate clusters	–	–	–	13-17-21

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