Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 47-57
Received: 02-12-2014
Accepted: 04-01-2015

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Leaf Morphoanatomy Studies of *Parmentiera cereifera* Seem., Family Bignoniaceae, Cultivated in Egypt

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Abstract
Parmentiera cereifera Seem. is one of the edible and medicinal plants. It is known as Candle tree, Candlestick tree and Panama Candle tree. Reviewing the available literature, only two studies could be traced concerning the microscopical features of *P. cereifera*. The current leaf morphoanatomy studies showed various standardized parameters such as macroscopic and microscopic characters. These various diagnostic features could be helpful in authentication and identification of *P. cereifera Seem.* leaf.

Keywords: *Parmentiera cereifera* Seem., Bignoniaceae, leaf, leaflet, rachis, morphoanatomy

1. Introduction
Family Bignoniaceae is rich in secondary metabolites and includes many genera of high economic and medicinal values [1]. It is known as the Bignonia family [2]. It is widely distributed in tropical and subtropical regions, with a few species in temperate climates [3]. It comprises 104 genera and 860 species, according to the last taxonomic revision [3]. One of these species is *Parmentiera cereifera* Seem. It is one of the edible and medicinal plants [4]. A study in the available botanical literature described the upper and lower epidermises of the leaf of *P. cereifera* [5]. Another study illustrated the macro- and microscopic characters of the stem [6]. Moreover, the features of the wood of genus Parmentiera were reported previously [7]. A thorough literature search showed that *P. cereifera* was not studied the botany of the leaf. The present study investigates leaf botanical characters not only morphologically but also anatomically, which could be helpful in authentication of the leaf of *P. cereifera* Seem. It is also give a tool for herbal quality control of the plant.

Fig 1: Photo of *Parmentiera cereifera* Seem.

2. Taxonomy
P. cereifera Seem. belongs to [8]:
Kingdom: Plantae, **Subkingdom:** Viridiplantae, **Infrakingdom:** Streptophyta, **Superdivision:** Embryophyta, **Division:** Tracheophyta, **Subdivision:** Spermatophytina, **Infradivision:** Angiospermae, **Class:** Magnoliopsida, **Superorder:** Asteranae, **Order:** Lamiales, **Family:** Bignoniaceae, **Genus:** Parmentiera DC. and **Species:** *P. cereifera* Seem. (Candle tree).

3. Experimental

3.1 Plant material

The plant material (leaves) of *P. cereifera* cultivated in El-Zohria botanical garden, Giza, Egypt, (Figures **1** and **2**). The plant material was collected in February 2009. The plant was kindly identified by Dr./ Mamdouh Shokry, director of El-Zohria botanical garden. A voucher specimen (Mn-Ph-Cog-003) was kept in the Herbarium of Pharmacognosy Dept., Faculty of Pharmacy, Minia University, Minia, Egypt. The plant material used for botanical study was taken from the fresh samples, as well as the samples preserved in alcohol (70%)-glycerine-water (1:1:1). The leaves were air-dried, reduced to fine powder suitable for microscopical examination and stored in well-closed containers.

3.2 Dyes

Safranin and light green were used for staining the plant sections and the powder.

3.3 Microscopic studies

Surface preparations, transverse sections (T.S.), longitudinal sections (L.S.) as well as the powder of the leaf were used for observation of various microscopic features**.** All microscopical investigations were done by using Microscope with camera, Leica® (Germany) and 10 megapixels digital camera, Samsung (Korea).

4. Results and Discussion

4.1 Macroscopical characters of the leaves

The leaves are palmate compound with opposite to subopposite phyllotaxis, rarely in whorls of 3 or 4. The leaves are formed of three leaflets on winged, narrow and greenish petioles. They measure 1.5-5.0 cm in length and 0.3 cm in width. The leaflets are elliptic to elliptic-rhomboidal in shape and the terminal leaflets are larger in size than the laterals. The leaflets measure 1.5-7.0 cm in length and 0.8-3.5 cm in width in the middle part. The margin of both lateral and terminal leaflets is entire when the tree matures, but when juvenile the margin is coarsely serrate. The apex is acute to acuminate and the base is decurrent on the petiole [4, 9]. The leaflet shows reticulate pinnate venation. The surface of the leaflet is hairy. Both the upper and lower surfaces are dark green in color (Figure **2**).

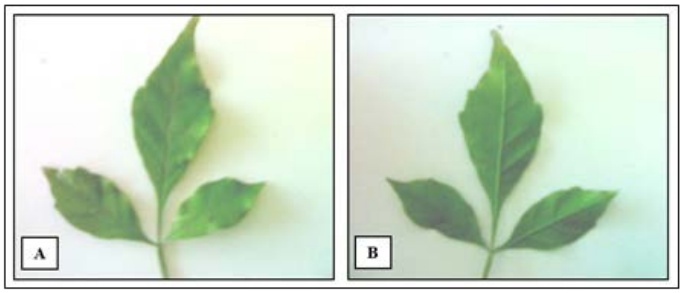

Fig 2: Leaf surfaces; **A**-Upper and **B**-Lower (Both x 0.5).

4.2 Microscopical characters of the leaf

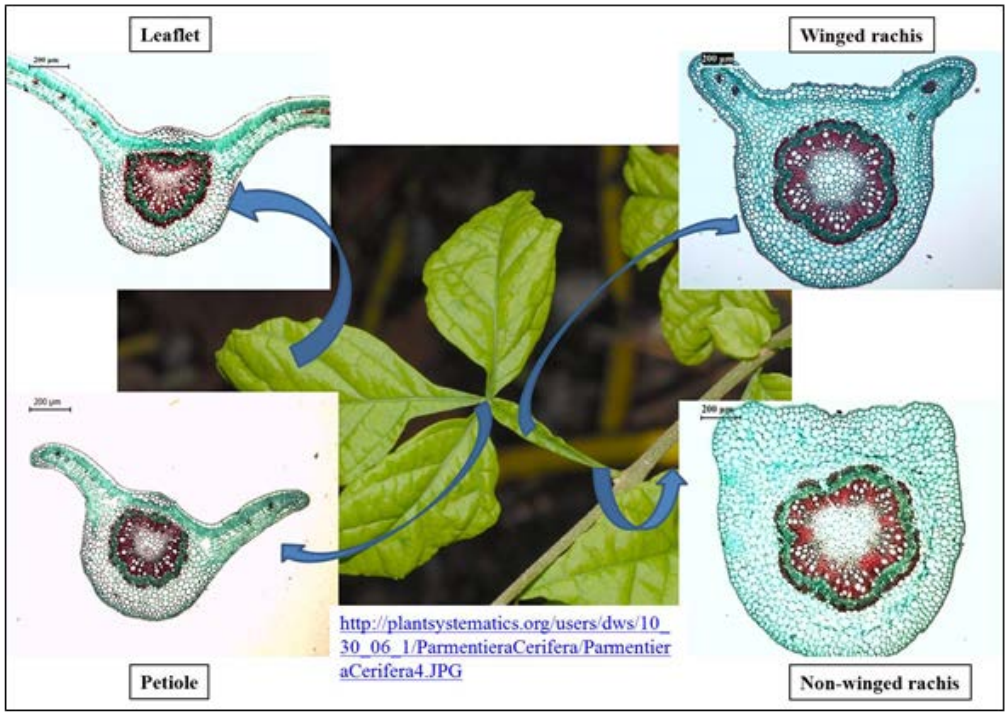

Fig 3: Different diagrammatic sections of the leaf and its parts.

4.2.1 Microscopical characters of the leaflet

A transverse section in the leaflet blade shows that the midrib is prominent on the lower surface and showing a small raised rounded ridge on the upper surface (Figure **4B**). The lamina has a dorsiventral structure and the palisade layer is continuous in the midrib region (Figure **4A** and **4B**). The transverse section also reveals a mass of subepidermal collenchyma under the upper and lower epidermises in the midrib region. The vascular system of the midrib is formed of a collateral vascular bundle with central pith surrounded by a quite or

almost continuous ring of xylem and phloem. The pericycle consists of groups of lignified pericyclic fibers lining the vascular bundle, interrupted by few parenchyma cells (Figures **4B** and **4C**).

4.2.1.1 The epidermis

4.2.1.1.1 The upper epidermis

It is formed of one row of subrectangular to square cells as seen in the transverse section (Figures **4A**, **4C** and **5C**). In surface view, the cells appear polygonal, with slightly sinuous anticlinal walls covered with thin, slightly striated cuticle and stomata are absent (Figure **5A, 5B** and **16F**). The cells of the upper epidermis contain cluster, acicular crystals and prisms of calcium oxalate in addition to starch granules, which are oval to round in shape with central point-like hilum (Figures **5** and **16F**). The granules are medium in size and the aggregation is mainly simple. The striations are indistinct.

The upper epidermis shows many types of hairs of both glandular and non-glandular types. It has two types of glandular hairs. The first one shows a multicellular head with indefinite number of cells and a unicellular stalk, which is called patelliform hair (Figure **6A**, **6B** and **16E**) [11]. The second type of glandular hair shows unicellular stalk and multicellular head of 16-22 radiating cells, which is called peltate hair (Figure **6C, 6D** and **16D**).

The non-glandular hairs present in 4 different types. Abundant unicellular conical, non-glandular hair covered with smooth cuticle (Figures **7A** and **16C**). Bicellular (Figures **7B** and **16A**) and multicellular uniseriate hairs of 4-8 cells with acute apices are present, but they are less abundant (Figures **7D** and **16B**). Another type of multicellular hair is presently having 3-4 uniseriate cells with blunt apex and the cells are nearly equal in size (Figure **7C**). The neural epidermis is slightly elongated carrying glandular and non-glandular hairs (Figure **8**).

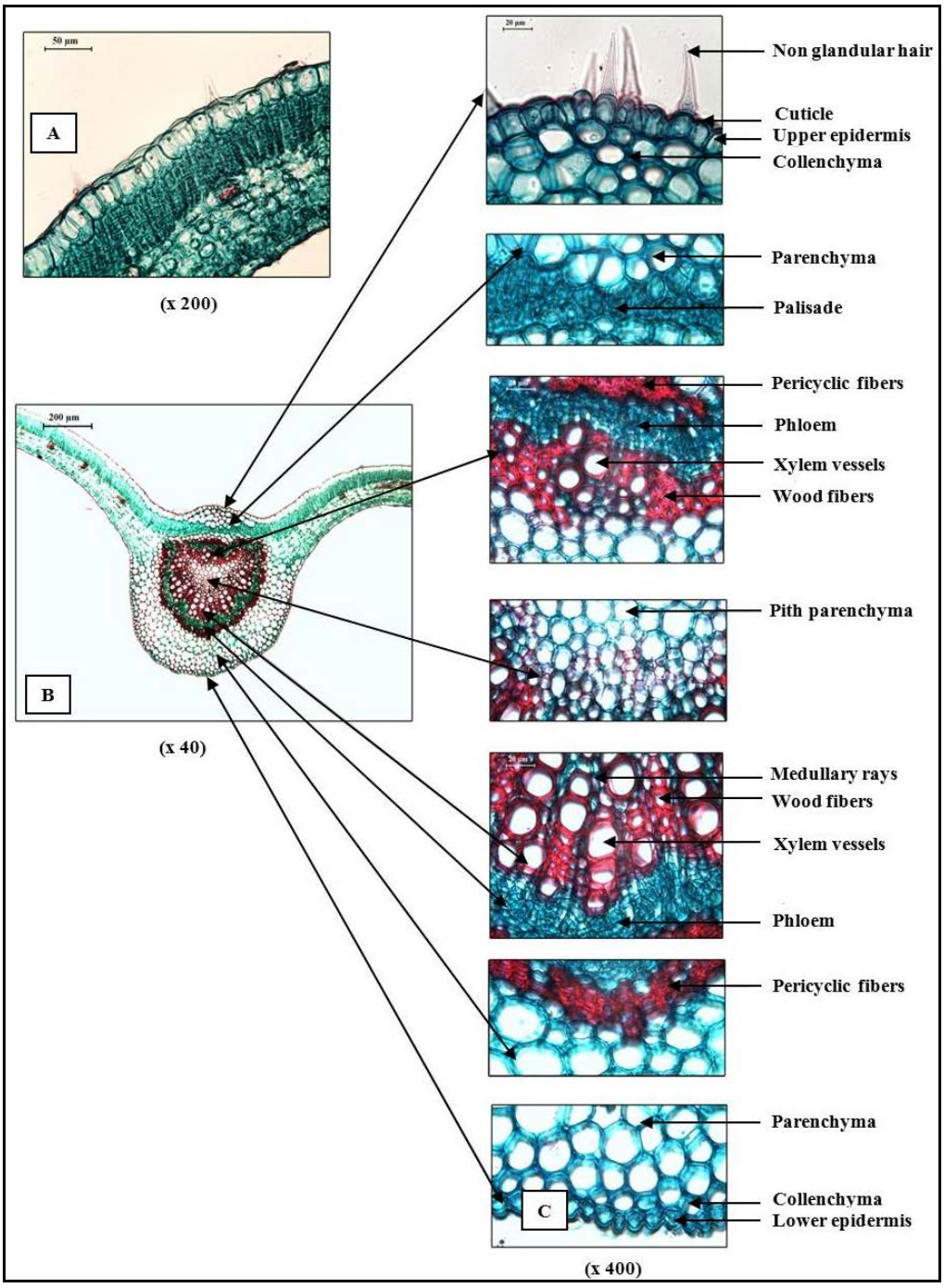

Fig 4: Detailed T.S. of the leaflet; **A-**Lamina and **B-**Midrib.

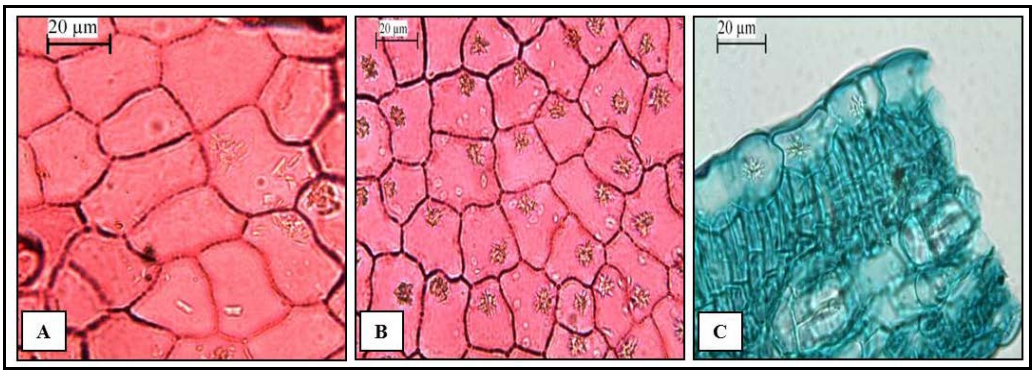

Fig 5: Upper epidermis showing; **A**-Prisms of calcium oxalate in surface preparation (x 200), **B**-Cluters of calcium oxalate and starch granules in surface preparation (x 400) and **C**-Clusters of calcium oxalate in T.S. in the lamina (x 400).

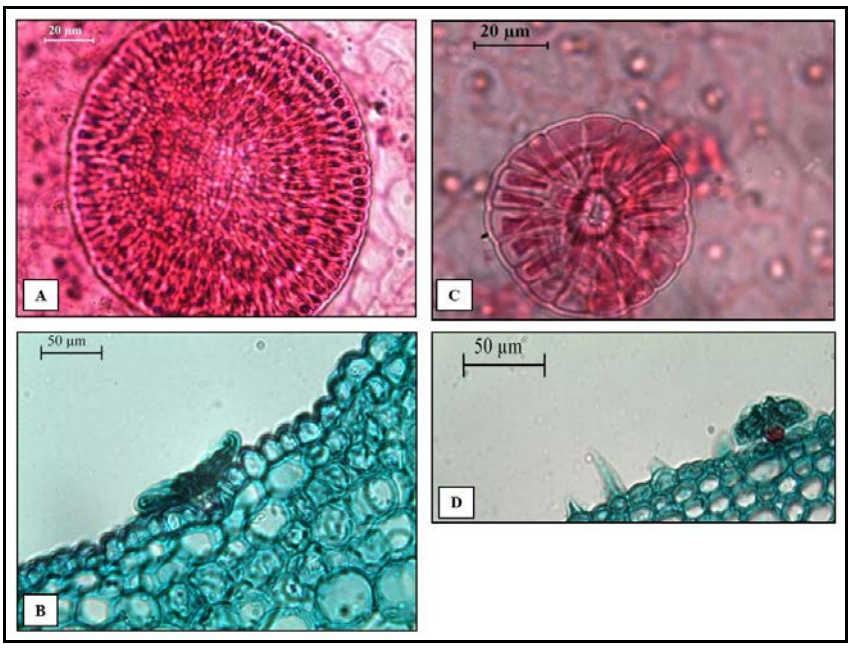

Fig 6: Upper epidermis showing glandular hairs; A-Top view of patelliform (x 400), B-Side view of patelliform (x 200), C-Top view of peltate (x 400) and D-Side view of peltate (x 200).

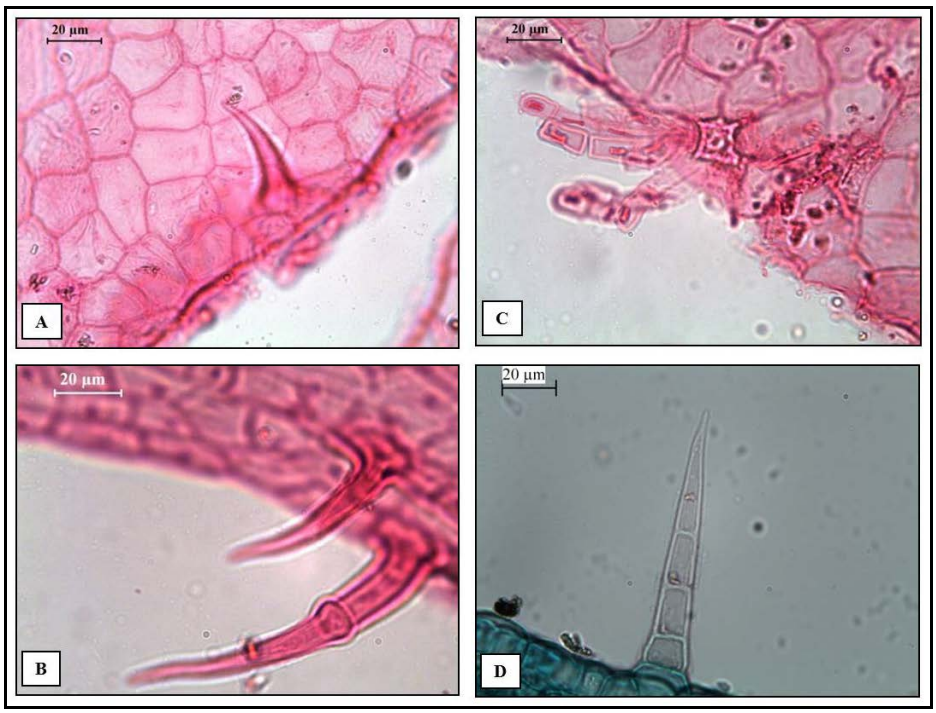

Fig 7: Upper epidermis showing side views of non-glandular hairs; A-Unicellular (x 400), B-Unicellular and bicellular hairs (x 400), C-Multicellular hairs with blunt apex (x 400) and D-Multicellular with acute apex (x 100).

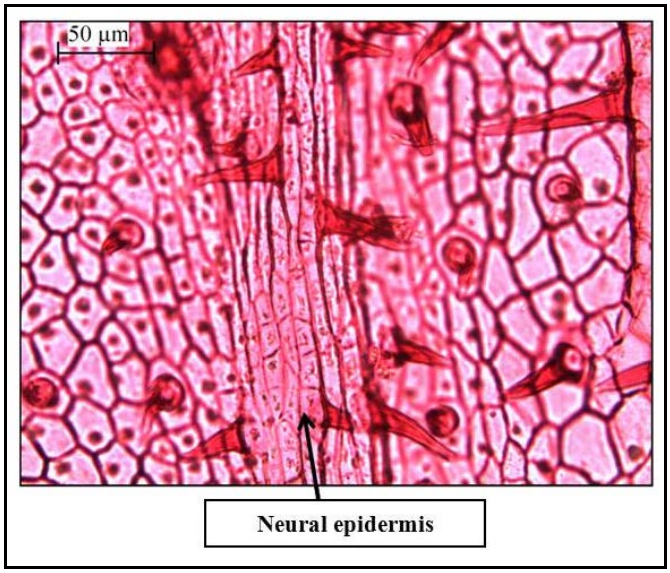

Fig 8: Surface preparation of the upper epidermis showing neural epidermal cells with hairs.

4.2.1.1.2 The lower epidermis

It is formed of one row of subrectangular to square cells as seen in the transverse section (Figures **4C** and **9C**). In surface view, the cells appear polygonal, with slightly more sinuous anticlinal walls than the upper epidermis, covered with thin smooth cuticle. The stomata are found only on the lower epidermis [11]. Its type is anomocytic (Figures **9A**, **9B** and **16G**). They are surrounded by 4-6 subsidiary cells. The cells of the lower epidermis also contain cluster, acicular crystals and prisms of calcium oxalate in addition to starch granules as the upper epidermis (Figure **9**).

Fig 9: Lower epidermis showing; **A**-Anomocytic stomata in surface preparation (x 400), **B**-Cluters and acicular crystals of calcium oxalate in surface preparation (x 400) and **C**-Prisms of calcium oxalate in T.S. in the lamina (x 400).

4.2.1.2 The mesophyll

It shows one row of the upper palisade consisting of columnar, cylindrical, thin walled cells and containing chloroplasts. The palisade layer represents approximately half of the distance between the two epidermises (Figures **4A**, **4B** and **5C**). This layer is continuous in the midrib region. The spongy tissue is formed of more or less rounded, thin walled chlorenchymatous cells with wide intercellular spaces, forming about 4-5 rows (Figures **5C** and **9C**). The mesophyll is traversed by separated strands of small vascular bundles representing the lateral veins (Figures **4A** and **4B**).

4.2.1.3 The cortex

It shows both upper and lower subepidermal collenchyma layers. The upper layer consists of 3-4 rows and the lower layer is formed of 1-2 rows (Figure **4C**). The collenchyma cells have thick cellulosic walls with no intercellular spaces. The rest of the cortical tissue consists of rounded to oval parenchyma cells having thin cellulosic walls surrounding the main vascular bundle of the midrib. It is wider below the bundle, consisting of 5-6 rows of parenchyma cells, while above the bundle, the parenchyma is formed of 1-2 rows of cells (Figure **4C**). The cortex parenchyma contains acicular, prism and cluster crystals of calcium oxalate (Figure **10**). The endodermis is parenchymatous and slightly distinguishable (Figure **4C**).

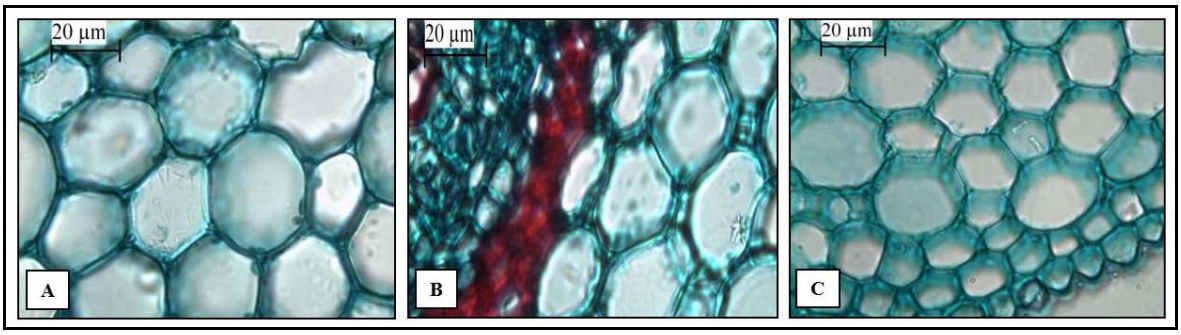

Fig 10: Leaflet cortex parenchyma showing calcium oxalate crystals; **A**-Acicular (x 400), **B**-Clusters (x 400) and **C**-Prisms (x 400).

4.2.1.4 The vascular tissue

4.2.1.4.1 The pericycle

It is formed of islets of strongly lignified pericyclic fibers separated by parenchyma cells (Figure **4C**). The pericyclic fibers are fusiform in shape, septate with thick lignified dentate walls (Figures **11** and **16L**)**.**

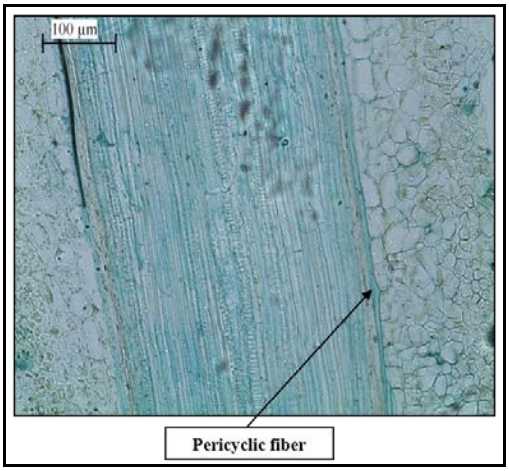

Fig 11: L.S. in the leaflet showing pericyclic fibers with dentate walls (x 100).

4.2.1.4.2 The phloem

It is formed of a narrow ring. It consists of thin walled, soft cellulosic elements; sieve tubes, companion cells and phloem parenchyma**.** The phloem region is free from any lignified elements (Figure **4C**).

4.2.1.4.3 The cambium

It is formed of 2**-**3 rows of thin walled, cellulosic, meristematic and rectangular cells (Figure **4C**).

4.2.1.4.4 The xylem

It is formed of lignified vessels, wood fibers, wood parenchyma and medullary rays (Figure **4C**). The vessels have mainly spiral and reticulate thickenings (Figures **12** and **16M**). Wood fibers are spindle-shaped with thick lignified walls, acute apices and slightly narrow lumena (Figure **12**). The wood parenchyma cells are rectangular in shape, with slightly lignified thin walls (Figures **4C** and **12**). The medullary rays are uni- to biseriate consisting of radially elongated thin**-** walled, slightly lignified cells (Figure **4C**).

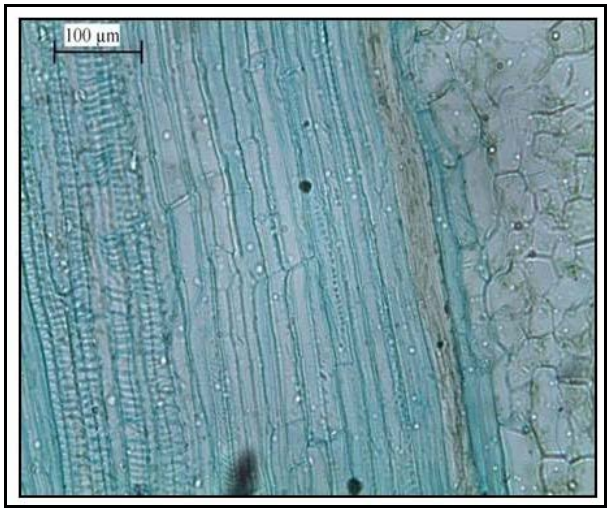

Fig 12: L.S. of the leaflet showing; wood fibers, spiral xylem vessels and wood parenchyma cells (x 100).

4.2.1.5 The pith
It is formed of rounded parenchyma cells with thin cellulosic walls (Figure **4C**).

4.2.2 Microscopical characters of the petiole of the leaflet
A transverse section in the petiole shows a similar structure to that of the leaflet except the presence of a small zone of collenchyma at the periphery of the wing (Figure **13**).

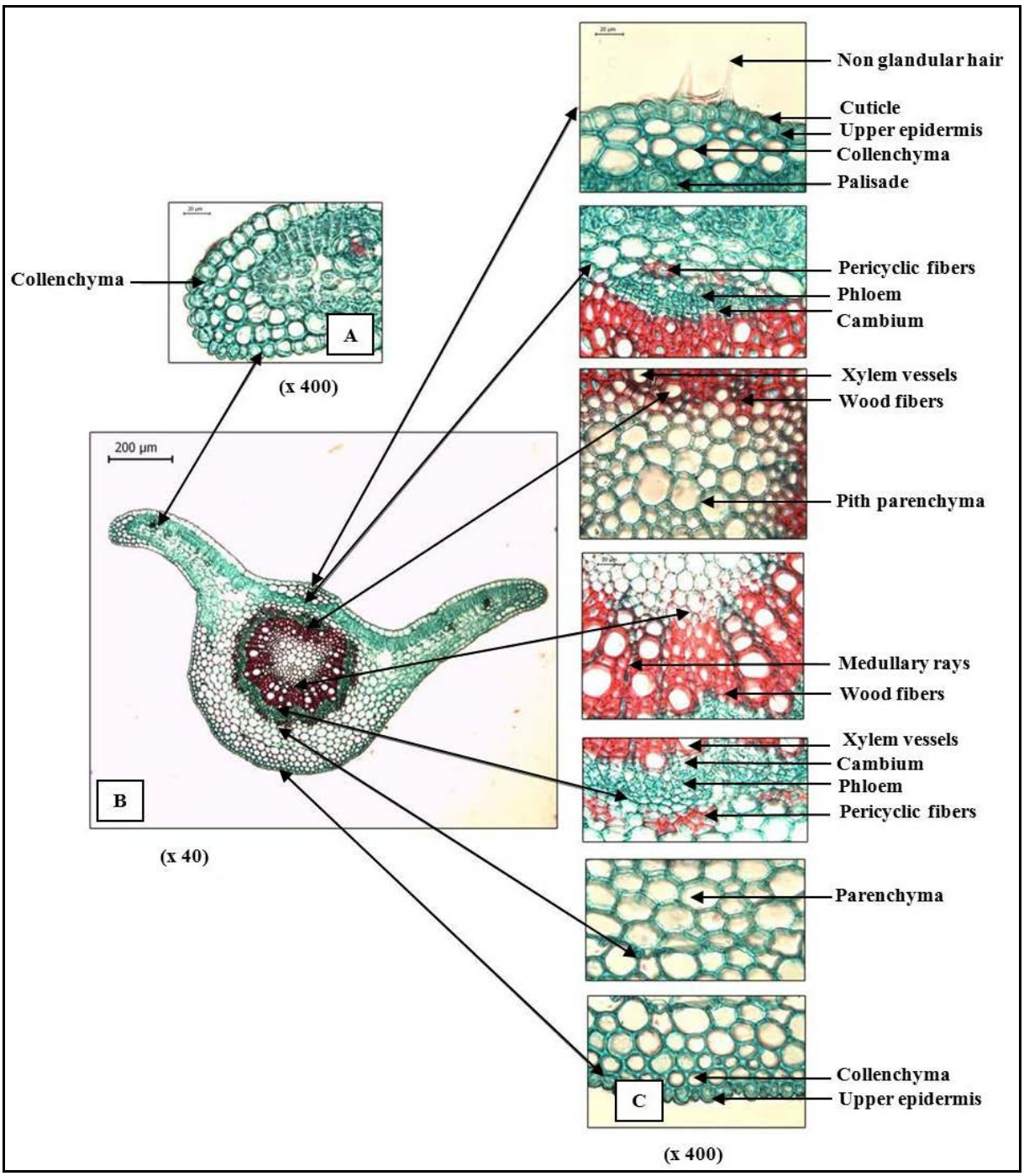

Fig 13: Detailed T.S. in the petiole of the leaflet.

4.2.3 Microscopical characters of the leaf rachis
It is recognized into two parts; a large winged part and a small non-winged part near its attachment to the stem.

4.2.3.1 Microscopical characters of the leaf rachis (winged part): A transverse section in the winged part of the rachis shows that it is plano-convex in outline with two wings on its upper side. The wings are similar in structure to those of the petiole and the vascular system is identical to that of the leaflet and the petiole (Figure **14**).

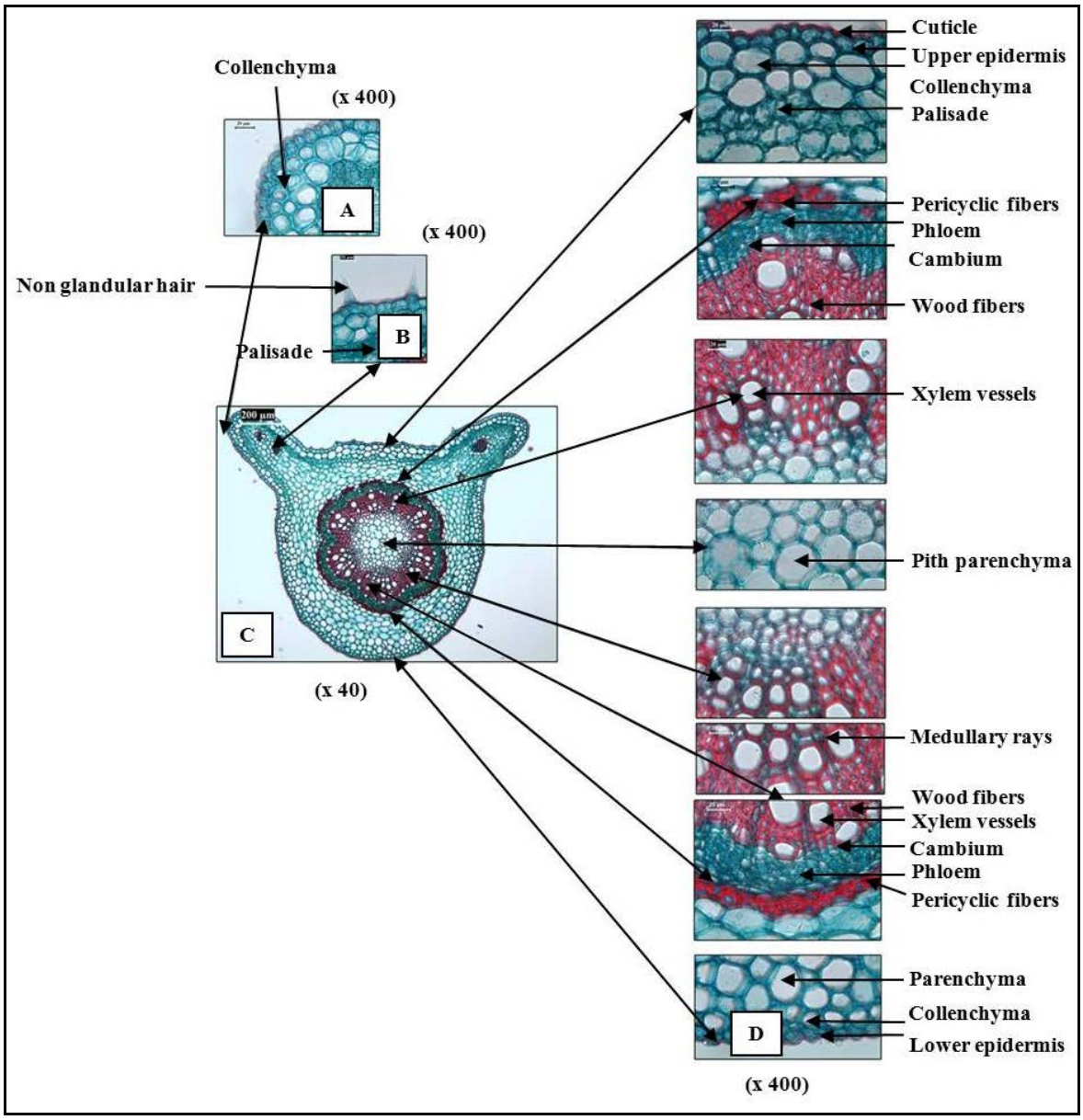

Fig 14: T.S. in the leaf rachis (winged part).

4.2.3.2 Microscopical characters of the leaf rachis (non-winged part)

A transverse section in the non-winged part of the rachis reveals that it is nearly circular in outline, more or less flattened at the upper surface with two slightly raised in small ridges on the upper side. The structure of the non-winged part of the rachis is similar to that of the winged part, except the two small zones of collenchyma cells under the ridges (Figure **15**).

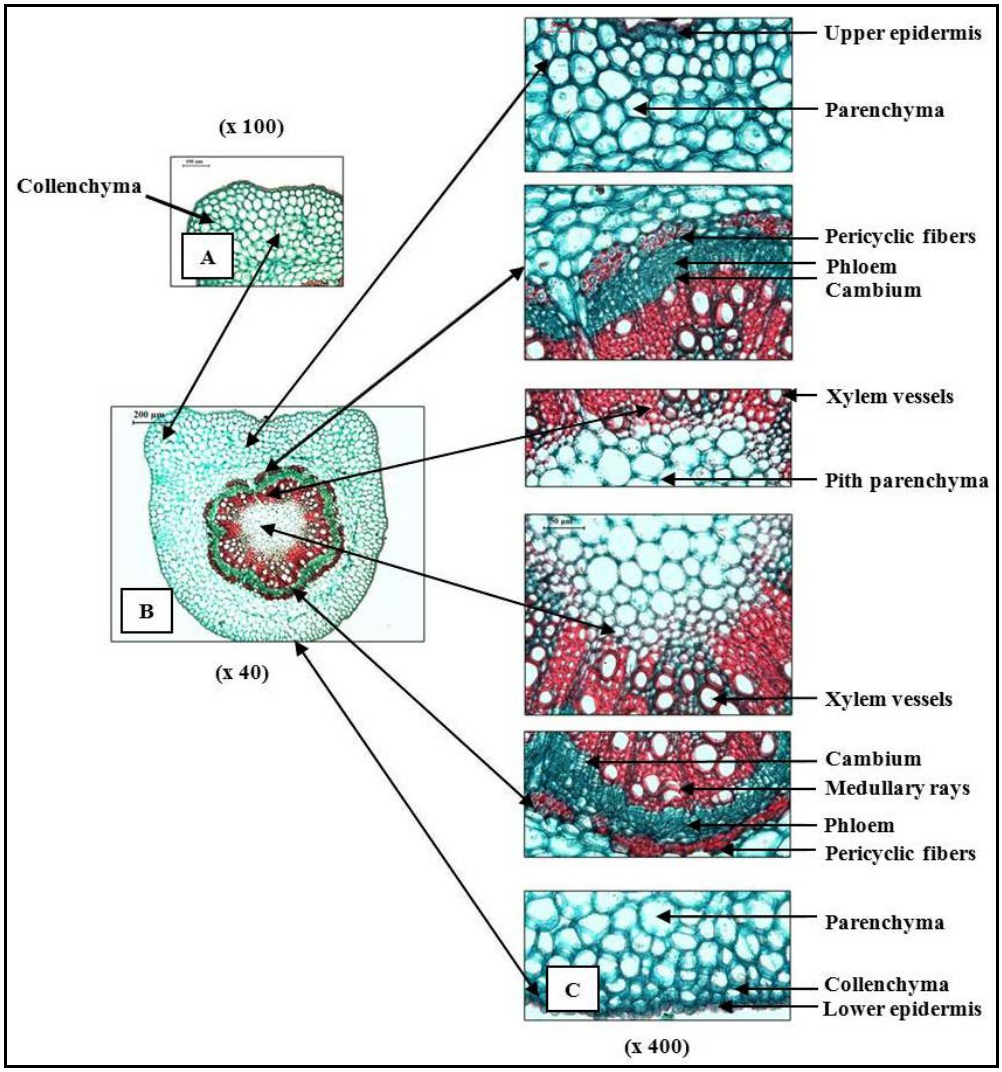

Fig 15: Detailed T.S. in leaf rachis (non-winged part).

4.2.4 The powder of the leaf

It is dark green in color with a faint aromatic odor and a slightly bitter taste. Its elements are shown in (Figure **16**).

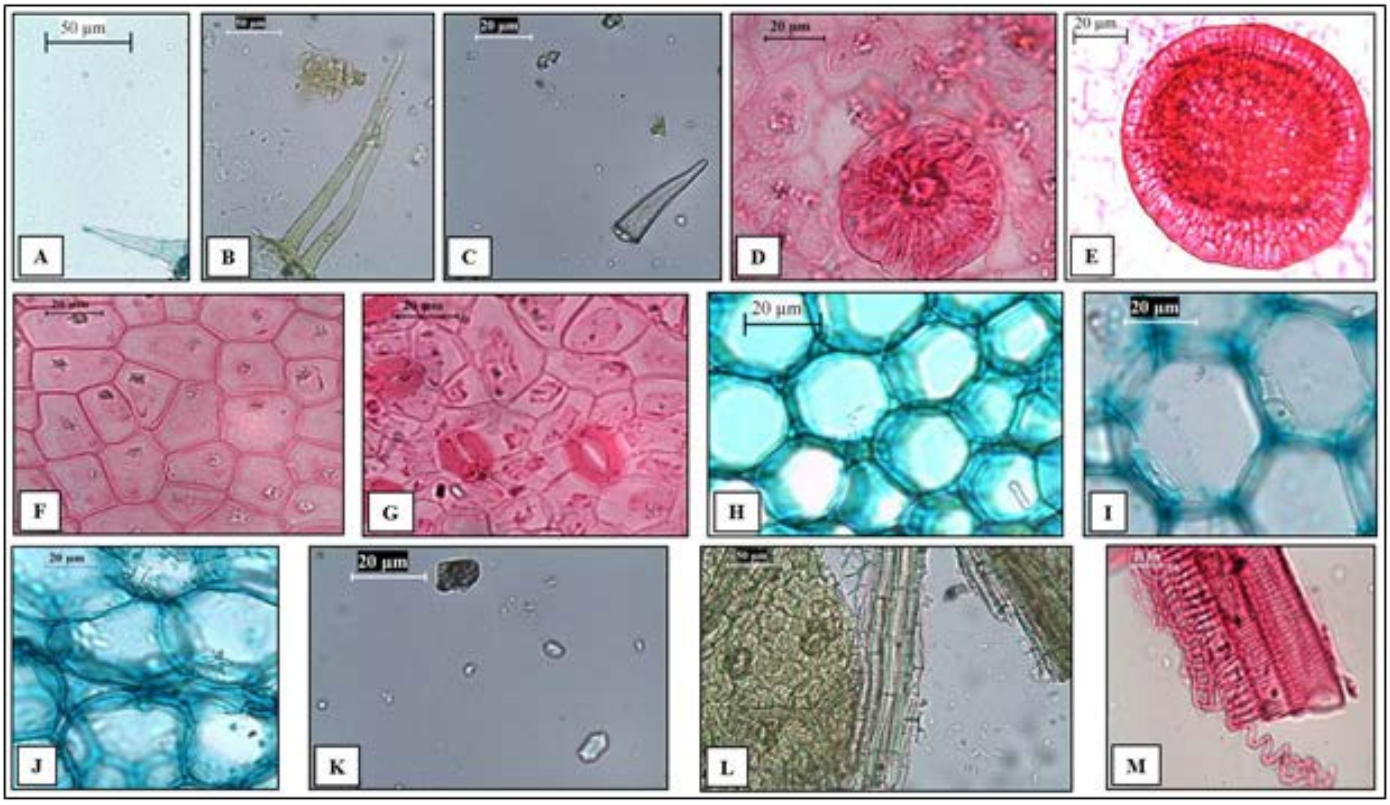

Fig 16: The powder of the leaf; **A-**Non-glandular bicellular hair (x 200), **B-**Non-glandular multicellular hairs with acute apex (x 200), **C-**Non-glandular unicellular hair (x 400), **D-**Glandular peltate hair (x 400), **E-**Glandular patelliform hair (x 400), **F-**Fragment of the upper epidermis with clusters of calcium oxalate (x 400), **G-**Fragment of the lower epidermis with anomocyctic stomata (x 400), (**H, I, J)-**Fragments of parenchyma cells containing prisms and clusters of calcium oxalate (x 400), **K-**Scattered prisms of calcium oxalate (x 400), **L-**Fragments of pericyclic fibers (x 200) and **M-**Fragments of spiral and reticulate xylem vessels (x 400).

Table 1: Microscopical dimensions of the leaflet elements of *P. cereifera* in (µm).

	Item	Length	Width	Height	Diameter
1.	Non-glandular unicellular hair	40-42-48	-----	-----	-----
2.	Non-glandular bicellular hair	50-53-56	-----	-----	-----
3.	Non-glandular multicellular hair with blunt apex	43-49-59	-----	-----	-----
4.	Non-glandular multicellular hair	90-100-154	-----	-----	-----
5.	Peltate hair	-----	-----	-----	43-53-60
6.	Patelliform hair	-----	-----	-----	90-148-150
7.	Stomata	42-50-54	28-40-46	-----	-----
8.	Upper epidermis	35-40-43	15-20-23	10-22-27	-----
9.	Lower epidermis	26-32-38	15-18-20	9-16-17	-----
10.	Palisade cells	-----	8-10-12	44-58-64	-----
11.	Collenchyma	-----	-----	-----	14-22-27
12.	Parenchyma of cortex	-----	-----	-----	17-30-37
13.	Pericyclic fibers	200-211-395	-----	-----	9-13-14
14.	Wood fibers	218-221-230	-----	-----	19-20-22
15.	Wood parenchyma	170-183-190	25-27-38	-----	-----
16.	Xylem vessels	-----	-----	-----	13-20-27
17.	Parenchyma of pith	-----	-----	-----	16-18-34
18.	Prisms of calcium oxalate	6-7-11	2-4-5	-----	-----
19.	Clusters of calcium oxalate	-----	-----	-----	8-10-12
20.	Acicular crystals of calcium oxalate	4-6-12	-----	-----	-----
21.	Starch granules	-----	-----	-----	3-4-5

5. Conclusion

From over present study entitled "Leaf Morphoanatomy Studies of *Parmentiera cereifera* Seem., Family Bignoniaceae, Cultivated in Egypt", it could be helpful in authentication of the leaf. Moreover, it is helpful in the identification of powdered drug prior using in any herbal formulations.

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