

Morphoanatomical Studies of *Markhamia Platycalyx* Leaves

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ABSTRACT

Markhamia platycalyx is one of Bignoniaceae plants. It is known as *Dolichandrone platycalyx* and its foliage leaves are eaten by primates such as chimpanzees. This study includes the botanical study of *Markhamia platycalyx* (Baker) Sprague leaves. The microscopical study of the leaflet indicates the presence of anomalous wedge shaped masses of interxylary phloem and numerous scattered vascular bundles at the periphery of the pith. Moreover, the presence of two concentric amphicribal vascular bundles on the cortical tissue of two ridges with a closed amphivasal vascular bundle at the periphery of pith of the rachis.

Keywords: *Markhamia platycalyx*, Bignoniaceae, leaf, leaflet, amphicribal, amphivasal, interxylary phloem.

INTRODUCTION

Bignoniaceae is a vast family of flowering trees and shrubs¹, comprised of 104 genera and 860 species². It is commonly called the trumpet vine or trumpet creeper family³ and also known as the Bignonia family⁴. It is rich in secondary metabolites and includes many genera of high economic and medicinal values⁵. It is found in tropical and subtropical areas with a few species in temperate climates². *Markhamia platycalyx* (Baker) Sprague (Syn. *Dolichandrone platycalyx* Baker) is one of Bignoniaceae species, which its foliage leaves are eaten by primates such as chimpanzees and black-and-white colobus monkeys in Kibale National Park^{6,7}. Some reviews consider *M. platycalyx* as a synonym of *M. lutea*, however, a recent study on cultivated plants in Egypt based on the morphological characters, classified the two plants in two different lineages⁸. The available botanical literature about *M. lutea* showed that it has isobilateral leaf, in addition to idioblasts of sclerenchyma in the mesophyll region⁹. There is no available literature about the botanical study of *M. platycalyx*. The present study was performed to investigate the morphological and the anatomical characters of *M. platycalyx* leaf, which could be helpful in authentication of the plant.

MATERIALS AND METHODS

Plant material

M. platycalyx was cultivated in El-Zohria botanical garden, Giza, Egypt (Fig. 1). The leaves were collected in May 2012. The plant was kindly identified by Mr. Mamdouh Shokry, director of El-Zohria botanical garden and confirmed by Prof. Mahmoud A. H. Abdo, Director of Floriculture Nursery (Aromatic and Medicinal plants), Faculty of Agriculture, Minia University. A voucher specimen (Mn-Ph-Cog-015) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia

University. The plant material used for the botanical study was taken from fresh samples, as well as from the samples preserved in ethanol-glycerin-water (1:1:1) and stored in well-closed containers. The leaves were air-dried and reduced to fine powder suitable for microscopical examination.

*Taxonomy M. platycalyx Sprague belongs to*¹⁰

Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakingdom: Streptophyta Superdivision: Embryophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Infradivision: Angiospermae, Class: Magnoliopsida, Superorder: Asteranae, Order: Lamiales, Family: Bignoniaceae, Genus: *Markhamia* Seem. ex Baill. and Species: *M. platycalyx* (Baker) Sprague.

Dyes

Safranin, light green, phloroglucinol and concentrated



Figure 1: A photo of *M. platycalyx*.

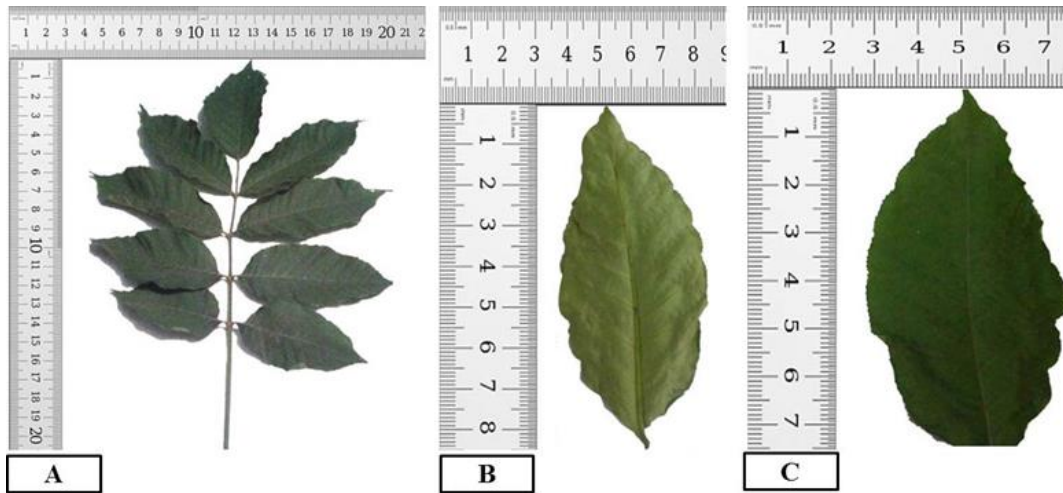


Figure 2: A photo of *M. platycalyx*; A: The leaf, B: Lower and C: Upper surface of the leaflet

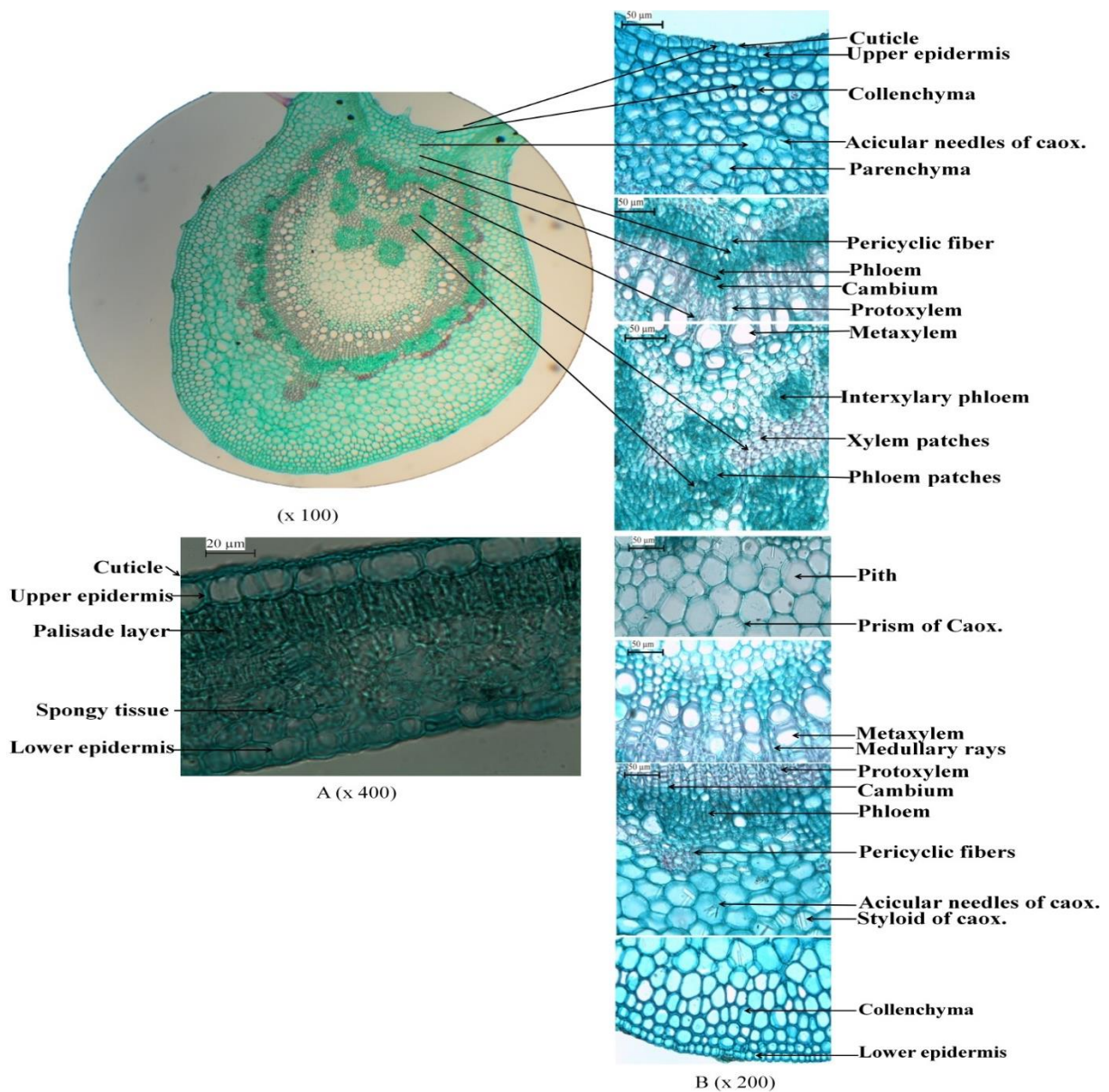


Figure 3: Detailed T.S. of the leaflet; A: Lamina region and B: Midrib region.

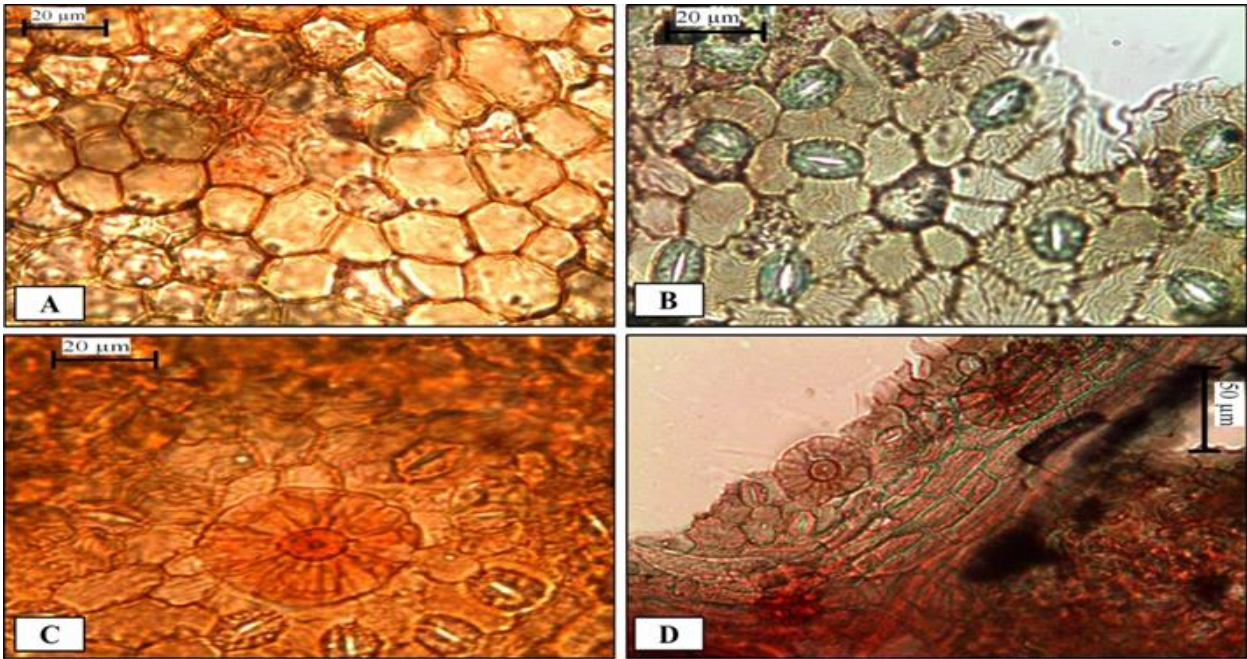


Figure 4: Surface preparation of the leaflet; A: Upper epidermis, B: Lower epidermis showing anomocytic stomata, C: Lower epidermis showing peltate hair (in surface view) and D: Peltate hair and neural epidermis. All (x 400) except D (x 200).

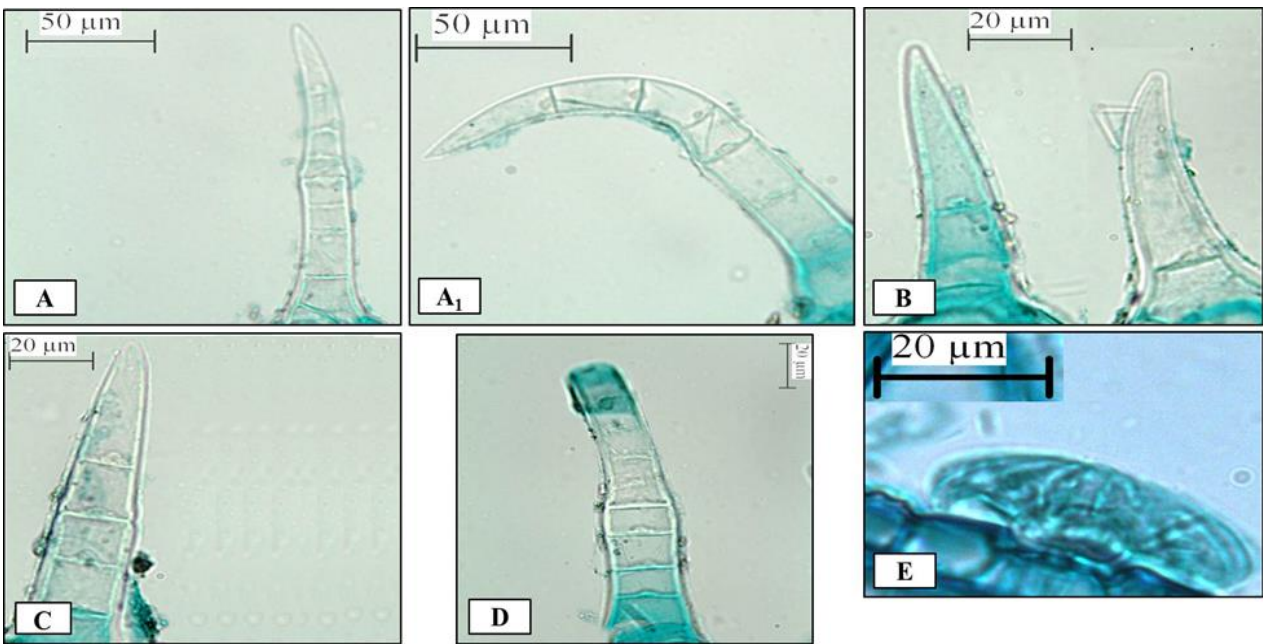


Figure 5: A: Non-glandular multicellular hair (3-8) cell with acute apices, B: Non-glandular multicellular hair (2-3) cell conical in shape with acute apices, C: Non-glandular multicellular hair (3-5) cell with rounded apices, D: Non-glandular multicellular hair (2-9) equal cells with blunt apices and E: Glandular hair of peltate type (in side view). All (x 400) except A (x 200).

hydrochloric acid were used for staining the leaflet sections and the powder.

Microscopic studies

Surface preparations, transverse sections (T.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 12.2 megapixels digital camera, Samsung (Korea).

RESULTS AND DISCUSSION

Macroscopical characters of the leaves

The leaves are normally arranged in groups at the ends of the branches (Fig. 1). They are imparipinnate compound with opposite phyllotaxis. Each leaf is composed of seven

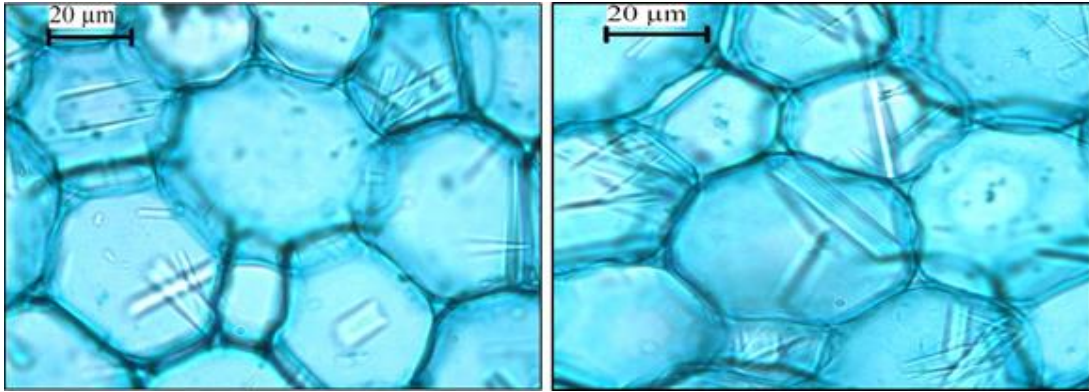


Figure 6: Parenchyma cells of the cortex, showing prisms, acicular needles, styloids and twin prisms of calcium oxalate (x 400).

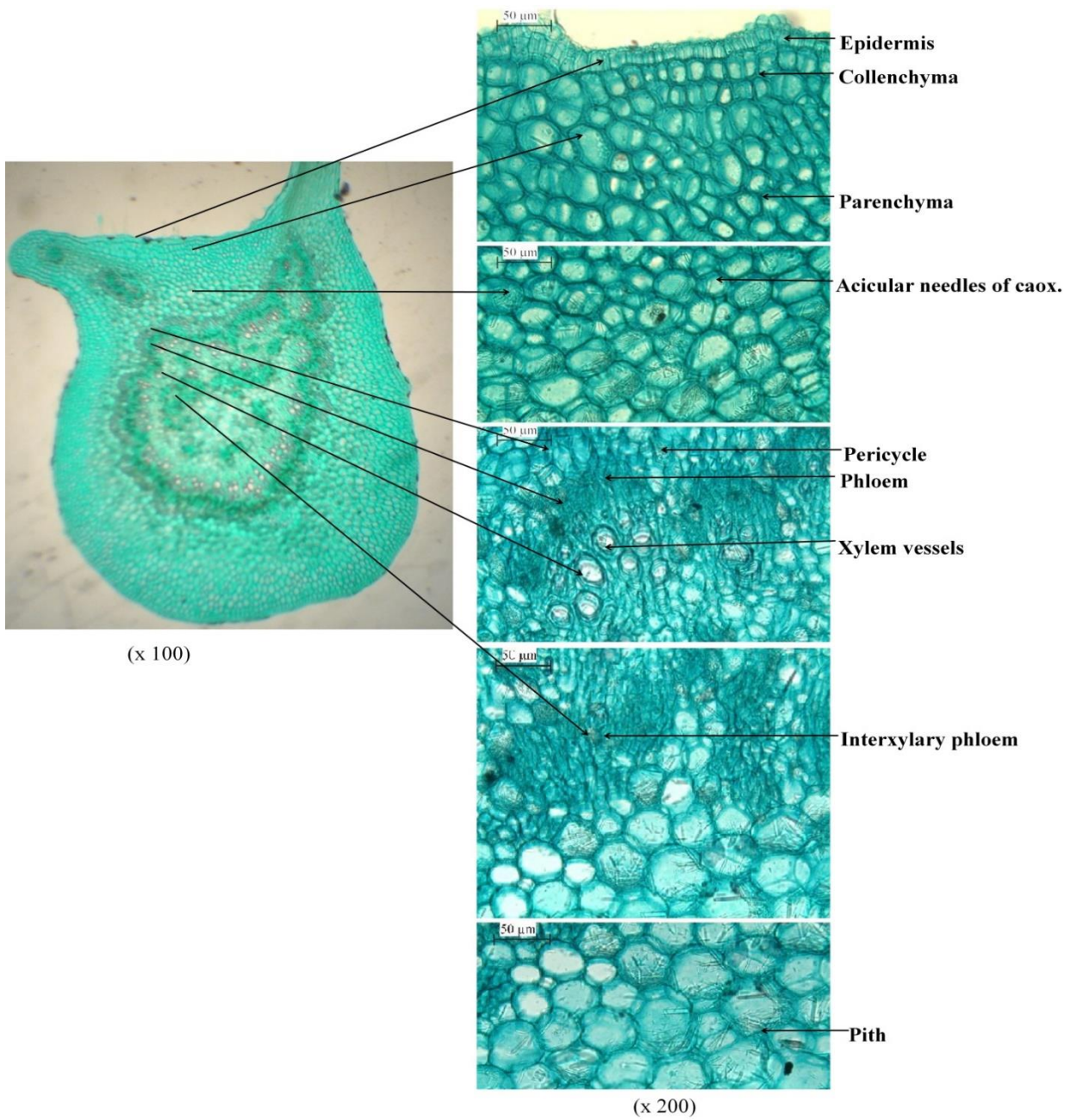


Figure 7: Detailed T.S. of the petiolule.

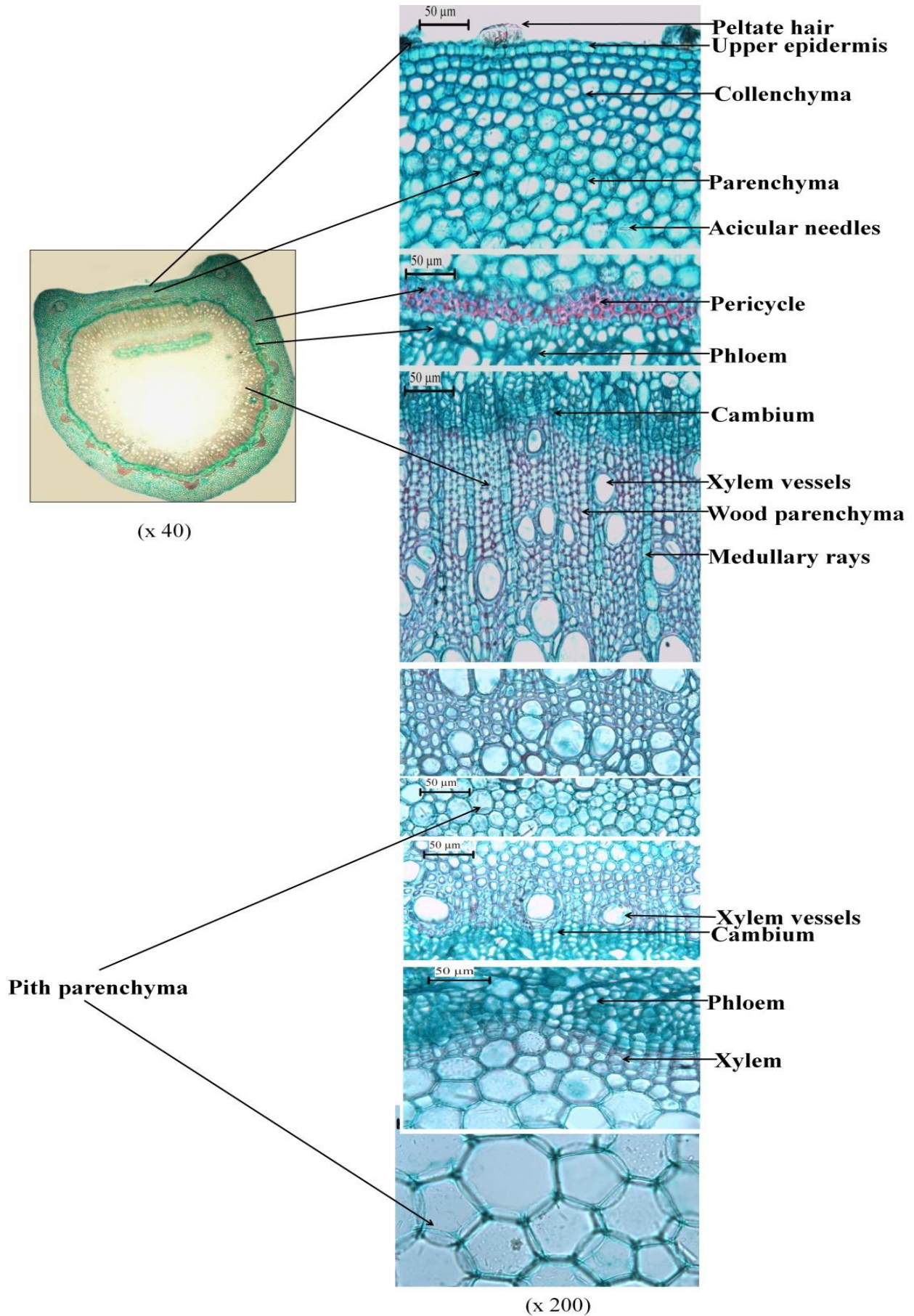


Figure 8: Detailed T.S. of the rachis in the middle region between the two ridges.

or nine leaflets, carried on long rachis, an elongation of the petiole measuring 10-15 cm in length. The lateral leaflets have very short petiolules nearly 0.1 cm, while the terminal leaflet has simple tendril of 2-4 cm in length. The leaflets are obovate in shape, measure 6.5-17 cm in length and 4-11.5 cm in width in the middle part. The leaflet has serrate margin, acuminate apex, asymmetric base and shows reticulate venation. The surface of the leaflet is hairy and green in color, where the upper surface is darker than the lower one (Fig. 2).

Microscopical characters of the leaflet

A transverse section in the leaflet blade is planoconvex in the outline with prominent midrib on the lower surface (Fig. 3). The lamina has a dorsiventral structure with a heterogeneous mesophyll. The palisade layer is disrupted in the midrib region with a mass of subepidermal collenchyma (Fig. 3A), in addition to the presence of another mass of collenchyma below the lower epidermis in the midrib region. The pericycle consists of groups of lignified pericyclic fibers lining the vascular bundle, interrupted by a few parenchyma cells (Fig. 3). The vascular system of the midrib is a quite continuous ring but somewhat flattened towards the adaxial side¹¹. It is formed of a collateral vascular bundle with central pith, surrounded by a continuous ring of phloem, cambium and xylem. The transverse section reveals the presence of anomalous wedge shaped masses of interxylary phloem¹², in addition to, numerous scattered vascular bundles at the periphery of the pith towards the upper epidermis (Fig. 3). The upper epidermis is formed of one row of square to subrectangular cells as seen in the transverse section (Fig.

3). In surface view, the cells appear isodiametric polygonal, with slightly wavy anticlinal walls covered with a thin, faintly striated cuticle with absence of stomata (Fig. 4A and 10A). Both of glandular and non-glandular trichomes are present. The glandular hair is peltate type with unicellular stalk and multicellular head of 6-18 thin walled radiated cells covered by a thin and smooth cuticle^{9,13} (Fig. 4C, 4D, 5E and 10C). The non-glandular hairs are less abundant, including four types; first type, multicellular, uniseriate hairs of 3-8 cells with an acute apex and sometimes curved at the apex (Fig. 5A and 5A₁). The second type is multicellular uniseriate conical-shaped hairs, having 2-3 cells, the terminal cell is longer than the others with acute apices (Fig. 5B), while the third type is of multicellular uniseriate hairs, having 2-9 cells with blunt apices and the cells are nearly equal in size (Fig. 5D) and lastly the fourth type is of multicellular uniseriate hairs of 3-5 cells with rounded apices (Fig. 5C). All the aforementioned hairs are covered with a smooth cuticle. The lower epidermis is formed of one row of square to subrectangular cells as seen in the transverse section (Fig. 3). In surface view, the cells appear polygonal, with more sinuous anticlinal walls, covered with a thin, striated cuticle. The stomata are abundant and of anomocytic type¹⁴, surrounded by 4-5 subsidiary cells (Fig. 4B and 10B). The hairs are similar to those of the upper epidermis, but more scattered on the midrib region. The neural epidermis consists of slightly elongated rectangular cells arranged in rows with straight anticlinal walls (Fig. 4D). The mesophyll is a heterogeneous mesophyll, showing one row of the upper palisade with columnar, cylindrical and

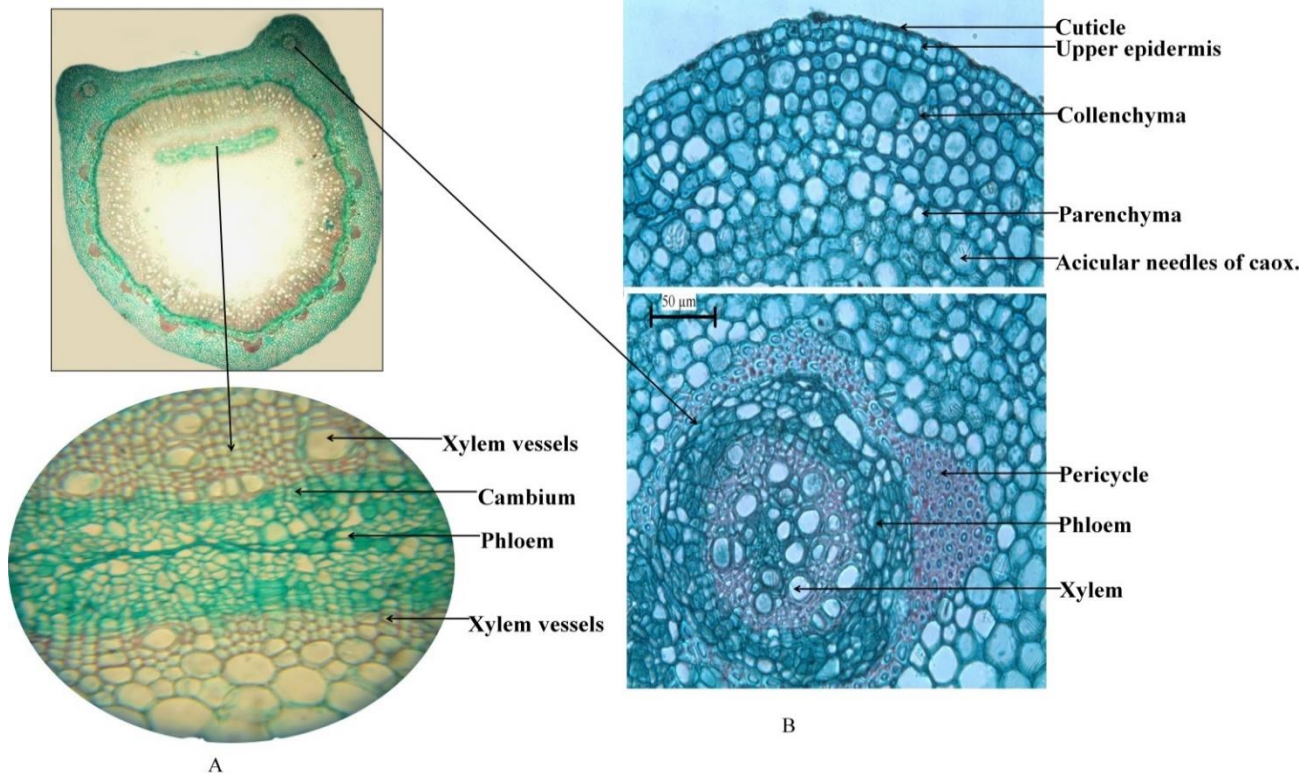


Figure 9: Detailed T.S. of the rachis; A: Amphivasal V.B. in the pith region and B: Amphicribial vascular bundle in the ridge region (x 200).

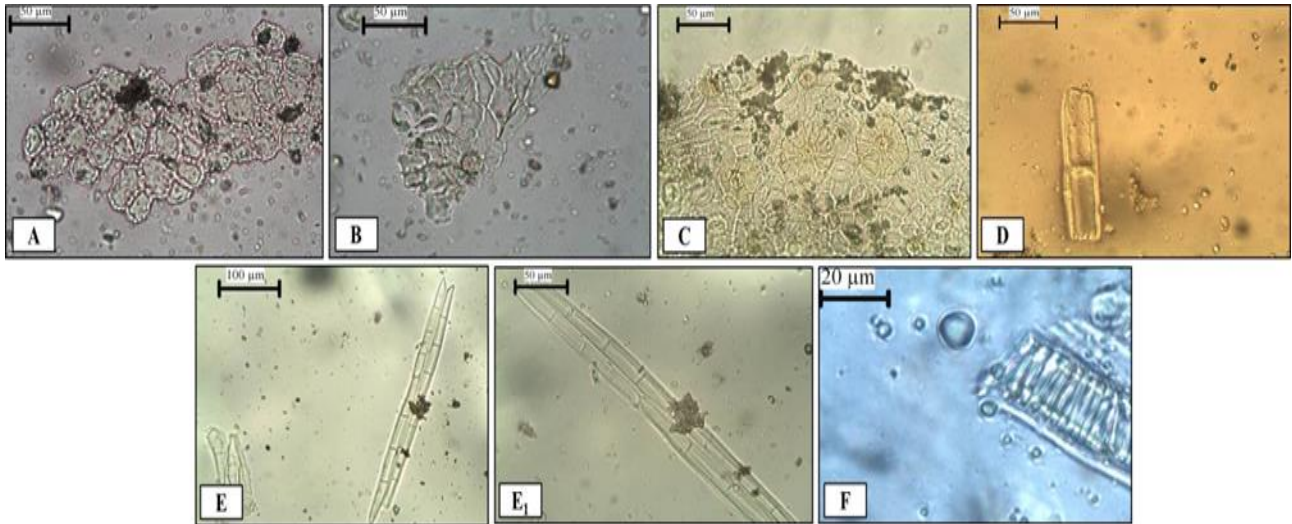


Figure 10: Leaf powder; A: Upper epidermis, B: Lower epidermis showing anomocytic stomata, C: Peltate hairs in surface view, D: Medullary ray cells, E, E₁: Pericyclic fibers and F: Spiral xylem vessel. All (x 200) except E (x 100) and F (x400).

Table 1: Microscopical dimensions of the leaf elements of in *M. platycalyx* (µm).

Item	Length	Width	Height	Diameter
1. Peltate hair			13-19-27	21-36-43
2. Non-glandular conical-shaped hair	62-64-71			
3. Non-glandular hair with blunt apex	116-129-132			
4. Non-glandular hair with rounded apex	64-96-99			
5. Non-glandular hair with acute apex	156-166-192			
6. Stomata	15-16-18	11-12-13		
7. Upper epidermis	21-23-28	12-16-18	8-10-14	
8. Collenchyma cells				15-19-31
9. Parenchyma cells				16-25-33
10. Pericyclic fibers	280-336-372	12-16-18		
11. Medullary ray cells	63-65-73	21-25-27		
12. Lower epidermis	16-20-23	10-13-17	6-8-10	

thin walled cells, containing chloroplasts. The palisade layer represents about one third of the distance between the two epidermises (Fig. 3A). This layer is disrupted in the midrib region with a mass of subepidermal collenchyma (Fig. 3). The spongy tissue is formed of more or less rounded, thin walled chlorenchymatous cells with wide intercellular spaces, forming about 4-5 rows (Fig. 3A). The mesophyll is transversed by separated strands of small vascular bundles representing the lateral veins. The cortex: The cortical tissue consists of both upper (4-5 rows) and lower (2-3 rows) subepidermal collenchymatous layers (Fig. 3B). The collenchyma cells have thick cellulose walls with no intercellular spaces. It is followed by rounded to oval parenchyma cells, having thin cellulose walls with intercellular spaces surrounding the main vascular bundle of the midrib. The parenchyma below the bundle is formed of 7-9 rows, wider than that above the bundle (5-6 rows) as shown in Fig. 3B. The cortical parenchyma contains acicular needles, prisms, twin prisms and styloids of calcium oxalate (Fig. 6). The endodermis is indistinguishable.

The vascular tissue

The pericycle is formed of islets of strongly lignified pericyclic fibers, separated by parenchyma cells (Fig. 3B).

The islets toward the upper surface consist of 7-10 fibers, much smaller than that of the lower (25-30 fibers). The fibers are fusiform in shape, septate with thick lignified walls, narrow lumena and narrow tapered ends as shown in the powder (Fig. 10E and 10E₁). The phloem consists of thin walled, soft cellulosic elements; sieve tubes, companion cells and phloem parenchyma, forming a narrow ring. The phloem region is free from any lignified elements (Fig. 3B). The cambium is thin walled, cellulose, meristematic and rectangular cells, formed of 2-3 rows (Fig. 3B). The xylem is formed of lignified proto and metaxylem vessels, wood parenchyma and medullary rays (Fig. 3B). The vessels have mainly spiral thickenings as shown in the powder (Fig. 10F). The medullary rays are uni- to biseriate consisting of radially elongated thin-walled, slightly lignified cells (Fig. 3B and 10D). Anomalous wedge shaped masses of interxylary phloem are present inside the xylem. In addition, numerous vascular bundles, formed of patches of phloem and xylem are scattered at the periphery of the pith towards the upper epidermis¹² (Fig. 3B). The pith is crescent shaped (Fig. 3B) and consists of polygonal to rounded thin-walled parenchymatous cells with intercellular spaces, where some of them contain a few prisms of calcium oxalate.

Microscopical characters of the petiolule

A transverse section in the petiolule (Fig. 7) appears more or less rounded in outline, showing two ridges on its upper side with a shallow groove in-between. The epidermis is formed of one row of square to subrectangular cells followed by the cortical tissue, which is formed of an outer collenchymatous layer (4-5 rows) and an inner parenchymatous one (6-7 rows) containing acicular needles and a few prisms of calcium oxalate. The epidermis carries both glandular and non-glandular hairs similar to those of the leaflet, the first type is fewer in number and more abundant on the lower epidermis while the second type is numerous and abundant on the upper epidermis. The vascular system shows certain similarity in structure to that of the midrib of the leaflet. It consists of a complete ring of slightly lignified xylem region surrounded by phloem with external slightly lignified patches of pericyclic fibers. Additionally two subsidiary small vascular bundles are present in the two ridges. In addition masses of interxylary phloem, which is more abundant than those of the leaflet midrib region and are present inside the xylem enclosing small central pith¹².

Microscopical characters of the rachis

A transverse section of the rachis (Fig. 8) is planoconvex in outline, showing two ridges on its upper side. Its structure is more or less similar to that of the midrib region of the leaflet. Below the epidermis, there is a hypodermal collenchyma layer of 3-4 rows which increases beneath the ridges to 5-6 rows. The cortical parenchyma is formed of 6-8 rows of thin walled rounded cells, containing prisms, styloids and acicular needles of calcium oxalate. The vascular system is an O-shaped vascular bundle¹⁵, represented by a central bundle consisting of a complete ring of xylem surrounded by cambium, phloem and pericycle and enclosing wide parenchymatous pith in the center, occupying approximately 1/2 of the whole section. The medullary rays are thin walled radially elongated cells of 2-3 rows¹⁵. There are two concentric vascular bundles on the cortical tissue of the two ridges, each bundle consists of a complete ring of xylem surrounded by phloem and pericycle named amphicribal vascular bundle (Fig. 9B), additionally, small scattered vascular bundles are located at the periphery of pith, but toward the upper surface some of them gathered together forming a closed amphivasal vascular bundle (Fig. 9A). The pith consists of parenchymatous cells, containing few prisms and acicular needles of calcium oxalate. Glandular hairs are present and similar to those of the leaflet.

The powder of the leaf

It is pale green in color with a faint characteristic odor and a disagreeable taste. The main diagnostic powder elements are:

Fragments of the upper epidermis in surface view, show polygonal isodiametric cells, with slightly wavy anticlinal walls covered with a faintly striated cuticle with no stomata (Fig. 10A).

Fragments of the lower epidermis of the lamina in surface view, show polygonal isodiametric cells, slightly elongated with sinuous anticlinal walls covered with a striated cuticle and anomocytic stomata (Fig. 10B).

Glandular hairs of peltate type: an unicellular stalk and multicellular head of 6-18 thin walled radiated cells covered by a thin and smooth cuticle (Fig. 10C).

Fragments of medullary rays: radially elongated thin-walled cells with slightly lignified walls (Fig. 10D).

Fragments of pericyclic fibers: fusiform in shape, septated with thick lignified walls, narrow lumina and narrow tapered ends (Fig. 10E and 10E₁).

Fragments of xylem vessels with spiral thickenings (Fig. 10F).

CONCLUSION

By comparing the available botanical literature about *M. lutea* with the present study, which showed dorsiventral leaf with no sclerenchyma in the mesophyll, confirming that *M. lutea* and *M. platycalyx* are two different plants.

CONFLICT OF INTEREST

We declare that no conflict of interest.

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