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FLORAL AND EXTRAFLORAL NECTARIES OF *MORUS MACROURA* MIQ. (FAMILY. MORACEAE) CULTIVATED IN EGYPT

Samia S. Hafez¹, Wafaa H. B. Hassan¹, Dalia I. Hamdan²* and Mai M. Karousa¹

¹Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt. ²Pharmacognosy Department, Faculty of Pharmacy, Menoufia University, Shibin Elkom 32511, Egypt.

*Corresponding Author: Dalia I. Hamdan

Pharmacognosy Department, Faculty of Pharmacy, Menoufia University, Shibin Elkom 32511, Egypt.

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ABSTRACT

The macro-and micromorphological characters of the inflorescence, infructescence, leaf and stem of *Morus macroura* Miq are presented with the aim of finding their diagnostic characters by which the plant can be easily identified in both the entire and powdered forms. The intriguing thing during the current research, is the presence of nectariferous tissues for the first time in floral and extra floral organs of *M. macroura* Miq.

KEYWORDS: Inflorescence, infructescence, leaf, stem, nectariferous tissues, Morus macroura Miq.

INTRODUCTION

Family Moraceae (Mulberry or fig family) is a family of flowering plants comprising about 40 genera and approximately 1000 species of monoecious rarely dioecious plants.^[1] It is distributed mostly in tropical and subtropical regions.^[2] Moreover, family moraceae specially all species of Ficus and Artocarpus are characterized by the presence of glandular spots with a waxy surface.^[3] The term of waxy gland in *Ficus* benjamina L. is substituted by phenolic gland after carrying out histo-chemical tests.^[4] The species belonging to this family may be trees, shrubs, woody climbers or herbs. The height of the trees is ranged between 20-60 m tall and formed of short trunk with several branches.^[5] The plant species are characterized by the presence of latex which is distributed in elongated cells called laticifers and secretory trichomes. Latex is usually fluid with various colours or may be milky white secretion, where, genus Morus contains a colourless fluid.^[6] Nectaries (external secretory structures) are either floral nectaries (responsible for pollination) situated within the flower in certain locations or extrafloral ones (antiherbivore protection) present on the vegetative organs.^[7-9] Moreover, the nectary cells are specialized small thin walled parenchyma cells containing dense cytoplasm, gologi apparatus, endoplasmic reticulum, small vacuoles and large nuclei.^[8] So, nectaries are stained red with neutral red or phenol red at PH at about 6.8 to 8.0.^[10,11] On ripening or by foraging vector, the cuticle of the secretory layer is ruptured causing the release of accumulated nectar beneath.^[12,13]

Economically, the mulberry leaves are considered a unique feeding for GR66 mutant larvae of silk worms.^[14]

Also, they used as food stuff and has important characteristic features in controlling environmental pollution, improving air quality, increasing water retention capacity and remediation of heavy metals from polluted soils.^[15] Morus species displayed many biological activities as anticancer.^[16] and antioxidant.^[17] which attributed to various classes of secondary metabolites specially flavonoids and other phenolic contents.

Morus macroura Miq. (King white mulberry, shahtoot mulberry, or long mulberry) is native to Pakistan and ubiquitous in tropical montane, forests of China, Malaysia, India, Thailand and Indonesia and is considered the most disease resistant species.^[18] The plant species is fast-growing deciduous tree up to 15 m tall and exhibited many significant biological activities as antioxidant, anti-inflammatory,^[19] antinematode and moderate antifungal.^[20] During the current study the authors found ingathering large number of ants on the leaves of the plant under investigation that indicating the presence of nectaries and this is in agreement with the reported data.^[21,22] According to the literature in hand, there is no direct report about the presence of nectariferous tissue in family Moraceae as well as no report about macro and micro morphological characters of Morus macroura Miq. So, it was deemed of interest to study morphological and anatomical features of this plant.

MATERIAL AND METHODS Plant material

The fresh plant materials were collected from private garden in Belbis (Sharkeya governorate, Egypt) on May, 2014 and kindly identified by Prof. Dr. Abdelhalem Abdelmogali, Taxonomy researcher, Ministry of Agriculture, Dokki- Cairo (Egypt). A voucher specimen (MM100) was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Zagazig University. Samples of different organs were preserved in a mixture of ethyl alcohol/ glycerin (1:1, v/v) and stored in a tightly closed container. Another sample of the same organs were separately air-dried and reduced to fine powder for phytochemical investigations. Sections in leaf, stem and peduncle are stained with safranin and fast green dye. While, the other sections of inflorescence and infructescence are stained with phloroglucinol and concentrated HCl. Elements of plant organs are stained with phenol red, methylene blue, Sudan III and Fehling's reagents.

RESULTS

I. Macro-morphology

Morus macroura Miq. is a perennial plant with medium sized tree. The main trunk "Fig. 1A" is erect and cylindrical reaches 1-1.8 m in height and 40-60 cm in diameter with greenish brown rough surface and showing deep longitudinal and transverse fissures. The stem branch "Fig. 1B" has greenish brown bark, relatively smooth, flexible when fresh, hard when dry, broken with fibrous fracture and shows small white lenticles, brown leaf scars measured about 2-5 mm in length, patches of cork and exuding latex on cutting. It has internodes that measure 3-9 cm in length and 0.3 -1.5 cm in diameter. The branch has pubescent axillary buds "Fig. 1C" measuring about 0.2-1 x 0.5 cm. The buds are green in colour changed brown at a later stage. Leaves "Fig. 1D" are thick, coriaceous, pubescent on the lower surface and on the petioles. They are arranged in two opposite vertical rows "Fig. 1B" and have linear-lanceolate lateral deciduous stipules "Fig. 1C" that measured 0.2-0.6 cm long and are sparsely to densely puberulous to hirtellous. The lamina is ovate to sub-ovate with entire or 2 to 3 lobed margins. The tips of lobes are well rounded. Lamina measures 7-15 cm in length and 3-8 cm in width. The leaf lamina shows serrate to denticulate margin, acute to acuminate apex and symmetric sub-cordate to rounded base. Leaf petiole is cylindrical, green with 1.5 - 6 cm long. The venation is brochidodromous with pinnately venation for all veins and palmately veined for three main lateral veins. Both leaves and young shoots contain colourless latex. The species under investigation is pistillate plant.

The inflorescence "Fig. 1E" is drooping catkin and comprises more than 70 florets arranged on a solid more or less cylindrical, green rachis with pubescent surface and measures 4 to 6 cm in length and 0.8 to 1 mm in diameter. Inflorescence is pedunculated in which peduncle is cylindrical in shape with 3-4 cm in length and 0.8 to 1 mm in diameter. The individual floret "Fig. 1F" is sessile, consists of green perianth with four tepals and gynoecium. Each tepal "Fig. 1G" is small inconspicuous in size which stimulated during fertilization and participate in fruit formation. It

measures 0.5 to 1 mm in length and 0.5 to 0.8 mm in width. Tepal is yellowish green in colour, membranous in texture, obovate in shape with entire margin. Each tepal has three main veins with palmate venation and the other lateral veins are pinnately veined. Gynoecium "Fig. 1H" is bicarpellary, syncarpus consists of superior, unilocular ovoid, slightly elongated ovary that is broad from the lower end and measures 0.3-0.7 mm in length and 0.15 to 0.35 mm in width. The ovary "Fig. 1I" carries one basal ovule, short single cylindrical style "Fig. 1H, J" that measured 0.1 to 0.2 mm in length and 0.05 to 0.1 mm in width and two long divergent papillosed stigma "Fig. 1H, J" measuring 2 to 3 mm in length and 0.5 to 1 mm in width. The infructescence "Fig. 1K" is sorosis with persistent style and stigma. It is formed of a group of drupelets. It is green when unripe but after ripening it becomes yellowish white with 6-12 cm in length. Each drupelet "Fig. 1L" is characterized by the presence of fleshy tepals "Fig. 1M" fleshy exocarp and ellipsoid to ovoid lignified endocarp that is resulted from the maturation of inner part of the ovary wall. The tepal "Fig. 1M" is colorless, fleshy with two large marginal groups of nectaries and other scattered at the apex of the adaxial surfaces. Each group is an aggregate of individual platiform glands vary in number, size and structure. The tepal shows also longitudinal and transverse secretory vascular strands connect nectaries and showing blisters due to the released nectar "Fig. 1N".



"Fig. 1" Macromorphology of *Morus macroura* **Miq.:** Photographs for trunk (A, x 0.228), stem branch (B, x 0.164), axially buds (C, x 0.4 small branch with leaves (D, x 0.366), the female inflorescence. (E, x 0.42), a floret(F), floret sepal (G), the gynoecium (H), Radial Longitudinal Section (RLS) of the floret (I), single style with papillosed stigma (J), the infructescence (K), a drupelet (L), the tepal showing longitudinal and transverse secretory vascular strands connecting nectaries (M) and L.C. of a part of infructescence tepal showing longitudinal secretory vascular strands with exudated nectar (N). from F- N (All x 100 except K x 0.37 and N x 400).

II- Micromorphology 1-The inflorescence

The peduncle

A transverse section of the peduncle "Fig. 2A, B" is circular in outline and shows outer epidermis, almost wide cortex formed of outer collenchyma and inner parenchyma. The vascular tissue is formed of a complete ring of collateral vascular bundles surrounding narrow parenchymatous pith. The pericycle is formed of alternative batches of parenchyma and collenchyma.

Epidermis

The epidermal cells of the peduncle "Fig. 2A, B, C" are polygonal, slightly axially elongated cells with straight anticlinal walls and covered with thin smooth cuticle. The cells measure about 10- 30μ in length, 6- 14μ in width & 2- 8μ in height. Stomata are absent.

Trichomes

The epidermis is characterized by the presence of covering trichomes as bristle type which is unicellular, terminating gradually into curved apex "Fig. 2D" and measured about 90μ - 140μ in length and 2- 8μ in width.

Cortex

The cortex "Fig. 2A, B" is wide, formed of two zones, the outer zone consists of 2-3 layers of more or less rounded collenchyma cells. The cells measure 2-6 μ in diameter. The inner zone is composed of 6-8 rows of rounded parenchyma cells, measure 8-20 μ in diameter and contain druses of calcium oxalate scattered randomly and measure 2-6 μ in diameter. Also, oval simple or compound (2-3 components) starch granules are present, which measures 2-8 μ in diameter as well as rosettes of calcium oxalate "Fig. 2E" which measure 2-16 μ in diameter.

Pericycle

The pericycle 'Fig. 2A, B" is formed of groups of collenchyma cells measure 4-6 μ in diameter, each group

is abutting the vascular bundle and separated by parenchyma that measure $10-12 \mu$ in diameter.

Vascular tissue

The vascular tissue "Fig. 2A, B" is formed of complete ring of collateral vascular bundles surrounding narrow parenchymatous pith.

The phloem "Fig. 2A, B" consists of polygonal, thin walled cellulosic elements.

The xylem "Fig. 2A, 2B, 2F" is formed of lignified spiral xylem vessel measuring 2- 8μ in diameter and moderately thick-walled cellulosic wood parenchyma measuring 2- 4μ in diameter.

The pith

The pith "Fig. 2A, B" is narrow and composed of rounded parenchyma cells with wide intercellular spaces measuring $10-14\mu$ in diameter. It contains druses of calcium oxalate measuring 4-6 μ in diameter.



"Fig. 2" The peduncle: Diagrammatic transverse section (A), Detailed transverse section (B), Epidermal cells (C), Bristle trichomes (D), Rosettes of calcium oxalates (E), Spiral xylem vessels (F). (All x 400 except A x 40). col., collenchyma; dr., druses; epi., epidermis; p., pith; par., parenchyma; per., pericycle; ph., phloem; ros., rosettes of calcium oxalates; xy., xylem.

The rachis

A transverse section of the rachis "Fig. 3A" is irregular in outline and notched at different regions due to loosed florets. It showed an epidermis surrounding a parenchymatous cortex followed by the pericycle. The vascular system consists of a ring of open collateral vascular bundles, where the phloem to outside and xylem to inner side surrounding a parenchymatous pith.

The epidermal cells

The epidermal cells "Fig. 3B" are rectangular and slightly axially elongated with straight anticlinal walls covered with thin smooth cuticle and measure 6 -12 μ in length, 6-10 μ in width and 2-6 μ in height. Stomata are absent.

Trichomes

The epidermal cells of rachis show covering trichomes "Fig. 3C" including bristle type which measure 140-180 μ in length and 2-10 μ in width.

The cortex

The cortex "Fig. 3A" is wide formed of several layers of thin walled rounded parenchyma cells measuring $4-10\mu$ in diameter and characterized by the presence of druses

of calcium oxalate (2-4 μ in diameter). The endodermis is undifferentiated.

Pericycle

The pericycle "Fig. 3A" consists of discontinuous band of collenchyma measures $4-6\mu$ in diameter separated by thin walled rounded parenchyma cells that measure $6-10\mu$ in diameter.

The vascular system

The vascular system "Fig. 3A" consists of open collateral vascular bundles surrounding narrow parenchymatous pith.

The phloem "Fig. 3A" consists of polygonal, thin walled cellulosic elements.

The xylem "Fig. 3A, D" is formed of lignified spiral xylem vessel measuring 2- 8μ in diameter and moderately thin walled cellulosic wood parenchyma.

The pith

The pith "Fig. 3A" is narrow and composed of thin cellulosic rounded parenchyma cells with narrow intercellular spaces.



"Fig. 3" The rachis: Diagrammatic transverse section of the rachis (A), Epidermal cells of the rachis (B), Bristle trichome (C), Spiral xylem vessels (D). (All x 400 except A x 40).

dr., druses; epi., epidermis; br.t., bristle trichomes; cor., cortex; n.g.t., non-glandular trichomes; p., pith; per., pericycle; ph., phloem; xy., xylem.

Tepals

A transverse section of the tepal "Fig. 4A" shows an outer and inner epidermises enclosing in between a parenchymatous mesophyll traversed longitudinally by three main vascular strands.

Outer and inner epidermises

The cells of the outer epidermis at the apex "Fig. 4B" showed polygonal, slightly curved anticlinal walls and covered with thin striated cuticle. While epidermal cells of apical region of inner epidermal surface cells "Fig. 4C" are polygonal with straight anticlinal walls and covered with thick smooth cuticle. Additionally, the epidermal cells at the marginal region of outer surface "Fig. 4D₁ &, D₂" are polygonal, axially elongated with straight anticlinal wall and covered with thin striated

cuticle. The cells at the marginal region of the inner surface "Fig. 4E" are polygonal, axially elongated with straight anticlinal walls and covered with thick smooth cuticle. Moreover, the cells of the middle region of outer surface "Fig. 4F" are polygonal with curved anticlinal wall, covered with striated cuticle. While those of the middle region of inner surface "Fig. $4G_1 \& G_2$ " are polygonal with straight anticlinal walls and covered with thick smooth cuticle. Finally, epidermal cells of the basal part of the outer surface "Fig. 4H" are polygonal with curved anticlinal wall and covered with striated cuticle. In contrast, the basal epidermal cells of inner surface "Fig. 4I" are axially elongated, more or less isodiametric showing sinuous anticlinal walls, covered with smooth cuticle. Dimensions of tepals epidermal cells are shown in table (1).

Stomata

Stomata are present on the outer surface "Fig. 4B, D2, F and H". They are almost rounded and of anomocytic type and measures $8-10\mu$ in length and $6-10\mu$ in width. They are numerous at the middle, less at the base and rare at the apex.

Trichomes

The inner epidermis are characterized by the presence of cystolith and covering trichomes, while bulbous trichomes are present on the outer ones. Cystolith (14-20 μ in length and 2-14 μ in width) contains calcium carbonate with swollen base sunken into mesophyll with very short mucronate apex and is numerous at the middle, less at the base and rare at the apex of the tepals "Fig. C, G₁, G₂ and I". Bulbous trichomes "Fig. 4D₁" measured 8-20 μ , 10-20 μ in length and 2-4 μ , 8-16 μ in width for stalk and head respectively and unicellular

trichomes "Fig.4E" measure 90-130 μ in length and 8-12 μ in width.

Calcium oxalate

Rosette crystals of calcium oxalate "Fig. 4J" are distributed in all over the mesophyll parenchyma with abundant presence in the middle region. They measure 10-14 μ in diameter.

Table (1):	Dimensions	of	tepals	epidermal	cells	(in
microns).						

Enidormia	Outer ep	oidermis	Inner epidermis		
Epiderinis	Length	Width	Length	Width	
At the apex	12-26	8-16	20-32	16-24	
At the margin	20-46	10-22	10-32	8-18	
At the middle	10-22	6-12	22-42	14-20	
At the base	18-32	10-16	10-30	8-16	



"Fig. 4" The perianth sepals: Diagrammatic transverse section (A), Epidermal cells of the outer surface at the apex (B), Epidermal cells of the inner surface at the apex (C), Epidermal cells of the outer surface at the margin (D1&D2), Epidermal cells of the inner surface at the margin (E), Epidermal cells of the outer surface at the middle (F), Epidermal cells of the inner surface at the middle (G1&G2), Epidermal cells of the outer surface at base (H), Epidermal cells of the inner surface at base (I), Rosettes crystal of calcium oxalates (J). (All x 400 except A x 40).

i. epi., inner epidermis; mes., mesophyll; n.g.t., non-glandular trichomes; o.epi., outer epidermis; v.b., vascular bundle.

The gynoecium

The stigma

The epidermal cells of stigma "Fig. 5A" are formed of polygonal, thin walled, axially elongated with straight anticlinal walls and covered with thin smooth cuticle. The outer periclinal walls of the cells are prolonged outwards into long conical papillae with rounded apices which measured $12-20\mu$ in length and $4-10\mu$ in width.

The style

The epidermal cells of the style "Fig. 5B" are polygonal axially elongated with straight anticlinal wall and covered with smooth cuticle. The cells measure $10-22\mu$ in length and 8 to 12μ in width.

The ovary

A transverse section of the ovary wall "Fig. 5C, D" is lenticular in outline. It consists of an outer and inner epidermises enclosing in between parenchymatous mesophyll which is traversed longitudinally by two vascular strands.

The outer epidermis of the ovary "Fig. 5C, D & E" are polygonal cells with straight anticlinal wall and covered with thick smooth cuticle. They measure $10-22\mu$ in length and $8-12\mu$ in width, while the inner epidermis is rectangular and measured about $4-12\mu$ in length and $4-8\mu$ in width. The basal part of ovary wall is characterized by thin sinuous lignified walled sclerides with wide lumen "Fig. 5F" measuring about $20-40\mu$ in diameter.

The mesophyll

The mesophyll "Fig. 5C, D" is formed of 6-8 layers of polygonal parenchyma cells with narrow intercellular spaces. They measure $10-20\mu$ in diameter. The ovary wall shows numerous small nectary glands "Fig. 5G" and this is suggested to be the inner most layer of mesocarp.



"Fig. 5" The gynoecium: Epidermal cells of stigma (A), Epidermal cells of style (B), Transvers section of the ovary wall (C), Detailed transverse section of ovary wall (D), Outer epidermis of the ovary with sclerides of basal part of ovary (E), Sclerides of basal part of ovary (F), Ovary wall showing numerous small nectary glands (G). (All x 400 except C x 100).

i.epi., inner epidermis; mes., mesophyll; o.epi., outer epidermis.

The powdered inflorescence

The powdered inflorescence is yellowish green in colour with slightly characteristic odour and slightly sweet taste. It is characterized microscopically by the following fragments:

- 1. Fragments of epidermal cells of the peduncle.
- 2. Fragments of epidermal cells of rachis.
- 3. Fragments of epidermal cells of apical part of the outer surface of tepals.
- 4. Fragments of epidermal cells of marginal region of the outer surface of tepals.
- 5. Fragments of epidermal cells of middle region of the outer surface of tepals showing anomocytic stomata.
- 6. Fragments of epidermal cells of basal part of the outer surface of tepals.
- 7. Fragments of epidermal cells of apical part of the inner surface of tepals.
- 8. Fragments of epidermal cells of marginal region of the inner surface of tepals.
- 9. Fragments of epidermal cells of middle part of the inner surface of tepals showing cystolith.
- 10. Fragments of epidermal cells of basal part of the inner surface of tepals.
- 11. Fragments of papillosed epidermal cells of stigma.
- 12. Fragments of epidermal cells of style.
- 13. Fragments of ovary wall showing numerous small nectary glands.
- 14. Fragments of outer epidermis of the ovary with sclerides of its basal part.
- 15. Sclerides of basal part of ovary.
- 16. Fragment of the mesophyll of the tepal with rosettes of calcium oxalate.
- 17. Bristle trichomes of the peduncle and rachis.
- 18. Spiral xylem vessels of the peduncle and rachis.

2-The infructescence

The four tepals and the ovary wall participating in fruit formation. They are enlarged and become succulent.

The tepal

A transverse section of the tepal "Fig. 6A, B" is formed of outer and inner epidermises enclosing in between the mesophyll which is traversed longitudinally by three main vascular strands. The outer and inner epidermises are more or less similar to those of the tepals of the inflorescence.

Mesophyll

The mesophyll "Fig. 6A, B" is formed of 3-4 layers of thin walled palisade like parenchyma cells with narrow intercellular spaces measuring 22-30 μ in length and 8-12 μ in width. It shows numerous rosettes of calcium oxalates "Fig. 6C" measuring 4-10 μ in diameter. Among the mesophyll, there are numerous nectary glands directly beneath the epidermis "Fig. 6D, E" measuring 10-20 μ in diameter for small glands and 24-45 μ in diameter for larger ones. Each gland consists of secretory zone of several rectangular cells covered with thick cuticle. The sub-secretory zone is about 3-5 layers of thin cellulosic walls measure $4-10\mu$ in diameter. These nectary glands are stained with phenol red and give positive results with Fehling's and Sudan III reagent "Fig. 6F, G & H", respectively. Fragments of mesophyll also showed the presence of secretory vascular strands and xylem vessels "Fig. 6I".

The pericarp

A transverse section of the pericarp "Fig. 7A" is formed of an epicarp, endocarp and parenchymatous mesocarp in between showing numerous nectariferous glands.

The epicarp

The epicarp "Fig. 7A, B" is formed of one row of polygonal, axially elongated cells with straight anticlinal walls covered with thick smooth cuticle. They measure $16-28\mu$ in length, $6-10\mu$ in width and $2-6\mu$ in height. Neither stomata nor trichomes are present.

The mesocarp

The mesocarp "Fig. 7A, B" is composed of wide rounded cellulosic thin walled parenchyma cells measured about 6-14 μ in diameter. They are traversed longitudinally by vascular strands with lignified xylem vessels and cellulosic phloem elements. In the mesocarp "Fig. 7B" there are numerous scattered large and small nectaries. The inner most layer of mesocarp "Fig. 7C, D, E, F" is formed completely of nectariferous tissues which are filled with mucilage. On ripening or by foraging vectors, the cuticle of the secretory layer is ruptured releasing the accumulated nectar beneath in the form of blisters "Fig. 7D, E, F"

The endocarp

The endocarp "Fig. 7G, H" is formed of polygonal isodiametric sclerides with moderately thick lignified walls and measure $10-16\mu$ in diameter.

Testa

Inside the pericarp, there is a single seed showing an outer seed coat on transverse section. It is composed of thin lignified sinuous sclerenchyma cells "Fig. 7I" with wide lumen measuring 17-22 μ in diameter, followed by parenchymatous endosperm and embryo "Fig. 7D" filled with fixed oil and proteins.



"Fig. 6" The tepal: Detailed transverse section of the tepal showing apart of nectaries (A), Diagrammatic transverse section of the tepal (B), Rosettes crystals of calcium oxalate (C), Fragment of large and small nectary glands (D), Nectary gland directly beneath the epidermis (E), Nectary gland with phenol red (F), Nectary gland with Fehling's reagent (G), Nectary gland with Sudan III (H), Secretory vascular strands and xylem vessels (I). (All x 400 except B x 100).

i.epi., inner epidermis; mes., mesophyll; nect., nectary; o.epi., outer epidermis; sec.z., secretory zone.



"Fig. 7" Fruit elements: Detailed transverse section of the pericarp (A), Epicarp and part of mesocarp with nectar gland in surface view (B), Nectary gland with methylene blue (C), Part of transverse section in fruit showing inner most layer of mesocarp with blisters (D), Part of transverse section of fruit showing complete layer of nectariferous tissues (E), Scattered ruptured nectaries of the inner most layer of mesocarp (F), Fragment of endocarp showing single scleride (G), Sclerides of endocarp (H), Sclerides of seed coat(I). (All x 400 except A, E, G x 100). cu., cuticle; end., endocarp; epic., epicarp; nect., nectary; t., testa.

The powdered infructescence

The powdered infructescence is yellowish white in colour with slightly characteristic odour and very sweet taste. It is characterized microscopically by the following fragments:

- 1. Fragments of polygonal, axially elongated cells of epidermis with straight anticlinal walls.
- 2. Fragments of polygonal thin walled cellulosic parenchymatous cells of mesocarp.
- 3. Fragments of isodiametric sclerides of endocarp.
- 4. Fragments of sinuous lignified sclerides of seed coat.

- 5. Necteriferous tissue stained with phenol red and give positive results with Fehling, methylene blue and Sudan III.
- 6. Fragments showing secretory vascular strands and xylem vessels.
- 7. Fragments of mesophyll showing rosettes crystals of calcium oxalate.

3-The leaf

A transverse section of the leaf "Fig. 8A, B & C" shows a dorsiventral structure with two layers of palisade under upper epidermis being discontinuous in the midrib region. The midrib which is more prominent on the lower surface is composed of parenchymatous cortical tissue with peripheral collenchyma cells. The center of cortical tissue shows the presence of horse shoe-like shaped vascular bundle which is formed of xylem directed towards adaxial and lateral surfaces and phloem directed towards the abaxial and lateral ones. The vascular bundle is surrounded by discontinuous collenchymatous pericycle which disrupted by parenchyma cells. Transverse section of the leaf revealed the presence of two patches of collenchyma cells, larger one in the center of vascular bundles and the smaller one at the periphery of vascular bundle towards the adaxial surface. Calcium oxalate in the form of druses, rosettes and prisms are scattered all over the tissue.

The epidermis: There were significant differences in the epidermal cells shape and dimension of both adaxial and abaxial epidermis of lamina and midrib region as follow:

The adaxial epidermis of lamina

The adaxial epidermal cells of lamina "Fig. 8D" are polygonal thin walled rectangular with slightly wavy anticlinal walls and covered with smooth cuticle. These cells contain deposit of mucilage on the inner tangential walls.

The abaxial epidermis of lamina

The abaxial epidermal of the "Fig. 8E" lamina formed of narrow lenticular cells with straight anticlinal walls and covered with thin smooth cuticle.

The adaxial neural epidermis

The adaxial neural epidermal cells "Fig. 8F" are polygonal axially elongated cells with straight anticlinal wall and covered with thin smooth cuticle.

The abaxial neural epidermis

The abaxial neural epidermal cells "Fig. 8G" are polygonal, axially elongated cells with straight anticlinal walls and covered with thin smooth cuticle. Dimensions in microns of the epidermal cells are shown in table (2).

Table	(2):	Dimensions	of	the	epidermal	cells	of	the
leaf (ir	ı mic	ron).						

Epidermis	Length	Width	Height
Adaxial lamina	10-20	6-10	6-10
Abaxial lamina	14-18	2-8	2-6
Adaxial neural	26-40	6-10	2-10
Abaxial neural	24-40	6-8	2-6

The stomata

The epidermis on the abaxial surface of the lamina "Fig. 8E" is characterized by the presence of anomocytic stomata (ranunculaceous type) which are oval in shape. They are either on the same level of the epidermis or may be slightly elevated.

Trichomes

The trichomes "Fig. 9F, G, H, I" of different types are present on both adaxial and abaxial surface of lamina and midrib. Different types of trichomes are classified into:

Glandular trichomes

Include bulbous trichomes "Fig. 9F" rarely present on the abaxial surface of lamina and midrib. This type of hair is composed of long unicellular stalk (26-34 μ in length and 8-12 μ in width) and biseriate multicelluar (2-4) head which measure 24-30 μ in length and 20-26 μ in width. This trichome contains fixed oil and tannin.

Non glandular types

Bracket trichomes "Fig. 9G" which is formed of unicellular hair with thin wall and hooked tapering apex. This type of hair is measured 60- 80μ in length and 2- 6μ in width.

Unicellular hair "Fig. 9H" with prominent base and exists on the abaxial surface of lamina, midrib and lateral epidermis. It is measured $100 - 110\mu$ in length and $2-14\mu$ in width.

Cystolith trichome "Fig. 9I" (80-100 μ in length and 2-20 μ in width is a unicellular, filiform hair with a bladder like base between normal epidermal cells. This trichome contains calcium carbonate which gives effervescence with concentrated hydrochloric acid.

The Mesophyll

The mesophyll "Fig. 8A, B" is a dorsiventral with upper palisade discontinuous in the midrib region and narrow spongy tissue. The palisade "Fig. 8A, B & 9A" consists of two layers of unequal length. The cells are cylindrical columnar and radially elongated "Fig. 8A, B". The longer layer is directed towards the adaxial surface and the cells measure 18-20 μ in length and 2-4 μ in width, while the shorter one is directed toward the abaxial surface with cells measured 12-14 μ in length and 2 – 4 μ in width.

The spongy parenchyma "Fig. 8A, B" is formed of small isodiametric parenchyma cells with wide intercellular

spaces and special arrangement containing scattered nectary glands "Fig. 9B" that measured 8-24 μ in diameter, stained red with phenol red and gave positive results with Fehling's reagents "Fig. 9L, M". The parenchyma cells measured 4-8 μ in diameter. The parenchymatous mesophyll contains rosette of calcium oxalate measuring 12-24 μ in diameter.

Furthermore; transverse section of the lamina showed idioblast of reproducible pyriform-shaped structures containing a vascular supply intermingled with a cystolith toward the adaxial epidermis "Fig. 8A". It is composed of triangular base that is formed of small parenchymatous cells measuring 12-16 μ in diameter. The body of this abnormal structure that faced towards the abaxial lamina surface is formed of numerous layers of very small parenchymatous cells measuring 2-8 μ in diameter and showed delicate vascular strand with small spiral xylem vessels. In addition, each parenchyma of this structure was occupied with rosette or prismatic crystal of calcium oxalate measuring 4-8 μ in diameter and 2-6 μ in diameter respectively.

The midrib

The cortical tissue

A transverse section in the midrib region "Fig. 8B, C" showed collenchyma with thick cellulosic wall below the upper and lower epidermises over the entire circumference. They are formed of 4-6 layers on the adaxial surface measuring 6-10 μ in diameter and 3-5 layers on the lateral and abaxial surfaces measuring 4-8 μ in diameter. They are followed by multilayered more or less rounded with thin cellulosic walled parenchyma cells with narrow intercellular spaces. They are measured 4 - 18 μ in diameter and contain simple or compound starch granules.

The endodermis is not differentiated.

The pericycle

The pericycle "Fig. 8B, C" composed of 3-5 layers of collenchymatous cells measured $2-4\mu$ in diameter, dissected by small rounded parenchymatous cells measuring $4-6\mu$ in diameter.

The vascular tissue

Table (3): The microscopical numerical values of the leaves of *M. macroura* Miq.

The vascular tissue system is recognized as an abaxial horse shoe-like shaped open collateral vascular bundles which composed of xylem "Fig. 8B, C", phloem (Fig. 8B, C). The xylem "Fig. 8B, C, 9J" is formed of lignified spiral vessels measuring 2- 8μ in diameter for protoxylem and 10-14 μ in diameter for metaxylem. In addition to, Polygonal cellulosic wood parenchyma measures 2-10 μ in diameter.

The phloem "Fig. 8B, C" consists of polygonal thinwalled cellulosic elements. In addition, phloem elements were associated with laticifers structure "Fig. 9K".

Moreover, there are two patches of collenchyma in the parenchyma region enveloped by U-shaped xylem.

The impressive thing revealed during microscopical examination of the midrib region was the deposition of different types of calcium oxalate which are described as follow:

- The cortical parenchyma "Fig. 8C" contained rosettes and prismatic crystals of calcium oxalate measuring 4-8μ in diameter, 2-4μ in diameter, respectively.
- However, the parenchymatous pericycle as well as that of phloem and xylem "Fig. 8C" exhibited the presence of rosette crystal of calcium oxalate only measuring 2-8µ in diameter.
- Moreover; star-like crystals called druses "Fig. 8C" and rosettes "Fig. 8C& 9C" were randomly scattered in the parenchymatous cell of cortical tissue and measure $4-6\mu$ in diameter, $2-8\mu$ in diameter, respectively.

Amazingly, it is found that, prismatic crystals "Fig. 9D" 2-4 μ in diameter are found in the intercellular spaces between parenchymatous cells and Y-shaped prismatic crystals in the cortical tissue "Fig. 9E" and measure 30-40 μ in length and 6-10 μ in width.

The microscopical numerical values of the leaf: Stomatal index, vein-islet number, veinlet termination number and palisade ratio data were determined and summarized in Table (3).

Toscopical numerical values of the leaves of <i>M. macroura</i> wild.				
The numerical value	Recorded value			
1. Stomatal Index: Lower surface of the lower Epidermis	11.1- 14.6			
2. Vein-Islet Number	24 - 32			
3.Veinlet-Termination Number	28-36			
4. Palisade Ratio: Upper surface	5-7			



"Fig. 8" The transverse section and epidermal cells of leaf: Detailed transverse section of the lamina (A), Diagrammatic transverse section of the leaf (B), Detailed transverse section of the midrib (C), Adaxial epidermal cells of the lamina showing cystolith trichome (D), Abaxial epidermal cells of the lamina showing stomata (E), Adaxial neural epidermal cells (G). (All x 400 except B x 40).



"Fig. 9" The leaf key elements: Palisade cells and spongy parenchyma (A), Nectary (B), Rosettes of calcium oxalate (C), Prisms of calcium oxalate in intercellular spaces (D), Y-shaped prismatic crystals of calcium oxalate (E), Bulbous trichomes (F). Bracket trichomes (G), Non glandular uniseriate unicellular trichomes (H), Cystolith trichomes (I), Spiral xylem vessels (J), Laticifers (K), Nectaries with phenol red (L), Nectaries with Fehling's reagent (M). (All x 400).

ab.epi., abaxial epidermis; ad.epi., adaxial epidermis; bul.tr., bulbous trichome; col., collenchyma; col.per., collenchymatous pericycle; cu., cuticle; dr., druses; idio., idioblast; lat.t., laticeferous tube; pal., palisade; par., parenchyma; ph., phloem; pr., prism; ros., rosette; xy., xylem., y-pr., y-shaped prismatic crystals.

The petiole

A transverse section of the petiole "Fig. 10A, B" is nearly rounded in outline on the abaxial surface and slightly channeled on the adaxial surface. It shows an outer epidermis surrounding a cortex formed of an outer collenchyma and inner parenchyma. The vascular system consists of five collateral vascular bundles separated by parenchymatous tissue. Additionally, there is a central pith formed of parenchyma with three patches of collenchyma.

Epidermis

The epidermis of the petiole "Fig. 10A, B and C" is covered with thin smooth cuticle. It is composed of

rectangular cells with straight anticlinal walls and measured about 26-48 μ in length, 4-10 μ width and 22-44 μ in height. Stomata are absent.

Trichomes

The epidermis of the petiole is characterized by the presence of non-glandular trichomes "Fig. 10D" formed of long unicellular stalk measuring 46-80 μ in length & 2-4 μ in width and unicellular swollen base which measure 22-28 μ in diameter. Moreover, bulbous trichomes "Fig. 10E" are present laterally and consist of long unicellular stalk that measure 24-30 μ in length & 4-16 μ in width and biseriate head measuring 22-26 μ in diameter. Also, few bracket hair "Fig. 10F" that measure 110- 130 μ in

length & 2-6 μ in width are found on the adaxial and laterally.

Cortical tissue

The cortical tissue "Fig. 10A, B" starts with 5-7 layers of collenchyma located beneath epidermis of the adaxial surface and 7-9 layers on abaxial side and laterally. Furthermore, the adaxial collenchyma cells measured 2- 6μ in diameter while the abaxial ones measured 2- 8μ in diameter. Additionally; multilayered parenchymatous cells were present on both adaxial (13-16 layers) and abaxial (5-7 layers) surfaces.

These are formed of thin walled, more or less rounded parenchyma cells with narrow intercellular spaces and measure $2-8\mu$ in diameter for adaxial surface and $2-6\mu$ in diameter for abaxial surface.

The pericycle

The pericycle "Fig. 10A, B" is formed of groups of collenchyma abutting the vascular bundles and separated by parenchyma.

The vascular tissue

The central part of the petiole "Fig. 10A, B" is occupied with five collateral vascular bundles separated by parenchymatous tissue. Each vascular bundle is formed of lignified Xylem and cellulosic phloem.

The xylem

The xylem "Fig. 10A, B" consists of lignified spiral vessels "Fig. 10G" measured 2-6 μ in diameter for protoxylem and 8-12 μ for metaxylem and thin walled polygonal cellulosic wood parenchyma measuring 2-6 μ in diameter.

The phloem

The phloem "Fig. 10A, B" is formed of polygonal thin walled cellulosic elements including phloem parenchyma measuring $2-4\mu$ in diameter.

Furthermore, there are three batches of collenchyma region in the central parenchymatous pith of the petiole with few scattered xylem vessels.

Calcium oxalate crystals

The calcium oxalate crystals in the petiole of *M.* macroura Miq. are mainly deposited as druses "Fig. 10A, B" (star-like crystals) measured 2-4 μ in diameter, prisms "Fig. 10H" measuring 2-6 μ in length and 2-4 μ in width, styloid "Fig. 10H" which measures 20-40 μ in length and 2-6 μ in width and rosette crystals of calcium oxalate "Fig. 10H" that measured 4-12 μ in diameter. They are irregularly scattered mainly in wood and phloem parenchyma. Moreover, starch granules are present in all parenchyma cells. Also, mucilage is present in the epidermis, wood parenchyma and phloem parenchyma of petiole and its presence was confirmed using methylene blue.







"Fig. 10" The petiole: Diagrammatic transverse section of the petiole (A), Detailed transverse section of the petiole (B), The epidermal cells of the petiole (C), Non glandular trichomes (D), Bulbous trichome (E), Bracket trichomes (F), Spiral xylem vessels (G), Rosettes, prisms and styloid crystals of calcium oxalate (H). (All x 400 except A x 40). ab.epi., abaxial epidermis; ad.epi., adaxial epidermis; col., collenchyma; col.pat., collenchyma patches; col.per., collenchymatous pericycle; dr., druses; p., pith; ph., phloem; pr., prism; ros., rosette; xy., xylem.

The powdered leaf

The powdered leaf is light green in colour with faint characteristic odour and slightly mucilaginous taste. It is characterized microscopically by presence of the following features:

- 1- Fragments of upper epidermis of the lamina; showing elongated cells with slightly wavy anticlinal walls and covered with thin smooth cuticle.
- 2- Fragments of upper epidermis of lamina.
- 3- Fragments of lower epidermis of the lamina; formed of rectangular cells with straight anticlinal walls and covered with thin smooth cuticle and showed anomocytic stomata.
- 4- Fragments of the upper and lower epidermis of the midrib in which the cells are polygonal axially elongated with straight anticlinal walls and covered with thin smooth cuticle.
- 5- Fragments of columnar palisade cells of the mesophyll.
- 6- Fragments of epidermal cells of the petiole.
- 7- Bulbous trichome.
- Bracket trichome with thin cellulosic wall and hooked apex.
- 9- Unicellular non glandular trichomes.
- 10- Cystolith trichomes.
- 11- Fragments of lignified spiral vessels.

- 12- Numerous rosettes and prismatic crystals of calcium oxalate.
- 13- Laticifers tube.
- 14- Fragments of nectary gland stained with phenol red and Fehling's reagent.

4-The stem branch

A transverse section of the stem "Fig. 11A, B" is circular in outline and consists of an outer cork followed by phelloderm (secondary cortex) which is differentiated into two parts separated by discontinuous band of sclerenchyma. The outer portion is formed of 6-8 layers of chlorenchyma cells containing prisms of calcium oxalate crystals while the inner portion of phelloderm is formed of 7-9 layers of parenchymatous cells. The pericycle is formed of isolated groups of non-lignified pericyclic fiber. The vascular tissue is open collateral and formed of an outer phloem, inner xylem and is traversed by medullary rays. The pith is very wide parenchymatous cells in the center and represents about 2/3 of whole transverse section of stem.

The cork

The cork "Fig. 11A, B, C" is formed of radially rows of tangentially elongated cells with suberized and lignified inner tangential walls. They measure about 2-10 μ in length, 2-8 μ in width and 2-6 μ in height.

The phelloderm

The phelloderm "Fig. 11A, B" is divided by discontinuous band of sclerenchymatous layers mixed with stone cells "Fig. 11D" (16-30µ in diameter, 50-60µ in length and 8-14µ in width respectively) into two portions. The outer part consists of 6-8 layers of chlorenchyma cells containing prismatic crystals of calcium oxalate "Fig. 11J" that measure 2- 6µ in diameter and characterized by the presence of nectaries which stained red with phenol red and measuring 2-10µ in diameter. However, the inner portion of phelloderm is formed of 7-9 layers of parenchyma cells with 2- 6µ in diameter and filled with oval-shaped starch grains. Sclerenchmatous layers are formed of isodiametric sclerides with thick lignified walls and narrow lumen. Also, this band showed the presence of elongated non lignified fusiform fiber with blunt, acute and truncate apex with thick wall and narrow lumen measuring 110- 120μ in length and $8-14\mu$ in width and stone cells measuring 50-60 μ in length and 8-14 μ in width. Additionally, chlorenchyma cells are filled with mucilage which stained blue with methylene blue.

The pericycle

The pericycle "Fig. 11A, B" is formed of isolated groups of non-lignified, fusiform, acute or blunt apices pericyclic fibers "Fig. 11H" with thick cellulosic wall and narrow lumen interrupted by thin cellulosic walled parenchymatous cells (4-6 μ in diameter) which characterized by the presence of nectaries measuring 2-10 μ in diameter. Pericyclic fibers measure 50-140 μ in length and 2 -14 μ in width.

The vascular tissue

The vascular tissue "Fig. 11A, B" is formed of a narrow phloem and wide xylem crossed by medullary rays.

The phloem

The phloem "Fig. 11A, B" consists of sieve tissue, companion cells, phloem parenchyma and small groups of non-lignified phloem fibers with 3 to 5 fibers in each group. It is characterized by the presence of laticifers secretory structures "Fig. 11K" that measure 2-6 μ in diameter.

The xylem

The xylem "Fig. 11A& B" consists of lignified metaxylem directed outward and measure 20-36 μ in diameter and protoxylem directed inward measuring 4-12 μ in diameter. Moreover, xylem vessels showed spiral and pitted lignified walls "Fig. 11E" and measured about 2-10 μ & 20-32 μ in diameter, respectively. Also, xylem is associated with tracheids, wood fibers and wood parenchyma.

The wood fibers

Wood fiber "Fig. 11A, B, I" are non-lignified, fusiform with thick walls, narrow lumens and acute to blunt apices. They are present as isolated groups between medullary rays measuring 80-140 μ in length and $2-10\mu$ in width.

The wood parenchyma:

Wood parenchyma "Fig. 11 A, B, F" are polygonal, usually axially elongated cells with lignified pitted wall and measured about 18- 28 μ in diameter, 26-40 μ in length & 8-18 μ in width. Also, there are non-lignified wood parenchyma around protoxylem and measure 10-14 μ in diameter. Furthermore, pitted tracheids (30-62 μ in length and 2-18 μ in width) and tracheidal vessels (46-50 μ in length and 6-14 μ in width) are present as xylem elements.

The medullary rays

Medullary rays "Fig. 11A, B, G" are mostly biseriate and formed of cellulosic, subrectangular cells in the phloem region and rectangular, radially elongated cells with lignified and pitted walls in the xylem region. The cells measure $4-10\mu$ in length and 2-10 μ in width.

The pith

Pith "Fig. 11A, B" is very wide parenchymatous region, represents about 2/3 of transverse section and characterized by the presence of large more or less rounded or polygonal, lignified and pitted parenchyma which constitute 3-4 layers of the whole pith and measure18-28 μ in diameter. This lignified parenchyma are characterized by the presence of prismatic crystals of calcium oxalate (6-10 μ in length and 6-8 μ in width). The rest of pith region is formed of large oval or nearly rounded thin-walled cellulosic parenchyma cells measuring 12-30 μ in diameter. Furthermore, all parenchyma cells of pith are filled with mucilage and starch.



"Fig. 11" The stem branch: Diagrammatic transverse section (A), Detailed transverse section (B), Cork cell (C), Sclerenchyma cells with stone cell (D), Spiral and pitted xylem vessels (E), Tracheids, tracheidal vessels and wood parenchyma (F), Medullary rays in phloem region (G), Non lignified pericyclic fibers (H), Non lignified wood fiber (I), Prisms of calcium oxalate (J), Laticifers (K), Nectaries with phenol red (L). (All x 400 except A x 40).

Chlor., chlorenchyma; ck., cork; lat.t., laticeferous tube; m.r., medullary rays; nect., nectary; n.l.p.f., non lignifiedpericyclic fiber; n.l.w.f., non-lignified wood fiber; n.l.w.par., non-lignified wood parenchyma; p., pith; par., parenchyma; ph., phloem; pr., prism; scl., sclerenchyma; st., stone cell; v., vessels

The powdered stem branches

The powdered stem branch is light brown in colour with slightly characteristic odour and slightly sweet taste. It is characterized microscopically by the following:

- 1. Fragments of rectangular polygonal cork cells with suberized and lignified walls.
- 2. Fragments of lignified sclerides and stone cells.
- 3. Fragments of non-lignified pericyclic fibers with acute or blunt apices and they had thick cellulosic wall and narrow lumen.
- 4. Fragments of non-lignified wood fibers with acute or blunt apices, thick cellulosic wall and narrow lumen.
- 5. Fragments of lignified pitted and spiral vessels.
- 6. Fragments of rectangular elongated wood parenchyma with lignified and pitted walls.
- 7. Fragments of biseriate medullary rays.
- 8. Fragments of parenchyma cells containing prasms of calcium oxalate numerous prisms of calcium oxalate in parenchymatous cells.
- 9. Fragments of laticifers.
- 10. Fragments of nectaries stained with phenol red.

CONCLUSION

The current study revealed the following features

1. Microstructure of nectariferous tissue is studied for the first time in *Morus macroura* Miq family Moraceae.

- 2. Histochemical tests were carried out using different reagents as follow:
- Sudan III revealed the presence of fixed oil and fats in the leaf (cortical tissue, pericycle and trichomes, stem (cortex and phloem parenchyma), inflorescence (outer surface of tepals) and infructescence (inner most layer of mesocarp).
- Methylene blue revealed the presence of neutral mucilage in the epidermis of leaf, cortical region in stem, outer surface of tepals in inflorescence and inner most layer of mesocarp in infructescence.
- Ferric chloride revealed the presence of phenolic contents in the leaf (cortical tissue of midrib, pericycle, vascular tissue and trichomes), stem (cortex and phloem parenchyma), inflorescence (tepals, basal part of ovary), infructrscence (tepals).
- Fehling's for reducing sugars in nectaries.
- Phenol red for nectariferous tissues.

Anatomical study of the current species revealed the presence of different types of hairs and calcium oxalate.

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