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Botanical studies of the leaf, stem and root of *Carissa macrocarpa*, (Apocynaceae), cultivated in Egypt

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Abstract

The present study examines various standardized parameters as morphological and histological characters which could be helpful in authentication of the leaf, stem and root of *Carissa macrocarpa*. Concerning the microscopical features of *Carissa macrocarpa*, the plant is characterized microscopically by presence of large calcium oxalate clusters, forked apex fibers as well as pericyclic fibers with balloon like structure.

Keywords: *Carissa macrocarpa*, Apocynaceae, leaf, petiole, stem, root, botanical studies

1. Introduction

Family Apocynaceae is a large family consist of 402 Genera and 18,497 species distributed all over the world [1], A variety of secondary metabolites with important biological and economic value were detected in Apocynaceae as; triterpenes and sterols [2], cardenolides [3, 4] and alkaloids [5, 6]. *C. macrocarpa* (Apocynaceae) is a shrub native to South Africa and cultivated in Egypt, a traditional food plant in Africa. It usually forms a dense thorny shrub but it can grow into a small tree up to 4 m height. The current study involves various parameters such as macroscopic and microscopic characters, which could be useful in authentication of leaf, stem and root of *C. macrocarpa*.

2. Taxonomy

C. macrocarpa belongs to Kingdom: Plantae, Subdivision: Angiosperms, Class: Asterids, Order: Gentianales, Family: Apocynaceae, Genus: *Carissa* and Species: *C. macrocarpa* [7].

3. Materials and Methods

3.1. Plant material

The leaf, stem and root of *C. macrocarpa* were collected in June 2012 from El-Orman Botanical Garden, Giza, Egypt and identified by Agr. Eng./ Tereez Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden. A voucher specimen has been deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University under registration number (Mn-Ph-Cog-020). The plant material used for botanical study was taken from the fresh samples, as well as the samples preserved in alcohol (70%)-glycerin-water (1:1:1). It left for air drying in the shade, reduced to a fine powder for microscopical examination and stored in well-closed containers.

3.2. Preparation of samples for microscopical examination

Safranin, light green, phloroglucinol, concentrated hydrochloric acid and iodine were used for preparation the plant sections and the powder.

3.3. Microscopic studies

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

4. Results and discussion

4.1. Macroscopical characters of the leaf

The leaf (Fig. 1B, C and D) is simple, opposite decussate, petiolate, ovate in shape with entire margin, symmetric base, mucronate apex and pinnately reticulate venation. The midrib is prominent on the lower surface. The upper surface is dark green in color, while the lower being paler. The surface is nearly glabrous. The leaf is about 1-3 cm long and 1-2 cm wide at the middle parts while the petiole is about 0.1-0.4 cm long and 0.1-0.2 cm diameter, having slightly astringent bitter taste and odorless.

4.2. Macroscopical characters of the Stem

Both young and old stems (Fig. 1E) are cylindrical to elliptical in shape, odorless and possess slightly bitter astringent taste, with monopodial branching, slightly hard to break, both of different age stems are breaking giving a fibrous ends and giving white latex on breaking. The young stem is green in color while the old stem is greenish brown in color. The stem of the shrub is erect and measure up to 70 cm in length and up to 1 cm in diameter.

4.3. Macroscopical characters of the root

The root (Fig. 1F) is yellow to yellowish brown in color. It shows lateral branching, where the main root is tough and harder than the smaller lateral ones. The surface is usually rough showing numerous wrinkles and longitudinal fissures. The cork is easily separated from the inner tissues exposing a yellow interior. The fracture of the root is fibrous on the inner part and smooth on the outer part. The main root measures 0.5-1 cm in diameter, while the lateral branch measures 0.1-3 cm in diameter and extends 10-30 cm laterally below the soil. The root has no odor and acrid taste.

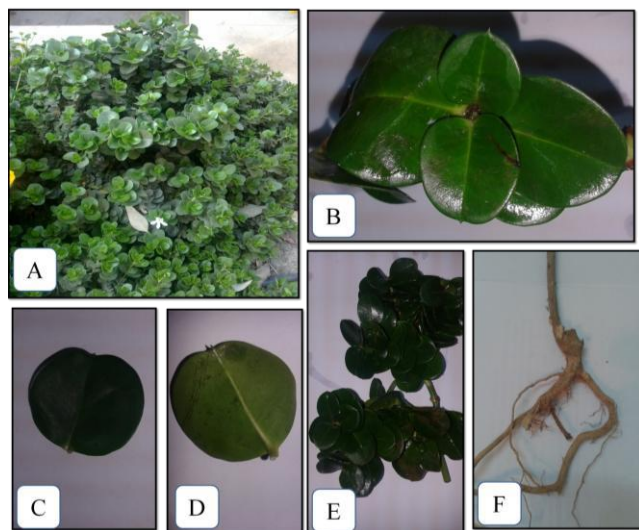


Fig 1: Photographs of *C. macrocarpa* (A) Photo of the shrubs, (B) Leaves showing opposite decussate phyllotaxis, (C) Upper surface of the leaf, (D) Lower surface of the leaf, (E) Stem carrying the leaves, (F) The root of the shrub.

4.4. Microscopical characters of the leaf

4.4.1. The Leaf blade

A transverse section in the leaf blade (Fig. 2) is convex, with dorsiventral mesophyll. The vascular bundle (Fig. 8) is collateral forming an open arc of xylem, phloem and perimedullary phloem. The pericycle is parenchymatous.

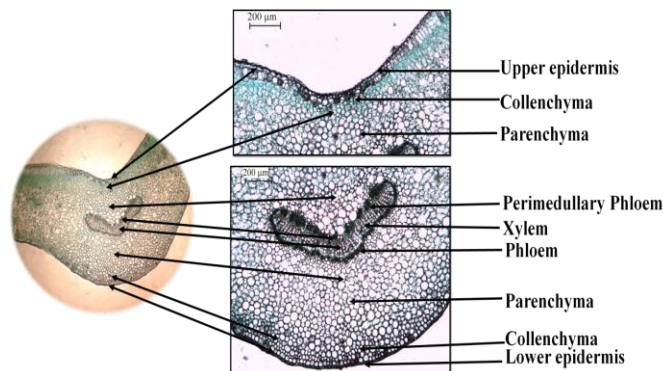


Fig 2: Detailed T.S.in Midrib region (x 40).

4.4.1.1. The epidermis

4.4.1.1.1. The upper epidermis

The Upper epidermis formed of one row of oblong to square cells covered with a thick cuticle as seen in the transverse section (Fig. 2). In surface view, the cells appear polygonal, with straight anticlinal walls, covered with slightly striated cuticle (Fig. 3). It shows unicellular, uniseriate short conical hair covered with slightly warty cuticle (Figs. 5 and 11A).

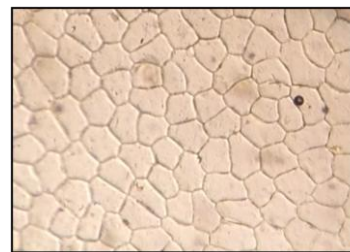


Fig 3: The Upper epidermis showing no stomata (x 400).

4.4.1.1.2. The lower epidermis

It is formed of one row of sub-rectangular to square cells as seen in the transverse section (Fig. 2). In surface view, the cells appear polygonal, with slightly sinuous anticlinal walls (Fig. 4), the hair is absent in the lower epidermis. The stomata are rounded or oval in shape of anomocytic type (3-5 subsidiary cells), being abundant on the lower surface and absent in the upper surface (Figs. 3 and 4)

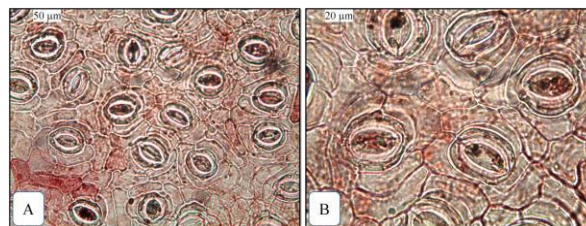


Fig 4: (A and B) The lower epidermis showing anomocytic stomata A-(x 200) and B-(x 400).

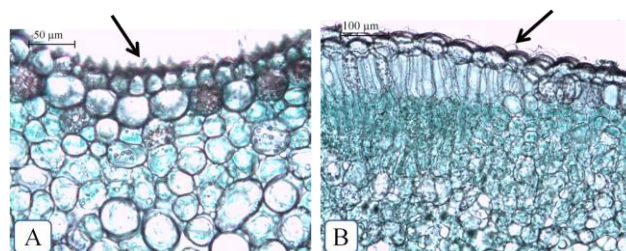


Fig 5: The short conical hair at the medrib region A-(x200) and at the lamina region B-(x100).

4.4.1.2. The mesophyll

The upper palisade layer consists of two rows of columnar, cylindrical, thin walled cells and containing chloroplasts and represents approximately one third of the distance between the two epidermises (Fig. 6A). This layer of palisade cells is absent in the midrib region (Fig. 2). The spongy tissue is formed of more or less large rounded, thin walled

parenchymatous cells with wide intercellular spaces, forming about 2-3 rows, some of them contain a large cluster crystals of Ca. Ox. (Fig. 6B). An aerenchyma layer is located below the palisade layer and represents the majority of the mesophyll (Fig. 6). The mesophyll is traversed by separated strands of small vascular bundles representing the lateral veins.

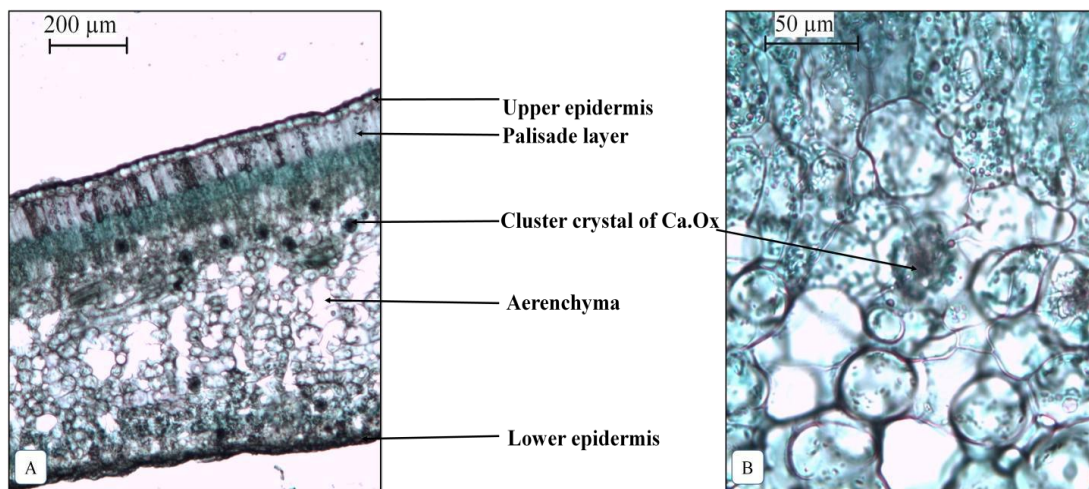


Fig 6: Detailed T.S. in the lamina of the leaf A-(x40) and B-(x200) showing the cluster crystal of Ca. ox. from lamina region.

4.4.1.3. The cortical tissue

The cortical tissue (Figs. 2 and 7) is represented by upper and lower hypodermal zones, each consisting of 3-6 rows of thick walled collenchyma cells with no intercellular spaces. The rest of the cortical tissue consists of rounded to oval parenchyma cells having thin cellulosic walls with intercellular spaces surrounding the main vascular bundle of the midrib. The parenchyma cells represent 12-15 rows above the vascular

bundle, while below the bundle they represent 10-12 rows (Fig. 2). The parenchymatous tissue of the cortex contains cluster crystals of calcium oxalate, brown content in addition to starch granules which are simple or semi-compound (2-3), rounded, without visible hila or striations (Fig. 7), the endodermis is indistinguishable and the pericycle composed of slightly thick walled rounded parenchyma cells.

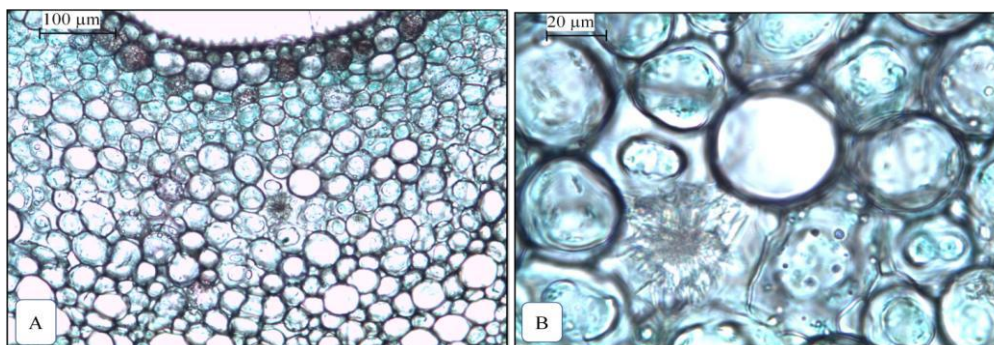


Fig 7: The cortical tissue A-(x 100) and B-(x 400) showing the cluster crystal of Ca. ox. from the midrib region.

4.4.1.4. The vascular system

The vascular system represented by an arc shaped strand of

collateral vascular bundles accompanied with patches of perimedullary phloem. (Figs. 2 and 8).

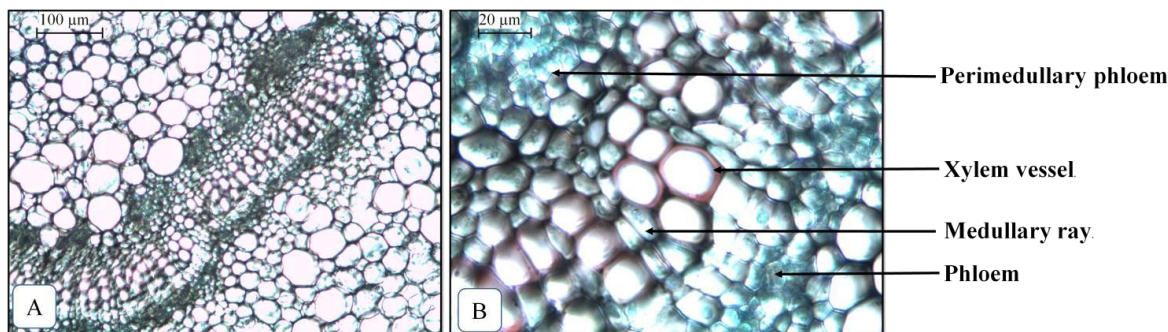


Fig 8: The vascular bundle of the leaf A-(x 100), and B-(x 400).

4.4.1.4.1. The phloem

The phloem consists of groups of thin walled, soft cellulosic elements alternating with parenchyma cells. It is formed of sieve tubes, companion cells and phloem parenchyma. The phloem region is free from any lignified elements, the cambium formed of 2-3 rows of thin walled, cellulosic, meristematic and rectangular cells.

4.4.1.4.2. The xylem

The xylem zone is formed of a band interrupted by medullary rays (Fig. 8), The xylem is formed of lignified vessel, wood fiber and wood parenchyma. The xylem vessels are lignified with pitted spiral thickening (Fig. 8). The medullary rays are

bi- to uniseriate consisting of radially elongated thin-walled, non lignified cells (Fig. 8). The wood fiber is spindle in shape, with pitted slightly lignified wall and slightly narrow lumen (Fig. 11F). The wood parenchyma cells are polygonal to sub-rectangular with pitted lignified walls.

4.4.2. The leaf petiole

A transverse section in the petiole shows that it is convex in outline, with glabrous surface and composed from the same layers as the leaf. The cortical tissue shows arc shaped vascular bundles similar in its structure to the midrib region of the leaf (Figs. 9 and 10).

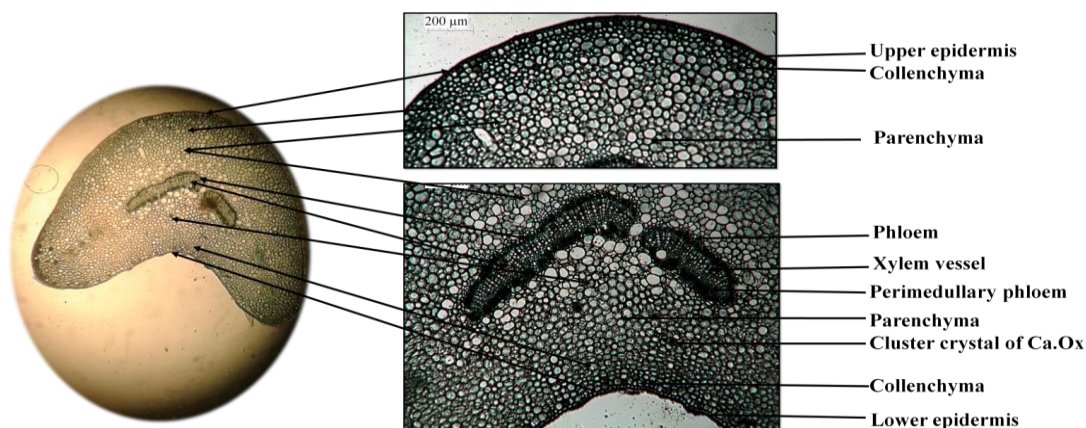


Fig 9: T.S. in the petiole (x 40).

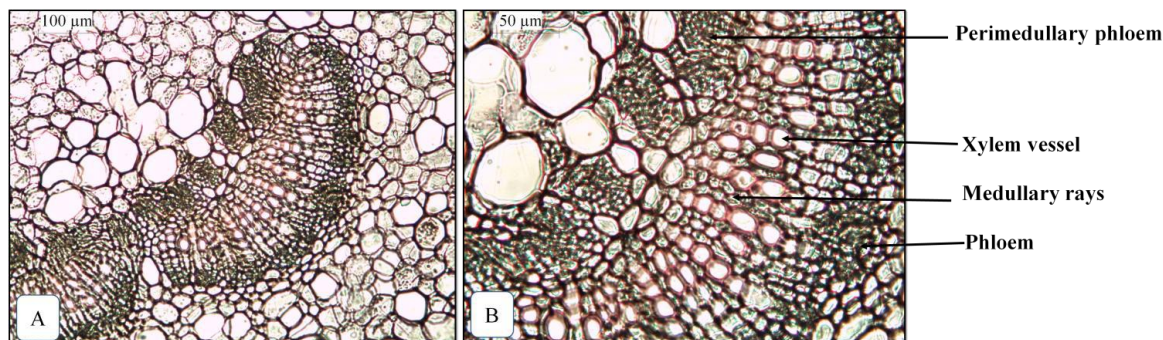


Fig 10: The vascular bundle of the petiole A-(x 100), and B-(x 200).

4.4.3. The powdered leaf

The powdered leaf has light green color with a faint odor and a slightly astringent bitter taste, the elements of the powdered leaf are shown in (Fig. 11).

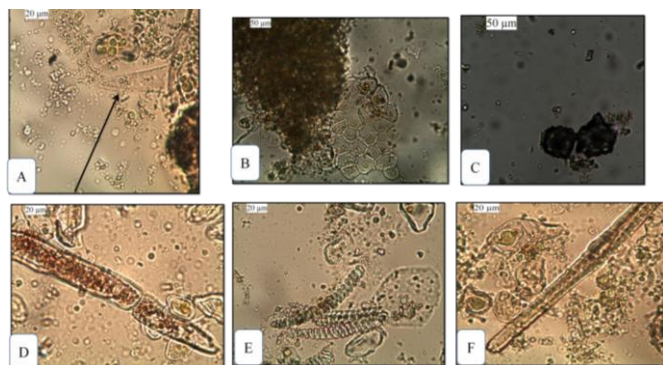


Fig 11: The powdered leaf; A-Short conical hair (x 400), B- Fragment of the upper epidermis (x 200), C- Large cluster crystal of Ca. ox. (x 200), D-Parenchyma cells contain starch granules (x 400), E-Spiral xylem vessels (x 400), F-Fragment of the wood fiber (x 400).

4.5. Microscopical characters of the Stem

A transverse section in the stem is cylindrical to elliptical in outline, different sections were made in different parts of the stem to clarify the anatomical features of the stem and to identify the differences between these different parts of the stem.

4.5.1. The young stem

A transverse section throughout young stem (Fig. 12) is cylindrical to elliptical in shape. It shows an epidermis enclosing a moderately narrow cortex. The pericycle is formed of parenchymatous tissue followed by continuous ring of vascular tissue traversed by medullary rays enclosing wide parenchymatous pith in the center.

4.5.1.1. The epidermis

The epidermis (Figs. 12 and 13) is composed of a single row of rectangular to sub-rectangular, tangentially elongated cells covered with thick smooth cuticle. In surface view they appear as polygonal cells, having straight anticlinal walls. The epidermal cells show no stomata and glabrous.

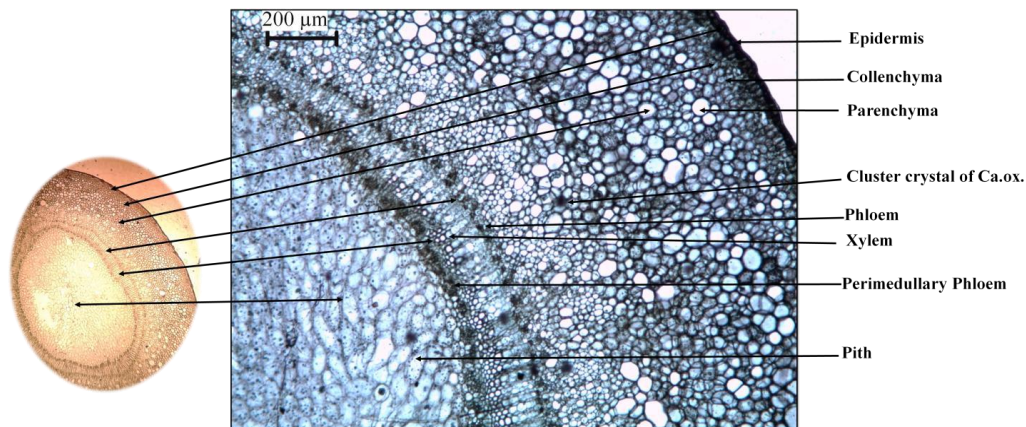


Fig 12: Detailed T.S. of the young stem (x 40).

4.5.1.2. The cortical tissue

The cortical tissue (Figs. 12 and 13) is composed of 3-5 rows of rounded collenchyma cells with no intracellular spaces followed by 10-12 rows of rounded to irregular shaped parenchyma cells with intracellular spaces. Some of them contain; large cluster crystal of Ca. ox., brown content and starch granules (stained blue with iodine solution in T.S.) The starch granules are simple or semi-compound (2-3), rounded in shape, without visible hila or striations. The endodermis is indistinguishable.

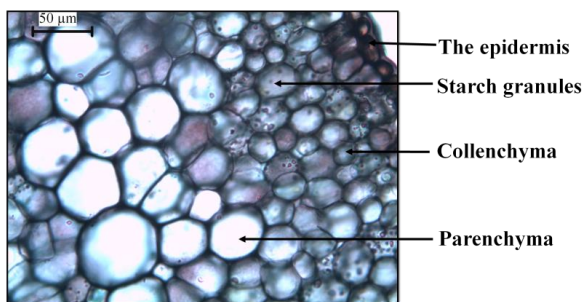


Fig 13: The epidermis and cortex region of the young stem (x 200).

4.5.1.3. The pericycle

The pericycle composed of slightly thick walled rounded parenchyma cells.

4.5.1.4. The Vascular system

The vascular system (Fig. 14) is composed of a complete ring of collateral vascular bundles accompanied by perimedullary phloem.

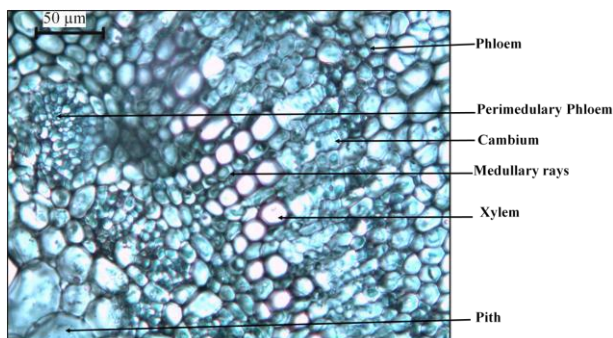


Fig 14: The vascular system of the young stem (x 200).

4.5.1.4.1. The phloem

The phloem consists of sieve tubes, companion cells and phloem parenchyma. It represents a wider zone above the

xylem than that below the xylem (Fig. 14), the phloem region shows non branched laticiferous tubes, which is responsible for the white milky exudates comes out upon breaking the stem (Figs. 19C and D).

4.5.1.4.2. The Cambium

The cambium (Fig. 14) formed of 3-4 rows of thin-walled, sub-rectangular, cellulosic, meristematic, tangentially elongated radially arranged cells.

4.5.1.4.3. The xylem

The xylem (Fig. 14) is formed of lignified vessels, wood parenchyma and wood fibers. The xylem vessels have mainly lignified spiral thickening as shown in the powder (Fig. 19A). The medullary rays are bi- to uniseriate consisting of radially elongated thin-walled, non lignified cells (Fig. 14). The Wood parenchyma is sub-rectangular cells with beaded non lignified wall and wide pitted lumen as shown in the powder (Fig. 19B), the wood fibers are present in groups; they are lignified spindle in shape, with narrow lignified lumen and acute apices and some of them are forked (Fig. 19E).

4.5.1.4.4. The Perimedullary phloem

The Perimedullary phloem (Fig. 14) is represented by isolated bundles at the margin of the pith.

4.5.1.5. The Pith

The Pith (Fig. 15) comprises the majority of the sector and is formed of thin walled, large, polygonal to irregularly shaped, water storing, parenchyma cells. the size of the cells increase toward the center. Cluster crystal of Ca. ox and starch granules are scattered in the pith.

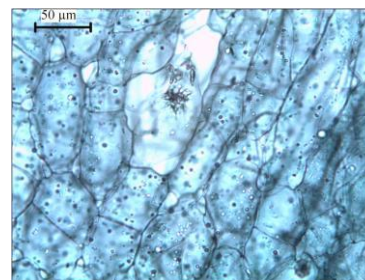


Fig 15: Cluster crystal of Calcium oxalate and starch granules scattered in the pith of the young stem (x 200).

4.5.2. The old stem

The structure of the old stem (Fig. 16) is almost similar to that of the young stem, with the following differences:

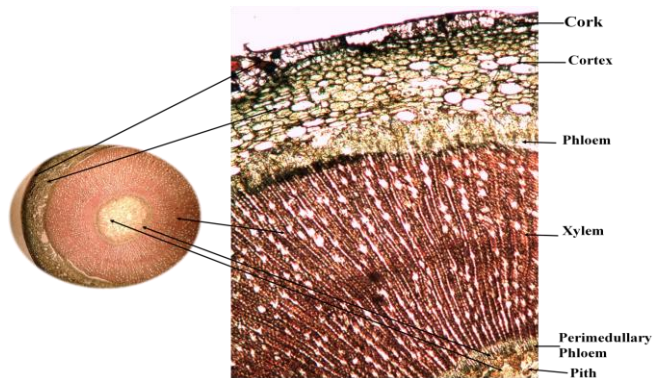


Fig 16: Detailed T.S. of the old stem (x 40).

1. The presence of cork cells (Figs. 17 and 19G) which are lignified polygonal cells radially arranged in several rows that may reach 3-5 rows.
2. The cortical tissue is narrower than that of the young stem, (Figs. 12, 16 and 17).
3. The presence of non or slightly lignified pericyclic fibers with beaded wall and slightly narrow lumen and with balloon like structure near the rounded tip (Fig. 18 and 20).

4. The presence of non lignified dentate pericyclic fibers with narrow lumen and dentate margin (Fig. 19F).
5. The vascular tissue is wider and of the same type while the pith is narrower compared to that of young stem, (Figs. 12 and 16).

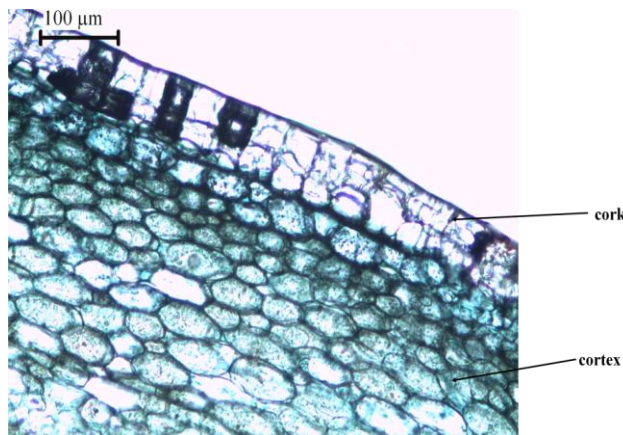


Fig 17: The layers of cork and cortex of the old stem (x100).

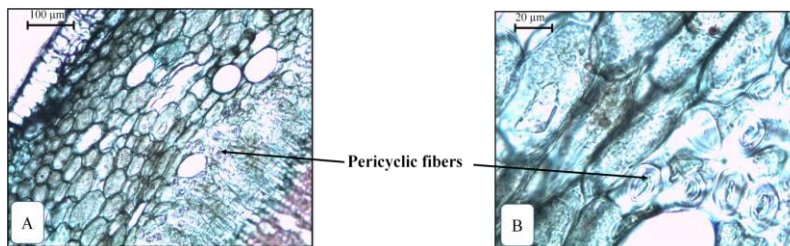


Fig 18: The pericyclic fibers of the old stem A-(x100) and B-(x400).

4.5.3. The powdered stem

The powdered stem is greenish yellow in color, a slightly astringent bitter taste, and odorless. The elements of the powdered stem are shown in (Figs. 19 and 20).

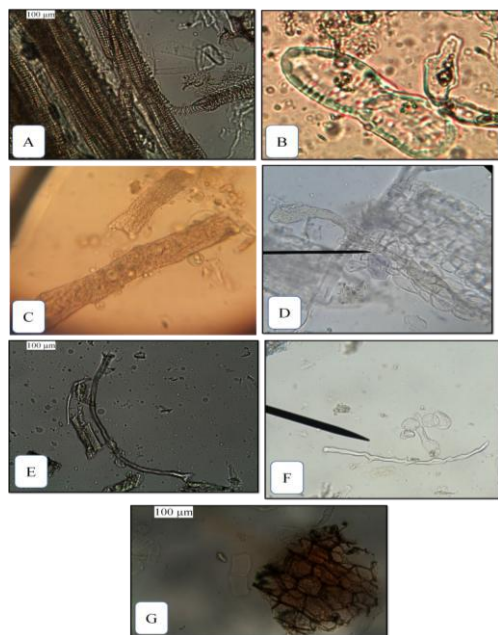


Fig 19: The powdered stem: A- Fragment of xylem vessels(x100), B-Fragment of wood parenchyma(x200), (C and D) - Fragment of the laticiferous tube (400), E-Wood fiber (x100), F-The dentate pericyclic fiber (x100), G-Fragment of cork cells (x100).

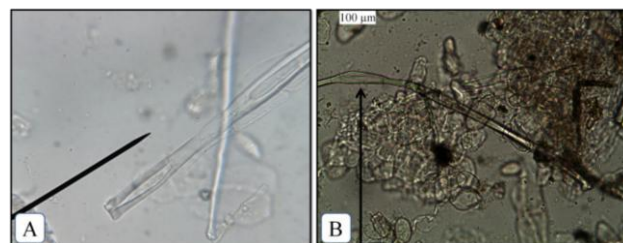


Fig 20: The powdered stem: The pericyclic fiber with balloon like structure A and B (x100).

4.6. Microscopical characters of the root

A transverse section in the root (Fig. 21) is nearly circular to elliptical in outline, It shows outer cork followed by moderately narrow cortex, the pericycle is parenchymatous, the vascular system consists of phloem outside, wide zone of xylem inside enclosing cambium in between.

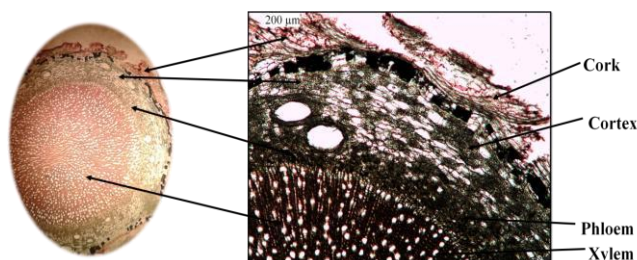


Fig 21: Detailed T.S. in the root (x 40).

4.6.1. The cork

The cork (Fig. 22) is formed of (8-10) rows of sub-rectangular, tangentially elongated, radially arranged, lignified cells. In surface view, they appear as polygonal cells with thick straight lignified walls contain dark brown content.

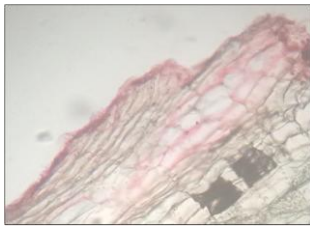


Fig 22: The layers of cork (x100).

4.6.2. The cortex

The cortex (Fig. 21) consists of (12-15) rows of oval to elongated thin walled parenchyma cells with narrow intercellular space. It shows starch granules which are simple or compound (2-3), rounded, without visible hila or striations. The endodermis is not distinct and the pericycle is polygonal isodiametric or elongated thin-walled parenchyma cells.

4.6.3. The vascular system

The vascular system (Fig. 21) is very wide and cross-sectioned by bi- to uniseriate medullary rays.

4.6.3.1. The phloem

The phloem represents a narrow layer surrounding the xylem and consists of thin walled phloem parenchyma, sieve tubes, and companion cells.

4.6.3.2 The cambium

The cambium is formed of 2 rows of thin-walled tangentially elongated meristematic cells.

4.6.3.3. The Xylem

The xylem is a wide cylinder of lignified radially arranged elements. The vessels are wide mostly scalariform as shown in the powder. Wood fibers are fusiform, with straight lignified walls, slightly wide lumen and acute apices.

4.6.4. The powdered root

The powdered root is yellow in color with no odor and acrid taste, The elements of the powdered root are shown in (Fig. 23).

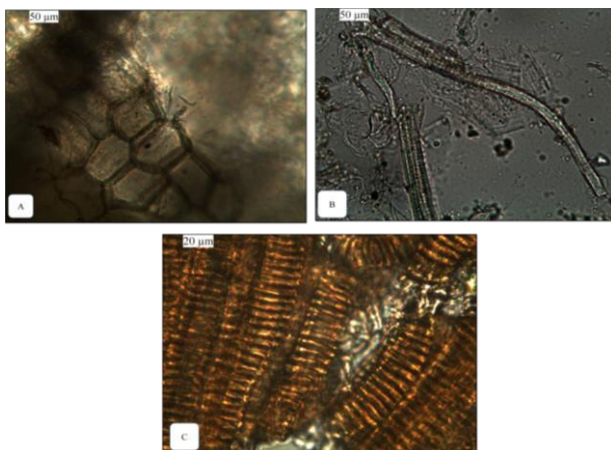


Fig 23: The powdered root: A-Fragment of the cork cells (x200), B-The wood fiber (x200) and C-The scalariform xylem vessels (x400).

Table 1: Microscopical measurements of the different organs of *C. macrocarpa* (in μm).

Item	Length	Width	Height	Diameter
The leaf				
Upper epidermis	25-26-28	7-10-12	5-9-12	
Lower epidermis	17-25-27	6-8-10	5-8-11	
Palisade cells			76-80-83	
Spongy parenchyma of lamina				38-46-52
Cluster crystals of Ca _{ox}				24-28-30
Cortex Parenchyma				15-37-37
Collenchyma				12-20-24
Xylem vessels				8-15-20
Conical hair	40-44-46	12-15-20		
The upper part of the stem				
Epidermis			8-10-12	
Collenchyma				12-17-23
Parenchyma of cortex				27-33-40
Xylem vessels				12-16-20
Cluster crystals of Ca _{ox} .				13-25-30
Parenchyma of pith				40-52-60
The lower part of the stem				
Cork	63-67-70	44-48-52	18-22-25	
Cortex parenchyma				22-28-35
Xylem vessels				15-18-22
Pith parenchyma				33-48-55
Pericyclic fibers				12-15-19
The root				
Cork	45-49-51	30-33-35	22-25-28	
Cortex parenchyma				22-25-28
Xylem vessel				18-19-23

5. Conclusion

Examination of both macroscopical and microscopical features of the leaf, stem and root of *C. macrocarpa* represents a good method in the identification of the plant. In addition, these characters will be helpful in the future investigation of the plant in many pharmacognostical and pharmacological studies.

6. Acknowledgement

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