



## **BOTANICAL STUDY AND DNA FINGERPRINT OF *CEIBA PENTANDRA* (L.) GAERTN. VAR. *PENTANDRA* CULTIVATED IN EGYPT**

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*Ceiba pentandra* (L.) Gaertn. (kapok) (F: Bombacaceae) is a large, fast growing tree of up to 50 m height. It grows naturally in the tropical and subtropical regions of the world and planted as a shade tree. In traditional medicine, it is mainly used as an emetic, diuretic and antispasmodic agent. Extracts of different morphological parts of the plant has been also recommended for the treatment of various ailments, include diabetes, bronchitis, skin diseases, diarrhea, dysentery, eye diseases, arthritis, insect bite and chronic fever. There are at least four common varieties of *Ceiba pentandra* species include; var. *caribaea* (DC.) Bakh., var. *guineensis* (Schumach. & Thonn.) H. G. Baker, var. *pentandra* and var. *indica* Bakhuisen.

However, there were no specific identification standards for such varieties in the previous researches. In this study, a detailed description for the morphological and anatomical characters of the leaves, stems and fruits of *Ceiba pentandra* var. *pentandra* is performed. Additionally, the DNA fingerprint of the variety *pentandra* was established to help in its future identification at the genomic level.

### **INTRODUCTION**

Bombacaceae (Bombax) is a small family containing about 28 genera and 200 species<sup>(1&2)</sup>. They grow naturally throughout tropical and subtropical areas of the world including tropical America<sup>(3&4)</sup>. Plants of Bombacaceae are mainly perennial large size woody trees, and rarely shrubs<sup>(5)</sup>.

The genus *Ceiba* comprises about 17 species that are large trees of 5-20 m height, and occasionally up to 50 m height. Among them, *Ceiba pentandra* (L.) Gaertn. (kapok) is a gigantic, fast-growing tree of up to 50 m height. It has a straight, cylindrical shaft of 20

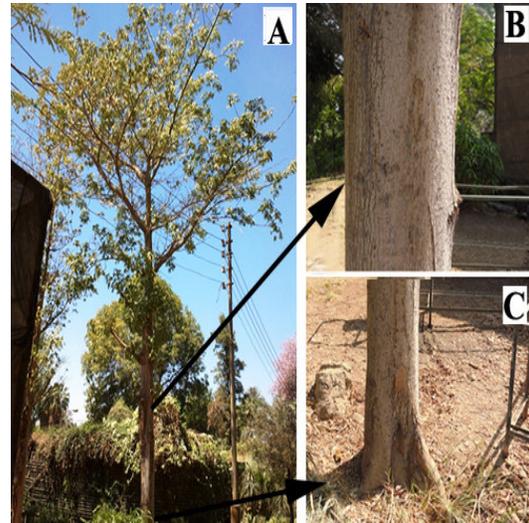
m height and up to 2 m diameter. It is distributed in the tropical, intertropical and subtropical regions of the world and planted as wayside and shade tree<sup>(3&6)</sup>. The plant is known traditionally as being good emetic, diuretic and antispasmodic agent<sup>(7)</sup>. It has been also prescribed for the treatment of diabetes, bronchitis, skin diseases, diarrhea, dysentery, painful eye diseases, arthritis, insect bite and chronic fever<sup>(8)</sup>. Modern pharmacological studies revealed that solvent extracts of various parts of the plant have anti-inflammatory<sup>(9)</sup>, hypoglycemic<sup>(10)</sup>, hypolipidemic<sup>(11)</sup>, anti-ulcerogenic<sup>(12)</sup>, and hepatoprotective effects<sup>(13)</sup>.

The species *Ceiba pentandra* includes at least four different varieties, var. *caribaea* (DC.) Bakh., var. *guineensis* (Schumach. & Thonn.) H. G. Baker, var. *pentandra*, and var. *indica* Bakhuisen, which are morphologically interconnected. Although little morphological differences among these varieties have been reported<sup>(14&15)</sup>, there was no specific study for the exact identification of this variety in the previous studies on the species<sup>(16-18)</sup>.

According to World Health Organization (WHO) guidelines, a herbal product should be standardized with respect to its identity and being free from adulterants to ensure its safety before releasing to the market<sup>(19&20)</sup>. In the same concern, pharmacognosy mainly addresses quality-related issues using routine botanical, organoleptic parameters of crude drugs, and chemo-profiling characterization by chromatographic and spectroscopic techniques<sup>(21)</sup>. In case of phytochemical, morphological or anatomical indistinguishable genuine drug from substituted or adulterated drug, plant genome analysis using different techniques such as hybridization based methods, polymerase chain reaction (PCR) based method and sequence based methods could be utilized for quality control of herbal medicines<sup>(22&23)</sup>. The DNA fingerprint has an advantage over chromatographic methods as being unchanged irrespective of the plant part used, while the phytochemical constituents may vary according to the part of plant used as well as physiological and environmental growth conditions<sup>(19&24)</sup>.

Randomly amplified polymorphic DNA (RAPD) fingerprint, a PCR-based DNA fingerprint method, is the fastest and easiest technique commonly used for primary screening the differences in DNA sequences; helps in differentiation of two species or varieties of plants<sup>(22&25)</sup>.

The aim of our investigations is to provide detailed morphological and anatomical description of leaflets, petioles, stems and fruits of the variety *pentandra*, and establishing a standard DNA fingerprint which could be useful for the correct identity and quality control of the drug either in the single form or in multiple herbal formulations.



**Fig. 1:** Photos of *Ceiba pentandra* (L.) Gaertn. var. *pentandra*.

A- The whole tree during new foliage (X 0.0032).

B- The spineless trunk of the tree.

C- Tree base showing buttresses at the tree base.

## EXPERIMENTAL

### General experimental procedures

Microscope with camera lucida Zeiss (Germany) was used for micro-morphological studies. Sigma 1-14X Microfuge (U.K.), Sensoquest Labcycler (Germany), GelHaus horizontal electrophoresis system (Korea) and UV transilluminator (India) were used for RAPD PCR technique.

### Material and methods

#### 1. Plant samples for DNA fingerprint study

*C. pentandra* fresh young leaves were collected in September 2014. The leaves were stored in sterile bags at -20°C and kept for DNA isolation.

#### 2. Plant samples for botanical study

Fresh plant materials of *C. pentandra* were collected in March 2013 from the experimental station of ornamental plants, Faculty of Agriculture, Assiut University and kindly identified by Dr. Esam Yousef, lecturer of Ornamental, Horticulture and Floriculture, Faculty of Agriculture, Assiut University. Samples of different organs were preserved in a mixture of ethyl alcohol/glycerin/water (1:1:1, v/v/v) and stored in a tightly closed container. Another sample of the same organs were

separately air-dried and reduced to fine powder.

## **Molecular characterization using RAPD technique**

### **1. DNA isolation**

*C. pentandra* leaves contain large amounts of mucilage<sup>(26)</sup>. Mucilage often interfere with PCR and the reaction enzymes such as Taq polymerase<sup>(27)</sup>. Because of the high viscosity of the leaf material due to the presence of mucilage content, commercially available kits as DNazol, Trizole and plant DNA extraction kits failed to extract DNA for PCR analysis. The DNA extraction protocol was done following Raju Ghosh method<sup>(27)</sup> with some modifications. Briefly, frozen leaf tissues (100 mg) was ground into a fine powder using pre-chilled mortar and pestle. The samples were then transferred to 30mL polypropylene tubes and 10 mL of pre-warmed (65°C) extraction buffer [100 mM Tris HCl (pH 8), 10 mM EDTA (pH 8), 1.4 M NaCl, 2% CTAB and 0.2% 1-mercaptoethanol] was added to the samples and incubated at 65°C for 30 min. During incubation, the contents were mixed three to four times by inverting the tubes gently. Samples were then removed from the water-bath and cooled to room temperature. For removing of the organic contaminants, 0.6 mL of chloroform/isoamyl alcohol (24:1, v/v) was added and mixed thoroughly for 5 min to form an emulsion. The samples were then centrifuged at 5000 rpm for 20 min at 10°C and the upper aqueous phase was transferred to a new microcentrifuge tube. The entire solution was treated with DNase free RNase at 37°C for 30 min. RNase contamination was removed by adding an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) and the aqueous phase was collected in a fresh microfuge tube after centrifugation at 3000 rpm for 5 min at room temperature. Again, an equal volume of chloroform/isoamyl alcohol (24:1, v/v) was added and mixed thoroughly to form an emulsion. The samples were centrifuged at 5000 rpm for 5 min at room temperature and the top aqueous phase was transferred to a fresh tube. A double volume of absolute ethanol was added into the collected aqueous phase and was mixed gently by inverting the tube. The samples were then centrifuged at 5000 rpm for 3 min at 4°C to collect the

nucleic acid precipitate. After centrifugation, the DNA pellet was washed with 70% ethanol, air-dried, and finally the purified DNA pellet was dissolved in 100 µL of TE buffer and used for amplification by PCR.

### **2. Qualitative estimation of DNA**

The quality of the DNA was estimated using agarose gel electrophoresis. The obtained DNA was run on 0.8% agarose gel stained with ethidium bromide at 80-90 V for 2 hrs followed by viewing the gel under UV transilluminator<sup>(28)</sup>.

### **3. PCR amplification using RAPD Markers**

The method uses oligonucleotide primers (8-10 nucleotides with a GC content of at least 50%) of randomly chosen sequences to prime DNA synthesis from pairs of sites to which it is matched or partially matched and results in plant specific arrays of DNA products<sup>(29&30)</sup>. Purified DNA samples (50-100 ng) were used for amplification by PCR with the same 5 RAPD primers sets (Metabion International AG, Germany) (Table 1) previously used in similar studies of three species of the family<sup>(18)</sup>. The total volume of the PCR reaction was 25 µL, which contained 1 µL of template DNA, 2.5 µL of 10 × Taq buffer with MgCl<sub>2</sub> (10 mM), 1.5 µL of 2.5 mM dNTPs, 2 µL of Taq-polymerase (fermentas), and 1 µL of 10 pmol RAPD primers. The PCR cycle was carried out with the initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 45 s, 35°C for 1 min, 72°C for 3 min and a final extension of 72°C for 10 min. The obtained PCR products alongside with a thermo scientific gene ruler 50 bp DNA ladder were viewed in 1.5% agarose gel stained with ethidium bromide and photographed on an UV transilluminator using a digital camera (12Mp, Sony).

### **4. Data analysis**

Agarose gel photos were scanned and all visible and unambiguous bands were scored and recorded by the gene profiler 4.03 computer software programs that use automatic lane and peak finding for detecting the presence of banding patterns, and calibrating them for size and intensity

**Table 1:** List of RAPD primers combination that used in PCR amplification.

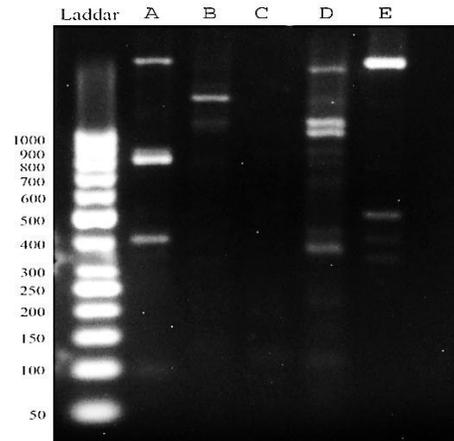
#	Primer pair	Sequence '5'-3'	GC content %
A	r34 r1302.1	GTCACCGGA GGAAATCGTG	60% 50%
B	r34 0PA1	GTCACCGGA CAGGCCCTTC	60% 70%
C	r34 OPP2	GTCACCGGA TCGGCACGAC	60% 70%
D	r34 OPM13	GTCACCGGA GGTGGTCAAC	60% 60%
E	r11 r1302.1	CCAAGCAGT GGAAATCGTG	50% 50%

## RESULTS

### DNA fingerprint (Fig. 2)

A PCR with 5 primer pairs produced a total of 32 fragments as shown in table 2 and figure 2. The molecular size of the produced fragments revealed the presence of a wide range of sequences. The maximum size was 1904 bp after using primer A which produced 5

randomly amplified polymorphic DNA fragments, while the minimum molecular size was 94 bp with the same primer. The primer B afforded sequences in the range 137 to 1550 bp. The primer D yielded sequences in the wide range, from 114 to 1770 bp. The primer E gave sequences in the range 346 to 1876 bp. The primer C didn't produce any band.

**Fig. 2:** RAPD electrophoretic profile of *C. pentandra* leaves generated by the 5 primers A-E.**Table 2:** The distribution and molecular weight of bands (markers) revealed by RAPD for *C. pentandra*.

Ladder (bp)	A	B	C	D	E
1000	1904	1550	-----	1770	1876
900	940	1419	-----	1420	1364
800	850	1158	-----	1192	1141
700	421	1089	-----	1089	994
600	94	940	-----	928	928
500	-----	336	-----	837	811
400	-----	137	-----	700	513
300	-----	-----	-----	448	421
250	-----	-----	-----	394	346
200	-----	-----	-----	221	-----
150	-----	-----	-----	114	-----
100	-----	-----	-----	-----	-----
50	-----	-----	-----	-----	-----

## **Macro- and micro-morphological studies of stems, leaflets, petioles, and fruits of *C. pentandra***

### **1. Macro- and micro-morphological studies of stems**

#### **1.1. Macro-morphology of the stem**

The stem of the old tree is medium-sized up to 30 m tall. The tree bole is straight, cylindrical and unbranched for up to half length of the tree. It usually has small plank-like buttresses (Fig. 1C). The outer surface of the bark is smooth, pale grey and usually spineless, while the inner surface is pale to pinkish red color with vertical stripes of white or yellowish tissue. The branching usually occurs in whorls of three branches either horizontal or ascending<sup>(31)</sup>.

#### **1.2. Micro-morphological study of the stem**

A transverse section (T.S.) through the young stem is nearly circular in outline. It shows an outer epidermis followed by hypodermal layer consists of 3-5 rows of parenchymatous cells. The cortex consists of 2-6 rows of collenchymatous cells and several rows of parenchymatous cells intersected by numerous cavities. The parenchyma cells contain cluster crystals and prisms of calcium oxalate, and starch granules. The endodermis is indistinct. The pericycle consists of groups of lignified fibers alternating with groups of parenchyma cells. The vascular tissue consists of a continuous ring of outer phloem and inner radiating xylem; both are traversed by medullary rays. The pith is formed of parenchymatous cells containing calcium oxalate crystals. The cell matrices are interrupted by some cavities (Figs. 3&4).

#### **The epidermis (Figs. 3-6)**

In sectional view, the epidermis appears as one row of square to sub-rectangular cells, covered with thick smooth cuticle. In surface view, the cells are polygonal, somewhat elongated with thick straight anticlinal walls, and contain mucilagenous matter (stained red with ruthenium red).

#### **The cortex (Figs. 3&4)**

It is formed of an outer region consists of hypodermis of three to five rows of polyhedral to sub-rectangular, sub-epidermal thin-walled

parenchymatous cells containing cluster crystals and prisms of calcium oxalate. The middle region consists of 2-6 rows of collenchymatous cells. The inner region is formed of several layers of thin-walled parenchyma cells intersected by numerous mucilagenous cavities. The inner cortical parenchyma contains oval to rounded starch granules which are mostly simple, few are compound of 2-3 components.

#### **The pericycle (Figs. 3-6)**

The pericycle is formed of groups of lignified fibers interrupted with parenchymatous cells. The fibers show thick lignified walls and moderately wide lumen.

#### **The vascular system (Figs. 3&4)**

The vascular system consists of continuous rings of vascular bundles which comprises of phloem, cambium and xylem.

#### **The phloem (Figs. 3&4)**

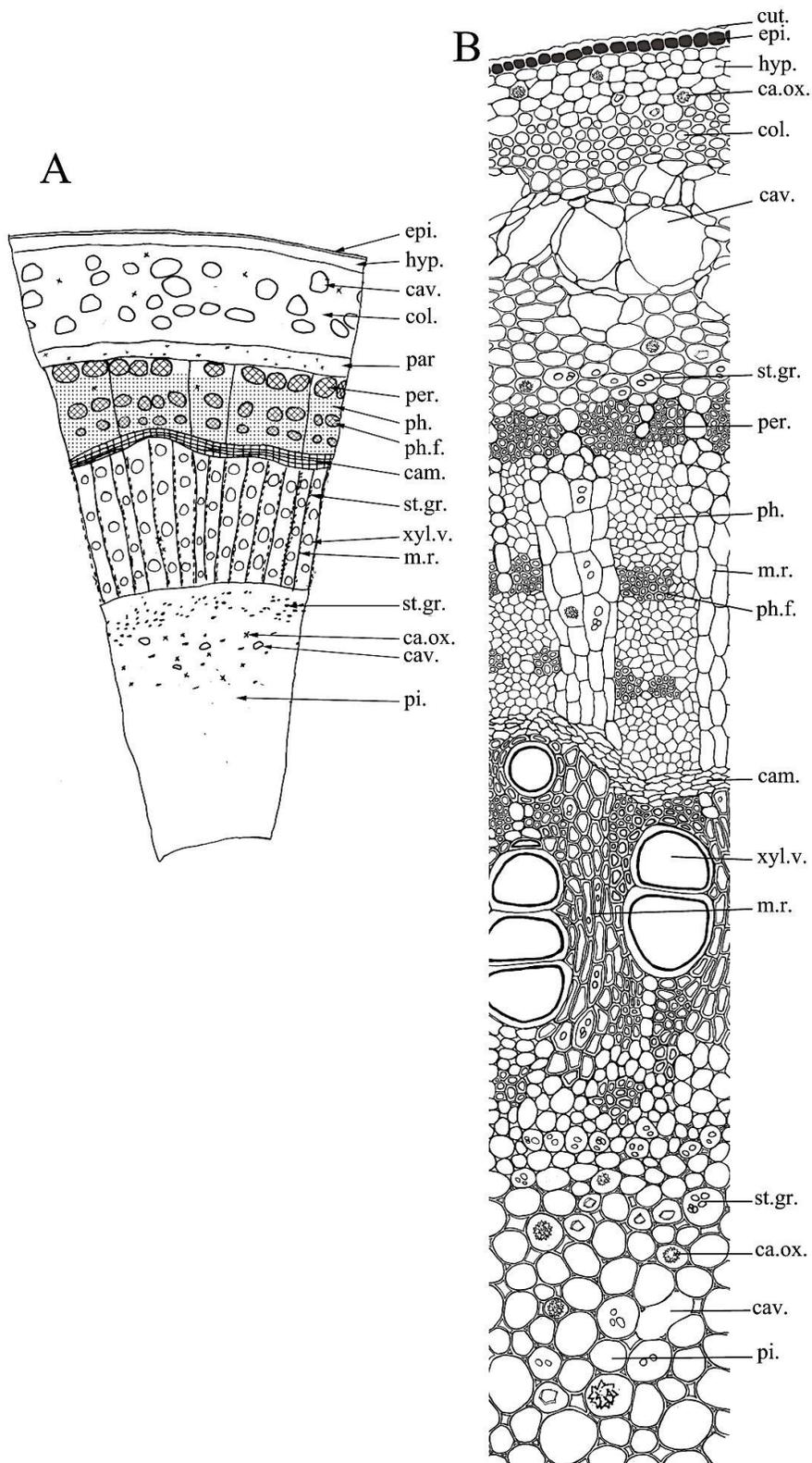
The phloem consists of phloem elements including shining thin-walled cellulosic soft elements of sieve tubes, companion cells and phloem parenchyma. These soft elements are interrupted by groups of lignified thick-walled phloem fibers and traversed by medullary rays. The fibers are similar to those of the pericycle.

#### **The cambium (Figs. 3&4)**

The phloem is separated from the xylem by a cambial zone formed of 4-6 rows of thin walled, collapsed tangentially elongated and radially arranged cellulosic cells.

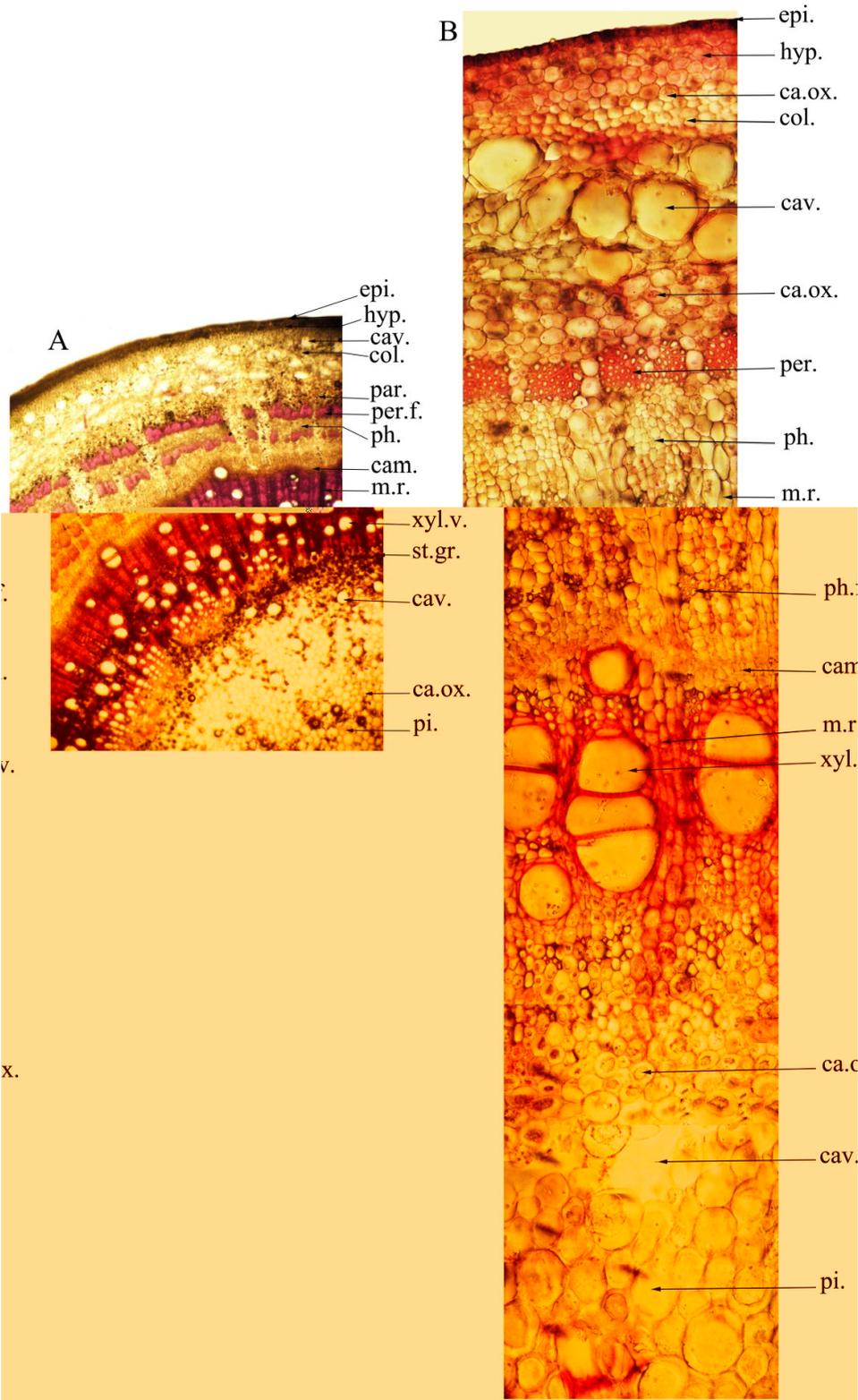
#### **The xylem (Figs. 3-6)**

The xylem consists of a comparatively wide zone of lignified thick-walled, radially arranged elements traversed by multi-seriate medullary rays with thin slightly lignified walls. The xylary elements include xylem vessels, wood fibers, wood parenchyma and tracheids. The vessels are arranged in radial rows and show reticulate, spiral and pitted thickening. The tracheids are slightly elongated with lignified pitted walls and rounded to blunt ends. The wood fibers have lignified walls, moderately narrow to wide lumen. Wood parenchyma consists of rectangular to sub-rectangular cells with lignified pitted walls.



**Fig. 3:** Hand drawing of diagrammatic (A) (X 42.51) and detailed T.S. of young stem (B) (X 138.78).

Ca.ox., calcium oxalate; cam., cambium; cav., cavity; col., collenchyma; cut., cuticle; hyp., hypodermis; epi., epidermis; m.r., medullary ray; par., parenchyma; per., pericycle; ph., phloem; ph.f., phloem fibers; pi., pith; st.gr., starch granules; xyl.v., xylem vessel.



**Fig. 4:** Photo of diagrammatic (A) and detailed T.S. of young stem (B).

Ca.ox., calcium oxalate; cam., cambium; cav., cavity; col., collenchyma; hyp., hypodermis; epi., epidermis; m.r., medullary ray; par., parenchyma; per., pericycle; per.f., pericyclic fiber; ph., phloem; ph.f., phloem fibers; pi., pith; st.gr., starch granules; xyl.v., xylem vessel.

**The medullary rays (Figs. 3-5)**

The medullary rays are multi-seriate. In the phloem region, they consist of slightly elongated parenchymatous cells with thin cellulose wall, while in the xylary region they consist of radially elongated cells with thin slightly lignified walls.

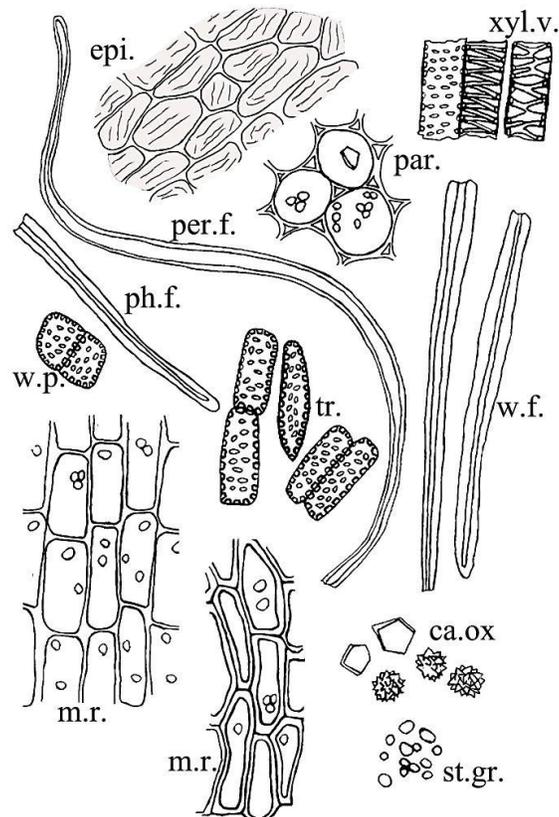
**The pith (Figs. 3&4)**

The pith is formed of wide central zone of rounded to oval thin-walled parenchymatous cells showing numerous cluster crystals of calcium oxalate. The cells neighboring to the primary xylem are much smaller and closely packed than that adjacent to the pre-medullary zone. Starch granules are numerous, simple, rarely compound of 2-3 components. The inner part of the pith is formed of thin-walled parenchyma cells with intercellular spaces and cavities.

**The powder of the stem (Figs. 5&6)**

The powdered stem of *C. pentandra* is tasteless, has yellowish brown color and faint odor. It is characterized microscopically by the following fragments:

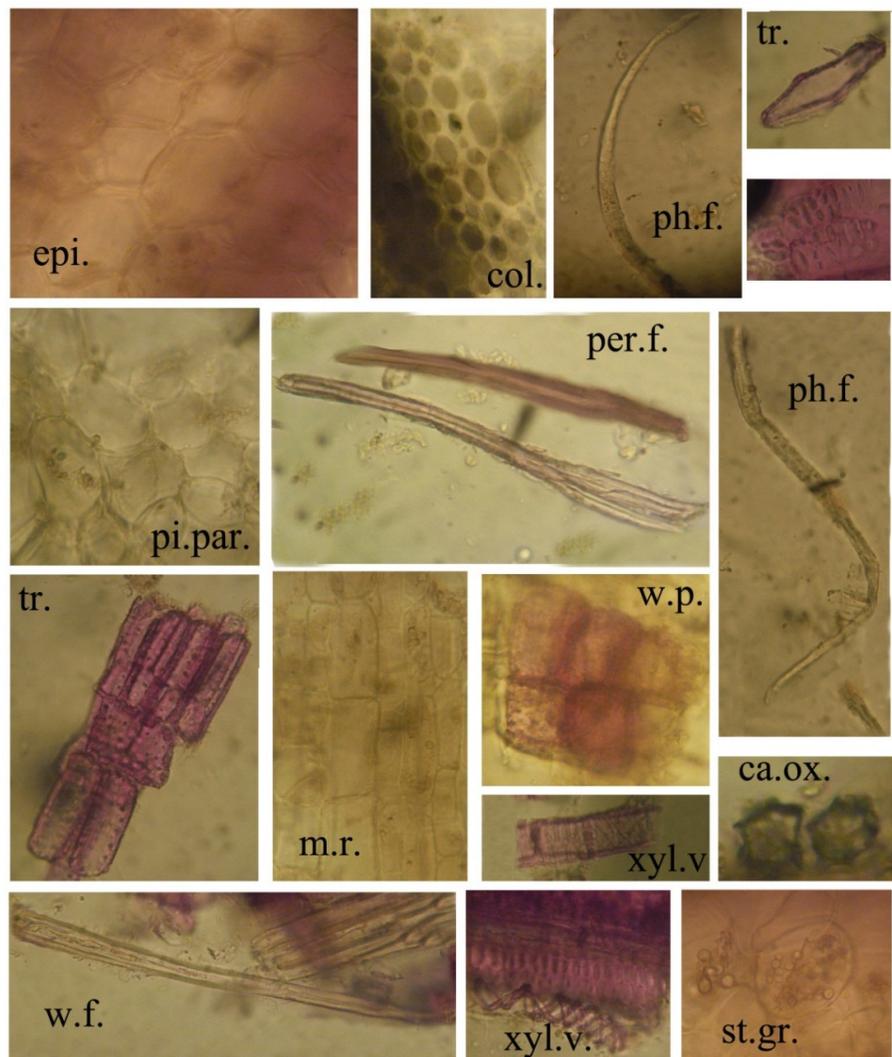
- 1- Fragments of polygonal, axially-elongated epidermal cells with brownish content, showing straight anticlinal walls and covered with thick striated cuticle. Stomata were not observed.
- 2- Fragments of thin-walled parenchyma cells, either from the cortex or the pith, containing prism and cluster crystals of calcium oxalate and starch granules.
- 3- Fragments of thin-walled radially elongated parenchyma of the medullary rays containing starch granules.
- 4- Fragments of pericyclic fibers with straight thick lignified walls, moderately wide lumen and blunt to rounded apices.



**Fig. 5:** Hand drawing of the isolated elements of the stem (X 172.77).

Ca.ox., calcium oxalate; epi., epidermis; m.r., medullary ray; par., parenchyma; per.f., pericyclic fiber; ph.f., phloem fiber; st.gr., starch granule; tr., tracheid; w.f., wood fiber; w.p., wood parenchyma; xyl.v., xylem vessel.

- 5- Fragments of lignified xylem vessels with spiral, reticulate and pitted thickenings.
- 6- Fragments of wood fibers with straight lignified walls, moderately wide lumen and blunt apices.
- 7- Fragments of thin-walled radially elongated parenchyma of the medullary rays containing starch granules.
- 8- Fragments of pericyclic fibers with straight thick lignified walls, moderately wide lumen and blunt to rounded apices.
- 9- Fragments of lignified xylem vessels with spiral, reticulate and pitted thickenings.
- 10- Fragments of wood fibers with straight lignified walls, moderately wide lumen and blunt apices.
- 11- Fragments of tracheids, with rounded apices and lignified pitted walls.
- 12- Numerous scattered prisms and clusters of calcium oxalate.
- 13- Numerous scattered starch granules which are mainly simple, but few are compound of 2-3 components.



**Fig. 6:** Photos of the isolated elements of the stem.

Ca.ox., calcium oxalate; col., collenchyma; epi., epidermis; m.r., medullary ray; per.f., pericyclic fiber; ph.f., phloem fiber; pi.par., pith parenchyma; st.gr., starch granule; tr., tracheid; w.f., wood fiber; w.p., wood parenchyma; xyl.v., xylem vessel.

## 2. Macro- and micro-morphological studies of the leaves

### 2.1. Macro-morphology of the leaflets (Fig. 7)

The leaf is palmately compound with long petiole. The petiole is about 5 to 25 cm long, reddish in color toward the base. It is thin, glabrous, and pulvinate on both ends with five to nine leaflets, each one has short petiolule. The leaflets are about 5-20 cm in length, glabrous, pinnately veined with lanceolate to oblanceolate lamina, entire margin and acuminate apices. The main vein is prominent on both sides and slightly reddish in color from the lower side, with 7-18 pairs of secondary lateral veins slightly anastomosed near the edge and slightly prominent on both sides. The base is cuneiform or attenuate with whole edges. The upper side of leaflet is dark green and the lower is pale green<sup>(32)</sup>.

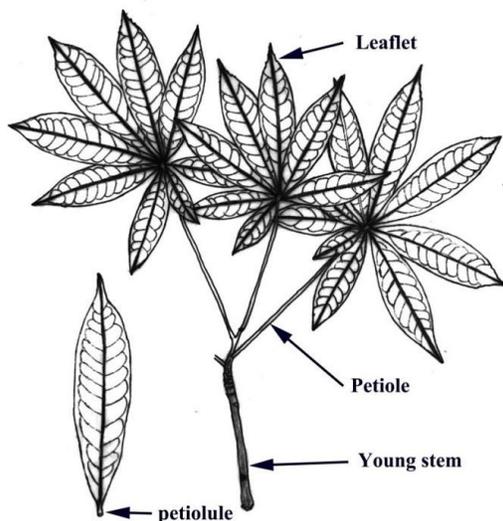


Fig. 7: Sketch of leaves of *C. pentandra* (X 0.37).

### 2.2. Micro-morphological study of the leaflet (Fig. 6)

A transverse section through the lamina of the leaflet shows a dorsiventral structure of one row of an upper palisade layer which is discontinuous in the midrib region being replaced by one row of hypodermal parenchyma and 6-8 rows of lignified parenchyma. Above the lower epidermis of the midrib, there are another 3-5 rows of collenchyma and 3-4 rows of tanniferous cells; distinguished by being stained bluish green with ferric chloride. The vascular system in the midrib region is represented by a large

crescent-shaped dissected vascular bundle accompanied by 3 separated, inverted smaller ones; contains groups of closed collateral vascular bundles. The pericycle is represented by parenchyma cells interrupted by groups of pericyclic fibers. The young leaflets are similar to the old ones except that they contain fewer cavities in lower region of the midrib.

### The epidermis (Figs. 8-11)

The upper epidermis in sectional view is formed of one row of square to sub-rectangular thin-walled cellulosic cells covered with thick cuticle. In surface view, they are polygonal, isodiametric to slightly-elongated with straight anticlinal walls and covered with thick, striated cuticle. Hairs and stomata are not observed. The upper epidermis in the midrib region is accompanied by one row of hypodermal parenchymatous cells. The upper epidermal cells of the lamina are larger in size than those present above the midrib region. The lower epidermal cells (Fig. 8C) are polygonal, nearly isodiametric to elongate with thin cellulosic, slightly-wavy anticlinal walls and covered with thick striated cuticle and show few glandular hairs with uniseriate stalk, multicellular head. The stomata are numerous, of the anisocytic type. The epidermal cells of the upper and lower surfaces contain mucilage (stained red with ruthenium red). The neural epidermal cells (Fig. 8C) appear in surface view as polygonal, axially-elongated with straight anticlinal walls and also containing mucilage (stained red with ruthenium red) and covered with thick striated cuticle.

### The mesophyll (Fig. 8B)

The mesophyll is dorsiventral, differentiated into upper palisade and spongy tissue. The palisade is formed of one layer of columnar cells containing chloroplast. It is interrupted in the midrib region by cortical tissue. The spongy mesophyll shows somewhat irregular thin-walled, more or less rounded chlorenchymatous cells interrupted by wide intercellular spaces. It shows scattered cluster crystals and prisms of calcium oxalate.

### The cortical tissue (Figs. 8&9)

The cortical tissue is formed of about 6-9 rows of lignified parenchyma with thick lignified walls and wide lumen, followed by a mucilagenous cavity and 1-2 rows of rounded

to oval thin, slightly lignified tanniferous cells. In the lower part of the midrib, the collenchyma is formed of 3-5 rows of rounded to oval collenchyma cells interrupted with cavities. The remaining cortical tissue is represented by several layers of slightly lignified tanniferous cells forming circle around the vascular bundle. The lower tanniferous layers are followed by a zone of parenchyma cells which are polygonal to round with thin cellulosic walls and showing intercellular spaces and contain starch granules.

#### **The pericycle (Figs. 8, 9, 11&12)**

The pericycle is formed of 3-8 rows of lignified pericyclic fibers which form an arc above the vascular bundle and patches below it. The fibers have tapering apices, wide lumen and slightly thick lignified walls. Some of the fibers are irregular in shape and bent. The pericyclic fibers in upper region penetrate into the phloem region to form arm like shape into the phloem of the separated vascular bundles.

#### **The vascular system (Figs. 8, 9, 11&12)**

The vascular system in the midrib region is represented by a large crescent-shaped, dissected vascular bundle accompanied by 3 separate, inverted, smaller ones (contains groups of closed collateral vascular bundles). Each system is surrounded by a pericycle.

#### **The xylem (Figs. 8, 9, 11&12)**

The xylary region consists of xylem vessels, wood fibers, wood parenchyma, and is traversed by 1-2 rows of rectangular lignified medullary rays. The vessels are lignified and have spiral, annular and pitted thickening. The wood fibers (Fig. 11) are few with blunt apices, wide lumen and slightly thick lignified walls. The wood parenchyma consists of rectangular to sub-rectangular cells radially elongated with lignified pitted walls and wide lumen.

#### **The phloem (Figs. 8&9)**

The phloem is formed of soft thin-walled cellulosic elements. The pericyclic fibers in the upper region penetrate the phloem region of the separated vascular bundles to form V-shaped phloem zone. Intra-xylary phloem is present in the lower vascular bundle which surrounded by an arc of thick-walled slightly lignified tanniferous cells. The phloem of the lower

region is presented by patches intersected by lignified cells.

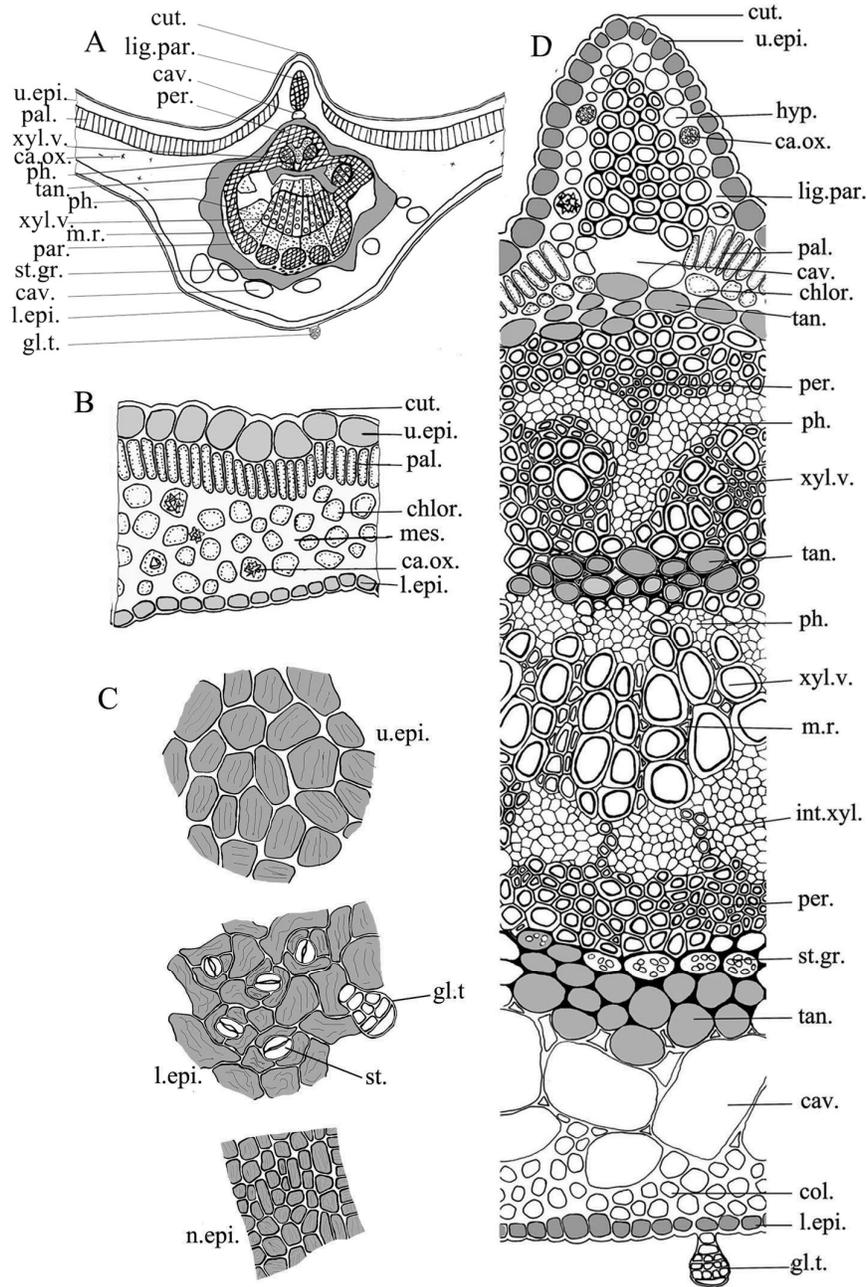
### **2.3. Micro-morphological study of petiolules of the leaflet (Fig. 10)**

A transverse section in the petiolules of the leaflets is nearly circular with three small projections at the upper side. It is similar to midrib of the leaflet but differs only in that, the cortex and the ground tissue are formed of polygonal to rounded collenchymatous cells with thin cellulosic walls and traversed by too much cavities than the midrib. It shows scattered cluster crystals and prisms of calcium oxalate and several rows of tanniferous cells around the vascular bundle.

#### **The powdered leaflets and petiolules (Figs. 11&12)**

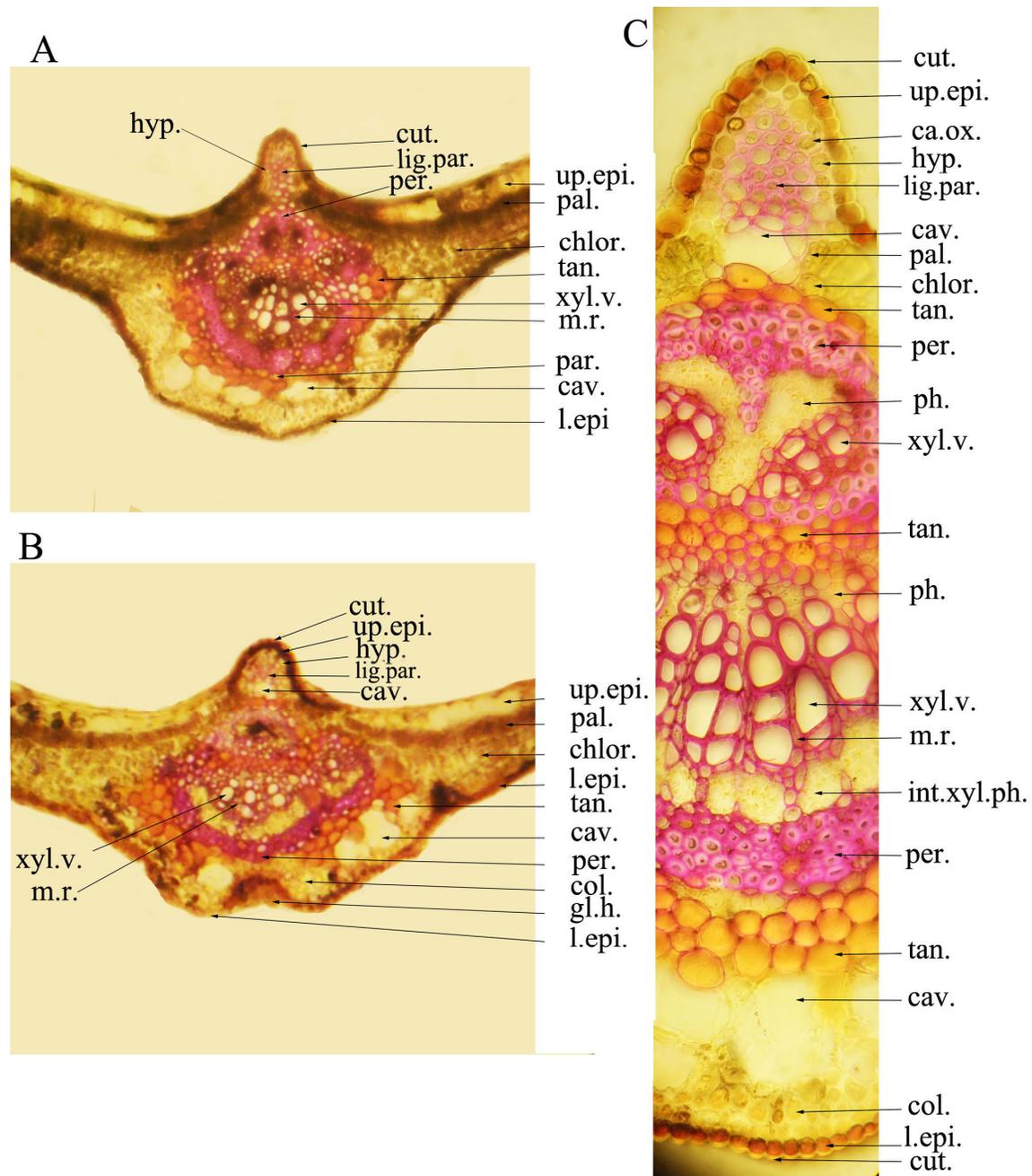
The powder is green in color with characteristic odor and mucilaginous taste. It is characterized microscopically by the followings:

- 1- Fragments of the upper epidermis of the leaflets showing polygonal, nearly isodiametric cells with straight anticlinal walls covered with thick striated cuticle.
- 2- Fragments of the lower epidermis of the leaflets showing polygonal, nearly isodiametric cells with wavy anticlinal walls and bearing anisocytic stomata and glandular hairs.
- 3- Few uniseriate stalk multicellular head glandular hairs.
- 4- Fragments of the columnar palisade cells of the mesophyll containing chloroplast.
- 5- Fragment of the cortical parenchyma cells containing crystals of calcium oxalate.
- 6- Fragments of pericyclic fibers with tapering apices, wide lumen and slightly thick lignified walls. Some of them are bent.
- 7- Fragments of spiral, annular and pitted lignified xylem vessels.
- 8- Fragments of wood fibers with straight, slightly thick lignified walls and blunt apices.
- 9- Fragments of radially elongated cells of the wood parenchyma with pitted lignified walls.
- 10- Numerous scattered prisms and cluster crystals of calcium oxalate.
- 11- Fragments of polygonal radially elongated tanniferous cells.
- 12- Fragments of thick-walled lignified parenchyma from the cortical region.



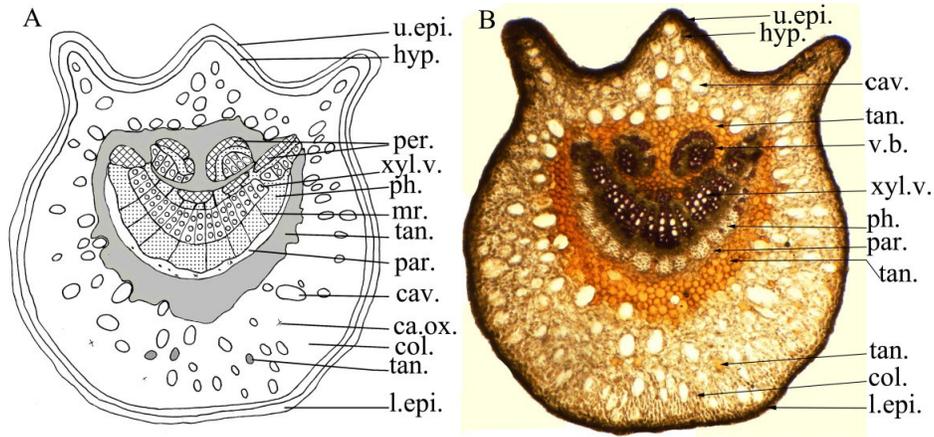
**Fig. 8:** Hand drawing of diagrammatic T.S. of the leaflet (A) (X 43.82), detailed T.S. of lamina of the leaflet (B) (X 99.9), surface preparation of the leaflet (C) (u.epi., X 151.2; l.epi. X 151.2; n.epi., X 147.66) and detailed T.S. of the leaflet (D) (X 201.5).

Ca.ox., calcium oxalate; cav., cavity; chlor., chlorenchyma; col., collenchyma; cut., cuticle; gl.t., glandular trichome; hyp., hypodermis; int.xyl.ph., intra-xylary phloem; l.epi., lower epidermis; lig.par., lignified parenchyma; mes., mesophyll; m.r., medullary rays; n.epi., neural epidermis; pal., palisade; par., parenchyma; per., pericycle; ph., phloem; st., stomata; st.gr., starch granules; tan., tanniferous cells; u.epi., upper epidermis; xyl.v., xylem vessels.



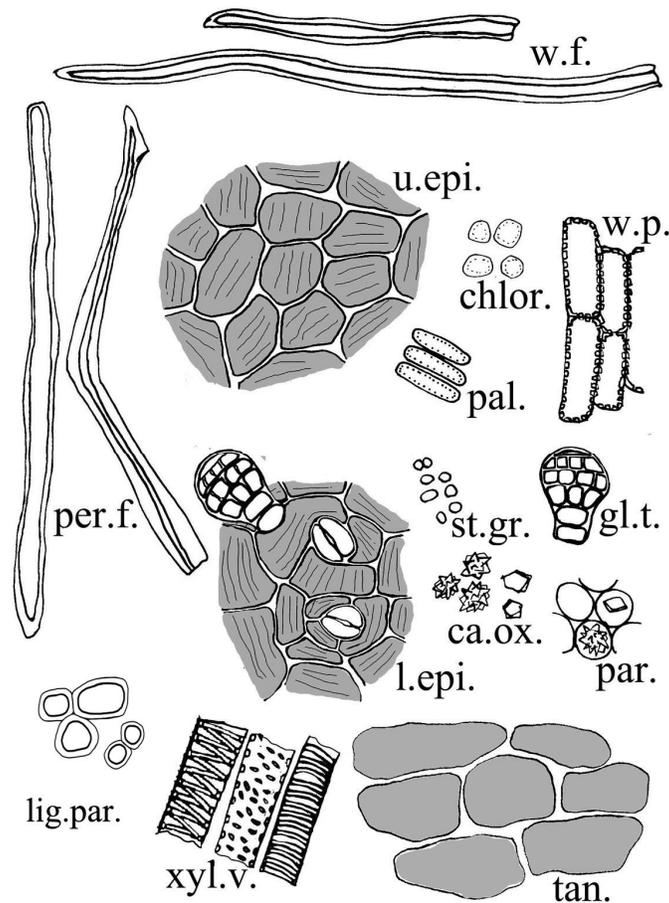
**Fig. 9:** Photos of diagrammatic T.S. in old leaflet (A), diagrammatic T.S. of young leaflet (B) detailed T.S. of the leaflet (C).

Ca.ox., calcium oxalate; cav., cavity; chlor., chlorenchyma; col., collenchyma; cut., cuticle; gl.h., glandular hair; hyp., hypodermis; int.xyl.ph., intra-xylary phloem; l.epi., lower epidermis; lig.par., lignified parenchyma; m.r., medullary rays; pal., palisade; per., pericycle; ph., phloem; st.gr., starch granules; tan., tanniferous cells; u.epi., upper epidermis; xyl.v., xylem vessels.



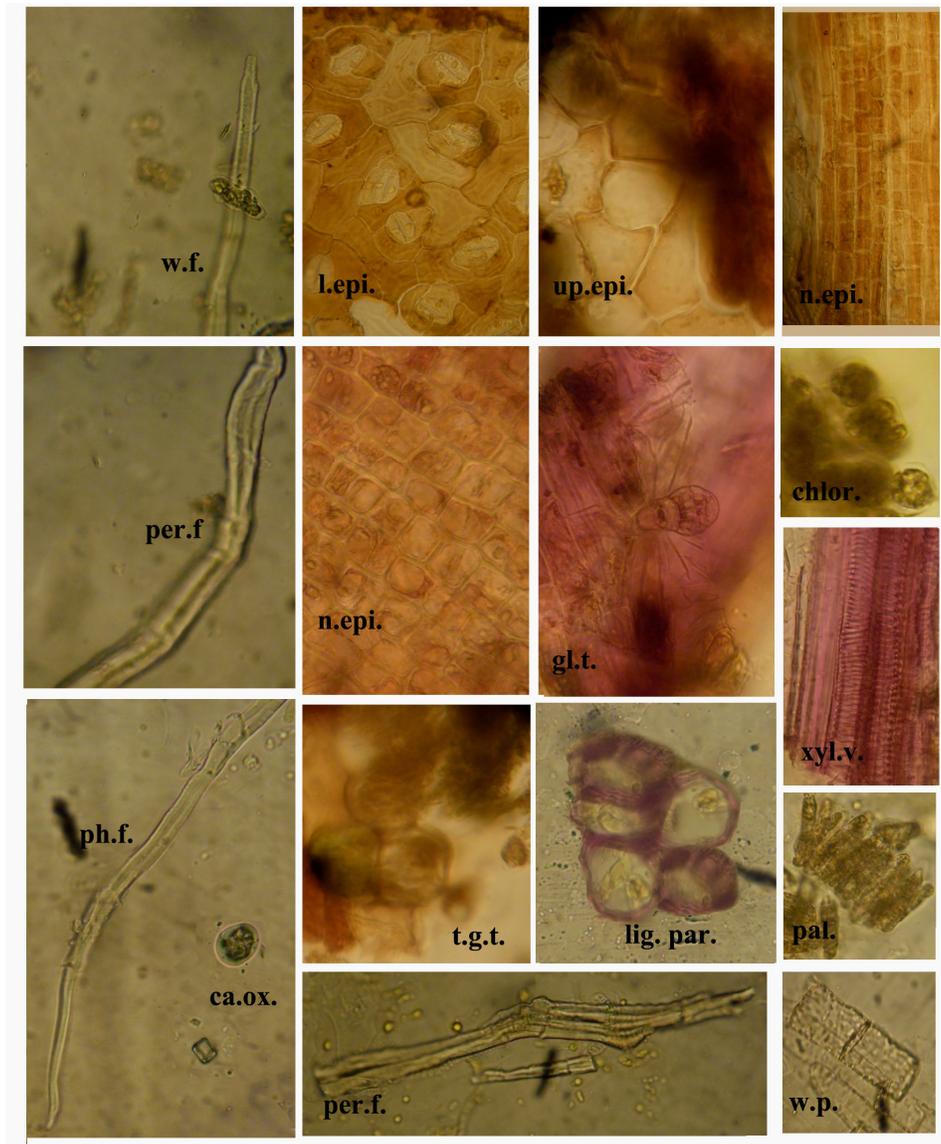
**Fig. 10:** Hand drawing of diagrammatic T.S. of the petiolule of the leaflet (A) (X 28.13), Photo of T.S. of the petiolule of the leaflet (B).

Ca.ox., calcium oxalate; cav., cavity; col., collenchyma; hyp., hypodermis; l.epi., lower epidermis; m.r., medullary rays; par., parenchyma; per., pericycle; ph., phloem; tan., tanniferous cells; u.epi., upper epidermis; v.b., vascular bundle; xyl.v., xylem vessels.



**Fig. 11:** Hand drawing of isolated elements of the leaflets (X 247.73).

Ca.ox., calcium oxalate; chlor., chlorenchyma; gl.t., glandular trichome; l.epi., lower epidermis; lig.par., lignified parenchyma; pal., palisade; par., parenchyma; per.f., pericyclic fibers; st.gr., starch granules; tan., tanniferous cells; u.epi., upper epidermis; w.f., wood fibers; w.p., wood parenchyma; xyl.v., xylem vessels.



**Fig. 12:** Photos of isolated elements of the leaflet.

Ca.ox., calcium oxalate; chlor., chlorenchyma; gl.t., glandular trichome; l.epi., lower epidermis; lig.par., lignified parenchyma; n.epi., neural epidermis; pal., palisade; per.f., pericyclic fibers; ph.f., phloem fibers; t.g.t., top view glandular trichome; u.epi., upper epidermis; w.f., wood fibers; w.p., wood parenchyma; xyl.v., xylem vessels.

#### **2.4. Micro-morphological study of leaf petiole (Figs. 13&14)**

A transverse section through the petiole is nearly circular with slightly wavy outline. It shows an outer epidermis accompanied by 1-2 rows of hypodermis consists of parenchyma cells. The cortex contains a ring of 9-12 rows of collenchymatous cells interrupted by mucilagenous cavities and three rows of tanniferous parenchymatous cells. The pericycle consists of groups of pericyclic

fibers. The vascular system is formed of dissected bundles; each consists of a radiating xylem and outer phloem. The vascular system is enclosing comparatively wide pith. Groups of intra-xylary phloem are scattered at periphery of the pith just beneath the xylem vessels.

#### **The epidermis (Figs. 13-16)**

The epidermis consists of one row of isodiametric to sub-rectangular cells. In surface

view, they are polygonal mostly sub-rectangular cells with straight anticlinal walls, covered with striated cuticle and containing mucilage (stained red with ruthenium red). The epidermal cells are followed by 1-2 rows of hypodermal parenchyma cells containing cluster crystals and few prisms of calcium oxalate. The epidermal cells also show anisocytic stomata and trichomes.

#### **The cortex (Figs. 13&14)**

It consists of ring of an outer zone of 7-9 rows of rounded collenchymatous cells interrupted by mucilagenous cavities and about 3-4 rows of tanniferous parenchymatous cells.

#### **The pericycle (Figs. 13-16)**

The pericycle is formed of lignified fibers forming a circle interrupted by thick, pitted-walled parenchyma cells. The pericycle forms continuous ring around the vascular bundle. The fibers have moderately thick lignified walls and narrow to moderately wide lumen with blunt to rounded apices and some of them are septated.

#### **The vascular system (Figs. 13-16)**

The vascular system is formed of dissected bundles interrupted by parenchyma cells. The phloem consists of shining thin-walled cellulosic soft elements of sieve tubes, companion cells and phloem parenchyma surrounding the xylem. The xylary elements include xylem vessels, wood fibers, wood parenchyma and tracheids. The vessels are lignified show annular, spiral, and pitted thickening. Wood fibers have blunt apices, wide lumen and slightly thick lignified walls. Tracheids are long with pitted lignified walls. The medullary rays are in the form of 1-3 radiating rows traversing the xylem and composed of sub-rectangular lignified cells. The pith consists of somewhat rounded to isodiametric, thick-walled lignified parenchyma cells with wide intercellular spaces and some of them are pitted. The cells contain starch, and prisms and cluster crystals of calcium oxalate. It also shows numerous tanniferous cells. Groups of intra-xylary phloem are scattered at periphery of the pith just beneath the xylem vessels.

#### **The powder of the petiole (Figs. 15&16)**

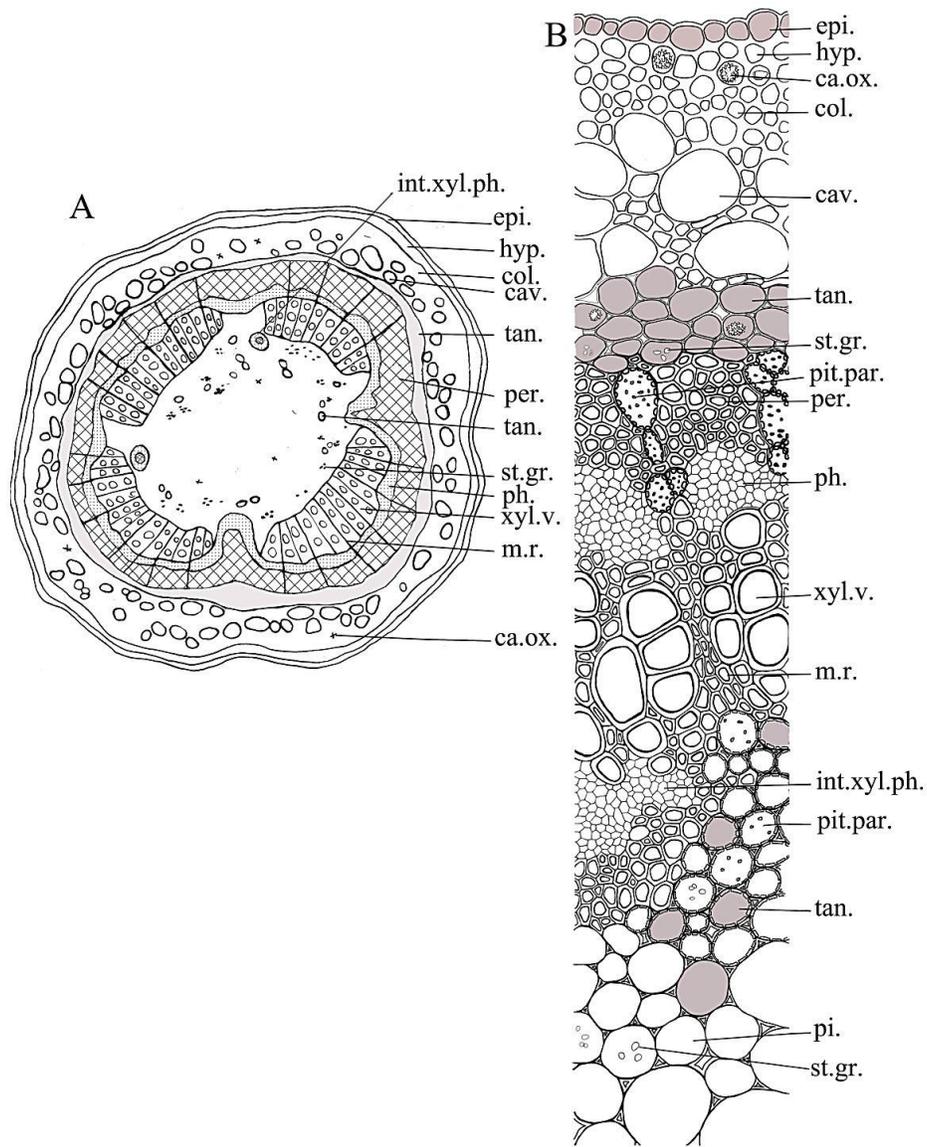
The powdered petiole is reddish brown in color with faint odor and slightly bitter taste. It is characterized microscopically by the followings:

- 1- Fragments of the upper epidermis show sub-rectangular to square; nearly isodiametric cells with straight anticlinal walls that covered with thin striated cuticle, and bearing anisocytic stomata and glandular trichomes.
- 2- Few uniseriate stalk multicellular head glandular hairs.
- 3- Fragment of the cortical tissues showing parenchyma cells contain prisms and clusters crystals of calcium oxalate, and starch granules.
- 4- Fragments of pericyclic fibers with tapering apices, wide lumen and slightly thick lignified walls.
- 5- Fragments of spiral, annular and pitted lignified xylem vessels. Fragments of wood fibers with straight, slightly thick lignified walls and blunt apices.
- 6- Fragments showing radially elongated wood parenchyma and pitted lignified tracheids.
- 7- Numerous scattered cluster crystals of calcium oxalate.
- 8- Fragments of polygonal tanniferous cells.
- 9- Fragments of lignified thick-walled, radially elongated medullary rays.
- 10- Fragments of pith parenchyma containing starch granules.
- 11- Fragments of parenchyma from the pith showing pitted wall.

### **3. Macro-and micro-morphological studies of the fruits**

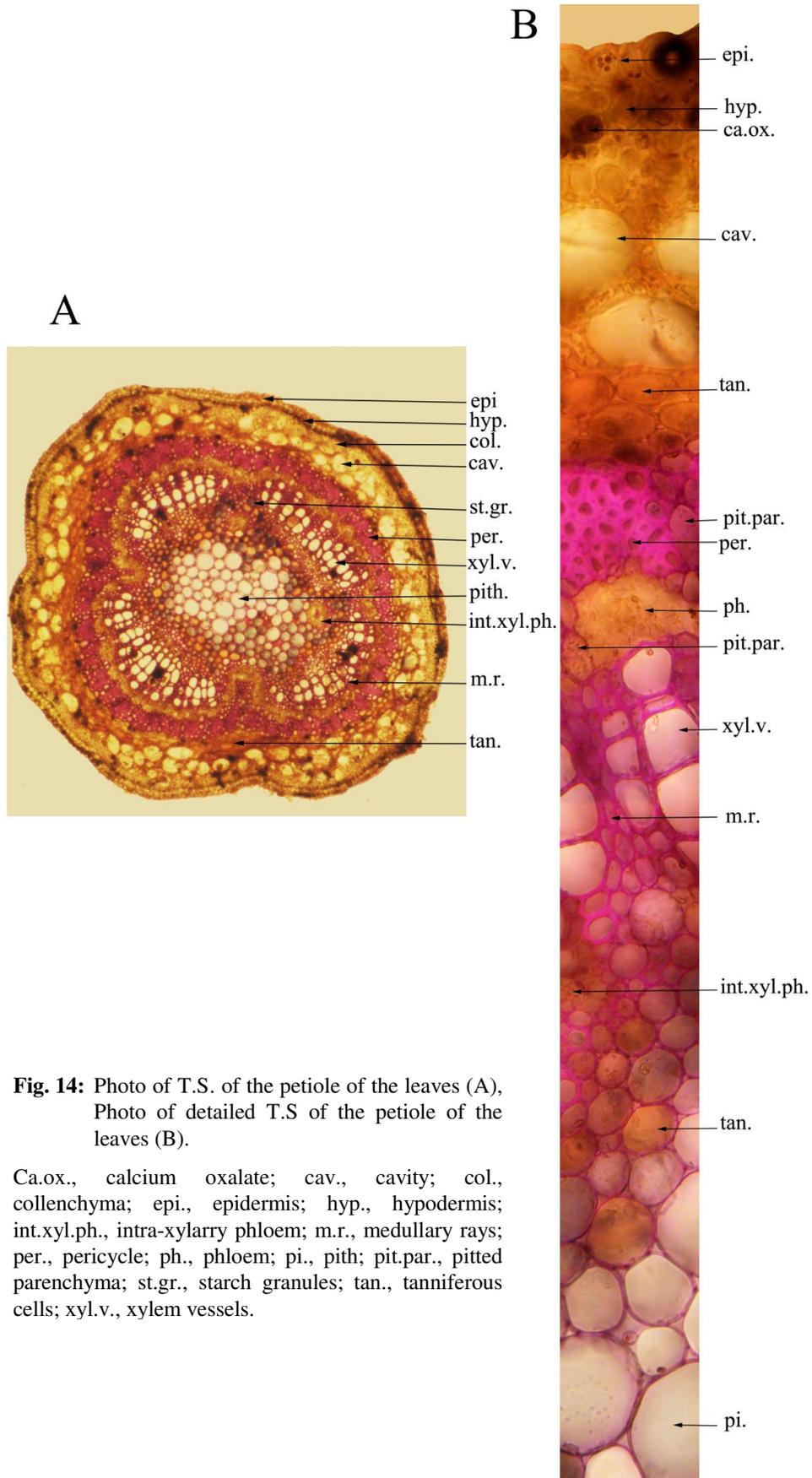
#### **3.1. Macro-morphology of the fruits (Fig. 17)**

The fruits are pendulous, oblong-ellipsoid capsule, measures about 7-30 cm length × 3-5 cm diameter, narrowed at both ends (banana-shaped). It is usually indehiscent and smooth-valved. The unripe fruits are green in color and become brown upon ripening and contain white flossy fibers and many seeds. Seeds are almost 4-6 mm in diameter, glabrous, dark brown or black in color and embedded in the copious white flossy of the fruits<sup>(32)</sup>.



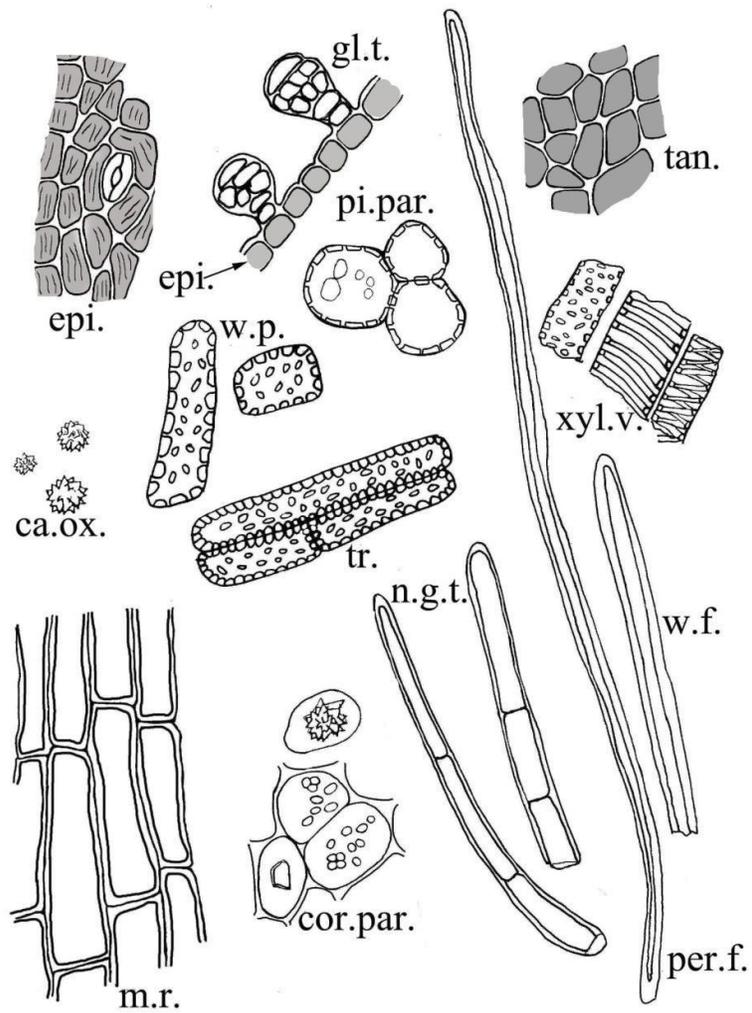
**Fig. 13:** Hand drawing of diagrammatic T.S. of the petiole of the leaves (A) (X 39.54), and detailed T.S. of the petiole of the leaves (B) (X 137.76).

Ca.ox., calcium oxalate; cav., cavity; col., collenchyma; epi., epidermis; hyp., hypodermis; int.xyl.ph., intra-xylary phloem; m.r., medullary rays; per., pericycle; ph., phloem; pi., pith; pit.par., pitted parenchyma; st.gr., starch granules; tan., tanniferous cells; xyl.v., xylem vessels.



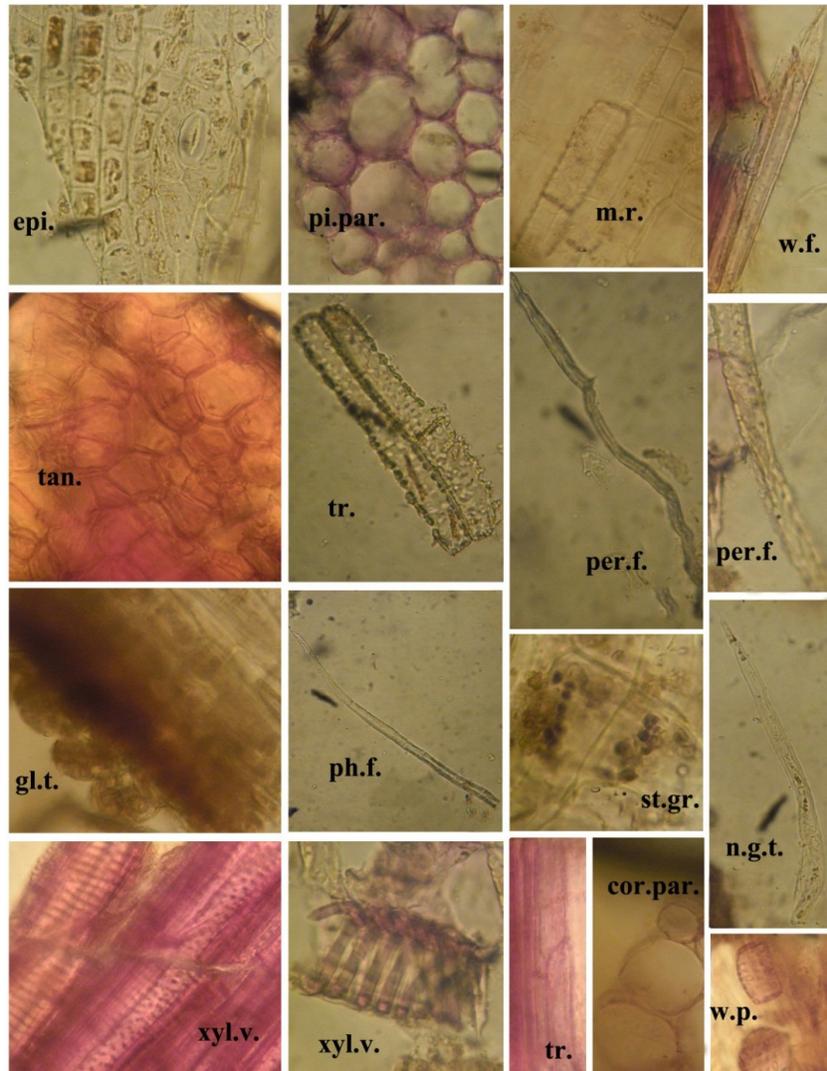
**Fig. 14:** Photo of T.S. of the petiole of the leaves (A), Photo of detailed T.S of the petiole of the leaves (B).

Ca.ox., calcium oxalate; cav., cavity; col., collenchyma; epi., epidermis; hyp., hypodermis; int.xyl.ph., intra-xylary phloem; m.r., medullary rays; per., pericycle; ph., phloem; pi., pith; pit.par., pitted parenchyma; st.gr., starch granules; tan., tanniferous cells; xyl.v., xylem vessels.



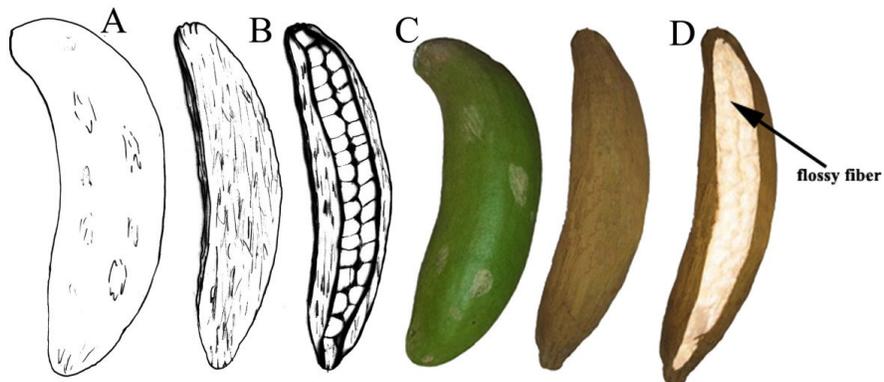
**Fig. 15:** Hand drawing of isolated elements of petiole (X 224).

Ca.ox., calcium oxalate; cor.par., cortical parenchyma; epi., epidermis; gl.t., glandular trichome; m.r., medullary rays; n.g.t., non-glandular trichome; pi.par., pith parenchyma; per.f., pericyclic fibers; st.gr., starch granules; tan., tanniferous cells; tr., tracheids; w.f., wood fibers; w.p., wood parenchyma; xyl.v., xylem vessels.



**Fig. 16:** Photos of isolated elements of the petiole.

Cor.par., cortical parenchyma; epi., epidermis; gl.t., glandular trichome; m.r., medullary rays; n.g.t., non-glandular trichome; pi.par., pith parenchyma; per.f., pericyclic fibers; ph.f., phloem fibers; st.gr., starch granules; tan., tanniferous cells; tr., tracheids; w.f., wood fibers; w.p., wood parenchyma; xyl.v., xylem vessels.



**Fig. 17:** Sketches of the unripe fruits (A), and the ripe fruits (B), Photos of the unripe fruits (C) and the ripe fruits (D) (X 0.33).

### **3.2. Micro-morphological study of the fruit pericarp**

A transverse section through the unripe pericarp of the fruit is nearly circular in outline. It shows an outer epicarp layer followed by seven to nine rows of sclerenchyma cells occasionally intersected by four to six layers of chlorenchyma cells. The mesocarp consists of parenchymatous cells traversed by numerous fibro-vascular bundles surrounded by sclereids. The endocarp is formed of five to seven rows of thin-walled parenchymatous cells ended with floss region. The floss silk inside the endocarp forms a large mass surrounding the seeds.

#### **The epicarp (Figs. 18-21)**

It is composed of thick, hard, single layer of square to sub-columnar polygonal cells. The cells of the epicarp have thick, pitted, cellulosic, straight anticlinal walls and covered with thick smooth cuticle and show few anomocytic stomata. The epidermal cells surround the stomata are thin-walled and containing mucilage.

#### **The mesocarp (Figs. 18&19)**

It is formed of an outer region consists of hypodermis of five to seven rows of polyhedral to sub-rectangular pitted, thick-walled sclerenchymatous cells, occasionally intersected by four to six layers of chlorenchyma cells. The inner region is formed of several layers of parenchymatous cells interrupted by numerous fibro-vascular bundles surrounded by sclereids. The sclereids are polygonal isodiametric, sometimes elongated, lignified, with generally slightly thickened, pitted walls and wide lumen.

#### **The fibro-vascular bundle**

#### **The pericycle (Figs. 18-21)**

The pericycle is formed of groups of lignified fibers. The fibers are straight with thick lignified walls, moderately wide lumen, and blunt to rounded apices.

#### **The vascular system (Figs. 18-21)**

The vascular system consists of enlarged parenchyma cells with thick cellulosic walls followed by vascular bundles (phloem, xylem).

#### **The phloem (Figs. 18-21)**

The phloem consists of shining cellulosic thin-walled phloem parenchyma, which occasionally traversed by thick-walled parenchyma cells. The phloem fibers are similar to those of the pericycle.

#### **The xylem (Figs. 18-21)**

The xylem consists of a comparatively wide zone of lignified thick-walled, radially arranged elements traversed by medullary rays with thin slightly lignified walls. The xylary elements include xylem vessels and wood parenchyma. The vessels are arranged in radial rows and show spiral and pitted thickenings.

#### **The medullary rays (Figs. 18&19)**

The medullary rays consist of multi-seriate, sub-rectangular, slightly elongated cells with thick lignified walls.

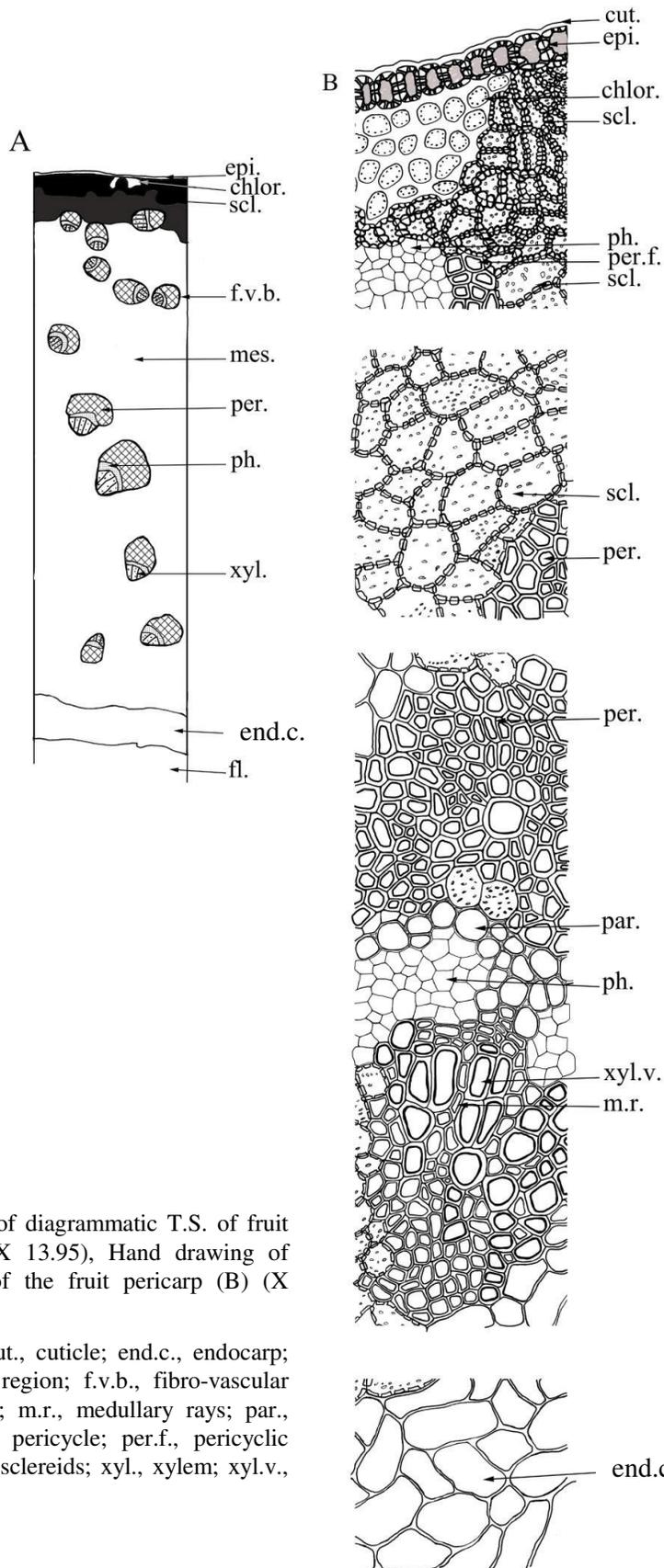
#### **The endocarp (Figs. 18&19)**

The endocarp is formed of five to seven layers of thin-walled polygonal elongated parenchyma cells ended with floss region. The floss silk inside the endocarp forms a large mass surrounding the seeds. The silky fibers are very long, thin-walled, cellulosic, having tapering ends with wide lumen.

#### **The powder of the fruits (Figs. 20&21)**

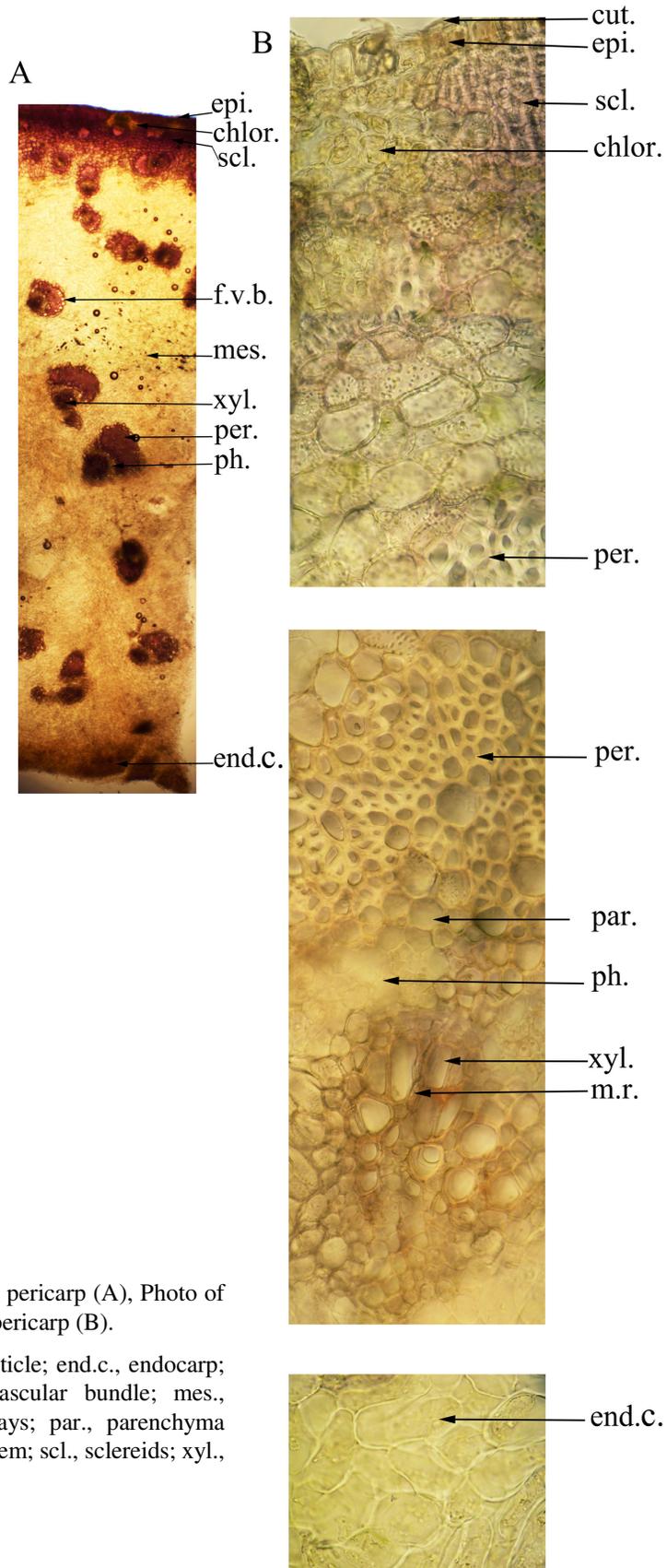
The powder of the dry ripe fruits is odorless, tasteless and brownish yellow in color. It is characterized microscopically by the presence of the following elements:

- 1- Fragments of the epicarp formed of square to sub-columnar polygonal cells with thick cellulosic, pitted, straight, anticlinal walls covered with thick smooth cuticle, and containing mucilage.
- 2- Fragments of slightly thick-walled pitted parenchyma.
- 3- Fragments of lignified vessels with spiral and pitted thickenings.
- 4- Fragments of polygonal isodiametric sclereids having thick-wall and narrow lumen.
- 5- Fragments of isodiametric sclereids with slightly thick, pitted, lignified wall and wide lumen accompanied, often, with fragments of pericyclic fibers.
- 6- Numerous elongated tortuous, forked pericyclic fibers with blunt apices and wide lumen.



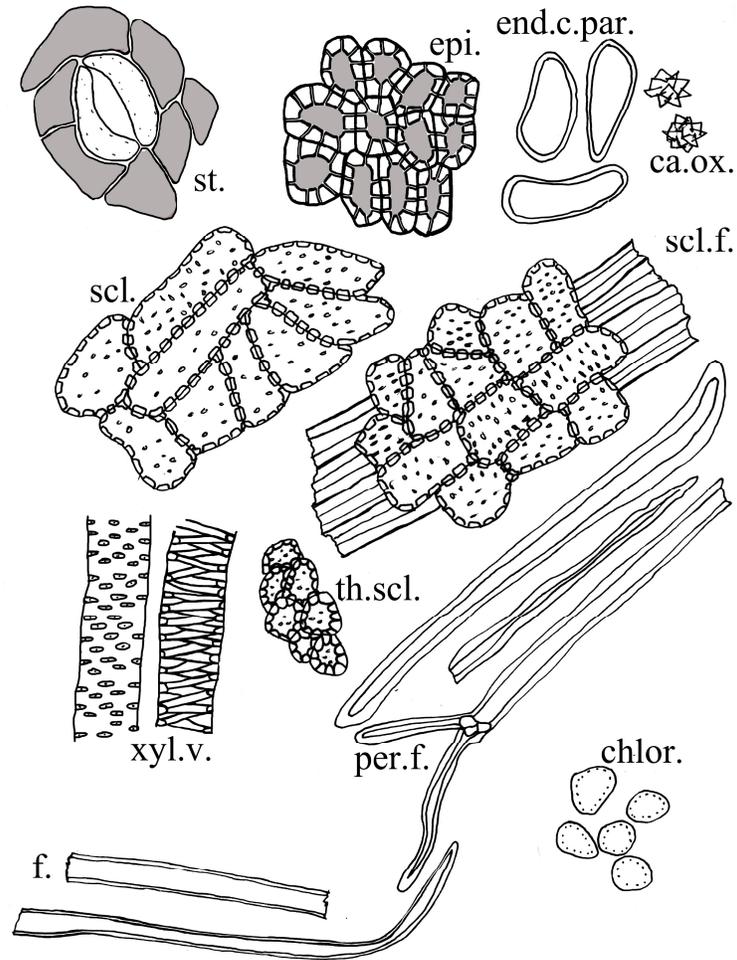
**Fig. 18:** Hand drawing of diagrammatic T.S. of fruit pericarp (A) (X 13.95), Hand drawing of detailed T.S. of the fruit pericarp (B) (X 164.85).

chlor., chlorenchyma; cut., cuticle; end.c., endocarp; epi., epicarp; fl., floss region; f.v.b., fibro-vascular bundle; mes., mesocarp; m.r., medullary rays; par., parenchyma cells; per., pericycle; per.f., pericyclic fiber; ph., phloem; scl., sclereids; xyl., xylem; xyl.v., xylem vessels.



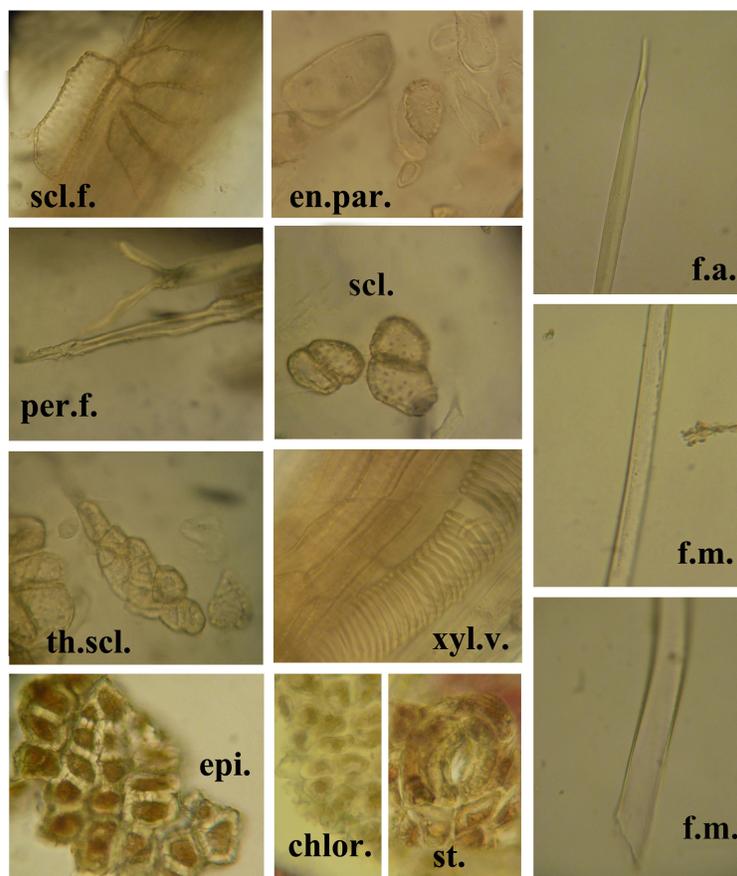
**Fig. 19:** photo of T.S. of fruit pericarp (A), Photo of detailed T.S of fruit pericarp (B).

chlor., chlorenchyma; cut., cuticle; end.c., endocarp; epi., epicarp; f.v.b., fibro-vascular bundle; mes., mesocarp; m.r., medullary rays; par., parenchyma cells; per., pericycle; ph., phloem; scl., sclereids; xyl., xylem.



**Fig. 20:** Hand drawing of isolated elements of the fruit pericarp (X 193).

Ca.ox., calcium oxalate; chlor., chloroplast; end.c.par., endocarp parenchyma; epi., epidermis; f., flossy fibers; per.f., pericyclic fiber; pi.par., pitted parenchyma; st., stomata; scl., sclereids; scl.f., sclereids with fibers; th.scl., thick walled sclereids; xyl.v., xylem vessels.



**Fig. 21:** Photos of isolated elements of the fruit pericarp.

chlor., chlorenchyma; en.par., endocarp parenchyma; epi., epicarp; f.a., flossy fibers apex; f.m., flossy fibers middle; per.f., pericyclic fiber; pi.par., pitted parenchyma; st., stomata; scl.f., sclereids with fibers; th.scl., thick walled sclereids; xyl.v., xylem vessels.

- 7- Occasional cluster crystals of calcium oxalate.
- 8- Fragments of parenchyma cells of the endocarp.
- 9- Fragments of chlorenchyma cells.
- 10- Fragments of epicarp showing anomocytic stomata.
- 11- Fragments of floss showing thin-wall, wide lumen fibers with blunt apices.

#### DISCUSSION AND CONCLUSION

*C. pentandra* (L.) Gaertn. is one of the famous plants used in traditional medicine in various localities of the world<sup>(7)</sup>. In addition, modern studies on solvent extracts of various parts of the plant revealed a wide range of pharmacological effects<sup>(2&8)</sup>. In the present

investigations, the different parts (leaves, stems and fruits) of *C. pentandra* were subjected to detailed morphological and anatomical studies which can be employed for identification, characterization and its differentiation from other related varieties. Likewise, the genomic DNA of the plant has been subjected to random amplified analysis. Five oligonucleotide primer pairs induced successive amplifications with a wide range of molecular size. The analysis of the amplified fragments generated by RAPD reactions revealed that the biotype of *C. pentandra* produces different molecular patterns from previously studied species of the family<sup>(18)</sup>. The primers A, B, D and E are thus usable for the future identification and genetic differentiation of *C. pentandra* from the other morphologically related varieties.

**Table 3:** Microscopical measurements of the different organs of *Ceiba pentandra* (L) Gaertn. var. *pentandra* (in micron).

Item	L			W			H			D		
<b>Young stem</b>												
Epidermis	43	<u>63</u>	83	19	<u>27</u>	36	8	<u>12</u>	16			
Hypodermis										12	<u>24</u>	35
Parenchyma of cortex										12	<u>33</u>	55
Collenchyma										8	<u>14</u>	20
Cavities	35	<u>65</u>	94	31	<u>57</u>	83						
Calcium oxalate clusters										16	<u>24</u>	31
Calcium oxalate prisms	12	<u>24</u>	36									
Starch										5	<u>10</u>	14
Xylem vessels										59	<u>79</u>	98
Pericyclic fibres	524	<u>589</u>	655							7	<u>13</u>	19
Phloem fibres	224	<u>281</u>	338							12	<u>15</u>	19
Wood fibres	238	<u>292</u>	345							10	<u>18</u>	26
Wood parenchyma	38	<u>43</u>	48	24	<u>27</u>	31						
Tracheids	64	<u>81</u>	98	19	<u>26</u>	33						
Medullary rays	19	<u>62</u>	105	8	<u>23</u>	38						
<b>Leaflet</b>												
Upper epidermis	36	<u>48</u>	60	21	<u>33</u>	45	9	<u>32</u>	55			
Lower epidermis	18	<u>43</u>	68	6	<u>27</u>	48	9	<u>13</u>	16			
Neural epidermis	9	<u>21</u>	33	6	<u>12</u>	19						
Stomata	18	<u>24</u>	30							15	<u>18</u>	21
Palisade	46	<u>58</u>	69	9	<u>14</u>	19						
Cavities	57	<u>78</u>	100	9	<u>33</u>	57						
Lignified parenchyma										11	<u>23</u>	34
Tanniferous cell	18	<u>55</u>	92	11	<u>22</u>	32						
Chlorenchyma										12	<u>27</u>	42
Collenchyma										7	<u>11</u>	16
Parenchyma										16	<u>21</u>	25
Pericyclic fibres	267	<u>296</u>	324							7	<u>18</u>	30
Wood fibres	185	<u>272</u>	359							8	<u>12</u>	16
Wood parenchyma	45	<u>54</u>	64	16	<u>20</u>	23						
Xylem vessels										14	<u>34</u>	55
Calcium oxalate clusters										12	<u>16</u>	21
Calcium oxalate prisms	10	<u>13</u>	16									
Starch granules										6	<u>8</u>	10
Glandular hair	39	<u>49</u>	60							37	<u>47</u>	57
Medullary rays	7	<u>15</u>	23									
<b>Petiole</b>												
Epidermis of petiole	21	<u>33</u>	45	10	<u>18</u>	26	14	<u>22</u>	31			
Collenchyma										9	<u>21</u>	34
Stomata	29	<u>33</u>	38							14	<u>19</u>	24
Glandular hair	45	<u>52</u>	60							33	<u>38</u>	43
Cavities										29	<u>45</u>	62
Tanniferous cell	17	<u>32</u>	48	12	<u>20</u>	29						
Starch granules										5	<u>10</u>	14
Pitted parenchyma										27	<u>46</u>	65
Pericyclic fibres	595	<u>652</u>	710							10	<u>20</u>	31

Item	L			W			H			D		
Xylem vessels										20	58	95
Medullary rays	93	<u>118</u>	143	17	<u>21</u>	26						
Parenchyma										20	<u>48</u>	75
Tracheids	71	<u>123</u>	174	19	<u>24</u>	29						
Calcium oxalate clusters										12	<u>20</u>	29
Calcium oxalate prisms	12	<u>17</u>	21									
Wood parenchyma	55	<u>87</u>	119	26	<u>32</u>	38						
Wood fibres	238	<u>252</u>	267	14	<u>18</u>	21						
Non-glandular hair	217	<u>245</u>	274	12	<u>18</u>	24						
Cortex parenchyma										20	<u>41</u>	62
<b>Fruit</b>												
Epicarp	31	<u>43</u>	54	23	<u>35</u>	47	25	<u>30</u>	36			
Chlorenchyma										10	<u>23</u>	36
Thick walled sclereids	21	<u>27</u>	34	16	<u>22</u>	28						
Thin walled sclereids	36	<u>83</u>	130	10	<u>21</u>	31						
Pericyclic fiber	231	<u>307</u>	383							11	<u>25</u>	39
Parenchyma cells										14	<u>29</u>	43
Xylem vessels										25	<u>38</u>	50
Calcium oxalate clusters										26	<u>32</u>	39
Stomata	60	<u>69</u>	78							36	<u>49</u>	62
Epicarp cells around stomata	49	<u>69</u>	88	23	<u>34</u>	44						
Flossy fibers	1100	<u>1240</u>	1380							8	<u>17</u>	26
Endocarp parenchyma	36	<u>63</u>	89	21	<u>38</u>	54						
Pitted parenchyma										31	<u>48</u>	65

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## نشرة العلوم الصيدلانية جامعة أسيوط



### دراسة نباتية وبصمة الحمض النووي لنبات سيبا بنتاندرا (ل. جارتن). صنف بنتاندرا المنزرع في مصر

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يتبع نبات سيبا بنتاندرا (ل. جارتن) (الكابوك) عائلة البومباكس وهو عبارة عن شجرة كبيرة، سريعة النمو وتنمو بشكل طبيعي في المناطق الاستوائية وشبه الاستوائية، وكذلك تزرع على جوانب الطرق كأشجار للظل. تستخدم الأجزاء المختلفة للنبات في علاج العديد من الأمراض بما في ذلك مرض السكري والتهاب الشعب الهوائية والأمراض الجلدية والإسهال والدوسنتاريا وأمراض العيون والتهاب المفاصل ولدغة الحشرات والحمى المزمنة. يوجد هناك أربعة أصناف من هذا النبات وبرغم ذلك لا توجد معايير محددة للتعرف على هذه الأصناف والتفرقة بينها في الدراسات السابقة، لهذه الأسباب فقد أجريت هذه الدراسة، لتحديد وصفاً مفصلاً للصفات العيانية والمجهريّة للساق والأوراق والثمار للصنف بنتاندرا محل الدراسة. بالإضافة إلى ذلك تمت دراسة بصمة الحمض النووي لهذا الصنف للمساعدة على التأكد من هويتها على المستوى الجيني.