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Research Article

Pharmacognostical Investigation of Leaf and Stem of *Persea americana*

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ABSTRACT

Persea americana Mill (family Lauraceae) is an evergreen tree native to Mexico and Central America and distributed in tropical and subtropical regions around the world. Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world. The present study deals with macro and micromorphological investigations of leaf and stem *Persea americana*, which assists in identification and standardization of the plant in both entire and powdered forms.

Keywords: Lauraceae, *Persea americana*, leaf, stem, macro and micromorphology.

INTRODUCTION

The Lauraceae is a great widely spread family, is also known as the Laurel family which includes the true laurel and its closest relatives. The family comprises over 3000 species of flowering plants in over 50 genera worldwide. They grow mainly in warm temperate and tropical regions, especially Southeast Asia and South America[1].

The genus *Persea* comprises approximately 150 species of evergreen trees. The best-known member of the genus is the avocado, *P. americana* Mill (synonym: *Persea gratissima*), which is an evergreen tree native to Mexico and Central America and distributed in tropical and subtropical regions around the world. Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world. The avocado fruit is large edible drupe containing a single seed and sometimes called an avocado pear or alligator pear (due to its shape and the rough green skin of some cultivars)[2,3].

Avocado trees are vulnerable to bacterial, viral, fungal, and nutritional diseases (excesses and deficiencies of key minerals). Disease can affect all parts of the plant, causing spotting, rotting, cankers, pitting, and discoloration[4].

The leaves are chewed by man as a remedy for pyorrhea, and the aqueous extract of the leaves has a prolonged hypotensive effect. It has been shown to possess anti-inflammatory, anti-convulsant, anti-diabetic, and vasorelaxant activities[5].

Avocado leaves, bark, skin, or pit are documented to be harmful to animals; cats, dogs, cattle, goats, rabbits[6], which can be severely harmed or even killed when they consume them. The avocado fruit is poisonous to some birds, and the American Society for the Prevention of Cruelty to Animals (ASPCA) lists it as toxic to many animals including cats, dogs, and horses. Avocado leaves contain a toxic fatty acid derivative named persin, which in sufficient quantity can cause colic in horses without veterinary treatment and finally death[7].

Taxonomy

The plant under investigation *Persea americana* (Fig. 1) according to the latest classification ("ITIS: Integrated Taxonomic Information System") belongs to:

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta – Vascular plants
Superdivision: Spermatophyta – Seed plants Division: Magnoliophyta – Flowering plants Class: Magnoliopsida – Dicotyledons Subclass: Magnoliidae
Order: Laurales
Family: Lauraceae – Laurel family Genus: *Persea* Mill. – bay
Species: *Persea americana* Mill. –avocado

MATERIALS AND METHODS

Plant material

The leaf and stem of *P. americana* were collected from El-Zohria botanical garden, Giza, Egypt, in April 2011. The plant was kindly identified by Prof. Dr. Mamdouh Shokry, director of El-zohriya garden. A voucher sample (Mn-ph-Cog-12) was kept in the Herbarium of Pharmacognosy Department, Faculty of pharmacy, Minia University, Minia, Egypt. The fresh samples of the plant preserved in a mixture of ethanol – glycerin – water (1:1:1). The plant material was air dried, reduced to fine powder and stored in closely tight containers.

Dyes

Safranin, xylol, Sudan III, iodine solution phloroglucinol and conc. HCl were used for staining the plant sections

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Table 1: Microscopical measurements of the different organs of *P. americana* (in microns)

Item	Length	Width	Height	Diameter
		Leaf		
Upper epidermis	29-32-48	16-29-35	14-12-10	
Lower epidermis	23-31-40	17-23-31	8-10-13	
Neural epidermis	17-27-33	12-14-16	15-20-23	
Non-glandular hair	104-113-104	8-11-12		
Stomata	18-20-22	16-18-21		
Collnchyma (above vascular bundle)				11-16-19
Collnchyma (below vascular bundle)				9-14-19
Parenchyma (above vascular bundle)				28-31-34
Parenchyma (below vascular bundle)				32-39-46
Spongy parenchyma of the lamina				20-24-31
Palisade cells (first row)	59-62-66	16-13-10		
Palisade cells (second row)	31-34-41	15-12-9		
Oil cells	72-80	34-38		
Xylem vessels				12-17-25
Wood parenchyma	200-220-230	20-25-27		
Phloem fibers	490-550-625	18-26-30		
Clusters of calcium oxalate				8-10-12
Prisms of calcium oxalate				9-12-14
Needles of calcium oxalate	8-10-16	1-2-5		
Petiole				
Epidermal cells	18-25-34	17-22-31	8-10-11	
Non-glandular hairs	96-160-200	8-10-12		
Stomata	17-20-23	16-18-22		
Collnchyma				16-24-28
Parenchyma				40-45-48
Oil cells	93-96	64-48		
Xylem vessels				11-18-25
Phloem fibers	490-550-625	18-26-30		
Clusters of calcium oxalate				8-10-12
Prisms of calcium oxalate				9-11-13
Needles of calcium oxalate	8-10-15	1-2-4		
Stem				
Cork cells	9-10-11	13-15-17	3-4-5	
Epidermal cells	15-17-20	10-15-21	16-20-24	
Non-glandular Hairs	78-93-109	9-10-11		
Parenchyma of cortex				40-48-56
Pericyclic fibers	195-280-312	18-20-22		
Tracheids	203-209-214	31-48-54		
Phloem fibers	250-265-270	11-12-14		
Tracheidal vesssels	234-250-265	43-46-48		
Xylem vessels				25-31-53
Wood fibers	312-343-375	12-15-18		
Wood parenchyma	40-54-62	21-25-28		
Cluster of calcium oxalate				8-10-13
Needles of calcium oxalate	9-10-15	1-2-4		
Prisms of calcium oxalate				9-12-14
Parenchyma of pith				46-53-65

and the powder[8].

Microscopic studies

Transverse, longitudinal sections and the powder of the leaf and stem were examined using microscope with camera, Leica® (Germany) and digital, 10 megapixels camera, Canon (Japan).

RESULTS AND DISCUSSION

Macromorphlogy of P. americana

Figure 1: *P. americana* tree (x0.02).

Figure 2: Leaves of *P. americana*

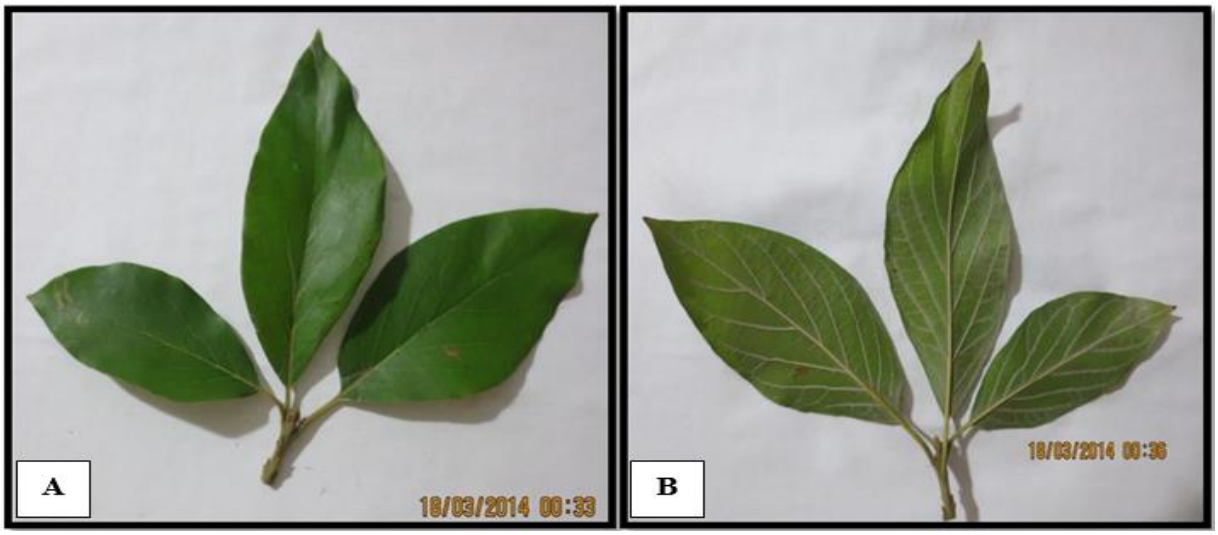

Figure 3: A: Upper surface of the leaf (x0.5); B: Lower surface of the leaf (x0.5)

Macromorphology of the leaf

The leaves (Fig. 2 and 3) are evergreen, simple, alternate, petiolate, exstipulate, elliptic, ovate, obovate or lanceolate. The upper leaves are small measuring about 6-8 cm in length and 2-5 cm in width while the lower ones measure 12-20 cm in length and 5-8 cm in width. The apex is mostly acute to short acuminate; the base is symmetric and the margin is entire. They have a dark green upper surface and a paler lower one and both surfaces are leathery with pinnate reticulate venation. The mid rib is more prominent on the lower surface and the lateral veins are much less prominent. The petiole is green in color and measures about1-2 cm in length for the upper leaves and 3-5 cm in length for the large lower ones. The avocado leaves have fragrant odor and slightly astringent taste when crushed.

Figure 4: Branch of *P. americana* (x0.3)

Macromorphology of the stem

The main stem is erect, cylindrical, solid and monopodially branched. It measures 3-4 m in length and

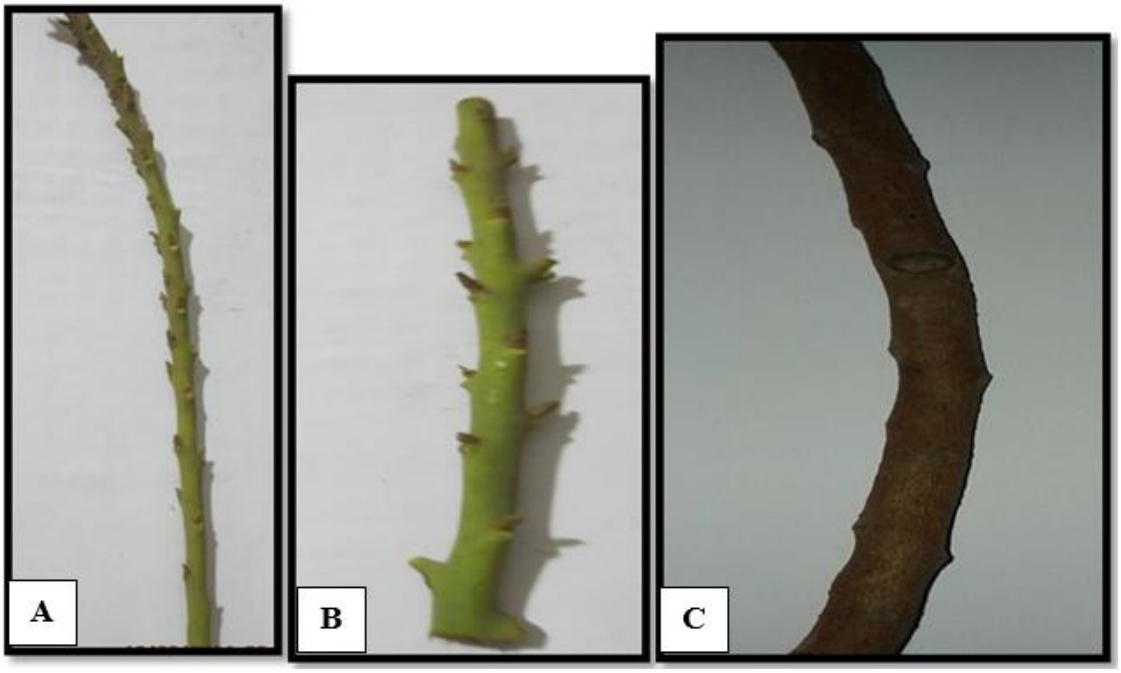

Figure 5: A: Upper part of stem (x0.4); B: Middle part of stem (x0.5); C: lower part of stem (x0.5)

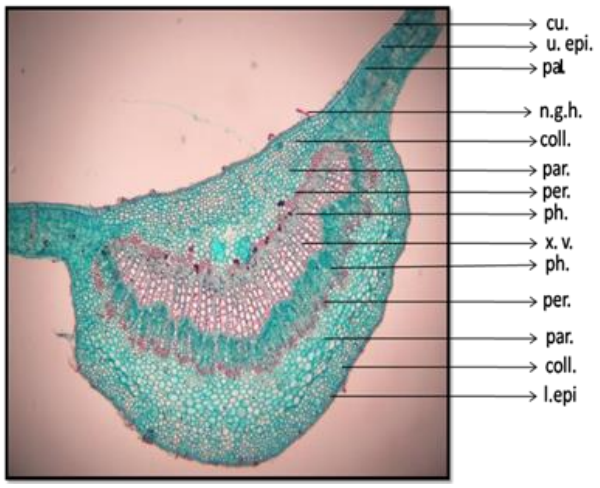

Figure 6: A: T.S of the leaf (x40)

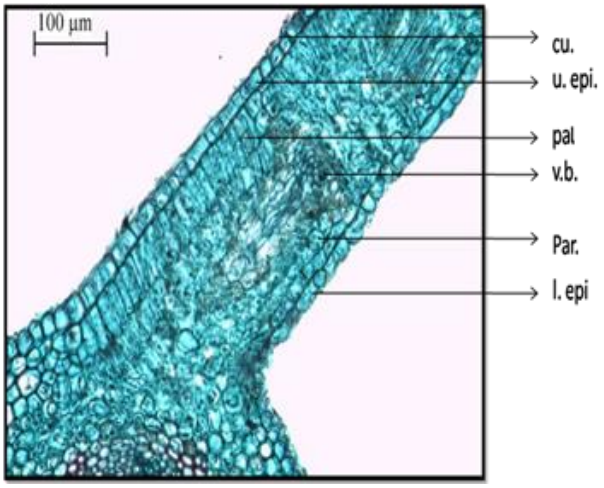

Figure 7: Detailed T.S of the leaf in the lamina region (x100).

20-30 cm in width at the middle part. The terminal and lateral branches (Fig. 4 and 5) are thinner, glabrous and green in color showing short internodes. The terminal young parts of the stem are smooth and bright green in color while the older parts are rough, yellowish brown and carrying the scars of fallen leaves. The stem breaks with a fibrous fracture when dried.

Micromorphology of P. americana

Micromorphology of the leaf

A transverse section through the leaf blade (Fig. 6) is planoconvex in the outline; the midrib is prominent on the lower surface while the upper surface is nearly planar. It reveals a dorsiventral structure with upper palisade cells consisting of two rows of columnar cells. In the midrib region, there are several layers of subepidermal collenchyma, together with another layers abutting on the lower epidermis. The vascular strand of the midrib is formed of fused bicollateral vascular bundles; consisting of two zones of phloem above and below the radiating xylem and lined towards both epidermises with pericycle. Numerous needles and prismatic crystals of calcium oxalate are scattered in the cortical tissue and the mesophyll. Both upper and lower epidermises are pubescent.

The epidermis

The upper epidermis

The upper epidermis (Fig. 7 and 8) is formed of one row of subrectangular to square cells covered with thin cuticle as seen in the transverse section, while in surface view (Fig. 11) the cells appear polygonal, usually isodiametric to slightly elongated with sinuous anticlinal walls covered with striated cuticle. Stomata are absent and only

non-glandular type of trichomes is present which is simple, unicellular, with acute apex, thick wall and covered with smooth cuticle.

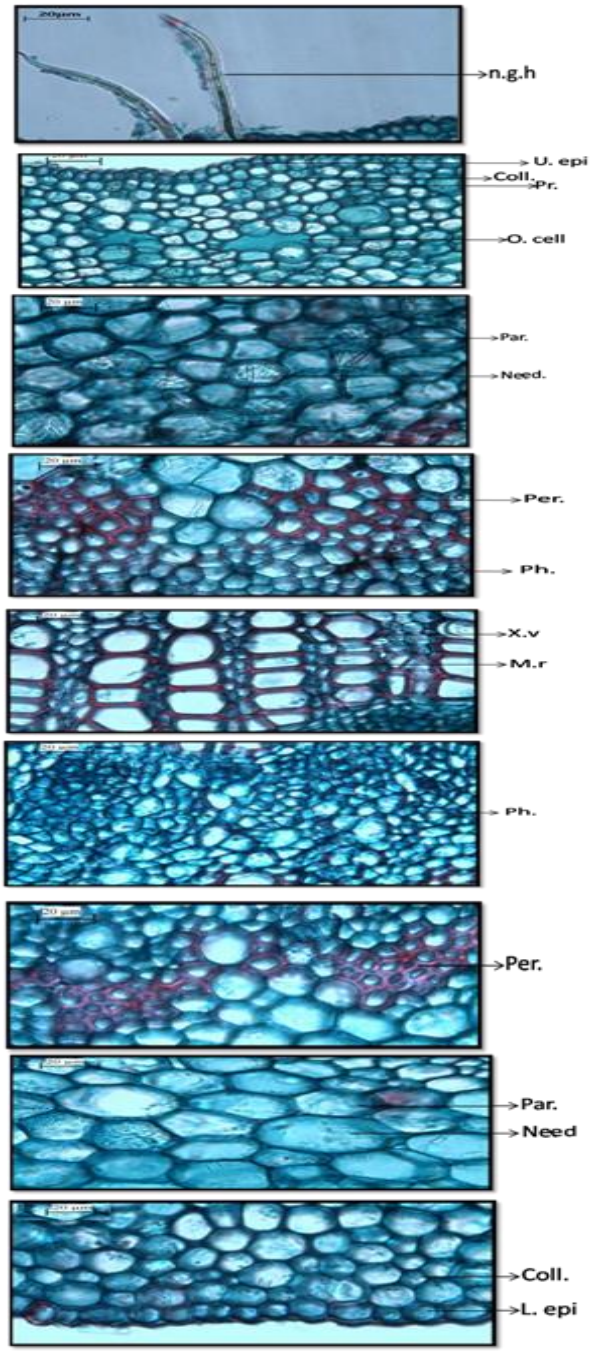

Figure 8: Detailed T.S of the leaf in the midrib region (x400).

The lower epidermis

The lower epidermis (Fig. 7 and 8) is formed of one row of subrectangular to square cells covered with thin cuticle as seen in the transverse section, while in surface view (Fig. 11) the cells appear polygonal, usually isodiametric to slightly elongated with beaded walls and sinuous anticlinal walls covered with striated cuticle. The cells are papillosed (Fig. 11) and the stomata are oval to round in shape, being of anomocytictype with a relatively wide osteole and surrounded with 4-5 cells. The trichomes are similar to those of the upper epidermis but more abundant.

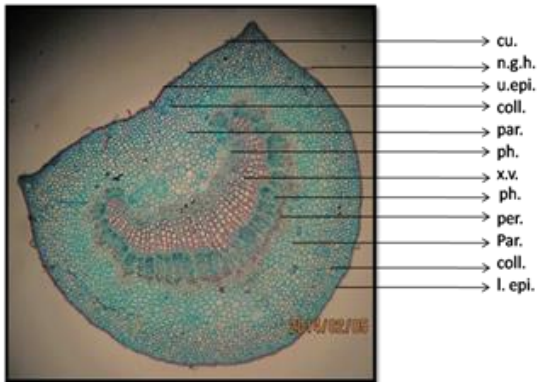

Figure 9: T.S of the petiole (x40)

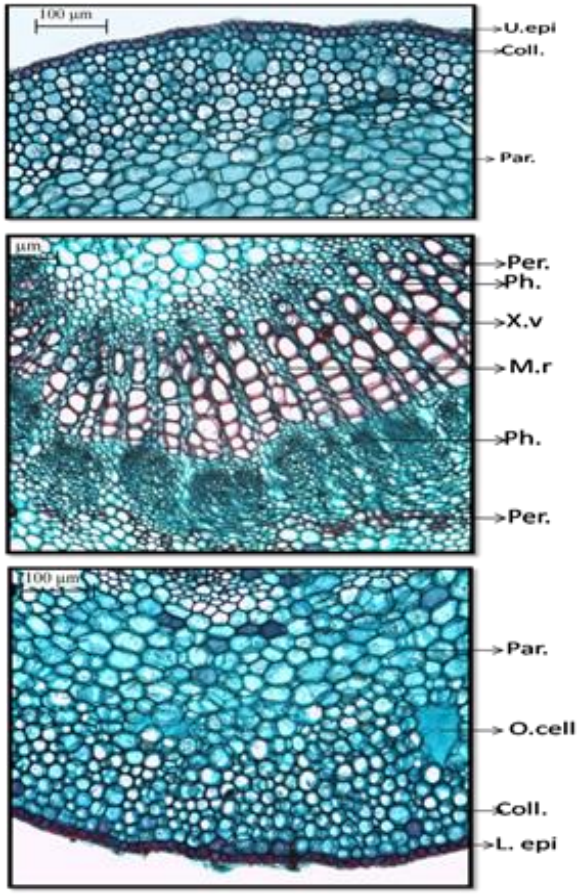

Figure 10: Detailed T.S of the petiole (x100).

The neural epidermis

The neural epidermis consists of square cells axially elongated as seen in the transverse section, while in surface view (Fig. 11) they appear polygonal, isodiametric to slightly elongated with straight anticlinal walls and covered with striated cuticle. They bear non-glandular hairs as those of the epidermis of the lamina. Anomocytic stomata are rare.

The mesophyll

The mesophyll (Fig. 7) is heterogenous consisting of upper zone of palisade cells which is formed of two rows of cylindrical, columnar thin walled cells with very narrow intercellular spaces and containing chloroplasts. The first row is somewhat longer than the

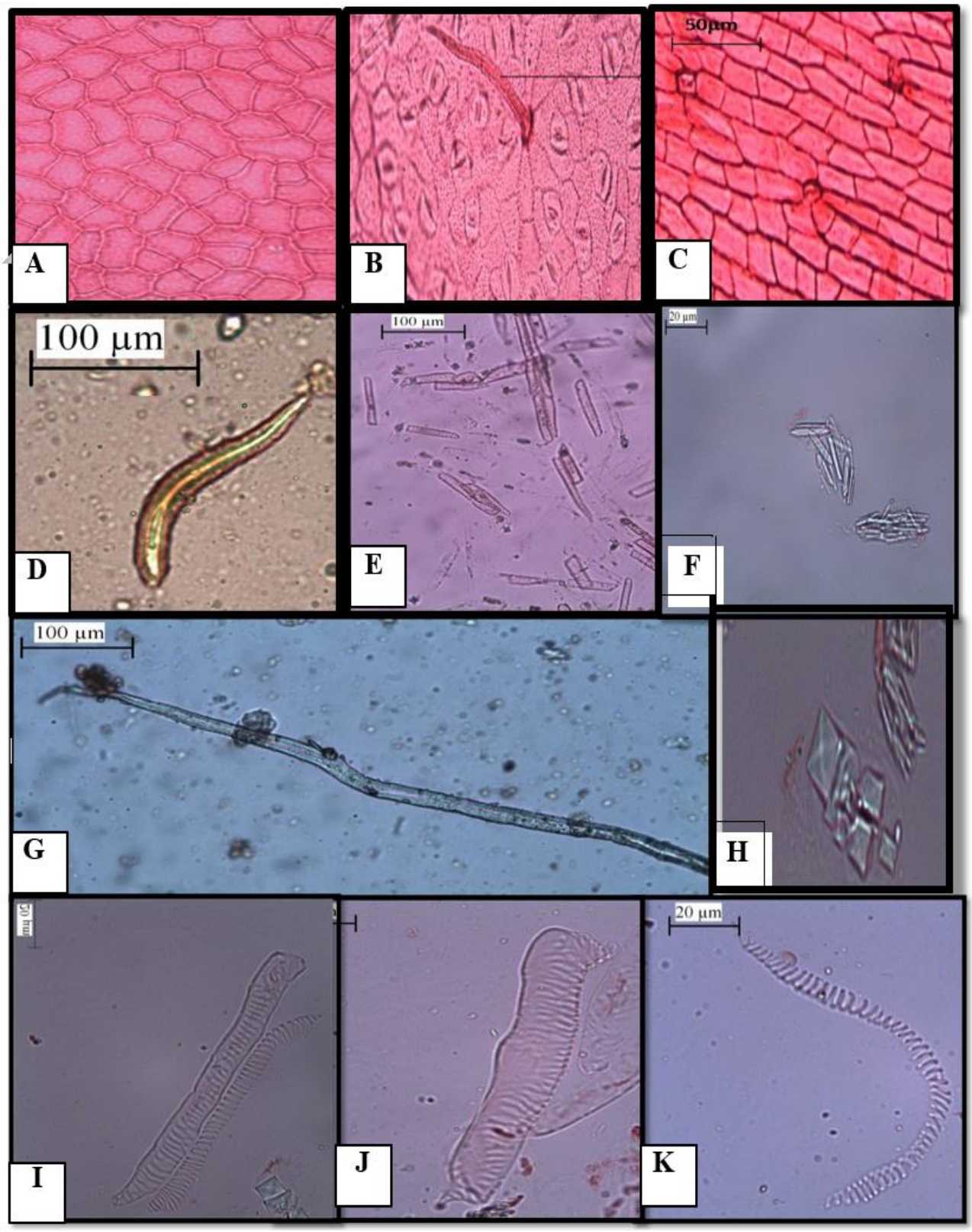

Figure 11: Powder elements of the leaf; A) Fragment of upper epidermis of leaf (x400); B) Fragment of lower epidermis of leaf (x200); C) Neural epidermis of leaf (x200); D) Non-glandular unicellular hairs of leaf (x200); E) Fragments of palisade cells (x100); F) Scattered needles of calcium oxalate in the powder of the leaf(x400); G) Fragment of phloem fiber (x100); H) Scattered prisms of calcium oxalate in the powder of the leaf(x400); I-K) Lignified xylem vessels (x400).

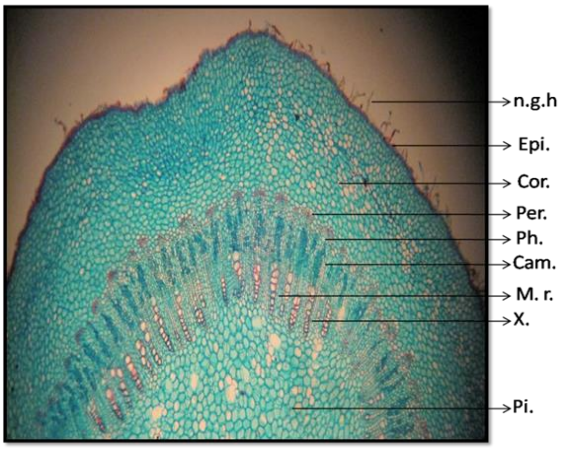

Figure 12: T.S. of the upper part of the stem (x40).

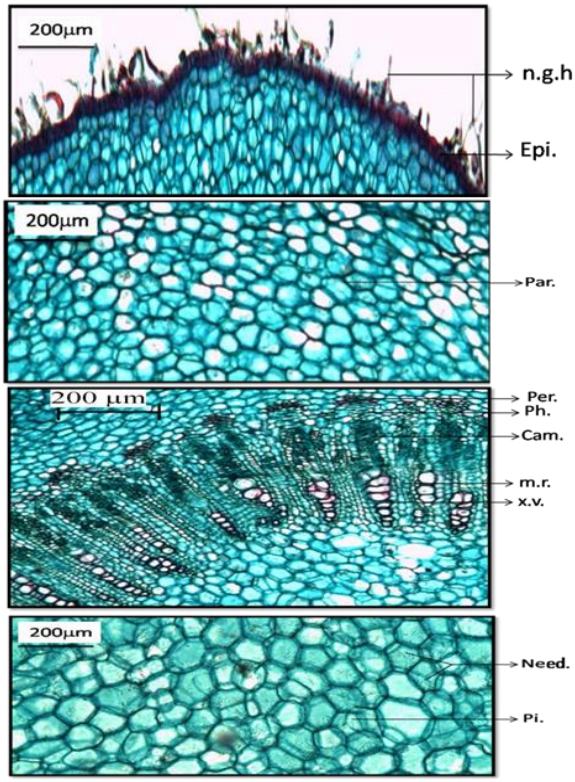

Figure 13: Detailed T.S of the lower part of the stem(x40).

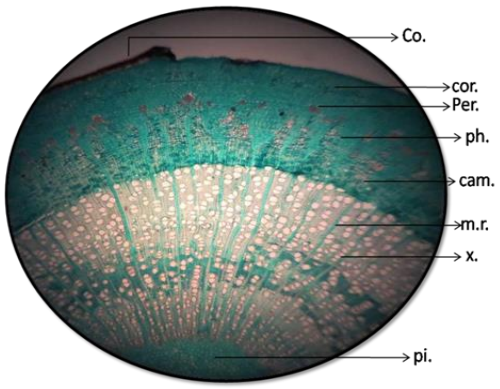

Figure 14: T.S of the lower part of the stem (x40).

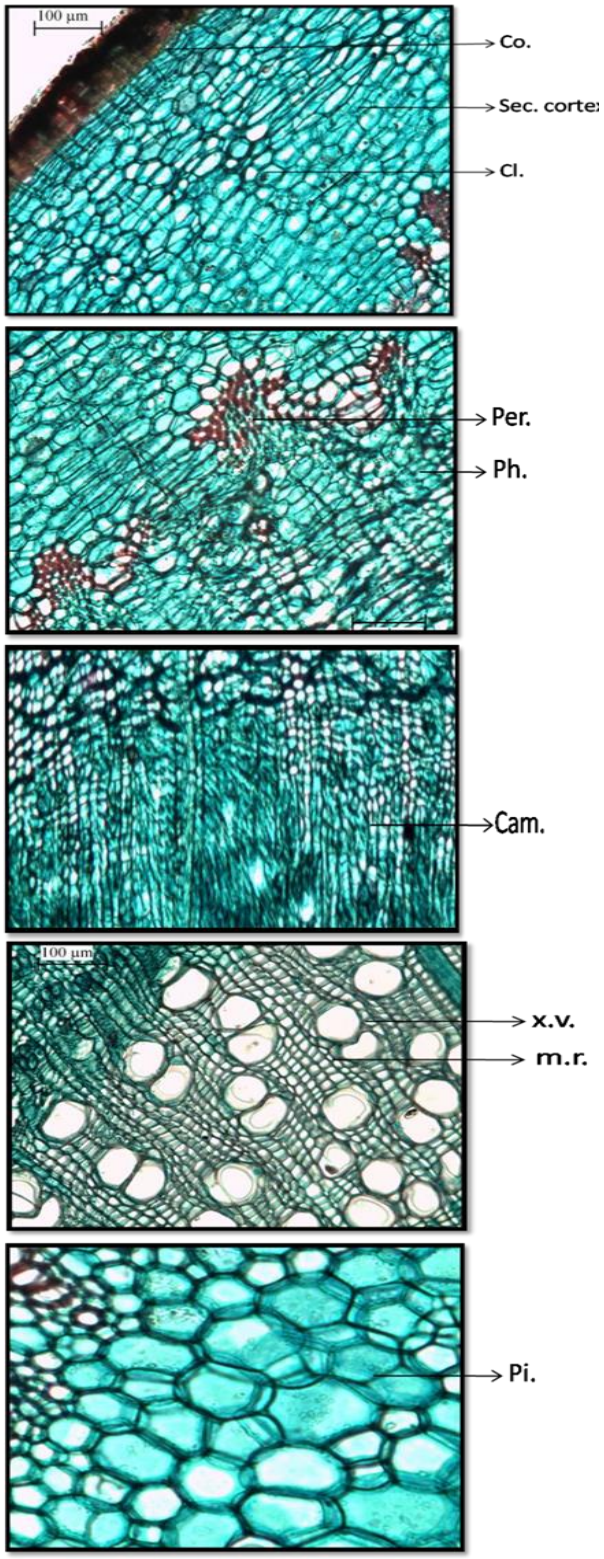

Figure 15: Detailed T.S of the lower part of the stem (x100).

second one. The spongy tissue is formed of tangentially elongated to more or less rounded parenchyma with nearly wide intercellular spaces. The mesophyll is traversed by separated strands of small vascular bundles representing the lateral veins.

The cortical tissue

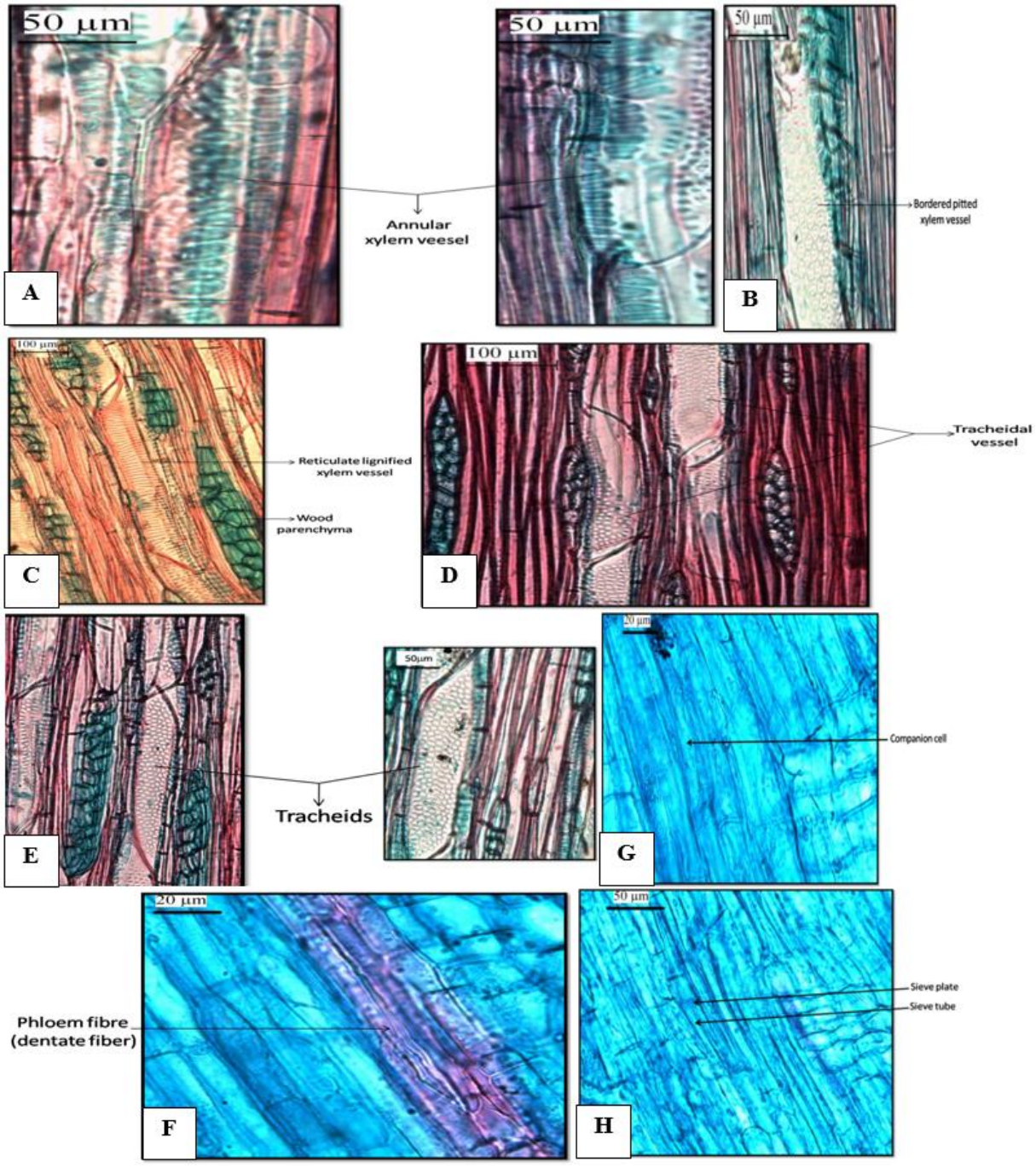

Figure 16: Longitudinal cut of old stem A) Annular lignified xylem vessels; B) Bordered pitted xylem vessel; C) Reticulate lignified xylem vessels & wood parenchyma; D) Tracheidal vessel; E) Tracheids; F) Phloem fiber; G) Companion cell; H) Sieve tube& sieve plate

The cortical tissue of the midrib (Fig. 8) shows an upper and lower subepidermal collenchymatous masses. The upper layer is formed of up nearly seven rows separated from the vascular tissue by up to ten layers of the ordinary parenchymatous cells. The lower layer of collenchymas is formed of up to five rows of small rounded cells with no intercellular spaces and separated from the vascular tissue by a layer of about eight rows of rounded to oval thin walled parenchyma cells with narrow intercellular spaces that contain numerous needles and some prisms of calcium oxalate. Large spherical to oval oil cells are also present.

The vascular tissue

The vascular strand (Fig. 8) is bi-collateral, slightly crescent-shaped, formed of two regions of phloem above and below the xylem region and lined with the pericycle.

The pericycle

The pericycle consists of a continuous two arcs of lignified sclerenchymatous cells interrupted by the pericylic parenchyma surrounding the vascular bundles.

The phloem

The phloem consists of wide zone of soft tissue

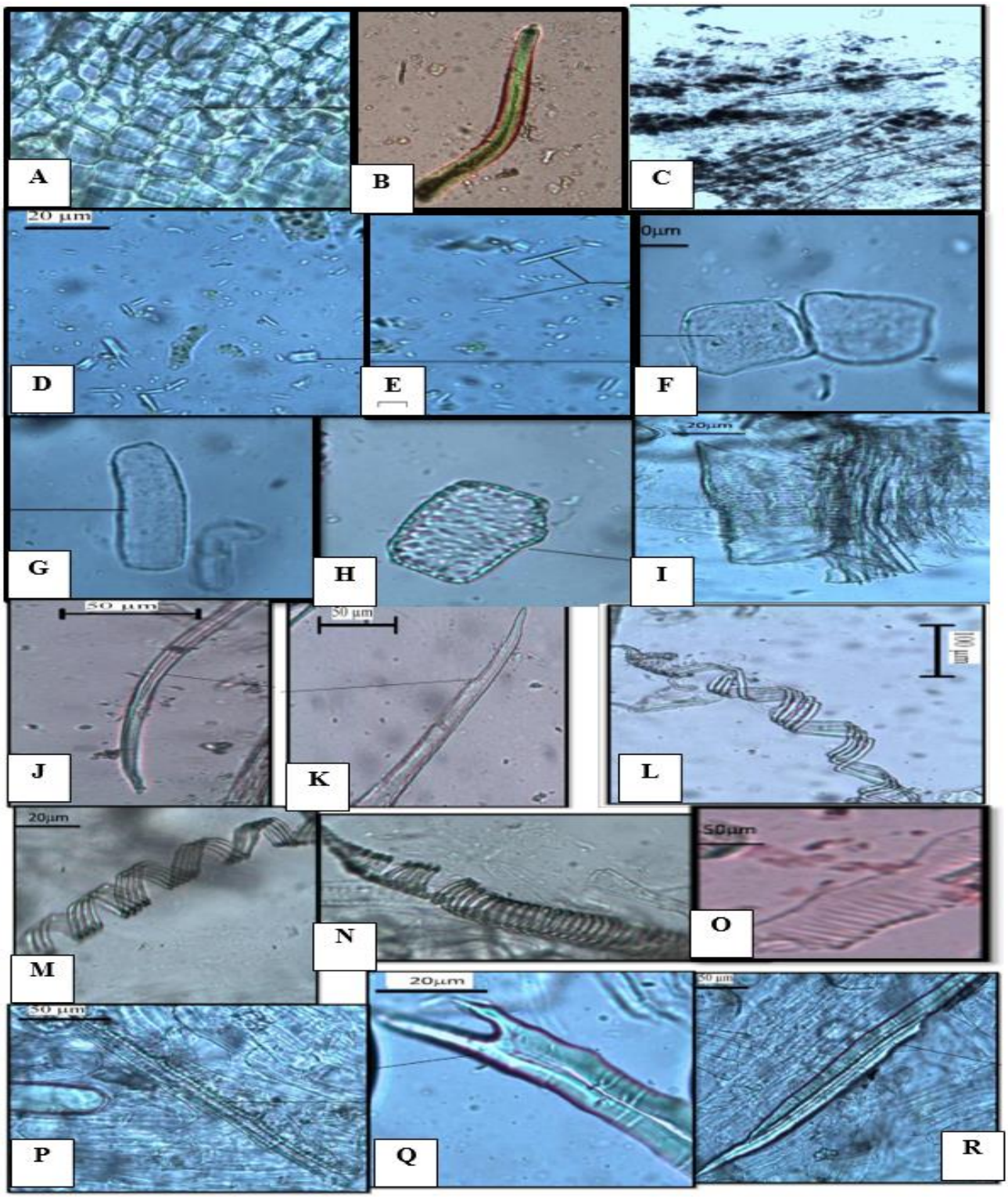

Figure 17: Powder elements of the stem. A) Fragments of cork cells (x400); B) Non- glandular unicellular hair (x200); C) Scattered cluster crystals of calcium oxalate; D) Scattered prisms of calcium; E) Scattered needles of calcium oxalate (x200); F) Fragments of pith parenchyma (x200); G) Fragments of medullary ray cells (x200); H) Fragments of wood parenchyma; I) Fragments of tracheids (x400). J&K) Fragments of wood fibers (x40); L, M,N) (Fragments of spiral lignified xylem vessels (x200); O) Fragments of reticulate lignified xylem (x200); Fragments of phloem fibers (x200); Q,R) Fragments of pericyclic fibers(x200).

consisting of sieve tubes, companion cells and phloem parenchyma.

The cambium

The cambium is undifferentiated.

The xylem

The xylem is formed of lignified vessels and thin walled

wood parenchyma. The medullary rays are uni or biseriate of radially elongated thin walled cellulosic cells. The thickening of xylem vessels have been identified from powdered leaves as annular, spiral and reticulated (Fig. 11).

Micromorphology of the petiole

A transverse section in the petiole (Fig. 9 and 10) is planoconvex in outline, showing two small wings on both sides. It shows two epidermises enclosing a cortical tissue which is formed of an outer collenchymatous zone and an inner parenchymatous one. The innermost layer of the cortex (endodermis) is indistinguishable. The vascular tissue is represented by a crescent shaped bi-collateral vascular strand lined toward both epidermises with pericycle.

The powder of the leaf

The powder of the leaf is green in color with fragrant odor and slightly astringent taste. The main diagnostic microscopical elements (Fig. 11) are:

- Fragments of the upper epidermis of the lamina in surface view consisting of polygonal, usually isodiametric to slightly elongated with sinuous anticlinal walls covered with striated cuticle The hairs are simple, non-glandular, and unicellular, with acute apex, with thick wall and covered with smooth cuticle. Stomata aren't observed.
- Fragments of the lower epidermis of the lamina in surface view. The cells appear polygonal usually isodiametric to slightly elongated with beaded walls and sinuous anticlinal walls covered with striated cuticle. The stomata are oval to round in shape and are of anomocytic type with a relatively wide osteole and surrounded with 4-5 cells. The trichomes are similar to those of the upper epidermis but more abundant.
- Fragments of the neural epidermis in surface view. They appeared polygonal, isodiametric to slightly elongated with straight anticlinal walls and covered with striated cuticle. They bear numerous non-glandular hairs as those of the epidermis of the lamina.
- Simple, non-glandular unicellular hairs with acute apex, thick wall and covered with smooth cuticle.
- Fragments of cylindrical columnar palisade cells.
- Fragments of parenchyma cells containing needles and prisms of calcium oxalate, sometimes these crystals are scattered.
- Fragments of lignified phloem fibers with wide lumen, acute apex and straight wall.
- Fragments of xylem vessels with annular, spiral and reticulated thickenings.

Micromorphology of the stem

A transverse section in the upper part of the stem (Fig. 12) is nearly circular in outline showing an epidermis followed by a cortex lined with islets of pericyclic fibers surrounding a continuous ring of vascular tissue with wide parenchymatous pith in the center. The phloem zone is separated from the xylem by several layers of thin walled cambial cells.

The epidermis

The epidermis is formed of one row of subrectangular cells as seen in transverse section (Fig. 13) while in surface view the cells appear polygonal, isodiametric with straight anticlinal walls covered with striated cuticle. Stomata are absent and only few non-glandular trichomes are present which are simple, unicellular, with acute apex, thick wall and covered with smooth cuticle.

The cortex

The cortical tissue is formed of 12-15 rows of rounded to oval thin walled parenchymatous cells with narrow intercellular spaces that contain few prisms of calcium oxalate. Large oval oil cells are also present (Fig. 13). The endodermis is indistinguishable from the other layers of the cortex.

The pericycle

The pericycle consists of islets of lignified fibers alternating with pericylic parenchyma (Fig. 13). The fiber has straight or slightly irregular walls, narrow lumen and tapering end or sometimes forked one (Fig. 17).

The phloem

The phloem formed of narrow zone of soft tissue consisting of sieve tubes, companion cells and phloem parenchyma (Fig. 13) as well as phloem fibers with wide lumen, acute apex and straight wall as shown in Fig. 17.

The cambium

The cambium is formed of many rows of radially arranged, tangentially elongated, cellulosic, and thin walled rectangular cells.

The xylem

The xylem is formed of lignified vessels, thick walled, pitted wood parenchyma (Fig. 13), wood fibers, tracheids and tracheidal vessels (Fig. 16). The medullary rays are multiseriate of radially elongated thin walled cellulosic cells. The thickening of xylem vessels was identified from the powdered stems as annular, spiral, bordered pitted and reticulate (Fig. 17). The tracheids are elongated with blunt ends, lignified walls with simple pits. The tracheidal vessels are similar to tracheids with wide openings at one of their ends. The wood fibers have straight walls, wide lumen and acute apex.

The pith

The pith is formed of wide parenchymatous zone, consisting of large thin walled nearly rounded cells containing needles of calcium oxalate (Fig. 13).

*Transverse section in the lower part of the stem (*Fig. 14 and 15) is almost similar to that of the upper part of the stem except the presence of several rows of tangentially elongated tabular cork cells. The secondary cortex becomes narrower, consists of nearly 10 rows of regular parenchymatous cells which contain cluster crystals of calcium oxalate. The pericycle consists of islets of lignified fibers alternating with pericylic parenchyma.The phloem shows scattered groups of phloem fibers followed by several layers of cork cambium. The wide zone of secondary xylem consists of larger xylem vessels surrounding narrower central pith.

The powder of the stem:

The powder of the stem is yellowish green in color, odorless and tasteless. The main diagnostic microscopical elements (Fig. 17) are:

- Fragments of cork cells consisting of tangentially

elongated, tabular cells with thick lignified walls.

- Non-glandular unicellular hairs with acute apex, thick wall and covered with smooth cuticle.
- Fragments of lignified pericyclic fibers, either solitary or in groups. The fiber has straight or slightly irregular walls, narrow lumen and tapering end or sometimes forked one.
- Fragments of phloem fibers with wide lumen, tapering ends and beaded walls showing few small projections (dentate-like).
- Fragments of lignified wood fibers with wide lumen, acute apex and straight wall.
- Fragments of elongated tracheids with lignified pitted walls and blunt ends.
- Fragments of tracheidal vessels with lignified pitted walls and wide openings at one of their ends.
- Fragments of xylem vessels with annular, spiral, bordered pitted and reticulate thickening.
- Fragments of thick walled, pitted wood parenchyma cells.
- Fragments of elongated thin walled cellulosic cells of the medullary rays.
- Fragments of oval to rounded thin walled parenchyma cells of the pith.
- Fragments of parenchyma cells containing needles, prisms and cluster crystals of calcium oxalate.
- Scattered free prisms, needles and heavy cluster crystals of calcium oxalate.

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