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Botanical investigation of the leaf and stem of *Forsskaolea tenacissima* Linn, family urticaceae, growing in Egypt

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Abstract
Forsskaolea tenacissima L. is a wild desert plant, growing in mountains and valley sides near water in stony soils road edges, never in open sand. Reviewing the available current literature, no detailed information could be traced concerning the macro and micromorphology of the different organs of the plant; this initiates the interest to study this plant. The current leaf and stem botanical studies showed various standardized parameters such as macroscopic and microscopic characters. These various diagnostic features could be helpful in authentication and identification of *Forsskaolea tenacissima* L.

Keywords: *Forsskaolea tenacissima* Linn, Urticaceae, leaf, petiole, stem, botanical studies

1. Introduction
Family Urticaceae comprises 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines [1]. *Forsskaolea* is a small genus of six species distributed primarily in tropical regions [2]. *Forsskaolea tenacissima* L. is a member of the non-stinging nettles genus *Forsskaolea* and is in the same family as the stinging kind Urticaceae, its synonyms: *Caidbeja adhaerens* Forssk. and *Forsskaolea cossoniana* Webb. [3]. It is distributed in South west Europe, North Africa, Palestine, Saudi Arabia, Iran, Afghanistan, Pakistan and India [4]. The flowering season is from March to June. Reviewing the available current literature, no detailed information could be traced concerning the macro and micromorphology of the different organs of the plant; this initiates the interest to study this plant. The current study investigates both macroscopical and microscopical features of the leaf and stem, which could be helpful in authentication of the plant stem. It is also supportive in the identification of entire and powdered drug prior using in any herbal preparations.

Fig 1: *Forsskaolea tenacissima* L. (x 0.9)

2. Taxonomy
Forsskaolea tenacissima Linn. was classified taxonomically as follow [5, 6]:

- **Kingdom:** Plantae,
- **Phylum:** Tracheophyta,
- **Class:** Magnoliopsida,
- **Order:** Rosales,
- **Family:** Urticaceae,
- **Genus:** *Forsskaolea* and
- **Species:** *tenacissima* Linn.

3. Materials and Methods
3.1. Plant materials
The aerial parts of *Forsskaolea tenacissima* L. were collected in the period from April to June 2013. It is collected from El-kawthar city, Sohag, Egypt. The plant identified and authenticated by Prof. Dr. Salah M. El-Naggar Professor of Botany and Plant Taxonomy, Faculty of Science, Assuit University, Assuit, Egypt. A voucher sample (Mn-Ph-Cog-07) was kept in the Herbarium of Faculty of Pharmacy, Minia University, Minia, Egypt. The plant material used for botanical study was taken from the fresh samples, as well as the samples preserved in alcohol (70%)-glycerine-water (1:1:1). The leaves were air-dried, reduced to fine powder suitable for microscopical examination and stored in well-closed containers.

3.2. Preparation of samples for microscopical examination
Safranin, light green, phloroglucinol, concentrated hydrochloric acid, iodine and chloral hydrate were used for preparation the plant sections and the powder.

3.3. Microscopic studies
Surface preparations, transverse sections as well as the powder of the stem were used for observation of various microscopic features. All sections and powder pictures were done by using Microscope with camera, Leica® (Germany) and digital camera (10 megapixels, Sony, Japan).

4. Results and Discussion

4.1. Macroscopical characters of the leaf

Leaves (1-4x) 0.5-3 cm, broadly obovate, serrate, green with hooked hairs on the upper surface, densely white-pubescent on the lower surface, the leaves are petiolate, the petiole 1-2 cm, the leaf base is symmetric in shape and the midrib prominent on the lower surface.

4.2. Macromorphology of the stem

Stems erect or ascending, rounded in shape sometimes have small projections, carrying dense non-glanular and hooked hairs in its surface. The lower part of stem is dark red in colour but the upper part of stem is greenish to red in colour.

4.3.1. Microscopical characters of the leaf

A transverse section through the midrib region (Figure 2) shows a prominent midrib on the lower surface. The lamina has a dorsiventral structure with upper palisade layer consisting of a single row of columnar palisade cells interrupted in the midrib region by subepidermal collenchyma. A layer of subepidermal collenchyma is also located on the lower surface of the midrib. The vascular system of the midrib is formed of a collateral vascular bundle forming large arc of vascular tissue with the xylem to the upper side and the phloem to the lower one, while the cambium is indistinct. The upper and lower epidermises carry non glandular hairs.

4.3.1.1. The epidermis

4.3.1.1.1. The upper epidermis

The upper epidermis is formed of one row of oblong to square cells covered with smooth cuticle as seen in the transverse section (Figure 2). In surface / top view, the cells appear polygonal, with sinuous anticlinal walls covered with smooth cuticle (Figure 3A) measuring (42-48-64 µm) in length, (22-25-35 µm) in width and (12-13-25 µm) in height, stomata are absent. Numerous cells of the epidermis are modified to cystoliths containing deposits of calcium carbonate which was identified by effervescence upon addition of HCL (Figure 5B).

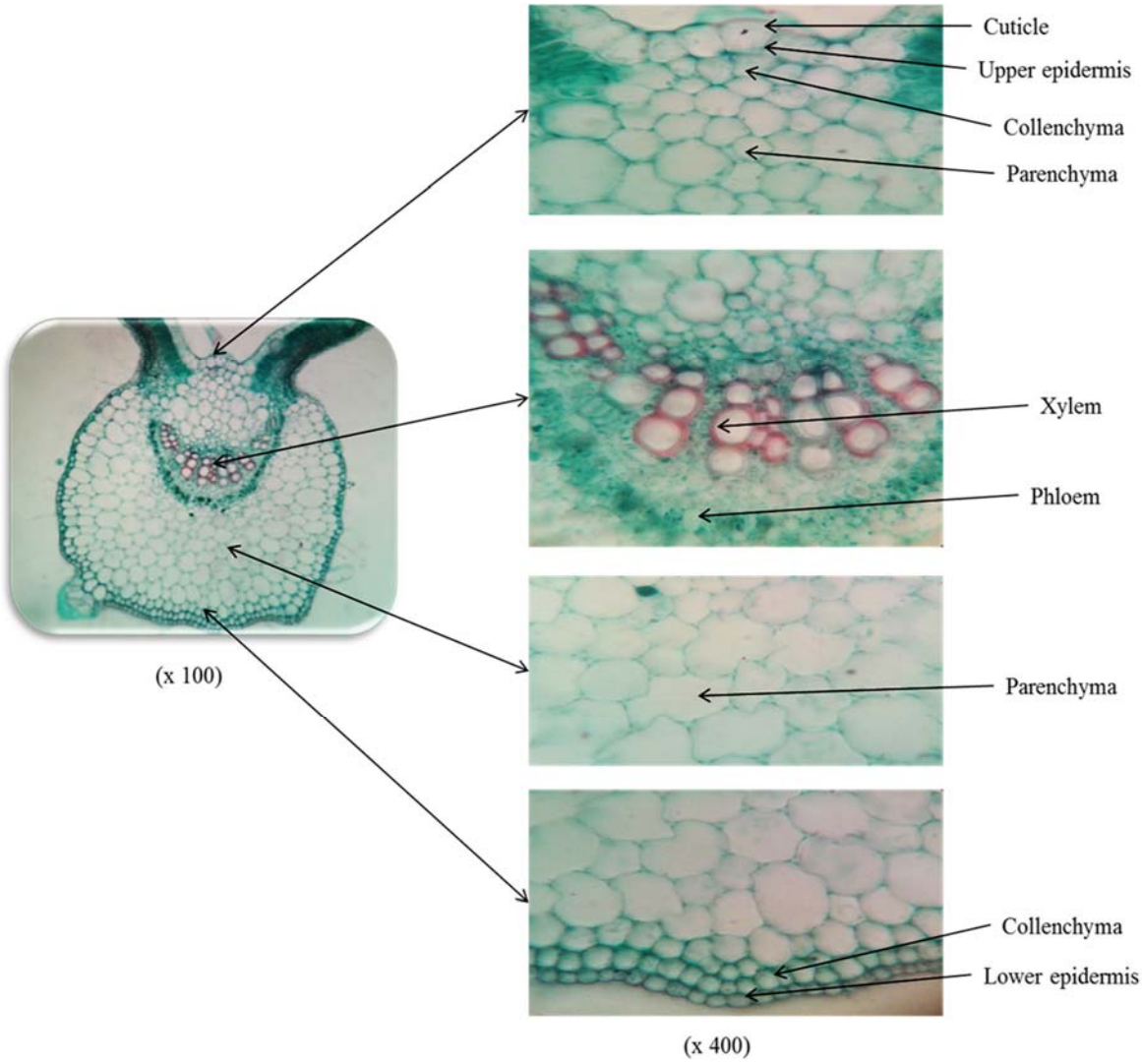


Fig 2: Detailed T.S of the leaf in the midrib region

4.3.1.1.2. The lower epidermis

The lower epidermis is formed of one row of subrectangular to square cells. In surface / top view, the cells appear polygonal, with slightly wavy anticlinal walls (Figure 3B), they measure about (35-52-62 µm) in length, (20-22-28 µm) in width, and (12-14-27 µm) in height. Stomata are anomocytic type, usually oval to round in shape measuring (14-20-24 µm) in diameter, surrounded by 4-6 subsidiary cells.

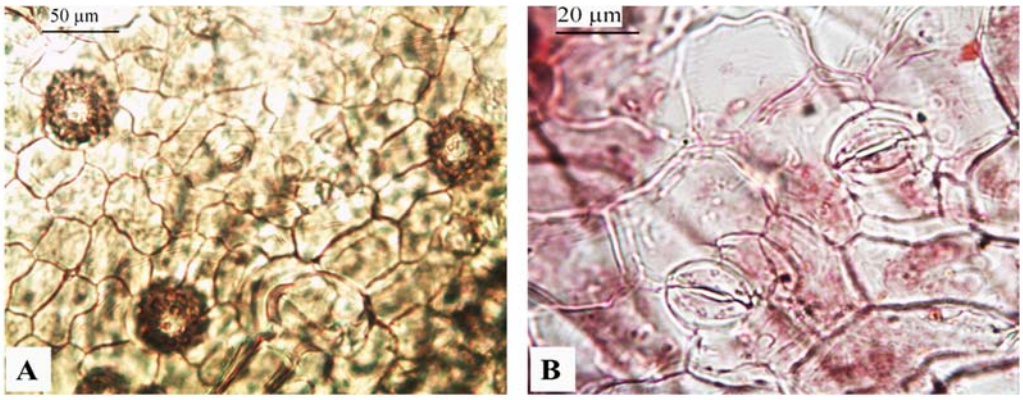


Fig 3: A- Surface view of the upper epidermis of the leaf (x 200) and **B-** Surface view of the lower epidermis of the leaf (x 400).

The upper and lower epidermises show abundant non glandular hairs of three types. Stiff hairs are unicellular, long, conical covered with warty cuticle, measuring (53-65-73 µm) in length and (7-10-14 µm) in width (Figure 4A). The second type, hook-shaped hairs are unicellular, curved near the top like arch, covered with smooth cuticle, measuring (67-93-114 µm) in length and (8-12-16 µm) in width (Figure 4C). The third type, unicellular conical, long, swollen at the base with narrow end, covered with smooth cuticle, measuring (73-110-132 µm) in length and (12-17-24 µm) in width (Figure 4B).

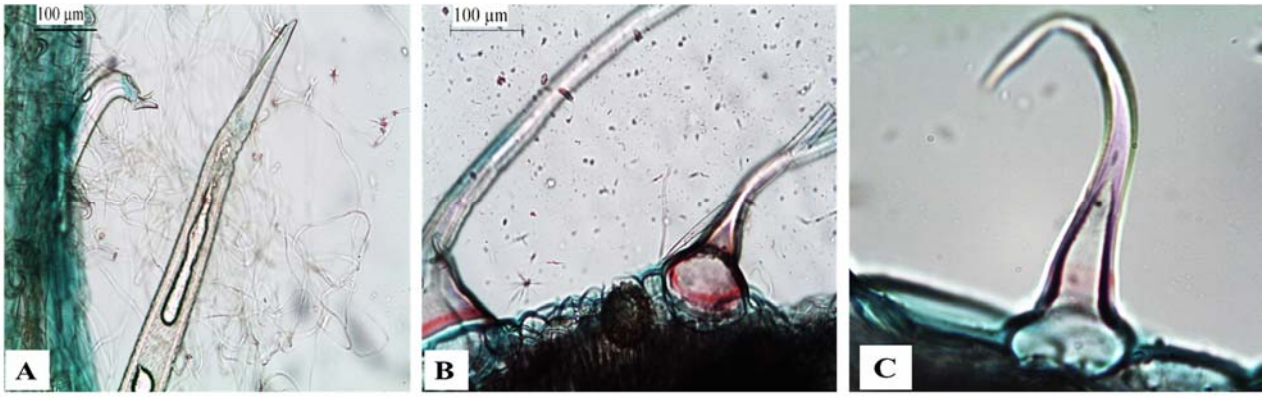


Fig 4: A- Stiff hair (x 100), **B-** Conical hair of the leaf (x 100) and **C-** Hook-shaped hair (x 200).

4.3.1.2. The mesophyll

The mesophyll differentiated into an upper palisade and spongy tissue (Figure 5A). The palisade is formed of one row of more or less compact, columnar cells, they measure (28-35-53 µm) in length and (7-10-13 µm) in width. Numerous cells of the epidermis are modified to cystoliths containing deposits of calcium carbonate (Figure 5B) which dissolve with effervescence in dil. HCl. The occurrence of these cystoliths is recorded as one of the most characteristic features of family Urticaceae [7]. The spongy tissue is formed of thin-walled, rounded, or slightly oval parenchymatous cells with wide intercellular spaces containing chloroplasts, measuring (18-23-32 µm) in diameter.

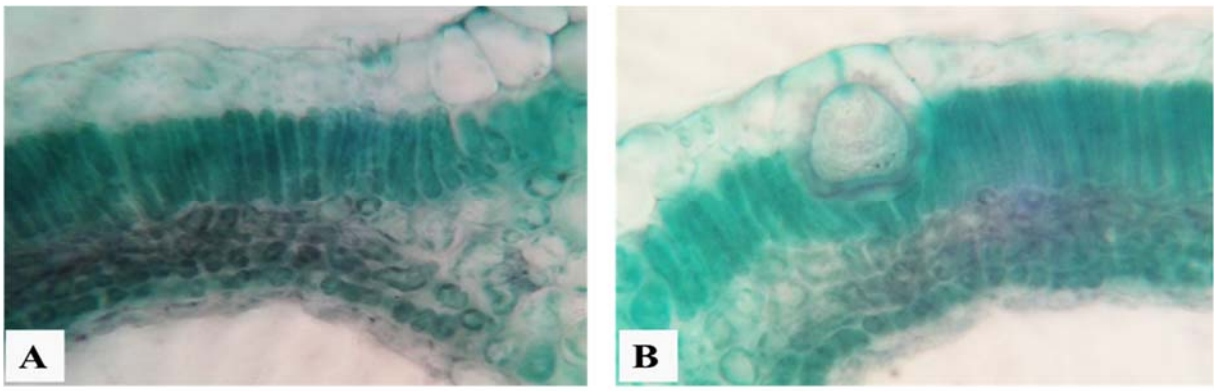


Fig 5: A- Detailed T.S of the leaf in the lamina region and **B-** Detailed T.S of the leaf in the lamina region containing cystoliths (Both x 400).

4.3.1.3. The cortical tissue

The cortical tissue shows both upper and lower subepidermal collenchyma layers (Figure 2). The upper and lower zones of collenchymatous cells, formed of 1-2 rows of nearly rounded, cellulosic cells with no intercellular spaces, measuring (7-12-20 µm) in diameter, followed by a mass of thin walled rounded and oval parenchymatous cells of about 7-9 rows, with intercellular spaces, surrounding the main vascular bundle of the midrib, measuring (15-43-62 µm) in diameter. Some cells are modified to cystoliths containing deposits of calcium carbonate measuring (18-25-33 µm) in diameter. The cortex parenchyma contains cluster crystals of calcium oxalate measuring (6-10-16 µm) in diameter. The cortex contains few schizolysigenous oil glands in the powder (Figure 7E).

4.3.1.4. The vascular tissue

The vascular system in the midrib region forming large arc of vascular tissue with the xylem to the upper side and the phloem to the lower one (Figure 2).

4.3.1.4.1. The pericycle

The pericycle is parenchymatous and indistinguishable.

4.3.1.4.2. The xylem

The xylem is formed of lignified vessels and non-lignified wood parenchyma. The vessels are lignified, with spiral thickening as shown in the powder (Figure 2), measuring about (14-25-29 µm) in diameter. The wood parenchyma cells are polygonal to sub rectangular, with thin walls; they measure about (7-18-30 µm) in length and (4-10-18 µm) in width.

4.3.1.4.3. The phloem

The phloem is formed of small, thin-walled, cellulosic cells, hardly differentiated into sieve tubes, companion cells, and phloem parenchyma. The phloem region is free from any lignified elements.

4.3.2. Micromorphological study of the petiole

A transverse section in the petiole shows that it is nearly rounded in outlying and deeply grooved in the upper (Figure 6). The petiole shows non-glandular hairs, like those of the lamina and midrib. The vascular bundles of the petiole appear as similar in structure to those of the midrib.

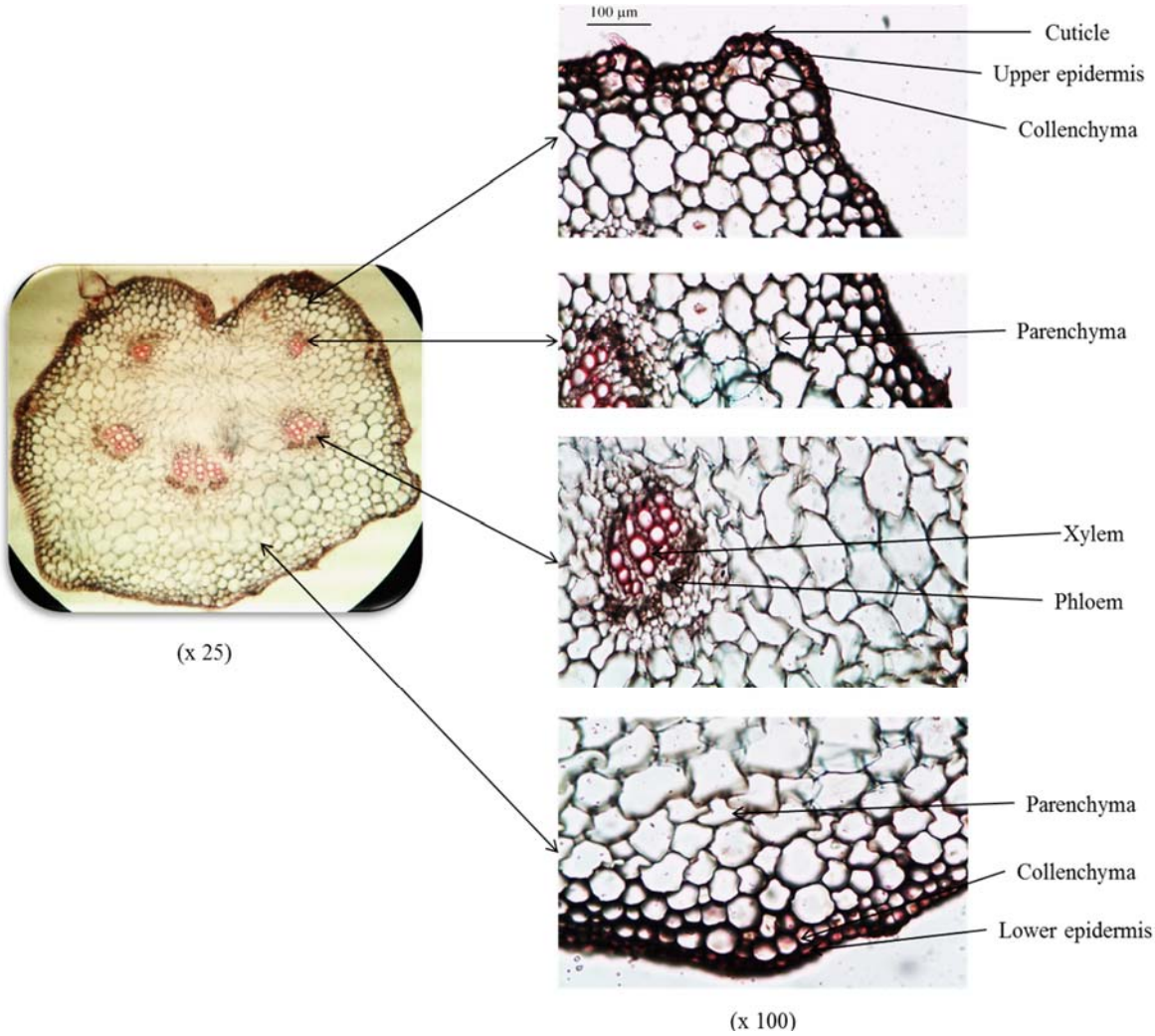


Figure 6: Detailed T.S of the petiole

4.3.3. The powder of the leaf

The powder of the leaf is dark green in colour, with faint odour and slightly bitter taste.

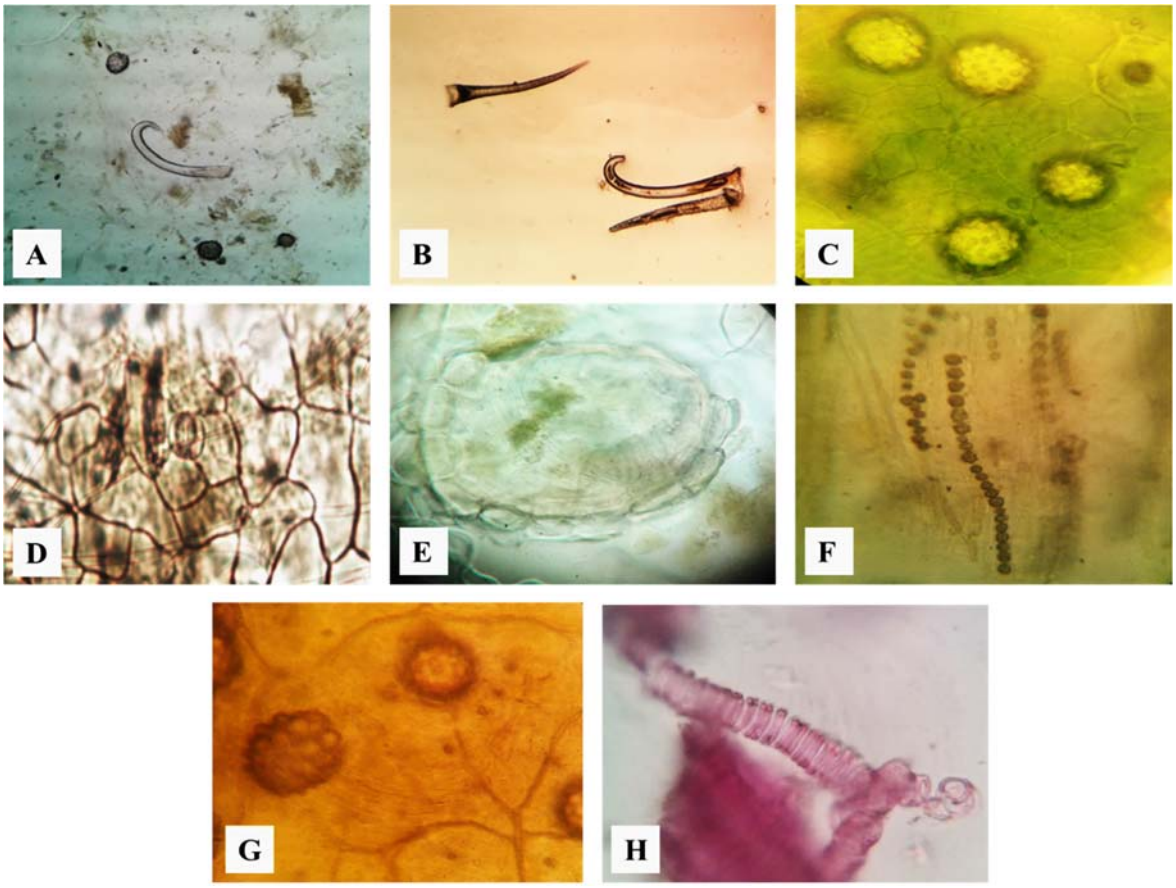


Fig 7: The powder of the leaf; **A**-Hook-shaped hair with scattered cystoliths (x 100), **B**-Stiff hair and hook-shaped hair (x 100), **C**-Epidermis of the leaf (x 100), **D**- Lower epidermis of the leaf with anomocytic stomata (x 200), **E**-Schizolysigenous oil gland (x 400), **F**- Cluster crystals of calcium oxalate in chains (x 400), **G**-Parenchyma cells with cluster of calcium oxalate and cystoliths (x 100), **H**- Lignified spiral xylem vessels (x 400).

4.4. Micromorphological investigation of the stem

4.4.1. Micromorphology of upper part of the stem

A transverse section in upper part of the stem (Figure 8) appears circular in shape. It shows an epidermis covered with thin cuticle, carrying non glandular hairs, continuous ring of vascular tissues transverse by medullary rays and central parenchymatous pith.

4.4.1.1. The epidermis

The epidermis in transverse section, is consists of square to slightly rectangular cells. In surface view, the cells are polygonal, subrectangular with straight anticlinal walls, covered with smooth cuticle and measuring (25-34-60 µm) in length, (12-16-29 µm) in width and (14-16-17 µm) in height. Non glandular hairs are present which similar to those of the leaf. Anomocytic stomata are observed and no cystoliths.

4.4.1.2. The cortex

The cortex is being formed of an outer continuous layer of collenchyma ranging from 2-4 rows of thick-walled cellulosic cells measuring (5-11-20 µm) in diameter, followed by parenchymatous region consists of about 5-7 rows and the cells show wide intercellular spaces measuring (10-29-46 µm) in diameter, some of cells containing starch granules rounded in shape, simple sometimes aggregated, hilum and striation are indistinguishable, measuring (2-3-4 µm) in diameter and cluster crystals of calcium oxalate measuring (6-10-16 µm) in diameter.

4.4.1.3. The pericycle

The pericycle consists of patches of non-lignified fibers with wide lumen, interrupted with parenchyma cells, but some fibers show narrow lumen. They have blunt to rounded apices acute sometimes acuminate and tapering apices measuring (270-392-545 µm) in length and (10-15-25 µm) in width.

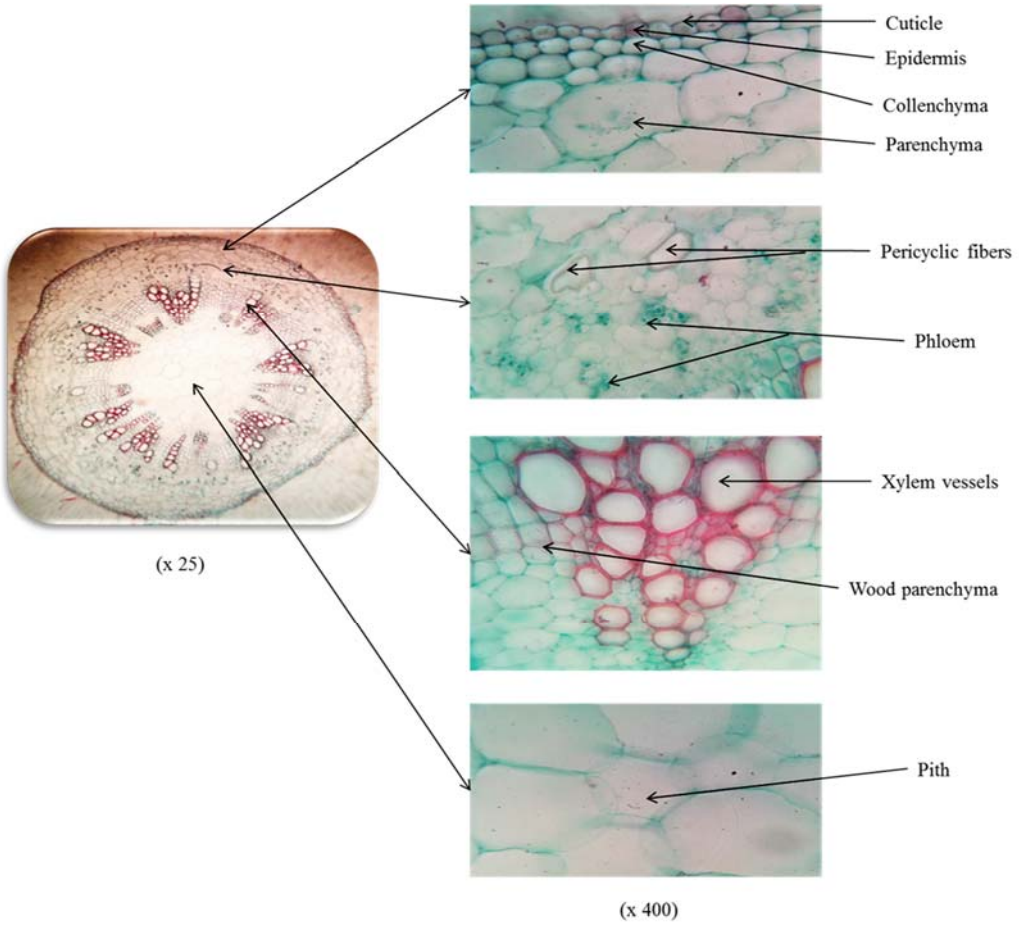


Fig 8: Detailed T.S of the upper part of the stem

4.4.1.4. The vascular system

The vascular system is formed of a continuous ring of phloem, cambium and xylem.

4.4.1.4.1. The phloem

The phloem is formed of phloem parenchyma, sieve tubes, companion cells and phloem fibers, the phloem parenchyma are polygonal to subrectangular, the phloem fibers (Figure 12) have moderately thick, non-lignified walls, comparatively narrow lumen and acute sometimes acuminate apices, measuring (295-412-565 µm) in length and (4-9-13 µm) in width.

4.4.1.4.2. The cambium

The cambium is formed of 2-3 rows of subrectangular, tangentially elongated, radially arranged meristematic cells.

4.4.1.4.3. The xylem

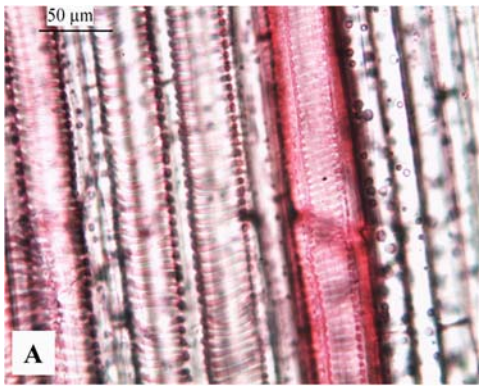
The xylem consists of a radiating zone of lignified vessels, wood parenchyma, wood fibers and tracheids. The vessels (Figures 12) are lignified with pitted, spiral, reticulate and sclariform thickenings, measuring (14-25-29 µm) in diameter. Some cells of xylem vessels (Figures 10) contains laticiferous duct appears different from other cells. The wood fibers are the main constituents and they have thick lignified walls, narrow lumen and mainly acute or rounded apices measuring (152-312-410 µm) in length and (10-15-22 µm) in diameter. The wood parenchyma are consists of subrectangular cells with lignified pitted walls and measure about (9-13-23 µm) in diameter and (128-152-188 µm) in length.

4.4.1.4.4. The medullary rays

The medullary rays are usually uniseriate, biseriate and triseriate. In the xylem region, they are radially elongated, lignified and pitted but not lignified in the phloem region, measuring (7-14-77 µm) in length, and (6-9-23 µm) in width.

4.4.1.4.5. The pith

The pith is formed of a wide central zone of rounded to oval parenchymatous cells. Size of the cells increase toward the center reaching about (19-38-59 µm) in diameter. They contain numerous clusters crystals of calcium oxalates as well as numerous starch granules.



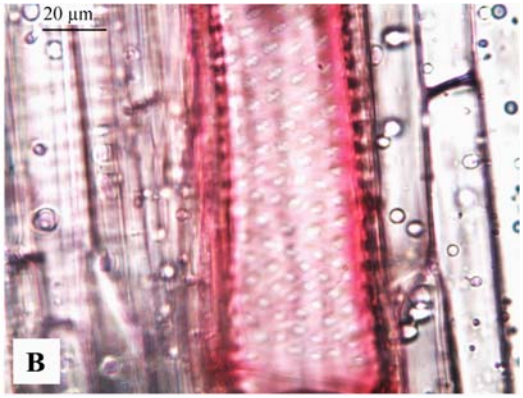
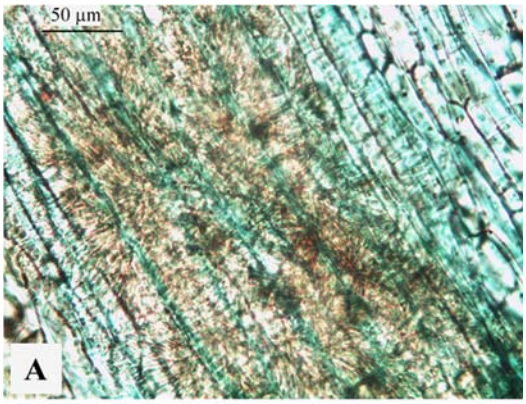


Fig 9: A- Detailed L.S of upper part of the stem (xylem vessels) (x 200) and **B-** Detailed L.S of upper part of the stem (pitted xylem vessels) (x 400).



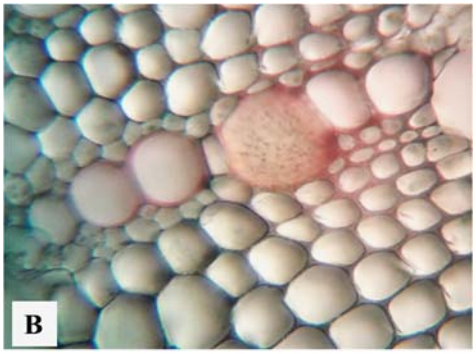


Fig 10: A- Detailed L.S of upper part of the stem (laticiferous ducts) (x 200) and **B-** Detailed T.S of upper part of the stem (xylem region showing laticiferous duct) (x 400).

4.4.2. Micromorphology of lower part of the stem

A transverse section in lower part of the stem (Figure 11) is nearly circular in outline with an outer cork layer. The epidermis is followed by a continuous layer of collenchyma and parenchyma cells. The cortex is very wide than upper part of stem and the pith is narrow containing cluster crystals of ca.ox and starch granules. The endodermis exhibits the same features of that of upper part of the stem. The endodermis is followed by a parenchymatous pericycle with abundant scattered batches of pericyclic fibers more than that present in upper part of the stem.

The vascular system is very wide formed of complete ring of secondary elements enclosing comparatively narrow pith. Both phloem and xylem are transversed by uni-to triseriate medullary rays.

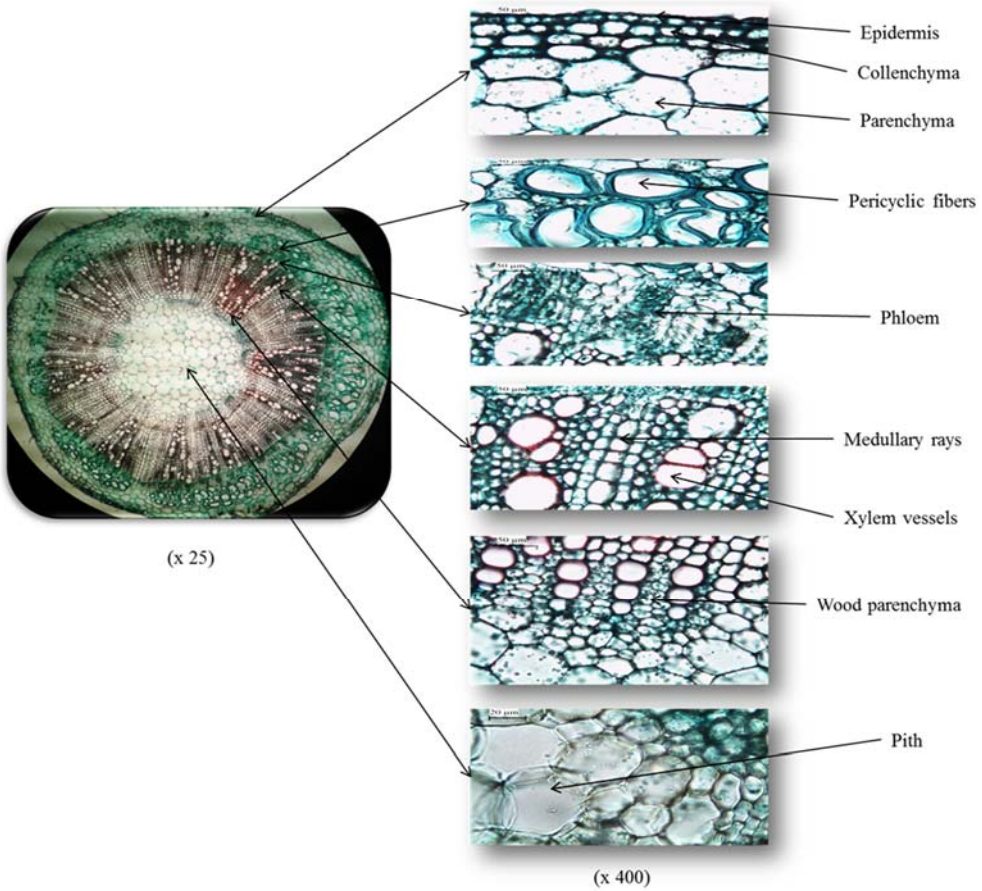


Fig 11: Detailed T.S of the lower part of the stem

4.4.3. The powder of the stem

The powder of the stem is pale green in colour, with faint odour and slightly bitter taste.

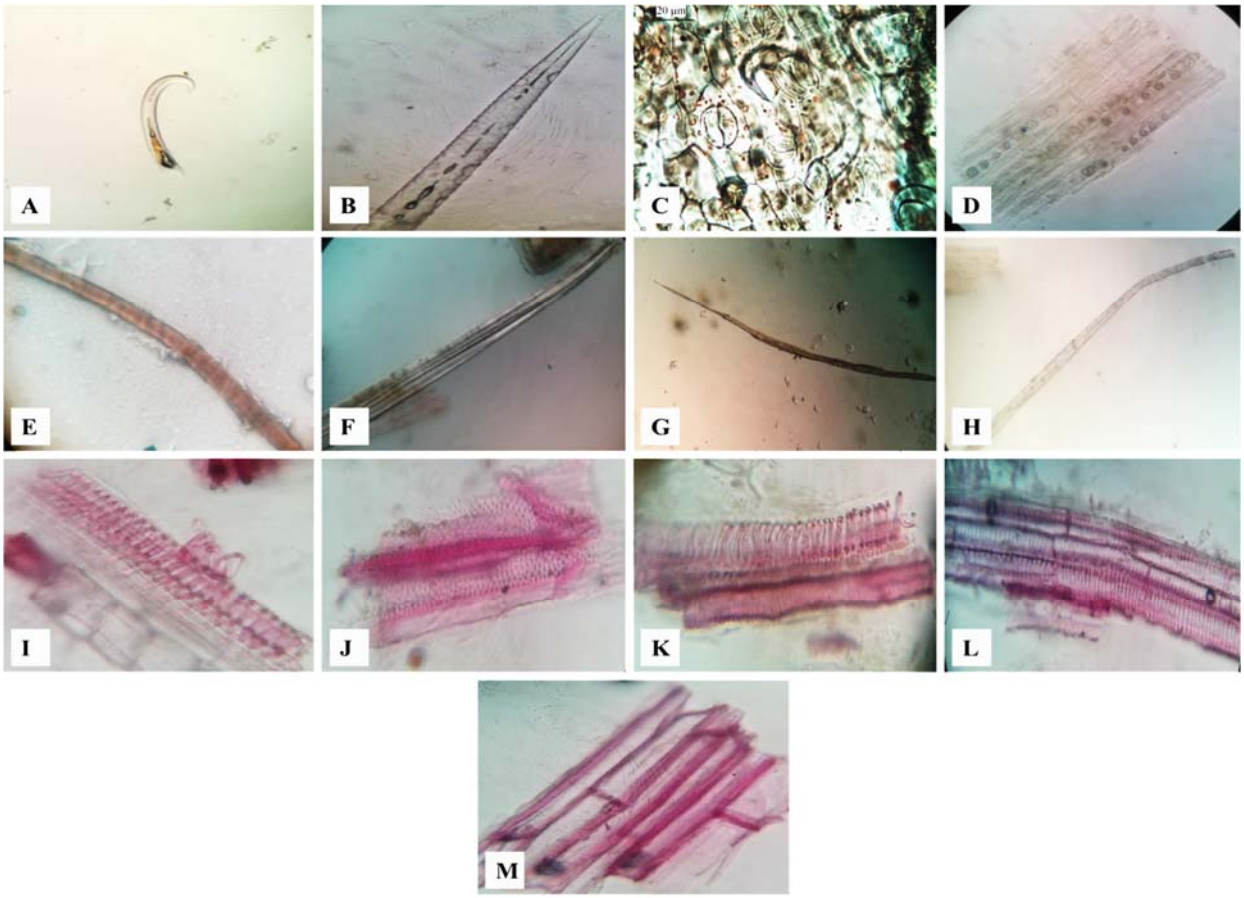


Fig 12: The powder of the leaf; **A-** Hook-shaped hair (x 100), **B**-Stiff hair (x 400), **C-** Epidermal cells with anomocytic stomata (x 400), **D-** Crystal sheath clusters of calcium oxalate (x 100), **E-** Laticiferous duSSct (x 400), **F-** Non-lignified pericyclic fibers (x 100), **G-**Non-lignified phloem fibers (x 100), **H-** Lignified wood fibers (x 100), **I-** Lignified spiral xylem vessels (x 400), **J-**Lignified pitted xylem vessels (x 400), **K-** Lignified reticulate xylem vessels (x 400), **L-**Lignified sclariform xylem vessels (x 400), **M-**Lignified wood parenchyma (x 400).

5. Conclusion

From the current botanical studies, it could be helpful in authentication of the leaf and stem of *Forsskaolea tenacissima* L. family Urticaceae. Moreover, it is helpful in the identification of powdered drug prior using in any herbal formulations.

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