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Botanical Studies of The Aerial Parts of Odontonema cuspidatum (Nees) Kuntze, Family Acanthaceae, Cultivated in Egypt.

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Abstract

Odontonema cuspidatum (Nees) Kuntze is commonly known as cardinal's guard, mottled toothedthread and fire spike. It is a perennial shrub belonging to family Acanthaceae. It is native to Mexico and widely cultivated in tropical and subtropical areas as an ornamental plant for its attractive red flowers. The present investigation attempts to study the Pharmacognostical features of the aerial parts (leaf, stem and flower) of *Odontonema cuspidatum* (Nees) Kuntze. Pharmacognostical evaluation includes macroscopic, microscopy (for leaf, stem and flower) and surface preparation (for leaf and stem). This study will be useful in establishing Pharmacognostical standard measure which helps in identification and classification of the plant.

Key words: Odontonema cuspidatum, Acanthaceae, leaf, stem and flower.

1. INTRODUCTION

Family Acanthaceae, a taxon of dicotyledonous flowering plants, deemed as one of the richest families with medicinal plants [1]. It includes about 346 genera and around 4300 species [2-4]. The most common genera are Barleria (230 spp.), Blepharis (129 spp.), Acanthus (50 spp.), Anisotes (23 spp.) [5], Andrographis (20 spp.) and Adhatoda (20spp.) [4] and Odontonema. The plant under investigation belongs to genus Odontonema which is a member of family Acanthaceae. Odontonema is native to the New World and comprises about 20-30 species distributed in Mexico, Central and South America and the Caribbean [6] and they are commonly cultivated as ornamentals in nurseries, greenhouses, and gardens in tropical and subtropical regions [6, 7]. The genus Odontonema are widely used in folk medicine as a remedy for several ailments: Odontonema callistachyum ground leaves and the stem applied on open wounds in order to heal them in Sierra Mazateca (Mexico) [8]. Odontonema tubiforme (Bertol.) kuntze leaves used by Kuna and Teribe Indians as an anti-inflammatory and for inducing child birth [9]. Odontonema cuspidatum the plant under investigation used in Burkina Faso for the treatment of hypertension [10] and recently, the methanolic extract of Odontonema cuspidatum aerial parts has reported for its antioxidant and hepatoprotective activity on Ccl4-induced liver injury in rats [11]. The plant reported to have flavonoids, saponin, glycosides, tannins, steroids and terpenoids in leaves extracts [12]. Odontonema cuspidatum is native to Mexico [6, 13]. It has been widely cultivated as an ornamental and can now be found naturalized in the southern United States (Florida), Central and South America, West Indies and several islands in the Pacific Ocean [6, 13-15]. Cardinal's crest, scarlet fire spike, scarlet flame and mottled toothedthread are known botanically as *O. strictum* (synonym: *Odontonema cuspidatum*) [13, 16, 17]. *Odontonema cuspidatum* is a perennial shrub and attains 6 ft in height. Stems are subquadrangular, pilose, older stems are glabrate. Leaves are opposite decussate, petiolate, blades elliptic to ovate, glabrous, margin entire to undulate. The inflorescence is a terminal raceme or panicle with 3 to many flowers in fascicles; the flowers are red in color. [15, 18]. The literature scrutiny revealed that nothing could be deeply traced concerning the anatomical features of *Odontonema cuspidatum* aerial parts. Thus, the present study will delineate the Pharmacognostical profile of the stem, flower and leaves of *Odontonema cuspidatum* to hasten its identification.

2. TAXONOMY

Odontonema cuspidatum (Nees) Kuntze can be classified according to United State Department of Agriculture (USDA) which Retrieved on 12 January 2015 from (http://plants.usda.gov/core/profile?symbol=ODCU) as follow: **Kingdom:** Plantae, **Subkingdom:** Tracheobionta (Vascular plants), **Superdivision**: Spermatophyta (Seed plants), **Division**: Magnoliophyta (Flowering plants), **Class:** Magnoliopsida (Dicotyledons), **Subclass:** Asteridea, **Order:** Scrophulariales, **Family:** Acanthaceae, **Genus:** *Odontonema* Nees (toothedthread) and **Species:** *O. cuspidatum* (Nees) Kuntze (mottled toothedthread).

3. MATERIALS AND METHODS

3.1. Collection of Plant materials

The fresh aerial parts of *Odontonema cuspidatum* (Nees) Kuntze collected from El- Orman Botanical Garden Giza, Egypt (on May 2013). The plant was botanically authenticated by Agr. Eng. Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden, Giza, Egypt. A voucher specimen has deposited at the herbarium of pharmacognosy department, faculty of pharmacy, Al-Azhar University, Assuit, Egypt under registration number (OC02-2013). Photos were taking using Panasonic digital camera 10 megapixels. Care was kept in mind to select healthy plants and for normal organs. The plant material used in this study was taken from the fresh samples, as well as the samples preserved in alcohol (70%)- glycerine-water (1:1:1). The required samples of different organs removed from the plant and transverse sections made, dyed and fixed for microscopically examination. The different parts of the plant were air-dried in the shade, reduced to a fine powder for microscopically examination and stored in well-closed containers.

3.2. Preparation of samples for microscopical examination

Light green, safranine, phloroglucinol, concentrated hydrochloric acid, iodine and chloral hydrate used for preparation the plant sections and the powder.

3.3. Microscopic studies

Transverse sections previously prepared using microtome (SLEE, Germany), surface preparations as well as the powder of the leaves, stem and flowers used for observation of various microscopic features. All sections and powder pictures were done by using microscope with camera, Leica® (Germany).

4. RESULTS AND DISCUSSION

4.1. Macromorphology

Odontonema cuspidatum (Nees) Kuntze (Fig. 1) is a member of the Acanthaceae family. The genus has 20-30 species. Odontonema cuspidatum occurs in tropical and subtropical regions of Mexico southward through Central America. It's a perennial, evergreen shrub grow up to 6 ft (1.8 m) tall. The stem is upright, green woody below and semi-woody above. The young stem is smooth while the older one is glabrate. It is subquadrangular in shape and develop few stiff branches. The leaves are opposite decussate, petiolate, blades elliptic to ovate, glabrous, margin entire to undulate, long tapering, pointed sharp tips and having a glossy dark green color. The inflorescence is a terminal raceme or panicle with 3 to many tubular flowers in fascicles; the flowers are red in color. The blooming appears from late summer to winter. The fruits are clavate capsules containing maximum of four flat seeds. [15, 18].

4.1.1. The leaf

The leaves are simple, opposite decussate, petiolate, petioles are up to 30-60 mm long, blades are ovate-elliptic to elliptic in shape and measure 39-310 mm long, 15-130 mm wide, glabrous, margin entire to undulate with acuminate to falcate at apex, acute to attenuate at base, surfaces pubescent with cualine-type trichomes (especially along midvein on the lower surface) or glabrate. Leaves are green in color, the upper epidermis more darker than the lower one. They measure about 8-15-20 cm long and 5-7-9 cm wide at the middle portion. The venation is pinnate, the veins being prominent in the lower surface, anastomose near the margin and leaving the midrib at an acute angle.

The texture is rough. The petiole has a green color, cylindrical to subcylindrical in shape and measures about 0.3-0.5-1.3 cm in length and 0.3-0.4-0.5 cm in diameter. The leaf has faint odour and slightly disagreable taste.

4.1.2. The stem

The main stem of the plant is erect, perennial, quadrate to quadrate-sulcate, sparsely pubscent, reaching about 1.8 meter in height and 0.5 cm in diameter. It is monopodially branched carrying numerous branches and showing more or less long internodes, up to 9-11 cm in the lower parts and shorter in the upper ones. The young upper part has smooth surface except at internodes, while the old one has rough surface covered with brownish cork. The stem is broken with complete fracture in its upper part and with fibrous fracture in its lower one. It has faint odour and slightly disagreeable taste.

4.1.3. The flower

The flower is up to 3 cm long, pedicellate, irregular, bracteolate, hermaphrodite, zygmorphic, pentamerous, hypogenous and possesses the following floral formula:

The calyx :

It is persistant, gamosepalous, comprising 5 fused sepals, green in color with a reddish cast. The outer surface is pubscent or nearly glabrous. The margin is entire and the apex is acuminate. The sepals measure about 2-5.5 mm in length and 0.8-1.3 mm in width.

The corolla :

The corolla is gamopetalous, formed of five united petals forming a funnel form, arranged in one whorl. The corolla is red in color. tubular, 25-35 mm long, glabrous, weakly bilabiate, lower lip 3-lobed, lobes elliptic-ovate, ciliolate, upper lip 2-lobed, lobes elliptic-ovate, apically rounded.

The androecium:

It is tetrandrous, didynamous (2 long and 2 short), one whorled and diadelphous. Filament is strap-like and white in color. The anther is oblong in shape, yellowish brown in color. It is bilobed, basifixed and epipetalous. stamens of pin flowers with filaments attached 17-19 mm from base of tube, free portions 2.5-3 mm long, stamens of thrum flowers with filaments attached 24-25 mm from base of tube, free portions 7-9 mm long, thecae 2.3-3.5 mm long, staminodes are 1.5 mm long.

The gynaecium:

The popular in Acanthaceae family is bicarpellary ovary but the plant under investigation is tricarpellary, trilocular, each carple has six ovules attached to axile placentation. The ovary is sessile or subsessile, superior, yellowish in color, oblong to subcylindrical in shape and nearly glabrous. The style is simple, yellowish white, slender in shape, style of pin flowers is 26-28 mm long, style of thrum flowers is 21 mm long, distal portion of style pale reddish. Stigma is funnel form or bifid, lobes are equal 0.2-0.3 mm long.

The inflorescence:

The inflorescence (Figs. 1(I) & 1(J)) is loose to dense, terminal, pedunculate, often basally branched dichasiate

commonly raceme or panicle (500 mm long). Rachis is pubsent with erect to flexuose-antrose eglandular trichomes. The flowered dichasia are opposite at nodes with 3 to many pedicellate flowers in fascicles and they are pubscent like rachis. Bracts subulate to triangular while bracteoles are triangular to triangular-subulate.

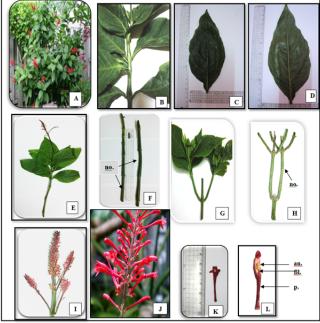


Fig.1: Photographs of Odontonema cuspidatum (A) O. cuspidatum shrub (X= 0.035), (B) Leaves showing opposite decussate phyllotaxis (X= 0.31), (C) Upper surface of the leaf (X= 0.26), (D) Lower surface of the leaf (X= 0.26). (E) Part of O. cuspidatum showing leaves, stem and inflorescence together (X= 0.116), (F, G and H) Stem of O. cuspidatum showing branching and nodes (F, X=0.128, G, X=0.25 and H, X=0.20), (I) Inflorescence of O. cuspidatum showing young dichasial raceme (X=0.37), (J) Inflorescence of O. cuspidatum showing mature flowers (X= 0.32), (K) Tubular petals of O. cuspidatum (X= 0.23), (L) Longitudinal cut in a petal (X= 0.47); an., anther; fil., filament; no., nodes; p., petal.

4.2. Micromorphology:

4.2.1. The leaf

4.2.1.1.The lamina:

A transverse section in the lamina (Figs. $2A_1 \& 2A_2$) shows the midrib which is more prominent on the lower surface than the upper. It shows a dorsiventral structure with two layers of palisade underlying the upper epidermis and continues in the midrib region, and the cells of the first layer are longer than the others. The midrib region showing a central arc of vascular tissue consisting of a radiating upper xylem and lower soft phloem. There are four small additional collateral vascular bundles at the side place. Both upper and lower epidermises show amphidiacytic type of stomata, abundant cystoliths of calcium carbonate and carrying glandular and nonglandular trichomes which are plentiful on the midrib region.

The epidermis:

The upper epidermis:

The upper epidermis (Figs. $2A_1 \& 2A_2$) in transverse section consists of one row of square to subrectangular cells, while in surface view (Fig. 5B) they seem polygonal

and isodiametric in shape. The epidermal cells are covered with thin smooth cuticle. They measure (21-30-55) µm in length, (18-21-27) µm in width and (10-13-16) µm in height. Numerous amphidiacytic type of stomata measuring about (17-20-26) µm in diameter are present. There are many epidermal cells modified to form lithocysts containing deposites of calcium carbonate which dissolved and disappeared on the addition of dilute HCl. The cystoliths (Fig. 2) are cylindrical, usually elongated elliptical with small projections and having generally a more tapered end than the other end, which has more rounded shape (Fig. 5B). The cystoliths have an average length of 125.8 microns. The existence of these cystoliths is registered as one of the most characteristic elements of family Acanthaceae [19]. The epidermal cells carry two types of trichomes; glandular (Figs. 2B & 5B) which have unicellular stalk, unicellular to multicellular globular head and nonglandular which are uniseriate, bicellular to multicellular with 3-4 cells and having acute to acuminate apices. Most of the trichomes covered with striated cuticle and others with smooth cuticle.

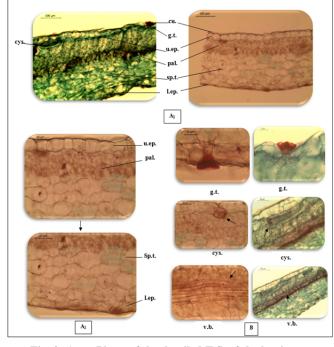


Fig. 2: A_{1&2}- Photo of the detailed T.S. of the lamina.
B- characteristic elements in the lamina.
cu., cuticle; cys., cystoliths of calcium carbonate; g.t., glandular trichome; l.ep., lower epidermis; pal., palisade; sp.t., spongy tissue; u.ep., upper epidermis.

The lower epidermis:

The lower epidermis (Figs. $2A_1 \& 2A_2$) consists of one row of cubical cells of different sizes as seen in transverse section, while in surface view (Fig. 5C) the cells seem polygonal, mostly isodiametric showing more sinuated anticlinal walls. The cells covered with smooth cuticle. Trichomes, stomata, and cystoliths are present, being properly identical to those of the upper epidermis.

The epidermal cells of both surfaces of the midrib region are polygonal in surface view, axially elongated with straight anticlinal walls. the cells carrying glandular trichomes formed by a basal cell and a multicellular head with four to eight cell. Multicellular trichomes (tector trichomes) are occur only on lower epidermis (Fig. 5C). They are composed of two or three cells decorated with surrounding cell forming wallpaper like shape .

The mesophyll

The mesophyll (Figs. $2A_1 \& 2A_2$) is dorsiventral, formed of parenchyma palisade comprising two distinct layers, The palisades are columnar cylindrical thin-walled cells containing chloroplasts. They measure about (54-64-77) µm in length and (18-26-32) µm in width. The spongy tissue is formed of 4-5 rows of more or less rounded, ovoid or irregular thin-walled parenchyma cells with intercellular spaces. The intercellular spaces are not very large, which gives this one aspect parenchyma more compact (Fig. 2A₁ & 2A₂). Small vascular bundles embedded within the spongy tissue (Fig. 2B).

4.2.1.2. The midrib

The cortical tissue

The cortical tissue of the midrib region (Figs. 3A & 3B) is represented by upper and lower zones of collenchymatous cells, formed of 4-5 rows of nearly rounded cellulosic collenchyma cells measuring about (17-35-54) µm in diameter. Numerous collenchymatous cells are modified to cystoliths (Figs. 3B₃ & 4) containing deposits of calcium carbonate measuring about (43-56-77) µm in diameter. The upper collenchyma is followed by 2 rows of columnar palisade tissue (Fig. 4), which is followed by a mass of thin-walled rounded and oval parenchymatous cells of about 11- 14 rows, measuring (35-50-89 µ) in diameter. Some of these cells containing acicular crystal of calcium oxalate (Fig. 5A₃) measuring (16-17-20) µm in length. Some cells are modified to cystoliths containing deposits of calcium carbonate measuring (40-51-74 μ) in diameter. Test results for demonstrate starch and phenolic compounds were negative [19].

The vascular system :

The vascular system (Figs.3A & $3B_2$) represented in the midrib region by an arc of vascular tissue with the xylem to the upper side and the phloem to the lower one, with four additional smaller lateral vascular bundles (Figs.3A and $5A_1$) usually present towards the upper surface.

The xylem:

The xylem (Figs. $3B_2 \& 4$) is formed of vessels and wood parenchyma. The vessels are lignified, with pitted, spiral and sclariform thickening, measuring about (14-23-33 μ) in diameter. The wood parenchyma cells seem to be polygonal to subrectangular, with thin walls; they measure about (24-45-72 μ) in length and (10-19-26 μ) in width. The cambium is hardly distinguishable. The xylem vessels traversed by uniseriate to tri-seriate rectangular cellulosic medullary rays (Figs 3B₂ & 4), They measure about (20-31-35) μ m in width and (25-34-47) μ m in length.

The phloem:

The phloem, (Figs. $3B_2$ and 4) is formed of small, thinwalled, cellulosic cells, hardly differentiated into sieve tubes, companion cells, and phloem parenchyma. Few scattered phloem fiber are also present, which have moderately thick, slightly lignified walls, comparatively narrow lumen and acute to rounded apices, measuring $(348-429-560 \ \mu)$ in length and $(9-13-18 \ \mu)$ in width. The phloem is followed by 2-3 layers of small thin-walled parenchymatous pericycle.

The pericycle

It is formed of thin-walled parenchyma cells beneath the vascular bundle. It is followed by11-13 rows of parenchyma cells which contain acicular crystals of calcium oxalate. The parenchyma cells are followed by 3-5 rows of lower collenchyma.

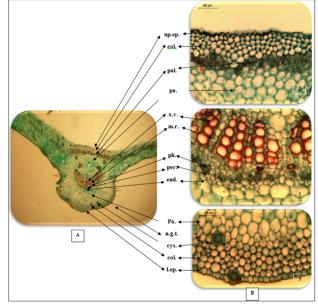


Fig. 3: A- low power view of the lamina and midrib (X= 40).
B- High power view of the midrib (B₁, X=100, B_{2&3}, X=200).
col., collenchyma; cys., cystoliths; g.t., glandular trichome; l.ep., lower epidermis; m.r., medullary ray; n.g.t., non glandular trichome; par., parenchyma; per., pericyclic; ph., phloem; u.ep.,

upper epidermis; xyl.v., xylem vessels.

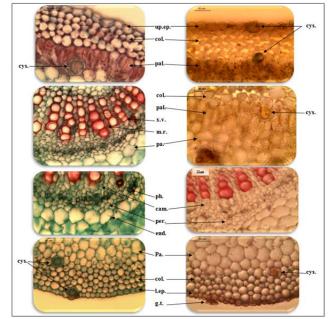


Fig. 4: Photo of the detailed T.S. of the leaf. cam., cambium; col., collenchyma; g.t., glandular trichome; l.ep., lower epidermis; m.r., medullary ray; par., parenchyma; per., pericyclic; ph., phloem; u.ep., upper epidermis; x.v., xylem vessels.

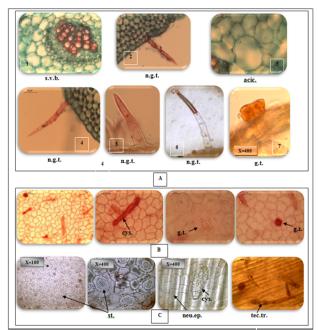


Fig. 5 : A- Photo of the characteristic elements in detailed T.S. of the leaf.

B- Photo of the upper epidermis of the leaf. **C-** Photo of the lower epidermis of the leaf.

 (A_1) s.v.b., side vascular bundle; (A_2) n.g.t., non glandular trichome; (A_3) acic., acicular crystal of calcium oxalate; $(A_{4, 5 \text{ and } 6})$ n.g.t., non glandular trichome; (A_7) ; g.t., glandular trichome; cys., cystoliths of calcium carbonate; neu.ep., neural epidermis st., stomata; tec.tri., tector trichome.

4.2.2. The petiole:

A transverse section in the petiole, (Fig. 6) shows that the epidermal cells at the position of the midrib are slightly immersed and the section has well-developed short lateral wings. It has an outer uniseriate epidermis coating the petiole covered with thin smooth cuticle carrying both glandular and nonglandular trichomes (Fig. 7) and followed by a continuous strip of angular chollenchyma comprising about eight layers small cells (Fig. 6) followed by comparatively wide parenchyma contains small acicular crystals of calcium oxalate (Fig. 7D). The vascular system is represented by a larger central side arc-shaped beam, with the opening facing the adaxial side. It is formed of large, collateral, central crescent-shaped vascular bundles. Four additional small collateral vascular bundles are present on both sides of the transverse section (Figs. 6, 7C and 7D). Abundant cystoliths of calcium carbonate are observed in the sub epidermal cell layers (Fig. 6), resembling those of the leaf in size and shape.

The epidermis:

It consists of uniseriate quadrangular with periclinal walls straight cells as seen in transverse section (Fig. 6) while in surface view, they seem polygonal, isodiametric with straight anticlinal walls. The cells are covered with thin smooth cuticle. Cystoliths of calcium carbonate (Fig. 6), glandular and nonglandular trichomes are also present (Fig. 7). The glandular trichomes have unicellular stalk and unicellular, bicellular to multicellular globular head (Figs. 7H, 7F and 7G). Numerous bicellular (Fig. 7J) and multicellular (Fig. 7I) non-glandular trichomes of different sizes are present. They measure about (81-97-155) µm in length and $(15-18-19) \mu m$ in width. The lower epidermal cells are longer, wider than the upper ones and have the outer periclinal walls slightly convex, as in the lower surface of the midrib. The epidermis measures about (22-34-59) μm in length, (13-15-19) μm in height and (18-24-30) μm in width.

The cortical tissue:

Internally to a track epidermis is continuous angular collenchyma, consisting of about 6-8 rows of irregular rounded collenchymatous cells with thick cellulosic walls and small intercellular spaces measuring about (13-27-45) µm in diameter. They are followed by a zone of 10-12 rows of oval to rounded parenchymatous cells with thin walls and wide intercellular spaces that measure about (25-47-74) µm in diameter. They contain acicular (Fig. 7E), prisms (Fig. 7A) and cluster crystals of calcium oxalate (Fig. 7B). Numerous cystoliths are dispersed among the collenchyma and parenchyma cell layers.

The vascular system:

The vascular system (Fig. 6) is formed of a large collateral vascular bundles. The xylem region consists of radial rows of lignified spiral, annular, pitted and reticulate vessels, wood fibers and wood parenchyma. The phloem forms a narrow zone of soft elements below the xylem. It is formed of small, thin-walled and cellulosic elements, interrupted by groups of non lignified fibers . The cambium is hardly distinguishable. The pericycle is formed of thin-walled parenchyma cells, formed of 2-3 rows. The endodermis is followed by 11-13 rows of parenchyma cells, containing acicular crystals of calcium oxalate. The parenchyma cells are followed by 6-8 rows of lower collenchyma cells. The two additional vascular bundles are collateral consisting of xylem and phloem (Figs. 7C & 7D).

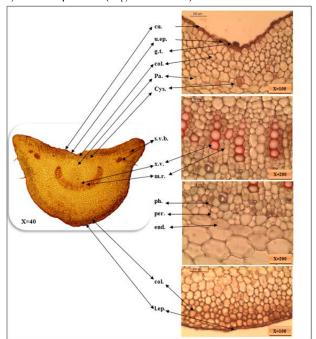


Fig. 6: Photo of diagrammatic T.S. of the petiole col., collenchyma; cu., cuticle; cys., cystoliths of calcium carbonate; end., endodermis; g.t., glandular trichome; l.ep, lower epidermis; m.r., medullary ray; pa., parenchyma; per., pericycle; ph., phloem; ph.f., phloem fiber; s.v.b., side vascular bundle; u.ep., upper epidermis; w.f., wood fiber; x.v., xylem vessel.

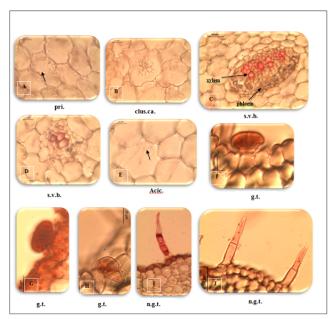


Fig. 7: Photo of the characteristic elements in detailed T.S. of the petiole.

(A) pri., prisms crystal of calcium oxalate; (B) clus.ca., cluster crystal of calcium oxalate; (C&D) s.v.b., side vascular bundle;
(E) acic., acicular crystal of calcium oxalate; (F, G and H) g.t., glandular trichome; (I&J); n.g.t., non glandular trichome.

4.2.3. The powdered leaf

The powder of leaf is dark green, with faint odour and slightly disagreeable taste. It is characterized microscopically (Fig. 8) by the following:

- Fragments of upper epidermal cells of the lamina (Fig. 1-8(I)) being polygonal to subrectangular with slightly sinuated anticlinal walls. The cells are covered with thin smooth cuticle. They show cystoliths of calcium carbonate. amphidiacytic type of stomata (caryophyllaceous) and nonglandular trichomes which are uniseriate, unicellular, bicellular and multicellular (Figs. 8F, 8G and 8H). They have acute to acuminate apices. Some of the trichomes covered with striated cuticle and others with smooth one. Fragments of glandular trichomes with unicellular stalk and unicellular, bicellular to multicellular radiating globular head (Figs. 8A, 8B, 8C and 8D).
- 2- Fragments of lower epidermal cells of the lamina (Fig. 8J)being polygonal, mostly isodiametric with more sinuated anticlinal walls. The cells are covered with thin smooth cuticle and showing cystoliths of calcium carbonate, diacytic type of stomata, as well as covering trichomes and glandular ones similar to those of the upper epidermal cells in all aspects.
- 3- Fragments of epidermal cells of both surfaces of the midrib region (Fig. 8K) which are polygonal in surface view, usually, axially elongated with straight anticlinal walls. They carry nonglandular trichomes and cystoliths of calcium carbonate.
- 4- Fragments of mesophyll cells showing columnar palisade cells (Fig. 8N) and spongy chlorenchyma.

- 5- Fragments of vascular bundles showing lignified xylem vessels with spiral, annular, pitted and reticulate thickening (Fig. 8M).
- 6- Scattered acicular crystals of calcium oxalate, either free or embedded in parenchyma cells of the cortical tissue (Fig. 8E).
- 7- Numerous scattered cystoliths of calcium carbonate, which are solitary, elongated with blunt end, irregular walls and striated surfaces (Fig. 8L). These cystoliths dissolved and disappeared on addition of dilute HCl.
- 8- Fragments of non-lignified phloem fibers (Fig. 8O).
- 9- Fragments of elongated, lignified and septated wood fibers with wide lumen and acute ends (Fig. 8P).

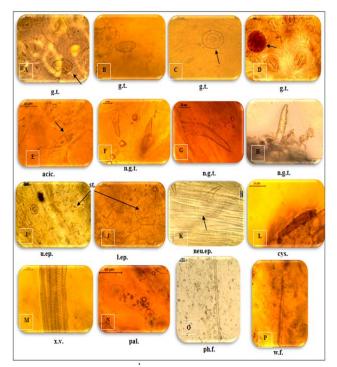


Fig. 8: The powder of the leaf.

(A, B, C and D) g.t., glandular trichome (A- unicellular head, B- bicellular head and C&D- multicellular head); (E) acic., acicular crystal of calcium oxalate; (F, G and H) n.g.t., non glandular trichome (F- unicellular, G-bicellular and H- multicellular); (I) u.ep., upper epidermis of the leaf; (J) l.ep., lower epidermis of the leaf; (K) neu.ep., neural epidermis of the leaf;
(L) cys., cystoliths of calcium carbonate; (M) x.v., xylem vessles; (N) pal., palisade; (O) ph.f., phloem fiber (X=100); (P) w.f., wood fiber (X=200).

4.2.4. The stem : 4.2.4.1. The young stem

A transverse section in young stem (Figs. 9 and10) is somewhat quadrangular in outline. It consists of an outer epidermis covered with thin cuticle and carrying glandular and nonglandular trichomes and showing cystoliths of calcium carbonate. The outer epidermis is followed by a nearly wide cortex consisting of collenchyma groups alternating with chlorenchyma ones, which are slightly elongated containing chloroplasts (Fig. 9). It is followed by parenchyma cells containing acicular crystals of calcium oxalate and starch granules. The endodermis is distinct and followed by the pericycle which is formed of 1-2 layers of parenchyma cells surrounding the central vascular bundle which is open surrounding wide region of pith.

The epidermis:

The epidermis in the transverse section of young stem (Figs. 9A, 9B and 10) consists of one layer of square to subrectangular cells, but in surface view (Fig. 16F) the cells are polygonal, usually isodiametric, sometimes axially elongated with more or less straight anticlinal walls. The cells are covered with thin smooth cuticle. Diacytic type of stomata is present (Figs. 16F and 17B). Glandular and nonglandular trichomes are observed. The glandular trichomes have unicellular stalk and unicellular, bicellular (Figs. 16A &16(I)) to multicellular globular head (Figs. 11A, 11B, 16D and 16G). The nonglandular trichomes are uniseriate, bicellular (Figs. 11C, 16B) to multicellular (Figs. 11D, 16E and 16H). They having acute to acuminate apices and covered with smooth or striated cuticle.

The cortex:

The cortex consists of about 4-8 rows of thick-walled cellulosic collenchymatous cells being rounded to angular in shape, alternating with chlorenchymatous cells followed by a parenchymatous region consisting of 3-8 layers (Figs.10A &10C). Numerous scattered cystoliths of calcium carbonate are also present (Figs.10A and 11F). Some cells contain acicular crystals of calcium oxalate (Fig.11E) and starch granules. The innermost layer of the cortex is the endodermis, distinct and formed of a single layer of tangentially elongated parenchyma cells and containing starch granules.

The pericycle:

The pericycle (Figs.10A $\&10C_1$) separates the central stele from the cortex and consists of 2-4 layers of thin-walled, non lignified parenchyma cells and containing lignified pericyclic fibers.

The phloem:

The phloem (Figs.10B & $10C_2$) consists of cellulosic elements of sieve tubes, companion cells, phloem parenchyma and phloem fibers. The phloem fibers are non-lignified and polygonal in shape.

The cambium:

The cambium (Figs.10B & $10C_2$) is formed of a band of cambiform cells consisting of 1-3 rows of thin-walled cellulosic cells, subrectanguar, tangentially elongated and radially arranged.

The xylem:

The xylem (Figs.10B & $10C_2$) consists of lignified, comparatively wide zone of distinctly radiating elements of vessels, wood fibers and wood parenchyma. The vessels are radially arranged and showing spiral and reticulate thickening in the isolated elements. The fibers have thick lignified walls, moderately wide lumen and acute to acuminate apices.

The medullary rays:

The medullary rays (Figs.10B & $10C_2$) are usually uniseriate or bi-seriate formed of cellulosic elongated cells with thin lignified walls.

The pith:

The pith (Figs. 9C & 9D) is formed of wide central zone of rounded to oval parenchymatous cells showing scattered acicular crystals of calcium oxalate, cystoliths of calcium carbonate and starch granule.

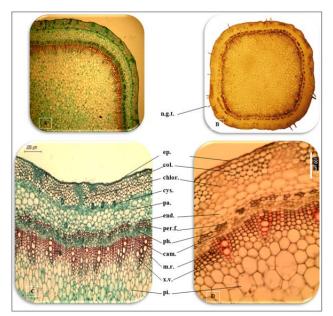


Fig. 9: Photo of the diagrammatic T.S. of the young stem: (A, B and C (X=40), D (X=100).

cam., cambium; chlor., chlorenchyma; col., collenchyma; cys., cystoliths of calcium carbonate; end., endodermis; ep., epidermis; m.r., medullary ray; n.g.t., non-glandular trichome; par.,

parenchyma; per.f., pericyclic fiber; ph., phloem; pi., pith; x.v., xylem vessel.

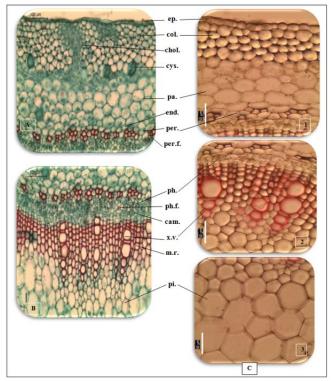


Fig. 10: Photo of the detailed sector of the young stem: (A&B (X=100) and C (X=200).

cam., cambium; chlor., chlorenchyma; col., collenchyma; cys., cystoliths of calcium carbonate; end., endodermis; ep., epidermis; m.r., medullary ray; per., pericycle; per.f., pericyclic fiber; par., parenchyma; ph., phloem; ph.f., phloem fiber; pi., pith; x.v., xylem vessel.

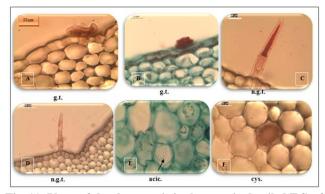


Fig. 11: Photo of the characteristic elements in detailed T.S. of the young stem

(A&B) g.t., glandular trichome; (C&D) n.g.t., non glandular trichome (C-bicellular, D- multicellular); (E) acic., acicular crystals of calcium oxalates; (F) cys., cystoliths of calcium carbonate.

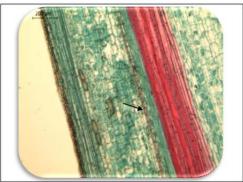


Fig. 12 : Longitudinal section (L.S.) in young stem showing phloem fiber (x 40).

4.2.4.2 The moderate and old stem:

A transverse section in the moderate (Figs.13 and 14) and old stem (Fig.15) is more or less circular in outline. The epidermis of moderate stem consists of one layer of square to subrectangular cells followed by the cortex which consists of an outer zone of collenchyma and inner parenchyma cells. While in old stem, the outer layer showing several rows of subrectangular, radially arranged and tangentially elongated cork cells (Fig.15) and cortex which consists of an outer zone of phelloderm and collenchyma in 8-10 rows, followed by 9-11 rows of parenchyma cells (Fig.15). In both stems, the parenchyma cells are slightly polygonal in outline, and some containing acicular crystals of calcium oxalate (Fig.16J) and starch granules. The innermost layer of the cortex is the endodermis which is distinct (Figs.13B & 13C). The pericycle consists of 2-3 rows of parenchyma cells interrupted by lignified fibers (Figs.14A & 14B). The fiber in the powdered form showing thick, lignified, striated wall with wide lumen and acute to acuminate apex. The phloem consists of cellulosic elements of sieve tubes, companion cells, phloem parenchyma and phloem fibers (Figs.14A, 14B and 15). The xylem consists of large vessels, wood fibers, tracheids, tracheidal vessels and wood parenchyma (Figs.14A and15). Some of these fibers in the powdered form are slightly tortuous with swellings, while others are septate. The pith consists of parenchyma cells containing acicular crystals of calcium oxalate, cystoliths of calcium carbonate and starch granules (Fig. 16L). The old stem

differs from the young stem in existence of the cork cells and wide cortex. The pericycle in the old stem consists of groups of lignified pericyclic fibers alternating with groups of parenchyma cells. The xylem vessels are more in number and wider. The medullary rays and wood parenchyma (Fig. 16K) are lignified and pitted. The pith in the old stem is wider than that of the young stem, showing the same contents (acicular crystals of calcium oxalate, cystoliths of calcium carbonate and starch granules).

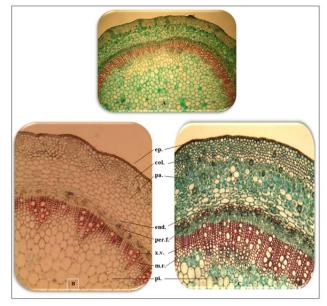


Fig. 13: Photo of the diagrammatic T.S of the moderate stem: (A&C (X=40), B (X=100)).

col., collenchyma; end., endodermis; ep., epidermis; m.r., medullary rays; pa., parenchyma; per.f., pericyclic fiber; pi., pith; xyl.v., xylem vessel.

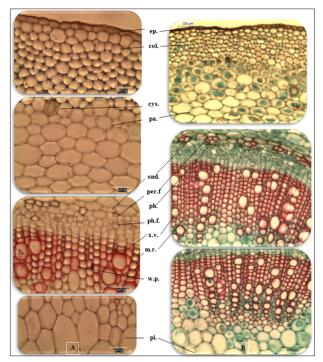


Fig. 14: Photo of the detailed sector of moderate stem: (A (X=200) and B (X=100)).

cam., cambium; col., collenchyma; cr., crystal of calcium oxalate; m.r., medullary rays; par., parenchyma; per.f., pericycle fiber; phl., phloem; ph.f., ph;oem fiber; pi., pith; xyl.v, xylem vessel.

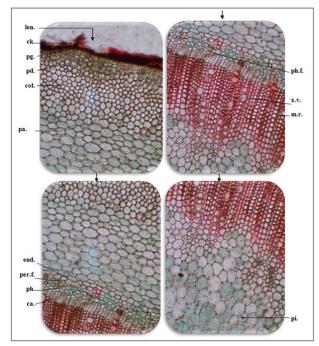


Fig. 15: Photo of the detailed sector of old stem: (X=100) cam., cambium; col., collenchyma; ck., cork; end., endodermis; len., lenticel; m.r., medullary rays; par., parenchyma; pd., phelloderm; per.f., pericycle fiber; pg., phellogen; ph., phloem; ph.f., phloem fiber; pi., pith; x.v, xylem vessel.

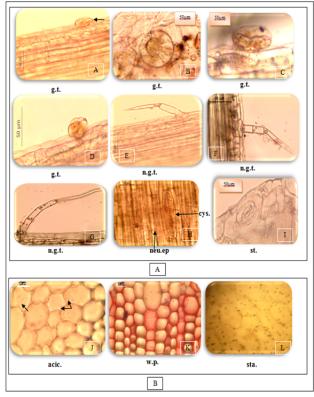


Fig. 16: A- Surface preparation in young stem
B- characteristic elements in the old stem
(A, B, C and D) g.t., glandular trichome; (E, F and G)) n.g.t., non glandular trichome (E- bicellular, F&G- multicellular); (H) neu.ep., neural epidermis; (I) st., stomata; (J) acic., acicular crystals of calcium oxalate (K) w.p., wood parenchyma; (L) sta., starch; cys., cystoliths of calcium carbonate.

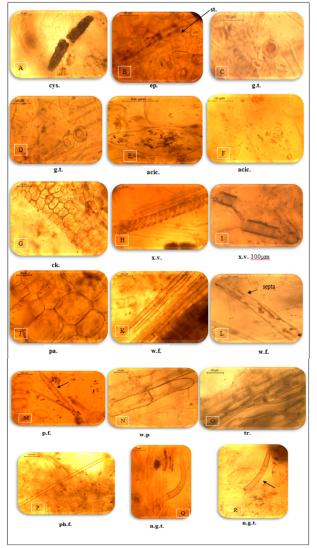


Fig. 17 : The powder elements of the stem (A) cys., cystoliths of calcium carbonate; (B) ep., epidermis; (C&D) g.t., glandular trichome; (E&F) acic., acicular crystals of calcium oxalate; (G) ck., cork; (H&I) x.v., xylem vessel; (J) parenchyma cells; (K&L) w.f., wood fiber; (M) p.f., pericyclic fiber; (N) w.p., wood parenchyma; (O) tr., tracheid; (P) ph.f., phloem fiber; (Q&R) n.g.t., non glandular trichome;

Powder of stem is dark green, has faint odour and slightly disagreeable taste and characterized microscopically (Fig. 17) by the following:

st., stomata.

- 1- Fragments of epidermal cells of young stem showing diacytic type of stomata. The cells are polygonal, usually isodiametric, sometimes axially elongated with more or less straight anticlinal walls (Fig. 17B). Some cells show cystoliths of calcium carbonate. Some fragments carry glandular trichomes which have unicellular stalk and unicellular (Figs. 17C & 17D) to multicellular head and other fragments carry few nonglandular trichomes which are uniseriate, multicellular cells having acute to acuminate apex and covered with smooth or striated cuticle (Figs. 17Q & 17R).
- Numerous scattered cystoliths of calcium carbonate (Fig. 17A).

4.2.4.3 The powdered stem

- 3- Fragments of phloem fibers, non-lignified and acicular in shape (Fig. 17P).
- 4- Fragments of pericyclic fibers, lignified, thick striated walls, wide lumen and acute to acuminate apex (from the old stem), (Fig. 17M).
- 5- Fragments of wood fibers with thick lignified walls, moderately wide lumen and acute to acuminate apices. Some of these fibers are slightly tortuous with swellings (from the old stem), (Fig. 17K) and others are septate (Fig. 17L).
- 6- Fragments of lignified spiral, annular and reticulate xylem vessels (Fig. 17H&I).
- 7- Fragments of tracheids and tracheidal vessels with lignified pitted walls (from the old stem), (Fig. 17O).
- 8- Fragments of wood parenchyma cells with thick pitted and lignified walls (from the old stem), (Fig. 17N).
- 9- Fragments of parenchyma cells (Fig. 17J) either showing scattered acicular crystals of calcium oxalate (Fig. 17E), cystoliths of calcium carbonate or starch granules.
- 10- Numerous scattered acicular crystals of calcium oxalate (Fig. 17F).
- 11- Fragments of polygonal cork cells with brownish contents (from the old stem), (Fig. 17G).

4.2.7. The flower:

4.2.7.1. The calyx

A transverse section in the sepals (Figs. $18B_1 \& 18B_2$) comprises of inner and outer epidermis, enclosing inbetween a wide mesophyll traversed by many vascular strands and showing cystoliths of calcium carbonate. Both the inner and outer epidermis carrying glandular and nonglandular trichomes and showing cystoliths of calcium carbonate. The mesophyll showing collenchyma and parenchyma cells traversed by vascular system which is formed of xylem and phloem.

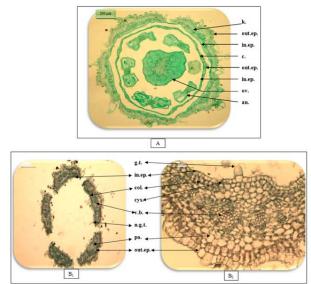


Fig. 18: A- Photo of the diagrammatic T.S of the flower (X=40)B-Photo of the diagrammatic T.S of the sepals $(B_1 (X=40), B_2 (X=200))$. an., anther; c., petals; cys., cystoliths of calcium carbonate; g.t., glandular trichome; in.ep., inner epidermis; k., sepals; n.g.t., nonglandular trichome; out.ep., outer epidermis; ov., ovary; v.b., vascular bundle.

The inner epidermis of sepal :

The cells are polygonal, isodiametric with straight anticlinal walls in surface view. They are covered with thin smooth cuticle and contain cystoliths of calcium carbonate (Figs. 19K & 19L). The epidermal cells show glandular trichomes have unicellular (Fig. 19C), bicellular (Fig. 19D) stalk and unicellular (Fig. 19C) to multicellular head with 4-8 cells (Figs. 19E, F and H). Multicellular stalk unicellular globular head glandular trichomes are also present (Fig. 19G). The nonglandular trichomes are uniseriate and unicellular (Figs. 19(I) & 19(J)), bicellular and multicellular. They have smooth and striated cuticle with acute to acuminate apices.

The outer epidermis:

The cells in the surface view are somewhat similar to those of the inner epidermis showing diacytic type of stomata and cystoliths of calcium carbonate. The glandular and nonglandular types of trichomes are also present.

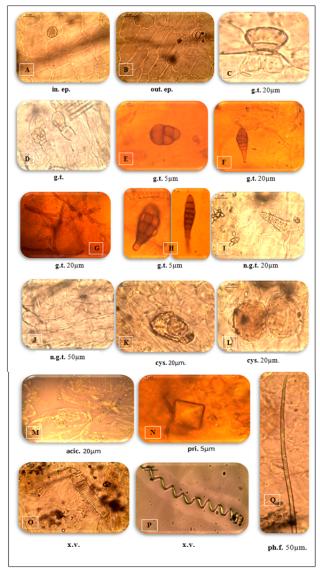


Fig. 19 : Powdered elements of the sepals (A) in.ep., inner epidermis; (B) out.ep., outer epidermis; (C, D, E, F, G,

(A) Intep, infer epiderinis, (B) out.ep., outer epiderinis, (C, D, E, F, G, and H) g.t., glandular trichome;
 (K&L) cys., cystoliths of calcium carbonate; (M) acic., acicular crystals of calcium oxalate; (O&P) x.v., xylem vessel; (Q) ph.f., phloem fiber.

4.2.7.2. The corolla :

A transverse section in the petals (Fig. 20) consisting of inner and outer epidermises, enclosing in between a wide parenchymatous homogenous mesophyll traversed by many vascular strands and showing cystoliths of calcium carbonate. Both the inner and outer epidermises carrying glandular and nonglandular trichomes and showing cystoliths of calcium carbonate.

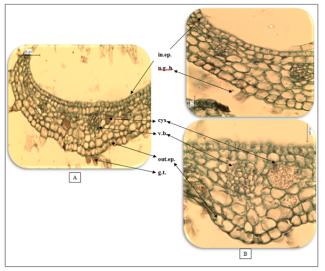


Fig. 20: Photo of the diagrammatic T.S of the petals: (A (X=200), B (X=400)).

cys., cystoliths of calcium carbonate; g.t., glandular trichome; in.ep., inner epidermis; n.g.t., nonglandular trichome; out.ep., outer epidermis; v.b., vascular bundle.

The inner epidermis:

The cells show some variations in size and shape in different parts of the surface. The cells at the apical part (Fig. 21A) have wavy anticlinal walls and are papillosed. They are covered with thin, smooth cuticle. The cells at the middle part (Fig. 21B) are with less wavy anticlinal walls and papillosed. They have both glandular and nonglandular trichomes. The lower part cells (Fig. 21C) are elongated, not papillosed and have straight anticlinal walls.

The outer epidermis:

The cells in surface view are somewhat larger than the inner ones. The cells of the apical part (Fig. 21D) are also with wavy anticlinal walls and papillosed. The cells at the middle part (Fig. 21E) are with less wavy anticlinal walls and papillosed. They carry both glandular and nonglandular trichomes. The cells at the lower part (Fig. 21F) are elongated, not papillosed and having straight anticlinal walls. They carrying both glandular and nonglandular trichomes. Both the inner and the outer epidermal cells are covered with thin smooth cuticle.

Trichomes :

There are two types of trichomes: glandular and nonglandular. The glandular type has multicellular stalk 3-4 cells with unicellular heads (Fig. 21(I)), unicellular stalk with unicellular head (Fig. 21G) and unicellular stalk with multicellular head (Fig. 21H). The nonglandular type is uniseriate either unicellular, bicellular (Fig. 21J) or multicellular 3-4 cells (Fig. 21K).

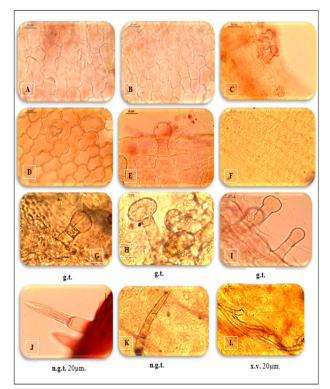


Fig. 21 : Powdered elements of the petals:

(A) Inner epidermis of the apical part of the petals; (B) Inner epidermis of the middle part of the petals; (C) Inner epidermis of the lower part of the petals; (D) Outer epidermis of the apical part of the petals; (E) Outer epidermis of the middle part of the petals; (F) Outer epidermis of the lower part of the petals; (G, H and I) g.t., glandular trichome; (J&K) n.g.t., nonglandular trichome; (L) x.v., xylem vessels.

4.2.7.3. The androecium:

The anther:

A transverse section in the anther (Figs. 22A & $22B_1$ & $22B_2$) shows two slightly unequal anther lobes which are attached together with connective tissue containing small vascular bundles. Each anther lobe is formed of one pollen sac containing pollen grains. The anther wall consists of one layer of epidermal cells followed by the fibrous layer (Figs. $22B_1$ & $22B_2$).

The filament:

The epidermis of the filament (Fig. 23A) consists of elongated cells with straight anticlinal walls in surface view. They are covered with smooth cuticle.

The epidermis of the anther:

The epidermis of the anther (Fig. 23B) consists of polygonal cells with straight anticlinal walls and covered with thin smooth cuticle in surface view. Stomata and trichomes are not spotted. A fibrous layer (Figs. $22B_1$ & $22B_2$) lies below the epidermis and consists of one layer of lignified bar-like thickening cells, while in surface view (Figs. 23C & 23D), they are elongated with slightly rounded ends.

The pollen grains

They are somewhat spherical with 4 corporate (Fig. 23G).

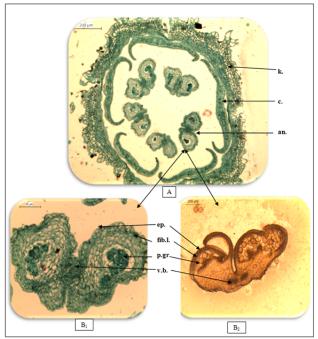


Fig. 22 : A- T.S. of the flower at the level of the anther lobes (X=40)

B- T.S. of the anther (B₁- X=200, B₂- X=40) an., anther; c., petals; ep., epidermis; fib.l., fibrous layer; k., sepals; p.gr., pollen grain; v.b., vascular bundle.

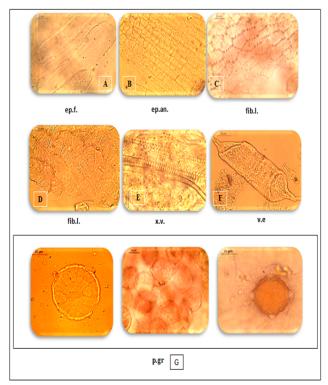


Fig. 23: Powdered elements of the androecium
(A) ep.f., epidermis of filament; (B) ep.an., epidermis of anther;
(C&D) fib.l., fibrous layer of anther; (E) x.v., xylem vessel; (F) v.e., vessel element with tail; (G) p.gr., pollen grain.

4.2.7.4. The gynaecium : The ovary:

A transverse section in the ovary (Fig. 24) shows that it is tricarpellary, trilocular with more or less circular outline. Each carple has six ovules with axile placentation. The epidermis formed of one layer of polygonal cells followed by wide parenchymatous ground tissue (Fig. 25J). The epidermis of the ovary, in surface view (Fig. 25F), is formed of polygonal, isodiametric cells with straight anticlinal walls. Diacytic type of stomata (Fig. 25H) and cystoliths of calcium carbonate (Fig. 25(I)) has observed in the isolated powder.

The style

The style is nearly glabrous and the epidermal cells (Fig. 25E) are polygonal in surface view. They are almost elongated with straight anticlinal walls and covered with smooth cuticle. Stomata are not observed. Abundant nonglandular (Figs. 25A & 25B) and slight glandular trichomes (Figs. 25C & 25D) cover the surface of the style. They are disposed throughout or restricted to proximal part. The nonglandular trichomes are uniseriate, unicellular and bicellular (Figs. 25A & 25B). They are covered with smooth cuticle and having acute to blunt apices. Acicular crystal of calcium oxalate (Fig. 25K) and xylem vessel (Fig. 25L) are also present.

The stigma

The stigma is funnel form or bi-lobed, lobes are equal. The epidermal cells of stigma are polygonal, papillosed and covered with smooth cuticle in surface view. Stomata and trichomes are not observed.

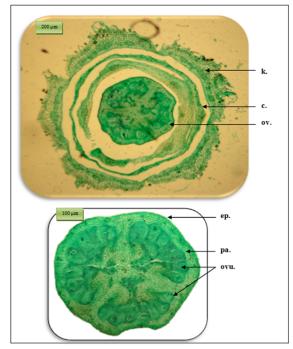


Fig. 24 : A- T.S. of the flower at the level of the ovary (X=40) **B- T.S. of the ovary** (X=100) c., petals; ep., epidermis; k., sepals; ov., ovary; ovu., ovules; pa., parenchyma.

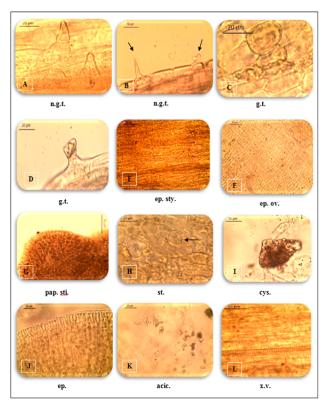


Fig. 25: Powdered elements of the gynaecium

(A&B) n.g.t., nonglandular trichome; (C&D) g.t., glandular trichome; (E) ep.sty., epidermis of the style; (F) ep.ov., epidermis of the ovary; (G) pap.sti., pappillosed stigma; (H) st., stomata; (I) cys., cystoliths of calcium carbonate; (J) ep., epidermis in transverse section; (K) acic., acicular crystal of calcium oxalate (L) x.v., xylem vessel.

4.2.7.5. The rachis of the inflorescence

A transverse section in the rachis (Figs. 26) is more or less quadrangular in outline. It has an outer epidermis followed by groups of collenchymatous cells alternated with chlorenchymatous ones (Fig. 26), followed by a wide parenchymatous cortex. The endodermis is distinguished from the surrounding parenchyma. The vascular cylinder is surrounded by parenchymatous pericycle interrupted by groups of lignified pericyclic fibers. The phloem tissue formed of soft cellulosic elements. The xylem region is radiating and formed of lignified elements. The central pith is wide and formed of parenchymatous cells showing acicular crystals of calcium oxalate and starch granules.

The epidermis

The epidermis (Fig. 26) is formed of one row of square to subrectangular cells. In surface view the cells are somewhat axially elongated, subrectangular, isodiametric with more or less straight anticlinal walls. Cystoliths of calcium carbonate are also present. The cells are covered by thin smooth cuticle. Diacytic type of stomata, few glandular and nonglandular trichomes (Figs. 26, 27A, 27B, 27C and 27D) are also spotted.

The cortex

The cortical tissue (Fig. 26) consists of 3-4 rows of collenchyma alternated with chlorenchyma followed by 5-6

rows of parenchyma cells which are somewhat polygonal in shape. Cystoliths of calcium carbonate are also present (Figs. 26 and 27(I)). The endodermis is clearly distinct.

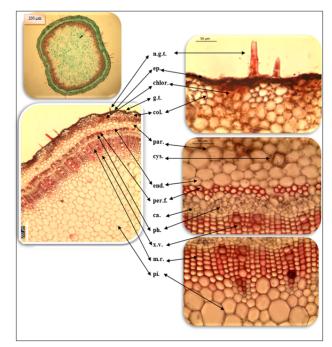


Fig. 26: Photo of diagrammatic and detailed T.S. of the rachis of the inflorescence

ca., cambium; chlor., chlorenchyma; col., collenchyma; cys., cystoliths of calcium carbonate; end., endodermis; ep., epidermis; g.t., glandular trichome; m.r., medullary rays; n.g.t., non glandular trichome; par., parenchyma; per.f., pericyclic fiber; ph., phloem; pi., pith; x.v., xylem

The pericycle

The pericycle (Fig. 26) is parenchymatous interrupted with lignified groups of pericyclic fibers. The fibers appear polygonal in outline in transverse section, while in powdered form, they have thick lignified walls, narrow lumens and blunt ends.

The vascular system

The vascular system presents phloem and xylem encircling a central wide pith. The phloem (Fig. 26) is formed of soft elements surrounding the xylem and including phloem parenchyma, sieve tubes and companion cells. The cambium is distinct. The xylem region (Fig. 26) is formed of lignified vessels, wood fibers and wood parenchyma. The xylem vessels are showing annular, pitted and spiral thickenings (Fig. 27H). The wood fibers having thick lignified walls, moderately wide lumen and acute to acuminate apices. The wood parenchyma are subrectangular with lignified pitted walls (Figs. 27J & 27K).

The pith

The pith (Fig. 26) forms a wide central zone of thin-walled parenchyma cells which are somewhat rounded in shape containing acicular crystals of calcium oxalate (Fig. 27F) and starch granules.

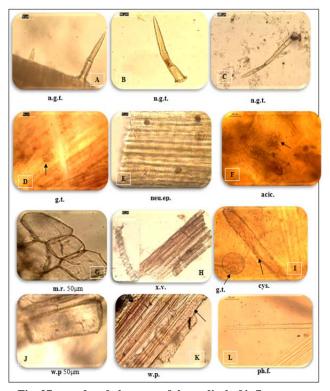


Fig. 27: powdered elements of the pedicel of inflorescence
(A, B and C) n.g.t., nonglandular trichome; (D) g.t., glandular trichome; (E) neu.ep., neural epidermis; (F) acic., acicular crystals of calcium oxalate; (G) m.r., medullary rays; (H) x.v., xylem vessel; (I) cys., cystoliths of calcium carbonate; (J&K) w.p., wood parenchyma; (L) ph.f., phloem fiber.

5. CONCLUSION

Examination of the different macroscopical and microscopical features of the aerial parts of *Odontonema cuspidatum* (Nees) Kuntze, family Acanthaceae dissects a good tool in the identification and authentication of the plant. These characters could be useful in identifying the plant earlier to its use in any pharmacological studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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