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Micromorphological Features of Stem, Root and Inflorescence of *Moluccella laevis* L., Family Lamiaceae (Labiatae)

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Abstract

Moluccella laevis L. belongs to family Lamiaceae (Labiatae or Mint family). It is an annual herb. It flowers from March to April. It bears dense spikes of small 2-lipped white fragrant florets with large, bell-shaped calyxes, known as "Shell flower" or "Bells of Ireland". It is cultivated as a decorative plant. By reviewing the literature, few botanical investigations were reported on this plant, which inspire us to explore both macro- and micro-morphological investigations of *M. laevis* L. stem, root and inflorescence, to aid the researchers in the authentication and identification of *M. laevis* in both entire and powdered forms.

Key words

Lamiaceae (Labiatae); *Moluccella laevis*; Micromorphological; Stem; Root; Inflorescence.

1. Introduction

Lamiaceae (Labiatae or Mint family) is one of the most important families containing essential oils. It comprises 236 genera and more than 7,000 species. Moreover, many plants are edible and can be used in the manufacture of perfume. These plants are used in folkloric medicine in gastrointestinal tract disorders as; antispasmodic, carminative, antiemetic and choleretic. Furthermore, they can be used as an anti-inflammatory, emmenagogue, diaphoretic and antimicrobial agents [1]. *Moluccella* (syn. *Lamium*) is considered one of the most noteworthy genera of Lamiaceae. Its geographical origin is in Asia, North Africa and Europe. It includes about forty species [2].

One important plant of this genus is *Moluccella laevis* L. due to its chemical and biological values. The essential oils and the saponifiable matters of the petroleum ether fraction of the total ethanolic extract (TEE) of *M. laevis* were analysed by GC-MS [3,4]. Moreover, the antibacterial activity of TEE and its various fractions was studied against Gram-positive and Gram-negative bacteria [4]. Furthermore, both of the aqueous and EtOAc fractions of TEE demonstrated the most potent cytotoxic effect against cancer cell lines as CACO-2 and MCF-7, respectively [5]. Additionally, 20 compounds were tentatively identified from LC-HR-ESI-MS metabolomic analysis. These compounds revealed that stachydrine is more likely to account for the cytotoxic activity of both the EtOAc and aqueous fractions *in silico* docking study [5]. Furthermore, 8 compounds: palmitic acid, tricosan-1-ol, pentadecan-1-ol, icosyl acetate, a mixture of β-sitosterol & stigmasterol, β-sitosterol-7-*O*-β-D-glucopyranoside and luteolin-7-*O*-β-D-glucopyranoside, were isolated and identified spectroscopically [5].

M. laevis is an annual herb, flowers from March to April. It bears white fragrant flowers, widely spread in West Asia. It is commonly known as "Shell flower" or "Bells of Ireland" (Figure 1A).

It is cultivated as a decorative plant [3]. The botanical study of *M. laevis* L. had been previously investigated using primitive methods [3]. Also, we performed a former botanical study on leaves [6]. This encouraged us to carry out the current morphoanatomical study including the detailed macroscopical and microscopical characters of the stem, root and inflorescence of *M. laevis* L., using advanced instruments that would be considered as a supportive tool for authentication and identification of this plant due to its great chemical and biological importance.

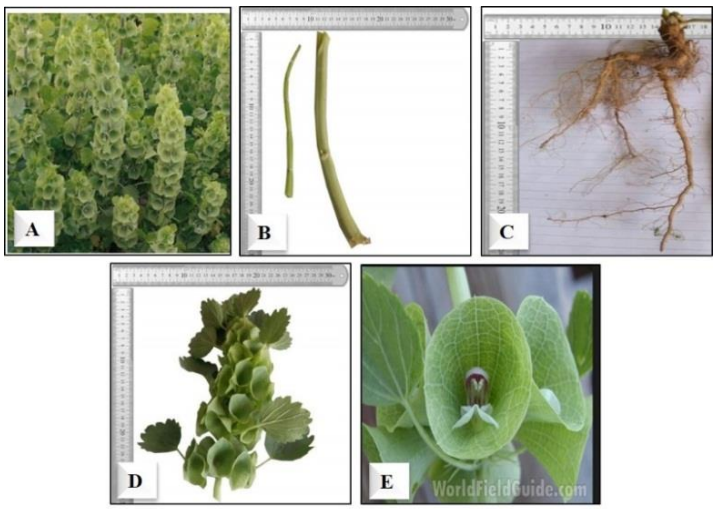

Figure 1: Photos of *M. laevis*; **(A)** The plant, **(B)** The stem, **(C)** The root and **(D& E)** The inflorescence. All **(x1)** except (**A) (x0.1)**.

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2. Material and Methods

2.1. Plant material

Stem, root and inflorescence of *M. laevis* L. were taken from the Nursery of the Faculty of Agriculture, Minia University in March 2016. The plant was identified by Prof. Nasser Barkat, Department of Botany, Faculty of Science, Minia University. A voucher specimen was preserved in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt, under registration number (Mn-ph-Cog-35). The plant parts that were utilized for the micromorphological investigation were taken from the fresh samples, as well as the preserved ones. They were kept in ethanol (70%)-glycerin-water (1:1:1). The different parts of the plant were also left to air dry in the shade, then were ground to a fine powder and deposited in well-closed bottles for microscopical investigation.

2.2. Samples preparation for microscopical examination

Safranin, phloroglucinol, light green and alcoholic potassium hydroxide were used for illustration of the plant sections, as well as their powders.

2.3. Microscopical examinations

Transverse sections (T.S.), longitudinal sections (L.S.), surface preparations and the powder of the different parts of the plant were utilized for observation of various microscopical characters. The microscopical examinations were performed by Leica camera attached to a microscope (Germany) and Samsung digital camera (10 megapixels, Korea).

3. Results and discussion

3.1. Macromorphology

3.1.1. Stem

It is erect, quadrangular and green in color with monopodial branching showing long internodes. It measures 30-60.0 cm (length) and 0.5-2.5 cm (diameter). It is longitudinally striated and breaks with a short fibrous fracture when dry. It has a lemon-like odor and a slightly bitter taste (Figure 1B).

3.1.2. Root

It has a fusiform tap root carrying several lateral rootlets and is cylindrical in shape. It measures 10.0-25.0 cm (length) and 1.0-3.0 cm (diameter). Externally, it is brown in color with a rough longitudinally wrinkled surface and shows a few small circular scars of removed rootlets. It has a short fibrous fracture after drying. It shows a yellowish-white solid interior. It is odorless and tasteless (Figure 1C).

3.1.3. Inflorescence

It is a raceme of verticillasters. Each verticillaster is formed of two cymose clusters of sessile florets. The floret has a floral diagram as illustrated in Figure 2. Each cluster has its oldest floret in the middle. It is a dichasial form which after the first branching passes into scorpioid. The florets are arranged in two groups of 3-5 florets arranged in opposite decussate form and each group is surrounded with 8-10 spiny bracts. The floret is a small whitish-violet, sessile, complete, zygomorphic, hermaphrodite and has the floral formula demonstrated in Figure 2. The inflorescence has a fragrant odor, measuring 0.5-2.5 cm (length) and 0.2-2.0 cm (diameter) at the widest part. The bract is a linear spiny, green in color, measuring 0.1-1.0 cm (length) and 0.1 mm width (Figure 1D and 1E).

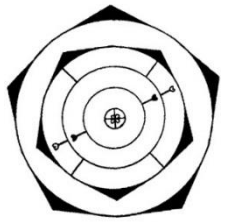

Figure 2: Floral diagram.

3.1.3.1. The calyx

It is persistent, green in color and consisting of five gamosepales forming a tube terminated by 5 small toothed mouth that is bell-shaped. The calyx tube measures 0.2-2.5 cm (length) and 0.2-2.0 cm (diameter) at the top and 0.1-0.3 cm (diameter) at the base.

3.1.3.2. The corolla

It is white to whitish-violet in color, bilipped, and consists of five united petals. It is formed of a straight tube, which widens toward the mouth. The corolla tube measures about 0.5-1.8 cm (length) and about 0.1-0.3 cm (diameter) near the mouth.

3.1.3.3. The androecium

It is formed of four epipetalous, didynamous stamens and the two long filaments measure 0.5-1.0 cm (length), while the other two are 0.4-0.8 cm long. The anthers are dorsifixed, bilobed, about 2.0 mm (length) and 1.0 mm (diameter), they dehisce entirely by a longitudinal slit.

3.1.3.4. The gynoecium

The ovary is superior, formed of two united carpels (syncarpous) showing four locules, bicarpillary. The ovary is elongated, measuring about 0.7 mm (length) and 0.4 mm (diameter), tetralocular, each locule contains a single basal ovule. The style is filliform, gynobasic, about 1.3 cm (length) and 1.0 mm (diameter) and ends with a bifid stigma.

3.2. Micromorphology

3.2.1. Stem micromorphology

3.2.1.1. The upper part of the stem

A transverse section in the upper part of the stem is almost quadrangular in shape, showing an epidermis followed by a relatively wide parenchymatous cortex with four groups of collenchymatous cells beneath the angles. The pericycle is parenchymatous interrupted with small groups of non-lignified fibers, enclosing a circle of vascular tissue with a wide parenchymatous pith and a narrow hollow central region (Figure 3).

3.2.1.1.1. The epidermis

It consists of polygonal to sub-rectangular axially elongated cells with straight anticlinal walls. It covers with a smooth cuticle. It shows diacytic stomata (Figures 3, 4 and 11A).

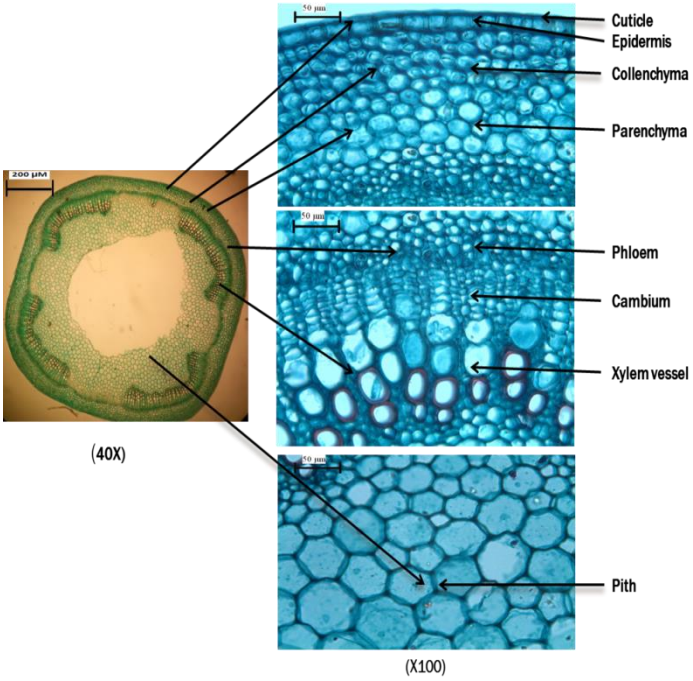

Figure 3: Detailed T. S. in the upper part of the stem.

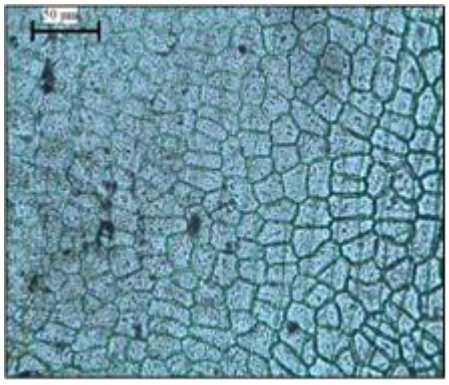

Figure 4: Surface preparation of the stem **(x200)**.

3.2.1.1.2. The cortex

The cortical tissue consists of collenchymatous cells (2-3 rows) beneath the ridges, while the cortex between the ridges consists of 6-12 rows of parenchymatous cells. The endodermis is indistinguishable (Figure 3).

3.2.1.1.3. The vascular tissue

3.2.1.1.3.1. The pericycle

It is formed of a continuous ring of parenchymatous cells interrupted with small groups of the dentate, non-lignified, wide lumen and acute to rounded apices fibers (Figures 5, 11B and 11C).

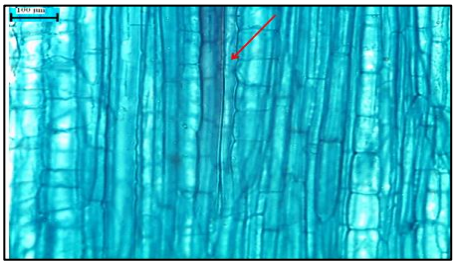

Figure 5: L.C. in the stem showing dentate pericyclic fibers **(x100)**.

3.2.1.1.3.2. The phloem

It is formed of soft, thin-walled cellulosic elements; sieve tubes, companion cells and phloem parenchyma. No lignified elements are found in the phloem region (Figures 3 and 6).

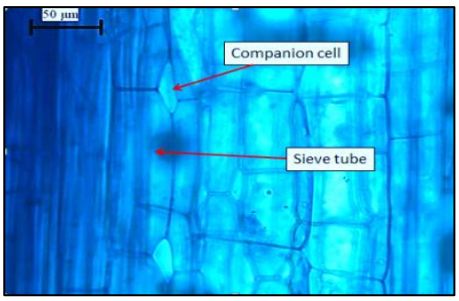

Figure 6: L.C. in the lower part of stem showing phloem **(x200)**.

3.2.1.1.3.3. The cambium

It is formed of several rows of tangentially thin-walled elongated meristematic cells (Figure 3).

3.2.1.1.3.4. The xylem

It is formed of lignified vessels with spiral thickening, tracheids, wood fibers and polygonal pitted walls wood parenchyma. The medullary rays are uni or biseriate transversing the xylem (Figures 3 and 9A).

3.2.1.1.4. The pith

The pith is formed of rounded thin-walled parenchyma cells (Figure 3).

3.2.1.2. The lower part of the stem

A transverse section in the lower part of the stem is almost quadrangular in outline. It is nearly resembling the upper part with the following differences:

A) The outer epidermis carrying glandular hairs with bicellular stalks and unicellular heads (Figure 7).

Figure 7: Epidermal cell carrying glandular hair **(x200)**.

B) The collenchymatous layer consists of 10-13 rows of small thick-walled rounded cells with no intercellular spaces (Figure 8).
C) The xylem consists of lignified vessels, tracheidal vessels, tracheids, wood parenchyma and wood fibers. The vessels have spiral, reticulate, sclariform, pitted and bordered pitted thickening (Figure 9). The tracheids are narrow with lignified pitted walls. The wood fibers are spindle-shaped demonstrating thick lignified walls, narrow lumens and tapering ends. The wood parenchyma is polygonal with lignified walls. The medullary rays are uni to biseriate formed of elongated cells with lignified and pitted walls (Figures 8 and 10C).
D) The pith is smaller than in the upper part of the stem (Figure 8).

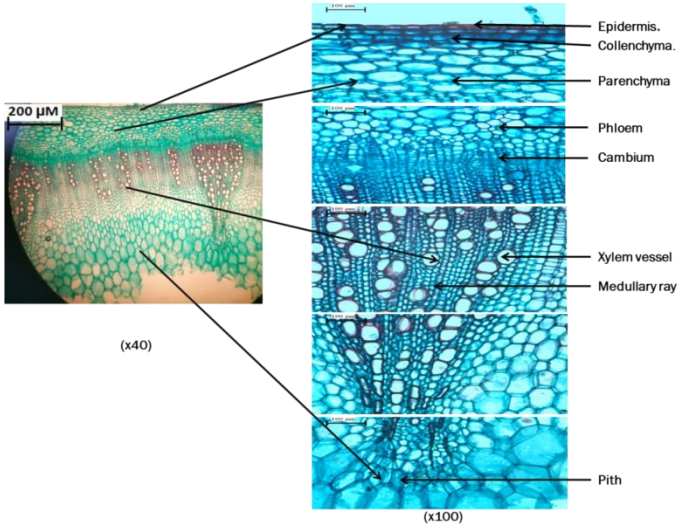

Figure 8: Detailed T. S. in the lower part of the stem.

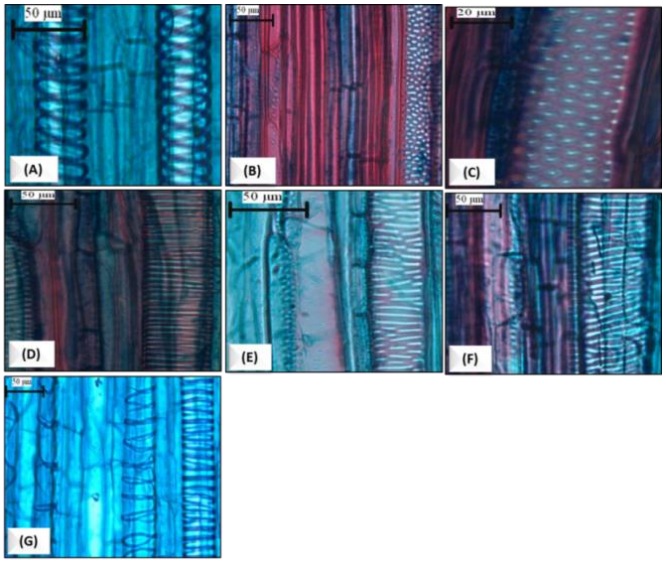

Figure 9: L.C. in the lower part of the stem showing **(A)** Spiral X.V., **(B)** Pitted X.V., **(C)** Borded pitted X.V., **(D)** Sclariform X.V., **(E) & (F)** Reticulated X.V., **(G)** Annular X.V. **All (x200) except (C) (x400)**.

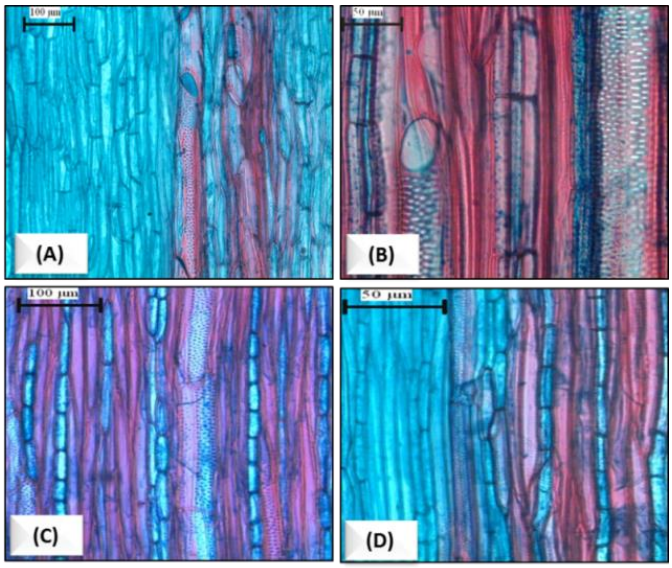

Figure 10: L.C. in the stem showing **(A) & (B)** Tracheid and tracheidal vessels, **(C)** Medullary rays, **(D)** Wood fiber. **(B) & (D) (x200) and (A) & (C) (x100)**.

3.2.1.3. The stem powder

It is pale green in color with faint lemon-like odor and a slightly bitter taste.
Its elements are demonstrated as follows (Figure 11):
A) The epidermis of the stem showing diacytic stomata.
B&C) Fragments of non-lignified dentate pericyclic fibers.
D) Fragment of xylem vessels showing reticulate xylem vessels (X.V.).
E) Fragment of X.V. showing bordered pitted X.V.
F) Fragment of wood parenchyma.
G&H&I) Fragment of wood fibers with thick lignified walls, narrow lumens and tapering ends.
J) Absence of calcium oxalate crystals.

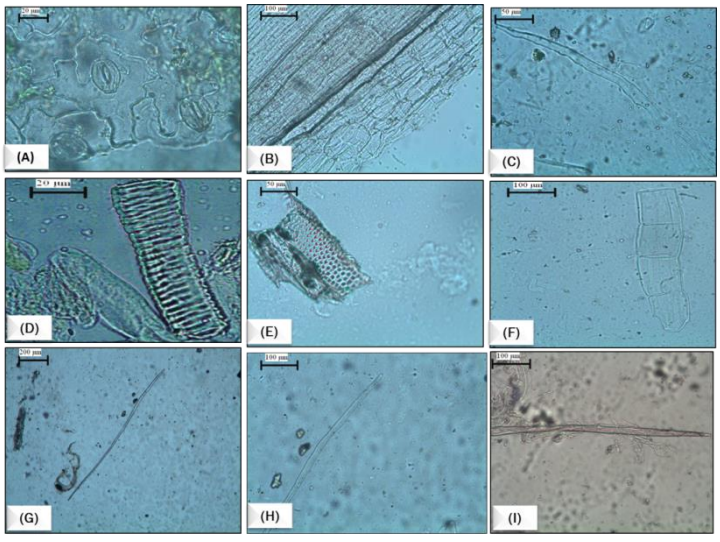

Figure 11: (A) Diacytic stomata, **(B) & (C)** Dentate fiber, **(D)** Reticulate X.V., **(E)** Bordered pitted X.V., **(F)** Wood parenchyma, **(G) & (H)** Wood fiber and **(I)** Wood fiber with phloroglucinol/HCl. **(A) and (D) (x400), (B), (F), (H) and (I) (x100), (C) and (E) (x200), (G) (x40)**.

3.2.2. Root micromorphology

A transverse section in the root has a nearly circular outline, showing a layer of brown cork cells surrounding a narrow secondary cortex followed by the vascular tissue, which consists of phloem and a wide zone of the xylem extending to the center (Figure 12).

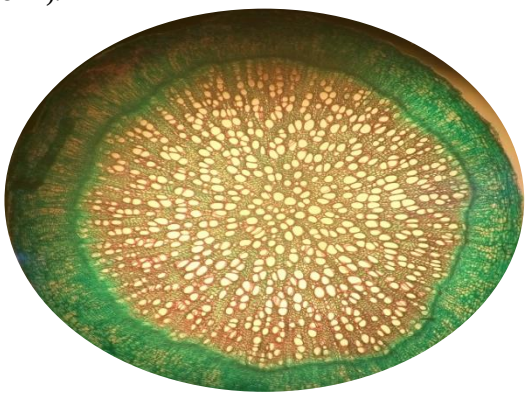

Figure 12: T. S. in the root **(x40)**.

3.2.2.1. The cork

It is formed of brown tangentially elongated tabular cells (3-4 rows) with thick walls as seen in the transverse section (Figure 13). While, in surface view, they appear as polygonal, isodiametric to slightly elongated thick-walled cells.

3.2.2.2. The secondary cortex

It consists of oval to elongated thin-walled parenchymatous cells (4-6 rows) with small narrow intercellular spaces (Figure 13).

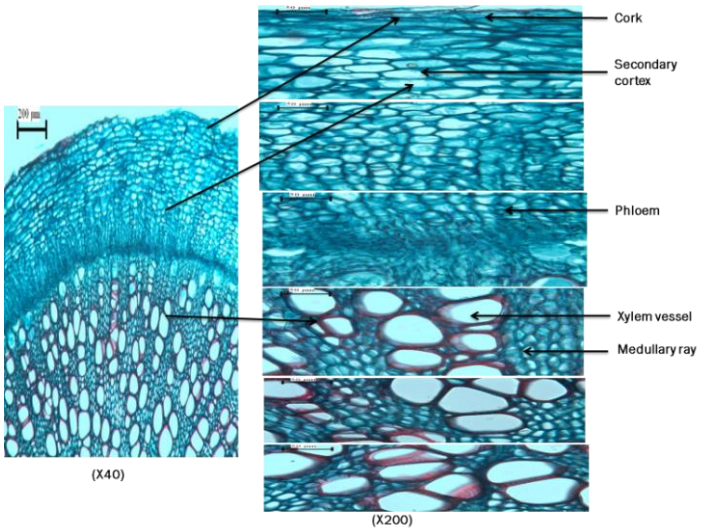

Figure 13: Detailed T. S. in the root.

3.2.2.3. The vascular tissue

3.2.2.3.1. The phloem

It consists of soft cellulosic elements; phloem parenchyma, sieve tubes and companion cells.

3.2.2.3.2. The cambium

It is not observed.

3.2.2.3.3. The xylem

It consists of lignified vessels, fibers, tracheids, tracheidal vessels, wood parenchyma and medullary rays (Figure 14). The xylem vessels are with spiral, annular and sclariform thickening while, the wood parenchyma are polygonal with thick lignified and pitted walls. The medullary rays are uni to biseriate have radially elongated cells in the phloem region and funnel-shaped cells in the xylem region (Figure 14).

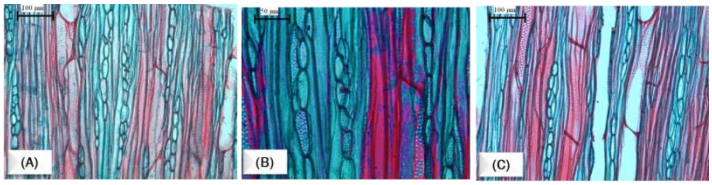

Figure 14: L.C. in the root showing **(A)** Tracheidal vessels **(B)** Medullary rays and **(C)** Tracheids. **A and C (x100), B (x200)**.

3.2.2.4. The root powder

It is pale greyish-brown to yellowish-brown in color, odorless and has a bitter taste. Its elements are listed as follows (Figure 15):

A) Fragments of parenchymatous cells of the secondary cortex.
B) Fragment of wood parenchyma.
C) Fragment of spiral X.V.
D) Fragment of sclariform X.V.
E) Fragment of annular X.V.
F) Fragment of medullary rays.

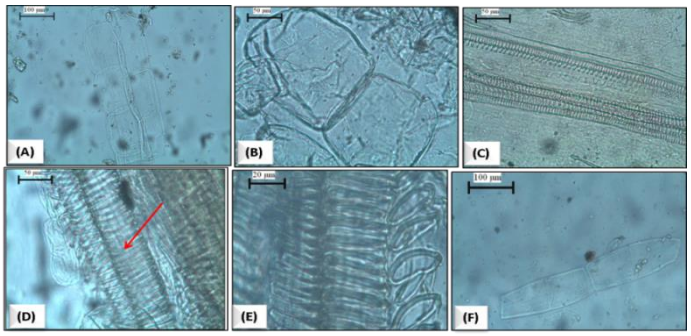

Figure 15: (A) Parenchyma cells of cortex, **(B)** Wood parenchyma, **(C)** Spiral X.V., **(D)** Sclariform X.V., **(E)** Annular X.V. and **(F)** Medullary ray. **All (x200) except A and F (x100), E (x400).**

3.2.3. Inflorescence micromorphology

It consists of one type of florets and each floret consists of five united green sepals, a corolla of five united white to whitish violet petals, a didynamous androecium of four stamens and a gynoecium of a superior tetralocular bicarpillary ovary (Figure 16).

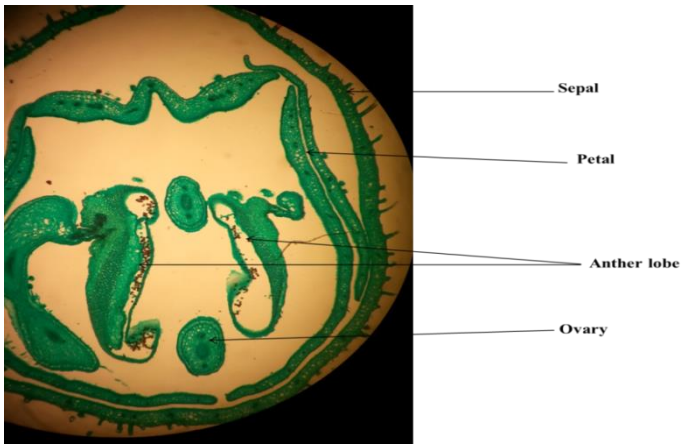

Figure 16: T.S. of the floret **(x40)**.

3.2.3.1. The calyx

3.2.3.1.1. Calyx micromorphology

A transverse section in the calyx shows hairy upper and lower epidermises enclosing a wide parenchymatous cortex and several vascular bundles (Figure 17). Glandular hairs with unicellular stalks and multicellular heads (labiaceous hair) with eight radiating cells and numerous non-glandular unicellular, bicellular and multicellular hairs are present on both upper and lower epidermis (Figure 18). The outer epidermis consists of one row of rectangular cells covered with a thin cuticle. While in surface view, the cells appear polygonal with straight anticlinal walls covered with a smooth cuticle showing diacytic stomata as demonstrated in the transverse section (Figure 17). The cortex is formed of several layers of rounded to slightly oval parenchymatous cells. The vascular tissue is formed of several collateral vascular bundles with xylem vessels towards the upper epidermis and phloem towards the lower one. The lower epidermis is similar to the upper one.

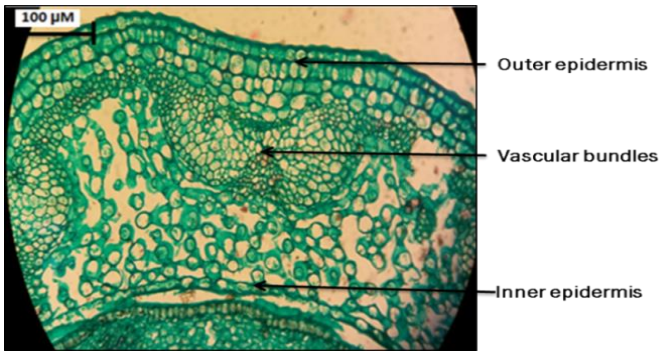

Figure 17: Detailed T. S. in sepal **(x100)**.

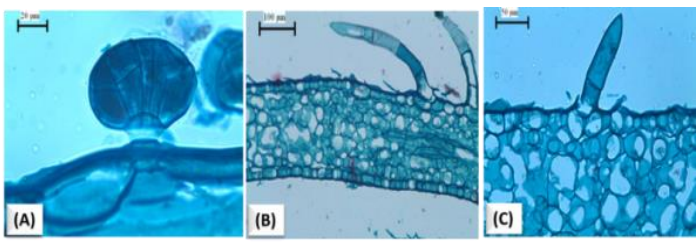

Figure 18: Epidermis of calyx showing **(A)** Labiaceous hair (side view). Non glandular hairs **(B)** Multicellular and **(C)** bicellular. **A (x400), B (x100) and C (x200)**.

3.2.3.1.2. The calyx powder

Its elements are listed as follows (Figure 19):
A) The upper epidermis polygonal cell with straight anticlinal wall showing diacytic stomata.
B) The lower epidermis polygonal cell with wavy anticlinal wall showing diacytic cell.
C) Non-glandular hairs.
D) Spiral X.V.

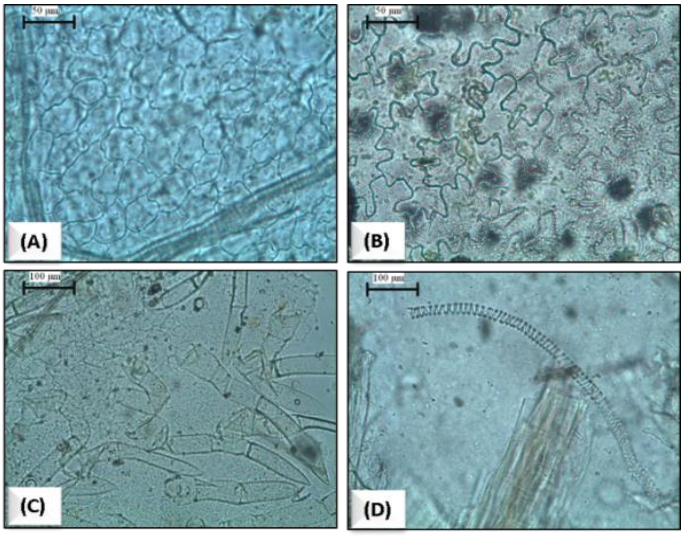

Figure 19: The powder element of the calyx **(A)** Upper epidermis, **(B)** Lower epidermis), **(C)** Non-glandular hairs and **(D)** Spiral xylem vessel. **A and B (x200), C and D (x100)**.

3.2.3.2. The corolla

3.2.3.2.1. Corolla micromorphology

A transverse section in the petals illustrates upper and lower epidermises enclosing a wide parenchymatous cortex, followed by small scattered vascular bundles. The upper and lower epidermises are formed of rectangular cells covered with a thin cuticle with both glandular and non-glandular trichomes. The glandular trichomes are labiaceous hair or unicellular head and unicellular or multicellular uniseriate stalk. The non-glandular trichomes are numerous, uni or multicellular uniseriate, covered with a smooth or slightly warty cuticle as shown in the powder of the corolla. While in surface view, the inner epidermis has polygonal, elongated cells with straight anticlinal walls covered with a smooth cuticle and the upper epidermis has polygonal cells (Figures 20 and 21).

3.2.3.2.2. The corolla powder

Its elements are listed as follows (Figure 22):
I) Non-glandular hairs: (**A**) Multicellular, (**B**) Bicellular and (**C**) Unicellular.
II) Glandular hairs: with unicellular head and unicellular stalk (**D**) Capitate hair.
III) Fragment of X.V. (**E**) Spiral and (**F**) Annular.

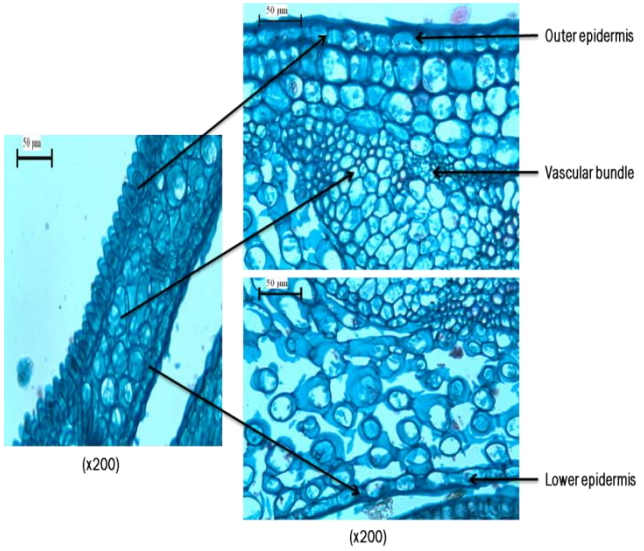

Figure 20: T. S. in the petal.

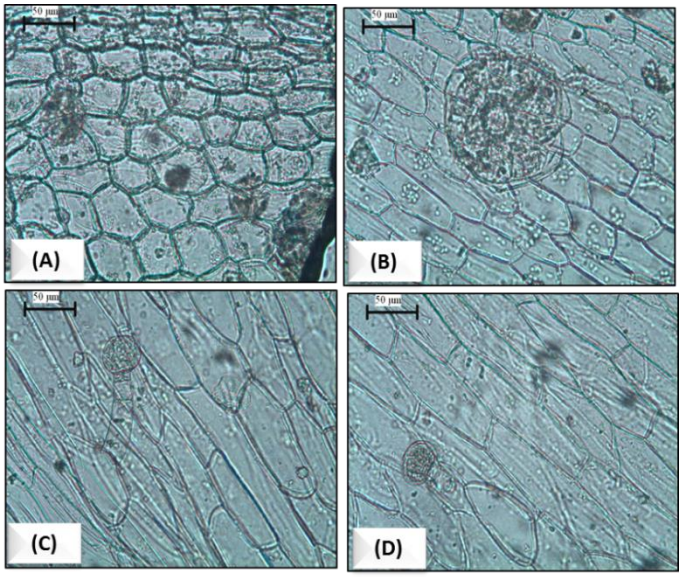

Figure 21: Surface preparation of the corolla: **(A)** Upper epidermis **(B)** Labiaceous hair (top view), **(C)** Lower epidermis, **(D)** Showing glandular hair with unicellular head and multicellular stalk **(C)**, Capitate hair **(D)**. **All (x200)**.

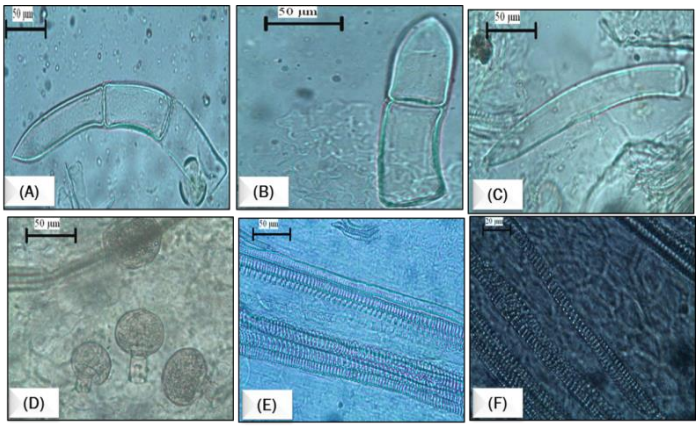

Figure 22: The powder elements of the corolla**: (A)** Non glandular multicellular hair, **(B)** Non glandular bicellular hair, **(C)** Non glandular unicellular hair, **(D)** Capitate hair, **(E)** Spiral X.V., **(F)** Annular X.V. **All (x200) except F (x400)**.

3.2.3.3. The androecium

3.2.3.3.1. The filament

The epidermal cells of the filament at the top appear in surface view as elongated cells with straight anticlinal walls and covered with smooth cuticles (Figure 23), while the epidermis of the middle part of the filament, is similar to that at the top but larger in size and at the base epidermal cells are polygonal with straight anticlinal walls. Stomata and trichomes are absent in the epidermis of the filament.

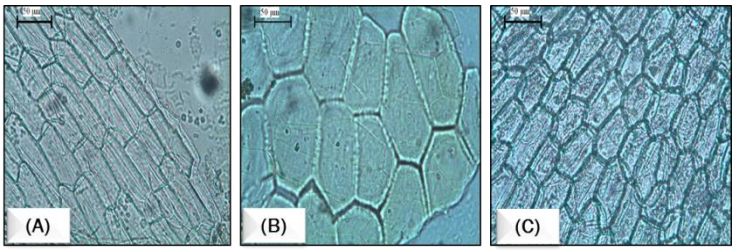

Figure 23: Surface preparation of the filament showing: **(A)** Epidermis of the filament in the upper region, **(B)** In the middle region and **(C)** In the lower region. All **(x200)**.

3.2.3.3.2. The anther

A transverse section in the anther illustrates two anther lobes attached together through connective tissue. The two anther lobes contain abundant spherical, finely pitted wall pollen grains. The anther wall is formed of an epidermis followed by a fibrous layer and the remaining tapetum (Figures 24 and 25).

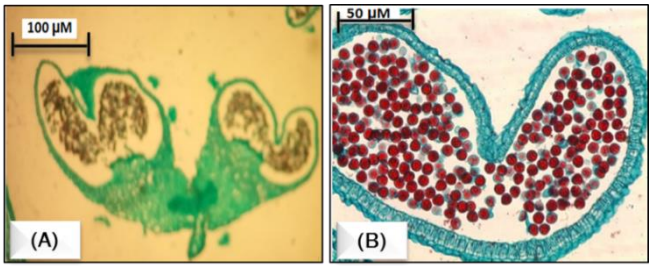

Figure 24: (A) Anther lobes, **(B)** Two pollen sacs with pollen grains. **A (x100) and B (x200)**.

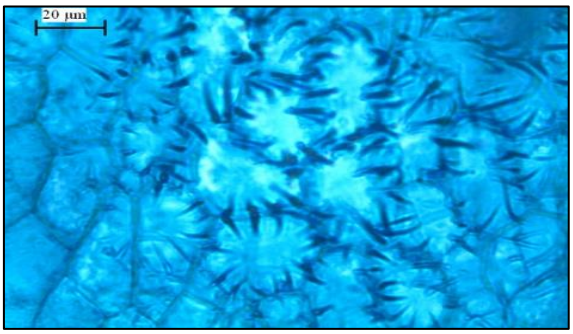

Figure 25: Fibrous layer of anther **(x400)**.

3.2.3.3.2.1. The fibrous layer of anther

It is formed of polygonal axially elongated cells with thick lignified walls showing bar-like thickening (Figure 25).

3.2.3.3.2.2. The pollen grains

They are spherical in shape, yellow in color showing smooth walls (Figure 26).

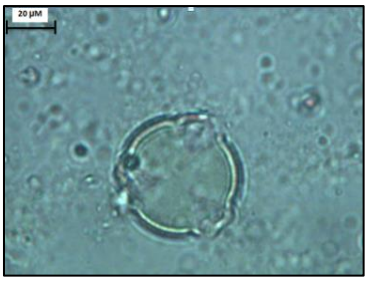

Figure 26: Pollen grain **(x400)**.

3.2.3.4. The gynoecium

3.2.3.4.1. The ovary

It has a circular outline in the transverse section. It shows an epidermis enclosing two united carpels with 4 locules; each contains one basal ovule. The ovary illustrates upper and lower epidermises enclosing a parenchymatous mesophyll in between (Figure 27).

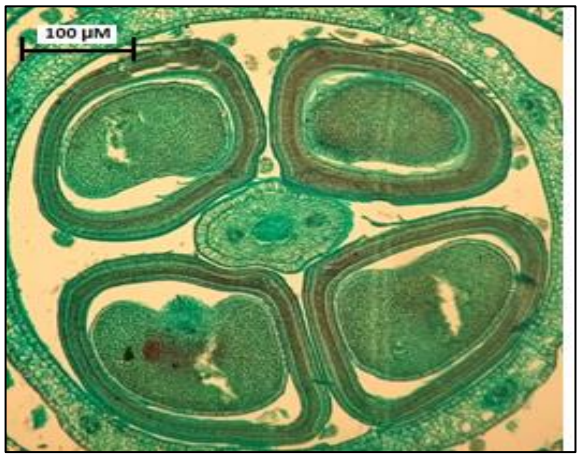

Figure 27: T.S. of the ovary **(x100)**.

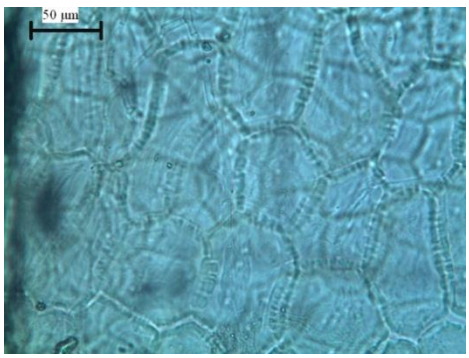

Figure 28: Surface view of the style **(x200)**.

3.2.3.4.2. The style

Its epidermal cells appear as polygonal, axially elongated cells with straight beaded anticlinal walls covered with a thin cuticle in surface view (Figure 28).

3.2.3.4.3. The stigma

Its epidermal cells consist of polygonal papillosed cells with straight anticlinal walls (Figure 29).

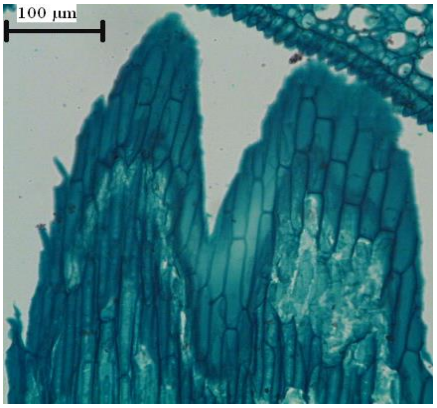

Figure 29: Bifid stigma **(x100)**.

Conclusion

Examination of the micromorphological features of *M. laevis* L. stem, root and inflorescence afford a good method for the identification of this plant. Furthermore, the botanical features could be supportive in future phytopharmacognostical investigations of this plant following appropriate authentication.

Conflict of interests

Authors declare no actual or potential conflict of interests.

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Table 1: Microscopical measurements of different organs of *M. laevis* in µm.

Item	Length	Width	Height	Diameter
		1) Stem		
Epidermal cells	12-20-30	10-15-20	12-16-21	
Glandular hair				
Head				9-13-16
Stalk	30-37-50	10-13-15		
Collenchyma cells				35-40-50
Parenchyma cells of the cortex				50-60-75
Wood parenchyma cells	32-41-48	35-52-60		
Wood fiber	300-400-500	9-12-15		
Xylem vessels				20-60-71
Tracheids	100-130-165	15-19-29		
Medullary rays	41-57-68	13-18-23		
Parenchyma cell of the pith				85-105-165
		2) Root		
Cork cell	9-18-38	10-15-23	2-5-7	
Parenchyma cells of the 2^{ry} cortex				20-33-50
Xylem vessels				18-35-55
Medullary rays	51-70-80			
Tracheids	85-105-160	9-12-15		
		3) Inflorescence (Floret)		
		3A) Calyx		
Upper epidermal cells	55-70-125	20-35-48	13-14-18	
Lower epidermal cells	18-39-61	11-19-28	10-13-17	
Glandular hair (Labiaceous hair)				50-60-70
Non-glandular hair	100-220-280	10-13-17		
Xylem vessel				13-15-23
		3B) Corolla		
Upper epidermal cells	45-70-95	35-43-50	10-15-18	
Lower epidermal cells	45-62-72	22-32-42	10-15-25	
Parenchyma cells				15-25-35
Non-glandular hair	45-55-70	13-19-26		
Glandular hair (Capitate hair)				
Head				10-13-16
Stalk	13-19-22	8-9-13		
		3C) Androecium		
Epidermis cells of the upper and lower part of the filament	14-18-22	36-50-64		
Fibrous layer of anther cells	11-15-18	18-36-54		
Pollen grains				15-18-20
		3D) Gynaecium		
Epidermis cells of style	76-95-115	13-16-22		
Epidermis cells of stigma	13-25-30	65-85-105		
Ovule cells	140-157-165	70-80-85		

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