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## Morphoanatomy studies of the leaves of *Moluccella laevis* L. Family: Lamiaceae (Labiatae), cultivated in Egypt

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### Abstract

*Moluccella laevis* L. belonging to family Lamiaceae. It is an annual herb, flowering from March to April, bearing dense spikes of small 2-lipped white fragrant flowers with large, bell-shaped calyxes, known as "Bells of Ireland" or "Shell flower". It is cultivated as an ornamental plant. By reviewing the literature few studies had been done on this plant, which encouraged us to investigate both macro and micro morphological examinations of *M. laevis* L. leaves, which could help the researchers in the identification and authentication of this plant leaves in both entire and powdered forms.

**Keywords:** *Moluccella laevis* L., Lamiaceae labiatae, leaf, petiole, morphology, histology.

### 1. Introduction

Lamiaceae is one of the most important families containing essential oils and was previously called Labiatae, or the mint family. It is one of the largest plant families comprising of 236 genera and more than 7,000 species. Owing to ease of cultivation, several members of Lamiaceae are cultivated for their aromatic properties, are used in medicine, food, and perfume industry [1]. Different secondary active constituents with important economic and biological values were found in Lamiaceae e.g. essential oils with (monoterpenes and sesquiterpenes), diterpenes triterpenes, tannins, phenolic acids and flavonoids [2]. *Moluccella* is a genus of eight species of annual and short-lived perennial plants intrinsic to Asia and the Mediterranean region [3]. *M. laevis* L. is an annual herb (Lamiaceae) flowering from March to April and bearing white fragrant flowers, widely spread in West Asia. It is commonly known as "Bells of Ireland" or "Shell flower". It is cultivated as ornamental plant [4]. Due to its valuable phytochemical and biological activities, *M. laevis* L. has a common medicinal and folkloric uses. The botanical study of *M. laevis* L. had been previously investigated using primitive method [4]. However, the current botanical investigation demonstrated the detailed macroscopical and microscopical features of the leaves of *M. laevis* L., using more advanced devices that would be considered as a helpful tool for identification and authentication of this plant.

### 2. Taxonomy

*M. laevis* L. belongs Kingdom: Plantae; Subkingdom: Tracheobionta; Superdivision: Spermatophyta; Division: Magnoliophyta; Class: Magnoliopsida; Subclass: Asteranae; Order: Lamiales; Family: Lamiaceae; Genus: *Moluccella*; Species: *Moluccella laevis* L. [5].

### 3. Materials and Methods

#### 3.1 Plant material

The plant material comprised of *M. laevis* L. leaves that were taken from the Nursery of the Faculty of Agriculture, Minia University in March 2016. The plant was recognized by Prof. Nasser Barkat, Department of botany, Faculty of Science, Minia University. A voucher specimen (Mn-ph-Cog-35) was located in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt. The plant material that used for the botanical study was taken from the fresh samples, as well as the samples preserved in alcohol (70%)-glycerin-water (1:1:1). Leaves were also left to air dry in the shade, then ground to a fine powder for microscopical investigation and deposited in well-closed containers.

#### 3.2 Preparation of samples for microscopical examination

Phloroglucinol, Safranin, light green, alcoholic potassium hydroxide and iodine were used for examination of the plant sections, as well as its powder.

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### 3.3 Microscopical studies

Surface preparations, transverse sections (T.S.), in addition to the powder of the leaf were used for observation of various microscopic characters. All microscopic examination were

performed by using a microscope with Leica camera (Germany) and 10 megapixels digital camera, Samsung (Korea).

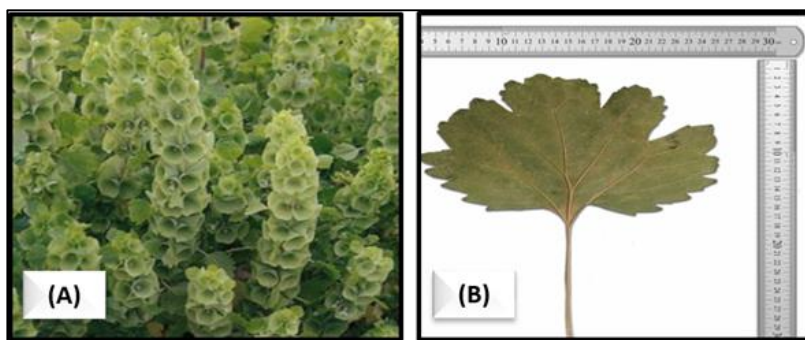


Fig. 1: Photos of *M. laevis* L.; (A) The plant (x0.1) and (B) The leaf (x1).

## 4. Results and discussion

### 4.1 Microscopical characters of the leaf

The leaf (Figure 1) is simple, palmate, with an acute to rounded apex<sup>[4]</sup>, opposite decussate, and petiolated. The petiole is rounded on the lower side, grooved on the upper one, hairy, pale green in color measuring 1.5-12 cm in length and 0.1-0.5 cm in width. A coarse teethed margin with a symmetric base and pinnate venation was demonstrated. The midrib is prominent on the lower surface more than the upper one. The leaf has a fine hairy surface with a green upper surface and a paler lower one. It measures 5-13 cm in length and 2.5-24 cm in width with a slight bitter taste and an aromatic odour.

### 4.2. Micro scopical characters of the leaf

#### 4.2.1 The leaf blade

The outline of the lamina in a transverse section is plano convex (Figure 2), with the midrib more prominent on the lower surface. It shows a dorsiventral mesophyll dis continued by the cortical tissue in the midrib region (Figure 3). Additionally, a group of sub-epidermal collenchyma is present under the upper and lower epidermises at the midrib region. The vascular system of the midrib consists of a large inverted collateral vascular bundle enclosed by non-lignified pericyclic fibers.



Fig. 2: A.T.S. in the leaf. (x40)

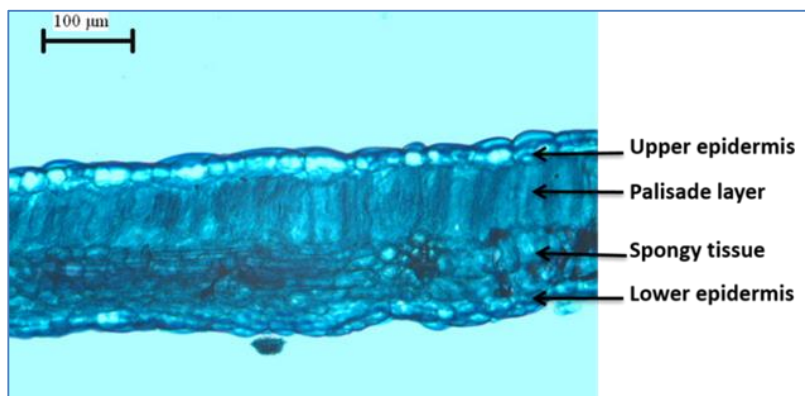


Fig. 3: A detailed T.S. in the lamina of the leaf (x100)

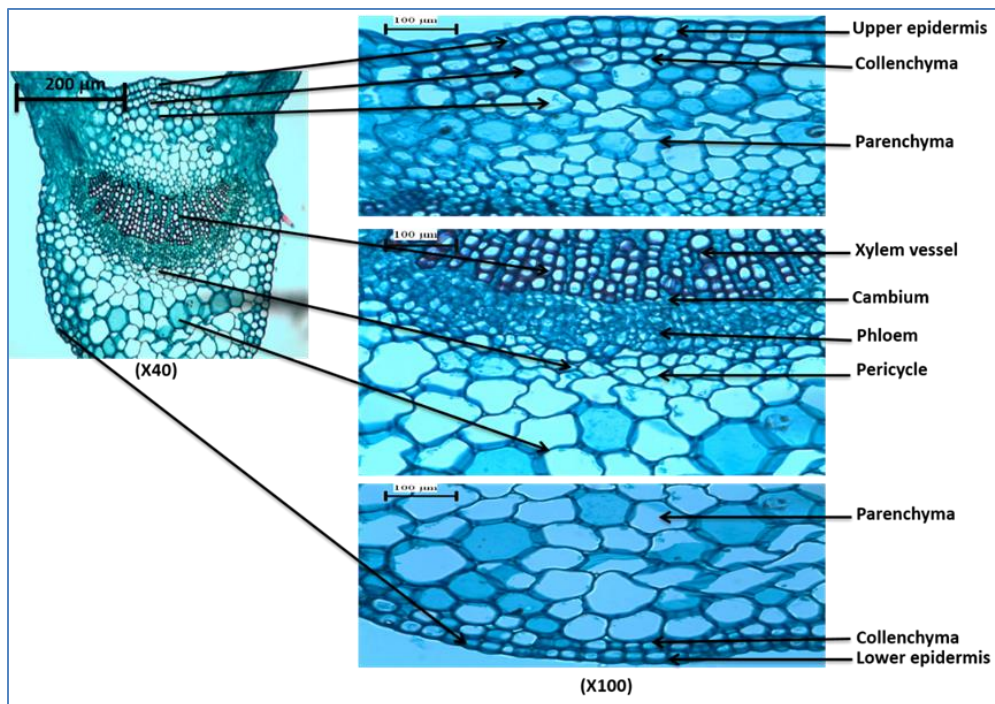


Fig. 4: A detailed T.S. in the midrib region of the leaf.

#### 4.2.2. The upper epidermis

It consists of one row of rectangular cells covered with a thin cuticle as shown in the transverse section (Figure 4). In top view, the cells are polygonal, isodiametric to slightly elongated with wavy anticlinal walls, showing diacytic stomata. Neural cells<sup>[4]</sup> are axially elongated with straight anticlinal walls (Figure 5). Diacytic stomata are observed on

both surfaces being more frequent on the lower one (Figure 5). Glandular and non-glandular trichomes are numerous on both surfaces. Labiate glandular trichomes are characterized by a small unicellular-stalk and an eight celled-head are present. Non glandular trichomes are unicellular, Bi cellular or multicellular, straight or curved and covered with a smooth or warty cuticle.

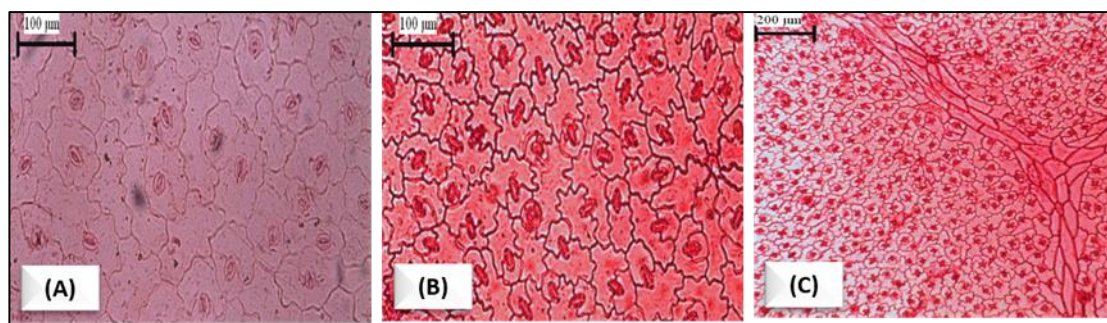


Fig. 5: Surface preparation of the leaf: (A) Upper epidermis, (B) Lower epidermis and (C) Lower epidermis showing neural cells. A & B (x100) and C (x40)

#### 4.2.3. The lower epidermis

It is consisted of one row of rectangular cells covered with a thin cuticle as shown in the transverse section (Figure 5). In top view, the cells appear polygonal, isodiametric with more wavy anticlinal walls covered with a smooth cuticle showing diacytic stomata, as in upper epidermis but more wavy. (Figure 5).

#### 4.2.4. The mesophyll

It is a dorsiventral, differentiated into a disconnected upper palisade layer and spongy tissue. The palisade layer consists of one row of cylindrical columnar cells, which contain chloroplasts and is interrupted by the cortical tissue in the midrib region. The spongy tissue consists of 3-5 rows of thin-walled, rounded or slightly irregular collenchyma with narrow intercellular spaces (Figure 3 and 4).

#### 4.2.5. The cortical tissue

The cortical tissue of the midrib region (Figure 4) reveals upper and lower sub-epidermal collenchymatous layers. The upper layer comprises of 3-6 rows whereas the lower one is consisted of 3-4 rows of small rounded cells with thick cellulosic walls without intercellular spaces followed by several rows of polygonal, large parenchymatous cells with thin cellulosic walls and large intercellular spaces. The endodermis is parenchymatous and is not distinct.

#### 4.2.6. The vascular tissue

##### 4.2.6.1. The pericycle

The pericycle is parenchymatous with small patches of non-lignified fibers (Figure 4). The fibers are septate with thick non-lignified walls, wide lumen and blunt apices as seen in the powder element (Figure 10 H and I).

#### 4.2.6.2. The xylem

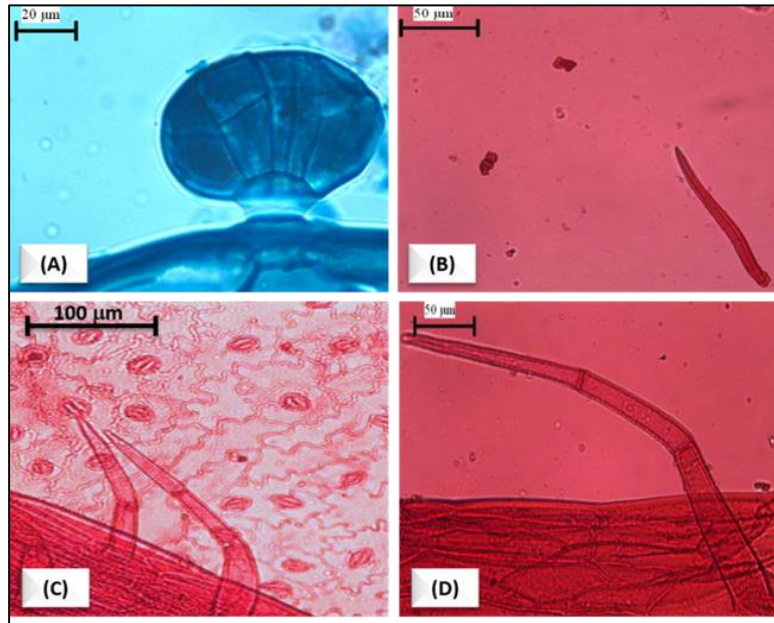
It consists of lignified vessels, fibers, and wood parenchyma (Figure 4). The vessels are mainly with spiral thickenings as seen in the powder (Figure 10J). The fibers have rounded apices, wide lumen and thick lignified walls as seen in the powder (Figure 10K). The wood parenchyma is formed of radially elongated cells with pitted walls. The medullary rays are uni, bi- or multiseriate forming radiating lines of elongated thin-walled cellulosic cells crossing the xylem (Figure 4).

#### 4.2.6.3. The cambium

It is consisted of 2-3 rows of tangentially elongated thin-walled meristematic cells.

#### 4.2.6.4. The phloem

It consists of thin-walled soft elements formed of sieve tubes, companion cells and phloem Parenchyma.



**Fig. 6:** Glandular and non-glandular hairs of the leaf: (A) Glandular hair with unicellular stalk and multicellular head (labiate hair in side view), (B) Non glandular unicellular hair, (C) Non glandular bicellular hair and multicellular hair and (D) Non glandular multicellular hair. A (x400), B & D (x200) and C (x100)

### 4.3. Micromorphology of the petiole

#### 4.3. The leaf petiole

A transverse section in the petiole is planoconvex in outline (Figure 7), rounded on the lower side and flat on the upper one with two prominent wings at each side. The petiole is nearly similar to the structure of leaf. However, the cortical tissue is transversely by two large collateral vascular bundles and two additional small vascular bundles one at each side.



**Fig. 7:** A diagrammatic T.S. in the petiole (x 40)

#### 4.3.1. The epidermis

The epidermis is the same as in the structure to the upper epidermis of the leaf, which is consisted of one row of

rectangular cells covered with a thin cuticle. In top view, the cells are polygonal, isodiametric to slightly elongated with straight anticlinal walls showing diacytic stomata.

#### 4.3.2. The cortical tissue

The cortex consists of 3-4 rows of small thick-walled collenchyma cells with narrow intercellular spaces followed by 3-7 rows of large thin-walled parenchyma cells with small intercellular spaces (Figure 8). In the wing region, the cortex consists of 6-9 rows of small thick-walled collenchyma cells without intercellular spaces (Figure 9).

#### 4.2.2.3 The vascular system

The pericycle is formed of parenchyma cells disconnected by non-lignified septate fibers surrounding the vascular bundle (Figure 8). The fibers are elongated, non-lignified septate walls, with wide lumens and acute apices (Figure 10H). The phloem consists of thin walled soft cellulosic elements around the xylem. The cambium is formed of 1-2 rows of tangentially elongated thin-walled meristematic cells. The xylem is formed of lignified vessels, wood fibers and wood parenchyma. The vessels are mainly with spiral thickening. The wood parenchyma is polygonal with thick lignified walls. The wood fibers are fusiform having thick lignified walls, wide lumens and tapering ends (Figure 10K). The medullary rays are uni to triseriate, which are formed of elongated thick-walled and lignified cells (Figure 8).

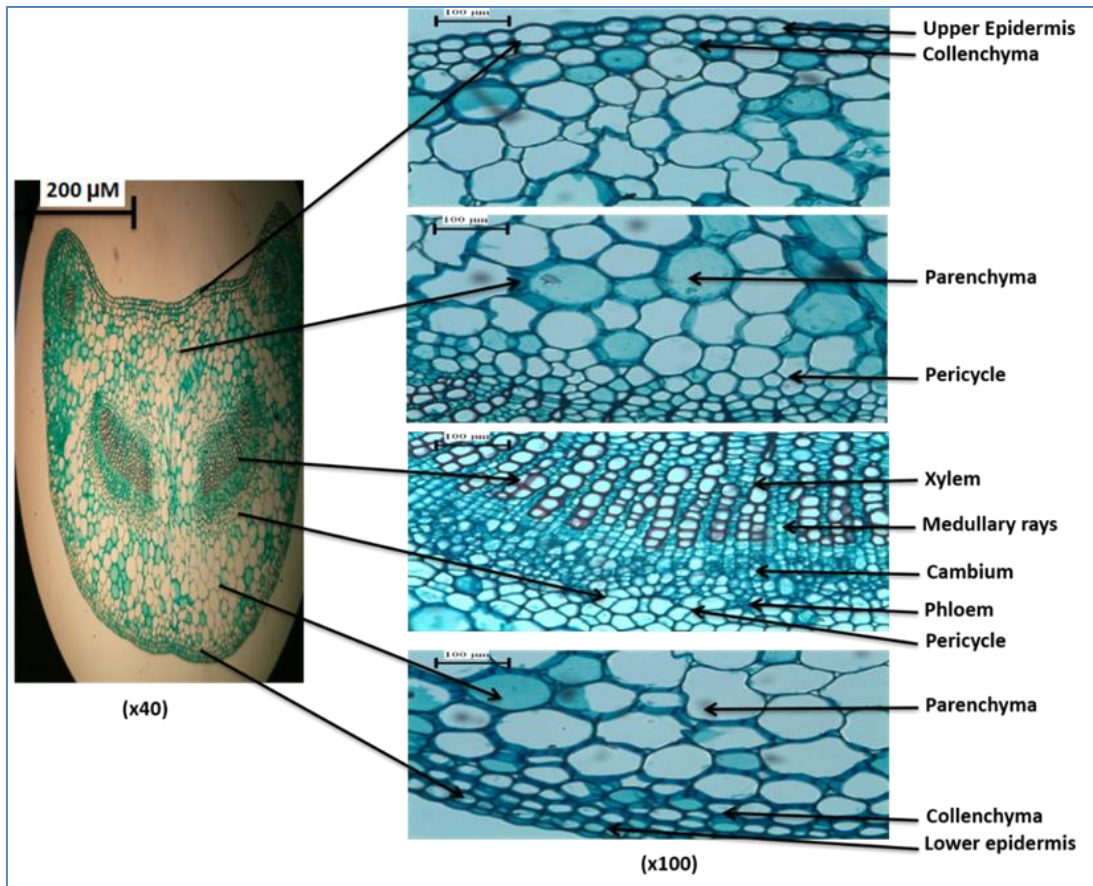


Fig. 8: A detailed T.S. in the middle part of the petiole.

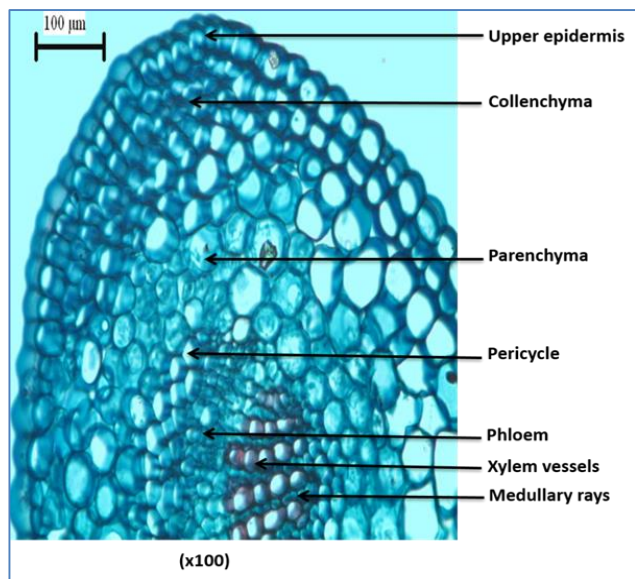


Fig. 9: A detailed T.S. in the petiole wing.

#### 4.3.3. The powdered leaf:

The leaf powder (Figure10) is dark green in color with a specific lemon-like odour and a bitter taste. Elements of the powdered leaf include:

##### 1-Fragments of the upper and lower epidermises:

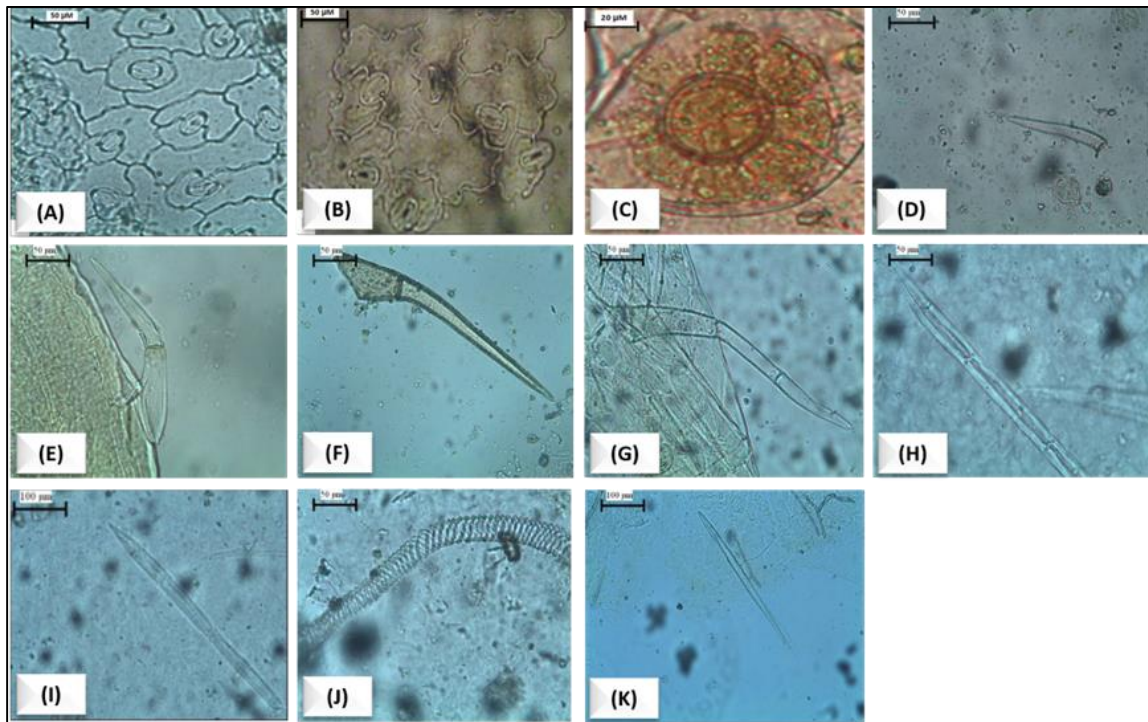
- A) The upper epidermis: polygonal, isodiametric to slightly elongated cells with slightly wavy anticlinal walls covered with a smooth cuticle, showing diacytic stomata.
- B) The lower epidermis: is similar to the upper one but with more wavy.

##### 2- Glandular hairs:

With unicellular stalk and multicellular head (8 radiating cells) in top view (labiate hair)

##### 3- Non glandular hairs

- D) Unicellular hair.
- E) Bicellular uniseriate covered with a smooth cuticle.
- F) Bicellular uniseriate covered with a warty cuticle.
- G) Multicellular uniseriate covered with a smooth cuticle.
- H & I) Fragments of non-lignified septate pericyclic fibers.
- J) Fragment of xylem vessels showing spiral thickening.
- K) Wood fibers with thick lignified walls, narrow lumens and tapering ends.



**Fig. 10:** (A) Upper epidermis, (B) Lower epidermis, (C) Labiate hair (top view), Non glandular hairs: (D) Unicellular covered with warty cuticle, (E) Bicellular covered with smooth cuticle, (F) Bicellular covered with warty cuticle, (G) Multicellular uniseriate covered with smooth cuticle, (H & I) Non-lignified septate pericyclic fiber, (J) Spiral xylem vessel and (K) Wood fiber. All (x200) except C (x400) and I & K (x100)

**Table 1:** Microscopical dimension of the leaf elements of *M. laevis* L. in (µm).

Item	Length	Width	Height	Diameter
Upper epidermis	20-33-50	30-33-43	33-43-50	
Lower epidermis	16-23-33	13-23-33	60-93-100	
Non glandular hairs	80-100-120	3-5-10		
A-Unicellular				
B-Bicellular with smooth cuticle	200-220-240	13-15-22		
C-Bicellular with warty cuticle	260-280-290	13-15-22		
D-Multicellular	300-320-330			
Labiate hair				50-60-70
Stomata	35-40-60	55-66-75		
Collenchyma				20-24-30
Parenchyma of the cortex				40-50-60
Pericyclic fibers	350-420-480	15-17-20		
Wood fiber	100-120-130	20-25-30		
Medullary rays				30-35-40
Palisade cells	80-100-120	5-10-20		
Sponge cells				4-10-15
Xylem vessels				33-45-56

## 5. Conclusion

Examination of both the macroscopical and microscopical features of *M. laevis* L. leaves provide a good method for the identification of this plant. In addition, the above mentioned botanical characters could be helpful in future phytopharmacognostical investigations of this species following appropriate authentication.

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