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Botanical studies on the stem and root of *Melissa officinalis* L. (lemon balm)

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Abstract

Melissa officinalis L. (family Lamiaceae) is a perennial herbaceous plant naturally found in southern Europe and central Asia, with worldwide culinary and medicinal applications. It shows an erect, quadrangular stem that grows up to 1.5 m in height, carrying simple ovate to cordate leaves in an opposite decussate pattern. Microscopically, *M. officinalis* is generally characterized by anomocytic stomata as well as a variety of non-glandular and glandular trichomes, exemplified by labiaceous and capitate hairs; giving the plant a hairy texture and distinctive lemon-like scent thanks to the considerable secretion of volatiles from the latter hair types. Additionally, different tissues of this herb are generally devoid of calcium oxalate crystals. As part of our ongoing phytochemical and botanical research on *M. officinalis*, which previously included the detailed macroscopical and microscopical analysis of its leaves, the present study describes the botanical features of stems and roots of *M. officinalis*. Such botanical data could be of value as standardized parameters for authentication purposes of this multipurpose plant species.

Key words
Botanical studies, Lemon balm, Melissa officinalis, Root, Stem

1. Introduction

Melissa is a small genus of aromatic perennial herbs in the family Lamiaceae (Labiatae) with a natural distribution in central and western Asia and Europe, especially the Mediterranean basin. The name of this genus originates from the Greek word *"Mélissa"*, which means "honeybee", owing to the copious floral nectary secretions [1, 2]. Out of four identified and accepted *Melissa* species, *Melissa officinalis* L. is the most abundant and commonly grown species worldwide, particularly after being naturalized in several other parts of the globe [3]. Like most other Lamiaceous plants, *M. officinalis* has a square or quadrangular stem growing to a height of 0.5–1.5 m, on which opposite decussate pairs of ovate to cordate green leaves are borne [4]. Axillary spikes of small white or pale pink flowers usually decorate the plant during the summer time [3]. Lemon balm shows a hairy root system with numerous lateral roots, making the plant more adaptable to varied environmental conditions. The upper part of the plant die off at the beginning of winter, but new shoots re-emerge from roots at spring [3]. *M. officinalis* is commonly referred to as honey balm, bee balm, and lemon balm; the latter name comes from the attractive lemony scent and flavor of its leaves [5]. Such characteristic odour is underlain by structurally diverse volatile mixtures of monoterpenes and sesquiterpenes, mostly predominated by citronellal, neral, geranial, geraniol, geranyl acetate, linalyl acetate, β-caryophyllene, and β-caryophyllene oxide [3]. Additionally, lemon balm has recently yielded a number of triterpenoids, saponins, phenyl propanoids, phenolic acids, and flavonoids [3, 6]. Biologically, *Melissa* plants exhibited wide range of important bioactivities, including antioxidant, anti-inflammatory, hepatoprotective, digestive, antibacterial, antifungal, antiviral, and antihistaminic effects [7]. Hence, in continuation of our interest in *M. officinalis* plant [4, 6, 8], the current study reports the macro- and microscopic characteristics of its stem and root, which could be applied as a valuable tool for authentication of this important medicinal herb.

2. Experimental

2.1. Plant material

Melissa officinalis was obtained from Sekeum botanical garden, Cairo, Egypt and identified by Dr. Saber Hendawy, the supervisor of Sekeum garden. A specimen (Mn-Ph-Cog-014) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Minia University. The plant material included in the current study was prepared from the fresh stems and roots, along with those kept in a mixture of equal proportions of ethanol (70%), glycerin, and water. For microscopical examination purposes, some of the stems and roots were air-dried and then finely powdered.

2.2. Chemicals and reagents

Various reagents, including phloroglucinol, conc. HCl, iodine, safranin, and light green were used for preparation of different plant sections and powders.

2.3. Microscopical studies

Transverse sections (T.S.) and powders of the stems and roots of *M. officinalis* were used for examination of various

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microscopical features employing a microscope provided with a Leica® camera (Germany) as well as another 10 megapixels digital camera, (Samsung, South Korea).

3. Result and discussion

3.1. Macroscopical characters of the stem

The stem of *M. officinalis* **(Figure 1)** is quadrangular, erect, and green in colour with monopodial branching. The upper part of the stem is herbaceous and sparsely hairy, whereas the lower one is harder and tough (*i.e.* suffruticose). It usually grows to a height of 0.5–1.5 m, carrying opposite decussate pairs of simple, ovate to cordate leaves. The stem possesses a characteristic lemon-like odour and taste.

Figure 1: The stem and root of *Melissa oficinalis* L.

3.2. Microscopical characters of the stem

3.2.1. The upper part of the stem

The transverse section of the upper part of the stem is quadrangular in outline with four corners. It consists of an outer epidermis, followed by a narrow cortex of sub-epidermal masses of collenchyma at the corners and parenchymatous cells towards the vascular tissue. The transverse section also reveals a parenchymatous pericycle surrounding a discontinuous ring of the vascular tissue, which is only present under each corner. Wide parenchymatous pith is also found in the center **(Figure 2)**.

Figure 2: A T.S. in the upper part of the stem (x 40).

3.2.1.1. Epidermis

It is represented by one row of tabular cells covered with thin smooth cuticle **(Figure 3)**, while in surface view; they appear as isodiametric polygonal cells with straight anticlinal walls, showing anomocytic stomata, as seen in the powder of stem.

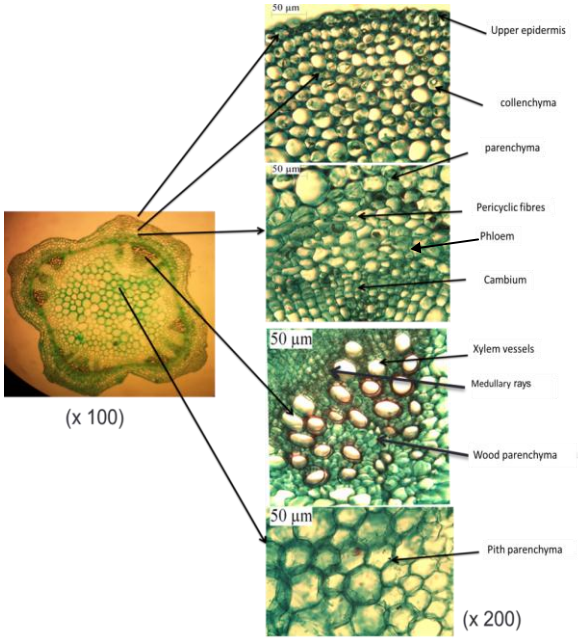

Figure 3: A detailed T.S. in the upper part of the stem (x 200).

3.2.1.2. Cortical tissue

It consists of a narrow zone of collenchyma at the corners, followed by a few rows of parenchyma cells. The collenchymatous layer is comprised of 7–10 rows of rounded thick-walled cells without intercellular spaces, while the parenchyma region shows 2–3 rows of polygonal or oval, thin-walled cells with marked intercellular spaces. The endodermis is parenchymatous and indistinguishable **(Figure 3)**.

3.2.1.3. Vascular system

Pericycle

It is represented by parenchyma cells interrupted by a few groups of non-lignified pericyclic fibers with narrow lumen **(Figure 3)**.

Phloem

It consists of a small ring of thin-walled cellulosic sieve tubes and companion cells associated with phloem parenchyma. No lignified elements are observed in the phloem tissues **(Figure 3)**.

Cambium

It is formed of 4–6 rows of thin tangentially elongated and radially arranged meristematic cells **(Figure 3)**.

Xylem

It includes lignified xylem vessels with spiral thickening, wood fibers, and wood parenchyma. Wood fibers show thick non-lignified walls, narrow lumina, and acute apices, whereas wood parenchyma is polygonal with slightly lignified walls. The xylem region is traversed by uni- or biseriate medullary rays consisting of thin-walled elongated cells **(Figure 3)**.

3.2.1.4. Pith

It approximately occupies three fourths of the transverse section of the stem showing a wide zone of rounded thin-walled parenchyma **(Figure 3)**.

3.2.2. The lower part of the stem

The transverse section of the lower part of the stem is typically quadrangular in outline, showing an outer epidermis carrying non-glandular trichomes in addition to a few numbers of glandular hairs, followed by a narrow cortex, which is differentiated into collenchyma and parenchyma. The vascular tissue is surrounded by a continuous ring of parenchymatous pericycle with isolated groups of lignified fibers. The transverse section also displays wide parenchymatous pith in the center. No cork is observed **(Figure 4)**.

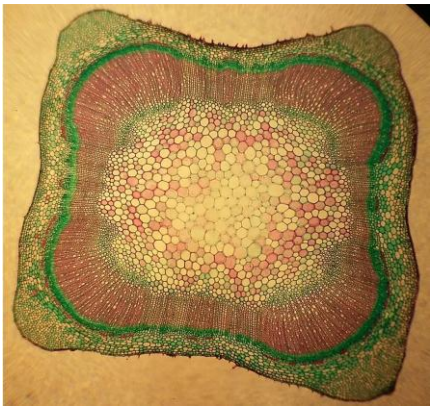

Figure 4: A T.S. in the lower part of the stem (x 40).

3.2.2.1. Epidermis

It consists of one row of tabular cells with thin smooth cuticle **(Figure 5)**, while in surface view they appear polygonal and isodiametric with straight walls. They show anomocytic stomata and carry different types of non-glandular uniseriate trichomes, such as unicellular, bicellular, and multicellular hairs, as also shown in the powder of stem **(Figure 8)**.

3.2.2.2. Cortical tissue

It is formed of an outer collenchymatous part and an inner parenchymatous one. The collenchyma consists of 10–18 rows of rounded and thick-walled cells showing no intercellular spaces, while the parenchymatous layer contains 4–7 rows of large polygonal or oval cells with thin walls and wide intercellular spaces. The endodermis is parenchymatous and indistinguishable **(Figure 5)**.

3.2.2.3. Vascular system

Pericycle

It is represented by parenchyma cells with isolated groups of fibers **(Figure 5)**; each group contains 5–15 fibers that are long with thick heavily lignified walls, wide lumina, and tapering ends, as also seen in the longitudinal cut **(Figure 6)**.

Phloem

It consists of soft cellulosic elements, including sieve tubes and companion cells associated with phloem parenchyma **(Figure 5)**.

Cambium

It is represented by tangentially elongated, thin-walled rectangular cells arranged in 4–6 rows **(Figure 5)**.

Xylem

It includes lignified xylem vessels, tracheids, and tracheidal vessels, in addition to wood fibers and parenchyma. Xylem vessels possess spiral, reticulate, sclariform, pitted, and bordered pitted thickened walls. Tracheids are narrow with lignified and pitted walls. Wood fibers are spindle-shaped and shorter than those of the pericycle, with thick lignified walls, narrow lumina, and tapering ends. Wood parenchyma cells are polygonal and lignified. Medullary rays are uni- to biseriate, showing elongated cells with pitted and lignified walls **(Figures 5 & 7)**.

3.2.2.4. Pith

It consists of large thin-walled, rounded parenchyma cells **(Figure 5 & 7)**.

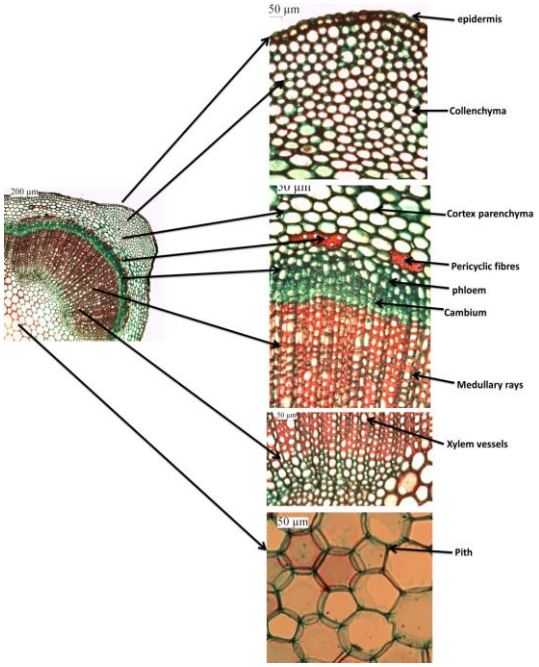

Figure 5: A detailed T.S. in the lower part of the stem (x 200).

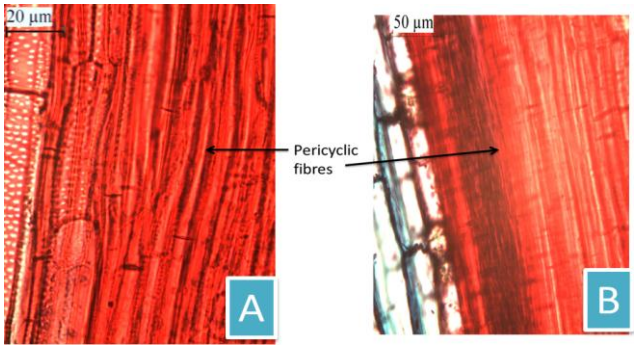

Figure 6: A longitudinal cut in the lower part of the stem showing pericylic fibers (A (x 400), B (x 200)).

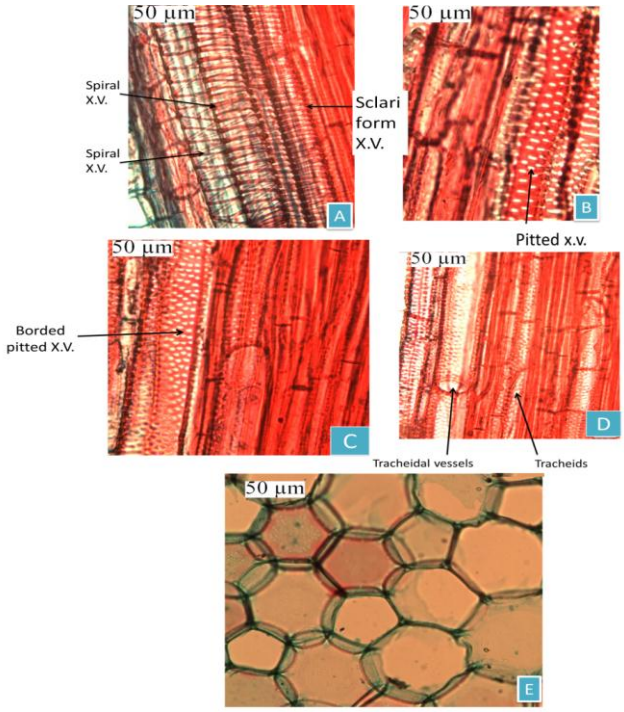

Figure 7: A longitudinal cut in the lower part of the stem showing A) spiral and sclariform xylem vessels, B) pitted xylem vessel, C) bordered pitted xylem vessels, D) tracheids and tracheidal vessels, and E) pith (x 200).

3.2.3. Powder of the stem

It is yellowish green with a characteristic lemon-like odour and flavor. It shows the following elements **(Figure 8)**:

- Fragments of epidermis showing anomocytic stomata with two guard cells surrounded by 3-4 subsidiary cells of equal size.
- Fragments of epidermal cells showing a labiaceous hair consisting of a short unicellular stalk and a multicellular head with eight radiating cells, covered with raised thin smooth cuticle.
- Fragments of epidermal cells showing a capitate hair formed of unicellular stalk and head covered with thin smooth cuticle.
- Non-glandular uniseriate hairs that are unicellular, bicellular, or multicellular, covered with thin smooth cuticle.
- Fragments of parenchyma cells from the cortex.
- Fragments of medullary rays with thin pitted walls.
- Fragments of pericyclic fibers, wood fibers, wood parenchyma, and lignified xylem vessels.

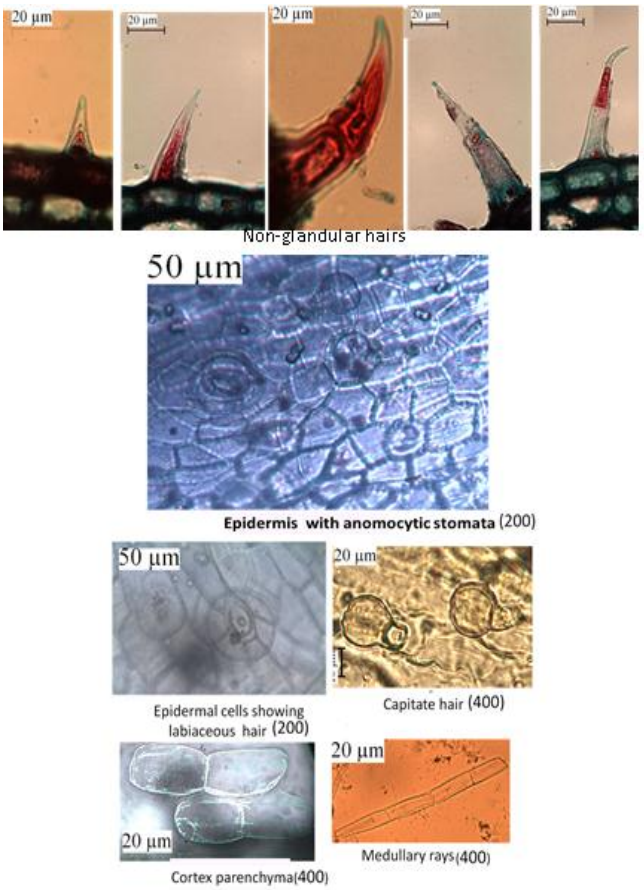

Figure 8: Elements of the powdered stem.

3.3. Macroscopical characters of the root

The root of *M. officinalis* **(Figure 1)** is represented by a cylindrical dark brown tap root carrying several lateral rootlets. The root is odourless and has a bitter taste.

3.4. Microscopical characters of the root

The transverse section of the root is nearly circular in outline, displaying an external layer of brown cork cells surrounding a narrow cortex. The vascular tissue consists of phloem and a wide xylary region extending to the center **(Figure 9)**.

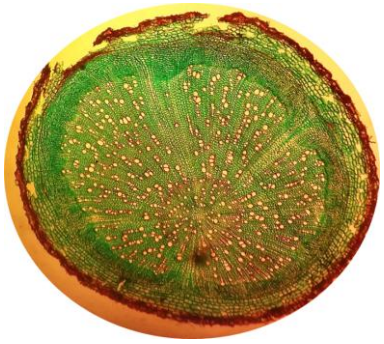

Figure 9: A T.S. in the root (x 40).

3.4.1. Cork

It shows 3–4 rows of brown tangentially elongated rectangular cells with thick walls **(Figures 10 & 11)**, while in surface view, they appear as isodiametric, slightly elongated polygonal cells with thick walls.

3.4.2. Cortex

It shows 4–6 rows of oval or rounded thin parenchyma with narrow intercellular spaces **(Figures 10–12)**.

3.4.3. Vascular system

Phloem is formed of soft cellulosic elements encompassing sieve tubes and companion cells, accompanied by phloem parenchyma and phloem fibers. Cambium is represented by a continuous ring of 4–6 rows of thin-walled, tangentially elongated, and radially arranged cells. The xylary tissue is formed of lignified xylem vessels, tracheids, and tracheidal vessels, wood fibers, and wood parenchyma. Xylem vessels show annular and spiral thickenings. Wood fibers have lignified walls, narrow lumen, and acute ends. Wood parenchyma is polygonal with thick and lignified pitted walls. Medullary rays are uni- or biseriate with radially elongated cells **(Figures 10 & 12)**. Oval, ovate, and rounded medium-sized starch granules are scattered in the medullary rays with faint hilum and invisible striations **(Figure 13)**.

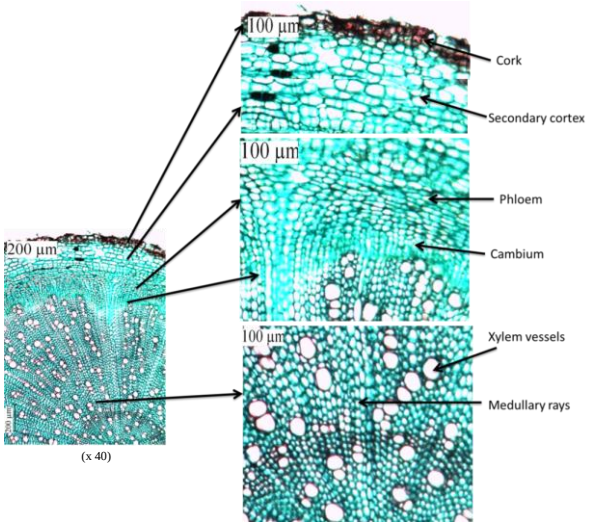

Figure 10: A detailed T.S. in the root (x 200).

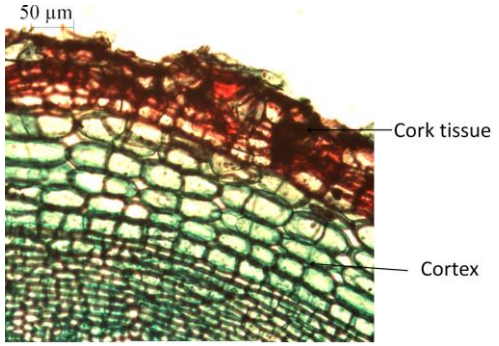

Figure 11: The cork and cortex tissues of the root (x 200).

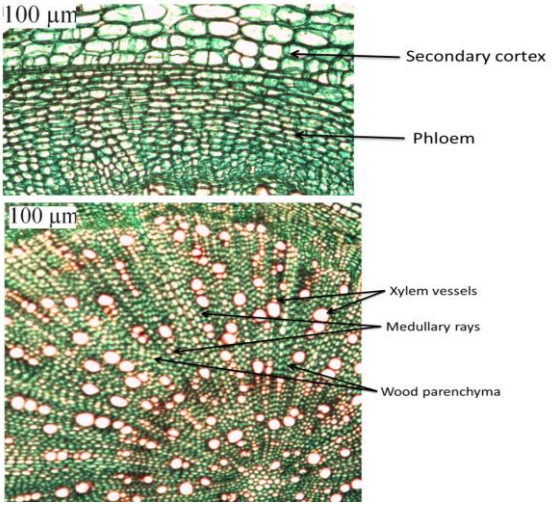

Figure 12: The secondary cortex and vascular tissue of the root (x 100).

3.4.4. Powder of the root

The powdered root is greyish brown to yellowish brown, odourless, and having a bitter taste. It displays the following microscopical elements **(Figure 13)**:

- Fragments of cork cells: polygonal, isodiametric or slightly elongated cells with thick brown walls.
- Fragments of medullary rays: elongated cells with thin non-lignified walls.
- Starch granules: oval, ovate, or rounded in shape, medium-sized, with faint hilum and invisible striations. They stain blue with iodine solution.
- Fragments of parenchyma cells of the cortex.
- Fragments of wood parenchyma.
- Fragments of xylem vessels with annular and spiral thickenings.

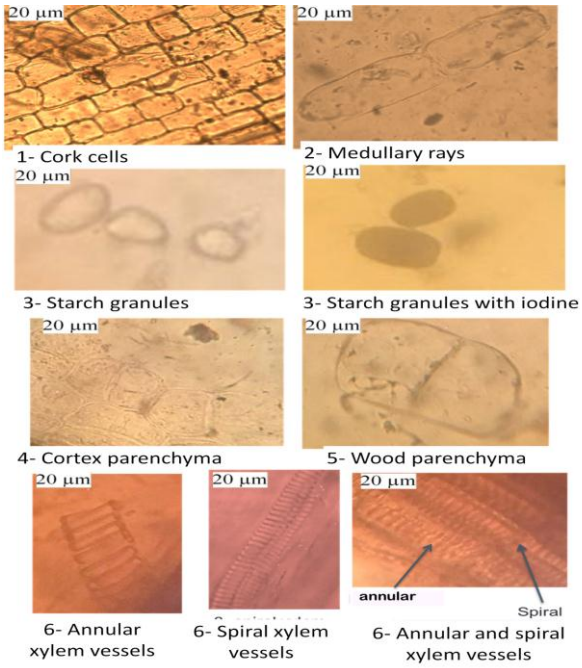

Figure 13: Elements of the powdered root (x 400).

Table 1: Microscopical measurements of different tissues of *M. officinalis* stem and root in micron.

Item	Length	Width	Height	Diameter
Stem				
Epidermal cells	15-24-33	7-10-15	9-11-13	–
Non-glandular hairs	50-144-182	15-20-28	–	–
Glandular hairs:	–	–	–	–
Stalk	14-20-26	8-10-12	–	–
Head	–	–	–	10-15-17
Collenchyma	–	–	–	6-18-28
Cortex parenchyma	–	–	–	26-54-60
Pericyclic fibers	450-1125-1800	10-15-19	–	–
Wood fibers	320-360-450	8-12-15	–	–
Wood parenchyma	30-40-51	31-40-50	–	–
Xylem vessels	–	–	–	26-52-75
Tracheids	94-105-120	10-12-15	–	–
Medullary rays	41-51-62	16-19-24	–	–
Pith parenchyma	–	–	–	26-56-95
Root				
Cork cells	22-27-42	10-15-20	3-5-8	
Cortex parenchyma	–	–	–	19-28-42
Phloem fibers	260-450-760	12-15-18	–	–
Wood fibers	157-184-280	11-13-15	–	–
Wood parenchyma	26-35-50	17-18-22	–	–
Xylem vessels	–	–	–	18-32-48
Tracheids	63-84-94	6-9-12	–	–
Medullary rays	52-60-95	10-13-16	–	–
Starch	–	–	–	12-16-18

3.5. Microscopical measurements

Microscopical measurements of various stem and root tissues of *M. officinalis* are mentioned in **(Table 1)**.

4. Conclusion

Studying the macro- and microscopical characters of the stems and roots of lemon balm provides a valuable method for the authentication of this important Lamiaceae species. Additionally, as an important quality control tool, such botanical data are also supportive to future chemical and pharmacological investigations of lemon balm.

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