

CANSU MÜKERREM DEMIREL – CSILLA TÓTH

COMPARATIVE MICROMORPHOLOGICAL EXAMINATION OF SECRETORY STRUCTURES OF STEM AND LEAF IN LAMIACEAE SPECIES

LAMIACEAE FAJOK MIRIGYSZŐREINEK ÖSSZEHASONLÍTÓ MIKROMORFOLÓGIAI VIZSGÁLATA

ABSTRACT

Microanatomical analyses of the essential oil storage glandular trichomes of six different field-cultivated medicinal plants, *Mentha x piperita* L., *Melissa officinalis* L., *Thymus vulgaris* L., *Salvia officinalis* L., *Rosmarinus officinalis* L. and *Lavandula officinalis* L. were carried out using light microscopy. In the case of all examined plant species, stem and leaf cross-sections were made. Non-glandular and two types of glandular trichomes (peltate and capitate) were described. The glandular hairs appearing on the stem and both the adaxial and abaxial leaf epidermis were typified, and their density was determined. The results showed that the number of glandular trichomes of the adaxial epidermis was higher than abaxial epidermis, except for sage. In the case of lemon balm and lavender, we observed an exceptionally high number of glandular hairs on both the upper and lower epidermis (lemon balm: $318 \pm 46.58 / 148 \pm 21.68$; lavender: $156 \pm 61.07 / 150 \pm 22.36$). In the matter of mint ($50 \pm 23.45 / 32 \pm 19.24$) and thyme ($46 \pm 23.97 / 36 \pm 15.17$) the number of glandular hairs was significantly lower compared to the values found in the other examined species. The volume density of capitate trichomes was higher than the volume density of peltate ones in every examined species. We found that thyme has a significant number of peltates and the largest peltate's diameter, while the smallest peltates were found in rosemary and lavender. In the case of these species, the random appearance of glandular hairs, and their uneven distribution on leaf surfaces could be established. In regards to mint, thyme, and lavender, the essential oil-storing gland hairs could be easily detected by microscope.

Keywords: Lamiaceae's species, cross-section of stem and leaf, adaxial and abaxial epidermis, type of glandular trichomes, number of glandular trichomes

ÖSSZEFOGLALÓ

Munkánk során 6 különböző termesztett gyógynövény, a *Mentha x piperita* L., *Melissa officinalis* L., *Thymus vulgaris* L., *Salvia officinalis* L., *Rosmarinus officinalis* L. és a *Lavandula officinalis* L. illóolajat raktározó mirigyszőreinek fénymikroszkópos vizsgálatát végeztük el. Valamennyi vizsgált növényfaj esetében szár- illetve levélkeresztmetszetet készítettünk, tipizáltuk a szár, valamint az adaxiális és az abaxiális levélepidermiszen megjelenő mirigyszőröket, meghatároztuk azok denzitását. A zsályát kivéve, ahol a mirigyszőrök száma az abaxiális oldalon volt a meghatározó, a legtöbb vizsgált növényfaj esetében megállapítható volt, hogy az adaxiális epidermiszen előforduló mirigyszőrök száma meghaladta az abaxiális oldal

mirigyszőreinek a számát. Megállapítottuk, hogy a citromfű és a levendula kiemelkedően magas mirigyszőrszámmal rendelkezik mind a színi, mind a fonáki epidermiszen (citromfű: $318 \pm 46,58 / 148 \pm 21,68$; levendula: $156 \pm 61,07 / 150 \pm 22,36$). A menta ($50 \pm 23,45 / 32 \pm 19,24$) és a kakukkfű ($46 \pm 23,97 / 36 \pm 15,17$) esetében a mirigyszőrök száma jelentősen elmaradt a többi vizsgált fajhoz képest. A kakukkfű jelentős számú és méretű pajzs alakú mirigyszőrrel rendelkezik, a legkisebb ilyen típusú mirigyszőröket a rozmaring és a levendula esetében találtuk. A mirigyszőrök véletlenszerű és egyenetlenül helyezkedtek el. A menta, a kakukkfű, valamint a levendula esetében a mirigyszőrök jelentős, mikroszkóppal jól detektálható mennyiségű illóolajat raktároztak.

Kulcsszavak: Lamiaceae fajok, szár- és levélkeresztmetszet, színi és fonáki epidermisz, mirigyszőr típusok, mirigyszőrök száma

1. Introduction

Plants disperse volatile chemicals via the trichomes' specialized physiological structures which have evolved to prevent herbivores, resist natural pests, reduce water loss through vaporization, beat off disease, attract pollinators, and take part in relations with a number of different organisms. Species related within the Mediterranean-regional species ample Lamiaceae are significant for many different industries. Lamiaceae species are abundant in aromatic chemicals, which are mostly released by glandular trichomes, therefore they are important assets in folk medicine, fragrance manufacturing, cuisine uses, and medical activities (Sota et al., 2019). Even yet, there has been still little research that look at the morphological, structural, histochemical, and essential oil contents of these glandular trichomes (Marin et al., 2006). The flowering axes, leaves, stems, and petioles of these plants have been utilized for the production of extracts that have antibacterial, antioxidant, anti-inflammatory, and antifungal properties along with calming effects on headaches, muscle spasms, and anxiety. These plants have been particularly notable for the composed biochemical mixtures described as essential oils, which originated from glandular and non-glandular structures named trichomes the fact that occur on stems, leaves, and flowering axis (Wagner, 1991). These essential oils have antimicrobial, antifungal, and anti-inflammatory properties. The synthesis of volatile compounds is mainly associated with the distribution and structure of these trichomes on leaf surfaces. Three main kinds of trichomes can be identified inside the Lamiaceae family: glandular, and non-glandular trichomes (Kowalski et al., 2019). Glandular trichomes can be classified into capitate and peltate (Salmaki et al., 2009). Capitate glandular trichomes can be classified into subtypes according to the length of the stalk cells, which have either one or two cells, along with a head with secretory cells (Boix et al., 2011). Peltate trichomes, on the other hand, have a wide stalk cell, a basal epidermal cell, and eight or more secretory cells (Sharma et al., 2003). Non-glandular trichomes

play a role in reducing transpiration, which is important for the species' adaptation to relatively arid habitats. Trichomes also have significance in the classification of species. In order to understand the physiology roles of the glandular trichomes better, both in the case of the stems and the leaves (Salmaki et al., 2009) we examined the glandular structures of several Lamiaceae species, including *Salvia officinalis* L., *Rosmarinus officinalis* L., *Mentha piperita* L., *Melissa officinalis* L., *Thymus vulgaris* L., and *Lavandula officinalis* L.. This study thus concerns the anatomy and morphology of secretory structures, characteristic of the species of Lamiaceae family.

2. Scientific overview

More than 7,200 species (240 genera in 7 subfamilies) belonging to the Lamiaceae family occur all over the world, especially in the Mediterranean countries (Harley et al. 2004, Bräuchler et al. 2010). Many aromatic species produce from this family have considerable quantities of essential oils, and some of them have great economic importance.

In the case of the Lamiaceae family, the essential oil biosynthesis, secretion and accumulation takes place in the glandular trichomes. Their structure has been studied by many authors (Werker et al., 1985; Husain et al., 1990; Corsi et al., 1999; Fahn, 2000). The glandular trichomes reserve lots of useful compounds that are attractants for pollinators, some are repellents for herbivores, and some are even attractants for natural enemies of herbivores (Jachula et al., 2018, Giuliani et al., 2020) They contain a variety of medicinally active ingredients and secondary metabolites (terpenes, flavonoids, alkaloids, polysaccharides, glycosides, fatty acids, and proteins) (Balcke et al. 2017, Konarska and Łotocka 2020). Environmental conditions and seasonal changes affect the growth and development of glandular trichomes, which has effects on their types, density, and morphology. These differences also depend on the environmental stress but the well-known thesis is that they also play an important role in plants' resistance to biotic and abiotic stresses (Soliman et al. 2019).

Epidermal trichomes are an important morphological characteristic of Lamiaceae species. Trichomes are defined as unicellular or multicellular appendages. They originate from epidermal cells only (Cantino, 1992), and can develop on all vegetative and generative parts of the plant. The essential oil production takes place in these trichomes, located on the adaxial and abaxial leaf surfaces. Three types of trichomes have been observed in this family: non-glandular trichomes, capitate glandular trichomes, and peltate glandular trichomes. The capitate glandular trichomes have one or two secretory disk cells and have a stalk that is twice as long as their head. Capitate trichomes are very variable in stalk length, head shape and secretion process, and can be subdivided into various types (Werker et al. 1985). The peltate glandular trichomes have a small, rounded, unicellular stalk and a globular or ovoid large head

composed of 4, 8, or 12 radiating cells with a raised common cuticle (Baran et al., 2010; Kahraman, 2010; Huang et al., 2008; McCaskill and Croteau, 1995). A large secretory head usually comprises of 1, 2, 4 central cells and 4, 6, 8–10, or 6–14 peripheral cells (Baran et al., 2010; Kahraman, 2010). There is a relation between the number and size of glands and the amount of their essential oil content. The diameter of the peltate trichomes secretory cavities are 40–60 µm, while the capitate trichomes have globular secretory cavities that have a diameter of 10–30 µm (Luo et al., 2010). The peltate trichomes produce most of the essential oils, and secrete only lipophilic substances, while capitate trichomes mainly secrete polysaccharide products (Huang et al., 2008). Most of the non-glandular trichomes are simple, branched, or star-shaped. In many cases, such trichomes are living cells, whereas in others they are dead and the protoplasm is replaced by air spaces (Fahn, 1988; Kondratenko, 1975). In the case of the *Thymus vulgaris*, the correlation between the number of glandular trichomes and the production of the essential oil can be determined.

Essential oils of Lamiaceae species contain lots of terpenoid-type components, such as monoterpenes, sesquiterpenes, and phenylpropanoids. The composition of the essential oil depends on the biosynthetic pathway and is related to climatic and cultivation factors (Dhifi et al. 2016).

Thanks to the properties mentioned above, numerous species of the Lamiaceae family are medicinal herbs and spices, such as the species we examined: peppermint (*Mentha × piperita* L.), lemon balm (*Melissa officinalis* L.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.) and lavender (*Lavandula officinalis* L.) (Carović-Stanko et al. 2016).

Based on the above-mentioned information, our experiment aims to examine the interspecies differences, as well as the numbers and size of glandular trichomes in some species of the Lamiaceae family.

3. Material and methods

The leaf samples of *Salvia officinalis* L., *Rosmarinus officinalis* L., *Mentha piperita* L., *Melissa officinalis* L., *Thymus vulgaris* L., and *Lavandula officinalis* L. for the microanatomical examinations were collected on September 28, 2023, from the Botanical Garden of the University of Nyíregyháza (N 47.98682° and E 21.720491°). Intact, healthy, mature leaves and stems were collected for the examinations from the upper third of the shoot. The collected leaves and stems were preserved in Strasburger-Flemming's preservative solution (a mixture of 96% ethanol, 99.5% glycerol, and distilled water, 1:1:1 v v-1 ratio (Barykina, 2004) until sectioning and preparation of the epidermis imprints.

Epidermis imprints and cross-sections were made from the leaf and stem samples, following the methods of Hilu and Randall (1984), Gardner et al.

(1995), and Elagöz et al. (2006). Imprints were made from the adaxial and abaxial surfaces of leaves, using clear nail polish on the one hand. After drying the nail polish, imprints were examined under a BX51-type Olympus light microscope (Olympus BioSystems, Munich, Germany). On the other hand, we used the method of García-Gutiérrez et al. (2020): Whole leaves and leaf segments (when the leaf lamina was very rigid) were partially digested with Franklin's solution (equal volumes of 35% hydrogen peroxide and glacial acetic acid) at room temperature for up to 24 hours. This solution digests the parenchyma tissue, leaving the epidermis and leaf veins intact. After washing the cleared leaf with water, leaf samples were placed in a weak solution of sodium hypochlorite (25–50% in water) to make the epidermis transparent. This procedure helped to separate the abaxial and adaxial epidermises. The samples were then washed with several changes of tap water to remove Franklin's solution and sodium hypochlorite. After that, the samples were stained with a 0.01% safranin aqueous solution (Merck KGaA, Darmstadt, Germany) and 0.05% aqueous toluidine blue (Merck KGaA, Darmstadt, Germany). The following micromorphometric parameters were examined: the density of capitate glandular trichomes (no/mm²), the density of peltate glandular trichomes (no/mm²), and the diameter of peltate glandular trichomes (µm).

Leaf and stem cross sections were made using razor blades, following the method of Sass (1951), the examination of the cross sections was done with the microscope described above. Preparations were stained with a 0.2% aqueous solution of toluidine blue (Merck KGaA, Darmstadt, Germany). The micromorphological parameters of the capitate and peltate glandular trichomes and non-glandular trichomes were examined, and based on that, the trichomes were classified. The general trichome terminology follows Metcalfe and Chalk (1972), Payne (1978) and Navarro and El Oualidi (2000).

A VSI RZ302 3M CMOS camera was used to prepare digital recordings, and a VSI RZ302 measuring program was applied to measure the above-mentioned micromorphometric parameters. Cross-sections and epidermal imprints and preparatums were digitally archived at 4x10, 10x10, 10x20, and 10x40 magnifications. All examined parameters were measured in 5 repetitions per species, and the obtained data was averaged.

Statistical analysis of experimental data was conducted with IBM SPSS Statistics 26.0 (Armonk, NY, USA) software, using analysis of a variance (ANOVA), followed by treatment comparison using Tukey's b-test.

4. Results

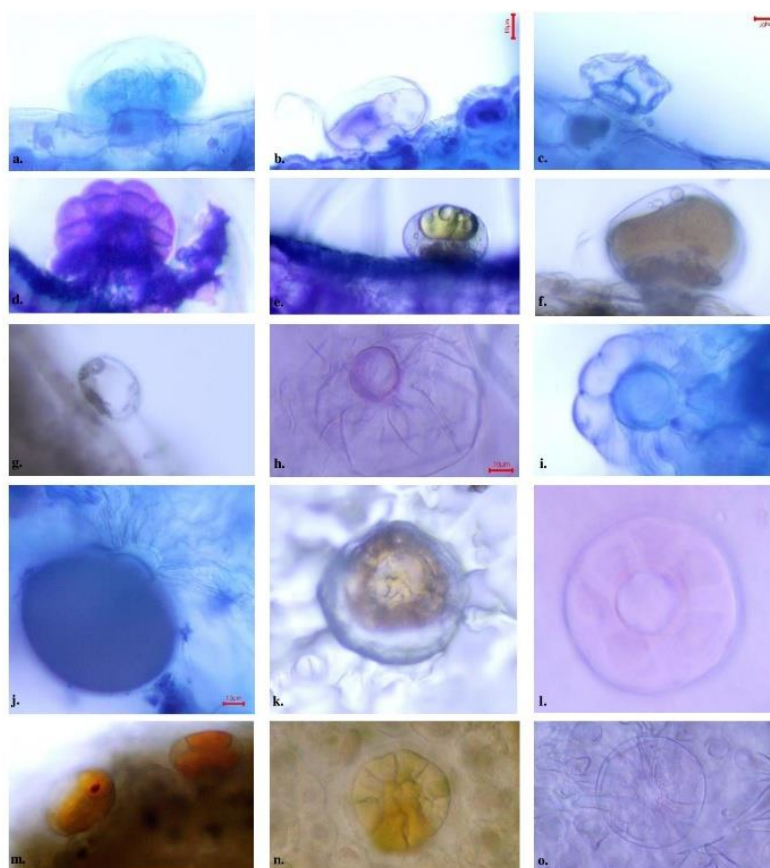
4.1. Microscopic description of trichomes

Three different trichome types on the stems and leaf blades of examined species were observed: peltate, capitate glandular and non-glandular trichomes

(Figs. 1–6.). We could establish four subtypes of capitate glandular and three subtypes of non-glandular trichomes.

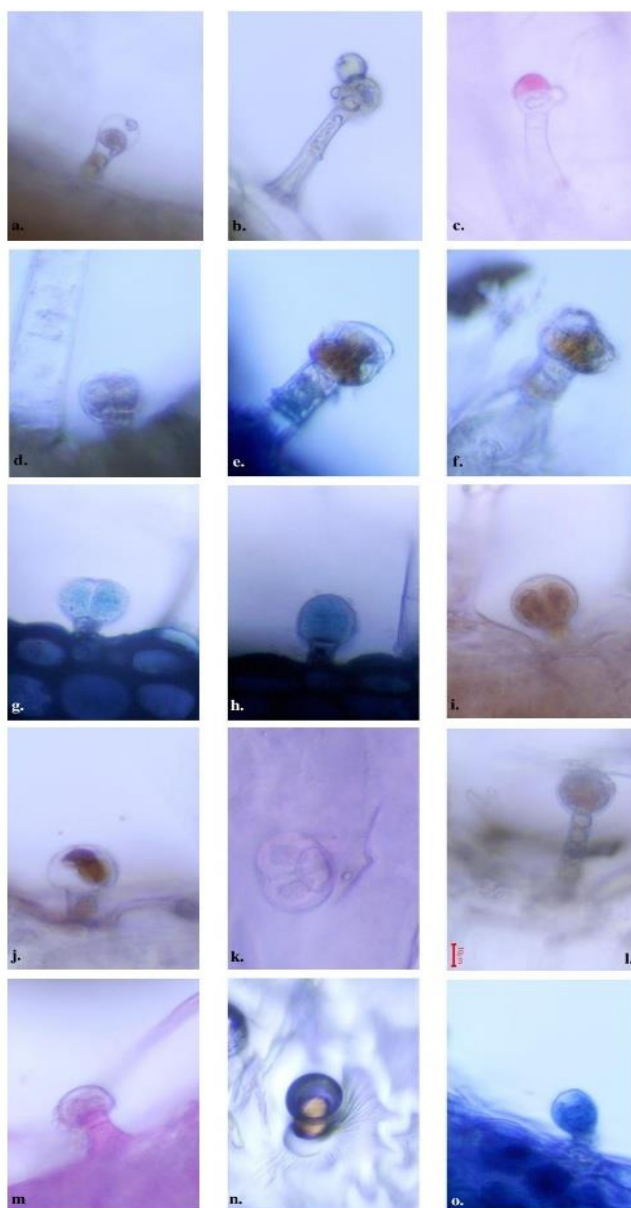
- *Type I*: typical peltate glandular trichome, which consists of a basal epidermal cell, a very short monocellular stalk, and a broad, round multicellular secretory head, consisting of four or twelve cells (one or four central cells surrounded by four or eight peripheral cells) in a single shield (Fig. 1a-o.).

Figure 1. The peltate glandular trichomes (Type I.) on the stem and leaf of examined Lamiaceae species



a. *Mentha piperita* L., leaf, **b.** *Mentha piperita* L., stem, **c.** *Mentha piperita* L., leaf, **d.** *Salvia officinalis* L., stem, **e.** *Salvia officinalis* L., stem, **f.** *Salvia officinalis* L., leaf, **g.** *Lavandula officinalis* L., leaf, **h.** *Lavandula officinalis* L., leaf, **i.** *Rosmarinus officinalis* L., leaf, **j.** *Rosmarinus officinalis* L., leaf, **k.** *Rosmarinus officinalis* L., leaf, **l.** *Rosmarinus officinalis* L., leaf, **m.** *Mentha piperita* L., leaf, **n.** *Mentha piperita* L., leaf, **o.** *Thymus vulgaris* L., leaf; Scale bar: 10µm

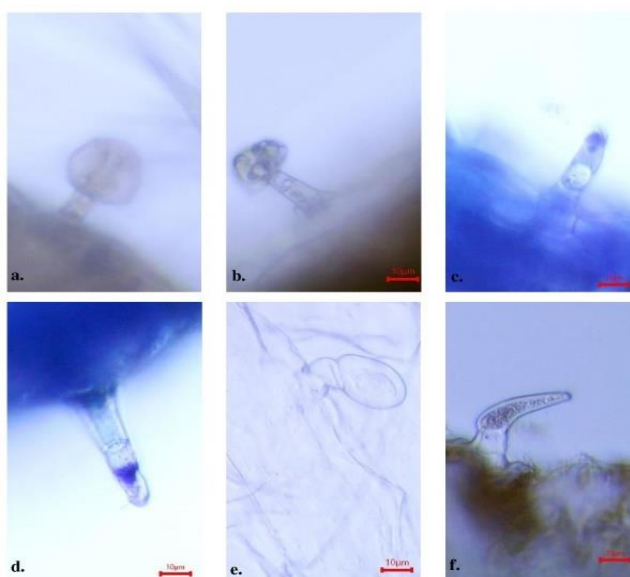
Figure 2. The capitate glandular trichomes (Subtype II/a.) on the stem and leaf of examined Lamiaceae species



a. *Salvia officinalis* L., leaf, **b.** *Salvia officinalis* L., leaf, **c.** *Salvia officinalis* L., leaf, **d.** *Melissa officinalis* L., leaf, **e.** *Melissa officinalis* L., leaf, **f.** *Melissa officinalis* L., leaf, **g.** *Lavandula officinalis* L., stem, **h.** *Lavandula officinalis* L., stem, **i.** *Lavandula officinalis* L., leaf, **j.** *Lavandula officinalis* L., leaf, **k.** *Lavandula officinalis* L., leaf, **l.** *Lavandula officinalis* L., leaf, **m.** *Rosmarinus officinalis* L., leaf, **n.** *Melissa officinalis* L., leaf, **o.** *Mentha piperita* L., leaf; Scale bar: 10µm

- *Type II*: capitate glandular trichomes, which are built from basal epidermal cell, unicellular or multicellular stalk. Their length varies (15–1000 μm). It consists of a neck cell (7–18 μm) and a large, cutinized, unicellular or bi-cellular secretory head (*Figs. 2–3*). These trichomes can be subdivided into four subtypes:
 - Subtype II/a.: globose unicellular/bicellular, stalk of one to four cells (30–900 μm) (*Fig 2*). A large percentage of these trichomes have one head cell
 - Subtype II/b.: cup-shaped unicellular head, one to five-celled stalk (35–1000 μm)
 - Subtype II/c.: hemispherical unicellular head, unicellular/bicellular stalk (35–500 μm) (*Fig 3*).
 - Subtype II/d.: oblong unicellular head, short unicellular stalk (15–90 μm), sometimes bicellular stalk (up to 150 μm) (*Fig 3*).

Figure 3. The capitate glandular trichomes (Subtype II/c-d.) on the leaf of examined Lamiaceae species

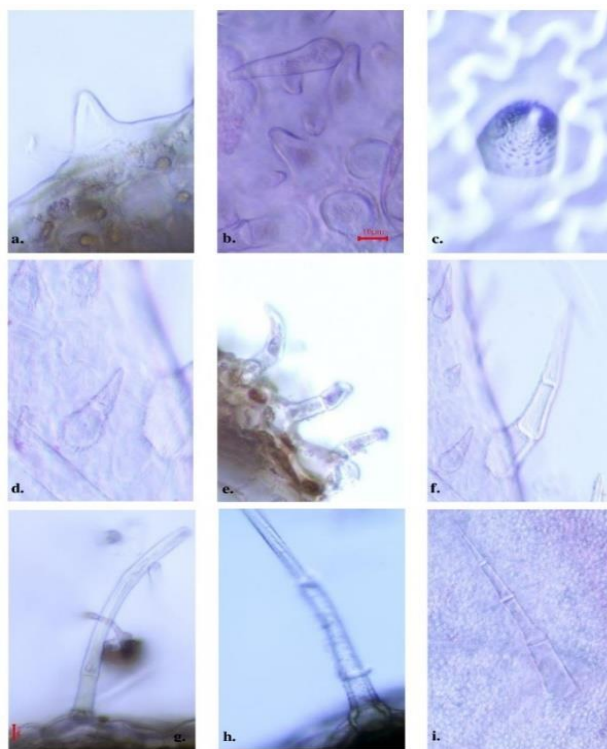


a. *Salvia officinalis* L., leaf, Subtype II/c. **b.** *Salvia officinalis* L., leaf, Subtype II/c., **c.** *Mentha piperita* L., leaf, Subtype II/d., **d.** *Mentha piperita* L., leaf, Subtype II/d., **e.** *Mentha piperita* L., leaf, Subtype II/d., **f.** *Thymus vulgaris* L., leaf, Subtype II/d.; Scale bar: 10 μm

- *Type III*: non-glandular trichome, composed of one basal epidermal cell. Unicellular to multicellular, uniseriate, unbranched, or branched (*Figs. 4–7*). It is quite variable in length (50–3000 μm). These trichomes can be subdivided into three subtypes.

- Subtype III/a.: Unicellular to multicellular (of up to seven cells) acicular trichomes in a single order (*Fig. 4.*). In particular, unicellular trichomes are thick-walled and densely covered by micro-papillae. The length of these trichomes vary between 50 and 1000 μm . Multicellular trichomes are curved or straight at the tip.
- Subtype III/b.: Multicellular (of up to thirteen cells), uniseriate flagelli-form trichomes with the distal end of the terminal cells delicate and much elongated (*Fig. 5.*). Their lengths are 1500–3000 μm .
- Subtype III/c.: Multicellular (of up to five to eight cells) trichomes with ridges and marked internodes (*Fig. 6.*). These trichomes are between 500 and 1400 μm long.
- Subtype III/d.: Dendroid hair - multicellular trichomes, branched to a tree in form (*Fig. 7.*). Having a stem-like part arising straight from the broadened base, and dividing to produce diverging branches which may be repeatedly forked in their turn. They are composed of living cells containing numerous plastids.

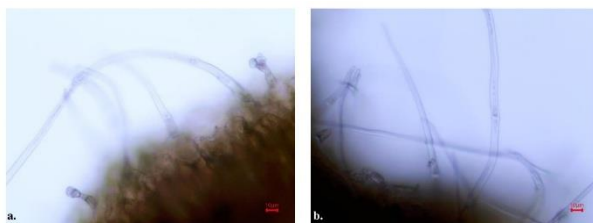
Figure 4. The non-glandular trichomes (Subtype III/a.) on the leaf of examined Lamiaceae species



a. *Mentha piperita* L., leaf, unicellular trichome, **b.** *Thymus vulgaris* L., leaf, unicellular trichome, **c.** *Melissa officinalis* L., leaf, two-celled trichome with protuberances on the wall, **d.**

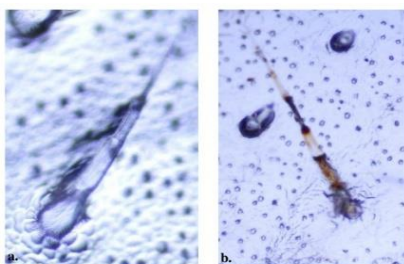
Melissa officinalis L., leaf, two-celled trichome with protuberances on the wall, **e.** *Thymus vulgaris* L., leaf, two-celled trichome with smooth walls, **f.** *Melissa officinalis* L., leaf, three-celled trichome, **g.** *Melissa officinalis* L., leaf, three-celled trichome, **h.** *Melissa officinalis* L., leaf, three-celled trichome, **i.** *Melissa officinalis* L., leaf, more than three-celled trichome; Scale bar: 10µm

Figure 5. The non-glandular trichomes (Subtype III/b.) on the leaf of examined Lamiaceae species



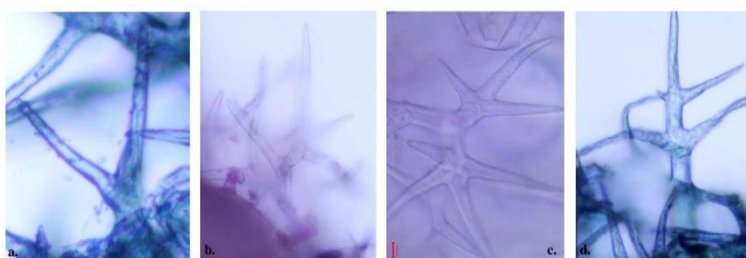
a. *Salvia officinalis* L., leaf, **b.** *Salvia officinalis* L., leaf; Scale bar: 10µm

Figure 6. The non-glandular trichomes (Subtype III/c.) on the leaf of examined Lamiaceae species



a. *Melissa officinalis* L., leaf **b.** *Melissa officinalis* L., leaf

Figure 7. The non-glandular trichomes (Subtype III/d.) on the leaf of examined Lamiaceae species



a. *Lavandula officinalis* L., leaf, biramous trichome, **b.** *Lavandula officinalis* L., leaf, stellate trichome with unicellular branches, **c.** *Rosmarinus officinalis* L., leaf, stellate trichome with unicellular branches, **d.** *Lavandula officinalis* L., leaf, dendroid trichome; Scale bar: 10µm

4.2. Secretory structures of stem and leaf in examined Lamiaceae species

The stem of *Melissa officinalis* is clearly quadrangular with 4 corners. The cuticle is thin. The epidermis consists of a single layer of oval cells. On the surface of the stem lots of glandular (capitate and peltate) and non-glandular trichomes can be observed. Below the epidermis, the number of angular collenchyma layers is present at the corners and between the corners of the stem as a continuous ring. Thin-walled, irregular in shape parenchymatous cells can be found below the collenchyma layer. Some oil ducts can be found in the primary cortex. The phloem is surrounded by sclerenchyma layers. The phloem is closely compressed, and the cambium is absent. The pith consists of large thin-walled parenchyma cells (Fig. 8a-d.).

In the leaf cross-section of *Melissa officinalis* the one-celled layer epidermis can be observed, the shape of the cells of the epidermis is oval. The epidermis is covered by thin cuticles. The leaf is dorsiventral and consists of two-rowed palisade parenchyma with a lot of chloroplasts and isodiametric spongy parenchyma with intercellular spaces. The main vein is surrounded by angular collenchyma layers (Fig. 8e.). Similar to other Lamiaceae species, kidney-shaped stomas are mostly found on abaxial epidermis (Fig. 9n.).

Table 1. Number of the whole glandular trichomes, the number of the peltate glandular trichomes and the diameter of peltate glandular trichomes

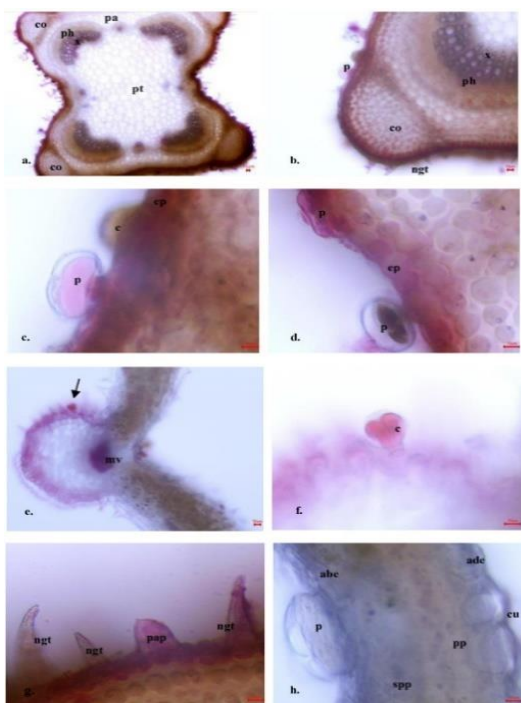
	<i>M. officinalis</i>	<i>M. piperita</i>	<i>R. officinalis</i>	<i>Th. vulgaris</i>	<i>L. officinalis</i>	<i>S. officinalis</i>
Adaxial epidermis						
Number of glandular trichomes (no/mm ²)	318±46.5 ^{8c}	50±23.45 ^a	88±25.88 ^{ab}	46±27.9 ^{3a}	156±61.0 ^{7b}	98 ± 38.98 ^{ab}
Number of peltate glandular trichomes (no/mm ²)	20±7.07 ^a	22±8.37 ^a	28±14.83 ^a	28±14.8 ^{3a}	30±7.07 ^a	14±5.47 ^a
Diameter of peltate glandular trichomes (µm)	37.80±4.4 ^{6a}	58.65±5.8 ^{3c}	45.98±13.3 ^{2abc}	67.63±4.79 ^d	65.15±2.2 ^{9d}	58.36±16.78 ^c
Abaxial epidermis						
Number of glandular trichomes (no/mm ²)	148±21.6 ^{8bc}	32±19.24 ^a	74±50.30 ^a	36±15.1 ^{7a}	150±22.3 ^{6c}	62 ± 26.83 ^a
Number of peltate glandular trichomes (no/mm ²)	8±8,37 ^a	8±4,47 ^a	34±5,47 ^c	26±5,47 ^b	34±5,47 ^c	14±5,47 ^a
Diameter of peltate glandular trichomes (µm)	56.59±7.1 ^{3a}	59.61±1.6 ^{5ab}	66.68±10.3 ^{8bcd}	78.8±6.2 ^{0d}	72.56±2.5 ^{3cd}	65.11±5.5 ^{0ac}

M. officinalis: *Melissa officinalis* L., *M. piperita*: *Mentha x piperita* L., *R. officinalis*: *Rosmarinus officinalis* L., *Th. vulgaris*: *Thymus vulgaris* L., *L. officinalis*: *Lavandula officinalis* L., *S. officinalis*: *Salvia officinalis* L.

Data are means of five replicates. ANOVA Tukey's b-test. Means within the lines followed by the same letter are not statistically significant at $p < 0.05$.

In the case of *Melissa officinalis*, the number of glandular trichomes on the adaxial and abaxial epidermis is the largest among the examined species, the difference is significant (Table 1., Fig. 10.). A large portion of these trichomes are capitate glandular trichomes. Capitate trichomes contain a head cell, a short stalk cell, and a basal cell surrounded by papillas (Fig. 8f., Fig. 9k-m.). The number of peltate glandular trichomes and the diameter of peltates is significantly the lowest among the examined species (Fig. 8c-d. and h., Fig. 9n., Fig. 11.). Non-glandular hairs are multicelled, Subtype III/a. and III/c.: two-celled trichomes with protuberances on the wall and three-celled trichomes (Fig. 9i. and o-r.). The basal cell of some trichomes can be surrounded by 12-16 cells (Fig. 9i-j.).

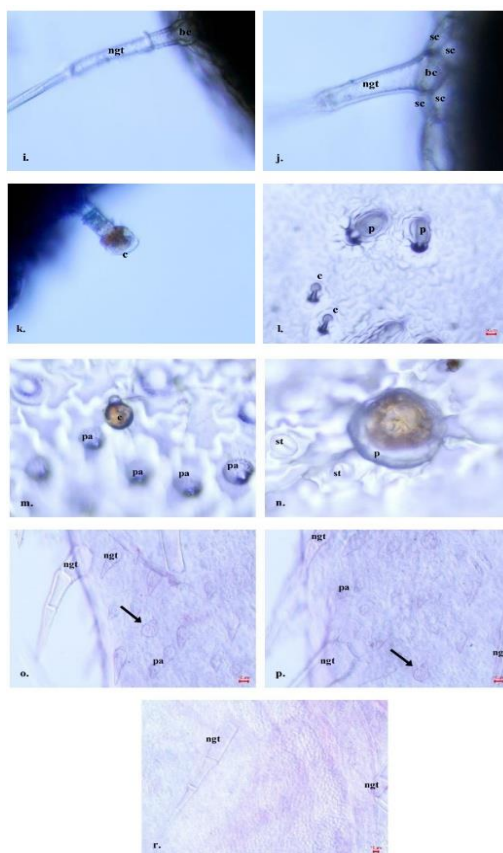
Figure 8. Cross-section of stem and leaf lamina of *Melissa officinalis* L.



(a) stem cross-section stained by safranin (4X), (b) stem cross-section stained by safranin (10X), (c) safranin stained peltate and capitate glandular trichomes in stem (40X), (d) safranin stained peltate glandular trichomes in stem (40X), (e) Safranin stained leaf lamina cross-section of *Melissa officinalis* (10X), (f) safranin stained capitate trichome in leaf cross-section

(40X) (g) safranin stained non-glandular trichomes and papilla in stem (40X), (h) peltate trichomes in leaf cross-section (40X), pt: pith, x: xylem, ph: phloem, co: collenchyma, pa: parenchyma, ep: epidermis, ade: adaxial epidermis, abe: abaxial epidermis, pap: palisade parenchyma, spp: spongy parenchyma, mv: main vein, c: capitate glandular trichome, ng: non-glandular trichome (two-celled trichome with smooth walls), pap: papilla (two-celled trichome with protuberances on the wall), p: peltate glandular trichome, black arrow: capitate glandular trichome; Scale bar: 10µm

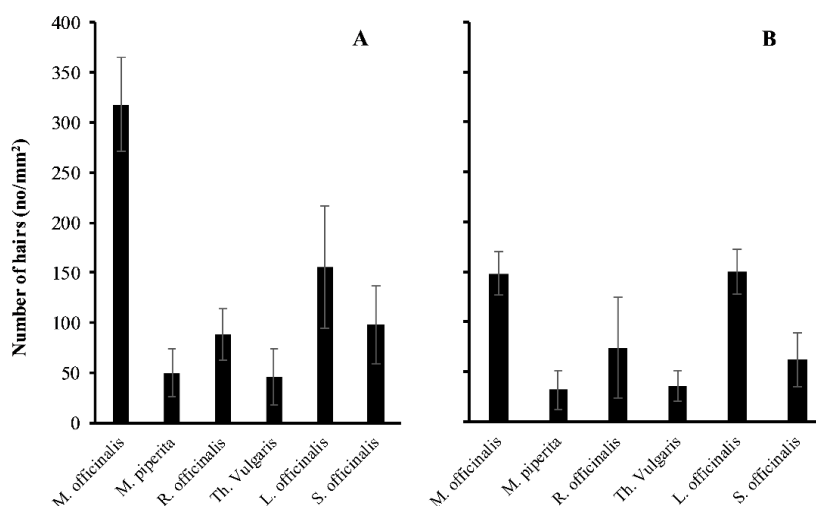
Figure 9. Glandular and non-glandular trichomes in leaf epidermis of *Melissa officinalis* L.



(i) non-glandular trichome (three-celled trichome) – adaxial epidermis (40X), (j) non-glandular trichome (three-celled trichome, of which basal cell is surrounded by 8 cells) – adaxial epidermis (40X), (k) toluidine blue stained capitate glandular trichome – adaxial epidermis (40X), (l) peltate and capitate glandular trichomes – adaxial epidermis (40X), (m) capitate glandular trichome and papillas (two-celled trichome with protuberances on the wall) – adaxial epidermis (40X), (n) peltate glandular trichome – abaxial epidermis (40X) (o) non-glandular trichome (three-celled trichome), papillas (two-celled trichome with protuberances on the wall) and capitate glandular trichome – adaxial epidermis (40X), (p) non-glandular trichomes, papillas and capitate glandular trichome – adaxial epidermis (40X), (r) non-glandular trichome

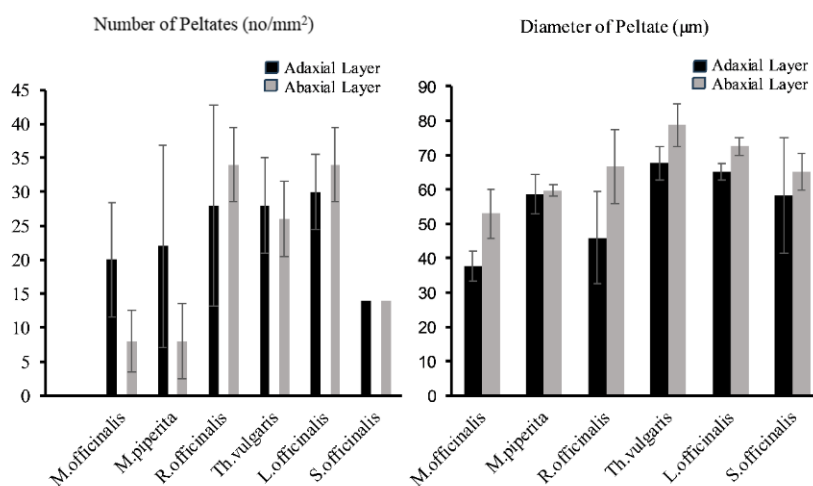
(more than three-celled trichome) – adaxial epidermis (40X), ngt: non-glandular trichome, bc: basal cell, sc: surrounding cell, c: capitate glandular trichome, p: peltate glandular trichome, st: stomata, pap: papilla, black arrow: capitate glandular trichome; Scale bar: 10µm

Figure 10. The number (no/mm²) of glandular trichomes of the examined Lamiaceae species



A: adaxial epidermis, **B:** abaxial epidermis

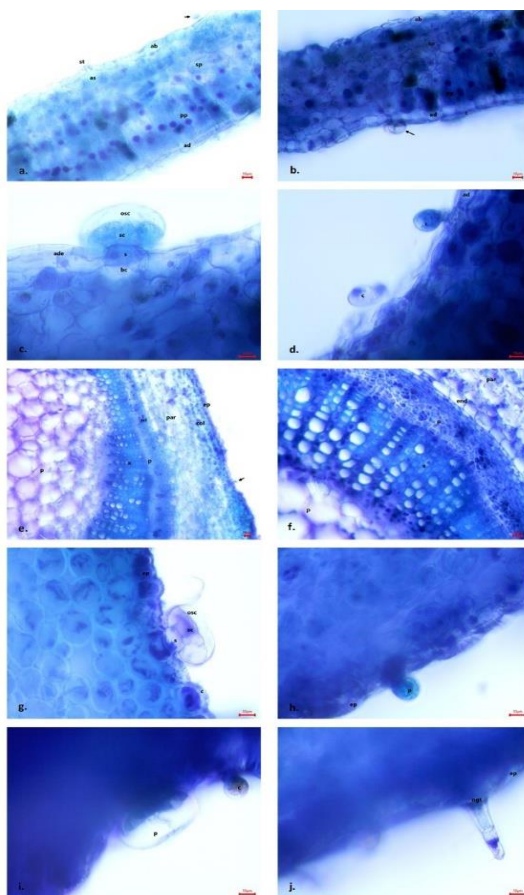
Figure 11. The number (no/mm²) and the diameter (µm) of the peltate glandular trichomes of the examined Lamiaceae species



In the cross-section of *Mentha x piperita*'s stem, we can observe that the stem is square-shaped. From the outside in, the following can be observed: the

epidermis, the primer cortex, and the central cylinder. The epidermis is formed from a single row of cells, covered by a cuticle. Inside the epidermis, lots of secretory hairs and non-glandular trichomes are situated (*Fig. 12b-d. and g-j.*). The secretory hairs are unicellular and also multicellular capitate glandular trichomes (Subtype II/a and Subtype II/c-d.) or have 4-8 cells in the secretory gland – peltate glandular trichomes (Type I.).

Figure 12. Cross-section of leaf lamina and stem of *Mentha piperata* L.



(a, b) cross-section of leaf lamina (20X), (c, d) adaxial surface of leaf, showing peltate and capitate glandular trichomes (40X), (e, f) cross-section of stem (10X, 20X), (g, h, i) peltate and capitate glandular trichomes of stem (40X), (j) non-glandular trichome of stem (40X). ab: abaxial epidermis, ad: adaxial epidermis, s: stoma, as: air space, sp: spongy parenchyma, pp: palisade parenchyma, *black arrow*: glandular trichome, c: cuticle, ade: adaxial epidermis, bc: basal cell, s: stalk cell, sc: secretory cell, osc: subcuticular oil storage cavity, c: capitate glandular trichome, ep: epidermis, col: collenchyma, par: parenchyma, scl: sclerenchyma, p: phloem, x: xylem, p: pith, end: endodermis, p: peltate glandular trichome, ng: non-glandular trichome; Scale bar: 10µm

The cortex is formed of angular chlorenchyma bundles (*Fig. 12e.*) and parenchyma (hypodermal chlorenchyma). Between the chlorenchyma and the central cylinder, the endodermis is clearly observable (*Fig. 12f.*). The vascular bundles are secondary origin, and they are built of secondary phloem, and secondary xylem. The primary origin xylem is reduced, and the wooden vessels are separated by parenchymatic medullary rays. The pith is parenchymatic.

Based on our examinations, we can conclude that the epidermis cells of the leaf have robust wavy walls. The leaf is hypostomatic, and it only has diacytic-type stomata in the lower epidermis.

The *Mentha x piperita* has a dorsiventral leaf, the mesophyll is of bifacial type, separated by palisade parenchyma and spongy parenchyma. The palisade parenchyma consists of a single layer of elongated cells, rich in chloroplasts. The spongy tissue, located under the abaxial epidermis, consists of 3-4 rows of isodiametric cells, which have thin walls with intercellular spaces. In the leaf mesophyll vascular collateral bundles were observed.

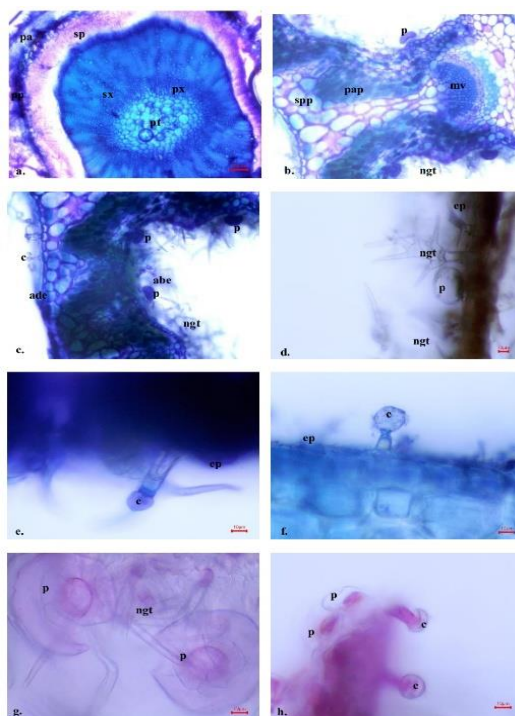
A lot of secretory hairs can be observed on the surface of the adaxial and abaxial epidermis too. The secretory hairs are unicellular or multicellular and are more frequent in the adaxial epidermis.

We can observe that the density of glandular trichomes is higher on the adaxial epidermis than on the abaxial epidermis (*Table 1., Fig. 10.*). The number of glandular trichomes of *Mentha x piperita* on both epidermis layers is significantly lower than in the other species. We can also conclude that the number of capitate glandular trichomes is higher than peltates. It was an interesting finding that the diameter of peltates of *Mentha* on the adaxial epidermis is significantly higher than in the case of *Melissa officinalis* and *Rosmarinus officinalis* (*Fig. 11.*). Meanwhile, on the abaxial epidermis among these species, we could not find significant differences.

The stem of *Rosmarinus officinalis* has a woody structure, thanks to secondary growth. This structure provides the plant with resistance to environmental conditions, temperature changes, drought, and wind. The epidermis of the stem has 3-4 cell layers, the angular collenchyma can be found below this. This area is followed by parenchyma, where some sclerenchymatic fibers may be observed. Near the endodermis (pericycle) 1-2 layers of cork cells are located. The secondary growth structures, such as the secondary xylem and secondary phloem can be observed. The residuals of the primary xylem and phloem can also be found (*Fig. 13a.*). The epidermis of the stem presents protective and secretory hairs as glandular and non-glandular trichomes. The distribution of non-glandular trichomes (Subtype III/d.) is denser than the distribution of glandular trichomes, which have branched shapes (*Fig. 13d.*). Capitate and multicellular head peltate glandular trichomes can also be found (*Fig. 13i-l.*).

The leaves of *R. officinalis* are covered by a thick cuticle, present on the surface of the adaxial and abaxial layers of the epidermis (Fig. 13b-c.). The adaxial and abaxial epidermis layers of the leaf are rich in glandular and non-glandular trichomes. Fig. 13b. shows lots of non-glandular and some peltate glandular trichomes surrounding the main vein. Non-glandular trichomes can be recognized as branched multicellular (Subtype III/d.) and unbranched unicellular trichomes (Subtype III/a.). The branched multicellular trichomes can be found mainly in a large abundance on the abaxial surface. While peltate glandular trichomes are dominantly located in the abaxial layer (Fig. 13g., Fig. 14l-n.), non-glandular and capitate glandular trichomes can be found in an adaxial epidermis layer of the leaf. Capitate trichomes are typical trichomes with globose unicellular/bicellular head and a stalk of one to four cells (Subtype II/a.). They consist of one basal cell, a neck cell, a stalk cell (1–4), and a head cell.

Figure 13. Cross-section of stem and leaf lamina of *Rosmarinus officinalis* L.



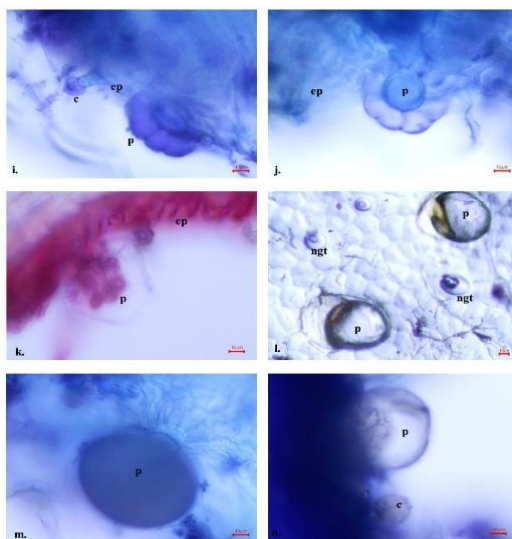
(a) stem cross-section stained by toluidine blue (4X), (b) cross-section of leaf lamina, peltate glandular trichomes and non-glandular trichomes (20X), (c) toluidine blue stained leaf lamina cross-section of *Rosmarinus officinalis* (20X), (d) non-glandular and peltate glandular trichomes in stem cross-section (20X), (e) capitate glandular trichome in stem cross-section (40X), (f) toluidine blue stained capitate glandular trichome in leaf cross-section (40X) (g)

safranin stained peltate glandular trichomes in the abaxial layer of leaf the epidermis (40X), (h) peltate and capitate glandular trichomes in stem cross-section (40X), pt: pith, px: primary xylem, sx: secondary xylem, sp: secondary phloem, pp: primary phloem, pa: parenchyma, ep: epidermis, ade: adaxial epidermis, abe: abaxial epidermis, pap: palisade parenchyma, spp: spongy parenchyma, mv: main vein, c: capitate glandular trichome, ng: non-glandular, p: peltate glandular trichome; Scale bar: 10µm

Capitate trichomes are situated among the non-glandular trichomes, and they have long stalk cells and contain oil (Subtype II/a.- *Fig. 13e*). The short-stalk-celled capitate trichome can be seen in *Fig. 13f*.

The number of glandular trichomes on the adaxial and abaxial epidermis of *Rosmarinus officinalis* is significantly lower than the number of glandular trichomes of *Melissa officinalis*. However, there is no significant difference compared to the number of glandular trichomes of other examined species (*Table 1*). The number of glandular hairs on the adaxial epidermis is higher than on the abaxial epidermis (*Fig. 10*). We can conclude that the number of capitate glandular trichomes is higher than the number of peltates. In the case of rosemary, the higher diameter of peltates can be measured on the abaxial epidermis (*Table 1*, *Fig. 11*).

Figure 14. Cross-section of stem and leaf lamina of *Rosmarinus officinalis* L.

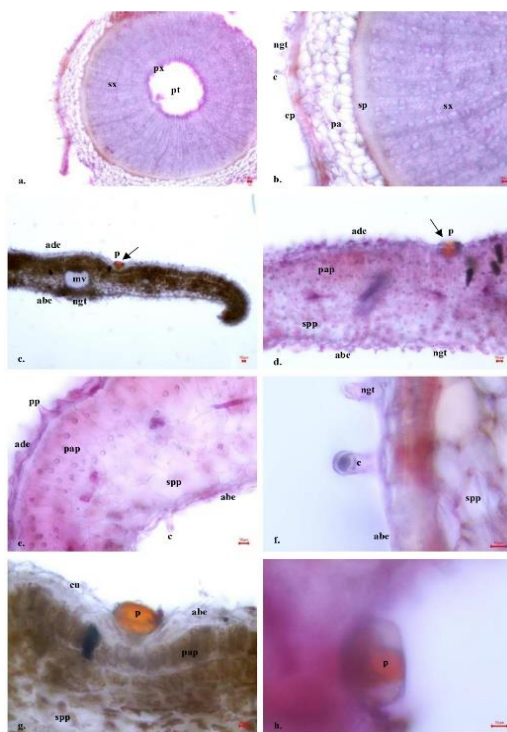


(i) capitate and multicellular head peltate glandular trichome in stem cross-section (40X), (j) multicellular head peltate glandular trichome in stem cross-section (40X), (k) multicellular head peltate glandular trichome in safranin stained stem cross-section (40X), (l) peltate and non-glandular trichomes in adaxial layer (20X), (m) peltate glandular trichome contains essential oil in toluidine blue stained stem cross-section (40X), (n) capitate and peltate

trichome in stem cross-section (40X), ep: epidermis, c: capitate glandular trichome, ng: non-glandular, p: peltate glandular trichome; Scale bar: 10µm

By analyzing the stem cross-section of *Thymus vulgaris*, it can be established that it has 3-4 layers of cork, the stele is thick with a secondary structure; between the secondary phloem and secondary xylem, the cambium ring can be found. The residue of the primary xylem also can be observed. On the cork, there are lots of capitate glandular and non-glandular trichomes (Fig. 15a-b.).

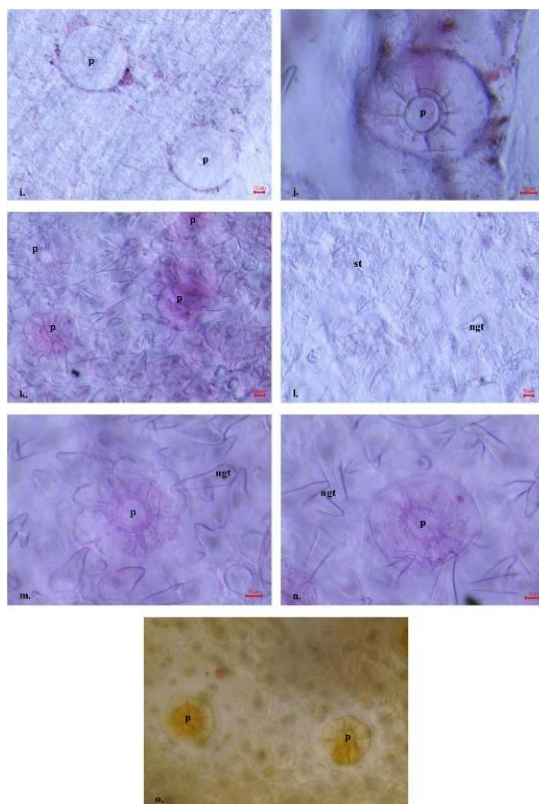
Figure 15. Cross-section of stem and leaf lamina of *Thymus vulgaris* L.



(a) cross-section of stem (stained by safranin) (4X), (b) cross-section of stem (stained by safranin) (10X), (c) cross-section of leaf lamina, peltate glandular trichome in adaxial layer and non-glandular trichomes in abaxial layer (4X), (d) cross section of leaf lamina, peltate glandular trichome is located on the adaxial layer, contains oil drops (stained by safranin) (10X), (e) palisade and spongy parenchyma of leaf lamina (20X), (f) capitate glandular and non-glandular trichomes located on the abaxial epidermis layer (40X), (g) undyed cross-section of leaf lamina (20X), (h) peltate glandular trichome stained by safranin (40X), ep: epidermis, pa: parenchyma, pt: pith, px: primary xylem, sx: secondary xylem, sp: secondary phloem, ade: adaxial epidermis, abe: abaxial epidermis, cu: cuticle, pap: palisade parenchyma, spp: spongy parenchyma, mv: main vein, ng: non-glandular, p: peltate glandular trichome, c: capitate glandular trichome; Scale bar: 10µm

Thymus vulgaris has a typical heterogen, dorsiventral leaf mesophyllum. The leaf epidermis consists of a single row of elongated-shaped cells, covered with a thin cuticle. The epidermis cells on the adaxial surface are wider. There are a lot of non-glandular and glandular trichomes in both epidermis layers (Fig. 15c-h.). Two different types of non-glandular trichomes were found: unicellular trichomes, and two-celled trichomes with smooth walls (Subtype III/a.).

Figure 16. Safranin stained leaf lamina cross-section of *Thymus vulgaris* L.



(a) peltate glandular trichomes in abaxial epidermis layer (20X), (b) peltate glandular trichome in abaxial epidermis layer (40X), (c) non-glandular trichomes, peltate glandula trichomes with contained oil in adaxial epidermis of leaf (d) stomatas and non-glandular trichomes in abaxial layer (20X), (e) multicelled head, peltate glandular trichome and non-glandular trichomes in adaxial layer (40X), (f) peltate and non-glandular trichomes in adaxial layer of epidermis (40X), ngt: non-glandular trichome, p: peltate glandular trichome, st:stoma; Scale bar: 10µm

Along the leaf surfaces, two different types of glandular trichomes were found. One of them is a typical capitate glandular trichome: it has a base, a stalk, and a unicellular head (Subtype II/a. and d.). The other is a peltate glandular trichome, which is composed of a base, a short sessile stalk, and a

multicellular head (Type I.). Peltate type of glandular trichomes have a large size and contain oil drops, and they can be found on both surfaces of the epidermis. The dispersion of peltate type of trichomes on the abaxial epidermis was more abundant than the dispersion of capitate glandular trichomes (Fig. 10. and Fig. 16i-o.). In the case of *Thymus*, the number of glandular trichomes (adaxial epidermis: 46 ± 27.93 , abaxial epidermis: 36 ± 15.17) is low, which is significantly lower than in the case of *Lavandula officinalis* and *Melissa officinalis*. We can conclude that the *Thymus vulgaris* has a significantly larger diameter of peltate glandular trichomes than the other examined species (Fig. 11., Table 1.) After examining the glandular trichomes dispersion on the adaxial and abaxial leaf surfaces, we can state that the number of glandular trichomes is larger on the adaxial epidermis.

Stomata are diacytic, and they were found both on the adaxial and abaxial epidermis layer. The stomata are denser and larger on the abaxial epidermis (Fig. 16l.).

The anatomical structure of the *Lavandula*'s stem is divided into three topographic zones: the epidermis/periderm (cork), the primer cortex, and the central cylinder. Under the periderm, the parenchyma can be found, which consists of 6-7 rows and remains throughout. Inside the cortex parenchyma groups of sclerenchymatic fibers are formed. We can observe the primary and the secondary phloem below the parenchymatic tissues. Between the secondary phloem and secondary xylem the cambium ring can be situated. The center of the stem is filled by pith (Fig. 17a.).

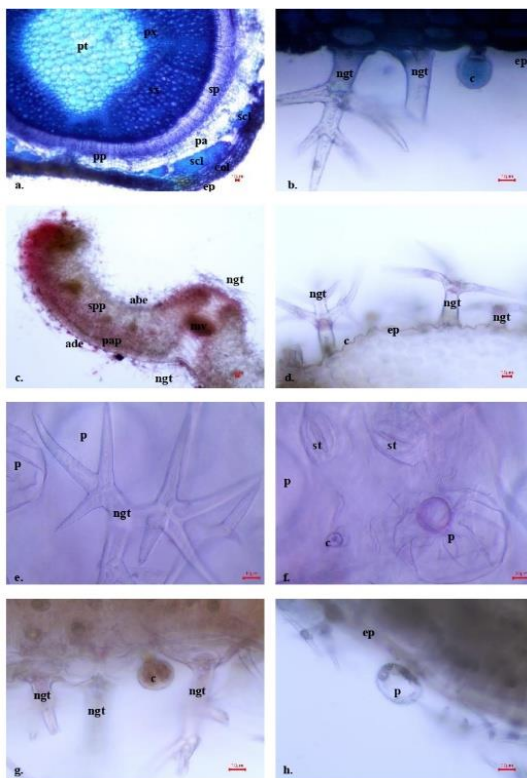
The leaf of *Lavandula officinalis* is dorsiventral. The epidermis layers are thick, one-celled layers, covered by thick-walled cuticle layer and lots of non-glandular (Subtype III/d.: abiramous trichome, stellate trichome with unicellular branches, dendroid trichome) and glandular trichomes (Type I., Subtype II/a.) (Fig. 17b-h.). The number of glandular trichomes on adaxial epidermis of *Lavandula officinalis* is significantly higher than in the case of *Mentha x piperita*, *Thymus vulgaris*, and significantly lower than *Melissa officinalis* (Table 1.). On the abaxial epidermis this value is significantly higher than the number of glandular trichomes of every examined species, except for *Melissa officinalis*. The number of capitate glandular trichomes is higher than peltates. We also can conclude that the diameter of *Lavandula*'s peltates on both epidermis layers is significantly higher than the peltates's diameter of *M. officinalis*, *M. piperita* and *R. officinalis* (Fig. 11.). The number of glandular trichomes on adaxial epidermis is higher than the glandular trichomes number of abaxial epidermis (Fig. 10.).

The leaf mesophyllum consists of 1-2 rows of palisade and 3-4 rows of sponge parenchyma (Fig. 17c). Drops of essential oil were found in epidermal and palisade cells. The vascular bundles are collateral. The leaves are

amphistomatic, stomata are located on both sides of the leaf, its shape is round-oval (*Fig. 17f*). The stomatas are submerged, diacytic types.

The contour of the stem cross-section of *Salva officinalis* is quadratic due to the angular collenchyma, present in the four ribs (*Fig. 18a-c*). The epidermis has isodiametric cells that have a very thick and cutinized outer wall. The cortical tissue is well-developed and consists of 7 parenchymatic cell layers. The collenchyma tissue in the two-celled layer can also be observed. Inside the parenchymatic tissues, lots of sclerenchymatic fibers are situated. The central cylinder contains the vascular tissues, arranged in the shape of rings. In the center of the stem, the parenchymatic pith can be seen. On the surface of the stem, a large amount of glandular (Type I., Subtype II/a, Subtype II/c-d.) and non-glandular trichomes (Subtype III/b.) can be observed (*Fig. 18b*. and *d*.).

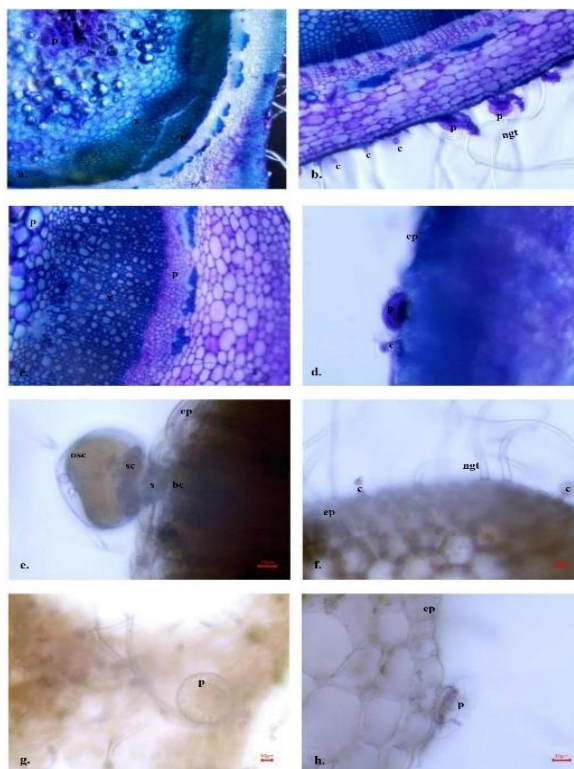
Figure 17. Cross-section of stem and leaf lamina of *Lavandula officinalis* L.



(a) cross-section of stem (stained by toluidine blue) (4X), (b) cross-section of leaf lamina, non-glandular and capitate glandular trichome (40X), (c) safranin stained leaf cross-section of *Lavandula officinalis* (4X), (d) non-glandular trichomes in leaf cross-section (40X), (e) non-glandular and peltate glandular trichomes in abaxial epidermis layer of *Lavandula officinalis* (40X), (f) stomatas and peltate and capitate glandular trichomes in abaxial epidermis layer (40X), (g) non-glandular and capitate glandular trichomes in leaf cross-section (40X), (h)

peltate glandular trichome in leaf cross-section (40X), pt: pith, px: primary xylem, sx: secondary xylem, sp: secondary phloem, pp: primary phloem, pa: parenchyma, scl: sclerenchymatic fibers, col: collenchyma, ep: epidermis, ade: adaxial epidermis, abe: abaxial epidermis, pap: palisade parenchyma, spp: spongy parenchyma, mv: main vein, c: capitate glandular trichome, ng: non-glandular, p: peltate glandular trichome, st: stomata; Scale bar: 10µm

Figure 18. Cross-section of stem of *Salvia officinalis* L.



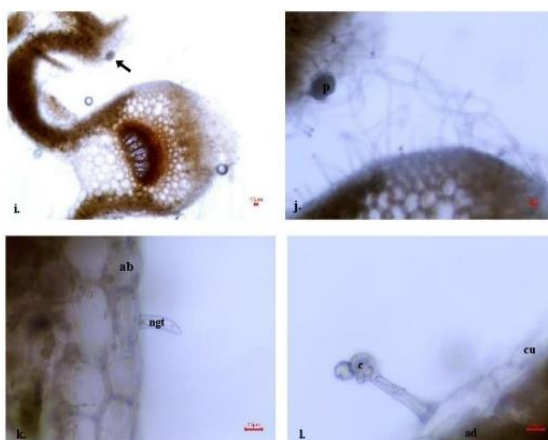
(a, b, c) secondary and primary growth of *Salvia officinalis* L. (20X), (d) peltate and capitate glandular trichomes of stem (20X), (e) peltate glandular trichome (40X), (f) glandular and non-glandular trichomes on leaf cross section (40X) (g), top view of the peltate glandular trichome and non-glandular trichomes on leaf cross section (20X), (h) peltate glandular trichome on leaf cross-section (40X). bc: basal cell, c: capitate glandular trichome, ep: epidermis, ng: non-glandular, osc: subcuticular oil storage cavity, p: pith, p: peltate glandular trichome, p: phloem, x: xylem, s: stalk cell, sc: secretory cell trichome; Scale bar: 10µm

Analyzing the internal structure of the leaf of *Salvia officinalis*, two one-celled layer epidermis was observed. These epidermis layers are covered by a thick cuticle layers. On both of the epidermis layers lots of glandular (capitate and mainly peltate – Fig. 18e-h., Fig. 19l.) and non-glandular trichomes (Subtype III/b. – Fig. 18f., Fig. 19j.) can be seen. The mesophyll is thick,

differentiating into a palisade parenchymatic tissue and a relatively compact spongy parenchymatic tissue on the abaxial side (Fig. 19i.). The vascular bundles have a primary structure (Fig. 19i.).

On the adaxial epidermis layer, we can observe more glandular trichomes than on the abaxial epidermis (Table 1., Fig. 10.). The number of capitate glandular trichomes is higher than peltates. The number of peltates is significantly lower than peltates number of *R. officinalis*, *Th. vulgaris* and *L. officinalis* (Fig. 11.). The diameter of peltate glandular trichomes is significantly larger than the peltates diameter of *Melissa officinalis* on both epidermis layers.

Figure 19. Cross-section of leaf and main vascular bundle of *Salvia officinalis* L.



(i), non-glandular hairs around main vascular bundle (j), (20X), non-glandular trichome on abaxial layer of epidermis (k), (40X), capitate glandular trichome with two secretory head trichome on adaxial layer of epidermis (l), (40X), ab: abaxial layer, ad: adaxial layer, cu: cuticle, black arrow: peltate glandular trichome; Scale bar: 10µm

5. Conclusion

According to data from Dunkic et al. (2013), on the epidermis of Lamiaceae species, mainly the subtypes of capitate trichomes, such as C1 and C2 can be found. C1 type of capitate trichomes consists of one basal epidermal cell, and one stalk cell, and the elliptically head cell can be found. C2 type of capitate trichomes is composed of one basal epidermal cell, two or three stalk cells, and a single-celled head. However, Dunkic et al. (2013) state that the C1 type of capitate trichomes are not very common in the Lamiaceae species. Based on the information discussed above, we can observe that the capitate glandular trichome mainly consists of the Subtype II/a. group (globose unicellular/bicellular, stalk of one to four cells) in case of examined species. Dunkić et al. (2001, 2007) also conclude that mainly the existence of peltate glandular trichomes is

common in Lamiaceae species. They stated, however, that the abundance of peltate-type glandular trichomes is different on adaxial and abaxial layers of the epidermis. A large percentage of these trichomes have one head cell. According to previous data of Ghonam et al. (2014), the abundance of peltate trichomes on leaves of *Lavandula hybrid*, *Mentha aquatic*, *Mentha citrate*, *Mentha longifolia*, *Mentha spicata*, *Ocimum basilicum*, *Origanum majorana*, *Rosmarinus officinalis*, *Salvia elegans*, *Salvia farinacea*, *Salvia splendens*, *Thymus capitatus*, and *Thymus vulgaris*, is higher, compared to non-glandular and capitate trichomes. They also state that the *Salvia splendens* has peltate trichomes of larger size, while *Thymus vulgaris* has smaller peltate trichomes. Compared to the above, we can establish that more capitate glandular trichomes can be found in both epidermis layers than peltates in the case of the species we examined. We found that the *Thymus vulgaris* has the largest peltates on both epidermis, whereas the other examined species have smaller peltates. Based on the diameter of peltates on the adaxial epidermis, we can state the following order: *Melissa officinalis*, *Rosmarinus officinalis*, *Salvia officinalis*, *Mentha x piperita*, *Lavandula officinalis*, and *Thymus vulgaris*. On the abaxial epidermis layer, the order is the next: *M. officinalis*, *M. piperita*, *S. officinalis*, *R. officinalis*, *L. officinalis*, and *Thymus vulgaris*.

In the case of *Mentha X piperita*, our results were similar to the results of Choi and Kim (2013) and Yu et al. (2018). They also found the number of peltate trichomes to be less in *Thymus vulgaris* compared to *Rosmarinus officinalis* and *Lavandula officinalis*. However, the diameter of the peltate trichomes is the highest on the abaxial layer in the case of the leaf of *Thymus vulgaris*, compared to other species we examined. We can conclude that in the case of *Mentha x piperita* the abundance of peltate trichomes is higher on the adaxial layer of leaf lamina compared to the abaxial layer. Opposed to our results, Choi and Kim (2013) stated that in the case of *Mentha* species, the distribution of peltate trichome on the abaxial layer of the leaf surface is higher compared to the adaxial layer. Choi and Kim (2013) also established, contrary to our results, that the capitate type of glandular trichomes is commonly found on the abaxial layer of the leaf. Similarly to our observations, Yu et al. (2018) concluded that in some *Mentha* species, the density of peltate and capitate trichomes on both sides of the leaf epidermis layers is high, and the diameter of peltate trichomes is higher on the adaxial layer than on the abaxial layer of leaf lamina.

Regarding the results of Marin et al. (2006), we can similarly conclude that in *Rosmarinus officinalis* the peltate glandular trichomes are predominantly located on the abaxial surface of the leaf. In our examination, the same result was obtained, but the diameter of the peltate glandular trichomes was found to be different in both leaf epidermis layers, and the diameter of the peltate glandular trichomes was measured to be higher in the abaxial layer of the epidermis.

According to Chwil et al. (2016), mainly non-glandular trichomes can be found on both surfaces of the *Melissa officinalis* leaf. Therefore, peltate trichomes were dominantly observed on the adaxial layer of the epidermis in *Melissa officinalis*. Based on their results, the diameter of peltate trichomes is around 56.78-80.16 μm , the mean is 67.54 μm and there were no significant differences between the size of the diameter of peltates on adaxial layer.

Boix et al. (2011) examined the dense coverage of peltate and capitate glandular trichomes on *Rosmarinus officinalis* leaves. They stated that on the leaves of *R. officinalis*, trichomes of one peltate and three subtypes of capitate were seen. As a result of their examination, Svidenko et al. (2018) conclude that the size of the trichomes is different on each side of the leaf. Nevertheless, they conclude that the primary component of volatile chemicals is located in the peltate trichomes. The results of González-Minero et al. (2020) show that numerous glandular (capitate and peltate) and non-glandular trichomes are present in the adaxial layer of the epidermis. On the adaxial layer, according to Boix et al. (2011), capitate trichomes can be found which have long and short stalk cells. On both surfaces of the leaf, capitate trichomes were dispersed randomly, but they were more common than peltate glandular trichomes. They noticed that the abaxial leaf surface grooves had a higher density of non-glandular trichomes. On the abaxial epidermis, peltate, capitate, and non-glandular trichomes are present (Boix et al., 2011). By comparing our results to the above, we can state that the distribution of capitate glandular trichomes is random, and they are present in a higher number than peltate-type trichomes.

Kowalski et al. (2019) examined some species from the Lamiaceae family and established that the abaxial layer of the epidermis has larger peltate glandular trichome diameters than the adaxial layer. In contrast to peltate glandular trichomes, capitate trichomes were dispersed more widely on the epidermis, per Kowalski et al. (2019). They also stated that more glandular trichomes were found on the leaves than on the stem. Based on their research, the adaxial side of the epidermis of *Mentha piperita* has peltate trichomes with the largest diameters (average diameter of peltate trichomes – 78.48 μm on the adaxial side of the leaf, up to 96.43 μm). In contrast to these findings, we concluded that the *Thymus vulgaris* has peltate trichomes with the biggest diameter on the epidermis adaxial layer. Additionally, their findings show that the peltate and capitate trichomes of lemon balm (*Melissa officinalis*) have the smallest diameters. In *Melissa officinalis*, our results are the same in terms of peltate diameter in the abaxial layer of the epidermis. They concluded that there were many trichomes in the epidermis of the abaxial leaf of lemon balm and the diameter of peltate glandular trichomes was the smallest, ranging from 44.88 to 74.36 μm . Their results also showed that the greatest density of glandular trichomes characterized the lemon balm compared to other plant species. To

compare this result to ours, we can state that in the case of *Melissa officinalis* on the abaxial layer larger peltate trichome diameter can be found than on the adaxial layer of the leaf. Additionally, they found that when comparing lemon balm to other Lamiaceae species, it had the highest density of glandular trichomes in the case of that species. According to their findings, the adaxial side of the peppermint (*Mentha x piperita*) leaf has the biggest diameter structures. Svidenko et al. (2018) also established that on the adaxial layer of the leaf of *Thymus vulgaris* has more peltate glandular trichomes. Our data is similar to this conclusion, and there are no statistically significant differences. They also stated that the diameter of peltate glandular trichomes is larger on the abaxial layer of the sage leaf, we can confirm this establishment. According to our data, it was reported that the diameter of peltate glandular trichomes of *S. officinalis* is min 40.1 μm and max 77.54 μm on the adaxial layer of the leaf, and the mean 58.36 ± 16.78 been reported. On the contrary, according to the results, the diameter of peltate min 58.97 μm and max 69.5 μm , and the mean was 65.10 ± 5.5 on the abaxial layer of the leaf reported.

6. Summary

Six field-grown therapeutic plants which belong to the Lamiaceae family like peppermint (*Mentha x piperita* L.), lemon balm (*Melissa officinalis* L.), thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), and lavender (*Lavandula officinalis* L.) were examined to study the microanatomical features associated with the glandular trichomes that store essential oils. For each species, samples were created with cross-sections from the stems and leaves. This allowed us to identify the non-glandular and two types of glandular trichomes (peltate and capitate glandular trichomes) in addition to determining the density of trichomes. In the case of sage (*Salvia officinalis*), we can conclude that the glandular trichomes predominate on the adaxial leaf epidermis. However, lemon balm (*Melissa officinalis*) and lavender (*Lavandula officinalis*) displayed particularly large glandular trichomes counts on both the upper and lower leaf surfaces. We also can determine that the mint (*Mentha x piperita*) and thyme (*Thymus vulgaris*) have a greater number of glandular trichomes than other species. Additionally, in all of the species we examined, the volume and density of capitate trichomes were greater than that of peltate trichomes. It became clear that the glandular trichomes were distributed independently on leaf surfaces. Particularly, mint, thyme, and lavender all had bigger peltate glandular trichomes that store essential oils that could be identified under a microscope. Additionally, differences in trichome density and morphology between the adaxial and abaxial leaf surfaces were noted, which helped to clarify the complex microanatomical characteristics of

the trichomes, which are glandular structures that store essential oils in these therapeutic plants.

REFERENCES

- Baran, P., Aktaş, K., Özdemir, C. 2010. Structural investigation of the glandular trichomes of endemic *Salvia smyrnea* L. In *South African Journal of Botany*, vol. 76, p. 572-578. <https://doi.org/10.1016/j.sajb.2010.04.011>
- Balcke, G. U., Bennewitz, S., Bergau, N., Athmer, B., Henning, A., Majovsky, P., Jiménez-Gómez, J. M., Hoehenwarter, W., Tissier, A. 2017. Multiomics glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites. *The Plant cell*, 29(5), 960-983. <https://doi.org/10.1105/tpc.17.00060>
- Barykina, R.P. 2004. *Guide on Botanical Microtechnique; Base and Methods*; MSU: Moscow, Russia; p. 312.
- Boix, Y., Fung, Y., Victório, C.P., Defaveri, A.C.A., Arruda, R.D.C.O., Sato, A., & Lage, C.L.S. 2011. Glandular trichomes of *Rosmarinus officinalis* L.: Anatomical and phytochemical analyses of leaf volatiles, 1-9. <https://doi.org/10.1080/11263504.2011.584075>
- Bräuchler, C., Meimberg, H., Heubl, G. 2010. Molecular phylogeny of Menthinaceae (Lamiaceae, Nepetoideae, Mentheae) - Taxonomy, biogeography and conflicts, *Mol. Phylogenet. Evol.*, 55, 501-523. <https://doi.org/10.1016/j.ympev.2010.01.016>
- Cantino, P.D. 1990. The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae, *J Arnold Arbor.*, 71(3), 323-370. <https://doi.org/10.5962/p.184532>
- Carović-Stanko, K., Petek, M., Grdiša, M., Pintar, J., Be-deković, D., Herak Čustić, M., Sato-vic, Z. 2016. Medicinal plants of the family Lamiaceae as functional foods a review. *Czech J. Food Sci.*, 34(5), 377-390. <https://doi.org/10.17221/504/2015-CJFS>
- Choi, J.S., Kim, E.S. 2013. Structural features of glandular and non-glandular trichomes in three species of *Mentha*. 43(2). <https://doi.org/10.9729/AM.2013.43.2.47>
- Chwil, M., Nurzyńska-Wierdak, R., Chwil, S., Matraszek, R., & Neugebauerová, J. 2016. Histochemistry and micromorphological diversity of glandular trichomes in *Melissa officinalis* L. leaf epidermis. *Acta Scientiarum Polonorum-Hortorum Cultus*, 15(3), 153-172.
- Corsi, G., Bottega, S. 1999. Glandular hairs of *Salvia officinalis*: New data on morphology, localization and histochemistry in relation to function, *Annals of Botany*, 84, 657-664. <https://doi.org/10.1006/anbo.1999.0961>
- Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., Mnif, W. 2016. Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines Basel*, 3(4), 25. <https://doi.org/10.3390/medicines3040025>
- DunkiĆ, V., Bezić, N., Mileta, T. 2001. Xeromorphism of trichomes in Lamiaceae species. *Acta Bot. Croat.* 60, 277-283.
- DunkiĆ, V., Bezić, N., Ljubešić, N., Bočina, I. 2007. Glandular hair ultrastructure and essential oils in *Satureja subspicata* Vis. ssp. *subspicata* and ssp. *liburnica* Šilić. *Acta Biol. Cracov. Ser. Bot.*, 49, 2, 45-51.
- Elagöz, V., Han, S.S., Manning, W.J. 2006. Acquired changes in stomatal characteristics in response to ozone during plant growth and leaf development of bush beans (*Phaseolus vulgaris* L.) indicate phenotypic plasticity. *Environ. Poll.* 140, 395-405. <https://doi.org/10.1016/j.envpol.2005.08.024>
- Fahn, A. 1988. Secretory tissues in vascular plants. *New Phytol.* 108, 229-257. <https://doi.org/10.1111/j.1469-8137.1988.tb04159.x>
- Fahn, A. 2000. Structure and function of secretory cells. *Advances in Botanical Research*, 31, 37-75. [https://doi.org/10.1016/S0065-2296\(00\)31006-0](https://doi.org/10.1016/S0065-2296(00)31006-0)

- García-Gutiérrez, E., Ortega-Escalona, F., Angeles, G. 2020. A novel, rapid technique for clearing leaf tissues. Applications in Plant Sciences 8(9): e11391. <https://doi.org/10.1002/aps3.11391>
- Gardner, S. D. L., Taylor, G., Bosac, C. 1995. Leaf growth of hybrid poplar following exposure to elevated CO₂. New Phytol. 131. <https://doi.org/10.1111/j.1469-8137.1995.tb03057.x>
- Ghonam, F.M., Turki, Z.A., Azazi, M.F. 2014. Morphological features of glandular and non-glandular trichomes in some species of family Lamiaceae. Journal of Environmental Studies and Researches. 1(1), 37-44.
- Giuliani, C.; Giovanetti, M.; Lupi, D.; Mesiano, M.P.; Barilli, R.; Ascrizzi, R.; Flamini, G.; Fico, G. 2020. Tools to Tie: Flower Characteristics, VOC Emission Profile, and Glandular Trichomes of Two Mexican Salvia Species to Attract Bees. Plants 2020, 9, 1645. <https://doi.org/10.3390/plants9121645>
- González-Minero, F.J., Bravo-Díaz, L., Ayala-Gómez, A. 2020. Rosmarinus officinalis L. (Rosemary): An Ancient Plant with Uses in Personal Healthcare and Cosmetics. Cosmetics, 7(4). <https://doi.org/10.3390/cosmetics7040077>
- Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R.J., Harley, M.M., Kok, R.P.J., de, Krestovskaja, T.V., Morales, R., Paton, A.J., Ryding, P.O. 2004. Labiatae. The Families and Genera of Vascular Plants. 7, 167-275. https://doi.org/10.1007/978-3-642-18617-2_11
- Hilu, K.W.; Randall, J.L. 1984. Convenient method for studying grass leaf epidermis. Taxon, 33, 413-415. <https://doi.org/10.1002/j.1996-8175.1984.tb03896.x>
- Huang, S.S., Kirchoff, B.K., Liao, J.P. 2008. The capitate and peltate glandular trichomes of Lavandula pinnata L. (Lamiaceae): Histochemistry, ultrastructure, and secretion. The Journal of the Torrey Botanical Society, 135(2), 155-167. <https://doi.org/10.3159/07-RA-045.1>
- Husain, S.Z., Marin, P.D., Šilić, Č., Qaser, M., Petković, B. 1990. A micromorphological study of some representative genera in the tribe Saturejeae. Bot. J. Linn. Soc., 103, 59-80. <https://doi.org/10.1111/j.1095-8339.1990.tb00174.x>
- Jachula, J., Konarska, A., Denisow, B. 2018. Micromorphological and histochemical attributes of flowers and floral reward in Linaria vulgaris (Plantaginaceae). Protoplasma 255, 1763-1776. <https://doi.org/10.1007/s00709-018-1269-2>
- Kahraman, A., Celep, F., Dogan, M. 2010. Anatomy, trichome morphology and palynology of Salvia chrysophylla Stapf (Lamiaceae). In South African Journal of Botany, 76(2), 187-195. <https://doi.org/10.1016/j.sajb.2009.10.003>
- Konarska, A., Łotocka, B. 2020. Glandular trichomes of Robinia viscosa Vent. var. hartwigii (Koehne) Ashe (Faboideae, Fabaceae)-morphology, histochemistry and ultra-structure. Planta, 252(6), 102. <https://doi.org/10.1007/s00425-020-03513-z>
- Kondratenko, L. M. 1975. About interconnection between external signs of flower and content of essential oil of thyme ordinary. (O vzaimosvyazi mezhdu vneshnimi priznakami tsvetka i sodержaniem efirnogo masla u timyana obyknovennogo). In Sb. nauch. rabot VNII lek. rast., vol. 8, p. 18.
- Kowalski, R., Kowalska, G., Jankowska, M., Nawrocka, A., Kałwa, K., Pankiewicz, U., Włodarczyk-Stasiak, M. 2019. Secretory structures and essential oil composition of selected industrial species of Lamiaceae. Acta Sci. Pol. Hortorum Cultus, 18(2), 53-69. <https://doi.org/10.24326/asphc.2019.2.6>
- Luo, S. H., Luo, Q., Niu, X. M., Xie, M. J., Zhao, X., Schneider, B., Gershenzon, J., Li, S. H. 2010. Glandular trichomes of Leucosceptrum canum harbor defensive sesquiterpenoids. Angew. Chem. Int. Ed. 49(26), 4471-4475. <https://doi.org/10.1002/anie.201000449>

- Marin, M., Koko, V., Duletić-Laušević, S., Marin, P.D., Rančić, D., Dajic-Stevanovic, Z. 2006. Glandular trichomes on the leaves of *Rosmarinus officinalis*: Morphology, stereology, and histochemistry. *South African Journal of Botany*, 72(3), 378-382, ISSN: 0254-6299. <https://doi.org/10.1016/j.sajb.2005.10.009>
- McCaskill, D., Croteau, R. 1995. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha×piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. *Planta* 197, 49-56. <https://doi.org/10.1007/BF00239938>
- Metcalf, C.R., Chalk, L., 1972. *Anatomy of the Dicotyledons*. Oxford University Press. Oxford. II.
- Navarro, T., El Oualidi, J. 1999. Trichome morphology in *Teucrium* L. (Labiatae), a taxonomic review. *Anales Jardin Botanico de Madrid*, 57, 277-297, ISSN 0211-1322. <https://doi.org/10.3989/ajbm.1999.v57.i2.203>
- Payne, W. W. 1978. A glossary of plant hair terminology. *Brittonia* 30, 239-255. <https://doi.org/10.2307/2806659>
- Salmaki, Y., Zarre, S., Jamzad, Z., Brauchler, C. 2009. Trichome micromorphology of Iranian *Stachys* (Lamiaceae) with emphasis on its systematic implication. *Flora-morphology, distribution, functional ecology of plants*, 204(5), 371-381, ISSN: 0367-2530. <https://doi.org/10.1016/j.flora.2008.11.001>
- Sass, J. E. 1951. *Botanical Microtechnique*, 2nd ed.; Iowa State College Press: Ames, IA, USA. <https://doi.org/10.5962/bhl.title.5706>
- Sharma, K., Dutta, N., Pattanaik, A., Hasan, Q.Z. 2003. Replacement Value of Undecorticated Sunflower Meal as a Supplement for Milk Production by Crossbred Cows and Buffaloes in the Northern Plains of India. *Tropical Animal Health and Production* 35, 131-145. <https://doi.org/10.1023/A:1022873402101>
- Soliman, S.S.M., Abouleish, M., Abou-Hashem, M.M.M., Hamoda, A.M., El-Keblawy, A.A. 2019. Lipophilic Metabolites and Anatomical Acclimatization of *Cleome amblyocarpa* in the Drought and Extra-Water Areas of the Arid Desert of UAE. *Plants (Basel, Switzerland)*, 8(5), 132. <https://doi.org/10.3390/plants8050132>
- Sota, V., Themeli, S., Zekaj, Z., Kongjika, E. 2019. Exogenous cytokinins application induces changes in stomatal and glandular trichomes parameters in rosemary plants regenerated in vitro. *Journal of Microbiology, Biotechnology and Food Sciences*, 9, 25-28. <https://doi.org/10.15414/jmbfs.2019.9.1.25-28>
- Svidenko, L., Grygorieva, O., Vergun, O., Hudz, N., Horčinová Sedláčková V., Šimková, J., Brindza. 2018. Characteristic of leaf peltate glandular trichomes and their variability of some lamiaceae martinov family species. *J. Agr. bio. div. Impr. Nut., Health Life Qual.*, 124-132. <https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.124-132>
- Wagner, G.J. 1991. Secreting glandular trichomes: more than just hairs. *Plant Physiology*, 96(3), 675-679. <https://doi.org/10.1104/pp.96.3.675>
- Werker, E., Ravid, U., Putievsky, E. 1985. Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae, Israel J. Bot., 34, 31-45, Corpus ID: 85614848. DOI: 10.1080/0021213X.1985.10677007.
- Yu, X., Liang, C., Fang, H., Qi, X., Li, W., Shang, Q. 2018. Variation of trichome morphology and essential oil composition of seven *Mentha* species. *Biochemical Systematics and Ecology*, 79, 30-36, ISSN: 0305-1978. <https://doi.org/10.1016/j.bse.2018.04.016>

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