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SECRETORY STRUCTURES AND ESSENTIAL OIL COMPOSITION OF SELECTED INDUSTRIAL SPECIES OF LAMIACEAE

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ABSTRACT

The objective of the study was to perform micromorphological analyses of the secretory structures of leaves and stems of oil-bearing industrial plants from the Lamiaceae family such as lemon balm (*Melissa officinalis* L.), peppermint (*Mentha* × *piperita* L.), sage (*Salvia officinalis* L.), marjoram (*Origanum majorana* L., syn. *Origanum dubium* Boiss.), rosemary (*Rosmarinus officinalis* L.) and common thyme (*Thymus vulgaris* L.) using light microscope and scanning electron microscope. In addition, an estimation of the content of volatile substances in the plant species under study was performed using GC-MS, as well as the qualitative and quantitative analysis of essential oil, that is an important component in terms of the estimation of raw material applicability for use in the industry. In the epidermal cells of studied plants, 2 types of Lamiaceae-type glandular trichomes were identified: short- and long-stalked capitate glandular trichomes with single- and bicellular secretory capitulum, and peltate glandular trichomes with eight- and over a dozen-cell secretory capitulum. Capitate trichomes were densely distributed on the surface of the epidermis, while peltate trichomes were sparse, though regular, and were situated in depressions. Glandular trichomes were found more frequently on leaves than on stems. The cuticle of the abaxial of leaf was characterized in most cases by the occurrence of larger average diameter peltate trichomes compared to the cuticle of the adaxial side of leaf. Peppermint produced the largest structures accumulating essential oil on the leaves (average diameter of peltate trichomes – 78.48 µm on the adaxial side of leaf, up to 96.43 µm), while on the stem, the highest average diameter of the peltate trichomes was observed in sage (an average of 75.53 µm, up to 85.99 µm). The lemon balm was characterized by the presence of capitate and peltate trichomes with the smallest diameter (an average of 44.26 µm). Lemon balm was characterized by the greatest density of glandular trichomes compared to other plant species. Among the plants studied, the highest content of oil was noted in the case of thyme and peppermint (2.22% and 2.20% v/w, respectively), and the lowest in green parts of lemon balm (0.17% v/w). The isolated essential oils contained predominantly components from the groups of monoterpenes and sesquiterpenes, and it is the presence of those substances that determines the possibility of utilizing the plants studied for a variety of purposes.

Key words: micromorphological analyses, trichomes, Lamiaceae, essentials oil

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INTRODUCTION

Systematics research on active plant substances has been related to the exceptionally rapid development of chemistry. Today, we observe a violent return of interest in natural methods of therapy and herbal medicine enjoys a revival. Moreover, special interest is focused on natural aromatic spices, that are used in our cuisine as well as in the food industry. The applications of plant species with medicinal effects and spice properties result from their content of biologically active substances. In this view, a very important group of compounds are essential oils secreted and accumulated in special glands situated in various plant organs (roots, rhizomes, bulbs, leaves, flowers, fruits, seeds) [Glas et al. 2012, Lange and Turner 2013]. Essential oils are especially abundant in plants from the families Lamiaceae, Apiaceae (Umbelliferae), Asteraceae, Zingiberaceae, Saxifragaceae and Geraniaceae [Glas et al. 2012, Lange and Turner 2013]. They are also common in gymnosperm plants (e.g. conifers). Hence the studies concerning the anatomy and morphology of secretory structures characteristic for the particular botanical families, combined with analysis of the composition of essential oil secreted and accumulated in those structures, are extremely interesting [Glas et al. 2012, Lange and Turner 2013].

More than 7000 species belonging to the Lamiaceae family occur all over the world, especially in the Mediterranean countries [Harley et al. 2004]. The flora of Poland includes over 75 taxons, mainly of herbal plants. In the vegetation cover, they play an important role as utility plants [Bukowiecki and Furmanowa 1972].

Various types, distribution, morphology, and density of glandular trichomes were observed in the Lamiaceae, which can be important taxonomic forms [Huang et al. 2008]. The glandular trichomes are called peltate or capitate, depending on the structure of the secretory head [Huang et al. 2008]. The capitate glandular trichomes have one or two secretory disk cells, while peltate trichomes may have up to eight cells in the disk. The secretory substance is accumulated in a subcuticular space, outside the cell wall. Peltate trichomes produce most of the essential oil [Huang et al. 2008].

Numerous taxa of the Lamiaceae are classified as medicinal herbs and spices including: peppermint (*Mentha* × *piperita* L.), lemon balm (*Melissa officinalis* L.), marjoram (*Origanum majorana* L. syn. *Origanum dubium* Boiss.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), hyssop (*Hyssopus officinalis* L.), basil (*Ocimum basilicum* L.), summer savory (*Satureja hortensis* L.), motherwort (*Leonurus cardiaca* L.), common horehound (*Marrudium vulgare* L.) and others [Carović-Stanko et al. 2016, Bekut et al. 2017]. Species belonging to the Lamiaceae are also planted in gardens for decorative purposes. Examples here can be narrow-leaved lavender (*Lavandula angustifolia* Mill.) and sage (*Salvia officinalis* L). The mentioned species are now mainly crop plants, where the raw material for industry is acquired primarily from specialized cultivations conducted on herb-growing farms. At present, Poland supplies more than 30% of herbal raw materials acquired from European cultivations. Polish agriculture has the largest area of the cultivation of herbal plants in Europe (30,000 ha), with the number of producers fluctuating around 20,000 farms a year [Spychalski 2013].

Species belonging to Lamiaceae are commonly grown all over the world as a source of raw material for the pharmaceutical, food and cosmetics industries [Uritu et al. 2018]. In the assessment of the quality of plant material for industry, various parameters are taken into account, the principal one being the chemical composition. One of the main active substances is the essential oil, the composition of which determines the possibility of using the raw material for various purposes [Sharifi-Rad et al. 2017]. Essential oil is a multi-component mixture of volatile compounds, which can be isolated from plants e.g. through distillation with water vapors [Dhifi et al. 2016]. Essential oils contain from several dozen to several hundred components, among which we can distinguish monoterpenes, sesquiterpenes and phenylpropanes [Dhifi et al. 2016]. Composition of the essential oil depends on the biosynthetic pathway of those components in the plant, i.e. it is a botanical trait, but it is also related to climatic factors, cultivation factors, as well as the conditions or raw material storage or the treatments applied during the processing of plant material [Góra and Lis 2107]. Usually, in the qualitative estimation of the essential oil, the main component or a group of components responsible for the aromatic properties (food industry, cosmetics industry) and medicinal properties (pharmaceutical industry), is identified [Dhifi et al. 2016]. In addition, essential oils may contain components with toxic properties, constituting a threat to human health and even life. The acceptable levels of individual components in the essential oil are often regulated by relevant norms [European Pharmacopoeia VIII 2015, ISO 9909 1997, ISO 856 2006, ISO 1342 2012, ISO 19817 2017] that allow to qualify the essential oil in terms of suitability for processing e.g. in food or pharmaceutical applications. Such norms are especially important in the case of standardization of raw materials originating from various geographic regions, frequently significantly different in the composition of the essential oils. To meet the quality requirements, the essential oil producers search for suitable plant material that contains essential oil of desired composition. Qualitative analyses of the essential oils are usually based only on chromatographic analysis of essential oil composition and, unfortunately, there is a scarcity of interdisciplinary studies in the area of the structure of secretory organs and analysis of chemical composition of volatile compounds produced by those glands. Available literature provides individual studies concerning particular species, without any comparative inter-species approach. In this situation, at the absence of a broader approach to the problem, we have undertaken such a study of the species from the Lamiaceae cultivated for industrial purposes.

The objective of this study was to perform micromorphological analyses of secretory structures of leaves and stems of selected oil-bearing industrial plants from the Lamiaceae (Labiatae). In addition, an estimation of the content of volatile substances in the plant species under study was performed, as well as the qualitative and quantitative analysis of essential oil, which is an important component in terms of the estimation of raw material applicability for use in the industry.

MATERIAL AND METHODS

Plant materials. The experimental material included leaves and stems of particular species of the Lamiaceae family:

– lemon balm (*Melissa officinalis* L.),
– peppermint (*Mentha piperita* L.),
– sage (*Salvia officinalis* L.),
– marjoram (*Origanum majorana* L. syn. *Origanum dubium* Boiss.),
– rosemary (*Rosmarinus officinalis* L.),
– common thyme (*Thymus vulgaris* L.).

The material was collected at the vegetative stage of development, just prior to blooming. All plants originated from the collection of the Botanical Garden of Maria Curie-Skłodowska University, Lublin (51°14'N 22°34'E), where a voucher specimen has been deposited.

Microscopic examinations. Leaves and stalks of plants of particular species were examined using light, fluorescence and scanning electron microscopy.

The surface of freshly gathered fully developed leaves and stems of individual species of Lamiaceae was examined with an Jenaval Contrast light microscope. The structural features of the trichome were examined in semi-permanent glycerine preparations from fresh material, observed with a light microscope. The observations were made on 1 mm^2 areas of the plant organs studied and the diameter of subcuticular cavity of the secretory trichomes (peltate trichome heads) was measured. Results were expressed as mean values calculated from 30 measurements of two leaves from five plants for each species.

Examinations of glandular trichomes with the fluorescence microscope were conducted after prior treatment of the fresh plant specimen with auramine, and then glycerine with water at 1 : 1 ratio. Due to auramine, the lipophilic substances contained in essential oil produced a fluorescence effect (the oil-containing cells of the glands glowed). The tissue samples were examined using a Nikon Eclipse 90i fluorescence microscope (FITC filter, excitation wavelength 465–495 nm, emission wavelength 515–555 nm).

Trichome shape, cuticle sculpture and surface waxes were observed with a Tescan Vega II LMU electron scanning microscope. Leaf fragments were fixed for 12 h at 4°C in 2% glutaraldehyde with 2.5% paraformaldehyde in 0.075 M phosphate buffer, pH 6.8 [Glauert 1974]. Then they were dehydrated in acetone series, critical-point dried in liquid CO_2, and gold-coated with a CS 100 sputter coater.

Distillation of essential oil. Twenty grams of plant material were placed in a round-bottom flask (1000 mL) and 400 mL of distilled water was poured over it – the installation was provided with a reflux condenser. The flask was put into a distillation apparatus (steam distillation method) and distillation was carried out for 3 h from the moment of the distillation of the first drop of essential oil [Polish Pharmacopoeia VIII 2008]. Achieved data of the oil volumes were calculated to initial weight of vegetable material subjected to the distillation process, expressed as a percentage of dry weight [Polish Pharmacopoeia VIII 2008]. The oil obtained was collected in dark-glass vessels, dried using anhydrous sodium sulphate, and stored at below –10°C.

Moreover, essential oils were appropriated for qualitative and quantitative analyses by gas chromatography-mass spectrometry GC-MS and gas chromatography with a flame-ionization detector GC-FID.

GC analysis. Essential oil was diluted 100 times using n-hexane to achieve 1 mL volume, then aliquot of 100 μL of C_{12} and C_{19} as internal standards mixture solution (1 mg/mL in toluene) was added to the diluted oil. Samples prepared in this way were subjected to GC-MS and GC-FID determinations.

GC-MS: ITMS Varian 4000 GC-MS/MS (Varian, USA) equipped with a CP-8410 autoinjector and a 30 m × 0.25 mm VF-5ms column (Varian, USA), film thickness 0.25 μm, carrier gas He 0.5 mL/min, injector and detector temperature were, respectively, at 250 and 200°C, respectively; split ratio 1 : 50; inject volume 5 μL. A temperature gradient was applied (50°C for 1 min, then incremented by 4°C/min to 250°C, 250°C for 10 min); ionisation energy 70 eV; mass range: 40–870 Da; scan time 0.80 s.

GC-FID: GC Varian 3800 (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm DB-5 column (J&W Scientific, USA), film thickness 0.25 μm, carrier gas He 0.5 mL/min, injector and detector FID temperatures were 260°C; split ratio 1 : 100; inject volume 5 μL. A temperature gradient was applied (50°C for 1 min, then incremented by 4°C/min to 250°C, 250°C for 10 min).

Qualitative analysis. The qualitative analysis was carried out on the basis of MS spectra, that were compared with those of the NIST library [2005] and with data available in literature [Joulain and König 1998, Adams 2004]. Identity of the compounds was confirmed by their retention indices [Van Den Dool and Kratz 1963] taken from literature [Joulain and König 1998, Adams 2004], and own data for standards (*p*-cymene, γ-terpinene, linalool, thymol, carvacrol, E-caryophyllene, caryophyllene oxide, 1,8-cineole, limonene, menhtone, menthol from Fluka, Sigma-Aldrich Chemie GmbH, Germany).

Quantitative analysis. Relative percentages of identified compounds were computed from the GC-FID peak area (assuming that the sum of peak areas for all identified constituents is 100%).

Statistical analysis. Data were analyzed by analysis of variance (Duncan's test) at 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.).

RESULTS AND DISCUSSION

The epidermis of tested species from the Lamiaceae family developed mechanical and glandular trichomes (Fig. 1). On the surface of the epidermis covering the leaf blade, different types of secretory trichomes were observed that differed in the structure of the capitulum and stalk. The capitate glandular trichomes had a single- or a bicellular capitulum (Fig. 1 Ic). Microscopic observations revealed that the leaf epidermis also developed trichomes with secretory capitulum composed of over a dozen cells. In most capitate trichomes, a single-celled stalk was observed (Fig. 1 II), but scarce trichomes with extended stalk composed of three cells were also identified (Fig. 1 IV).

The plants studied were characterized by the occurrence of capitate glandular trichomes of various structure, both in terms of shape and the number of cells comprising their capitula and stalks. Metcalfe [1957] demonstrated that the number of cells in the capitulum of peltate trichomes of plants from the Lamiaceae amounts to 16 and more. That diversity of structure of trichomes may constitute an important trait in plant taxonomy. On the surface of epidermis covering the leaf blade of plants under study, several types of trichomes were identified. The peltate glandular trichomes, characteristic of the Lamiaceae, appear on the surface of leaves in the form of large spherical "beads", as opposed to long and sharp-tipped mechanical trichomes [Polish Pharmacopea VI 2002]. They are notably larger than other types of glandular trichomes (Fig. 1 Ia,b; IIa,b; IIIa,b; IVa,b; Va,b; VIa,b). Moreover, capitate trichomes with single- or bicellular secretory capitulum were identified (Fig. 1 Ic). The stalks of trichomes of that type were situated at the level of other cells of the epidermis. Pichersky and Gershenzon [2002] report that peltate trichomes of basil grow in depressions below the level of other epidermis cells – it is a common feature of Labiatae taxa.

Microscopic observations revealed differences in the size and location of glandular trichomes. The presence of structures accumulating essential oil was noted on the adaxial and abaxial epidermis of the leaf and on epidermis of the stem. Variation in the diameter of peltate glandular trichomes is presented in Table 1.

Cells of the adaxial and abaxial epidermis of lemon balm leaves were with sinuous walls (Fig. 1 Ia,b). Characteristic Lamiaceae-type glandular trichomes (Fig. 1 Ia) occurred in depressions, mainly on abaxial epidermis. On both sides of leaf blade, but more often on the adaxial surface, there occurred short single- or bicellular conical glandular trichomes (Fig. 1 Ib). The capitate trichomes had single- or bicellular capitula and single- or multicellular stems (Fig. 1 Ic). Epidermis of the abaxial leaf side of lemon balm (*M. officinalis*) was abundant in trichome-type secretory structures with diameters in the range from 44.88 µm to 74.86 µm. In the case of epidermis of the upper surface of leaf, smaller trichomes were observed than those described above, with average diameter of 34.17 µm, while the stem epidermis was characterized by trichomes with mean diameter of 43.48 µm. Lemon balm was characterized by the presence of trichomes with the smallest diameter (an average of 34.17 µm) and by the greatest density of glandular trichomes compared to other plant species.

Turner et al. [2000] reported that in epidermis covering the leaves of peppermint, glandular trichomes with unicellular stalks were identified, which is supported by our observations (Fig. 1 IIc). In addition, those authors observed eight-celled secretory capitula. Cells of the adaxial and abaxial epidermis of peppermint leaves were with sinuous walls. On both adaxial and abaxial epidermis of leaves of peppermint, there occurred characteristic Lamiaceae-type peltate glandular trichomes (Fig. 1 IIa,b), most often in depressions on the leaf blade, and capitate trichomes with unicellular capitulum and single- or multicellular stalk. According to the Polish Pharmacopeia VI [2002], on leaf veins there appear capitulum-less thin-walled multicellular trichomes, composed of eight-to-fifteen cells, and on the edge of the leaf blade – monocellular trichomes. The trichomes on peppermint stem displayed a similarity to those on the leaf, but they appeared less frequently. Glandular trichomes of peppermint appeared on its leaves and stems, and the largest ones were formed on the epidermis of the upper side of leaf, with diameters of 78.48 µm. Notable smaller trichomes were observed on the lower side of leaf – mean diameters of 66.48 µm. Glandular trichomes on the stem were characterized by mean diameter of 66.86 µm. Peppermint produced the largest structures accumulating essential oil on leaves (an average of 78.48 µm on the adaxial side of leaf, up to 96.43 µm), while on the stems, the highest average diameter of secretory trichomes were observed in sage (an average of 75.53 µm, up to 85.99 µm).

According to Serrato-Valenti et al. [1997], on the surface of *Salvia aurea* epidermis, there were diverse glandular trichomes with two- and eight-celled capitula set on single- or multi-celled stalks. As reported by Tirillini et al. [1999], salvia capitate glandular trichomes and eight-celled peltate glandular trichomes were sampled randomly on both the abaxial and adaxial leaf surfaces. In *S. officinalis*, one-to-four-celled peltate glandular trichomes were not present in adult leaves [Tirillini et al. 1999]. Cells of adaxial epidermis of sage leaf were straight- and thick-walled, and those of abaxial epidermis – undulating and thin-walled. On both sides of the leaf blade of sage, there appeared Lamiaceae-type glandular trichomes: capitate trichomes and capitulum-less trichomes. Peltate glandular trichomes of Lamiaceae type (Fig. 1 IVa) were not indented into the epidermis. Capitate trichomes (white arrows) had stalks built of one to four cells and a small single-celled capitulum (Fig. 1 IVc). It was noted that the largest trichomes appeared on stem epidermis (68.07–85.99 µm). In the case of trichomes on the stem, the glands of the largest mean diameter (average 75.53 µm) were found in sage as compared to other plants.

On the leaves of marjoram (*O. majorana*), distribution of glandular trichomes was non-uniform (Fig. 1 VIa,b,c). They were the most abundant on the youngest leaves and close to the leaf petiole. On the abaxial side, the number of trichomes was similar as on the adaxial side (Tab. 1). The multicellular basal stem was short and had a spherical bicellular capitulum [Svoboda and Svoboda 2000]. On the stems, glandular trichomes were less abundant compared to the leaves [Svoboda and Svoboda 2000]. The glandular trichomes observed on all plant organs studied were of similar diameters (from a mean of 50.14 µm for the stem to a mean of 54.45 µm for the upper side of the leaf). Pimple et al. [2012] reported that both surfaces showed presence of numerous covering trichomes, diacytic stomata and thin walled, wavy epidermal cells. The covering trichomes were multicellular, uniseriate, thin walled and pointed.

In rosemary (*R. officinalis*), numerous multicellular and highly branched covering trichomes (Fig. 1 IIIa) appeared on the abaxial leaf side, while on the adaxial side, sparse conical trichomes were observed. There were two types of glandular trichomes. Peltate trichomes had short monocellular stem and a capitulum built of eight radially-arranged cells. Capitate trichomes with a monocellular stem and a spherical single- or bicellular capitulum were less numerous (Fig. 1 IIIc). Sagawa et al. [2013] observed peltate glandular trichomes on leaves and stems of fresh rosemary. Peltate glandular trichomes were distributed widely over the stems, and on the upper and lower surfaces of leaves [Sagawa et al. 2013], which it was confirmed in the study. Choi et al. [2011] showed that the rosemary leaves were characterized by an abundance of two types of glandular trichomes – small capitate and large peltate glandular trichomes. In addition to glandular trichomes, numerous non-glandular trichomes were present on the abaxial surface of the leaf. These trichomes mainly predominated on the midrib, whereas glandular trichomes occurred on non-vein area, which was confirmed also in the study presented here. Secretory structures of rosemary were the most abundant on the adaxial side of leaf, where they attained diameters up to 72.15 µm. On the abaxial side of the leaf, secretory structures with an average diameter of 74.19 µm were observed, while on stem epidermis, the mean diameter of glandular trichomes was 61.49 µm.

Table 1. Diameter and density of the peltate glandular trichomes on the leaves and stems of plant species of the Lamiaceae

Species	The diameter of trichomes (range) (µm)				Trichome density (no./mm^2)	
	abaxial leaf side	adaxial leaf side	stem	mean	abaxial leaf side	adaxial leaf side
M. officinalis	55.13CDa (44.88–74.86)	34.17Db (33.30–35.03)	43.48Dab (37.70–66.02)	44.26	87–136	100–165
M. piperita	66.48BCb (50.24–84.35)	78.48Aa (64.54–96.43)	66.86ABab (50.18–89.82)	70.61	6–40	11–17
S. officinalis	58.10CDb (36.12–83.91)	59.95Bb (38.85–94.30)	75.53Aa (68.07–85.99)	64.53	22–25	24–42
O. majorana	51.11DEa (27.85–75.39)	54.45BCa (26.92–86.82)	50.14Da (26.12–75.70)	51.90	5–30	4–43
R. officinalis	74.19Aa (66.83–79.32)	50.90BCb (32.28–72.15)	61.49BCb (56.76–68.34)	62.19	10–37	20–56
Th. vulgaris	44.90Ea (23.59–69.63)	45.97Ca (17.29–77.68)	54.34CDa (28.96–68.07)	48.40	31–130	14–53
Mean	58.32	53.99	58.64	56.98	–	–

Values designated with the same letters (A, B, C, D…) within columns do not significantly differ at $P < 0.05$ (Duncan's test) Values designated with the same letters (a, b) within rows do not significantly differ at $P < 0.05$ (Duncan's test)

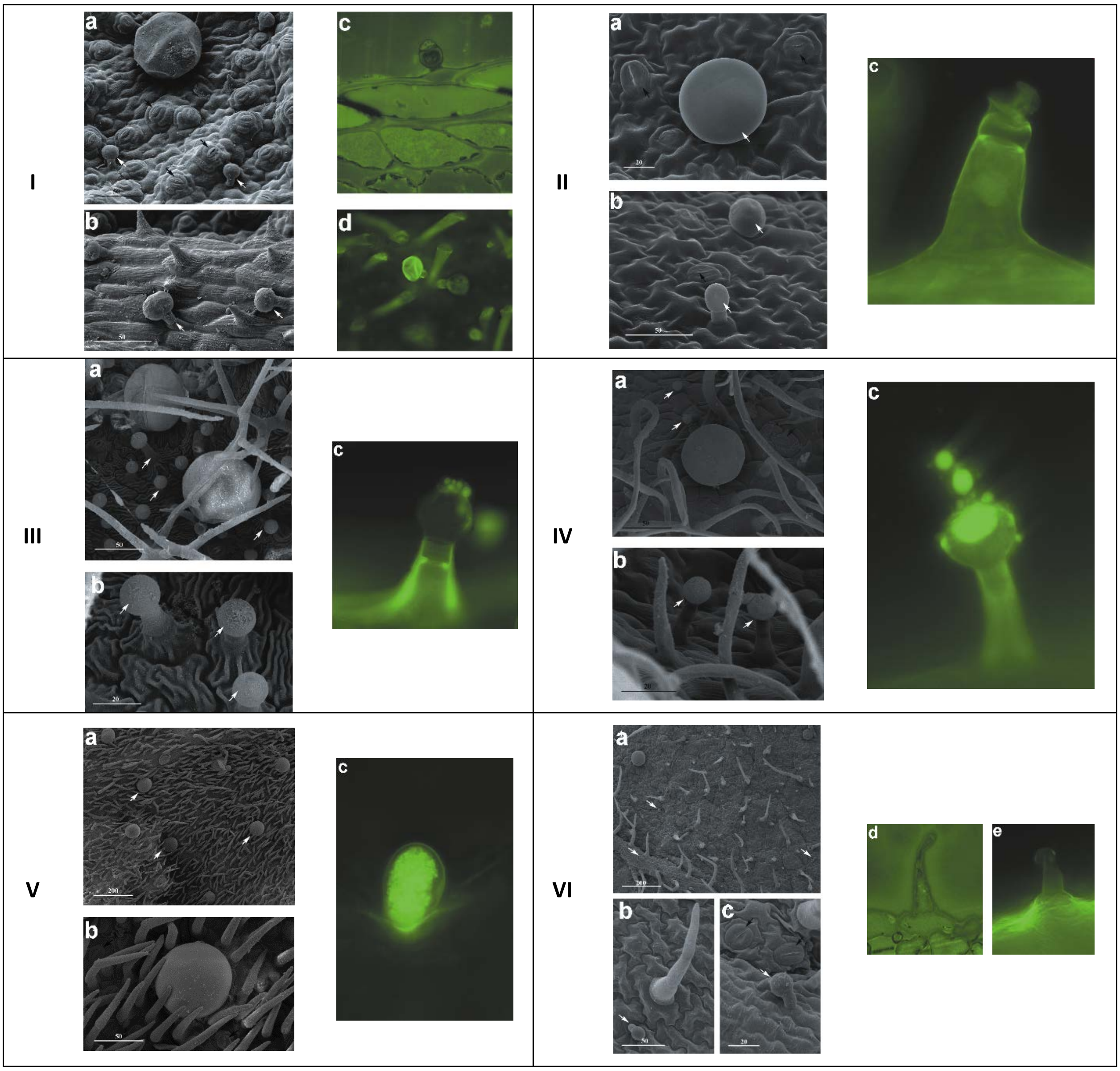

Figure 1. Scanning electron micrographs (SEMs) of leaf trichomes and fluorescence micrographs (FMs) of free-hand cross-sections of leaf and stem trichomes of selected commercially important aromatic plants of the Lamiaceae

I. Lemon balm (*M. officinalis*): a, b – abaxial surface of leaf, showing peltate and capitate (*white arrows*) glandular trichomes (SEM, scale bars: 50 µm); c – cross-section of a leaf with capitate trichome (FM, 40×), d – trichomes on adaxial side of leaf (FM, 20×)
II. Peppermint (*M. piperita*): a – peltate glandular trichomes (white arrow) on abaxial surface of leaf, b – adaxial surface of leaf with peltate and capitate glandular trichomes (white arrows) (SEM, scale bars: 20 and 50 µm, respectively); c – capitate trichome on adaxial side of leaf epidermis (FM, 40×)
III. Rosemary (*R. officinalis*): a, b – abaxial surface of leaf (SEM, scale bars: 50 and 20 µm, respectively); note peltate and capitate (white arrows) glandular trichomes, and highly branched non-glandular trichomes in a; c – cross-section of capitate trichome on stem epidermis, with small drops of intensely fluorescent material in the subcuticular space (FM, 40×)
IV. Sage (*S. officinalis*): a – adaxial surface of leaf, b – abaxial side of leaf (SEM, scale bars: 50 and 20 µm, respectively); note peltate and capitate (white arrows) glandular trichomes, and elongated non-glandular trichomes; c – cross-section of a capitate trichome on adaxial side of leaf, showing strong fluorescence on the surface and within the capitulum (FM, 40×)
V. Common thyme (*Th. vulgaris*): a – abaxial surface of leaf showing peltate trichomes (white arrows) and numerous non-glandular trichomes; b – detail of a (SEM, scale bars: 200 and 50 µm, respectively); c – cross-section of trichome capitulum on adaxial side of leaf (FM, 40×)
VI. Marjoram (*O. majorana*): a, c – abaxial surface of leaf, b – adaxial surface of leaf (SEM, scale bars: 200, 50 and 20 µm, respectively); note peltate and capitate (white arrows) glandular trichomes, and uniseriate non-glandular trichomes; d,e – cross-sections of capitate trichome (FM, 40×)
Black arrows in Ia, IIa,b, IVa, VIc denote stomata

On the adaxial surface of leaves of common thyme (*Th. vulgaris*), short conical warty thick-walled monocellular trichomes were observed, while on the edges and on the abaxial side, we observed bi- or tricellular elbow-shaped trichomes, which has also been described in the Polish Pharmacopea VI [2002]. Apart from these, peltate glandular trichomes (Fig. 1 Vb), and capitate trichomes (Fig. 1 Va) with monocellular capitulum and monocellular stem (Fig. 1 Vc) appeared on both leaf sides. Blažeković et al. [2006] observed characteristic warty bicellular (or tricellular) elbow-shaped covering trichomes on the lower surface of the thyme leaf. In our study, the epidermis of common thyme (*Th. vulgaris*) produced glandular trichomes with average diameter of 54.34 µm, while secretory trichomes observed on the abaxial side of thyme leaves had diameters in the range from 23.59 to 69.63 µm. The adaxial side of the leaf was characterized by the smallest glandular trichomes ranging in diameter from 17.29 to 77.68 µm (average 45.97 µm).

Table 2. Content of the essential oil in studied plant species from the Lamiaceae

Species	The yield of essential oil (% v/w)
M. officinalis	0.17 ±0.01e
M. piperita	2.20 ±0.08a
S. officinalis	1.32 ±0.05d
O. majorana	1.25 ±0.05c
R. officinalis	1.99 ±0.08b
Th. vulgaris	2.22 ±0.10a

Values designated with the same letters (a, b, c, d, e) do not significantly differ at $P < 0.05$ error (Duncan's test)

The presence of organs secreting essential oils analyzed with regard to the location of their occurrence, undoubtedly indicates the epidermis of leaf, on which the structures studied were observed in all plant species under study. Glandular trichomes were found more frequently on leaves than on stems. The abaxial side of leaf was characterized in most cases by the occurrence of larger trichomes compared to the upper side of the leaf.

The essential oil yields in studied plant species from the Lamiaceae are presented in Table 2. The highest content of essential oil was noted in thyme (2.22%) and peppermint (2.20%). The lowest levels (0.17%) of volatile substances were characteristic of the leaves of lemon balm.

Table 3. Major compounds of the essential oil from lemon balm (*M. officinalis*) leaves

Compound	RI*	Concentration (%)
α-pinene	937	1.5
δ-2-carene	1002	2.0
citronellal	1153	5.6
neral	1240	16.0
geranial	1272	23.3
bornyl acetate	1291	1.6
geranyl acetate	1382	2.4
β-caryophyllene	1421	2.4
δ-amorphene	1519	1.0
spathulenol	1585	2.1
caryophyllene oxide	1591	15.8
epi-α-cadinol	1645	2.4
epi-α-muurolol	1651	2.4
α-cadinol	1662	4.3
SUM	–	82.8

*RI – retention indices (from temperature-programming), using definition of Van den Dool and Kratz [1963]

Table 4. Major compounds of the essential oil from peppermint (*M. piperita*) leaves

Compound	RI*	Concentration (%)
1,8-cineole	1033	4.5
menthone	1158	33.8
iso-menthone	1168	7.6
neo-menthol	1172	2.4
menthol	1177	28.0
piperitone	1260	1.2
menthyl acetate	1298	9.3
β-caryophyllene	1425	2.7
germacrene D	1494	2.0
SUM	–	91.5

*Explanation as in Table 3

Table 5. Major compounds of the essential oil from sage (*S. officinalis*) leaves

Compound	RI*	Concentration (%)
α-pinene	937	1.3
camphene	946	2.7
β-pinene	973	1.7
1,8-cineole	1029	7.0
cis-thujone	1098	19.3
trans-thujone	1109	2.1
camphor	1141	15.3
borneol	1166	2.7
β-caryophyllene	1421	2.5
α-humulene	1457	7.5
viridiflorol	1596	11.6
humulene epoxide II	1611	1.5
manool	2060	18.9
SUM	–	75.2

*Explanation as in Table 3

The analyzed lemon balm oil (Tab. 3) was characterized by the highest content of geranial (23.3%), followed by neral (ca. 16.0%) and caryophyllene oxide (15.8%). Góra and Lis [2017] reported that the qualitative composition of lemon balm oil is subject to notable variation and that large differences were observed in the levels of main components, which finds support in this study. The authors cited state that the dominant components of lemon balm oil are citronellal (from 0.2%, Cuba, to 46.8%, Poland), neral (from 10%, Lithuania, to 39.3%, India) and geranial (from 6.3%, Poland, to 41.1%, Italy). In lemon balm oils obtained from leaves and green parts of plants grown in Poland, the following main components were assayed: β-caryophyllene (5.88–31.73%), β-caryophyllene oxide (0.01–12.2%), geranial (6.27–32.92%), neral (4.59–17.37%) and citronellal (1.33–15.18%) [Patora et al. 2003]. In essential oils isolated from lemon balm grown in Greece, 45 components were identified, the dominant ones being β-pinene, sabinene, E-caryophyllene and caryophyllene oxide [Basta et al. 2005].

Table 6. Major compounds of the essential oil from marjoram (*O. majorana*)

Compound	RI*	Concentration (%)
α-thujene	926	1.4
α-pinene	937	1.1
sabinene	976	5.8
myrcene	991	2.1
α-terpinene	1019	6.2
p-cymene	1026	1.3
limonene	1032	2.0
β-phellandrene	1033	2.3
γ-terpinene	1061	8.9
cis-sabinene hydrate	1074	5.6
terpinolene	1088	2.7
linalool	1105	3.4
trans-sabinene hydrate	1107	18.3
cis-*p*-menth-2-en-1-ol	1123	1.3
trans-*p*-menth-2-en-1-ol	1146	2.1
terpinen-4-ol	1186	14.8
α-terpineol	1198	5.0
linalyl acetate	1264	3.3
β-caryophyllene	1422	2.6
bicyclogermacrene	1508	1.5
SUM	–	90.2

*Explanation as in Table 3

Table 7. Major compounds of the essential oil from rosemary (*R. officinalis*)

Compound	RI*	Percentage (%)
α-pinene	937	15.4
camphene	953	4.7
β-pinene	980	1.1
myrcene	988	1.6
p-cymene	1025	2.9
limonene	1029	3.4
1,8-cineole	1039	31.3
camphor	1151	10.5
borneol	1176	7.5
terpinen-4-ol	1184	1.3
α-terpineol	1196	4.8
β-caryophyllene	1425	2.6
SUM	–	84.5

*Explanation as in Table 3

Table 8. Major compounds of the essential oil from common thyme (*Th. vulgaris*)

Compound	RI*	Concentration (%)
myrcene	991	1.3
δ-2-carene	1002	1.1
p-cymene	1026	24.3
γ-terpinene	1063	3.9
linalool	1105	2.0
borneol	1176	1.3
thymol	1299	51.7
carvacrol	1308	3.4
β-caryophyllene	1423	2.1
SUM	–	91.1

*Explanation as in Table 3

As it results from our own analyses, the main components of mint oil (Tab. 4) include menthone (33.8%) and menthol (28.0%) and, at lower content levels, menthyl acetate (9.3%) and isomenthone (7.6%). Góra and Lis [2017] reported that up till now, approximately 300 components of peppermint essential oil have been identified, the main ones being menthol (from 24.1%, France, to 59.4%, Russia) and menthone (from 4.5%, France, to 32.1%, Poland). The following components have been identified in *M. piperita* essential oil: menthol (33–60%), menthone (15–32%), isomenthone (2–8%), 1,8-cineole (5–13%), menthyl acetate (2–11%), menthofuran (1–10%), limonene (1–7%), β-myrcene (0.1–1.7%), β-caryophyllene (2–4%), pulegone (0.5–1.6%) and carvone (1%) [McKay and Blumberg 2006]. In the essential oil obtained from Polish mint, Freire et al. [2012] identified the following main components: menthol (54.2%), menthone (7.3%), neomenthol (6.3%), carvone (5.0%) and menthyl acetate (4.0%).

According to Ożarowski and Jaroniewski [1989], the most valuable component of the essential oil from sage is *cis*-thujone. In our study, *cis*-thujone was the dominant component (19.30%) in the essential oil distilled from sage (Tab. 5). Among the main components of sage oil, we should mention manool (18.9%), camphor (15.3%), viridiflorol (11.6%), α-humulene (7.5%) and 1,8-cineole (7.0%). Kowalski and Wawrzykowski [2009b] report that the main components of sage oil were manool (17.15%), viridiflorol (14.39%), borneol (7.75%), camphor (13.60%), *cis*-thujone (6.66%), and *trans*-thujone (3.26%). Góra and Lis [2017] report that sage oil is characterized by the occurrence of the following main components: *cis*-thujone (from 13.9%, USA, to 45.8%, Italy) and camphor (from 1.9%, USA, to 22.9%, Turkey).

In essential oil from marjoram (Tab. 6), the dominant component was *trans*-sabinene hydrate (18.3%). In addition, the main components of that oil included terpinene-4-ol (14.8%) and γ-terpinene (8.9%). As reported by Vági et al. [2005], the essential oil obtained by steam distillation contains mainly terpinene-4-ol (>20%), which along with (+)-*cis*-sabinene hydrate (3–18%), is responsible for the characteristic flavor and fragrance of marjoram oil. In addition to these compounds, α- and γ-terpinene and terpinolene were other major components [Vági et al. 2005]. The oil composition of *O. majorana* growing in India showed terpinene-4-ol (31.15%), *cis*-sabinene hydrate (15.76%), *p*-cymene (6.83%), sabinene (6.91%), *trans*-sabinene hydrate (3.86%) and α-terpineol (3.71%) as the main constituents [Raina and Negi 2012]. The main compounds of essential oil of marjoram are the epimeric monoterpene alcohols: *trans*-sabinene hydrate, *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate [Novak et al. 2000]; *cis*-Sabinene hydrate is an intensively spicy and marjoramy aroma compound, whereas *trans*-sabinene hydrate has no typical and marjoramy properties [Lossner 1968, Oberdieck 1981, Franz 1990]. Góra and Lis [2017] reported that marjoram oil was characterized by the occurrence of the following main components: terpinene-4-ol (from 7.3%, Germany, to 51.7%, Finland), *cis*-sabinene hydrate (from 0.4%, France, to 44.2%, Turkey), and linalool (from 1.6%, Turkey, to 41.6%, Finland).

The main component in the essential oil obtained from rosemary was 1,8-cineole, with the content of 31.3% (Tab. 7). The dominant components of the oil include α-pinene (15.4%), camphor (10.5%) and borneol (7.5%). Zaouali et al. [2013] assayed the following main components in the composition of rosemary oil: 1,8-cineole (35.80%), camphor (14.50%), α-pinene (10.10%) and borneol (9.20%). Góra and Lis [2017] report that rosemary oil is characterized by the occurrence of the following main components: 1,8-cyneol (from 3.4%, Corsica, to 52.4%, Algeria), camphor (from 2.9%, Corsica, to 33.6%, Algeria), and α-pinene (from 3.4%, Algeria, to 37.2%, Italy).

The dominant components of thyme oil were thymol (51.71%) and *p*-cymene (24.30%), whereas γ-terpinene and carvacrol were less abundant (3.9% and 3.5%, respectively; Tab. 8). Kowalski and Wawrzykowski [2009a] obtained similar results: thymol (54.5–55.2%), *p*-cymene (18.0–21.0%), γ-terpinene (4.3–7.3%) and carvacrol (2.9–3.2%). Lis [2017] reported that thyme oil was characterized by the occurrence of the following main components: thymol (from 16.6%, Italy, to 83.2%, Finland), *p*-cymene (from 9.7%, Finland, to 36.4%, Poland), and *γ*-terpinene (from 0.1%, Germany, to 12.3%, Italy).

Due to the high diversity of composition of the essential oil in individual species of plants from the Lamiaceae, in the estimation of the essential oil quality, limit reference values are used, given in relevant norms [European Pharmacopoeia VIII 2015, ISO 9909 1997, ISO 856 2006, ISO 1342 2012, ISO 19817 2017]. In the case of non-conformance of the analyzed oil with the normative values in force, the composition of a given essential oil can be modified through mixing with other essential oils from the same species, thus obtaining a desired quality and quantity profile, which allows the application of the essential oil for industrial purposes. Figure 2 presents the main profiles of the essential oils obtained from the species of the experiment presented herein, in comparison to the literature data and to the relevant normative requirements.

The research presented here as well as the literature data indicate that monoterpenes are the dominant group of compounds in the essential oils from the studied group of Lamiaceae family plants (Fig. 3). Biosynthesis of terpenes is often limited to specialized morphological structures such as idioblasts (e.g. oil cells of *Laurus* sp.), or secretory ducts (e.g. resin ducts of *Pinus* sp.) or secretory trichomes (e.g. glandular trichomes in Lamiaceae and Asteraceae) that can accumulate those lipophilic substances at high concentrations [Fahn 1988]. Studies on Lamiaceae species such as mint (*Mentha* sp.) and basil (*Ocimum basilicum* L.) provided a lot of information on the biosynthesis of essential oils. In both species (mint and basil), the essential oil is produced in secretory trichomes distributed on the aboveground parts of plants. Those secretory trichomes are built of a group of secretory cells covering a subcuticular storage cavity, where the essential oil accumulates (Fig. 4) [Gershenzon et al. 1989, Turner et al. 1999].

The flavor, taste and pharmaceutical properties of plants from the Lamiaceae result from the presence of essential oil that is composed mainly of monoterpenes and sesquiterpenes [Skoula and Harborne 2002, Stahl-Biskup 2002]. The essential oils, they secrete, are used in the production of perfumes, cosmetics, and also for medicinal and pharmaceutical purposes, e.g. as antibacterial or antiseptic preparations [Kintzios 2002, Stahl-Biskup 2002]. Mono- and sesquiterpenes, as substances from the group of secondary metabolites, play the role of a defensive mechanism of a plant against the attack of various pathogens or herbivorous insects [Gershenzon and Dudareva 2007].

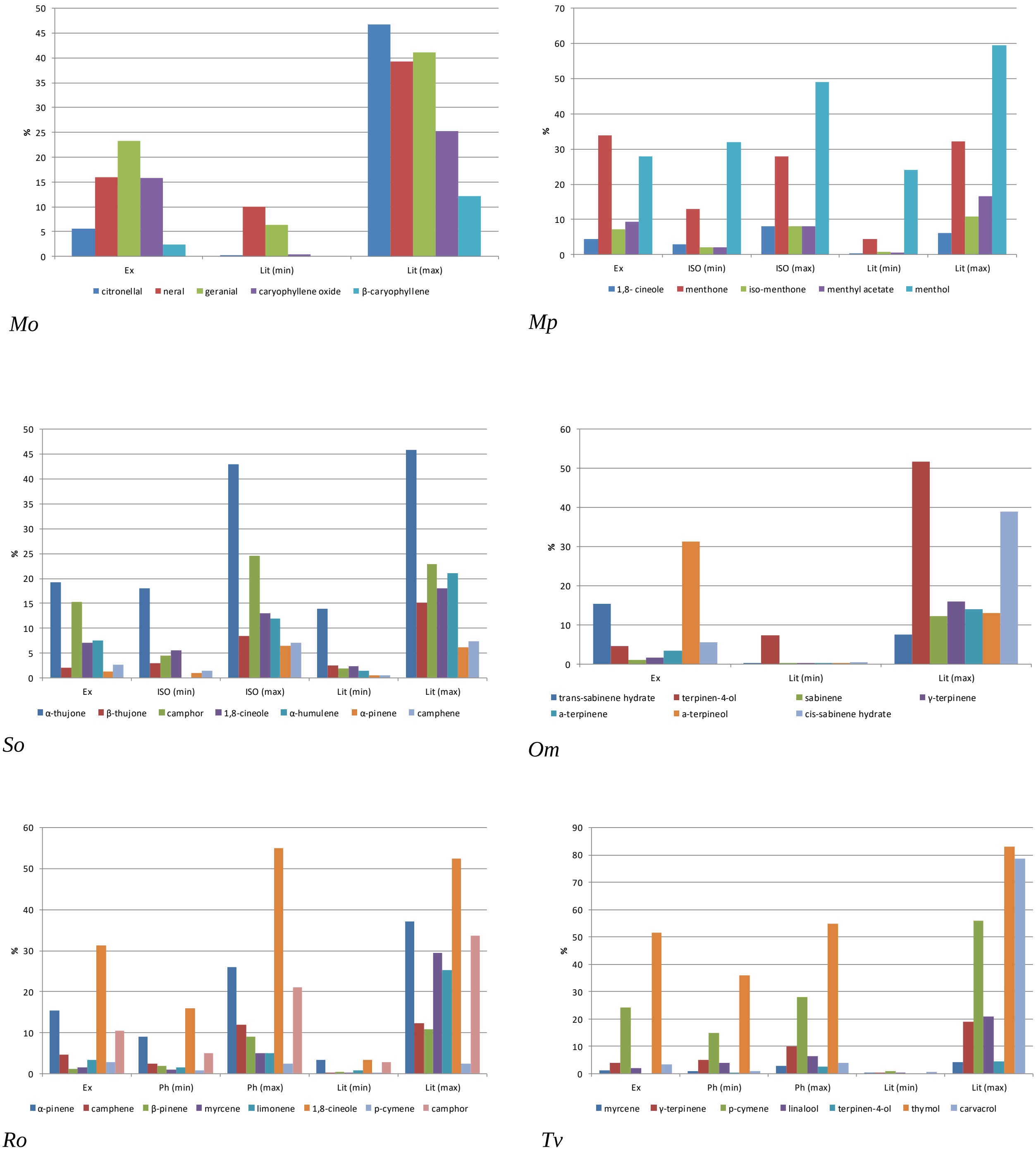

Figure 2. Profiles of the major compounds of the essential oils for the tested Lamiaceae species in comparison to literature data [Góra and Lis 2017, Lis 2017] and to the relevant normative requirements [European Pharmacopoeia VIII 2015, ISO 9909 1997, ISO 856 2006, ISO 1342 2012, ISO 19817 2017]

M. officinalis (*Mo*), *M. piperita* (*Mp*), *S. officinalis* (*So*), *O. majorana* (*Om*), *R. officinalis* (*Ro*), *Th. vulgaris* (*Tv*)
Ex – experimental data, Lit – literature data, Ph – pharmacopoeial standards, ISO – ISO standard, min – minimum percentage, max – maximum percentage

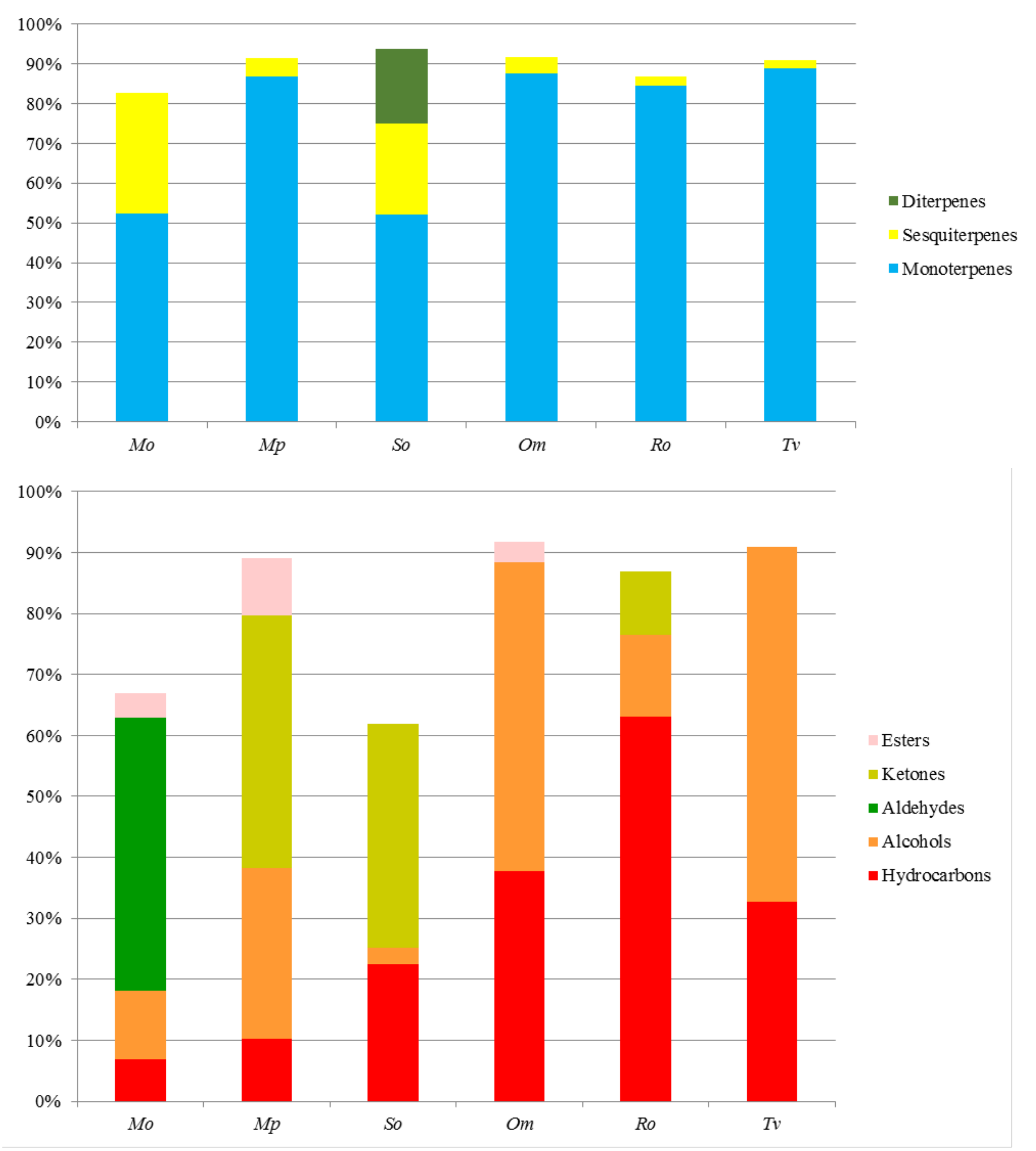

Figure 3. Profile of major groups of chemical constituents of essential oils for the tested Lamiaceae species: *M. officinalis* (*Mo*), *M. piperita* (*Mp*), *S. officinalis* (*So*), *O. majorana* (*Om*), *R. officinalis* (*Ro*), *Th. vulgaris* (*Tv*)

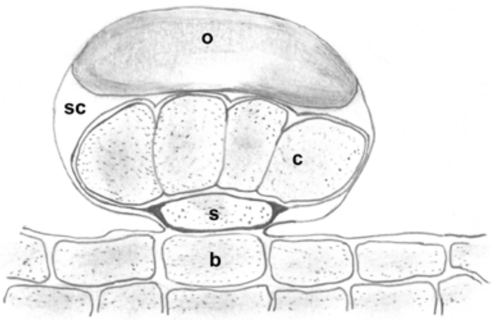

Figure 4. Spherical glandular trichomes of Lamiaceae type. Schematic drawing of spherical trichome built of basal cell (b) and secretory cells (c) comprising capitulum based on one stalk cell (s). Secreted terpenes are accumulated in the subcuticular cavity (sc) in the form of oil drop (o). Modified after Fahn [1988]

CONCLUSIONS

The following conclusions result from the conducted research:

1. In the epidermis of the studied plants, two types of Lamiaceae-type glandular trichomes were identified: capitate glandular trichomes with single- and bicellular secretory capitulum, and peltate glandular trichomes.

2. Capitate trichomes were densely distributed on the surface of epidermis, while peltate trichomes were sparse, though regular, and were situated in depressions.

3. Glandular trichomes were found more frequently on leaves than on stems. The cuticle of the lower leaf side was characterized in most cases by the occurrence of larger trichomes compared to the upper side of leaf. Peppermint produced the largest structures accumulating essential oil on the leaves, while on the stems, the greatest average diameter of secretory trichomes was observed in sage. Lemon balm was characterized by the presence of trichomes of the smallest diameter.

4. Among the plants studied, the highest content of oil was noted in thyme and peppermint, and the lowest in green parts of lemon balm.

5. The isolated essential oils contained predominantly components from the groups of monoterpenes and sesquiterpenes, and it is the presence of those substances that determines the possibility of utilizing the plants studied for a variety of purposes.

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