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HISTOCHEMISTRY AND MICROMORPHOLOGICAL DIVERSITY OF GLANDULAR TRICHOMES IN *Melissa officinalis* L. LEAF EPIDERMIS

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Abstract. Due to the presence of glandular trichomes in their epidermis and production of essential oils, plants from the family Lamiaceae, including *Melissa officinalis* L., are commonly cultivated in most European countries and across Poland. With its diverse composition of essential oils and the wide range of pharmacological effects, the raw material of the lemon balm is widely applied in various branches of industry. In literature, there are no data presenting histochemical assays of glandular trichomes in the lemon balm; therefore, the aim of the study was to detect some substances with the use of selected stains and to characterise the micromorphology of the trichomes. The investigations were conducted using light, fluorescence, and scanning electron microscopes. The histochemical tests were based on Sudan Red 7b, Sudan black B, Nile blue, Nadi reagent, ferric chloride, potassium dichromate, magnesium acetate, Ruthenium red, and periodic acid-Schiff reagent. Digitiform as well as morphologically diverse capitate and peltate glandular trichomes were distinguished in the leaf epidermis. The histochemical tests showed heterogeneity of the composition of the lemon balm essential oil. They were applied to determine lipids, fatty acids, neutral fats, terpene compounds, polyphenols, flavonoids, and polysaccharide compounds in the analysed glandular trichomes. Improvement of the histochemical methods for analysis of glandular trichomes will expand the knowledge of the metabolism of secretory cells and facilitate future modification of their secretion products.

Key words: Lamiaceae, epidermis micromorphology, anatomy, histochemical tests, SEM

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INTRODUCTION

The well-known *Melissa officinalis* L. species used in herbal therapy originates from southern Europe. Three subspecies have been reported from the continent, i.e. *M. officinalis*: ssp. *officinalis* L., ssp. *altissima* Arcangeli, and ssp. *indora* Boran, with the first taxon having important applications [Bağdat and Cosge 2006, Moradkhani et al. 2010]. The lemon balm is cultivated in a majority of countries of Europe, North Africa, and West Asia [Basar and Zaman 2013]. In Poland, the species is grown on an area of ca. 5000 ha across the country [Seidler-Łożykowska et al. 2015]. The herbal raw material comprises leaves and leaved shoot apices (*Melissae folium*) containing essential oil, tannins, flavonoids, bitters, polyphenolic compounds, triterpene compounds, organic acids, and minerals [Moradkhani et al. 2010, Basar and Zaman 2013]. The concentration of essential oils in fresh and dry lemon balm raw material ranges from 0.01 to 0.15 and from 0.1 to 0.3%, respectively [Saeb and Gholamrezaee 2012, Nurzyńska-Wierdak et al. 2014]. Given its broad spectrum of pharmacological properties, the lemon balm is used in medicine, food production, and cosmetic and perfume industry [Moradkhani et al. 2010].

The lemon balm raw material is used in the pharmaceutical industry as an ingredient of herbal blends and teas as well as a component of medicinal formulations [Bağdat and Coşge 2006, Abdellatif et al. 2014]. Lemon balm extracts have sedative, carminative, diaphoretic, antiviral, and antioxidant activity [Moradkhani et al. 2010, Koksal et al. 2011, Basar and Zaman 2013]. *Melissa* essential oil is used for production of perfumes, creams, and emulsions. Its leaves serve as flavouring of sauces and salads as well as liqueurs, wine, and beer; they are also ingredients of meat marinades. Essential oils and extracts of lemon balm herb are used in food industry as antioxidants [Moradkhani et al. 2010].

As a medicinal, seasoning, and honey-bearing plant, the lemon balm is commonly grown in gardens. The sugar-rich nectar and lemon scent of this perennial plant attract insect pollinators, especially honeybees [Chwil 2009]. The aroma is released in a process of exosecretion carried out by glandular trichomes, which not only store phytotoxic oils but also are the first line of defence against insect pests, and the mixture of substances produced by these structures is applied as a pesticide. The chemical composition of the secretion accumulated in the subcuticular space varies in different Lamiaceae species [Nurzyńska-Wierdak and Dzida 2009, Nurzyńska-Wierdak et al. 2011, Dzida et al. 2015]. The concentration and composition of specific substances have chemotaxonomic and phylogenetic relevance [Kiliç 2013, Mirzaei et al. 2015]. Similarly, the micromorphology, anatomy, and ultrastructure of glandular trichomes are taken into account in identification of related species [Satil et al. 2011, Celep et al. 2014, Eiji and Salmaki 2016]. Improvement of methods for isolation and analysis of glandular trichomes will provide knowledge of the metabolism and enzymology of secretory cells. In turn, analyses based on the use of mutants with altered phenotypes of trichomes will reveal the genetic background of their development, which may serve as a basis for further research on the modification of trichome secretion products [Tisser 2012].

The literature provides no data on histochemical assays of *Melissa officinalis* L. glandular trichomes. The information about the trichome structure is also incomplete.

Therefore, the aim of the study was to determine the location of biologically active substances secreted via the exosecretion process using appropriate stains and the micromorphology of the trichomes in the *M. officinalis* leaf epidermis. Additionally, some anatomical features of these organs were characterised.

MATERIAL AND METHODS

Plant material. Lemon balm (*Melissa officinalis* L.) leaves used in the investigations were collected in the initial flowering stage from plants growing in the Botanical Garden of Maria Curie-Skłodowska University, Lublin (N 51.2615737964403° and E 22.5141835212708°). Margins and the central part of the lamina (a 5-6-mm square) were dissected from the leaves. Hand-made transverse sections were made from the fresh leaf fragments.

Fixation of the plant material. Prior to the observations of the leaf epidermis surface and preparation of semi-thin sections, the sampled lamina fragments were fixed in 4% glutaraldehyde for 6 hours at room temperature and next in 0.01 M phosphate buffer, pH 7.0, at 4°C for 48 hours. The plant material was dehydrated for 15 min in an ethanol series at concentrations of 15, 30, 50 70, 90, 96, and 99.8% and twice in absolute ethanol. The dehydrated plant samples were embedded in Spurr Low Viscosity resin and polymerised at 60°C for 48 hours.

Transverse, semi-thin sections were made from the fixed material. 0,8-1-µm thick sections were cut with a glass knife using a Reichert Ultracut S microtome. The sections were stained with 1% toluidine blue and 1% Azure II (1:1) at a temperature of 60°C for 5 min. The preparations were rinsed with distilled water and 5% ethanol and dried.

Microscopy. The trichomes were observed under stereoscopic, fluorescence, light, and scanning electron microscopes.

Stereoscopic microscope (SM). Preliminary investigations of the leaf epidermis surface were carried out under a stereoscopic microscope SMT 800. The photographic documentation was done using a Nicon Coolpix 4500 camera.

Fluorescence microscope (FM). Transverse leaf sections were placed in a drop of 0.01% auramine O fluorochrome and embedded in a 50% glycerol solution [Heslop-Harrison and Heslop-Harrison 1981]. The slides were observed under a Nikon Eclipse 90i fluorescence microscope equipped with an FITC filter (excitation light 465–495 nm) and a barrier filter (wavelength 515–555 nm). Autofluorescence of the glandular trichomes and their stain reactions were observed in the presence of magnesium acetate or lead acetate using a Cy5 filter.

Light microscope (LM). In order to carry out histochemical assays of the content of the selected bioactive compounds in the glandular trichomes, the leaf sections were stained with appropriate stains:

– Sudan Red 7B and Sudan black B for lipids [Jensen 1962, Brundrett et al. 1991],

– Nile blue for neutral and acidic lipids [Cain 1947],

– Nadi reagent for terpenoids [David and Carde 1964],
– ferric chloride and potassium dichromate for phenols [Johansen 1940, Gabe 1968],
– magnesium acetate for flavonoids [Charrière-Ladreix 1976],
– ruthenium red for pectins and mucilage [Johansen 1940],
– periodic acid-Schiff reagent (PAS) for polysaccharides [Jensen 1962].

Scanning electron microscope (SEM). The fixed leaf fragments were dehydrated in increasing acetone concentrations, i.e. 15, 30, 15, 30, 50, 70, 90, and 99.5%. The plant samples were kept for 15 minutes in each concentration. The plant material was critical-point dried in liquid CO_2 in an Emitech K850 dryer and next sputter-coated with gold using an Emitech K550X sputter coater. The observations of the adaxial and abaxial leaf surfaces and the photographic documentation were performed under a Tescan Vega II LMU scanning electron microscope.

Morphometric measurements and statistical analyses. The height of the digitiform, peltate, and capitate (with a unicellular stalk and a bicellular head) glandular trichomes and the diameter of the secretory head were measured. Additionally, the length of the protective trichomes in the leaf epidermis was determined. Each trait was measured in 16 replicates with a computer program for Nikon NIS-Elements image analysis, version 3.0 Advance Research. The standard deviation was calculated in Excel software.

RESULTS

Micromorphology of the leaf epidermis surface. Hipostomatic *M. officinalis* leaves (figs 1 A–D, 2 A) exhibited opposite arrangement and had an oval shape, a cordate base, a serrated margin, and a pointed apex. Diacytic stomata were located above the other epidermis cells (fig. 2 A–C, E). Their length and width were 20 and 16 μm, respectively. The cuticle on both leaf epidermis surfaces was smooth or striated. Cuticular striae were arranged radially on the surface of the protective trichome basal cells (fig. 1 D) or formed bands on some guard cells (fig. 2 E). Both surfaces of the epidermis were covered by non-glandular trichomes (figs 1 A–D, 2 A–D) as well as digitiform and capitate glandular trichomes (figs 1 B, C, 2 A–D, 3 A, B, 5 A, B); in turn, peltate trichomes were only observed on the adaxial epidermis (figs 2 A–C, 3 A, 5 A–C). Glandular trichomes emitted autofluorescence (fig. 3 A). Secretion products in the form of droplets emitted light blue fluorescence at the application of the DAPI filter (fig. 3 C, D). In turn, the cuticle on the trichome surface emitted green fluorescence in the presence of auramine (fig. 3 B).

The following types of glandular trichomes were distinguished:

I – digitiform trichomes composed of 3 linearly arranged cells, ending with a single cylindrical secretory cell (figs 4 M, N, 5 F) (7-μm width/15-μm height). The trichomes were located at the level of the other epidermis cells. Their length ranged from 33 to 43 μm (tab. 1).

II – capitate trichomes; this group comprised: (i) – trichomes composed of a 1-celled stalk and a 1-, 2-, 3-, or 4-celled head (fig. 4 A–D); (ii) – trichomes composed of a 2- or 3-celled stalk and a 1-celled head (fig. 4 K, L). These structures were located at the

level of the other epidermis cells. The secretory head contained dense cytoplasm and a large cell nucleus with a visible nucleolus. Dark stained plastids were located near the nucleus. The 1-celled stalk of the capitate trichomes was 22.5 μm high and exhibited typical cytological traits of trichomes with a characteristic thick cell wall (fig. 5 G, H). The diameter of the 2-celled secretory head was in the range of 21–26 μm (tab. 1).

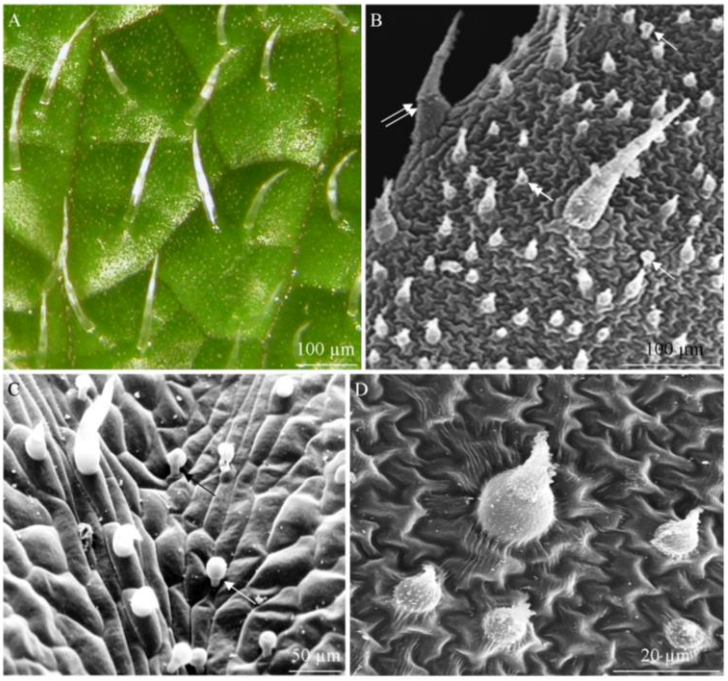

Fig. 1 A–D. Adaxial surface of *M. officinalis* leaves: A – multicellular protective trichomes, B – lamina margin, visible capitate glandular (arrow) and conical trichomes (double-headed arrow) as well as multicellular protective trichomes (two arrows), C – central part of the lamina, visible capitate glandular trichomes (arrow) and smooth cuticle, D – smooth or striated cuticle, conical protective trichomes with visible microvilli on the cell wall. A – SM; B–D – SEM

III – peltate trichomes (fig. 4 F–J) composed of a short stalk and a multicellular head (usually 8- or 12-celled) (figs 3 J–L, 4 I, J). These trichomes were sparsely located in small depressions of the epidermal cells. Various stages of oil exosecretion were observed in the secretory head. Initially, small secretion droplets were accumulated in the subcuticular space; in the subsequent stage, they merged into larger drops (fig. 3 C–H) and filled the subcuticular space (fig. 3 I). The diameter of the secretory head ranged from 38 to 95 μm (tab. 1).

The protective trichomes differed in the number and size of their linearly arranged cells. The following types were distinguished among them:

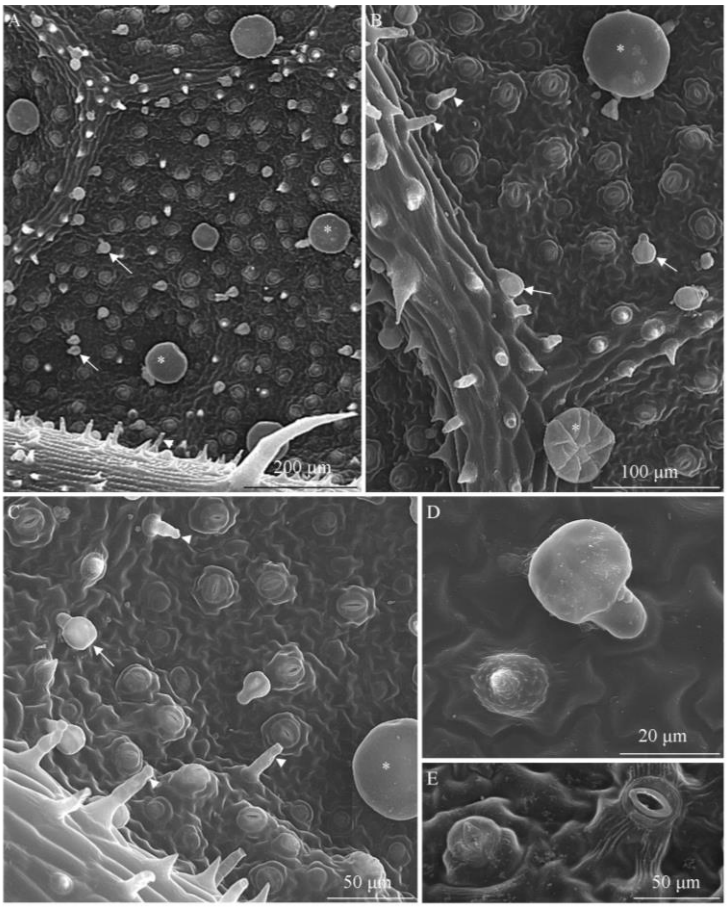

Fig. 2 A–E. Abaxial surface of *M. officinalis* leaf epidermis: A, B – glandular trichomes: peltate (asterisk), capitate (arrow), and digitiform (arrowhead), C – glandular trichomes: digitiform (arrowhead), bicellular capitate (arrow) and peltate (asterisk) as well as diacytic stomata, D – smooth surface of the cuticle with delicate striation at the base of a conical protective trichome witch microvilli on the cell wall; visible a capitate trichome, E – stomata witch smooth or striated cuticle. A–E – SEM

IV – 1-celled trichomes, dilated at the base (short 15–26 µm) (tab. 1). They had microvilli on the surface (figs 1 D, 4 O, P) and a thick cell wall (fig. 5 D, E).

V – conical, sharply pointed, composed of 2–8 linearly arranged cells (figs 1 A–C, 4 R). Depending on the number of constituent cells, the length of the trichomes ranged from 43 µm (2-celled) to 802 µm (6-celled) (tab. 1). 7- and 8-celled trichomes were noted less frequently.

The anatomical structure of the leaves was characterised by a thicker periclinal outer epidermis cell wall than other walls (fig. 5 D, E). The stomata had well-developed, thick cuticular ledges (fig. 5 E). Under the epidermis, there was a single layer of palisade parenchyma cells and 2–3 layers of spongy parenchyma (fig. 5 C–E).

Table 1. The size of glandular and protective trichomes in *M. officinalis* leaf epidermis (µm)

	Analysed trait			Min.–Max.	Mean	SD
Glandular trichomes	height of	trichomes		33.40–43.42	39.62	2.94
		glandular cell	digitiform	13.36–16.70	15.22	2.29
	width of			6.68–8.35	7.33	0.81
	height of	trichomes		23.38–31.73	28.58	2.72
		one-celled sta	capitate	17.92–25.06	22.48	1.89
	diameter of two-celled head			6.88–13.36	10.39	2.20
	height of trichomes		peltate	30.06–56.78	42.31	9.22
	diameter of secretory head			56.78–80.16	67.54	7.62
Protective trichomes	length of	one-		15.36–25.60	22.56	3.53
		two-		38.40–56.32	43.20	6.33
		three-	celled	110.08–250.88	176.48	50.61
		four-	trichomes	192.00–355.84	302.40	53.52
		five-		348.16–524.80	471.68	60.35
		six-		599.04–1177.60	801.76	169.49

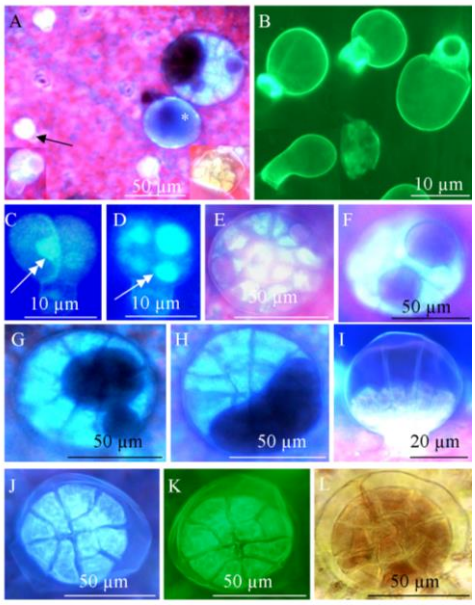

Fig. 3 A–L. Fragments of the surface of *M. officinalis* leaf epidermis*:* A – abaxial epidermis, visible autofluorescence of capitate (arrow) and peltate (asterisk) trichomes, B – adaxial epidermis, visible green fluorescence of the cuticle on the surface of glandular trichomes, C, D – fluorescent secretion droplets (two arrows), E–H – trichome head with secretion droplets, I – capitate trichome with visible secretion, J–L – multicellular glandular head. A–K – FM, L – LM

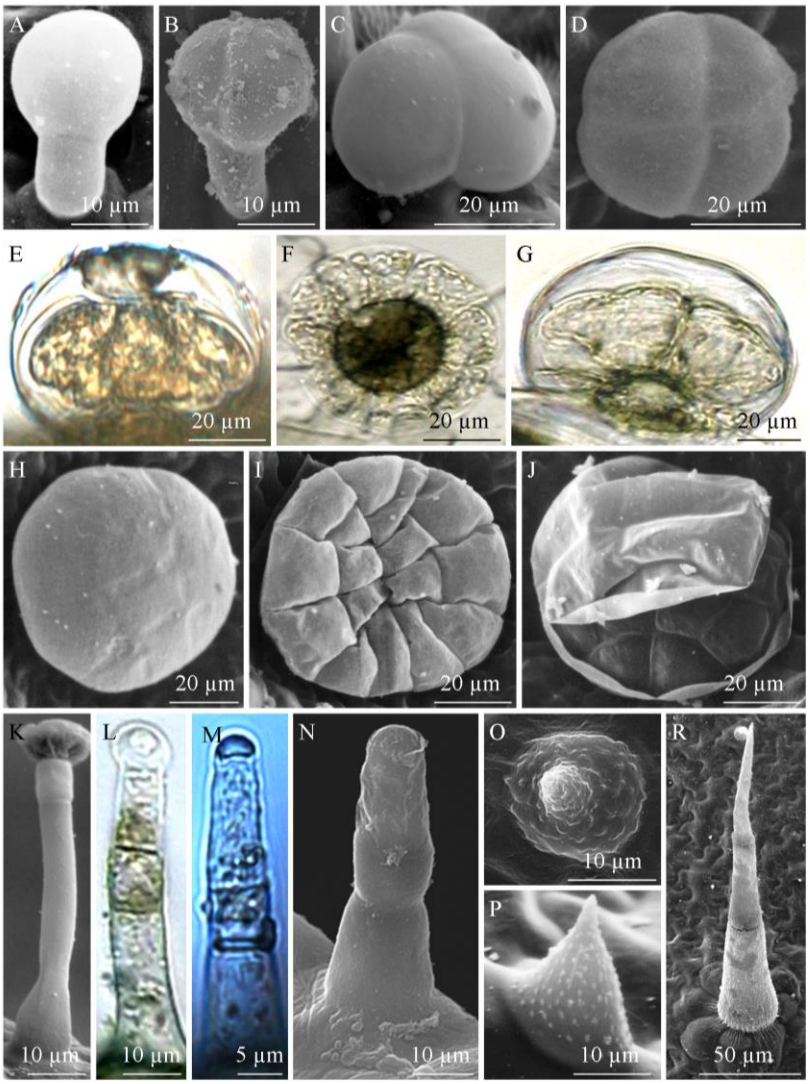

Fig. 4 A–R. Glandular (A–N) and protective (O–R) *M. officinalis* trichomes: A–D – capitate with a 1-, 2-, 3-, and 4-celled head and a short stalk, E–J – peltate in different stages of secretion, K, L – capitate with a 1-celled head and an elongated stalk, M, N – digitiform, O, P – conical with microvilli on the cell wall, R – elongated protective trichome

Histochemistry of glandular trichomes. The histochemical assays showed a characteristic colour, which resulted from the reaction based on selective solubility or specific binding of the stains in the detected substances. The histochemistry of the glandular trichomes revealed the presence of various groups of biologically active compounds contained therein.

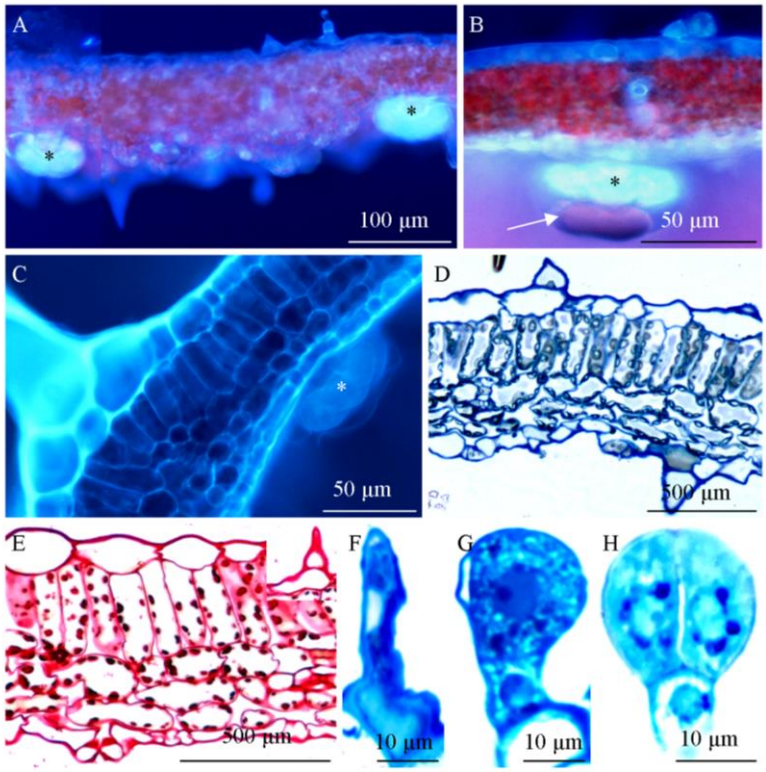

Fig. 5 A–H. Fragments of cross-sections of *M officinalis* leaves*:* A – autofluorescence of peltate glandular trichomes (asterisk), B – stronger fluorescence of abaxial epidermis cells and a head of a glandular trichome (asterisk), visible secretion accumulated in the subcuticular space (asterisk), C – epifluorescence of adaxial and abaxial epidermis, visible peltate glandular trichome (asterisk), D, E – thick outer cell wall of the adaxial epidermis and protective trichomes, single layer of palisade mesophyll cells, 2–3 layers of parenchyma cells, stomata above other epidermis cells, F – digitiform trichome, G, H – capitate glandular trichomes with a 1- or 2-celled head, visible large centrally located nucleus, dense cytoplasm, and plastids

The orange-red colour of the secretory head of the digitiform, capitate, and peltate trichomes stained with Sudan Red indicated the presence of lipids (fig. 6 A–C), which was confirmed by the dark blue colour noted after the application of Sudan black B (fig. 6 D–F). In turn, the blue colour appearing upon the application of Nile blue indicated the presence of fatty acids, while the purple colour evidenced the content of neutral fats (fig. 6 G–I). Additionally, in all three analysed types of glandular trichomes, the purple stain produced by Nadi reagent (fig. 6 J–L) confirmed the presence of terpene compounds. The polyphenols contained in the glandular trichomes of the lemon balm stained black in the presence of ferric chloride (fig. 7 A–C) and brown after application of potassium dichromate (fig. 7 D–F). The presence of flavonoids in all types of glandu-

lar trichomes was shown by the yellow colour visible after the application of magnesium acetate (fig. 7 G–I). Furthermore, the polysaccharide compounds contained in the analysed trichome types stained pink in the PAS (periodic acid-Schiff reagent) reaction (fig. 7 J–L), likewise pectins or mucilage treated with Ruthenium red (fig. 7 M–O).

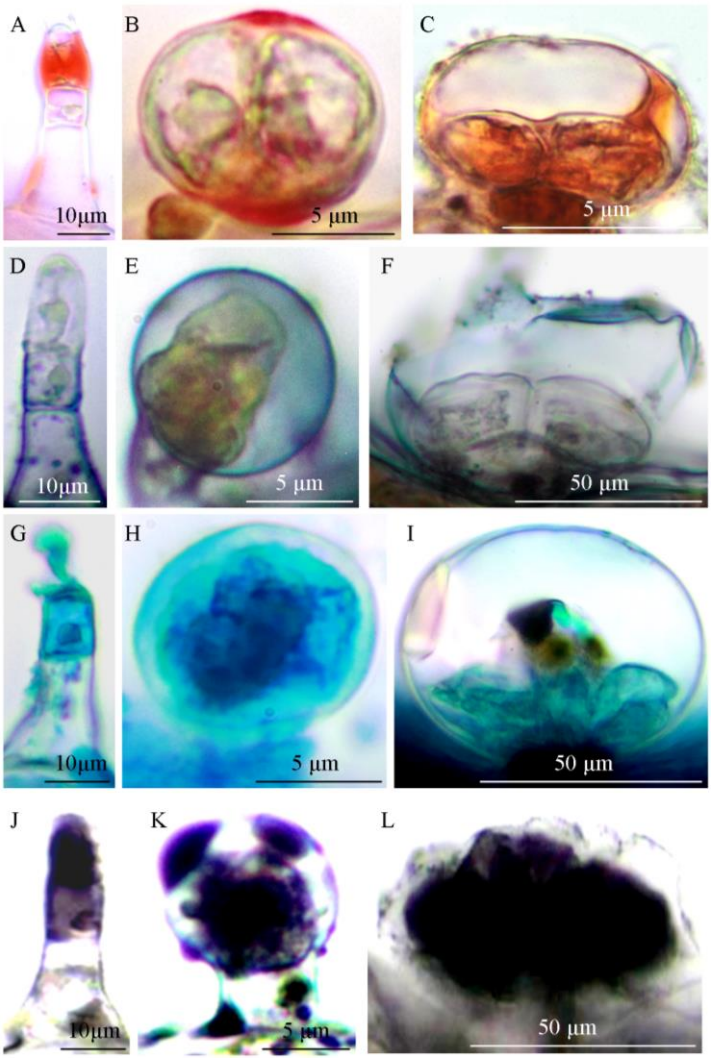

Fig. 6 A–L. Histochemical assays of selected compounds in digitiform, capitate, and peltate glandular trichomes: A–D – orange-red (Sudan Red – A–C) and dark blue-stained (Sudan Black – D–F) lipid compounds, G–I – blue stained acidic lipids and purple stained neutral lipids (Nile blue), J–L – purple-blue stained terpenes (Nadi)

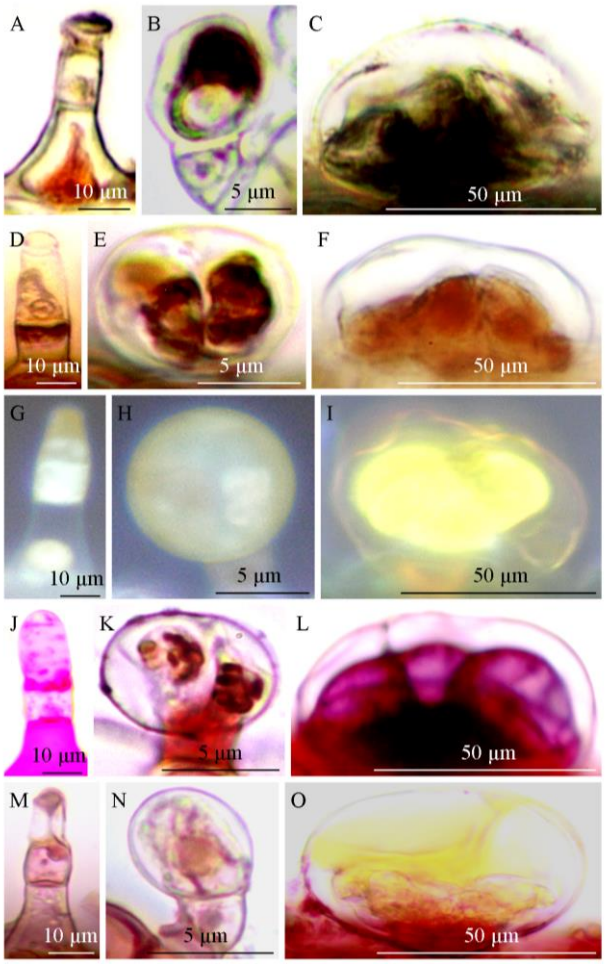

Fig. 7 A–O. Histochemical assays of selected compounds in digitiform, capitate, and peltate glandular trichomes: A–F – black (ferric chloride – A–C) and brown stained polyphenols (potassium dichromate – D–F), G–I – flavonoids stained yellow with magnesium acetate, J–L – pink stained neutral polysaccharides (PAS), M–O – pink stained pectins (ruthenium red) A–F, J–O – LM; G–I – FM

DISCUSSION

Trichome structure and oil secretion. In the presented investigations, the third type of glandular trichomes, i.e. digitiform, was identified in the *M. officinalis* leaf epidermis, besides the two other types, i.e. capitate and peltate trichomes, which had been previously described in this species [Moradkhani et al. 2010, Antal et al. 2014]. The glandular trichomes in the representatives of the family Lamiaceae are characterised by great diversity in the shape, size, and number of their constituent cells (tab. 2).

Table 2. Characteristic of glandular trichomes of chosen Lamiaceae species

Species	Glandular trichomes						Author
	digitiform	peltate		capitate			
		stalk	head	subtype	stalk	head	
Melissa officinalis	–	short	8	I	1–2	1 – 2	Pădurariu et al. 2009
				I	2	1	Petenatti et al. 2014
Micromeria thymifolia	3	wide	12	I	1	1	Marin et al. 2013
				II	2	1	
Pogostemon cablin	3–4	short	spherical	I	1	2	Rusydi et al.2013
				II	2–3	1	
Salvia fruticosa	3–4	short	8	I	1	2	Allahverdiyeva et al. 2004
				II	2	2	
				III	1	1	
				IV	2	1	
				V	3	1	
Satureja horvatii	2–3	wide	12	I	1	1	Marin et al. 2012
				II	1	1	
Stachys lavandulifolia	1–2	1	8	I	2	2	Rezakhanloa and Talebi 2010
Thymus quinqueco	3–4	1	12	I	1–2	1	Jia et al. 2013

In the epidermis of the examined leaves, many capitate trichomes had a 1- or 2-celled and sometimes 3- or 4-celled secretory head. In turn, the head of the peltate trichomes was composed of 8 or 12 cells. Literature data (tab. 2) show a 1- or 2-celled head in capitate trichomes and an 8-celled head in peltate trichomes in the *M. officinalis* epidermis. In other Lamiaceae representatives, the trichome secretory head was composed of 1–3 (capitate) and 8 or 12 (peltate) cells [Pădurariu et al. 2009, Rezakhanloa and Talebi 2010, Marin et al. 2012, Allahverdiyeva et al. 2004, Jia et al. 2013, Marin et al. 2013, Rusydi et al. 2013, Petenatti et al. 2014].

The lemon balm trichomes were characterised by a thick, cutinised cell wall of the stalk, which is a typical trait in Lamiaceae representatives [Gersbach 2002]. This modification was observed already in the first phase of growth of young trichomes in *Satureja subspicata* leaf epidermis [Dunkić et al. 2007]. Cutin incrustation stiffens the trichome structure, prevents influx of water into secretory cells, and provides a barrier against apoplastic transport of secretion to neighbouring cells and its autotoxicity to other plant parts [Hallahan et al. 2000, Gersbach 2002].

Our observations have shown that, in the first exosecretion phase in the peltate trichomes, essential oil accumulated in the form of small droplets, which merged during the process and filled the subcuticular space. Literature data indicate that secretion products present in the cytoplasm of secretory cells transport rough endoplasmic reticulum and dictyosomal vesicles fused with the membrane into the plasmalemma [Huang et al. 2008]. This confirms that granulocrine secretion is the major mode of transfer of substances from glandular cells into the subcuticular space [Gersbach 2002, Turner and Croteau 2004, Huang et al. 2008]. The accumulated secretion is characterised by a taxon-specific chemical composition [Nurzyńska-Wierdak and Dzida 2009, Nurzyńska-Wierdak et al. 2011, Dzida et al. 2015].

Exosecretion products. The histochemical assays of the glandular trichomes have confirmed the presence of lipo- and hydrophilic compounds. These substances serve a variety of functions in plants and exert diverse pharmacological effects.

Fatty compounds stained positively in all the analysed peltate, capitate, and digitiform glandular trichomes. Similar results of trichome histochemistry in different Lamiaceae representatives have been reported by other authors [Marin et al. 2010, Jia et al. 2012, Teixeiria et al. 2013, Liu and Liu 2014]. From this compound group, palmitic, stearic, and oleic acids have been detected in the cuticle. The lipid character of this layer ensures protection against environmental factors [Yeast and Rose 2013]. The type and concentration of lipid compounds modify the properties of cell membranes. Membrane fluidity depends on the level of unsaturated fatty acids [Upchurch 2008]. Fatty acids can be oxidised, leading to formation of oxylipins as an endogenous signal evoked in response to plant wounding as well as biotic and abiotic stress [Mithöfer et al. 2004, Tsitsigiannis et al. 2007]. Oxylipins regulate colonisation of the host and production of mycotoxins by fungi from the genera *Aspergillus* and *Candida* [Tsitsigiannis et al. 2007]. In terms of pharmacological properties, oxylipins have anti-inflammatory activity and have a beneficial effect on the immune system [Benavides et al. 2009], whereas unsaturated fatty acids inhibit growth of breast, colon, and prostate tumour cells, prevent obesity, and reduce inflammations [Nowak 2010, Vaughan et al. 2013].

Terpene compounds were detected in the three types of the analysed glandular trichomes. Literature data show varied location of these compounds in the glandular trichomes of Lamiaceae species. They were detected in capitate, conoidal, and digitiform trichomes of *Plectranthus ornatu* [Ascensão et al. 1999], in peltate and capitate trichomes of *Mentha pulegium* [Rodrigues et al. 2013] and *Salvia aurea* [Serrato-Valenti et al. 1997], in peltate and some capitate trichomes of *Salvia officinalis* [Corsi and Bottega 1999], *Lavandula stoech*, and *L. pedunculata* [Teixeira et al. 2013], and in capitate trichomes of *Melittis melissophyllum* [Maggi et al. 2010] and *Isodon rubescens* [Liu and Liu 2014]. The presence of terpenes in lemon balm raw material is indicated by the lemon odour produced by citral [Bağdat and Coşge 2006, Moradkhani et al. 2010]. Volatile fragrant substances in essential oil including mono-, sesqui-, and triterpenes attract insect pollinators and deter herbivorous animals, thereby influencing the ecosystem by the relations between organisms [Chizzola 2013]. From this compound group, monoterpenes dominate in the lemon balm essential oil [Saeb and Gholamrezaee 2012, Abdellatif et al. 2014, Nurzyńska-Wierdak 2014]. These compounds are used in the food industry as flavouring and fragrant substances and in chemical industry for production of disinfectants and plant protection agents [Bohlmann and Keeling 2008]. Additionally, they are used in medicine, due to their antioxidant, antiviral, antibacterial, and antifungal activity [Abdellatif et al. 2014, Tantry et al. 2014]. The group of sesquiterpenes in the lemon balm essential oil is mainly represented by ß-caryophyllene and germacrene D [Moradkhani et. al. 2010, Kazemi and Esmaili 2014, Abdellatif and Hassani 2015]. The presence of caryophyllene allows discrimination of lemon balm essential oil from other oils with a similar odour [Moradkhani et al. 2010]. The compound has antispasmodic and relaxant activity [Pinho-da-Silva et al. 2012]. In turn, germacrene D has been reported to have insecticidal effects against mosquitoes [Kiran et al. 2007] and to repel aphids [Bruce et al. 2005]. However, it is an attractant to the Tobacco

Mudworm Moth, *Heliothis virescens* [Mozuraitis et al. 2002]. Triterpenoids are common plant isoprenoids. These structurally diverse polycyclic compounds serve different biological functions in primary and secondary metabolism. Triterpene skeletons are precursors of the synthesis of plant sterols, i.e. phyto-, sigma-, and campesterol, which mediate the synthesis of brassinosteroid hormones, steroidal saponins, and other specific triterpenes and saponins, i.e. bioactive compounds that help plants survive in their environment. Triterpenes play an important role as wax and cuticle components [Moses et al. 2015]. Triterpenes and saponins exhibit diverse pharmacological activities beneficial to humankind, for example poleanolic and ursolic acids have anti-hepatotoxic, antibacterial, anti-inflammatory, and cytotoxic activity [Mencherini et al. 2007, Basar and Zaman 2013].

In addition, tri- and sesquiterpenes as well as phenolic compounds, which have also been detected in the *M. officinalis* glandular trichomes, exhibit antioxidant activity [Mimica-Dukic et al. 2004, Mencherini et al. 2007, Bağdat and Coşge 2006]. Phenolic compounds in Lamiaceae are contained in various types of glandular trichomes, e.g. in capitate and peltate trichomes of *Thymus quinquecostatus*, *Micromeria thymifolia*, and *Isodon rubescens*, peltate trichomes of *Rosmarinus officinalis*, and capitate trichomes of S*atureja subspicata* and two species from the genus *Lavandula* [Marin et al. 2006, 2010, Jia et al. 2012, Teixeira et al. 2013, Liu et al. 2014]. Phenols constitute a numerous and diverse group of secondary metabolites in plants [Ferguson 2001]. The interspecific variability of production of these compounds is associated with a defence response against herbivores and pathogens as well as the ability to absorb UV radiation [Pandey and Rizvi 2009, Agati and Tattini 2010].

The phenolic group in the lemon balm essential oil is represented by e.g. flavonoids and phenolic acids [Ibragić et al. 2014, Nurzyńska-Wierdak et al. 2014]. The presence of flavonoids in the *M. officinalis* trichomes presented in this study was confirmed by the positive reaction with magnesium acetate. Phenolic compounds in capitate and peltate glandular trichomes have also been detected in other species from the family Lamiaceae, e.g. *Ocimum obovatum, Salvia officinalis*, and *Thymus quinquecostatus* [Corsi and Bottega 1999, Jia et al. 2012, Naidoo et al. 2013]. The lemon balm essential oil is dominated by apigenin, quercetin, isoquercitrin, luteolin, kaempferol, cynaroside, cosmosin, rhamnocitrin, and quercitrin [Moradkhani et al. 2010, Kazemi and Esmaili 2014]. Apigenin exhibits anti-proliferative, anti-inflammatory, and anti-allergic activity. It reduces production of inflammatory cytokines in monocytes and modulates immune response *in vivo* [Nicholas et al. 2007]. Quercetin exhibits antioxidant activity [Zhang et al. 2011], and luteolin has an anti-inflammatory, antibacterial, and anti-tumour effect [Lopez-Lazaro 2009]. Similar activity has been detected in the case of phenolic acids [Dai and Mumper 2010, Shekarchi et al. 2012, Taiwo et al. 2012]. Rosmarinic acid was found to inhibit herpes virus infection [Astani et al. 2014]. In turn, polyphenols are a source of natural antioxidants and protect against the development of cancer, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases [Pandey and Rizvi 2009, Ferrazzano et al. 2011].

The presence of polysaccharides, i.e. the basic structural plant constituents, in the *M. officinalis* glandular trichomes was confirmed by the positive result of the PAS and Ruthenium red reactions. The compounds have also been reported in other species of

the family Lamiaceae, i.e. in capitate and peltate glandular trichomes of *Rosmarinus officinalis* [Marin et al. 2006], *Ocimum obovatum* [Naidoo et al. 2013], and *Thymus lykae* [Marin et al. 2008], in capitate trichomes of *Lavandula stoechas* and *L. pedunculata* [Teixeira et al. 2013], and in peltate trichomes of *Thymus quinquecostatus* [Jia et al. 2012]. Pectins, representing polysaccharides, have been detected mainly in trichome cell walls [Jia et al. 2012]. These compounds have been found in peltate and capitate glandular trichomes of *Mentha pelegium* [Rodrigues et al. 2013], *Satureja subspicata* [Marin et al. 2010], and *Lavandula pedunculata* and in capitate trichomes of *Lawendula stoechas* [Teixeira et al. 2013].

CONCLUSIONS

Digitiform, capitate, and peltate glandular trichomes and protective trichomes formed of 1–8 linearly arranged cells were present in the *M. officinalis* leaf epidermis. The present paper provides the first description of digitiform glandular trichomes in the analysed species and shows the morphological diversity of capitate and peltate trichomes. A novelty in the determination of the structure of *M. officinalis* glandular trichomes was the use of the cytochemical reactions of the selected bioactive compounds. The histochemical tests showed a heterogeneous nature of the lemon balm essential oil. Lipids, fatty acids, neutral fats, terpene compounds, polyphenols, flavonoids, and polysaccharide compounds were detected in the three types of glandular trichomes.

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HISTOCHEMIA I MIKROMORFOLOGICZNE ZRÓŻNICOWANIE WŁOSKÓW GRUCZOŁOWYCH W EPIDERMIE LIŚCI *Melissa officinalis* L.

Streszczenie. Rośliny z rodziny Lamiaceae, w tym (*Melissa officinalis* L.), ze względu na obecność w epidermie włosków gruczołowych i produkcję olejków eterycznych są powszechnie uprawiane w większości krajów Europy i na terenie całej Polski. Surowiec zielarski melisy o zróżnicowanym składzie chemicznym olejku i szerokim zakresie działania farmakologicznego znajduje szerokie zastosowanie w różnych dziedzinach przemysłu. W literaturze brak danych dotyczących histochemicznych testów włosków gruczołowych melisy lekarskiej, dlatego celem badań było określenie wybranych substancji przy użyciu wybranych barwników oraz określenie mikromorfologii włosków. Badania przeprowadzono przy użyciu mikroskopu świetlnego, fluorescencyjnego i skaningowego elektronowego. W testach histochemicznych zastosowano: Sudan czerwony 7B, Sudan czarny B, błękit Nilu, odczynnik Nadi, chlorek żelaza, dichromian potasu, octan magnezu, czerwień rutenową i odczynnik Schiffa. W epidermie liści wyróżniono włoski gruczołowe: palcowate i zróżnicowane morfologicznie głowiaste oraz tarczowate. Histochemiczne testy wykazały heterogenny charakter olejku melisowego. Przy ich użyciu wyznakowano w badanych włoskach gruczołowych lipidy, kwasy tłuszczowe, tłuszcze obojętne, związki terpenowe, polifenole, flawonoidy i związki polisacharydowe. Doskonalenie histochemicznych metod badań włosków gruczołowych umożliwi poznanie metabolizmu komórek wydzielniczych, a w przyszłości być może pozwoli na modyfikację produktów sekrecji włosków.

Słowa kluczowe: Lamiaceae, mikromorfologia epidermy, anatomia, histochemiczne testy, SEM

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