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A Comparative Study on Glandular Trichomes of *Lavandula* × *intermedia* ‚Budrovka' and *L. angustifolia*

By

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With 6 Figures

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Key words: *Lavandula* × *intermedia* Emeric ex Loisel. ‚Budrovka', *Lavandula angustifolia* Mill.; *Lamiaceae*. – Trichomes, histochemistry, phenolic compounds, essential oil.

Summary

Blažeković B., Stabentheiner E., Brantner A. & Vladimir-Knežević S. 2012. A comparative study on glandular trichomes of *Lavandula* × *intermedia* ‚Budrovka' and *L. angustifolia*. – Phyton (Horn, Austria) 52 (2): 227–244, with 6 figures.

Lavandula species have a long history of use in traditional medicine and fragrance industry and Croatian's lavender industry bases on the cultivar *Lavandula* × *intermedia* Emeric ex Loisel. ‚Budrovka'. This taxon was investigated in comparison to *L. angustifolia* Mill. studying morphology, trichome types and trichome distribution by light and scanning electron microscopy and by applying thin layer chromatography for phytochemical analyses. Morphological characteristics clearly distinguished *L.* × *intermedia* ‚Budrovka' from *L. angustifolia*. Three distinct classes of trichomes (non-glandular trichomes, peltate and four types of capitate glandular trichomes) were observed on leaves and inflorescence of both taxa. Trichomes

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showed distinct organ specific differences concerning occurrence and density but no taxa specific differences could be observed. Long stalked capitate trichomes with distinct knobs formed by the upper stalk cells, a final neck cell and a unicellular head, were reported for the first time as the exclusive glandular trichome type found on the inner surface of the corolla. Histochemical tests identified both lipophilic and phenolic substances as main products secreted by the glandular trichomes. Furthermore, comparative TLC fingerprint profiles of individual plant parts of both taxa regarding the presence of flavonoids and essential oil components were established.

Zusammenfassung

Blažeković B., Stabentheiner E., Brantner A. & Vladimir-Knežević S. 2012. A comparative study on glandular trichomes of *Lavandula* × *intermedia* ‚Budrovka' and *L. angustifolia*. [Eine vergleichende Studie der Drüsenhaare von *Lavandula* × *intermedia* ‚Budrovka' und *L. angustifolia*]. – Phyton (Horn, Austria) 52 (2): 227–244, mit 6 Abbildungen.

Morphologie, Vorkommen, Verteilung und Histochemie der Trichome und Phytochemie von *Lavandula x intermedia* Emeric ex Loisel. „Budrovka", der Basis der kroatischen Lavendellindustrie, wurden im Vergleich mit *L. angustifolia* Mill. untersucht. Die beiden untersuchten Taxa unterschieden sich deutlich in verschiedenen morphologischen Merkmalen, wohingegen es bei den Trichomtypen und deren Verteilung keine signifikanten Unterschiede gab. Borstenhaare, Schildhaare und vier verschiedene Typen von Köpfchenhaaren konnten auf den Blättern, den Stängeln und den Blüten beobachtet werden, wobei auf den Lippenblüten ein neuartiger Trichomtyp beschrieben wurde: die oberen Stielzellen des langstieligen Köpfchenhaares wiesen armartige Fortsätze auf, darauf folgte dann eine Halszelle und das einzellige Köpfchen. Lipophile als auch phenolische Substanzen konnten als Bestandteil der Sekrete der Drüsenhaare nachgewiesen werden. Organspezifische Profile („fingerprints") von Flavonoiden und ätherischen Ölen beider Taxa wurden mittels Dünnschichtchromatographie erstellt.

1. Introduction

The genus *Lavandula* is an important member of the *Lamiaceae* family, subfamily *Nepetoideae*, comprising a total of 39 species, 30 infraspecific taxa and 17 hybrid species that are widely distributed from the Canary and Cape Verde Islands and Madeira, across the Mediterranean Basin, North Africa, South-West Asia, the Arabian Peninsula and tropical NE Africa with a disjunction to India. Considering the large morphological and chemical diversity Upson & Andrews 2004 proposed a subgeneric classification of eight sections and three subgenera. section *Lavandula* (subgenus *Lavandula*) contains the economically most important species, *L. angustifolia* (true or common lavender), *L. latifolia* (spike lavender) and their sterile hybrid *L.* × *intermedia* (lavandin). They grow as perennial evergreen shrubs, mainly on arid rocky hillsides. Besides essential oil, *Lavandula* species are reported to contain phenolic compounds, such as tannins, flavonoids (Upson & al. 2000), phenolic acids, coumarins (Areias & al. 2000, Torras-Claveria & al. 2007) as well as triterpenes (Papanov & al.

1992, HARBORNE & WILLIAMS 2002). *Lavandula* essential oil is worldwide used in fragrance, cosmetic and food industry as well as for aromatherapy.

Nowadays, *L. angustifolia* and *L.* × *intermedia* are extensively cultivated all over the world for their aromatic flowers, which contain at least 1.5 percent (v/w) essential oil with linalool and linalyl acetate as its main components (BLUMENTHAL & al. 1998). While *L.* × *intermedia* gives the larger yield of oil and is therefore economically important, *L. angustifolia* produces the highest quality oil, with higher linalyl acetate contents and less camphorous aroma (CHARLES & al. 2002) and, therefore, is very much appreciated in perfumery. *L. angustifolia* is also generally accepted as the most effective medicinal plant belonging to the genus *Lavandula*. It has been reported to possess anxiolitic, sedative (BUCHBAUER & al. 1991, LEWITH & al. 2005, BRADLEY & al. 2007), anti-inflammatory, analgesic (HAJHASHEMI & al. 2003), antimicrobial (MUYIMA & al. 2002, ROTA & al. 2004, D'AURIA & al. 2005), and antiparasitic activity (MOON & al. 2006).

Lavandin (*L.* × *intermedia*) plays a very important role in the world's production of lavender oil all over the world (UPSON & ANDREWS 2004). ‚Budrovka' is an indigenous cultivar of lavandin which forms the basis of the Croatian lavender industry. In spite of its economic importance, however, scientific data remain sparse (KUŠTRAK & BEŠIĆ 1975, BLAŽEKOVIĆ & al. 2010). The presented study aimed to characterize morphological, anatomical and phytochemical features of *L.* × *intermedia* ‚Budrovka' in comparison to *L. angustifolia*. Moreover, considering the interest in secreted material of *Lavandula* for pharmaceutical purposes, special attention was devoted to elucidate morphology and distribution of glandular trichomes, since a detailed organographic study in any *Lavandula* taxa has not been reported before and histochemical analysis of secreted products is still limited.

2. Materials and Methods

2.1. Plant Material

The above-ground parts of cultivated plants of *L.* × *intermedia* EMERIC ex LOISEL. ‚Budrovka' and *L. angustifolia* MILL. were collected at full blooming stage in July 2008, from a three-year old plantation on a farm in the village Dragovanščak near to the city Jastrebarsko (Central Croatia, 45°70′ N, 15°55′ E). The plantation is located at about 300 m above sea level in the continental agricultural region, on hill slopes with predominant submediterranean vegetation. Thirty specimens of each taxon were used in this study and analyses were performed on fresh as well as air-dried material. Morphological observations and biometric measurements were performed for leaves, flowers and inflorescence stems of each taxon.

2.2. Light Microscopy (LM) and Histochemistry

All specimens were prepared for LM by standard techniques and then observed under a light microscope. The main classes of plant metabolites were investigated in

fresh plant sections, using the following histochemical tests: Sudan III for localization of lipids (SASS 1951), vanillin-hydrochloric acid for general detection of phenols (excitation 420 nm; COMBRINCK & al. 2007), Natural products reagent (β-aminodiethylester of diphenylboric acid) for detection of flavonoids (excitation 365 nm; KOLB & MÜLLER 2004), and safranin-astrablue (SA) was used to distinguish between lignified (red) and non-lignified cell walls (blue) (HOROBIN 2002). Investigations were carried out using a light microscope equipped with an epifluorescence unit (Zeiss Axioplan) and a colour video camera.

2.3. Scanning Electron Microscopy (SEM)

Samples were fixed in a mixture of absolute ethanol and glacial acetic acid (3:1) for 24 hours at 4 °C, and subsequently prepared for SEM. The specimens were dehydrated using ethanol series, and dried at the critical point using CO_2 as the drying agent (Bal-Tec CPD 030, Bal-Tec Union Ltd., Lichtenstein). The samples were mounted on aluminium stubs using double-sided carbon impregnated tape, coated with a thin layer of gold (Agar sputter coater, Agar Scientific, U.K.), and investigated with a scanning electron microscope Philips XL 30 ESEM (FEI, The Netherlands) (high vacuum; acceleration voltage 20 kV).

2.4. Thin Layer Chromatography

Thin layer chromatography (TLC) was used for analysis of plant bioactive constituents as described by WAGNER & BLADT 2009. Briefly, essential oils, obtained by hydrodistillation of fresh flowers and leaves, respectively, were studied by TLC using toluene/ethyl acetate (93:7) as a mobile phase and separated compounds were visualized with vanillin/sulphuric acid, followed by heating at 110 °C. For flavonoid analysis, methanolic extracts of flowers, stems and leaves as well as standard solutions were applied to a TLC plate and developed with a mixture of ethyl-acetate/formic acid/acetic acid/water (100:11:11:27). The plate was examined in UV light at 365 nm after spraying with the Natural products-polyethylene glycol reagents (NP/PEG) (Fluka Chemie, Switzerland).

TLC analyses were carried out on Silica gel 60 F_{254}-precoated plates (Merck, Germany), samples were applied using Camag automatic TLC sampler 4 and the chromatograms were scanned by Camag Reprostar 3 (Camag, Muttenz, Switzerland).

2.5. Statistical Analysis

The significance of differences between the taxa studied was calculated by Student's *t*-test, using commercial software (GraphPad Software, San Diego, CA), and the level of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Morphological and Anatomical Features

L. × *intermedia* ‚Budrovka' and *L. angustifolia* are perennial shrubs with a woody base (Fig. 1). Morphological measurements for both taxa are summarized in Table 1, while general descriptions of morphological characteristics for each taxon are listed below.

Table 1. Morphological measurements of *Lavandula* × *intermedia* ‚Budrovka' and *L. angustifolia*.

	L. × *intermedia* ‚Budrovka'	*L. angustifolia*
Inflorescence		
Inflorescence length (mm)	95.30 ± 13.64	51.39 ± 6.34*
Gap length (mm)	43.89 ± 8.00	25.67 ± 5.89*
Number of verticillasters per spike	7 ± 1	5 ± 0*
Number of flowers per verticillaster	11 ± 2	5 ± 1*
Corolla length (mm)	8.80 ± 0.75	8.92 ± 1.16^{ns}
Calyx length (mm)	5.50 ± 0.25	5.12 ± 0.16*
Bract lenght (mm)	5.16 ± 0.66	5.23 ± 0.54^{ns}
Bract width (mm)	2.60 ± 0.39	3.45 ± 0.48*
Bracteole lenght (mm)	2.41 ± 0.58	–
Leaf		
Leaf length (mm)	28.04 ± 7.43	15.46 ± 5.32*
Leaf width (mm)	2.42 ± 0.36	1.68 ± 0.39*
Inflorescence stem		
Stem diameter (mm)	1.38 ± 0.16	0.78 ± 0.06*

Data are given as mean ± SD (n=30); * $p < 0.001$; ^{ns} no significant difference

L. × *intermedia* ‚Budrovka' is a large and robust plant that grows to heights up to 105 cm (Fig. 1, Table 1). Leaves are opposite, linear to lanceolate-linear, 15–40 mm x 1.5–3 mm, with a greenish grey look. The older foliage is green while the new growth is silvery. The leaf base attenuates to a very short petiole; the leaf is entire, revolute and obtuse with a prominent midrib on the abaxial surface. Inflorescence stems are up to 80 cm long, 1–2 mm in diameter, dark green with light green margins, semi-upright to upright. Frequently occurring lateral branches above the main foliage are long and terminated by small spikes, rarely one-sided or absent. The flowers are distinctly stalked, arranged in 2 cymes giving verticillasters, each 8- to 26-flowered, 5–9 verticillasters forming the terminal cylindrical spike-like inflorescence. Spikes are 7–15 cm long, lax, with a basal verticillaster 2–11 cm below the main spike. Bracts are light brown, broadly ovate-rhombic, 3.5–7 mm × 2–4 mm, while bracteoles, marking each branching point of the cyme, are 1–4 mm long and brown. The densely tomentose calyx is tubular, 5–7 mm long, 13-veined, with 5 teeth, with a small circular appendage, green with the upper half being violet-blue. Corollas are 7.5–11 mm long, bright violet-blue, two-lipped, the lower lip with 3 lobes, upper lip 2-lobed and notched.

L. angustifolia is an erect and compact plant, up to 60 cm high, with dense green foliage (Fig. 1, Table 1). Leaves are linear, 10–35 mm × 1–2 mm. Those in leaf axils are smaller, highly revolute with a dense felt-like indumentum. Inflorescence stems are light green, up to 40 cm long, thin (0.5–1 mm), erect, bearing more or less compact terminal spikes. Lateral

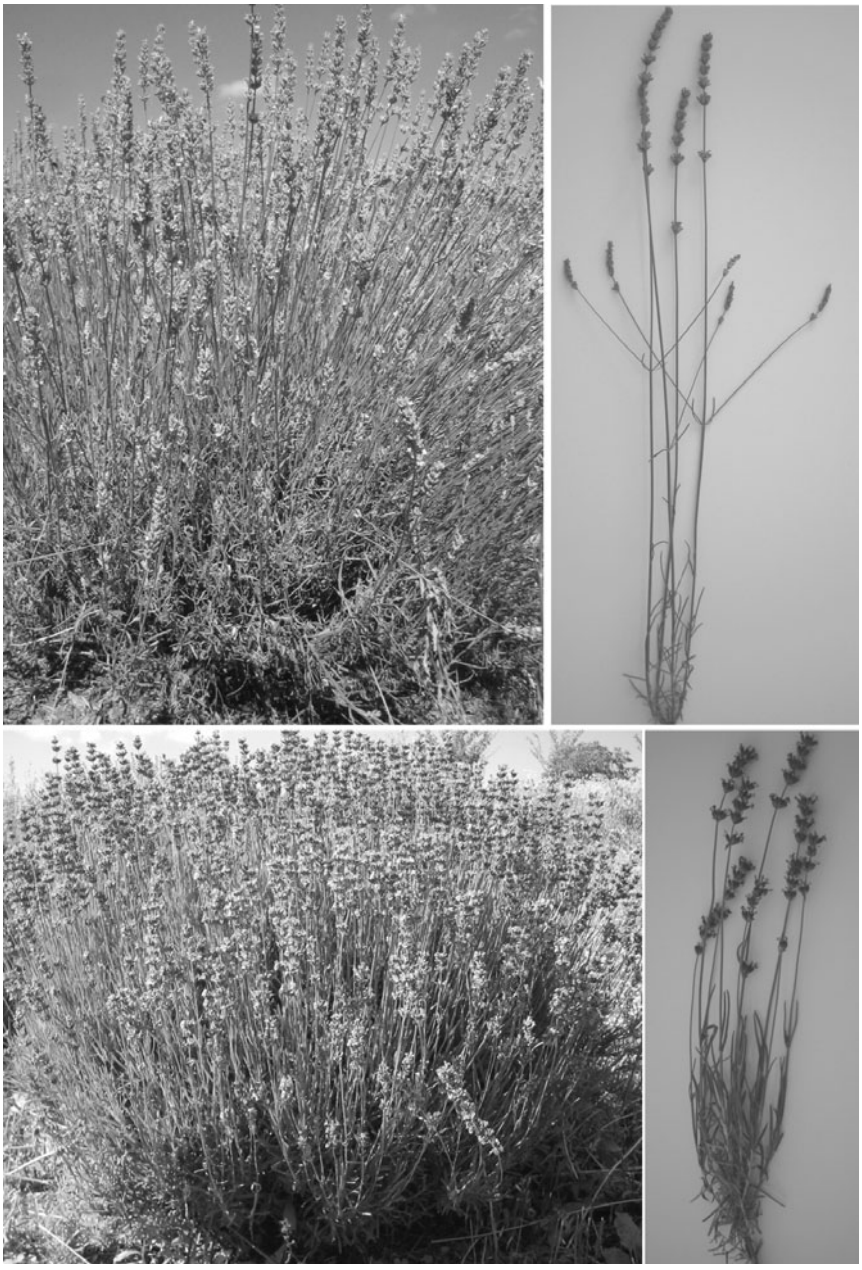

Fig. 1. Photographs of *Lavandula* × *intermedia* ‚Budrovka' (top) and *L. angustifolia* (bottom) cultivated in Croatia.

branching is uncommon, but occasionally semi-stalked laterals can be found. Spikes are 4–8 cm long with 4–6 verticillasters, up to 9-flowered each, occasionally with 1–4 cm remote basal verticillasters. Each of two opposite cymes of the verticillaster is subtended by a broadly rhomboidal bract, 4.5–6.5 mm × 2.5–4.5 mm, with a long cuspidate apex, mid brown, dry, while bracteoles are minute and not clearly visible. Flowers are very short pedicellate. Calyces (4.5–6 mm) are tubular, 13-veined, pubescent, and green with a dark violet-blue suffusion. The corolla is typically bilabiate with two lobes forming an upper lip and three lobes forming a lower lip, 7–12 mm long, purplish-violet.

With the exception of the length of the corolla and the bracts (no significant differences), and the bract width, *L.* × *intermedia* is significantly taller than *L. angustifolia* (Table 1). Overall anatomical features (cross sections of leaf, inflorescence stem and calyx) did not differ between *L.* × *intermedia* ‚Budrovka' and *L. angustifolia*.

3.2. Trichomes

Leaves, inflorescence stems and flowers of both investigated taxa were densely covered with trichomes of the glandular and non-glandular typ. The glandular trichomes could further be divided into peltate and capitate types. Peltate trichomes consisted of a basal cell, a short unicellular stalk, and a large head of eight secretory cells with a large subcuticular space (Fig. 2A). On some trichomes the cuticle was removed and the number of cells was countable. The trichome was often darkly coloured and the subcuticular space filled with oily droplets (Fig. 5A, B). Four types of capitate trichomes could be observed. Type I consisted of a short unicellular stalk and a distinct, spherical unicellular head (Fig. 2B), type II was a short-stalked trichome with two head cells (Fig. 2A), while type III had a small unicellular head on a stalk consisting of two to three cells (Fig. 2C). All three types (I-III) were characterized by a smooth surface. Type IV was characterized by a long multicellular stalk with a warty cuticle, a smooth neck cell and a distinct unicellular head. A characteristic ring of four protruding knobs could often be observed between stalk and neck cell (Fig. 2D). The stalk was often purple coloured due to anthocyans (Fig. 5J). Non-glandular trichomes were uniseriate (Fig. 2E), as well as one- to three branched multicellular (Fig. 2A, F), all of them having a warty cuticle. Some of them were coloured by anthocyans (Fig. 5E).

The distribution of the various trichomes differed between organs (Table 2, Fig. 3, 4), but no distinct difference between *L.* × *intermedia* ‚Budrovka' and *L. angustifolia* could be observed. Young leaves (Fig. 3A) and inflorescence stems (data not shown) were densely covered with two- and three-branched non-glandular trichomes with the density decreasing progressively with organ maturity (compare Fig. 3B – mature leaf). The

Table 2. Trichome types and their distribution on the plant parts of *Lavandula* × *intermedia* ‚Budrovka'.

Trichome	Leaf		Bract		Calyx		Corolla		Infl. stem
	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	
Non-glandular									
branched multicellular	+	+	–	+	+	–	+	+	+
long unicellular	–	–	–	–	–	–	–	+	–
short unicellular or papillae	–	–	–		–	–	–	+	+
Glandular									
Peltate	+	+	–	+	+	–	+	–	+
Capitate									
type I	+	+	–	–	+	–	–	–	+
type II	+	+	–	–	–	–	–	–	+
type III	–	–	–	–	+	–	–	–	–
type IV	–	–	–	–	–	–	–	+	–

outer surfaces of the calyx (Fig. 4D) and the corolla (Fig. 4B) were also intensively covered with branched non-glandular trichomes.

Peltate trichomes could be observed on the vegetative as well as on the reproductive organs (Fig. 3, 4), dominating on leaves, bracts and flower parts (corolla and calyx) and being only sparsely scattered on the inflorescence stem (Fig. 3D). Being present on both leaf surfaces, they were much more frequent on the abaxial leaf surface (Fig. 3B as compared to Fig. 3C); on the bracts they occurred only on the abaxial leaf surface. Those on the calyx and the corolla were generally limited to the outer surface and were mainly localized in the indentations whereas the ribs were dominated by non-glandular branched trichomes (Fig. 4B, D).

Whereas the peltate trichomes were present on all investigated organs, the four types of capitate trichomes showed characteristic organ specificity (Table 2). Type I occurred on ad- and abaxial leaf surfaces, on the stem and also on the outer side of the calyx, but, in general they were rare. Much more common and uniformly distributed was type II on both leaf surfaces and on the inflorescence stem. In contrast, type III of the capitate trichomes could only be observed on the calyx. There, however, it was abundant. Type IV of the capitate trichomes occurred exclusively on the inner surface of both corolla lips (Fig. 4A, B, C).

According the distribution of the non-glandular trichomes the branched ones covered nearly all investigated plant organs (inflorescence stem, both leaf surfaces, abaxial bract surface, and outer surface of calyx and corolla, respectively). On the inner corolla surface long uniseriate trichomes together with branched non-glandular trichomes could be observed. Short unicellular trichomes occurred on the stems only, while the corolla was densely papillose inside.

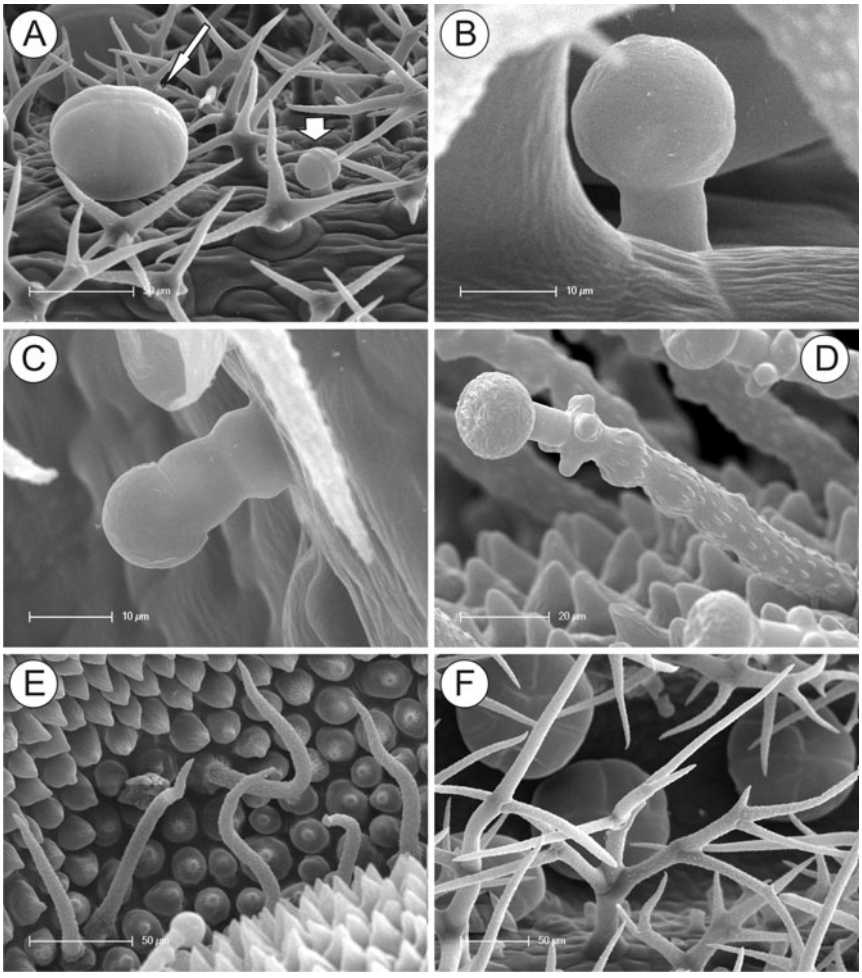

Fig. 2. Scanning electron microscopic micrographs of trichomes. (A) Peltate trichome (long arrow), capitate trichome type II (short arrow) and non-glandular branched trichomes on leaves. (B) Capitate trichome type I. (C) Capitate trichome type III on the inner calyx surface. (D) Capitate trichome type IV on the inner surface of the corolla. (E) Uniseriate non-glandular trichomes on the corolla. (F) Multibranched non-glandular trichome on the calyx.

3.3. Histochemistry

All non-glandular trichomes were characterized by thick and lignified cell walls (red staining with safranin) whereas the normal epidermal cells showed a thick but non-lignified cell wall (blue with astrablue) (data not shown). The staining results for the glandular tri-

chomes of *L.* × *intermedia* ‚Budrovka' are presented in Table 3 and in Fig. 5. The products in the subcuticular space of the peltate trichomes (Fig. 5B) and compounds in the head cells as well as the stalk or neck cell of type I (Fig. 5F), III (Fig. 5I) and IV (Fig. 5K) reacted positive for lipids (red with Sudan III) while type II of the capitate trichomes showed only a very faint reaction (Fig. 5 H). Flavonoids (yellow fluorescence following staining with Natural product reagent) could be detected exclusively in the head cells of the trichomes with the strongest colour reaction in the head cells of type I (Fig. 5C) and type IV (Fig. 5L) capitate trichomes. The flavonoids of the peltate trichomes seemed to be concentrated in the cells (cell sap) whereas a droplet filled subcuticular space resulted in a reduced and more diffuse colour reaction (Fig. 5C). Comparably, a strong positive reaction after staining with vanillin-HCl (yellow fluorescence) indicated varying amounts of phenols, especially in the peltate trichomes (Fig. 5D).

Table 3. Histochemistry of glandular trichomes on vegetative and reproductive organs of *Lavandula* × *intermedia* ‚Budrovka'.

Staining procedure	Target compound	Observed colour	Peltate trichomes	Capitate trichomes			
				type I	type II	type III	type VI
Sudan III	Total lipids	Red	+++	+	(+)	+	+
Natural product reagent	Flavonoids	Yellow to orange	+	++	+	+	++
Vanillin-HCl	Flavonoids	Yellow	+++	+	+	+	+
Safranin-astrablue	Lignified cell walls	Red	–	–	–	–	–
	Non-lignified cell walls	Blue	+	+	+	+	+

–, negative; (+) slightely positive, + positive. +++ strongly positive

3.4. Phytochemical Analysis

The phytochemical constituents of *L.* × *intermedia* ‚Budrovka' and *L. angustifolia* as well as their distribution among the different parts of each species were investigated by TLC. Chromatograms obtained for the essential oils isolated from the flowers of both *Lavandula* taxa (Fig. 6A) showed two very strong spots with the same colour and retention factors (R_f) as the linalool and linalyl acetate standards (R_f = 0.41 and 0.59, respectively) indicating their presence. TLC of the essential oils from the leaves displayed weak spots of linalool and linalyl acetate or even their absence. The strong brown spot corresponding to the 1,8 cineole with R_f 0.41 appeared on the TLC plate of the essential oils from *L.* × *intermedia* ‚Budrovka', while a specific unidentified brown spot below linalool (R_f = 0.23) was detected in *L. angustifolia* essential oil only. Phytochemical screening of the leaf, in-

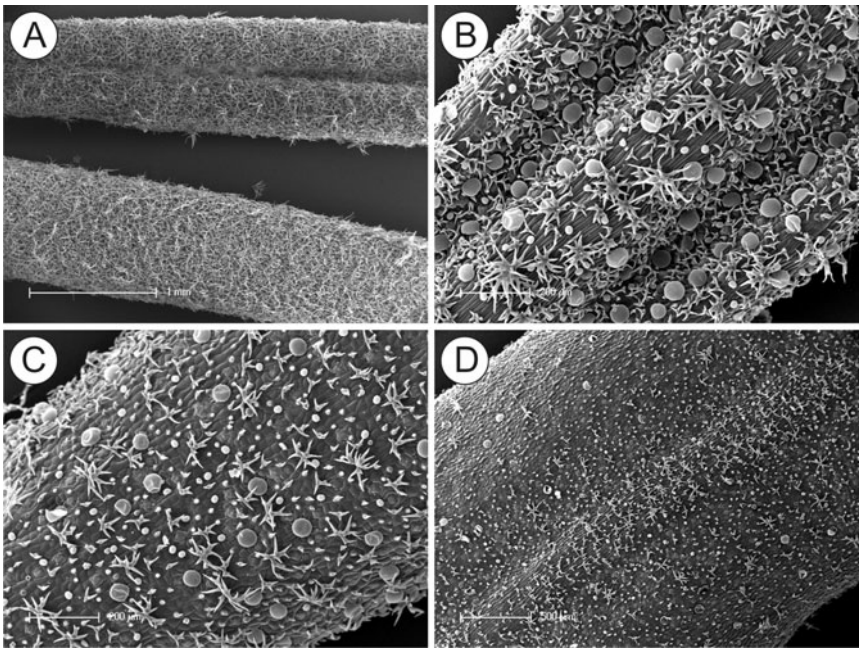

Fig. 3. Scanning electron microscopic micrographs showing the distribution of trichomes on leaves and inflorescence stem, *L.* × *intermedia* ‚Budrovka'. (A) Abaxial (top) and adaxial (bottom) surface of young leaves. (B) Abaxial surface of a mature leaf. (C) Adaxial surface of a mature leaf. (D) stem.

florescence stem and flower extracts of *L.* × *intermedia* ‚Budrovka' and *L. angustifolia* displayed the presence of phenolic compounds in all parts of the plants (Fig. 6B). At least two flavonoid compounds (R_f 0.47 and 0.62, yellow fluorescence) and four phenolic acids with R_f values of 0.45, 0.75, 0.83 and 0.95, respectively, (blue fluorescence) were found to be constituents of both *L.* × *intermedia* ‚Budrovka' and *L. angustifolia*. Among the examined plant organs of the two taxa, there were noticeable organ specific differences in the quantity of the compounds. In general, flavonoids (yellow fluorescence) were most prominent in leaves, followed by the inflorescence stem and were much less present in the flower extracts. Phenolic acids (blue fluorescence) were much less prominent in inflorescence stems as compared to flowers and leaves. The spot corresponding to luteoline-7-glucoside was intense in leaf extracts of both taxa as well as in stem extracts of *L.* × *intermedia* ‚Budrovka', while it was very faint or even absent in flower and stem extracts of *L. angustifolia*. The chromatographic profiles of all *Lavandula* extracts showed spots with the characteristic blue fluorescence of rosmarinic acid (R_f = 0.95).

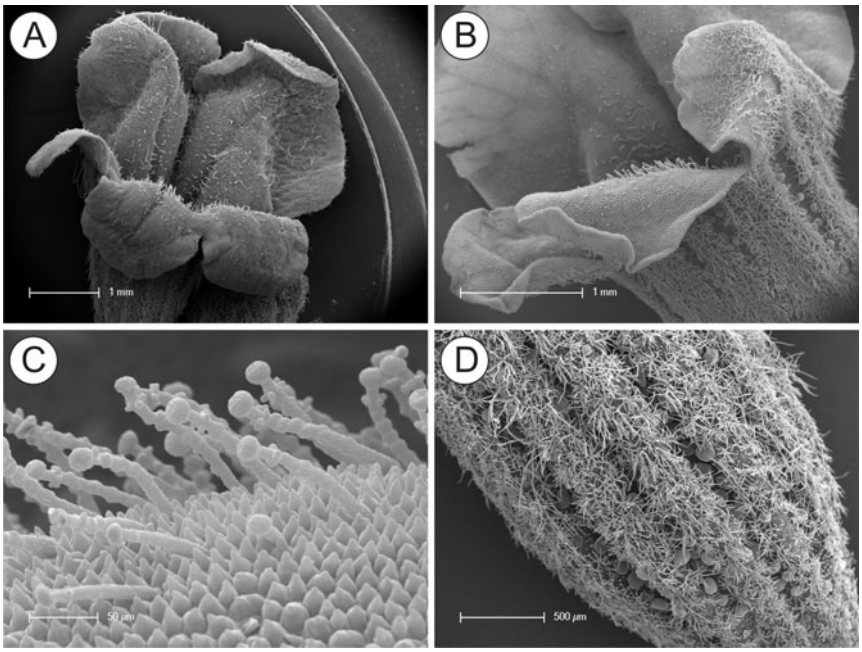

Fig. 4. Scanning electron microscopic micrographs showing the distribution of trichomes on floral parts. (A) Overview of the corolla. (B) Corolla. (C) Capitate trichomes type IV on the lower lip. (D) Calyx.

4. Discussion

This study presents morphological, anatomical and phytochemical features of *L.* × *intermedia* ‚Budrovka', which was studied in comparison to the closely related *L. angustifolia*.

The results of the morphological analyses of *L. angustifolia* were consistent with the data given by Flora Europaea (Tutin & al. 1972) while the morphological features of the cultivar ‚Budrovka' fit the general description of *L.* × *intermedia* Emeric ex Loisel. (Upson & Andrews 2004). *L.* × *intermedia* ‚Budrovka' was significantly larger than *L. angustifolia* and was characterized by thicker stems, larger leaves and flowers with larger calyces and a greater number of individual flowers per cyme, and bracteoles that are missing in *L. angustifolia*.

Both investigated taxa were characterized by a more or less dense indumentum of non-glandular and glandular trichomes. These trichomes are of special importance for taxonomy in the *Lamiaceae* family (Metcalfe & Chalk 1972, Giuliani & Maleci Bini 2008) and are the source of essential oils that are the responsible for economical importance of lavender.

A characteristic feature of *Lamiaceae* is the presence of peltate and capitate glandular trichomes (Ascensão & al. 1999, Giuliani & Maleci

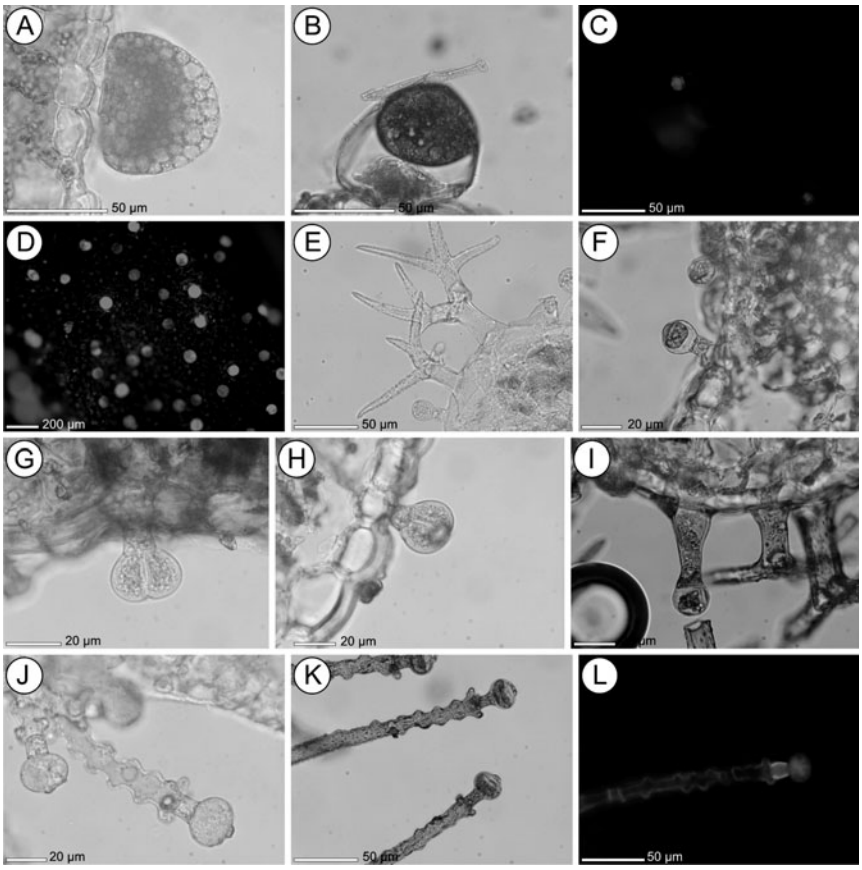

Fig. 5. Light microscopic micrographs of trichomes and results of histochemical staining; *L.* × *intermedia* ‚Budrovka'. (A) No staining; peltate trichome. (B) Sudan III; peltate trichome, the secretion products in the subcuticular space are intensively red coloured. (C) Natural product reagent; intensive yellow colouring in the head cells of capitate trichome type I and more diffuse colouring of the head cell of a peltate trichome. (D) Vanillin-HCl; intensive colouring of peltate trichomes. (E) No staining; capitate trichome type I and branched non-glandular trichomes with purple cell sap. (F) Sudan III; capitate trichome type I. (G) Safranin-astrablue; capitate trichome Type II. (H) Sudan III; capitate trichome type II. (I) Sudan III; capitate trichome type III. (J) No staining; capitate trichome type IV with purple cell sap in the stalk. (K) Sudan III; capitate trichome type IV. (L) Natural product reagent; capitate trichome type IV.

Bini 2008). Peltate and four different types of capitate trichomes could be observed on the investigated *Lavandula* taxa, However, there was no distinct difference, neither in trichome morphology nor in the organ specific distribution, between *L.* × *intermedia* ‚Budrovka' and *L. angustifolia*. The peltate glandular trichomes, consisting of a short unicellular stalk and a

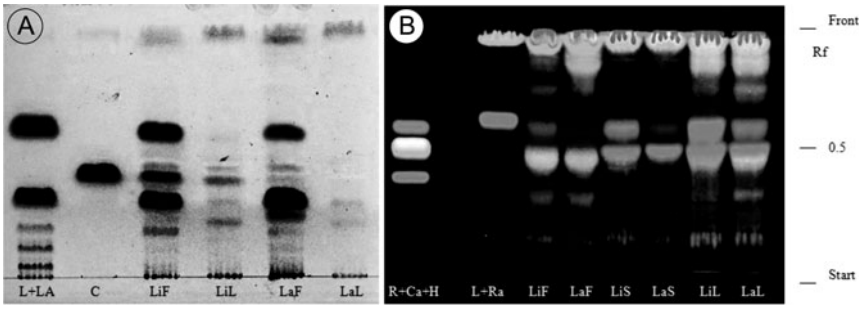

Fig. 6. Thin layer chromatograms. (A) TLC separation of essential oil isolated from flowers (LiF) and leaves (LiL) of *L.* × *intermedia* ‚Budrovka' and flowers (LaF) and leaves (LaL) of *L. angustifolia*; standards: L – linalool, LA – linalyl acetate, C – 1,8 cineole. (B) Comparative TLC analysis of the methanolic extract of flowers, inflorescence stems and leaves of *L.* × *intermedia* ‚Budrovka' (LiF, LiS and LiL, respectively) and *L. angustifolia* (LaF, LaS and LaL, respectively); standards: R – rutin, Ca – chlorogenic acid, H – hyperoside, L – luteoline-7-glucoside, Ra – rosmarinic acid.

secretory head composed of eight cells arranged in a single circle, were similar to those reported by Iriti & al. 2006 for *L. angustifolia*, for *L. pinnata* (Huang & al. 2008) as well as some other *Lamiaceae* (Serrato-Valenti & al. 1997, Ascensão & al. 1999, Bottega & Corsi 2000, Turner & al. 2000, Jurišić Grubešić & al. 2007). In the presented study it was shown that this trichome type was also densely distributed on the outer surface of the corolla, the abaxial surface of the bracts and on both leaf surfaces, while they were only sparsely scattered on the inflorescence stem. These findings are in agreement with a recent study of Guitton & al. 2010.

The complex nature of the material secreted by *Lavandula* peltate trichomes was revealed by histochemical studies. The presence of polyphenols, already indicated by the dark colour of the trichome head, was confirmed by vanillin-HCl staining. Secretion products in the subcuticular space intensively stained for lipids, and flavonoids were localized in the cells beneath the subcuticular space. The presented results confirm and complement other reports on secretion products of peltate trichomes (Ascensão & al. 1999, Bottega & Corsi 2000, Iriti & al. 2006, Marin & al. 2006, Giuliani & Maleci Bini 2008, Rodrigues & al. 2008). In conformity with Maffei 2010 the peltate glandular trichomes of *Lavandula* taxa were found to produce and store a bulk of essential oil. Single oil gland analysis of *Salvia sclarea* also showed that peltate trichomes are the main source of essential oils in *Lamiaceae*, although they contain a very complex mixture of several compounds and different substance classes. Moreover, from *S. sclarea* it was reported that oil composition varied according to the plant organ and even revealed a high variation on a single organ (Schmiderer & al. 2008). More detailed work will be necessary to elucidate organ specific

differences and also developmental variations in the composition and secretion processes of this trichome type.

Whereas the peltate trichomes are quite uniform in the *Lamiaceae*, capitate glandular trichomes show much more variation in structure and size (Ascensão & al. 1999). Four types of capitate trichomes were observed in the examined *Lavandula* taxa. The short-stalked capitate trichomes, type I and II trichomes, with unicellular stalk and unicellular or bicellular head, respectively, are supposed to be common in most members of the *Lamiaceae* family (Metcalfe & Chalk 1972). Leaves of *L.* × *intermedia* ‚Budrovka' and *L. angustifolia* were covered with type I and type II capitate trichomes affirming previous observations on *L. angustifolia* (Urvin & al. 2007). Type II trichomes were the dominating capitate trichomes on the leaves and, though sparsely distributed, also present on the inflorescence stem. Type I capitate trichomes were also found on the calyx. In addition, capitate trichomes of type III with a two-celled stalk and a unicellular head were detected on the calyx. This is the first evidence on this trichome type for the genus *Lavandula*. Exclusively on the inner surface of the corolla type IV of the capitate trichomes could be observed. The morphology of this trichome type is very unique. It is characterized by a long stalk with prominent warty knobs, a distinct neck cell and a one-celled spherical head. This trichome type is reported to be present in powdered dried flowers of *L. angustifolia* (European Directorate for the Quality of Medicines & Health Care 2002). To our knowledge this is the only report of this trichome type. A recent investigation of flowers of *L.* × *intermedia* ‚Super' did not detect any trichomes on the inner corolla surface (Cerpa & al. 2008). The characteristic morphology and the exclusive distribution on the corolla lips suggest a role in pollination ecology of lavender. This would be an interesting field for future work.

According to histochemistry the capitate hairs also differ in their secretion products. All four types of capitate trichomes stained positively for lipids, though there were distinct differences in staining intensity. The lipid staining was most intensive in the head cells of type IV and only very faint in the head cells of type II. All types stained positively for flavonoids, but again type II trichomes showed a lesser staining intensity.

Since TLC was recognized as a potential tool for preliminary investigation of phytochemistry and still remains the reference for Pharmacopoeia, this method was employed for a rapid qualitative analysis of the essential oils and phenolic constituents attempting to elucidate the chemistry of secretory products. Our phytochemical work revealed the monoterpenes linalool and linalyl acetate as the major constituents of the flower essential oils from the two *Lavandula* taxa. These results were consistent with the well-investigated chemical composition of the *L. angustifolia* essential oil (Harborne & Williams 2002) while the preliminary analysis of essential oil from cultivar ‚Budrovka' suggested similar chemical profile to

cultivars ‚Super' and ‚Special' (CHATZOPOULOU & GOLIARIS 2003), but different in regard to *L. angustifolia* due to the prominent amount of 1,8 cineole detected. Hence, this difference could be considered as an intertaxa marker. In contrast to the flowers, TLC chromatograms indicated that the leaves of both *Lavandula* taxa produce linalool and linalyl acetate in only trace amounts. Different chemical composition of flower and leaf essential oils could be, at least in part, attributed to the exclusive presence of type IV glandular trichomes on the corolla and type III on the calyx. Moreover, according to TURNER & al. 2000 who supported the statement that the changes in leaf oil composition are related to leaf and gland maturation, we may assume that the compositional differences between organs follow ontogenetic development. Similar findings were reported for essential oil of leaves and flowers in the genus *Achillea* (NEMETH 2005). TLC separation of phenolic constituents revealed the presence of at least two flavonoids, probably luteolin derivatives (HARBORNE & WILLIAMS 2002) and four phenolic acids in *L.* × *intermedia* ‚Budrovka' and *L. angustifolia*. Although the two investigated taxa seem to have equal phenolic profile, our comparative analysis of their distribution among the different plant organs displayed visible differences within taxa and between them. Detected phenols were especially concentrated in the leaves, while inflorescence stems were characterized by relatively simpler phenolic acid composition.

In conclusion, the presented study gives the first and extensive description of the morphological and anatomical features of the cultivar *L.* × *intermedia* ‚Budrovka', which is of great economic importance for fragrance, flavour, and pharmaceutical industries in the whole Mediterranean area. The comparison with *L. angustifolia*, the most common taxa of the genus *Lavandula*, resulted in some differences in morphology and essential oil composition but showed conformity in structure, occurrence and distribution of non-glandular and various types of glandular trichomes. Especially, the exclusive type of capitate glandular trichomes discovered on the corolla provides a new insight into the *Lavandula* essential oil producing structures. Obtained results encourage more detailed chemical studies of the glandular secretion with regard to the trichome structure, organographic distribution and ontogeny.

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