



## Article

# Essential Oil Quality of Lavender Grown Outside Its Native Distribution Range: A Study from Serbia

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**Abstract:** The aim of this study was to test the quality (physicochemical and sensory odor properties) and bioactivity (antimicrobial and antioxidant activities) of the essential oils (EO) obtained from the most frequently cultivated lavender and lavandin varieties in Serbia, whose cultivation areas were previously reserved for warmer climates, outside the agroecological region of Serbia. Seven EO from true lavenders (*L. angustifolia* Mill. and cultivars: ‘Hidcote blue’, ‘Munstead’, ‘Primorska’), Croatian indigenous lavandin cultivar (*L. × intermedia* ‘Budrovka’), lavandin ‘Grosso’ and one undetermined lavender sample (*Lavandula* sp.) showed compliance with standard requirements for lavender EO composition (contents of linalool 23.9–30.2% and 28.9–36.9%, and of linalyl acetate 22.2–32.2% and 6.9–20.7% in true lavender and lavandin samples, respectively). All EO were characterized as pleasant, with a floral aroma as a prominent odor. Samples exhibited high antimicrobial activities (3.5–14.2  $\mu\text{L mL}^{-1}$  MIC and MBC values) against important Gram-positive (*B. cereus* and *L. monocytogenes*) and Gram-negative bacteria (*E. coli*) and yeasts (*C. albicans*), and high antioxidant capacity (IC<sub>50</sub> values of 0.23–0.59  $\mu\text{g AAE mL}^{-1}$  EO). This preliminary research on the quality of lavender EOs reveals the potential of this species for the future of medicinal and aromatic plant species production and further diversification of agriculture in the area.

**Keywords:** antimicrobials; antioxidants; aroma; GC-MS; essential oil; lavender; lavandin; *Lavandula* cultivars; sensory odor evaluation



**Citation:** Kiprovski, B.; Zeremski, T.; Varga, A.; Čabarkapa, I.; Filipović, J.; Lončar, B.; Aćimović, M. Essential Oil Quality of Lavender Grown Outside Its Native Distribution Range: A Study from Serbia. *Horticulturae* **2023**, *9*, 816. <https://doi.org/10.3390/horticulturae9070816>

Academic Editor: Laura Santagostini

Received: 20 June 2023

Revised: 9 July 2023

Accepted: 13 July 2023

Published: 15 July 2023



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## 1. Introduction

The genus *Lavandula* L. (*Lamiaceae*) counts 41 accepted species native to Macaronesia, the Mediterranean basin, Northern and North-Eastern Africa, South-Western Asia, the Arabian Peninsula, Central and Southern India, or introduced to Eastern Europe, Australia, and New Zealand, according to the World Checklist of Vascular Plants [1]. Commonly grown representatives of the *Lavandula* genus used for the distillation of essential oil are *L. angustifolia* Miller, *L. latifolia* Medikus, and lavandin (a sectional hybrid of *L. angustifolia* Mill.  $\times$  *L. latifolia* Medik.), which belong to the *Lavandula* section *Spica* Ging., along with *L. lanata* Boiss. and an additional hybrid *L. lanata* Boiss.  $\times$  *L. angustifolia* Miller, with many varieties produced throughout the years with different commercial names [2].

True lavender (*L. angustifolia* Miller subsp. *angustifolia* or *L. spica* L. var.  $\alpha$ , *L. officinalis* Chaix) or English lavender [3,4] can be found as a spontaneous population or clonal. There is also the Spanish type of lavender, which can be the native species *L. latifolia* Medikus (*L. spica* L. var.  $\beta$ , *L. latifolia* Villers), but also a hybrid [3,5]. Additionally, the French type of lavender [3,6,7], or a sterile cross between the English and spike lavender, is known as lavandin (*L. × intermedia* Emeric. ex Loiseleur syn. *L. hybrida* Reverchon). There are

two lavandin cultivars, ‘Abrial’ and ‘Grosso’, which are recognized by ISO standards, and several others selected for essential oil production and horticultural purposes. Lavandin has a much larger habitus than true lavender and a higher content of essential oil (6.2–10% and 2.3–5%, respectively) [8], the reason for which it has become very popular; however, when it comes to essential oil composition, these species are quite different (Table 1).

**Table 1.** Gas chromatography profiles (flame ionization detector) and sensory odor properties of lavender essential oils: English type (*L. angustifolia* Mill.), Spanish type (*L. latifolia* Medik.) and French type (*L. × intermedia* Emeric. ex Loiseleur) ‘Abrial’ and ‘Grosso’ lavandin, according to lavender essential oil standards [4–7,9]. Compounds are expressed as min–max content (%).

Compound	English Type, True Lavender *	Spanish Type, Spike Lavender	French Type, Lavandin ‘Abrial’	French Type, Lavandin ‘Grosso’	Odor Type
myrcene	nd	nd	0.4–0.9	0.3–1.0	balsamic, spice [10]
$\beta$ -phellandrene	tr-1.0	nd	nd	nd	minty terpenic [11]
<i>cis</i> - $\beta$ -ocimene or <i>Z</i> - $\beta$ -ocimene	1.0–10.0	nd	1.4–3.0	0.5–1.5	sweet, floral, herbal [10]
<i>trans</i> - $\beta$ -ocimene or <i>E</i> - $\beta$ -ocimene	1.0–6.0	nd	2.5–6.0	nd-1.0	sweet herbal [11]
limonene	0.3–1.0	0.5–3.0	0.5–1.5	0.5–1.5	terpene, pine, herbal, peppery [11]
1,8-cineole	0.5–3.0	16.0–39.0	6.0–12.5	4.0–8.0	floral, minty, fruity [10]
linalool	22.0–45.0	34.0–50.0	28.0–38.0	24.0–37.0	floral [12]
camphor	tr-1.5	8.0–16.0	7.0–11.0	6.0–8.5	camphor [10]
borneol	nd	nd	1.5–3.5	1.5–3.5	camphor [10]
lavandulol	nd	nd	0.4–1.2	0.2–1.0	herbal-rosy scent [12]
trepinen-4-ol	1.2–8.0	nd	0.3–1.2	1.5–5.0	peppery, woody [11]
3-octanone	tr-5.0	nd	nd	nd	herbal, mushroom [11]
$\alpha$ -terpineol	0.5–2.0	0.2–2.0	0.3–1.2	0.3–1.3	oil, anise, mint [10]
hexyl butyrate	nd	nd	0.2–0.5	0.3–0.5	sweet, fruit [10]
linalyl acetate	25.0–47.0	nd-1.6	19.0–29.0	25.0–38.0	sweet, citrus, floral, woody [10,12]
lavandulyl acetate	1.0–8.0	nd	1.0–2.0	1.5–3.5	herbal-rosy scent [12]
$\beta$ -caryophyllene	nd	nd	1.5–2.5	nd	sweet, woody, spicy [11]
<i>trans</i> - $\alpha$ -bisabolene	nd	0.4–2.5	nd	nd	fruity, citrus, woody [11]
Odor type [11]	lavender, floral, herbal, woody	camphor, eucalyptus, fresh, herbal, rosemary, woody, floral	herbal, spicy, camphoreous, soapy, floral, balsamic, woody	camphoraceous, lavender, herbal, floral, fruity	

\* Spontaneous and clonal lavenders of all origins are reported in the standard, nd—not detected.

The essential oil (EO) is accumulated in the aerial parts of lavender plants, mostly in the flowers, and the major compounds of the essential oil are oxygenated monoterpenes, alcohol linalool, and ester linalyl acetate [4–7,9]. Additionally, there are eucalyptol (1,8-cineole), terpineols, camphor, borneol, and unique irregular oxygenated monoterpenes, such as lavandulol and lavandulyl acetate. Regardless of the high yield of lavandin EO, the quality of true lavender EO is more appreciated in the perfume industry due to its lower camphor content and higher linalyl acetate content, and has an advantage in the market trade [13–15].

The quality and content of an essential oil depend on the species, environmental factors, agronomic practices, and methods of plant-material processing. The semi-dry Mediterranean climate type is dominant in the region of Vojvodina (Northern Serbia) [16], and with an increase in severity of environmental change, such as heat waves, droughts, and extreme precipitations, the distribution area of typically Mediterranean plant species expanded, which provided an opportunity to grow wild and cultivated plant species outside the area of their native distribution or formerly optimal climate. Growing MAPS as a crop diversification strategy could also restore and harness agrobiodiversity, increasing the overall resilience of farming systems.

According to a comparative, financial, and economic study of lavender production in the world (mainly in Bulgaria, France, China, Ukraine, Spain, and Morocco) reported by Giray [14] and Kontic et al. [17], there is a high possibility of production of lavender in other countries (even in Serbia) when profitability is concerned, due to the low production costs and high profit rate, especially by small farmers. However, their focus should be on the quality of the essential oil, since the quality is their main advantage when it

comes to competitiveness in the world market trade [14]. The cultivation of lavandin is cheaper, and currently its production areas are larger than those of true lavender, yet the price of true lavender's EO is 3–7 times higher, which provoked the adulteration of the EO, mainly through the addition of synthetic linalool and linalyl acetate to improve its sensory quality [15,18]. According to Renaud et al. [19], useful indicators of the purity of lavender oils are the enantiomeric distribution of (R)-(–) and (S)-(+)-forms of linalool and linalyl acetate.

Lavender EO is widely used in perfumery, cosmetics, and food processing. It is recognized for its medicinal properties, including antimicrobial (due to camphor as a potent antimicrobial compound), antispastic, anti-inflammatory, and general tonic action [20]. According to sensory analysis, lavender essential oil has a strong floral note, and it also has moderate spicy, herbal, woody, hay, camphor-, pine-, clove- and medicine-like odors [12]. It is used in aromatherapy for mental stress relief (traditionally used and approved by the European Medicines Agency—EMA), convulsion anxiety, and depression relief, along with a positive effect in the treatment of several neurological disorders [21,22]. Linalool alone and the essential oil, as a mixture of volatile lavender compounds, affect neural firing activity in a concentration-dependent manner [23]. Furthermore, lavenders are proposed as excellent phytoextractors for heavy-metal-contaminated soils—hyperaccumulators of lead and accumulators of cadmium and zinc [24]—since the accumulation of these heavy metals does not affect the development of the plant or the quality of their essential oils. This multipurpose quality of lavenders is of great importance to current EU policy and strategy in the Food, Bioeconomy, Natural resources, Agriculture, and Environment sectors, since its cultivation can contribute to the circular bioeconomy of rural and marginal areas.

Having in mind climate change and the expansion or shift of cultivation areas for many cultivated plant species, as well as financial gain from growing lavenders, the aim of this study was to test the EO quality (physicochemical and sensory properties) and bioactivity (antimicrobial and antioxidant activities) of the most frequently cultivated lavender varieties when grown outside their natural distribution and commonly grown areas, with a view to testing compliance of their quality with the standard requirements for lavender EO composition as well as available literature data.

## 2. Materials and Methods

### 2.1. Essential Oil Samples

In this research, seven samples of *Lavandula* sp. essential oils (EO) were analyzed: L1—*L. angustifolia* Mill. (Central Serbia), L2—*L. angustifolia* 'Hidcote blue' (Central Serbia), L3—*L. angustifolia* 'Munstead' (Central Serbia), L4—*L. angustifolia* 'Primorska' (Vojvodina, Bački Petrovac), L5—*L. × intermedia* 'Budrovka' (Vojvodina, Bukovac), L6—*Lavandula* sp. (Vojvodina, Irig), and L7—*L. × intermedia* 'Grosso' (Vojvodina, Sremska Kamenica). Varieties 'Hidcote blue' and 'Munstead' are grown in England, while 'Primorska' represents domesticated *L. angustifolia* from the Adriatic Sea coast, and 'Budrovka' represents the Croatian indigenous lavandin cultivar. All samples were obtained directly from farmers who grow and process lavender for essential oil for the market. All essential oils are obtained by steam distillation of the flowering top parts of the fresh lavender plant material collected in 2022. All lavender plantings were 4–7 years old.

### 2.2. Physicochemical and Sensory Properties of Lavender Plants Essential Oils

#### 2.2.1. Refractive Indices and TLC Analysis of Lavender Essential Oils

The refractive index at 20 °C was determined for all tested samples of lavender essential oil (refractometer Carl Zeiss, Jena, Germany), and compared to standard reference values for lavender essential oils [4–7,9]. Qualitative analysis was performed by thin-layer chromatography (TLC) and compared with linalool and linalyl acetate as standards. The procedure was conducted under conditions explained in the European Pharmacopoeia [9]. Lavender essential oils (20 µL) and a standard solution (linalool, 10 µL) were diluted in 1 mL of toluene and applied on the TLC plates (Merk, DC-Alufolien Kieselgel 60 F254,

20 × 20 cm, 0.2 mm) with capillary tubes (Scorex micropipettes with interchangeable glass capillary tubes, 10 µL). Samples and the standard were developed by mobile phase (toluene:ethyl acetate, 95:5) in a chromatography tank with a cover, and after reaching 10 cm, the plates were dried at room temperature and sprayed with sulfuric vanillin solution as a visualization reagent. Plates were heated in a laboratory oven at 105 °C and examined in daylight. In order to classify the tested individuals, their profiles were distinguished according to the presence or absence of discriminant spots with similar profiles, by the calculation of retention factor (R<sub>f</sub>) values in a visible light. Measured R<sub>f</sub> values were further compared with GC-MS results, as well as qualitative descriptions in Ph. Eur. VIII [9] and the TLC atlas [25].

### 2.2.2. GC-MS Analysis of Lavender Essential Oils

Lavender essential oils were analyzed with the same method and instrument as in our previous works [26,27] using an Agilent 6890 gas chromatograph with an Agilent 5973 Network mass-selective detector (MSD) (Agilent, Santa Clara, CA, USA) in the positive ion–electron impact (EI) mode. The instrument was equipped with an Agilent 19091S-433 HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), which was used for the separation. Essential oil (20 µL) was diluted with 1 mL of absolute ethanol and directly analyzed. A sample (1 µL) was injected in an injector at 250 °C in a splitless mode. Helium was used as a carrier gas with a 20.3 kPa inlet pressure and a 1 mL min<sup>-1</sup> linear velocity at 210 °C. The oven program was from 60 °C to 285 °C at a rate of 3 °C min<sup>-1</sup>. MS scan conditions were MS source temperature, 230 °C; MS Quad temperature, 150 °C; energy, 70 eV. The mass range analyzed by the mass spectrometer was 35.00–500.00 amu. The identification of the EO constituents was performed by the computer matching of mass spectra with ADAMS and NIST mass spectral databases [28] and by the comparison of their linear retention indices (LRI) relative to a series of n-hydrocarbons (C<sub>9</sub>–C<sub>40</sub>).

### 2.2.3. Sensory Odor Analysis

Seven lavender essential oils were evaluated in a sensory laboratory by an experienced panel consisting of eight members. The sensory attributes were determined as previously described by Xiao et al. [10], with slight modifications. Twelve aroma terms (spicy, camphor-like, herby, clove-like, woody, medicine-like, pine-like, hay, floral, watery, green, and earthy) were chosen for further descriptive analysis. Tested lavender essential oils were evaluated in triplicate on a 10-point interval scale (0 = none, 9 = extra strong). For the detection of the odor, smelling stripes were used by dipping one end (about 1 cm) into the essential oil sample. Three deep and quick sniffs were achieved from the smelling strip, which was then removed from the odor source. Clean air was obtained between each assessment. There was a gap of 20 s between the individual odor assessments.

## 2.3. Bioactivity of Lavender Essential Oils

### 2.3.1. Antimicrobial Activity of Lavender Essential Oils

The antimicrobial activity of the lavender essential oil was evaluated using nine strains from the American Type Culture Collection: *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 10536, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19111, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231. The modified broth microdilution method (described in detail in [29]) with serial doubling dilutions was prepared in a 96-well microtiter plate over the range of 454.4–0.22 µL mL<sup>-1</sup> of essential oil, in order to determine minimal inhibitory (MIC) and minimum bactericidal (MBC) concentrations.

### 2.3.2. Antioxidant Activity of Lavender Essential Oils

The ability of essential oil solution to reduce DPPH-radical (2,2-diphenyl-1-picrylhydrazyl, purple color) into its reduced yellow form DPPH-H, as a measure of the antioxidant capacity

of the solution, was determined for all EO samples [30]. A stock ethanolic (absolute ethanol) solution of DPPH-radical was diluted with 100% MeOH (24 mg DPPH in methanol) to obtain an absorbance of approx. 0.980 ( $\pm 0.02$ ) at 515 nm. To 500  $\mu\text{L}$  of diluted DPPH-radical solution, different volumes of dissolved lavender essential oil solutions (20–1000  $\mu\text{L}$ ) and 100% MeOH (to reach a final volume of 3 mL) were added, and absorbance was measured at 515 nm after incubation at 30 °C for 30 min. For blank samples, MeOH was used instead of the essential oil samples. DPPH-radical scavenging capacity (RSC) was calculated using the following Equation (1) and expressed in %:

$$\% \text{ RSC} = \frac{(A \text{ blank} - A \text{ sample}) \times 100}{A \text{ blank}} \quad (1)$$

Values of  $\text{IC}_{50}$  (50% inhibitory concentration) were determined for all EO samples graphically (calculated RSC data were plotted against the EO concentrations in the reaction medium) and expressed as  $\mu\text{g}$  ascorbic acid equivalents per ml of the EO ( $\mu\text{g AAE mL}^{-1}$ ).

#### 2.4. Statistical Analysis

The values of the RI and antioxidant parameters were expressed as means  $\pm$  standard error of determinations made in duplicates. Antioxidant parameters were tested by ANOVA, followed by comparison of the means by Duncan's multiple range test ( $p < 0.05$ ). To discover natural groupings of EO samples according to their composition and bioactivity, principal component analysis (PCA) and cluster analysis were carried out. All statistical analyses were performed by StatSoft Statistica 12 (StatSoft Inc., Tulsa, OK, USA), except for the sensory evaluation, which was performed using a balanced factorial design in the Experiment design for sensory analysis with XLSTAT-MX (XLSTAT 2018.7. Addinsoft., <http://www.xlstat.com>, accessed on 26 August 2022).

### 3. Results

#### 3.1. Physicochemical and Sensory Properties of Lavender Essential Oils

##### 3.1.1. Refractive Indices and TLC Analysis of Lavender Essential Oils

As already known, *L. angustifolia* (L1–L4) and lavandin (L5 and L7) samples were compared to representative standards; however, 'Budrovka' had RI values somewhat higher in comparison to lavandin 'Abiral' and Grosso', while all samples of *L. angustifolia* were at the maximum of the standard RI values. *Lavandula* sp. sample (L6) was within the limits for the standard RI values according to the European Pharmacopoeia and ISO standards for lavender essential oils [4–7,9] (English type: from 1.455 to 1.466; French type or lavandin: from 1.460 to 1.466 for 'Abrial' and from 1.458 to 1.462 for 'Grosso'; and Spanish type or spike lavender: from 1.461 to 1.468). The results of measured refractive indices are presented in Table 2.

**Table 2.** Refractive indices at 20 °C for tested lavender essential oils.

EO Sample	RI $\pm$ Se
<i>L. angustifolia</i> Mill.	1.465 $\pm$ 0.33
<i>L. agustifolia</i> 'Hidcote blue'	1.466 $\pm$ 0.00
<i>L. angustifolia</i> 'Munstead'	1.466 $\pm$ 0.00
<i>L. angustifolia</i> 'Primorska'	1.466 $\pm$ 0.33
'Budrovka' lavandin	1.468 $\pm$ 0.33
<i>Lavandula</i> sp.	1.467 $\pm$ 0.33
'Grosso' lavandin	1.464 $\pm$ 0.00

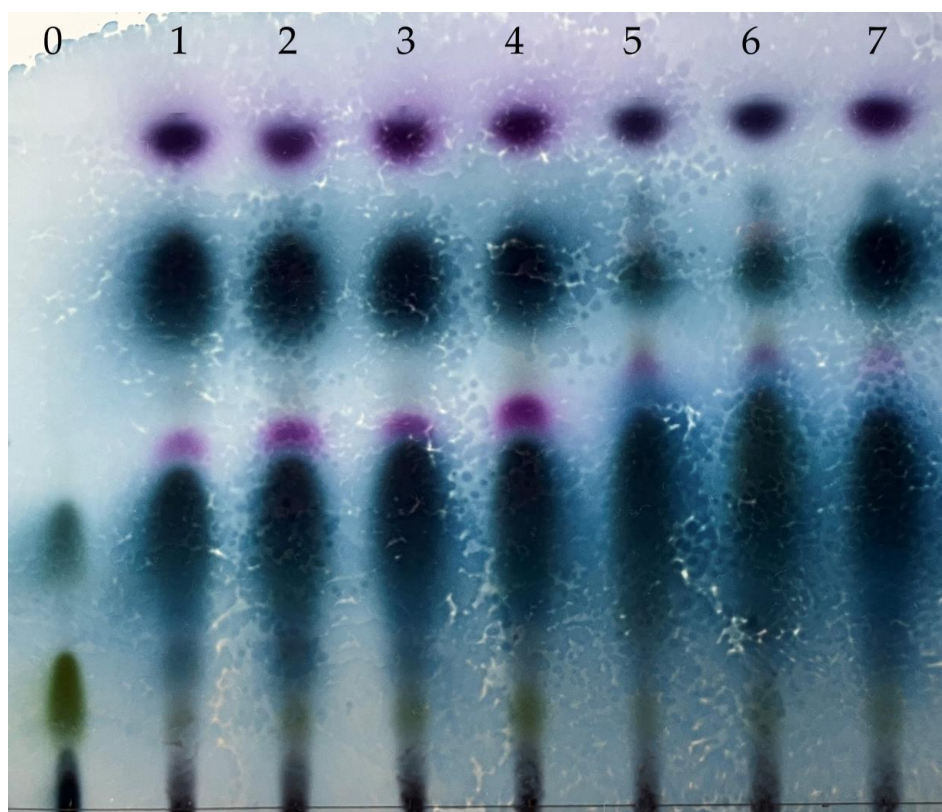
According to the examination of TLC profiles of lavender EO samples (Figure 1), two patterns of composition were defined according to the presence or absence of discriminant spots. All samples contain linalool ( $R_f = 0.30$ ); however, samples L5 ('Budrovka') and L6 (*Lavandula* sp.) clearly differed from the other five samples in the presence of the major



lavender essential oil compounds (bigger linalool (Rf = 0.30) and smaller linalyl acetate (Rf = 0.59) spots). This was confirmed by the following GC-MS analysis (Table 3).

**Table 3.** Qualitative and quantitative analysis of lavender EO (L1–L7). L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’. Reference LRI values were sourced from [28,31], experimental LRI were calculated relative to C9–C40 alkanes.

Compound	LRI <sub>ref</sub>	LRI <sub>exp</sub>	L1	L2	L3	L4	L5	L6	L7
α-Thujene	930	927	0.13	0.23	0.25	0.12	0.15	0.21	0.25
α-Pinene	937	934	0.32	0.47	0.49	0.28	0.97	1.13	1.07
Camphene	952	949	0.22	0.36	0.37	0.30	0.45	0.52	0.61
Sabinene	975	973	nd	0.18	0.10	0.06	0.30	0.40	0.27
β-Pinene	979	978	0.19	0.48	0.44	0.54	1.48	1.91	1.25
3-Octanone	986	985	0.35	0.36	0.87	0.49	0.08	0.04	0.05
Myrcene	991	989	0.68	1.07	0.81	0.52	0.59	0.65	0.96
α-Phellandrene	1004	1005	0.04	0.12	0.08	0.03	0.07	0.08	0.06
δ-3-Carene	1011	1010	0.15	1.16	0.39	0.16	0.38	0.27	0.05
Hexyl acetate	1011	1011	0.40	0.53	0.28	0.31	0.13	0.26	0.17
δ-2-Carene	1015	1016	0.05	0.05	0.08	0.06	0.09	0.11	0.12
o-Cymene		1020	0.07	0.18	0.11	0.23	0.11	0.05	nd
p-Cymene	1025	1023	0.27	0.49	0.40	0.71	0.53	0.39	0.27
Limonene	1030	1027	0.42	2.05	0.82	0.85	nd	nd	nd
1,8-Cineole	1032	1029	0.74	1.83	1.29	1.91	nd	nd	nd
Limonene + 1,8-Cineole			6.87	Nd	nd	nd	15.32	14.61	10.25
β-Z-Ocimene	1038	1036	2.91	4.20	6.11	2.80	2.93	3.99	0.46
β-E-Ocimene	1049	1046	0.16	2.92	2.94	0.94	0.35	0.39	0.38
γ-Terpinene	1060	1056	0.06	0.16	0.28	0.17	0.27	0.30	0.32
cis-Sabinene-hydrate	1068	1064	0.17	0.05	0.07	0.05	0.06	0.23	0.11
cis-Linalool oxide	1074	1070	0.04	0.18	0.26	0.43	0.18	0.23	0.25
Terpinolene	1088	1087	0.23	0.30	0.36	0.46	0.40	0.45	0.56
Linalool	1099	1105	23.90	25.15	29.31	30.22	31.94	36.89	28.86
1-Octen-3-yl-acetate	1111	1112	1.03	0.34	0.57	0.82	nd	0.04	0.34
Camphor	1144	1142	0.30	0.34	0.36	1.04	5.07	2.25	8.25
Borneol	1166	1164	0.86	2.02	1.36	3.55	nd	nd	3.59
Lavandulol	1168	1166	0.79	0.72	0.98	1.55	11.53	10.57	nd
Terpinen-4-ol	1177	1176	5.62	3.46	6.32	4.19	5.95	6.71	5.75
p-Cymen-8-ol	1183	1181	0.10	0.13	0.09	0.23	0.11	0.04	nd
Cryptone	1184	1184	0.24	0.66	0.30	0.91	0.27	0.20	0.07
α-Terpineol	1189	1190	1.78	1.94	1.29	1.02	1.38	0.94	1.66
Hexyl butanoate	1196	1192	nd	0.64	0.46	0.45	0.67	0.73	0.46
Eucarvone		1206	nd	0.09	0.05	0.21	0.10	0.06	0.06
Nerol	1228	1228	0.27	0.33	0.19	0.17	0.48	0.26	0.19
Cuminaldehyde	1239	1237	0.11	0.31	0.16	0.50	0.40	0.39	0.18
Linalyl acetate	1257	1259	32.22	28.90	24.07	22.18	7.16	6.90	20.68
Z-Isocitral		1272	0.05	0.09	nd	0.13	0.05	nd	nd
Bornyl acetate	1285	1284	0.34	0.17	0.21	0.18	0.12	0.05	0.04
Lavandulyl acetate	1289	1292	4.84	5.52	5.05	6.53	0.77	0.93	3.43
Neryl acetate	1364	1364	0.57	0.70	0.44	0.41	0.18	0.07	0.40
α-Copaene		1373	0.07	Nd	nd	nd	nd	nd	nd
Geranyl acetate	1382	1383	1.07	1.30	0.89	0.86	0.35	0.22	0.87
Sesquithujene			0.11	0.05	0.07	0.08	0.16	0.15	0.11
E-Carryophyllene	1405	1417	5.18	4.11	5.48	5.53	1.23	0.76	1.51
α-cis-Bergamotene	1415	1433	0.23	0.34	0.17	0.26	0.13	0.12	0.16
α-Humulene	1454	1450	0.14	0.10	0.16	0.14	0.04	nd	0.05
β-E-Farnesene	1457	1456	3.75	1.22	2.00	2.04	3.14	3.00	1.23
Germacrene D	1480	1477	0.42	0.36	0.27	0.54	0.53	0.40	0.50
Sesquisabinene		1482	0.10	0.13	0.06	0.12	0.14	nd	0.04
γ-Cadinene	1513	1510	0.14	0.24	0.53	0.27	0.46	0.37	0.65
Caryophyllene oxide	1581	1578	0.32	0.73	0.49	1.33	0.21	0.13	0.17
epi-α-Cadinol	1640	1637	nd	Nd	0.35	0.14	0.04	nd	0.24
Total identified			98.76	96.81	98.15	96.13	97.22	98.20	96.90
Total monoterpenes			88.15	89.03	88.14	85.82	90.59	92.48	91.67
Total sesquiterpenes			10.45	7.27	9.57	10.46	6.09	4.93	4.67
Total esters			0.40	1.17	0.74	0.76	0.80	0.98	0.63



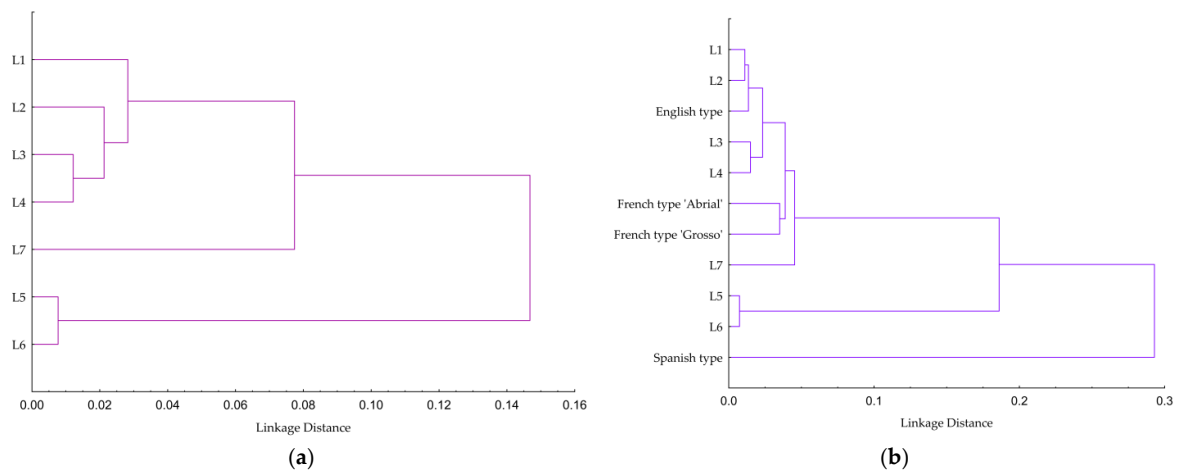
**Figure 1.** TLC plate of lavender essential oils. (0) linalool, (1) L1—*L. angustifolia* Mill., (2) L2—*L. angustifolia* 'Hidcote blue', (3) L3—*L. angustifolia* 'Munstead', (4) L4—*L. angustifolia* 'Primorska', (5) L5—*L. × intermedia* 'Budrovka', (6) L6—*Lavandula* sp., (7) L7—*L. × intermedia* 'Grosso'.

### 3.1.2. GC-MS Analysis of Lavender Essential Oils

The composition of the essential oils of the seven lavenders grown in Serbia is presented in Table 3. There is a very good correlation between the reference and experimentally obtained linear retention indices, which confirms that identification based on compounds' mass spectra analyses was accurate.

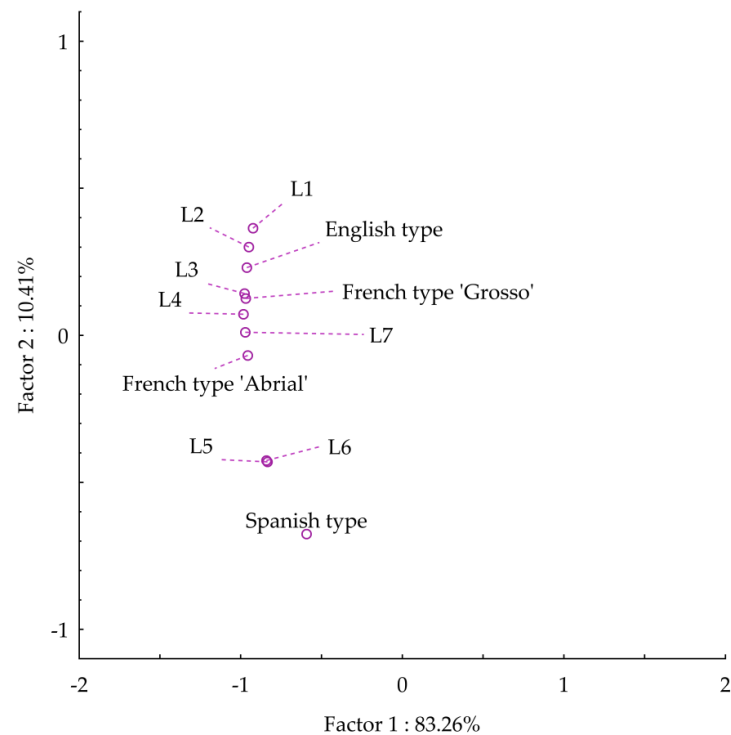
The major compounds were linalool (23.9–39.9%) and linalyl acetate (6.9–32.2%), with the highest content in true lavender and lavandin samples, respectively. 'Budrovka' and L6 had a lower content of linalyl acetate (6.9–7.2%) when compared to standard values (Table 1). However, none of the samples were similar to the Spanish type of lavender (since low contents of 1,8-cineole and no trans- $\alpha$ -bisabolene was detected for these samples). True lavender samples (L1–L4) had lower total monoterpene content and higher total sesquiterpene content than other analyzed EOs in the research.

Multivariate cluster analysis (Figure 2a), based on the composition of essential oils, showed a clear division of true lavender samples (L1–L4): *L. angustifolia*, *L. angustifolia* 'Hidcote blue', *L. angustifolia* 'Munstead', *L. angustifolia* 'Primorska') from lavandin 'Budrovka' and L6 samples, while lavandin 'Grosso', was positioned between these. When compared to standard EO profiles of English, Spanish, and French types of lavenders (maximum contents according to standards in Table 1 are used as values), four groups can be distinguished (Figure 2b): (1) true lavender samples with standard English type lavender and tested *L. angustifolia*, *L. angustifolia* 'Hidcote blue', *L. angustifolia* 'Munstead', *L. angustifolia* 'Primorska'; (2) French types of lavenders or lavandins; (3) lavandin 'Budrovka' and L6; and (4) the Spanish type of lavender.



**Figure 2.** Dendrograms of the results of cluster analysis using composition analysis of estimated lavender EO samples—all determined compounds (a) and compounds proposed by standards in Table 1 (b). L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.

According to the principal component analysis (Figure 3) of lavender EO standard compounds (listed in Table 1) for values of EO samples used in this research and standard EO types, the major positive influence on the first principal component (Factor 1) had linalool (57.86%) and on the second principal component (Factor 2) had linalyl acetate (49.10%), based on correlations. The grouping of samples points to a significantly high correlation among all samples ( $r = 0.8–0.9$ ), except lavandin ‘Budrovka’ and L1 ( $r = 0.6–0.8$ ), and, expectedly, the Spanish type.

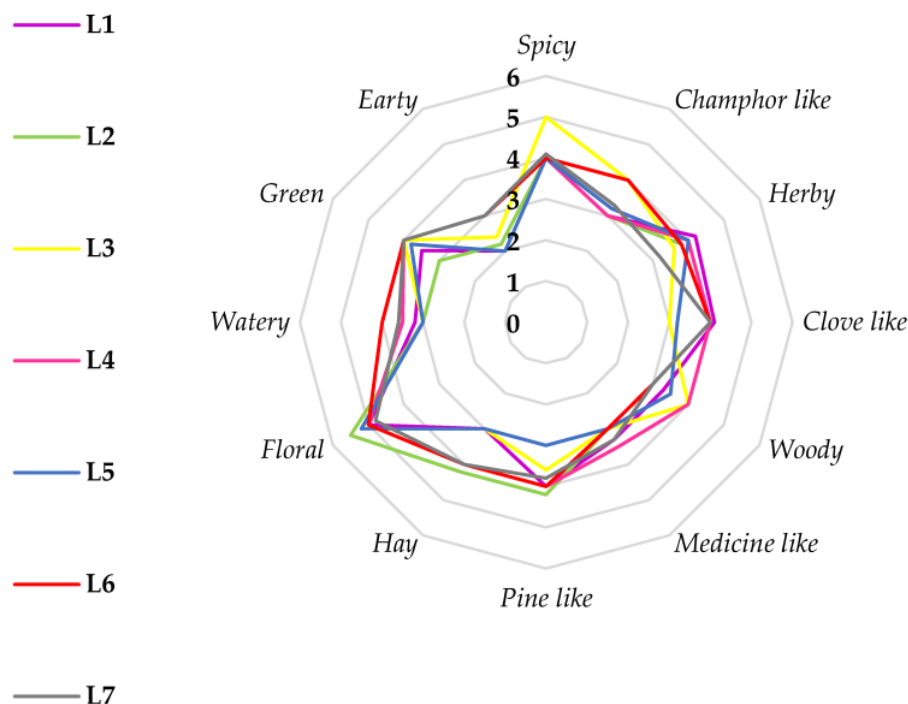


**Figure 3.** PCA analysis of tested lavender EO samples and standard lavender EOs using values obtained in the research and values of the compounds proposed by standards in Table 1. L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.



### 3.1.3. Sensory Analysis

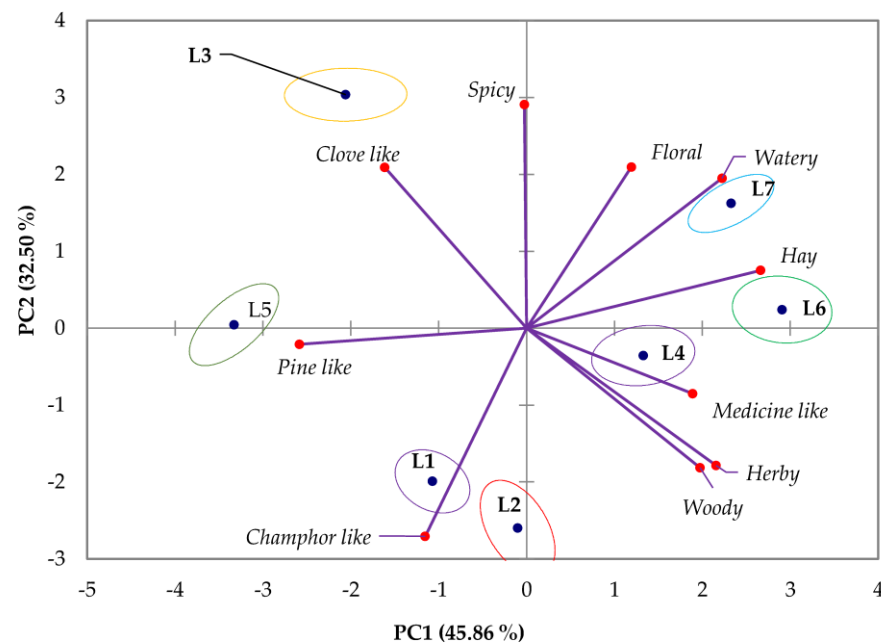
The sensory analysis results are presented in Figure 4, showing slight differences in the observed EOs. All evaluators seem to find a floral smell in all observed samples, while true lavender cultivars ‘Hidcote blue’ and ‘Munstead’ had prominent floral and spicy aromas, respectively. The herbal odor was more profound in *L. angustifolia* Mill., *L. angustifolia* ‘Primorska’, and *L. × intermedia* ‘Budrovka’, while a fresh green odor was noticed in *L. angustifolia* ‘Munstead’, *L. angustifolia* ‘Primorska’, *Lavandula* sp., and *L. × intermedia* ‘Grosso’. It is difficult to draw clear conclusions and determine the best sample of essential oil because all samples were appreciated and characterized by a pleasant odor.



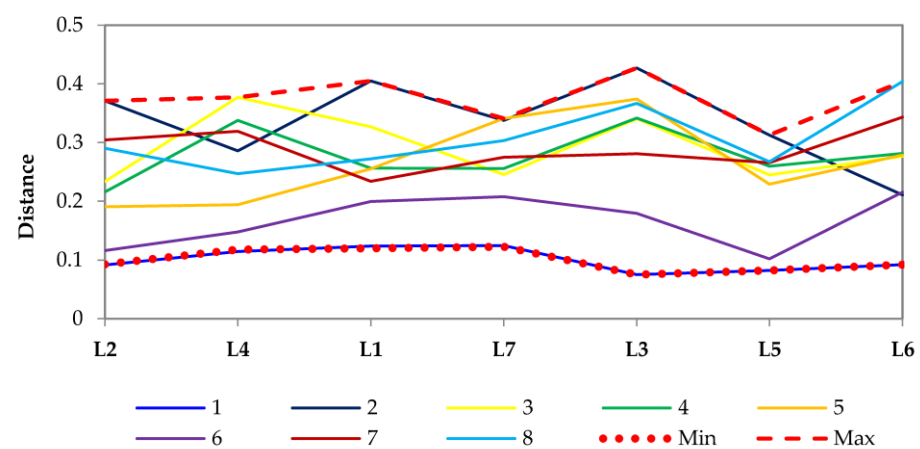
**Figure 4.** Results of the sensory analysis of the lavender EOs. L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.

Principal component analysis (PCA) was applied to investigate the relationships between the essential oil samples according to the sensorial analysis, and the results are illustrated in Figure 5. The nearness of marks in the PCA figure reveals pattern similarity [32]. By examining Figure 5, one can efficiently determine the correlation between the observed samples, as the angles between corresponding variables reflect the degree of correlation, with smaller angles corresponding to stronger correlations. The first two PCs demonstrated 78.36% of the total variance in the recorded data. The first PC explained 44.86% and the second explained 32.50% of the total variance between the analyzed data.

The Euclidean distance was considered per the assessor’s sensory evaluation for every sample compared to the average score for all assessors and descriptors. Figure 6 shows these distances for each sample for all assessors, enabling the identification of the assessor’s sensory score distance from the consensus. If the Euclidean distance is lower, the assessor is closer to the consensus [33]. According to the presented results, assessor 2 exerts the most elevated distance from the consensus for essential oil evaluation.



**Figure 5.** The PCA biplot diagram, depicting the relationships among observed samples according to the sensory profiles. L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.



**Figure 6.** Euclidean distance of the assessor’s (1–8) evaluation to consensus.

### 3.2. Bioactivity of Lavender Essential Oils

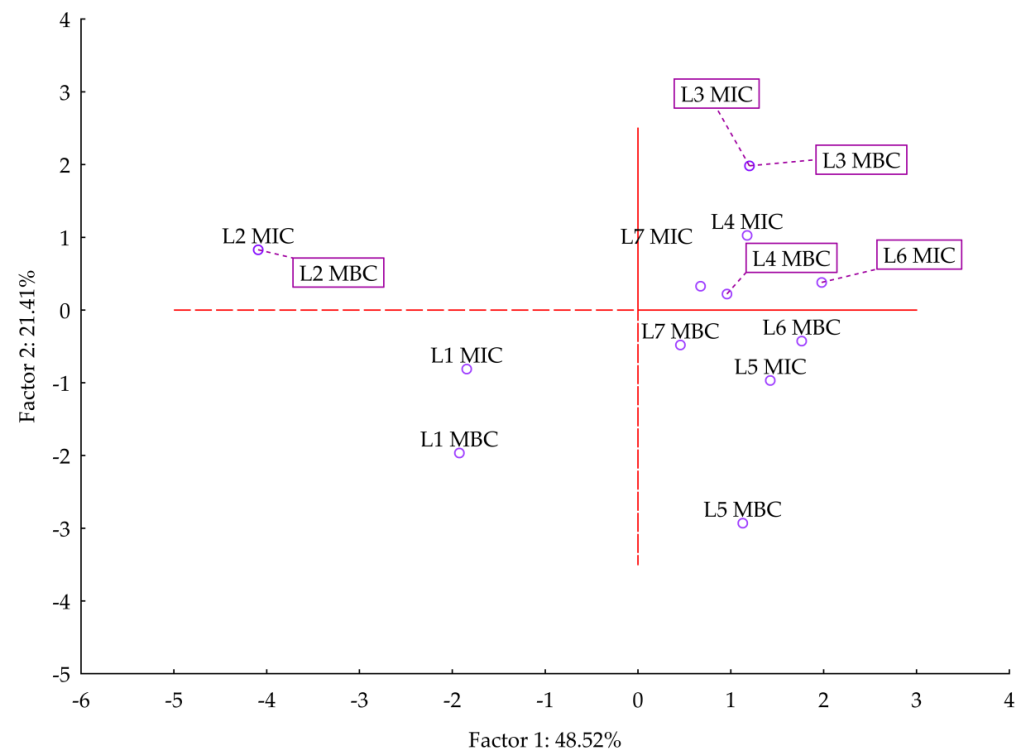
#### 3.2.1. Antimicrobial Activity of Lavender Essential Oils

All samples showed different antimicrobial activities (Table 4). The essential oil of *L. angustifolia* only had higher antimicrobial activity against *Bacillus cereus*; however, chemically distinctive samples of ‘Budrovka’ and L6 had marked activities against *Enterococcus faecalis*, *Listeria monocytogenes*, and, especially, *Salmonella enteritidis* (Table 4). Similarly, ‘Primorska’ was highlighted for antimicrobial activity against *B. cereus*, but the sample additionally showed the highest antimicrobial activity against *Candida albicans*. Sample ‘Munstead’ dominated in antimicrobial activity towards *Escherichia coli*, in addition to *Listeria monocytogenes*. None of the samples showed distinguished antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. typhimurium*, and *Staphylococcus aureus*.

**Table 4.** Antimicrobial activity of tested lavender essential oils (L1–L7). MIC—minimum inhibitory, MBC—minimum bactericidal concentration in  $\mu\text{L mL}^{-1}$ ; L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.

		Gram-Positive Bacteria					Gram-Negative Bacteria			Yeast
		<i>B. cereus</i>	<i>E. faecalis</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. enteritidis</i>	<i>S. Typhimurium</i>	<i>C. albicans</i>
L1	MIC	3.5	227.2	14.2	227.2	14.2	227.2	454.5	227.2	14.2
	MBC	3.5	227.2	14.2	227.2	14.2	227.2	454.5	454.5	14.2
L2	MIC	14.2	454.5	28.4	227.2	14.2	227.2	454.5	113.6	14.2
	MBC	14.2	454.5	28.4	227.2	14.2	227.2	454.5	113.6	14.2
L3	MIC	7.1	227.2	7.1	113.6	3.5	113.6	113.6	56.8	7.1
	MBC	7.1	227.2	7.1	113.6	3.5	113.6	113.6	56.8	7.1
L4	MIC	3.5	227.5	7.1	227.2	7.1	227.5	113.6	113.6	3.5
	MBC	3.5	227.5	14.2	227.2	7.1	454.5	113.6	113.6	3.5
L5	MIC	7.1	28.4	7.1	56.81	14.2	227.2	56.81	227.2	7.1
	MBC	7.1	28.4	14.2	56.81	14.2	454.5	56.81	454.5	7.1
L6	MIC	7.1	28.4	7.1	56.81	7.1	227.2	56.81	113.6	7.1
	MBC	7.1	28.4	14.2	56.81	7.1	454.5	56.81	113.6	7.1
L7	MIC	7.1	227.2	7.1	113.6	7.1	227.2	227.2	227.2	7.1
	MBC	7.1	227.2	14.2	113.6	7.1	454.5	227.2	227.2	7.1

The grouping of samples according to their antimicrobial activities was performed by PCA analysis (Figure 7). Grouping across both principal components (PC1 and PC2) was carried out in such a way that four groups were observed: (1) *L. angustifolia* or L1; (2) ‘Hidcote blue’ or L2; (3) lavandin ‘Budrovka’ or L5; and (4) all other samples: L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L6—*Lavandula* sp., and L7—*L. × intermedia* ‘Grosso’.



**Figure 7.** Principal component analysis two-dimensional scatter plot based on the first two principal components (Factor 1 and Factor 2) generated for the antimicrobial activity of lavender essential oils. L1—*L. angustifolia* Mill., L2—*L. angustifolia* ‘Hidcote blue’, L3—*L. angustifolia* ‘Munstead’, L4—*L. angustifolia* ‘Primorska’, L5—*L. × intermedia* ‘Budrovka’, L6—*Lavandula* sp., L7—*L. × intermedia* ‘Grosso’.

### 3.2.2. Antioxidant Activity of Lavender Essential Oils

The antioxidant test (DPPH-test) showed that true lavender samples (L1–L4) had lower IC<sub>50</sub> values than lavandin samples, which points to a higher scavenging capacity of true lavenders EO (Table 5). Tested lavender essential oils (with volumes of 100 µL) exhibited activity from 31 to 76.8% of scavenged DPPH radicals. The highest values of the DPPH-test were for *L. angustifolia* (L1), ‘Hidcote blue’ (L2), and ‘Munstead’ (L3). Compared to the other tested samples, lavandin ‘Budrovka’ and ‘Grosso’, and sample *Lavandula* sp. had significantly lower antioxidant activities (Table 5).

**Table 5.** Antioxidant activity of lavender essential oils. IC<sub>50</sub> values expressed as µg AAE mL<sup>-1</sup> EO. Each value is the mean of all tested samples ± standard error. Results marked with different letters differ significantly at *p* < 0.05 (Duncan’s test).

EO Sample	$\bar{X} \pm Se$	Duncan’s Test
<i>L. angustifolia</i> Mill.	0.237 ± 0.003	<i>a</i>
<i>L. angustifolia</i> ‘Hidcote blue’	0.280 ± 0.012	<i>ab</i>
<i>L. angustifolia</i> ‘Munstead’	0.240 ± 0.006	<i>a</i>
<i>L. angustifolia</i> ‘Primorska’	0.423 ± 0.032	<i>cd</i>
‘Budrovka’ lavandin	0.567 ± 0.015	<i>e</i>
<i>Lavandula</i> sp.	0.497 ± 0.064	<i>de</i>
‘Grosso’ lavandin	0.593 ± 0.052	<i>e</i>

## 4. Discussion

Results obtained for the refractive index of lavender essential oils were in accordance with previously published data (1.458 to 1.475) of commercial lavender essential oil samples [20,34], European Pharmacopoeia [9] and ISO standards for lavender essential oils [4–7]. The same stands for TLC analyses, which were in accordance with the results of reference method descriptions in Ph. Eur. VIII and the TLC atlas [9,25]. The composition of lavender EOs tested in this research was also within the values for standard EOs [4–7,9], except for lavandins ‘Budrovka’ and ‘Grosso’ and the unknown lavender sample (L6), which undoubtedly belonged to one of the lavandins; however, lavandin ‘Budrovka’ is quite different from standard *L. × intermedia* cultivars.

Boelens (1995) [35] explained in great detail the publication timeline of the chemical and sensory evaluation of lavender and lavandin EOs and their sensory odor characteristics, where it is explained, citing Naef and Morris (1992) [36], that lavender EO general odor characterization is herbal, floral, and fresh with a green, hay-like, and fruity top note, and is sweet and slightly woody when dried. They explained the following: fresh and floral notes come from linalool and lavandulol (and their esters); the fruity top note is based on lower aliphatic esters (1%); the earthy, green herbal, sweet-warm, and floral notes are provided by functionalized C8 compounds (3-octyl derivatives, 4.7%); fresh aromatic and spicy notes come from the ocimenes (14.5%); soft and warm woody tones come from sesquiterpene derivatives (functionalized santalenes, 2.3%); and butyl benzoate (0.01%) provides balsamic notes; while cryptone (0.2%) provides warm herbal and cuminic notes. As for lavandin EO, it has additional camphoraceous and fresh tones due to 1,8-cineole (10%), camphor (12%), and borneol (3%). Xiao et al. [10] concluded that lavender EO odor coincided with the geographical distribution and that the main compounds of the EO (according to ISO standards [4–7]), limonene, linalool, linalyl acetate, and camphor, form the characteristic aromas floral, woody, and herbal, with additional sweet, camphor, and fruity types. The same authors confirmed positive and negative correlations to individual compounds and odor sensory attributes; however, there was no significant correlation to floral, herby, woody, or camphor notes.

Available literature [34,37–44] reported that the composition of lavender EOs, as well as the content of the major compounds, linalool and linalyl acetate, greatly varied depending on the variety, species, and country where the experiments were conducted. Contents of linalool and linalyl acetate in EO of true lavender (*L. angustifolia*) and its cultivars were

(respectively): 18.4–39.5% and 25.6–29.8% for lavender grown in Romania [40]; 28.1–35.4% and 28.4–36.8% for lavender grown in Turkey [34]; 53.9% and 11.6% for lavender grown in Croatia [39]; 22.4–40.5% and 25.6–28.7 for lavenders ‘Hidcote blue’ and ‘Munstead’ grown in Hungary [41]; 49.9% and 17.9% for lavender grown in Italy [42]; 19.8–55.1% and 7.5–39.2% for different, new lavender cultivars grown in Ukraine [15,43]; 21.8% and 34.2 for lavender ‘Hidcote blue’ grown in Poland [44]. Also, the contents of linalool and linalyl acetate in the EO of lavandin (*L. × intermedia*) and its cultivars were (respectively): 21.5% and 22.5% for lavandin grown in Romania [40]; 29.1–46.9% and 4.6–33.1% for lavandin grown in Turkey [34]; 57.1% and 9.8% for lavandin ‘Budrovka’ grown in Croatia [39]; 51.4% and 5.3% for lavandin ‘Grosso’ grown in Hungary [41]; 29.5% and 18.5% for lavandin ‘Grosso’ grown in Poland [44]. It is important to note that one additional factor should also be considered when determining the variability in content of the major compounds of lavender EO, and that is the year in which the plant materials were collected (since lavenders are perennial species). Experiments conducted for two consecutive years showed that there was no clear trend for inter-year compositional changes, but there was a significant change in linalool and linalyl acetate contents for some cultivars between observed years (up to 13–14%) [41,43].

According to EUCAST [45], a ratio of MBC/MIC  $\leq 4$  points to the bactericidal property of antimicrobial substances, while substances with a MBC/MIC ratio  $> 4$  are regarded as bacteriostatic. The MBC/MIC ratios of lavender essential oils tested in this research were below 4 (1–2), which positions these essential oils as bactericidal. Samples tested in this research had higher antimicrobial activities than those reported in the published literature, since the average MIC and MBC values determined in this research were (respectively): 0.006–0.171 and 0.006–0.183 mg mL<sup>-1</sup> against Gram-positive bacteria (*B. cereus*, *E. faecalis*, *L. monocytogenes*, and *S. aureus*), 0.008–0.190 and 0.008–0.291 mg mL<sup>-1</sup> against Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. enteritidis*, and *S. typhimurium*) and 0.008 mg mL<sup>-1</sup> against *C. albicans*. Linalool had the following MIC and MBC values: 0.4–2.0 and 1.0–3.0 mg mL<sup>-1</sup> against Gram-positive bacteria (*B. cereus*, *S. aureus*, and *L. monocytogenes*), 0.5–0.75 and 0.6–1.7 mg mL<sup>-1</sup> against Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. enteritidis*), and 0.50 and 0.75 mg mL<sup>-1</sup> against *C. albicans*, respectively [39,46]. However, linalyl acetate showed lower antimicrobial activity since its MIC and MBC values were 2.5–5.0 and 5.0–10.0 mg mL<sup>-1</sup> against Gram-positive bacteria (*B. cereus*, *S. aureus*, and *L. monocytogenes*), 5.0–20.0 and 7.5–25.0 mg mL<sup>-1</sup> against Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. enteritidis*), and 15 and 20 mg mL<sup>-1</sup> against *C. albicans*, respectively [39].

Essential oils of ‘Budrovka’ lavandin and *L. angustifolia* grown near Zagreb in central Croatia [39] showed higher MIC and MBC values to samples tested in this research for Gram-positive bacteria *B. cereus*, *S. aureus* and *L. monocytogenes* (0.75 and 3.0, 1.0–4.0 mg mL<sup>-1</sup>, respectively), Gram-negative bacteria *E. coli*, *P. aeruginosa* and *S. enteritidis* (0.25–1.50 mg mL<sup>-1</sup> and 0.5 and 2.0 mg mL<sup>-1</sup>, respectively) and yeasts *C. albicans* (0.75–1.0 mg mL<sup>-1</sup>), which could be due to the much higher camphor content in ‘Budrovka’ lavandin in this research (42-times higher). Higher eucalyptol and camphor contents in lavandin essential oils result in a lower oil quality for the perfumery industry, but when it comes to antimicrobial properties, the presence of camphor is preferable, since it is effective as an antimicrobial agent against bacteria such as *S. aureus*, methicillin-resistant *S. aureus*, *E. coli* and *C. albicans* [47].

Another study [44] on the antimicrobial activity of essential oils of different cultivars of *L. angustifolia* (‘Hidcote blue’) and lavandin (‘Phenomenal’ and ‘Grosso’) grown in Poland against the tested reference bacteria (MIC 2.5–10 mg mL<sup>-1</sup>) and yeasts (MIC 0.3–1.25 mg mL<sup>-1</sup>) also showed a lower antimicrobial activity than essential oils tested in this research. However, Garzoli et al. [42] reported 0.19% against *B. cereus* and 0.39% against *E. coli* values of MIC and MBC (the values were the same for both parameters) for the essential oil of *L. angustifolia* grown in Tuscany, Italy, which were lower but more in accordance with the results presented in this research (0.7% against *B. cereus* and 0.9% against *E. coli*).



There are several papers that investigated the antioxidant activities of essential oils, since most of the published literature mostly reports the antioxidant activity of extracts of medicinal and aromatic plant species and rarely their essential oils, due to the solubility of oils and the inability to perform clear mediums for measurement in aqueous solutions, which are mostly used for the antioxidant tests. However, the DPPH-test can be performed for EO samples, and the results are similar across other reported data. An antioxidant capacity of 80% ethanol flower extracts of *L. angustifolia* and lavandin 'Budrovka' grown in Croatia had the IC<sub>50</sub> of 10.62 µg mL<sup>-1</sup> [48], while aqueous leaf and flower extracts of *L. angustifolia* grown in North Iran had the SC<sub>50</sub> (50% scavenging concentration) value of 29.2 µg mL<sup>-1</sup> [49]. Ethanolic extract (c = 100 mg/mL) from flowering aerial parts of *L. angustifolia* from Portugal inhibited 23% of DPPH radicals [50]. Essential oil samples of *L. angustifolia* and lavandin cultivars grown in western Anatolia, Turkey [30], showed SC<sub>50</sub> values (SC<sub>50</sub> 89.8–105.0 µg α-tocopherol equivalents mL<sup>-1</sup>) of samples between 5.5 and 6.4 times higher than that of standard α-tocopherol, while the essential oil of *L. angustifolia* grown in Italy (Tuscany), had the IC<sub>50</sub> of 7.75 µg Trolox equivalents mL<sup>-1</sup>. Ferreira et al. [50] reported <5% inhibited DPPH radicals for the essential oil of *L. angustifolia* from Portugal, and Tița et al. [51] reported 12.76% inhibited DPPH radicals for essential oil (for a volume of 30 µL) of *L. angustifolia* from Romania, which was previously encapsulated. All elaborated values point to variable results due to the inconsistency of the standard compounds that are used for the calculation of the DPPH test. When compared to ethanolic or methanolic extracts, the % of inhibited DPPH radicals by essential oils was quite low. As for individual terpenoid standards, Wang et al. [46] determined IC<sub>50</sub> values for some of the most common terpenoids in comparison to butylated hydroxytoluene, or BHT (the IC<sub>50</sub> of BHT is below 20 µg mL<sup>-1</sup>) in the following order: α-pinene 12.57 < limonene 13.35 < terpineol 30 < linalool ~35 < myrcene 40.8 mg mL<sup>-1</sup>.

## 5. Conclusions

Results on the physicochemical properties and biological activities obtained in this study confirm that the quality of true lavender, lavandin, as well as their cultivars, have not been affected by growing conditions in Central and Northern Serbia (Vojvodina Province). Tested EOs, especially true lavender samples, showed compliance with standard requirements for EO composition and exhibited high antimicrobial activities against important Gram-positive and negative bacteria and yeasts, as well as a high antioxidant capacity. Further research on the optimization of agrotechnology for lavender growing could improve the stability of the yield of this species. Nevertheless, this preliminary research on the quality of EOs from lavenders grown in Serbia reveals the potential of this species for the future of medicinal and aromatic plant species production and further diversification of agriculture in the area. The cultivation of lavenders would be an innovative approach to further scale-up the revenue of small farmers and primary producers in the period of climate change.

**Author Contributions:** Conceptualization, B.K. and M.A.; formal analysis, B.K., T.Z., A.V. and I.Č.; data analysis and visualization, B.K., J.F. and B.L.; writing—original draft preparation, B.K., M.A., J.F. and B.L.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers: 451-03-47/2023-01/200032, 451-03-47/2023-01/200222, 451-03-47/2023-01/200134, and HUSRB/1903/42/0059 'Enhancing the entrepreneurship and employment potential in cross-border region through innovation driven agricultural practices AGRINNO 2', Interreg-IPA Cross-border Cooperation Programme Hungary-Serbia (2021–2022).

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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