



Article

Weather Conditions Influence on Lavandin Essential Oil and Hydrolate Quality

Milica Aćimović¹ , Biljana Lončar^{2,*} , Jovana Stanković Jeremić³ , Mirjana Cvetković³ , Lato Pezo⁴ ,
Milada Pezo⁵ , Marina Todosijević⁶ and Vele Tešević⁶

- ¹ Institute of Field and Vegetable Crops Novi Sad—National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia; acimovicbabcimilica@gmail.com
- ² Faculty of Technology Novi Sad, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
- ³ Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia; jovana_stankovic@hotmail.com (J.S.J.); miracvet13@gmail.com (M.C.)
- ⁴ Institute of General and Physical Chemistry, Studentski trg 10–12, 11000 Belgrade, Serbia; latopezo@yahoo.co.uk
- ⁵ Department of Thermal Engineering and Energy, “Vinča” Institute of Nuclear Sciences—National Institute of the Republic of Serbia, University of Belgrade, P.O. Box 522, 11001 Belgrade, Serbia; milada@vin.bg.ac.rs
- ⁶ Department of Organic Chemistry, Faculty of Chemistry, Studentski trg 16, 11000 Belgrade, Serbia; marinab@chem.bg.ac.rs (M.T.); vtesevic@chem.bg.ac.rs (V.T.)
- * Correspondence: cbiljana@uns.ac.rs; Tel.: +381-(0)21-485-3708

Abstract: *Lavandula* sp. essential oil and hydrolate are commercially valuable in various industry branches with the potential for wide-ranging applications. This study aimed to evaluate the quality of these products obtained from *L. x intermedia* cv. ‘Budrovka’ for the first time cultivated on Fruška Gora Mt. (Serbia) during three successive seasons (2019, 2020, and 2021). Essential oil extraction was obtained by steam distillation, and the composition and influence of weather conditions were also assessed, using flowering tops. The obtained essential oils and hydrolates were analysed by gas chromatography with a flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). A linear regression model was developed to predict *L. x intermedia* cv. ‘Budrovka’ essential oil volatile compound content and hydrolate composition during three years, according to temperature and precipitation data, and the appropriate regression coefficients were calculated, while the correlation analysis was employed to analyse the correlations in hydrolate and essential oil compounds. To completely describe the structure of the research data that would present a better insight into the similarities and differences among the diverse *L. x intermedia* cv. ‘Budrovka’ samples, the PCA was used. The most dominant in *L. intermedia* cv. ‘Budrovka’ essential oil and hydrolate were oxygenated monoterpenes: linalool, 1,8-cineole, borneol, linalyl acetate, and terpinene-4-ol. It is established that the temperature was positively correlated with all essential oil and hydrolate compounds. The precipitations were positively correlated with the main compounds (linalool, 1,8-cineole, and borneol), while the other compounds’ content negatively correlated to precipitation. The results indicated that Fruška Gora Mt. has suitable agro-ecological requirements for cultivating *Lavandula* sp. and providing satisfactory essential oil and hydrolate.

Keywords: lavandin; precipitations; temperature; volatile compounds



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1. Introduction

The genus *Lavandula* (order Lamiales, family Lamiaceae) originates from the Mediterranean. It is cultivated worldwide as an ornamental and essential oil-bearing plant [1,2]. There are 39 species, but only three are commercially important: true or English lavender (*L. angustifolia* Mill.), spike or Spanish lavender (*L. latifolia* Medic.), and their hybrid known as lavandin (*L. x intermedia* Emeric ex Loisel). With more than 400 registered cultivars and hybrids, *Lavandula* sp. cultivation, essential oil production, and consumption

rapidly has increased due to certain profits [3–6]. Major producing regions of *Lavandula* sp. are in Europe, and the dominant countries are Bulgaria (with 3700 ha) and France (with 3500 ha) [1,3], but it is crucial in Mediterranean countries, especially Greece, Spain, and Turkey, as well [7]. Croatia is traditionally associated with *Lavandula* sp. cultivation and essential oil production on the *Adriatic coast*, but continental parts have become a commercial cultivation region [8,9]. In Hungary, a country with a continental climate, the cultivation of *Lavandula* sp. has expanded considerably in recent years [10], and in Romania [5]. Serbia follows the same path, with more than 100 ha under this crop in 2021, with an increasing tendency for the future (Aćimović, personal communication).

The *Lavandula* sp. essential oil is used in cosmetic (soaps, bath, colognes, perfumes, skin lotions, and after-shaves), pharmaceutical (mild sedative and analgesic, rubefacient and wound healing, and antioxidant and antimicrobial agents), and food industries (flavourings in baked goods, beverages, puddings, ice creams, candies, and chewing gums), in household products (detergents and hygiene products) and aromatherapy (for decreasing stress and anxiety, as well as pain intensity and against migraine), but it is also used as a biopesticide [3,4,11–15]. However, the application of lavender depends on its chemical composition. The common criteria for determining *Lavandula* sp. essential oil quality are camphor, linalool, and linalyl acetate percentage [4]. Essential oil of *L. angustifolia* is highly valued due to the low content of camphor (up to 1.2% according to European Pharmacopoeia) and is much more expensive. Therefore, it is often mixed with cheaper oils of *L. latifolia* and *L. x intermedia* to achieve better quality that satisfies ISO 8902 standard [15,16]. However, *L. latifolia* and *L. x intermedia* achieve higher essential oil yields [17,18]. Essential oil content and composition are primarily determined by plant genotype. However, it may be influenced by environmental factors (climate, but also weather conditions during growth year, soil conditions, and nutrient supply), harvest time, post-harvest treatments, and extraction methods [10,19–21].

Hydrolates are accrued as by-products during the steam distillation of essential oils. Essential oils contain volatile, lipid-soluble, and partially water-soluble compounds, generally of lower density than water [22,23]. However, volatile water-soluble compounds remain in condensate water in a Florentine flask (oil–water separator) and give a specific fragrance. This water is called hydrolate, hydrosol, aromatic or floral water [24–27]. Since hydrolates contain only a small amount of dissolved essential oil components in water, the amount of volatile organic compounds affects their biological properties [28,29]. Principally, they are by-products of essential oil distillation that could be useful as raw material in many industries, such as the food, and beverage industry (for flavouring and preservation, as well as in soft drinks), cosmetics (replacement for water phase in cosmetics, lotions, creams, soaps, and tonics) and aromatherapy (skincare or as massage products, as facial and body sprays to feel relaxed and refreshed, as air fresheners) [24]. Although the quality standards for hydrolates are not defined, their global economic impact is increasing [30]. Therefore, it is necessary to develop norms and standards for plant-derived components as there is growing interest in using them. Furthermore, due to volatile organic compounds, *Lavandula* sp. hydrolate has a pleasant lavender aroma and biological activities. Therefore, it has the potential for wide-ranging applications [28,29,31].

Fruška Gora Mt. (in Latin Alma Mons) is situated in northern Serbia, at the confluence of the Danube and Sava Rivers. This is the oldest national park in Serbia (with 25,525 ha of protected area), with almost 90% of the linden, oak, and beech forest. The rims of the Fruška Gora Mt. are used for grape cultivation, and this tradition dates from the Roman period. However, medicinal and aromatic plants were not cultivated in this region until recently. As a result, many wild species of medicinal plants, are mainly collected by gatherers [32]. This region is suitable for organic cultivation, and previous research indicated promising results in organically grown *Lavandula* sp. through Europe [33–35]. *Lavandula* sp. is a perennial, heliophyte, and drought-resistant shrub [10]. It requires well-drained soil, but it can adapt to poor soils with low fertility [36]. Croatian cultivar of *L. x intermedia* ‘Budrovka’ is widely

grown in the former Yugoslavia region. This cultivar is well adapted to the continental climate and low temperatures during winter, up to $-20\text{ }^{\circ}\text{C}$ [37,38].

Considering the great commercial importance of *Lavandula* sp. flowers and essential oil in different industry branches and the increasing trend of cultivation lavender and lavandin through Europe, this paper aimed to evaluate the quality of introduced *L. x intermedia* cv. ‘Budrovka’ on Fruška Gora Mt. (Serbia). The main goal of this investigation was the influence of microclimatic conditions in the foreground temperature and precipitations in a selected location on *L. x intermedia* essential oil and hydrolate quality. The second goal of this investigation is to evaluate the essential oil quality of cv. ‘Budrovka’ compared to other samples *L. x intermedia* reported in the literature and ISO standards. Further, results of the chemical composition of *L. x intermedia* ‘Budrovka’ hydrolates from this study and other from literature were used for setting ranges of the main compounds of *Lavandula* sp. hydrolate.

2. Materials and Methods

2.1. Plant Material

The plantation of *Lavandula x intermedia* Emeric ex Loisel cv. ‘Budrovka’ was established in autumn 2014, at a certified organic farm in the village of Bukovac (area Beljevo; 45.111744 N, 19.530732 E) on Fruška Gora region (Figure 1). One-year-old seedlings were planted at a 1.5 m interrow distance and 0.5 m between plants.

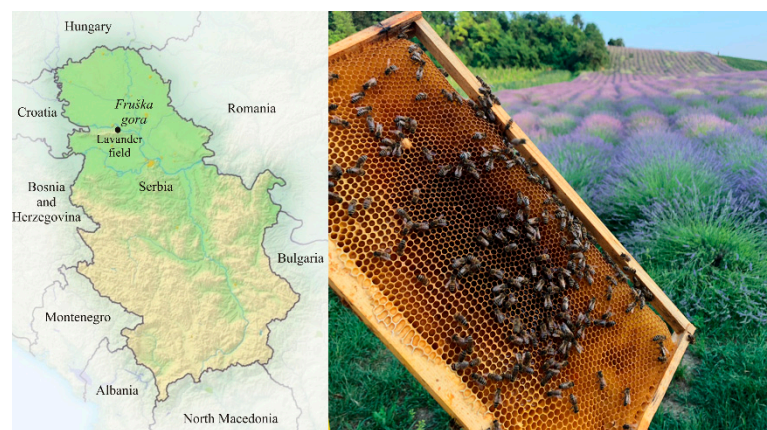


Figure 1. Field location and lavender field in full blooming with honeybees.

2.2. Soil Characteristics

Basic chemical properties of the soil where *L. x intermedia* was cultivated were determined before establishing the experiment (October 2014) in samples of the top layer (0 to 40 cm) and are shown in Table 1. The soil samples were analysed at the Agriculture Extension Service Novi Sad, following standardized methods adopted in Serbia [39].

Table 1. Basic chemical characteristics of the soil.

	pH		CaCO ₃	Humus (%)	Total Nitrogen	P ₂ O ₅		K ₂ O
	1MKCl	H ₂ O				(mg·100 g ⁻¹ Soil)		
Hill	7.13	8.22	11.27	1.03	0.051	3.6	8.0	
Middle	7.19	8.34	8.10	0.56	0.028	7.4	8.0	
Valley	7.41	8.31	7.89	0.96	0.048	7.4	8.0	

2.3. Weather Conditions

The climate of Fruška Gora, as an isolated mountain, significantly differs from its surroundings and modifies the local meteorological conditions. Although the mountain is small, the wooded slopes and the E–W direction of the ridge influence the passing air masses greatly [40]. The weather conditions during the three successive growing years are presented in Figure 2.

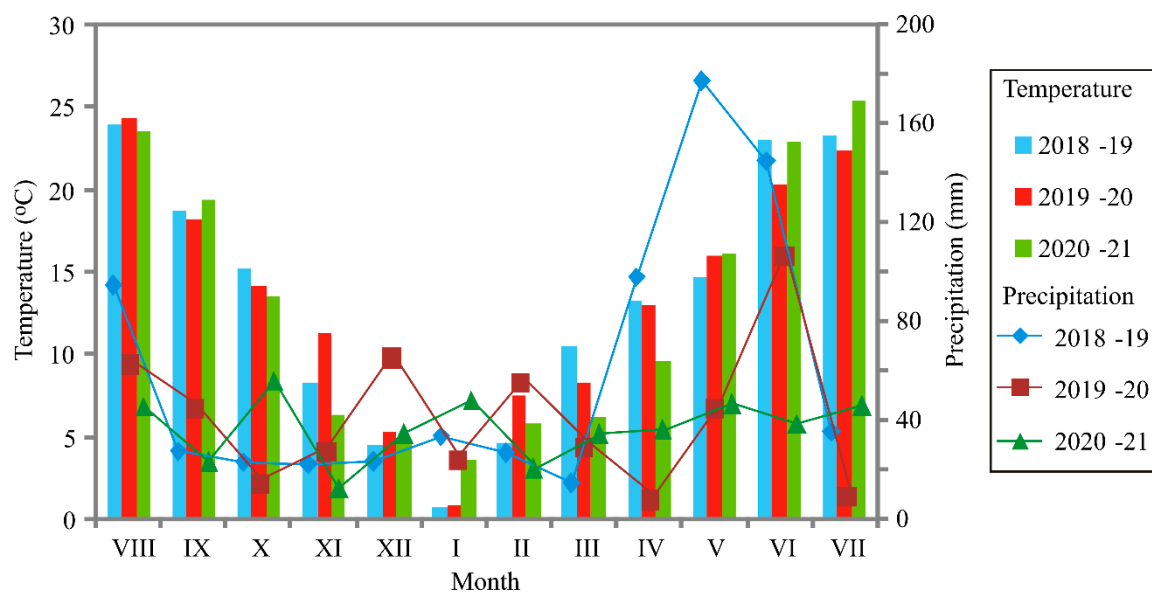


Figure 2. The weather conditions during the three successive growing seasons.

2.4. Essential Oil and Hydrolate Extraction

The small-scale distillation unit at the Institute of Field and Vegetable Crops Novi Sad, consists of a cylindrical distillation vessel with a conical bottom of stainless-steel pipe, which connects the vessel with a water-cooled condenser Florentine flask. The steam was produced externally, by a steam generator, and supplied the vessel with plant material via a pipe on the bottom. Our previous paper provides detailed information about capacity and conditions [41].

Briefly, 100 kg of fresh flowers of *L. x intermedia* cv. 'Budrovka' were placed in a distillation vessel, routed upwards through a plumbing system, and supplied with steam. After 20 min, the condensed vapours started to collect in the Florentine flask. After 2 h the distillation process was over. Accumulated essential oil floated on the water phase (hydrolate). The hydrolate was collected in sterile plastic bottles via filter paper, while essential oil was dried applying sodium sulphate and stored in amber glass bottles. The same extraction process was repeated three times.

2.5. Analysis of Volatile Compounds

The hydrolates before analysis were subjected to simultaneous distillation and extraction with dichloromethane via a Likens–Nickerson apparatus. Obtained essential oils and hydrolates samples were analysed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). GC-FID/MS analyses were performed according to Acimovic et al. [42] with some modifications of the split ratio. Injection volume was 1 μ L, split ratio 10:1 for essential oils and 50:1 for hydrolates.

2.6. Statistical Analysis

A linear regression model was developed to predict the *L. x intermedia* cv. 'Budrovka' essential oil active compound content and hydrolate composition during three years according to temperature and precipitation data, and the appropriate regression coefficients were calculated using the linear formula: $x = a + b_T \cdot T + b_P \cdot P$, where x was EO active compound content or hydrolate content, a was the intercept, b_T and b_P were temperature and precipitation coefficients, while T and P were temperature and precipitation amounts, respectively.

The collected data were processed statistically using the software package STATISTICA 10.0.

3. Results

3.1. Fresh Herb Yield and Essential Oil Content

This variety (Vouch No 2-0987, Herbarium BUNS, Serbia), obtained from local nursery garden, is a shrub ranging up to 150 cm, with linear-lanceolate to spatulate leaves, often tomentose. Its inflorescence stalk is branched, and flowers show a corolla with bilateral symmetry and vary in colour from lilac-purple to white, blooming from late June to July. Flowering tops were collected at the full bloom stage. Harvest dates and yields are given in Table 2.

Table 2. Date of harvest, fresh herb yield and essential oil content for *L. x intermedia* cv. ‘Budrovka’ cultivated in Fruška gora Mt. during 2019–2021.

	2019	2020	2021
Date of harvest	11th–17th July	27th June–2nd July	9th–15th July
Fresh herb yield (kg/ha)	4.840	5.040	5.670
Essential oil content (%)	1.26	1.19	1.03

3.2. Chemical Composition of Essential Oil

The essential oil from *L. x intermedia* cultivated on Fruška Gora Mt. contains 56 compounds (Table 3). The most abundant compounds were: linalool (33.3–42.0%), 1,8-cineole (12.9–19.0%), borneol (8.1–16.1%), linalyl acetate (5.3–8.8%), and terpinene-4-ol (3.5–6.6%). All these compounds belong to oxygenated monoterpenes, which are the dominant compound class in essential oils.

Table 3. *L. x intermedia* cv. ‘Budrovka’ essential oil composition during three growing years and related multiple correlation coefficients with the observed temperature and precipitation amounts.

No	Compound	RI	2019	2020	2021	Temp. Coeff.	Prec. Coeff.
			EO19	EO20	EO21		
6	<i>n</i> -hexanol ^O	861	0.2	-	-	0.70	-0.25
7	α -thujene ^{MT}	924	0.1	0.1	0.1	0.70	-0.25
8	α -pinene ^{MT}	931	0.9	0.6	1.4	0.71	-0.23
9	camphene ^{MT}	945	0.4	0.7	0.5	0.71	-0.24
12	sabinene ^{MT}	970	0.3	0.1	0.4	0.70	-0.24
14	β -pinene ^{MT}	974	1.6	0.9	2.1	0.71	-0.21
15	3-octanone ^O	984	0.1	-	-	0.70	-0.25
16	dehydro-1,8-cineole ^{OMT}	989	-	0.1	-	0.70	-0.25
17	myrcene ^{MT}	990	0.5	0.1	0.7	0.70	-0.24
18	δ -3-carene ^{MT}	1008	0.3	0.1	0.4	0.70	-0.24
19	hexyl acetate ^O	1010	0.1	-	-	0.70	-0.25
20	α -terpinene ^{MT}	1013	-	-	0.1	0.70	-0.25
22	<i>p</i> -cymene ^{MT}	1022	0.4	0.8	0.3	0.71	-0.24
23	limonene ^{MT}	1025	1.1	0.7	1.1	0.71	-0.23
24	1,8-cineole ^{OMT}	1028	12.9	16.4	19.0	0.82	0.11
25	<i>cis</i> - β -ocimene ^{MT}	1033	1.9	-	3.6	0.72	-0.21
27	<i>trans</i> - β -ocimene ^{MT}	1044	0.2	-	0.4	0.70	-0.24
28	γ -terpinene ^{MT}	1055	0.1	-	0.2	0.70	-0.25
29	<i>cis</i> -sabinene hydrate (IPP vs OH) ^{OMT}	1063	0.2	0.2	-	0.70	-0.25
30	<i>cis</i> -linalool oxide (furanoid) ^{OMT}	1069	0.1	2.4	-	0.71	-0.23
32	<i>trans</i> -linalool oxide (furanoid) ^{OMT}	1085	-	2.1	-	0.71	-0.23
33	terpinolene ^{MT}	1086	0.2	-	0.3	0.70	-0.24
34	linalool ^{OMT}	1101	41.8	33.3	42.0	0.98	0.59
36	<i>allo</i> -ocimene ^{MT}	1126	0.3	-	0.6	0.70	-0.24
38	<i>trans</i> -pinocarveol ^{OMT}	1134	-	0.3	-	0.70	-0.25
40	camphor ^{OMT}	1141	3.7	4.6	3.9	0.73	-0.16

RI—Retention index (relative to C8–C36 n-alkanes on HP-5MSI column).

Table 3. Cont.

No	Compound	RI	2019	2020	2021	Temp. Coeff.	Prec. Coeff.
			EO19	EO20	EO21		
41	hexyl isobutanoate ^O	1145	0.1	0.1	-	0.70	-0.25
43	borneol ^{OMT}	1164	11.4	16.1	8.1	0.79	0.01
45	<i>trans</i> -linalool oxide (pyranoid) ^{OMT}	1168	-	0.2	-	0.70	-0.25
46	terpinen-4-ol ^{OMT}	1175	6.6	3.5	5.3	0.74	-0.14
48	cryptone ^O	1180	-	0.3	-	0.70	-0.25
49	α -terpineol ^{OMT}	1188	0.8	0.5	-	0.70	-0.24
50	hexyl butanoate ^O	1189	0.4	0.4	0.5	0.70	-0.24
51	myrtenal ^{OMT}	1190	-	0.2	-	0.70	-0.25
55	hexyl 2-methyl butanoate ^O	1234	0.2	0.3	0.1	0.70	-0.24
56	cumin aldehyde ^O	1236	-	0.1	-	0.70	-0.25
57	carvone ^{OMT}	1238	-	0.1	-	0.70	-0.25
58	hexyl isovalerate ^O	1239	0.1	-	-	0.70	-0.25
61	linalyl acetate ^{OMT}	1254	6.5	8.8	5.3	0.75	-0.10
63	bornyl acetate ^{OMT}	1284	0.1	0.2	-	0.70	-0.25
64	lavandulyl acetate ^{OMT}	1289	0.6	1.2	0.3	0.71	-0.23
65	neryl acetate ^{OMT}	1363	0.1	-	-	0.70	-0.25
66	daucene ST	1377	0.1	-	-	0.70	-0.25
67	geranyl acetate ^{OMT}	1382	0.2	-	-	0.70	-0.25
68	hexyl hexanoate ^O	1384	0.2	0.2	-	0.70	-0.25
69	7- <i>epi</i> -sesquithujene ST	1388	0.1	-	-	0.70	-0.25
70	sesquithujene ST	1403	0.1	-	-	0.70	-0.25
71	α -santalene ST	1414	-	0.3	-	0.70	-0.25
72	<i>trans</i> -caryophyllene ST	1417	0.9	-	0.6	0.70	-0.24
73	<i>trans</i> - α -bergamotene ST	1433	0.1	-	-	0.70	-0.25
74	<i>trans</i> - β -farnesene ST	1455	2.0	0.3	1.1	0.71	-0.23
75	germacrene D ST	1480	0.3	-	0.1	0.70	-0.25
76	lavandulyl isovalerate ^{OST}	1508	0.3	0.4	-	0.70	-0.24
77	γ -cadinene ST	1513	tr	0.1	-	0.70	-0.25
79	caryophyllene oxide ^{OST}	1580	0.2	0.8	-	0.70	-0.24
80	<i>epi</i> - α -bisabolol ^{OST}	1682	0.1	-	-	0.70	-0.25
Monoterpene hydrocarbons (MT)			8.3	4.1	12.2		
Oxygenated monoterpenes (OMT)			85.0	90.2	83.9		
Sesquiterpene hydrocarbons (ST)			3.6	0.7	1.8		
Oxygenated sesquiterpens (OST)			0.6	1.2	-		
Other (O)			1.4	1.4	0.6		
Total identified			98.9	97.6	98.5		

RI—Retention index (relative to C8–C36 n-alkanes on HP-5MSI column).

Comparing rainfall among years (Figure 2), the growing season 2018/19 had significantly higher rainfall (694.2 mm) in comparison with the other two growing seasons (458.8 mm for 2019/20, and 416.9 mm for 2020/21). On the other hand, comparing average year temperatures for all three investigated seasons, the third growing season (2020/21) was slightly cooler (13.2 °C) in comparison to the other two (13.5 °C on average). Therefore, it could be said that temperature was in positive correlation with all essential oil compounds (temp. coeff. varied between 0.70 and 0.98), while precipitations were in negative correlation with a large number of essential oil compounds (prec. coeff. varied between -0.10 and -0.25), except linalool (0.59), 1,8-cineole (0.11), and borneol (0.01).

3.3. Chemical Composition of Hydrolate

There were 40E compounds identified in *L. x intermedia* cv. 'Budrovka' hydrolate (Table 4). The most abundant was linalool (21.9–32.1%), 1,8-cineole (12.7–26.2%), borneol (10.6–24.4%), terpinen-4-ol (6.4–12.2%), and *cis*- and *trans*-linalool oxides (1.4–11.5% and 1.3–10.9%, respectively).

Table 4. *L. x intermedia* cv. ‘Budrovka’ hydrolate composition during three growing years and related multiple correlation coefficients with the observed temperature and precipitation amounts.

No	Compound	RI	2019	2020	2021	Temp. Coeff.	Prec. Coeff.
			EO19	EO20	EO21		
1	3-methyl-2-butenal ^O	772	0.1	-	0.4	0.70	-0.24
2	hexanal ^O	798	-	-	0.2	0.70	-0.25
3	2,2-dimethyl-3(2H)-furanone ^O	829	-	-	0.1	0.70	-0.25
4	furfural ^O	832	-	-	0.1	0.70	-0.25
5	<i>cis</i> -3-hexenol ^O	847	-	-	0.3	0.70	-0.25
6	<i>n</i> -hexanol ^O	858	1.0	0.6	2.2	0.71	-0.22
10	4-methyl pent-2-enolide (impure) ^O	949	0.1	-	0.3	0.70	-0.25
11	2-ethenyltetrahydro-2,6,6-trimethyl-2H-pyran ^O	968	0.1	0.1	-	0.70	-0.25
13	1-octen-3-ol ^O	974	0.9	0.6	0.9	0.71	-0.23
15	3-octanone ^O	982	-	-	0.1	0.70	-0.25
16	dehydro-1,8-cineole ^{OMT}	990	tr	-	0.1	0.70	-0.25
21	1,4-cineole ^{OMT}	1014	0.1	-	-	0.70	-0.25
24	1,8-cineole ^{OMT}	1030	12.7	14.4	26.2	0.84	0.15
26	lavender lactone ^O	1036	-	0.2	0.1	0.70	-0.25
30	<i>cis</i> -linalool oxide (furanoid) ^{OMT}	1071	1.4	11.5	2.7	0.74	-0.13
31	camphenilone ^{NOMT}	1080	-	-	0.1	0.70	-0.25
32	<i>trans</i> -linalool oxide (furanoid) ^{OMT}	1088	1.3	10.9	2.4	0.74	-0.14
34	linalool ^{OMT}	1102	26.0	21.9	32.1	0.90	0.33
35	hotrienol ^{OMT}	1102	-	1.0	-	0.70	-0.24
37	nopinone ^{NOMT}	1136	0.2	0.2	0.2	0.70	-0.24
38	<i>trans</i> -pinocarveol ^{OMT}	1134	-	0.2	0.1	0.70	-0.25
39	<i>trans</i> -sabinol (trans for OH vs. IPP) ^{OMT}	1136	0.1	-	-	0.70	-0.25
40	camphor ^{OMT}	1143	7.1	4.5	6.3	0.74	-0.12
42	<i>neiso</i> -3-thujanol ^{OMT}	1146	-	0.2	-	0.70	-0.25
43	borneol ^{OMT}	1166	24.4	16.8	10.6	0.81	0.10
44	<i>cis</i> -linalool oxide (pyanoid) ^{OMT}	1168	0.6	1.0	0.5	0.71	-0.23
45	<i>trans</i> -linalool oxide (pyanoid) ^{OMT}	1173	0.4	0.8	0.4	0.71	-0.24
46	terpinen-4-ol ^{OMT}	1177	12.2	6.4	9.7	0.77	-0.05
47	<i>p</i> -cymen-8-ol ^O	1179	-	0.2	0.2	0.70	-0.24
48	cryptone ^O	1180	-	1.0	0.3	0.71	-0.24
49	α -terpineol ^{OMT}	1190	6.0	4.5	1.4	0.73	-0.17
51	myrtenol ^{OMT}	1191	-	0.1	-	0.70	-0.25
52	verbenone ^{OMT}	1208	0.2	0.3	0.1	0.70	-0.24
53	<i>trans</i> -carveol ^{OMT}	1216	-	0.1	-	0.70	-0.25
54	nerol ^{OMT}	1222	-	0.3	-	0.70	-0.25
57	carvone ^{OMT}	1243	0.1	0.1	-	0.70	-0.25
60	geraniol ^{OMT}	1248	-	0.9	-	0.70	-0.24
61	linalyl acetate ^{OMT}	1248	-	-	0.2	0.70	-0.25
64	lavandulyl acetate ^{OMT}	1286	-	0.1	-	0.70	-0.25
78	<i>cis</i> -nerolidol ^{OST}	1526	-	-	0.2	0.70	-0.25
Nor oxygenated monoterpenes (NOMT)			0.2	0.2	0.3		
Oxygenated monoterpenes (OMT)			92.6	96.0	92.8		
Sesquiterpene hydrocarbons (ST)			-	-	-		
Oxygenated sesquiterpens (OST)			-	-	0.2		
Other (O)			2.2	2.7	5.2		
Total identified			95.0	98.9	98.5		

RI—Retention index (relative to C8–C36 n-alkanes on HP-5MSI column).

During all three years oxygenated monoterpenes were the dominant class, with 92.6–96.0%. Similar to essential oils, differences in climate conditions during tree investigated years have not impacted hydrolate composition variations. In addition, the temperature was in positive correlation with all hydrolate compounds (temp. coeff. varied between 0.70 and 0.84), while precipitations were in negative correlation with a large number of essential oil compounds

(prec. coeff. varied between -0.10 and -0.25), except linalool (0.33), 1,8-cineole (0.15), and borneol (0.10).

3.4. Correlation between Chemical Compounds of Essential Oil and Hydrolate

The correlation analysis was employed to examine the relations in hydrolate and essential oil compounds of *L. x intermedia* cv. 'Budrovka' samples from three growing years (2019, 2020, and 2021), and the results were displayed in Figure 3. It can be noticed from the figure that the darker blue colour of the squares, which indicates the two active compounds 'content similarity, presents a more significant correlation linking observed active compounds. In contrast, the lighter tone indicates a particular dissimilarity active compound. Therefore, if the colour tone is lighter, consequently the correlation is lower. On the other hand, the red colour symbolizes a negative correlation between active compounds.

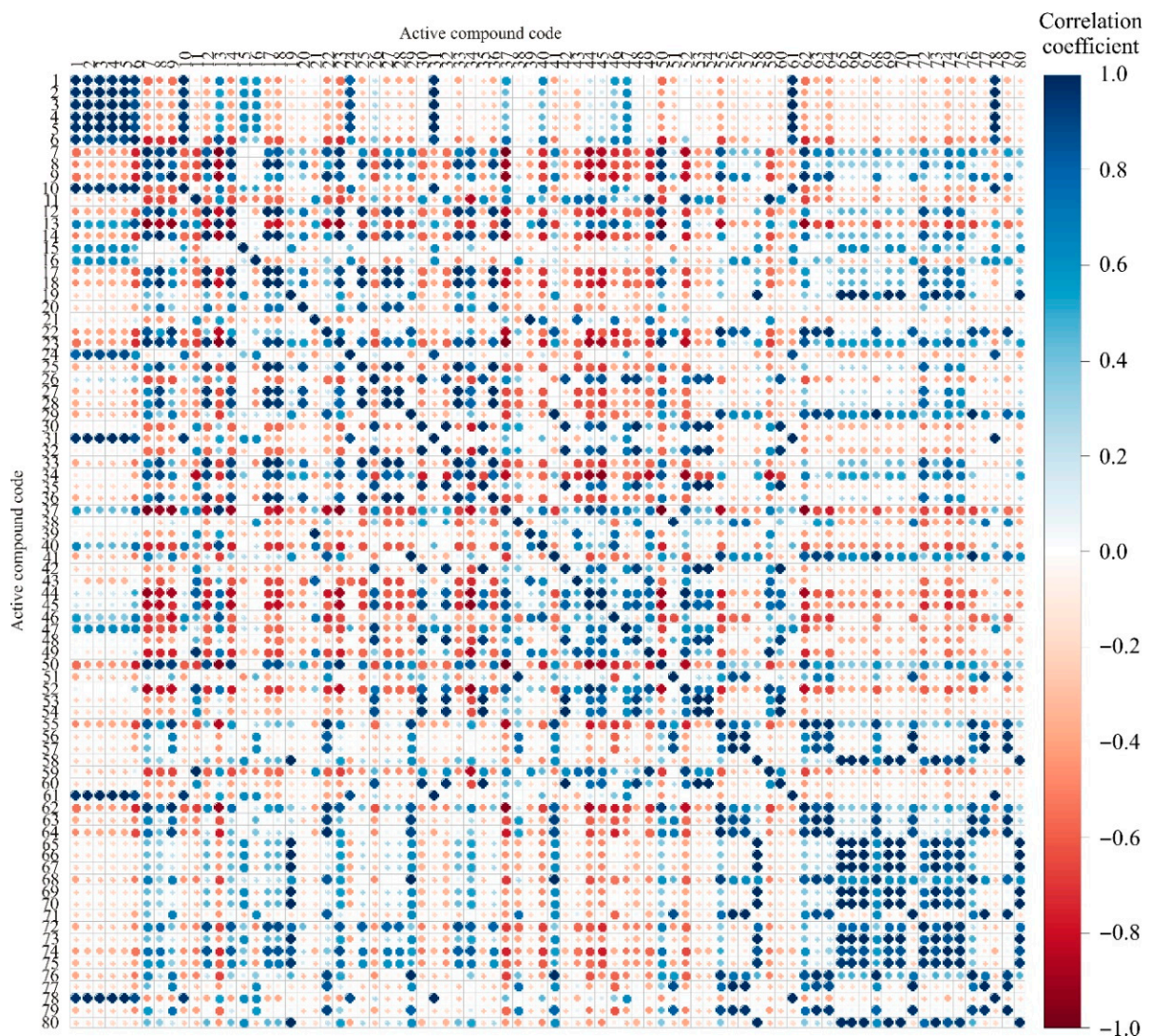


Figure 3. Correlation between active compounds content in hydrolate and essential oil of *L. x intermedia* cv. 'Budrovka' samples from 2019, 2020, and 2021 (active compounds codes are listed in Tables 3 and 4).

To thoroughly explain the structure of the research data that would provide a better perception of similarities and differences among diverse the *L. x intermedia* cv. 'Budrovka' samples from 2019, 2020, and 2021, PCA was used, and the results are presented in Figure 4. The first PC explained 43.46%, the second 19.10%, and the third 16.53% of the total variance within the experimental data. The separation between samples could be recognized

from the PCA figures, where the samples from *L. x intermedia* cv. ‘Budrovka’ hydrolate composition during 2019, 2020, and 2021 are grouped on the right side of the graphic, while the samples from the *L. x intermedia* cv. ‘Budrovka’ essential oil composition during three growing years are grouped on the graphic’s left side. Table 5 represents the correlation matrix among active compounds content in hydrolate and essential oil of *L. x intermedia* cv. ‘Budrovka’ samples from 2019, 2020, and 2021.

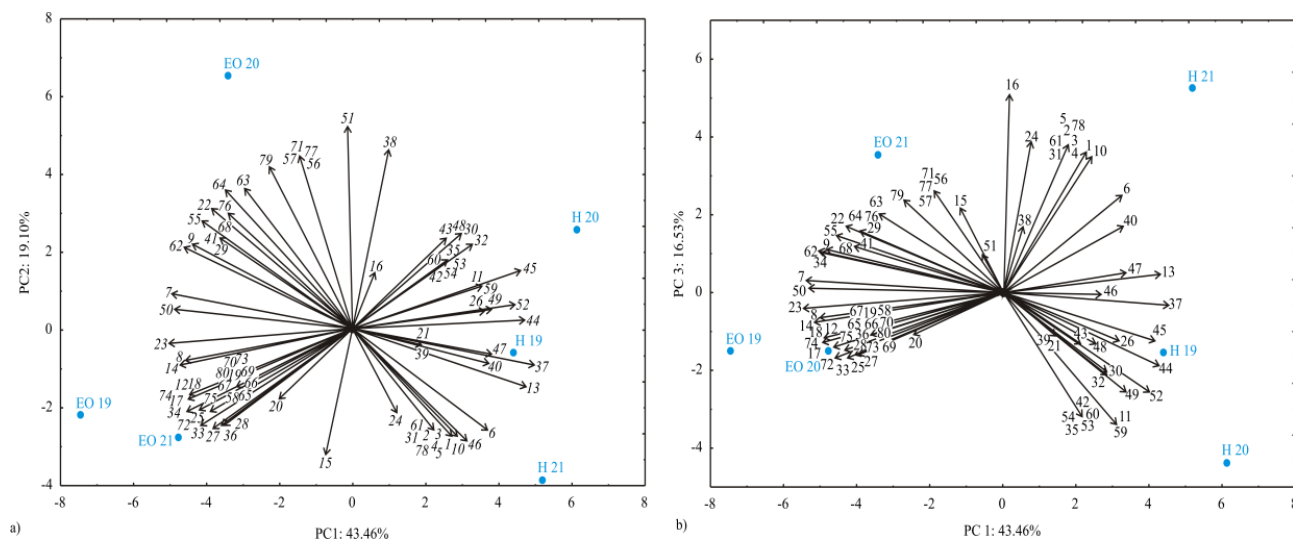


Figure 4. The PCA biplot diagram depicting the relationships among hydrolate and essential oil compounds of *L. x intermedia* cv. ‘Budrovka’ samples from three growing years: (a) projection of variables and cases in the PC1 and PC2 plane, (b) projection of variables and cases in PC1 and PC3 plane (active compounds codes and samples codes are listed in Tables 3 and 5).

Table 5. Correlation matrix between active compounds content in hydrolate and essential oil of *L. x intermedia* cv. ‘Budrovka’ samples from 2019, 2020, and 2021.

	EO20	EO21	H19	H20	H21
EO19	0.967	0.987	0.855	0.795	0.908
EO20		0.959	0.896	0.868	0.928
EO21			0.820	0.788	0.940
H19				0.893	0.874
H20					0.870

EO20—essential oil active compounds from 2020, EO21—essential oil active compounds from 2021, H19—hydrolate active compounds from 2019, H20—hydrolate active compounds from 2020, H21—hydrolate active compounds from 2021.

4. Discussion

4.1. Essential Oil

Investigations conducted with *L. latifolia* showed that the environment significantly affected the essential oil quality, especially altitude [43]. Further, in the case of *L. angustifolia*, agronomical practices such as irrigation [44], variety [19], and substrate [45] significantly impact essential oil composition. In addition, investigations show that during the blooming period, linalool content was influenced by temperature; although, rainfalls remarkably decreased its production [46]. As it can be seen from Figure 4, our long-term experiment (three successive years) shows that on accumulation linalool and linalyl acetate precipitation, and the temperature are in positive relations, while in the case of linalyl acetate, the temperature is in positive (0.75), and precipitation in negative (−0.10) correlations.

The isolated essential oil of *L. x intermedia* cv. ‘Budrovka’ cultivated in Fruška gora Mt. did not comply with international standards requirements for the lavandin (ISO 8902), similar is with the same cultivar grown in Croatia, which contained linalool (57.1%), linalyl acetate (9.8%), and 1,8-cineole (8.5%) as dominant compounds [9]. One of the main criteria

for *Lavandula* sp. essential oil quality is the ratio between linalool and its ester form, linalyl acetate. A ratio lower than one indicates the high quality of essential oil [47]. In this study, the linalool: linalyl acetate ratio ranged from 3.78 (in 2020) and 6.43 (in 2019) to 7.92 (in 2021). On average, this value was 6.04, while in Croatia, this ratio was 5.81 for the same cultivar [9].

Table 6 shows 28 samples of *L. x intermedia* essential oil from literature and average values from this study (total 29 accessions) with ten compounds from ISO standard specification. As can be seen, no sample satisfies all values required for essential oil quality. However, it is known that *L. x intermedia* is a cheaper hybrid, 6–7 times than *L. angustifolia*, and usually is mixed with them to obtain a higher quality of essential oil [16]. In addition, the accumulation of main compounds in *Lavandula* sp. plants are genetically influenced, i.e., by a key gene involved in controlling the production of linalyl acetate, camphor, 1,8-cineole, and borneol [48].

Table 6. Comparison results of main *L. x intermedia* essential oil components and ISO 8902 standard.

No.	Cultivar	Origin	Limonene	1,8-cineole	Cis-B-Ocimene	Trans-B-Ocimene	Camphor	Linalool	Linalyl Acetate	Terpinene-4-OI	Lavandulyl Acetate	Lavandulol	Reference
1	Hidcote Giant	Canada	1.4	17.1	7.8	12.2	13.3	23.8	0.3	4.1	0.0	1.6	[19]
2	Grosso	Canada	2.0	10.7	3.4	4.4	10.8	30.6	8.3	3.3	3.7	0.0	[19]
3	Super	Canada	2.7	13.1	3.5	6.2	5.5	28.3	10.0	0.0	3.4	0.0	[19]
4	OK-Farms Super	Canada	2.4	4.8	3.1	8.0	2.1	35.2	11.6	0.0	1.8	0.0	[19]
5	French Super	Canada	2.5	5.2	4.0	9.8	4.8	25.2	12.2	0.5	2.7	0.0	[19]
6	Super	Turkey	0.0	2.6	0.0	0.0	4.8	34.0	47.7	0.0	0.0	0.0	[49]
7	n.s.	Macedonia	0.0	6.7	2.3	0.8	6.6	39.0	2.1	5.1	1.9	1.7	[11]
8	Dutch	Turkey	1.1	7.6	0.0	0.0	11.3	44.8	8.2	1.5	0.0	0.1	[50]
9	Giant Hidcote	Turkey	1.3	12.0	0.0	0.0	6.6	41.6	3.9	1.5	0.0	0.2	[50]
10	Super A	Turkey	0.8	2.1	0.0	0.0	7.5	38.1	36.2	0.5	0.0	0.0	[50]
11	Super A	Turkey	0.4	3.2	1.5	6.6	43.7	24.6	5.4	0.0	0.0		[51]
12	Sumiens	Italy	0.7	12.0	1.9	1.0	7.1	40.4	9.9	0.2	0.3	0.0	[3]
13	Super A	Italy	0.6	6.9	1.3	0.7	6.6	36.2	18.4	3.3	4.5	0.1	[3]
14	Grosso	Italy	0.5	8.1	1.2	0.8	8.1	38.4	15.7	3.6	4.1	0.1	[3]
15	Super	Turkey	0.7	0.0	2.6	1.5	5.3	36.8	33.1	0.0	1.2	0.1	[4]
16	Grey Hedge	Turkey	2.3	0.0	1.8	5.0	6.4	28.5	4.6	6.9	0.8	0.5	[4]
17	Budrovka	Croatia	4.0	8.4	0.0	0.4	0.1	57.1	9.8	2.3	0.2	1.1	[9]
18	Grosso	Turkey	2.5	33.1	0.0	0.0	21.8	0.2	0.0	1.0	0.2	0.4	[18]
19	Dutch	Turkey	2.9	35.8	0.0	0.0	22.2	0.2	0.0	0.8	0.1	0.2	[18]
20	Abriel	Turkey	2.5	35.9	0.0	0.0	22.8	0.1	0.0	1.0	0.2	0.1	[18]
21	Grosso	Italy	1.0	5.2	0.9	0.7	6.0	41.6	23.0	4.8	3.2	0.0	[52]
22	n.s.	Serbia	0.4	14.6	0.4	0.0	16.3	23.1	10.0	0.7	2.6	0.9	[53]
23	Grappenhall	Hungary	1.4	9.0	9.3	3.4	2.8	46.8	3.3	3.0	0.9	1.2	[10]
24	Grosso	Hungary	0.7	3.0	4.1	1.9	14.8	55.2	4.4	0.9	1.2	1.4	[10]
25	n.s.	China	0.0	44.8	0.0	0.5	12.2	7.6	0.0	0.6	0.0	0.0	[21]
26	Abrialis	Italy	0.5	7.0	3.0	8.3	9.4	40.3	18.4	0.6	1.4	0.8	[54]
27	Rinaldi Cerioni	Italy	0.4	10.0	0.0	0.0	11.5	65.8	0.5	2.9	0.0	0.6	[54]
28	Sumiens	Italy	0.7	12.1	3.0	0.4	6.8	48.0	14.9	0.3	0.0	0.0	[54]
29	Budrovka	Serbia	1.0	16.1	1.8	0.2	4.1	39.0	6.9	5.1	0.7	0.0	TS
AVERAGE			1.3	12.0	2.0	2.4	9.1	34.1	11.6	2.1	1.2	0.4	
RANGE			≤4.0	≤44.8	≤9.3	≤12.2	0.1–22.8	0.1–65.8	≤47.7	≤6.9	≤4.5	≤1.7	
ISO STANDARD			0.5–1.5	4.0–7.0	0.5–1.5	tr-1.0	6.0–8.0	24.0–35.0	28.0–38.0	1.5–5.0	1.5–3.0	0.2–0.8	

n.s.—not specified; TS—this study (average value for three investigated year).

The primary source of variability in chemical composition and oil yield among the various populations of *L. x intermedia* are differences in environmental conditions [14].

According to 29 accessions of *L. x intermedia* from the literature, unrooted cluster tree (Figure 5) shows the presence of four chemotypes: (1) linalool + linalyl acetate, (2) linalool + 1,8-cineole, (3) linalool + camphor, and (4) 1,8-cineole + camphor. The first one, with dominant linalool and linalyl acetate, could be divided into three subgroups according to linalool:linalyl acetate ratio: (1) ratio ranged between 0.7 and 1.1—all three accessions from Turkey [4,49,50]; (2) ratio 1.8—one accession from Turkey [51]; and (3) ratio ranged from 1.8 to 5.5—five accessions from Italy [3,52,54], and one from Turkey [50]. In the second chemotype, the dominant compounds were linalool, and 1,8-cineole, with two subgroups: (1) 39.0–46.8% of linalool and 6.7–16.1% of 1,8-cineole [3,10,11,50], and this study; and (2) 55.2–65.8% of linalool and 3.0–10.0% of 1,8-cineole [9,10,54]. Linalool+camphor chemotype is present in Canada (five accessions, [19]) as well as with one sample from Turkey and Serbia [4,53]. Chemotype with dominant 1,8-cineole, and camphor is noted in Turkey (three accessions, [18]) and China [21].

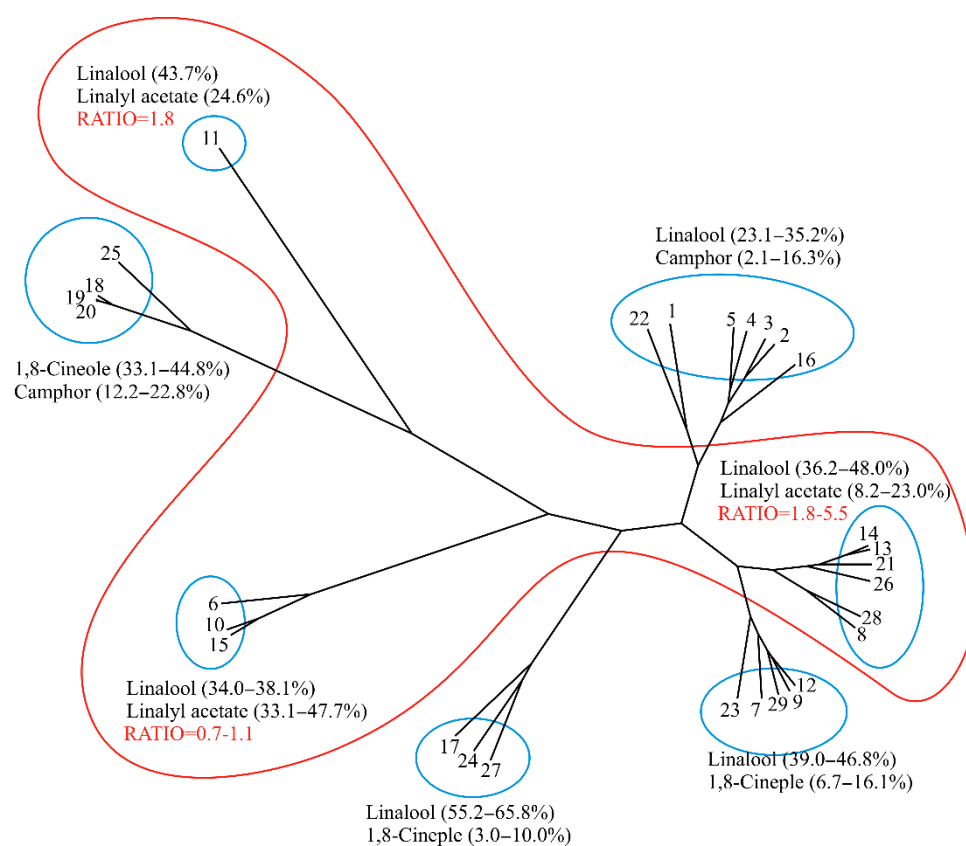


Figure 5. Unrooted cluster tree for chemical composition of *L. x intermedia* essential oil (samples are coded according to Table 6).

4.2. Hydrolate

Lavandula sp. hydrolates have a characteristic delicate lavender scent [28]. However, the chemical composition of hydrolates and essential oil, depends on plant part (herb or flower), postharvest processing (fresh or dry plant material), and isolation technique [55,56]. The chemical composition of different *Lavandula* sp. hydrolates found in literature and from the study is shown in Table 7. As can be seen, all *Lavandula* sp. hydrolates contain linalool (ranged between 7.7% and 55.6%). The content of other significant compounds (average content more than 1.5% according to 19 samples from literature, and average value from this study) are: linalool oxides ($\leq 67.3\%$), 1,8-cineole ($\leq 52.9\%$), camphor ($\leq 19.6\%$), borneol ($\leq 17.3\%$), α -terpineol ($\leq 13.0\%$), terpinen-4-ol ($\leq 9.4\%$), and geraniol ($\leq 5.0\%$). The most abundant group of compounds are oxygenated monoterpenes, mainly monoterpene alcohols [28]. The hydrosols were not found to contain linalyl acetate or sesquiterpenes, which are present in the essential oil [28,49].

Table 7. Chemical composition of different *Lavandula* sp. hydrolates according to literature and this study.

No.	Species/ Variety/ Cultivar	Origin	Extraction Technique/ Plant Material	Linalool	1,8-cineole	Linalool Oxides	α -terpineol	Camphor	Borneol	Terpinen-4-Ol	Geraniol	Reference
1	LI cv "Super"	Turkey	f.f., SD	55.6	9.8	6.0	0.0	13.4	13.5	0.0	1.6	[49]
2	LO	Morocco	n.s.	45.0	14.8	0.4	11.8	15.7	11.3	0.0	0.0	[57]
3	LA	Poland	HV400	39.2	0.0	19.2	7.1	1.3	4.8	4.6	2.9	[58]
4	LA	Poland	HV3200	29.0	0.0	19.1	12.7	3.0	9.3	6.9	0.0	[58]
5	LA	Poland	f.h.	53.0	2.9	0.4	8.3	0.9	5.3	3.9	4.0	[55]
6	LA	Poland	d.h.	48.0	2.7	0.6	8.8	1.5	5.8	6.6	5.0	[55]
7	LA	Poland	f.f.	43.6	4.4	1.0	7.5	0.0	6.6	5.6	3.4	[55]
8	LA	Poland	d.f.	39.2	0.0	19.2	7.0	1.3	4.8	4.6	2.9	[55]
9	LA	Poland	d.f., VH400	43.6	4.4	0.0	7.5	0.0	6.6	5.6	0.0	[28]
10	LA	Poland	d.f., VH800	44.9	2.0	0.0	8.5	1.3	5.2	5.4	3.4	[28]
11	LA	Poland	d.f., VH1200	43.9	4.0	1.2	5.8	2.7	4.0	3.9	4.5	[28]
12	LA	Poland	d.f., VH1600	25.7	2.7	1.4	4.1	1.0	4.6	3.7	1.1	[28]
13	LI	Italy	f.f.	43.8	25.4	0.1	1.8	12.8	4.3	4.5	0.0	[30]
14	LI	Italy	f.s.	34.4	28.9	0.0	2.2	15.4	4.0	2.7	0.2	[30]
15	LI	Serbia	d.f.	7.7	6.8	67.3	2.7	7.2	0.0	0.4	0.0	[53]
16	LA	Croatia	d.f., SD	7.9	20.6	21.0	10.4	0.4	0.0	1.1	1.0	[56]
17	LA	Croatia	d.f., HD	23.2	19.5	13.8	13.0	0.5	0.0	1.2	2.3	[56]
18	LI cv „Grosso“	Italy	n.s.	12.6	52.9	0.7	4.8	19.6	3.0	5.4	0.0	[29]
19	LA	Italy	f., SD	42.9	11.8	0.1	12.6	18.4	5.8	8.4	0.0	[31]
20	LI cv „Budrovka“	Serbia	f.f., SD	26.7	17.8	11.3	4.0	6.0	17.3	9.4	0.3	TS
AVERAGE				35.5	11.6	9.1	7.0	6.1	5.8	4.2	1.6	
RANGE				7.7– 55.6	≤52.9	≤67.3	≤13.0	≤19.6	≤17.3	≤9.4	≤5.0	

LI—*Lavandula × intermedia*; LO—*Lavandula officinalis*; LA—*Lavandula angustifolia*; TS—This Study (average values for all three years); VH—Volume of Hydrosols in ml; SD—Steam Distillation; HD—Hydro Distillation; n.s.—not specified; f.f.—fresh flowers; f.h.—fresh herb; d.h.—dry herb; d.f.—dry flower; f.s.—fresh stem; f.—flowers.

Moroccan hydrolate of *L. officinalis* contained linalool as the dominant compound, followed by camphor, 1,8-cineole, α -terpineol, and borneol [57]. The main component of the volatile fraction of *L. angustifolia* from Poland was linalool, followed by α -terpineol, borneol, and geraniol [55], while in another study from Poland, it was linalool, followed by α -terpineol and terpinen-4-ol [28,58]. Hydrolate obtained from *L. angustifolia* buds from Croatia had the most significant proportion of 1,8-cineole, linalool oxide, and linalool [56]. Turkish *L. x intermedia* hydrolate had linalool, borneol, and camphor as the main compounds [49], while Italian *L. intermedia* samples contained linalool, 1,8-cineole, and camphor in different proportions [29–31]. Serbian *L. x intermedia* hydrolate had a high percentage of linalool oxides. A higher abundance of monoterpene alcohols oxides (*cis*- and *trans*-linalool) in hydrolate compared to the corresponding oil is probably due to their better solubility in water [53].

According to the unrooted cluster tree (Figure 6), it could be said that in *Lavandula* sp. hydrolate linalool dominated in almost all samples (in different proportions, from 25.7% to 55.6%), while in one sample, the dominant compound was linalool oxide [53], and in three it was 1,8-cineole [29,56]. Moreover, there is no difference between the chemical compositions of *L. angustifolia* and *L. x intermedia* hydrolates.

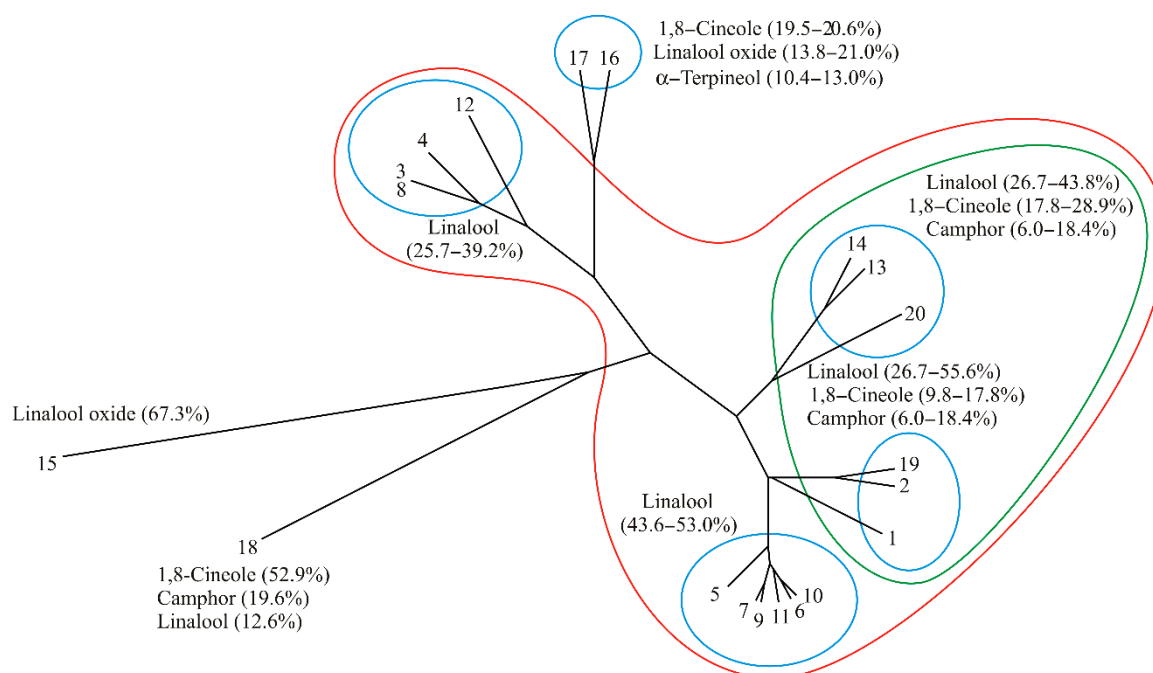


Figure 6. Unrooted cluster tree for chemical composition of *L. x intermedia* hydrolate according to literature and this study (samples are coded according to Table 7).

5. Conclusions

This research has proved that Fruška Gora (Serbia) has good agro-ecological conditions for cultivating *Lavandula* sp. and producing acceptable quality essential oil and hydrolate. Growing this species in this region could be recommended as Fruška Gora is a protected area suitable for organic farming. Since *Lavandula* sp. is a pollen and nectar-producing plant, it can positively affect the ecosystem's biodiversity. Honeybees are the most common visitors to *Lavandula* sp. fields and produce lavender honey. This type of unifloral honey has a high commercial value, which further supports the cultivation of this species. Tourism on Fruška Gora is quite developed due to many Orthodox monasteries, vineyards, and small wineries. Therefore, lavender fields could improve touristic content in this region following examples of other countries.

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