GC/MS chemical analysis of lavandin (*Lavandula x intermedia*) hydrolat: successive extraction fractions

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Hydrolats are valuable co-products of the essential oil distillation process, whose volatile compounds can be quantified by various methods. In this paper, we have tried to estimate the liquid-liquid extraction cycle number threshold for volatile compounds quantification of lavandin (*Lavandula x intermedia*) hydrolat. For this purpose, ten consecutive hydrolat extractions with *n*-hexane were analyzed GC/MS with hexadecane (C16) as an internal standard and compared with the lavandin essential oil. The chemical composition of the lavandin hydrolat showed similarity with its essential oil to the great extent, while volatile compounds dissolved in hydrolat exclusively belonged to the class of oxygenated monoterpenes. The total amount of extracted compounds has been estimated to 2174.2 mg/L, where the most dominant compounds in lavandin hydrolat were *cis*- and *trans*-furanoid linalool oxide (676.3 and 634.1 mg/L, respectively), followed by much smaller amounts of linalool, camphor, and 1,8-cineole (167.6, 157.0, and 148.2 mg/L, respectively). Cumulative recoveries of total compounds yield after the third, fifth, and eighth extractions were 88%, 96%, and 99%, respectively. Combined fraction analysis confirmed that in the first 5 cycles more than 95% of the total yield (from 10 cycles) of extracted volatile compounds can be collected. Based on the results of this study, for volatile compounds quantification in lavandin hydrolat, 5 cycles of *n*-hexane liquid-liquid extraction can be recommended.

Key words: *hydrosol, essential oil, volatile compounds, solubility, hydrolat, gas chromatography*

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1. INTRODUCTION

Hydrolats are valuable co-products obtained from aromatic and other plants by steam distillation. During this process, some components of the essential oil are dissolved in water in a certain ratio. Beside hydrolat, there are many different names for this co-product among which hydrosol, aromatic water, floral water, essential water, and herbal distillates are the most common. Hydrolats have many industrial applications such as cosmetics, perfumery, pesticides, aromatherapy, pharmaceutical, medical, and religious (Rajeswara Rao, 2013). Since some essential oils have a relatively high proportion of water-soluble compounds, a significant amount of the essential oil could retain in the water during the distillation process. Therefore, hydrolats of excellent quality are obtained when cohobation is an integral part of the distilling process (Price and Price, 2007). Essential oil components are lipophilic and have different solubility in water at room temperature, mostly below 2 % (Chen et al., 2014). However, due to different water-solubility of the components, the chemical analysis of the hydrolate will show a different profile from the essential oil from which it originates (Catty, 2001). The quality of the hydrolate may also depend on the collection time so that the early collected contain more low and the later collected more terpenoids with higher boiling points. (Rajeswara Rao, 2013). Some research indicates that for the therapeutic value of hydrolates, separation of fractions is more desirable than the entire distillation water collected at the end of the distillation process (Rose, 1999).

Lavandin (*Lavandula* × *intermedia*) is an artificially created hybrid of true lavender (*Lavandula augustifolia*) and spike lavender (*Lavandula latifolia*), which is widely cultivated due to its desirable characteristics such as oil yield and cultivation benefits (Lesage-Meessen et al., 2015; Lis-Balchin, 2002; Tucker et al., 1984).

Considering that it is generally accepted that less than 2 % of essential oil is dissolved in hydrolats and that there is a lack of methods for quantification of individual components in the literature, the aim of this paper was to test the method of hydrolat compounds quantification using an internal standard on the example of lavandin distillation. In order to evaluate the yield of total and individual extracted volatile compounds dissolved in the hydrolate, individual and combined *n*-hexane liquid-liquid extractions were analyzed.

2. MATERIALS AND METHODS

2.1. Standards and reagents

Sodium sulfate anhydrous (Na_2SO_4) and hexadecane were purchased from Sigma Chemicals Co. (USA), while *n*-hexane and distilled water were purchased from Zorka Pharma, Šabac (Serbia). All chemicals used in the experimental procedure were of analytical grade purity.

2.2. Plant material

Flowers of lavandin (*Lavandula* × *intermedia*) were purchased from the Production sector of the Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade.

2.3. Essential oil and hydrolat extraction procedures

Isolation of essential oil and hydrolat was performed using the hydro-distillation method using a Clevenger type apparatus according to the procedure I of the Ph. Jug. IV (1984). Lavandin flowers (40 g) were placed in flat-bottom flask (1 L) and filled with tap water until mass of 493 g. After setting the Clevenger apparatus on the heating body, an additional 7 mL of water was introduced into the system through a pipe above the burette, making a total mass of water of approximately 460 g, and together with the sample 500 g. Hydrolat was collected from the burette after the distillation process. The essential oil was collected and dried over anhydrous sodium sulfate. The essential oil yield, expressed as a percentage, was calculated on a moisture-free basis.

2.4. Sample preparation

Oil samples (20 μL) were dissolved in *n*-hexane (2 mL) and stored at 4 °C until further analysis. Hydrolat was extracted with *n*-hexane. For optimization of the number of extraction cycles liquid-liquid extraction (LLE) of volatile compounds dissolved in water was performed ten times (5 mL of hydrosol with 10×1 mL *n*-hexane). Hexadecane (C16) was used as an internal standard for quantification of volatile compounds dissolved in hydrolat. For this purpose, an internal standard stock solution has been made (5.5 mg in 1 mL *n*-hexane). In each extraction party, 100 µL of internal standard has been added so that the final concentration of internal standard was 500 mg/L. All samples were stored in the freezer until further analysis.

In order to validate the yield ratio of the extracted compounds from the lavandin hydrolat, the first five (1-5) and the last five (6-10) LLE fractions were combined into separate groups. Extractions were performed on newly prepared hydrolat from the same plant material and by the same procedure as for the analysis of individual fractions. In 1 mL of combined hexane fractions, 100 µL of the internal standard has been added so that the final concentration of internal standard was 500 mg/L. Samples were stored in the freezer until further analysis. Chemical analysis of combined fractions was performed under the same GC/MS conditions, as for analysis of individual fractions.

2.5. Chemical analysis

The chemical composition of the essential oil and hydrolat extraction parties was analyzed using GC/MS technique. GC/MS analyses were performed on a Shimadzu GCMS-QP2010 ultra mass spectrometer fitted with a flame ionic detector and coupled with a GC2010 gas chromatograph. The InertCap5 capillary column (60.0 m×0.25 mm×0.25 μm) was used for separation. Helium (He), at a split ratio of 1:5 and a linear velocity of 35.2 cm/s was used as a carrier gas. Initially, the oven temperature was 60 °C, which was held for 4 min, then increased to 280 °C at a rate of 4 °C/min, and held for 10 min. The injector and detector temperatures were 250 °C and 300 °C, respectively. The ion source temperature was 200 °C. The identification of the constituents was performed by comparing their mass spectra and retention indices (RIs) with those obtained from authentic samples and/or listed in the NIST/Wiley mass-spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data (Adams, 2007).

3. RESULTS AND DISCUSSION

3.1. Essential oil chemical composition

The yield of the essential oil was 6.2 %, which corresponds to the lower values of the previously published data on oil yields of lavandin (Arabaci et al., 2007; Kara and Baydar, 2013; Pistelli et al., 2017; Renaud et al., 2001) Chromatographic analysis of the essential oil showed a typical lavandin profile, where among 57 integrated peaks 46 were identified, representing 98.7 % of total compounds (Table 1). The most abundant compounds were oxygenated monoterpenes (91.2 %) such as linalool (23.1 %), camphor (16.3 %), 1,8-cineole (14.5 %), linalool acetate (10.0 %), borneol (5.2 %) and caryophyllene oxide (2.1 %). Similar oil patterns have been reported in previously published papers (Bajalan et al., 2017; Blažeković et al., 2018; Carrasco et al., 2016; Garzoli et al., 2020). Sesquiterpenes and their oxygenated forms are much less abundant in the essential oil than oxygenated monoterpenes with total values of 0.7 % and 3.3 %, respectively.

3.2. Hydrolat chemical composition

The chemical composition of the lavandin hydrolat is generally similar to the main components of the essential oil (Table 2). In our experiment, no sesquiterpene component was detected in the aqueous solution. The volatile compounds dissolved in water exclusively belonged to the class of oxygenated monoterpenes, which is mostly similar to previously published analyzes (Baydar and Kineci, 2009; Politi et al., 2020; Śmigielski et al., 2013). The most abundant compounds in lavandin hydrolat were *cis*- and *trans*-furanoid linalool oxide (676.3 and 634.1 mg/L, respectively), followed by much smaller amounts of linalool, camphor, and 1,8-cineole (167.6, 157.0, and 148.2 mg/L, respectively). The next compounds regarding quantity were *cis*-pyranoid linalool oxide, which co-eluted together with borneol at the same retention time, hotrienol, α-terpineol, and *trans*-pyranoid linalool oxide (89.7, 79.7, 67.0, and 63.4 mg/L, respectively). Two furanoid linalool oxide isomers, which amount in essential oil fluctuated about 4 % each, were expressed in hydrolat in the highest amounts among all water-soluble compounds. Tannous et al. (2004) reported that oxygenated compound are more water-soluble. So, further oxidation of the monoterpene alcohols, *cis*- and *trans*-linalool to the corresponding oxides, probably leads to their better solubility in water, which is why they are the compounds with the highest share. The higher amount of *cis*- and *trans*-linalool oxide in lavandin hydrolat than in essential oil was reported by Śmigielski et al. (2013). Baydar and Kineci (2009) reported the much higher content of linalool oxide in hydrolat than in essential oil, also. Furthermore, two pyranoid linalool oxides, which were not observed in the essential oil, were detected in hydrolat in significant amounts. Also, in the essential oil, co-elution of *cis*-pyranoid linalool oxide with borneol was not detected. This indicates that the total amounts of these pyranoid linalool oxide isomers from the plant material are dissolved in the hydrolat. Our results of lavandin hydrolat chemical composition are not in accordance with the majority of previously published reports, where linalool was the most abundant compound (42-56 %), followed by camphor (13-24 %) and 1,8-cineole (8-24 %), while linalool oxide isomers were detected in smaller amounts (3-6 %) (Baydar

Table 1. Chemical profile of lavandin (*Lavandula* × *intermedia*) essential oil

#	Compound[a]	RI[b]	%m/m
1	*α*-pinene	931.5	0.26
2	camphene	948.5	0.56
3	1-octen-3-ol	974.4	0.26
4	*β*-pinene	978.5	0.32
5	3-octanone	981.3	0.19
6	myrcene	987.4	0.45
7	dehydro-1,8-cineole	990.0	0.18
8	hexyl acetate	1004.8	0.30
9	pinocarvone	1009.5	0.15
10	*p*-cymene	1019.7	0.14
11	limonene	1025.0	0.37
12	1,8-cineole	1029.1	14.55
13	*trans*-*β*-ocimene	1039.3	0.43
14	*γ*-terpinene	1052.6	0.12
15	*cis*-sabinene hydrate	1062.6	0.20
16	*cis*-furanoid linalool oxide	1067.7	4.09
17	*trans*-furanoid linalool oxide	1083.4	3.64
18	linalool	1096.1	23.07
19	hotrienol	1099.0	1.51
20	n.i.	1124.2	0.09
21	n.i.	1137.6	0.36
22	camphor	1147.1	16.29
23	lavandulol	1159.8	0.90
24	borneol	1166.7	5.23
25	terpinen-4-ol	1176.6	0.72
26	*p*-cymen-8-ol	1182.0	0.89
27	*α*-terpineol	1191.0	3.05
28	myrtenol	1197.8	0.24
29	n.i.	1202.1	0.28
30	verbenone	1211.8	0.14
31	*trans*-carveol	1219.0	0.17
32	nerol	1225.2	0.47
33	isobornyl formate	1232.2	0.37
34	carvone	1246.5	0.15
35	linalool acetate	1253.1	9.97
36	lavandulyl acetate	1286.4	2.61
37	bornyl acetate	1289.7	0.19
38	hexyl tiglate	1315.9	0.21
39	n.i.	1324.4	0.09
40	verbanol acetate	1336.5	0.38
41	neryl acetate	1347.2	0.64
42	geranyl acetate	1364.7	1.33
43	*α*-santalene	1409.9	0.18
44	*trans*-caryophyllene	1416.2	0.39
45	*cis*-*β*-farnesene	1438.6	0.11
46	lavandulyl isovalerate	1495.7	0.26
47	n.i.	1516.9	0.24
48	n.i.	1553.9	0.12
49	spathulenol	1575.9	0.16
50	caryophyllene oxide	1582.3	2.10
51	*epi*-*α*-cadinol	1638.2	0.50
52	n.i.	1659.9	0.10
53	14-hydroxy-9-epi-*trans*-caryophyllene	1672.3	0.25
	SUM od identified		98.72
	Monoterpenes		2.85
	Oxigenated monoterpenes		91.25
	Sesquiterpenes		0.68
	Oxigenated sesquiterpenes		3.27
	Other		1.94

[a] n.i. - stands for not identified compounds
[b] RI - retention index relative to C_8-C_{40} *n*-alkanes on InertCap5 column

and Kineci, 2009; Politi et al., 2020; Yohalem and Passey, 2011). Some other sources reported components such as 1,8-cineole and camphor as the most dominant in lavandin hydrolat (53 and 67 %, respectively) (Garzoli et al., 2020; Jeon et al., 2013). Reports dealing with the chemical composition of true lavender (L. angustifolia) hydrolats also indicate a high content of linalool (26-52 %)(Kaloustian et al., 2008; Prusinowska et al., 2016; Śmigielski et al., 2013). However, none of the previously published reports list linalool oxides as the dominant compounds in the hydrolat, while pyranoid types of linalool oxides were not even mentioned. These differences can arise as a consequence of different starting plant material, as well as different distillation techniques. Kunicka-Styczyńska et al. (2015) reported different chemical composition of true lavender hydrolats depending on whether fresh or dried flowers were distilled. No linalool oxides were detected in the fresh flower hydrolat, while in the case of dried flowers they were detected in a significant amount (3-10 %). The distribution of the components dissolved in the hydrolat largely depends on the presence of that component in the oil, but also on the water-solubility of that component. Yalkowsky et al. (2016) reported different water-solubilities for 1,8-cineol (3.1-3.4 g/L), linalool (1.5-1.8 g/L), camphor (1.5-2.0 g/L), and borneol (0.7 g/L) at 25 °C.

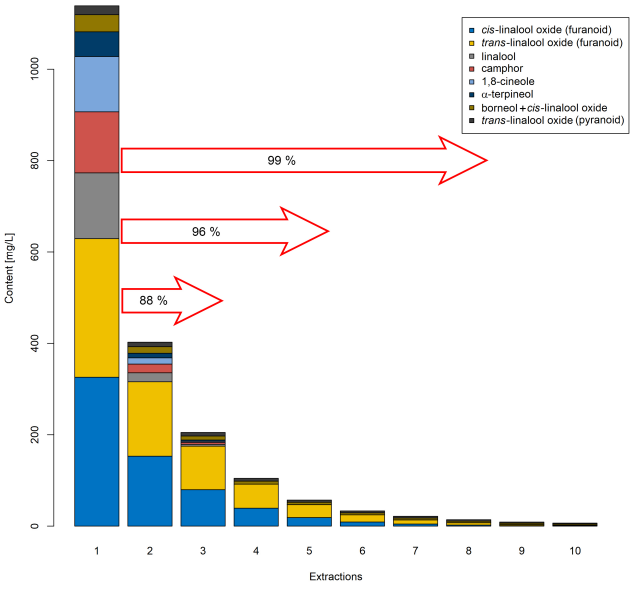

Fig. 1. Content of eight most abundant lavandin (*Lavandula* × *intermedia*) hydrolat compounds in ten consecutive extractions.

3.3. Fractions

In ten consecutive LLE of lavandin hydrolat about 2 g/L of dissolved oxygenated monoterpene compounds have been quantified (Table 2). This amount of dissolved essential oil in water is in the accordance with previously published reports that dissolved compounds contents were in range from less than 1 g/L (Labadie et al., 2015) to about 3,5 g/L (Martínez-Gil et al., 2013).

Table 2. Content of dissolved essential oil compounds in ten consecutive liquid-liquid lavandin (*Lavandula* × *intermedia*) hydrolat extractions

			Extractions												Validation		
			1	2	3	4	5	6	7	8	9	10	SUM	SUM	1-5	6-10	SUM
#	Compound[a]	RI[b]	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	%m/m	mg/L	mg/L	mg/L
1	1,8-cineole	1029.2	121.0	13.6	2.4	2.1	1.9	1.7	1.6	1.5	1.2	1.2	148.2	6.8	104.4	-	104.4
2	lavender lactone	1033.2	4.6	3.1	2.6	-	-	-	-	-	-	-	10.3	0.5	15.1	5.26	20.4
3	*cis*-linalool oxide (furanoid)	1071.8	325.9	153.1	79.7	39.3	18.7	9.0	4.6	2.3	1.1	0.6	634.3	29.2	705.3	28.13	733.4
4	*trans*-linalool oxide (furanoid)	1087.9	303.3	162.8	95.2	52.6	33.4	11.6	8.1	5	2.6	1.6	676.2	31.1	755.2	44.01	799.2
5	linalool	1096.0	143.9	19.7	4.0	-	-	-	-	-	-	-	167.6	7.7	106.3	-	106.3
6	hotrienol	1100.4	63.4	12.8	3.4	-	-	-	-	-	-	-	79.6	3.7	98.0	-	98.0
7	lilac aldehyde B	1138.4	9.3	2.0	-	-	-	-	-	-	-	-	11.3	0.5	12.3	-	12.3
8	camphor	1147.1	133.7	19.1	4.2	-	-	-	-	-	-	-	157.0	7.2	130.4	-	130.4
9	sabina ketone	1155.5	1.8	-	-	-	-	-	-	-	-	-	1.8	0.1	-	-	-
10	lavandulol	1160.7	7.9	1.6	-	-	-	-	-	-	-	-	9.5	0.4	8.8	-	8.8
11	*cis*-linalool oxide (pyranoid) + borneol	1167.0	37.4	14.4	8.9	6.6	5.1	4.5	4.1	3.5	2.8	2.4	89.7	4.1	70.6	14.75	85.4
12	*trans*-linalool oxide (pyranoid)	1169.7	19.5	9.8	7.7	6.1	4.8	4.1	3.7	3.1	2.4	2.0	63.2	2.9	45.2	17.98	63.2
13	terpinen-4-ol	1177.5	7.9	1.5	-	-	-	-	-	-	-	-	9.4	0.4	8.6	-	8.6
14	*p*-cymen-8-ol	1183.7	10.3	2.4	-	-	-	-	-	-	-	-	12.7	0.6	10.7	-	10.7
15	α-terpineol	1192.2	54.1	1.0	2.8	-	-	-	-	-	-	-	57.9	2.7	40.1	-	40.1
16	myrtenol	1198.6	2.5	2.9	1.5	-	-	-	-	-	-	-	6.9	0.3	3.7	-	3.7
17	*cis*-4-caranone	1202.8	2.9	-	-	-	-	-	-	-	-	-	2.9	0.1	2.1	-	2.1
18	verbenone	1213.0	5.4	1.8	-	-	-	-	-	-	-	-	7.2	0.3	8.8	-	8.8
19	*trans*-carveol	1220.1	2.8	-	-	-	-	-	-	-	-	-	2.8	0.1	1.6	-	1.6
20	nerol	1225.8	4.7	-	-	-	-	-	-	-	-	-	4.7	0.2	4.3	-	4.3
21	linalool acetate	1251.8	15.0	1.7	-	-	-	-	-	-	-	-	16.7	0.8	7.8	-	7.8
22	lavandulyl acetate	1286.4	2.9	-	-	-	-	-	-	-	-	-	2.9	0.1	1.9	-	1.9
23	limonen-10-ol	1288.4	1.4	-	-	-	-	-	-	-	-	-	1.4	0.1	-	-	-
24	hexadecane (C16)[c]	1575.3															
	SUM od identified		1281.6	423.3	212.4	106.7	63.9	30.9	22.1	15.4	10.1	7.8	2174.2		2141.3	110.1	2251.4
	Recovery ratio [%]		58.9	19.5	9.8	4.9	2.9	1.4	1.0	0.7	0.5	0.4			95.11	4.89	
	Cumulative recovery [%]		58.9	78.4	88.2	93.1	96.0	97.5	98.5	99.2	99.6	100.0					
	Monoterpenes												0.0				0.0
	Oxigenated monoterpenes												2174.2				2251.4
	Sesquiterpenes												0.0				0.0
	Oxigenated sesquiterpenes												0.0				0.0

[a] n.i. - stands for not identified compounds
[b] RI - retention index relative to C_8-C_{40} *n*-alkanes on InertCap5 column
[c] Internal standard

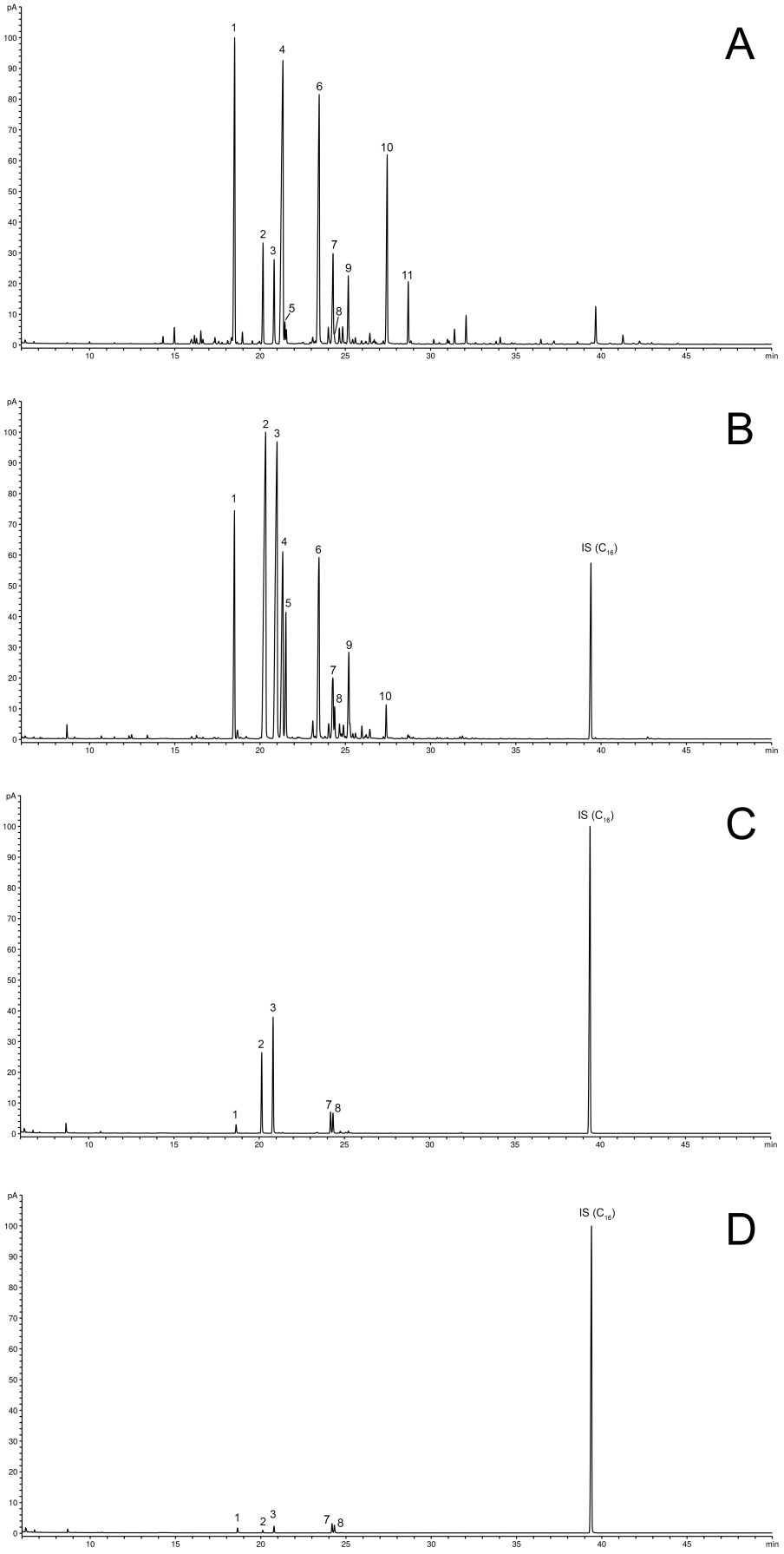

Fig. 2. GC-chromatograms of lavandin (Lavandula × intermedia) A - essential oil; B – hydrolat 1st extraction; C – hydrolat 5th extraction; D – hydrolat 10th extraction; 1 – 1,8-cineole; 2 - *cis*-furanoid linalool oxide; 3 - *trans*-furanoid linalool oxide; 4 – linalool; 5 - hotrienol; 6 – camphor; 7 – *cis*-pyranoid linalool oxide + borneol; 8 - *trans*-pyranoid linalool oxide; 9 - α-terpineol; 10 - linalool acetate; 11 - lavandulyl acetate; IS(C16) – internal standard (hexadecane).

The first extraction with *n*-hexane had the highest yield of all dissolved compounds, while each subsequent extraction had a decreasing amount. The recovery of the compound from the hydrolat did not proceed uniformly during the extractions. The number of extracted compounds decreased from the first to the fourth extraction, after which traces of five compounds were retained until the last extraction. Those compounds were 1,8-cineole, *cis*- and *trans*-furanoid linalool oxides, and *cis*- and *trans*-pyranoid linalool oxides. It may be important to note here that borneol did not co-eluted with *cis*-pyranoid linalool oxide after the third extraction. Figure 1 shows the *n*-hexane recovery of the eight most dominant compounds from lavandin hydrolat. Hereby, the recovery ratio of total compounds yield after the third, fifth, and eighth extractions is presented here (88 %, 96 %, and 99 %, respectively). Śmigielski et al. (2013) also reported higher volatile compounds recovery with an increased number of extraction cycles. They also reported that, extraction of 97 % of the volatile compounds from the hydrolat was achieved with five LLE cycles.

In Figure 2, chromatograms of essential oil and chosen hydrolat extraction cycles (1st, 5th, and 10th) have been shown. This presentation provides insight into the similarity of the chemical composition of essential oil and hydrolat, as well as the gradual depletion of compounds from aqueous solution in higher extraction cycles. The main weakness of this method is that even after ten consecutive *n*-hexane extractions of volatile compounds from the hydrolat, not all components are depleted to level below the detection on the gas chromatogram. Therefore, other non-polar solvents should be explored in further research. Furthermore, the choice of aliphatic internal standard could be further considered since hexadecane (C16) in our GC method elutes at a time very close to eluation time of *trans*-caryophyllene (RT ~40 min, Figure 2A). This did not interfere with the hydrolat analysis, as only oxigenated monoterpene compounds dissolved in the hydrolat and no traces of sesquiterpenes were detected, but it would be desirable to design an internal standard elution at the time where other components do not elute nearby.

3.4. Combined fractions

In order to verify the obtained results of quantification of volatile compounds in individual extraction cycles, we quantified the extractions combined into two groups of 1-5 cycles and 6-10 cycles (Table 2). The chemical composition of the combined extractions differed slightly from that of the individual fractions study. The most important difference is that the entire amount of 1,8-cineole was extracted in the first five cycles, while the lavander lactone was abundant in the second five cycles. This discrepancy may be due to sampling, as a new distillation was performed for grouped fractions yield validation purposes. However, the total yield of extracted volatile compounds from the hydrolat almost coincides with the total yield of components from the ten cycles of the dynamic study (2174.2 mg/L and 2251.4 mg/L). Judging by the cumulative recovery, it can be concluded that in the first 5 cycles more than 95 % of the total yield (from 10 cycles) of extracted volatile compounds has been collected.

CONCLUSION

The chemical composition of the lavandin hydrolat showed high similarity with its essential oil. However, analysis reviled that the volatile compounds dissolved in water exclusively belonged to the class of oxygenated monoterpenes. In ten consecutive liquid-liquid extractions of lavandin hydrolat total amount of 2174.2 mg/L extracted compounds has been quantified. The most dominant compounds in lavandin hydrolat were *cis*- and *trans*-furanoid linalool oxide (676.3 and 634.1 mg/L, respectively), followed by much smaller amounts of linalool, camphor, and 1,8-cineole (167.6, 157.0, and 148.2 mg/L, respectively). The number of extracted compounds decreased from the first to the fourth extraction, after which traces 1,8-cineole, *cis*- and *trans*-furanoid linalool oxides, and *cis*- and *trans*-pyranoid linalool oxides were retained until the last extraction. Cumulative recoveries of total compounds yield after the third, fifth, and eighth extractions were 88 %, 96 %, and 99 %, respectively. Method verification confirmed that in the first 5 cycles more than 95 % of the total yield (from 10 cycles) of extracted volatile compounds can be collected. Therefore, based on the results of this study, for the purpose of volatile compounds quantification in lavandin hydrolat 5 cycles of *n*-hexane liquid-liquid extraction can be recommended.

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