

Essential oil composition of five Basil cultivars (*Ocimum basilicum*) from Albania

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Abstract

Basil (*Ocimum basilicum* L., fam. Lamiaceae) is an important medicinal and aromatic plant with very wide range of uses. This paper presents qualitative and quantitative analyses of essential oils obtained from five types of Italian basil cultivars: Napolitan, Red basil, Fino Verde, Limonez, and Genoveze cultivated in Albania and collected during the summer 2012.

The hydrodistilled BEO (Basil essential oil) content ranged from 0.11% to 3.40%. Within the total of 65 identified compounds with GC/FID/MS, nine were considered as predominant (1,8-cineole, linalool, *cis*-thujone, methyl chavicol, eugenol, *trans*-(E)-caryophyllene, *trans*-(α)-bergamotene, germacrene D, and *epi*- α -cadinol) representing 49.20 to 85.43% of the components in the analysed essential oils. In all cultivars, linalool was detected as the most abundant component (36.20-46.59%).

BEO's from all five cultivars differ in their chemical composition but generally conform to EO's from Sweet Basil grown in the Mediterranean region. The Napolitan cultivar showed the largest similarity to Sweet Basil, not only for the morphology, also due to the essential oil composition that comprises Basil's most dominant chemical components (linalool, methyl chavicol and eucalyptol) in particular percents.

Keywords: Napolitan, Red basil, Fino Verde, Limonez, Genoveze, volatile oil, GC-MS analysis

Introduction

Common basil (*Ocimum basilicum* L.), a member of the Lamiaceae family is an annual herb which grows in several regions around the world. Among more than 150 species of the genus *Ocimum*, basil is the major crop which is cultivated commercially in many countries (Sajjadi, 2006). Different authors have grouped basil into a subgenus based on its chemical composition or morphol-

ogy (Imeri et al., 2014). There are many cultivars of basil which vary in their leaf color (green or purple), flower color (white, red, purple) and aroma (Morales and Simon, 1996), but most of commercial basil cultivars available in the market belong to the species *O. basilicum*. According to Darrah (1980), these cultivars are classified in seven types: (1) tall slender types, which include the sweet basil group; (2) large-leafed, robust types, including 'Lettuce Leaf' also called 'Italian' basil; (3) dwarf types, which are short and small leafed, such as 'Bush' basil; (4) compact types, also described, *O. basilicum* var. *thyrsiflora*,

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commonly called 'Thai' basil; (5) *purpurascens*, the purple-colored basil types with traditional sweet basil flavor; (6) purple types such as 'Dark Opal', a possible hybrid between *O. basilicum* and *O. forskolei*, which lobed-leaves and a sweet basil plus clove-like aroma; and (7) *citriodorum* types, which include lemon-flavored basil. The most popular and commonly cultivated is Sweet Basil (Genovese, Large leaf, Lettuce Leaf, Mammoth).

Traditionally, basil has been extensively utilized in food, as a flavoring agent, as well as in perfumery and pharmaceutical or cosmetic industries (Telci et al., 2006). In folk medicine, leaves and flowering tops of the plant are perceived as carminatives, galactogogues, stomachics and antispasmodics (Sajjadi, 2006). However, recently the potential uses of basil essential oil (BEO), particularly as antimicrobial and antioxidant agents have also been investigated (Politeo et al., 2007; Sartoratto et al., 2004; Wannissorn et al., 2005). The *O. basilicum* essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, as well as aroma and origin of the plants (Sajjadi, 2006).

However, only a few articles have examined the yield and composition of essential oils of Basil cultivars, especially from Albania (Cheliku et al., 2015; Keita et al., 2000; Morales and Simon, 1996), therefore the aim of this study was chemical characterization of essential oils of Napolitan, Red basil, Fino Verde, Limonez, and Genoveze cultivars planted in Albania.

Experimental

Plant collection

The over-ground parts of the flowering plants (20-25 cm from the top) were obtained from five types of Italian basil cultivars: Napolitan, Red basil, Fino Verde, Limonez, and Genoveze cultivated and collected in Albania, during the summer 2012. The plant material was air dried, packed in paper bags and kept in a dark and cool place until analysis. Voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje (Napolitan - Ob/Al-N/12, Red basil - Ob/Al-RB/12, Fino Verde Ob/Al-F/12, Limonez - Ob/Al-L/12, and Genoveze - Ob/Al-G/12).

Essential oil isolation

Essential oil isolation from basil was performed by hydrodistillation in all-glass Clevenger apparatus for 2.5 h, according to the method described in the European Pharmacopoeia (Ph. Eu. 8.0., 2014).

Analysis of essential oil's chemical composition

EO samples in hexane (1:1000) were analyzed on Agilent 7890A Gas Chromatography system equipped with

FID detector and Agilent 5975C mass spectrometer. For this purpose, HP-5 ms capillary column (30 m x 0.25 mm, film thickness 0.25 μ m) was used. Adams's analytical conditions were as follows: oven temperature at 60 °C (0 min), 3 °C/min to 240 °C (1 min) and at the end increased to 280 °C at a rate of 10 °C/min (1 min) (Adams, 2007); helium, as carrier gas, at a flow rate of 1 ml/min; injector temperature 220 °C and that of the FID detector 270 °C. One μ l of each sample was injected at a split ratio of 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50 to 550 Da. The MS was operated in scan mode.

Identification of the components

The components were identified according to the available literature considering their retention times (Adams, 2007), as well as Kovat's (retention) indices determined using a homologous mixture of normal alkanes (C9 - C25) analysed under Automated Mass Spectral Deconvolution and Identification System (AMDIS) conditions (AMDIS ver.2.1.).

Confirmation was done by comparing the mass spectra of components present in the BEOs with the reference spectra obtained from Nist, Wiley and Adams' mass spectra libraries. Quantification of the BEOs components was performed using the normalisation method of the GC/FID peak areas without any correction factors.

Results and discussion

Essential oil yield and composition

The BEO yield of five basil cultivars from Albania ranged from 1.10 ml/kg to 34.00 ml/kg (0.11-3.40 %) (Table 1). The highest yield was recorded in one Fino Verde cultivar (No.1) while the lowest was found in one Napolitan cultivar (No.2).

GC/FID/MS analyses of the isolated BEOs revealed, a total of 65 compounds representing 89.23-99.95% of the oils (Table 1). Nine components were considered as predominant: 1,8-cineole (1.25-13.00%), linalool (26.17-50.16%), *cis*-thujone (0.25-6.56%), methyl chavicol (0.66-33.05%), eugenol (0.17-13.73%), *trans*-(E)-caryophyllene (0.29-4.03%), *trans*-(α)-bergamotene (1.81-12.58%), germacrene D (1.29-5.50%), and *epi*- α -cadinol (0.27-6.01%) that represented 49.20 to 85.43% of the chemical composition of the analysed essential oils. However, linalool, eugenol, farnesene and elemol, were reported as major components of the oils of different *O. basilicum* chemotypes (Chalchat et al., 1999; Grayer et al., 1996; Marotti et al., 1996). According to our findings, linalool was the most abundant component identified in all investigated cultivars which complies with all literature data (Cheliku et al., 2015; Labra et al., 2004; Sajjadi 2006). Further-

Table 1. Chemical composition (%) and essential oil yields (%) from five Basil cultivars (*Ocimum basilicum*) from Albania.

No.	Constituents	Cultivars BEO yield (%) KIL	Neapolitan			Red Basil			Fino Verde			Limonese			Genovese		
			No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
1	α -thujene	924	0.24	0.46	0.28	0.22	0.18	0.00	0.15	tr.	0.43	0.23	0.29				
2	α -pinene	932	0.26	0.10	0.26	0.16	0.99	0.23	0.37	0.32	0.22	0.30	tr.				
3	camphene	946	0.16	0.00	0.29	0.00	0.12	0.16	0.00	0.90	0.24	0.00	0.00				
4	sabinene	969	0.36	0.17	0.52	0.21	0.55	0.19	tr.	tr.	0.37	0.37	0.21				
5	β -pinene	974	0.88	0.41	1.11	0.44	1.48	0.50	1.14	1.14	0.81	0.84	0.41				
6	myrcene	991	1.02	0.53	0.51	0.27	0.88	0.43	0.22	0.48	0.78	0.66	0.38				
7	α -phellandrene	1005	0.00	0.00	0.00	0.00	tr.	0.00	0.00	0.00	0.00	0.00	0.00				
8	α -terpinene	1018	0.00	0.00	0.00	0.00	0.13	0.00	tr.	tr.	0.00	0.00	0.00				
9	(<i>o+p</i>)-cymene	1020	0.00	0.00	0.00	0.00	0.51	0.00	0.23	0.23	0.00	0.00	0.00				
10	limonene	1024	0.15	tr.	0.16	0.11	0.63	0.25	0.28	0.28	0.18	0.21	0.13				
11	1,8-cineole	1033	8.92	5.43	9.02	5.33	13.00	5.21	1.25	1.25	7.46	8.62	5.75				
12	B-(E)-ocimene	1044	0.87	0.37	0.00	0.00	0.10	tr.	0.00	0.00	0.35	0.29	0.22				
13	γ -terpinene	1054	tr.	tr.	0.00	0.00	0.38	tr.	0.49	0.61	0.00	tr.	0.00				
14	<i>cis</i> -sabinene hydrate	1065	0.18	0.12	0.10	0.13	1.19	0.23	0.60	0.92	0.16	0.21	0.13				
15	linalool oxide	1067	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00				
16	terpinolene	1086	0.13	tr.	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.14	0.00				
17	linalool	1095	33.92	43.49	40.73	43.71	26.17	47.73	33.33	28.69	43.63	45.88	50.16				
18	<i>cis</i> -thujone	1110	1.93	0.00	2.56	6.56	0.25	0.00	0.34	0.29	0.16	0.00	0.00				
19	<i>trans</i> -thujone	1111	0.77	0.00	0.45	0.00	1.08	0.00	tr.	0.00	0.00	0.00	0.00				
20	camphor	1143	tr.	0.25	0.00	0.00	0.00	0.75	0.57	0.18	0.14	0.15	0.19				
21	borneol	1165	0.45	0.00	0.23	0.39	0.75	0.83	0.00	0.00	0.00	0.00	0.40				
22	terpinen-4-ol	1177	0.12	0.10	0.11	0.41	4.54	1.04	3.66	6.70	0.48	0.19	0.24				
23	α -terpineol	1189	0.87	0.58	1.01	0.51	0.98	0.82	0.00	0.00	0.91	1.07	0.77				

No.	Constituents	Cultivars	Napolitan			Red Basil			Fino Verde			Limonese			Genovese				
			No.1	No.2	No.1	No.2	No.1	No.2	No.1	No.2	No.1	No.2	No.1	No.2	No.1	No.2	No.1	No.2	No.3
		BEO yield (%)	1.30	0.11	0.47	1.70	3.40	0.50	2.50	1.00	1.00	1.00	1.23	1.40	1.00	1.00	1.00	1.00	1.00
		KIL																	
24	<i>p</i> -menth-1-en-8-ol	/	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	methyl chavicol	1195	33.05	23.70	0.66	0.00	1.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.72
26	nerol	1227	0.00	0.00	0.00	0.00	0.00	0.14	1.57	0.53	3.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	neral	1235	0.00	0.00	0.00	0.56	0.00	0.80	13.71	12.52	5.02	0.00	0.33	0.28	0.00	0.00	0.00	0.00	0.00
28	geraniol	1249	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.17	2.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	geranial	1264	0.00	0.12	0.00	0.71	0.00	1.11	18.22	16.40	6.56	0.00	0.42	0.37	0.00	0.00	0.00	0.00	0.00
30	bornyl acetate	1285	0.72	0.00	0.10	tr.	1.09	1.58	0.00	0.21	tr.	0.87	1.13	0.58	0.00	0.00	0.00	0.00	0.00
31	α -cubebene	1345	0.00	0.10	0.00	0.00	tr.	0.11	tr.	tr.	0.16	0.11	tr.	0.10	0.00	0.00	0.00	0.00	0.00
32	eugenol	1356	0.70	0.58	2.23	1.74	8.09	5.37	0.17	0.43	0.41	13.73	9.41	6.91	0.00	0.00	0.00	0.00	0.00
33	neryl acetate	1359	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.70	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34	α -copaene	1374	0.05	0.24	0.17	0.00	0.29	0.26	0.17	0.16	0.53	0.19	0.16	0.24	0.00	0.00	0.00	0.00	0.00
35	geranyl acetate	1379	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.12	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36	β -elemene	1389	0.30	1.64	3.17	2.69	2.17	2.47	0.40	0.53	0.35	1.83	1.69	1.67	0.00	0.00	0.00	0.00	0.00
37	methyl eugenol	1403	0.19	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.07	0.12	0.00	0.00	0.00	0.00	0.00	0.00
38	<i>cis</i> -(α)-bergamotene	1411	tr.	tr.	0.16	0.00	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.10	0.00	0.00	0.00	0.00	0.00
39	<i>trans</i> -(E)-caryophyllene	1418	0.83	0.44	2.54	1.57	0.29	0.91	2.44	2.41	4.03	0.29	0.33	0.60	0.00	0.00	0.00	0.00	0.00
40	<i>trans</i> -(α)-bergamotene	1432	1.81	5.59	12.24	12.58	4.50	5.69	3.67	4.73	2.09	3.45	2.43	4.78	0.00	0.00	0.00	0.00	0.00
41	α -guaiene	1437	0.00	0.00	1.07	1.07	1.25	1.35	0.00	0.00	0.14	1.03	0.73	1.32	0.00	0.00	0.00	0.00	0.00
42	aromadendrene	1439	0.13	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
43	<i>cis</i> -muurola-3,5-diene	1448	0.00	0.15	0.00	0.00	0.12	0.13	0.00	0.00	0.00	0.13	0.16	0.14	0.00	0.00	0.00	0.00	0.00
44	α -humulene	1452	0.76	0.76	1.75	1.39	0.71	1.02	1.09	1.47	1.09	0.85	0.92	0.89	0.00	0.00	0.00	0.00	0.00
45	<i>allo</i> -aromadendren	1458	0.00	tr.	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	γ -muurolene	1478	0.00	0.00	tr.	0.34	0.00	0.00	0.00	tr.	tr.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	germacrene D	1484	1.29	2.83	4.40	5.33	3.69	4.04	2.06	1.89	5.50	3.55	3.40	4.78	0.00	0.00	0.00	0.00	0.00
48	β -selinene	1489	0.00	0.21	0.24	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

No.	Cultivars	Neapolitan			Red Basil			Fino Verde			Limonese			Genovese		
		No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
	BEO yield (%)	1.30	0.11	0.47	1.70	3.40	0.50	2.50	1.00	1.00	1.00	1.23	1.40	1.00	1.00	
	KIL															
	Content (%)															
49	bicyclogermacrene	0.30	1.09	1.47	1.70	1.80	0.89	0.36	0.34	0.47	1.65	1.59	1.32			
50	α -bulnesene	0.33	1.90	2.82	3.37	2.50	2.65	0.00	0.74	0.00	2.15	1.83	1.83			
51	γ -cadinene	1.18	1.62	1.50	1.50	2.55	1.74	0.18	0.47	0.60	2.81	2.77	2.52			
52	<i>trans</i> -calamenene	0.22	0.00	0.00	0.98	0.53	0.52	0.00	0.31	0.00	0.63	0.48	0.57			
53	α -cadinene	tr.	tr.	0.00	0.00	tr.	tr.	0.00	0.00	0.00	tr.	tr.	0.00			
54	α -bisabolene	0.00	0.00	0.00	0.00	0.00	0.80	2.27	2.11	4.27	0.00	0.00	0.00			
55	E-nerolidol	tr.	0.00	0.22	0.14	0.17	0.00	tr.	0.00	0.11	0.14	0.21	0.00			
56	maaliol	0.00	0.00	1.25	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
57	spathulenol	0.15	0.13	0.24	0.18	0.70	0.34	0.00	0.00	0.00	0.63	1.06	0.35			
58	caryophyllene oxide	0.14	0.00	0.30	0.00	0.00	0.12	0.91	1.02	0.26	0.00	0.00	0.00			
59	viridiflorol	0.17	0.00	0.17	0.00	tr.	tr.	0.00	0.00	0.00	0.00	0.00	0.00			
60	humulene epoxide II	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
61	1,10-di- <i>epi</i> cubebol	0.44	0.52	0.49	0.59	0.74	0.78	0.00	0.00	tr.	0.77	0.79	0.75			
62	<i>epi</i> - α -cadinol	3.22	3.13	3.60	3.64	5.41	5.42	0.27	0.88	0.77	5.28	6.01	5.35			
63	β -eudesmol	tr.	0.00	0.00	0.25	0.19	0.19	0.00	0.00	0.00	0.15	0.18	0.21			
64	α -cadinol	tr.	0.33	0.38	0.36	0.33	0.44	0.00	0.00	0.30	0.35	0.51	0.59			
65	α -bisabolol	tr.	0.00	0.00	0.00	0.00	0.00	tr.	0.14	0.00	0.11	tr.	0.00			
	TOTAL	97.79	97.52	98.56	99.95	94.57	97.59	92.77	89.23	96.98	97.24	96.11	99.30			

1) KIL - Kovat's Index Literature (Adams, 2007); tr. = traces (< 0.10%).

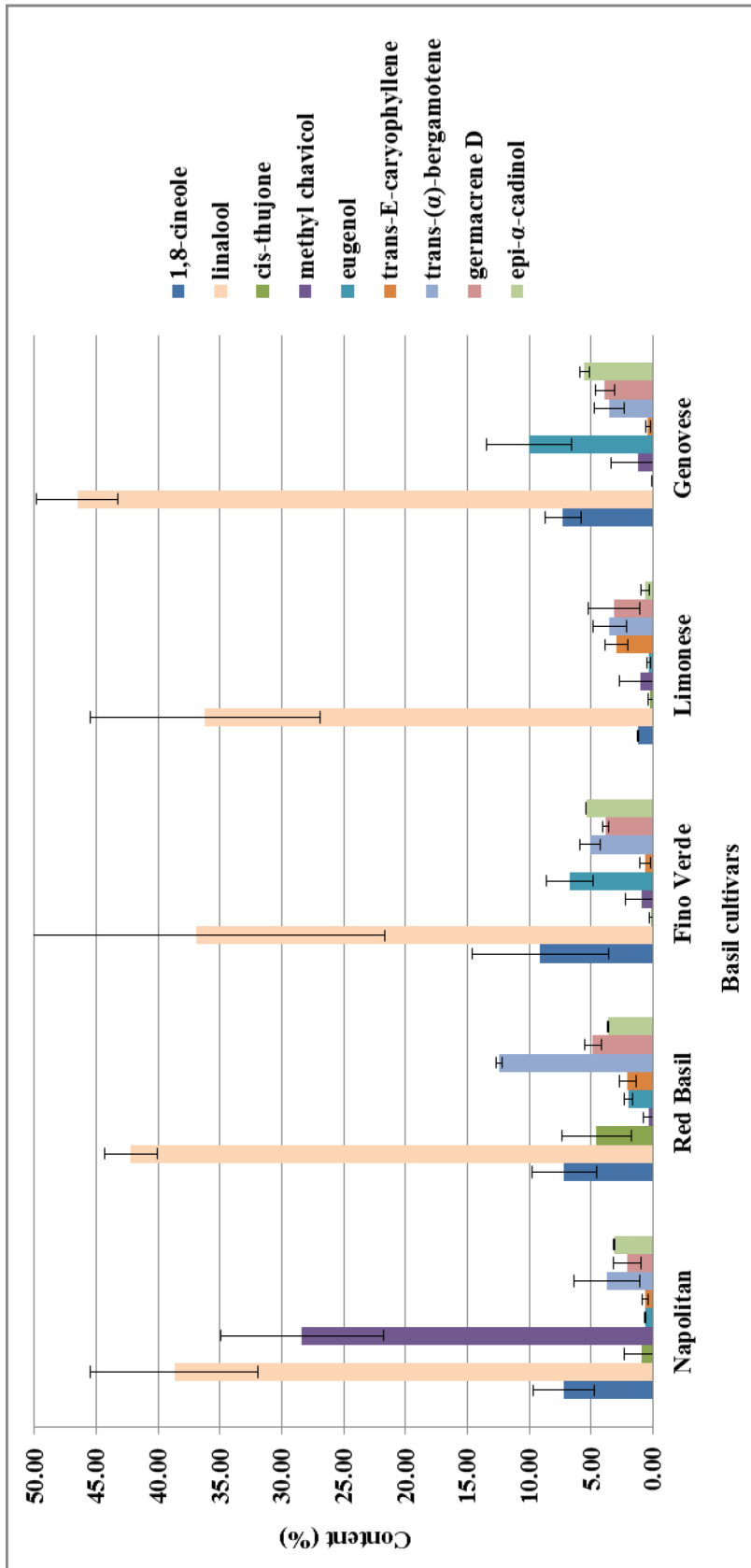


Figure 1. Relative presence of predominant BEO constituents identified in all five Basil cultivars from Albania.

more, linalool was declared as dominant constituent in the two green and purple cultivars, with different leaves morphology (Raguso and Pichersky, 1999). In the volatile oils, obtained from aerial parts of *O. basilicum* grown in Colombia and Bulgaria, linalool was also reported as a major component (Benitez et al., 2009; Jirovetz and Buchbauer, 2001; Vina and Murillo, 2003). On the other hand, 1,8-cineole can be considered as the third main component in all examined samples that conforms with the data from the study of 65 Italian different BEO's samples (Labra et al., 2004) (Fig. 1).

Regarding gas chromatography analyses, it is evident that the examined five basil cultivars had variances in the chemical composition of the essential oil (Fig. 1). The Napolitan cultivars often known as wide leaf cultivars, were rich in methyl chavicol (23.70-33.05%), which is second abundant compound in the BEO thus it differs from all the other investigated cultivars. Red or purple basil is the only cultivar where *trans*-thujone (up to 6.56%) and *trans*-(α)-bergamotene (up to 12.58%) were identified in significant amount. On the other hand, *trans*-(E)-caryophyllene was present in both, the Red basil and Limones cultivars. Also, characteristic for the Limonese cultivars was α -bisabolene, which was absent in the other types of basil. In the composition of the BEO's of Fino Verde and Genovese samples, eugenol was found in significant amount (5.37-13.73%) but was absent from the other examined cultivars.

High contents of linalool and methyl chavicol in Napolitan basil indicated that this cultivar could be considered as European originated chemotype with some influences of the North African (the Egyptian) chemotypes which are with high methyl chavicol contents (Telci et al., 2005). Alike, geraniol and methyl chavicol were the main components in the oil of *O. basilicum* L. cv. purple from Turkey and Iran (Özcan and Chalchat, 2002; Sajjadi, 2006). Although the EO's of some Turkish and Bulgarian green basil were characterized by a high content of citral (neral and geraniol) (46.10%) and methyl cinnamate (Sajjadi, 2006), these were barely detected in our BEO's.

Conclusion

The yield of essential oils isolated from five different basil cultivars (Napolitan, Red basil, Fino Verde, Limonez, and Genoveze) from Albania ranged from 0.11% to 3.40%. With GC/FID/MS analysis, total of 65 compounds were identified and nine of them were considered as predominant (1,8-cineole, linalool, *cis*-thujone, methyl chavicol, eugenol, *trans*-(E)-caryophyllene, *trans*-(α)-bergamotene, germacrene D, and *epi*- α -cadinol), present in the BEO's with more than 5%. The most abundant component detected in all cultivars was linalool.

BEO's from all five investigated cultivars differ in their chemical composition but generally conform to EO's from Sweet Basil grown in the Mediterranean region. The Napolitan cultivar showed the largest similarity to Sweet

Basil, not only for the morphology, also due to the essential oil composition that comprises Basil's most dominant chemical components (linalool, methyl chavicol and eucalyptol) in particular percents.

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Резиме

Состав на етерично масло од пет култивари на босилек (*Ocimum basilicum*) од Албанија

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Клучни зборови: Napolitan, Red basil, Finoverde, Limonez, and Genoveze, испарливи масла, GC-MS анализа.

Босилек (*Ocimum basilicum* L., фамилија Lamiaceae) е важно лековито и ароматично растение со многу широк опсег на употреба. Во овој труд е претставена квалитативната и квантитативната анализа на етеричните масла добиени од пет сорти на италијански босилек: Napolitan, Red basil, Finoverde, Limonez, и Genoveze, одгледани и собрани во Албанија, во текот на летото во 2012-та година.

Содржината на етеричното масло (ЕМ) добиена по дестилација со водена пара се движи од 0,11% до 3,40%. Со GC/FID/MS анализа, идентификувани се вкупно 65 соединенија, од кои девет се сметаат како доминантни (1,8-цинеол, линалол, *cis*-тујон, метил кавикол, еугенол, *trans*-(E)-кариофилен, *trans*-(α)-бергамотен, гермакрен D, и *epi*- α -кадинол) и претставуваат 49,20-85,43% од вкупниот број на компоненти во анализираните етерични масла. Кај сите испитувани сорти, линалолот е идентификувана како најзастапена компонента (36,20-46,59%).

Очигледно е дека ЕМ од сите пет различни сорти се разликуваат во однос на хемискиот состав, но генерално сите покажуваат голема сличност со ЕМ од типичниот *Ocimum basilicum* што се одгледува во медитеранскиот регион. Култиварот Napolitan може да се смета за најсличен со типичниот босилек, како поради неговата морфологија така и според хемискиот состав на маслото, за кое се поврзуваат хемиските компоненти линалол, метил кавикол и еукалиптол во сооднос што е карактеристичен за медитеранскиот босилек.