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Variability of the Essential Oil Composition in *Origanum vulgare* subs. *vulgare* Collected in Albania

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Abstract

The aim of this study was to analyse the chemical composition and the variability of essential oils obtained from seven native accessions of *Origanum vulgare* L. subs.*vulgare* taken from the Albanian Gene Bank and cultivated in the experimental field of the Agricultural University of Tirana. Essential oils were obtained by steam distillation and analysed using gas chromatography equipped with a flame ionization detector (GC-FID) and examined for their volatile constituents by gas chromatography-mass spectroscopy (GC-MS). The results showed that the yield of essential oils (v/w dry weight) varied depending on accessions and ranged from 0.08 to 0.33%. Forty-nine volatile constituents were identified in total. The main constituents were: Germacrene D (18.05-37.23%), E-Caryophyllene (12.80-21.50%), α-Cadinol (2.39-5.62%), Carvacrol (1.81-5.24%), Elemol (1.63-4.88%), BornylAcetate (0.82-6.09%), Thymol (0.41-8.06%), α-Humulene (2.20-3.35%), Sabinene (0.7-7.41%), E,E-α-Farnesene(1.31-4.54%), Caryophyllene Oxide (1.67-3.04%) and Z-β-Ocimene (0.13-6.89%).

Keywords: *Origanum vulgare L. subs. vulgare,* gas chromatography, clusters, essential oils.

1. Introduction

The genus *Origanum* belongs to the *Lamiaceae* family distributed around the Mediterranean region [13] where *Origanum vulgare* L. is the most variable species and the only one commonly known as "Oregano" in most European countries [4, 22]. Several subspecies were discriminated according to the taxonomic studies of morphological characters. i.e. *hirtum*, *vulgare*, *virens*, *viride*, *gracile* and *glandulosum* [12, 6, 8, 9]. In Flora of Albania, two subspieces of *Origanum vulgare* are found: subs.*hirtum* and *vulgare.* [23]. *Origanum vulgare* L. subs.*vulgare* is a perennial herb (30-90 cm) with purple bracts and flowers, fine leaves and with little push glandular stomata [13]. Wild population of *Origanum vulgare* subs.*vulgare* are intensively exploited leading to genetic erosion of the specie. Extended research on germplasm is urgently needed because genetic resources, diversity and potential for utilization of *Origanum vulgare* have not yet been fully explored [17]. Numerous studies have shown that *Origanum vulgare* subs.*vulgare* is poor in sources of volatiles [3, 14] and rich in acyclic compounds and sesquiterpenoids [21]. Its principal constituents are Sabinene, (Z)-β-Ocimene, β-Caryophyllene and Germacrene D, while the phenols Thymol and Carvacrol are nearly absent [3]. There was found that the essential oil of this sub specie in Turkey was rich in Terpinen-4-ol+β-Caryophyllene(21%) and Germacrene D (17.8%) [20]. From a study of the seeds of *Origanum vulgare* subs.*vulgare* in Italy four chemotypes were identified: β-Cariophyllene, Thymol, Terpinen-4-ol and p-Cymene-β–Cariophyllene [15]. Meanwhile in France, studies classified the subspecies into six groups: Sabinene, Germacrene D,Germacrene D–β –Cariophyllene, cis-Sabinene hydrate, Terpinen-4-ol and β-Ocimene [7]. In Iran a study for the variation of the essential oils composition of four accessions of the sub specie identified four chemotypes consisting on Carvacrol, Sabinene, Caryophyllene oxide and Linalyl acetate [2]. The essential oils of *Origanum vulgare* subsp. *vulgare* have a high antimicrobial activity against bacteria,fungi and yeast [19].

This paper presents the variability of essential oil compositions of seven accessions of *Origanum vulgare* subs.*vulgare* collections of the Albanian Gene Bank, cultivated in the Agricultural University of Tirana's experimental field.

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2. Materials and Methods

2.1. Plant material

Seven accessions of *Origanum vulgare* subs. *vulgare* from the Albanian GenBank collected from natural are in Albania were cultivated at the Agricultural University of Tirana's experimental field in 2014 (Table 1). In summer 2015 the aerial part of 5 individual plants of each accession were cut at 30 cm during the flowering period. The collected samples were dried in the shadow at room temperature and cut in pieces before analysis.

Table 1. Main characteristics of the sites from where the plant materials of *Origanum vulgare subs. vulgare* accessions were collected.

No	Collsite	Region	Latitude	Longitude	Elevation	Colldate
1	Bicaj	Kukës	415941N	0202428E	464	20050823
2	Drobonik	Berat	404000N	0200000E	520	20030725
3	Mali me Gropa	Tiranë	412256N	0200100E	1294	20030828
4	ZallBastar	Tiranë	412500N	0195500E	210	20030828
5	Klos	Dibër	413000N	0201000E	300	20030820
6	Maqellarë	Dibër	413704N	0203015E	460	20050822
7	Sohodoll	Dibër	414031N	0202714E	490	20050822

2.2. *Essential oil isolation*

Dried *Origanum vulgare* subs.*vulgare* plants (flowers, stems, and leaves) from the seven accessions were submitted to hydrodistillation(100 g of cut tissue in 0.5 litres of water contained in a 1 litre flask) in a Clevenger apparatus for 3h at a distillation rate of 3 mL.min−1. The essential oils obtained were stored in the dark at -18°C in the deep freezer until further analysis.

2.3. Gas Chromatography (GC) analysis

GC/FID analyses were performed using an Agilent 7890A GC system equipped with an FID detector (Agilent Technologies). The separation was conducted on a HP-5MS column (30 m × 0.25 mm with a 0.25 μm film thickness). Helium was used as the carrier gas with an initial flow rate of 0.6 mL/min and then at a constant pressure of 50.0 psi. The front inlet was maintained at 250°C in a split ratio of 50:1. The GC oven temperature was increased from 60°C to 260°C at a rate of 5°C/min, and the FID was operated at 250°C with an air flow of 350 mL/min and a hydrogen flow of 35 mL/min. The injection volume was 1.0 μl.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC/MS analyses were performed using an Agilent 7890A GC system coupled to a 5975C MSD (Agilent Technologies). The ionization energy was 70 eV with a mass range of 40-400 *m/z*. The separation was conducted using the same column and temperature program as for the analytical GC.

2.5. Identification of components

The identification of each of the components of the essential oil was done by comparing their retention time with respect to the *n*-alkane series (C9-C28) internal standards under identical experimental conditions [1]. The components were also identified by comparing the mass spectra of each constituent with those stored in the NIST 08.L [18].) and WILEY MS 9th databases and with mass spectra from the literature [1]. Furthermore, some of the main peaks were identified by comparing the retention times and mass spectra with those of authentic constituents. The percentage composition of the oils was computed using the normalization method from the GC peak areas, calculated as the mean of three samples, without correction factors.

3. Results and Discussion

The EO quantity expressed in g/100 g dw (%) was variable from one accession to another at the ranges of 0.08–0.33%. The lowest values were observed in Sohodoll accession (7) and the highest in the Klos accession (5). Regarding the assessment of essential oil for *O.vulgare* ssp. *vulgare* it is once more confirmed that the sub-species is poorer in essential oil [11]. This feature of the sub-species was confirmed from a previous work for the estimation of the essential oil content in *O. vulgare* (ssp. *vulgare* and ssp. *hirtum*) from wild collection in Albania [10]. Instead high values of essential oil (0.1%-1.8%) for *O.vulgare* ssp. v*ulgare* have been recorded in Austria [14].

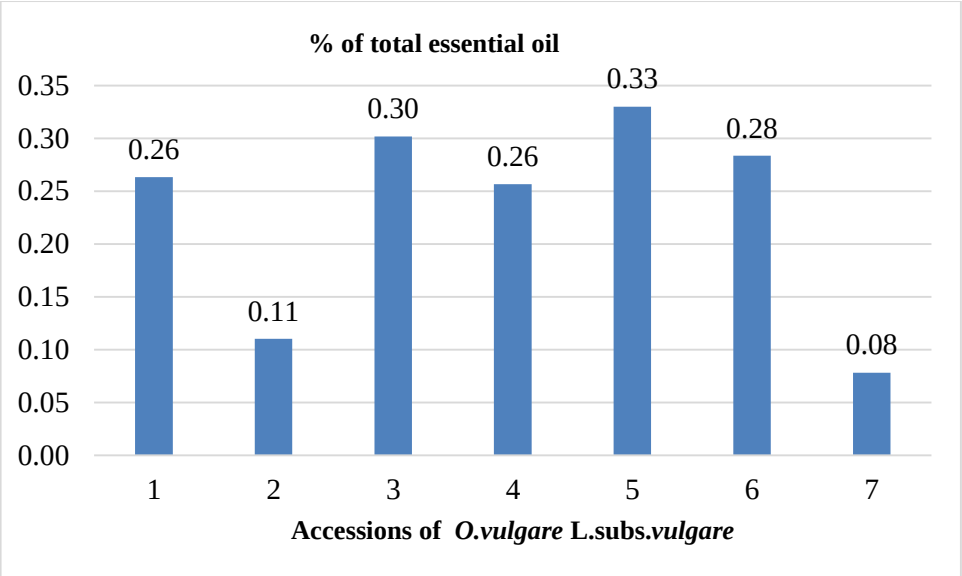

Figure 1. Mean essential oil yields (%, g/100 g dw) of the seven *Origanum vulgare*. subs. *vulgare* accesions of the Albanian GenBank

Forty-nine volatile constituents were identified (Table 2) representing 99.62-95.39% of total oil. Different investigation has shown various numbers of components regarding the subspecie, where a higher number of components (53) has been recorded in *O.vulgare* subs. *vulgare* in Austria [14].

The essential oil major components in the *O. vulgare* ssp. *vulgare* accessions assessed are found to be Germacrene D (18.05-37.23%), E-Caryophyllene (12.80-21.50%), α-Cadinol (2.39-5.62%), Carvacrol (1.81-5.24%), Elemol (1.63-4.88%), BornylAcetate (0.82-6.09%) Thymol (0.41-8.06%), α-Humulene (2.20-3.35%), Sabinene (0.7-7.41%), E-α-Farnesene (1.31-4.54%), Caryophylleneoxide (1.67-3.04%) and Z-β-Ocimene (0.13-6.89%). Similar findings regarding the major components in *O.vulgare*.subs.*vulgare* in other countries have shown different values. In Kosovo, the major components of the essential oil isolated from the *O. vulgare* collected in wild flora include: Sabinene, 1,8-Cineole, Caryophyllene oxide, β-Caryophyllene, p-Cymene, α-Terpineol, and Germacrene D [16]. Meanwhile in Turkey, the major components of the subspecie are found to be Caryophyllene oxide (34.44%), β-Caryophyllene (20.40%), and δ-Cadinol (7.02%) [4].

In accession number one: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from 0.02% to 35.25% with Germacrene D as the component with the highest percentage, followed by oxygenated Sesquiterpenes (0.07-3.29%) with Elemol as the main component, oxygenated Monoterpenes (0.07-3.89%) with Carvacrol as the main component and hydrocarbon Monoterpenes (0.02-2.12%) with Sabinene.

In accession number two: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from (0.07%-21.72%) with Germacrene D as the component with the highest percentage, followed by oxygenated Monoterpenes (0.03-6.97%) with Thymol, oxygenated Sesquiterpenes (0.09-3.09%) with α-Cadinol and hydrocarbon Monoterpenes (0.05-4.26%) with Sabinene.

In accession number three: the most abundant structure was hydrocarbon Sesquiterpenes ranging from (0.02-37.23%) with Germacrene D, followed by oxygenated Sesquiterpenes (0.10-4.78%) with α-Cadinol, oxygenated Monoterpenes (0.09-5.29%) with Bornyl acetate and hydrocarbon Monoterpenes (0.04-1.58%) with γ-Terpinen.

In accession number four: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from (0.03- 30.75%) with Germacrene D, followed by oxygenated Sesquiterpenes (0.02-5.62%) with α-Cadinol, oxygenated Monoterpenes (0.15-4.41%) with Bornyl acetate and hydrocarbon Monoterpenes (0.05-1.53%) with γ-Terpinen.

In accession number five: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from(0.07 - 18.05%) with Germacrene D, followed by hydrocarbon Monoterpenes (0.05-7.4%) with Sabinene, oxygenated Sesquiterpenes (0.08-4.79%) with α-Cadinol and

oxygenated Monoterpenes (0.06-5.24%) with Carvacrol.

In accession number six: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from (0.02-27.60%) with Germacrene D, followed by oxygenated Monoterpenes (0.05-8.06%) with Thymol, oxygenated Sesquiterpenes (0.02-3.29%) with α-Cadinol and hydrocarbon Monoterpenes (0.05-7.4%) with Sabinene.

In accession number seven: the most abundant chemical structure within components was hydrocarbon Sesquiterpenes ranging from (0.10 - 27.79%) with Germacrene D, followed by oxygenated Sesquiterpenes (0.02-3.29%) with α-Cadinol, oxygenated Monoterpenes (0.06-1.96%) with Bornyl acetate and hydrocarbon Monoterpenes (0.02-1.10%) with γ-Terpinen.

Table 2. Chemical composition of the essential oil from aerial parts of *Origanum vulgare*s ubsp. *vulgare* in seven accessions of the Albanian GenBank

			Accessions						
No	**KI**	**Components**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	930	α-Thujene	0.08	0.11	0.12	0.05	0.12	0.26	0.04
2	939	α-Pinene	0.20	0.21	0.21	0.94	0.38	0.30	0.06
3	954	Camphene	0.22	0.25	0.36	0.15	0.18	0.31	0.05
4	975	Sabinene	**2.12**	**4.26**	**1.28**	**1.17**	**7.41**	**2.63**	**0.70**
5	979	β-Pinene	0.30	0.31	0.14	0.12	0.80	0.24	0.09
6	990	Myrcene	0.02	0.05	0.04	-	0.05	0.10	0.02
7	1001	δ-2-Carene	0.33	0.53	0.41	0.19	0.92	0.66	0.13
8	1017	α-Terpinene	0.19	0.37	0.27	0.14	0.27	0.45	0.13
9	1026	o-Cymene	0.12	0.52	0.45	0.38	0.21	1.52	0.54
10	1031	1,8-Cineole	0.48	2.14	0.35	0.64	0.68	0.57	0.54
11	1037	Z-β-Ocimene	**1.21**	**0.24**	**1.41**	**0.79**	**6.89**	**1.48**	**0.13**
12	1050	E-β-Ocimene	0.23	0.88	0.24	0.71	1.22	0.81	0.50
13	1059	γ-Terpinen	1.88	0.47	1.58	1.53	2.24	1.40	1.10
14	1070	cis-Sabinen hydrate	0.11	0.17	0.28	1.58	0.31	0.74	0.06
15	1096	Linalool	0.07	0.83	0.09	0.22	0.11	0.12	0.65
16	1169	Borneol	0.75	1.14	3.30	1.16	1.60	1.73	0.15
17	1177	Terpinen-4-ol	0.19	0.03	0.10	0.15	0.06	0.05	0.06
18	1188	α-Terpineol	0.30	2.36	1.19	1.38	1.03	0.82	0.62
19	1235	Thymol methyl ether	0.12	5.75	0.38	0.18	1.12	1.08	0.83
20	1285	Bornyl acetate	1.68	0.82	5.29	4.41	1.81	6.09	1.96
21	1290	Thymol	**0.41**	**6.97**	**1.58**	**1.70**	**1.97**	**8.06**	**1.27**
22	1299	Carvacrol	**3.98**	**4.62**	**1.81**	**2.41**	**5.24**	**4.42**	**1.87**
23	1376	α-Copaene	0.27	1.36	0.31	0.07	0.09	0.19	0.30
24	1388	β-Bourbonene	0.50	0.86	0.56	0.12	0.48	0.74	1.17
25	1390	β-Elemen	0.89	0.65	1.20	0.45	0.77	0.68	0.82
26	1400	Sibirene	-	0.07	-	-	0.72	-	0.10
27	1419	E-Caryophyllene	**21.50**	**16.63**	**6.14**	**19.36**	**16.60**	**12.80**	**16.82**
28	1432	β-Copaene	1.41	0.31	0.30	0.15	0.71	0.25	0.38
29	1454	α-Humulene	2.33	2.90	2.70	3.28	2.20	3.85	3.20
30	1460	Allo-Aromadendrene	0.67	0.60	0.83	0.47	1.31	0.45	1.15
31	1479	γ-Muurolene	0.19	0.61	0.30	0.24	0.31	0.29	0.32
32	1481	Germacrene D	**35.25**	**21.72**	**37.23**	**30.75**	**18.05**	**27.60**	**27.79**
33	1496	Viridiflorene	1.83	0.57	0.02	0.16	0.30	0.19	0.55

No	KI	Components	Accessions						
			1	2	3	4	5	6	7
34	1500	Bicyclogermacrene	0.02	1.26	0.15	0.03	0.14	0.02	2.20
35	1505	E,E-α-Farnesene	2.06	1.31	3.93	2.89	2.44	1.98	4.54
36	1513	γ-Cadinene	3.05	1.38	2.76	2.16	2.59	0.92	1.15
37	1523	delta-Cadinene	1.01	0.55	1.07	1.24	2.22	0.42	1.00
38	1548	Elemol	3.29	2.16	3.51	4.88	4.07	1.63	2.92
39	1561	Germacrene B	0.11	0.42	0.28	0.33	0.07	0.40	0.21
40	1572	Unknown	-	3.15	0.09	0.26	0.00	0.32	2.37
41	1575	Germacrene D-4-ol	0.46	0.34	4.02	0.38	0.39	2.25	0.94
42	1579	Spathulenol	0.07	0.09	0.41	0.94	0.11	1.28	0.72
43	1583	Caryophyllene oxide	**2.19**	**1.65**	**2.77**	**1.93**	**1.67**	**1.97**	**3.04**
44	1608	Humulene epoxide II	1.17	0.93	1.09	0.08	0.30	0.24	1.77
45	1640	Caryophylla-4-(12),8(13)-dien-5alfa-ol	1.49	0.87	0.22	0.12	0.77	0.94	2.11
46	1640	Caryophylla-4-(12),8(13)-dien-5beta-ol	0.14	0.63	0.83	0.02	0.13	0.63	0.84
47	1646	alfa-Muurolol	1.71	1.25	2.09	3.74	2.90	1.51	2.75
48	1652	Unknown	0.19	0.25	0.89	0.06	0.26	0.28	0.53
49	1655	alfa-Cadinol	**2.39**	**3.09**	**4.78**	**5.62**	**4.79**	**3.29**	**5.46**
50	1677	Unknown	0.33	0.60	0.18	0.06	0.68	0.31	1.70
51	1687	Eudesma-4(15),7-dien-beta-ol	0.20	0.16	0.10	0.05	0.08	0.02	0.30
52	1841	(E)-pseudoisoeugenyl 2-methylbutyrate	0.31	0.63	0.33	0.16	0.21	0.70	1.39
		Identified	99.49	96.00	98.84	99.62	99.06	99.09	95.39
		Unknown	0.52	4	1.16	0.38	0.94	0.91	4.61
		Oxygenated monoterpenes	8.09	24.8	14.4	13.8	13.9	23.7	8
		Hydrocarbon monoterpenes	6.89	8.17	6.51	6.18	20.7	10.2	3.47
		Oxygenated Sesquiterpenes	13.1	11.17	19.84	17.76	15.21	13.77	20.84
		Hydrocarbon Sesquiterpenes	71.1	51.2	57.8	61.7	49	50.8	61.7
		Others	0.31	0.63	0.33	0.16	0.21	0.70	1.39

KI-Kovats Index; (-) did not identify

All the studied accessions presented high levels of Germacrene D that recorded the values of 18.05% to 37.23%. The second major component was β-Caryophyllene that ranged from 12.80 to 21.50% (Table 2). Regarding Thymol as an important component of the subspecies it has been found at low concentrations in the oil of our accessions ranging from 0.41 to 8.06%. Similar values for the subspecies have been recorded in Hungary [24].

The main components of these essential oil, from accession one were Germacrene D, E-Caryophyllene and Carvacrol (Figure 2) ranged respectively from 25.14 to 45.21%, 5.87 to 57.70% and 0.72 to 22.27%.

For accessiontwo the main compounds were Germacrene D, E-Caryophyllene and Thymol ranged from 2.23 to 31.76, 8.99 to 36.18% and 0.74 to 51.20% respectively.

For accession three the main compounds were Germacrene D, E-Caryophyllene and BornylAcetate ranged from 27.76 to 52.56%,5.38 to 22.23% and 0.52 to 20.64% respectively.

For accession four the main compounds were Germacrene D, E-Caryophyllene and alfa-Cadinol ranged from 8.93 to 42.24%,4.61 to36.05% and 2.11 to 24.41% respectively.

For accession five the main compounds were Germacrene D, E-Caryophyllene and Sabinene ranged from 7.56 to 26.77%, 6.37 to 26.61% and 3.32 to3 8.90% respectively.

For accession six the main compounds were Germacrene D, E-Caryophyllene and Thymol ranged from 3.93 to 40.61%, 3.59 to 35.86% and 0.82 to 52.86% respectively.

For accession seven the main compounds were Germacrene D, E-Caryophyllene and alfa-Cadinol ranged from 15.51 to 38.30% ,6.31 to 26.08% and 2.94 to 7.84% respectively.

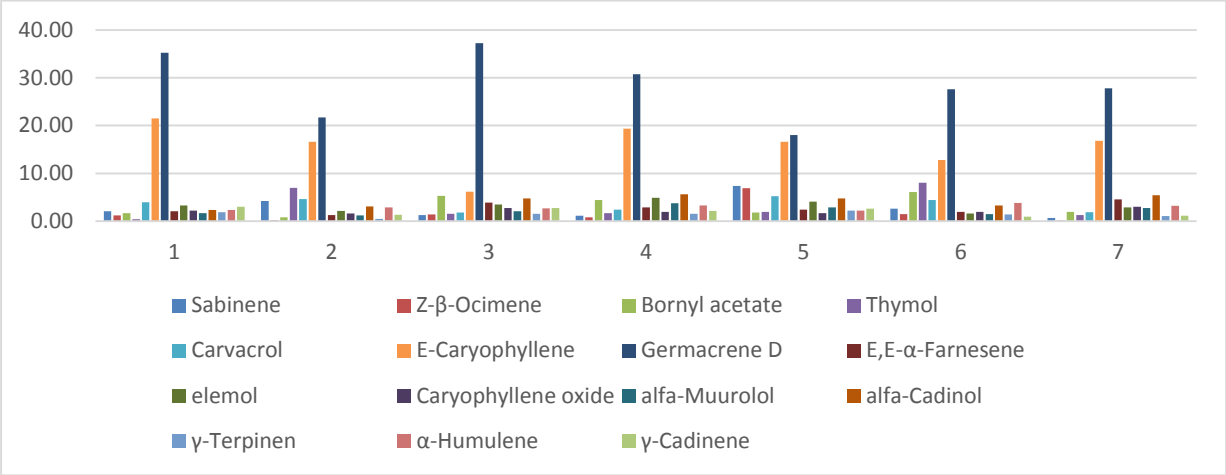

Figure 2. Total percentage for compounds of *essential oils content* (%) in seven accessions of *O. vulgare* L. subs. *vulgare*

4. Conclusions

The content of essential oil in seven *Origanum vulgare* L. subs *vulgare* accessions in Albania varies between 0.08 to 0.33%. Qualitative and quantitative analyses performed using GC GC-MS techniques have found 49 volatile constituents representing 99.62 to 95.39% of total oil. The essential oil major components in the *Origanum vulgare* ssp. *vulgare* accessions assessed are found to be: Germacrene D (18.05-37.23%), E-Caryophyllene (12.80-21.50%), α-Cadinol (2.39-5.62%), Carvacrol (1.81-5.24%), Elemol (1.63-4.88%), Bornyl acetate (0.82-6.09%), Thymol (0.41-8.06%), α-Humulene (2.20-3.35%), Sabinene (0.7-7.41%), E,E-α-Farnesene (1.31-4.54%), Caryophyllene Oxide (1.67-3.04%) and Z-β-Ocimene (0.13-6.89%). It could be concluded that the chemical composition of the essential oil of Albanian *Origanum vulgare* L. subsp.*vulgare* may be categorized in the "Germacrene D" chemo-type which is known for its antimicrobial, antifungal and insecticidal properties and also plays a role as insect pheromone.

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