



## Phytochemical analysis of volatiles and biological activities of *Chaerophyllum bulbosum* L. essential oils

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**Abstract:** The present study reports for the first time the chemical composition of the headspace volatiles (HS) and essential oils obtained from fresh *Chaerophyllum bulbosum* roots and aerial parts, as well as biological activities of the essential oils. Generally, monoterpene hydrocarbons were found to be the main class of all investigated samples, with (*E*)- $\beta$ -ocimene being the most abundant component. The results of antibacterial assay showed that both investigated samples exhibit bactericidal activity against two tested Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis* subs. *spizizenii*) while they were inactive against tested Gram-negative bacteria (*Escherichia coli* and *Salmonella abony*). Regarding the antioxidant activity, it was found that the essential oils showed low antioxidant capacities in comparison with standard antioxidant compounds (such as butylated hydroxytoluene). The obtained results were expected given the fact that the main components in both samples were monoterpene hydrocarbons.

**Keywords:** chemical composition; GC/MS; antibacterial assay; antioxidant activity.

### INTRODUCTION

Apiales, carrot order of flowering plants, consist of about 5,489 species. There are seven families in the order, of which the largest are *Apiaceae* (carrot or parsley family), *Araliaceae* (ginseng family) and *Pittosporaceae*. *Apiaceae*, also called *Umbelliferae* (the parsley family) comprising between 300 and 400 genera of plants distributed worldwide, principally in the north temperate regions of the world. Many species of the *Apiaceae* are poisonous, some are widely used vegetables, others are used as herbs and spices, while some species are grown for their ornamental value. The genus *Chaerophyllum* belongs to the *Apiaceae*, and includes about 40 species widespread in Europe, Asia and North America, six of

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which are described in the flora of Serbia.<sup>1</sup> *Chaerophyllum bulbosum* L. which is known by several common names, including turnip-rooted chervil, tuberous-rooted chervil, bulbous chervil and parsnip chervil, is one of the vegetables that is more commonly grown in the past than it is today. This is a tall annual herb with fringelike divided leaves and large umbels of white flowers. The plant is cultivated on a small scale in parts of Europe for the edible root, which is similar to carrot. Because of its biochemical composition<sup>2,3</sup> and specific chestnut-like flavor,<sup>4</sup> tuberous-rooted chervil is considered as a “gourmet” vegetable. The root is characterized by the accumulation of starch and sucrose. It is interesting that at harvest time the root is not edible and traditionally, storage at 10 °C during four to five months is necessary to reach the stage at which it is edible. Storage enables changes in carbohydrate reserves responsible for taste. *C. bulbosum* is also reported to be a traditional medicinal plant used by locals in Eastern Turkey (the rhizomes were consumed raw to increase appetite, to treat diabetes and high cholesterol levels).<sup>5</sup>

Literature survey showed that there are several phytochemical investigations that have been published on *C. bulbosum*<sup>2–9</sup> with only a few reports regarding the essential oil isolated from air dried aerial parts of the plant.<sup>10–12</sup> It is interesting that even though the root is the only part of the plant that has an application in human diet, there are no data considering the chemical composition of the root essential oil, so this is the first report about the chemical composition, antimicrobial and antioxidant activity of the essential oil obtained from *C. bulbosum* roots as well as fresh aerial parts of the plant.

## EXPERIMENTAL

### *Plant material*

Root maturity is reached when leaves turn yellow in June. The plant material at flowering stage was collected on Vlasina plateau, Serbia, at an altitude of 1250 m, in July 2018. Around 75 plant individuals were collected at the same location in the diameter of approximately 150 m to give a composite sample. The plant material was identified by Bojan Zlatković and the voucher specimen was deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, under the acquisition number 7232. The fresh plant material was divided into parts (aerial parts and root) and they were analyzed separately.

### *Headspace sample preparation*

The fresh plant material was analyzed immediately after harvest. Five hundred mg of milled plant material was put into 20 mL HS vial and then soaked with 2 mL of distilled water. The sample was heated at 80 °C for 20 min with the following mixing program: shaking for 5 s, pause for 2 s. 500 µL of vapor generated from the samples was drawn out from the vial using a gas-tight syringe (90 °C) and injected directly in the chromatographic column *via* a transfer line (75 °C).

### *Hydrodistillation*

Fresh samples (473 g of aerial parts and 636 g of roots) were subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus and yielded 0.15 and 0.02 mass% (based on the weight of fresh plant material) of essential oil, respectively. The obtained essential oils were decanted; extracted with hexane and treated with anhydrous magnesium sulfate to remove any residue of moisture, then filtered and solvent was evaporated in a stream of nitrogen. Samples were analyzed immediately after isolation.

### *Identification of volatile compounds*

The samples were analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, Santa Clara, CA, USA) equipped with a Combi PAL sampler and Headspace for G6501B/G6509B. A fused silica capillary column HP-5MS (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) was used. The injector and interface operated at 250 and 300 °C, respectively. Temperature program: from 50 to 290 °C at a heating rate of 4 °C/min. The carrier gas was helium (99.9999 %) with a flow of 1.0 mL/min. The samples (500 µL) were injected for HS and of essential oil solutions (1 µL), prepared by mixing 20 µL of essential oil with 980 µL of *n*-hexane, were injected (split ratio 40:1) for liquid analyses. Post run: back flush for 1.89 min, at 280 °C, with helium pressure of 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 40–440, scan time 0.32 s. GC analysis equipped with FID detector was carried out under the same experimental conditions using the same column as described for the GC/MS. The relative percentage composition of the samples (analysis was performed in triplicate) was computed from the GC peak areas without any corrections. The median and standard deviation was performed as statistical analysis. Essential oil constituents were identified by comparison of their linear retention indices (relative to C<sub>8</sub>-C<sub>32</sub> *n*-alkanes on the HP-5MS column) with literature values and their MS with those from Adams,<sup>13</sup> Wiley 6, NIST11, Agilent Mass Hunter Workstation B.06.00 software and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils by application of the automated mass spectral deconvolution and identification system (AMDIS software, ver. 2.1, DTRA/NIST, 2011).

### *Antibacterial activity*

Antibacterial activity was evaluated against two Gram-positive and two Gram-negative bacteria by using the disk diffusion assay.<sup>14</sup> The Gram-positive bacteria used were: *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The Gram-negative bacteria utilized in the assay were: *Escherichia coli* ATCC 8739 and *Salmonella abony* ATCC 6017. The inocula of the bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The suspensions of the tested microorganisms (0.1 mL) were spread on the solid Mueller-Hinton agar plates. All agar plates were prepared in 90 mm Petri dishes giving the final depth of 4 mm. Sterile filter paper disks ("Antibiotica Test Blättchen", Macherey-Nagel, Düren, Germany, 9 mm in diameter) were impregnated with 30 µL of the essential oil (essential oils were dissolved in hexane at concentration of 100 mg/mL) and placed on the inoculated plates. These plates, after standing at 4 °C for 2 h, were incubated at 37 °C for 24 h. Chloramphenicol (30 µg) and streptomycin (10 µg) were used as positive reference standards to determine the sensitivity of a strain of each tested microbial species while disks containing hexane were used as the negative control. The diameters of the inhibition zones were measured in millimeters using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co., USA). Each test was performed in triplicate.

### *Antioxidant activity*

ABTS radical “scavenging” activity was performed according to the method of Re *et al.*<sup>15</sup> The ABTS radical was produced by the reaction of ABTS stock solution with potassium persulfate and the mixture was allowed to stand in the dark at room temperature for 12–16 h before use. The solution was then diluted by mixing 7 mL ABTS<sup>•+</sup> solution with 120 mL methanol to obtain an absorbance of 0.7±0.02 units at 734 nm. An aliquot of each sample (0.1 mL), concentration 100 mg/mL, was mixed with 1.8 mL of diluted ABTS solution in the concentration of 7 mmol/L and diluted with methanol to a total volume of 4 mL. After 6 min at room temperature, the reduction in absorbance was measured at 734 nm. Results are expressed as mg of Trolox equivalents (TE) per mg of essential oil (mg TE per mg EO).

The quantitative assays of the essential oils (EO) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were performed according to the method of Dimitrijević *et al.*<sup>16</sup> 1.5 mL of 100 mmol/L 2, 2-diphenyl-1-picrylhydrazylradical methanol solution, 0.1 mL of 100 mg/mL EO and 4 mL of methanol were shaken, left to react for 60 min in darkness, and the absorbance was measured at 515 nm, and expressed in µg of Trolox equivalents (TE) per mg of essential oil (µg TE/mg EO).

The ferric reducing antioxidant power (FRAP) assay was performed using method of Benzie and Strain.<sup>17</sup> 1 mL of FRAP reagent was mixed with 0.1 mL of EO, concentration 100 mg/mL, and diluted with water to a volume of 4 mL. After 5 min of incubation at 37 °C, the absorbance was recorded at 595 nm. The ferric ion reducing antioxidant power value was expressed as µg of Fe(II) equivalents per mg of essential oil (µg Fe/mg EO).

The reducing power (TRP) of essential oils was determined by the method of Oyaizu.<sup>18</sup> Reaction mixtures were prepared by mixing of EO (0.01 mL), 1 mL of 1% K<sub>3</sub>[Fe(CN)<sub>6</sub>], phosphate buffer (pH 6.6) and water. The mixtures were incubated at 50 °C for 30 min and after incubation 10 % trichloroacetic acid (1 mL) and FeCl<sub>3</sub> (0.6 mL) were added. The absorbance was measured at 700 nm against a blank sample and results were expressed as mg ascorbic acid equivalents per mg of essential oil (mg AAE/mg EO).

The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed using the method of Apak *et al.*<sup>19</sup> This assay involved the addition of EO (0.1 mL), phosphate buffer (pH 7.0, 1 mL), neocuproine ( $7.5 \times 10^{-3}$  mol/L), copper(II)chloride (0.01 mol/L) and dilution with water to a total volume of 4.1 mL. The mixture was left for 30 min at 25 °C and the absorbance was measured at 450 nm. Trolox was used as a standard and results were expressed as µg Trolox equivalents per mg of essential oil (µg TE/mg EO).

Trolox (TE) was used as a standard for ABTS, DPPH and CUPRAC assays, ascorbic acid (AAE) for TRP and fero sulfat for FRAP assay.

## RESULTS AND DISCUSSION

### *Chemical composition*

Chemical compositions of the hydrodistillated essential oils (EO) as well as the volatiles obtained by static headspace method (HS) of *C. bulbosum* were analyzed by GC and GC/MS, and the results are presented in Table I.

In general, monoterpene hydrocarbons were found to be the main class of all investigated samples, with (*E*)-β-ocimene being the most abundant component.

Headspace volatiles of the aerial parts of the plant consisted 100 % of monoterpene hydrocarbons with (*E*)-β-ocimene accounting 96.1 % of the sample. In

the root sample, for which twenty compounds were identified (representing 99.9 % of the total), the main class was also represented by monoterpene hydrocarbons (98.7 %) with (*E*)- $\beta$ -ocimene (46.1 %), followed by limonene (14.9 %),  $\gamma$ -terpinene (12.5 %), *p*-cymene (11.1 %) and  $\alpha$ -pinene (10.9 %) as the main components. Sesquiterpene hydrocarbons were also present but with the contribution of only 0.4 %.

TABLE I. Chemical compositions (average of triplicates $\pm$ SD) of the *Chaerophyllum bulbosum* essential oils and headspace volatiles; compounds listed in order of elution on a HP-5MS column. RI: experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C<sub>8</sub>–C<sub>32</sub>; RA: Adams retention indices; \*: identified by NIST Chemistry WebBook Retention indices; tr: trace (<0.05 %); -: not detected. Headspace samples: HS1 – aerial parts, HS2 – root; essential oil samples: EO1 – aerial parts, EO2 – root

RI	RA	Compound	Content, %			
			HS1	HS2	EO1	EO2
928	924	$\alpha$ -Thujene	–	0.1 $\pm$ 0.01	–	–
935	932	$\alpha$ -Pinene	0.3 $\pm$ 0.02	10.9 $\pm$ 1.05	0.4 $\pm$ 0.02	2.0 $\pm$ 0.2
950	946	Camphene	–	0.1 $\pm$ 0.04	–	–
975	969	Sabinene	–	0.7 $\pm$ 0.05	tr	0.9 $\pm$ 0.05
978	974	$\beta$ -Pinene	0.4 $\pm$ 0.05	0.2 $\pm$ 0.01	0.3 $\pm$ 0.02	0.2 $\pm$ 0.01
991	988	Myrcene	0.1 $\pm$ 0.01	1.0 $\pm$ 0.35	0.2 $\pm$ 0.02	0.9 $\pm$ 0.07
1004	998	<i>n</i> -Octanal	–	0.2 $\pm$ 0.05	–	0.5 $\pm$ 0.03
1006	1002	$\alpha$ -Phellandrene	–	tr	–	–
1018	1014	$\alpha$ -Terpinene	–	0.1 $\pm$ 0.02	–	0.1 $\pm$ 0.02
1026	1020	<i>p</i> -Cymene	0.6 $\pm$ 0.05	11.1 $\pm$ 0.55	0.8 $\pm$ 0.02	3.3 $\pm$ 0.54
1030	1024	Limonene	0.3 $\pm$ 0.02	14.9 $\pm$ 1.13	0.3 $\pm$ 0.02	15.2 $\pm$ 1.25
1039	1032	( <i>Z</i> )- $\beta$ -Ocimene	0.4 $\pm$ 0.05	0.9 $\pm$ 0.32	1.6 $\pm$ 0.24	1.0 $\pm$ 0.03
1051	1044	( <i>E</i> )- $\beta$ -Ocimene	96.1 $\pm$ 2.17	46.1 $\pm$ 1.15	91.5 $\pm$ 1.18	38.5 $\pm$ 0.95
1061	1054	$\gamma$ -Terpinene	1.9 $\pm$ 0.35	12.5 $\pm$ 0.95	4.1 $\pm$ 0.08	11.7 $\pm$ 0.55
1091	1089	<i>p</i> -Cymenene	–	0.7 $\pm$ 0.11	0.1 $\pm$ 0.01	0.7 $\pm$ 0.05
1121	1118	<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	–	–	–	0.6 $\pm$ 0.02
1140	1137	( <i>E</i> )-Epoxy-ocimene	–	–	–	1.6 $\pm$ 0.16
1158	1157	( <i>E</i> )-2-Nonen-1-al	–	–	–	0.4 $\pm$ 0.02
1178	1174	Terpinen-4-ol	–	–	–	0.3 $\pm$ 0.01
1186	1184	Dill ether	–	–	–	0.2 $\pm$ 0.01
1187	1184	Cryptone	–	–	–	tr
1191	1186	$\alpha$ -Terpineol	–	–	–	tr
1196	1195	<i>cis</i> -Piperitol	–	–	–	0.1 $\pm$ 0.02
1198	1195	Methyl chavicol	–	–	–	0.2 $\pm$ 0.02
1208	1207	<i>trans</i> -Piperitol	–	–	–	0.5 $\pm$ 0.01
1220	1215	<i>trans</i> -Carveol	–	–	–	tr
1229	1232	Thymol, methyl ether	–	–	–	0.2 $\pm$ 0.02
1234	1232*	Isothymol, methyl ether	–	–	–	0.7 $\pm$ 0.05
1256	1249	Piperitone	–	–	–	tr
1260	1260	( <i>E</i> )-2-Decenal	–	–	–	0.2 $\pm$ 0.04
1287	1287	Bornyl acetate	–	tr	–	0.4 $\pm$ 0.01

TABLE I. Continued

RI	RA	Compound	Content, %			
			HS1	HS2	EO1	EO2
1293	1289	Thymol	—	—	—	tr
1315	1315	( <i>E,E</i> )-2,4-Decadienal	—	—	—	tr
1358	1356	Eugenol	—	—	—	tr
1379	1374	α-Copaene	—	—	—	tr
1382	1380	Daucene	—	—	—	0.2±0.02
1402	1396	α-Chamipinene	—	—	—	0.2±0.02
1415	1407	α-Barbatene	—	—	—	tr
1422	1413	β-Funebrene	—	0.1±0.04	—	2.3±0.34
1426	1419	β-Cedrene	—	—	—	0.5±0.05
1436	1429	cis-Thujopsene	—	—	—	0.1±0.02
1441	1436	Isobazzanene	—	—	—	tr
1444	1440	( <i>Z</i> )-β-Farnesene	—	—	—	tr
1452	1450	β-Barbatene	—	0.1±0.01	—	1.2±0.08
1456	1454	( <i>E</i> )-β-Farnesene	—	—	—	0.5±0.01
1461	1456*	Amorpha-4,11-diene	—	0.1±0.01	—	1.5±0.05
1483	1476	β-Chamigrene	—	—	—	0.5±0.03
1486	1484	Germacrene D	—	—	0.1±0.01	—
1496	1493	α-Zingiberene	—	—	—	tr
1499	1498	β-Alaskene	—	—	—	0.4±0.05
1501	1500	Bicyclogermacrene	—	—	0.2±0.01	—
1502	1501	Epizonarene	—	—	—	0.1±0.01
1512	1505	β-Bisabolene	—	0.1±0.02	—	1.5±0.12
1516	1512	α-Alaskene	—	—	—	0.5±0.02
1524	1517	Myristicin	—	—	—	5.1±0.64
1534	1530	Dauca-4(11),8-diene	—	—	—	0.3±0.02
1537	1532	γ-Cuprenene	—	—	—	0.2±0.01
1556	1555	Elemicin	—	—	—	0.2±0.01
1583	1577	Spathulenol	—	—	—	tr
1596	1589	allo-Cedrol	—	—	—	0.2±0.03
1608	1600	Cedrol	—	—	—	0.6±0.05
2034	2035	( <i>Z</i> )-Falcarinol	—	—	—	1.9±0.12
Number of constituents			8	20	12	57
Total identified			100	99.9	99.6	98.4
Monoterpene hydrocarbon (MH)			100	99.3	99.3	74.5
Monoterpene oxygenated (MO)			—	—	—	4.8
Sesquiterpenes hydrocarbon (SH)			—	0.4	0.3	9.8
Sesquiterpenes oxygenated (SO)			—	—	—	0.8
Others (O)			—	0.2	—	3.2
Phenylpropanoids (PP)			—	—	—	5.3

Similar situation was noticed regarding essential oil samples. In the essential oil obtained from the aerial parts, monoterpene hydrocarbons make up over 99 % of the sample with (*E*)-β-ocimene as the main component. Although, both of the samples were dominated by (*E*)-β-ocimene, two main features distinguished

essential oil obtained from the root; the number of identified constituents (fifty-seven vs. twelve) and the presence of the oxygenated fraction which was not even detected in aerial parts. In the root sample (*E*)- $\beta$ -ocimene was present with the contribution of 38.5 %, followed by limonene (15.2 %) and  $\gamma$ -terpinene (11.7 %), unlike the aerial parts were (*E*)- $\beta$ -ocimene make up over 90 % of the total (91.5 % to be exact). These differences could be attributed to the fact that the essential oils which have been isolated from root and aerial parts of the plant have significantly different chemical composition.

If we compare our results with the previously published results it is obvious that the composition of the essential oils of the aerial parts of the *C. bulbosum* collected from different geographic regions was found to be quite different. The analysis of the volatile fraction of *Chaerophyllum bulbosum* L. ssp. *bulbosum* (Apiaceae) growing wild in Greece demonstrated the presence of apiole (37 %), 3,7,11-trimethyldodeca-1,6,10-trien-3-ol (8.5 %), linalool (7.7 %), myristicine (6.9 %) and eugenol (5.8 %).<sup>10</sup> The essential oil of *C. bulbosum* from Iran had (*E*)- $\beta$ -farnesene (22.3 %), (*Z*)- $\beta$ -ocimene (18.8%), and myristicin (17.1%) as the major components in this oil. The other notable compounds in the oil of the plant were caryophyllene oxide (6.6 %), *allo*-ocimene (5.1%), and (*E*)- $\beta$ -ocimene (4.0 %).<sup>12</sup> In the oil from the epigeal part of *C. bulbosum* grown in Azerbaijan,<sup>11</sup> linalool (18.3 %) and  $\alpha$ -pinene (7.8 %) were predominant. Since this is the first report considering the chemical composition of the essential oil obtained from the root, comparison cannot be done.

The differences in chemical composition can be explained by many factors including genetic variation, plant ecotype or variety, plant nutrition, application of fertilizers, geographic location of the plants, climate conditions, seasonal variations, stress during growth or maturity and the post-harvest drying and storage.<sup>20</sup> All these factors influence one another so it is difficult to say with certainty which of these factors have the greatest effect on the chemical composition of essential oils.

#### *Antibacterial activity*

The results of antibacterial assay showed that both investigated samples exhibit inhibitory activities against two tested Gram-positive bacteria (Table II).

Root essential oil showed bactericidal activity against *Staphylococcus aureus* and *Bacillus subtilis* subs. *spizizenii* (diameter of zone of inhibition 10 and 16 mm, respectively), while diameter of zone of inhibition for aerial parts oil was 12 and 14 mm, respectively. Chloramphenicol (30  $\mu$ g) and streptomycin (10  $\mu$ g) were used as positive reference standards, and diameter of zone of inhibition for chloramphenicol against *Staphylococcus aureus* and *Bacillus subtilis* subs. *spizizenii* was 24 and 28 mm, respectively, while the diameter of zone of inhibition for streptomycin was 14 and 24 mm, respectively. Previous studies of the essen-

tial oil obtained from epigeal parts of *C. bulbosum* from Iran<sup>12</sup> also showed significant activity against Gram-positive bacteria, (*Staphylococcus aureus*, *Bacillus anthracis* and *Streptococcus pyogenes*), moderate inhibitory activity against *Escherichia coli*, and inactivity against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

TABLE II. Antibacterial activity of *Chaerophyllum bulbosum* essential oils and positive controls; values are the mean inhibition zone of three replicates including disc; Bc: bactericidal activity; Bs: bacteriostatic activity; n.a.: not active. Active amounts: *C. bulbosum* root and aerial parts essential oils (30 µg/disc), chloramphenicol (30 µg/disc), streptomycin (10 µg/disc). Hexane used to dissolve the samples, was employed as negative control and didn't have any activity. Essential oil samples: EO1 – aerial parts, EO2 – root

Sample	Zone of inhibition, mm							
	<i>B. spizizenii</i>		<i>S. aureus</i>		<i>S. abony</i>		<i>E. coli</i>	
	Bc	Bs	Bc	Bs	Bc	Bs	Bc	Bs
EO1	14	n.a.	12	n.a.	n.a.	n.a.	n.a.	n.a.
EO2	16	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.
Chloramphenicol	28	n.a.	24	n.a.	25	n.a.	28	n.a.
Streptomycin	24	n.a.	14	n.a.	17	n.a.	16	n.a.

These differences in antibacterial activity can be explained by the fact that our and their sample has significantly different chemical composition. Obtained results showed that the antibacterial activities of the same plant species differed significantly depending on taxonomic characteristics as well as biological characteristics of the tested bacteria.

#### Antioxidant activity

This is the first report on the evaluation of the antioxidant activity of *C. bulbosum* essential oils. The antioxidant activity was evaluated by five different methods: DPPH, ABTS, total reducing power (TRP), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC). The results are given in Table III.

TABLE III. Antioxidant activity of *Chaerophyllum bulbosum* essential oils; values are means ± SD (n = 3). Essential oil samples: EO1 – aerial parts, EO2 – root

Sample	ABTS µg TE / mg EO	DPPH µg TE / mg EO	TRP µg AAE / mg EO	FRAP µg Fe / mg EO	CUPRAC µg TE / mg EO
EO1	0.3±0.04	1.34±0.04	0.163±0.003	0.162±0.006	6.1±0.2
EO2	0.85±0.02	1.30±0.02	0.379±0.009	0.512±0.008	7.79±0.08

Since there are no any data regarding antioxidant activity of *C. bulbosum* essential oils, the obtained results were compared with activity of the commercial standard antioxidants and it was found that both essential oils showed low antioxidant capacities in comparison with standard antioxidant compounds.

Free radical scavenging activity of samples was investigated by DPPH and ABTS assays. According to the results obtained, DPPH “scavenging” radical capacity of samples were similar ( $1.30\pm0.02$  and  $1.34\pm0.04$  µg TE/mg oil for the root and aerial parts, respectively), while the antioxidant capacity estimated in terms of the ABTS<sup>+</sup> radical scavenging activity, was  $0.85\pm0.02$  and  $0.30\pm0.04$  µg TE / mg oil, respectively. Reducing power of the samples was estimated by FRAP and CUPRAC assays. The results of FRAP assay, expressed as µg Fe / mg of essential oils, indicates that the root essential oil was almost 4 times more active than aerial parts essential oil ( $0.512\pm0.008$  and  $0.162\pm0.006$  µg Fe / mg EO). The higher CUPRAC capacity was observed for root sample ( $7.97\pm0.08$  mg TE / mg EO) then for aerial parts ( $6.1\pm0.2$  mg TE / mg EO). The total reducing power of the oil was found to be  $0.379\pm0.009$  µg AAE / mg oil for the root sample and  $0.163\pm0.003$  µg AAE/mg oil for the aerial parts.

Given the fact that the main components in both samples were monoterpene hydrocarbons, it is not unexpected that the oils exhibit no significant antioxidant activity.<sup>21</sup>

#### CONCLUSION

This is the first report about the chemical composition, antimicrobial and antioxidant activity of the essential oils obtained from *C. bulbosum* roots as well as fresh aerial parts of the plant. In general, monoterpene hydrocarbons were found to be the main class of all investigated samples, with (*E*)-β-ocimene being the most abundant component. As can be seen from the results, (*E*)-β-ocimene make up over 90 % of the essential oil of *C. bulbosum* aerial parts which points to the fact that it can be used in perfumery industries. Comparison of ours with previously published results considering the antimicrobial activities led to the conclusion that the antibacterial activities of the same plant species depend on taxonomic characteristics as well as biological characteristics of the tested bacteria. Since there are no previously published investigations of the antioxidant activity, comparison cannot be done. On the other hand, low antioxidant activity of the examined samples was expected, since the major compounds in both samples were monoterpene hydrocarbons.

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## И З В О Д

ФИТОХЕМИЈСКА АНАЛИЗА ИСПАРЉИВИХ КОМПОНЕНАТА И БИОЛОШКА  
АКТИВНОСТ ЕТАРСКИХ УЉА БИЉНЕ ВРСТЕ *Chaerophyllum bulbosum*

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У овом раду је, по први пут, испитиван хемијски састав лако испарљивих састојака и етарских уља изолованих из свежег корена и надземних делова биљне врсте *Chaerophyllum bulbosum* L. као и биолошка активност етарских уља. Испитивања су показала да су угљоводонични монотерпени доминантна класа једињења у свим испитваним узорцима, при чему је као главна компонента идентификован (*E*)- $\beta$ -оксимен. Резултати антибактеријског теста показали су да оба испитивана узорка показују бактерицидно дејство на две тестиране Грам-позитивне бактерије (*Staphylococcus aureus* и *Bacillus subtilis subs. spizizenii*), док су Грам-негативне бактерије (*Escherichia coli* и *Salmonella abony*) биле резистентне. Што се тиче антиоксидативне активности, утврђено је да су етарска уља показала слабу антиоксидативну активност у поређењу са стандардним антиоксидативним једињењима. Добијени резултати су очекивани с обзиром на чињеницу да су главне компоненте у оба узорка угљоводонични монотерпени.

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