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Chemical Composition, Antimicrobial and Antioxidant Activities of *Seseli pallasii* Besser. (syn *Seseli varium* Trev.) Essential Oils

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Abstract: The chemical composition, antimicrobial and antioxidant activities of essential oils obtained from vegetative parts (roots, stems and fruits) of *Seseli pallasii* Besser. were studied. *S. pallasii* essential oils obtained by hydrodistillation of different vegetative parts, were analyzed by GC and GC-MS, applying the liquid injection mode. These results were compared with the corresponding chemical composition of volatiles achieved by the "headspace" injection mode, followed by GC and GC-MS (HS-GC-MS). The main constituents identified in *S. pallasii* stems and fruits essential oils were monoterpenes, with *α*-pinene as major constituent (27.3% and 84.7%, respectively). Saturated hydrocarbons comprise 58.5% of *S. pallasii* root essential oil, while 34.5% were monoterpenes. The major constituents identified in *S. pallasii* root essential oil were *n*-nonane (45.2%), (*Z*)-*β*-ocimene (34.5%) and *n*-undecane (13.3%).The antimicrobial activity of the essential oils was screened against a panel of four bacterial and two fungal strains (*E. coli*, *P. aeruginosa*, *B. cereus*, *S. aureus*, *C. albicans* and *A. niger*). The oils showed moderate to high activity against all strains estimated via minimum inhibitory concentration (MIC) ranging from 21.9 to 416 μg mL^{-1} and minimum bactericidal concentration (MBC) ranging from 54.2 to 582.4 μg mL^{-1}. On the other hand, *S. pallasii* essential oils showed very low antioxidant activity.

Keywords: Essential oil, GC-MS; head-space; antimicrobial; antioxidant; *Seseli pallasii*.

1. Introduction

Apiaceae Lindl. (Umbelliferae Juss.) represents a large family of flowering plants, with up to 455 genera distributed primarily in temperate regions [1]. A curious feature about the Apiaceae species is that more than 50% of species are placed in small number of large genera (*Angelica*, *Ferula*, *Peucedanum*, *Pimpinella* and *Seseli*); other species are placed in a large number of small genera [2]. Family Apiaceae is represented by 82 genera and 334 species in Balkan Peninsula [3].

Genus *Seseli* L. (Apiaceae) contains herbaceous perennial plants, with flowers arranged in compound umbels, with bracts few or absent. Petals are white or yellow, and the fruit ovoid or ellipsoid. Genus *Seseli* is distributed in the Irano-Turanian, Euro-Siberian and East Mediterranean

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geographical region [4,5]. Total number of *Seseli* taxa throughout the world is about 125-140, with 101 species found in Asia and 9 in Turkey [1]. According to [6] genus *Seseli* contribute with 10 species to flora of Serbia.

Seseli pallasii Besser. (syn. *Seseli varium* Trev.) is a glabrous biennial or perennial plant that reaches a height of 30-120 cm. Its leaves vary from 2- to 4-pinnate linear to almost filiform. Its petals are white and glabrous, and its fruit is ellipsoid or oblong, glabrous or slightly tuberculate-verrucose. This taxon is distributed in the north of Italy, the Czech Republic and Slovakia eastwards to central Ukraine [7].

On the basis of previously conducted chemical studies, its known that *Seseli* species are source of coumarins [8-11], sesquiterpenes and phenyl propanoides [12-14]. Previous analyses of the essential oils from various *Seseli* species demonstrated that the main components of essential oils are quite different from species to species: sabinene, *α*-pinene and *β*-phellandrene in *S. globiferum* aerial parts [15]; *α*-pinene, *β*-pinene, and limonene in *S. peucedanoides* (MB) Kos.-Pol [16]; germacrene D, sabinene, (*Z*)-*β*-ocimene and limonene in *S. annuum* aerial parts [17]; *β*-pinene, 4-*α*-hydroxygermacra-1(10)-5-diene and *α*-pinene in *S. resinosum* fruits and (*E*)-sesquilavandulol, sabinene, *α*-pinene and *β*-phellandrene in *S. tortuosum* fruits [18].

Essential oils may play active role through exhibiting cytotoxic but usually nongenotoxic effects, because of evidenced pro oxidant effects on the cellular level [19]. Among plant secondary metabolites, polyphenols are known to have a high capacity to scavenge free radicals due to the hydrogen and electron transfer abilities and in that way are considered as prevailing responsible for antioxidant action. High content of non-phenolic compounds (monoterpenes and sesquiterpenes) in most essential oils might be related to their weak antioxidant activity [20]. Lately, one of the major problems in medicine has become increasingly prominent bacteria resistance to the antibiotics, mostly due of pretty good bacterial mechanisms of genetic adjustment, resulting with antibiotic resistance [21]. However, while affecting the pathogenic bacteria, antibiotics also non-selectively affect non-pathogenic bacteria, causing unpredictable genetic changes [22]. This is the main reason of increased interest in ongoing search for new agents with antibiotic effects. As a fairly potent sources of antimicrobial agents are aromatic and essential oil rich plants, traditionally used for the treatment of many diseases, primarily infectious ones [23].

To the best of authors' knowledge, this study is the first report on the chemical composition of the essential oils and volatiles of vegetative parts (roots, stems and fruits) of *S. pallasii*, accomplished by GC, GC-MS of essential oils and head-space GC-MS of corresponding plant parts volatiles. Also, the study of antimicrobial and antioxidant action of the essential oils of vegetative parts of *S. pallasii* was conducted for the very first time.

2. Materials and Methods

2.1. Plant material

Roots and aerial parts of *S. pallasii* were collected in August 2013 from area of Kravlje, Eastern Serbia. The plant material was collected and identified by Marija Marković, PhD of botany, and the voucher specimen was deposited in the Herbarium Moesiacum Nis (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Nis under the acquisition number 7211.

Air dried plant material was cut into small pieces and subjected to hydrodistillation for 2 hours using a Clevenger type apparatus.

2.2. GC-MS and GC-FID analyses

The GC-MS analysis of the samples was carried out by a 7890/7000B GC/MS/MS triple quadrupole system (Agilent Technologies, USA) equipped with a CombiPal sampler and Headspace Upgrade for G6501B/G6509B). The fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30m × 0.25 mm, film thickness 0.25 μm) was used.

The injector and interface operated at 250°C and 300°C, respectively. Temperature program: from 50°C to 290°C at a heating rate of 4°C min^{-1}. The carrier gas was helium with a flow of 1.0 mL min^{-1}. The 500 μL sample were injected for HS and, 1 μL of the oil solutions in hexane (1:100), were

injected (the flow rate was 1.0 mL min^{-1}, split ratio 40:1). Post run: back flush for 1.89 min, at 280°C, with helium at 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-500 *m/z*, scan time 0.32 s.

The GC-FID analyses were carried out under the same experimental conditions and using the same column as described for the GC-MS analyses. The relative content in percent of each component was computed from the GC peak areas without the use of correction factors.

For head space (HS) experiment 300 mg of grained dried plant material was put into 20 mL HS vial than soaked with 2 mL of distilled water. The sample was heated at 80°C for 20 minutes with the next mixing program: shaking for 3 seconds, pause for 2 seconds. 500 μL of vapor generated from the aerial parts 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).

2.3. Identification of volatile compounds

Oil constituents were identified by comparison of their linear retention indices (relative to C_8-C_{20} and C_{21}-C_{44} alkanes [24] on the HP-5MS column) with literature values [25], and their MS with those of authentic standards, as well as those from Wiley 6, NIST11, Agilent Mass Hunter Workstation B.06.00 software [26], and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils by the application of the AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA/NIST, 2011). Some components were identified by co-injection with an authentic sample.

2.4. Antimicrobial activity

Antimicrobial activity determination was performed by a microdilution method, presented as a consensus standard by the NCCLS (National Committee for Clinical Laboratory Standards, 2003). Six overnight culture of laboratory control strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 16404 and *Aspergillus niger* ATCC 10231 obtained from the American Type Culture Collection, were used for the preparation of suspension (0.5 McFarland standard turbidity) [27]. A serial doubling dilution of the extracts (in 10% DMSO) was prepared in a 96/well microtiter plate in inoculated nutrient broth (the final volume - 100 mL and final concentration 106 CFU/mL in each well). The plate was incubated for 24 h at 37°C. All determinations were performed in triplicate, and two growth controls consisting of nutrient broth and medium with solvent were included. Tetracycline and nystatine served as a positive control. Microbial growth was determined by adding 20 μL of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution [28]. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of extracts at which microorganisms showed no visible growth. In order to determine minimal bactericidal concentration (MBC), broth was taken from each well and inoculated on Mueller Hinton agar (MHA) for 24 h at 37°C. The MBC is defined as the lowest concentration of the extracts at which 99.9% of inoculated microorganisms were killed.

2.5. Antioxidant activity

DPPH "scavenging" radical capacity of samples was determined using DPPH radical [29]. Reaction mixtures of samples were prepared by mixing appropriate amounts of essential oil, 1.5 mL of DPPH and methanol to a total volume of 4 mL. The absorbance at 515 nm was measured after the solution had been allowed to stand in the dark for 30 min. Blank sample was analyzed in the same manner, only instead of sample solution, pure methanol was used and it served as negative control. Butylated hydroxytoluene (BHT) was used as a positive control, performing the experiment as described above. All determinations were performed in triplicate. The EC_{50} values (concentration of essential oil and BHT in the reaction mixture needed to decrease by 50% the initial $DPPH^{\cdot}$ concentration) were determined by polynomial regression analysis of the obtained DPPH-RSC values.

The ferric reducing power was determined according to the method of Oyaizu [30]. The reductive ability of essential oil was measured trough an electron transfer reaction (Fe^{3+}-Fe^{2+}) using a ferric salt as an oxidant agent. Mixtures of appropriate amounts of essential oil, 1 mL of 1% $K_3[Fe(CN)_6]$ solution and 1 mL phosphate buffer (pH 6.6), were incubated at 50°C for 30 minutes. After cooling, 1 mL of 10% trichloroacetic acid were added to mixture in order to stop the reaction,

and after that 0.6 mL of $FeCl_3$ were added and diluted with water to a total volume of 5 mL. Absorbance of obtained mixtures was measured at a wavelength of 700 nm. Increased absorbance of reaction mixture indicates higher reducing capacity. Phosphate buffer was used as blank solution. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. Total reducing power of essential oils was calculated using ascorbic acid calibration curve and expressed as μg of ascorbic acid equivalent per 1 mg of essential oil (μg AAE/1 mg EO).

ABTS radical "scavenging" activity was measured using a modification of the method of Re et al. [28]. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark, to yield a dark colored solution containing ABTS radical cation. Prior to use in the assay, the ABTS radical cation was diluted with methanol to obtain absorbance of about 0.700 (±0.020) at 734 nm. An aliquot of essential oil was mixed with 1.8 mL of diluted ABTS radical cation solution and diluted with methanol to a total volume of 4 mL. After reaction at room temperature for 6 min, the decrease in absorbance was measured at 734 nm. Results are expressed as μg of Trolox equivalents per 1 mg of essential oil sample (μg TE/1 mg EO).

3. Results and Discussion

3.1. Chemical composition of essential oils

Hydrodistilled essential oils of different plant parts of *S. pallasii*, as well as volatiles of the same parts were analyzed and obtained results are summarized in Table 1.

Total of 38, 100 and 49 components were identified in root, stem and fruit essential oils of *S. pallasii*, respectively. The main constituents identified in *S. pallasii* stems essential oil were: *α*-pinene (27.3%), limonene (9.5%), caryophyllene oxide (4.8%), (*E*)-verbenol (3.6%), camphene hydrate (3.3%), camphene (2.8%), (*E*)-caryophyllene (2.7%), *α*-campholenal (2.6%), (*Z*)-*β*-ocimene (2.5%) and (*E*)-pinocarveol (2.0%). *S. pallasii* fruits essential oil consist mainly of monoterpenes (98.7%). Main constituents of *S. pallasii* fruits essential oil were: *α*-pinene (84.7%), *β*-pinene (3.5%), limonene (2.7%) and camphene (2.6%).

Table 1. Chemical composition of the *S. pallasii* roots, stems and fruits essential oils/volatiles achieved by GC, GC-MS and HS-GC-MS.

				Composition (%) - calculated from FID data						
				Roots		**Stems**		**Fruit**		
No	**RL**	**RI**	**Compound**	**GC-MS**	**HS/GC-MS**	**GC-MS**	**HS/GC-MS**	**GC-MS**	**HS/GC-MS**	**Id**
1	765	779	3-Methyl-2-buten-1-ol	-	-	t	-	t	-	a,b
2	800	800	*n*-Octane	-	0.2	-	-	-	-	a,b
3	801	800	Hexanal	0.1	-	0.1	0.3	t	-	a,b
4	846	850	(2*E*)-Hexenal	-	-	t	0.2	t	-	a,b
5	863	865	*n*-Hexanol	t	-	0.1	-	t	-	a,b
6	900	900	*n*-Nonane	45.2	63.6	0.4	1.3	t	-	a,b,c
7	901	901	Heptanal	-	-	t	-	t	-	a,b
8	907	909	(2*E*,4*E*)-Hexadienal	-	-	0.2	-	-	-	a,b
9	921	923	Tricyclene	t	t	t	-	-	-	a,b
10	924	928	*α*-Thujene	-	-	t	-	0.1	t	a,b
11	932	936	*α*-Pinene	0.5	2.8	27.3	71	84.7	93.7	a,b,c
12	946	950	Camphene	0.5	1.2	2.8	2.4	2.6	2	a,b,c
13	953	956	Thuja-2,4(10)-diene	-	-	0.2	-	t	-	a,b
14	952	961	Benzaldehyde	-	-	0.1	-	-	-	a,b
15	969	975	Sabinene	-	-	0.3	t	0.8	0.3	a,b,c

16	974	979	*β*-Pinene	t	t	1.3	2	3.5	2.1	a,b,c
17	981	987	6-methyl-5-hepten-2-one	-	-	0.1	-	-	-	a,b
18	988	992	Myrcene	0.7	0.8	1.9	4.9	1.2	0.4	a,b,c
19	1000	1000	*n*-Decane	0.1	0.1	0.2	-	-	-	a,b
20	998	1004	*n*-Octanal	0.2	-	-	-	-	-	a,b
21	1002	1007	*α*-Phellandrene	-	-	0.2	-	0.5	0.1	a,b
22	1008	1013	*δ*-3-Carene	t	1.3	2.1	5.9	0.9	0.4	a,b
23	1020	1027	*p*-Cymene	0.1	0.1	1.7	0.9	t	-	a,b
24	1022	1027	*o*-Cymene	-	-	-		0.4	0.1	a,b
25	1024	1031	Limonene	0.6	0.8	9.5	10.6	2.7	0.8	a,b,c
26	1032	1039	(*Z*)-*β*-Ocimene	34.5	22.3	2.5	-	0.1	-	a,b
27	1030	1039	(3*E*)-Octen-2-one	-	-	t	-	-	-	a,b
28	1036	1045	Benzeneacetaldehyde	0.1	-	0.4	-	t	-	a,b
29	1044	1049	(*E*)-*β*-Ocimene	0.4	0.2	0.3	-	0.1	-	a,b
30	1049	1058	(2*E*)-Octen-1-al	-	-	0.2	-	-	-	a,b
31	1054	1061	*γ*-Terpinene	t	-	0.5	-	0.2	t	a,b
32	1063	1070	*n*-Octanol	-	-	0.1	-	t	-	a,b
33	1073	1074	(*Z*)-Linalool oxide	-	-	0.1	-	-	-	a,b
34	1086	1091	Terpinolene	t	t	0.4	-	0.1	-	a,b
35	1092	1094	(*Z*)-4-Undecene	0.3	-	-	-	-	-	a,b
36	1095	1092	*α*-Pinene oxide	-	-	0.3	-	-	-	a,b
37	1100	1100	*n*-Undecane	13.3	6.5	0.5	-	-	-	a,b
38	1100	1104	*n*-Nonanal	0.1	-	0.4	-	t	-	a,b
39	1114	1115	endo-Fenchol	-	-	0.1	-	-	-	a,b
40	1119	1122	(*E*)-*p*-mentha-2,8-dien-1-ol	-	-	0.3	-	-	-	a,b
41	1122	1128	*α*-Campholenal	-	-	2.6	-	0.1	-	a,b
42	1128	1130	allo-Ocimene	0.7	-	-	-	-	-	a,b
43	1128	1132	(*Z*)-Epoxy-ocimene	0.1	-	-	-	-	-	a,b
44	1132	1133	(*Z*)-Limonene oxide	-	-	0.2	-	-	-	a,b
45	1133	1137	(*Z*)-*p*-mentha-2,8-dien-1-ol	-	-	0.2	-	-	-	a,b
46	1135	1142	(*E*)-Pinocarveol	-	-	2.0	-	0.2	-	a,b
47	1140	1142	neo-allo-Ocimene	0.1	-	-	-	-	-	a,b
48	1137	1144	(*Z*)-Verbenol	-	-	0.6	-	-	-	a,b
49	1140	1148	(*E*)-Verbenol	-	-	3.3	-	0.2	-	a,b
50	1145	1152	Camphene hydrate	-	-	0.5	-	-	-	a,b
51	1150	1154	(2*E*,6*Z*)-Nonadienal	-	-	0.2	-	-	-	a,b
52	1152	1159	pentyl-Benzene	0.2	-	-	-	-	-	a,b
53	1154	1157	*β*-Pinene oxide	-	-	0.1	-	-	-	a,b
54	1156	1160	Pentyl cyclohexa-1,3-diene	0.2	-	-	-	-	-	a,b
55	1157	1160	(2*E*)-Nonen-1-al	-	-	0.2	-	t	-	a,b
56	1158	1164	(*E*)-Pinocamphone	-	-	t	-	-	-	a,b
57	1160	1166	Pinocarvone	-	-	0.4	-	0.1	-	a,b
58	1166	1169	*p*-Mentha-1,5-dien-8-ol	-	-	1.4	-	-	-	a,b
59	1174	1180	Terpinen-4-ol	-	-	0.9	-	0.1	-	a,b
60	1179	1186	*p*-Cymen-8-ol	-	-	0.6	-	t	-	a,b
61	1187	1189	(*E*)-*p*-Mentha-1(7),8-dien-2-ol	-	-	0.1	-	-	-	a,b

62	1186	1192	*α*-Terpineol	-	-	1.1	-	t	-	a,b
63	1194	1195	Myrtenol	-	-	1.3	-	0.1	-	a,b
64	1195	1199	Myrtenal	-	-	-	-	t	-	a,b
65	1212	1204	Homomyrtenol	-	-	0.5	-	-	-	a,b
66	1204	1212	Verbenone	-	-	0.9	-	t	-	a,b
67	1215	1220	(*E*)-Carveol	-	-	1.5	-	t	-	a,b
68	1227	1230	(*Z*)-*p*-Mentha-1(7),8-dien-2-ol	-	-	0.1	-	-	-	a,b
69	1226	1232	(*Z*)-Carveol	-	-	0.1	-	-	-	a,b
70	1232	1236	(3*Z*)-Hexenyl 3-methyl butanoate	-	-	0.3	-	-	-	a,b
71	1241	1241	Hexyl isovalerate	-	-	0.2	-	-	-	a,b
72	1241	1246	Carvacrol, methyl ether	-	-	-	-	t	-	a,b
73	1239	1247	Carvone	-	-	0.3	-	-	-	a,b
74	1260	1262	(2*E*)-Decenal	0.1	-	0.2	-	-	-	a,b
75	1292	1294	(2*E*,4*Z*)-Decadienal	t	-	0.3	-	-	-	a,b
76	1294	1300	Perilla alcohol	-	-	0.1	-	-	-	a,b
77	1300	1300	Tridecane	t	-	-	-	-	-	a,b
78	1315	1317	(2*E*,4*E*)-Decadienal	0.2	-	0.6	-	t	-	a,b
79	1334	1336	Linalool propanoate	-	-	0.1	-	-	-	a,b
80	1357	1364	(2*E*)-Undecenal	-	-	0.1	-	-	-	a,b
81	1374	1382	*α*-Copaene	-	-	0.2	-	-	-	a,b
82	1387	1391	*β*-Bourbonene	-	-	0.3	-	t	-	a,b
83	1424	1426	2,5-dimethoxy-*p*-Cymene	t	-	-	-	-	-	a,b
84	1417	1427	(*E*)-Caryophyllene	-	-	2.7	-	0.2	-	a,b
85	1440	1439	Butanoic acid, 3-methyl-, octyl ester	-	-	0.3	-	-	-	a,b
86	1432	1440	*α*-(*E*)-Bergamotene	0.1	-	-	-	-	-	a,b
87	1439	1449	Aromadendrene	-	-	0.1	-	-	-	a,b
88	1453	1454	Geranyl acetone	-	-	0.2	-	-	-	a,b
89	1452	1461	*α*-Humulene	-	-	0.2	-	-	-	a,b
90	1478	1483	*γ*-Muurolene	-	-	0.5	-	-	-	a,b
91	1484	1488	Germacrene D	-	-	1.2	-	0.4	-	a,b,c
92	1490	1494	Phenyl ethyl 3-methyl butanoate	-	-	0.8	-	-	-	a,b
93	1492	1497	*δ*-Selinene	-	-	-	-	0.1	-	a,b
94	1496	1498	*α*-Zingiberene	t	-	-	-	-	-	a,b
95	1495	1501	*γ*-Amorphene	-	-	0.1	-	-	-	a,b
96	1500	1504	Bicyclogermacrene	-	-	0.5	-	0.1	-	a,b
97	1500	1506	*α*-Muurolene	-	-	0.2	-	t	-	a,b
98	1505	1512	*β*-Bisabolene	0.1	-	-	-	-	-	a,b
99	1513	1521	*γ*-Cadinene	-	-	0.3	-	-	-	a,b
100	1521	1528	*β*-Sesquiphellandrene	t	-	-	-	-	-	a,b
101	1522	1530	*δ*-Cadinene	-	-	0.8	-	t	-	a,b
102	1529	1536	(*E*)-*γ*-Bisabolene	0.1	-	-	-	-	-	a,b
103	1537	1544	*α*-Cadinene	-	-	0.1	-	-	-	a,b
104	1544	1551	*α*-Calacorene	-	-	0.1	-	-	-	a,b
105	1549	1560	Salviadienol	-	-	0.3	-	-	-	a,b
106	1571	1579	Caryolan-8-ol	-	-	0.5	-	-	-	a,b
107	1597	1579	Widdrol	-	-	-	-	0.1	-	a,b

108	1577	1587	Spathulenol	-	-	1.4	-	0.1	-	a,b
109	1582	1592	Caryophyllene oxide	-	-	4.8	-	0.2	-	a,b,c
110	1594	1611	Butanoic acid, 3-methyl-, 3-phenylpropyl ester	-	-	0.4	-	-	-	a,b
111	1608	1618	Humulene epoxide II	-	-	0.3	-	-	-	a,b
112	1630	1636	Muurola-4,10(14)-dien-1-β-ol	-	-	0.5	-	-	-	a,b
113	1632	1643	3-butyl hexahydro-Phthalide	0.1	-	-	-	-	-	a,b
114	1639	1645	Caryophylla-4(12),8(13)-dien-5-α-ol	-	-	0.6	-	-	-	a,b
115	1644	1649	α-Muurolol	-	-	0.3	-	-	-	a,b
116	1645	1653	Cubenol	-	-	0.1	-	-	-	a,b
117	1652	1662	α-Cadinol	-	-	0.2	-	-	-	a,b
118	1666	1665	14-hydroxy-(*Z*)-Caryophyllene	-	-	0.2	-	-	-	a,b
119	1668	1678	14-hydroxy-9-epi-(*E*)-Caryophyllene	-	-	0.4	-	-	-	a,b
120	1685	1694	Germacra-4(15),5,10(14)-trien-1-α-ol	-	-	0.3	-	-	-	a,b
121	1722	1734	(*E*)-Neocnidilide	1	-	-	-	-	-	a,b
122	1846	1846	Hexahydrofarnesyl acetone	-	-	0.6	-	-	-	a,b
			Total	**99.7**	**99.9**	**95.1**	**99.5**	**99.9**	**99.9**	
			Monoterpenoids (M)	38.2	29.5	70.6	97.7	98.7	99.9	
			hydrocarbons	38.1	29.5	51	97.7	97.9	99.9	
			oxygenated	0.1	-	19.6	-	0.8	-	
			Sesquiterpenoids (S)	0.3	-	16.9	-	1.2	-	
			hydrocarbons	0.3	-	7.3	-	0.8	-	
			oxygenated	-	-	9.6	-	0.4	-	
			Others (O)	61.2	70.4	7.6	1.8	t	-	

*Compounds listed in order of elution on HP-5MS column; RL: literature retention indices [25]; RI: experimentally determined retention indices on the mentioned column by co-injection of a homologous series of n-alkanes C_8–C_{20} and C_{21}–C_{44}; a: constituent identified by mass spectral comparison; b: constituent identified by retention index matching; c: constituent identity confirmed by co-injection of an authentic sample; t: trace (< 0.05%); -: not detected.

Comparing *S. pallasii* aerial parts essential oils main constituents with other previously investigated species of *Seseli* genus we noticed that there are some similarities in the main constituent composition between different species. The main similarity between aerial parts essential oils from different species of *Seseli* genus and *S. pallasii* appears to be occurrence of α-pinene as main constituent, while presence of the other constituents varies between different species. α-pinene (35.9%), sabinene (8.8%), (*E*)-sesquilavandulol (8.4%) and β-pinene (7.0%) are the main constituents of *S. tortuosum* grown in Turkey [31]; Balkan endemic species *Seseli rigidum* Waldst. & Kit in the volatile oil from aerial parts and fruit also contains α-pinene as predominant component(57.4% and 23.3%, respectively). In the aerial parts of the same species, limonene (6.7%), camphene (5.8%) and sabinene (5.5%) are present in high amounts, while in the fruit oil, β-phellandrene (17.4%) and sabinene (12.9%) were found in significant quantities [32]; The main components of *S. campestre* Besser essential oil are α-pinene (38.6%), β-pinene (17.5%) and (*E*)-sesquilavandulol (10.3%) [33]; essential oils of crushed fruits and herbal parts of *S. campestre* Besser mainly contain α-pinene (26.2% and 35.8%, respectively) and (*E*)-sesquilavandulol (11.8% and 3.2% respectively) [34].

However, main constituents of aerial parts essential oils of many other species of *Seseli* genus are different in comparison to *S. pallasii*. Marongui at al. [35] investigated composition of leaves essential oils from *S. bocconi* Guss. subsp. *praecox* Gamisans, collected from different zones of Sardinia. *S. bocconi* from Buggerru main constituents are: himachalol (16.4%), sabinene (14.8%), β-phellandrene (8.1%) and (*Z*)-sabinene hydrate (4.5%); dominant components of *S. bocconi* from Carloforte are β-phellandrene (29.2%), undecane (9.6%), α-pinene (6.1%) and β-guaiene (5.7%), while in *S. bocconi* from Oligastra, α-humulene (17.7%), γ-himachalene (9.3%), β-phellandrene (8.0%) and bicyclogermacrene (7.7%) are the most represented. In the essential oil of aerial parts of *S. annuum*

grown in Serbia [17], *β*-phellandrene is the major component in the flower and stem oils (47.5 and 63.1%). Sabinene is the main compound in the leaf, stem and flower essential oils oils from the *S. rigidum* Waldst from Bulgaria (39.8%, 6.5% and 19.8%, respectively) [36]. *S. petraeum* fruits essential oil main constituents are carotol (20.7%), *γ*-terpinene (11.3%), sabinene (9.5%) and germacrene D (7.8%) and for *S. andronakii* fruit essential oil: carotol (52.7%) and germacrene D (8.7%) [37].

Our findings about *S. pallasii* root essential oil main constituents were: *n*-nonane (45.2%), (*Z*)-*β*-ocimene (34.5%) and *n*-undecane (13.3%). Comparing *S. pallasii* root essential oil main constituents to the rest of *Seseli* species, there are no obvious similarities between them. The principal component of root essential oil of *S. rigidum* Waldst. from Bulgaria is (*Z*)-falcarinol (48.7%), followed by sabinene (12.4%) and elemol (8.7%) [36]. On the contrary, the root essential oil of *S. rigidum* Waldst. & Kit. from different regions of Serbia is composed almost entirely of the falcarinol (88.8%) [32]. Saturated hydrocarbons comprise 58.5% of *S. pallasii* root essential oil, and among them 34.5% are monoterpenes. In contrast, *S. rigidum* Waldst. Root essential oil mainly consists of polyacetylene falcarinol.

HS-GC-MS volatiles analysis resulted in total of 15, 11 and 11 components identified in roots, stems and fruits, respectively. *n*-nonane and (*Z*)-*β*-ocimene (63.7% and 22.3%) were the main constituents of root volatiles; *α*-pinene, limonene, *δ*-3-carene and myrcene (71%, 10.6%, 5.9% and 4.9% respectively) were the main constituents of stem volatiles while *α*-pinene and *β*-pinene (93.7% and 2.1%) were identified as main constituents of fruit volatiles.

Comparing the chemical composition of essential oils obtained by GC, GC-MS and volatiles chemical composition obtained by HS-GC-MS, we found out that a number of components identified by GC, GC-MS is considerably higher in comparison to number of components identified by HS-GC-MS, which lead us to observation that only the most volatile components were identified by HS-GC-MS.

3.2. Antimicrobial activity

As shown in Table 2, the essential oils exhibited moderate to high antimicrobial effect against all strains tested, with minimum inhibitory concentration (MIC) ranging from 21.9 to 416 μg mL^{-1} and minimum bactericidal concentration (MBC) ranging from 54.2 to 644.8 μg mL^{-1}. Manifested activity can be considered as a very good in comparison to the effects of referent antibiotics (tetracycline was active against bacterial strains in the range of 0.7-4.0 μg mL^{-1} while nystatin expresses activity against fungal strains in the range of 8.0-12.0 μg mL^{-1}). Although the essential oil of the present species showed both antifungal and antibacterial activity, MIC values for bacteria were lower than that of fungi which indicates the higher antibacterial potential of the essential oils against tested bacteria. Root essential oil was the most active against all tested bacterial and fungal strains, followed by fruit essential oil and as the least active was stem essential oil. The only exception was *S. aureus*, against which as the most active was proven fruit essential oil, followed by root and finally as the least active-stem essential oil.

S. pallasii root and fruit essential oils showed remarkable antimicrobial effect against all Gram-positive and Gram-negative bacteria tested. The best activity, root oil demonstrated against bacterial strain *B. cereus* (MIC=21.9 μg mL^{-1}, MBC=87.8 μg mL^{-1}), while the strongest effect of fruit oil was against *S. aureus* (MIC=27.1 μg mL^{-1}, MBC=54.2 μg mL^{-1}). The Gram-negative bacteria, *E. coli*, appears to be less sensitive in comparison to *P. aeruginosa* to the action of root and stem EO, and equally susceptible to fruit EO. Among Gram-positive bacteria as more sensitive to the action of root EO was *B. cereus*, the same in case of stem EO and the less sensitive to fruit EO. These results could be associated with major components present in essential oils of *S. pallasii* vegetative plant parts. Major component of fruit essential oil, *α*-pinene (84.7%), have been reported to display strong antibacterial effects against several microorganisms [19, 38, 39]. In addition, the components present at lower amounts such as, limonene, camphene, *δ*-carene, *β*-pinene and *p*-cymene could also contribute to the antimicrobial activity of plant essential oils by synergism with the other active components [40]. It was also proved that type of alkyl group influences activity, favoring alkenyl over alkyl, which further can be exploited to explain higher activity of limonene than *p*-cymene. On the other side, presence of hydroxyl group in compound moiety is strongly associated to increased antimicrobial activity [38, 41, 42]. It seems, action of hydroxyl derivatives, presented in stem EO was

neutralized and even exceeded by alkyl and alkenyl derivatives, predominantly represented in fruit and root EO, resulting in better antimicrobial properties of last two EO. The possible mechanism of action is through accumulation of the lipophilic hydrocarbon molecules in the cell lipid bi-layer, distorting lipid-protein interaction or directly interacting with hydrophobic protein parts [43,44], enabling to the rest of constituents of EO easier transfer to the cell interior. Considering abundance of non-polar hydrocarbons in the root EO, previously discussed mechanism could be applied for explication of strong activity of root EO against tested bacteria. The present study indicates that *S. pallasii* essential oils exhibited considerable antimicrobial activities and potentials for possible application in various area associated with food, medicinal and pharmaceutical issues.

Table 2. Antimicrobial activity of essential oils of different plant parts of *S. pallasii* and referent antibiotics.

Essential oils		**Gram (-) bacteria**		**Gram (+) bacteria**		**Fungi**	
	Plant part	*Escherichia coli* *ATCC 25922* (MIC/MBC) μg mL^{-1}	*Pseudomonas aeruginosa* ATCC 27853 (MIC/MBC) μg mL^{-1}	*Staphylococcus aureus* *ATCC 6538* (MIC/MBC) μg mL^{-1}	*Bacillus cereus* ATCC 10876 (MIC/MBC) μg mL^{-1}	*Candida albicans* ATCC 16404 (MIC/MBC) μg mL^{-1}	*Aspergillus niger* *ATCC 10231* (MIC/MBC) μg mL^{-1}
	Root	43.9/175.6	35.1/70.2	57/228.3	21.9/87.8	87.8/263.4	175.6/245.8
	Stem	83.2/166.4	104.0/166.4	104.0/208	104.0/208	416.0/644.8	416.0/582.4
	Fruit	54.2/216.8	54.2/108.4	27.1/54.2	54.2/216.8	216.8/433.6	216.8/433.6
Tetracycline		2.0/4.0	2.0/4.0	0.7/1.4	1.8/1.8	-	-
Nystatine		-	-	-	-	8.0/8.0	12.0/12.0

3.3. Antioxidant activity

Obtained results for antioxidant activity of essential oils from different parts of *S. pallasii* are summarized in Table 3. According to the results, root essential oil of *S. pallasii* exhibited the highest DPPH radical scavenging activity, manifested with the lowest EC_{50} value of 107.1 mg mL^{-1}, though in comparison with positive control BHT (EC_{50}=3.45±0.17 mg mL^{-1}), essential oils showed very low DPPH radical scavenging activity.

Table 3. DPPH radical scavenging activity (EC_{50}), reducing power (AAE) and ABTS radical scavenging activity of essential oils of different plant parts.

Plant part	**EC_{50}** mg mL^{-1}	**AAE** μg AAE/1 mg EO	**ABTS** μg TE/1 mg EO
Roots	107.10±1.32	0.38±0.05	1.11±0.11
Stems	329.11±2.36	0.31±0.03	1.32±0.10
Fruits	451.23±1.56	0.32±0.04	1.20±0.12

Results for reducing power and ABTS radical scavenging activity showed that there is a little difference in antioxidant activity between essential oils from different parts of *S. pallasii*. In general, essential oils of *S. pallasii* plant parts have proven to act as very weak antioxidants, which is not surprising, having in mind their chemical composition in which are mostly prevailing monoterpene hydrocarbons, the class of compounds with evidenced very low antioxidant activity.

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