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Composition and Antimicrobial Activity of *Seseli globiferum* Essential Oil

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The essential oil from aerial parts of *Seseli globiferum* Vis. obtained by hydrodistillation with Clevenger-type apparatus was analyzed by GC-MS. Twenty-eight compounds were identified, representing 99.4% of the total oil. The main components of the oil were sabinene (38.0%), α-pinene (21.2%) and β-phellandrene (13.5%). The microbial growth inhibitory properties of the isolated essential oil were determined using the broth microdilution method against seven bacterial species: *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (clinical isolates), *Bacillus cereus* (clinical isolates), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus flavus* (ATCC 10240) and three fungal species: *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Trichoderma viride* (IAM 5061) and *Penicillium funiculosum* (ATCC 36839). The essential oil showed activity against bacteria *P. aeruginosa*, followed by *M. flavus, L. monocytigenes* and *E. coli*, and all investigated fungal species.

Keywords: *Seseli globiferum*, Apiaceae, essential oil, antibacterial activity, antifungal activity.

The genus *Seseli* L. is comprised of 55 species distributed mainly in Europe. *Seseli globiferum* Vis. (Apiaceae) is native to western part of Balkan Peninsula (Croatia, Bosnia and Herzegovina and Montenegro), where it inhabits dry rocky places on limestone, at altitudes up to 1000 m above sea level [1].

Species from the genus *Seseli* L. have long been used in tradition medicine in Mediterranean region [2]. *Seseli* species are well-known source of the linear or angular pyranocoumarins possessing antiproliferative, antiviral and antibacterial activities [3]. Traditional uses of *Seseli* species as anti-inflammatory agents were supported by the results of Kupeli [4]. *S. tortuosum* was used as a contraceptive in the 13th century AD in Saudi Arabia [5]. Pharmacodynamic effects of volatile fractions isolated from *S. sibiricum* [6] and *S. indicum* [7] have been studied. Essential oil of *S. annuum* was reported to have significant cytotoxic capacity [8], as well as fungicidal effect [9]. Chemical composition, antimicrobial and antiradical properties of essential oil from fruits of *S. globiferum* have also been investigated [10].

Even though some reference data exist, scientific study concerning aspects of the therapeutic uses of *S. globiferum* essential oil, as well as its chemical composition, remains scarce and incomplete. Because of these facts, the aim of this investigation was to determine the chemical composition of the essential oil of *S. globiferum*, and to assess its antimicrobial activity.

Air-dried flowering parts of *S. globiferum* yielded 0.28% of pale yellow essential oil with pungent scent. GC-MS analysis resulted in the identification of 25 compounds, representing 99.4% of total oil. All components are listed in Table 1, in order of their elution. The essential oil of *S. globiferum* was characterized by exceptionally high percentage of sabinene (38.0%) and α-pinene (21.2%), followed by the β-phellandrene (13.5%), myrcene (6.0%), terpinen-4-ol (3.6%), β-pinene (3.0%) and camphene (2.6%).

Table 1: Composition of *Seseli globiferum* essential oil.

Compound	KI	%
α-Pinene	923	21.2
Camphene	939	2.6
Sabinene	978	38.0
β-Pinene	980	3.0
Myrcene	996	6.0
α-Phellandrene	1008	1.5
Kar-3-en	1013	0,5
α-Terpinene	1018	0.5
p-Cymene	1021	1.1
β-Phellandrene	1032	13.5
trans-β-ocimene	1046	1.1
γ-Terpinene	1056	1.1
Terpinolene	1084	0.5
Terpinen-4-ol	1191	3.6
Citronellal	1232	0.3
Bornyl acetate	1289	0.4
α-Ulangene	1378	0.1
β-caryophyllene	1424	1.6
Germacrene D	1486	0.2
Bicyclogermacrene	1501	0.4
Elemol	1555	1.0
Caryophyllene oxide	1587	0.3
Guaiol	1602	0.2
α-Eudesmole	1656	0.3
γ-Eudesmole	1660	0.4
Total		99.4

KI, Kovats Indices relative to n-alkanes on HP-5 MS.
%, Relative percentage obtained from peak area.

In recent report on the essential oil composition of unripe and ripe fruits of *S. globiferum* a total of 42 and 35 compounds were identified respectively [10]. Principle components of essential oil of unripe fruits were sabinene (53.1%), γ-terpinene (7.7%), α-pinene (7.2%), β-phellandrene (5.0%), α-terpinene (4.1%) and α-thujene (3.8%). The essential oil of ripe fruits also contained sabinene as main component (65.3%), followed by γ-terpinene (6.6%), β-phellandrene (4.9%), α-thujene (4.5%), α-pinene (4.4%) and α-terpinene (4.3%)[10]. β-pinene (37.5%), 4α-hydroxygermacra-1(10)-5-diene (21.7%) and α-pinene (13.7%) were main constituents of *S. resinosum* Freyn et Sint. essential oil, while (*E*)-sesquilavandulol (37.0%), sabinene (19.7%), α-pinene (13.5%) and β-phellandrene (7.8%) were the principal constituents of *S. tortuosum* L. essential oil [11]. Essential oil from the aerial parts of *S. rigidum* W et K. contained α-pinene (53.3%), limonene (10.0%) and germacrene D (9.3%) as major constituents [12]. Analyses of *S. peucedanoides* (MB) Kos. –Pol. essential oil revealed 46 compounds representing 96.3% of the oil, with α-pinene (69.4%), β-pinene (4.9%) and limonene (4.6%) as principal compounds [13]. Essential oils of *S. buchtormense* (Ficsh. ex Sprengel) Koch. obtained from the Altai Region, possessed sabinene (17.7-25.1%), α-pinene (5.3-14.6%), (*E*)-nerolidol (5.5-11.6%) and β-phellandrene (2.5-7.0%) as major compounds [14]. The principal compounds in the essential oil from the aerial parts of *S. macrophyllum* Regel ex Schmalh were *p*-cymene (27.2%), thymol(15.1%), carvacrol (12.2%) and pulegone (8.2%) [15]. Himachalol (16.4%) and sabinene (14.8%) were the major compounds of the essential oil of *S. bocconi* Guss [16]. From *S. libanotis* (L.) W.D.J. Koch, concentrations of the dominant compounds were – sabinene 46.2% (vs. 37.4% by HD), β-phellandrene 23.5% (vs. 19% by HD)[17] respectively. Also (+)-spatulene (7.7%) and (-)-bornyl acetate (5.2%) were found in this species [18].

Table 2: Antibacterial activity of *Seseli globiferum* essential oil and streptomycin.

Bacteria	Essential oil		Streptomycin	
	MIC	MBC	MIC	MBC
M. flavus	1.25	10.0	50	100
B. cerues	2.5	5.0	50	50
L. monocytogenes	1.25	10.0	100	100
S. aureus	-	-	50	100
E. coli	1.25	2.5	50	100
En. cloacae	-	-	50	100
S. epidermidis	-	-	100	200
P. aeruginosa	0.5	1.0	200	400

MICs and MBCs (μL/mL), mean value of two measurements.
*Streptomycin was used as stock solution 0.1 mg mL^{-1}.

Table 3: Antifungal activity of *Seseli globiferum* essential oil and bifonazole.

Fungi	Essential oil		Bifonazole	
	MIC	MFC	MIC	MFC
A. flavus	2.5	10.0	100	100
A. niger	5.0	10.0	100	100
A. versicolor	2.5	5.0	100	100
P. funiculosum	5.0	10.0	150	200
T. viride	5.0	10.0	150	200

MICs and MFCs (μL/mL), mean value of two measurements.
*Bifonazole was used as a stock solution 1 mg mL^{-1}.

The antimicrobial activity results are expressed as minimal inhibitory concentration (MIC) for the essential oil applied in four concentrations for bacteria (Table 2) and seven for fungi (Table 3) and minimum bactericidal and fungicidal concentration (MBC/MFC). The best inhibitory and bactericidal effect was detected against *Pseudomonas aeruginosa* (MIC 0.5 μL/mL; MBC 1.0 μL/mL), followed by *M. flavus, L. monocytigenes* and *E. coli* (MIC 1.25 μL/mL), while it had no effect on *Enterobacter cloacae* and *Staphylococcus epidermidis* in tested concentrations. The oil also showed significant activity against analyzed micromycetes. Strongest antifungal activity was found against *Aspergillus versicolor* where MIC and MFC were 2.5 μL/mL and 5 μL/mL, respectively. Such significant bactericidal and fungicidal activity can be attributed to a considerable degree of relativly high concentrations of alpha-pinene (21.2%) and sabinene (38.0%) presence on one hand, and beta-phellandrene (13.5%) and myrcene (6,0%), on the other [19,20]. The volatile compounds from fruits of *S. libanotis*, wild-growing in Poland, showed better antibacterial activity against Gram-positive bacteria (MICs between 0.15 to 1.25 mg/mL) when compared with Gram-negative bacteria (MICs between 1.25 to 2.5 mg/mL[17].

Experimental

Plant material and isolation of essential oil: *Seseli globiferum* was collected at full flowering during mid

October 2008 from calcareous rocks of Morača canyon in Montenegro. A voucher specimen (BEOU Sgl102008) was deposited in the Herbarium of Institute of Botany, University of Belgrade - Faculty of Biology. The essential oil was isolated by 3-hours hydrodistillation using Clevenger-type apparatus, according to the procedure described in Pharmacopeia Europaea 6 [21].

Gas chromatography-mass spectrometry: GC analysis was performed on Agilent 7890A GC system equipped with 5975C MSD and FID, using DB-5 MS column (30 m × 0.25 mm × 0.25 μm). Injection volume was 1 μL and injector temperature was 220°C with 10:1 split ratio. Carrier gas (He) flow rate was 1.0 mL/min at 210°C (constant pressure mode). Column temperature was linearly programmed in a range of 60-240°C at a rate of 3°C/min. Transfer line was heated at 240°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in m/z range 30-550. A library search and mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software version 2.64.113.71, using retention index (RI) calibration data analysis parameters with 'strong' level and 10% penalty for compounds without an RI. The retention indices were experimentally determined using the standard method involving retention times of *n*-alkanes, injected after the essential oil under the same chromatographic conditions. The search was performed against our own library, containing 4972 spectra. Percentage (relative) of the identified compounds was computed from GC peak area.

Antimicrobial activity: Antimicrobial activity of isolated oil was assayed using broth microdilution methods [22]. Antimicrobial activity was determined using a panel of following microorganisms: Gram (+) bacteria *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (clinical isolates); Gram (–) bacteria *Bacillus cereus* (clinical isolates), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus flavus* (ATCC 10240); Fungi: *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730) *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839). Microorganisms were provided by the Institute of Immunology and Virology, Torlak, Belgrade. For the determination of the minimum inhibitory (MIC) and minimum bactericidal and fungicidal concentrations (MBC and MFC) a broth microdilution assay was used, as recommended by the NCCLS [22]. Test strains were suspended in medium to give a final density of $5x10^5$ cfu/mL. Oil was diluted in DMSO (15mg/mL) to concentrations ranging from 1.25 to 10μL for bacteria, 0.078 to 5μL for fungi. After incubation at 28°C for 24 hours for bacteria, and 72 hours for fungi, the growth of the microorganisms was determined. For MIC the lowest concentration where there was no significant growth of bacteria/fungi was taken, and for MBC/MFC – the lowest concentration where no visible growth was recorded.

The MIC and MBC of the reference antibiotics were determined in parallel experiments, with positive controls of growth included. The MIC and MBC/MFC of standard antibiotic Streptomycin and fungicide Bifonazole were determined in parallel experiments, with positive controls of growth included.

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