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Chemical Composition, Antimicrobial and Antiradical Properties of the Essential Oils of *Seseli globiferum* Fruits

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The chemical composition and antimicrobial and antiradical activities of the essential oils isolated from unripe and ripe fruits of *S. globiferum* Vis. (Apiaceae) have been determined. The most abundant constituent in the essential oils of unripe and ripe fruits was sabinene (53.1% and 65.3%), followed by γ-terpinene (7.7% and 6.6%), α-pinene (7.2% and 4.4%), and β-phellandrene (5.0% and 4.9%). Antibacterial and antifungal properties of these oils were evaluated using a modified microdilution technique. Scavenging activity was determined by the DPPH radical assay. The essential oils exhibited significant antimicrobial, but low antiradical activity.

Keywords: Essential oil, fruits, *Seseli globiferum*, chemical composition, antimicrobial activity, DPPH.

The emergence of antibiotic resistance among pathogenic and commensal bacteria has become a serious problem worldwide. The use and overuse of antibiotics in a number of settings are contributing to the development of antibiotic-resistant microorganisms [1a]. Many pathogenic organisms, such as *Escherichia coli*, and some species of *Fusarium*, *Aspergillus*, and *Penicillium* are well known causal agents of food-borne diseases and food spoilage. Food-borne diseases are still a major and very important problem in the World [1b]. Spices in different types of food have been well known for their antioxidant capacities since ancient times [1c].

The genus *Seseli* L. contains 55 species, most of which are represented in the flora of Europe [2a]. Coumarins have been isolated from *S. tortuosum* L. [2b-2e], *S. devenyense* Simonkai [3a], *S. libanotis* [3b] and *S. hartvigii* [3c]. Pharmacodynamic effects have been studied of a volatile fraction isolated from *S. sibiricum* Benth. [3d]. Traditional uses of *Seseli* species as an anti-inflammatory agent were supported by the results of Kupeli [3e].

There are no previous reports on the chemical constituents, antimicrobial and antiradical activity of the essential oils of *S. globiferum* fruits, and so these have been investigated. The yield of essential oils obtained by hydrodistillation of unripe and ripe fruits was 2.5% and 2.0% v/m, respectively. GC and GC-MS analyses showed a total of 42 compounds in the essential oil of unripe fruits of *S. globiferum*, and a total of 35 compounds in that of the ripe fruits, representing 100% of the oils. In the essential oil of unripe fruits the principal compound was sabinene (53.1%), followed by 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 the 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%) (Table 1). β-Pinene (37.5%), 4α-hydroxygermacra-1(10)-5-diene (21.7%) and α-pinene (13.7%) were the 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 main constituents of *S. tortuosum* essential oil [4a].

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 [4b]. Analyses of *S. peucedanoides* (MB) Kos.- Pol.

Table 1: Chemical composition of the essential oils (EO) of *Seseli globiferum* unripe and ripe fruits.

Compound	unripe fruits EO %	ripe fruits EO %	KI*
α-Thujene	3.8	4.5	924
α-Pinene	7.2	4.4	932
Camphene	0.8	0.4	946
Sabinene	53.1	65.3	969
β-Pinene	2.4	1.1	974
β-Myrcene	1.6	0.7	988
α-Phellandrene	0.1	ND**	1002
Δ^3-Carene	1.1	1.1	1008
α-Terpinene	4.1	4.3	1014
p-Cymene	1.5	1.1	1020
β-Phellandrene	5.0	4.9	1025
trans-β-Ocimene	0.2	ND	1044
γ-Terpinene	7.7	6.6	1054
cis-Sabinene hydrate	0.4	0.2	1065
α-Terpinolene	1.9	1.9	1082
trans-Sabinene hydrate	0.3	0.1	1098
β-Thujone	0.1	ND	1112
cis-Menth-2-en-1-ol	0.1	ND	1118
α-Campholenal	0.1	ND	1122
trans-Pinocarveol	ND	0.1	1135
trans-Verbenol	0.1	0.1	1140
1,4-Dimethyl-3-cyclohexenyl methyl ketone	0.3	0.1	n/a
Citronellal	0.1	ND	1148
trans-2-Nonen-1-al	0.1	0.2	1157
Borneol	0.1	0.1	1165
p-Mentha-1,5-dien-8-ol	ND	0.1	1166
Terpinen-4-ol	2.5	0.5	1174
p-Cymene-8-ol	0.1	0.1	1179
α-Terpineol	0.1	ND	1186
trans-Chrysanthenyl acetate	0.1	ND	1214
Thymol methyl ether	0.1	ND	1232
Bornyl acetate	0.5	0.2	1287
δ-Elemene	0.1	0.1	1335
β-Elemene	ND	0.1	1389
Methyleugenol	0.2	0.1	1403
β-Caryophyllene	0.1	ND	1417
γ-Elemene	0.3	0.4	1434
Germacrene D	0.1	0.1	1484
β-Selinene	ND	0.04	1490
α-Muurolene	ND	0.1	1500
γ-Cadinene	0.1	0.03	1513
Myristicin	1.9	0.3	1517
Elemol	1.4	0.6	1548
1,10-Di-*epi*-cubenol	0.1	0.1	1619
γ-Eudesmol	ND	0.1	1630
α-Eudesmol	0.1	ND	1647
Bulnesol	0.1	ND	1665
α-Bisabolol	0.1	ND	1685
Total	100	100	

*KI: Kovats index on DB-5 column; **ND: Not detected

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 [4c]. Essential oils of *S. buchtormense* (Fisch. ex Sprengel) Koch. obtained from various samples from the Altai Region, had as major compounds, sabinene (17.7-25.1%), α-pinene (5.3-14.6%), (*E*)-nerolidol (5.5-11.6%) and β-phellandrene (2.5-7.0%) [4d]. 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%) [4e]. Himachalol (16.4%) and sabinene (14.8%) were the major compounds of the essential oil of *S. bocconi* Guss [4f].

Table 2: Antibacterial activity of *Seseli globiferum* essential oils and streptomycin (MICs and MBCs in μL/mL).

Bacteria	unripe fruits MIC MBC	ripe fruits MIC MBC	streptomycin MIC MBC
M. flavus	4.8±* 0.3	10.3±0.3	50.0±0
	9.7±0.3	24.8±0.3	100.0±0.5
S. typhimurium	24.8±0.3	50.0±0.5	100.0±0
	50.0±0.5	100.3±0.3	100.3±0.3
S. enteritidis	10.0±0.0	24.8±0.3	50.0±0
	10.3±0.3	50.0±0.0	100.0±0.5
S. epidermidis	4.8±0.3	10.3±0.3	100.0±0
	9.7±0.3	24.8±0.3	100.3±0.3

Table 3: Antifungal activity of *Sesile globiferum* essential oils and bifonazole (MICs and MFCs in μL/mL).

Fungi	unripe fruits MIC MFC	ripe fruits MIC MFC	bifonazole MIC MFC
T. viride	24.0±* 0.5	24.8±0.3	150.0±0.5
	50.0±0.5	50.8±0.5	250.3±0.3
A. flavus	0.5±0.0	4.8±0.3	100.0±0.0
	1.0±0.5	5.0±0.5	100.3±0.3
A. fumigatus	5.0±0.5	5.3±0.3	100.0±0.0
	9.7±0.3	10.0±0.0	100.3±0.3
A. niger	4.8±0.3	5.0±0.5	100.0±0.0
	10.0±0.3	10.8±0.3	100.3±0.3
A. ochraceus	1.0±0.0	1.3±0.3	100.0±0.0
	1.3±0.3	4.8±0.3	100.3±0.3
P. funiculosum	1.0±0.0	0.5±0.0	150.0±0.5
	1.3±0.3	1.0±0.5	200.3±0.3
P. ochrachloron	0.5±0.0	0.5±0.0	150.0±0.5
	0.5±0.0	0.50±0.0	150.0±0.5
F. tricinctum	9.7±0.3	10.0±0.0	100.0±0
	10.0±0.0	10.3±0.3	100.3±0.3

In the microdilution antibacterial test, the effect of *S. globiferum* oils was most prominent against *Micrococcus flavus* (MIC=4.8–10.3 μL/mL; MBC=9.7–28.3 μL/mL). The most resistant bacterial species was *Salmonella typhimurium* with a MIC= 24.8–50.0 μL/mL and MBC=50.0–100.3 μL/mL (Table 2). Results obtained after 24 and 48 h were the same. It is evident that the essential oil of unripe fruits exhibited a higher antibacterial activity than that of ripe fruits. In all cases, the essential oils were more effective than the commercial drug streptomycin.

Fungi were more sensitive than bacteria to the effect of the essential oils. Those from unripe and ripe fruits of *S. globiferum* exhibited strong fungistatic and fungicidal effects. In both cases, the most sensitive species was *Penicillium ochrochloron* (MIC=MFC=0.5 μL/mL), whereas *Trichoderma viride* was the most resistant, with a MIC=24.8 μL/mL and MFC=50.0 μL/mL. The investigated essential oils were more efficient than the commercial drug bifonazole (Table 3).

The essential oil of *S. libanotis* seeds evidenced a strong antimicrobial effect on 5 different bacteria [5]. The antimicrobial activity of the essential oil of *S. indicum* was also strong at a very low concentration [6a], and that of *S. annuum* showed antifungal

activity against 15 fungi tested [6b], but the antiradical activities of both oils were low, with SC_{50} values in the DPPH radical scavenging test of 446.7 and 373.2 μL/mL for unripe and ripe oil, respectively. In conclusion, considering the broad spectrum of antimicrobial action of the essential oils of *S. globiferum*, these might have application in agriculture as antimicrobial preparations, as well as in the food industry, as food preservatives.

Experimental

Plant material: Unripe fruits were collected in September 2006, and ripe fruits in October 2006 in Herceg Novi (Montenegro). Plant material was dried at room temperature. A voucher specimen (No S06051986) has been deposited at the Institute for Biological Research 'Siniša Stankovic'.

Isolation of essential oil: Essential oils were isolated by hydrodistillation from the unripe and ripe fruits of *S. globiferum* in a Clevenger-type apparatus for 3 h according to the procedure of the Yugoslav Pharmacopeia IV [6c]. The obtained essential oils were stored at +4°C until further analyses.

Chemical analyses: Qualitative and quantitative analyses of the essential oils were performed using GC and GC/MS. The GC analysis of the oils was carried out on a GC HP-5890 II apparatus, equipped with split-splitless injector, attached to a HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to a FID. The carrier gas was H_2 (1 mL/min). A sample (1 μL) in ethanol (0.2%) was injected in split mode (1:30) at 250°C. The detector temperature was 300°C (FID), while the column temperature was linearly programmed from 40-260°C, at 4°C/min. As for GC/MS analysis, a Hewlett-Packard, G 1800C Series II GCD model and a HP-5MS column (30 m × 0.25 mm × 0.25 μm) were used. Mass spectra were recorded in EI mode (70 eV), in the m/z range 40-400. The transfer line was heated at 260°C. Identification of the individual essential oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those of MS libraries (NIST and Wiley 275.l) using a computer search and literature [6d]. For the purpose of quantitative analysis, area percentages obtained by FID were used.

Antimicrobial activity

Microorganisms and culture conditions: For the bioassays, five bacterial strains: *Micrococcus flavus* (ATCC 9341), *Salmonella typhimurium* (ATCC 13311), *Salmonella enteritidis* (ATCC 13076) and *Staphylococcus epidermidis* (ATCC 2228); and eight fungi: *Trichoderma viride* (IAM 5061), *Aspergillus flavus* (ATCC 9643), *A. fumigatus* (ATCC 9142), *A. niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *P. Ochro-chloron* (ATCC 9112), and *Fusarium tricinctum* (CBS 514478) were used. All of the organisms tested were from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia. The micromycetes were maintained on malt agar (MA), bacteria on Mueller-Hinton agar (MH); cultures were stored at +4°C and subcultured once a month [7a].

***Microdilution method*:** In order to investigate the antimicrobial activity of the isolated essential oils, the modified microdilution technique was used [7b,7c]. Bacterial species were cultured overnight at 37ºC in LB (Luria broth) medium. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The fungal and bacterial cell suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μL per well. The inocula were stored at +4°C for further use. Dilutions of the inocula were cultured on solid MH for bacteria and solid MA for fungi to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MICs) determinations were performed by a serial dilution technique using 96-well microtiter plates. The investigated essential oil was added to either Luria medium broth (bacteria) or Malt medium broth (fungi) containing inoculum. The microplates were incubated for 24 h and 48 h at 37°C for bacteria, or 72 h at 28°C for fungi, respectively. The lowest concentrations without visible growth (binocular microscope) were defined as MICs. The minimum bactericidal (MBCs) and minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μL in microtitre plates containing 100 μL of broth per well and further incubation for 24 h and 48 h at 37°C or 72 h at 28°C, respectively. The lowest concentration with no visible growth was defined as MBC/MFC, respectively, indicating = 99.5% killing of the original inoculum. Each experiment was repeated in triplicate. Streptomycin and bifonazole were used as positive controls (0.1-2 mg/mL).

***DPPH radical assay*:** Essential oil was dissolved in 1.2 mL of absolute EtOH and then 0.3 mL of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in

MeOH was added. Mixtures were vigorously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using MeOH as a blank. One mL of 0.5 mM DPPH diluted in 4 mL of MeOH was used as control. Scavenging of DPPH radical was calculated using the equation: $S(\%)=100\times(A_0-A_s)/A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the tested sample. The SC_{50} value represented the concentration of the essential oil that caused 50% of neutralization (scavenging) of DPPH radical [8]. Results were compared with the activity of L-ascorbic acid.

Statistical analysis: The results of the antimicrobial tests of the essential oils were analyzed by two factorial analysis of variance (ANOVA). The package program Statistica (release 4.5, Copyright StatSoft, Inc. 1993) was used for statistical evaluation. Experiments were replicated twice on the same occasion. All analyses were run in triplicate for each replicate ($n = 2 \times 3$).

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