Chemistry of Natural Compounds, Vol. 45, No. 2, 2009

CHEMICAL COMPOSITION AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *Seseli rigidum* FLOWER ESSENTIAL OIL

S. Stojkovic,[1] S. Petrovic,[1*] J. Kukic,[1]
A. Dzamic,[2] M. Ristic,[3] M. Milenkovic,[4]
J. Glamoclija,[5] M. Sokovic,[5] and D. Stojkovic[5]

UDC 547.913

The natural products industry is currently looking for natural therapeutics and preservatives that can replace synthetic preparations. The scientific literature has identified new applications and uses of both traditional and exotic essential oils.

Fungal and bacterial infections are important problems in phytopathology, agriculture, the food industry, and especially in medicine. Bacterial resistance to antimicrobial agents has become a serious problem worldwide, with resistance mechanisms having been identified and described for all the known antibiotics currently available for clinical use [1].

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA), which leads to cell injury and death [2, 3]. The cytotoxic effect of free radicals is deleterious to mammalian cells [4].

Plants belonging to Apiaceae family are widespread and they are represented by 455 genera and 3750 species [5]. This family is represented by 82 genera and 334 species in the flora of Balkan peninsula; 49 species are Balkan endemits [6].

The latin name of the genus *Seseli* L. originates from the words seseli, seselis, or sesili and it was used by Hippocrates and Dioscorides [7]. Plants of this genus are perennials [6], with numerous species that have been used in traditional medicine since ancient times [8]. Three angular-type pyranocoumarins and two linear-type furocoumarins were isolated from the *n*-hexane extract obtained from the roots of *S. resinosum* [9]. Coumarin compounds were also isolated from different plant tissues of *S. rigidum* (coumarins osthol, suberosin, and furocoumarins psoralen, pranferol) [10]. A new tetrahydrofuranoid lignan seselinone and one known lignan eudesmin have been isolated from *S. annuum* and showed cytotoxic activity against C6 rat glioma cell cultures [11]. It has been reported that ethyl acetate and methanol extracts of different *Seseli* species possess anti-inflammatory and antinociceptive activities [12]. Seselidiol, a new polyacetylene, has been isolated from the roots of *S. mairei*. Seselidiol and its acetate have demonstrated moderate cytotoxicity against KB, P-388, and L-1210 tumor cells [13].

However, there are no data on the antimicrobial and antioxidant activity of *S. rigidum* essential oil.

The objectives of this study were to investigate the chemical composition and antimicrobial and antioxidant activity of the essential oil isolated by hydrodistillation from the flowers of *S. rigidum*.

The yield of the *S. rigidum* essential oil was 0.6% (v/w). The results of chemical analysis of the isolated essential oil are presented in Table 1. Fifty-four components (98.6% of the oil) were identified. The most abundant component was α-pinene (48.5%), followed by camphene (4.6%), β-pinene (4.2%), and limonene (4.1%).

Previous chemical analyses had shown that the main component of the *S. rigidum* var. *rigidum* essential oil was also α-pinene [14, 15]. The main components of the essential oil of *S. annuum* were: germacrene D (29.8%), sabinene (10.3%), β-*o*-cymene Z (9.8%), and limonene (8.6%) [16]. Analysis of *S. bocconi* essential oil showed himahalol (16.4%) as the most abundant component, followed by β-phellandrene (29.2%) and sabinene (14.8%) [17]. α-Pinene (26.2% and 35.8%) and (*E*)-sesquilavandulol (11.8% and 3.2%) were the main constituents in the *S. campestre* fruit and herb essential oils, respectively [18].

1) Institute of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia, e-mail: silvana@pharmacy.bg.ac.yu; 2) Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Studentski Trg 16, 11000, Belgrade, Serbia, e-mail: simicana@bfbot.bg.ac.yu; 3) Institute for Medicinal Plant Research "Josif Pancic", Tadeusa Koscuska 1, 11000 Belgrade, Serbia, e-mail: iplb@sezampro.yu; 4) Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, 11221 Belgrade, Serbia, e-mail: marinama@pharmacy.bg.ac.yu; 5) Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Sinisa Stankovic", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia, e-mail: mris@ibiss.bg.ac.yu. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 216–218, March–April, 2009. Original article submitted July 12, 2007.

0009-3130/09/4502-0253

TABLE 1. Chemical Composition of *Seseli rigidum* Essential Oil

Compound	Percentage	KI	Compound	Percentage	KI
Tricyclene	0.1	923	Bornyl acetate	3.1	1289
α-Thujene	0.3	939	α-Elemene	0.1	1381
α-Pinene	**48.5**	954	β-Bourbonene	0.2	1388
Camphene	4.6	946	β-Elemene	0.3	1391
Thuja-2,4(10)-diene	0.1	975	β-Caryophyllene	0.4	1419
Sabinene	4.0	979	γ-Elemene	0.2	1437
β-Pinene	4.2	991	α-Humulene	0.2	1455
β-Myrcene	2.1	1003	γ-Muurolene	0.2	1480
α-Phellandrene	0.2	1017	Germacrene D	1.1	1485
α-Terpinene	0.1	1025	Bicyclogermacrene	0.5	1500
p-Cymene	1.3	1029	β-Sesquiphellandrene	1.0	1523
Limonene	4.1	1060	*cis*-Nerolidol	0.3	1533
γ-Terpinene	0.7	1070	Elemol	0.3	1550
cis-Sabinene hydrate	0.3	1089	Germacrene B	0.3	1561
α-Terpinolene	0.2	1091	Mintoxide	1.9	1565
Linalool	0.7	1114	Spathulenol	2.0	1578
β-Thujone	0.1	1126	Caryophyllene oxide	0.9	1583
α-Campholene aldehyde	0.5	1139	Carotol	2.0	1595
trans-Pinocarveol	0.7	1145	β-Oplopenone	0.5	1608
trans-Verbenol	1.6	1169	Isospathulenol	0.7	1625
Borneol	1.5	1177	Muurola-4,10(14)-dien-1-ol	1.7	1626
Terpinen-4-ol	0.9	1183	3(15)-Cedren-4-ol	0.4	1647
p-Cymen-8-ol	0.1	1189	*allo*-Himachalol	0.8	1650
α-Terpineol	0.2	1196	Amorpha-4,9-dien-2-ol	0.3	1679
Myrtenal	0.6	1205	γ-Costol	0.4	1732
Verbenone	0.2	1217	β-Costol	0.4	1754
trans-Carveol	0.3	1235	Total	98.6	
Thymol methyl ether	0.2				

*KI: kovats index on DB-5 column.

TABLE 2. Antibacterial Activity of *Seseli rigidum* Essential Oil and Streptomycin (MICs and MFCs in µL/mL)

Bacteria	*Seseli rigidum* essential oil		Streptomycin*	
	MIC	MBC	MIC	MBC
Escherichia coli (ATCC 25922)	50.0	100.0	50.0	100.0
Pseudomonas tolaasii (isolated from *Agaricus bisporus*)	50.0	100.0	100.0	200.0
Bacillus subtilis (ATCC 10707)	50.0	75.0	50.0	50.0
Micrococcus flavus (ATCC 9341)	100.0	100.0	50.0	100.0
Staphylococcus epidermidis (ATCC 2228)	100.0	100.0	100.0	100.0

*1 mg of streptomycin in 1 mL of DMSO.

The antibacterial effect of *S. rigidum* essential oil in the microdilution test was the most prominent against *Bacillus subtilis* (MIC 50 µL/mL; MBC 75 µL/mL). The most resistant bacterial species were *Micrococcus flavus* and *Staphylococcus epidermidis* with MIC=MBC of 100 µL/mL (Table 2).

The essential oil from the flowers of *S. rigidum* showed high antifungal activity against *Aspergillus fumigatus* (MIC 10 µL/mL; MFC 25 µL/mL). *Penicillium ochrochloron* and *Aspergillus niger* were the most resistant fungal species with MIC=MFC at 50 µL/mL. In all cases the essential oil was more efficient than the commercial antifungal drug bifonazole (Table 3).

TABLE 3. Antifungal Activity of *Seseli rigidum* Essential Oil and Bifonazole (MICs and MFCs in μL/mL)

Fungi	*Seseli rigidum* essential oil		Bifonazole*	
	MIC	MFC	MIC	MFC
Aspergillus flavus (ATCC 9643)	25.0	50.0	100.0	100.0
Aspergillus fumigatus (ATCC 9142)	10.0	25.0	100.0	100.0
Aspergillus niger (ATCC 6275)	50.0	50.0	100.0	100.0
Penicillium ochrochloron (ATCC 9112)	50.0	50.0	150.0	200.0
Penicillium funiculosum (ATCC 36839)	25.0	50.0	150.0	150.0
Trichoderma viride (IAM 5061)	25.0	50.0	150.0	250.0

*Commercial drug bifonazole (1 g/500 mL).

TABLE 4. Scavenging Activity of *Seseli rigidum* Essential Oil Against DPPH Radical

Concentration, μL/mL	Scavenging, %	Concentration, μL/mL	Scavenging, %
13.3	21.9	23.3	45.4
20.0	41.5	26.7	55.3

Previous investigation showed that the essential oil of *S. annuum* also possesses antifungal activity [16]. The essential oils from the seeds of *S. indicum* [19] and *S. libanotis* [20] exhibited strong antibacterial activity.

Most of the terpenoids were found to inhibit microbial oxygen uptake and oxidative phosphorylation. In particular, the phenolic and non-phenolic alcohols had the strongest inhibitory effects, followed by aldehydes and ketones. The monoterpene hydrocarbons had lower activity. It was suggested that the free OH-group of the phenol and alcohol might be a key to their activity [21].

α-Pinene, γ-terpinene, and limonene were found to affect the structural and functional properties of microbial artificial membranes. These compounds were shown to permeabilize the membranes, making them swell. This inhibited respiratory enzymes, which led to a partial dissipation of the pH gradient and electrical potential, each being crucial to the energy system in a cell [22].

In previous investigation it was found that α-pinene acts as an antifungal agent between 30 μL/mL and 85 μL/mL, tested on the same fungal species as we used in this experiment [21]. From our results it is evident that the essential oil tested in this experiment exibited higher antifungal potency than its main constituent α-pinene. It might be concluded that the essential oil from the flowers of *S. rigidum* had a synergistic effect on the microorganisms tested.

The free radical scavenging capacities of the investigated essential oil measured by DPPH assay are shown in Table 4. The concentration of the oil at which 50% of DPPH scavenging was achieved (SC_{50}) was 24.5 μL/mL. When compared to ascorbic acid (SC_{50} 4.1 μg/mL) or gallic acid (SC_{50} 1.5 μg/mL), it is evident that the oil tested showed a lower capacity for "scavenging" free radicals.

Essential oils are, from the chemical point of view, quite complex mixtures consisting of many components, and this complexity often makes it difficult to explain the activity pattern. Even though the antioxidant activity of some phenolic compounds [23] and some other examples of pure compounds [24] are well known, many reports on the antioxidant potentials of the essential oils often refer to concepts such as synergism, antagonism, and additivity. Phenols were confirmed to possess strong antioxidant activity [25]. In particular, oxygenated monoterpens are mainly responsible for the antioxidant potential of the plant oils which contain them [26]. The presence of strongly activated methylene groups in monoterpene hydrocarbons could be taken as a reason for the antioxidant activity of these molecules. The antioxidant potential of monoterpene hydrocarbons is obviously lower than oxygenated monoterpenes. Sesquiterpene hydrocarbons and their oxygenated derivatives have very low antioxidant activity [25].

ACKNOWLEDGMENT

The authors are grateful to the Ministry of Science of Serbia for financial support (Grants No. 143012 and 143041) and scholarship (Grant No. 107).

REFERENCES

1. A. C. Fluit, M. E. Jones, F.-J. Schmitz, J. Acar, R. Gupta, and J. Verhoef, *Clin. Infect. Dis.*, **30**, 454 (2000).
2. J. M. McCord, *Am. J. Med.,* **108**, 652 (2000).
3. M. Zheng and G. Storz, *Biochem. Pharmacol.,* **59**, 1 (2000).
4. B. Tepe, M. Sokmen, H. A. Akpulat, D. Daferera, M. Polissiou, and A. Sokmen, *J. Food Eng.,* **66**, 447 (2005).
5. G. H. M. Lawrence, *Taxonomy of Vascular Plants*, Macmillan, New York, 1969, pp. 642–646.
6. V. Nikolic, *Apiaceae*, in: M. Josifovic (Ed.), *The Flora of FR Serbia*, Vol. **5**, Serbian Academy of Sciences and Arts, Belgrade, 1973, pp. 242–243.
7. D. Simonovic, *Botanical Dictionary*, Serbian Academy of Sciences and Arts, Belgrade, 1959, pp. 434–435.
8. V. N. Florya and L. G. Kretsu, *Izv. Akad. Nauk Mold. SSR, Biol. Khim. Nauki,* **2**, 89 (1980).
9. A. Tosun, M. Baba, O. Bahadir, and T. Okuyama, *Pharm. Biol.,* **44**, 528 (2006).
10. G. A. Kuznetsova, V. N. Medvedev, S. Pavlovic, and R. Jancic, *Acta Biol. Med. Exp.*, **12**, 93 (1987).
11. I. Vuckovic, V. Trajkovic, S. Macura, V. Tesevic, P. Janackovic, and S. Milosavljevic, *Phytother. Res.*, **21**, 790 (2007).
12. E. Kupeli, A. Tosun, and E. Yesilada, *J. Ethnopharmacol.*, **104**, 310 (2006).
13. C. Q. Hu, J. J. Chang, and K. H. Lee, *J. Nat. Prod.*, **53**, 932 (1990).
14. K. P. Savikin-Fodulovic, G. M. Zdunic, and S. R. Tasic, *J. Essent. Oil Res.,* **18**, 156 (2006).
15. G. A. Kuznetsova, A. L. Shevarda, S. Pavlovic, and R. Jancic, *Arh. Farm.*, **32**, 291 (1982).
16. S. Milosavljevic, V. Tesevic, I. Vuckovic, M. Jadranin, V. Vajs, M. Sokovic, P. Janackovic, and A. Jovanovic, *Fitoterapia,* **78**, 319 (2007).
17. B. Marongiu, A. Piras, S. Porcedda, E. Tuveri, and A. Maxia, *Nat. Prod. Res.*, **20**, 820 (2006).
18. K. H. C. Baser, T. Ozek, and M. Kurkcuoglu, *J. Essent. Oil Res.*, **12**, 105 (2000).
19. G. Singh, I. P. Kapoor, S. K. Pandey, U. K Singh, and R. K. Singh, *Phytother. Res.*, **16**, 680 (2002).
20. S. Meene, F. M. Chaudhary, and M. K. Bhatty, *Pakistan J. Sci. Ind. Res.*, **32**, 316 (1989).
21. M. Sokovic and L. J. L. D. Van Griensven, *Eur. J. Plant. Pathol.,* **116** (3), 211 (2006).
22. D. Kalemba and A. Krunicka, *Curr. Med. Chem.,* **10**, 813 (2003).
23. I. M. Halendar, H. L. Alakomi, K. Latva-Kala, T. Mattila-Sandhom, I. Pol, E. J. Smid, L. G. M. Goris, and A. von Wright, *J. Agric. Food Chem.*, **46**, 3590 (1998).
24. H. L. Madsen and G. Bertelsen, *Trends Food Sci. Technol.,* **6**, 271 (1995).
25. G. Ruberto and M. T. Baratta, *Food Chem.*, **69**, 167 (2000).
26. M. T. Baratta, H. J. D. Dorman, S. G. Deans, D. M. Biondi, and G. Ruberto, *J. Essent. Oil Res.,* **10**, 618 (1998).