Chemical Composition and Antibacterial Activity of Essential Oil from Bulgarian Wild Growing *Seseli rhodopeum*

M. Todorova and A. Trendafilova
Institute of Organic Chemistry
Centre of Phytochemistry
Bulgarian Academy of Sciences
Sofia
Bulgaria

R. Ivanova and S. Stoitsova
Institute of Microbiology
Bulgarian Academy Sciences
Sofia
Bulgaria

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Abstract

The essential oil composition of *Seseli rhodopeum* was studied by GC and GC/MS. The oil was characterized by a high amount of monoterpene hydrocarbons (84.7%), of which sabinene was the major component with a concentration of 51.3%. α-Pinene (11.8%) and α-phellandrene (10.3%) were also dominant compounds in the oil. The antibacterial activity of the oil was tested against a set of four reference strains of Gram-positive and Gram-negative bacteria on Müller-Hinton agar. Moderate activity was obtained against *S. aureus* ATCC 27853.

INTRODUCTION

The genus *Seseli* L. (family *Apiaceae*) comprises 34 species distributed in Europe (Ball, 1976), with 9 species in Bulgaria (Andreev et al., 1992). Some *Seseli* species are reported to possess various healing effects against human inflammation, swelling, rheumatism, pain, common cold as well as to exhibit anthelmintic and carminative activity (Kupeli et al., 2006). *Seseli rhodopeum* Velen. is one of the three endemic species of *Seseli* in Bulgaria, with a limited distribution in Western and Central Rhodope Mountain. It is included in the Red book of Bulgaria (1984) as a rare species. The literature survey revealed only one article dealing with essential oil content of the species (Robeva, 1970). However, its chemical composition has not been studied so far. There are publications on essential oil composition of many *Seseli* species of different origin and plant organs, some of which have been found to exhibit antimicrobial and antifungal activities (Ozturk and Ercisli, 2006; Stojkovic et al., 2008; Stojković et al., 2009; Šiljegović et al., 2011).

The objectives of this study were to investigate chemical composition and antibacterial activity of the essential oil of *S. rhodopeum*.

MATERIALS AND METHODS

Plant Material

Leaves of *S. rhodopeum* were collected from the vicinity of Trigrad (Western Rhodope Mountain, Bulgaria) at flowering stage in July 2010. The species was identified by Dr. L. Evstatieva of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Preparation of Essential Oil

Essential oil was prepared by micro steam distillation-extraction of the air-dried plant material for 2 h in Lickens-Nickerson apparatus using diethyl ether as a solvent.

Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC/MS)

GC analysis was performed on HP 5890 gas chromatograph (FID), carrier gas nitrogen, linear velocity 25 cm/s, split ratio 1:100, fused silica capillary column HP-5MS (poly-5%-diphenyl-95%-dimethylsiloxane), 30 m x 0.25 mm, 0.25 μm film thickness.

The injector and detector temperature was 260°C, column temperature was programmed from 50-240°C at rate of 4ºC/min, and 10 min at 240°C. The quantitative estimation was determined by relative peak area (electronic integration). GC/MS analysis was performed on HP 6890 instrument. All chromatographic conditions and the column were as described above, but the carrier gas was helium. The oil components were identified by comparison of their retention indices and mass spectra, with those published in the literature (Tkachev, 2008; Adams, 2009) and presented in NIST 98 as well as a library developed by us. A homologues series of n-alkanes (C_5-C_{20}) under the same conditions were used as reference points for calculation of RI.

Evaluation of Antibacterial Activity

Antibacterial activity of the plant extracts was tested on reference strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Streptococcus saprophyticus* NCTC 7292. The strains were stored frozen in Trypticase soy broth (TSB) (Difco) supplemented with 30% glycerol. Before use, samples were streaked on agar, and single colonies were inoculated into TSB and incubated overnight at 37°C. One hundred µl from the overnight cultures were spread on Müller-Hinton agar (NCIPD, Sofia, Bulgaria).

The classical disk-diffusion assay was applied to characterize the antibacterial activities of the essential oil. The oil was dissolved in ethanol. Disks were loaded with 50, 100, 200, and 300 µg of oil, air dried and applied onto the Müller-Hinton agar plated with the test strains. After 24 hours, the results were recorded by visual estimation. The test was repeated three times.

In another experiment, the oil was suspended as a stock solution in either ethanol, or 0.5% Tween 80. Aliquot from the suspensions were added to Müller-Hinton broth to final concentrations of 100, 200, 300, and 400 µg/ml. Overnight culture of *S. aureus* ATCC 25923 was diluted 1:10 with the thus prepared samples and was applied onto 96-well microtitre plates. Control samples included broth with a similar amount of the respective solvent. After 24 hours, OD_{620} of the samples was measured.

RESULTS AND DISCUSSION

The essential oil obtained by hydrodistillation from the aerial parts of the plant was colorless with a specific odor. The oil yield was 0.7% w/w. The chemical composition of the isolated oil is presented in Table 1. Thirty six components representing 99.9% of the oil were identified. Out of them, 30 compounds were in concentration over 0.1% and 6 are present in traces. The oil was characterized by a high amount of monoterpene hydrocarbons (84.7%), while sesquiterpene hydrocarbons were only 7.3%. Oxygenated terpenes were also in relatively low concentrations (7.9%). Sabinene was the major component among the monoterpene hydrocarbons and reached 51.3%. α-Pinene (11.8%) and α-phellandrene (10.3%) were also dominant compounds, while germacrene D, the main sesquiterpene, was found in moderate concentration (4.3%). Terpinene-4-ol (2.9%) was found as the main oxygenated monoterpene. Besides, some terpenes as cryptone, α-terpineol, δ-elemene and α-copaene, hexenyl valerate and hexenyl tiglate were registered in traces. The main components of the studied oil - sabinene, α-pinene and β-phellandrene, have been reported to dominate in oils from *S. globiferum* (Stojković et al., 2008), *S. buchtormense* (Tkachev et al., 2006), *S. tortuosum* (Habibi et al., 2003), although in the latter sesquilavandulol has been the major compound. Sesquilavandulol (*S. tortuosum*) (Habibi et al., 2003), (*S. campestre*) (Baser et al., 2000), asarone (*S. libanotis*) (Ozturk and Ercisli, 2006), carotol (*S. petraeum* and *S. andronaxii*) (Tosun et al., 2006), limonene (*S. rigidum*) (Šavikin-Fodulović et al., 2006), (*S. pencedanoides*) (Bulatović et al., 2006), (*S. annuum*) (Milosavljević et al., 2007) found in the respective species in significant concentrations were not detected even in traces in *S. rhodopeum*. The observed differences in oil composition could be of taxonomical interest of the genus *Seseli*.

The disk diffusion assay applied on the reference strains showed that the essential

oil was inactive against three of the reference strains, or with apparently low activity against the Gram-positive strain *Staphylococcus aureus* ATCC 25923. This was recorded upon application of 200 and 300 μg per disk (Table 2). Our preliminary results are in agreement with those reported for other essential oils, which are often active against Gram-positive bacteria, especially *S. aureus* (Burt, 2004).

While these values illustrate an apparently low inhibition activity of *S. rhodopeum* essential oil against *S. aureus*, hydrophobicity could be a possible cause for the low outcome of the present trials. Hydrophobicity could be a reason for low diffusion rate of the tested oil on the agar. For this reason, we tried to estimate the activity of the oil in solute using different solvents for stock solution preparations. While in samples suspended in ethanol the oil separated rapidly from the bacterial culture medium, some more satisfactory stability was obtained in the initial stock suspension with Tween 80. About 50% of suppression of *S. aureus* growth was registered with essential oil amounts 200-400 μg/ml as compared to the values in Müller-Hinton broth supplemented with a corresponding amount of Tween 80 alone. Notably, a 25% inhibition of bacterial growth was obtained also with the 100 μg/ml dilution. However, due to problems with solubility in bacterial growth media, to this moment we were unable to estimate the MIC. This implies that for local applications (e.g. skin) the oil might be significantly more effective than illustrated by the present data set.

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Tables

Table 1. Essential oil composition of *S. rhodopeum* in percentages.

KI	Compound	%	KI	Compound	%
	C_{10} hydrocarbons	**84.7**	1191	α-Terpineol	t
928	α-Thujene	0.5	1207	M=152	0.6
937	α-Pinene	11.8	1290	Bornyl acetate	0.3
954	Camphene	0.3		**C_{15} hydrocarbons**	**7.3**
976	Sabinene	51.3	1343	δ-Elemene	t
984	β-Pinene	3.0	1380	α-Copaene	t
999	Myrcene	2.3	1395	β-Bourbonene	0.1
1011	δ-2-Carene	1.0	1395	β-Elemene	0.1
1021	α-Terpinene	0.6	1427	β-Caryophyllene	1.4
1029	p-Cymene	0.4	1489	α-Humulene	0.1
1034	β-Phellandrene	10.3	1492	Germacrene-D	4.3
1042	*cis*-Ocimene	1.0	1505	Bicyclogermacrene	1.3
1052	*trans*-Ocimene	0.7		**C_{10} oxygenated**	**2.2**
1062	γ-Terpinene	1.2	1580	Spathulenol	1.3
1091	Terpinolene	0.3	1585	Caryophyllene oxide	0.3
	C_{10} oxygenated	**5.7**	1640	Guaia-6,10(14)-dien-11-ol	0.2
1071	*cis*-Sabinene hydrate	0.7	1648	Isospathulenol	0.4
1102	Linalool	0.8		**Others**	**t**
1104	*trans*-Sabinene hydrate	0.4	1279	Hexenyl valerate	t
1185	Terpinen-4-ol	2.9	1315	Hexenyl tiglate	t
				Total	99.9

Table 2. Inhibition zones of *S. rhodopeum* essential oil against different bacterial strains.

Bacterial strain	Concentration of essential oil (μg/disk)			
	50	100	200	300
	Inhibition zone (mm±SD)			
E. coli ATCC 25922	na	na	na	na
P. aeruginosa ATCC 27853	na	na	na	na
S. aureus ATCC 25923	na	na	7±0.5	9±0.3
S. saprophyticus NCTC 7292	na	na	na	na

na: Not active.