Antibacterial and Antioxidant Activity and Essential Oil Composition of *Grammosciadium scabridum* Boiss. from Iran

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The *in vitro* antibacterial and antioxidant activity of the essential oil and its two main components of *Grammosciadium scabridum* Boiss. (Apiaceae) growing wild in Iran, as well as the composition of its essential oil were studied. A total of 19 compounds representing 99.9% of the oil has been identified. γ-Terpinene (73.5%), *p*-cymene (14.2%) and (*E*)-β-farnesene (5.3%) were characterized as the main components. The oil showed remarkable activity against three Gram-negative and four Gram-positive test bacteria, with minimal inhibitory concentration (MIC) values ranging from 0.31 to 10.00 mg/ml. The oil and its two main components were also subjected to screening for their possible antioxidant activity by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The free radical scavenging capacity of the oil was determined with an IC_{50} value of 6.6 mg/ml.

Key words: Grammosciadium scabridum, Essential Oil, Antibacterial and Antioxidant Activity

Introduction

Essential oils are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition (Buchbauer, 2000). There is an increasing worldwide attempt to screen plants for studying the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects (Sokmen *et al.*, 1999, 2004; Hammer *et al.*, 1999; Dorman and Deans, 2000; Tzakou *et al.*, 2001; Oumzil *et al.*, 2002; Bassole *et al.*, 2003; Salgueiro *et al.*, 2003a, b; Skaltsa *et al.*, 2003; Tzakou and Skaltsa, 2003). Although, there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ascorbic acid, which are commonly used in processed foods, it has been showed that these compounds have some side effects (Ito *et al.*, 1983). Therefore, research on the identification of the natural sources of antioxidants and antioxidant potentials of plants is important. Members of the genus *Grammosciadium* are among the most important aromatic plants and the commercial value of the essential oils of this genus has already been reported (Tamamschian, 1987).

The genus *Grammosciadium* DC. (Apiaceae) consists of three species in Flora of Iran. *G. scabridum* Boiss. is a native plant growing wild in Iran and also Iraq. In our previous study (Sonboli *et al.*, 2005) the antibacterial activity and composition of the oil of *G. platycarpum* were documented. Linalool (79.0%–81.8%) and limonene (10.0%–5.8%) were found to be the major compounds of the oils of *G. platycarpum* collected from two different localities with notable antibacterial activity. To the best of our knowledge, *G. scabridum* has not been the subject of previous investigation. Here, we now report the chemical composition and *in vitro* antibacterial and antioxidant activity of the essential oil of *G. scabridum* and its main compounds from Iran.

Materials and Methods

Plant material

The aerial parts of *G. scabridum* were collected during flowering stage on June 27, 2004 from Aghbolagh village, Thakht-e Soleiman district, at an altitude of 2250 m, Takab, Iran. A voucher specimen (mp-390) was deposited at the herbarium of

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Essential oil isolation and analysis

The air-dried and ground aerial parts of the plant were subjected for 4 h to hydrodistillation using a Clevenger-type apparatus. The obtained oil was dried over anhydrous sodium sulphate and stored at 4 °C until tested and analyzed. GC analysis was performed by using a Thermoquest gas chromatograph equipped with a flame ionization detector (FID). The analysis was carried out on fused silica capillary columns with two different stationary phases [DB-1 (60 m × 0.25 mm i.d., film thickness 0.25 μm) and DB-wax (30 m × 0.25 mm i.d., film thickness 0.25 μm)]. The operating conditions were as follows: injector and detector temperatures, 250 °C and 300 °C, respectively; carrier gas, N_2 at a flow rate of 1 ml/min; oven temperature programme, 60 °C–250 °C at a rate of 5 °C/min, and finally held isothermally for 10 min. GC-MS analysis was accomplished by using a Thermoquest-Finnigan gas chromatograph coupled with a TRACE MS. Helium was used as carrier gas at a flow rate of 1.1 ml/min. Ion source and interface temperatures were kept at 200 °C and 250 °C, respectively. The quadrupole mass spectrometer was scanned from 43–456 mass unit with an ionization voltage of 70 eV. Gas chromatographic conditions were the same as given above for GC.

Retention indices (RI) for all constituents were calculated according to Van den Dool approach, using *n*-alkanes (C_6–C_{24}) as standards and the essential oils on DB-1 and DB-wax columns under the same chromatographic conditions. The identification of the components was made based on comparison of their mass spectra with those of the internal computer reference mass spectra libraries (Wiley 7 and NIST), as well as by comparison of their retention indices with the published data (Davis, 1987; Shibamoto, 1987), and in some cases by co-injection with authentic compounds.

Antibacterial activity

The *in vitro* antibacterial activity test was carried out using the disk diffusion method (Baron and Finegold, 1990). The potency of the oil and its major components, γ-terpinene and *p*-cymene, were determined against four Gram-positive bacteria: *Bacillus subtilis* (ATCC 9372), *Enterococcus faecalis* (ATCC 15753), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228); and also three Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27852) and *Klebsiella pneumoniae* (ATCC 3583). The micro-dilution broth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC), as recommended by NCCLS (1999). After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibitiory concentration (MIC). Ampicillin was used as standard antibacterial agent.

Antioxidant activity: Free radical scavenging capacity (RSC)

The free radical scavenging capacity of the essential oil and its two major constituents, and also two positive controls, butylated hydroxytoluene (BHT) and ascorbic acid, was measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The various volumes of the samples were mixed with 1 ml of 0.004% DPPH solution and filled up with 95% methanol to a final volume of 4 ml. After a 30 min incubation period at 30 °C, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent was calculated as follows:

$$\text{RSC}\ (\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}),$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the oil) and A_{sample} is the absorbance of the sample. The oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentages against oil concentrations.

Results and Discussion

Essential oil analysis

The hydrodistillation of the shade-dried aerial parts of the plant at full flowering stage gave a yellow oil with yields of 0.7% (v/w) and 0.44% (w/w) based on the dry weight of plant. Analysis of the essential oil was conducted by GC-FID and GC-MS using fused silica capillary columns with two different stationary phases, polar and apolar. The constituents were identified and their percentages listed according to their elution order on the apolar DB-1 column (Table I). A total of 19 compounds was identified, amounting to 99.9% of the oil. The oil was characterized by a high amount of

Table I. Constituents of the essential oil of *Grammosciadium scabridum.*

Compound[a]	RI		Percent	Identification method
	Apolar[b]	Polar[c]		
α-Thujene	0929	1019	1.6	d-e
α-Pinene	0938	1014	0.3	d-e-f
Sabinene	0972	1111	0.3	d-e
β-Pinene	0979	1097	0.5	d-e-f
Myrcene	0985	1155	0.7	d-e
α-Terpinene	1013	1167	0.1	d-e
p-Cymene	1020	1264	14.2	d-e-f
β-Phellandrene	1028	1195	0.3	d-e
γ-Terpinene	1063	1246	73.5	d-e-f
Terpinen-4-ol	1171	1583	0.4	d-e
α-Terpineol	1182	1690	0.1	d-e
Carvacrol methyl ether	1232	–	0.1	d-e
Thymol	1273	2189	0.1	d-e-f
Carvacrol	1283	2198	1.2	d-e
β-Caryophyllene	1429	1570	0.8	d-e
(*E*)-β-Farnesene	1452	1655	5.3	d-e
Germacrene-D	1488	1685	0.1	d-e
Bicyclogermacrene	1503	–	0.2	d-e
Spathulenol	1581	2105	0.1	d-e
Monoterpene hydrocarbons			91.5	
Oxygenated monoterpenes			1.9	
Sesquiterpene hydrocarbons			6.4	
Oxygenated sesquiterpenes			0.1	
Total identified			99.9	

[a] Compounds listed in order of their elution on DB-1 column.
[b] Retention indices relative to C_6–C_{24} *n*-alkanes on the apolar DB-1 column.
[c] Retention indices relative to C_6–C_{24} *n*-alkanes on the polar DB-wax column.
d, Comparison with published retention indices in literature; e, comparison of mass spectra with mass libraries; f, coinjection with authentic compounds.

monoterpene hydrocarbons (91.5%), γ-terpinene (73.5%) and *p*-cymene (14.2%) being the principal components. Oxygenated monoterpenes represented five of the 19 compounds, corresponding to 1.9% of the total oil with carvacrol (1.2%) as the main constituent. Spathulenol (0.1%) was the only oxygenated sesquiterpene present. In contrast, sesquiterpene hydrocarbons constituted 6.4% of the total oil with (*E*)-β-farnesene (5.3%) as the predominant component. The essential oil composition of *G. scabridum* was totally different compared to that of *G. platycarpum* (Sonboli *et al.*, 2005). Concerning the main components of these oils, it is noteworthy that linalool and limonene, which are present in high percentages in the latter species, were completely absent in the former oil, while γ-terpinene and *p*-cymene, two major constituents of the oil of *G. scabridum* were also present in low concentrations in the oil of *G. platycarpum.*

Antibacterial activity

The antibacterial activity (zones of growth inhibiton and minimal inhibitory concentrations) of the essential oil and its two major components is shown in Table II. As can be seen, *Staphylococcus epidermidis, Bacillus subtilis* and *Escherichia coli* with 20 mm, 19 mm and 18 mm zones of growth inhibition and MIC values of 0.31 mg/ml, 1.25 mg/ml and 1.25 mg/ml, respectively, seemed to be more sensitive to the oil than other examined strains. The antibacterial activity of the two main components of the oil (γ-terpinene and *p*-cymene) was also assayed against the same bacteria. From our results obtained, it is clear that the activity of the oil can mainly be associated with the significant contribution of γ-terpinene. The oil exhibited marked inhibition of three Gram-negative tested bacteria compared with the standard, ampicillin, and two major components of the oil. γ-Terpinene and *p*-cymene showed no activity against *Kleb-*

Table II. Antibacterial activity (inhibition zone and minimal inhibitory concentration) of the essential oil of *Grammosciadium scabridum* and its two main compounds.

Microorganism	Inhibition zone [mm][a]				MIC[b]		
		Main compounds		Standard		Main compounds	
	Oil (10 μl/disk)	γ-Terpinene (10 μl/disk)	*p*-Cymene (10 μl/disk)	Ampicillin (10 μg/disk)	Oil	γ-Terpinene	*p*-Cymene
Bacillus subtilis	19	19	17	15	1.2	3.8 (27.9)	3.8 (28.3)
Staphylococcus aureus	14	10	9.5	13	0.6	15.0 (110.1)	15.0 (111.8)
Staphylococcus epidermidis	20	14	9	19	0.3	7.5 (55.1)	15.0 (111.8)
Enterococcus faecalis	14	11	–	11	2.4	7.5 (55.1)	nt
Escherichia coli	18	12	11	–	1.2	7.5 (55.1)	7.5 (55.9)
Klebsiella pneumoniae	12	–	–	–	4.8	nt	nt
Pseudomonas aeruginosa	9.5	–	–	–	9.6	nt	nt

[a] Diameter of inhibition zones (mm) including sterile disk diameter of 6 mm.
[b] Minimum inhibitory concentration as mg/ml for essential oil and mg/ml (mM) for pure compounds.
–, Inactive; 7–14, moderately active; > 14, highly active; nt, not tested.

siella pneumoniae and *Pseudomonas aeruginosa.* It may be concluded that other compounds such as (*E*)-β-farnesene and carvacrol could also contribute to antibacterial activity of the oil. In addition, the oil showed the similar type of inhibitory activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* like the standard ampicillin.

Free radical scavenging activity

In the DPPH assay the radical scavenging ability of the oil and its two main components and also the positive controls (BHT and ascorbic acid) was measured spectrophotometrically (Table III). In general, the oil was able to reduce the stable radical DPPH to the yellow coloured DPPH-H with an IC_{50} value of 6.6 mg/ml. To illustrate the relation between activity and main components, radical scavenging capacity of two major components, γ-terpinene and *p*-cymene was also studied. The IC_{50} value of γ-terpinene was 15.5 mg/ml, while *p*-cymene showed very weak activity with an IC_{50} value of 148.5 mg/ml. *p*-Cymene has already been reported to exhibit low antioxidant activity (Tepe *et al.*, 2004; Burits and Bucar, 2000). BHT and ascorbic acid as two positive controls exhibited high antioxidant activity with IC_{50} values of 2.5×10^{-2} mg/ml and 7.2×10^{-3} mg/ml, respectively. Combining the results obtained with antioxidant activities of the oil and its two major constituents, we could suggest that the free radical scavenging capacity of the oil may in part be attributed to the presence of γ-terpinene and/or also other phenolic and alcoholic components which constituted 2% of the total oil.

Table III. Antioxidant activity of the essential oil of *G. scabridum* and its two main components and positive controls (BHT and ascorbic acid) on DPPH assay.

Sample	IC_{50} [mg/ml]
Essential oil	6.6
γ-Terpinene	15.5
p-Cymene	148.5
BHT	2.5×10^{-2}
Ascorbic acid	7.2×10^{-3}

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