Antibacterial Activity and Chemical Composition of the Essential Oil of *Grammosciadium platycarpum* Boiss. from Iran

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The chemical composition of the essential oils obtained from two samples (GP1 and GP2) of *Grammosciadium platycarpum* Boiss. was analyzed by GC and GC-MS. The analysis of the oils resulted in the identification of twenty-two constituents. Linalool (79.0% – GP1, 81.8% – GP2) and limonene (10.0%, 5.8%) were found to be the major components, respectively. The *in vitro* antibacterial activities of these oils and their main compounds against seven Gram-positive and Gram-negative bacteria were investigated. The results exhibited that the total oils and their major components possess strong to moderate activities against all the tested bacteria except for *Pseudomonas aeruginosa.*

Key words: Grammosciadium, Essential Oil, Antibacterial Activity

Introduction

There is an increasing worldwide attempt to screen plants 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, 2003b; Skaltsa *et al.*, 2003; Tzakou and Skaltsa, 2003). Essential oils are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition (Buchbauer, 2000). Members of the genus *Grammosciadium* DC. are among the most important aromatic plants and the commercial potential of this genus as a source of essential oils has already been reported (Tamamschian, 1982).

The genus *Grammosciadium* belonging to the family Apiaceae is represented in the Flora Iranica area by 5 species, 3 of them grow in Iran, which are characterized by setaceous leaf lobes and the persistent and often prominent sepals. *G. platycarpum* is a perennial plant growing up to 40 cm high, which is found in sandy mountain areas of Iran. The species has short sturdy styles in comparison with other species. The wings of the fruit of *G. platycarpum* are not obvious until maturity (Tamamschian, 1982). This species is called *samoureh* among people in the Iranian collection area and is known to be used in various food preparations with carminative and relief stomachache properties. Fresh or dried herbal parts of this plant are used as a local vegetable and flavoring in soups and foods in the collection site (Safiarkhaneh village of Takab district). As far as our literature survey could ascertain, there have been no attempts to investigate the chemical composition and biological activities of the essential oils of *Grammosciadium* species grown in Iran. For this, the objectives of the present study are aimed to assess the chemical composition and *in vitro* antibacterial activity of two samples of *G. platycarpum* essential oils from Iran and their main components.

Materials and Methods

Plant material

The aerial parts of *G. platycarpum* were collected during the fruiting stage on June 27, 2004 from Blooz protected area, the Safiarkhaneh village, Takab, West Azerbaijan, at an altitude of 2150 m (GP1), and at Zareshouran village, Thakht-e Soleiman district, Takab, at an altitude of 2250 m (GP2), Iran. Voucher specimens of the samples were deposited at the Herbarium of Medicinal Plants Research Institute, Shahid Beheshti University, Tehran, Iran.

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Isolation procedure

The air-dried and ground aerial parts of the samples were submitted for 4 h to hydrodistillation using a Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at 4 °C until tested and analyzed.

Analytical techniques

The GC analysis was accomplished by a Thermoquest GC instrument with a flame ionization detector (FID), using a fused silica capillary column Rtx-1 (60 m × 0.25 mm i.d., film thickness 0.25 μm). The analytical program was as follows: The temperature was increased from 60 °C to 250 °C at a ramp of 5 °C/min and was then held constant at 250 °C for 10 min; the injector and detector temperatures were 250 °C and 280 °C, respectively; carrier gas was nitrogen at a flow rate of 1.1 ml/min; split ratio was 1/50. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization without using response factors. The GC-MS analysis was carried out on a Thermoquest GC-MS instrument operating in the EI mode at 70 eV (split ratio 1/50), adjusted with a Rtx-1 (60 m × 0.25 mm i.d., film thickness 0.25 μm) fused silica capillary column, with the same thermal program as above mentioned for GC analysis. Helium was used as the carrier gas at a flow rate of 1.1 ml/min. The identification of the individual constituents was based on comparison of their retention indices with those from the literature (Davis, 1987) and by matching their mass spectra with those obtained from authentic compounds and/or the NIST library spectra of the GC-MS system.

Antibacterial activity

The antibacterial activity of the essential oils of the samples and their major compounds, linalool

Table I. Constituents of the essential oils of two samples of *Grammosciadium platycarpum.*

Compound	RI[a]	GC area (%)		Identification method[c]
		GP1	GP2	
α-Pinene	0937	0.1	tr[b]	RI, MS, CoI
β-Pinene	0977	2.2	2.1	RI, MS, CoI
Myrcene	0982	0.3	0.2	RI, MS
p-Cymene	1016	0.7	0.4	RI, MS, CoI
Limonene	1026	10.0	5.8	RI, MS, CoI
(*Z*-β)-Ocimene	1039	0.1	–	RI, MS
γ-Terpinene	1052	1.8	1.9	RI, MS, CoI
Linalool oxide	1063	0.1	–	RI, MS
Linalool	1093	79.0	81.8	RI, MS, CoI
α-Terpineol	1180	0.2	0.2	RI, MS,
Carvon	1226	0.1	–	RI, MS, CoI
Thymol	1270	–	tr	RI, MS, CoI
δ-Elemene	1340	tr	tr	RI, MS
β-Elemene	1391	–	tr	RI, MS
β-Caryophyllene	1426	0.7	0.8	RI, MS
α-Humulene	1454	0.3	–	RI, MS
(*Z*/*E*)-α-Farnesene	1483	1.4	1.6	RI, MS
Bicyclogermacrene	1500	1.0	2.8	RI, MS
Spathulenol	1575	0.3	0.3	RI, MS
Caryophyllene oxide	1580	–	0.1	RI, MS
Cedr-8-en-15-ol	1634	–	0.2	RI, MS
Santalol	1652	0.5	1.3	RI, MS
Monoterpene hydrocarbons		15.2	10.4	
Oxygenated monoterpenes		79.4	82.0	
Sesquiterpene hydrocarbons		3.4	5.2	
Oxygenated sesquiterpenes		0.8	1.9	
Total identified		98.8	99.5	

[a] RI, retention indices relative to C_6–C_{24} *n*-alkanes on the Rtx-1 column.
[b] tr, trace (< 0.1%).
[c] MS, mass spectra (matching with library spectra of GC-MS system); CoI, co-injection with an authentic component.

and limonene, against four Gram-positive bacteria: *Bacillus subtilis* (ATCC 9372), *Enterococcus faecalis* (ATCC 15753), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), and three Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27852) and *Klebsiella pneumoniae* (ATCC 3583), were determined using a disk diffusion method (Baron and Finegold, 1990).

The micro-dilution broth susceptibility assay was used for the evaluation of minimum inhibitory concentrations (MICs), as recommended by NCCLS (1999). A stock solution was made in dimethyl sulphoxide (DMSO) and then the dilution series were prepared, using sterile distilled water in 96-well microtiter plates. Overnight grown bacterial suspensions in Mueller Hinton broth were standardized to approximately 10^6 CFU/ml and 100 μl of each bacterial suspension was added to each well. The last row containing only the sterile dilutions of the antibacterial agent without microorganisms was considered as negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibitory concentration. Ampicillin was used as standard antibacterial agent.

Results and Discussion

Essential oil composition

Essential oil yields of the two air-dried samples of *Grammosciadium platycarpum* (GP1 and GP2) were 1.01% and 0.72% (w/w) on a dry weight basis of the plant, respectively. The composition of the essential oils is reported in Table I, where compounds are listed in order of their elution from an Rtx-1 column. The GC-MS analysis of the oils resulted in the identification of twenty-two components, amounting to about 99% of the whole oils (see Table I). The two samples were characterized by a high percentage of oxygenated monoterpenes (79.4% – GP1, 82.0% – GP2) with linalool (79.0%, 81.8%) as the main constituent followed by monoterpene hydrocarbons (15.2%, 10.4%) with limonene (10.0%, 5.8%) as the principal compound. Both oils were similar regarding the qualitative pattern but displayed some quantitative differences especially for limonene (10.0% – GP1 vs. 5.8% – GP2). From these results obtained, it is clear that there is no chemical polymorphism at least between the two oil samples studied. The high content of linalool (79.0%, 81.8%) of this plant may be of commercial value as an essential oil source.

Antibacterial activity

The antibacterial assay by a disk diffusion method, used in the preliminary screening of the antibacterial activity of the essential oils, showed that the two samples were strongly to moderately active against all the tested Gram-positive and Gram-negative bacteria, except for *Pseudomonas aeruginosa* which was found to be resistant in all experiments. *Bacillus subtilis, Staphylococcus epidermidis* and *Escherichia coli* with inhibition zones of (35.2 mm – GP1, 33.5 mm – GP2), (35.5 mm, 39.7 mm) and (22.4 mm, 26.3 mm), and minimum inhibitory concentrations (MICs) of (0.46 mg/ml, 0.93 mg/ml), (0.93 mg/ml, 0.93 mg/ml) and (1.87 mg/ml, 1.87 mg/ml), respectively, were the most sensitive strains. *Enterococcus faecalis* and *Klebsiella pneumoniae* showed a lower zone of inhibitions, (12.6 mm, 11.5 mm) and (14.8 mm, 13.5 mm), and MICs of (15.0 mg/ml, 15.0 mg/ml) and (7.5 mg/ml, 15 mg/ml), respectively. Because limonene, the second main compound of the oils, at a dose of 1 μl, corresponding about to its percentage in the oils, showed a weak growth inhibition only against two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus epidermidis*, it is possible to state that the effectiveness of the essential oils is apparently due to linalool, that at a dose of 8 μl, corresponding to its percentage in the oils, maintained a high inhibitory activity with MICs ranging from 0.156 mg/ml to 2.5 mg/ml. The antibacterial activity of the mixed two main compounds, linalool and limonene, at a final volume of 9 μl (Lin, 8 μl, + Lim, 1 μl), corresponding to their percentage in the oil, showed no significant differences to the active compound linalool alone, and hence it might be concluded that these two major constituents have no antagonistic interaction (Table II).

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Table II. Antibacterial activity (Inhibition zone and MIC) of the oils and their main compounds of two samples of *Grammosciadium platycarpum*.

Test Organism	Inhibition zone [mm][a,c]						MIC[b,c]				
							[mg/ml]		[mg/ml (mM)]		
	GP1 (10 µl)	GP2 (10 µl)	Lin (8 µl)	Lim (1 µl)	Lin + Lim (8 µl + 1 µl)	Ampicillin (10 µg/disk)	GP1	GP2	Lin	Lim	Lin+Lim
Bacillus subtilis	35.2 ± 0.7	33.5 ± 0.9	28.5 ± 0.6	10.6 ± 0.4	26.6 ± 0.8	14.2 ± 0.4	0.5 ± 0.2	0.9 ± 0.3	0.2 (1.3) ± 0.1	0.6 (4.4) ± 0.1	0.3 (1.0) ± 0.1
Staphylococcus aureus	18.3 ± 0.8	17.6 ± 0.3	17.6 ± 0.9	–	19.3 ± 1	13.1 ± 0.8	1.9 ± 0.3	3.7 ± 0.5	0.6 (3.9) ± 0.3	2.5 (18.4) ± 0.2	0.6 (2.1) ± 0.2
Staphylococcus epidermidis	35.5 ± 0.8	39.7 ± 1	26.6 ± 1	10.2 ± 0.8	27.4 ± 0.9	19.0 ± 0.4	0.9 ± 0.2	0.9 ± 0.2	0.2 (1.3) ± 0.2	0.6 (4.4) ± 0.2	0.3 (1.0) ± 0.1
Enterococcus faecalis	12.6 ± 0.4	11.5 ± 0.5	10.1 ± 0.6	–	10.3 ± 0.9	11.2 ± 0.3	15.0 ± 1	15.0 ± 0.8	2.5 (16.2) ± 0.4	5.0 (36.8) ± 0.9	2.5 (8.6) ± 0.3
Escherichia coli	22.4 ± 0.6	26.3 ± 0.5	20.4 ± 0.9	–	20.2 ± 0.6	12.0 ± 1	1.9 ± 0.2	1.9 ± 0.2	1.2 (7.8) ± 0.2	5.0 (36.8) ± 0.4	0.6 (2.1) ± 0.2
Klebsiella pneumoniae	14.8 ± 0.7	13.5 ± 0.8	13.1 ± 0.9	–	12.5 ± 0.5	–	7.5 ± 0.5	15.0 ± 0.9	0.6 (3.9) ± 0.3	1.2 (9.2) ± 0.3	1.2 (4.3) ± 0.2
Pseudomonas aeruginosa	–	–	–	–	–	9.7 ± 0.6	nt	nt	nt	nt	nt

Values are presented as mean ± standard deviation.
[a] Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm).
[b] Minimum inhibitory concentration as mg/ml for essential oils and mg/ml (mM) for pure compounds.
[c] Lin, linalool; Lim, limonene; Lin+Lim, mixture of linalool and limonene; GP, *Grammosciadium platycarpum*.
(–), Inactive; (7–14); moderately active; (> 14), highly active; nt: not tested.

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