Antimicrobial Activity and Composition of the Essential Oil of *Gontscharovia popovii* from Iran

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The aerial parts of *Gontscharovia popovii* (B. Fedtsch. and Gontsch.) Boriss. were collected at full flowering stage. The essential oil was isolated by hydrodistillation and analyzed by a combination of capillary GC and GC-MS. Thirty-one components were identified with the main constituent being carvacrol (71.9%), followed by linalool (5.5%), *p*-cymene (4.5%) and γ-terpinene (4.4%). The *in vitro* antimicrobial activity of the essential oil of *G. popovii* was studied against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The results of the bioassays showed that the oil exhibited strong antimicrobial activity against all the tested fungi and bacteria except for the resistant bacterium *Pseudomonas aeruginosa*.

Key words: Gontscharovia popovii, Labiatae, Antimicrobial Activity

Introduction

Gontscharovia Boriss. is one of the genera of the Lamiaceae family, closely related to *Satureja* but with some morphological differences. *Gontscharovia popovii* (B. Fedtsch. and Gontsch.) Boriss. is an aromatic species distributed in Tadjikistan (Central Asia), Afghanistan, Pakistan (Rechinger, 1982) and has recently been reported in Southern Iran as a new record for flora of Iran (Jamzad *et al*., 2004). It is a small aromatic shrub up to 45 cm. It differs from *Satureja* in having the following characteristics: long, slender spike inflorescence; 15-nerved calyx and nutlets apically attenuate into a short beak. The chemical composition of the essential oils and biological activities of different *Satureja* species have already been reported (Capone *et al*., 1989; Deans and Svoboda, 1989; Tumen *et al*., 1998; Sefidkon and Jamzad, 2000; Sefidkon and Ahmadi, 2000; Ciani *et al*., 2000; Ghannadi, 2002; Sajjadi and Baluchi, 2002; Tzakou and Skaltsa, 2003; Goren *et al*., 2004). Aerial flowering parts of *Satureja* species have frequently been used as a flavouring agent in food and also in traditional medicine for treating of different infectious diseases. In general, thymol, carvacrol and their precursors, *p*-cymene and -terpinene, were found to be the main constituents of the savory oil, from which it is possible to conclude that the phenolic compounds are responsible for the strong biological and pharmacological properties. Because of the high percentage of carvacrol in *G. popovii* essential oil, we were interested in studying its antimicrobial activity as well as its composition.

Material and Methods

Plant material and isolation procedure

The aerial parts of *Gontscharovia popovii* were collected from Bokhon Mountain (Hormozgan province) at full flowering stage in September 2005. Air-dried aerial parts of the plant were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored in sealed vials at low temperature until analysis and other tests.

Gas chromatography

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.,

film thickness 0.25 μm). Column temperature was held at 40 °C for 5 min and then programmed to 250 °C at a rate of 4 °C/min. Injector and detector (FID) temperatures were 260 °C; helium was used as carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-mass spectrometry

GC-MS analysis was carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.). Column temperature program was 40 °C to 240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, injector temperature 250 °C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/min, ionization energy 70 eV, scan time 1 s, mass range 40–350 amu.

Identification of components

The components of the oil were identified by comparison of their mass spectra with those in a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Shibamoto, 1987; Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

Bioassay procedure

The *in vitro* antibacterial and antifungal activity of the oil and its main compounds were evaluated by a disc diffusion method using Mueller-Hinton agar for bacteria and Sabouraud Dextrose agar for fungi (Baron and Finegold, 1990). Discs containing 2.5 μl and 5.0 μl of the oil were used and growth inhibition zones were measured after 24 h and 48 h of incubation at 37 °C and 24 °C for bacteria and fungi, respectively. Gentamicine and tetracycline for bacteria and nystatine for fungi susceptibility were used as positive controls. The microorganisms used were as follows: *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, *Klebsiella pneumoniae* ATCC 3583, *Candida albicans* ATCC 5027, *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404. Minimum inhibitory concentrations (MICs) were measured by a microdilution broth susceptibility assay recommended by NCCLS (1999).

Results and Discussion

Essential oil analysis

The hydrodistilation of the aerial parts of *G. popovii* gave a yellow oil in 0.9% (w/w) yield based on the dry weight of plant. The results obtained by GC and GC-MS analysis of the essential oil can be seen in Table I, where compounds are listed in order of their elution from a DB-5 column. Thirty-one components were identified, representing more than 99.3% of the total oil. The chemical composition of the essential oil includes a high content of monoterpenes (94.5%) from

Table I. Percentage composition of the essential oil of *Gontscharovia popovii.*

Compound	RI	(%)	Method of identification[a]
α-Thujene	0930	0.4	MS, RI
α-Pinene	0938	0.5	MS, RI, CoI
Camphene	0952	0.2	MS, RI
Sabinene	0975	t[b]	MS, RI
β-Pinene	0980	0.2	MS, RI
Myrcene	0990	0.7	MS, RI, CoI
α-Phellandrene	1004	0.2	MS, RI
α-Terpinene	1017	0.9	MS, RI, CoI
p-Cymene	1025	4.5	MS, RI, CoI
Limonene	1030	0.2	MS, RI, CoI
1,8-Cineole	1033	0.5	MS, RI, CoI
(*E*)-β-Ocimene	1050	t	MS, RI, CoI
γ-Terpinene	1061	4.4	MS, RI, CoI
cis-Sabinene hydrate	1068	t	MS, RI
Terpinolene	1087	t	MS, RI
Linalool	1096	5.5	MS, RI, CoI
α-Thujone	1101	t	MS, RI, CoI
β-Thujone	1113	t	MS, RI, CoI
trans-Pinocarveol	1137	t	MS, RI
Camphor	1143	t	MS, RI, CoI
Borneol	1164	1.4	MS, RI, CoI
Terpinen-4-ol	1177	0.5	MS, RI
p-Cymen-8-ol	1181	t	MS, RI
α-Terpineol	1189	t	MS, RI
Carvone	1241	0.3	MS, RI
trans-Myrtanol	1256	0.3	MS, RI
Geranial	1268	t	MS, RI, CoI
Thymol	1290	1.9	MS, RI, CoI
Carvacrol	1297	71.9	MS, RI, CoI
β-Caryophyllene	1418	4.0	MS, RI, CoI
Spathulenol	1576	0.8	MS, RI
Monoterpene hydrocarbons		12.2	
Oxygenated monoterpenes		82.3	
Sesquiterpene hydrocarbons		4.0	
Oxygenated sesquiterpenes		0.8	
Total		*99.3*	

[a] RI, retention indices in elution order from a DB-5 column; MS, mass spectroscopy; CoI, co-injection.
[b] t, trace (< 0.05%).

which 82.3% and 12.2% are attributed to the oxygenated and hydrocarbon monoterpenes, respectively. *p*-Cymene (4.5%) and γ-terpinene (4.4%) were the major monoterpene hydrocarbons found in the oil. The oxygenated monoterpenes fraction of the oil is characterized by the presence of high content of carvacrol (71.9%), followed by linalool (5.5%). β-Caryophyllene (4.0%) and spathulenol (0.8%) were the only sesquiterpenes present in the oil.

Table II. Antimicrobial activity of the essential oil of *Gontscharovia popovii.*

Microorganism	Essential oil		Antibiotics		
	IZ[a]	MIC[b]	Tetracycline (30 μg/disc)	Gentamicine (10 μg/disc)	Nystatine
Bacillus subtilis	40 ± 0.8	0.93	21 ± 0.8	–	nt
Staphylococcus epidermidis	35 ± 0.8	0.93	34 ± 0.8	–	nt
Enterococcus faecalis	18 ± 0.4	7.44	9 ± 0.4	–	nt
Staphylococcus aureus	30 ± 0.8	1.86	20 ± 0.8	–	nt
Klebsiella pneumoniae	22 ± 0.4	3.72	–	20 ± 0.8	nt
Pseudomonas aeruginosa	–	nt	–	12 ± 0.4	nt
Escherichia coli	28 ± 0.4	1.86	–	23 ± 0.8	nt
Aspergillus niger	40 ± 0.4	0.16	nt	nt	16 ± 0.8
Candida albicans	38 ± 0.8	0.16	nt	nt	18 ± 0.4
Saccharomyces cerevisiae	33 ± 0.4	0.32	nt	nt	18 ± 0.4

[a] Zone of inhibition includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml.
Essential oil tested at 2.5 μl for bacteria and 5.0 μl for fungi.
(–), Inactive; (7–14), moderately active; (>14), highly active; nt, not tested.
Values are given as mean ± standard deviation.

Table III. Antimicrobial activity of the main compounds of the essential oil of *Gontscharovia popovii.*

Microorganism	Carvacrol		*p*-Cymene		γ-Terpinene		Linalool	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC
Bacillus subtilis	43 ± 0.8	0.2 (1.3) ± 0.1	17 ± 0.6	3.75 (27.9) ± 0.6	19 ± 0.8	3.75 (27.5) ± 0.6	29 ± 0.8	0.2 (1.3) ± 0.1
Enterococcus faecalis	25 ± 0.4	0.8 (5.3) ± 0.4	–	nt	11 ± 0.4	7.5 (55.1) ±0.4	12 ± 0.4	3.2 (20.7) ± 0.8
Staphylococcus aureus	35 ± 0.6	0.4 (2.6) ± 0.2	9 ± 0.4	15 (55.9) ±0.8	9 ± 0.4	>15 (>110.1)	18 ± 0.4	0.8 (5.2) ± 0.4
Staphylococcus epidermidis	45 ± 0.8	0.2 (1.3) ± 0.1	8 ± 0.4	15 (55.9) ±0.4	14 ± 0.4	7.5 (55.1) ± 0.4	27 ± 0.6	0.2 (1.3) ± 0.1
Escherichia coli	34 ± 0.4	0.4 (2.6) ± 0.2	11 ± 0.6	15 (55.9) ±0.8	12 ± 0.6	7.5 (55.1) ±0.4	22 ± 0.8	0.4 (2.6) ± 0.2
Klebsiella pneumoniae	30 ± 0.6	0.8 (5.3) ± 0.4	–	>15 (>55.9)	–		14 ± 0.4	0.8 (5.2) ± 0.4
Pseudomonas aeruginosa	12 ± 0.4	6.4	–	nt	–		–	nt
Aspergillus niger	28 ± 0.4	0.8 (5.3) ± 0.4	–	nt	12 ± 0.4	>15 (>110.1)	14 ± 0.4	4.8 (31.1) ± 0.8
Candida albicans	40 ± 0.8	0.4 (2.6) ± 0.2	12 ± 0.4	>15 (>55.9)	15 ± 0.8	7.5 (55.1) ± 0.4	30 ± 0.8	0.6 (3.8) ± 0.1
Saccharomyces cerevisiae	35 ± 0.6	0.4 (2.6) ± 0.2	12 ± 0.8	>15 (>55.9)	13 ± 0.4	>15 (>110.1)	26 ± 0.6	1.2 (7.7) ± 0.2

[a] Zone of inhibition includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml (mM).
Main compounds tested at 10 μl/disc.
(–), Inactive; (7–14), moderately active; (> 14), highly active; nt, not tested.
Values are given as mean ± standard deviation.

Antimicrobial activity

The essential oil of *G. popovii* and its main constituents were tested against four Gram-positive and three Gram-negative bacteria as well as three fungi. The results of the bioassays showed that the oil exhibited strong antimicrobial activity against all the tested microorganisms except for the resistant bacterium *Pseudomonas aeruginosa* (Table II). Three tested fungi, *Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae* were found to be more sensitive to the oil than bacteria with inhibition zones of 40 mm, 38 mm and 33 mm, and MIC values of 0.16 mg/ml, 0.16 mg/ml and 0.32 mg/ml, respectively. Gram-positive bacteria, *Bacillus subtilis, Staphylococcus epidermidis* and *Staphylococcus aureus*, were also more sensitive to the oil than other bacteria with MIC values of 0.93 mg/ml, 0.93 mg/ml and 1.86 mg/ml, respectively. From Gram-negative bacteria, *Pseudomonas aeruginosa* was resistant to the oil, while *Escherichia coli* and *Klebsiella pneumoniae* showed high sensitivity towards the oil with inhibition zones of 28 mm and 22 mm and MIC values of 1.86 mg/ml and 3.72 mg/ml.

Based on obtained results (Table II), it is evident that the essential oil has a stronger activity than the standard antibiotics. The antimicrobial nature of the oil investigated is apparently related to the presence of a high phenolic content; carvacrol together with linalool was studied and their antimicrobial properties are documented in Table III. Owing to its potent antibiotic property exhibited in the antimicrobial test, the essential oil of *G. popovii* could be considered as a natural source that can be freely used in food and pharmaceutical industries as a natural antibiotic and culinary spice.

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