Composition and Antimicrobial Activity of the Essential Oil of *Dicyclophora persica* Boiss. from Iran

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The chemical composition of the essential oil of *Dicyclophora persica* Boiss. was identified by GC and GC-MS analysis. The analysis of the oil resulted in the identification of forty-five components constituting 98.6% of the total oil. The main constituents were α-pinene (31.5%), (*Z*)-β-ocimene (23.3%), *p*-cymene (6.7%) and (*E*)-β-ocimene (5.4%). The antimicrobial activity of the oil was tested by the disk diffusion method against four Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis)* and three Gram-negative (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa)* bacteria together with a fungus *(Aspergillus niger)*. The oil showed strong inhibition activity toward all the tested microorganisms except for *Pseudomonas aeruginosa*.

Key words: Dicyclophora persica, Antimicrobial Activity, Essential Oil Composition

Introduction

The monotypic genus *Dicyclophora* Boiss. belonging to the Apiaceae family consists of only one species in the world. *D. persica* Boiss. is a species endemic to southern parts (Fars, Bushehr, Khuzestan, Hormozgan and Balochestan provinces) of Iran (Mozaffarian, 1996). Morphologically, this species is easily recognized by the tall annual habit, the thickened flat rays, the showy outer petals and the sterile purplish-black central umbellule (Hedge and Lamond, 1987).

In the framework of our studies on the essential oil compositions and biological activities of Iranian aromatic plants (Sonboli *et al*., 2005), here we report the composition and antimicrobial activity of the essential oil of *D. persica* against four Gram-positive and three Gram-negative bacteria and also a fungus which has not been the subject of previous investigations.

Material and Methods

Plant material

The aerial parts of *Dicyclophora persica* were collected at full flowering stage from Darab towards Fasa road in Fars province at an altitude of 1400 m on May 14, 2004. A voucher specimen (MP-736) has been deposited in Medicinal Plants and Drugs Research Institute Herbarium of Shahid Beheshti University, Tehran, Iran.

Oil isolation procedure

Air-dried aerial parts of the plant (50 g) were hydrodistilled for 3.5 h using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate and kept in a sealed vial at 4 °C until analyzed and tested.

GC and GC-MS analysis

GC analysis was performed on a Thermoquest-Finnigan Trace GC instrument equipped with a capillary DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min. Split ratio was adjusted at 1/50. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively.

GC-MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-1 fused silica capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min, and then kept at 250 °C for

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10 min. Transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio of 1/50. A quadrupole mass spectrum was scanned over 45–465 amu with an ionizing voltage of 70 eV and an ionizing current of 150 A.

The constituents of the oil were identified by calculation of their retention indices under temperature programmed conditions for *n*-alkanes (C_6–C_{24}) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with a computer library (Wiley 7.0) or authentic compounds and confirmed by comparison of their retention indices with authentic compounds or of those published in the literature (Shibamoto, 1987; Davies, 1990; Adams, 2001).

Antimicrobial activity assay

The *in vitro* antimicrobial activity of the essential oil and its main compounds was evaluated by a disc diffusion method using Mueller-Hinton Agar for bacteria and Sabourod Dextrose Agar for the fungus, with determination of inhibition zones (Baron and Finegold, 1990). The microbial species used in this research were: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, *Klebsiella pneumoniae* ATCC 3583 and *Aspergillus niger* ATCC 16404. The essential oil (10 μl for bacteria and 20 μl for the fungus) was applied on the paper discs which subsequently were placed on inoculated plates. After 24 h of incubation at 37 °C for bacteria and 48 h at 24 °C for the fungus the diameters of growth inhibition zones were measured. For the determination of MIC (minimum inhibitory concentration) values, a microdilution broth susceptibility assay was used as recommended by NCCLS (1999). Standard reference antibiotics (ampicillin for bacteria and nystatine for the fungus) were used as positive controls. All experiments were performed in triplicate.

Results and Discussion

Essential oil analysis

The essential oil of *D. persica* was obtained in the yield of 0.5% (v/w) and 0.3% (w/w) based on the dry weight of the plant. Forty-five components were identified which were 98.6% of the total oil.

Table I. Essential oil composition of *Dicyclophora persica* from Iran.

Identification method[a]	%	RI	Compound
RI, MS	0.2	0926	α-Thujene
RI, MS, Co-I	31.5	0938	α–Pinene
RI, MS, Co-I	1.8	0950	Camphene
RI, MS, Co-I	0.5	0970	Sabinene
RI, MS, Co-I	2.9	0977	β-Pinene
RI, MS, Co-I	4.7	0984	Myrcene
RI, MS	2.5	1002	α-Phellandrene
RI, MS, Co-I	6.7	1017	*p*-Cymene
RI, MS	4.7	1026	Limonene
RI, MS	23.3	1029	(*Z*)-β-Ocimene
RI, MS	5.4	1040	(*E*)-β-Ocimene
MS	0.1	1078	1-Methyl-4-iso-propenyl benzene
RI, MS	0.2	1082	6-Camphonenone
RI, MS	1.6	1084	*p*-Mentha-1,4(8)-diene
RI, MS	0.2	1111	6-Camphenol
RI, MS	0.5	1120	*allo*-Ocimene
MS	0.1	1124	*trans*-Epoxy ocimene
RI, MS	0.8	1131	*cis*-Verbenol
RI, MS	0.3	1134	*trans*-Verbenol
RI, MS	0.1	1152	*p*-Mentha-1,5-dien-8-ol
RI, MS	0.5	1165	*p*-Cymen-8-ol
RI, MS, Co-I	0.1	1168	4-Terpineol
RI, MS	0.2	1178	Myrtenol
RI, MS	0.6	1188	α-Phellandrene epoxide
RI, MS	0.1	1203	*trans*-Carveol
RI, MS, Co-I	0.1	1223	Carvone
RI, MS, Co-I	0.1	1270	Thymol
RI, MS, Co-I	0.1	1280	Carvacrol
RI, MS	0.1	1375	Methyl eugenol
RI, MS	0.4	1383	α-Copaene
RI, MS	0.2	1393	β-Cubebene
RI, MS	0.6	1427	*trans*-Caryophyllene
RI, MS	0.1	1460	α-Humulene
RI, MS	0.1	1474	ar-Curcumene
RI, MS	0.3	1485	Germacrene D
RI, MS	0.2	1491	β-Selinene
RI, MS	0.1	1501	α-Selinene
RI, MS	0.2	1522	δ-Cadinene
RI, MS	0.1	1533	Ledol
MS	0.1	1536	3-Methoxy piperanol
RI, MS	0.1	1575	Spathulenol
RI, MS	0.3	1582	Caryophyllene oxide
MS	4.9	1593	*trans*-Isomyristicin
RI, MS	0.1	1636	α-Cadinol
MS	0.8	1937	*trans*-3-Caren-2-ol
	98.6		Total identified
	86.3		Monoterpene hydrocarbons
	3.6		Oxygenated monoterpenes
	2.2		Sesquiterpene hydrocarbons
	6.4		Oxygenated sesquiterpenes

[a] RI, retention indices relative to C_6–C_{24} *n*-alkanes on DB-1 column; MS, mass spectrum; Co-I, co-injection with authentic compounds

Table II. Antimicrobial activity of the essential oil and main compounds of *Dicyclophora persica*.

Microorganism	Essential oil		Ocimene		α-Pinene		Antibiotics	
	IZ[a]	MIC[b]	IZ	MIC[c]	IZ	MIC[c]	Ampicillin[d]	Nystatine[e]
Bacillus subtilis	23.2 ± 0.5	0.6 ± 0.1	14.2 + 0.3	1.8 (13.2) ± 0.4	10.1 ± 0.6	7.5 (55.1) ± 0.2	14 ± 0.4	nt
Enterococcus faecalis	14.4 ± 0.3	2.4 ± 0.2	8.3 + 0.2	7.2 (52.4) ± 0.3	–	nt	11 ± 0.3	nt
Staphylococcus aureus	20.2 ± 0.2	1.2 ± 0.2	9.1 + 0.2	7.2 (52.4) ± 0.2	8.3 ± 0.4	>15 (>55.1) ± 0.4	13 ± 0.3	nt
Staphylococcus epidermidis	26.1 ± 0.3	0.3 ± 0.1	12.1 + 0.5	3.6 (26.5) ± 0.4	9.4 ± 0.5	15 (110.2) ± 0.3	19 ± 0.5	nt
Escherichia coli	17.5 ± 0.2	2.4 ± 0.2	9.6 + 0.3	7.2 (52.9) ± 0.3	11.5 ± 0.1	15 (110.2) ± 0.4	12 ± 0.2	nt
Klebsiella pneumoniae	11.4 ± 0.6	4.8 ± 0.4	–	nt	–	nt	–	nt
Pseudomonas aeruginosa	–	nt	–	nt	–	nt	9.7 ± 0.2	nt
Aspergillus niger	26.2 ± 0.3	0.6 ± 0.1	–	nt	–	nt	nt	16 ± 0.4

[a] Inhibition zone includes diameter of the disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml.
[c] Minimum inhibitory concentration values in mg/ml (mM).
[d] Tested at 10 μg/disc.
[e] Tested at 30 μg/disc.
Essential oil and main compounds tested at 10 μl/disc on bacteria and 20 μl/disc on fungus.
(–), Inactive; (7–14), moderately active; (>14), highly active; nt, not tested.
Values given as mean ± standard deviation.

The oil was dominated by monoterpene hydrocarbons with 86.3% of the total oil including α-pinene (31.5%), (*Z*)-β-ocimene (23.3%) and (*E*)-β-ocimene (5.4%) as the major constituents. The summarized results are represented in Table I, where all compounds are listed according to their elution from a DB-1 column.

Antimicrobial activity

The antibacterial activity of the oil of *D. persica* was tested against four Gram-positive and three Gram-negative bacteria. As shown in Table II, the oil strongly inhibited the growth of *Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis.* The oil also showed moderate antibacterial activity against *Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae. Pseudomonas aeruginosa* was resistant to the oil at 10 μl/disc content. An inhibition zone of 26 mm was observed for *Aspergillus niger* with a MIC value equal to 0.6 mg/ml.

We also tested the antibacterial activities of α-pinene and β-ocimene in order to explore if they are responsible for the observed antibacterial activity of the constituents. The results showed that in addition to effects of these components other components of the oil with smaller quantities like carvacrol and thymol should be effective for growth inhibitions of microorganisms.

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Adams R. (2001), Identification of Essential Oil Components by Gas Chromatography/Quadroupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, USA.

Baron E.-J. and Finegold S.-M. (1990), Methods for testing antimicrobial effectiveness. In: Diagnostic Microbiology (Stephanie M., ed.). C. V. Mosby Co., Baltimore, pp. 171–194.

Davies N. N. (1990), Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. **503**, 1–24 (1990).

Hedge I. C. and Lamond J. M. (1987), *Dicyclophora.* In: Flora Iranica, Umbelliferae, No. 162 (Rechinger K. H. and Hedge I. C., eds.). Akademische Druck and Verlagsanstalt, Graz, Austria, pp. 67–68.

Mozaffarian V. (1996), A Dictionary of Iranian Plant Names. Farhang-e Moaser, Tehran.

NCCLS (National Committee for Clinical Laboratory Standards) (1999), Performance Standards for Antimicrobial Susceptibility Testing. 9th International Supplement, Wayne, PA, M100-S9.

Shibamoto T. (1987), Retention indices in essential oil analysis. In: Capillary Gas Chromatography in Essential Oil Analysis (Sandra P. and Bicchi C., eds.). Huethig Verlag, New York.

Sonboli A., Salehi P., Kanani M. R., and Nejad Ebrahimi S. (2005), Antibacterial and antioxidant activity and essential oil composition of *Grammosciadium scabridum* Boiss. from Iran. Z. Naturforsch. **60c**, 534–538.