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Original Article

Antimicrobial Activity and Composition of the Essential Oil of *Cymbopogon Olivieri* (Boiss.) Bor from Iran

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Abstract

The *in vitro* antimicrobial activity of the essential oil isolated from the aerial parts of *Cymbopogon Olivieri* (Boiss.) Bor, an aromatic grass of Iran was tested against three Gram-negative and four Gram-positive bacteria and also three fungi. The results of the bioassays showed that the oil has a remarkable antimicrobial activity. *Bacillus subtilis* and *Candida albicans* were more sensitive to the oil than other microorganisms with inhibition zones of 20 mm and MIC values of 3.75 mg/ml and 2.5 mg/ml, respectively. The Gram-negative bacteria, *Pseudomonas aeruginosa* was resistant and *Klebsiella pneumoniae* showed less sensitivity to the oil with MIC value of >15 mg/ml. GC-MS analysis of the oil confirmed the determination of 40 compounds representing 95.0% of the oil. The main identified constituent was piperitone (48.9%).

Keywords: *Cymbopogon Olivieri*; Poaceae; Essential oil; Antimicrobial activity; Iran.

Introduction

The genus *Cymbopogon* Spreng. is an important member of aromatic grasses, belonging to the Poaceae family which are widely distributed and cultivated in tropical and subtropical regions in the world especially in southeast of Asia (1). The genus *Cymbopogon* comprises two perennial species in flora of Iran, *C. Olivieri* (Boiss.) Bor and *C. parkeri* Stapf., which are distributed in tropical regions of Iran including southern parts of Fars, Kerman, Hormozgan, Khuzestan, Bushehr and Baluchestan provinces (2, 3). *C. Olivieri* is known as “Kah Makki”, “Potar” and “Nagerd” in different areas which this plant is gathered (4). In traditional medicine, leaves and roots are widely used as antiseptic and for the treatment of stomachache (5). Evaluation of repellent activities of *Cymbopogon* essential oils against mosquito vectors of malaria, filariasis and dengue fever in India has been reported (6). The antimicrobial action of palmarosa oil (7) and antibacterial activity of the oil of *C. densiflorus* (8) has been the subject of earlier studies. As far as our literature survey could ascertain, the essential oil composition of the aerial parts of *C. Olivieri* has already been analyzed and piperitone (53.3%), α-terpinene (13.6%) and elemol (7.7%) were found to be the major constituents (9). In another study, the composition and antimalarial efficacy of the essential oil of *C. Olivieri* have been reported and it has exhibited interesting activity against larvaes of *Anophel stephensi* (10). To the best

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of our knowledge, the *in vitro* antimicrobial activity and composition of the essential oil of *C. Olivieri* reported here, has not been the subject of previous investigation.

Experimental

Plant Material

Aerial parts of *Cymbopogon Olivieri* were collected at full flowering stage in April 2003, from Hormozgan: Bandar-Abbas - Hajiabad road, Siahoo village, at an altitude of 1900 m, Iran. A voucher specimen (MP-489) was deposited at the Medicinal Plants and Drugs Research Institute Herbarium, Shahid Beheshti University, Tehran, Iran.

Essential Oil Isolation and Analysis Procedure

The essential oil obtained by hydrodistillation from the air-dried aerial parts of plant using a Clevenger-type apparatus for 3 h. The distilled oil was dried over anhydrous sodium sulfate and stored in sealed vial at 4 ºC until bioassay and GC-MS analyses.

GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS equipped with a fused silica capillary DB-1column (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.; transfer line temperature, 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; split ratio, 1/50. The quadruple mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library Wiley 7.0 or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (11). For quantification purposes relative area percentages obtained by FID were used.

Bioassay Procedure

The *in vitro* preliminary antimicrobial activity of the essential oil (dissolved in 0.1%

Table 1. Essential oil composition of *C. olivieri* from Iran.

No.	Compound	%	RI
1	tricyclene	0.6	924
2	α-pinene	1.2	933
3	camphene	2.2	948
4	methyl-4-methylene-bicyclo [3.2.1] oct-2-en	1.7	964
5	myrcene	0.7	980
6	α-terpinene	13.8	999
7	p-cymene	0.4	1013
8	limonene	6.3	1023
9	(Z)-ocimene	0.1	1035
10	fenchone	0.1	1073
11	linalool	0.5	1083
12	(Z)-p-menth-2-en-1-ol	1.0	1110
13	2-carene	t	1116
14	(E)-p-menth-2-en-1-ol	0.7	1126
15	borneol	0.5	1154
16	(E)-carveol	0.4	1161
17	4-terpineol	0.5	1165
18	α-trpineol	1.5	1175
19	(Z)-piperitol	0.3	1182
20	(E)-piperitol	0.3	1192
21	piperitone	48.9	1237
22	thymol methyl ether	0.1	1240
23	p-mentha-1,3-dien-7-al	0.1	1254
24	thymol	0.1	1263
25	isoborneol	t	1270
26	geranyl actate	0.4	1355
27	β-elemene	1.4	1389
28	α-yelangen	0.1	1418
29	β-caryophyllene	0.1	1423
30	germacrene D	0.3	1480
31	valencene	0.2	1486
32	bicyclogermacrene	0.2	1491
33	γ-cadinene	0.4	1509
34	δ-cadinene	0.2	1515
35	elemol	3.8	1537
36	spathulenol	0.3	1570
37	α-cadinol isomer=1	2.2	1610
38	γ-eudesmol	0.7	1624
39	guaiol	0.8	1626
40	β-eudesmol	1.9	1642
	Total identified	95.0	

[a] RI, retention indices relative to C_6 - C_{24} *n*-alkanes on a DB-1 column;
MS, mass spectrum; Co-I, co-injection with authentic compound.
[b] t, trace (<0.1%).

Table 2. Antimicrobial activity of *Cymbopogon olivieri* essential oil.

Microorganism	Essential oil[a]		Inhibition zone (mm)[b]	
	IZ[b]	MIC[c]	Ampicillin (10 µg/disc)	Nystatine (30 µg/disc)
Bacillus subtilis	20	3.75	14	nt
Enterococcus faecalis	11	15	11	nt
Staphylococcus aureus	15	7.5	13	nt
Staphylococcus epidermidis	13	7.5	19	nt
Pseudomonas aeruginosa	-	nt	9.7	nt
Escherichia coli	12	15	12	nt
Klebsiella pneumoniae	8	>15	-	nt
Candida albicans	20	2.5	nt	18
Aspergillus niger	14	5	nt	16
Microsporium gypsium	15	10	nt	17

[a] Essential oil tested at a concentration of 15 µl/disc for bacteria and 30 µl/disc for fungi.
[b]Diameter of inhibition zones including diameter of sterile disc (6 mm).
[c] Minimal inhibitory concentration, values are given as mg/ml
(-), Inactive; (7-14), moderately active; (>14), highly active; nt, not tested.

tween 80, 1:1) was evaluated by disc diffusion method using Mueller-Hinton Agar for bacteria and Sabourod Dextrose Agar for fungi (12) with determination of inhibition zones. Three Gram-negative and four Gram-positive bacteria and also three fungi 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, *Microsporium gypsium* ATCC 5070 and *Aspergillus niger* ATCC 16404. All bacteria strains as well as two yeasts, *Candida albicans* and *Microsporium gypsium* were sub-cultured from their original lyophilized stocks (ATCC) on nutrient agar and then the obtained single colonies were used for future bioassays. For *Aspergillus niger* a concentration of 1×10^6 /ml spore was used. Ampicillin for bacteria and nystatine for fungi were used as positive controls. Tween 80 (0.1%) was used as negative control. The incubation conditions used were 24 h at 37 °C for bacteria and 48 h at 24 °C for fungi. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the oil that resulted in a complete inhibition of visible growth in the broth which was measured by microdilution broth susceptibility assay recommended by NCCLS (13).

Results and Discussion

The yield of the essential oil of *C. Olivieri* was 0.6% (*w/w*) and 0.9% (*v/w*) based on the dry weight of plant. The oil was yellow in colour. GC-MS analysis of the oil confirmed the determination of 40 compounds representing 95.0% of the oil (Table 1). The main identified constituents were piperitone (48.9%), α-terpinene (13.8%), limonene (6.3%) and elemol (3.8%), which constituted 72.9% of total oil. The results obtained from essential oil analysis showed some quantitative and qualitative differences with the previous investigation (9), which could be attributed to geographical origin and chemotype of them.

Antimicrobial activity of the oil measured by disc diffusion and minimal inhibitory concentration (MIC) methods showed that the oil of *C. Olivieri* was active against most of the tested microorganisms except for *Pseudomonas aeruginosa* that was resistant to the oil. Table 2 shows *in vitro* antimicrobial property, growth inhibitory zones and MIC values, of the oil of *C. olivieri* and the inhibition zones formed by standard reference antibiotic discs. The oil exhibited moderate to high activity towards the microorganisms among which *B. subtilis* and *C. albicans* with inhibition zones of 20mm and MIC values of 3.75mg/ml and 2.5mg/ml, respectively, being more sensitive than the others. *Klebsiella pneumoniae* exhibited less

sensitivity to the oil with inhibition zone of 8mm and MIC value of >15mg/ml. The results revealed that the oil exhibited the same type of activity compared to positive standard controls (Table 2). The essential oil composition and the observed antimicrobial property showed that the essential oil of plant has a good potential for use in aromatherapy and pharmacy and supports its uses in traditional medicine.

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