VOLATILE CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF ZATARIA MULTIFLORA, POPULATION IRAN

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ABSTRACT: *The essential oils of Zataria multiflora (Labiatae) from four regions of Iran were investigated by GLC and GC-MS methods. The main constituents were carvacrol, thymol, linalool and p-cymene. The percentage of the constituents (in dry and wet plant) of different regions have been compared. Antibacterial and antifungal effects of these essential oils have been studied. The essential oil with highest percentage of thymol and carvacrol had a significant antimicrobial activity.*

KEY WORDS: *Zataria multiflora, Labiatae, Essential oil, Antibacterial, Antifungal.*

INTRODUCTION

The constituents of the essential oil of *Zataria multiflora* of one region in Iran (Yazd, central Iran) was reported previously [1].

Thymol and carvacrol have been shown to have antimicrobial activity [2]. In addition, essential oils have been used as antifungal in folk medicine. In the present work, constituents of the essential oil of the plant collected from Kerman and Fars provinces were compared, and the antimicrobial activity of these essential oils were determined.

RESULTS AND DISCUSSION

Table 1 shows the composition of the essential oil of *Zataria multiflora* growing in different regions of Kerman and Fars Provinces. The 35 compounds have been identified by Kovats indices and MS spectrums [3-8]. One unknown component with MW=152 and Neryl acetate were detected in one region (III_A and III_B). Neroloxide was also detected in some regions.

The percentage of the constituents depended upon the age of the plant and the regions of growth. When leaves, collected from region III were very fresh, linalool was the main constituent of the essential oil (see Table 1).

The essential oil of the dry plant showed high percentages of carvacrol and thymol. Comparison

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Table 1: Constituents of the essential oil of Zataria multiflora collected from different regions of Kerman and Fars provinces

Peak No.	Compound	% in Oil								
		IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB	RI
1	α-Thujene	0.06	0.68	0.02	0.12	0.03	0.03	0.03	0.03	926
2	α-Pinene	0.42	1.64	0.18	0.61	0.19	0.10	0.29	0.14	935
3	Camphene	0.04	0.11	0.02	0.04	0.01	0.01	0.02	0.01	950
4	1-Octen-3-ol	0.07	0.35	0.02	0.21	0.02	0.02	0.04	0.03	966
5	3-Octanone	0.10	0.95	0.06	0.49	0.07	0.04	0.10	0.14	970
6	β-Pinene	0.37	0.30	0.02	0.08	0.09	0.02	0.03	0.05	974
7	Myrcene	0.71	0.85	0.18	0.32	0.11	0.03	0.20	0.08	983
8	α-Phellandrene	–	–	0.01	0.01	0.01	0.01	0.02	0.01	999
9	α-Terpinene	0.05	0.46	0.04	0.21	0.04	0.02	0.02	0.03	1013
10	p-Cymene	0.85	11.4	0.61	5.45	0.80	0.88	0.83	4.61	1016
11	1,8-Cineol	–	–	0.07	0.60	0.06	0.04	0.50	0.21	1023
12	Limonene+β-	0.91	5.60	0.31	1.50	0.08	0.05	0.18	0.06	1025
13	phellandrene									
14	γ-Terpinene	0.16	0.34	0.27	0.65	0.16	0.21	0.06	0.44	1055
15	*trans*-Linalool oxide[a]	0.01	0.01	0.07	0.04	0.02	0.01	0.01	0.01	1068
16	*cis*-Linalool oxide[a]	0.01	0.01	0.05	0.03	0.09	0.33	0.01	0.01	1080
17	Linalool	8.20	9.60	9.88	25.10	13.32	80.57	5.17	25.06	1087
18	Neroloxide	–	–	0.42	0.65	0.26	0.15	–	–	1140
19	Borneol	–	–	0.19	0.28	0.13	0.27	0.12	0.57	1155
20	4-Terpineol	0.95	1.58	0.56	0.48	0.32	0.85	0.21	1.13	1168
21	α-Terpineol	1.66	0.24	1.45	4.82	0.65	2.06	0.92	4.56	1178
22	Unknown Mw=152	–	–	–	–	0.08	0.19	–	–	1188
23	Thymol Me ether	0.87	0.69	0.41	0.52	0.32	2.15	0.30	0.06	1217
24	Carvacrol Me ether	0.83	1.84	1.98	2.18	2.05	0.21	1.20	0.35	1223
25	Thymol	58.60	52.50	14.15	7.13	28.30	2.81	17.20	28.30	1273
26	Bornyl acetate	1.82	0.15	0.18	0.09	–	–	0.04	0.02	1274
27	Carvacrol	12.20	1.85	65.10	44.12	46.31	4.12	66.30	27.60	1282
28	Carvyl acetate	2.30	0.14	0.08	0.19	0.18	0.54	0.03	0.04	1345
29	Neryl acetate	–	–	–	–	0.09	0.05	–	–	1347
30	β-Caryophyllene	4.18	1.65	2.21	1.46	3.06	1.02	2.36	2.75	1424
31	Aromadendrene	0.32	0.26	0.10	0.09	0.03	0.03	0.17	0.14	1442
32	Alloaromadendrene	0.14	0.08	0.01	0.01	0.15	0.03	0.16	0.11	1453
33	α-Humulene	0.32	0.26	0.10	0.09	0.03	0.03	0.17	0.14	1462
34	Valencene	0.76	0.75	0.18	0.11	0.25	0.09	0.29	0.17	1490
35	Spathulenol	1.10	0.61	0.10	0.07	0.57	0.71	0.79	0.95	1568
36	Widdrol	1.52	0.63	0.09	0.05	0.78	0.90	0.98	1.49	1596

IA= Essential oil of dry plant in Tanbour; IB= Essential oil of wet plant in Tanbour; IIA= Essential oil of dry plant in Zarand; IIB=Essential oil of wet plant in Zarand; IIIA= Essential oil of dry plant in Shirkuh; IIIB= Essential oil of wet plant in Shirkuh; IVA= Essential oil of dry plant in Dehbid; IVB=Essential oil of wet plant in Dehbid.

a) see Kries, P., Dietrich, A., Mosandl, A. J. Essent. oil Res., 8, 339(1996).

Table 2: Antimicrobial activities of Zataria multiflora

Organisms	mg of sample oil/paper disc	Zone of inhibition in mm(mean ±SD, n=3) Essential oil of different regions 1	2	3	4
	0.15	8.0±0.35	–	6.9±0.28	–
Staphylococus aureus	0.20	9.07±0.62	7.9±0.42	8.0±0.56	–
(PTCC 1337)	0.50	14.1±0.28	9.0±0.30	8.3±0.5	–
	1.0	31.9±1.5	10.1±0.7	9.7±0.7	8.0±0.41
Gentamycin 30 μg= 10 mm inhibition					
	0.15	–	–	–	–
Bacillus subtillis	0.20	14.0±1.05	8.0±0.90	7.9±0.45	–
(PTCC 1023)	0.50	16.1±0.75	14.1±0.80	11.3±0.54	–
	1.0	34±1.4	31.8±0.82	17.2±0.78	7.0±0.33
Gentamycin 30 μg= 30 mm inhibition					
	0.15	–	–	8.0±0.5	–
Proteus mirabilis	0.20	10.1±0.56	–	10.0±1.03	–
(PTCC 1076)	0.50	11.8±1.05	10.06±0.8	14.3±1.2	–
	1.0	13.7±0.9	11.8±0.6	22.06±0.6	7.1±0.26
Gentamycin 30 μg= 22 mm inhibition					
	0.15	7.9±0.60	–	10.1±0.66	–
Klebsiella Pneumonia	0.20	9.0±0.52	8.0±0.63	13.1±0.92	–
(PTCC 1053)	0.50	11.9±0.91	10.2±0.73	13.9±0.80	–
	1.0	16.2±0.85	12.1±1.0	17.9±0.86	8.1±0.25
Gentamycin 30 μg= 8 mm inhibition					
	0.15	9.9±0.71	8.1±0.38	–	–
Escherichia coli	0.20	12.3±0.90	9.2±0.29	8.1±0.40	–
(Endemic)	0.50	16.0±0.95	16.3±0.95	10.3±0.85	7.9±0.25
	1.0	16.1±1.1	16.2±1.1	11.9±0.95	10.3±0.73
Gentamycin 30 μg= 20 mm inhibition					
	0.15	8.9±0.61	–	–	–
Candida albicans	0.20	10.3±0.65	8.0±0.30	12.1±0.75	–
(PTCC 5027)	0.50	22.0±0.95	21.9±1.0	14.2±0.55	7.0±0.20
	1.0	32.1±1.3	28.3±1.1	30.0±0.90	8.1±0.28
Ketoconazol 30 μg= 26 mm inhibition					
	0.15	–	7.9±0.5	7.8±0.3	–
Aspergillus niger	0.20	10.0±0.61	9.9±0.85	10.0±0.51	–
(PTCC 5013)	0.50	18.2±0.80	19.8±0.76	11.9±0.88	–
	1.0	34.0±1.2	22.1±0.90	15.8±0.95	8.0±1.0
Ketoconazol 30 μg= 28 mm inhibition					

1: The essential oil of dry plant from Khormiz.

2: The essential oil of wet plant from Khormiz.

3: The essential oil of dry plant from Shirkuh.

4: The essential oil of wet plant from Shirkuh.

between the percentage of constituents in dry and wet plants demonstrated that in the drying process, the percentage of linalool, thymol and carvacrol changes.

Antimicrobial activity

Four oil samples with different major components have been selected.

1: The essential oil of the dry plant from Khormiz, the constituents of it have been previously reported [1]. In this essential oil carvacrol was the major component (carvacrol 61.29% and thymol 25.8%) and showed significant antimicrobial activity (Table 2).

2: The essential oil of wet plant from Khormiz with thymol as the major component (thymol 48.4% and carvacrol 12.6%) showed a moderate antimicrobial activity.

3: The essential oil of the dry plant from Shirkuh with carvacrol as a major compound (carvacrol 46.3% and thymol 28.3%), also showed a moderate antimicrobial activity (Tables 1 and 2).

4: The essential oil of the wet plant from Shirkuh, with linalool as a major compound (linalool 80.57%, thymol 2.8% and carvacrol 4.12%) had the lowest antimicrobial activity (Tables 1 and 2).

From these antimicrobial properties it can be assumed that the sum of carvacrol and thymol (in percent) is important for antimicrobial activity.

The antimicrobial activity of the essential oil of *Zataria multiflora* collected from different regions are shown in Table 2.

EXPERIMENTAL SECTION

Plant materials

The plant materials were collected in June and July 1995 from south of Iran, Tanbour and Zarand mountains in Kerman and Shirkuh and Dehbid mountains in Fars (south central).

Aerial parts were divided in two parts, the first part being hydrodistilled while the plant was fresh (sample B). The second part was air dried in the shade and hydrodistilled by using a clevenger type apparatus (Sample A). The yields of oils are shown in Table 3.

Analytical techniques

Gas Chromatography (GC): Capillary GC was carried out on a Varian GC 3600 Chromatograph with DB1 column (fused silica 60m×0.32mm i.d.) and flame ionization detector. Temperature programming was performed from 60°C to 230°C at 3°C/min and finally isothermal for 5 minutes, injector temperature 230°C.

Table 3: The amount of oil (V/W, %) of Zataria multiflora from different regions (based on dry plant)

(mean ±SD, n=3)	Kerman		Fars	
	Tanbour (I)	Zarand (II)	Shirkuh (III)	Dehbid (IV)
Dry plant(A)	0.97±0.05	0.88±0.05	0.88±0.08	0.82±0.02
Wet plant(B)	1.45±0.04	0.90±0.07	0.97±0.05	1.0±0.09

GC-MS: A Varian GC 3400 was interfaced with a quadropole mass spectrometer (Finnigan-Mat TSQ 70). A fused silica capillary column (DB1 60m×0.32 mm i.d.) was used with helium as carrier gas. The temperature was programmed as in the GC analysis. Identification was carried out by using Kovats indices and MS spectrums [3-8].

Antimicrobial testing

The antimicrobial effects of the essential oil were tested by the disc diffusion method [9,10]. The following microorganisms were used in this experiment; *Staphylococcus aureus* (PTCC 1337), *Bacillus subtilis* (PTCC 1023), *Escherichia coli* (Endemic), *Proteus mirabilis* (PTCC 1076), *Klebsiella pneumoniae* (PTCC 1053), *Candida albicans* (ATCC 5027) and *Aspergillus niger* (ATCC 5013).

Paper discs (6 mm diam.) containing 0.15, 0.20, 0.50, 1.0 mg of samples to be assayed were deposited on the surface of the seeded nutrient agar (for bacterial assay) and sabouraud dextrose agar (for fungal assay) in petri dishes. The bacterial petri dishes were incubated for 24 hours at 37°C while the fungal petri dishes were incubated for 24-48 hours at 20°C. Four samples with different major components and percentages were selected and their antimicrobial activities compared (Table 2).

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