Analysis of the flower essential oil at different stages of plant growth and *invitro* antibacterial activity of *Perovskia abrotanoides Karal*, In Iran

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Abstract

The essential oils of the flower were isolated by hydrodistillation from *Perovskia abrotanoides karel*, at different stages of plant growth. The oils were studied by GC/MS. The major components in the flower oils were: Camphor,1,8 – cineol, α-pinene, o-cimene, β-pinene, camphene, caryophyllene, α-humulene and borneol. The results show that, the oil yield and the major constituent's percentage of the flower were different at stages of plant growth. The essential oils of the flowers *Perovskia abrotanoids Karel*, were tested against two strains of Gram positive bacteria (Bacillus cereus, Staphylococcus aureus) and two strains of Gram negative bacteria (Escherichia coli, Klebsiella pneumonia). The average MICs and MBCs of essential oils of the flowers *Provskia abrotanodies*, were tested against the organisms by agar dilution.

Kew words: *Perovskia abrotanoides Karel*, essential oil, GC/MS, Bacillus cereus, Staphylococcus aureus, Escherichia coli

Introduction

Perovskia is a genus belongs to the *Lamiaceae* family witch is allied to *Salvia*, *Nepeta* and *Rosmarinus* [1-2]. *Provskia* is represented in Flora Iranica by only three species [3]. *Perovskis abrotanoides. Karel*, is an aromatic erect herb which is used in Iranian Folk medicine as an analgesic in rheumatic pains. Previous investigations on this plant resulted in isolation of bioactive tanshinones with leishmani cidal, antiplasmodial and cytotoxic activities [4]. There are also other reports of isolation and identification of two triterpenes with a novel carbon skeleton from *P.abrotanoides* [5-6]. The phytochemical study on chemical composition of essential oil back to 1978-2005, from aerial parts of *poabrotanoides* [7-9]. In this study we investigated the chemical compositions of volatile oil of the Iranian native plant, also the antibacterial activity of the flower oil were studied.

Results and Discussion

The yellow color essential oils From flowers of *Provskia abrotanodies Karel*, collected from Tivan-Darrehgaz Road (Northeast of Khorassan Razavi Province of Iran),was obtained in 1.69(w/w)%yield at beginning of flowering(maximum amount),and the end of flowering

stage the results show that increasing of the percentage yield of the oil (Fig. 1). This behavior could be deepened to beginning of fruiting stage. Essential oils isolated by hydrodistillation from flower parts of *P. abrotanoides Karel*, included 37 components (Table1). The major components were camphor 1,8 – cineol, α-pinene, o-cimene, β-pinene, camphene, caryophyllene, α-humulene and borneol. These results are agreement with the components that obtained for aerial parts of the plant in previous studies [8].

Table 2 shows the average MIC and MBC values of essential oils from flowers of *Perovskia abrotanoides Karel* in vitro antibacterial activity experiments. Table 2 shows the antibacterial activity of the oil against the selected tested bacteria. Results shown in table 2 indicates that of all tested bacteria, B. cereus had the lowest MIC, i.e. the essential oil had more activity against B. cereus than average other tested bacteria. The average MBC of both essential oils against B.cereus (4.37 µg/mL) and S.aureus (>60 µg/mL) were higher than MIC values. So, the essential oils showed bacteriosatatic activity against tested Gram positive bacteria contrast in Gram negative bacteria (Table 2). The essential oils had bactericidal effects against tested Gram negative bacteria (the MIC and MBC values were the same) .

Table1. The chemical composition of the flower essential oil of *Perovskia abrotanoides Karel* at different stages of plant growth

No.	Compound	R.I	% Percentage			
			Sample 1	Sample 2	Sample 3	Sample 4
1	tricyclene	923	0.13	--	0.08	0.06
2	α- thujene	926	--		0.1	0.15
3	α- pinene	940	9.49	31.76	9.91	14.83
4	Camphene	953	3.14	6.42	3.34	4.4
5	β-pinene	982	3.17	5.62	2.94	4.09
6	myrcene	998	0.74	1.88	0.56	0.81
7	α-phellandrene	1010	0.11	0.20	0.07	0.11
8	*E*-ocimine	1019	6.3	9.08	4.81	3.62
9	α - terpinene	1022	0.33	0.32	0.19	0.07
10	1,8- cineole	1043	18.1	14.83	21.60	22.94
11	*z*- ocimine	1046	0.3	0.53	0.38	0.3
12	α-terpinene	1063	0.66	0.69	0.47	0.67
13	cis- sabinene hydrate	1070	0.16	--	0.13	0.16
14	terpinolene	1090	0.72	0.66	0.53	0.53
15	Linalool	1108	0.14	--	0.06	0.06
16	Camphor	1153	19.08	14.67	29.15	29.61
17	borneol	1171	3.3	2.35	2.7	2.29
18	α- terpineol	1193	1.81	1.82	2.01	1.80
19	bornyl acetate	1284	1/89	0.08	1.96	1.78
20	terpineyl acetate	1349	1.85	--	1.94	2.31
21	α- copaene	1366	0.15	--	0.15	0.17
22	α- gurgunene	1396	0.44	0.18	0.38	0.37
23	*Z*-β- caryo phyllene	1405	4.43	2.69	3.23	3.52
24	α- humulene	1441	3.89	2.28	2.85	3.1
25	β- gurgunene	1449	0.40	--	0.13	0.03
26	germacrene D	1465	0.07	--	0.02	--
27	*A*- transe bergamatene	1473	0.13	--	0.12	--
28	Valencene	1488	0.34	--	0.08	--
29	α- muurolene	1499	2.54	--	1.09	1
30	α- cadinene	1513	0.87	0.34	0.85	1.06
31	caryophyllene oxide	1575	1.07	0.49	0.89	--

32	viridiflorol	1602	1.96	0.45	1.6	--
33	cadionl	1630	6.41	1.88	2.91	--
34	eudesmol	1633	0.18	--	0.08	--
35	α- eudesmol	1636	0.28	--	0.17	--
36	cadionlepi	1640	0.14	0.15	0.09	--
37	bisabolol	1675	2.78	--	0.04	--

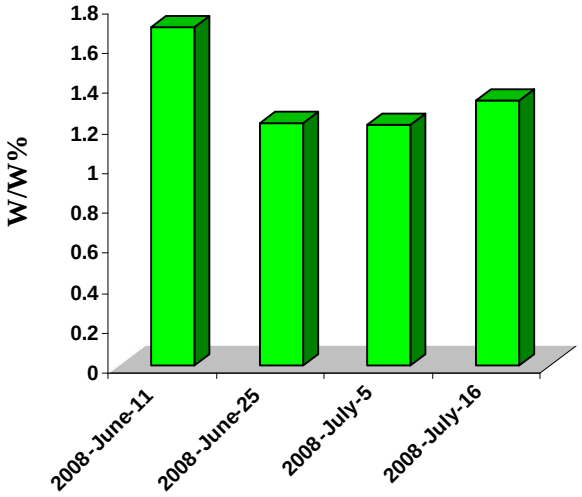

Figure1. The percentage yield of essential oils obtained by hydrodistillation from flowers of *Provskia abrotanodies karel*, at different stages of plant growth

Table 2. The average MICs and MBCs of essential oil from flowers of *Provskia abrotanodies* on tested organisms by agar dilution method

Bacteria	average* MIC (µg/ml)	average MBC (µg/ml)
E. coli	5	5
K. pneumonia	6.25	6.25
S. aureus	4.37	>60
B. cereus	1.87	4.37

* values are mean of triplet experiments.

Materials and Methods

Plant material

The flowers of *Perovskia abrotanoides Karel*, at different stages of plant growth were collected from Tivan-Darrehgaz Road (Northeast of Khorassan Razavi Province of Iran) in June – July 2008 (for 2 mounts) at an altitude of 1517m. The plant was identified at the Islamic Azazd University of Mashhad. A Voucher specimen (N.1954) was deposited at the Herbarium of Islamic Azazd University of Mashhad.

Isolation of essential oil

The plant flowers were dried at room temperature for 5 days. The flowers were hydrodistillated in a Clevenger-type apparatus for 3h according to the method recommended in the British Pharmacopoeia. The oil was dried over anhydrous sodium sulphate and deoxygenated under nitrogen gas and stored in sealed vial at frizzier until the analysis time.

Gas chromatography – mass spectrometry (GC-MS) analysis

The essential oil was analyzed by GC/MS. The GC/MS analysis was carried out on a Shimadzu GC/MS (QP5050). The capillary conditions were as follows; carrier gas, helium with a flow rate of 1.7mL/min; injected 0.1µLof the essential oil and ionization potential 70ev. The initial temperature of column was 60 ^{o}C (held 1min) then heated to 280 ^{o}C with a rate 3 ^{o}C/ min then heated to 250 ^{o}C and kept constant for 4min. The same condition of temperature programming used for n-alkenes mixture to calculate the retention indexes (RI). The identification of each component was studied t by mass spectral data, literature and NIST computer library. The relative percentage of the oil constituent was calculated.

Bacterial Strains

The bacterial strains that used in this study were obtained from the Microbiology Laboratory, School of pharmacy, Mashhad University of Medical Sciences.

The essential oils of the flowers of *Perovskia abrotanoids Karel*, were individually tested against two strains of Gram positive bacteria (Bacillus cereus ATTC 10876 and Staphylococcus aureus PTCC 1112) and two strains of Gram negative bacteria (Escherichia coli PTCC 1330 and Klebsiella pneumonia).

Evaluation of the antibacterial activity

At a first screening, the essential oils were tested against the above mentioned bacteria. Minimal inhibitory conentrations (MICs) were determined by the agar serial dilution method [10] at concentration ranging from 0.93 to 60 µg/mL . Two fold serial dilution were made from essential oil in molten Mueller Hinton agar (Pronadisa- Madrid) cooled to 45-50 ^{o}C in a water bath. The essential oil was dispersed in mixture using dimethyl sulfoxide (DMSO). The amount of 0.01 mL of every bacterial suspension, equivalent to McFarland tube No. 0.5 (10^{8} CFU/mL), inoculated on the agar of every well. The culture plates were then incubated at 37 ^{o}C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed [11]. The Mueller Hinton agar were contained DMSO without essential oil was used a negative control while gentamycine and kanamycin were used as positive control.

MBC determination

The minimum bactericidal concentrations (MBCs) were established by lack of growth upon re-inoculation from essential oil treated microplates to Mueller- Hinton agar plates. The lowest concentration showing lack of growth represents the MBC [10,12]. The experiments were repeated three times and the average value of MIC and MBC were considered.

Conclusion

Considering the increased development of resistance of bacteria to antibiotics; searching for new antimicrobial agents with natural origin is valuable. In the present study, it was shown that the essential oil from flowers of *Perovskia abrotanoids* had in vitro antibacterial

activity. Further studies are necessary to evaluate the in vivo effects of active compounds of this plant.

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