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DETERMINATION OF BIOACTIVE MOLECULES FROM FLOWERS, LEAVES, STEMS AND ROOTS OF *PEROVSKIA ABROTANOIDES* KAREL GROWING IN CENTRAL IRAN BY NANO SCALE INJECTION

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The chemical variation of the essential oils from flowers, leaves, stems and roots of *Perovskia abrotanoides* Karel have been studied. The essential oils were isolated by hydrodistillation in July and December. Analyses of the oils by GC and GC-MS spectrometry were performed and sixty compounds were identified. α-Cadinol, 1,8-cineole and camphor were the common major components of the oils. Essential oils showed antimicrobial activity against Microsporum gypseum, Candida albicans (yeast), Aspergillus fumigatus (fungi) and Salmonella typhi (Gram negative bacteria).

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Keywords: Perovskia abrotanoides Karel; Labiatae; Essential oil; α-Cadinol; 1,8-Cineole; Camphor, Antibacterial Activity

1. Introduction

The genus *Perovskia*, which belongs to the tribe stachyo ideae-Nepeteae, family Lamiaceae, is distributed in various regions of Asia, Afghanistan, Himalaja, Turkestan, Pakistan and Tibet. The Iranian flora includes four species of *Perovskia* which one of them is *Perovskia abrotanoides* Karel [1]. Some other members of this genus are *P. atriplicifolia Benth., P. scrophulariifolia* and *P. angustifolia* [2-5].

P. abrotanoides is an herb used to treat leishmaniasis in Iranian folk-medicine tradition. Thus, villagers in the Isfahan province of Iran apply a poultice, made of crushed roots of the plant, water, sesame oil, and wax, on lesions caused by cutaneous leishmaniasis [6]. The essential oil of this plant from Pakistan possessed antibacterial activity against salmonella typhi, which was comparable to that of chloromphenicol but was much less than that of streptomycin [7]. A new triterpene, perovskone with a novel carbon skeleton was isolated from the whole plant of *P. abrotanoides* [8], a novel triterpene, peradione was isolated from the Pakistani medicinal plant *P. abrotanoides* [9], and a quinoid diterpene with a nor-abietane skeleton, and three new natural products were isolated from roots of *P. abrotanoides*, have leishmanicidal, antiplasmodial , and cytotoxic activity [6].

In this communication, we report on the volatile oil contents and composition of different plant organs of *P. abrotanoides* Karel and its biological activity. To our knowledge the oil of *P. abrotanoides* from kashan area (Isfahan province, Iran) has not been studied previously.

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2. Experimental

Plant material

The plant material of *Perovskia abrotanoides Karel.* (Persian name: brazambel) were collected at an altitude of 2100 m in the area between Natanz and kashan, Isfahan province, Iran, in July and December 2008 and were dried in the shade at room temperature. A voucher specimen (Alt. 11015) was deposited at the herbarium of the Isfahan Research Centre of Natural Resources and Animal Science, Isfahan, Iran.

Isolation of the essential oil

Air dried flowers, leaves, stems and roots were hydrodistilled in a Clevenger type apparatus for 4 hours. The percentage of the oil for each organ was found to be 3.3, 3.3, 1 and 1 v/w yield, respectively in July and 1.33, 1.33, 0.66 and 0.66 v/w yield in December. The oils were dried over anhydrous sodium sulphate and kept at 4ºC in sealed brown vials for analysis. The quantitative and qualitative analyses of the oils were performed by GC and GC-MS, respectively.

Gas chromatography

Capillary gas chromatography was carried out using a Hewlett-Packard (Model 6890) chromatographic system with a flame ionization detector (FID), equipped with a capillary columns HP 5MS (30m × 0.25 mm; film thickness 0.25 μm). Oven temperature was held at 60 ºC for 3 min and then programmed to 220ºC at a rate of 6 ºC/min with helium as carrier gas (1 ml/min). Injector and detector were heated to 220 and 230 ºC respectively. Volume injected of the sample was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of *n*-pentane, v/v) in the splitless mode [10-12].

Gas chromatography and mass spectrometry

GC/MS analysis was performed on a HP-6890 mass selective detector coupled with a HP-6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30m × 0.25 mm i.d., film thickness, 0.25 μm) and operating under the same condition as described above. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2A; ion source temperature, 200 ºC; resolution, 1000. Identification of the constituents was performed by computer library search (Wiley 275.L), retention indices and visual interpretation of mass spectra with those found in the literature [13, 14] or with authentic samples. The percentage composition of the samples was computed from the GC peak areas.

Table l. Percentage composition of stem, flower, root and leaf essential oils of perovskia abrotanoides karel in July

No	Compounds	% in Stem	% in Flower	% in Root	% in Leaf	RI	Identification
1	α-Pinene	-	3.99	-	2.85	935	GC^1-MS
2	Camphene	-	2.30	-	-	950	GC^2-MS
3	β-pinene	-	3.54	-	-	961	GC^1-MS
4	Myrcene	-	4.20	-	4.15	981	GC^2-MS
5	δ-3-Carene	-	2.77	-	1.94	1006	GC^2-MS
6	1,8-Cineole	3.04	7.87	-	12.18	1025	GC^1-MS
7	γ-Terpinene	-	1.01	-	-	1055	GC^2-MS
8	Camphor	10.15	8.41	2.17	10.85	1126	GC^1-MS
9	Borneol	2.79	11.49	1.15	9.74	1155	GC^2-MS
10	α-Terpineol	-	1.18	-	-	1178	GC^2-MS
11	Bornyl acetate	4.62	5.72	2.48	3.83	1263	GC^2-MS

No	Compounds	% in Stem	% in Flower	% in Root	% in Leaf	RI	Identification
12	2-Carene	-	5.25	1.76	2.70	1320	GC^2-MS
13	Terpinolene	2.71	-	-	-	1382	GC^2-MS
14	α-Gurjunene	1.39	1.73	2.03	3.25	1412	GC^2-MS
15	β -Caryophyllene	6.62	5.58	8.83	10.54	1420	GC^2-MS
16	α-Humulene	7.49	6.40	8.01	10.67	1453	GC^2-MS
17	Alloaromadendrene	-	-	5.78	-	1462	GC^2-MS
18	β-Selinene	-	-	5.54	-	1484	GC^2-MS
19	α-Muurolene	1.74	-	-	-	1495	GC^2-MS
20	*trans-β*-Faranesene	-	-	4.38	-	1499	GC^2-MS
21	Isoledene	-	-	2.60	-	1503	GC^2-MS
22	γ-Cadinene	2.62	2.59	3.28	2.23	1512	GC^2-MS
23	δ-Cadinene	4.13	2.75	4.17	4.40	1516	GC^2-MS
24	7-β-Acetyl-4a. β-methyl-1a. β -octahydron	-	-	-	3.84	1518	GC^2-MS
25	8-(1-Pentenylcarbonyl) bicyclo[6.1.0] nonane	-	-	-	5.96	1573	GC^2-MS
26	3,5-Diisopropenyl-1,2-dimethyl cyclohexane	1.37	-	-	-	1577	GC^2-MS
27	β-Bourbonene	2.85	-	-	-	1586	GC^2-MS
28	Viridiflorol	-	-	-	5.19	1588	GC^2-MS
29	Illudol	-	-	-	5.68	1589	GC^2-MS
30	9-Isopropenyl,7,7,8trimethyl-4-oxatricyclo[6.1.0]nonane	-	8.91	-	-	1596	GC^2-MS
31	Caryophyllene oxide	2.84	-	7.39	-	1599	GC^1-MS
32	Patchulane	3.87	-	-	-	1610	GC^2-MS
33	Torreyol	2.22	-	3.75	-	1631	GC^2-MS
34	Verbenol	-	-	2.62	-	1634	GC^2-MS
35	trans-2-Carene-4-ol	3.15	-	-	-	1637	GC^2-MS
36	*α*-Cadinol	15.20	14.30	23.27	-	1642	GC^2-MS
37	*trans*- Muurolol	4.42	-	-	-	1652	GC^2-MS
38	δ-Guaiene	-	-	4.94	-	1671	GC^2-MS
39	3-Ethenyl-3-methyl-2-(1-methylethen) Cyclohexanol	5.80	-	-	-	1675	GC^2-MS
40	Hasmigone	10.97	-	5.85	-	1681	GC^2-MS
	Total identified	99.99	99.99	100	100		

Components are listed in order of elution from a HP 5 MS column.
MS; mass spectrum. GC^1, retention index according to authentic standards; GC^2, retention index according to literature. RI; Kovats retention index according to n-alkanes (C10–C23) on the HP 5 MS column

Table 2. Percentage composition of stem, flower, root and leaf essential oils of perovskia abrotanoides karel in December

No	Compounds	% in Stem	% in Flower	% in Root	% in Leaf	RI	Identification
1	α-Pinene	-	-	4.96	6.17	935	GC^1-MS
2	Myrcene	-	-	-	5.82	981	GC^2-MS
3	1,8-Cineole	0.13	20.99	7.66	12.99	1025	GC^1-MS
4	γ-Terpinene	-	0.43	0.27	0.46	1055	GC^2-MS
5	Linalool	-	-	-	0.63	1087	GC^1-MS
6	Plinol	-	0.66	-	-	1114	GC^2-MS
7	Camphor	5.34	17.84	6.05	15.71	1126	GC^1-MS
8	Borneole	-	-	-	4.69	1155	GC^1-MS
9	Coriandrol	-	0.16	-	-	1179	GC^2-MS
10	Geranyl acetate	-	-	-	0.26	1214	GC^1-MS
11	Bornyl acetate	-	5.37	1.28	3.05	1263	GC^2-MS
12	α-Terpinenyl acetate	1.65	-	-	3.09	1335	GC^2-MS
13	α-Copaene	0.84	0.74	0.19	1.85	1379	GC^2-MS
14	β-Caryophllene	4.96	6.02	3.19	5.62	1420	GC^2-MS
15	α-Gurjunene	0.62	-	-	-	1422	GC^2-MS
16	α-Humulene	-	-	-	7.15	1453	GC^2-MS
17	Alloaromadendrene	-	-	10.13	-	1462	GC^2-MS
18	Eremophylene	-	-	6.84	-	1465	GC^2-MS
19	β-Selinene	-	5.11	-	0.77	1484	GC^2-MS
20	epi-Bicyclo sesquiphellandrene	-	-	14.95	-	1496	GC^2-MS
21	δ-Cadinene	3.95	6.36	-	5.45	1516	GC^2-MS
22	Iso-germacrone epoxide	-	-	1.91	-	1527	GC^1-MS
23	Caryophyllene oxide	4.54	-	-	-	1599	GC^1-MS
24	α -Amino-α - methylbenzene propanoic acid	-	-	5.91	-	1622	GC^2-MS
25	Torreyol	-	-	-	21.79	1631	GC^2-MS
26	trance-Cadinol	9.88	-	-	-	1636	GC^2-MS
27	α-Cadinol	-	23.89	-	-	1642	GC^2-MS
28	Dehydro aromadendrane	-	1.07	-	-	1651	GC^2-MS
29	Hexenyl cyclopentanone	6.04	-	-	-	1681	GC^2-MS
30	α -Bisabolol	22.37	-	-	-	1682	GC^2-MS
31	4-(4'-methyl-3'-pentenyl)-3-cyclohexenylpentylketon	-	3.31	-	1.28	1689	GC^2-MS
32	Phenol,4,4'-(1-methylethyliden)-bis	15.22	-	-	-	1838	GC^2-MS
33	Elaegine	-	-	16.15	-	1874	GC^2-MS
34	13-epirimuene	-	2.17	-	0.78	1876	GC^2-MS
35	Ferruginol	-	1.58	10.99	0.89	1972	GC^2-MS
36	N -Valeryl-alanine methyl ester	9.29	-	-	-	2021	GC^2-MS
37	Phenanthrene	-	1.6	-	-	2022	GC^2-MS
	Total identified	84.83	97.30	90.48	98.45		

Components listed in order of elution from a HP 5 MS column.
MS; mass spectrum. GC^1, retention index according to authentic standards; GC^2, retention index according to literature. RI; Kovats retention index according to n-alkanes (C10–C23) on the HP 5 MS column

Antimicrobial activity

Antimicrobial activity was assayed via agar diffusion method [15] using Nystatin and Streptomycin as the reference compounds. Tested microorganisms were Microsporum gypseum, Candida albicans (yeast); Aspergillus fumigatus (fungi); Salmonella typhi, Escherichia coli (Gram negative bacteria) and Staphylococococcus aureus (Gram positive bacteria). The microorganisms were obtained from the stock cultures of the Department of Microbiology Faculty of Pharmacy Isfahan University, Isfahan, Iran. Small cups were taken out of the agar, which could take approximately 60 µl of oil solutions. Each cup was filled accurately with 50 µl of oil solutions (20 mg oil was dissolved in 1 ml dimethylformamide, DMF), as well as DMF as a control. The plates were incubated overnight at 37 ºC for bacteria and 25 ºC for fungi. After incubation, a clear zone of growth inhibition around the disk demonstrates the relative susceptibility of the microorganisms to the potential antimicrobial agent (Table 3). The inhibition zones caused essential oil on microorganisms were measured and the activity rated on the basis of the size of inhibition zone. In the screening standards of pure α-cadinol, 1,8-cineole and camphor were tested on the same cultures under identical conditions to compare their activity with that of the investigated oils.

Table 3. Results of antimicrobial activity tests of the essential oils of perovskia abrotanoides karel

	Fungi			Gram – bactrial		Gram+ bactrial
	C.albicans	M.gypseum	A.fumigatus	S.typhi	E.coli	S.aureus
Oils in July	++	++	++	+++	-	-
Oils in December	++	++	++	+++	-	-
α-cadinol	+	+	+	++	-	-
1,8-cineole	+++	+++	+++	+	-	+
Camphor	++	++	++	+	-	+
Nystatin	++	++	++	+++	+++	+++
Streptomycin	++	++	++	+++	+++	+++

Key to symbols:

Highly active = +++ (inhibition zone > 12 mm)
Moderately active = ++ (inhibition zone 9-12 mm)
Slightly active = + (inhibition zone 6-9 mm)
Inactive = - (inhibition zone <6 mm)

3. Results and discussion

Tables 1 and 2 show retention indices and relative percentages of the oil constituents from the identified compounds in July and December. Sixty components were identified by GC and GC-MS representing about 84.83-100% of the oils. Fourty components are shown in Table 1 and thirty-seven components in Table 2. The major components in Table 1 were α-cadinol (23.70% in root, 14.30% in flower and 15.20% in stem), 1,8-cineole (12.18% in leaf), borneol (11.49% in flower) and camphor (10.15% stem and 10.85% in leaf). In Table 2 the main components were α-cadinol (23.89% in flower), α-bisabolol (22.37% in stem), torreyol (21.79% in leaf), 1,8-cineole (20.99% in flower and 12.99% in leaf) and camphor (17.84% in flower and 15.71% in leaf).

The volatile oil of aerial parts of this plant were analysed by GC in Holland and the major components were methylcitronellate (25.4%) and nerol (20.7%) [16]. Ethyl acetate extracted from

the flowers and leaves of *Perovskia abrotanoides* in the Karakorame Himalaya district were analyzed by GC/MS. The major volatile constituents were 1,8-cineole (24.4-27.1%) which is one of the major component in leaf and α-pinene (18.2-23.2%) [17]. The comparison of the results with the literature showed significant differences for oils, which can be attributed to either climatological factors or genetic differences or development stages or plant parts analysed.

4. Conclusions

Growing tendency for replacing synthetic compounds by natural ones has emerged great interest on the evaluation of antimicrobial properties of plants products in phamaceutical industry. Essential oils of *Perovskia abrotanoides* Karel showed (Table 3) significant level of antimicrobial activity against Candida albicans, Microsporum gypseum, Aspergillus fumigatus and Salmonella typhi. The comparison of oil with α-cadinol, 1,8-cineole and camphor suggests that the activity of the oil could be attributed, to a considerable degree, to the existence of these constituents and particularly to 1,8-cineole.

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