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Short Communication

Essential Oil Composition and Antimicrobial Activity of the Oil and Extracts of *Bunium persicum* (Boiss.) B. Fedtsch.: Wild and Cultivated Fruits

Arezoo Rustaie[1,2], Roya Keshvari[1], Nasrin Samadi[3], Farahnaz Khalighi-Sigaroodi[4], Mohammad Reza Shams Ardekani[1,5], Mahnaz Khanavi[1,2,6*]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[2]*Persian Medicine and Pharmacy Research Centre, Tehran University of Medical Sciences, Tehran, Iran.*
[3]*Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[4]*Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.*
[5]*Department of Traditional Pharmacy, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
[6]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.*

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ABSTRACT

Background: Fruits of *Bunium persicum* (Boiss.) B. Fedtsch (Apiaceae) has been used as spice, anti-flatulence and antiseptic agent for many years. In recent years the wild resources of the plant have been threatened by extinction. Domestication of such a plant saves its genetic resources from depletion. However, concerns remain about the possible changes due to development of chemotypes and changes in the composition and biological and pharmacological potentials.
Methods: Analyses of essential oils from fruits of wild and cultivated types was performed using Gas Chromatography/Mass Spectroscopy. Antimicrobial assessment was done by agar diffusion method
Results: The main compounds of both oils were included γ-terpinene (30.77% and 27.57%), cuminaldehyde (20.49% and 21.1%), ρ-cymene (20.1% and 18.32%) and γ-terpinen-7-al (8.29% and 7.84%) respectively. Analytical results of both tested oils exhibited very close similarities in major compounds, whereas some differences in their percentages were observed. In vitro antimicrobial evaluation of volatile oils, total extract and the resultant fractions against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* demonstrated some similarities and differences. Minimum inhibitory concentrations (MICs) of wild grown fruits essential oils ranged between 0.375-1.5 mg/ml, while those of cultivated one were 0.75-6.25 mg/ml. All extracts and fractions showed similarly minor antibacterial potential while anti-*Candida albicans* activity was much remarkable with MICs calculated 2.5-5 mg/ml for cultivated and 5 mg/ml for wild grown extracts and fractions.
Conclusion: In conclusion, despite the substantial similarities in composition of both oils, the alteration in antimicrobial results may be caused by variety in concentration of major and minor compounds and their synergism or antagonism in mixture.

Introduction

In recent decades there has been an increasing tendency toward herbal medicine use in prevention, control and treatment of diseases. Anthropogenic interferences with natural habitats and ecosystems, and threats posed by human to comply the market request, make many medicinal plants endangered. Medicinal plants cultivation, provides required resources and makes avoidance of wild resources depletion, furthermore it may control the growing condition, harvesting at the right time, and reducing the possibility of adulteration.[1] There are several studies on the chemotype development in various plants' cultivars and differences in essential oils composition and biological activities between the cultivated and wild ones.[2-7] The research

***Corresponding Author:** Mahnaz Khanavi, E-mail: khanavim@tums.ac.ir

implemented by Chatzopoulou *et al*., has shown the same major components in both wild and cultivated *Hypericum perforatum* L., however there was significant difference in the amount of main components.[4]

Bunium persicum (Boiss.) B. Fedtsch (Apiaceae) is a perennial herb, native to Iran, Pakistan and Afghanistan.[8] The small odorant fruits of *B. persicum* ("Zireh siah" or "Wild Caraway") are traditionally used as antiseptic, carminative and condiment.[9] Unfortunately, due to climate changes and indiscriminate harvesting of the whole plant instead of collecting fruits, this invaluable species has become an endangered species.[10] Several studies have previously determined essential oil composition of *B. persicum* and commonly reported γ-terpinene, cuminaldehyde and ρ-cymene as major compounds.[11-14] Moreover, the oil has demonstrated strong antibacterial activity against some gram positive and gram negative bacteria and also considerable insecticide effects.[14,15] Another research has investigated the essential oil composition of wild and cultivated *B. persicum* from India and shown some similarities and differences in oils, mainly the higher level of cuminaldehyde in cultivated fruits.[6] In a study exploring antioxidant components of the fruits, kaempferol, caffeic acid and p-coumaric acid were introduced as active ingredients from extract.[16] Also, antimicrobial properties of these three compounds against some bacterial and fungal strains have been shown in some studies.[17-20] In recent years, *B. persicum* is cultivated in limited areas in Iran especially in Khorasan Razavi province. The aim of this study is to compare the essential oils composition and antimicrobial activity of one domesticated and one wild grown plants' fruit in Iran.

Materials and Methods

Plant materials

Fruits of wild grown were purchased from Kerman Bazaar and domesticated one were supplied from agricultural research fields of Ferdowsi University of Mashhad (2013). The fruit samples were authenticated in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences and voucher specimens were deposited at the herbarium (PMP-649 and PMP-689).

Essential oil preparation

The essential oils were obtained by hydrodistillation from 100g of powdered fruits, using Clevenger type apparatus. Then the essential oils were dried over anhydrous sodium sulfate and kept in sealed vials at 4ºC until analysis and antimicrobial evaluations.

Extraction and fractionation

The dried powdered fruits (250g) also were subjected to extraction using methanol (5×1.5L) at room temperature. The resulting total extracts were concentrated under vacuum by means of a rotary evaporator at 40ºC, and then were lyophilized by freeze dryer at -40ºC for 24h (Lyotrap Ultra, LTE Scientific Ltd., Oldham, UK). A portion of dried extracts were fractionated using solid-liquid fractionation method with appropriate volumes of petroleum ether and ethyl acetate.

GC-MS analyses

Gas chromatography was performed with an Agilent Technologies gas chromatograph 6890 equipped with a BPX5 capillary column (30m×0.25mm id, 0.25µm film thickness) coupled to an Agilent mass spectrometer 5973 worked with electron ionization method operating at 70 eV. The ionization source temperature set at 220ºC. The initial oven temperature was 50ºC that was kept for 5 minutes, then was raised to 240ºC at constant velocity of 3ºC/min, then increased by 15ºC/min to 300ºC. The GC injector temperature was 290ºC and 1µl of the diluted essential oils were injected separately with a split ratio of 1:25. The flow rate of Helium, as carrier gas, was 0.5ml/min. The essential oils were also analyzed for relative quantification of components using Agilent 6890 gas chromatograph coupled to a FID detector. The operation was conducted under the same condition as described for GC-MS analyses. The FID detector temperature set at 290ºC.

Identification of components was based on GC retention indices and computerized comparison of their mass spectra with those in Wiley library as well as collation of the mass spectra with those reported in the literature.[21]

Microorganisms and growth conditions

The minimum inhibitory concentrations (MICs) of the essential oils, extracts and fractions of the fruits of the cultivated and wild types *B. persicum* were determined by agar diffusion method against test microorganisms including one Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538), one Gram-negative bacteria (*Escherichia coli* ATCC 8739) and one yeast (*Candida albicans* ATCC 10231). Test microorganisms were stocks of the Department of Drug and Food Control, School of Pharmacy, Tehran University of Medical Sciences. Two-fold dilution of the essential oils, fractions and extracts were prepared in dimethylsulfoxide (DMSO; 1ml). Each dilute was added to 14 ml of the Caso agar (CA) for bacteria and Sabouraud dextrose agar (SDA) for yeast to give the final concentrations of 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156mg/ ml.

The bacteria inocula were prepared by suspending

overnight colonies from CA media in 0.9% saline. The *C. albicans* inoculum was prepared by suspending colonies from 48 h old SDA cultures in 0.9% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standard (1.5×10^8CFU/ml). Then microbial suspensions were diluted in 0.9% saline to give 10^7CFU/ml. The plates were spot-inoculated with 3 µL of each suspension (10^4CFU/spot); including a control plate containing 1 ml DMSO without any antimicrobial agent. The plates containing bacteria were incubated at 30-35ºC for 24 h and those containing yeast were incubated at 20-25ºC for 48 h.

Results and discussion

Determination of essential oils composition

The yield of both essential oils were approximately equal and estimated 2.25% and 2.5% (w/w) for cultivated and wild grown *B. persicum* respectively. Gas chromatograms are represented in figures 1 and 2. A total of twenty eight compounds were identified which accounted for 93.73% and 95.72% of cultivated and wild volatile oils respectively. Hydrocarbon monoterpenes and oxygenated monoterpenes were found as the main groups of constituents in both analyzed samples, among them γ-terpinene, cuminaldehyde, p-cymene, γ-terpinen-7-al, α-terpinen-7-al and limonene were determined as major components (Table 1). The results demonstrated that the level of oxygenated monoterpenes was slightly higher in cultivated plant, whereas the amount of hydrocarbon monoterpenes was higher in wild grown.

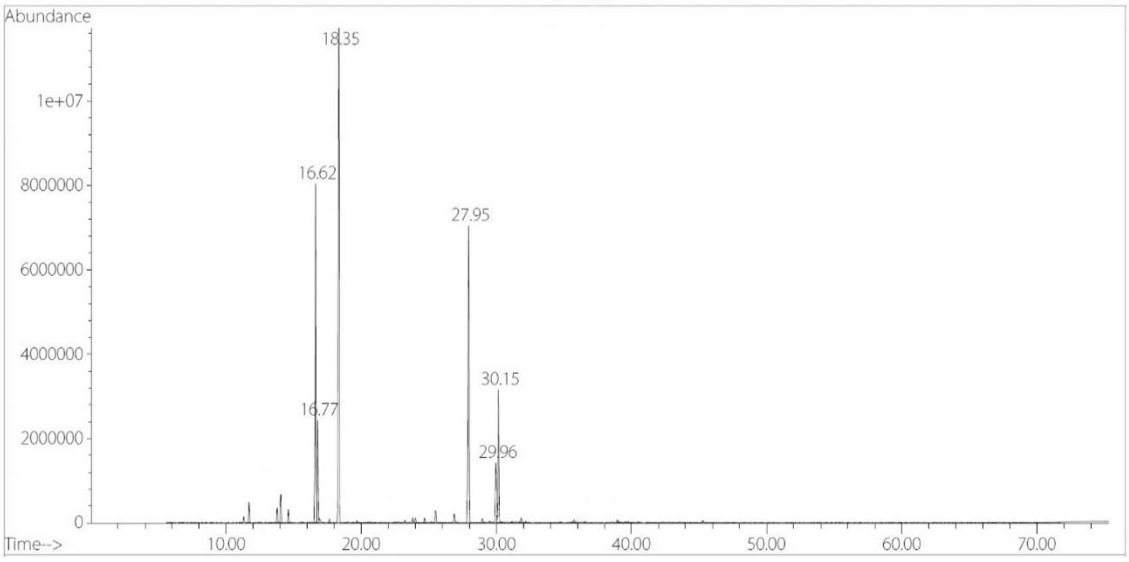

Figure 1. Gas chromatogram of essential oil obtained from the fruits of wild *Bunium persicum.*

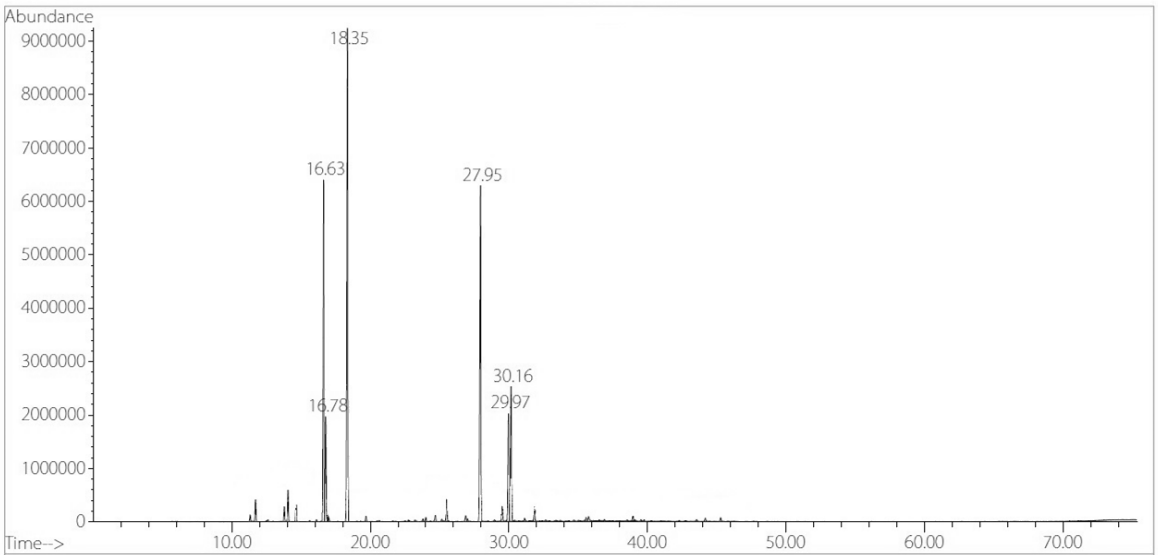

Figure 2. Gas chromatogram of essential oil obtained from the fruits of cultivated *Bunium persicum.*

Table 1. Chemical composition of essential oils obtained from the fruits of cultivated and wild *B. persicum*.

No.	Compounds[a]	RI[b]	Composition (%)		RT[e]
			Cultivated[c]	Wild[d]	
1	α-Thujene	927	0.37	0.36	11.3
2	α-Pinene	934	1.14	1.13	11.7
3	Camphene	952	0.11	-	12.6
4	Sabinene	975	0.78	0.85	13.8
5	β-Pinene	981	1.69	1.76	14.0
6	β-Myrcene	992	0.67	0.71	14.6
7	δ-3-Carene	1011	0.08	0.07	15.6
8	α-Terpinene	1021	0.12	0.05	16.1
9	ρ-Cymene	1031	18.32	20.1	16.6
10	Limonene	1033	5.38	5.51	16.8
11	β-Phellandrene	1036	0.2	0.46	16.9
12	(E)-β-Ocimene	1050	-	0.29	17.6
13	γ-Terpinene	1064	27.57	30.77	18.3
14	cis-Sabinene hydrate	1076	-	0.12	19.0
15	α-Terpinolene	1089	0.33	0.12	19.7
16	Linalool	1102	-	0.05	20.5
17	Limonene oxide	1141	-	0.07	22.5
18	Terpinene-4-ol	1189	0.42	0.3	24.7
19	ρ-Cymen-8-ol	1199	0.16	0.04	25.1
20	Cuminaldehyde	1258	21.1	20.49	27.9
21	Bornyl acetate	1291	0.93	0.14	29.5
22	α-Terpinen-7-al	1301	6.34	3.77	29.9
23	γ-Terpinen-7-al	1305	7.84	8.29	30.2
24	Carvacrol	1325	-	0.08	30.8
25	trans-Caryophyllene	1426	0.07	-	35.5
26	β-Farnesene	1463	-	0.08	39.0
27	Zingiberene	1497	0.07	-	39.1
28	β-Bisabolene	1514	0.04	0.11	39.7
Hydrocarbon monoterpenes			56.76	62.18	
Oxygenated monoterpenes			36.79	33.35	
Hydrocarbon sesquiterpenes			0.18	0.19	
Total identified			93.73	95.72	

[a] Identified compounds listed in order of elution from BPX-5 column; [b] Relative retention indices to C8-C24 n-alkanes series on BPX5 column; [c] mean values in fruits of cultivated *Bunium persicum*; [d] mean values in fruits of wild *Bunium persicum*; [e] Retention time in minute

Qualitative and quantitative comparison between the two studied samples revealed some slight differences among major compounds, for example higher level of α-terpinen-7-al in cultivated *B. persicum* fruits and somewhat higher concentration of γ-terpinene and p-cymene in wild one. In the case of minor compounds, a few components were absent in one sample and the concentration of some others showed some differences in cultivated and wild types respectively (Table 1).

Antimicrobial activity of essential oils and extracts

Results of antimicrobial experiments are represented in Table 2 and Table 3. The essential oils have shown stronger antimicrobial effect in comparison with extracts and fractions. In a screening study, total methanol extract obtained by soxhlet extraction method from *B. persicum* fruits did not show antimicrobial activity against tested microorganism, including *C. albicans*, *S. aureus*, *E. coli* and some other bacteria except *Bacillus subtilis*.[22] In our experiment, total extracts, petroleum ether and methanol fractions obtained from both cultivated and wild types showed anti-*Candida albicans* activity, however they didn't demonstrate any antibacterial activity against *S. aureus* and *E. coli* (Table 3).

Table 2. Minimum inhibitory concentration of cultivated and wild types of *B. persicum* essential oils against tested microorganisms.

Test microorganism	Cultivated[a](mg/ml)	Wild[b] (mg/ml)	Ciprofloxacin(µg/ml)	Fluconazole(µg/ml)
S. aureus ATCC 6538	6.25	1.5	0.39	-
E. coli ATCC 8739	6.25	1.5	0.01	-
C. albicans ATCC 10231	0.75	0.375	-	128

[a]MIC of cultivated type *Bunium persicum* fruits; [b] MIC of wild type *Bunium persicum* fruits.

Table 3. Minimum inhibitory concentration of cultivated and wild types of *B. persicum* extracts against tested microorganisms.

	Cultivated[a] (mg/ml)			Wild[b] (mg/ml)			Ciprofloxacin	Fluconazole
Test microorganism	TE[c]	M[d]	E[e]	TE	M	E	(µg/ml)	(µg/ml)
S. aureus ATCC 6538	>10	>10	>10	>10	>10	>10	0.39	-
E. coli ATCC 8739	>10	>10	>10	>10	>10	>10	0.01	-
C. albicans ATCC 10231	5	2.5	2.5	5	5	5	-	128

[a]MIC of cultivated type *Bunium persicum* fruits; [b] MIC of wild type *Bunium persicum* fruits; [c] Total extract; [d] Methanol fraction; [e]Petroleum ether fraction.

Also, in present research, minimum inhibitory concentrations (MIC) of both essential oils were determined and showed considerable antimicrobial activity, particularly against *C. albicans*. According to our results, the wild grown essential oil showed higher inhibitory activity on microorganisms, while only a slight difference was determined in essential oil major compounds such as the level of ρ-cymene and γ-terpinene. Both essential oils were rich in compounds that their singly antimicrobial potentials have already been assessed in many studies.[23-25] Several studies have confirmed potent antimicrobial activity of cuminaldehyde which was a major component in both essential oils in comparable levels.[26,27] Although there is considerable resemblance between the two essential oils profiles, the differences in antimicrobial properties could be due to slight variations in major and minor components through more complex mechanisms such as antagonism and synergism among them.[28]

Conclusion

Similarities between cultivated and wild types essential oils composition, such as aldehyde content, especially comparable levels of cuminaldehyde, which has been shown to be responsible for specific aroma of *Zireh*,[6] prove that cultivation of this invaluable plant won't cause a major change in essential oil profile. Furthermore, remarkable anti-*Candida albicans* activity in both oils is another considerable aspect, however, more precise phytochemical investigation, biological and pharmacological experiments are required to introduce *B. persicum* as a remarkable commercial food preservative.

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Conflict of interests

The authors claim that there is no conflict of interest.

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