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Original Article

Chemical Constituents and Antibacterial Activity of Essential Oil of *Peucedanum ruthenicum* M. Bieb. Fruits

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Abstract

The essential oil of *Peucedanum ruthenicum* fruits, obtained by hydrodistillation, was analyzed by gas chromatography and gas chromatography-mass spectrometry. Among the 31 identified constituents accounting for 83.9% of the total oil, the major components were 1,8-cineole (11.15%), camphor (5.86%), *Z*-carveol (6.88%), l-carvone (5.61%), 8,9-dehydroisolongifolene (11.35%), caryophyllene oxide (13.65%), and caryophylla-4(12),8(13)-dien-5-β-ol (5.19%). Antimicrobial activity of the essential oil was investigated against various gram-positive and gram-negative bacteria. The essential oil of *P. ruthenicum* showed activity against gram-positive bacteria but had no effect on the tested gram negative bacteria.

Keywords: Antibacterial activity; *Z*-Carveol; 8,9-Dihydroisolongifolene; Essential oil; *Peucedanum ruthenicum*.
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1. Introduction

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials play a major role in primary health care as therapeutic remedies in many countries [1, 2]. Plants still continue to be almost the exclusive source of drugs for the majority of the world's population [3-5].

The genus *Peucedanum* S.L. comprises about 100 to 120 species, mainly distributed in Europe and Asia. In Europe, *Peucedanum* comprises 29 species [6], and the 4 species existing in Iran include: *P. glaucopruinosum*, *P. knappii*, *P. translucens* and *P. ruthenicum*, which are distributed in the northern and central provinces of Iran [7, 8]. *P. ruthenicum* Bieb. is a glabrous perennial 40-100 cm; stock *ca* 1 cm in diameter, with abundant fibers; stem terete, striate, solid; leaves 3(-4)-ternate; lobes 20-90 mm; rays 7-28; bracts 1-3, subulate; bracteoles several, filiform; petals pale yellow; fruit 6-7.5 mm [6]. Since some species of this genus have been used traditionally in the treatment of cold [9], cough due to pathogenic wind-heat, accumulation of phlegm, heat in the lung [10], anti-tussive, anti-asthma and as a remedy for angina [11], the essential oil of *P. ruthenicum* was subjected to investigation for antibacterial properties.

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Table 1. The composition of *Peucedanum ruthenicum* fruits' essential oil.

No.	Compounds name	%	RRI[a]
1	α-Pinene	0.46	943
2	Camphene	t	957
3	Sabinene	t	981
4	β-Pinene	0.12	985
5	α-Terpinene	0.13	1021
6	*p*-Cymene	0.89	1028
7	dl-Limonene	0.11	1031
8	**1,8-Cineole**	**11.15**	**1034**
9	Unknown (MW=136)	0.38	1040
10	β-Ocimene	0.86	1051
11	Unknown (MW=136)	0.21	1057
12	γ-Terpinene	0.25	1064
13	**Camphor**	**5.86**	**1139**
14	Unknown (MW=150)	3.61	1154
15	Terpinene, 4-ol	4.87	1174
16	Unknown (MW=136)	3.40	1186
17	*Z*-Dihydrocarvone	0.89	1190
18	Unknown (MW=152)	0.79	1202
19	*E*-Carveol	3.97	1214
20	***Z*-Carveol**	**6.88**	**1226**
21	**l-Carvone**	**5.61**	**1247**
22	Unknown (MW=150)	1.10	1302
23	Unknown (MW=151)	1.65	1318
24	Naphthalene, 1-methyl	1.28	1332
25	2-Methylnaphthalene	1.05	1349
26	Unknown (MW=152)	0.73	1355
27	Unknown (MW=174)	0.34	1375
28	Tetradecane	0.71	1402
29	Naphthalene, 1,5-dimethyl	1.06	1424
30	Naphthalene, 1,8-dimethyl	1.72	1434
31	Naphthalene, 2,7-dimethyl	1.36	1442
32	Naphthalene, 2,3-dimethyl	0.27	1455
33	Naphthalene, 1,3-dimethyl	0.43	1467
34	β-Ionone	0.68	1485
35	Germacrene B	0.45	1507
36	Phenol, 2,5-bis[1,1-dimethylethyl]	1.38	1528
37	**8,9-Dehydroisolongifolene**	**11.35**	**1576**
38	**Caryophyllene oxide**	**13.65**	**1585**
39	Farnesol (*Z*-*E*)	1.18	1699
40	**Caryophylla-4(12),8(13)-dien-5-β-ol**	**5.19**	**1727**
41	Unknown (MW=204)	3.86	1748
	Hydrocarbon monoterpenes	2.91	
	Oxygenated monoterpenes	39.23	
	Hydrocarbon sesquiterpenes	19.65	
	Oxygenated sesquiterpenes	20.02	
	Nonterpenes	2.09	
	Unknown	16.10	
	Total identified	83.90	

[a]RRI: relative retention indices as determined on a DB-5 column using the homologous series of n-alkanes (C8-24); t: trace(<0.1%).

Table 2. Antimicrobial activity of *Peucedanum ruthenicum* fruits' essential oil using disc diffusion assay.

	Inhibition zone diameter (mm)	
Strains	**Essential oil (2 mg/disc)**	**Neomycin (200 μg)**
S. aureus	13[a]	20
S. epidermidis	10	18
B. cereus	7	15

[a](n = 4)

Previous phytochemical studies on this species indicated the presence of furanocoumarins and their glycoside derivatives, linear-type furanocoumarin glucosides and simple coumarin glucosides [12, 13]. A phytochemical experiment on *P. ruthenicum*, a native Bulgarian Umbelliferae, showed the presence of peucedanin (furanocoumarin) and a new coumarin (peuruthenicin) in the roots and rutin (flavonol glycoside) in the flowers [14]. Several new coumarins from *P. praeruptorum* e.g. qianhucoumarin I, have been reported [10]. There are some reports on the chemical analysis of volatile oil of this genus in the literature. The reported compounds of the essential oil from herb and rhizome of *P. ostruthium* include: sabinene (35.2%), 4-terpineol (26.6%), β-caryophyllene (16.1%) and α-humulene (15.8%) [15]. Major constituents were found to be sabinene and trans-anethole in the essential oil of the leaf and branch of *P. verticillare*. β-Caryophyllene, α-phellandrene, *cis*-β-farnesene and β-bisabolene were found in the essential oil of the dried fruit, and sabinene in the essential oil of the fresh fruit of *P. verticillare* [16].

The present study reports the composition of the essential oil isolated from the dried fruits of *P. ruthenicum* by gas chromatography (GC) and GC/ mass spectrometry (MS). The antimicrobial activities of the essential oil against some gram-positive and gram-negative bacteria are also investigated.

2. Materials and methods

2.1. Plant material

Fresh plant of *P. ruthenicum* with flower and fruits were collected in October 2003 from Arak (Markazi province), Rasband mountains, 16 km north-west of Shahzad, rocky slopes west of Babakhodadad, 33° 55' N, 49° 19' east, and 2200 m. The plant was identified by Dr. H. Akhani and voucher specimen (hb. Akh. 15487) was deposited in the personal herbarium of Dr. H. Akhani (Plant Science Department of Tehran University, Iran). The fruits were isolated from the plant and dried in the shade.

2.2. Isolation of the essential oil

The dried fruits were submitted to water distillation for 4 hours using a Clevenger type apparatus. The obtained essential oil (yield: 1.8 % v/w) were dried over anhydrous sodium sulfate and stored at +4 °C until GC/MS analyzing.

2.3. Antimicrobial activity

The disc-diffusion assay was used to determine the growth inhibition of bacteria by the essential oil [17]. The following bacteria were used: *Staphylococcus aureus* ATCC 29737, *Staphylococcus epidermidis* ATCC 14990, *Bacillus cereus* ATCC 1247, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhi* ATCC 19430. They were obtained from the department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical sciences. Base plates were prepared by pouring 10 ml Mueller-Hinton (MH) agar into sterile Petri dishes (9 cm) and allowed to set Mueller-Hinton agar held of 48 °C was inoculated with a broth cultured (1×10^8 cfu/ml) of the test organism and poured over the base plates forming a homogenous top layer. Aliquots of 2.5 μl [2 mg, (d=0.8)] of plant essential oil were applied per filter paper

Table 3. The minimum inhibitory concentrations of *Peucedanum ruthenicum* fruits' essential oil against some gram-positive bacteria.

Strains	MIC (mg/ml)	
	Essential oil	Neomycin
S. aureus	0.03	4×10^{-3}
S. epidermidis	0.29	1.2×10^{-4}
B. cereus	0.10	1.2×10^{-4}

disc (Whatman No.3, 6 mm diameter). Discs were placed on to the second top layer of the agar plates. The essential oil was tested in quadruplicate (4 disc/plate) with neomycin (200 µg)/ discs as reference or positive control. The plates were evaluated after incubation at 37 °C for 18 h. Antibacterial activity was expressed as the inhibition zone (mm) was produced [18]. The activity of neomycin was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain. Minimum inhibitory concentrations (MICs) of essential oil was determined against the tested microorganisms. The agar dilution method [19] was used against *S. aureus*, *S. epidermidis*,*B. cereus*,*E. coli*, *P. aeruginosa* and *S. typhi*, with two full serial dilutions of plant essential oil from 0.001 to 0.5 mg/ml of the medium. Dimethylsulfoxide (DMSO) was used as solvent for mixing of essential oil with the medium. MIC values were taken as the lowest concentration of essential oil which completely inhibited bacterial growth after 18 h of incubation at 37 °C. Neomycin and DMSO with no essential oil were used as the positive and negative controls, respectively.

2.4. GC/MS analysis

Analysis of the essential oil was performed using a Hewlett Packard 6890 GC equipped with a HP-5MS capillary column (30 m x 0.22 mm i.d., 0.25 µm film thickness) and a mass spectrometer 5973 from the same company for GC/MS detection with an electron ionization system energy (70 ev) was used. Helium was the carrier gas, at a flow rate of 1 ml/min, injector and detector MS transfer line temperatures were set at 250 and 290 °C, respectively. Column temperature was initially kept at 60 °C for 5 min, then gradually increased to 220 °C at the rate of 6 °C/min. Identification of essential oil components was based on comparison of their mass spectra with those of Wiley library data of mass spectroscopy, and literature data as well as on comparison of their retention indices with normal alkanes (C_8-C_{24}).

3. Results and discussion

The composition of *P. ruthenicum* fruits essential oil (Table 1) consisted of 41 compounds, which 31 of them were accounting for 83.9% of the total oil. The most important compounds were monoterpenes (42.14%), consisting of hydrocarbon monoterpenes (2.91%) and oxygenated monoterpenes (39.23%), sesquiterpenes (39.67%) consisting of hydrocarbon sesquiterpenes (19.65%) and oxygenated sesquiterpenes (20.02%). The major component of monoterpenes were 1,8-cineole (11.15%), *cis*-carveol (6.88%), camphor (5.86%), l-carvone (5.61%), and those of sesquiterpenes were caryophylene oxide (13.65%), 8,9-dehydroisolongifolene (11.35%), and caryophylla-4(12), 8(13)-dien-5-β-ol (5.19%) [20].

The essential oil was tested against 6 standard bacteria (gram-positive and gram-negative) strains. Antibacterial activities were found against *S. aureus*, *S. epidermidis* and *B. cereus* (Table 2). The antibacterial activity of essential oil was mainly against the gram-positive bacteria and didn't show any activity against the gram-negative bacteria such as *E.coli*, *P. aeruginosa*, and *S. typhi*. The MIC values (0.03–0.29 mg/ml) of essential oil for

the sensitive bacteria (Table 3), confirmed the bacteriostatic activity of essential oil against some bacterial strains. These values are high compared with those of neomycin.

In summery, the data summarized in Table 3 indicate that the essential oil of *P. ruthenicum* fruits were shown to have antibacterial activity against gram-positive bacteria, which may justify the use of these species in traditional medicine and underline the importance of the bioactive ethnobotanical approach for the selection of plants for the discovery of the new antibacterial substances. Some compounds of this essential oil such as 1,8-cineol, camphor, and carveol have been reported to possess antibacterial activity [21]. However, as there was often no correlation between the antibacterial activity and the main chemical components, it is possible that either there is a more complex relationship with the chemical composition (which includes the minor components) or substantial adulteration had occurred in some essential oil samples [22].

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