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Original Article

Chemical Constituents and Antibacterial Activity of Essential Oil of *Prangos ferulacea* (L.) Lindl. Fruits

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Abstract

The essential oil of *Prangos ferulacea* (Apiaceae) fruits was obtained by hydrodistillation and analyzed by gas chromatography (GC) and GC-mass spectrometery (MS). Among the 39 identified constituents accounting for 99.99% of the total oil, the major components were chrysanthenyl acetate (26.53%), limonene (19.59%), alpha pinene (19.50%), delta-3-carene (6.56%), mesitaldehyde (6.09), and germacrene-B (3.55%). Antimicrobial activity of the essential oil was investigated against some gram positive and gram negative bacteria. The essential oil of *P. ferulacea* showed activity against *Staphylococus aureus, S. epidermis, Eschrechia coli* and *Pseudomonas aeroginosa*.

Keywords: Antibacterial activity; Chrysanthenyl acetate; Essential oil; *Prangos ferulacea*.
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1. Intorduction

Many infectious diseases have been 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 an important source of drugs for majority of the world population [3-5]. The genus of *Prangos* with the common Persian name of "Jashir" include 15 species which are growing wildly in many regions of Iran. Some species are distributed in Anatolia, Central Asia and Caucasian. Five species delicated to Iran include: *P. gaubae, P. calligonoides, P. cheilantifolia, P. tuberculata,* and *P. crossoptera* which are distributed in the north and central provinces of Iran [6].

Previous phytochemical studies on *P. ferulacea* (L.) Lindl. have indicated the presence of coumarin and its derivatives [7, 8]. The fruits of endemic *P. unchtritzii* Boiss. and Hausskn. (Apiaceae) are subjected to hydrodistillation and microdistillation. The resulting volatiles were investigated by gas

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Table 1. The essential oil *Prangos ferulacea* fruits' essential oil.

No	Compound name	%	RI[a]
1	Methae, sulfoxide	0.01	928
2	alpha-Thujene	0.01	941
3	alpha-Pinene	19.50	948
4	Sabinen	0.32	982
5	beta-Pinene	1.12	986
6	beta-Myrcene	2.94	1023
7	alpha-Phellandrene	0.46	1030
8	delta-3-Carene	6.56	1034
9	Limonene	19.59	1038
10	1,3-cyclopentadiene,1,2,3, 4, 5 pentamethyl	0.20	1055
11	alpha-Terpinene	0.82	1057
12	Camphene	0.47	1060
13	Terpinene-3-ol	0.07	1062
14	*cis*-Limonene oxide	0.04	1126
15	trans-Limonene oxide	0.08	1132
16	trans-Verbenol	0.55	1148
17	Safranol	1.04	1156
18	Terpinene-4-ol	0.21	1173
19	trans-Carveol	1.06	1182
20	Chrysanthenyl acetate	26.53	1202
21	Bornyl acetate	0.80	1226
22	Pinocarveyl acetate, trans	0.18	1244
23	m-Acetoaminophenol	0.19	1306
24	Mesitaldehyde	6.09	1335
25	beta-Elemene	0.99	1348
26	Methyl eugenol	0.07	1375
27	trans-Caryophyllene	0.14	1424
28	gamma-Elemene	0.99	1443
29	Aromadendron	0.12	1452
30	alpha- Humulene	2.84	1466
31	Germacrene- D	0.72	1482
32	Bicyclogermacrene	0.73	1492
33	Germacrene-B	3.55	1506
34	Spanthulenol	0.22	1575
35	Caryophyllene oxide	0.18	1584
36	Junipene	0.06	1658
37	alpha-Bisabolol	0.30	1710
38	Iso-aromadendron epoxide	0.48	1731
39	Osthol	0.37	1742

[a] RI: Retention indices as determined on a DB-5 column using the homologous series of n-alkanes (C_{8-24}).

chromatography-mass spectrometery (GC-MS) to determine the composition of the essential oil and 109 compounds representing 86.7% and thirty two compounds representing 90.0% were identified and isolated by two different techniques, respectively. Column chromatography of the essential oil yielded a new bisabolene ethe (7-epi-1,2-dehydro sesquicineole), which was characterized by spectral methods (GC- FTIR- 1D-, 2D NMR and HRESIMS) [9].

The water distilled essential oil from crushed dry fruits of *P. heyniae*, a recently described endemic in Turkey, collected from two localities were analyzed by GC/MS and 61 and 79 compounds representing 92.2%, 89.8% of the oils were characterized with β-bisabolenal (53.3% and 18.0%), β-bisabolene

Table 2. Antimicrobial activity of *Prango ferulacea* fruits' essential oil using disk diffusion assay.

Strain	Inhibition zone diameter (mm)	
	Essential oil (2 mg/ disc)	Neomycin (200µg)
S. aureus	14	21
S. epidermidis	13	18
E. coli	12	17
B. subtilis	7	15
P. aeroginosa	5	14

(14.6% and 2.3%) and β-bisabolene (12.1% and 10.1%) as the main constituents [10].

The present study reports the composition of the essential oils isolated from the dried fruits of *P. ferulacea* by GC and GC/MS. The antimicrobial activities of the essential oil against some gram positive and gram negative bacteria were also investigated.

2. Materials and methods

2.1. Plant material

Fresh plants of *P. ferulacea* with fruits and roots collected in May and June 2006 from Tehran (Tehran province): between Shemshak and Dizin, 30°02'30"N, 51°27'41"E, 2500 m, the plant was identified by Yousef Ajani and a voucher specimen (2055) was deposited at the herbarium of Tehran University of Medical Sciences.

2.2. Isolation of the essential oil

The dried fruits were submitted to water distillation for 4 h using a Clevenger type apparatus. The obtained essential oil (yield 1.50% v/w) were dried over anhydrous sodium sulfate and stored at +4 °C until GC/MS analysis.

2.3. Antimicrobial activity

The disk diffusion assay [11] was used to determine the growth inhibition of bacteria by the essential oil. The following bacteria were used: *Staphylococus aureus* ATCC 29737, *S. epidermis* ATCC 14990, *Bacillus subtilis* ATCC 6633, *Eschrechia coli* ATCC 8739 and *Pseudomonas aeroginosa* ATCC 9027. The bacteria were obtained from 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 at 80 °C was incubated with a broth cultured (1×10^8 cfu/ml) of the test organism and poured over the base plates forming a homogenous top layers. Aliquots of 2.5 µl [2 mg, (d= 0.8)] of plant essential oil were applied on filter paper disc. 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) per disc 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 [12]. The activity of neomycin was introduced in this equation to adjust for plate-to-plate variation in the sensitivity of particular bacterial strain. Minimum inhibition of concentration (MICs) of the essential oil was determined against the tested microorganisms. The agar dilution method [13] was used for *S. aureus, S. epidermis, B. subtilis, E. coli, P. aeroginosa* with two full serial dilutions of plant essential oil from to 0.5 mg/ ml of medium.

DMSO was used as the solvent for mixing essential oil with the medium. MIC was taken as the lowest concentration of the 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

Table 3. The minimum inhibitory concentration of *Prangos ferulacea* fruits' essebtial oil against some Gram- positive and Gram-negative bacteria.

Strain	Inhibition zone diameter (mm)	
	MIC (mg/ml)	NeomycinMIC (mg/ml)
S. aureus	6.5×10^{-3}	4×10^{-3}
S. epidermidis	6.8×10^{-3}	4×10^{-3}
E. coli	12.5×10^{-3}	9×10^{-3}
B. subtilis	11×10^{-3}	8×10^{-3}
P. aeroginosa	10×10^{-3}	8.2×10^{-3}

using a Hewlett Packard 6890 GC equipped with a HP-5MS capillary column (30 m×0.22 mm i.d., 0.25 μm film thickness) and a mass spectrophotometer 5973 from the same company for GC/MS detection with an electron ionization system energy (10 eV) was used. Helium was the carrier gas, at a flow rate of 1 ml/min., injector and detector MS transfer line temperature 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 the essential oil components was based on comparisons of their mass spectra with those of Wiley library data of MS and the 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. ferulacea* fruits essential oil (Table 1) was consist of 39 compounds; 11 of them accounting for 85.25% of the total oil. The most important compounds were monoterpene (more than 85%). The major components were chrysantenyl acetate (26.53%), limonene (19.59%), α-pinene (19.50%), α-humulene (2.84%).

The essential oil was tested against five standard bacterial strains (gram positive and gram negative). The MIC values (0.5×10^{-3}-12.5×10^{-3} mg/ ml) of the essential oil for the sensitive bacteria are represented in Table 3.

In summary, the data summarized in Table 3 indicate that the essential oil of *P. ferulacea* fruits have antibacterial activity against both gram positive and gram negative 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 limonen, α-pinene and α-humulene have been reported to possess antibacterial activity [14].

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