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Ferula gummosa FRUITS: AN AROMATIC ANTIMICROBIAL AGENT

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Ferula gummosa Boiss. *(Apiaceae) fruit volatile oil was analyzed by GC/MS. Seventy-three components (96.89%) were identified, and the major components were* β*-pinene (43.78%),* α*-pinene (27.27%), and myrcene (3.37%). The antimicrobial activity of the oil was tested on three strains of Gram positive bacteria (*Staphylococcus aureus, S. epidermis, *and* Bacillus subtilis*), three strains of Gram negative bacteria (*Escherichia coli, Salmonella typhi, *and* Pseudomonas aeruginosa*), and two strains of fungi (*Candida albicans *and* C. kefyr*). The essential oil remarkably inhibited the growth of the tested microorganisms. The results indicate that the fruits have potential for use as an aromatic antimicrobial agent.*

Key words: *Ferula gummosa*, Apiaceae, antimicrobial, essential oil, GC/MS.

Plants and their derivatives such as essential oils and oleoresins have long been used as food flavoring, beverages, and antimicrobial agents [1]. A number of studies on the antimicrobial effects of the essential oils and their components have been reported [1–3]. *Ferula* genus (Apiaceae) consists of 133 species distributed throughout the Mediterranean area and Central Asia [4, 5]. The flora of Iran comprises 30 species of *Ferula*, where some species are endemic, e.g., *F. persica, F. tabasensis, and F. gummosa* [5, 6]. *Ferula gummosa* Boiss., Barije in Persian, is a wild plant indigenous to Iran, growing in the northern and western parts of the country [7]. *F. gummosa* shows several pharmacological activities, including antispasmodic, expectorant, anticonvulsant, anticatarah, and antinociceptive [8]. Little work has been done to study the fruit essential oil components [9, 10] or antimicrobial activities [11].

This paper reports the GC/MS analysis of essential oil chemical constituents of *F. gummosa* fruits collected from Iran. The antimicrobial activity of the essential oil against three strains of Gram positive bacteria (*Staphylococcus aureus* (PTCC 1112), *S. epidermis* (PTCC 1114), and *Bacillus subtilis* (PTCC 1023)), three strains of Gram negative bacteria (*Escherichia coli* (PTCC 1338), *Salmonella typhi* (PTCC 1609), and *Pseudomonas aeruginosa* (PTCC 1074)), and two strains of fungi (*Candida albicans* (ATCC 14053) and *C. kefyr* (ATCC 38296)) were examined. The yield of the oil was 4.0% (v/w) based on the dry weight of the plant.

Seventy-three components were identified in the oil, representing 96.89% of the total oil (Table 1). The main group was monoterpene hydrocarbons (77.14%) with β-pinene (43.78%), α-pinene (27.27%), and myrcene (3.37%) as the major components. Moreover, the main components of the fruit oils collected from the Tehran region were reported as β-pinene (82%), α-pinene (5.4%), and myrcene (3.4%) [9], or β-pinene (50.1%), α-pinene (18.3%), δ-3-carene (6.7%), α-thujene (3.3%), and sabinene (3.1%) [10]. Differences in the oil composition and quantities may be due to the collection time and geographic factors. Table 2 shows *in vitro* bacteriostatic and fungistatic activities of *F. gummosa* essential oil and the inhibition zones formed by standard antibiotic discs. In our experiment a strong antibacterial and antifungal activity was observed at 7ml for Gram positive and negative bacteria and fungi tested. The bacteriostatic and fungistatic properties of the oil are suspected to be associated with the high α-pinene and β-pinene content, which has been tested previously and was found to have a significant antibiotic activity [12]. The observed antimicrobial properties show that the plant has potential for use as an antimicrobial agent.

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TABLE 1. Constituents of *Ferula gummosa* Fruit Essential Oil

RI[a]	Compound	%[b]	References[c]	RI[a]	Compound	%[b]	References[c]
944	α-Pinene	27.27	9, 10	1438	Unknown	0.15	-
952	Camphene	0.30	10	1442	3,7-Guaidiene	0.15	-
988	β-Pinene	43.78	9, 10	1451	α-Humulene	0.11	-
997	Myrcene	3.37	9	1473	γ-Selinene	0.10	-
1006	α-Phellandrene	0.05	9, 10	1476	γ-Muurolene	0.06	-
1012	δ-3-Carene	0.46	9, 10	1480	Germacrene-D	0.63	9, 10
1027	*p*-Cymene	0.04	9	1484	β-Selinene	0.17	-
1032	Limonene	1.00	9	1493	α-Selinene	0.35	-
1034	1,8-Cineole	0.20	9	1496	Dihydroagarofuram	0.19	-
1042	*cis*-Ocimene	0.16	-	1498	α-Muurolene	0.19	10
1052	*trans*-β-Ocimene	0.05	9	1502	Cuparene	0.05	-
1089	Terpinolene	0.66	9	1505	Germacrene-A	0.05	-
1115	Fenchol	0.05	-	1509	β-Bisabolene	0.09	-
1127	α-Campholene-aldehyde	0.05	-	1513	γ-Cadinene	0.38	-
1141	*trans*-Pinocarveol	0.36	-	1522	Cubebol	1.39	-
1148	*trans*-Verbenol	0.15	9	1525	δ-Cadinene	1.13	9, 10
1159	Unknown	0.05	-	1528	Liguloxide	0.50	-
1164	Pinocarvone	0.18	-	1532	Cadina-1,4-diene	0.10	-
1177	(3E,5Z)-1,3,5-Undecatriene	0.65	-	1537	α-Cadinene	0.08	-
1179	Terpin-4-ol	0.06	-	1540	Selina-3,7(11)-diene	0.05	-
1185	(E,E)-1,3,5-Undecatriene	0.19	-	1551	Elemol	0.10	-
1189	*p*-Cymen-8-ol	0.07	-	1555	Germacrene-B	0.71	-
1192	α-Terpineol	0.07	-	1576	Apofarnesol<(z)-Dihydro->	0.89	-
1194	Myrtenal	0.13	9, 10	1599	Guaiol	1.44	9
1197	Myrtenol	0.14	-	1605	Eudesmol (isomer)	0.07	-
1208	Verbenone	0.05	-	1614	Cebenol (1,10,di,epi)	0.08	-
1222	α-Fenchyl acetate	1.07	9	1618	Eudesmol (10-epi-γ)	0.40	-
1237	Citronellol	0.81	-	1627	Unknown	0.11	-
1246	Carvacrol methyl ether	0.15	-	1631	γ-Eudesmol	0.08	-
1286	Bornyl acetate	0.28	9	1640	Hinesol	0.09	-
1298	*trans*-Verbenyl acetate	0.08	-	1651	β-Eudesmol	0.60	-
1336	δ-Elemene	0.06	-	1654	α-Eudesmol	0.65	-
1352	α-Terpinenyl acetate	0.74	9	1668	Bulnesol	0.61	-
1357	Citronellyl acetate	0.35	-	1694	Unknown	2.66	-
1365	1,2,4-Metheno, 1H-indene	0.06	-	1739	Unknown	0.12	-
1370	α-Ylange	0.06	-	1845	Unknown	0.02	-
1374	α-Copaene	0.19	-		Identification, %	96.89	
1387	Geranyl acetate	0.22	-		Grouped components		
1390	β-Elemene	0.14	-		Monoterpene hydrocarbons	77.14	
1409	β-Cubebene	0.11	-		Oxygen-containing monoterpenes	5.21	
1416	*trans*-Caryophyllene	0.22	-		Sesquiterpene hydrocarbons	6.96	
1427	Thujopsene	0.06	-		Oxygen-containing sesquiterpenes	6.74	
1434	γ-Elemene	1.31	-		Others	0.84	

[a]The retention index of compounds on the HP-5MS was determined.

[b]Percentage were calculated based on the concentration obtained on the same column.

[c]Reported in the literature as volatile compound in *Ferula gummosa* Boiss. fruits.

TABLE 2. Antimicrobial Activity of *Ferula gummosa* Boiss. Fruits

Microorganisms	Inhibition zone[a], µL					Gentamicin	Ampicillin	Nistatin
	3	4	5	6	7			
Staphylococcus aureus	+	++	+++	+++	+++		++++	
Staphylococcus epidermis	+++	+++	++++	++++	++++		++++	
Bacillus subtilis	+++	++++	++++	++++	++++		++++	
Escherichia coli	+++	+++	++++	++++	++++	+++		
Salmonella typhi	+++	+++	++++	++++	++++	+++		
Pseudomonas aeruginosa	+++	+++	++++	++++	++++	+++		
Candida albicans	++	++	++	+++	++++			+++
Candida kefyr	++	++	+++	+++	+++			++++

[a]+: 1-4 mm, ++: 5-9 mm, +++: 10-14 mm, ++++ > 14 mm.
[b]Micro liters of the F. gummosa fruit essential oil were applied to the discs.

EXPERIMENTAL

Plant Material. *F. gummosa* Boiss. fruits were collected from plants growing wild in: Daran, 40 km to Tiran, Gord-e Olia, Tcheshmeh Tangeh, altitude: 2500–2700 m, Isfahan, Iran, on July 30, 2001. Plant material was identified by I. Mehregan and a voucher specimen deposited in the Shiraz Faculty Pharmacy herbarium.

Distillation. Air-dried fruits were powdered and subjected to hydrodistilation for 4 h using a Clevenger-type apparatus.

Antimicrobial Assay. *In vitro* antimicrobial activities were determined by the agar disc diffusion method. Each microorganism was produced in suspension physiological saline solutions 0.9% w/v. The 0.5 McFarland standard (1.5×10^8 CFU/ml) was used to adjust the turbidity of the inoculum concentration for the antimicrobial test. Muller-Hinton agar was used as growth media and inoculated with a lawn of the test microorganisms. The obtained essential oil (3, 4, 5, 6, and 7 ml) of *F. gummosa* was injected into sterilized discs 6.4 mm in diameter and placed on Muller-Hinton agar plates. Gentamicin (10 µg), ampicillin (10 µg), and nistatin (10 µg) were used as positive controls against Gram positive bacteria, Gram negative bacteria, and fungi test microorganism, respectively. Plates were incubated at an appropriate temperature for fungi (25°C) and bacteria (37°C) for a period of 18–24 h. Studies were performed in triplicate. The sample that had antimicrobial activity produced a distinct, clear, and circular zone of inhibition around the disc and was used as an indication of antimicrobial activity.

Analysis of Essential Oil. The GC/MS analyses were carried out using a Hewlett-Packard 6890. The gas chromatograph was equipped with a HP-5MS capillary column (phenylmethylsiloxane, 25 m × 0.25 mm i.d.). The oven temperature was programmed from 50°C (3 min) to 250°C at a rate of 3°C/min and finally 10 min at 250°C. The carrier gas was helium with a flow rate of 1.2 ml/min, and the split ratio was 1:30. The mass spectrometer was operating in the EI mode at 70 eV. The interface temperature was 250°C; mass range was 30–600 *m/z*. Identification of components was based on a comparison of their RI and mass spectra with Wiley (275) and Adams libraries spectra [13].

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