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Original article

Essential oil analysis and antibacterial activity of *Ferula assa-foetida* L. aerial parts from Neishabour mountains

N. Samadi [1], S. Shahani[2], H. Akbarzadeh[3], S. Mohammadi-Motamed[3], E. Safaripour[4], F. Farjadmand[5], M. Eftekhari[6], H.R. Monsef-Esfahani[7], M. Khanavi[6,7*]

[1]*Department of Drug and Food Control, Faculty of Pharmacy and Pharmaceutical Quality Assurance Research Center, Tehran University of Medical Sciences, Tehran 14155-6451, Iran.*
[2]*Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*
[3]*Pharmaceutical Sciences Branch, Islamic Azad University, Tehran 19419-33111, Iran.*
[4]*Department of Drug and Food Control, Faculty of Pharmacy and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran 14155-6451, Iran.*
[5]*Food and Drug Organization, Ministry of Health and Medical Education of Iran & Department of Traditional Pharmacy, Faculty of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
[6]*Department of Pharmacognosy, Faculty of Pharmacy and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran14155-6451, Iran.*
[7]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.*

Abstract

Background and objectives: *Ferula assa-foetida* (asafoetida) is a native Iranian species which grows in different regions and climates in Iran. The plant is well known in Iranian Traditional Medicine as well as folk medicine for treatment of diseases. Several studies have been carried out on the essential oil of this species collected from different areas of Iran. This study is the first report about the essential oil of the plant collected from Neishabour mountains that is a potent area for growing this valuable plant species. **Methods:** Essential oil of the aerial part of *Ferula assa-foetida* which was collected from Neishabour, Iran, was analyzed by gas chromatography-mass spectroscopy (GC/MS). The minimum inhibitory concentrations of the essential oil was investigated against both Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia)* bacteria using microdilution method. **Results:** Twenty three components representing 97.06% of the total oil were identified. (*E*)-1-propenyl *sec*-butyl disulfide (53.77%), (*Z*)-1-propenyl *sec*-butyl disulfide (35.6%) and α-pinene (3.4%) were identified as major components. The MIC of the essential oil ranged from 12-24 mg/mL against all tested bacteria. **Conclusion:** The results indicated that among various compounds identified in the essential oil *of F. assa-foetida* L. from Neishabour mountains, disulphide compounds were the major constituents of the oil. In comparison to other reports of this plant around the country, disulphide compounds could be the reason of its moderate antibacterial effect.

Keywords: antibacterial activity, essential oil, *Ferula assa-foetida*, GC/MS

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*Corresponding author: *khanavim@sina.tums.ac.ir /khanavi@mail.ubc.ca*, Tel: +98 21-66954706, Fax: : +98 21 66461178

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Introduction

The genus *Ferula* from Apiaceae family comprises about 170 species in the world. *Ferula* genus is represented by 30 species In Iran, while *Ferula assa-foetida* L. (asafoetida) is a native one [1] and is called "*Koma*" [2]. This herbaceous and perennial plant with a height of 2 m [3], enriched with sesquiterpenes, sesquiterpene coumarins and sulfur-containing compounds [4]. Investigations on the *Ferula* species have indicated antinociceptive, anti-inflammatory, antipyretic [5], contraceptive [6,7] and smooth muscle relaxant activities [8]. In Iranian folk medicine, an oleo-gum-resin called "*Anghouzeh*" obtained from this plant is used as antispasmodic, anti-seizure, anti-constipation in elderly and also for asthma [9]. Besides, the oleo-gum-resin is used as a culinary spice and has shown several activities such as antioxidant, antiviral, antifungal, anti-diabetic, hypotensive, molluscicidal and cancer chemopreventive activities in recent pharmacological studies [10]. Moreover, several biological activities such as antibacterial and antifungal properties have been reported from the essential oil of *Ferula* species [2,11]. Previous studies have shown that the essential oils of asafoetida obtained from Kerman [12] and Isfahan provinces of Iran [13] were rich in sulfur compounds and various monoterpenes. Also asafoetida essential oil obtained from Pakistan [14], and India [15] had antimicrobial activities against Gram-positive and Gram-negative bacteria.

The aim of the present study was to determine the essential oil composition and antibacterial effects of aerial parts of asafoetida collected from Neishabour, Khorasan-Razavi Province, Iran for the first time.

Experimental

Plant material

The aerial part of asafoetida was collected from Neishabour, Khorasan-Razavi Province, Iran, at an altitude of 2000 m, in March 2009 during the flowering stage. The plant was identified and authenticated and the voucher specimen was deposited at the Herbarium of the Institute of Medicinal Plants, ACECR, Karaj, Iran (No. 307 ACECR).

Isolation of the volatile oil

Air-dried flowering aerial parts (500 g) were subjected to hydrodistillation, using a Clevenger type apparatus [16], for 5 h. After decanting, the oil was dried over anhydrous sodium sulfate and kept at 4°C in a sealed brown vial.

Gas chromatography-mass spectroscopy

Analytical gas chromatography was carried out using a termoquest 2000 GC with capillary column DB-5 (30 m×0.25 mm i.d., 0.25 µm film thicknesses); carrier gas, helium; split ratio, 1:25; and using a flame ionization detector (FID); flow rate, 1.5 mL/min. The column was held at 50 °C for 1 min and programmed up to 265 °C at a rate of 2.5 °C/min, then kept constant at 265°C for 20 min.

GC/MS was performed on a termoquest 2000 with a quadruple detector, on capillary column DB-5; carrier gas, helium with the same condition for GC. The MS operated at 70/eV ionization energy. Retention indices were calculated by using retention times of *n*-alkenes that were injected after the oil at the same chromatographic conditions.

The compounds were identified by comparing retention indices (RI, DB-5) with those reported in the literature and by comparing their mass spectra with the Wiley library or the published mass spectra [13,17-20].

Antibacterial activity determination by cup plate method

Antibacterial activity of the essential oil was screened using cup plate method against different Gram-positive (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC

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6538, *Bacillus subtilis* ATCC 6633) and Gram-negative (*Eecherichia coli* ATCC 8739, *Pseudomonas aeruginosae* ATCC 9027, *Klebsiella pneumoniae* ATCC 10031) bacteria. The bacteria inocula were prepared by suspending overnight colonies in 0.9% saline and adjusted photometrically to a cell density equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL). Mueller-Hinton (MH) agar plates were seeded individually with bacterial suspensions using a sterile cotton swab. Wells were prepared by punching a stainless steel cylinder onto the agar plates. The essential oil was dissolved in MH broth containing 1% Tween 80 and diluted in a two-fold manner to make a series of concentrations ranged from 6.25 to 100 mg/mL). Then, 100 µL of each dilute was placed individually in three wells. The MH broth containing 1% Tween 80 was used as negative control. After incubation at 35 °C for 24 h, the mean inhibition zone diameter for each concentration was determined [21].

Minimum inhibitory concentration (MIC) determination

The MIC of the essential oil was determined by microdilution method using 96 U-shaped wells plates with respect to the above mentioned Gram-positive and Gram-negative bacteria. A stock concentration of the essential oil was prepared in MH broth containing 1% Tween 80 in the first well in each row and serially diluted by mixing with 100 µL of MH broth in subsequent wells to reach a concentration range of 100-3.12 mg/mL. Final concentration of bacteria in each well was adjusted to about 5×10^5 CFU/mL. After 24 h incubation at 35 °C, the microplates were examined for possible bacterial turbidity and MIC of the essential oil was determined as the lowest concentration that could inhibit visible bacterial growth [22-23].

Results and Discussion

The aerial part of asafoetida yielded 1.7% (w/w) yellowish essential oil with a volumetric mass density of 0.96 (g/cm^3 in ambient condition). Twenty three components were identified in the essential oil which represented 97.06% of the total oil (table 1). The oil was rich in sulfur-containing compounds (90.17%). Also, eight monoterpene hydrocarbons (5.32%) and five sesquiterpene hydrocarbons (1.07%) were found in the oil.

Table 1. Chemical composition (%) of the oil of aerial part of *Ferula assa-foetida* L.

No	Compound	Percentage	RI[a] Sample	RI[a] Standard
1	Dimethyl disulfide	0.40	783	783
2	α-Pinene	3.40	937	939
3	Dimethyl trisulfide	0.40	970	977
4	β- Pinene	1.40	977	980
5	Myrcene	0.01	990	991
6	Limonene	0.10	1026	1031
7	(*Z*)-β-Ocimene	0.10	1035	1040
8	(*E*)-β-Ocimene	0.28	1048	1050
9	Tetra methyl pyrazine	0.10	1085	1085
10	Borneol	0.04	1162	1165
11	(*Z*)-1-propenyl *sec*-butyl disulfide	35.60	1174	1177
12	(*E*)-1-propenyl *sec* butyl disulfide	53.77	1179	1181
13	α-Terpineol	0.03	1188	1189
14	Bornyl acetate	0.30	1286	1285
15	β-Elemene	0.04	1391	1391
16	(*Z*)-Caryophyllene	0.16	1404	1404
17	(*E*)-Caryophyllene	0.19	1415	1418
18	α-Humulene	0.10	1453	1454
19	γ-Curcumene	0.07	1478	1480
20	β-Selinene	0.08	1485	1485
21	δ-Cadinene	0.13	1508	1513
22	(*Z*)-Asarone	0.06	1616	1622
23	β-Eudesmol	0.30	1644	1649
	Sulfur compounds	90.17	-	-
	Monoterpenes	5.32	-	-
	Sesquiterpenes	1.07	-	-
	Non-terpenoid	0.16	-	-
	Unknown	2.94	-	-
	Total	97.06	-	-

RI[a]: Retention Index on DB-5 in reference to *n*-alkanes injected after the oil at the same chromatographic conditions

(*E*)-1-propenyl *sec*-butyl disulfide (53.77%), (*Z*)-1-propenyl *sec*-butyl disulfide (35.6%) and α-pinene (3.4%) were identified as the major components. Dimethyl disulfide (0.4%) and dimethyl trisulfide (0.4%) were found as the other sulfur-containing compounds.

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The results of the antibacterial activity of the essential oil are shown in table 2 and 3. In the present study, the essential oil of asafoetida was found active against all tested strains. As shown in table 2, wider inhibition zones were found against Gram-positive than Gram-negative bacteria and no inhibition zones were observed against Gram-negative bacteria at the concentration of 12 mg/mL essential oil. The data showed that the MIC values of the essential oil against all strains ranged from 12-24 mg/mL.

Table 2. Antibacterial activity of the essential oil of *Ferula assa-foetida* by cup plate method

Bacterial strain	Concentration (mg/mL)/ Inhibition zone diameter (mm)				
	100	**50**	**25**	**12.5**	**6.25**
Staphylococus aureus	16	14	12	11.5	NZ
Staphylococus epidermidis	13	12	10	10	NZ
Escherichia coli	9	8	8	NZ	NZ
Bacillus subtilis	15	12	11	10	NZ
Klebsiella pneumoniae	20	15	10	NZ	NZ
Pseudomonas aeruginosa	15	10	9	NZ	NZ

NZ: No inhibition zone was observed

Table 3. Minimum inhibitory concentration (MIC) of the essential oil of *Ferula assa-foetida* L. determined by microdilution method

Bacteria	MIC (mg/mL)	Ciprofloxacin (µg/mL)
Gram-positive		
Bacillus subtilis	12	0.2
Staphylococcus	12	0.2
Staphylococcus	12	0.2
Gram-negative		
Klebsiella	24	0.005
Escherichia coli	24	0.01
Pseudomonas	24	0.05

The overall objective of the current study was to determine the essential oil composition and antibacterial effects of the aerial parts of asafoetida. Several studies have been carried out on the essential oil composition of different parts of *F. assa-foetida* collected from different places and climates of Iran. Table 4 compares the results of the present study with other researches on the essential oil composition of *F. assa-foetida* collected from different regions in Iran. Differences in places of collection and the examined parts of the plant could explain the observed variety in the essential oil composition. (*E*)-1-propenyl *sec*-butyl disulfide and/or (*Z*)-1-propenyl *sec*-butyl disulfide were the two common sulfur compounds which were mentioned in several studies and mostly as the major components [12,13,24,26,29,30]; however, there are some other reports with no detected sulfur compound in the essential oil [3,27]. In this study, 90.17% of the essential oil components were identified as sulfur containing compounds in which (*E*)-1-propenyl *sec*-butyl disulfide and (*Z*)-1-propenyl *sec*-butyl disulfide with 89.37% seemed to be responsible for the observed antimicrobial effect. The current study represented the highest reported amount of sulfur-containing compounds in the essential oil of Persian species of *F. assa-foetida* (table 4). Moreover, apart from the above mentioned compounds, α-pinene was found to be the third major compound (3.40%). In most available reports from different regions, monoterpene and/or sesquiterpene compounds were found to be the most abundant compounds after sulfur compounds [12,13, 24-26, 29, 30]. The amount of sesquiterpenoids in our sample was reported only 1.07%.

A few studies have been performed about the antibacterial activity of asafoetida. In the study of Kavoosi and Rowshan from Fars province of Iran the essential oils of three different kinds of gum represented antibacterial activity against both Gram-positive and Gram-negative bacteria [29]. The minimal inhibitory concentration (MIC) for Gram-positive and Gram-negative bacteria growth were 27–111, 23–107 and 15–65 µg/mL of the essential oil. In detail, the sample with high level of acyclic sulfide compounds exhibited lower antibacterial effect compared to the one with heterocyclic sulfide compounds [29]. Investigation of antibacterial activity of asafetida seeds oil from Pakistan showed that

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Table 4. Comparison of major essential oil compounds of *Ferula assa-foetida* collected from different areas of Iran

Place of collection	Plant part	Time of collection	Major component (More than 10% (w/w)) (%)	Total sulfur compounds (%)	Monoterpene compounds (%)	Sesquiterpene compounds (%)	Reference
Neishabur	Aerial part	March	(*E*)-1-Propenyl *sec*-butyl disulfide (53.77), (*Z*)-1-Propenyl *sec*-butyl disulfide (35.6)	90.17	5.32	1.07	The current study
Kerman	Aerial part	June	1-Methylpropyl (1*E*)-prop-1-en-1-yl disulfide (32.80), α-Pinene (11.30),	45.30	23.50	12.10	[25]
Sari	Aerial part	Summer	Phenol, 2-methyl-5-(1-methyl ethyl) (18.2), α-Bisabolol (10.4)	0.70	9.20	30.80	[31]
Yazd	Oleo-gum resin	May	(*E*)-1-Propenyl *sec*-butyl disulfide (30.45), 1-(Propylthio)-propyl propyldisulfan (22.68), (*Z*)-1-Propenyl *sec*-butyl disulfide (18.68)	82.84	ND	3.02	[26]
Tabas	Oleo-gum resin	July	(*E*)-1-Propenyl *sec*-butyl disulfide (40.15), (*Z*)-1-Propenyl *sec*-butyl disulfide (23.93)	77.71	2.93	10.88	[30]
Yazd	Oleo-gum resin	July	(*E*)-1-Propenyl *sec*-butyl disulfide (44.36), (*Z*)-1-Propenyl *sec*-butyl disulfide (27.98)	72.36	3.59	19.66	[30]
Esfahan	Oleo-gum resin	August	(*E*)-1-Propenyl *sec*-butyl disulfide (35.1), (*Z*)-1-Propenyl *sec*-butyl disulfide (22.1), α-Pinene (12.2)	71.00	22.00	0.10	[13]
Fars, Larestan	Oleo-gum resin	May	Bis (1-methyl–thio) propyl disulfide (29.68), (*Z*)-1–Propenyl *sec*–butyl disulfide (19.34), (*E*)-1–Propenyl *sec*–butyl disulfide (15.73)	65.39	17.57	12.85	[26]
Fars, Larestan	Oleo-gum resin	15 June	(*E*)-1-Propenyl *sec*-butyl disulfide (23.922), 10-*epi*-γ- Eudesmol (15.091)	33.20	24.75	40.83	[29]
Fars, Larestan	Oleo-gum resin	30 June	(*Z*)-1-Propenyl *sec*-butyl disulfide (27.77), (*E*)-1-Propenyl *sec*-butyl disulfide (20.289), α-Pinene (10.775), β-Pinene (10.238)	50.518	36.45	12.690	[29]

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Table 4. Continued

Place of collection	Plant part	Time of collection	Major component (More than 10% (w/w)) (%)	Total sulfur compounds (%)	Monoterpene compounds (%)	Sesquiterpene compounds (%)	Reference
Fars, Larestan	Oleo-gum resin	15 July	β-Pinene (47.1) , α-Pinene (21.36), 1, 2- Dithiolane (18.63)	22.27	73.26	1.09	[29]
Gonabad	Root	July	(*E*)-1-Propenyl *sec*-butyl disulfide (30.7), (*Z*)-1-Propenyl *sec*-butyl disulfide (12.4), Eudesmol (10-epi-γ) (12.70)	64.00	ND	32.30	[24]
Tabas	Root	Julie	(*E*)-1-Propenyl *sec*-butyl disulfide (18.80), Eudesmol (10-epi-γ) (18.70)	40.50	ND	47.30	[24]
Khorramabad	Leaves	July	Eremophilene (31.28), δ-Cadinene (22.05), Longiborneol (12.09)	ND	1.70	82.78	[27]
Kerman	Fruit	October	(*E*)-1-Propenyl *sec*-butyl disulfide (40.00)	53.50	20.40	18.80	[12]
Kermanshah	Fruit	July	epi-α-Cadinol (23.15), Germacrene B (10.98)	11.18	6.14	81.25	[28]
Kashan	Fruit	June	γ-Elemene (32.21), *Trans*, 2-Undecene-1-ol (17.26)	ND	57.26	30.42	[3]

ND: Not detected.

the oil was active against Gram-positive and Gram-negative strains in a MIC range of 80-200 µg/mL against the susceptible bacteria [14]. In another study, *B. subtilis* was the most susceptible strain to the oil of asafoetida from India [15].

Similar to our results, the fruit oil of *F. latisecta* was reported rich in polysulphide compounds of which (Z)-1-propenyl *sec*-butyl disulfide (65.2%) and (*E*)-1-propenyl *sec*-butyl disulfide (6.8%) were the main compounds [32]. The antibacterial spectrum of the fruit oil of *F. latisecta* resembled that of our sample and was more effective against Gram- positive than Gram negative strains. It was reported that disulfides exhibited much lower antimicrobial activity than other sulfur-containing compounds [33], and presence of about 90% disulfides in this essential oil might be responsible for the lower antibacterial activity compared to the above mentioned studies.

The results obtained from the present study indicated that among various compounds in the essential oil *of F. assa-foetida* L. from Neishabour mountains, disulphide compounds were the major constituents of the oil which could be the reason of its moderate antibacterial effect. Also, the highest amount of sulfur-containing compounds in the plant was present in the current study particularly comparing to all other reports from Iran. The variety of the

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antibacterial potency of the essential oil of *F. assa-foetida* might be due to the differences in geographical area of plant collection and various examined parts of the plant which have led to the significant differences in composition of the essential oils.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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