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Essential Oil Composition of *Ferula Assa-Foetida* L. Fruits from Western Iran

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A B S T R A C T

Chemical composition of the essential oil of the fruit of *Ferula assa-foetida* L. obtained by steam distillation solvent extraction methodhas been studied by GC/MS for the first time. Fifty-four components, comprising 96.9% of the total oil, were identified.epi-α-Cadinol (23.15 %), germacrene B (10.98 %), α-gurjunene (6.18 %), (Z)-1-propenyl *sec*-butyl disulfide (5.89 %), 5-epi-7-epi-α-eudesmol (4.89 %), δ-cadinene (4.78 %), γ-cadinene (3.36 %) and germacrene D (3.09 %) were found to be the major constituents of the oil. The oil of the fruit of *F. assa-foetida* consisted of ten monoterpene hydrocarbons (6.14%), twenty-six sesquiterpene hydrocarbons (43.48%), nine oxygenated sesquiterpenes (37.77%), one oxygenated hydrocarbon (0.35%), and nine volatile sulfides (11.18%).

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Introduction

The genus *Ferula* (Apiaceae) consists of about 170 species worldwide with thirty species found in Iran, of which fifteen are endemics.[1]. Phytochemical analyses of *Ferula* spp. have confirmed the presence of sesquiterpene coumarins [2,3], sesquiterpenes [4-7], sulfides and volatile oils [8-10].

The exudates from this plant are locally known as "anghuzeh", "Heng" and "Buganeh", and have been traditionally used for treatment of vast range of diseases (urinary, gasterointestinal and respiratory infections, and epilepsy), as well as an aphrodisiac, an emmenagogue, and also to treat snake and insects bites, with the best documented folk use being the management of intestinal worm infections [11-14]. There are several reports on thepharmacological activities of Asafoetida such as antiviral (HSV, HRV, H1N1, HIV), antispasmodic, hypotensive and antidiabetic [12]. One of the characteristics of its essential oil is the existence of volatile sulfide constituents, a type of non-ubiquitous compounds with significant pharmacological effects [15,16].

Since Asafoetida is a commercial name with no specific botanical connection, it is difficult to assign the occurrence of the various compounds to a particular *Ferula* species. So, in accordance with our systematic study of various Iranian *Ferula* species [3,10,17], here we are reporting chemical profile of fruit of *F. assa-foetida* growing wild in Mount Telesm, Kermanshah province, west of Iran. Available information indicates that the essential oil of *F. assa-foetida*L. fruit growing wild in western Iran has not been the subject of phytochemical research up to now. Besides, a systemic comparison of major chemical ingredients of different populations of Iranian asafetida based on literature has been performed.

Materials and methods

Plant material

The fruit of *F. assa-foetida*L. were collected in July 2011 from Mount Telesm in Kermanshah province (Fig. 1) (longitude: 47° 00' 41" E; latitude: 34° 34' 49" N; altitude: 2280 m above sea level). The sample (Fig. 2) was identified by one of the authors (M. K.), and compared to voucher specimens (MPH-1251) deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran.

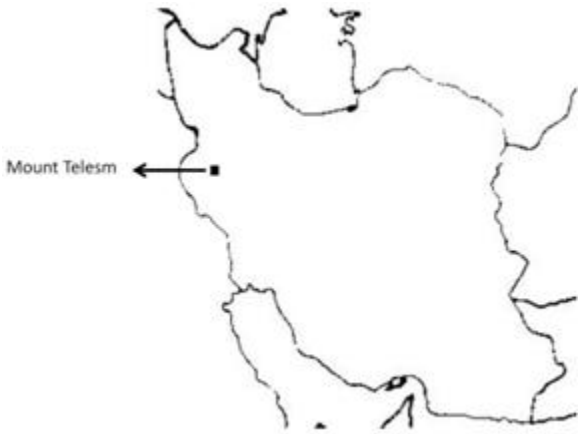

Fig. 1. Plant material collection location, Mount Telesm, Kermanshah, Iran. longitude: 47° 00' 41" E; latitude: 34° 34' 49" N; altitude: 2280 m above sea level

Fig 2. *Ferula assa-foetida* growing wild in Mount Telesm, Kermanshah, Iran

Isolation of the essential oil

The essential oil of air dried fruits (20 g) of *F. assa-foetida* was isolated using steam distillation solvent extraction (SDE) for 3h. The SDE was a modification of the apparatus of Linkens and Nickerson [18] and Godefroot and co-workers (Fig. 3). It was constructed for the use of organic solvents with density lower than water [19,20]. Hexane (Merck, Germany) was used

as the extraction solvent. The volatile oil was dried over anhydrous sodium sulfate and stored in sealed vial at 4° C until analysis.

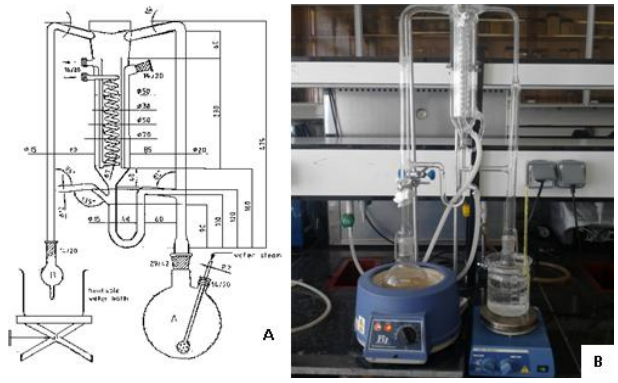

Fig 3. Steam distillation solvent extraction (SDE) apparatus of isolation of essential oil; **A:**Scale drawing (dimension in millimeters) of the SDE apparatus [18]. Vessel A, sample in water; Vessel B, Organic solvent, total volume 30 ml; **B:**SDE instrument on lab bench.

GC/MS analysis

Gas chromatography combined with mass spectrometry was used for identification of the components. The analysis was performed on a Hewlett-Packard 5972A mass selective detector coupled with a Hewlett-Packard 6890 gas chromatograph, equipped with a HP-5MS capillary column (30 m x 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed from 60-280°C at the rate of 4°C per min. Helium was used as the carrier gas at a flow rate of 2 mL/min. Injector and detector temperatures were 280°C. The MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 230°C; mass range, 35-425. The MSD ChemStation was used as operating software.

Retention indices were calculated by using retention times of *n*-alkanes (C_8-C_{24}) that were injected after the oil at the same conditions. Components of the oil were identified by comparison of their retention indices (RI) with those reported in the literature [10,15,21], two latter references especially for organosulfurs, and also by computer matching with NIST and Wiley275.L libraries. The fragmentation patterns of the mass spectra were also compared with those reported in the literature [21,22].

Results and discussion

The fruit of *F. assa-foetida* yielded 0.3% (v/w) of a yellowish oil with a sulphorous odor. Fifty-four components, comprising 96.9% of the total oil, were identified in the fruit of *F. assa-foetida*. The identified components and their percentage are shown in table 1, where the components are listed in order of their elution on the HP-5MS column. As it is clarified, epi-α-cadinol (23.15 %), germacrene B (10.98 %), α-gurjunene (6.18 %), (Z)-1-propenyl *sec*-butyl disulfide (5.89 %), 5-epi-7-epi-α-eudesmol (4.89 %), δ-cadinene (4.78 %), γ-cadinene (3.36 %) and germacrene D (3.09 %) (Fig. 4.) were found to be the major constituents of the oil.

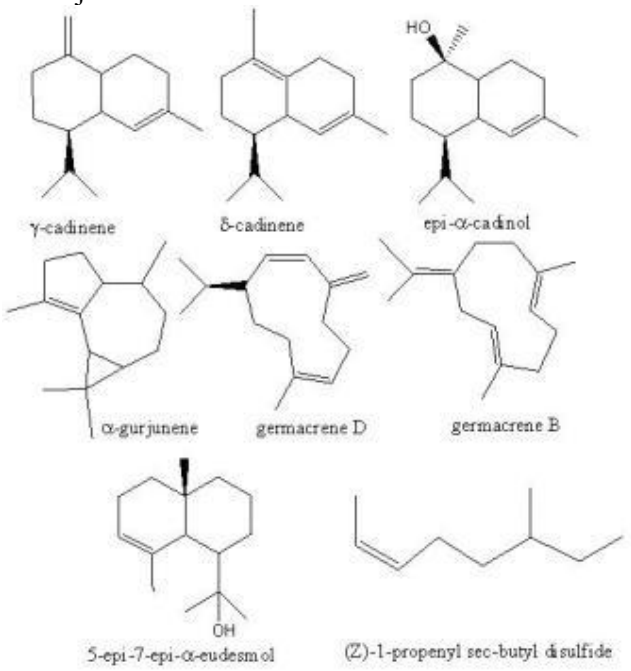

Fig 4. Structures of major compounds of essential oil of the fruits of *Ferula assa-foetida* L. growing wild in Mount Telesm, Kermanshah, Iran

The oil of the fruit of *F. assa-foetida* L. consisted of ten monoterpene hydrocarbons (6.14%), no oxygenated monoterpenes (0%), twenty-six sesquiterpene hydrocarbons (43.48%), nine oxygenated sesquiterpenes (37.77%), no phenylpropanoids (0%), one oxygenated hydrocarbon (0.35%), and nine volatile sulphides (11.18%).

There are several reports on essential oil composition of aerial parts of different *Ferula* species whereas a few of them describe volatile oil composition of fruit.

Comparative data on essential oils obtained from different *F. assa-foetida* populations from different regions of Iran have been presented in table 2.

Essential oil of *F. assa-foetida* have exerted anti-parasitic [23], antifungal and antibacterial [24] effects,and Those are besides other pharmacological effects related to asafetida total extracts which may contain considerable essential oil inside [12]. One of the special characteristics of its essential oil is volatile sulfide constituents (VSC), not a ubiquitous type of compound. They are found in a number of families including Apiaceae [15]. Another less common type of compounds in this family is phthalides[25].

Table 1. Composition of the essential oil of the fruits of *Ferula assa-foetida* L. growing wild in Mount Telesm, Kermanshah, Iran

No.	RI	Compound	Percentage (Area)%
1	937	α-pinene	1.66
2	952	camphene	0.22
3	972	sabinene	1.03
4	979	2-β-pinene	0.57
5	991	myrcene	0.14
6	1005	α-phellandren	0.45
7	1026	*p*-cymene	0.15
8	1031	limonene	0.93
9	1039	(*Z*)- β-ocimene	0.51
10	1050	(*E*)- β-ocimene	0.48
11	1056	1-propyl *sec*-butyl disulfide	0.54
12	**1169**	**(*Z*)-1-propenyl *sec*-butyl disulfide**	**5.89**
13	1172	(*E*)-1-propenyl *sec*-butyl disulfide	1.07
14	1209	bis (1-methylpropyl1) disulfide	0.51
15	1372	α-copaene	0.66
16	1388	β-elemene	0.27
17	1391	α-cubebene	0.53
18	1407	(*Z*)-caryophyllene	0.63
19	**1414**	**α-gurjunene**	**6.18**
20	1423	bis (1-methyl thio) propyl disulfide	1.09
21	1427	β-gurjunene	0.50
22	1431	γ-elemene	0.91
23	1437	(*Z*)-1-propenyl propyl trisulphide	1.04
24	1442	(*E*)-1-propenyl trisulphide	0.33
25	1449	α-humulene	1.17
26	1453	α-acoradiene	0.31
27	**1459**	**germacrene D**	**3.09**
28	1463	β-acoradiene	0.30
29	1472	γ-gurjunene	0.73
30	1479	β-selinene	1.34
31	1490	α-selinene	0.91

Continue of Table 1. Composition of the essential oil of the fruits of *Ferula assa-foetida* L. growing wild in Mount Telesm, Kermanshah, Iran

No.	RI	Compound	Percentage (Area)%
32	1494	cadina-1,4-diene	1.07
33	1499	cuparene	0.40
34	1504	β-bisabolene	1.08
35	**1509**	**γ-cadinene**	**3.36**
36	1513	methyl pentyl tetra sulphide	0.50
37	**1520**	**δ-cadinene**	**4.78**
38	1527	Calamenene	2.21
39	1533	(*E*)-γ-bisabolene	0.60
40	1542	α-cadinene	0.26
41	1547	Elemol	0.51
42	**1555**	**germacrene B**	**10.98**
43	1570	α-amorphene	0.30
44	1587	β-humulene	0.91
45	1593	longipinene epoxide	0.84
46	1596	guaiol	2.74
47	**1609**	**5-epi-7-epi-α-eudesmol**	**4.89**
48	1617	γ-eudesmol	1.08
49	**1642**	**epi-α-cadinol**	**23.15**
50	1648	β-eudesmol	1.88

RI= Retention indices on HP-5MS capillary column, calculated by using retention times of *n*-alkanes (C_8-C_{24}). Percentages calculated from TIC data.

However, plant- derived organic sulfides are subdivided structurally according to number of sulphur atoms and being linear or cyclic and bearing heteroatm [15]. In recent years, they have been assigned for various biological properties, including antioxidant, cancer chemopreventive, blood lipid reducing,having antibacterial, neuroprotective and immunomodulatory effects [15] in accordance with reported pharmacological effects of asafetida [12]. Only *Ferula* spp. contains VSC in Apiaceae, and from *Ferula* species, only essential oils obtained from *F. assa-foetida*, *F. fukanensis*, *F. latisecta*, *F. persica* and *F. sinkiangensis* contained sulfur compounds [26]. *sec*-Butyl-(*Z*)-propenyl disulfide and *sec*-butyl-(*E*)-propenyl disulfide were found to be the most prevalent sulfur-containing compounds in the essential oils of some *Ferula* species specially asafetida gum producing *Ferula* spp[26]. The former compound is the major VSC of our investigated plant, as well.

Table 2. Comparison of major volatile compounds of *Ferula assa-foetida* populations in Iran

Plant part	Time of collection	Place of collection	Major compounds (percent)	Biological evaluation	Reference
oleo-gum-resin	15th June	Larestan, Fars	(*E*)-1-propenyl *sec*-butyl disulfide (23.9%) and 10-*epi*-γ-eudesmol (15.1%)	-	[27]
oleo-gum-resin	30th June	Larestan, Fars	(*Z*)-1-propenyl *sec*-butyl disulfide (27.7%) and (*E*)-1-propenyl *sec*-butyl disulfide (20.3%)	-	[27]
oleo-gum-resin	15th July	Larestan, Fars	β-pinene (47.1%),α-pinene (21.3%)	-	[27]
N.R.*	October	Kerman	*E*-1-propenyl *sec*-butyl disulfide (40.0%), germacrene B (7.8%).	-	[32]
aerial parts	Summer	Sari	phenol, 2-methyl-5-(1-methyl ethyl) (18.2%), α-bisabolol (10.4%), arsine triethyl (8.7%)	antioxidant	[33]
oleo-gum-resin	Summer	Kerman	(*E*)-1-propenyl *sec*-butyl disulfide (58.9%), (*Z*)-β-ocimene (11.9%), (*E*)-β-ocimene (9.0%)	-	[34]
fruits	June	Kashan	trans-2-undecen-1-ol (17.26%), γ-elemene (32.21%)	-	[28]
oleo-gum-resin	August	Isfahan	(*Z*)-1-propenyl *sec*-butyl disulfide (35.1), (*E*)-1-propenyl *sec*-butyl disulfide (22.1) and α-pinene (12.2)	Antispasmodic on rat ileum	[35]
root	July	Gonabad	*E*-1-propenyl *sec*-butyl disulfide (30.7%), *Z*-1-propenyl *sec*-butyl disulfide (12.4%) eudesmol (10-epi-γ) (12.7%)	-	[36]
root	July	Tabas	*E*-1-propenyl *sec*-butyl disulfide (18.8%), *Z*-1-propenyl *sec*-butyl disulfide (9.2%), eudesmol (10-epi-γ) (18.7%)	-	[36]
aerial parts	May/June	Kerman	1-Methylpropyl (1*E*)-prop-1-en-1-yl disulfide (32.8%),α-pinene (11.3%), germacrene B (5.5%)	-	[10]

*N.R. Not reported

It has been proposed that VSC amount decreased with sample gathering in warmer months [27], so it may exemplify our sample lower sulfur content to some other investigations. On the other hand, the only other research on fruit of the plant showed no VSC as main compounds. They reported trans-2-undecen-1-ol and γ-elemene as major volatile constituents [28].

The terpenoid compounds have been almost the most abundant components of *Ferula* oils and

phenylpropanoids are rarely reported from *Ferula spp*[10,26], a fact with complete agreement with our results; Because, sesquiterpenes (81.25%) are the dominant terpenoids and monoterpenes represented only 6.14 % of the essential oil. Data are in consistence with other species of *Ferula,* but not similar even to other Apiaceaous genera [29-31]. *epi*-α-cadinol and α-pinene were found to be the most abundant components of the sesquiterpenoid and monoterpenoid fractions, respectively. However, no phenylpropanoids was identified in the essential oil of *F. assa-foetida*.

The most frequent terpenoid compounds that occurred as main components in the *Ferula* sppessential oils were α-pinene [26], and first rated non-sulphidic compound in essential oil of asafetida (table 2) were reported to be 10-epi-γ-eudesmol, β-pinene, α-pinene [27], germacrene B [32], phenol, 2-methyl-5-(1-methyl ethyl) [33], trans-2-undecen-1-ol and γ-elemene [28].

sec-Butyl disulfide and trisulfide derivatives have only been reported in the genus *Ferula*[15]. Overall, a comprehensive databank of VSCs retention indices and other useful analytical issues have been recently presented in a review by Iranshahi [15].

Conclusion

Essential oil of fruit of *F. assa-foetida* growing wild in western Iran was obtained by steam distillation solvent extraction method proved to contain fifty-four constituents in which epi-α-cadinol (23.15 %), germacrene B (10.98 %), α-gurjunene (6.18 %), (Z)-1-propenyl *sec*-butyl disulfide (5.89 %), 5-epi-7-epi-α-eudesmol (4.89 %) and δ-cadinene (4.78 %) were the major compounds.

Conflict of Interests

I certify that no actual or potential conflict of interest related to this article exists.

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