

Chemical Composition and Antioxidant Properties of *Ferula-assa-foetida* Leaves Essential Oil

HASSAN AHMADVAND, HAMZE AMIRI, ZAHRA DEGHANI ELMI, and SHAHROKH BAGHERI

For author affiliations, see end of text.

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ABSTRACT

Recent investigations have shown that plants have antioxidant properties and could decrease oxidative stress in different human diseases. The present work was identified the chemical composition compounds and evaluated the various antioxidative activities of *Ferula-assa-foetida* leaves hydroalcoholic extract (FLHE) and *Ferula-assa-foetida* leaves essential oil (FLEO). Radical scavenging activity of samples was assessed using Diphenylpicrylhydrazyl (DPPH). Total antioxidant capacity was assessed by methods phosphomolybdat. The amount of total phenol and flavonoid was assessed by Folin-Ciocalteu and Zhishen methods. Also, the components of FLEO were analyzed with gas chromatography/mass spectrometry (GC/MS). In FLHE and FLEO, total antioxidant capacity were 1.55 ± 0.13 and 1.09 ± 0.43 nmol, ascorbic acid equivalents/g, phenol content were 441.37 ± 2.50 and 76.56 ± 9.65 mg of Gallic acid equivalents (GAE)/g, and flavonoid content was 12.53 ± 3.20 and 0.015 ± 0.002 mg of quercetin equivalents/100 g respectively. In the DPPH scavenging assay, the IC_{50} values of FLHE, FLEO and Butylated hydroxytoluene (BHT) as reference were 787.13 ± 3.66 ; 2375.66 ± 5.13 and 3.88 ± 1 $\mu\text{g/mL}$ respectively. Also GC/MS data and retention indices for reference essential oil leaves samples were used to identify 12 constituents. These compounds made up a total of 85.57% of FLEO: Eremophilene; -cadinene; Longiborneol; Dehydro aromadendrene; Isoledene; -Gurjunene; J-Guaiene. This study showed that *ferula-assa-foetida* has good antioxidant properties. As it is an easily-accessible source of natural antioxidants such as Eremophilene and -cadinene, it may be suitable for use in food and pharmaceutical applications.

Keywords: Total phenols, Total flavonoids, Antioxidant activity, Radical scavenging activity, DPPH; Essential oil, Chemical composition, *Ferula-assa-foetida*

Oxidative stress is an imbalance between productions of free radicals and the body's antioxidant defense mechanisms. Lipid peroxidation in the walls of living cells is a marker of free radicals attack. The presence of peroxides, especially free radicals play a key role in the number of virulent diseases such as diabetes, heart disease, cancer, aging and other diseases [1,2]. Hence the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of such inflammatory disorders.

Ferula assa-foetida grows in Afghanistan and Kashmir. Also *ferula assa-foetida* grows wildly in the

central and southern mountains of Iran [3,4]. It is traditionally used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza [4,5]. Recent pharmacological and biological studies have also shown several activities, such as antioxidant [3], antiviral, antifungal, cancer chemopreventive [6], anti-diabetic, and antispasmodic and hypotensive from *Ferula assa-foetida* [4,7]. *Ferula-assa-foetida* has good effects as herbal drug. These effects are related to chemical composition of *Ferula-assa-foetida*. Since the various antioxidative activities of Lorestan FLHE and

FLEO and chemical composition compounds of Lorestan *ferula-assa-foetida* leaves essential oil have not previously been reported; the objective of the present study is to investigate various antioxidative activities of Lorestan FLHE and FLEO and chemical composition compounds of Lorestan FLEO.

MATERIALS AND METHODS

Isolation of the Hydroalcoholic extract and essential oil from Ferula-assa-foetida leaves

Ferula-assa-foetida were prepared in July 2012 from cultivated plant in Khoram Abad (Lorestan province, western Iran). Leaves of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade separately. The voucher specimen (no. 1209) was deposited at Herbarium of the Agriculture and Natural Resources Research Center of Lorestan Province, Khoramabad, Iran. Leaves were hydro-distilled using a Clevenger apparatus for 4 h, giving yellow oil in 0.38% yield. The oil was dried over anhydrous sodium sulfate and stored at 4°C. Also, hydroalcoholic extract of leaves at the Research Center of Lorestan University of Medical Sciences was prepared.

DPPH free radical-scavenging activity

DPPH free radical-scavenging activity of the test samples was determined according to the method of Blois (1958) [8]. In brief, 4 mL of DPPH radical solution in ethanol (1 mM) was mixed with 1 mL of *Ferula-assa-foetida* extract or essential oil solution in ethanol containing 0.01–1000 µg/mL of FLHE or FLEO; and after 30 min, the absorbance was measured at 517 nm. This activity was given as percentage DPPH scavenging that is calculated as %DPPH scavenging = [(control absorbance - FLHE or FLEO absorbance) / (control absorbance)] × 100. The 50% inhibition concentration (IC₅₀), i.e. the concentration of FLHE or FLEO that was required to scavenge 50% of radicals, was calculated [8].

Total antioxidant activity

Total antioxidant activity of the test samples was determined according to a previously-described procedure [9]. In brief, 0.3 mL of sample was mixed with 3.0 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695 nm. The total antioxidant activity was expressed as the number of equivalents of ascorbic acid (µmol g⁻¹).

Total phenolic content measurement

Total phenols content in the obtained extracts was determined using the Folin-Ciocalteu's phenol reagent, according to a previously-described procedure [10], with some modifications. Briefly, 200 µL of the extract

solution was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water). After 3 min, 1.5 mL sodium bicarbonate solution (60 g L⁻¹) was added to the mixture. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytic Jena 200-2004 spectrophotometer). Gallic acid was used for constructing the standard curve (1–3500 µg/mL). The results were expressed as mg of Gallic acid equivalents (GAE)/g of extract.

Determination of total flavonoids

Total flavonoid contents were determined using the method of Ordon ez et al. (2006) [11]. A volume of 0.5 ml of 2% AlCl₃ ethanol solution was added to 0.5 mL of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Quercetin was used for constructing the standard curve (1–50 µg/mL). The results were expressed as mg of quercetin equivalents /g of extract [11].

Gas chromatography/ mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane. 25 m, 0.25 mm i.d., ratio, 1:25, and flame ionization detector. Temperature programme: 60°C (2 min) rising to 240°C at 4°C/min: injector temperature 250°C, detector temperature, 260°C. GC-MS was performed using a Hewlett-Packard 6859 with a quadrupole detector, on a HP-5 column, operating at 70 eV ionization energy, using the same temperature programme and carrier gas as above. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic to Van Den Dool method.

Identification of components

The linear retention indices for all the compounds were determined by conjection of the sample with a solution containing the homologous series of C8-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

Statistical analysis

The data were presented as mean ± SD of three experiments performed in duplicate. These parameters were obtained using the student's *t*-test (using SPSS 13.0 statistical software) for independent data and the differences were considered significant when *p* < 0.05.

RESULTS AND DISCUSSION

The polyphenol compounds exhibit a wide variety of beneficial activities in mammals including anti-diabetic [12], anti-bacterial [13], immune stimulating [14], anti-allergic [15], anti-hypertensive [16], anti-ischemic,

Table 1. The IC₅₀ (the concentration required to scavenge 50% of radical) values of *Ferula-assa-foetida* leaves hydroalcoholic extract *ferula-assa-foetida* leaves hydroalcoholic extract (FLHE), *ferula-assa-foetida* leaves essential oil (FLEO) and BHT. Each point represents the mean of five experiments

Parameter	Mean	S.D.
FLHE (µg/ml)	787.13*	3.66
FLEO	2375.66	5.13
BHT (µg/ml)	3.88*	1.00

**p* < 0.05 as compared with FLEO

antiarrhythmic [17], anti-thrombotic [18], hypocholesteromic, hepatoprotective [19], and anti-inflammatory [20], anticarcinogenic [21,22]. Flavonoids are an important group of polyphenols with antioxidant, anti-viral, anti-bacterial [23] and anti-inflammatory activities [20,24]. The present investigations have demonstrated a strong correlation between antioxidant activities and chemical composition content of *ferula-assa-foetida* leaves. A stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) has widely been used in the assessment of radical scavenging activity of plant extracts, natural compounds and foods [25].

DPPH radical scavenging capacity

The antioxidant activity of *ferula-assa-foetida* leaves was evaluated by the DPPH radical scavenging capacity. Table 1 shows the percentage of DPPH radicals scavenging capacity FLHE, FLEO and BHT as reference. In the DPPH scavenging assay, the IC₅₀ (the concentration required to scavenge 50% of radical) values of FLHE, FLEO and BHT as reference were 787.13 ± 3.66; 2375.66 ± 5.13, 3.88 ± 1 µg/ml, respectively. The data obtained show that *ferula-assa-foetida* leaves is free radical scavenger and may act as primary antioxidant, which can react with free radicals by donating hydrogen. Nabavi et al. (2011) reported the IC₅₀ values of *ferula-assa-foetida* leaves ethanol/water

(70:30; V/V) extract prepared from cultivated in Gadouk area (central Elburz, Iran) was 1925 ± 8.6 µg/ml [3]. Also Zia-UL-Haq et al. (2012) reported the IC₅₀ values of *ferula-assa-foetida* leaves aqueous methanolic mixture (80:20; V/V) extract prepared from Pakistan was 99.36 ± 0.28 µg/ml [26].

Our findings shows that the anti-oxidant properties of *ferula-assa-foetida* leaves ethanol/water (50:50; V/V) extract are higher than those of *ferula-assa-foetida* leaves ethanol/water (70:30; V/V) extract and less than those of *ferula-assa-foetida* leaves aqueous methanolic mixture (80:20; V/V) extract. Researches showed the presence of different flavonoides, phenolic compound, Eremophilene and -cadinene in *ferula-assa-foetida* leaves [27]. These compounds may be the main cause of its considerable radical-scavenging activity.

Researchers are recently get interested in investigation and research into extraction of natural antioxidants such as coenzyme Q10, rosmarinic acid, tannins and flavonoids, from medical herbs to replace synthetic antioxidants [2,28]. Natural antioxidants are safer and more beneficial and have fewer side effects than synthetic antioxidants [29]. Phytochemical with antioxidant effects include some cinnamic acids, oleuropein, coumarins, falvonoids, eremophilene and tannins [30,31]. Therefore, the herbs which have high amount of these compounds are taken into consideration in order to inhibit diseases related to oxidative stress such as coronary heart disease and diabetes [32]. Conducting research on herbal antioxidants and evaluating and comparing their antioxidant effects, as well as newer and more valuable sources of natural antioxidants can be found and used in food and pharmaceutical applications.

Total antioxidant activity

The phosphomolybdenum method has been widely used in the assessment of total antioxidant activity of plant extracts, natural compounds and foods. The total antioxidant activity of FLHE and FLEO were 1.55±0.13 and 1.09±0.43 nmol of ascorbic acid equivalents/g respectively (Table 2). The difference in the amount of antioxidant of extracts may be attributed to the

Table 2. The total antioxidant activity, total phenols content and total flavonoids content of *ferula-assa-foetida* leaves hydroalcoholic extract (FLHE) and *ferula-assa-foetida* leaves essential oil (FLEO). Abbreviations as in Table 1. Each point represents the mean of five experiments

Parameter	FLHE	FLEO
Total antioxidant activity (nmol ascorbic acid equivalents/g FLHE or FLEO)	1.55 ± 0.13*	1.09 ± 0.43
Total phenols (mg of gallic acid equivalents (GAE)/g FLHE or FLEO)	441.37 ± 12.03*	76.56 ± 9.65
Total flavonoids (mg of of quercetin equivalents/100g FLHE or FLEO)	12.53 ± 3.20*	0.015 ± 0.002

**p* < 0.05 as compared with FLEO

Table 3. Chemical composition of the *Ferula-assa-foetida* leaves essential oil (FLEO)

Compound	Retention Time (min)	Yield %
Eremophilene	39.35	31.28
δ-cadinene	37.67	22.05
Longiborneol	33.90	12.09
Dehydro aromadendrene	39.70	3.99
Isoledene	36.43	3.98
τ-Gurjunene	35.83	3.93
J-Guaiene	38.11	3.53
-pinene	6.08	1.47
Ledenoxid	34.77	1.04
Trans-caryophyllene	22.70	0.98
-Gurjunene	37.98	0.95
2-J-pinene	7.39	0.23

differences in the amount and kind of existing antioxidant compounds.

Total phenols

The total phenol of FLHE and FLEO were 441.37 ± 12.03 and 76.56 ± 9.65 of Gallic acid equivalents/g respectively (Table 2). Total phenol shown by the FLHE or FLEO may be due to the presence of different phenolic compounds. Nabavi et al. (2011) reported the total phenol of *ferula-assa-foetida* leaves ethanol/water (70/30) extract prepared from cultivated in Gadouk area (central Elburz, Iran) was 49.4 ± 2.1 of Gallic acid equivalents/g extract [3]. Also Zia-UL-Haq et al. (2012) reported the total phenol of *ferula-assa-foetida* leaves aqueous methanolic mixture (central: 20; V/V) extract

prepared from Pakistan was 437.67 ± 1.44 of Gallic acid equivalents/g extract [26]. Our findings on the total phenol of *ferula-assa-foetida* leaves ethanol/water (50:50; V/V) extract was higher than that of *ferula-assa-foetida* leaves ethanol/water (70:30; V/V) extract and less than that of *ferula-assa-foetida* leaves aqueous methanolic mixture (80:20; V/V) extract. Our recent results indicated that *ferula-assa-foetida* leaves extract possess a good antioxidant activity.

Total flavonoids

The total flavonoid of FLHE and FLEO were 12.53 ± 3.2 and 0.015 ± 0.002 mg of quercetin equivalents/g respectively (Table 2). Total flavonoid shown by the FLHE or FLEO may be due to the presence of diferent

Table 4. Bioactive and fragrance components percentage of some *Ferula* species essential oils

Plant Name [Ref.]	Major Components (%)
<i>F. szowitsiana</i> leaf [19]	-Eudesmol (32.0), -Eudesmol (18.2), -Pinene (8.6)
<i>F. arrijonii</i> leaf [28]	-Phellandrene (16.6), Germacra-1 (10),5-dien,11-ol- (15.4)
<i>F. communis</i> leaf [29]	Myrcene (53.5), Aristolene (8.5)
<i>ferula-assa-foetida</i> leaves [our study]	Eremophilene (31.28%) and -cadinene (22.05)

flavonoides such as catechin, epicatechin and myricetin. Nabavi et al. (2011) reported the total flavonoid of *ferula-assa-foetida* leaves ethanol/water (70:30; V/V) extract prepared from cultivated in Gadouk area (central Elburz, Iran) was 20.9 ± 1.4 mg of quercetin equivalents/g extract [3]. Also, Zia-UL-Haq et al. (2012) reported the total flavonoid of *ferula-assa-foetida* leaves aqueous methanolic mixture (80:20; V/V) extract prepared from Pakistan was 416.33 ± 1.61 mg of quercetin equivalents/g extract [26]. Our findings on the total flavonoid of *ferula-assa-foetida* leaves ethanol/water (50:50; V/V) extract are less than that of *ferula-assa-foetida* leaves ethanol/water (70:30) extracts and *ferula-assa-foetida* leaves aqueous methanolic mixture (80:20; V/V) extract. Our recent results indicated that FLHE or FLEO possess a good antioxidant activity.

Chemical composition of *ferula-assa-foetida* leaves essential oil

The yield of the essential oils obtained from leaves was 0.38% (W/W) respectively. Results of the GC-MS analysis of the oils are shown in Table 3. Twelve compounds of FLEO were identified (85.57% of the total oils respectively).

The main constituents found in the *Ferula-assa-foetida* leaves were Eremophilene (31.28%); -cadinene (22.05%); Longiborneol (12.09%); Dehydro aromadendrene (3.99%); Isolatedene (3.98%); -Gurjunene (3.93%); J-Guaiene (3.53%); -pinene (1.47%); Ledenoxid (1.04%); Trans-caryophyllene (0.98%); -Gurjunene (0.95%); 2-J-pinene (0.23%).

Kose et al. (2010) reported, the main constituents found in the *Ferula-assa-foetida* aerial parts prepared from Turkey were -Pinene (59.89%); -Pinene (13.48%); Limonene (3.21%) and Bornyl acetate (2.10%) [33]. Also, another study reported, the main constituents found in the *Ferula-assa-foetida* aerial parts prepared from Sari forest (mazandaran, Iran) were Phenol, 2-methyl-5-(1-methylethyl) (18.2%); Bisabolol (10.4%); Fenchyl acetate (4.7%); γ -Gurjunene (2.3%) and -Pinene (0.6%) [27]. Also, according to a similar experiment on the essential oil from of *F. assa-foetida* leaves in the province of Kerman, south of Iran, in August 2005 [34], the major components were reported as *E*-1-Propenyl sec-butyl disulfide (40.0%) and Germacrene B (7.8%), while in our work, Eremophilene (31.28%) and -cadinene (22.05%) are presented as major components in oils from leaves in Lorestan species, respectively. As can be seen, the chemical composition of *F. assa-foetida* in our results is different from the other one. These differences between the oil compositions of one species of *Ferula* from two different areas can be related to climatological factors (from south to central Iran) and is discussed in phytological studies. Previous studies on essential oils of members of *Ferula* genus showed various compositions. Table 4 shows the diversity of major components among oils of some *Ferula* species which have been analyzed before [34-37]. -Eudesmol (32.0),

-Eudesmol (18.2), -Pinene (8.6) in *F. szowitsiana* leaves [35], -Phellandrene (16.6), Germacrene-1(10),5-dien,11-ol- (15.4) in *F. arrijonii* [36] and Myrcene (53.5), Aristolene (8.5) in *F. communis* [37] oils are the major components.

The results of this study indicate that the composition of volatile oil of *Ferula-assa-foetida* leaves is not similar to those which are reported in other studies. The observed differences may be probably due to using of different parts of plant for analysis, different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition.

In conclusion, This study showed that essential oil of *ferula-assa-foetida* leaf is an easily-accessible source of natural antioxidants such as eremophilene, -cadinene and longiborneol and it may be suitable for use in food and pharmaceutical applications and a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical-associated health problems.

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CURRENT AUTHOR ADDRESSES

Hassan Ahmadvand, Razi Herbal Medicine Research Center; Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khoramabad, Iran. Email: hassan_a46@yahoo.com (Corresponding author)

Hamze Amiri, Department of Bology, Faculty of Basic Science, Lorestan University, Khoramabad, Iran.

Zahra Dehghani Elmi4, 4Department of Biology, Islamic Azad University, Brojerd Branch, Brojerd, Iran.

Shahrokh Bagheri, Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khoramabad, Iran.