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Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition

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RESUMEN

Actividad antioxidante de extractos metanólicos de *Ferula assafoetida* y la composición de su aceite esencial.

La actividad antioxidante de las partes aéreas de *Ferula assafoetida* se determinó empleando varios sistemas de ensayos *in vitro*. El IC_{50} de la actividad captadora de radicales de DPPH fue 380 ± 12 mg ml^{-1}. Los extractos también mostraron una buena actividad captadora de óxido nítrico (IC_{50} fue 270 ± 3) y capacidad quelatante de Fe^{2+} (IC_{50} fue 0.57 ± 0.02 mg ml^{-1}). La inhibición de la peroxidación (actividad antioxidante) de los extractos mostró valores del 82% (a las 24 horas) y 88% (a las 72 horas). El extracto mostró un ligero descenso del poder reductor con 25- 800 μg ml^{-1} de extracto que no fue comparable con la vitamina C ($p < 0.001$). Los extractos ensayados mostraron una muy baja actividad antioxidante. Además, la composición química del aceite esencial de las partes aéreas fue determinada. Los principales compuestos fueron fenol, 2-metil-5-(1-metil etilo) (18.2 %), α.-Bisabolol (10.4%) and Arsine trietilo (8.7 %). Los fenoles totales, determinados por el método de Folin-Ciocalteau, fue de 94.8 ± 5.9 mg de equivalentes de ácido gálico/g de extracto en polvo y el contenido total de flavonoides (por el método del $AlCl_3$) fue de 90.9 ± 6.3 mg de equivalentes de quercitina/g de extracto en polvo.

PALABRAS CLAVE: Aceite esencial – Actividad antioxidante – Capacidad quelatante – DPPH – Ferula assafoetida.

SUMMARY

Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition.

The antioxidant activity of the aerial parts of *Ferula assafoetida* was determined by employing various in vitro assay systems. IC_{50} for DPPH radical-scavenging activity was 380 ± 12 mg ml^{-1}. The extracts showed good nitric oxide-scavenging activity (IC_{50} was 270 ± 3) and Fe^{2+} chelating ability (IC_{50} was 0.57 ± 0.02 mg ml^{-1}). The peroxidation inhibition (antioxidant activity) of the extracts exhibited values from 82% (at 24 hrs) and 88% (at 72 hrs). The extract exhibited a fairy weak reducing power at 25-800 μg ml^{-1} of extracts which was not comparable with Vitamin C ($p < 0.001$). The tested extracts exhibited very low antioxidant activity. In addition, the chemical composition of the essential oil of the aerial parts was determined. The major compounds were phenol, 2-methyl-5-(1-methyl ethyl) (18.2 %), α.-Bisabolol (10.4%) and Arsine triethyl (8.7 %). Total phenol compounds, as determined by the Folin Ciocalteu method, were 94.8 ± 5.9 mg gallic acid equivalent/g of extract powder and the total flavonoid content (by $AlCl_3$ method) was 90.9 ± 6.3 mg quercetin equivalent/g of extract powder.

KEY-WORDS: Antioxidant activity – Chelating ability – DPPH – Essential oil – Ferula assafoetida.

1. INTRODUCTION

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell *et al*. 1992). The harmful action of free radicals can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Cao *et al*. 1996; Wang *et al*. 1996; Zheng and Wang 2001). The search for newer natural antioxidants, especially of plant origin, has been increasing ever since. Plants have been a constant source of drugs and recently, much emphasis has been placed on finding new therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs.

The Ferula genus from the family of Umbelliferae has been found to be a rich source of gum-resin (Fernch 1971). This resin enjoys a reputation as a folklore medicine (Abd El-Razek *et al*. 2001). It is considered to be a sedative, carminative, antispasmodic digestive, expectorant, laxative, analgesic, anthelminitic, antiseptic and a diuretic in its properties. It is also believed to have aphrodisiac properties and increase sexual appetite (Eigner and Scholz 1990). This genus presents interesting phytochemical features, such as the occurrence of sesquiterpenes and sesquiterpene coumarins (Abd El-Razek *et al*. 2001; Kojima *et al*.

2000; Su *et al*. 2000). *F. assafoetida* L. is an herbaceous wild plant native to Iran. It is herbaceous and perennial and grows up to 2 m high. In Iranian traditional medicine, *F. assafoetida* gum extract has been used as a remedy for abdominal pain, constipation and diarrhea and as an antihelminthic (Fatehi *et al*. 2004). Although there is some evidence for the anticoagulant action (Leung 1980), antispasmodic and hypotensive effects of *F. assafoetida* gum (Fatehi *et al*. 2004) and pregnancy interceptive activity (Keshri *et al*. 2004). The essential oil composition of this plant has been published recently (Khajeh *et al*. 2005). In this study, the essential oil composition and antioxidant activity of the *Ferula assafoetida* aerial parts were examined by employing various in vitro assay systems, such as DPPH and nitric oxide radical scavenging, reducing power, linoleic acid and iron ion chelating power, in order to understand the usefulness of this plant.

2. MATERIALS AND METHODS

2.1. Plant materials

The aerial parts of *Ferula assafoetida* were collected from Sari forest, Iran, in the summer of 2007. After identification of the plant by Dr. Bahman Eslami, a voucher (No. 340) has been deposited in the Sari School of Pharmacy herbarium.

2.2. Chemicals

Ferrozine, Linoleic acid, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA) and Ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or more pure.

2.3. Isolation of the essential oil

The air-dried and ground aerial parts of the plant collected were submitted for 3 h to water-distillation using a British-type Clevenger apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at 4°C until tested and analyzed.

2.4. Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS was carried out using a Hewlett-Packard 5975B series instrument and an Agilent 19091J-433 HP-5 capillary column (30 m., 250 μm i.d., film thickness 0.25μm) which was set at 50°C for 10 min, then increased 4°C/min to 300°C; using helium as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was *m/z* 40–400. The compounds were identified according to their retention indexes and by comparison of their mass spectra with those of a computer library or with authentic compounds. a-pinene, Decane, Benzene and Limonene were identified by co-injection.

2.5. Preparation of methanolic extract

The aerial parts were dried at room temperature and coarsely ground before extraction. A known amount of each part was extracted at room temperature following the percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained (10.8 %), which was then freeze-dried for complete solvent removal.

2.6. Determination of total phenolic compounds and flavonoid content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ebrahimzadeh *et al.* 2008a,b). The extract sample (0.5 ml) was mixed with 2.5 ml of a 0.2 N Folin-Ciocalteau reagent for 5 min and then 2.0 ml of 75 g l^{-1} sodium carbonate were added. The absorbance of the reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoids were estimated using the method of Ordonez *et al.* (Ebrahimzadeh *et al.* 2008a,b). Briefly, 0.5 mL solution of plant extract in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). The total flavonoid contents were calculated as quercetin from a calibration curve.

2.7. DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of the free radical-scavenging activity of the extracts (Ebrahimzadeh *et al.* 2008a,b; Nabavi *et al*. 2008a,b). Different concentrations of extract were added, at an equal volume, to a methanolic solution of DPPH (100 mM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

2.8. Reducing power determination

The reducing power of *F. assafoetida* was determined according to the method of Yen and Chen (Ebrahimzadeh *et al*. 2008a,b; Nabavi *et al*. 2008a,b). 2.5 ml of extract (25-800 $mgml^{-1}$) in water were mixed with a phosphate buffer (2.5 ml, 0.2M, pH6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a positive control.

2.9. Assay of nitric oxide-scavenging activity

For the experiment, sodium nitroprusside (10 mM), in a phosphate-buffered saline, was mixed with different concentrations of each extract dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without extract but with an equivalent amount of water, served as the control. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as a positive control (Ebrahimzadeh *et al*. 2008a,b; Nabavi *et al*. 2008a).

2.10. Metal chelating activity

The chelating of ferrous ions by *F. assafoetida* was estimated according to the method of Dinis *et al*., (Ebrahimzadeh *et al*. 2008c). Briefly, the extract (0.2-3.2 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as $[(A_0-A_s)/A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/standard. Na_2EDTA was used as a positive control.

2.11. Determination of Antioxidant Activity by the FTC Method

This method was adopted from Osawa and Namiki (Ebrahimzadeh *et al.* 2008 a,b; Nabavi *et al.* 2008a). Twenty mg/mL of sample dissolved in 4 mL of 95% (w/v) ethanol were mixed with linoleic acid (2.51%, v/v) in 99.5% (w/v) ethanol (4.1 mL), 0.05 M phosphate buffer pH 7.0 (8 mL) and distilled water (3.9 mL) and kept in screwcap containers at 40°C in the dark. 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate were then added to 0.1 mL of this solution. Precisely 3 min after the addition of 0.1 mL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured; and it was measured again every 24 h until the day when the absorbance of the control reached its maximum value. The percent inhibition of linoleic acid peroxidation was calculated as: (%) inhibition = 100 - [(absorbance increase of the sample/absorbance increase of the control) $\times$ 100]. All tests were run in duplicate and analyses of all samples were run in triplicate and averaged. Vit C and BHA were used as positive controls.

2.12. Statistical analysis

The experimental results are expressed as means $\pm$ SD. All measurements were taken in triplicicate. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range tests. The EC_{50} values were calculated from a linear regression analysis.

3. RESULTS

3.1. Chemical composition of the essential oil

The results obtained by the GC-MS analysis of the essential oil of the *F. assafoetida* aerial parts are presented in Table 1. Sixty one compounds were identified, representing 98.8% of the total oil. The oil yield of the plant was determined as 0.94% v/w. As determined from the GC-MS analysis, the major compounds were Phenol, 2-methyl-5-(1-methyl ethyl) (18.2 %). Additionally, other major compounds were α.-Bisabolol (10.4%), Arsine triethyl (8.7%) and Cyclopropa [a] naphthalene-octahydro-tetramethyl (6.6%).

3.2. Total phenol and flavonoid contents

The total phenolic content was 94.8 $\pm$ 5.9 mg gallic acid equivalent/g of extract powder in reference to the standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total flavonoid content was 90.9 $\pm$ 6.3 mg quercetin equivalent/g of extract powder, in reference to the standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$).

3.3. DPPH radical-scavenging activity

IC_{50} for DPPH radical-scavenging activity was 380 $\pm$ 12 mg ml^{-1}. The IC_{50} values for Ascorbic acid, quercetin and BHA were 1.26 $\pm$ 0.11, 1.32 $\pm$ 0.07 and 13.49 $\pm$ 1.04 mg ml^{-1}, respectively.

3.4. Reducing power of extract

Figure 1 shows the dose– response curves for the reducing powers of the extracts from *F.*

Table 1
Chemical composition of the essential oil of *F. assafoetida* aeial parts

No.	K. I	Components	Composition (%)
1	1113	α-Pinene	0.6
2	1175	2-(Bromomethyl)trimethylcyclohexene	1.7
3	1177	trimethyl-4-methylidene-cyclohexene	0.2
4	1179	Bicyclo[3.1.1]heptane	0.3
5	1195	β-Myrcene	0.6
6	1201	Decane	0.6
7	1221	Benzene	0.3
8	1225	dl-Limonene	0.8
9	1235	Octatriene, 3,7-dimethyl-E,	0.9
10	1243	Octatriene, 3,7-dimethyl-,Z	0.4
11	1289	Undecane	0.4
12	1322	Alloocimene	0.2
13	1379	Arsine triethyl	8.7
14	1382	Trans propenyl s-butyl disulfide	0.7
15	1465	Fenchyl acetate	4.7
16	1640	Phenol, 5-methyl-2-(1-methylethyl)	2.0
17	1663	Phenol, 2-methyl-5-(1-methylethyl)	18.2
18	1682	Cyclohexene,methyl-3-(methylethenyl)	0.4
19	1708	(+)-2-Carene	0.6
20	1730	Carvacryl acetate	0.2
21	1746	b-Elemene	0.3
22	1758	Benzene, 1,2-dimethoxy-4-(2-propenyl)	0.2
23	1775	trans-Caryophyllene 0.3	
24	1778	Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	0.1
25	1782	10-Oxatetracyclotetradecane-9,11,14-trione 4-[(2-methoxyethoxy)methoxy]-5-methyl	0.2
26	1788	β.-Elemene	0.3
27	1799	β-Selinene	0.8
28	1802	Camphene	1.2
29	1806	1H-Cyclopropa[a]naphthalene, decahydro-trimethyl-7-methylene-	0.3
30	1811	Dodecatriene-dimethyl-3-methylene-	0.9
31	1821	(+)-Epi-bicyclosesquiphellandrene	0.4
32	1828	Cyclohexane, methylene	0.3
33	1840	g.-curcumene	0.3
34	1847	Spiro[5.5]undec-2-ene,trimethyl-methylene-	1.9
35	1850	b-Selinene	0.4
36	1856	Lepidozene	0.7
37	1859	Valencene	0.7
38	1969	Albicanol	2.5
39	1876	b.-Bisabolene	1.3
40	1884	Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	0.4
41	1897	Benzodioxole, methoxy-6-(propenyl) 4.4	
42	1914	Trans-.γ.- bisabolene	2.3
43	1925	Cis-α.- bisabolene	1.5
44	1933	Spirobi(4-phenyl-3H-oxathiazoline	0.8
45	1938	Elemol	0.4
46	1942	Benzene, trimethoxy-5-(2-propenyl)	0.5
47	1949	Methano-1-benzoxepin, octahydro tetramethyl	0.6
48	1956	Dodecatrien-3-ol, trimethyl	0.5
49	1989	(2,6-Dimethyl-hepta-1,5-dienyl) -trimethyl-bicyclo[4.2.0]oct- 2-ene	0.4
50	2021	Guaiol	1.7
51	2038	2-naphthalenemethanol -octahydro-. tetramethyl-	1.3
52	2072	Cyclopropa[a]naphthalene-octahydro-tetramethyl-	6.6
53	2087	Selina-4(19),11-DIENE	0.6
54	2101	Naphthalene,octahydro-dimethyl-7-(1-methylethenyl)	1.6
55	2123	Neoisolongifolene	2.2
56	2136	2-Naphthalenemethanol, octahydro-8-tetramethyl	0.7
57	2145	Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,	2.4
58	2157	Naphthalene, octahydro-4a,8-dimethyl-2-(1-methylethylidene)	0.6
59	2206	γ.-Gurjunene	2.3
60	2215	Azulene, octahydr1,4-dimethyl-7-(1-methylethenyl)	3.0
61	2242	α.-Bisabolol	10.4
total			98.8

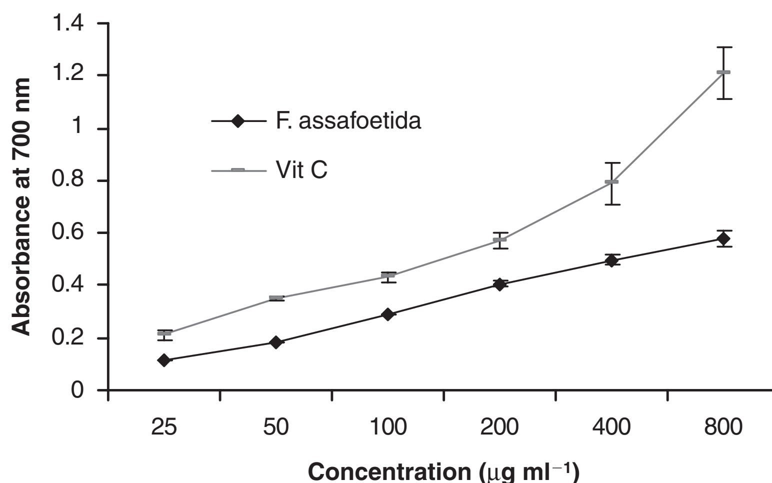

Figure 1
Reducing power of *F. assafoetida*

assafoetida. It was found that the reducing powers of the extracts also increased with anincrease in their concentrations. The extract exhibited a fairy weak reducing power at 25 and 800 μg ml^{-1} which was not comparable with Vit C ($p < 0.001$).

3.5. Assay of nitric oxide-scavenging activity

The extracts also showed weak nitric oxide-scavenging activity between 0.1 and 800 mg ml^{-1}. The % inhibition increased with increasing concentration of the extract. IC_{50} was 270 $\pm$ 3 for *F. assafoetida* vs. 17.01 $\pm$ 0.03 mg ml^{-1} for quercetin.

3.6. Fe^{2+} chelating activity of extract

The absorbance of Fe^{2+} -ferrozine complex decreased dose-dependently, i.e. the activity increased with increasing concentration from 0.2 to 3.2 mg ml^{-1}. *F. assafoetida* extract showed good Fe^{2+} chelating ability. IC_{50} was 0.57 $\pm$ 0.02 mg ml^{-1}. EDTA showed very strong activity (IC_{50} = 18 mg ml^{-1}).

3.7. FTC Method

Figure 2 shows the time-course plots for the antioxidative activity of the *F. assafoetida* extract using the FTC method. The peroxidation inhibition (antioxidant activity) of the extract exhibited values from 82% (at 24th hrs) and 88% (at 72nd hrs). The extracts tested exhibited very low antioxidant activity. There were significant differences between the extracts and controls ($p < 0.01$).

4. DISCUSSION

Sixty one compounds were identified in the present study, representing 98.8% of the total oil. Only 25 compounds have been a identified in recently published paper (Khajeh *et al.* 2005). E-1-propenyl sec-butyl disulfide was the major component of the plants in that report (40.0%) but this component was only a minor one in our study (0.7%). Phenol, 2-methyl-5-(1-methyl ethyl) with 18.2 % and α-Bisabolol with 10.4% were the major compounds. Maybe local climate and/ or the increase in temperature to 300°C (instead of 250°C in the Khajeh report) have played a major role in the number of identified components.

Total phenol compound, as determined by the Folin Ciocalteau method, was reported as gallic acid equivalents and total flavonoid content was reported as the quercetin equivalent/g of extract powder by AlCl3 colorimetric method. This plant showed high total phenol and flavonoid contents. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (van Acker *et al*. 1996). Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (Hertog *et al*. 1993).

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al*. 2003). DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams *et al*. 1995). It was found that the radical- scavenging activity of the extracts increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity was 380 $\pm$ 12 mg ml^{-1}. The high total phenol and flavonoid contents of this plant may lead to its good DPPH-scavenging activity.

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important

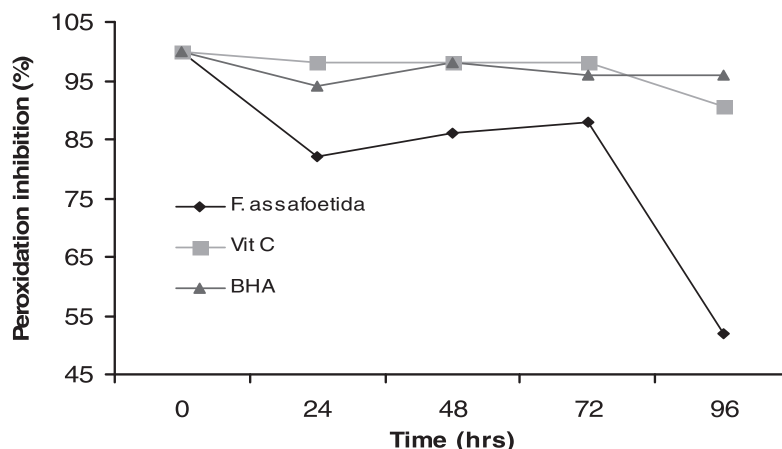

Figure 2
Antioxidant activity of *F. assafoetida* in FTC method at different incubation times. Plant methanolic extract (0.4 mg/ml), Vit C and BHA (0.1 mg/ml).

mechanism of phenolic antioxidant action (Yildirim *et al*. 2001). In this assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability.

Figure 1 shows the dose– response curves for the reducing powers of the extracts from *F. assafoetida*. The extracts exhibited a fairy weak reducing power at 25 and 800 $\mu g\ ml^{-1}$ which was not comparable with Vit C ($p < 0.001$). It was evident that *F. assafoetida* did not show reductive potential and could not serve as electron donors for terminating the radical chain reaction.

The extracts also showed weak nitric oxide-scavenging activity between 0.1 and 800 mg ml^{-1}. The procedure is based on the principle that sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The % inhibition increased with an increasing concentration of the extract. IC_{50} was 270 $\pm$ 3 for *F. assafoetida* vs. 17.01 $\pm$ 0.03 mg ml^{-1} for quercetin. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Moncada *et al*. 1991). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in humans and thereby improves quality of life and overall survival of some diseases such as Thalassemia major (Hebbel *et al*. 1990). In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's disease (AD) pathology and so iron chelation could be considered a rational therapeutic strategy for AD (Reznichenko *et al*. 2006). Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts in oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell 1997). These processes can be delayed by iron chelation and deactivation. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (Ebrahimzadeh *et al*. 2008c; Nabavi *et al*. 2008b). Because Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by the method of Dinis *et al*., (Ebrahimzadeh *et al*. 2008 a,b,c; Nabavi *et al*. 2008a). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The absorbance of Fe^{2+}- ferrozine complex decreased dose-dependently, i.e. the activity increased with the increasing concentration from 0.2 to 3.2 mg ml^{-1}. Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al*. 1999). It was reported that chelating agents are effective as secondary

antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon 1990). *F. assafoetida* extract showed good Fe^{2+} chelating ability. IC_{50} was 0.57 ± 0.02 mg ml^{-1}.

Figure 2 shows the time-course plots for the antioxidative activity of the *F. assafoetida* extract using the FTC method. Membrane lipids are rich in unsaturated fatty acids which are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu 2001). The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Nabavi *et al*. 2008a). Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The tested extracts exhibited very low antioxidant activity. There were significant differences between extracts and controls ($p < 0.01$).

Be complete but concise in discussing findings, comparing results with previous work and proposing explanations for the results observed. Avoid speculation unsupported by the data obtained. Concluding statements may be either incorporated at the end of this section or under a separate Conclusions section. Citations should be according to the described above.

5. CONCLUSIONS

The aerial parts of the extract of *F. assafoetida* exhibited good but different levels of antioxidant activity in all the models studied. The extracts had good Fe^{2+} chelating ability, DPPH radical and nitric oxide scavenging activity. Further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is needed.

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