Journal of Medicinal Plants Research Vol. 6(16), pp. 3254-3258, 30 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.141
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antioxidant potential of various parts of *Ferula assafoetida* L.

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Accepted 14 February, 2012

***Ferula assafoetida* Linn, (*Apiaceae*) is used in herbal medicine globally for cure and prevention of many diseases. Current research has been carried out to investigate antioxidant potential of this plant. Results indicated that sufficient amount of total phenolic contents and total flavonoid contents are present in its leaves, flowers and seeds. Some top-benched bioassays like 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity and superoxide radical scavenging activity confirmed the radical scavenging and antioxidant potential of this plant. Since *F. assafoetida* hold immense therapeutic potential besides food uses, its usage in dietary supplementation can be exploited largely as a functional food.**

Key words: *Ferula assafoetida*, total phenolic contents, antioxidant potential, Pakistan.

INTRODUCTION

Plant kingdom is a consecration and boon from nature to humans living on this planet. For centuries herbal potions have been concocted with the goal of preventing, and or curing various ailments. Pakistan is considered a treasure house of many exotic medicinal plant species many of which are unique in extent, composition and endemism. *Ferula assafoetida* (*Apiaceae*) is a native plant of Pakistan found in Quetta and nearby areas like Hazarganji where it grows wild. Commonly known as “hing”, this plant is well- recognized for their medicinal and nutritional attributes. Leaves of plant are used as anthelmintic, carminative and diaphoretic. Stem is used as brain and liver tonic, root as antipyretic (Chatterjee and Pakrashi, 1995). Its oleo-gum resin is believed to possess sedative, expectorant, analgesic, carminative, stimulant, antiperiodic, antidiabetic, antispasmodic, emmenagogue, vermifuge, laxative, anti-inflammatory, contraceptive and antiepileptic properties (Chevallier, 1996; Chiej, 1984; Duke, 1985; Garg et al., 1980). It is also traditionally used to treat many women diseases as it’s believed to increase secretion of progesterone hormone. The resin is used as opium antidote and also used to treat ear diseases after heating with ginger oil.

Its paste along with lemon juice when applied to aching teeth, gives immediate relief. Aqueous paste is applied on chest during whooping cough for relief and also used to treat skin diseases and scorpion bites. It is popular in natural food cuisine as a garlic substitute. It is used to make meat tender and also preserve it. The resin is added to add aroma and flavor in meatballs, curries, dishes, dal, sauces, pickles, vegetarian soups, many fish dishes and some pappadums (Abdel-razek, 2001). Plant roots, young shoots and leaves are used as vegetable (Grieve, 1984). The cabbage-like folded heads are eaten raw as a delicacy. A starch extracted from the roots is used to make porridge (Komarov, 1968). Despite its multipurpose and wide uses no study exists on antioxidant potential of its leaves, seeds and flowers. So the current study has been designed to investigate antioxidant capacity of these parts of this plant as part of

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our series of studies on flora of Pakistan (Zia-Ul-Haq et al., 2007a, b; 2008 a, b; 2009, 2010, 2011 a, b, c, d and e; 2012).

MATERIALS AND METHODS

The plant material (*F. assafoetida* seeds, leaves, and flowers) was air-dried in shade and crushed to coarse powder separately with the help of pestle and mortar. Plant material (0.5 kg each) was macerated with aqueous methanolic mixture (80:20; v/v) 1 L, at room temperature for fifteen days with occasional shaking. The process was repeated for three times with same quantity of solvent mixture. The extracts so obrtained, were then filtered through filter paper under vacuum and concentrated under reduced pressure in a rotary evaporator (model Q-344B – Quimis, Brazil) using a warm water bath (model Q-214M2 - Quimis, Brazil) to obtain a thick gummy mass, which was further dried in a dessicator and stored in air- tight vial till further use.

Chemicals

Methanol, catechin, gallic acid, quercitin, folin and ciocalteau's reagent, ferric chloride, DPPH, trichloroacetic acid (TCA), thiobarbituric acid (TBA), butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), L-Ascorbic acid and 2,4,6-tri (2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma (St Louis, MO), USA. All the chemicals used were of analytical grade.

Determination of total phenolic (TPC) and flavonoid (TFC) contents

For determination of total flavonoid contents, each extract (200 µl) was oxidized with Folin-Ciocalteu reagent (1 ml; 0.5 N) and then the reaction was neutralized with 1 ml of saturated sodium carbonate (75 g/L). After incubation for 2 h at room temperature,the absorbance of the resulting blue color was measured at 760 nm with spectrophotometer. Quantification was done on the basis of the standard curve of gallic acid and results were expressed as milligram of gallic acid equivalent (mg GAE/g) (Yafang et al., 2011). For determination of total flavonoid contents, the examined extracts (200 µl) were mixed with 2% $AlCl_3 \times 6H_2O$ (0.5 mL). After incubation at room temperature for half an hour, the absorbance of the reaction mixtures was measured at 430 nm. The blank sample was a 1:1 mixture of the examined extracts and distilled water. A catechin standard curve was used to calculate the flavonoid content of the sample extracts and results were expressed as milligram of catechin equivalent (mg CE/g) (Jia et al., 1999; Heimler et al., 2005).

DPPH free radical scavenging activity

The ability of the extract to scavenge DPPH radicals was assessed as described by Ohinishi et al. (1994). Freshly prepared ethanolic DPPH (0.1 mM; 1 ml) solution was added to different concentrations of extract (20 to 200 µg/ml), solution was added. After half an hour, the absorbance was recorded at 517 nm. Results were expressed as percentage inhibition as:

$$\text{Inhibition (\%)} = \frac{A0-A1}{A0} \times 100$$

Where A_0 = absorbance of the control and A_1= absorbance of the extract. The percentage inhibition was plotted against the sample extract concentration in order to calculate the IC_{50} values, which is the concentration (µg/ml) of the extract that causes 50% loss of DPPH activity. Results were compared with the positive control, ascorbic acid.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was measured using modified method as described previously (Halliwell et al., 1987). The assay was performed by adding Ethylenediaminetetraacetic acid (EDTA) (0.1 ml; 1 mM), $FeCl_3$ (0.01 ml; 10 mM), H_2O_2 (0.1 ml;10 mM), deoxyribose (0.36 ml; 10 mM), 1.0 ml of extract (20 to 200 µg/ml) dissolved in distilled water, phosphate buffer (0.33 ml; 50 mM; pH 7.4) and 0.1 ml of ascorbic acid in sequence. After ambient incubation, for 1 h, about 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of TCA (10%) and 1.0 ml of (0.5%) TBA (in 0.025 M NaOH containing 0.025 M NaOH BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{A0-A1}{A0} \times 100$$

Where A_0 = absorbance of the control and A_1= absorbance of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan et al., 2003). Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract (20 to 200 µg/ml) dissolved in methanol and incubated at ambient conditions. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequentcoupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standard.

Superoxide radical scavenging activity

This activity was measured as described by Sabu and Ramadasan (2002). Test solutions of extract (20 to 200 µg/ml) were taken in a test tube. To this, reaction mixture consisting of sodium carbonate (1 ml; 50 mM), nitro-blue tetrazolium (NBT) (0.4 ml; 24 mM) and EDTA solutions (0.2 ml; 0.1 mM) were added to the test tube and immediate reading was taken at 560 nm. About 0.4 ml of hydroxylamine hydrochloride (1 mM) was added to initiate the reaction then reaction mixture was incubated at 25°C for 15 min and reduction of NBT was measured at 560 nm. Ascorbic acid was used as the reference compound. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage of inhibition was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{A0-A1}{A0} \times 100$$

Table 1. TPC and TFC contents.

F. assafoetida	TPC	TFC
Flowers	456.12a±1.33	443.06a± 1.89
Leaves	437.67b±1.44	416.33b± 1.61
Seeds	389.31c ± 1.04	396.214c± 1.14

Data are expressed as the mean ± standard deviation; values having different letters differ significantly ($p<0.05$).

Table 2. *In vitro* antioxidant activity (IC_{50} (µg/ml).

F. assafoetida	DPPH scavenging activity	OH scavenging activity	O˙scavenging activity	NO scavenging activity
Flowers	78.19c±0.89	75.95c±0.29	133.41b±0.33	117.16c±0.81
Leaves	99.36b±0.28	88.43b±0.56	153.29a±0. 78	142.91b±0.22
Seeds	107.55a±1.44	112.32 a ±0.63	169.45a±0.92	156.21a±1.11
Ascorbic acid	70.69d±0.11	72.51c±0.73	69.07c±0.11	36.73d±0.72

Where A_0 = absorbance of the control and A_1= absorbance of the extract.

Statistical analysis

Analyses were performed in triplicate. Data analysis was carried out using the analysis of variance and least significant differences (LSD) test using the “MSTATC” statistical computer package.

RESULTS AND DISCUSSION

In biological systems, several diverse mechanisms are involved in generation of reactive oxidative species (ROS) from biomolecules like carbohydrates, proteins and lipids. These of reactive oxidative species (ROS) are harmful if in excess. Human body has a built-in defense mechanism to counter these species. Besides various synthetic antioxidants are available to coup attack these radicals. Synthetic antioxidants like BHA, BHT, propyl gallate and tert-butylhydroquinone are believed to be source of cancer and liver damage. Scientists are turning towards natural sources of antioxidants due to their safety and efficacy. It is imperative to assess the antioxidant activity by characterizing plant extracts from different dimensions by using a battery of antioxidant assays. Current study has been designed to investigate antioxidant potential of *F. assafoetida*. Total phenolic and flavonoid content determination from herbal extracts and phytochemicals are considered necessary to study potential of plants in disease prevention. Significant amount of total phenolic content (TPC) and total flavonoid content (TFC) were found in investigated extract.

Total phenolic observed were 437.67 ± 1.44, 456.12a ± 1.33, 389.31c ± 1.04 mg/g; and flavonoid contents were 416.33b ± 1.61, 443.06a ± 1.89 and 396.214c ± 1.14 for leaves, flowers and seeds, respectively. It is documented in several reports that TPC is directly associated with antioxidant activity (Amarowicz et al., 2004). IC_{50} value for DPPH activity was 78.19 to 107.55 and 70.69 µg/ml for extracts of various parts of *F. assafoetida* and ascorbic acid, respectively. DPPH is a stable lipophilic free, nitrogen-centered radical with adsorption maxima at 517 nm and is most commonly used to assess antioxidant capacities of herbal extracts due to its rapidity, sensitivity, simplicity and reproducibility. As it can be seen from Table 1, all extracts were capable of scavenging DPPH free radical. DPPH radical scavenging activity of the extracts is concentration dependent and a lower IC_{50} value reflects better protective action. Nitric oxide is a vital molecule essential for several physiological processes like neural signal transmission, immune response, control vasodialation and control of blood pressure (Gold et al., 1990). However, its excess may lead to several pathological conditions, including cancer.

Nitric oxide has an unpaired electron, hence is a free radical nitric oxide. Plant extracts and natural products isolated may counteract the ill-effect of nitric oxide *in vivo*. Our results (Table 2) indicated that extracts have significant potential to quench nitric oxide and order of activity is flowers>leaves>seeds. Superoxide anion is a hyper-reactive oxygen species, which may harm cells and deoxyribonucleic acid (DNA) resulting in various diseases. Superoxide anion plays an important role in the formation of other reactive oxygen-species, such as hydrogen peroxide, or singlet oxygen in living systems (Stief et al., 2003). So extracts ability was measured to scavenge the superoxide radical. Our results indicated that extracts have significant potential to quench nitric oxide and order of activity is flowers>leaves>seeds. Same order was observed for hydroxyl radical scavenging activity. The results of the different antioxidant assays used in the present study of different

Table 3. Comparison among antioxidant assays as represented by correlation coefficient.

Parameter	TPC	TFC	NO scavenging activity	OH scavenging activity	DPPH scavenging activity	O˙scavenging activity
TPC	-	0.945	0.906	0.997	0.999	0.951
TFC	0.945	-	0.994	0.966	0.985	0.999
NO scavenging activity	0.906	0.994	-	0.935	0.997	0.992
OH scavenging activity	0.997	0.966	0.935	-	0.909	0.971
DPPH scavenging activity	0.876	0.985	0.997	0.909	-	0.981
O˙scavenging activity	0.951	0.999	0.992	0.971	0.981	-

extracts were compared and correlated with each other. Correlation between results of different antioxidant assays is represented in Table 3. The content of TFC showed good correlation with most of the antioxidant assays, such as TFC (r = 0.945), nitric oxide scavenging activity (r = 0.906), hydroxyl radial scavenging activity (r = 0.997), DPPH (r = 0.999) and superoxide anion scavenging activity (r = 0.951).

Many scientists have reported outstanding linear correlations between antioxidant activity tests and total phenolic content (Sultana et al., 2007). The content of TFC showed a good correlation with most of the antioxidant assays like nitric oxide scavenging activity (r = 0.994), hydroxyl radial scavenging activity (r = 0.966), DPPH (r = 0.985) and superoxide anion scavenging activity (r = 0.999). There was also good relation among different antioxidant assays. Correlation analyses indicated that nitric oxide scavenging activity showed good correlation with hydroxyl radical scavenging activity (r = 0.935), DPPH (r = 0.997) and superoxide anion scavenging activity (r = 0.992). Hydroxyl radical scavenging activity showed good correlation with DPPH (r = 0.909) and superoxide anion scavenging activity (r = 0.971). DPPH radical scavenging assay showed close relation with superoxide anion scavenging activity (r = 0.981).

This may be due to the reason that many other compounds such as carotenoids, tocopherol and vitamin C other than total phenols and total flavonoids also contribute to antioxidant activity (Mccune and Johns, 2002). Pakistan has one of the richest assemblages of medicinal plants with an extraordinary variety of geological, topographical soil conditions, has bounteous scope in gaining a footholding the global herbal pharmaceutical market.

Despite the fact that antioxidants are included in food in the form of vegetables, fruits and beverages, however wild sources of antioxidants which are mostly more rich than cultivated species in form of medicinal flora are neglected. The results obtained in this study clearly indicate that *F. assafoetida* has a sufficient potential use as a natural antioxidant agent. Well conducted biological studies are still needed for several indications of this species.

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