

## Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss

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### RESUMEN

#### Actividad antioxidante de extractos de flores, tallos y hojas de *Ferula gummosa* Boiss.

La actividad antihemolítica y antioxidante de extractos hidroalcohólicos de flores, tallos y hojas de *Ferula gummosa* Boiss fueron investigados empleando diferentes ensayos *in vitro*. Los extractos de hojas mostraron una mejor actividad captadora de radicales libres. Además, mostraron una mejor actividad captadora de óxido nítrico y H<sub>2</sub>O<sub>2</sub> y actividad quelatante de Fe<sup>2+</sup> que las otras partes. Los extractos exhibieron una buena actividad antioxidante en el ensayo con ácido linoleico pero no comparable con la vitamina C (p < 0.001). Los extractos mostraron una débil actividad de poder reductor. Los extractos de tallos de *F. gummosa* mostraron una mejor actividad antihemolítica contra la hemólisis inducida con H<sub>2</sub>O<sub>2</sub>. Entre los extractos, las flores tienen los más altos contenidos de fenoles y flavonoides. Esta planta es muy prometedora para futuros experimentos bioquímicos.

**PALABRAS CLAVE:** Actividad antioxidante – Antihemolítica – *Ferula gummosa* – Flavonoides.

### SUMMARY

#### Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss.

The Antioxidant and antihemolytic activities of hydroalcoholic extracts of the flowers, stems and leaves of the *Ferula gummosa* Boiss were investigated employing different *in vitro* assay systems. Leaf extract showed better activity in DPPH radical scavenging. In addition it showed better activity in nitric oxide and H<sub>2</sub>O<sub>2</sub> scavenging and Fe<sup>2+</sup> chelating activity than the other parts. The extracts exhibited good antioxidant activity in linoleic acid system test but were not comparable with vitamin C (p < 0.001). The extracts showed weak reducing power activity. The *F. gummosa* stem extract showed better antihemolytic activity against H<sub>2</sub>O<sub>2</sub> induced hemolysis. Among the extracts, the flowers had higher phenolic and flavonoid contents. This plant is very promising for further biochemical experiments,

**KEY-WORDS:** Antihemolytic – Antioxidant activity – *Ferula gummosa* – Flavonoid.

### 1. INTRODUCTION

Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis aiding in the aging process as well as leading to several human diseases such as cancer and inflammatory disorders, (Halliwell 1994). Antioxidants provide protection to living organisms from damage caused by the uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal *et al.* 1996). Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant and/or radical scavenging mechanisms as part of their activity (Ebrahimzadeh *et al.* 2009f). Synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes. However, due to their unstable and highly volatile nature, they have frequently brought up some questions about their safety and efficiency ever since their initial introduction into the food industry (Nabavi *et al.* 2008a). Consequently, the need to identify alternative natural and safe sources of food antioxidants arose and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (Ebrahimzadeh *et al.* 2009e; Ghasemi *et al.* 2009). *Ferula gummosa* Boiss. (Apiaceae) is a perennial plant native to central Asia, growing in the northern and western parts of Iran and blooms once in its several years' life span (Zargari 1991). Nomads of southwest Iran call this plant 'Barijeh' and traditionally use its resin for the treatment of diarrhea. They eat a small piece of the resin and believe it to be a very effective anti-diarrheal herbal medicine (Sadraei *et al.* 2001). In Iranian ancient medicine, the gum obtained from the aerial parts of this plant has been used for stomach pain, chorea, epilepsy and as a wound-healing remedy (Sayyah *et al.* 2002; Zargari 1991). In recent years there are some reports regarding the main effects of this plant. An antinociceptive activity has been shown for the

hydroalcoholic extract of aerial parts (Fazly Bazaz *et al.* 1997) and acetone extract of *F. gummosa* seed and root has been reported previously (Mandegary *et al.* 2004). Furthermore, a methanol-chloroform (1:1) extract of *F. gummosa* and its fractions have alleviated the morphine withdrawal syndrome induced by naloxone (Ramezani *et al.* 2001). The anticonvulsant potential of an essential oil (Sayyah *et al.* 2001) and the antibacterial activity of the seed (Eftekhar *et al.* 2004) and anti-inflammatory activity of the seed and root of *F. gummosa* (Mandegary *et al.* 2004) have been reported previously. The composition of the essential oil of the fruit of the plant has been determined and it has been shown that terpenoid compounds such as alpha-pinene, betapinene, 3-carene, alpha-thujene and sabinene are abundant in this plant (Sayyah *et al.* 2001). We recently reported good antioxidant activity from a methanol extract of *Ferula assafoetida*, another species from the *ferula* genus (Dehpour *et al.* 2009). According to our knowledge there is no scientific report on the antioxidant activity of *Ferula gummosa* leaves, flowers or stems. The aim of this study was to determine the antioxidant and antihemolytic activities of the hydroalcoholic extract of *Ferula gummosa* Boiss flowers, stems and leaves in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials and preparation of freeze-dried extract

*Ferula gummosa* Boiss was collected from the Gadouk area, north of Sari, Iran, in 2009. The samples were Identified By Dr. Bahman Eslami (Assistant. Prof.of plant systems, Islamic Azad university of Ghaemshahr, Iran). Voucher specimens are deposited with the faculty of pharmacy herbarium (No 712-714). The Samples were dried at room temperature and coarsely ground before extraction. A known amount of sample (120, 250 and 200g for flowers, stems and leaves, respectively) was extracted at room temperature by percolation method using Ethanol/water (70: 30). The resulting extract was concentrated over a rotary vacuum until a crude extract was obtained, which was then freeze-dried for complete solvent removal. Yields were 16, 9 and 11% for flowers, stems and leaves, respectively.

### 2.2. Chemicals

Trichloroacetic acid (TCA), 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Potassium ferricyanide and Hydrogen peroxide H<sub>2</sub>O<sub>2</sub> were purchased from Sigma Chemicals Co. (USA). Butylated hydroxyanisole (BHA), Vitamin C, Sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, Ethylenediaminetetraacetic acid (EDTA) and Ferric chloride were purchased from

Merck (Germany). All other chemicals were of analytical grade or purer.

### 2.3. Determination of total phenolic compounds and flavonoid content

Total phenolic compound contents were determined by the Folin-Ciocalteu method (Nabavi *et al.* 2009b; Esmaeili *et al.* 2009). The extract samples (800 mg ml<sup>-1</sup>, 0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent for 5 min and 2.0 ml of 75 g l<sup>-1</sup> sodium carbonate were then 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 as previously described (Ebrahimzadeh *et al.* 2008a ; Nabavi *et al.* 2009a). Briefly, 0.5 mL solution of each extract in methanol (800 mg ml<sup>-1</sup>), 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). Total flavonoid contents were calculated as quercetin from a calibration curve.

### 2.4. Antioxidant activity

#### *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.* 2008b). Different concentrations of extracts ( 2 ml, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) were added, at an equal volume, to a methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamine C, BHA and Quercetin were used as standard controls. IC<sub>50</sub> values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

#### *Determination of metal chelating activity*

The ability of the *Ferula gummosa* Boiss extracts to chelate ferrous ions was estimated in our recently published paper (Ebrahimzadeh *et al.* 2008a; Nabavi *et al.* 2009a). Briefly, different concentrations of each extracts (1 ml, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) were added to a solution of 2 mM FeCl<sub>2</sub> (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixtures were then shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solutions was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe<sup>2+</sup> complex formation was calculated as [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] x100, where A<sub>0</sub> was the absorbance of the control, and A<sub>1</sub> of the mixture

containing the extract or the absorbance of a standard solution. EDTA was used as a standard.

#### *Assay of nitric oxide-scavenging activity*

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 the Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of *Ferula gummosa* Boiss extracts dissolved in water (25, 50, 100, 200, 400, 800 and 1600  $\mu\text{g ml}^{-1}$ ) and incubated at room temperature for 150 min. The same reaction mixture, without the extracts but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2%  $\text{H}_3\text{PO}_4$  and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) were added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as a positive control (Nabavi *et al.* 2009b; Ebrahimzadeh 2009c).

#### *Scavenging of Hydrogen Peroxide*

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Dehpour *et al.* (Dehpour *et al.* 2009). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. All the Extracts in distilled water (2 ml, 25, 50, 100, 200, 400, 800 and 1600  $\mu\text{g ml}^{-1}$ ) were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged  $[\text{H}_2\text{O}_2] = [(A_0 - A_1)/A_0] \times 100$  where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance in the presence of the sample of extract and standard.

#### *Reducing power determination*

The reducing powers of *F. gummosa* extracts were determined according to the method of Yen and Chen (Nabavi *et al.* 2008a, Ebrahimzadeh *et al.* 2010). Different concentrations of extracts in water (2.5 ml; 50, 100, 200, 400 and 800  $\mu\text{g ml}^{-1}$ ) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (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 and was then centrifuged at 3000 rpm for 10 min.

The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and  $\text{FeCl}_3$  (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a positive control.

#### *Antioxidant activity in a hemoglobin-induced linoleic acid system*

The antioxidant activity of the extracts was determined by a modified photometry assay (Yuan *et al.* 2005). Reaction mixtures (200 ml) containing 10 ml of each extract (12.5, 25, 50, 100 and 200 mg), 1 mmol/l of linoleic acid emulsion, 40 mmol/l of phosphate buffer (pH 6.5), and 0.0016% hemoglobin, were incubated at 37°C for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the lipid peroxidation. The amount of peroxide value was measured in triplicate using the thiocyanate method by reading the absorbance at 480 nm after coloring with 100 ml of 0.02 mol/l of  $\text{FeCl}_2$  and 50 ml of ammonium thiocyanate (30 g/100 ml). Vitamin C was used as a positive control.

## **2.5. Antihemolytic activity**

#### *Preparation of rat erythrocytes*

All the animal experiments were carried out with the approval of the institutional animal ethical committee. Male Wistar rats in the body weight range of 180–220g were housed in individual polypropylene cages and had free access to food and water. The animals were fed with a standard diet. The animals were sacrificed under anesthesia and blood was collected by heart puncture in heparinized tubes. Erythrocytes were isolated and stored according to the method described by Yuan *et al.* (Yuan *et al.* 2005) and Yang *et al.* (Yang *et al.* 2006). Briefly blood samples collected were centrifuged (1500 $\times$ g, 10 min) at 4°C, erythrocytes were separated from the plasma and buffy coat and were washed three times by centrifugation (1500 $\times$ g, 5 min) in 10 volumes of 10 mM phosphate buffered saline (pH 7.4; PBS). The supernatant and buffy coats of white cells were carefully removed with each wash. Washed erythrocytes were stored at 4°C and used within 6 h for further studies.

#### *Antihemolytic activity of extracts against $\text{H}_2\text{O}_2$ induced hemolysis*

The inhibition of rat erythrocyte hemolysis by the extracts was evaluated according to the procedure described by Ebrahimzadeh *et al.* (Ebrahimzadeh *et al.* 2009 d). The rat erythrocyte hemolysis was performed with  $\text{H}_2\text{O}_2$  as free radical initiator. To 100  $\mu\text{l}$  of 5% (v/v) suspension of erythrocytes in PBS, 50  $\mu\text{l}$  of each extract with different concentrations (5, 10, 15, 20 and 25  $\mu\text{g}$  in PBS pH 7.4), which

corresponds to 100– 3200  $\mu\text{g}$  of extracts, were added. To this, 100  $\mu\text{l}$  of 100 IM  $\text{H}_2\text{O}_2$  (in PBS pH 7.4) were added. The reaction mixtures were shaken gently while being incubated at 37°C for 3 h. The reaction mixtures were diluted with 8 ml of PBS and centrifuged at 2000 $\times$ g for 10 min. The absorbance of the resulting supernatants was measured at 540 nm by spectrophotometer to determine the hemolysis. Likewise, the erythrocytes were treated with 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and without inhibitors (plant extracts) to obtain a complete hemolysis. The absorbance of the supernatants was measured at the same condition. The inhibitory effect of the extracts was compared with standard antioxidant Vitamin C. To evaluate the hemolysis induced by *F. gummosa* extracts, erythrocytes were preincubated with 50  $\mu\text{l}$  of extracts corresponding to 25  $\mu\text{g}$  extracts for 1 h and the hemolysis was determined. The percentage of hemolysis was calculated by taking hemolysis caused by 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  as 100%. The  $\text{IC}_{50}$  values were calculated from the plots as the antioxidant concentration required for the inhibition of 50% hemolysis.

## 2.6. Statistical analysis

Experimental results are expressed as means  $\pm$  SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ( $p < 0.05$ ) and the means separated by Duncan's multiple range tests. The  $\text{EC}_{50}$  values were calculated from linear regression analysis.

## 3. RESULTS AND DISCUSSION

Total phenolic contents of the extracts are shown in Table 1. The maximum of extractable polyphenolic content was recorded in flower extract with  $20.77 \pm 0.91$  mg gallic acid equivalent/g of extract, by reference to standard curve ( $y = 0.0063x$ ,  $r^2 = 0.987$ ). Also, the maximum flavonoid

content was recorded in the flower extract with  $9.22 \pm 0.26$  mg quercetin equivalent/g of extract, by reference to standard curve ( $y = 0.0067x + 0.0132$ ,  $r^2 = 0.999$ ). 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). 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 (Ebrahimzadeh *et al.* 2009g; Nabavi *et al.* 2008b).  $\text{IC}_{50}$  for DPPH radical-scavenging activity exists in Table 1. The  $\text{IC}_{50}$  values for Vitamin C, quercetin and BHA were  $5.05 \pm 0.1$ ,  $5.28 \pm 0.2$  and  $53.96 \pm 3.1$   $\mu\text{g ml}^{-1}$ , respectively. The phenol and flavonoid contents of this plant seem to have direct roles in its good DPPH-scavenging activity (Ebrahimzadeh *et al.* 2010). 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 improves quality of life and overall survival in some diseases such as Thalassemia major, cancer, HIV or Wilson's disease (Hebbel *et al.* 1990; Grazul and Budzisz 2009). In addition it is implicated in Alzheimer's disease (AD) pathology and so iron chelation could be considered a rational therapeutic strategy for AD (Ebrahimzadeh *et al.* 2009f). Bivalent transition metal ions play an important role as catalysts in oxidative processes, leading to the formation of radicals via Fenton chemistry (Halliwell 1997). The chelating of ferrous ions by the extract was estimated according to our recent paper (Ebrahimzadeh *et al.* 2009f). Ferrozine can quantitatively form complexes with  $\text{Fe}^{2+}$ . In the presence of other chelating agents, the complex formation is disrupted and the red color of the complexes decreases. Both extract

Table 1  
Phenol and flavonoid contents and antioxidant activities of flowers, stems and leaves extract of *F. gummosa* Boiss

Sample name	Total phenol contents ( $\text{mg g}^{-1}$ ) <sup>a</sup>	flavonoid Contents ( $\text{mg g}^{-1}$ ) <sup>b</sup>	DPPH radical scavenging, $\text{IC}_{50}$ ( $\mu\text{g ml}^{-1}$ )	Nitric oxide scavenging, $\text{IC}_{50}$ ( $\mu\text{g ml}^{-1}$ )	$\text{H}_2\text{O}_2$ scavenging $\text{IC}_{50}$ ( $\mu\text{g ml}^{-1}$ )	$\text{Fe}^{2+}$ chelating ability $\text{IC}_{50}$ ( $\mu\text{g ml}^{-1}$ )	Antihemolytic activity ( $\mu\text{g ml}^{-1}$ )
<i>F. gummosa</i> flowers	$20.8 \pm 0.91$	$9.2 \pm 0.46$	$906^{***}$	$407 \pm 14.1^{***}$	$194 \pm 9.6^{***}$	$726 \pm 28.4^{***}$	$451 \pm 22.5^{***}$
<i>F. gummosa</i> leaves	$18.5 \pm 0.58$	$8.2 \pm 0.23$	$798^{***}$	$96 \pm 2.8^{***}$	$106 \pm 8.3^{***}$	$534 \pm 21.5^{***}$	$318 \pm 17.2^{***}$
<i>F. gummosa</i> stems	$12.9 \pm 0.39$	$6.9 \pm 0.31$	$1130^{***}$	$570 \pm 19.3^{***}$	$150 \pm 6.4^{***}$	$634 \pm 19.8^{***}$	$277 \pm 11.4^{***}$
BHA	-	-	$54 \pm 3.1$	-	-	-	-
Vitamin C	-	-	$5 \pm 0.1$	-	$21 \pm 1.1$	-	$235 \pm 9.1$
EDTA	-	-	-	-	-	$18 \pm 0.5$	-
Quercetin	-	-	$5 \pm 0.2$	$20 \pm 0.01$	$52 \pm 2.6$	-	-

<sup>a</sup>mg gallic acid equivalent/g extract. <sup>b</sup> mg quercetin equivalent/g extract. Data are Means  $\pm$  SD (n=3). \*\*\*  $p < 0.001$  vs. positive control.

and EDTA interfered with the formation of ferrous and ferrozine complexes. Results were presented in Table 1. Among the different extracts, leaf extract showed better activity than the others ( $IC_{50} = 533.8 \pm 21.5 \mu\text{g ml}^{-1}$ ). Metal chelating capacity was good since the extracts reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al.* 1999). The nitric oxide assay 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 the Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to the reduced production of nitrite ions. The % inhibition increased by increasing the concentration of the extracts. The *F. gummosa* leaf extract also showed potent nitric oxide-scavenging activity ( $IC_{50} = 96.3 \pm 2.8 \mu\text{g ml}^{-1}$  vs. Quercetin  $20 \pm 0.01 \mu\text{g ml}^{-1}$ ). Although Quercetin showed very potent NO radical scavenging, its **carcinogenic** activity has been reported (Dunnik and Hailey, 1992). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Moncada *et al.* 1991). A number of disease states including sepsis and hepatic failure are characterized by abnormally high NO production and removing the excess NO could have salutary effects (Shah *et al.* 2004). 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. Further, the scavenging activity may also help to arrest the chain of reactions initiated by the excess generation of NO that are detrimental to human health. The scavenging of  $H_2O_2$  by *F. gummosa* extracts may be attributed to their phenolics and other active components which can donate electrons to  $H_2O_2$ , thus neutralizing it to water (Halliwell and Gutteridge, 1990; Ebrahimzadeh *et al.* 2009h). The extracts were capable of scavenging  $H_2O_2$  in a concentration dependent manner. Results are shown in Table 1. Leaf extract showed better activity than the others ( $IC_{50}$  was  $105.7 \pm 8.3 \mu\text{g ml}^{-1}$ ). The  $IC_{50}$  values for Vitamin C and BHA were  $21.4 \pm 1.1$  and  $52 \pm 2.6 \mu\text{g ml}^{-1}$ , respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing  $H_2O_2$  is very important throughout food systems. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Particularly, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu 2001; Ebrahimzadeh *et al.* 2009i). In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of  $Fe^{3+}$  to  $Fe^{2+}$  by donating an electron. The amount of  $Fe^{2+}$  complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Dehpour *et al.* 2009) 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. It was found that the reducing powers of all the extracts also increased by increasing concentrations. There were no significant differences ( $p > 0.05$ ) among the extracts in reducing power that were not comparable with Vitamin C ( $p < 0.001$ ). The polyphenolic contents of all the sample extracts appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reactions by converting free radicals and reactive oxygen species to more stable products. Similar observations between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for several plant extracts (Dehpour *et al.*, 2009).

Erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the  $O_2$  transport associated with redox active hemoglobin molecules, which are potent promoters of reactive  $O_2$  species. Specifically, 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 to singlet oxygen and hydroxyl radical (Ebrahimzadeh *et al.* 2009 a, Ebrahimzadeh *et al.* 2009 b). Tested extracts show good activity in hemoglobin-induced linoleic acid systems. There were no significant differences between extracts ( $p > 0.05$ ). Vitamin C showed higher inhibition (Figure 2). The effect of the extracts were tested and found to not show any harmful effects on erythrocytes. Results are shown in table 1. *F. gummosa* stem extract showed better antihemolytic activity than the others ( $IC_{50}$  was  $277 \pm 11.4$  vs.  $235 \pm 9.1 \mu\text{g ml}^{-1}$  for vitamin C,). The antihemolytic activity of quercetin and other flavonoid have been previously reported and good activity of the extracts maybe result in high flavonoid content especially quercetin (Chaudhuri *et al.* 2007).

#### 4. CONCLUSION

Our studies indicate that the hydroalcoholic extract of *F. gummosa* Boiss flowers, stems and leaves have remarkable antioxidant and antihemolytic effect that maybe results of its high phenol and flavonoid contents. It is very promising for further biochemical experiments, which will be focused on evaluating other biological effects or improving mechanism of these activities.

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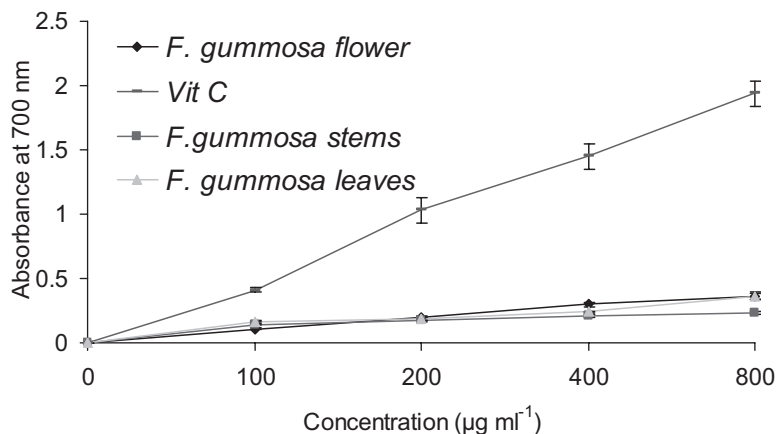


Figure 1  
Reducing power of ethanol/water (70: 30) extract of *F. gummosa* Boiss flowers, stems and leaves. Vitamin C used as control.

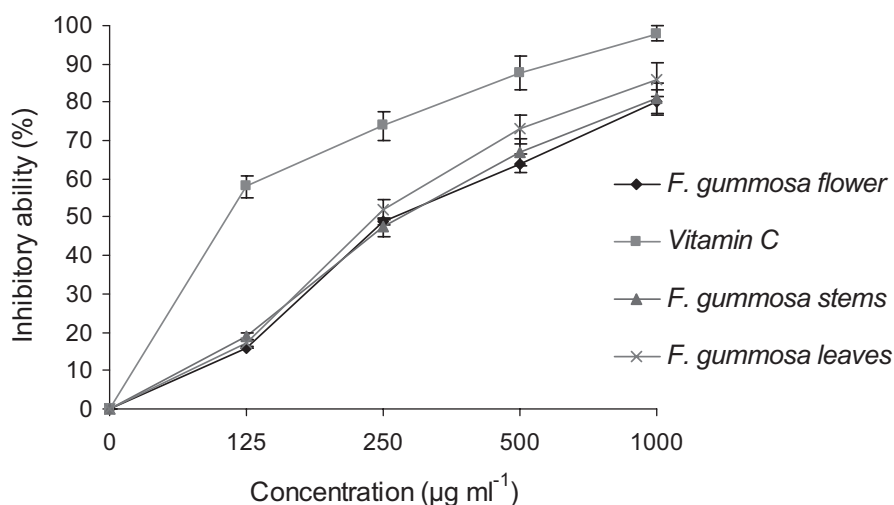


Figure 2  
Hemoglobin-induced lipid peroxidation of ethanol/water (70: 30) extract of *F. gummosa* Boiss flowers, stems and leaves. Vitamin C used as control.

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