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Evaluation of the Antioxidant Potential of Bioactive Compounds in Methanolic Extracts of Aerial Parts of *Fagonia glutonosa*

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***Fagonia glutonosa* (L.) (Zygophyllaceae) is a medicinal plant traditionally used as an mainly as a popular remedy for the treatment of various skin lesions and for the treatment of various other digestive diseases. Preliminary phytochemical screening of the plant showed the presence of large amounts of phenolics and flavonoids, anthraquinones, terpenes, saponins, alkaloids, coumarins and tannins. Subsequent quantification showed the presence of 0.74% (m/m) phenolics (calculated as gallic acid) and 0.13% (m/m) flavonoids calculated as catechin equivalents per 100 g of fresh mass. The presence of phenolic compounds prompted us to evaluate its antioxidant activity. In the present study, methanolic extract of aerial parts of *Fagonia glutonosa* was screened to evaluate its free - radical scavenging effect. The highest antioxidant and free radical scavenging ability of the extract was observed at a concentration of 2500 μg mL^{-1}.**

Key words: *Fagonia glutonosa*, medicinal plants, skin lesions.

Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases (Halliwell, and Gutteridge, 1999; Finkel and Holbrook, 2000). Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant-based drugs/ formulations for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades (Devasagayam *et al.*, 2004). This has attracted a great deal of research interest in natural antioxidants. Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic,etc. (Miller, 1996). The aim of the present investigation was to evaluatein vitro antioxidant and free radical scavenging activity of the *Fagonia glutonosa* extract. Plants belonging to the genus *Fagonia* are often used in folk medicine, mainly as a popular remedy for the treatment of various skin lesions. Additionally, the aerial plant is claimed to be a remedy for cancer in its early stages and for the treatment of various other diseases of digestive and blood vascular system. The medicinal properties of the plant were attributed due to its variety of active phytochemical constituents.

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MATERIALS AND METHODS

Chemicals

Chemicals used in this study were 1,1-diphenyl-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, NADH and sulfanilamide obtained from Gulf scientific Laboratories, Saudi Arabia,

Folin- Ciocalteu reagent, potassium ferricyanide and sodium nitroprusside obtained from Qualigens FineChemicals, Glaxo Smithkline Pharmaceutical Ltd., naphthylethylenediamine dihydrochloride,N-1-naphthylethylenediamine dihydrochloride, sodium nitrite, trichloroacetic acid, butylated hydroxy anisole (BHA), ascorbic acid,a-tocopheryl acetate, ethylenediamine tetraacetic acid, phosphoric acid, nitro blue tetrazolium, phenazine methosulfate, ferrous ammonium sulfate, DMSO are obtained from Sd Fine Chemicals Ltd, India. All reagents used in the study were of analytical grade.

Plant material

Fagonia glutonosa (Zygophyllaceae) plants were collected from the areas near to Riyadh, Saudi Arabia and authenticated at the Department of Botany, King Saud University, Riyadh, Saudi Arabia. The plants were cleaned and dried in the shade, then powdered to 0.422 mm mesh size and stored in an airtight container at 25°C.

Extraction

Fagonia glutonosa (100 g) in powdered form were extracted with methanol using a Soxhlet assembly for 48 h, filtered and last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield was 2.78 g of dry extract.

Total phenolic content

The total phenolic content of *Fagonia glutonosa* methanolic extract was determined spectrometrically (Singleton and Rossi, 1965). Folin- Ciocalteu's reagent, 1 mL previously diluted with 20 mL distilled water, was added to 1 mL of sample (250 mg mL^{-1}) and mixed thoroughly. To the mixture, 4 mL of sodium carbonate (75 g L^{-1}) and 10 mL of water were added and mixed well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000g for 5 min and the absorbance of the supernatant was taken at 760 nm using a double beam spectrophotometer 2202 (Jenway, UK). A standard curve was obtained using various concentrations of gallic acid. Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g fresh mass.

Total flavonoid assay

Expressed as quercetin equivalent (mg/100 g *Fagonia glutonosa* extract) Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al*., 2002). Therefore, 0.1 ml of each extract (10 mg/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M CH_3COOK and 2.8 ml of distilled water and kept at room temperature for 30 min. The Abs. of the reaction mixture was measured at 415 nm. A calibration curve of quercetin was prepared by using concentration from 12.5 to 100µg/ml in methanol, and the total flavonoids were expressed as quercetin equivalent (mg QE/100 g arak root extract).

DPPH free radical scavenging activity

The free-radical scavenging activity of *Fagonia glutonosa* extract was measured by the decrease in absorbance of methanolic solution of DPPH (Sreejayan and Rao, 1996). A stock solution of DPPH (33 µg L^{-1}) was prepared in methanol and 5 mL of this stock solution was added to 1 mL of the *Fagonia glutonosa* extract solution at different concentrations (250, 500, 1000, 1500, 2000 and 2500 µg mL^{-1}). After 30 min, absorbance was measured at 517 nm and compared with the standards, i.e., ascorbic acid, BHA and a-tocopherol (10–50 mg mL^{-1}). Scavenging activity was expressed as the percentage inhibition.

Hydroxyl radical scavenging activity

Methanolic extract at different concentrations was placed in a test tube and evaporated to dryness. One mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1 mL of DMSO (0.85%,V/V, in 0.1 mol L^{-1} phosphate buffer, pH 7.4) and 0.5 mL of 0.22% ascorbic acid were added to each tube (Klein, *et al*., 1981). The tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min. The reaction was terminated by adding 1 mL of ice-cold TCA (17.5% m/V). Three ml of Nash reagent (75.0 g ammonium acetate, 3 mL glacial acetic acid and 2 mL acetyl acetone were mixed and water was added to a total volume of 1 L) was added to each tube; the tubes were left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured at 412 nm against a blank of the reagent. Percentage inhibition was determined by comparing the results of the test and standard compounds.

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (40 m mol L^{-1}) was prepared in phosphate buffer (pH 7.4). Different concentrations (250–2500 µg mL^{-1}) of *Fagonia glutonosa* were added to the hydrogen

peroxide solution (40 m mol L^{-1}, 0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide[10]. Percentage scavenging of hydrogen peroxide of the *Fagonia glutonosa* extract and standard compounds was calculated.

Superoxide radical scavenging assay

The reaction mixture consisting of 1 mL of nitro blue tetrazolium (NBT) solution (156 m mol L^{-1} NBT in phosphate buffer, pH 7.4), 1 mL NADH solution (468 m mol L^{-1} NADH in phosphate buffer, pH 7.4), and 1 mL of sample solution of *Fagonia glutonosa* extract was mixed. The reaction was started by adding 100 mL of phenazine methosulfate (PMS) solution (60 m mol L^{-1} PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with the standards (Gülçin *et al*., 2005; Nishikimi *et al*., 1972). Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan *et al*., 2003). Sodium nitroprusside (5 m mol L^{-1}) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the *Fagonia glutonosa* extract (250–2500 µg L^{-1}) prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. 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). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

Statistical analysis

Values were represented as meanSD of three parallel measurements and data were analyzed using the t-test.

RESULTS AND DISCUSSION

From the results on the total phenolic content, it was found that there was 0.74% of gallic acid equivalents of phenolic compounds while the total flavonoid content was 0.13% of catechin equivalent of fresh mass of *Fagonia glutonosa* extract. The results of antioxidant and free radical scavenging activity are given in Table I. The free radical scavenging activity was evaluated by using various in vitro assays. DPPH radical was used as a substrate to evaluate the free radical scavenging activity of *Fagonia glutonosa* extract. The scavenging effect of *Fagonia glutonosa* extract on the DPPH radical was 89.0±2.4% ($p< 0.005$), at a concentration of 2500 µg mL^{-1} compared to the scavenging effects of ascorbic acid, BHA and a-tocopherol at 50 µg mL^{-1} of 90.5±1.3, 71.2±1.2 and 57.4±0.7% ($p< 0.05$) respectively.

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage (Aurand *et al*., 1977). The percentage of hydroxyl radical scavenging increased with the increasing concentration of *Fagonia glutonosa* extract. The percentage of H_2O_2scavenging activity of *Fagonia glutonosa* was found to be 66.4±2.3 ($p< 0.001$) at 2500 mg mL^{-1}, and antioxidant activity of BHA and ±-tocopherol

Table 1. Antioxidant activities of methanolic extract of the aerial parts of *Fagonia glutonosa*

Sample	Conc. µg mL^{-1}	Scavenging Activity of (DPPH) (%)	Scavenging Activity of (·OH) (%)	Scavenging Activity of H_2O_2(%)	Superoxide anion scavenging (%)	Scavenging Activity of (NO) (%)
Fagonia glutonosa	2500	89±2.4	74.2±2.6	66.4±2.3	74.3±3.1	63.2±3.6
Ascorbic acid	50	90.5±1.3	66.4±2.1	55.2±1.7	88.6±2.6	88.4±3.4
Butylated hydroxy anisole(BHA)	50	71.2±1.2	84.6±3.3	92.4±2.7	71.8±3.8	56.2±1,7
α-tocopherol	50	57.4±0.7	65.1±1.9	95.4±3.2	79.7±2.6	59.2±1.5

was 92.4±2.7% ($p< 0.05$) and 95.4±3.2% ($p< 0.05$), respectively at a concentration of 50 µg mL^{-1}.H_2O_2 itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems.

The superoxide anion radical scavenging activity of *Fagonia glutonosa* was assayed using the PMS-NADH system. The percentage inhibition of superoxide generation by *Fagonia glutonosa* at 2500 µg L^{-1} concentration was found to be 74.3±3.1% ($p< 0.005$). On the other hand, ascorbic acid, BHA and ±-tocopherol at 50 µg mL^{-1} exerted 88.6±2.6, 71.8±3.8and 79.7±2.6% ($p< 0.05$) inhibition of the superoxide radical. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity (Hagerman *et al*., 1992). The percentage inhibition of nitric oxide generation by *Fagonia glutonosa* at 2500 µg mL^{-1} concentration was found to be 63.2±3.6% ($p< 0.005$). On the other hand, ascorbic acid at 50 mg mL^{-1} concentration showed 88.4±3.4% ($p< 0.05$) inhibition of nitric oxide.

CONCLUSIONS

Antioxidant potential of methanolic extract of *Fagonia glutonosa* increases with increasing concentration and maximum antioxidant activity was observed at 2500 µg mL^{-1}. Antioxidant activity may be due to phenolic compounds in *Fagonia glutonosa*.

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