Original Article

Antioxidant Activity and Phytochemical Screening of *Ficus benghalensis* Aerial Roots Fractions

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Abstract

Context: *Ficus benghalensis (Moraceae)* is an evergreen tree found in south and southeast of Iran as wild and cultivated plants. Different parts of this plant have different effects such as antitumor, antipyretic, analgesic and anti-inflammatory. **Aims:** The aim of this study was investigated the phytochemical screening and antioxidant activities of different fractions of plant roots. **Materials and Methods:** Phytochemical investigation was done by different methods in references. Antioxidant activity was evaluated by DPPH and FRAP assay. All chemical materials and solvents were prepared from Sigma-Aldrich, Scharlau and Merk. **Statistical Analysis:** All measurements were carried out in triplicate and the data were expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) and tukey test. **Results:** Phytochemical screening showed steroids, flavonoids, tannins, phenolic compounds, and anthraquinone glycoside are *F. benghalensis* constituents. This plant had antioxidant activity, but it was lower than the Indian kinds. **Conclusion:** This study elucidated *Ficus benghalensis* could be useful plant with antioxidant activity. Further investigation needs for details.

Keywords: *2,2-diphenyl-picrylhydrazyl, antioxidant activity, ferric reducing antioxidant power, Ficus benghalensis, phytochemical screening*

Introduction

Ficus includes 800 species that is found in most tropical zones all over the world.[1] *Ficus benghalensis* (*Moraceae*) is one of them and it is a large evergreen tree 23–34 m tall[2] that is found in south and southeast of Iran as wild and cultivated plant. Various studies show that different parts of this plant can be effective as traditional medicine because of the presence of different chemical constituents such as triterpenoids, flavonoids, tannin, different glucosides, steroids, resin, albumin, and malic acid.[2,3] Methanolic extracts of root, bark, and leaf of *F. benghalensis* were studied for antimicrobial effects, and all three parts of plant extracts inhibited the growth of Gram-positive bacteria as well as Gram-negative bacteria. Among the tested microbial strains, bacteria were found to be more sensitive than fungi. The methanolic extracts of root and leaf are potent against *Candida albicans*.[2] This plant has antipyretic activities, analgesic effects,[4] antitumor activities,[5] and anti-inflammatory[6] activities. The bark of this plant has anti-inflammatory and analgesic properties in animal models,[7] and it is useful for burning sensation, ulcers, and painful skin diseases.[8] The aqueous and methanolic extracts of this plant kill the earthworm and it was comparable by antihelminthic drugs.[9] Antidiabetic activity of the aqueous extract of *F. benghalensis* at a dose of 500 mg/kg/day was shown with histological studies in normal and streptozotocin-induced diabetic rats.[10] Indian researchers study various biological activities of different parts of *F. benghalensis*. This plant is one of the Iranian traditional medicine that is used in different traditional formulations There is not enough study about Iranian kind besides useful uses of it. Therefore, the aim of this study was preliminary phytochemical screening and investigation of antioxidant activity of Iranian *F. benghalensis* root as a traditional medicine for comparing with Indian kind to further pharmaceutical investigations.

Materials and Methods

Plants materials

The roots of *F. benghalensis* were purchased from botanical market of Tehran. The dried roots (300 g) were powdered and extracted by ethanol 80% in percolator at room temperature (3 × 48 h). The extract was concentrated using a rotary evaporator

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at 37°C (190 g). Twenty-nine grams of dried extract was fractionated based on increasing polarity by chloroform, ethyl acetate, and methanol at room temperature by silica gel (mesh 35–70) and filtration set. The different fractions were concentrated using a rotary evaporator at 37°C. All fractions, i.e., chloroform (8.3 g), ethyl acetate (5.5 g), and methanol (7.1 g), were stored at 4°C until use.

Chemicals

2,2-diphenyl-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), and 2,4,6-tripyridyl-striazine (TPTZ) were bought from Sigma-Aldrich (Germany). Sodium carbonate, sodium acetate, ferrous sulfate, and FeCl3 were prepared from Scharlau, Spain. Other chemicals and all solvents were purchased from Merck (Germany).

Phytochemical screening

Standard phytochemical methods were used to evaluate the presence of different constituents such as saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenic glycosides, amino acid, and protein and flavonoids.[11-15]

Antioxidant activity

Free radical-scavenging assay 2,2-diphenyl-1-picrylhydrazyl assay

The DPPH assay is a popular method in natural product antioxidant studies. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers.[16] The assay was carried out according to Sarker *et al*.[17,18] The stock solution of DPPH was prepared at the concentration of 8.0×10^{-2} mg/mL in methanol. The extract dilutions were made in methanol to get the concentrations of 5.0×10^{-1}, 2.5×10^{-1}, 1.25×10^{-1}, 6.25×10^{-2}, 3.13×10^{-2}, and 1.6×10^{-2} mg/mL. The prepared solutions of extracts (2.0 mL each) were mixed with DPPH solution (2.0 mL). After 30 min, ultraviolet absorbances of the solutions were recorded at 517 nm. BHA, a synthetic antioxidant, and Vitamin C, a natural antioxidant, were used as the positive control. All experiments were carried out at least three times. Inhibition of DPPH-free radical was calculated as:

Inhibition % = 100 – ([Sample absorption − control absorption]/Blank absorption) × 100.

The concentration that caused 50% decrease in the initial DPPH radical concentration was defined as IC_{50}. The experiments were repeated three times, and the IC_{50} values were expressed as mean ± standard deviation.[17,18]

Ferric reducing antioxidant power assay

The antioxidant activity of the plant fractions was evaluated according to the method of Benzie and Strain.[12] The ferric reducing antioxidant power (FRAP) reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl3 solution, and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10 (v/v). Fifty microliters of each diluted extract was mixed with 3 mL of freshly prepared FRAP reagent, and the reaction mixtures were incubated at 37°C for 30 min. The absorbance was determined at 593 nm against distilled water as blank. Aqueous solutions of ferrous sulfate (100–2000 μM) were used for calibration. Triplicate measurements were taken, and the FRA*P* values were expressed as μmol of Fe (II)/g dry weight of plant powder.[19,20]

Results

Phytochemical screening of *Ficus benghalensis* aerial roots

The total extract and different fractions of the plant were used for preliminary phytochemical screening that is shown in Table 1.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

All results are reported in Table 2. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH-free radical and was calculated by plotting a graph of concentration and % inhibition. The calibration curve for the quantification of DPPH assay was linear over the range of standard concentrations of 500–2000 μg/ml with correlation coefficient of $R^2 = 0.957$ about the validity, accuracy, and precision of the method being also in the acceptable range. Methanolic fraction was more effective than the others.

Ferric reducing antioxidant power radical scavenging activity

All results are reported in Table 3. Calibration curve of standard solution of sulfate iron is shown in Figure 1. The calibration curve for the quantification of FRAP assay was linear over the range of standard concentrations of 100–1000 μM/L with correlation coefficient of $R^2 = 0.987$ about the validity, accuracy, and precision of the method being also in the acceptable range. Ethyl acetate fraction was more effective than the others.

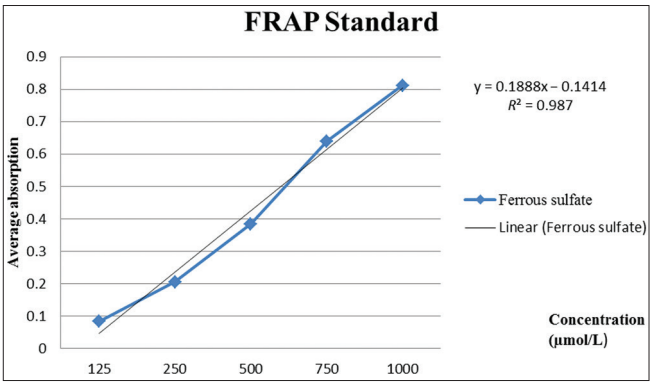

Figure 1: Calibration curve and regression equation calculated standard solution of sulfate iron