FREE RADICAL SCAVENGING ACTIVITY OF EXTRACTS OF YOUNG PROP ROOTS OF *FICUS BENGALENSIS*

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Summary

The various parts of Indian medicinal plant *Ficus bengalensis* Linn.(Moraceae) is used in the Indigenous System of Medicine and known for its wide therapeutic benefits.The extracts of young prop roots of *Ficus bengalensis* was evaluated for its free radical scavenging activity in different methods viz. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay and iron chelation activity using orthophenathroline. The results were analyzed statistically by regression method. In all the methods the alcoholic and aqueous extracts showed concentration dependent radical scavenging activity and the IC_{50} values were 50.87 and 95.7 µg/ml for DPPH assay, 49.23 and 103.08 µg/ml for iron chelating activity, 19.89 and 392 µg/ml for ABTS scavenging. The alcoholic extract exhibited promising antioxidant activity compared to aqueous extracts and the results were comparable with standard antioxidant ascorbic acid.

Key words: *Ficus bengalensis,* Prop roots, Moraceae, Antioxidant, Free radical scavenging

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Introduction

Free radicals and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several physiological threats such as cancer, inflammatory disorders, as well as in aging process (1). Antioxidants provide protection to living organisms from oxidative damage caused by free radicals. The synthetic antioxidants have been suspected to cause or promote negative health effects and there is a trend to substitute them with naturally occurring antioxidants (2). Herbs contain different phytoconstituents that can act as free radical scavengers which is an important characteristic of antioxidant. Some natural antioxidants are been used commercially as antioxidant additives or as nutritional supplements.
Ficus bengalensis Linn. (Moraceae) is widely distributed throughout the forests tracts of India, both in the sub-Himalayan region and in the deciduous forests of Deccan and South India (3). The research on *Ficus bengalensis* began much earlier in order to establish the scientific base for the traditional claims but, only in recent years much attention is paid towards exploring the therapeutic benefits of young prop roots. The aerial roots are useful in obstinate vomiting and leucorrhea and are said to be used in osteomalacia of the limbs (4). It is also used to treat intrinsic hemorrhage, bone fracture (externally), ulcers, enhance memory (5-7). Recent study has been carried out on prop roots for hepatoprotective (8), growth promotion (9) and antidiabetic (10) activity. The present study was aimed to evaluate the antioxidant activity of alcoholic and aqueous extracts of dried young prop roots of *Ficus bengalensis* in different radical scavenging methods.

Materials and Methods

Chemicals and Instruments

1, 1-Diphenyl-2 picryl hydrazyl (DPPH) from Hi media laboratories Ltd., Mumbai, ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] from Sigma-Aldrich Co. USA. O-phenathroline from Spectrochem, Mumbai. Ascorbic acid, Ferric chloride and EDTA from NICE Chemicals Pvt. Ltd., Cochin and other chemicals were procured from E.Merck (India) Ltd., Mumbai, India. All the chemicals and solvents used were of analytical grade. The instruments used for the study were UV spectrophotometer (Shimadzu 160 IPC) and pH meter (Elico Ltd., India).

Plant material

The young prop roots of the plant material *Ficus bengalensis* were collected around the region of Manipal, Udupi, Karnataka, India. The plant was authenticated by Dr.Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. The voucher specimen (PP-04/2004-05) has been deposited in the Departmentof Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal, India.

Preparation of alcohol and aqueous extracts

About 100 g of coarse powder of the young prop roots were extracted in the Soxhlet extractor with ethanol as solvent. After complete extraction the solvent was recovered by distillation *in vacuo* and the yield was 5.7% w/w and for aqueous extract, the coarsely powdered drug was macerated in chloroform water for 7 days. After maceration, the residue (yield 7.2% w/w) was obtained by evaporation. The extracts obtained were then stored in a dessicator and used for subsequent experiments.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on the petroleum ether, benzene, chloroform, acetone, methanol and aqueous fractions of young prop roots of *Ficus bengalensis* (11,12).

***In-vitro* antioxidant studies**

The antioxidant activity of the extracts was evaluated using simpler and rapid free scavenging assays viz, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay, iron chelation activity using orthophenathroline and ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation assay.

DPPH (1, 1, diphenyl 2-picryl hydrazyl) assay

The antioxidant activity by DPPH assay was assessed using the stable free radical DPPH. To 1 ml of various concentration of the extract and 1 ml of DPPH 0.1mM was added to the test tube. Ascorbic acid was used as the standard for comparison. After incubation 30 min in dark at room temperature, absorbance was recorded at 517 nm. The percent DPPH radical scavenging, which is calculated with the equation: % DPPH radical scavenging = [(control absorbance - sample absorbance)/control absorbance] × 100. The experiment was carried out in triplicate and IC_{50} values (the concentration required to scavenge 50% DPPH free radicals) were calculated (13,14).

Reduction of ferric ions by ortho-phenanthroline colour method

The reaction mixture contains 1ml phosphate buffer of pH 7.4, ferric chloride 200 μM, 0.5 ml of various concentrations of the extract, 0.3 ml EDTA 1 mM, 0.9ml ortho-Phenanthroline (41.66 mg in 50ml distilled water). The mixture was incubated at ambient temperature for 10 min and the absorbance was measured at 510 nm. Ascorbic acid was used as the standard for comparison. The percent reduction calculated with the equation: % Reduction = [(sample absorbance - control absorbance) / sample absorbance] X 100. The experiment was carried out in triplicate and IC_{50} values were calculated (15).

ABTS radical scavenging activity

To the 0.5 ml of various concentrations of extract, 0.3 ml of ABTS radical cation prepared using 2mM ABTS and 70 mM potassium persulphate (used after 6 hrs), 1.7 ml of Phosphate buffer, pH 7.4 was added. The absorbance was measured at 734 nm. Blank was performed in the same manner without the drug. The percent ABTS radical scavenging, which is calculated with the equation: % scavenging = [(control absorbance - sample absorbance) / control absorbance] × 100. The experiment was performed in triplicate and IC_{50} values were calculated (16).

Statistics

The results of all experiments were expressed as mean ± S.E.M. The concentrations of the extracts that cause 50% of inhibition (IC_{50}) were determined by the linear regression analysis using Microsoft Excel programme for Windows, v.XP.

Results

Preliminary phytochemical screening on different fractions revealed the presence of alkaloids, steroids, phenolic compounds and tannins. The free radical scavenging capacity of alcoholic and aqueous extracts of young prop roots of *Ficus bengalensis* was evaluated on DPPH assay, reduction of ferric ions by ortho-Phenanthroline method and ABTS radical cation assay.

In DPPH assay, the electron donating ability of the extracts was determined from the bleaching of purple colored methanol solution of DPPH to light yellow and measured spectrophotometrically. Both extracts were able to reduce DPPH radical in concentration dependent manner (Figure 1).

The IC_{50} values of the alcoholic and aqueous extracts were found to be 50.87 and 95.7 μg/ml respectively (Figure 4).

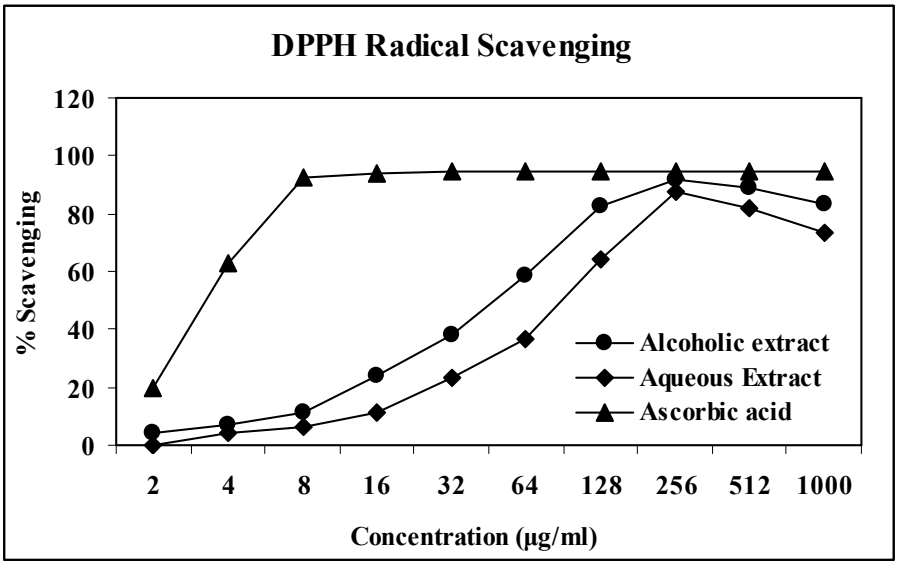

Figure: 1 Effect of DPPH radical scavenging activity of extracts of young prop roots of *Ficus bengalensis*. Plot of % scavenging Vs concentration. The values are expressed in Mean ± SEM; n =3

Ortho substituted phenolic compounds may exert pro-oxidant effect by interacting with iron. Antioxidant donates electrons for the reduction of ferric ion to ferrous ion, which forms a coloured complex with orthophenanthroline, which is measured at 510 nm.The percentage of reduction increases in a concentration dependent manner (Figure 2).The IC_{50} values of the extracts were calculated and is shown in Figure 4.

ABTS [2,2-azino bis (3-ethyl benzo-thiazoline-6-sulphonic acid)] is converted to a cation radical using potassium persulphate, which shows absorbance at 734 nm. The scavenging of this free radical by test compound was measured. The IC_{50} values of the alcoholic and aqueous extracts were 19.89 and 392 µg/ml respectively which is comparable with ascorbic acid (Figure 4).

The comparison of antioxidant activity (IC_{50} values) showed variable results, depending on the model system used for evaluation and showed in Figure 4.

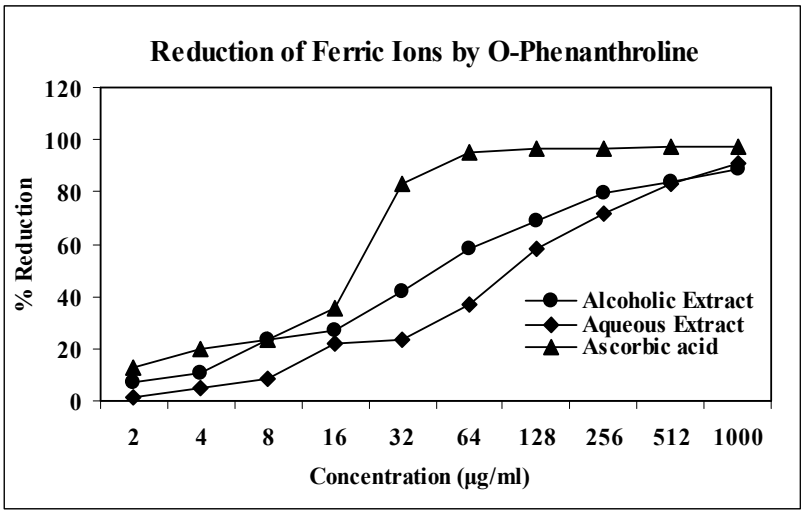

Figure:2 Effect of extracts of young prop roots of *Ficus bengalensis* on Reduction of ferric ions by ortho-Phenanthroline method. Plot of % reduction Vs concentration. The values are expressed in Mean ± SEM; n =3

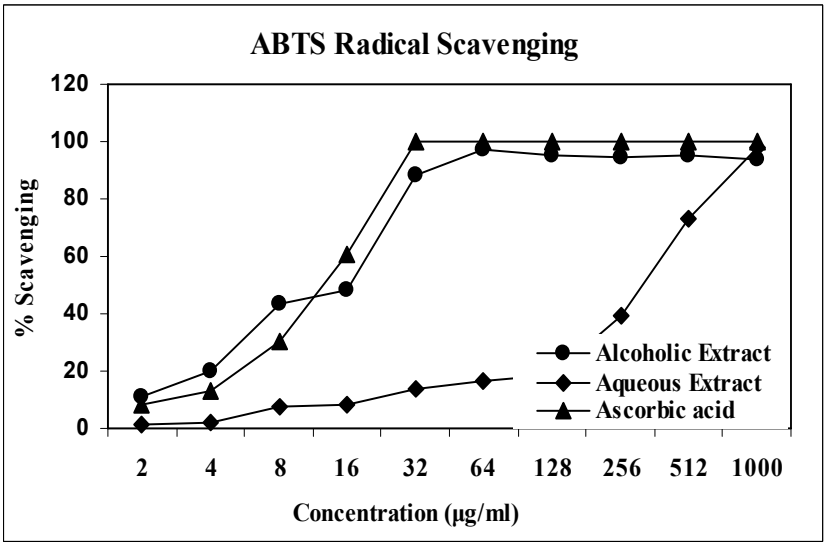

Figure: 3 Effect of extracts of young prop roots of *Ficus bengalensis* on ABTS radical scavenging activity. Plot of % scavenging Vs concentration. The values are expressed in Mean ± SEM; n =3

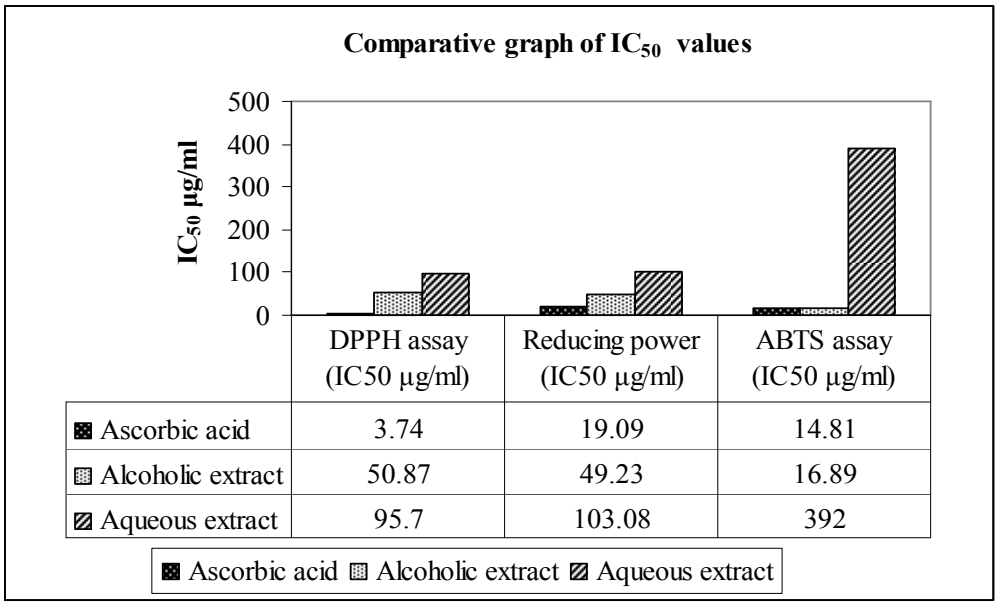

Figure: 4 Comparison of antioxidant activity (IC_{50} values) exhibited by extracts of young prop roots of *Ficus bengalensis* in various *Invitro* free radical scavenging methods.

Discussion

Free radicals are involved in the development and progression of many diseases. Highly reactive free radicals and oxygen species are present in the biological systems oxidizes nucleic acids, proteins, lipids and can initiate degenerative diseases (16). Scientific evidence showed that antioxidants reduce the risk of development of many chronic diseases. The main marker and characteristic of an antioxidant is its ability to trap free radicals. Most of the antioxidant compounds are derived from plant sources. Plants with antioxidant potential play an important role in protecting living organisms from the oxidative damage and act as life style enhancers.

The study showed that the alcoholic and aqueous extracts of young prop roots of *Ficus bengalensis* showed a remarkable antioxidant activity in all the assessed cell free radical scavenging methods and the results were comparable with the standard antioxidant ascorbic acid. The aerial roots of *Ficus bengalensis is* used traditionally for the treatment of diabetes and

many inflammatory diseases where free radicals play a major role as causative factor. So, the free radical scavenging and antioxidant nature of the extracts proved in this study may be, or at least in part, can provide a beneficial effect in the above conditions. The study also provides a scientific support for the ancient claims of their health benefits.

Preliminary phytochemical studies on the plant revealed the presence of steroids, alkaloids, tannins and phenolics. A number of scientific papers indicate the phenolics as effective contributor to antioxidant activity and inhibit oxidative mechanisms and inturn depends on the availability of the phenolic hydrogen as hydrogen donating scavengers(8,17-18). Here, the antioxidant activity could be attributed at least in part, to the presence phenolic compounds. In conclusion, the present study reveals the promising and potent free radical scavenging activity of extracts of dried young prop roots of *Ficus bengalensis* in different *in vitro* models and proves to be an excellent plant for detailed investigation.

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