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In vitro Antioxidant Activities of Aqueous Extract of *Ficus Bangalensis* Linn. Root

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Abstract: In this study, antioxidant activity of aqueous extract of *Ficus bengalensis* Linn. (FBWE) root was investigated for its free radical scavenging activity by adopting various *in vitro* models. The extract was investigated for its antioxidant activity by 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity, hydrogen peroxide activity, determination of total phenolic content using Folin-Ciocalteu's phenolic reagent. FBWE showed maximum scavenging of DPPH radical (96.07%) at 250 μg mL^{-1} concentration and hydrogen peroxide (69.23%) at 1000 μg mL^{-1} concentration. Reducing power was also dose dependent and total phenolic content evaluated that 1 mg of FBWE contains 25.34 μg equivalent of gallic acid. The extract showed significant results when compared with the standard compounds.

Key words: Antioxidant, aqueous extract, *Ficus bengalensis*, free radicals, *in vitro*

INTRODUCTION

Oxidative stress due to free radicals may lead to a number of ailments (Gutteridgde, 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001). Superoxide anion, hydrogen peroxide, peroxyl radicals, reactive hydroxyl radicals are the most commonly reactive oxygen species and nitric oxide and peroxynitrite are the nitrogen derived free radicals. These ROS have been implicated in the pathogenesis of a number of diseases/disorders including arthritis, carcinogenesis, aging, physical injury, infection, acquired immunodeficiency syndrome, etc. Antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals (Nayana and Janardhanan, 2000). The medicinal properties of plants have been centre of attraction of the researchers in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Auddy *et al*., 2002).

The present study has been designed to investigate the *in vitro* antioxidant potential of *Ficus bengalensis* Linn. roots. The plant, *Ficus bengalensis* Linn. is found throughout India, grows wildly on the sub Himalayan tracts, mountains, lower slopes of the deccan hills and Malabar coasts in deciduous and semi evergreen forests. It is found near temples and roadsides for shade (Sharma, 1996). Different parts of the plant are used for various medicinal purposes. Leaf bud is used in dysentery, diarrhea and to cure burns, thirst, haemorrhage. The

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concentrated juice of young leaf bud and fruit exhibit aphrodisiac properties and also useful in gonorrhea. Leaf is also good for ulcers, aerial root in gonorrhea, seed and fruit are reported to have cooling and tonic properties. Aqueous extract of the bark possesses hypoglycaemic, hypochoesterolaemic and hypolipidaemic effects (Shrotrim and Aiman, 1960; Shukla, 1994, 1995a, b; Shukla *et al*., 2004).

MATERIALS AND METHODS

Plant Material

The roots of the plant *Ficus bengalensis* Linn. were taken in April 2009 from Mandi Dabwali, Dist. Sirsa, Haryana, India and authenticated by Dr. P. Jayaraman, Scientist, Plant Anatomy Research Centre, Chennai. One voucher specimen has been procured in our lab for future reference (Voucher No. PARC/2006/14).

Preparation of the Extracts

The roots were dried under shade, coarsely powdered and root powder was successively extracted with petroleum ether (60-80°C), ethyl acetate, alcohol (hot continuous extraction method) and distilled water (cold maceration). The water extract was concentrated under reduced pressure and dried in vacuum. The dried extract (FBWE) thus obtained was used for the assessment of antioxidant activity through various *in vitro* models.

Chemicals

All chemicals except 1,1-diphenyl, 2-picry hydrazyl (DPPH) and solvents were of analytical grade and were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. DPPH was obtained from Sigma Chemicals, USA. The other chemicals used were, potassium ferricyanide, trichloroacetic acid, gallic acid, hydrogen peroxide, Ferric chloride, ascorbic acid, potassium iodide, ammonium molybdate, sodium thiosulfate, Folin-Ciocalteu's phenol reagent, etc.

Preliminary Identification of Chemical Constituents

Mainly, polyphenolic compounds like flavonoids, tannins are responsible for antioxidant activity. Preliminary chemical tests (Khandelwal, 2000; Geissman, 1995) were performed to detect the presence of polyphenolic compounds. So, the qualitative chemical tests performed were Shinoda test, ammonia fuming test, lead acetate test, boric acid test for flavonoids and ferric chloride test, nitric acid test, ammonia hydroxide-potassium ferricyanide test, lead acetate test for tannins. All the tests confirmed the presence of flavonoids and tannins.

Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Blois, 1958; Lai *et al*., 2001). The 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (8-250 μg mL). It was incubated at room temperature for 45 min and the absorbance was measured at 517 nm against the corresponding blank solution. The assay was performed in triplicates. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contain DPPH and distilled water without any extract using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in μg mL^{-1}) of extracts that inhibits the formation of DPPH radicals by 50%.

Determination of Reduction Capability by Fe^{3+}-Fe^{2+} Transformation

The different concentration of the extracts (100-1000 μg mL^{-1}) in 1 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged for at 1000 x g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm (Oyaizu, 1986; Jayaprakasha *et al.*, 2001). Ascorbic acid was taken as a reference.

Determination of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration. The assay was performed by adding 1.0 mL of Hydrogen peroxide (0.1 mM) and 1 mL of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 mL of sulfuric acid (2 M) and 7 mL of potassium iodide (1.8 M). The mixed solution was titrated with 5.09 mM sodium thiosulfate until yellow color disappeared (Zhang, 2000). The percentage of scavenging of hydrogen peroxide was calculated as:

$$H_2O_2 \text{ scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

where, V_{cont} was volume of sodium thiosulfate used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_{test} was the volume of sodium thiosulfate solution used in the presence of extract.

Determination of Total Phenolic Compounds using Folin-Ciocalteu Phenolic Reagent

One milliliter of extract solution (1000 μg of the extract) in a volumetric flask diluted with distilled water (46 mL). Folin-Ciocalteu reagent (1 mL) was added and the contents of the flask were mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (Slinkard and Singleton, 1977). The amount of total phenolic compounds in the FBWE extracts was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.00816 \times \text{Total phenols [Gallic acid equivalents (μg)]} - 0.0135$$

Statistical Analysis

Tests were carried out in triplicate. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Instat software. Results were expressed as graphically/Mean±SD (Gorinstein *et al.*, 2004).

RESULTS AND DISCUSSION

Scavenging Effect on DPPH Radicals

The DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity (Chang *et al*., 2002). Figure 1 shows a significant ($p<0.01$) decrease in the concentration of DPPH radicals due to the scavenging ability of both aqueous extracts and standards. A 250 μg mL^{-1} of FBAE and ascorbic acid (std.) exhibited 96.07 and 90.41% inhibition, respectively and the IC_{50} values were found to be 93.22 and 22.54 μg mL^{-1} for FREA and ascorbic acid (std.), respectively.

Reducing Power

Figure 2 shows the reductive capability of the FREA to ascorbic acid (standard). For the measurement of the reductive ability, we investigated the Fe^{3+} - Fe^{2+} transformation in the presence of FREA using the method of Oyaizu (1986) and Jayaprakasha *et al*. (2001). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). Like the antioxidant activity, the reducing power of the extracts increased with increasing the concentration (0.1-1.0 mg mL^{-1}). The reducing power showed by the extract is statistically significant ($p<0.01$). The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the

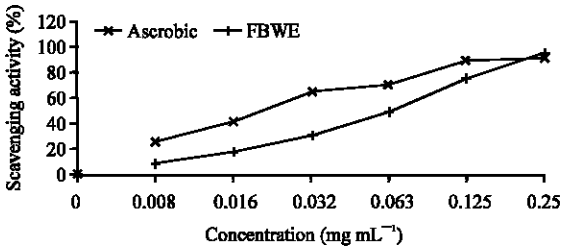

Fig. 1: The DPPH radical scavenging activity of Ficus bengalensis aqueous extract at different concentrations. Each value represents Means±SD (n = 3)

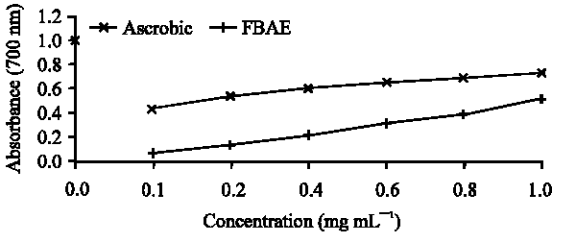

Fig. 2: Reducing power of Ficus bengalensis aqueous extract at different concentrations. Each value represents Means±SD (n = 3)

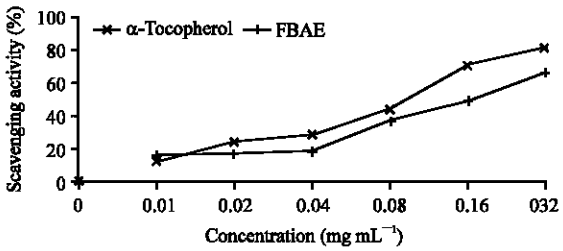

Fig. 3: H_2O_2 radical scavenging activity of Ficus racemosa ethyl acetate extract at different concentrations. Each value represents Means±SD (n = 3)

binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging (Diplock, 1995).

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly, once inside the cell, hydrogen peroxide can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Miller *et al*., 1993). Figure 3 clearly shows that extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with an IC_{50} of 0.65 mg mL^{-1}, while of standard (ascorbic acid) as 0.507 mg mL^{-1}.

Phenolic Content

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). One milligram of extract contained 25.34 μg gallic acid equivalents of phenols, respectively. Phenolic compounds are famous powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992). It has been suggested that up to 1.0 g polyphenolic compounds (from diet rich fruits or vegetables) ingested daily have remarkable inhibitory effects on mutagenesis and carcinogenesis in humans (Tanaka *et al*., 1998). In addition, it has been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation (Yen *et al*., 1993).

CONCLUSIONS

The findings of the present study explored the antioxidant potential of the plant extract by 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity and hydrogen peroxide activity. The polyphenolic content responsible for antioxidant activity may be the mechanism of action, justifying the therapeutic effectiveness of the drug.

REFERENCES

Auddy, B., M. Ferreira, F. Blasina, L. Lafon and F. Arredondo *et al*., 2002. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J. Ethnopharmacol., 84: 131-138.

Blois, M.S., 1958. Antioxidant determination by the use of stable free radicals. Nature, 181: 1199-2000.

Chang, L.W., W.J. Yen, S.C. Huang and P.D. Duh, 2002. Antioxidant activity of sesame coat. Food Chem., 78: 347-354.

Diplock, A.T., 1995. Will the good fairies please prove to us that vitamin E lessens human degenerative disease. Free Radical Res., 27: 511-532.

Geissman, T.A., 1995. Modern methods of plant analysis. Springer Verlag, Berlin.

Gorinstein, S., R. Haruenkit, Y.S. Park, S.T. Jung and Z. Zachwieja *et al*., 2004. Bioactive compounds and antioxidant potential in fresh and dried Jaffa sweeties, a new kind of citrus fruit. J. Sci. Food Agric., 84: 1459-1459.

Gutteridgde, J.M.C., 1995. Free radicals in disease processes: A complication of cause and consequence. Free Radic. Res. Comm., 19: 141-158.

Hatano, T., R. Edamatsu, A. Mori, Y. Fujita and E. Yasuhara, 1989. Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl radical. Chem. Pharm. Bull., 37: 2016-2021.

Jayaprakasha, G.K., R.P. Singh and K.K. Sakariah, 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73: 285-290.

Khandelwal, K.R., 2000. Practical Pharmacognosy, Techniques and Experiments. 2nd Edn., Nirali Prakashan, Pune, India.

Lai, L.S., S.T. Chou and W.W. Chao, 2001. Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. J. Agric. Food Chem., 49: 963-968.

Meir, S., J. Kanner, B. Akiri and S.P. Hadas, 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem., 43: 1813-1819.

Miller, M.J., Sadowska-Krowicka, S. Chotinaruemol, J.L. Kakkis and D.A. Clark, 1993. Amelioration of chronic ileitis by nitric oxide synthase inhibition. The J. Pharmac. Experi. Thera., 264: 11-16.

Nayana, J. and K.K. Janardhanan, 2000. Antioxidant and antitumour activity of *Pleurotus folrida*. Curr. Sci., 79: 941-943.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucosamine. Jap. J. Nutr., 44: 307-315.

Shahidi, F. and P.K.J.P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103.

Sharma, P.V., 1996. Classical Uses of Medicinal Plants. Chaukhamba Visvabharti Oriental Publishers and Distributors, Varanasi, India, pp: 218.

Shrotrim, D.S. and R. Aiman, 1960. The relationship of the post absorptive state to the hypoglycaemic action studies on *Ficus bengalensis*. Ind. J. Med. Res., 48: 162-163.

Shukla, R., K.M. Prabhu and P.S. Murthy, 1994. Hypoglycaemic effect of the water extract of *Ficus bengalensis* in alloxan recovered, mildly diabetic and severely diabetic rabbits. Int. J. Diabetes Dev. Count., 14: 78-81.

Shukla, R., K. Anand, K.M. Prabhu and P.S. Murthy, 1995a. Hypocholesterolemic effect of water e xtract of the bark of banyan tree, *Ficus bengalensis*. Ind. J. Clin. Biochem., 10: 14-18.

Shukla, R., K. Anand, K.M. Prabhu and P.S. Murthy, 1995b. Hypolipidaemic effect of water extract of the bark of *Ficus bengalensis* in alloxan induced diabetes mellitus in rabbits. Ind. J. Clin. Biochem., 10: 119-121.

Shukla, R., S. Gupta, J.K. Gambhir, K.M. Paha and P.S. Murthy, 2004. Antioxidant effect of aqueous extract of the bark of *Ficus bengalensis* in hypercholest-erolaemic rabbits. J. Ethanopharmacol., 92: 47-55.

Slinkard, K. and V.L. Singleton, 1977. Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic, 28: 49-55.

Soares, J.R., T.C.P. Dins and A.P. Cunha, 1997. Antioxidant activity of some extracts of *Thymus zygis*. Free Radic. Res., 26: 469-478.

Tanaka, M., C.W. Kuei, Y. Nagashima and T. Taguchi, 1998. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi, 54: 1409-1414.

Tiwari, A., 2001. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. Curr. Sci., 81: 1179-1187.

Yen, G.C., P.D. Duh and C.L. Tsai, 1993. Relationship between antioxidant activity and maturity of peanuts hulls. J. Agric. Food Chem., 41: 67-70.

Zhang, X.Y., 2000. Principles of Chemical Analysis. China Science Press, Beijing, China, pp: 275-276.