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In vitro antioxidant studies of *Ficus racemosa* Linn. root

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ABSTRACT

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases. Modern research is now directed towards natural antioxidants originated from plants due to safe therapeutics. Antioxidant activity of ethyl acetate extract of *Ficus racemosa* (FREA) root was investigated for its free radical scavenging activity by adopting various *in vitro* models. The extract was investigated for its antioxidant activity using Barotene-linoleate oxidation method, 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity, hydrogen peroxide scavenging activity and determination of total phenolic content using Folin-Ciocalteu's phenolic reagent. FREA clearly indicated the polyphenolic content (hR_f 89.3, 84.0, 74.6, 86.6). FREA at 250 µg/ml concentration showed maximum scavenging activity of DPPH radical upto 73.11% and for hydrogen peroxide upto 65.42% at 1000 µg/mL.Reducing power of FREA was also dose dependent. The measurement of total phenolic content by Folin-Ciocalteu's phenol reagent indicated that 1mg of FREA contains 8.8 µg equivalent of gallic acid. The antioxidant property of the extract may be due to presence of phenolic content.

KEYWORDS: *Antioxidants, Free radicals, Ficus racemosa Linn., Moraceae*

INTRODUCTION

Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite(1-2).When the balance between ROS production and antioxidant defenses is lost, 'Oxidative stress' results which through a series of events deregulates the cellular functions and leads to various pathological conditions including aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurodegenerative diseases(3).Antioxidants can neutralize the ill effects of free radicals by scavenging or chain breaking (like vitamin A, C, beta carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are "used up" in the process of neutralizing free radicals(4).The antioxidative potential of phenolic compounds can be attributed to their strong capability to transfer electron to ROS/free radicals, chelating metal ions and to activate antioxidant enzymes(5).DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers(6,7). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have restricted use in food industry as they are suspected to be carcinogenic(8).Therefore, the antioxidants with natural origin have been the centre of attraction for the modern researchers.

Ficus racemosa Linn. syn. *Ficus glomerata* Roxb. of family Moraceae also known as gular (Hindi), is a very common plant and distributed throughout India. It has long been used in Indian medicinal practice as astringent, carminative, stomachic, vermicide, etc. It is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhoea, circulatory and respiratory disorders and menorrhagia(9,10).The root is very useful in dysentery, as a powerful tonic and in diabetes(11).The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties(12,13).It is well established that diabetes is associated with low level of antioxidants and many plants show hypoglycemic property due to their antioxidant potential(14-16).So,for establishing a correlation between the antioxidant potential and therapeutics the *in vitro* antioxidant studies were carried out. Polyphenolic compounds like flavonoids, tannins are mainly responsible for the antioxidant activity. Hence, qualitative chemical tests were performed to determine the flavonoids and tannins. The antioxidant potential of the extract was evaluated by thin layer

chromatography on *B*-carotene-linoleate oxidation model, 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity, hydrogen peroxide scavenging activity and determination of total phenolic content using Folin-Ciocalteu's phenolic reagent.

MATERIALS AND METHODS

Chemicals

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

Plant material and extract

The roots of the plant *Ficus racemosa* Linn. were taken from the outskirts of Dist. Sirsa, Haryana and authenticated (Voucher No. PARC/2007/21) by Dr. P. Jayaraman, Scientist, Plant Anatomy Research Centre, Chennai. A voucher specimen has been retained in department of Pharmacognosy, Guru Jambheshwar University of Science & Technology, Hisar. The roots were dried under shade, coarsely powdered and 50 g root powder was extracted with 400mL of ethylacetater 72 h by hot continuous extraction method using soxhlet apparatus. The ethyl acetate extract was filtered. The extract was concentrated by evaporating the solvent under reduced pressure and dried in vacuum. The dried extract (FREA) thus obtained was used for the assessment of antioxidant activity through various *in vitro* models. Preliminary qualitative analysis(17,18)showed the presence of flavonoids, tannins, steroids, proteins and starch. FREA was used for antioxidant studies.

B-Carotene-linoleate oxidation method(19)

The extract was subjected to TLC plates on 20 X 20 cm precoated silica gel G plates. The developing solvent system used was chloroform : methanol (9:1 v/v) for flavonoids and chloroform : ethylacetate : formic acid (5 : 4 :1 v/v) for free phenolic compounds. The locations of the spots were marked under UV light. *B*-carotene-linoleate (a mixture of *B*-carotene (6 mg) in 30 ml of chloroform and 2 mL of purified linoleic acid in 60 ml of 95% ethanol) was sprayed uniformly on the plates and exposed to daylight for about 4 h. The background was bleached and the spots which contained the flavanoids and phenolic compounds retained the yellow color which is indicative of antioxidant activity.

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(20,21).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 forty-five minutes 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) of extracts that inhibits the formation of DPPH radicals by 50%.

Reducing power assay(22,23)

The different concentration of the extracts (100-1000 µg/mL) in 1 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and 1% potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL). The mixture was incubated at 50°C for 20 minutes. The reaction was stopped by adding trichloroacetic acid (2.5 mL, 10%) to the mixture, which was then centrifuged for at 1000 x g for 10 minutes. 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. Ascorbic acid was taken as a reference.

Determination of hydrogen peroxide scavenging activity(24)

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 (2M) and 7 mL of potassium iodide (1.8 M). The mixed solution was titrated with 5.09 mM sodium thiosulfate until yellow

color disappeared. The percentage of scavenging of hydrogen peroxide was calculated as:

$$\text{Hydrogen peroxide Scavenged (\%)} = \frac{(V_{cont}-V_{test}) \times 100}{V_{cont}}$$

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 content using Folin-Ciocalteu phenolic reagent (25)

1 mL 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 minutes, 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. The amount of total phenolic content in the FREA extracts was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard gallic acid graph:

Absorbance = 0.00816 X 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 statistics software. Results were expressed as graphically/mean ± standard deviation.

RESULTS AND DISCUSSION

B-Carotene-linoleate oxidation method

Qualitative chemical tests confirmed the presence of polyphenolic contents (flavonoids, tannins) in the extract, further, when antioxidant property was assessed by TLC method, three (hR_f 89.3, 84.0, 74.6) spots were obtained for free phenolic compounds when run in chloroform : ethyl acetate : formic acid (5:4:1) developing solvent system and one spot was obtained for flavonoids (hR_f 86.6) in chloroform : methanol (9:1) developing solvent system as described by Geissman(19).

DPPH radical scavenging activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule(26).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 (27).

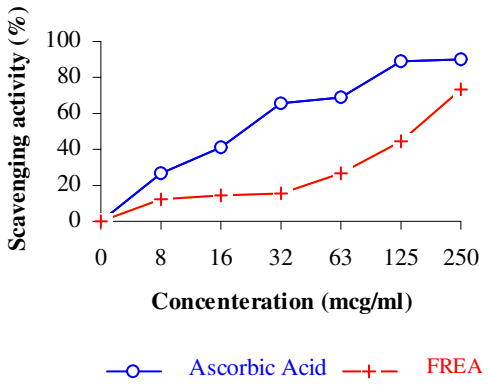

Fig. 1. DPPH radical scavenging activity of Ficus racemosa ethyl acetate extract at different concentrations. Each value represents means ± SD (n=3)

Figure 1 illustrates a significant ($p < 0.01$) decrease in the concentration of DPPH radicals due to the scavenging ability of both ethyl acetate extracts and standards. A 250 µg/mL of FREA and ascorbic acid (std.) exhibited 73.11% and 90.41% inhibition, respectively and the IC_{50} values were found to be 155.81 µg/mL and 22.4 µg/mL for FREA and ascorbic acid (std.) respectively. The results indicate that FREA reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles(28).DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electron taken up(21).

Reducing power activity

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(22)and Jayaprakash(23).The reducing capacity of a compound may serve as a

significant indicator of its potential antioxidant activity(29).

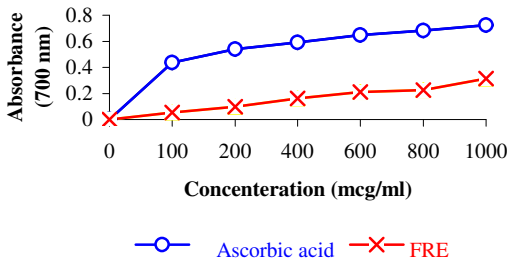

Fig. 2. Reducing power of Ficus racemosa ethyl acetate extract at different concentrations. Each value represents means ± SD (n=3)

Like the antioxidant activity, the reducing power of the extracts increased with increasing the concentration. 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 binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging(30).

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(31).So, it is biologically beneficial for the cells to control the accumulated hydrogen peroxide. Figure 3 clearly shows that extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with an IC_{50} of 0.706 mg/mL, while of standard (ascorbic acid) as 0.507 mg/mL.

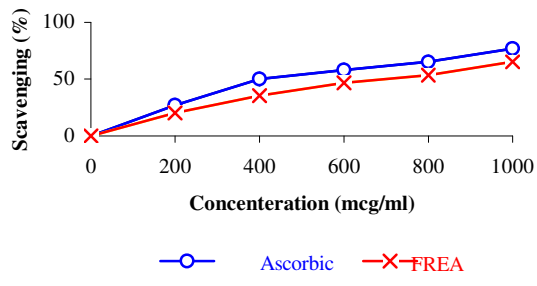

Fig. 3. H_2O_2 radical scavenging activity of Ficus racemosa ethyl acetate extract at different concentrations. Each value represents means ± SD (n=3)

Determination of total phenolic content using Folin-Ciocalteu phenolic reagent(25)

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups(32).One milligram of extract contained 8.8 µg gallic acid equivalents of phenols, respectively. Phenolic compounds are famous powerful chain breaking antioxidants(33).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(34).In addition, it has been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation(35).

CONCLUSION

The results of the present study show that the ethyl acetate extract of *Ficus racemosa* Linn. root possess antioxidant activity through the DPPH radical scavenging activity, hydroxyl radical scavenging activity, reducing power, hydrogen peroxide scavenging activity. Preliminary phytochemical analysis and *B*-carotene linoleate oxidation modes indicates the presence of polyphenols (tannins, flavonoids) in FREA. Polyphenols like flvonoids and tannins are the well known natural antioxidants(36).So, the antioxidant potential of FREA may be due to the presence of polyphenolic content.

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REFERENCES

1. N. Dasgupta and B. De. Antioxidant activity of *Piper betle* L. leaf extract *in vitro*. *Food Chem*. **88**: 219-224 (2004).
2. J.M. David, A.L.B.S. Barreisors and J.P. David. Antioxidant phenyl propanoid esters of triterpenes from *Dioclea lasiophylla*. *Pharm. Biol.* **42**: 36-38 (2004).
3. V.K. Gupta and S.K. Sharma. Plants as natural antioxidants. *Nat. Prod. Rad.* **5(4)**: 326-334 (2006).
4. V. Kumar and S.K. Sharma. Antioxidant studies on some plants: a review. *Hamdard Medicus* (Pakistan) **XLIX (4)**: 25-36 (2006).
5. P. Cos, L. Ying, M. Calomme, J.P. Hu, K. Cimanga, B. Van Poel, L. Pieters, A.J. Vlietinck and D.V. Berghe. Structurally-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.* **61**: 71-76 (1998).

6. J.R. Totter. Spontaneous cancer and its possible relationship to oxygen metabolism. *Proc. Natl. Acad. Sci.* USA **77(4)**: 1763-1767 (1980).
7. B.N. Ames, M.K. Shigenaga and T.M. Hagen. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* USA **90**: 7915-7922 (1993).
8. G.K. Jayaprakasha, T. Selvi and K.K. Sakariah. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extract. *Food Res. Int.* **36**: 117-122 (2003).
9. A.K. Nadkarni. Indian Materia Medica, 3rd ed., (Popular Book Depot, Bombay, 1954).
10. R.N. Chopra, I.C Chorpra, K.L. Handa and L.D Kapin. Indigenous Drugs of India, (U. N. Dhur & Sons Private Ltd., Calcutta, 1950).
11. K.R. Kiritikar and B.D. Basu. Indian Medicinal Plants, 2nd ed, Vol III, (Bishen Singh, Mahendra Pal Singh Dehradun, 1975) pp. 2328.
12. M.G. Hertog, E.J. Feskens, P.C. Hollman, M.B. Katan and D. Kromhout. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **342**: 1007-1011 (1993).
13. Z. Zhang, Q. Chang, M. Zhu, Y. Huang, W.K.K. Ho and Z.Y. Chen. Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem.* **12**: 144-152 (2001).
14. U.K. Mazumdar, M. Gupta and Y. Rajeshwar. Antihyperglycemic effect and antioxidant potential of *Phyllanthus niruri* (Euphorbiaceae) in streptozotocin induced diabetic rats. *European Bull. Drug Res.* **13**: 15-23 (2005).
15. L.M. McCune and Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous people of the North American boreal forest. *J. Ethnophamacol.* **82**: 197-205 (2002).
16. M.C. Garg and D.D. Bansal. Protective antioxidant effect of vitamin C and E in streptozotocin induced diabetic rats. *Indian J. Exp. Biol.* **38(2)**: 101-104 (2000).
17. S.H. Lee. Antioxidative activity of browning reaction products isolated from storage-aged orange juice. *J. Agric. Food Chem.* **40**: 550-552 (1992).
18. Khandelwal KR. Practical Pharmacognosy, Techniques and Experiments, 2nd Ed. (Nirali Prakashan, Pune, 2000).
19. Geissman TA. Modern methods of plant analysis, 3, (Berlin, Gottingen, Heidelberg. Springer Verlag, 1995) pp. 450.
20. Blois MS. Antioxidant determination by the use of a stable free radical. *Nature*. **181**: 1199-1200 (1958).
21. Lai LS, Chous ST & Chao WW. Studies on the antioxidant activities of hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *J. Agric. Food Chem.* **49**: 963-968 (2001).
22. M. Oyaizu. Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.* **44**: 307-315 (1986).
23. G.K. Jayaprakash, R.P. Singh and K.K. Sakariah. Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. *J. Agric. Food Chem.*, **55**: 1018-1022 (2001).
24. X.Y. Zhang. Principles of Chemical Analysis. (China Science Press, Beijing, 2000) pp. 275-276.
25. K. Slinkard and V.L. Singleton. Total phenol analysis:automation and comparison with manual methods. *Am. J. Enol. Vitic.* **28**: 49-55 (1977).
26. J.R. Soares, T.C.P. Dins, A.P. Cunha and L.M. Ameida. Antioxidant activity of some extracts of *Thymus zygis*. *Free Rad. Res.* **26**: 469-478 (1997).
27. L.W. Chang, W.J. Yen, S.C. Huang and P.D. Duh. Antioxidant activity of sesame coat. *Food Chem.* **78**: 347-354 (2002).
28. C. Sanchez-Moreno. Review: Methods used to evaluate the free radical scavenging activity in foods & biological systems. *Food Sci. Tech. Int.* **8**: 121-137 (2002)
29. S. Meir, J. Kanner, B. Akiri and S.P. Hadas. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J. Agric. Food. Chem.* **43**: 1813 – 1815 (1995).
30. A.T. Diplock. Will the 'good fairies' please proves to us that vitamin E lessens human degenerative of disease? *Free Rad. Res* **27**:511-532 (1997).
31. M.J. Miller, Sadowska-krowicka, S. Chotinaruemol, J.L. Kakkis and D.A. Clark. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmac. Exp. Thera.* **264**: 11-16 (1993).
32. T. Hatano, R. Edamatsu, A. Mori, Y. Fujita and E. Yasuhara. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem. Pharm. Bull*. **37** 2016-2021 (1989).
33. F. Shahidi and P.K.J.P.D. Wanasudara. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **32**: 67-103 (1992).
34. Tanaka M, Kuei CW, Nagashima Y and Taguchi T. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products, *Nippon Suisan Gakk* **54**: 1409-1414 (1998).
35. G.C. Yen, P.D. Duh and C.L. Tsai. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.* **41**: 67-70 (1993).
36. I.E. Dreosti. Antioxidant polyphenols in tea, cocoa and wine. *Nutr.* **16**: 692-694 (2000)