Study on Anti-oxidant Activity of Unripe Fruit of *Ficus glomerata* (Roxb.) using *In-vitro* Models

Yusufuddin Irfan[1], Mohd Asif khan[2], Shivakumar H.[3]

[1]Dept. of Pharmacology, S.C.S. College of Pharmacy, Harapanahalli, Davangere Dist. (Karnataka) 583131. India
[2]Dept. of Pharmacology, Faculty of Pharmacy, VCTE, Bijnor- 246701 (U.P.) India.
[3]Dept. of Pharmacology, Faculty of Pharmacy, BLD University, Bijapur – 586101, (Karnataka), India

ABSTRACT

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. India is blessed with enormous biodiversity resources, but plagued with several diseases, including those with ROS as the etiological factor. The present study was designed to evaluate the antioxidants activities of unripe fruits of *Ficus glomerata* Roxb and three complementary test systems, namely superoxide anion scavenging activity, reducing power, hydroxyl radical scavenging activity were used for the antioxidant analysis. In this study *Ficus glomerata* exhibited strong antioxidant activity.

Keywords: *Ficus glomerata,* reducing power, hydroxyl radical, superoxide anion, antioxidant.

Corresponding author: Mohd Asif Khan, Assit. Professor, Dept. of Pharmacology, Faculty of Pharmacy VCTE, Bijnor- 246701 (U.P.) India, E-mail: asifmpharma@gmail.com, Phone: +91 7376762117

DOI: 10.5530/ax.2011.4.11

INTRODUCTION

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA[1-2]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others[3-8]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders[9-11]. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. For instance in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[12-16]. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention[17].

In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value[18]. Antioxidant derived from fruits, vegetables, spices and cerials are very effective and have reduced interference with the body's ability to use free radicals constructively[19-20].

One of such plant is *Ficus glomerata* (Roxb) has been used in traditional system of medicine for treating diabetes, liver diseases, piles, asthma, leprosy and diarrhea[21]. The hepatoprotective activity of leaves of *Ficus glomerata* has been reported [22]. Leaves shows anti-bacterial activity[23], stem bark shows anti-tussive potential[24], anti-diuretic activity[25], anti-pyretic potential[26], anti-inflammatory activity of the leaves, bark and unripe fruit[27-29], hypoglycemic activity of roots, leaves and fruit[30-32] and anti-filarial activity of the fruits[33].

However, there is no scientific claims has been made regarding the antioxidant activity of unripe fruits of *Ficus glomerata*. In view of this, in the present investigation an attempt will be made to study of antioxidant activity unripe fruit extract of *Ficus glomerata*.

MATERIAL AND METHODS

Plant material

The unripe fruits of *Ficus glomerata* were collected from the surrounding fields of Harapanahalli. The identification of plant was made by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of our college.

Prepration of Extract

The unripe fruits were collected in the month of April. The unripe fruits were dried in shade at room temperature. The dried unripe fruits were powdered by using grinder, to coarse powder and this powder was packed into soxhlet column and the extracted 70% ethanol (60 – 80^0) for 24 hrs. The extract was concentrated under reduced pressure with the use of rotatory flash evaporator. Further the extracts were concentrated by using hot water bath (70 – 80^0). The dried extract was stored in airtight container in refrigerator below 10^{0}C.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the 70% ethanolic extract of unripe fruit of *Ficus glomerata* for qualitative identification. The tests for common phytochemicals were carried out by standard methods described in practical pharmacognosy by K.R. Khandelwal[34].

Antioxidant activity assays

Superoxide anion scavenging activity [35]

About 1 ml of nitroblue tetrazolium (NBT) solution (156 M NBT in 100 mM phosphate buffer, pH 7.4). 1 ml NADH solution (468 M in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of ethanolic extract of *Ficus glomerata* fruit in water was mixed. The reaction was started by adding 100 l of Phenazine Methosulphate (PMS) solution (60 M PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25^{0}C for 5 minutes and the absorbance at 560 nm was measured against blank. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The results are compiled in Table 1 and graphically represented in Fig. 1.

Total Reducing power [36]

Different doses of ethanolic extract of *Ficus glomerata* unripe fruit were mixed in 1 ml of distilled water so as to get 10 g, 25 g, 50 g and 100 g concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50^{0}C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 100 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power. The percentage increase of reducing power was calculated by using the formula mentioned in the estimation of superoxide anion scavenging activity in the earlier page. The results are compiled in Table 1 and graphically represented in Fig. 2.

Hydroxyl radical scavenging activity [37]

Hydroxyl radical generation by phenyhydrazine has been measured by the 2-deoxyribose degradation, assay of Hathwell and Gutterridge in 56 mM phosphate buffer (pH 7.4) containing 1 mM deoxyribose, 0.2 mM phenylhydrazine hydrochloride and other additions as necessary in a total volume of 1.6 ml incubating was terminated after 1 hr or 4 hrs and 1 ml each of 2.8% TCA and 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 20 min in a boiling water bath. The tubes were cooled and absorbance taken at 532 nm. Decrease in absorbance is indicating the increase in the hydroxyl free radical scavenging activity.

Table 1. Anti-oxidant activity of unripe fruit of Ficus glomerata (Roxb.) using in-vitro models

	Superoxide anion scavenging activity		Total Reducing power activity		Hydroxyl radical scavenging activity			
Group	Absorbance Mean ± SEM	% Inhibition	Absorbance Mean ± SEM	% Inhibition	Absorbance Mean ± SEM (after 1 hr.)	% Inhibition	Absorbance Mean ± SEM (after 4 hr.)	% Inhibition
Control	0.585 ± 0.0045	--	0.146 ± 0.0005	--	0.078 ± 0.001	--	0.069 ± 0.0005	--
Standard 25 g (Sodium Metabisulphate)	0.287 ± 0.0038	71%	0.250 ± 0.005	75%	0.029 ± 0.0015	66.95%	0.027 ± 0.0005	69.15%
Ethanolic extract 10 g	0.437 ± 0.0052	43%	0.0159 ± 0.0005	12%	0.062 ± 0.0025	25%	0.057 ± 0.0045	32.17%
Ethanolic extract 25 g	0.368 ± 0.005	56%	0.099 ± 0.0005	38%	0.051 ± 0.0018	38.96%	0.048 ± 0.0060	40.88%
Ethanolic extract 50 g	0.450 ± 0.0005	41%	0.154 ± 0.0005	62%	0.038 ± 0.0022	55.65%	0.033 ± 0.005	59.75%
Ethanolic extract 100 g	0.272 ± 0.0049	73%	0.184 ± 0.0005	67%	0.028 ± 0.0011	68.47%	0.027 ± 0.0040	69.15%

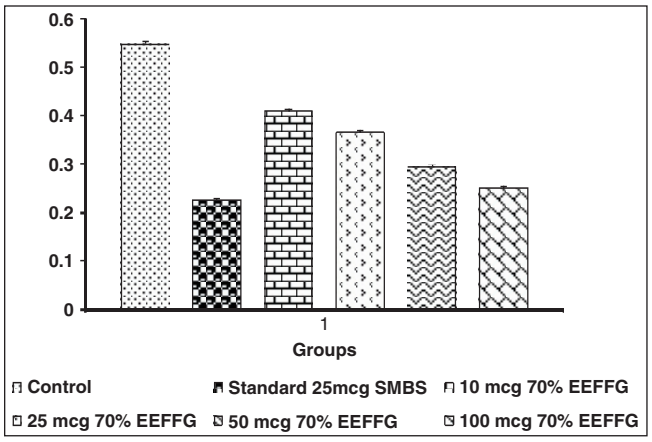

Figure 1. Superoxide anion scavenging activity of unripe fruits of *Ficus glomerata.*

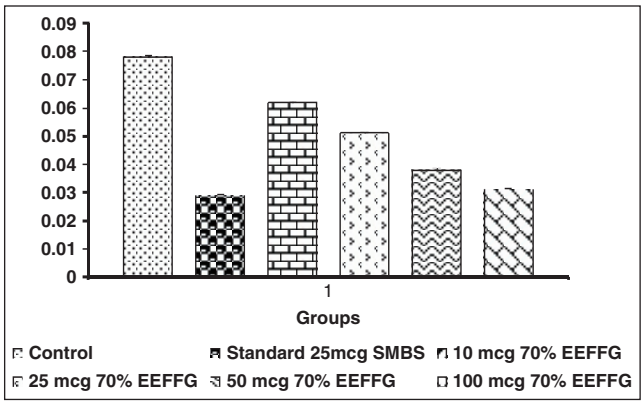

Figure 3. Hydroxyl radical scavenging activity of unripe fruits of *Ficus glomerata.*

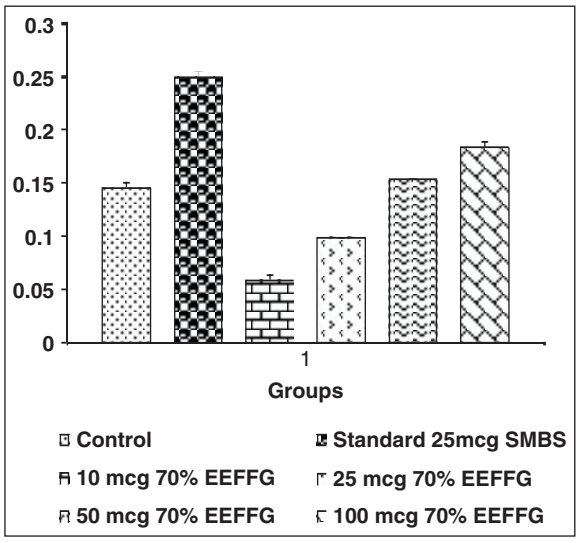

Figure 2. Reducing power activity of extract of unripe fruits of *Ficus glomerata.*

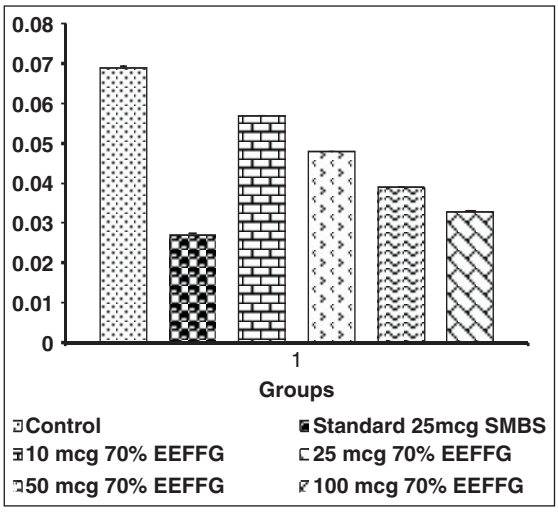

Figure 4. Hydroxyl radical scavenging activity of unripe fruits of *Ficus glomerata*

The percentage reduction in the absorbance is calculated and results are compiled in Table 1 graphically represented in Fig. 3, 4.

% inhibition was calculated by using the following formula

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and At was the Absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Statistical analysis

The replicates of each sample were used for statistical analysis. Analysis of the data was performed on the original data by one-way analysis of variance (ANOVA). Differences at $P < 0.05$ were considered significant

RESULTS

Superoxide anion scavenging activity of unripe fruit extract of Ficus glomerata

It was observed that the test extract demonstrated significant percentage increase in the superoxide anion scavenging activity at the higher dose. 25 g sodium metabisulphate (standard) showed 71% superoxide anion scavenging property whereas the test extract at 25 g and 100 g had shown 56% and 73% superoxide anion scavenging property. On comparison with standard, the test extract at 100 g has shown a better activity.

Total Reducing power activity of unripe fruit extract of Ficus glomerata

It was observed that the test extract demonstrated dose dependent increase in reducing property. 25 g sodium metabisulphate (standard) showed 75% reducing property, whereas the test extract at 100 g had more reducing property than standard i.e. 87%.

Hydroxyl radical scavenging activity of extract of unripe fruit of Ficus glomerata

It was observed that the test extract demonstrated dose dependent percentage increase in hydroxyl radical scavenging activity. 25 g sodium metabisulphate (standard) showed 66.95% (for 1hr. incubation period) and 69.15% (for 4hr. incubation period). The test extract showed a significant activity in 1 hr. incubation period i.e. 68.47% at the concentration 100 g. whereas in 4 hr. incubation period the test extract at the dose of 100 g showed the same percentage increase as that of the standard i.e. 69.15%.

DISCUSSION AND CONCLUSION

The reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical has been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, diabetes mellitus, and cancer[38]. They play an important role in inflammation process after intoxication by ethanol and CCl_4 [39]. The potential mechanism of oxidative damage is the nitration of tyrosine residue of proteins, peroxidation of lipids, degradation of DNA, and also oligonucleosomal fragments[38]. Antioxidant reactions involve multiple steps initiation, propagation, branching, and termination. Antioxidants fall into two mechanistic groups: those that inhibit or retard the formation of free radicals from their unstable precursors (initiation) and those that interrupt the radical chain reaction (propagation and branching). The former are called as preventive antioxidant and the latter as chain-breaking antioxidants[40].

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress[41]. Reducing power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity[42]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants[43]. Anti-oxidant effect of polyphenols (flavonoids) on lipid peroxidation is the result of scavenging of hydroxyl radicals at the stage of initiation and termination of peroxyl radicals[44]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell[45].

From the present study, it was found that the percent inhibition of lipid peroxidation by ethanol fruit extract decreased after a certain concentration which may be due to the degradation or peroxidation of the source. Plant breeders and food producers are increasingly identifying specific genotypes and varieties of fruits and vegetables rich in functional ingredients comprising of nutritive and non-nutritive antioxidants.

The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant materials.

In conclusion, the 70% ethanolic extract of unripe fruits of *Ficus glomerata* demonstrated dose dependent reduction in reducing power activity, superoxide anion scavenging activity, hydroxyl radical scavenging activity.

REFRENCES

1. Gutteridge, JMC. Free radicals in Disease Processes: A Compilation of cause and Consequence. Free radic. Res. Comm. 1995; 19:141.
2. Halliwell, B. How to characterize an antioxidant: an update. Biochem. Soc. Symp. 1995; 61:73-101.
3. Squadriato GL, Pelor WA. Free Rad. Oxidative chemistry of nitric oxide: The roles of super oxide, peroxynitrite, and carbon dioxide. Biol. Med. 1998; 25:392-403.
4. Devasagayam T PA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Review: Free radicals and antioxidants in human health: Current status and future prospects. J. Assoc. Phys. India 2004; 52:794- 804.
5. Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu OI. In vitro antioxidant properties of dantrolene sodium. Pharmacol. Res. 2001; 44:491-595.
6. Shahidi F, Wanasundara PKD. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 1992; 32:67-103.
7. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L.). Ach. J. Ethnopharmacol. 2002; 79:325-329.
8. Branien AL. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. JAOCS. 1975; 52:59-63.
9. Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J. Natl. Cancer Inst. 1983; 70:343-347.

10. Rice-Evans CA, Sampson J, Bramley PM, Hollowa DE. Why do we expect carotenoids to be antioxidants in vivo. Free Rad. Res. 1997; 26:381-398.
11. Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr. Sci. 2001; 81:1179.
12. Joyce DA. Oxygen radicals in disease. Adv. Drug Reac. Bull. 1957; 127: 476.
13. Gamble JS. Flora of the presidency of Madras. 1957; 1:32.
14. Nair NC. Henry A.N. Flora of Tamilnadu, India Series 1, Analysis, 1983; 13.
15. Mali RG, Hundiwale JC, Sonawane RS, Patil RN, Hatapakki BC. Evaluation of Capparis deciduas for Anthelmintic and Antimicrobial activities. Indian J. Nat. Prod. 2004; 20(4):10-12.
16. Chaudhary SR, Chavan MJ, Gaud RS. Anti inflammatory and Analgesic activity of *Capparis zeylanica* root extracts. Indian J. Nat. Prod. 2004; 20(1):36-39.
17. Ekramul H, Haque MM, Raheman M, Satyajit DS. 2004. E-Octodec-7-en-5 ynoic acid fom the root of *Capparis zeylanica*. Fitoterapia 75:130-133.
18. Ajila CM, Naidu KA, Bhat UJS, Rao P. *Food Chemistry.* 2007; 105: 982-988.
19. Kahkonen MP, Hopia AI, Vuorela HJ, Raucha JP, Pihlaja K, Kujala T S *et al*. *Journal of Agricultural and Food Chemistry***.** 1999; 47: 3954-3962.
20. Wolfe K, Xianzhong WU, Liu RH. *Journal of Agricultural Food Chemistry.* 2003; 51:609-614.
21. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants, Madras. Oriental Longman Ltd., 1996; (3): 34p.
22. Mandal SC, Maity TK, Das J, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rat. Phytotherapy Research 1999; 13(5): 430-32.
23. Mandal SC, Shah BP, Pal M. Studies on antibacterial activity of *Ficus glomerata* Linn. leaf extract. Phytotherapy Research 2000; 14(4): 278-80.
24. Bhaskara Rao R, Murugesan T, Pal M, Saha BP, Mandal SC. Antitussive potential of methanol extract of stem bark of *Ficus glomerata* Linn. Phytotherapy Research 2003; 17(9): 1117-18.
25. Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Biology Hung 2003; 54(3-4): 357-63.
26. Rao RB, Anupama K, Swaroop KR, Murugesan T, Pal M, Mandal SC. Evaluation of anti-pyretic potential of *Ficus glomerata* bark. Phytomedicine 2002; 9(8): 731-733.
27. Mandal SC, Maity TK, Das J, Saha BP, Pal M. Antiinflammatory evaluation of *Ficus racemosa* Linn.leaf extract.J Ethnopharmacol 2000; 72(1-2): 87-92.
28. Rachel W Li, Stephen P Myers, David N Leach, David Lin G, Greg Leach. Across-cultural study: Anti-inflammatory activity of Australian and Chinese plants. J Ethnopharmacol 2003; 85(1): 25-32.
29. Shivakumar H, Sankara SLVJ, Vaidya VP. Anti-inflammatory activity of the unripe fruits of *Ficus glomerata*. Indian Drugs 2007; 44(1): 48-50.
30. Ajit Kar, Choudhary BK, Bandyopathdhy NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003; 84(1): 105-08.
31. Mandal SC, Mukharjee PK, Das J, Pal M, Saha BP. Hypoglycemic activity of *Ficus racemosa* Linn leaves in streptozotocin induced diabetic rat. Nat Prod Sci 1997; 3(1): 38-41.
32. Patil KS, Varke PD, Chaturvedi SC. Hypoglycemic properties of *Ficus glomerata* fruits in alloxan induced diabetic rat. J Nat Rem 2006; 6(2): 120-23.
33. Vandan M, Nizam UK, Singhal KC. Potential antifilarial activity of fruit extracts of *Ficus racemosa* Linn. against Setaria cervi in vitro. Indian J Exp Biol 2005; 43: 346-350.
34. Khandelwal KR. Practical Pharmacognosy. 11th ed. Pune: Nirali Prakashan 2004: 149p.
35. Ilhas Gulcin, Munir Oktay, Irfan KVO, Ali Aslan. Determination of antioxidant activity of *lichen Cetraria islandica* (L) Ach. J Ethnopharmacol 2002; 79: 325-329.
36. Oyaizu M. Studies on product of browing reaction preparation from glucose amine. Jap J Nutrition 1986; 44: 307-09.
37. Barry halliwell, John GMC. Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of Iron salts. FEBS Letters 1981; 128(2): 347-52.
38. Hemnani T, Parihar MS. Reactive oxygen species and oxidative DNA damage. Indian J Physiology Pharmacology. 1998; 42: 440-52.
39. Yoshikawa T, Tanaka H, Yoshida N, Seto O, Sunigo N, Kondo M. Adjuvant arthritis and lipid peroxide protection by suproxide dismutase. Lipid peroxide research 1983; 7: 108-110.
40. Vinqvist MR, Barclay LR. On the antioxidants mechanism of curcumin: classic methods are needed to determine antioxidant mechanism and activity. Org Lett 2000; 2: 2841-2843.
41. Meyer AS, Isaksen A. Application of enzymes as food antioxidants. Trends in Food Science and Technology 1995; 6: 300-304.
42. Oktay M, Gulcin I, Kufre Vioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Feoniculum vulgare)* seed extracts. Lebanon Wissen Technology 2003; 36: 263-271.
43. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenecity. Journal of Agriculture and Food Chemistry 1995; 43: 27-32.
44. Hussain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987; 26: 2489-2491.
45. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. Anal. Biochem. 1987; 165: 215-219.