Research Article

ESTIMATION OF ANTIOXIDANT POTENTIAL OF AQUEOUS EXTRACT OF *FICUS BENGALENSIS* LEAF ON GASTRIC ULCER

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ABSTRACT

In this study, antioxidant activity of aqueous extract of *Ficus bengalensis (FBE)* leaf extract was investigated for its free radical scavenging activity by adopting various *in vitro and in vivo* models. The extract was investigated for its antioxidant activity by DPPH radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity, hydrogen peroxide activity, determination of total phenolic content using Folin-Ciocalteu's phenolic reagent showed maximum scavenging of DPPH radical (89.26%) at 250 mg ml^{-1} concentration and hydrogen peroxide (50.61%) at 1000mg ml^{-1} concentration, respectively and the IC_{50} were to be 67.10 and 23.54 µg/ mL, for FREA and ascorbic acid (Std.) Reducing power was also dose dependent and total phenolic content evaluated that 1 mg of FBE contains 5.2µg equivalent of gallic acid. In case of in vivo antioxidant activity, on lipid peroxidation (LPO), $P<0.05$, superoxide dismutase (SOD), $P<0.01$, and catalase (CAT), $P<0.001$ activity were found to be significant at various level in both models.

Keywords: Antioxidant, Aqueous extract, *Ficus bengalensis*, Free radicals, In vitro, SOD.

INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thioles, ascorbic acid or polyphenols[1]. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. The present study has been designed to investigate the antioxidant potential of *Ficus bengalensis* Linn. leaf. It has now been established, that oxygen derived free radicals, primarily superoxide anion and hydroxyl radical, play an important role in the pathogenesis of acute experimental gastric lesions induced by stress, ethanol and non steroidal anti-inflammatory drugs (NSAIDs), viz. Indomethacin, aspirin etc[2-4]. Ficus compound showed significant antioxidant effects which may be attributed to their polyphenolic nature[5], the bark of *Ficus bengalensis* decreased fasting blood sugar[6], anti-tumor activity[7], antihelmentic activity[8], anti-inflammatory[9], antistress and antiallergic[10], antidiarrhoeal[11], antidiabetic and ameliorative[12], anti-inflammatory[13], Hypolipidemic[14], analgesic & antipyretic[15], wound healing[16].

MATERIALS AND METHODS

Animals

Swiss albino rats weighing (150-240 gm) were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D.

Collection of plant material

The leaves plant of *Ficus bengalensis* (Family -Moraceae) were collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of September 2010. The plant materials were authenticated by Dr. Sayeeda Khatoon, Chemotaxonomist at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

Extraction of *Ficus bengalensis* leaf

The fresh leaves were cleaned by rinsing in clean water, rendered free of adulterants and ground. The aqueous leaf extract was obtained by macerating the ground leaves (2 kg) in distilled water (2 l) for 24 h. The resulting decoction was decanted, filtered and concentrated under pressure in a rotary evaporator (R110 Buchi, Switzerland) at 60°C and dried to a constant weight in an oven set at

40°C. The dried extract gave a yield of 20.14% (w/w) and was stored in an air-tight container at about 4°C until required. The extracts obtained were further subjected pharmacological investigation.

Chemicals

All chemicals except I,I-diphenyl, 2-picry hydrazyl (DPPH), ferricyanide, trichloroacetic acid, gallic acid, hydrogen peroxide, ferric chloride, ascorbic acid, potassium iodide, ammonium molybdate, sodium thiosulfate, Folin-Ciocalteu's phenol reagent, etc. were supplied by research institute.

Preliminary identification of chemical constituents[17-18]

Mainly, polyphenolic compounds like flavonoids, tannins are responsible for antioxidant activity. Preliminary chemical tests were performed to detect the presence of polyphenolic compounds. So, the qualitative chemical tests performed were Shinoda test, ammonia 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.

1) *In-vitro* antioxidant activity

a) Determination of DPPH radical scavenging activity[19-20]

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. 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 run 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:

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

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_{so}. The IC_{so} value was defined as the concentration mg/ml of extracts that inhibits the formation of DPPH radical by 50%.

b) Determination of reduction fe^{2+} transformation on capability by fe^{3+} [21-22]

The different concentration of the extracts (100-1000 mg/ml) in 1 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1.0 % potassium ferricyanide (2.5 ml). The mixture was incubated at 50 cc for 20 min. trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for at 1000 xg for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl, (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Ascorbic acid was taken as a reference.

c) Determination of hydrogen peroxide scavenging activity[23]

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 3.0 % 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. The percentage of scavenging of hydrogen peroxide was calculated as:

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

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

2) *In-vivo* antioxidant activity

a) Lipid peroxidation

Allow all reagents to reach room temperature before use. SDS Solution will take at least one hour if stored at 2-8°C. Heating the SDS Solution at 37°C briefly will dissolve precipitated SDS. SDS Solution can then be stored at room temperature.

Step 1: Collect EDTA plasma, for preparation of other sample types, see sample preparation section of this insert.

Step 2: Label each disposable glass test tube with the standard number or sample identification.

Step 3: Add 100 µl sample or standard to properly labeled tube.

Step 4: Add 100 µl SDS Solution to each tube and swirl to mix.

Step 5: Add 2.5 ml TBA/buffer reagent forcefully down the side of each tube.

Step 6: Cover each tube with a glass marble and incubate at 95°C for 60 min.

Step 7: Remove from incubation and cool to room temperature in an ice bath for 10 min.

Step 8: Centrifuge samples at 3000 rpm for 15 min.

Step 9: Remove supernatant from samples for analysis.

Step 10: Spectrophotometer Analysis*:* Read absorbance of supernatants at 532 nm.

b) SOD determination[24]

SOD was estimated by following the method of. The inhibition of reduction of nitro blue tetrazolium (NBT) to blue coloured formation in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using *n*-butanol as blank. One unit of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the defined assay conditions and the results have been expressed as units (U) of SOD activity/mg protein.

C) Catalase (CAT)[25]

Decomposition of H_2O_2 in the presence of catalase was followed at 240 nm. A 50 µm sample was added to buffered substrate (50 mM phosphate buffer, ph 7.0 containing 10 Mm H_2O_2) to make total volume 3 ml and decrease in the absorbance was monitored at 37^0 c for 2.5 min. at an interval of 15 sec at 240 nm. Results are expressed as units (U) of CAT activity/mg protein.

3) Determination of total phenolic compounds using folin-ciocalteu phenolic reagent[26]

One millilitre of extract solution (1000).lg 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_2C03 (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 compounds in the FBWE 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 (fig)] - 0.013

RESULTS

In-vitro antioxidant activity of ficus bengalensis (leaf)

DPPH (1,1-diphenyl-2-picrylhydrayl) radical scavenging activity[27]

Fig.1 show, 250mg/ml of FBAE and ascorbic acid (std.) exhibited 89.26% and 86.22% inhibition. The DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. 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.

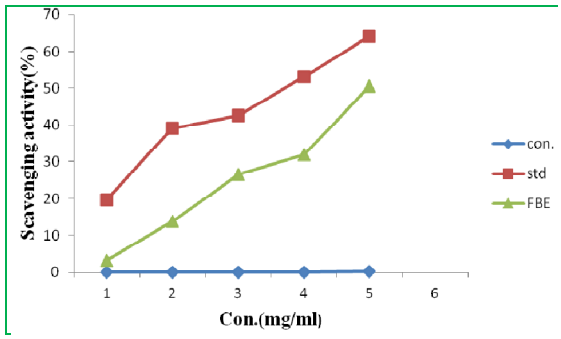

Figure 1: The DPPH radical scavenging activities of *Ficus bengalensis* aqueous extract at different concentrations

Reducing power[28]

Figure 2 shows the reductive capability of the FREA to ascorbic acid (standard). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Like the antioxidant activity, the reducing power of the extracts increased with increasing the concentration (0.1-1.0 mg ml-1).

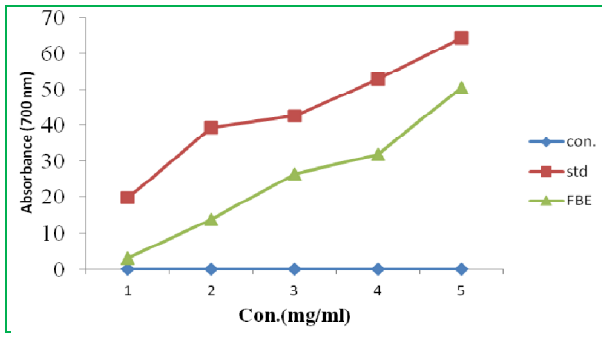

Figure 1: Reducing power of Ficus bengalensis aqueous extract at different concentrations.

Hydrogen peroxide scavenging activity[29]

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiole (-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. Figure 3 clearly shows that extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner.

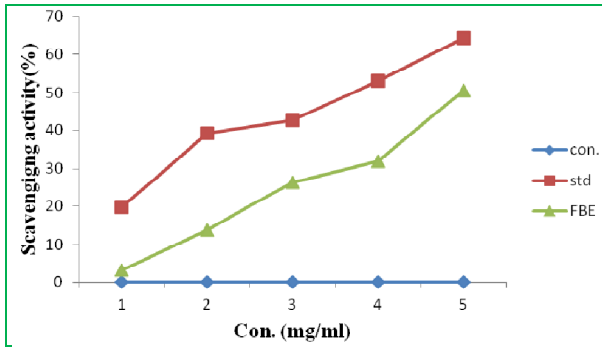

Figure 1: H_2O_2 radical scavenging activity of *Ficus bengalensis* aqueous extract at different concentrations.

2) *In-vivo* antioxidant activity

Table 1: Effect of *Ficus bengalensis* leaf extract (twice daily for five days) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Pylorus ligation induced gastric ulcers.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Control	_	0.52±0.01	98.21±0.29	35.6±0.32
Pylorus ligation	_	0.77±0.04	170.41±22.7	21.5±0.42***
FBA	250	0.44±7.7*	153.3±2.2**	31.8±0.40***
FBA	500	0.39±6.9	132.3±0.6*	26±0.83***

Values are mean± SEM for 6 rats; Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test
Where * represents significant at p<0.05; **represents highly significant at p< 0.01; ***represents very significant at p<0.001. when compared to control group.

Table 2: Effect of *Ficus bengalensis* leaf extract (twice daily for five days) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Ethanol induced gastric ulcers.

Treatment	Dose (mg/kg)	LPO	SOD	CAT
Control	-	0.42±6.9	83.5±16.5	31.6±0.48***
Ethanol	-	0.56±9.8	134.6±0.55	15.5±0.26***
FBA	250	0.45±4.5 *	86.6±0.55***	27.9±0.35***
FBA	500	0.38±0.01	77.8±0.66	25.8±0.84***

Values are mean± SEM for 6 rats; Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test
Where *represents significant at p<0.05; **represents highly significant at p< 0.01; ***represents very significant at p<0.001. when compared to control group.

3) Phenolic content

One milligram of extract contained 5.2µg gallic acid equivalents of phenols respectively.

CONCLUSION

The findings of the present study explored the antioxidant potential of the plant extract by 1,l-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity and hydrogen peroxide activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[30]. Like the antioxidant activity, the reducing power of the extracts increased with increasing the concentration (0.1-1.0 mg/mL^{-1}). 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]. The polyphenolic content responsible for antioxidant activity may be the mechanism of action, justifying the therapeutic effectiveness of the drug. In our present study, the antioxidant property of aqueous extract of leaves of *Ficus bengalensis* were found to make changes in SOD, CAT, and LPO levels in rat gastric mucosa. During the ulcer condition there is increase in gastric mucosal SOD and LPO activities. This indicated that the generation of reactive oxygen species during stress might be the causative factor for the inactivation of gastric peroxidase. *Ficus bengalensis* exerts their antioxidant defensive mechanism probably by metabolising lipid peroxidase scavenging endogenous hydrogen peroxide. One milligram of extract contained 5.2µg gallic acid equivalents of phenols respectively. Phenolic compounds are famous powerful chain breaking antioxidants[32]. 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[33].

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