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Evaluation of *In-Vitro* Antioxidant Activity in *Ficus religiosa* (L.) Leaves

Kumar Hemant[1*], Goswami Mradul[1], Yadav Sanjay[1], Rao Ch. V.[2]

[1]Department of Pharmacy, Saroj Institute of Technology and Management, Lucknow
[2]Division of Pharmacognosy and Ethnopharmacology,
National Botanical Research Institute, Lucknow

ABSTRACT

Medicinal plants have been used in traditional medicine for the treatment of several diseases. In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases and physiological abnormalities under practice such as Ayurveda, Siddha and Unani. Research in herbal medicine has recently been revolutionized with the identification of several botanical plants with established physiological effect and efficacy for clinical condition either alone or combination with pharmaceuticals. Ayurveda is a comprehensive natural health care system that organized in India more than 5000 year ago. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid and tocopherols, polyphenolic compounds, or terpenoids. They perform several important functions in plants and humans. In this study, antioxidant activity of ethanolic extract of *Ficus religiosa* Linn. (EEFR) leaf 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. EEFR showed maximum scavenging of DDPH radical 91.20% at 250 µg/ml concentration and hydrogen peroxide and reducing power were also dose dependent. The IC_{50} values were found to be 71.10µg/ml and 22.5 µg/ml of EEFR and ascorbic acid respectively. The total phenolic content evaluated that 1 mg of extract contained 3.2µg Gallic acid equivalents of phenols respectively. The extract showed significant results when compared with standard groups.

KEYWORDS: In vitro, *Ficus religiosa*, Antioxidant, Ethanolic extract, Free radicals, DPPH

***Corresponding Author**
Hemant Kumar
Department of Pharmacy;
Saroj Institute of Technology and Management, Lucknow
Phone no. +91 8687731655, 9760122018
E- mail: hemant_ch17@rediffmail.com

INTRODUCTION

An antioxidant is a molecule that slows or prevents the oxidation of the molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often considered as reducing agents such as thiols, ascorbic acid, and polyphenols. Oxidation refers to transfer of electrons from a substance to an oxidizing agent[1]. Oxidation reactions results in free radicals, which immediately start chain reactions that result in damage to the living cells. Oxidative stress due to free radicals may lead to a number of ailments. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism.[2, 3]

Ficus religiosa Linn (Moraceae) commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart shaped long tipped leaves on long slender petioles and purple fruits growing in pairs. The tree is regarded as a sacred tree to both Hindus as well as Buddhists. It has got mythological, religious and medicinal importance in Indian culture since ancient times.[4,5,6] Leaves yield campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan.[7-8]

The leaf of *F. religiosa* contained glycosides and tannins, when prepared as ointment form exhibited wound healing activity in rats.[10] Recent study has also revealed that the methanolic leaf extract of *F. religiosa*, which contain high total phenolic and exhibited high antioxidant activity.[11,12]

Figure 1: *Ficus religiosa* plant leaves

MATERIALS AND METHODS

CHEMICALS

All chemicals except 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) and solvents were of analytical grade and were obtained from Research Institute. DPPH and other chemicals used were potassium ferricyanide, trichloroacetic acid, gallic acid, hydrogen peroxide, ascorbic acid, potassium iodide, ammonium molybdate, sodium thiosulfate, Folin-Ciocalteu's phenol reagent, etc.

COLLECTION OF PLANT MATERIAL

The leaves plant of *F.religiosa* were collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of October 2010. The plant materials were authenticated by Dr Tariq Husain, Head & Scientist, Biodiversity & Angiosperm Taxonomy at National Botanical Research Institute; Lucknow and voucher specimens (98145) were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

PREPARATION OF HERBAL EXTRACT:

Ficus religiosa leaves were washed with fresh water to remove adhering dirt and foreign particles and dried at 35 -40^{0}c in an oven. The dried leaves were crushed and grinded to get powder and weighed. The weighed powder was then placed with ethanolic solution in a cylinder. 500g of *Ficus religiosa* powder in 1.0 liter of ethanolic solution were macerated for 7 days. The mensturm was removed and concentrated by vaccum distillation. Again the crude material was allowed to undergo maceration for 4 days followed by 2 days for complete extraction. The mensturm was collected and concentrated using Rotary evaporator at 50°C. This mixture was cooled and filtered by Buchner funnel and filter paper and then air dried in an evaporating dish till constant weight was obtained.[13,14]

IN-VITRO ANTIOXIDANT ACTIVITY

DETERMINATION OF DPPH SCAVENGING ASSAY:

DPPH radical scavenging activity of ethanolic. extract of *Ficus religiosa* L. leaves were determine according to the method reported by Blois. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using

UV-VIS spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.[15]

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

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%.

DETERMINATION OF TOTAL PHENOLICS:

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method as described earlier. An aliquot (100 µl) of the extracts was mixed with 5 ml Folin- Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was recorded against reagent blank at 765 nm using the Simadzu UV-VIS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic contents were expressed as mg/g gallic acid equivalent.[16]

Absorbance ~ 0.00816 x Total phenols [Gallic acid equivalents (sample)] -0.0135

DETERMINATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Hydrogen peroxide scavenging activity of the ethanolic 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 sodiun thiosulfate until yellow colour disappeared.[18] The percentage of scavenging of hydrogen peroxide was calculated as:

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

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

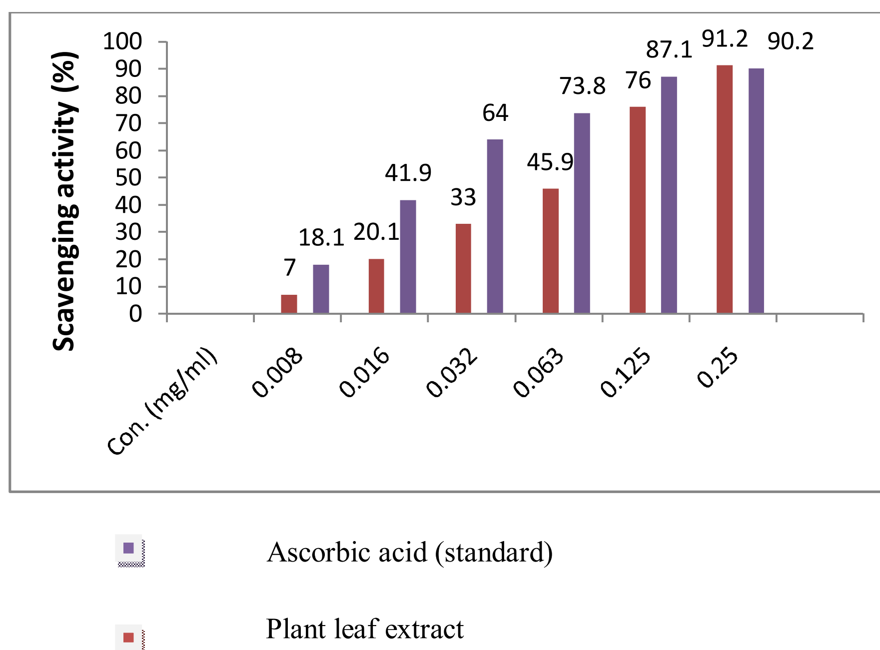
DETERMINATION OF REDUCTION CAPABILITY BY FE_3^+- FE_2^+ TRANSFORMATION

The different concentration of the extracts (100-1000 µg rnL^{-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 [$K_4Fe(CN)_6$] (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 rpm 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. Ascorbic acid was taken as a reference.[19, 20]

RESULTS & DISCUSSION

IN VITRO ANTIOXIDANT ACTIVITY OF *Ficus religiosa* L. (LEAF)

DPPH (1, 1 – DIPHENYL-2-PICRYLHYDRAZYL) RADICAL SCAVENGING ACTIVITY



Ascorbic acid (standard)

Plant leaf extract

Figure 2: The DPPH radical scavenging activity of *Ficus religiosa* ethanolic leaf extract at different concentrations

Line graph show that, EEFR and ascorbic acid (std.) exhibited 91.2% and 90.2% inhibition of free radicals respectively. The DPPH, free radical is a stable 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,

which results in the scavenging of the radical by hydrogen donation. The IC_{50} values were found to be 71.10 µg/ml and 22.5 µg/ml of EEFR and ascorbic acid respectively.

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. Line graph clearly shows that extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner.

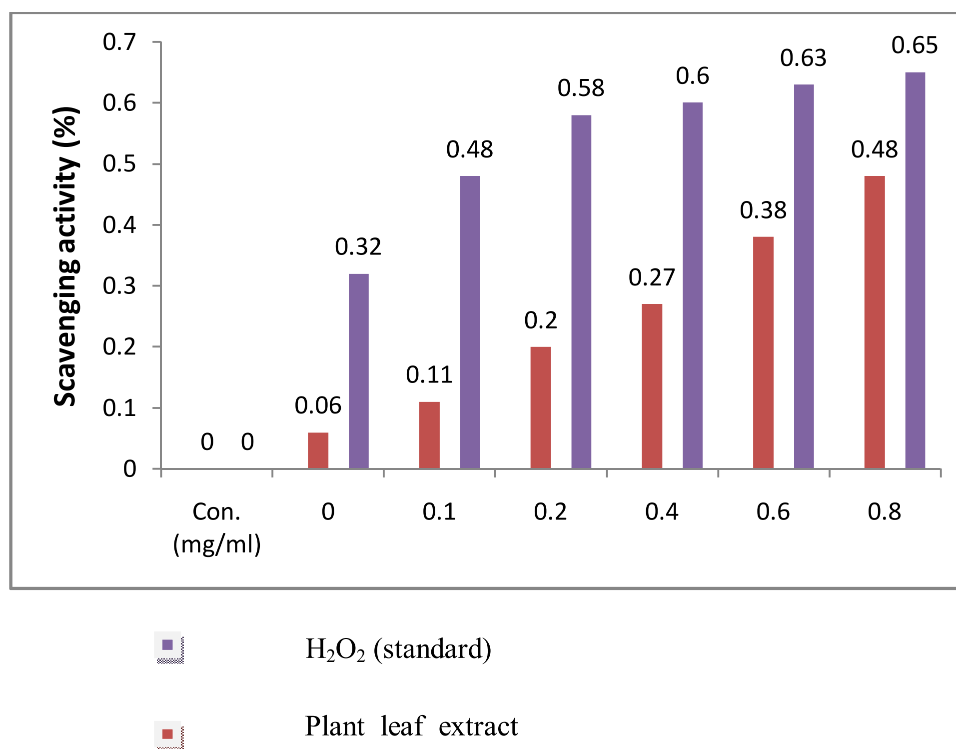


Figure 3: H_2O_2 radical scavenging activity of *Ficus religiosa* ethanolic leaf extract at different concentrations

REDUCING POWER OF *FICUS RELIGIOSA* LEAF EXTRACT:

Line graph shows the reductive capability of the EEFR 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}).

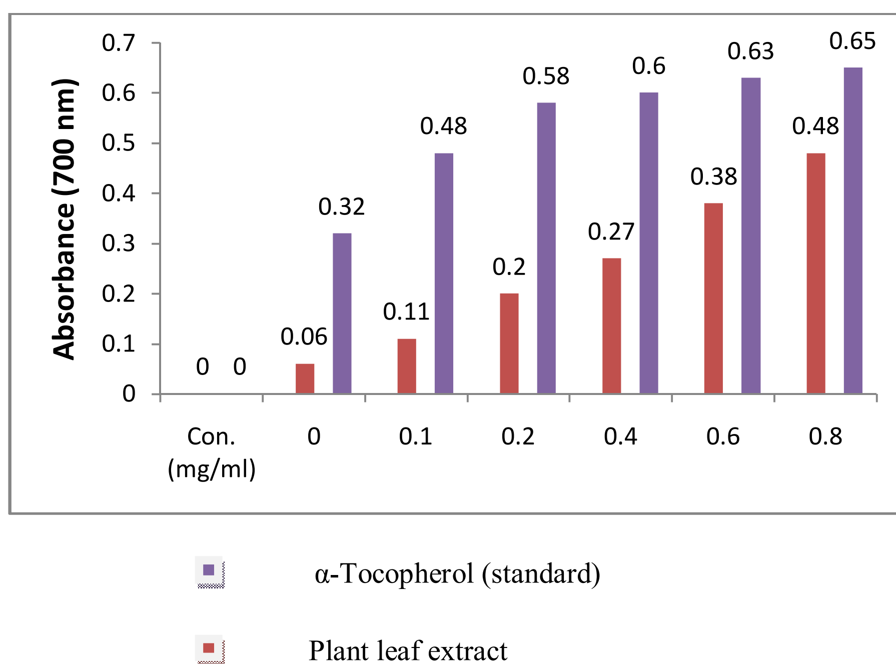


Figure 4: Reducing power of *Ficus religiosa* ethanolic leaf extract at different concentrations.

PHENOLIC CONTENT

One milligram of extract contained 3.2µg gallic acid equivalents of phenols respectively.

CONCLUSION:

Antioxidant activity of ethanolic extract of *Ficus religiosa* (EEFR) leaf extract was investigated as free radical scavenging activity by adopting various *in vitro* methods. 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. The findings of the present study explored the antioxidant potential of the plant extract by 1,I-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.

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