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Research Article

Assessment of anti-oxidant activity of *Ficus benghalensis* leaves: an *In-vitro* design

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ABSTRACT

Background: Medicinal plants represent a rich source of natural anti-oxidants. The traditional medicine involves the use of different plant extracts of bioactive constituents. *Ficus benghalensis* is a large evergreen tree found throughout India. It is commonly called Banyan tree. It is used in traditional system of medicine like Ayurveda and homoeopathy. *Ficus* is a huge tropical deciduous or evergreen tree with more than 800 species.

Objective: Anti-oxidant effect of ethnomedicinal important plant species *Ficus benghalensis* (Family-Moraceae) was screened. The aim of this study was to assess the *in-vitro* potential of methanolic extract of *Ficus benghalensis* leaves as a natural anti-oxidant.

Method: Assessment of *in vitro* anti-oxidant activity of methanolic leaves extract of *Ficus benghalensis* was determined by DPPH free radical scavenging assay.

Results: The methanolic extract of *Ficus benghalensis* leaves demonstrates a remarkable amount of phenolic content (54 µg/ml of GAE/100gm of extract) by using Folin-ciocalteu reagent. *In-vitro* anti-oxidant activity of methanolic leaves extract of *Ficus benghalensis* was determined by DPPH free radical scavenging assay, which showed significant anti-oxidant activity compared to standard anti-oxidant (ascorbic acid). A direct correlation between total phenol and anti-oxidant activity indicates polyphenols are the main anti-oxidants.

Conclusion: These findings suggest the excellent medicinal bio-activity of *Ficus benghalensis* and explain the popularity of this plant in the folk medicine as a remedy for different disorders, thus supporting its folklore application as preventive remedy as an anti-oxidant. The present study shows that *Ficus benghalensis* leaves have radical scavenging activity and can be considered as good sources of natural anti-oxidants for medicinal and commercial uses.

Keywords: *Ficus benghalensis*, anti-oxidant, DPPH scavenging activity, Folin-ciocalteu reagent.

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INTRODUCTION

The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity. Plant based drugs are having a revived interest now-a-days because of awareness of deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in pharmaceutical industry as drug them or as drug carrier or bio-enhancers or excipients. The importance of herbal/plant medicines is well documented in Vedas, which proved to be the ancient literature. The properties of the plants and their remedies are given in detail and in fact

Ayurveda is the very principle root for the emergence of Ancient medical science in India that gave origin to branches like Sushruta and Charka Samhita^{1,2}.

Anti-oxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Free radicals or ROS formed in the body as a result of biological oxidation. Over production of free radicals contributes to oxidative stress associated with various degenerative diseases. Oxidation is very important for life to survive but harmful effects of oxygen are believed to be due to the formation of reactive oxygen species (ROS) known as free radicals, these free radicals have been implicated in the pathogenesis of various diseases including myocardial and cerebral ischemia, arteriosclerosis, diabetes, cataracts, atherosclerosis, rheumatoid arthritis,

inflammation and cancer-initiation as well as in the aging process result in tissue damage and cell death^{3,4}.

Free radical and Reactive Oxygen Species (ROS) like superoxide, hydroxyl radical, peroxy radical as well as non-radical species such as hydrogen peroxide (H₂O₂). These free are derived from the normal metabolism or exogenous agent like chemical or medicine. Reactive species are act as oxidative damage of cellular tissue that is implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging. *In vivo*; such reactive species is reduced by endogenous anti-oxidative defences, so as to preserve optimal cellular function. In pathological conditions, however, the detoxifying mechanisms are often inadequate as excessive in finding anti-oxidant phytochemical, because they can inhibit the propagation of free radical reactions; protect the human body from disease. The anti-oxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, and singlet oxygen quenchers and metal chelators⁵⁻⁷.

Ficus benghalensis (Moraceae, Mulberry family) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, most of which are native to the old world tropics. It is used in Ayurveda for the treatment of diarrhea dysentery and piles; teeth disorders Rheumatism, skin disorders like sores to boost immune system as a hypoglycemic. The extracts of *Ficus benghalensis* were also reported to inhibit activity from liver and kidney fruit extracts exhibited anti-tumor activity in the potato disk bioassay. The English name Banyan is given by the Britishers to this tree because under the tree Baniyas that is the Hindu merchants used to assemble. The seed of banyans are dispersed by fruits-eating birds. The seeds are small and most banyans grow in forests, so that a plant germinating from a seed that land on the ground is unlikely to survive. However many seeds land on branches and germinate they send roots down towards the ground and may envelop parts of the host tree or building structure giving banyans the casual name of "Strangler". The "Strangling" growth habit is found in a number of tropical forest species particularly of the genus, that complete for light. Any *Ficus* species showing that habit may be termed a stringier. The leaves of the banyan tree are large, leathery, glossy green and elliptical in shape, the leaf bud is covered by two large scales. Young leaves have an attractive reddish tinge⁸⁻¹¹.

Phenolic compounds are derived from phenylalanine and considered to be secondary metabolites. Phenolic possess a unique structure containing an aromatic ring with one or more hydroxyl groups. Flavonoids, tannins, benzoic acids and cinnamic acid derivatives are the examples of phenolic derivatives which can be found in plants. About 8000 plant phenolics are found naturally and most of them are flavonoids. Flavonoids also have unique structure which is based on the C₁₅ heterocyclic nucleus of flavones and they usually vary in the number of phenols, such as phenolics quinines, phenolics acid and phenyl propanoids. Anti-oxidants are considered important nutraceuticals on account of many health benefits. Due to the high anti-oxidant activity, *F. benghalensis* leaves might be considered in the management and prevention of degenerative diseases associated with free radical damage. The requirement of a standard assay is very important in order to compare the results of different laboratories and validation of the conclusions. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of Nitrogen Bridge. Scavenging of DPPH radical is the basis of the popular DPPH anti-oxidant assay. Due to

simplicity, flexibility, high throughput, TLC technique is used for qualitative anti-oxidant test¹²⁻¹⁴.



Figure 1: *Ficus benghalensis* plant



Figure 2: *Ficus benghalensis* leaves

The present aim of this study was to assess the *in-vitro* potential of methanolic extract of *Ficus benghalensis* leaves as a natural anti-oxidant.

materials and METHODS

Plant collection

The leaves of *Ficus benghalensis* were collected from local area of Medchal dist. Telangana, India.

Preparation of extract

300 g dried powder was weighed and taken in an aspirator (2.5 L). Before placing powder into the aspirator, the jar was washed properly with acetone and then dried. 800 ml of solvent i.e. methanol was added gradually. The container with its content was sealed and kept for 20 days with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. Then whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation in dry and clean air. The entire process was repeated using ethyl acetate and petroleum ether as the solvent to obtain ethyl acetate and petroleum ether extract. But here 225 g of dried leaves was taken with 675 ml ethyl acetate and petroleum ether separately. And it was kept for 15 days^{15,16}.

Chemicals and reagents

Methanol, Folin-ciocalteu reagent, gallic acid, DPPH solution (1,1-Diphenyl-2-picryl-hydrazyl) are used for the experiment.

Determination of Total Phenolic Component (TPC)

By using Folin-ciocalteu reagent total phenolic component were determined colorimetrically. In 10ml methanol 150 mg extract was dissolved and 2 ml of this solution was filled up with 0.3% HCl to 5 ml. A 100- μ l aliquot of the resulting solution was added to 2 ml of 2% Na₂CO₃ and 100 μ l of Folin-ciocalteu reagent was added after 2 minutes. The absorbance was measured at 750 nm using a spectrophotometer after 30 minutes. Total phenolic component were quantified by measuring the calibration curve total phenolic content was quantified. The calibration curve was obtained from measuring the absorbance of a known concentration of gallic acid standard. The final results were expressed as micrograms gallic acid equivalents per 100 ml extract^{17,18}.

In-vitro anti-oxidant activity

Flavonoids are known to be highly effective anti-oxidant by scavenging oxygen radical, by having interesting anti-cancer, hypolipidemic, anti-ageing, and anti-inflammatory activities. Moreover, the protective effects of flavonoids in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate anti-oxidant enzymes, reduce alpha tocopherol radicals, and inhibit oxidases^{19,20}.

Preparation of extract solution

The anti-oxidant activity of leaf extract was determined by *in-vitro* method (DPPH free radical scavenging assay). The free radical scavenging capacity of the methanolic extract of *Ficus benghalensis* was determined using DPPH. DPPH solution (0.004% w/v) was prepared in methanol. Methanol extract of *Ficus benghalensis* was mixed with methanol to prepare the stock solution (10 mg/100 ml). The concentration of this *Ficus benghalensis* methanolic extract solution was 10 mg/100 ml from stock solution, 500 μ g/ml, 250 μ g/ml, 125 μ g/ml, 62.5 μ g/ml, 31.25 μ g/ml, 15.625 μ g/ml, 7.812 μ g/ml, 3.906 μ g/ml, 1.953 μ g/ml, 0.96 μ g/ml solution was prepared respectively. Freshly prepared DPPH

solution (0.004% w/v) was added in each of these test tubes containing *Ficus benghalensis* methanolic extract and after 10 min, the absorbance was taken at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10 mg/100 ml) of methanolic and ethyl acetate extract of *Ficus benghalensis*. Control sample was prepared without adding any extract and ascorbic acid, methanol was used as blank, ascorbic acid solution was used as a standard²¹. The scavenging activity of DPPH (%) was calculated by using the following equation in anti-oxidant activity assays, different concentrations of leaf extract were used to find out the concentrations which could include 50% inhibition to calculate IC₅₀.

$$\% \text{ DPPH radical scavenging} = [(A_0 - A_1)/A_0] \times 100$$

Where A₀ is the absorbance of the control;

A₁ is the absorbance of test sample.

Statistical Analysis

The data was statistically analyzed by using one-way ANOVA. The values are reported as mean $\pm$ SD.

RESULTS

Total Phenolic Contents (TPC)

Folin-ciocalteu method was used to determine the total phenolic content and results were showed in gallic acid equivalents (Figure 3). Our experiment showed the results of the total phenolic content 54 μ g/ml for methanolic extract of *Ficus benghalensis* leaves (Table 1).

Anti-oxidant test

In this study, the anti-oxidant capacity of methanolic extract of *Ficus benghalensis* leaves and ascorbic acid was systematically evaluated. Anti-oxidant activity of selected plant extract compared with standard anti-oxidant ascorbic acid showed that selected plants have potential anti-oxidant activity like ascorbic acid. Methanolic extract of *Ficus benghalensis* showed 51.57% DPPH scavenging activity and IC₅₀ was 0.814 where as 1 in ascorbic acid (Table 2).

Table 1: Determination of total phenolic content of methanolic extract of *Ficus benghalensis* leaves

Sample	Absorbance	Average	Phenolic content
Methanolic	0.478	0.489	54 μ g/ml
	0.486		
	0.489		
	0.487		

Table 2: Radical scavenging activity of methanolic extract and standard anti-oxidant on DPPH free radical

Sample	IC ₅₀ (μ g/ml) Mean $\pm$ SD	% Inhibition
Methanolic extract	90 $\pm$ 11.56	51.57
Ascorbic acid	73.26 $\pm$ 17.80	46.66

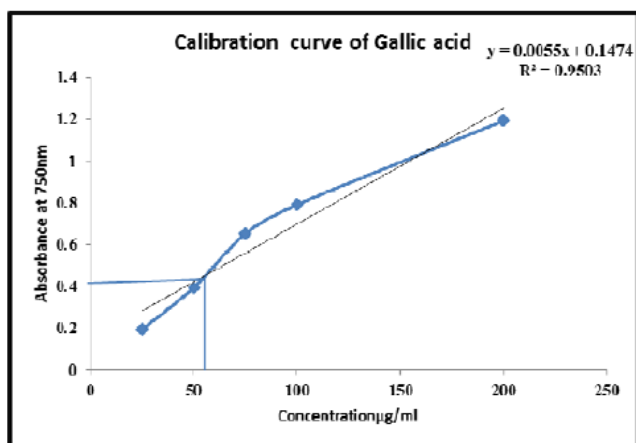


Figure 3: Standard curve of gallic acid

DISCUSSION

Calculating the total phenolic content in plant species is important. Phenolics and polyphenolic compounds are directly related to anti-oxidative action and they involve in the main class of natural anti-oxidants present in plants. Because of differences in analytical methodologies, sample material and origin and the development of novel sophisticated methods, it is hardly possible to compare with the data obtained in the present study. From the results of our study it can be definite that there is a direct relationship between total phenolics contents and anti-oxidant activity of *Ficus benghalensis* leaves.

Previous study done by also reported different results about the relationship between phenolic content and anti-oxidant activity. Some previous studies found correlation between the total phenolic contents and the anti-oxidant activity while some found no such relationship. During germination of *Pangium edule* there was a parallel increase in between phenol content and antioxidant activity which was done by Andarwulan (1999)²². An increase in the antioxidant activity of lupin seed was also observed by Tsaliki *et al.* (1999)²³. Javanmardi *et al.* (2003) also found a linear positive relationship between the anti-oxidant activity and total phenolic content of the tested *Ocimum* accessions²⁴. Maillard and Berset (1995) showed no correlation between phenolic contents and anti-oxidant activity in malts and suggested that other compounds are also responsible for the anti-oxidant activity²⁵. Another study done by citrus residues also showed no relationship between antioxidant activity and phenolic composition. Further research should be needed to find out the proper relationship between phenolic content and antioxidant activity of *Ficus benghalensis* leaves.

CONCLUSION

From the results of our study it is clear that due to the presence of total phenolic contents of *Ficus benghalensis*, it possess significant anti-oxidant activity. A strict linear relationship was also observed in the study between total phenolics and anti-oxidant activity. It needs further studies to assess their potential components as effective natural remedies. From the above study, it is concluded that the leaves of *Ficus benghalensis* may represent a new source of anti-oxidant with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local ethno-medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the

phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind. These findings suggest a new pathway in elucidating a potent anti-oxidative agent from *Ficus benghalensis* leaves. These local ethno medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. A large scale isolation and further spectral techniques are required to isolate and identify a particular compound responsible for scavenging free radicals.

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AUTHOR'S CONTRIBUTION

All authors contributed to the design and implementation of the research.

CONFLICTS OF INTEREST STATEMENT

Authors declare that they have no conflicts of interest.

SPONSORSHIP

Nil.

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