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Original Article

QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *FICUS BENGHALENSIS* LINN SEED

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ABSTRACT

Objective: To evaluate the phytochemical constituents and the antioxidant activity of ethanolic extract of *Ficus benghalensis* seed locally prescribed as a diet for peptic ulcer.

Methods: Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics, flavonoids and tannins were determined by the well-known test protocol available in the literature. The antioxidant and free radical scavenging activity of ethanolic extract of the plant was assessed against DPPH, nitric oxide, lipid peroxidation and reducing power assay using standard protocols.

Results: Phytochemical analysis revealed the presence of phenols, flavones, tannins, carbohydrates, saponins, alkaloids, cardiac glycosides, steroids, quinones, terpenoids and coumarins. The total tannin content of the ethanolic seed extract was high (127.12±1.508 TE/g), followed by phenolics (80.46±1.868 GE/g) and flavonoids (39.23±1.205 QE/g). The activities of plant extract against DPPH, nitric oxide and lipid peroxidation were concentration dependent with IC_{50} values of 446.9, 596.0 and 557.0 µg/ml respectively. The reducing ability of *F. benghalensis* was found to be 418.34 at 1000 µg/ml.

Conclusion: Our findings provide evidence that the crude extract of *Ficus benghalensis* is a potential source of natural antioxidants, and this justifies its use in folkloric medicine.

Keywords: *Ficus benghalensis*, Phytochemical, Antioxidant, Scavenging, Free radicals, DPPH, Nitric oxide, Lipid peroxidation.

INTRODUCTION

A free radical is a chemical species which contains an unpaired electron spinning on the peripheral layer around the nucleus. Free radicals generated from the oxygen are called Reactive oxygen species (ROS) which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS include superoxide radical, hydroxyl radical, nitric oxide radical, lipid peroxyl radical and non free radical species such as hydrogen peroxide, singlet oxygen, ozone, lipid peroxide are different forms of activated oxygen [1-3]. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA.

This ROS can generate oxidative stress and produce many pathological diseases such as arthritis, diabetes, inflammation, cancer, aging and genotoxicity [4, 5]. Sources of free radicals include metabolism by-products, neutrophils, UV radiation, air and water pollutants, fatty foods, hazardous chemicals, and cigarette smoke.

Antioxidants are any substance that when present at low concentration compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate [6]. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. 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 reducing agents such as thiols, ascorbic acid or polyphenols [7]. However, this natural antioxidant mechanism can be inefficient for severe or continued oxidative stress.

Based on this idea, there has been a strong demand of therapeutic and chemo preventive antioxidant agents with limited cytotoxicity to enhance the antioxidant capacity of the body and help attenuate the damage induced by ROS. There are some synthetic antioxidants like butylated hydroxyl toluene, butylated hydroxyl anisole and tetra butyl hydroquinone that have been commonly used. However, it has been suggested that these compounds are carcinogens [8]. This led to an increased interest in natural antioxidants from plant sources.

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignin, tannins, flavonoids, quinones, coumarins, alkaloids, amines and other metabolites, which are rich in antioxidant activity [9, 10]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antiulcer, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities [11, 12]. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, ulcer and other diseases associated with ageing [13, 14]. In recent years, there has been a worldwide trend towards the use of natural phytochemicals since natural antioxidants are presumed to be safe as they occur in plants. Evidence suggests that compounds, especially from natural sources are capable of providing protection against free radicals. This has attracted a great deal of research interest in natural antioxidants. It is necessary to screen out medicinal plants for their antioxidant potentials.

Ficus benghalensis Linn (*F. benghalensis*, syn. *Ficus indica*) belongs to the family Moraceae. *F. benghalensis* is commonly known as Indian banyan tree. It is called as Aal, Alam, Aalam vizhudhu, Alamaram in Tamil. This plant is native from the Indian subcontinent, Burma to Malaya. It is the national tree of India [15]. Various parts of *F. benghalensis* used in Ayurveda for diabetes, tonic, diuretic, diarrhea and ulcer [16]. Bark used in the treatment of ulcers, erysipelas, diabetes, vaginal disorders. Milky juice used for piles, tonic and gonorrhea, and applied externally for rheumatic pains and lumbago. Decoction of leaf buds used for hemorrhages. Leaves applied as poultice to abscesses. Aerial roots used for gonorrhea, diuretic, syphilis, dysentery and liver inflammation [17]. The seed of *F. benghalensis* is refrigerant, demulcent, diuretic, pectoral and tonic, and they are prescribed as a diet for peptic ulcer in the Ayurvedic system of medicine [18]. Protein, pentose, mucilage and tannins are reported in the seeds [19]. But no scientific reports are available at phytochemical and antioxidant activity of *F. benghalensis* seed, so it is very essential to develop natural antioxidants to meet up the

challenges and demands, especially in case of lipid oxidation to avoid carcinogenic synthetic antioxidants.

Hence, this is the first attempt ever made to investigate the phytochemical and antioxidant activity of ethanolic extract of the seeds of *F. benghalensis*. For this purpose, the factors responsible for the potent antioxidant ability of *F. benghalensis* ethanolic seed extract was evaluated by preliminary phytochemical assay, DPPH scavenging activity, nitric oxide scavenging activity, lipid peroxidation inhibitory activity and reducing power assay. The content of important phytoconstituents such as phenolics, flavonoids and tannins was also quantitatively determined.

MATERIALS AND METHODS

Plant collection and authentication

The seeds of *F. benghalensis* were collected (from a forest in the Thennampattu village of Thiruvannamalai district of Tamilnadu, India). The plant was identified and authenticated by Prof. Dr. Jayaraman, Plant Anatomy Research Centre, Chennai by comparing with the voucher specimen.

Powder preparation

The seeds were collected, washed thoroughly with fresh running water, dried under shade with room temperature (25±1) ℃ for a few weeks and coarsely powdered in a blender. The powdered sample was separately kept in an airtight container until use [20].

Extraction

Extraction was performed by a hot percolation method using soxhlet apparatus. About 500 g of the coarsely powdered raw materials of *F. benghalensis* seeds were extracted in 96 % ethanol by the continuous hot extraction method at 50℃. The extract with 96 % ethanol was decanted from the soxhlet apparatus and the filtrate was evaporated for the total elimination of alcohol using a rotaflash vacuum evaporator. The concentrated liquid extract obtained was then transferred to a china dish and kept in a water bath at 50℃ to concentrate to dryness. The residual extract, *F. benghalensis* ethanolic seed extract was transferred and stored in an airtight container free from any contamination until it was used. The % yield was calculated [21].

Preliminary phytochemical analysis

The *F. benghalensis* ethanolic seed extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Harbone [22]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavones, glycosides, cardiac glycosides, saponins, alkaloids, anthraquinones, quinones, terpenoids, coumarins, steroids, phlobatannins and tannins.

Total phenol determination

The total phenolic content was determined using the method of McDonald *et al.* [23]. A volume of 1 ml of *F. benghalensis* ethanolic seed extract or standard was mixed with 5 ml of Folin Ciocalteau reagent and 4 ml of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue color developed was read at 765 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoid determination

The total flavonoid content was determined using the method of Chang *et al.* [24]. A volume of 0.5 ml of *F. benghalensis* ethanolic seed extract or standard was separately mixed with 4.5 ml of methanol. To the mixture, 0.1 ml of 10 % aluminium chloride and 0.1 ml of 1 mol/l sodium acetate was added. Then allowed the mixture at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Total tannin determination

The total tannin content was determined using the method of Schanderl [25]. One milliliter of the *F. benghalensis* ethanolic seed extract or standard was taken and the volume was made up to 1 ml with distilled water. To the mixture, 0.5 ml Folin's phenol reagent followed by 5 ml of 35 % sodium carbonate was added and kept at room temperature for 5 min. The blue color formed was read at 640 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The content of tannins was calculated using standard graph of tannic acid and the results were expressed as tannic acid equivalent (mg/g).

DPPH Radical scavenging activity

The DPPH radical scavenging assay was performed by the method of Koleva *et al.* [26]. About 10 µl of each concentration (100-1000.00 µg/ml) of *F. benghalensis* ethanolic seed extracts or standard was added to 190 µl DPPH solution. After vortexing, the mixture was incubated for 20 min at 37 °C. The decrease in absorbance of the test mixture due to quenching of DPPH free radical was measured at 517 nm. The IC_{50} value was determined as the concentration of the test mixture that gave 50 % reduction in the absorbance from control blank. Vitamin C was used as a reference standard. The percentage inhibition was calculated as follows:

$$(\%)\ \text{Inhibition} = [(\text{control} - \text{test})/\text{control}] \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging assay was performed by the method of Green *et al.* [27]. Sodium nitroprusside (5 m mol/ℓ) in phosphate buffered saline was mixed with 3 ml of different concentrations (100−1000.00 µg/ml) of the *F. benghalensis* ethanolic seed extract dissolved in water and incubated at 25℃ for 150 min. The samples from the above were allowed to react with Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). Quercetin was used as a reference standard. The percentage of nitric oxide radical scavenging activity was calculated by the formula below and the results were computed. The IC_{50} value was determined.

$$(\%)\ \text{Inhibition} = [(\text{control} - \text{test})/\text{control}] \times 100$$

Lipid peroxidation inhibition assay

The lipid peroxidation inhibition assay was performed by the method of Okhawa *et al.* [28]. The test system contained 1 ml of homogenate with *F. benghalensis* ethanolic seed extract (100−1000.00 µg/ml). To 1 ml of homogenate, lipid peroxidation was initiated by the addition of 0.1 ml of $FeSO_4$ (25 m mol/l), 0.1 ml of ascorbate (100 m mol/l) and 0.1 ml of KH_2PO_4 (10 m mol/ℓ) and the volume was made up to 3 ml with distilled water and incubated at 37℃ for 1 h. Then, 1 ml of 5 % trichloroacetic acid and 1 ml of 2-thiobarbituric acid was added to this reaction mixture and the tubes were boiled for 30 min in a boiling water bath. This was centrifuged at 3500 r/min for 10 min. The extent of inhibition of lipid peroxidation was evaluated by the estimation of thiobarbituric acid reactive substances (TBARS) level by measuring the absorbance at 532 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). Quercetin was used as a reference standard. The IC_{50} value was determined. The percentage inhibition of lipid peroxidation was calculated by the following formula:

$$(\%)\ \text{Inhibition} = [(\text{control} - \text{test})/\text{control}] \times 100$$

Reducing power assay

Reducing ability was performed by using potassium ferricyanide-ferric chloride system. 1 ml of extract solution (100−1000.00 µg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1 %). The content was mixed and heated at 50°C for 20 min and cooled; then trichloroacetic acid (2.5 ml, 10 %) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %), and the absorbance was

measured at 700 nm against a blank. Increasing absorbance of the reaction mixture indicates increasing, reducing power [29].

Statistical analysis

All the experiments were done in triplicates. The experimental results are expressed as mean±SD of triplets. Statistical analysis was performed using the Graph Pad Prism Software, Version 4.0.3 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Extract yield

The yield of *F. benghalensis* ethanolic seed extracts was 9.2 % w/w.

Qualitative phytochemical analysis

Preliminary phytochemical screening of *F. benghalensis* ethanolic seed extract revealed the presence of various components such as carbohydrates, phenols, tannins, flavones, saponins, steroids, quinones, terpenoids, coumarins, cardiac glycosides and alkaloids among which phenols, tannins and flavones were the most prominent ones and the results are summarized in table 1.

Table 1:Phytoconstituents in *F. benghalensis* ethanolic seed extract

Phytoconstituents	Presence/Absence
Carbohydrate	++
Tannin	+++
Saponins	+
Flavones	+++
Alkaloid	+
Quinones	+++
Glycosides	-
Cardiac glycoside	++
Terpenoids	++
Phenol	+++
Coumarins	++
Steroid	++
Phlobatannins	-
Anthraquinones	-

+: Presence (+mild,++moderate,+++high), −: Absence

Quantitative phytochemical analysis

Among the secondary metabolites that were quantified, the total tannin content was the highest with (127.12±1.508) TE/g of the extract followed by the total phenolic content with (80.46±1.868) GE/g of the extract and the total flavonoid content with (39.23±1.205) QE/g of the extract. The results are tabulated in table 2.

Table 2:Quantified phytochemical compounds

Compounds	Amount	Equivalents
Total phenolics	80.46±1.868 GE/g*	Gallic acid
Total flavonoids	39.23±1.205 QE/g*	Quercetin
Total tannins	127.12±1.508 TE/g*	Tannic acid

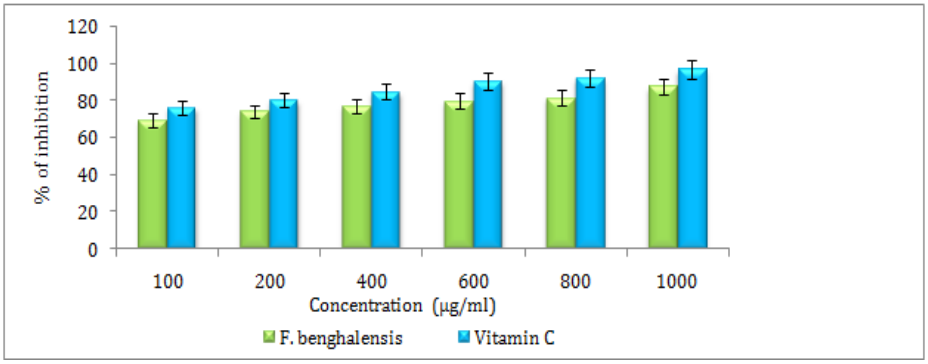
*: Values are expressed as mean±SD (n=3)

GE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Tannic acid equivalent

DPPH Scavenging activity

The results of DPPH radical scavenging activity of the *F. benghalensis* ethanolic seed extract and the standard vitamin C are presented in fig. 1. The percentage inhibitory activity of free radicals by 50 % has been used widely as a parameter to measure antioxidant activity. In this study, both the plant extract and the standard vitamin C significantly scavenged the DPPH radical with increasing concentrations.

The percentage inhibition of the DPPH radical by the *F. benghalensis* ethanolic seed extract and vitamin C at100 µg/ml was 69.46 % and 76.42 %, while the IC_{50} values were 446.9µg/ml and 430.6µg/ml respectively. The scavenging activity of the *F. benghalensis* ethanolic seed extract was found to be lower than that of vitamin C standard. The percentage of inhibition reached a maximum of 87.56 % at 1000 µg/ml of *F. benghalensis* ethanolic seed extract.



n=3

Fig. 1: DPPH radical scavenging activity of the ethanolic seed extract of *F. benghalensis* in comparison with vitamin C

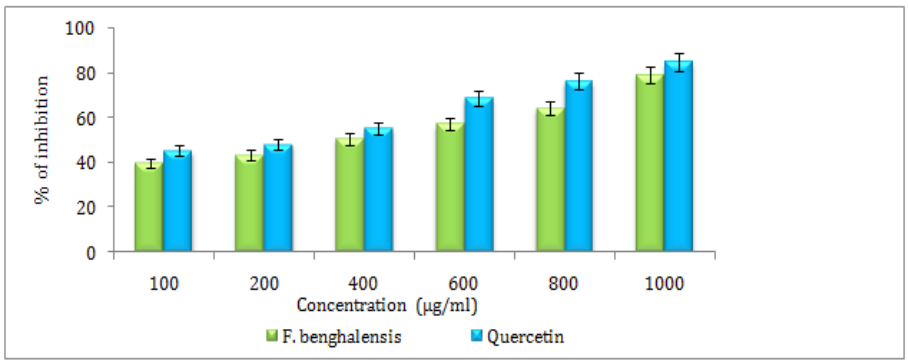
Nitric oxide radical scavenging activity

Fig. 2 shows the scavenging activity of *F. benghalensis* ethanolic seed extract against nitric oxide radical released by sodium nitroprusside in a concentration dependent manner. A comparable scavenging activity was observed between the extract and the standard quercetin. At 100µg/ml, the percentage inhibitions of the *F. benghalensis* ethanolic seed extracts and quercetin were 39.62 % and 45.26 % respectively. The IC_{50} value of the standard was 535.8µg/ml while that of the extract was 596.0µg/ml. The standard and the extract recorded a gradual dose-dependent inhibitory activity tested in an increasing order. And in the case of *F. benghalensis* ethanolic seed extract, the maximum scavenging activity of 79.25 % was observed at 1000 µg/ml concentration.

Lipid peroxidation inhibition assay

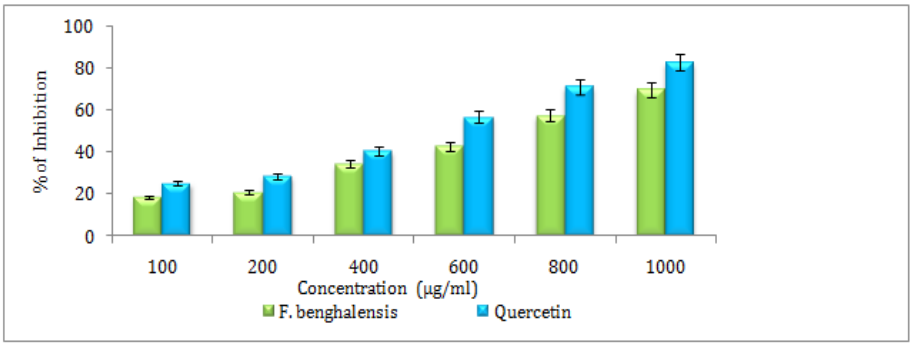
The lipid peroxide inhibitory activity of the extract against TBARS initiated by ferric chloride was investigated and the result is shown in fig. 3. The percentage inhibitory activity of the *F. benghalensis* ethanolic seed extracts was compared with the standard quercetin. The extract showed the prevention of formation of TBARS in a dose dependent manner. At 100 µg/ml concentration of the extract and quercetin, the percentage of inhibition was 18.36 % and 25.16 % respectively. The IC_{50} value of the extract was 557.0µg/ml while that

of the standard was lesser of 543.2µg/ml. The extract showed a maximum of 69.77 % inhibition at 1000 µg/ml concentration. The *F. benghalensis* ethanolic seed extract was found to be only a mild inhibitor of lipid peroxidation *in vitro* in contrast to the standard quercetin, which showed a maximum of 82.95 % inhibition at 1000 µg/ml concentration.



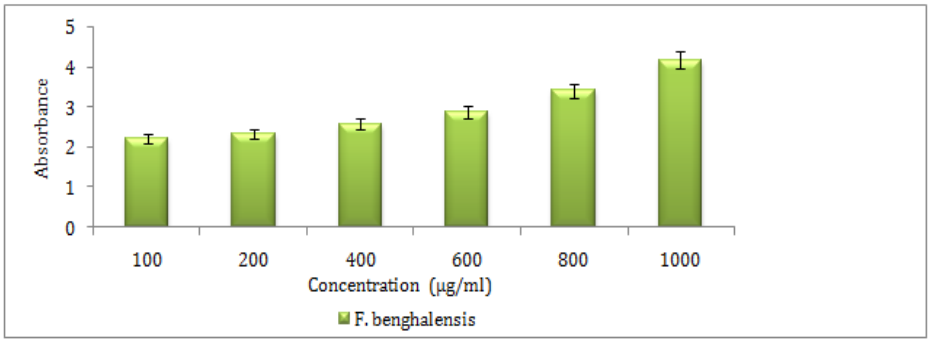
n=3

Fig. 2: Nitric oxide scavenging activity of the ethanolic seed extract of *F. benghalensis* in comparison with quercetin



n=3

Fig. 3:Lipid peroxidation inhibitory activity of the ethanolic seed extract of *F. benghalensis* in comparison with quercetin



n=3

Fig. 4:Reducing ability of the ethanolic seed extract of *F. benghalensis*

Reducing power assay

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form.

By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. At 1000 µg/ml concentration of the extract the absorbance obtained was 4.183 (fig. 4). The results are expressed as absorbance × 100. The reducing ability of *F. benghalensis* was found to be 418.3 at 1000 µg/ml.

DISCUSSION

Preliminary phytochemical tests showed the presence of various phytochemical compounds in the seeds of *F. benghalensis* which are known to have various therapeutic importance in medicinal sciences. Alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities. Saponins have properties of precipitating and coagulating red blood cells and

they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity. Steroids are known important for their cardio-tonic activities and also used in nutrition, herbal medicine and cosmetics [30]. Terpenoids promotes glutathione-S-transferase and cancer cell apoptosis; hence, terpenoids have been used for anti-cancer properties. Flavonoids are well known for their anti-viral, anti-inflammatory, antioxidant activity and also used in the treatment of hypertension, diabetes, ulcer, rheumatic fever [31]. Polyphenols are active in curing kidney and stomach problems and have been found to be helpful in protection and prevention against many diseases. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [32]. An extensive phytoconstituent study has been made previously in *F. benghalensis* leaf, stem bark and root bark. The aqueous extracts of the plant have revealed some potential phytochemicals like alkaloids, glycosides, terpenoids, flavonoids and tannins [33]. This conforms to research demonstrated by Babu [34] in the preliminary phytochemical screening of *F. benghalensis* bark. They showed the presence of tannins, saponins, flavonoids, and terpenoids. In another study reported by Neena Unnikrishnan [35] showed the phytochemical screening on qualitative analysis revealed that the bark of Ficus is rich in popular phytochemical substances like flavonoids, steroids, glycosides, saponins and tannins. A study by Diwan [36] demonstrated that areal roots of *Ficus benghalensis* revealed the presence of alkaloids, flavonoids, simple phenolics, steroids and saponins. Previous studies on the phytochemical screening of *F. benghalensis* revealed the presence of saponins, tannins and flavonoids in aqueous and methanol extract [37]. Nidhi Chowdhary [38] reported that *F. benghalensis* leaf extracts showed the presence of alkaloids, flavonoids, saponins, phenols, tannins, diterpenes, phytosterols and proteins. A Study by Yogesh Chand Yadav [39] exhibited that methanolic extraction of *Ficus benghalensis* latex has revealed the presence of glycoside, alkaloids, tannin, flavonoids and amino acids.

Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant activity of phenolic compound is due to the hydroxyl functional group, however, other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties [40]. In the current study total phenolic content was found to be 80.46±1.868 mg GE/g for *F. benghalensis* seed. This shows that *F. benghalensis* seed has high content of phenolic compounds. Research by Sharma [41] exhibited that aerial roots of *Ficus benghalensis* had levels of total phenolics, total flavonol and total flavonoid compounds in 70 mg/g, 3 mg/g, 5 mg/g respectively. A Study by Bandekar [42] demonstrated that total polyphenols in *Ficus benghalensis* fruits was 276 mg GE/g. A study by Xia [43] exhibited that total phenolic content in the methanolic extract of *S. arvensis* was 38.8 g GE/100 g, while total flavonoid in *S. oleraceus* was 14.85 g rutin equivalent/100 g.

Flavonoids have also been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 [44]. Flavonoids serve as health promoting compound as a result of its anion radicals [45]. Total flavonoid contents were found to be 39.23±1.205 mg QE/g for *F. benghalensis* seed. The compounds such as flavonoids, which hold hydroxyl groups, are responsible for the radical scavenging activity in the plants. It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness. It is known that flavonoids act through scavenging of chelating process [46]. Nidhi Chowdhary [38] reported that *F. benghalensis* leaf extracts contain total phenols and total flavonoids were 5.55 µg/mg gallic acid equivalents and 5.11 µg/mg quercetin equivalents respectively. A Study by Yogesh Chand Yadav [39] exhibited that the methanolic extract of *Ficus benghalensis* latex has revealed the presence of total phenolic content 276 mg GE/g and total flavonoid content 1.84 mg QE/g. Previous study revealed that total flavonoid and total phenolic content in the ethanolic herb extract of *B. pilosa* were 20.90 g rutin equivalent/100 g and 9.53 g GE/100 g [47].

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and as anticancer agents [48]. Thus, *F. benghalensis* seed extract containing this compound (127.12±1.508 mg TE/g) may also serve as a potential source of bioactive compounds in the treatment of cancer and various diseases. Tannins do not act as pro-oxidants and in fact react very rapidly to quench the hydroxyl radical. The tannins in *F. benghalensis* seed extract may contain both hydrolysable and condensed tannins, since both have got a wide array of antioxidant mechanism like free radical scavenging activity, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation [49]. A previous study by Gayathri [50] exhibited that quantitative analysis of phytochemicals in aqueous extracts of the barks of *F. benghalensis* showed the presence of tannins and saponins were 7.75 mg/g and 2.03 mg/g respectively. A Study by Bandekar [42] demonstrated that *Ficus benghalensis* fruits had tannins, alkaloids and saponins were 1.88 mg/g, 9.7 mg/g and 4.25 mg/g respectively. Another study by Mabel Parimala [51] reported that *N. nouchali* hydroethanolic seed extract showed total tannin content was the highest with 195.84 GE/g followed by the total phenolic content with 179.56 GE/g and the total flavonoid content with 23.55 QE/g of the extract. Gracelin [52] carried out a phytochemical quantification study of methanol extracts of five *Pteris* species. The result revealed, the highest amount of alkaloids 16 mg, flavonoids 17 mg, phenol 13 mg, saponins 11 mg and tannins 06 mg are quantified in the selected *Pteris* species.

An antioxidant, in general, is a substance capable of preventing or slowing the oxidation of other molecules. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals, acting as electron donors, preventing the formation of peroxides, breaking the auto oxidative chain reaction and/or reducing localized O_2 concentrations [53]. Antioxidant activities of the plant extracts are generally studied and evaluated by using *in vitro* systems wherein ROS are generated by certain chemical reactions which liberate free radicals. Therefore, the quenching or scavenging ability or the inhibition of the generation of radicals is being taken as the antioxidant capacity of the plant extract.

The DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude extracts of plants. DPPH is a relatively stable free-radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [54]. DPPH radical is scavenged by antioxidants present in the *F. benghalensis* ethanolic seed extract through the donation of proton forming the reduced DPPH. Radical scavenging activity of *F. benghalensis* ethanolic seed extract increased with increasing percentage of the free radical inhibition. The maximum inhibition, which occurred at 1000 µg/ml indicates the maximum free radical scavenging potential of *F. benghalensis* ethanolic seed extract by their hydrogen donating ability. A Study by Nidhi Chowdhary [38] in *F. benghalensis* aqueous leaf extracts showed the DPPH scavenging activity was 47.24% inhibition at 25 µg/ml concentration. IC_{50} values for scavenging of DPPH was 21.59 µg/ml. A Study by Bandekar [42] revealed that *Ficus benghalensis* fruits had IC_{50} of DPPH scavenging activity was 28.67 µg/ml. Yogesh Chand Yadav [39] exhibited that the methanolic extract of *Ficus benghalensis* latex had IC_{50} of DPPH scavenging activity was 28.63 µg/ml. While the previous study by Erel [55] stated that the DPPH scavenging activity of methanolic extract of *A. vulgaris* by soxhlet extraction was 43.38%. Karabegovic [56] revealed that the methanolic extract of *A. vulgaris* that was extracted by soxhlet extraction had IC_{50} 28.1 µg/ml, while *A. campestris* had IC_{50} 28.1 µg/ml. A study by Deba [57] demonstrated that IC_{50} of DPPH scavenging activity of essential oil from leaves and flower of *B. pilosa* were 47.5 and 49.7 µg/ml, respectively, while aqueous flower and leaf extracts had IC_{50} 172 and 61 µg/ml respectively. Ethyl acetate fraction of *B. pilosa* had IC_{50} of DPPH scavenging activity was 43.53 µg/ml.

Nitric oxide radical scavenging capacity of *F. benghalensis* ethanolic seed extract was detected by sodium nitroprusside in aqueous solution which at physiological pH spontaneously generated nitric oxide [58]. Scavengers of nitric oxide from the *F. benghalensis*

ethanolic seed extracts competed with oxygen, leading to reduced production of nitrite ions, but showed only a mild inhibitory activity. A Study by Mabel Parimala [51] exhibited that the percentage inhibition of the nitric oxide radical by the *N. nouchali* hydroethanolic seed extract and quercetin at1. 95 µg/ml were 21.87% and 30.20%, while the IC_{50} values were 23.58 µg/ml and 13.54 µg/ml respectively.

Assay of TBARS measures malondialdehyde present in the sample, as well as malondialdehyde generated from lipid hydro peroxides by the hydrolytic conditions of the reaction [59]. *F. benghalensis* ethanolic seed extracts was found to inhibit lipid peroxidation to a certain extent. Though this method is slower than DPPH, the test environment is more similar to the *in vivo* conditions [60]. A previous study by Nidhi Chowdhary [38] in *F. benghalensis* aqueous leaf extracts showed the lipid peroxidation inhibition was 67.1% at 25µg/ml. IC_{50} values for percentage inhibition of lipid peroxidation was 13.67µg/ml. A Study by Mabel Parimala [51] exhibited that the percentage inhibition of the lipid peroxidation by the *N. nouchali* hydroethanolic seed extract and quercetin at1. 95 µg/ml were 24.69% and 37.50%, while the IC_{50} values were 54.65 µg/ml and 21.34 µg/ml respectively.

The reducing capacity of the *F. benghalensis* ethanolic seed extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of $Fe^{3+}$to $Fe^{2+}$by donating an electron. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. 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 antioxidants. The increased absorbance of the reaction mixture indicated increased, reducing power of the extracts. The reducing power of the extracts is increased with the increase in their concentration [61]. The present study was correlated with the study by Anjali Soni [62] revealed the reducing power of *Mentha spicata* extract was found to have the absorbance value 1.92 at 1 mg/ml concentration.

CONCLUSSION

To conclude, the findings of the present study support that the crude ethanolic extract of *F. benghalensis* seed has indicated strong antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stresses induce diseases such as ulcer, diabetes, which would be beneficial to the human health. This may be related to the high amount of phenolic, flavonoid and tannin compounds present in this plant extract. We have also established the relationship of total phenolic, flavonoid and tannin contents and the free radical scavenging activity. Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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