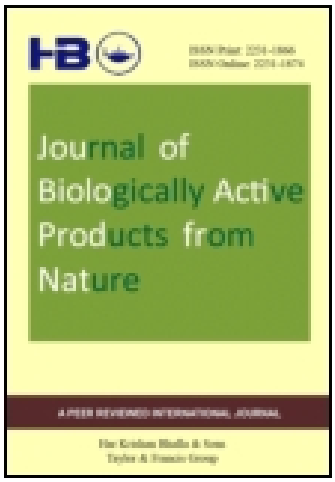

Journal of Biologically Active Products from Nature
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbap20

Phytochemical Composition and Antioxidant Activity of Ficus benghalensis (Moraceae) Leaf Extract

K.V. Bhaskara Rao[a], V. Ojha[a], Preeti[a], G. Kumar[b] & L Karthik[c]
[a] VIT University-SBST Vellore, Tamil Nadu India
[b] Jaipur National University-School of Life Sciences Jaipur, Rajasthan India
[c] Shri JJT University Jhunjhunu, Rajasthan India
Published online: 17 Sep 2014.

To cite this article: K.V. Bhaskara Rao, V. Ojha, Preeti, G. Kumar & L Karthik (2014) Phytochemical Composition and Antioxidant Activity of Ficus benghalensis (Moraceae) Leaf Extract, Journal of Biologically Active Products from Nature, 4:3, 236-248, DOI: 10.1080/22311866.2014.936902

To link to this article: http://dx.doi.org/10.1080/22311866.2014.936902

Taylor & Francis
Taylor & Francis Group

ISSN Print: 2231-1866
ISSN Online: 2231-1874

Phytochemical Composition and Antioxidant Activity of *Ficus benghalensis* (Moraceae) Leaf Extract

K.V. Bhaskara Rao [1]*, V. Ojha [1]., Preeti [1], G. Kumar [2], L, Karthik [3]

[1] VIT University-SBST Vellore, Tamil Nadu India
[2] Jaipur National University-School of Life Sciences Jaipur, Rajasthan India
[3] Shri JJT University Jhunjhunu, Rajasthan India

Received 06 January 2014; accepted in revised form 23 February 2014

Abstract: The aim of this study was to evaluate the phytochemical composition and antioxidant potential of the *Ficus benghalensis* (*F. benghalensis*) by *in vitro* methods. Methanolic extract of *F. benghalensis* leaves was evaluated for the presence of carbohydrates, proteins, phenolic compounds, oil and fats, saponins, flavonoids, alkaloids and tannins by using standard protocols. Antioxidant activity of the extract was screened by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, total antioxidant assay, iron chelating assay and reducing power assay. Cytotoxic activity of the extract was tested by brine shrimp lethality assay. Estimation of total phenolic content was performed by Folin-Ciocalteau reagent method and estimation of total flavonoid content was performed by aluminium chloride method. Chromatographic detection of polyphenolic compounds was estimated by High performance thin layer chromatography (HPTLC) and High performance liquid chromatography (HPLC). *F. benghalensis* showed the presence of carbohydrates, phenolic compounds, oil and fats, saponins, flavonoids, alkaloids, proteins and tannins as major phytochemical groups. The extract exhibited significant antioxidant activity in all methods performed. The extract also found to exhibit significant cytotoxic activity towards brine shrimp larvae. Extract exhibited significant amount of polyphenolic compounds which were further characterized by HPTLC which showed the presence of six bands of polyphenolic compounds. Further analysis of extract with HPLC showed the presence of Gallic acid, Rhein, Anthraquinone, Gallocatechin, Theaflavin-3, 3'-digallate and Flavone. The results of the present study emphasize that the methanolic extract of *F. benghalensis* is a good source of antioxidant compound and can be used in the field of therapeutics.

Key words: Phytochemical composition; Antioxidant potential; *Ficus benghalensis*; Cytotoxicity; Polyphenolic compounds.

Introduction

Free radicals are atoms or a group of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules during the natural cellular metabolic process of oxidation in body. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane as a result of this cells may function poorly or may die in some case. There are many types of radicals, but those of most concern in biological systems are derived from oxygen, and known collectively as reactive oxygen species (ROS). Oxygen has two unpaired electrons in separate orbitals in its outer shell. This electronic structure makes oxygen especially susceptible to radical formation. Sequential reduction of molecular oxygen (equivalent to

*Corresponding author (K.V. Bhaskara Rao)
E-mail: < kokatibhaskar@yahoo.co.in >

sequential addition of electrons) leads to formation of a group of reactive oxygen species: superoxide anion, hydrogen peroxide, hydroxyl radical. One of the best known toxic effects of oxygen free radicals is damage to cellular membranes (plasma, mitochondrial and endomembrane systems), which is initiated by a process known as lipid peroxidation [1-2].

In a normal cell there is balance between formation and removal of free radicals. However this balance can be shifted towards free radicals due to excessive generation of free radicals or depletion of natural antioxidants. This condition is commonly known as oxidative stress and can result in serious cell damage if left untreated. Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [3,4].

The human body has several mechanisms to counteract oxidative stress by producing antioxidants which are either naturally produced in body, or externally supplied through food supplements. Endogenous and exogenous antioxidants act as free radical scavengers and therefore protect the body from oxidative stress and lower the risk of cancer and degenerative diseases [5]. An antioxidant is any substance that when present at low concentrations significantly delays or prevent oxidation of cell contents like protein, lipid, fats, carbohydrates and DNA. Medicinal plants have been widely acknowledged for their antioxidant potential worldwide. The antioxidant potential of the plants is mostly due to the presence of polyphenolic group of compounds, which occurs abundantly in all parts of the plant-wood, bark, stems, leaves, fruit, roots, flowers, pollen and seeds. These compounds are potential scavengers of free radicals; however their potential varies with plants [6].

Ficus benghalensis is a large tree belonging to family Moraceae. It is widely present in Indian subcontinent and grows in plains from the coast to 1000 m. *F. benghalensis* is commonly known as banyan tree, Vata or Vada tree. It is a remarkable tree, which is known to sends down its branches and great number of shoots, which take root and become new trunk. This tree is considered to be sacred in many places in India [7]. Different parts of the tree have been used in Ayurvedic and Unani medicine for the treatment of biliousness, vaginal complaints, fever, ulcers, erysipelas, vomiting, inflammation, leprosy, aphrodisiac and piles. In recent past this plant has been extensively screened for its pharmacological properties and reported to possess anthelmintic activity, wound-healing activity, carbohydrate hydrolyzing enzymes inhibitory activity, antimicrobial activity, anti-inflammatory activity, antiulcer activity, Antimutagenic and antioxidant activity [8-14]. However most of these studies were conducted using the stem bark or roots of this plants, and leaves remains relatively less explored, therefore this study was planned to investigate the leaves of *F. benghalensis* for its phytochemical composition and antioxidant activity by various *in vitro* methods. In addition, cytotoxic activity of the leaves was also evaluated by brine shrimp lethality test.

Materials and methods

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Quercetin was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium carbonate (Na_2CO_3), Sodium phosphate (NaH_2PO_4) was purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Methanol, Ferric chloride ($FeCl_3$), Potassium Ferricyanide ($K_3Fe(CN)_6$), Trichloroacetic acid, Folin-Ciocalteau reagent, Methanol, Ascorbic acid, Gallic acid were purchased from SRL Pvt. Ltd. (Mumbai, India). Ammonium molybdate ($(NH_4)_2MoO_4$) and Aluminium chloride ($AlCl_3$) were purchased from SD Fine-Chem. Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Plant material

Leaves of *F. benghalensis* were collected from the tree growing in the VIT University, Vellore (Lat.12.9202ÚN, Long.79.1333ÚE), Tamil Nadu, India, during month of August 2012.

Processing of plant

Fresh and mature leaves of *F. benghalensis* were collected and washed thoroughly in distilled water and shade dried at room temperature. Dried leaves

were powdered uniformly using a mechanical grinder. The powder was extracted in methanol using a soxhlet extractor. Dried extract was collected in air tight container and stored at 4°C until for further use.

Phytochemical screening

Phytochemical screening of the methanolic extract of *F. benghalensis* leaves was carried out by using the standard protocols for the presence of carbohydrates, proteins, phenolic compounds, oil and fats, saponins, flavonoids, alkaloids and tannins [15].

Antioxidant activity

DPPH radical scavenging activity

The extract was diluted in distilled water to make 5, 10, 15, 20, 25, 30 µg/ml dilutions. Two millilitres of each dilution was thoroughly mixed with 1 ml of DPPH solution (0.2 mM/ml in methanol). The mixed solution was incubated in dark at 20°C for 40 minutes. Absorbance was measured at 517 nm using UV-Vis spectrophotometer with methanol as blank. Ascorbic acid was used as experimental control. Experiment was performed in triplicates at each concentration [16]. The percentage scavenging of DPPH by the extract was calculated according to the following formula:

$$\% \text{ DPPH Radical scavenging} = [(Ac-At)/Ac] \times 100$$

where, Ac is the absorbance of the control (DPPH), At is the absorbance of the test sample.

Determination of total antioxidant activity

A volume of 1 ml of the extract (50, 100, 150 and 200 µg/ml) was mixed with 3 ml of the reaction mixture (containing 10 ml of concentrated H_2SO_4, 1.005 g of sodium phosphate monobasic and 1.47 g of ammonium molybdate which was dissolved in 290 ml of water). The mixture was kept in water bath for one hour at 95°C. The solution containing 3 ml of reaction mixture and 1 ml of distilled water was used as blank and the absorbance was measured using UV-Vis spectrophotometer at 695 nm. Ascorbic acid was used as experimental control. Experiment was performed in triplicates at each concentration [17].

Iron chelating activity

The extract was diluted to 50, 100, 150 and 200 µg/ml. A reaction mixture having 1 ml of 0.05 % O-Phenanthroline and 2 ml of 200 µM Ferric chloride was mixed with 2 ml of the plant extract of the above mentioned concentrations. The mixed solution was kept for incubation for 10 minutes at room temperature. Solution containing 1 ml of 0.05% O-Phenanthroline, 2 ml of 200 µM Ferric chloride and 2 ml of distilled water was used as blank. The absorbance was measured at 510 nm using UV-Visible spectrophotometer. Ascorbic acid was used as experimental control. Experiment was performed in triplicates at each concentration [18].

Reducing power assay

A volume of 1 ml of plant extract at different concentrations (125, 250, 500 and 1000 µg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1 % Potassium Ferricyanide. The mixture was incubated at 50°C for 20 min.

A volume of 2.5 ml of Trichloroacetic acid (10 %) was added to the mixture, and was centrifuged at 3000 rpm for 10 min in a cooling centrifuge. 2.5 ml of the supernatant was mixed with equal volume of distilled water and 0.5 ml Ferric chloride (0.1 %). Absorbance was measured at 700 nm using a UV-Visible spectro-photometer. Higher absorbance of the reaction mixture indicated greater reductive potential. Ascorbic acid was used as experimental control. Each experiment was performed in triplicates at each concentration [19].

Cytotoxic activity (Brine shrimp lethality assay)

Brine shrimp (*Artemia saline* Leach) eggs (Ocean Star International, Inc., Snowville, USA) were placed in a hatching tank containing sea water for 48 hours. After hatching, 4 ml of sea water containing 20 brine shrimp nauplii was mixed with 1 ml of methanolic extract of *F. benghalensis* (50, 100, 150 and 200 µg/ml in sea water). Survival of brine shrimp nauplii was recorded at 1, 2, 3 and 4 hours of the exposure. Experiment was performed in triplicates at each concentration [20].

Estimation of polyphenolic compounds

Estimation of total phenolic content

The determination of total phenolic content of the methanolic extract of *F. benghalensis* was carried out using Folin-Ciocalteau reagent method. Extract was diluted to obtain different concentrations (125, 250, 500 and 1000 µg). 100 µl of each extract was mixed with 2.5 ml of Folin-Ciocalteau reagent (1/10 dilution in distilled water) and 2 ml of 7.5 % Na_2CO_3 (w/v in distilled water). The mixture was incubated at 45°C for 15 min. The absorbance was measured at 765 nm using a UV-Visible spectrophotometer. Na_2CO_3 solution (2 ml of 7.5 % Na_2CO_3 in 2.60 ml of distilled water) was used as blank. The result was expressed as Gallic acid equivalence in µg. Each experiment was performed in triplicates at each concentration [21].

Determination of total flavonoid content

The determination of total flavonoids of the methanolic extract of *F. benghalensis* was carried out using aluminium chloride method. A volume of 1 ml extract (containing 125, 250, 500 and 1000 µg/ml) was mixed with 1 ml of Aluminium chloride (2 % in ethanol). The mixture was incubated at room temperature for 60 minutes. Aluminium chloride solution (1 ml of 2 % $AlCl_3$ + 1 ml of water) was used as blank. The absorbance was measured at 420 nm using UV-Vis spectrophotometer. Total flavonoid content was expressed as Quercetin equivalence (QE) in µg. Experiment was performed in triplicates at each concentration [22].

Estimation of polyphenolic compounds: HPTLC analysis

Estimation of polyphenolic compounds in methanolic extract of *F. benghalensis* was carried out by HPTLC analysis. An amount of 100 mg extract was dissolved in methanol and the total volume was made up to 1 ml. The solution was centrifuged and the supernatant liquid was collected carefully. A volume of 2 µl solution was loaded as 5 mm band length in the 2 x 10 Silica gel 60F 254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The plate loaded with samples was kept in TLC twin trough developing chamber (after saturation with solvent vapour) with mobile phase Toluene-Acetone-Formic acid (4.5 : 4.5 : 1) and the plate was developed in the mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and the images captured at White light, UV 254 nm. Finally, the plate was fixed in scanner stage and scanning was done at 254 nm and 366 nm. The peak densitogram was noted [23].

Estimation of polyphenolic compounds: HPLC analysis

Analysis of polyphenolic compounds present within the methanolic extract of *F. benghalensis* was performed using the polyphenolics reference standard for HPLC [24]. HPLC analysis was carried out using a Waters 2487 HPLC system consisting of a dual λ detector and a Waters 1525 binary pump, and equipped with a Waters Symmetry C18 column (5 µm, 4.6 × 150 mm) with Waters Sentry™ universal guard column (5 µm, 4.6 × 20 mm) (Waters Corporation, Milford, MA, US. Gradient elution was performed with Solution A (50 mM sodium phosphate in 10 % methanol; pH 3.3) and Solution B (70 % methanol) in the following gradient elution program: 0-15 min 100 % of Solution A; 15-45 min 70 % of Solution A; 45-65 min 65% of Solution A; 65-70 min 60 % of Solution A; 70-95 min 50 % of Solution A; 95-100 min 0 % of Solution A. The dried extract was dissolved in HPLC grade methanol (10 mg/ml), filtered through sterile 0.22 µm syringe filter and 10 µl volume waµs injected to the HPLC. Flow rate was maintained 1 ml/min and temperature was adjusted to 25°C. Detection was monitored at diverse wavelengths 250, 280, 320, 370 and 510 nm

Statistical analysis

The results of DPPH radical scavenging activity, total antioxidant activity, iron chelating activity, reducing power activity, total phenolic content, total flavanoids content and brine shrimp lethality are reported as mean ± standard deviation of three replicates. Results were analyzed using Microsoft Excel 2007 and GraphPad Prism 5.

Results

Yield of the extract

10 gm of dried leaves powder was extracted in methanol to obtain the test extract. After drying, *F. benghalensis* yielded 0.58 gm of extract that is 5.8 % of the initial plant powder.

Phytochemical analysis

The results of the phytochemical analysis of the methanolic extract of *F .benghalensis* leaves showed the presence of tannins, carbohydrates, phytosterols, flavonoids and phenolic as major phytochemical groups; whereas saponins, oil and fats and alkaloids were find to be absent. In past, it has been widely accepted that phenolics and flavonoids are two important phytochemical groups contributing significantly for antioxidant activity of the plants. Results of phytochemical screening are summarized in Table 1.

Antioxidant activity

DPPH radical scavenging activity

The DPPH assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. It was observed that upon adding the reaction mixture into the methanolic plant extract, colour changed from purple to yellow. The extract showed a concentration dependent increase in DPPH radical scavenging activity with an IC_{50} value 11.21 µl. The results are graphically represented as percentage DPPH radical scavenging (Figure 1).

Table 1. Phytochemical composition of methanolic extract of *F. benghaliansis* leaves

Compound	*F. benghaliansis*
Saponins	-
Oil and fats	-
Tannins	+ + + + +
Alkaloid	-
Carbohydrates	+ + + +
Phytosterols	+ + + + +
Flavonoids	+ + + + +
Phenolics compounds	+ + + + +

- (Negative), + (Positive)

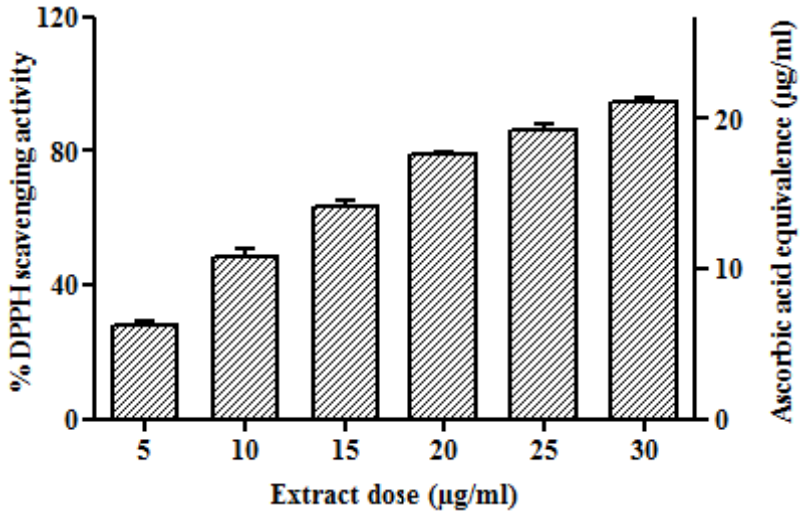

Fig 1. Percentage DPPH Ï% radical scavenging potential of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) with ascorbic acid equivalence

Results were compared with ascorbic acid and represented as ascorbic acid equivalence. The results of DPPH radical scavenging activity are reported in Figure 1.

Total antioxidant activity

The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of extract. In the presence of the antioxidants, the Mo(VI) is reduced to Mo(V) and forms a green coloured phosphomolybdnum V complex which shows maximum absorbance at 695 nm. The methanolic plant extract showed a good dose dependent increase in the total antioxidant activity and it was compared with the standard ascorbic acid equivalence. The results for total antioxidant activity of *F. benghalensis* are summarized in Figure 2.

Iron chelating assay

The antioxidant activities of phenolic compounds are also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as the catalysts for the initial formation of reactive oxygen species. Chelating agents may stabilize pro-oxidative metal ions in living systems by complexing them. In iron chelating assay, O-phenanthroline quantitatively forms complexes with Fe^{+2} which get disrupted in the presence of chelating agents. The chelating agents interfered with the formation of a ferrous-o-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. In this respect, methanolic extracts of the *F. benghalensis* was assessed for their ability to chelate transition activity. Extract showed a high iron chelating activity in a dose dependent manner. The results of iron chelating activity are compared with the standard ascorbic acid equivalence and are graphically represented in Figure 3.

Reducing power activity

Reducing power assay is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, extract was assessed to reduce ferric ions are into ferrous ions with the colour of the reaction mixture changes from yellow to bluish green due to formation of Perl's Prussian blue. Extract demonstrated a dose dependent increase in absorbance which is corresponding to the increase in the reducing power of the extract. The results of reducing power activity are compared with the standard ascorbic acid equivalence and are graphically represented in Figure 4.

Brine shrimp lethality assay

The brine shrimp cytotoxicity assay was

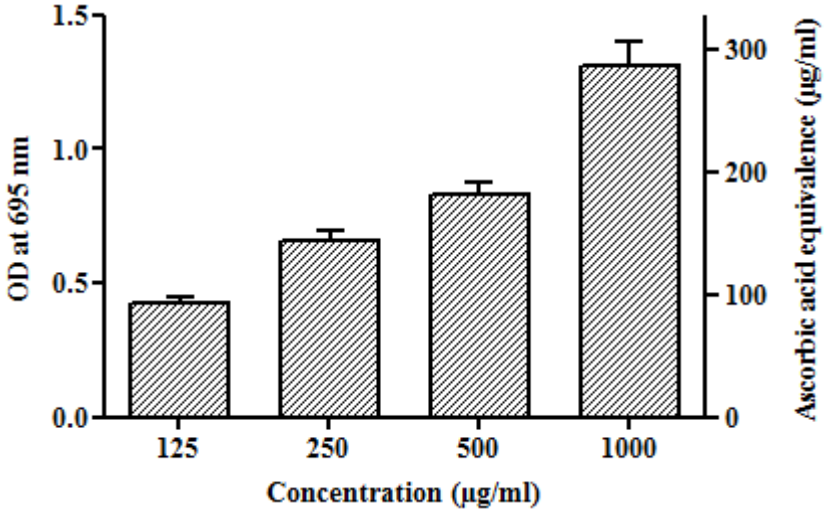

Fig. 2. Total antioxidant activity of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) with ascorbic acid equivalence

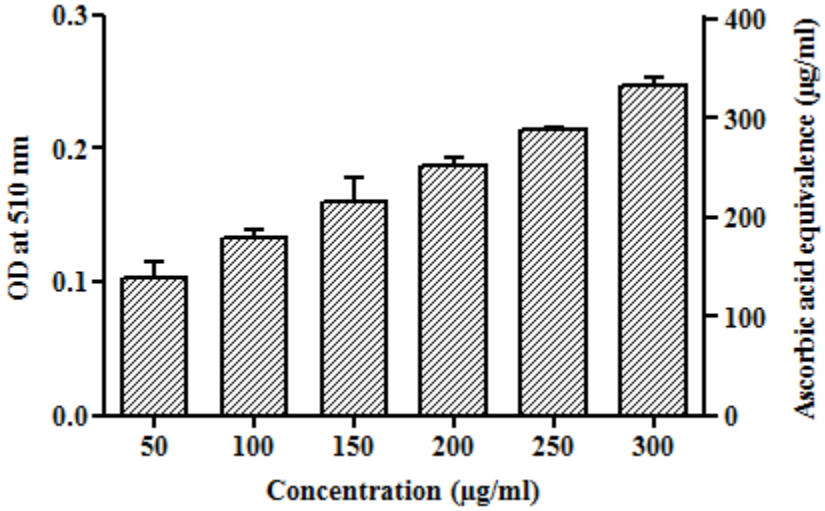

Fig. 3. Iron chelating activity of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) with ascorbic acid equivalence

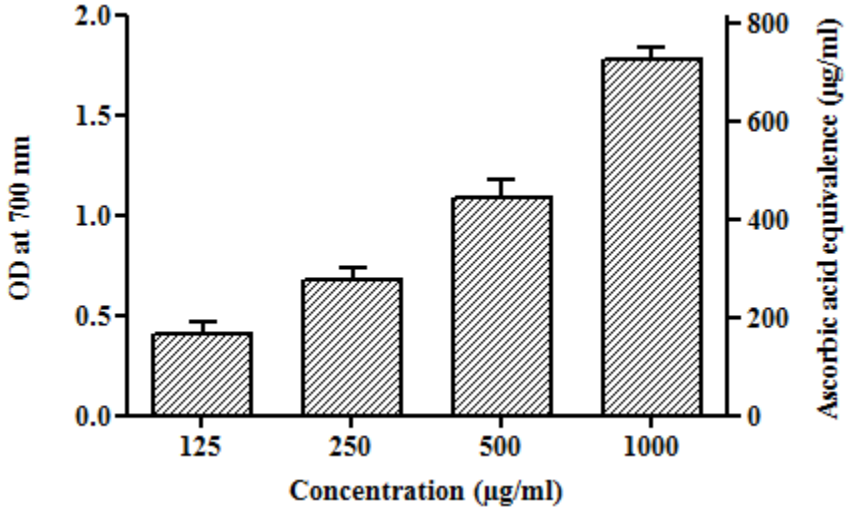

Fig. 4. Reducing power activity of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) with ascorbic acid equivalence

considered as a convenient probe for preliminary assessment of cytotoxicity of the plant extracts. Methanolic extract of *F. benghalensis* leaves demonstrated a dose dependent and time dependent increase in cytotoxic activity towards brine shrimp nauplii. Results of the cytotoxic activity are depicted in graphical form Figure 5.

Estimation of polyphenolic compounds

Estimation of total phenolic and total flavonoid content

Phenolics and flavonoids are two groups of phytochemicals contributing significantly in the antioxidant potential of plant material as these two inhibit the chain reaction started by the free radicals thereby preventing the body from various diseases. The total phenolic and flavonoid content of *F. benghalensis* was found to be 53.004 mg gallic acid equivalence/gm dry weight of the extract and 142.99 mg quercetin equivalence/gm dry weight of the extract, respectively. The results of total phenolic content and total flavonoid content are reported in Figure 6 and Figure 7. On the basis of the results obtained it can be concluded that *F. benghalensis* possess large amount of phenolics and flavanoids which may

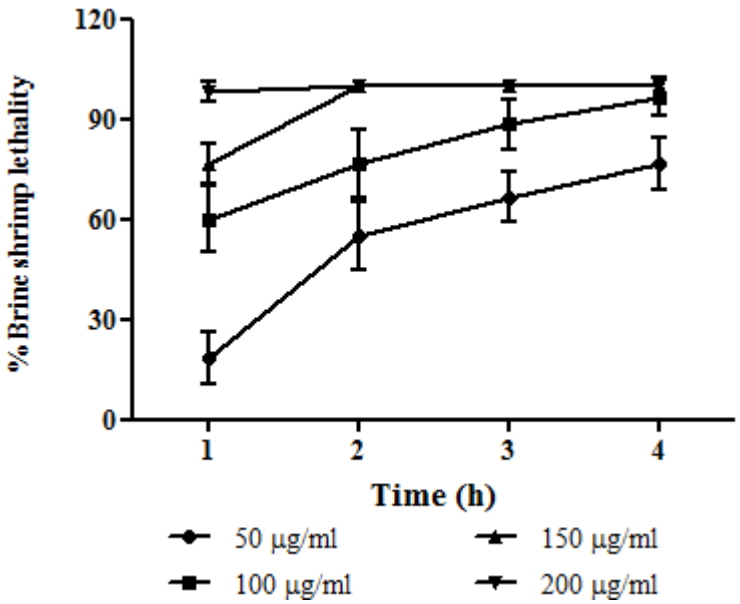

Fig. 5. Brine shrimp lethality potential of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test)

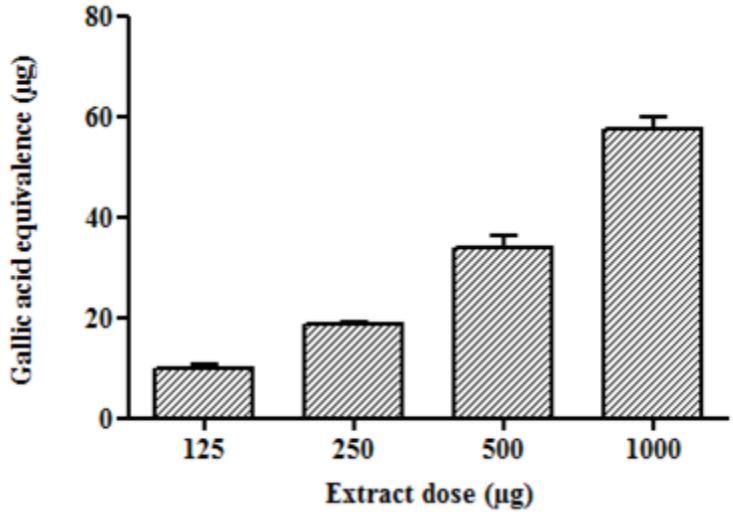

Fig. 6. Total phenolic content of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) and expressed as gallic acid equivalence (GAE)

be the candidates towards the antioxidant activity of the *F. benghalensis*.

HPTLC analysis of polyphenolic compounds

HPTLC analysis was performed to check the presence of polyphenolic compounds in methanolic extract of *F. benghalensis*. HPTLC chromatogram of aqueous extract of *E. ganitrus* is reported in Figure 8 A. 3D peak densitogram displayed the presence of 13 peaks, among them 6 peaks (peak 2, 4, 6, 8, 9 and 13) corresponding to polyphenolic compounds (Figure 8 B). Height, area and Rf values of the peaks appeared in peak densitogram are reported in Table 2. Based on area, peak 2, 9 and 13 appeared as major peaks while peak 4, 6 and 8 as minor peaks.

HPLC analysis of polyphenolic compounds

Polyphenolic compounds can be defined as a large series of chemical constituents possessing

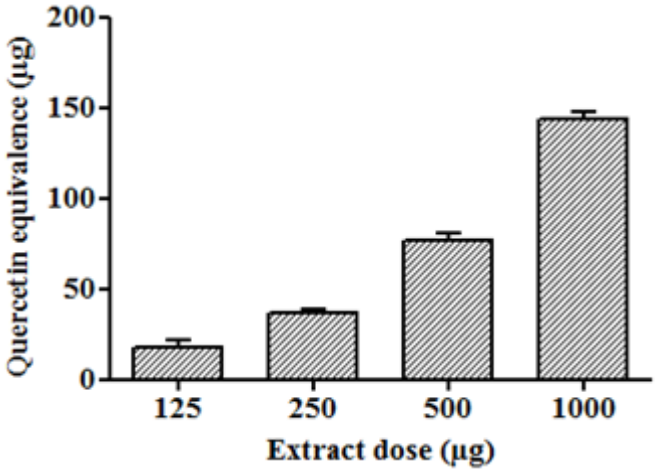

Fig. 7. Total flavonoids content of varying concentrations of methanolic extract of *F. benghaliansis*. Data is given in mean ± SD (n = 3 test) and expressed as quercetin equivalence (QE)

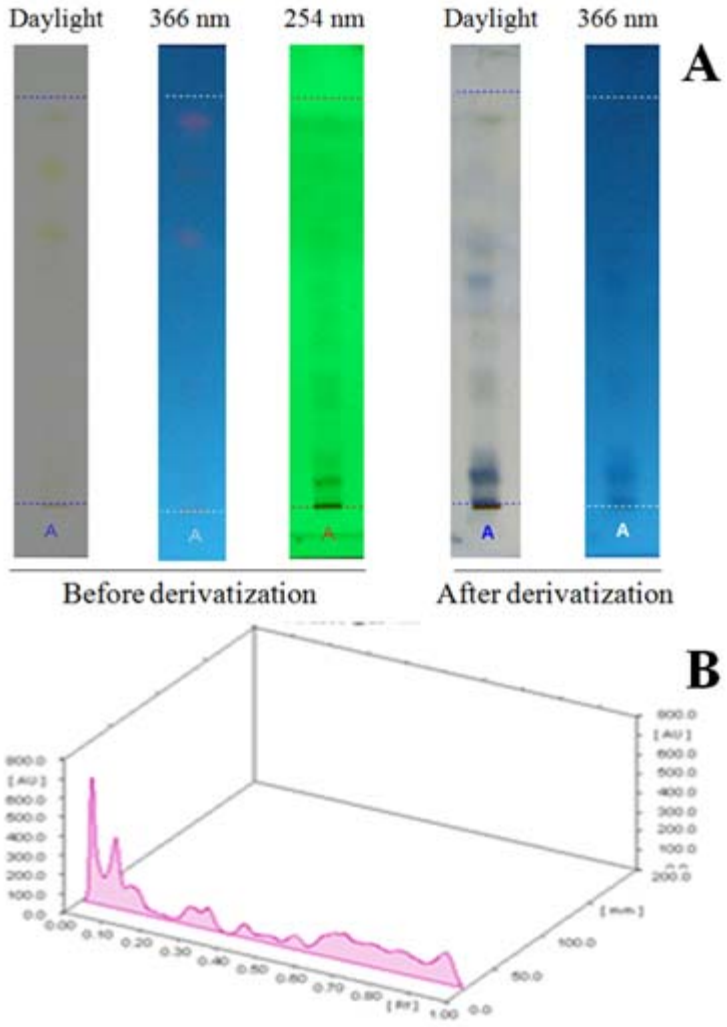

Fig. 8. HPTLC analysis of methanolic extract of *F. benghaliansis* for polyphenolic compounds. (A) HPTLC chromatogram, (B) 3D Peak densitogram display at 254 nm

Table 2. HPTLC analysis of methanolic extract of *F. benghaliansis* for polyphenolic compounds-peak assignment

Peak	Rf	Height	Area	Assigned substance
1	0.03	650.4	10958.1	Unknown
2	0.09	363.5	10009.4	Polyphenol 1
3	0.13	133.6	5014.4	Unknown
4	0.29	92.0	3558.2	Polyphenol 2
5	0.33	107.0	3000.1	Unknown
6	0.42	70.0	2128.6	Polyphenol 3
7	0.49	38.8	1390.1	Unknown
8	0.56	70.5	2629.4	Polyphenol 4
9	0.66	132.7	5859.8	Polyphenol 5
10	0.68	140.5	4617.1	Unknown
11	0.76	120.2	6454.8	Unknown
12	0.83	118.5	8063.8	Unknown
13	0.95	164.0	8358.1	Polyphenol 6

at least one aromatic ring, bearing hydroxyl and other sub-constituents. RP-HPLC analysis is the most used method for the identification of plant phenolic compounds. Because of the diversity and complexity of natural phenolics in medicinal plants, it is difficult to characterize every compound and elucidate its structure. In the present study, the identification of polyphenolic compounds was estimated by HPLC and by the comparison of their retention time with reference standards [25]. The extract showed the presence of Gallic acid (3,4,5-OH), Rhein (1,8-OH, 3-COOH), Anthraquinone (none), (-)-gallocatechin (R_1 = OH, R_2 = H, R_3 = OH), Theaflavin-3,3'-digallate (R_1 = OG, R_2 = OG), Flavone (none). The results are summarised in Table 3.

Discussion

Concern over the safety of synthetic anti-oxidants has shifted the global interests towards exploration of antioxidant compounds from natural sources. A plethora of polyphenolic compounds extracted from several plant species have been reported to possess strong antioxidant activities [25-26]. Polyphenolic compounds are ubiquitously present in plants, and when plants

Table 3. Polyphenolic compounds identified in methanolic extract of *F. benghaliansis* leaves by HPLC

Compounds	Type of compound	λ^a (nm)	Et_R^b (min)	Rt_R^c (min)
Gallic acid (3,4,5-OH)	Benzoic acids	250	5.95	5.8
Rhein (1,8-OH, 3-COOH)	Theaflavins	250	85.43	85.3
Anthraquinone (none)	Theaflavins	250	88.62	88.5
(-)-gallocatechin (R_1 = OH, R_2 = H, R_3 = OH)	Catechins	280	7.99	8.1
Theaflavin-3,3'-digallate (R_1 = OG, R_2 = OG)	Theaflavins	280	81.47	81.3
Flavone (none)	Flavones	280	88.65	88.8

[a] Wavelenghth for determination

[b] Experimental retention times

[c] Reference retention times [25]

are consumed as foods, these phytochemicals contribute to the intake of natural antioxidants in the diets of human. Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of medicinal plants, fruits and vegetables. The antioxidant properties of polyphenolic compounds are attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities [27], therefore, in the present study four different assays were employed in order to determine the antioxidant properties of the methanolic extract of *F. benghalensis*. During this study methanolic extract of *F. benghalensis* leaves exhibited significant antioxidant activity in all the tests performed which proves the strong antioxidant property of the extract. Antioxidant activity of methanolic extract of *F. benghalensis* may be due to the presence of high amount of polyphenolic compounds in the extract. HPLC analysis of extract showed the presence of some of the commonly known antioxidant compounds such as Gallic acid, Rhein, Anthraquinone, (-)-gallocatechin, Theaflavin-3,3'-digallate and Flavone [28-33] in the extract, which further justifies the antioxidant nature of the extract. Earlier also several studies have reported a strong correlation between the presence of phenolic contents and the antioxidant potential of the plants [34-35]. Brine shrimp lethality assay is a very simple and widely used preliminary method for the evaluation of cytotoxicity of natural products [20]. During the study methanolic extract of *F. benghalensis* leaves found to exhibit significant cytotoxic activity toward brine shrimp nauplii. Though it is very important to study the effect of the extract on the cancer cell lines before making any strong statement regarding the cytotoxicity activity of methanolic extract of *F. benghalensis* leaves, however extract demonstrate a promising cytotoxic potential in brine shrimp lethality assay.

The results from the present study demonstrated that *F. benghalensis* can be used as a source of safe and natural antioxidant compound. A significantly good amount of flavonoids and phenolic compounds could be obtained from *F. benghalensis*, which could be used for the development of important therapeutic or nutritional supplements. Further, more advanced studies could be conducted to isolate the principle molecule(s) and to establish the mechanism of action of the molecule(s).

Acknowledgement

The authors wish to thank the Management and Staff of VIT University, Vellore, TN, India for providing necessary facilities to carry out this study.

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