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Antioxidant activities and GCMS analysis fruits of *Ficus benghalensis* L

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Abstract

Ficus benghalensis belongs to the family Moraceae is a giant evergreen tree with spreading branches which are supported by aerial roots that go down into the soil to form the 'props'. The milky latex is applied externally for treating pains in rheumatism and in lumbago. The infusion of the bark is used against dysentery, diarrhoea and diabetes. The study aims to evaluate its antioxidant activities and GC-MS analysis of ethanol fruit extract of *F. Benghalensis*. The antioxidant assays such as DPPH[•] radical, ABTS^{•+} radical cation, OH[•] radical and NO[•] radical scavenging assays, phosphomolybdenum reduction and Fe³⁺ reducing power assays were carried out for fruit extract. The maximum DPPH[•] radical scavenging activity was 75.74±5.30% at 60 µg/mL concentration and the IC₅₀ was 32.20 µg/mL concentration. The maximum NO[•] radical scavenging activity was 51.96±3.64% at 60 µg/mL concentration and the IC₅₀ was 57.74 µg/mL concentration. The maximum ABTS^{•+} radical cation scavenging activity was 79.57± 5.57% at 30 µg/mL concentration and the IC₅₀ was 13.69 µg/mL concentration. The maximum OH[•] radical scavenging activity was 57.02±3.99 % at 60 µg/mL concentration and the IC₅₀ was 34.37 µg/mL concentration. The maximum phosphomolybdenum reduction was 80.48±5.63 % at 60 µg/mL concentration and the RC₅₀ was 13.78 µg/mL concentration. The maximum Fe³⁺ reduction was 69.19±4.84 % at 60 µg/mL concentration and the RC₅₀ was 18.71 µg/mL concentration. GC-MS analysis showed different ester derivative compounds present in the fruit extract of *F. Benghalensis*.

Keywords: *Ficus benghalensis*, DPPH[•] radical, ABTS^{•+} radical cation, GC-MS analysis

Introduction

Ficus benghalensis is commonly known as Banyan tree and is a large evergreen tree distributed all over India from sub Himalayan region to the deciduous forest in South India [1, 2]. It grows up to 30 m height, with spreading branches and many aerial roots. The leaves are stalked, ovate-corate, 3-nerved entire, when young downy on both sides. The petioles are broad smooth greasy gland at the apex, compressed and downy. The fruits are in auxiliary pairs as the size of a cherry. The external features of the bark are 12-18 mm thick, grey, closely adhered ashy white, light bluish-green or grey patches, slightly curve, thickness varies with the age of the tree. The surface of bark is deeply fissured and rough due to the presence of longitudinal and transverse row of lenticels, mostly circular and prominent, fracture short in outer 2/3 of bark while inner portion shows a fibrous fracture [3, 4]. It is used for treatment of neuralgia, rheumatism, lumbago, bruises, nasitis, gonorrhoea, inflammations, cracks of the sole and skin diseases and in ayurveda for diarrhoea, dysentery, and piles [5].

Taxonomic classification of *Ficus benghalensis*

Kingdom: Plantae
SubKingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Hamamelidae
Order: Urticales
Family: Moraceae
Genus: *Ficus*
Species: *F.benghalensis*

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Fig 1: *Ficus benghalensis* fruit

Materials and Methods

Collection of plant material and preparation of the extract

Fruits of *F. benghalensis* were collected at Perungudi, Chennai, India, washed with tap water and subjected to mild crushing. The squashed fruits were soaked in ethanol for 72 h. The supernatant was filtered by filter paper and condensed at 50°C in a hot plate.

Qualitative phytochemical analysis

The fruit extract of *F. benghalensis* was subjected to preliminary phytochemical analysis for different classes of phytoconstituents using specific reagents [6, 7].

Estimation of total phenolic content

Folin-Ciocalteu reagent method was used to determine the total phenolic compounds with slight modifications [8]. One hundred μL of fruit extract (1mg/mL) of *F. benghalensis* was mixed with 900 μL of ethanol and 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) Na_2CO_3 solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent ($\mu\text{g}/\text{mg}$ of extract), which is a common reference compound.

Estimation of total flavonoids

The total flavonoid content of ethanol fruit extract of *F. benghalensis* was determined using aluminium chloride reagent method with slight modification [9]. Five hundred μL of extract (1mg/mL) was mixed with 500 μL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.3 mL 10% (w/v) aluminium chloride solution was added and incubated for further 5 min at room temperature followed by 1 mL of 1 M NaOH solution was added. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using spectrophotometer. The result was expressed as ($\mu\text{g}/\text{mg}$ of extract) quercetin equivalent

In vitro antioxidant activity

DPPH $^{\bullet}$ radical scavenging activity

The antioxidant activity of ethanol fruit extract of *F. benghalensis* was measured on the basis of DPPH free radical scavenging activity [10]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (10-60 $\mu\text{g}/\text{mL}$) of fruit extract. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used

as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

ABTS $^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was estimated in terms of the ABTS $^{\bullet+}$ radical cation scavenging activity [11]. ABTS $^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS $^{\bullet+}$ solution (stable for 2 days) was diluted with distilled water to reach an absorbance of 0.70 ± 0.02 at 734 nm. Ethanol fruit extract of various concentrations (5-30 $\mu\text{g}/\text{mL}$) was mixed with 500 μL of diluted ABTS $^{\bullet+}$ solution and the absorbance was measured at 734 nm after 10 min. The ABTS $^{\bullet+}$ radical-scavenging activity was expressed as

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Hydroxyl radical (OH $^{\bullet}$) scavenging activity

The hydroxyl radical scavenging activity of ethanol fruit extract of *F. benghalensis* was evaluated by the method of Ganu *et al* [12]. Various concentrations (10-60 $\mu\text{g}/\text{mL}$) of fruit extract was added with 1.0 mL of iron EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22% w/v) and incubated at 80-90°C for 15 min in a water bath. After incubation, the reaction was terminated by the adding 1.0 mL of ice cold TCA (17.5% w/v) solution. Then 3 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and made up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured at 412 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated as:

$$\% \text{ of OH}^{\bullet} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Nitric oxide radical (NO $^{\bullet}$) scavenging activity

Nitric oxide scavenging activity was carried out by Griess reagent method [13]. Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations (10- 60 $\mu\text{g}/\text{mL}$) of ethanol fruit extract and incubated at 30°C for 3 h. The same reaction mixture without the extract but the equivalent amount of methanol served as the control. After incubation period, 0.5 mL of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore was immediately read at 550 nm. Inhibition of nitrite formation by the fruit extract by means of NO $^{\bullet}$ radical scavenging activity was calculated as

$$\% \text{ of NO}^{\bullet} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of ethanol fruit extract of *F. benghalensis* was assessed by the method of Jan *et al* [14]. The fruit extract with different concentrations ranging from 10 to 60 µg/mL was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Mo}^{6+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Ferric (Fe³⁺) reducing power assay

The reducing power of ethanol fruit extract of *F. benghalensis* was determined by slightly modified method of Yildirim *et al* [15]. One mL of fruit extract of different concentrations (10 - 60 µg/mL) was mixed with 1 mL of 1% (w/v) potassium ferricyanide [K₃Fe(CN)₆] solution and 1 mL of 0.2 M phosphate buffer (pH 6.6) and. The mixture was then incubated at 50°C in a water bath for 30 min. One mL of 10% (w/v) trichloroacetic acid was added to each mixture. Then 1 mL 0.1% (w/v) of freshly prepared FeCl₃ solution was added and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Gas chromatography–Mass Spectrometry (GC–MS)

For GC-MS analysis, the fruit extract was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units [16].

Results and Discussion

Total Phenol and Flavonoid content

Flavonoids, and phenolic compounds are known to play major roles in the antioxidant and prooxidant capacities exhibited by plant extracts. The antioxidant effect conferred by these compounds are due to the phenolic hydroxyl groups attached to their respective ring structures that can act as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers, and as metal chelators. They are also said to reduce α-tocopherol radicals or tocopheroxyls, activate antioxidant enzymes, mitigate nitrosative stress, and inhibit oxidases [17]. After proton donation, these compounds are oxidized to resonance-stabilized radicals that can further act as prooxidants at high concentrations, high pH, and in the presence of metal ions [18].

Flavonoids and phenolic acids such as quercetin, myricetin, gallic acid, caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid have been proven to exhibit oxidant and prooxidant activities [19]. The total phenol and flavonoid content of ethanol fruit extract of *F. benghalensis* were 421.54 ± 3.29 µg/mg and 15.54 ± 0.28 µg/mg respectively.

DPPH[•] radical scavenging assay

The DPPH radical scavenging activity was assessed by H donating ability of ethanol fruit extract of *F. Benghalensis*. The maximum DPPH[•] radical scavenging activity was 75.74 ± 5.30 % at 60 µg/mL. Fruit extract of *F. benghalensis* demonstrated high capacity to deactivate free radicals by reducing the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC₅₀ value was found to be 32.20 µg/mL concentration (Figure 2 and Table 1) and was compared with standard (Ascorbic acid, IC₅₀ = 11.98 µg/mL concentration).

ABTS^{•+} radical cation scavenging assay

ABTS^{•+} is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the fruit extract, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum ABTS^{•+} radical cation scavenging activity was 79.57 ± 5.57 % at 30 µg/mL concentration. The experiment demonstrated high antioxidant activity the IC₅₀ of 13.69 µg/mL concentration (Figure 4 and Table 3) and was compared with standard Ascorbic acid (IC₅₀ = 4.21 µg/mL concentration).

Hydroxyl (OH[•]) radical scavenging activity

The hydroxyl radical is a highly reactive free radical formed in biological systems and reacts with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Scavenging of hydroxyl radical is an important antioxidant activity. The maximum hydroxyl radical scavenging activity was 57.02 ± 3.99 % at 60 µg/mL concentration. This experiment also showed reliable antioxidant activity with IC₅₀ of 34.37 µg/mL concentration (Figure 2, Table 1) and was compared with standard ascorbic acid (IC₅₀ = 3.26 µg/mL). The ethanol extract of *F. benghalensis* significantly inhibited generation of OH[•] radicals in a dose-dependent manner.

Nitric oxide radical (NO[•]) scavenging activity

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO[•]. Under aerobic conditions, NO[•] reacts with oxygen to produce stable products such as nitrate and nitrite ions and the quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen and to reduce production of nitrite ions formed during diazotization of the nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes [20]. Excess concentration of NO is associated with several diseases [21]. NO is generated in biological tissues by specific nitric oxide synthesis, which metabolizes arginine to citrulline with the formation of NO via a five electron

oxidative reaction [22]. These compounds are responsible for altering the structural and functional behaviour of many cellular components. NO· radical scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. The maximum nitric oxide radical scavenging activity was $51.96 \pm 3.64\%$ at 60 $\mu\text{g/mL}$ concentration and the IC_{50} was 57.74 $\mu\text{g/mL}$ concentration (Figure 2, Table 1) and was compared with standard ascorbic acid ($\text{IC}_{50} = 3.26 \mu\text{g/mL}$). The ethanol extract of *F. benghalensis* significantly inhibit generation of NO· radicals in a dose-dependent manner.

Phosphomolybdenum reduction assay activity

The total antioxidant activity of ethanol extract of *F. benghalensis* was measured spectrophotometrically by phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum reduction was $80.48 \pm 5.63\%$ at 60 $\mu\text{g/mL}$ concentration and the RC_{50} was 13.78 $\mu\text{g/mL}$ concentration (Figure 3 and Table 2). It was

compared with the standard ascorbic acid ($\text{RC}_{50} = 5.97 \mu\text{g/mL}$).

Ferric (Fe^{3+}) reducing power activity

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [23]. 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.

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+} /ferricyanide complex used in this method the reducing power of Fe^{3+} to Fe^{2+} by the ethanol fruit extract of *F. benghalensis* showed the reduction ability in a dose dependent manner (Figure 3 and Table 2). The maximum reduction was $69.19 \pm 4.84\%$ at 60 $\mu\text{g/mL}$ concentration and the RC_{50} was 18.71 $\mu\text{g/mL}$ concentration, which was compared with the standard ascorbic acid ($\text{RC}_{50} = 29.11 \mu\text{g/mL}$).

Table 1: DPPH· radical, OH· radical and NO· radical scavenging activities of ethanol fruit extract of *F. benghalensis*

S. No	Concentration ($\mu\text{g/mL}$)	% of inhibition		
		DPPH·	OH·	NO·
1	10	30.18 ± 2.11	16.44 ± 1.15	08.82 ± 0.62
2	20	41.34 ± 2.89	25.88 ± 1.81	22.06 ± 1.54
3	30	46.58 ± 3.26	35.09 ± 2.46	25.98 ± 1.82
4	40	58.43 ± 4.09	43.64 ± 3.06	28.92 ± 2.02
5	50	70.04 ± 4.90	48.03 ± 3.36	40.20 ± 2.81
6	60	75.74 ± 5.30	57.02 ± 3.99	51.96 ± 3.64

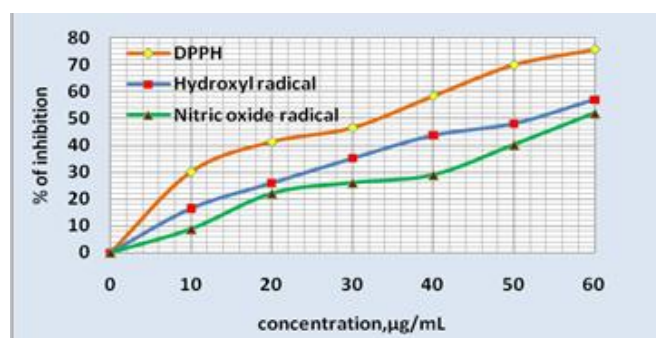


Fig 2: DPPH· OH· and NO· radical scavenging assay

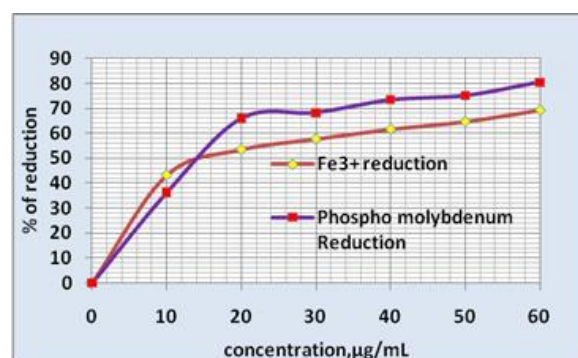


Fig 3: Phosphomolybdenum reduction and Fe^{3+} reducing power assay

Table 2: Phosphomolybdenum reduction and Fe^{3+} reducing power of ethanol fruit extract of *F. benghalensis*

S. No	Concentration ($\mu\text{g/mL}$)	% of reduction	
		Phosphomolybdenum reduction assay	Fe^{3+} reducing power assay
1	10	36.27 ± 2.54	43.17 ± 3.02
2	20	65.97 ± 4.62	53.44 ± 3.74
3	30	68.29 ± 4.78	57.64 ± 4.04
4	40	73.36 ± 5.14	61.55 ± 4.31
5	50	75.09 ± 5.26	64.60 ± 4.52
6	60	80.48 ± 5.63	69.19 ± 4.84

Table 3: ABTS·⁺ radical cation scavenging activity of ethanol fruit extract of *F. benghalensis*

S. No	Concentration ($\mu\text{g/mL}$)	% of inhibition
		ABTS· ⁺
1	5	28.17 ± 1.97
2	10	44.89 ± 3.14
3	15	54.80 ± 3.84
4	20	55.11 ± 3.86
5	25	74.92 ± 5.24
6	30	79.57 ± 5.57

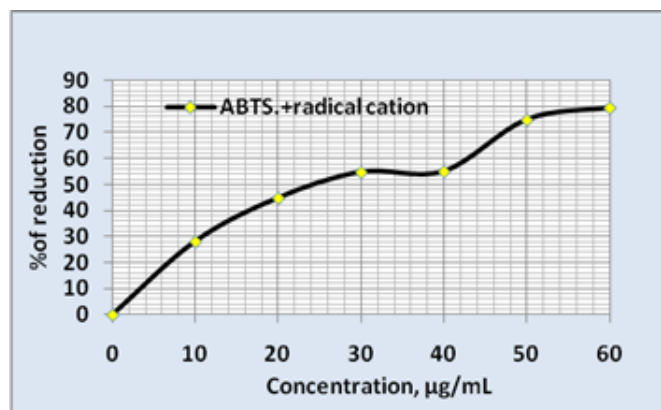


Fig 4: ABTS^{•+} radical cation scavenging assay

GC-MS analysis

GC-MS analysis was carried out for ethanol fruit extract of *F. benghalensis* and the eluted compounds were showed in Table 4. The compound 14, 17-octadecadienoic acid, methyl ester is reported to posses skin scaling and wound healing property.

The compound heptadecanoic acid, 16 methyl, methyl ester is reported to posses antioxidant, anti-inflammatory, antifibrotic and vasodilator property and could be one of the reasons for the antioxidant property of the extract.

Table 4: GC-MS analysis of ethanol fruit extract of *F. benghalensis*.

S. No	RT	Compound structure	Mol. Formula	Mol. weight g/mol	Compound name	Biological activity
1	14.17		C ₁₉ H ₃₄ O ₂	294.479	14,17-octadecadienoic acid,methyl ester	Prevent hair loss and Skin scaling ^[24] , Wound healing ^[25] .
2	15		C ₁₃ H ₂₆ O ₂	214.3443	Undecanoic acid,10methyl,methyl ester	
3	16.13		C ₁₄ H ₂₄ O ₄	256.33796	5-Decenedioic acid,5,6-dimethyl,dimethyl ester	
4	17.08		C ₁₇ H ₃₄ O ₂	270.4507	Hexadecanoic acid,methyl ester	Antioxidant, Antifungal and Antimicrobial ^[26] .
5	18.13		C ₁₈ H ₃₆ O ₂	284.4772	Hexadecanoic acid,14-methyl,methyl ester	Anti-inflammatory ^[27] , Hypocholesterolemic, Antioxidant, nematicide, pesticide, anti-androgenic ^[28] .
6	19.07		C ₁₉ H ₃₈ O ₂	298.5038	Heptadecanoic acid,16 methyl,methyl ester	Antioxidant ^[29] , Anti-inflammatory ^[30] , Anti-fibrotic ^[30] , Vasodilator ^[31] .
7	20.55		C ₁₉ H ₃₆ O ₃	312.494	Oxiraneoctanoic acid,3 octyl,methyl ester	

Conclusion

The study showed that the ethanol fruit extract *F. benghalensis* has significant antioxidant and radical scavenging activities. The antioxidant compounds present in the fruit extract can be used to prevent oxidative stress related degenerative disease which may cause damage to cells. Further research work is required to find out bioactive molecules and their mechanism of action to explore their therapeutic potential before it can be recommended for clinical use.

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