



Research Article

ANTI-OXIDANT, ANTI-MICROBIAL ACTIVITIES AND PHYTOCHEMICAL ASSAY OF AERIAL ROOTS OF FICUS BENGHALENSIS

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Abstract: A country's glory is determined by the resources present in it, especially flora and fauna. India is one of those countries of the world, which is bestowed with vivid and diverse resources. The medicinal plants, which are indigenous to this country, are perhaps one of the most desired gifts of the nature. A few trees like *Ficus benghalensis* are so precious that every part of their entity can be used as the panacea for many chronic malaises. This paper focuses on the importance of aerial roots of the tree. The various important pharmaceutical activities like anti-oxidant and anti-microbial are studied. However, prior to these studies phyto-chemical assay of the aerial roots is also done. The various standard anti-oxidant studies include: Total phenolic content of the sample (absorbance at 750nm), DPPH radical scavenging activity, Nitric oxide free radical scavenging activity and reducing power ability. The anti microbial activities against *K. pneumoniae* and *S.aureus* were studied and the inhibition zones were recorded.

Keywords: *Ficus benghalensis*, Aerial Roots Anti-oxidant & Anti-microbial studies *K. pneumoniae*, *S. aureus*

INTRODUCTION

The medicinal plants have been playing a prominent role in therapeutic uses [1]. *Ficus benghalensis*, one of the species of family Moraceae (Mulberry), plays an important role among the traditionally used medicinal plants in India [2]. It is referred as *Avaroha*, *Bahupada*, *Vaisravana*, *Nyagrodha*, and *Vat* (in Sanskrit language) and has been used as the potent medicine for many malaises. It is a part of the mythology of the land and a member of four scared trees: *Nalpamara* [3] (*Ksirivraksas*). The Fig tree is even described in *Rigveda*, *Charaka Samhita* and *Sushrusa Samhita* [4] with enlightening notes about its medicinal uses. Every part of the tree is considered to be useful, especially as an antibacterial and antifungal agent [5-7]. According to a study by WHO [8], 80 percentage of the world population still depends upon the conventional and traditional method of treatment. In developing countries like India, which are ancient civilizations in the world follow the methodologies like Ayurveda and Unani [9] to cure common diseases and *Ficus benghalensis* is used in them. Although there is extensive research going on all the other parts of Fig tree like Leaves, Bark, Figs and Stem, we concentrated our studies on Aerial roots. Apart from phytochemical studies we conducted anti-oxidant and anti-microbial studies. We selected *Klebsiella pneumoniae*, one of the prominent microbes causing urinary tract infections, pneumonia, septicemias and soft tissue infections [10] and *Staphylococcus aureus* which causes skin infections like pimples, boils etc. in human beings [11]. The chief reason for this study is to rediscover effective medicine from traditionally important *Ficus benghalensis* as many new drug resistant bacterial strains are evolving [12].

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MATERIALS AND METHODS**Extract preparation**

The dried aerial roots (sunlight), which were collected within Vishakhapatnam (urban), Andhra Pradesh, were cut and ground to powder. Then a calculated amount of material (500 grams) was taken against two liters of Ethanol (95% w/v), transferred into a Round Bottom (RB) flask. It was heated in a hot water bath for about five hours. The concentrated plant extract, in ethanol solvent, was then subjected to distillation process and pure extract was collected in the beaker. This was stored in airtight container for further biological and phytochemical studies.

Biological activity**Antioxidant Activities****Total Phenolic content determination assay:**

Total natural phenolic content of plant extract was determined using *Folin-Ciocalteu* assay (Singleton and Rossi, 1965) with slight modifications. Test tube containing 500ul of standard solutions of gallic acid (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125ug/ml) and crude extracts (diluted 400-fold with distilled deionized water) was taken. 500ul of 10% *Folin-Ciocalteu's* phenol reagent (in DDW) was added into the test tube and mixed. After 20min, 350ul of 1M Na_2CO_3 solution was added into the mixture. After incubation for 20min at room temperature, the absorbance was determined at 750nm against the parallel-prepared blank (500ul of DDW +500ul of 10% FC reagent + 350ul of 1M Na_2CO_3 solution).



Determination of DPPH radical scavenging activity: The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (250-2500 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = (\text{A control} - \text{A test} / \text{A control}) \times 100$$

Where **A control** is the absorbance of the control reaction and **A test/std** is the absorbance in the presence of the extract. The antioxidant activity of the extracts was expressed as IC₅₀. The IC₅₀ value was defined as the concentration in µg/ml of extracts that inhibits the formation of DPPH radicals by 50%.

Reducing power assay: The reducing power of methanolic extracts was determined according to the method of Oyaizu (1986). Different concentrations of pod extract (10– 100 µg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standard. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of Nitric oxide radical scavenging activity: Nitric Oxide radical inhibition was estimated by the use of Griess Illosvoy reaction (Garrat, 1964). Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10-100 µg /ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore

formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm. The percentage scavenging of nitric oxide of Plant extract and standard compounds were calculated using the following formula:

$$\text{NO Scavenged (\%)} = (\text{A cont} - \text{A test}) / \text{A cont} \times 100$$

Where **A cont** is the absorbance of the control reaction and **A test** is the absorbance in the presence of the sample of the extract.

Antibacterial Activities

Bacterial strains: The antibacterial activity of the aerial root extract was determined on gram negative (*Klebsiella pneumonia*) and gram-positive bacteria (*Staphylococcus aureus*).

Materials Used: Nutrient agar (Hi-Media), Nutrient Broth (Hi-Media), Petri plates, Autoclave, Conical flasks 250ml, Laminar airflow, and Required Bacterial strains (Clinically isolated Pathogens).

Procedure: The determination of the antibacterial activity of surfaces was conducted by the following norms: ISO 22196 and JIS Z 2801. The agar diffusion test, or the Kirby-Bauer disk-diffusion method (well diffusion method), was used to study the antibacterial activity of extract on the bacteria culture. The required numbers of Nutrient agar plates were prepared. The extract 5mg/ml (5000µg/ml) is made in DMSO and the required organisms (*S. aureus*, *K. pneumoniae*) are made ready in nutrient broth in proper dilution such that 10⁸ cells/ml. About 0.1ml of the organisms in the broth was evenly spread on the labelled petri plates. Then wells were made in the plates using 6mm cork borer, 60µl of the sample loaded. Ampicillin was used as standard and DMSO and Ethanol as control. The plates were incubated at 37°C for 18 -24hrs and zones of inhibition were measured.

Phytochemical analysis of the plant extract

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, flavonoids, alkaloids and glycosides in accordance with Allan (1976) and Harborne (1998) with slight modifications.

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water

bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4 . A yellow coloration observed in each extract indicated presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Alkaloids

(A) Dragendroffs reagent: 8g of bismuth nitrates $Bi(NO_3)_3 \cdot 5H_2O$ was dissolve in 20ml of HNO_3 and 2.72g of Potassium iodide in 50ml of H_2O . These were mixed and allowed to stand for deposition of KNO_3 Crystals. The Supernatant was decanted off and made up to 100ml with distilled water. Procedure: To 0.5ml of leaf extract 2ml of HCl was added. To this acidic medium 1ml of dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

(B) Mayers test: 1.36g of Mercuric chloride was dissolved in 60ml of distilled water and 5g of Potassium iodide in 10ml of water. These two solutions were mixed and diluted to 100 ml with distilled water.

Procedure: 1.2 ml of plant extract was taken in a test tube and to this 0.2 ml of dilute HCl and 0.1 ml of Mayers reagent were added. Formation of yellowish Puff colored precipitate indicates the presence of alkaloid.

RESULTS

Antioxidant activity

Table I: Total Phenolic Content

Conc. in ug/ml	Absorbance at 750nm
1.953	0.117
3.906	0.189
7.812	0.313
15.62	0.587
31.25	1.134
62.5	2.142
125	3.746

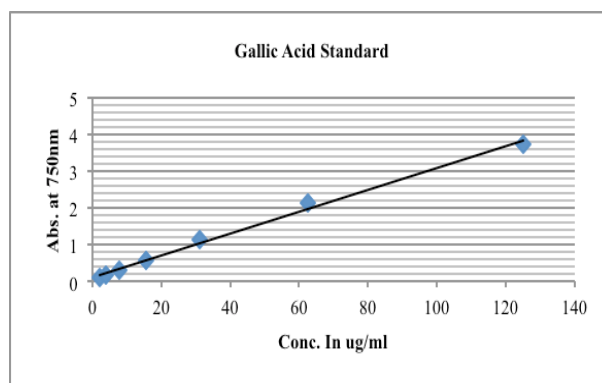


Table II: DPPH Assay

Conc. in ug/ml	% of Inhibition activity	
	Ascorbic acid	Sample
1000	97.76	77.60
500	96.20	72.02
250	95.68	47.17
125	91.36	36.90
62.5	88.98	34.37

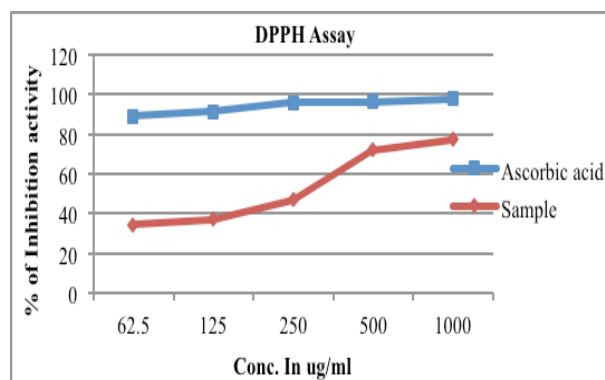


Table III: Nitric oxide Free Radical Scavenging Activity

Conc. in ug/ml	in Ascorbic acid	Sample
1000	48.1	28.1
800	47.8	17.4
400	31.7	6.8
200	21.1	0.5

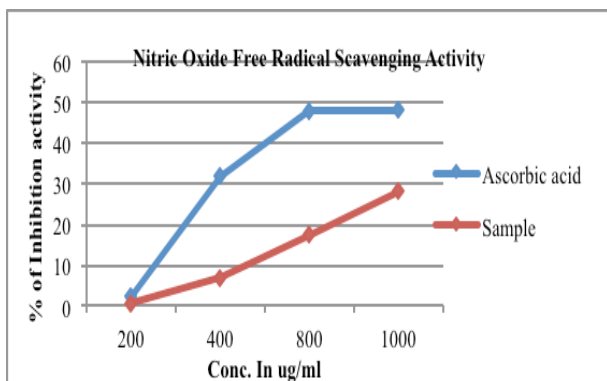
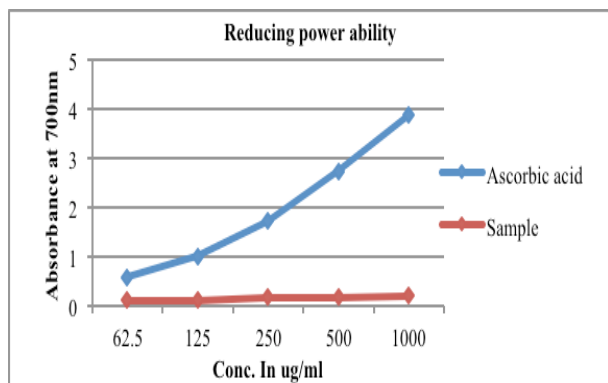


Table IV: Reducing Power

Conc. in ug/ml	Absorbance at 700nm	
	Ascorbic acid	Sample
1000	3.868	0.216
500	2.721	0.175
250	1.712	0.164
125	1.002	0.121
62.5	0.577	0.113



Antibacterial activity

Table V: Zones of Inhibition

S.No.	Organism	Zones of Inhibition			
		Sample	DMSO	Ethanol	Ampicillin
1	<i>K. pneumoniae</i>	10mm	nil	Nil	Nil
2	<i>S. aureus</i>	8mm	7mm	Nil	nil



Figure I: *Klebsiella pneumoniae*



Figure II: *Staphylococcus aureus*

DISCUSSIONS AND CONCLUSIONS

- The extract was subjected to phytochemical tests for tannins, saponins, flavonoids, alkaloids and glycosides and they were detected in the extract.
- The total Phenolic content of the sample is 12.875 µg GAE/mg of crude dry extract.
- The Anti-oxidant activity of the extract. i.e. DPPH study of the extract shows that the inhibition activity increases with concentration of the sample and the activity is highest at 1000ug/ml(Ascorbic acid is taken as reference)
- The Nitric Oxide Free Radical Scavenging Activity also increases with the concentration of the sample and the graph is almost linear.
- The reducing power of the sample increases is sparse. i.e. very low activity is observed.
- The extract shows anti-bacterial activity i.e. against the bacteria *Staphylococcus aures* with an inhibition Zone of about 8mm and *Klebsiella pneumoniae's* inhibition zone of 10mm.

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