# Phytochemical Screening, Antimicrobial and Antioxidant Activity of Bark Extract of *Ficus benghalensis* L

J. Ramalakshmana[1], S. Padmavathi*, V. Medan Mohan Rao[2], N. Chandra Babu[#] and S. B. Padal[3]

[1] (Guest Faculty in Botany, Department of Botany, Andhra University, Visakhapatnam-530 003 (AP), India).
[*] (Lecturer in Botany, Dr. V. S. Krishna Government Degree College (A), Visakhapatnam 530013 AP, India).
[2] (Lecturer in Botany, Government Degree College, Chintalapudi, Eluru -534460, A.P, India).
[#] (Lecturer in Botany, Government Degree College, Tiruvuru, NTR District - 521235, A.P, India).
[3] (Professor in Botany, Department of Botany, Andhra University, Visakhapatnam-530003 (AP), India).
Corresponding author: J. Ramalakshmana

**Received:** July 15, 2025 **Accepted:** Aug 16, 2025 **Published:** Aug 16, 2025

## Abstract

The present study investigated the phytochemical composition, antimicrobial activity, and antioxidant potential of bark extracts of *Ficus benghalensis* L. collected from Visakhapatnam district, Andhra Pradesh, India. The bark was extracted using acetone, methanol, and aqueous solvents through soxhlet extraction, and preliminary phytochemical screening was conducted using standard protocols. The methanol extract exhibited the highest diversity of phytochemicals, including alkaloids, flavonoids, tannins, phenolic compounds, and fixed oils and fats. Total phenolic content (TPC) estimation revealed higher values in methanol extracts, which strongly correlated with antioxidant activity (PCC = 0.918, $R^2$ = 0.843). Antimicrobial assays demonstrated that acetone extracts showed maximum inhibition against *E. coli* and *S. aureus*, while methanol extracts displayed moderate antibacterial and antifungal effects. Antioxidant evaluation using the DPPH assay indicated that methanol extract had the highest radical scavenging activity (94.59 ± 0.39% at 120 µg/mL) with the lowest $IC_{50}$, comparable to ascorbic acid. These results suggest that *F. benghalensis* bark, particularly methanol extracts, is a rich source of phenolic antioxidants with significant antimicrobial potential, supporting its ethnomedicinal applications.

**Key Words:** Phytochemicals, Methanol, Antimicrobial activity, Antioxidant, ascorbic acid.

## Introduction

One of the major genera in the family Moraceae, which has around 800 species worldwide and about 115 species distributed in India [I]. *F. benghalensis* L., frequently called the "Indian Banyan Tree" grows huge up to several meters in length and breadth. It is an evergreen tree that predominates in parks and botanical gardens all over the world's tropics. Being an Indian indigenous plant, it is considered sacred and can be found planted around

homes and religious places everywhere. It thrives well in semitropical, tropical, and regions with moderate amounts of precipitation and forest cover. The production of the primary metabolites (sugars, amino acids and fatty acids) is crucial to the plant's survival. However, secondary metabolites are necessary for their defense. Secondary metabolism provides protection from different stresses, which initiates secondary metabolite synthesis, which further activates the plant's mechanism of defense [2, 3, 4] Alkaloids, terpenes, and polyphenols are plants' most diverse secondary metabolites [5]. Flavonoids are produced in almost all the plant parts and cause flower pigmentation [6, 7]. They have an astringent flavor that makes them resistant to predators, including insects, birds, and herbivores [7, 8]. The world is passing through a phase that could be critical since antibiotics are losing their efficacy and the previously treatable common microbial diseases are becoming more challenging to treat.

The aqueous and the other organic extracts of *Ficus benghalensis* L. were discovered to exhibit a variety of pharmacological effects, including anti-diabetic, antitumor, hypolipidemic, hypocholesterolemic, anthelmintic, antiinflammatory, and antimicrobial [9]. It has also been found to promote pregnancy and acts as a natural plasma disinfectant. It has a significant historical role in indigenous medical systems including Ayurveda, Siddha, Unani, and homeopathy. Ficus is one of the most loved bonsai [1]. It has immune modulatory action and is good for wound healing, stress, and allergy relief [10]. The present work was carried out to study the differential phytochemical activity, antimicrobial and antioxidant activity of bark of *F. benghalensis* L.

## Materials and Methods

The barks of the *Ficus benghalensis* L. was simultaneously collected from Visakhapatnam district, Andhra Pradesh, India in January- 2024. Herbarium was submitted to AUV, Andhra University with Herbarium numbers MV 23402. These parts of the plants were identified and authenticated before phytochemical analysis. The bark of the two species was cleaned with water and dried at room temperature for two weeks and was ground and kept for future analysis. Preparation of an extract Thirty grams of the powdered bark material of the *Ficus benghalensis* was extracted with 3 distinct solvents (acetone, methanol, aqueous) in Soxhlet equipment in 250 ml of each solvent separately for 48 hours. After extraction, crude liquids were collected and they were utilised for the preliminary phytochemical analysis and later they were concentrated by a slow evaporation process by using a hot water bath at 60°C to remove the excess solvents. The obtained crude extracts were retained for later use in sealed containers at 4 °C. Qualitative Phytochemical screening Crude extracts were subjected to preliminary phytochemical screening by following standard procedures [11]. Quantitative phytochemical screening Here we are estimating the total phenol content of both species. The protocol employed [11] was used to estimate the TPC of selected Indian medicinal plants by using the Folin-Ciocalteu phenol reagent. The standard here used is Gallic acid. The TPC calculation was done by using the following formula; Calculation of TPC $= \frac{\text{GAE (Gallic acid equivalents)in mg}}{\text{grams of extrac}}$

For anti-bacterial activity, the cup plate agar diffusion method is used. A Petri plate was filled with the prepared nutrient agar medium after being inoculated with 18-hour-old

cultures of the test organism. In a laminar airflow, at room temperature, the plates' medium was given time to harden. Four 5mm-diameter cups were created on each plate at an identical spacing when the media solidified. The stock solutions with different concentrations were prepared (800 µL, 600 µL and 400 µL). Using sterile micropipettes, 50 µL of each concentration was added to the cups. Each plate containing one cup was utilised for the standard drug. The prepared Petri plates were placed in an incubator and kept at 37°C for 24 hours before being measured for inhibitory zones and analysed [1, 12]. Anti-fungal activity For anti-fungal activity, potato dextrose agar medium is utilised and 50 µL of the fungal test organism, Aspergillus niger, which was made from 48-hour-old cultures, is inoculated with it and transferred into sterile petri plates. The same procedure is repeated as anti-bacterial activity, from solidification of the medium to preparing different concentrations of stock solutions. Stock concentrations were put into the cups using sterile pipettes. In each plate, one cup is left for control. As a result, the plates were incubated at 35 °C for 24 to 48 hours. The studies were carried out in duplicate, and tabular records of the average diameters of the zones of inhibition were made.

## Results and Discussion

While the *Ficus benghalensis* bark the acetone extract shows the presence of alkaloids, triterpenoids and flavonoids. The methanol extract also shows triterpinoids, alkaloids, flavonoids, tannins, phenolic compounds, fixed oils and fats. The aqueous extract shows the presence of Carbohydrates, Proteins, amino acids, alkaloids, Flavonoids and tannins. The phytochemical results were presented in (Table 1).

Table 1. Phytochemical analysis of *Ficus benghalensis* Bark

| S.No | Name of the test | Acetone | Methonol | Aqueous |
|---|---|---|---|---|
| Test for Carbohydrates | | | | |
| 1 | Molisch’s test | - | - | + |
| 2 | Fehling’s test | - | - | + |
| 3 | Benedict’s test | - | - | - |
| 4 | Barfoed’s test | - | - | - |
| Test for Proteins and aminoacids | | | | |
| 5 | Biuret test | - | - | - |
| 6 | Millon’s test | - | - | + |
| 7 | Xanthoprotein test | - | - | - |
| 8 | Ninhydrin test | - | - | - |
| Test for Triterpinoids | | | | |
| 9 | Salkowski test | + | + | - |
| 10 | Libermann Burchard | + | + | - |
| Test for Alkaloids | | | | |
| 11 | Dragendorff’s test | + | + | + |
| 12 | Mayer’s test | + | + | + |
| 13 | Hager’s test | + | + | + |
| 14 | Wagner’s test | + | + | + |

| | | | | |
|---|---|---|---|---|
| Test for Cardiac glycosides | | | | |
| 15 | Baljet's test | - | - | - |
| 16 | Legal's test | - | - | - |
| 17 | Keller killiani test | - | - | - |
| Test for Anthraquinone glycosides | | | | |
| 18 | Borntrager's test | - | - | - |
| Test for Saponin glycosides | | | | |
| 19 | Foam test | - | - | - |
| 20 | Haemolysis test | - | - | - |
| Test for Flavonoids | | | | |
| 21 | Shinoda test | + | + | + |
| Test for tannins and phenolic compounds | | | | |
| 22 | Fecl3 test | - | + | + |
| Test for fixed oil and fats | | | | |
| 23 | Spot test | - | + | - |

+ = Present, - = Absent

The methanol extract of bark showed the presence of flavonoids, saponins, steroids, wax, terpenoids, cardiac glycosides and tannins [13].

While the present study *Ficus benghalensis* bark the acetone extract shows the presence of alkaloids, triterpenoids and flavonoids. The methanol extract also shows triterpinoids, alkaloids, flavonoids, tannins, phenolic compounds, fixed oils and fats. The aqueous extract shows the presence of Carbohydrates, Proteins, amino acids, alkaloids, Flavonoids and tannins. The phytochemical results were presented in (Table 5). Previous studies on the phytochemical screening of *F. benghalensis* revealed the presence of saponins, tannins and flavonoids in aqueous and methanol extract [14]. Levels of total phenolics, total flavonol and total flavonoid compounds in aerial roots in 70 mg/g of extract, 3 mg/g quercetin equivalent and 5 mg quercetin equivalent/g extract have also been reported [15].

## Antimicrobial activity

Antimicrobial activity of *Ficus benghalensis* was investigated by the agar well diffusion method. Acetone, methanol and aqueous extracts of *Ficus benghalensis* stem bark was tested against two bacterial strains *E. coli* and *S. aureus* and two fungal pathogens like *A. niger* and *S. cerevisiae***.** The highest antibacterial activity of Acetone extract was 1.8 ± 0.1mm and 1.6 ± 0.5mm diameter of zone of inhibition at the concentration of 800µg/mL against *E. coli* and *S. aureus***.** On the other hand, the lowest activity of acetone extract of *Ficus benghalensis* stem bark was1.3 ± 0.1 mm and 1.1 ± 0.1mm diameter of zone of inhibition at the concentration of 400µg/mL against *E. coli* and *S. aureus*. The anti-fungal activity was determined by agar well diffusion method. The highest anti-fungal activity of acetone extract of *Ficus benghalensis* stem bark was 1.2 ± 0.1 mm and 1.0 ± 0.5mm diameter of zone of inhibition at the concentration of 800µg/mL against *A. niger* and *S. cerevisiae.*

The methanol extract of *Ficus benghalensis* stem bark showed maximum zone of inhibition 1.6 ± 0.1mm and 1.5 ± 0.1 mm diameter of zone of inhibition against *E coli* and *S. aureus* at the concentration of 800µg/mL followed by 1.4 ±0.1 mm diameter of zone of inhibition at concentration of 600µg/mL against *E coli*. The lowest zone of inhibition 1.2 ± 0.1 mm and 1.0 ± 0.1 mm diameter of zone of inhibition against *E. coli* and *S. aureus* at the concentration of 400µg/mL. The highest antifungal activity of methanol extract of *Ficus benghalensis* stem bark was 1.4 ± 0.1 mm diameter of zone of inhibition against *A. niger*.

The aqueous extract of *Ficus benghalensis* stem bark showed maximum antibacterial activity was 1.6 ± 0.1mm and 1.3 ± 0.5mm diameter of zone of inhibition against *E. coli* and *S. aureus*. When compared to the all the solvents acetone extract showed maximum zone of inhibition.

Figure:1 The zone of inhibition by acetone extract of *Ficus* Bark

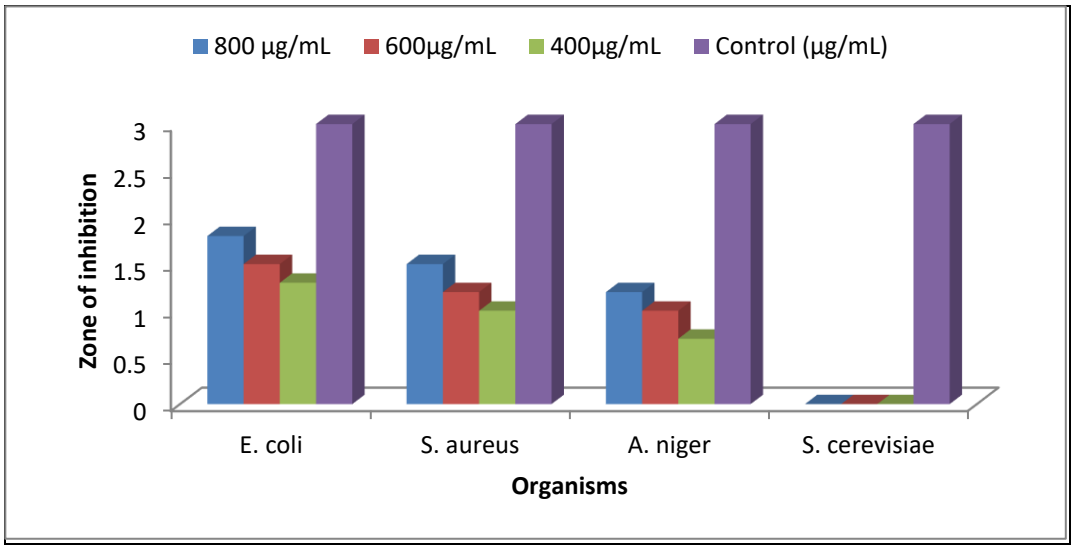



Figure: 2 The zone of inhibition by Methanol extract of *Ficus* Bark

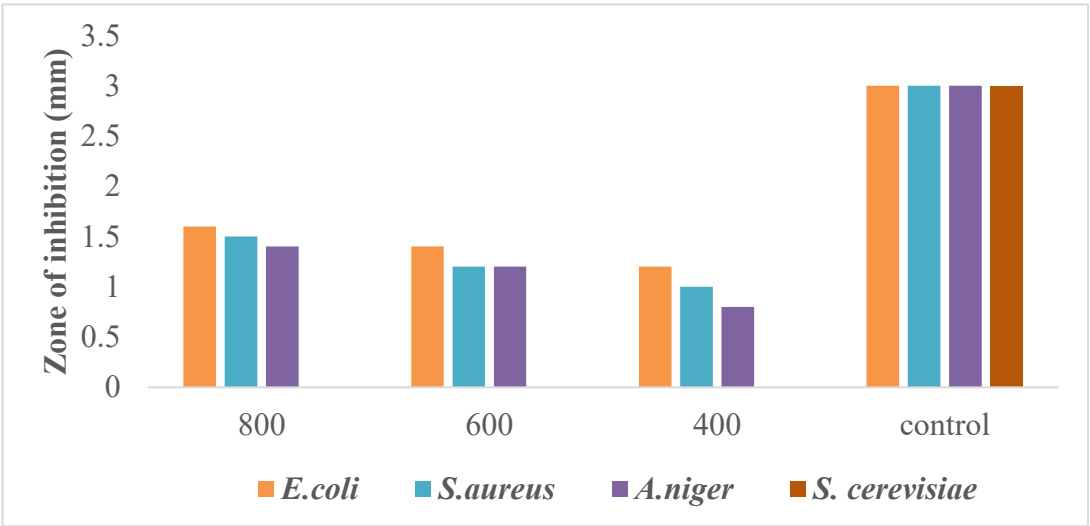
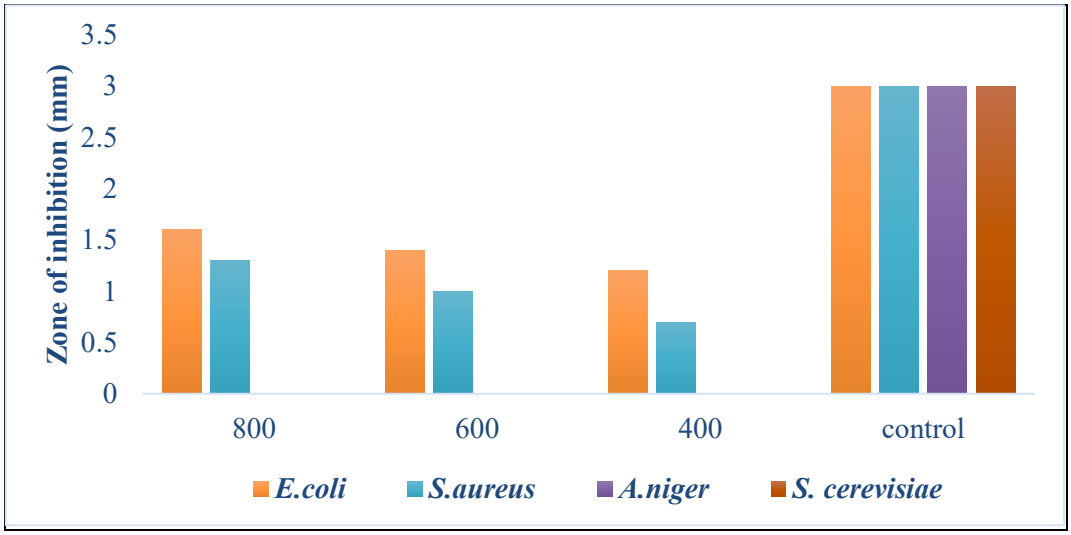



Figure:3 The zone of inhibition by Aqueous extract of *Ficus* Bark.




## Antioxidant Characteristics

The bark extracts of *Ficus benghalensis* exhibited remarkably high antioxidant activity in all three tested solvents, with the methanolic extract showing the most potent radical scavenging capacity. In the DPPH assay, the methanol extract achieved a radical scavenging activity (RSA) of 94.59 ± 0.39% at 120 µg/mL, which was almost comparable to the standard antioxidant ascorbic acid (96.54 ± 0.05%). The $IC_{50}$ values, which indicate the concentration required to scavenge 50% of the DPPH radicals, further confirmed this trend to methanolic extract values were the lowest among all solvents, highlighting superior antioxidant potency. In contrast, aqueous and acetone extracts showed higher $IC_{50}$ values, indicating comparatively lower, but still significant, activity.

The high antioxidant potential of *F. benghalensis* is strongly correlated with its elevated total phenolic content (TPC), particularly in methanol extracts. The methanol extract displayed one of the highest TPC values among all the studied species, and statistical analysis revealed a very strong positive correlation between TPC and RSA (PCC = 0.918, $R^2$ = 0.843). This suggests that the phenolic compounds present are major contributors to the observed antioxidant capacity. The phenolic constituents, rich in hydroxyl functional groups, act as efficient hydrogen donors, effectively neutralizing free radicals and preventing oxidative damage.

Phytochemical literature supports these findings, with compounds such as lupeol, lanosterol, bergaptol, β-sitosterol-d-glucoside, and leucocyanidin-3-O-β-D-glucopyranoside previously identified in methanolic bark extracts of *F. benghalensis*. These bioactive molecules are known for their potent antioxidant, anti-inflammatory, and medicinal properties. The strong correlation between high TPC and low $IC_{50}$ values in methanolic extracts of *F. benghalensis* underscores its potential as a rich source of phenolic antioxidants with significant therapeutic relevance.

## Conclusion

The findings of this study highlight *Ficus benghalensis* bark as a valuable source of bioactive compounds with notable antioxidant and antimicrobial properties. Methanol extracts, characterized by high phenolic content, exhibited the strongest antioxidant activity, indicating that phenolic constituents play a key role in free radical scavenging. Acetone extracts demonstrated superior antibacterial activity, suggesting solvent-specific bioactivity. These results provide scientific validation for the traditional medicinal uses of *F. benghalensis* and emphasize its potential as a natural source of therapeutic agents. Further studies focusing on the isolation and characterization of individual bioactive compounds are recommended to explore their pharmacological applications.

## Acknowledgement

I am thankful to my dearest friend Dr. Vasudevarao Penugurti Ph. D, Postdoctoral Associateat Duke University School of Medicine, Durham, NC, USA for his guidance, financialsupport in need, and suggestions on the manuscript writing, and moral help throughout mystudy.

## References

1. Ramalakshmana, J., Sowmithri, C., Babu, Y. R., & Padal, S. B. (2023). Phytochemical Screening, Antioxidant, Antimicrobial activities and GC-MS Analysis of Ficus benjamina L. and Ficus hispida L. f. *International Journal of Engineering and Management Research*, *12*, 100-115.
2. Kliebenstein, D.J. & Osbourn, A. (2012). Making New Molecules—Evolution of Pathways for Novel Metabolites in Plants. Current Opinion in Plant Biology, 15, 415–423. Doi: 10.1016/j.pbi.2012.05.005.
3. Isah, T. (2019). Stress and Defense Responses in Plant Secondary Metabolites Production.Biological Research, 52(39). Doi: 10.1186/s40659-019-0246-3.
4. Singh, P. et al. (2023). A comparative study on GC-MS analysis and antimicrobial activity of bioactive compounds present in aerial parts (leaf and fruit) of Ficus benghalensis L.. Journal of Applied and Natural Science, 15(2), 870 - 883. https:// doi.org/10.31018/jans.v15i2.4618
5. Rai, A., Saito, K. & Yamazaki, M. (2017). Integrated Omics Analysis of Specialized Metabolism in Medicinal Plants. Plant Journal, 90, 764–787. Doi: 10.1111/ tpj.13485.
6. Durazzo, A., Lucarini, M., Souto, E.B., Cicala, C., Caiazzo, E., Izzo, A.A., Novellino, E. & Santini, A. (2019). Polyphenols: A Concise Overview on the Chemistry, Occurrence, and Human Health. Phytotherapy Research, 33, 2221–2243. Doi: 10.1002/ptr.6419
7. Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U., Miesbauer, O. & Eisner, P. (2021). Common Trends and Differences in Antioxidant Activity Analysis of Phenolic Substances Using Single Electron Transfer Based Assays. Molecules, 26:1244. Doi: 10.3390/molecules2605 1244.
8. Pizzi, A. (2019). Tannins: Prospectives and Actual Industrial Applications. Biomolecules, 9:344. Doi: 10.3390/ biom9080344.

9. Ahmad, S., Rao, H., Akhtar, M., Ahmad, I., Hayat, M.M., Iqbal, Z., & Nisar-ur, Rahman. (2011). Phytochemical composition and pharmacological prospectus of Ficus benghalensis Linn. (Moraceae) – A review. Journal of medicinal plant research, (5), 6393–6400. Doi: 10.5897/ JMPR11.455
10. Papitha, R., Ravi, L., Kaviyarasi, R. & Bhuvaneswari, M. (2017). Phytochemical investigation, gas chromatography –mass spectrometry, and Fourier transform infrared analysis in adventitious roots of Ficus benghalensis L. International Journal of Green Pharmacy, 11(2), 127- 131.
11. Chandramouli, B., Rao, V. M. M., & Ramalakshmana, J. (2023). *Preliminary phytochemical analysis and evaluation of anti-bacterial activities of Morinda coreia* Buch.-Ham.
12. Rao, P. J., Ramalakshmana, J., Babu, Y. R., & Padal, S. GC-MS Chemical Profiling and In vitro Biological Activities of Leaf Extracts of Cymbidium aloifolium (L.) Sw.(Orchidaceae).
13. Uma, B., Prabhakar, K. & Rajendran, S. (2009). In vitro antimicrobial activity and phytochemical analysis of Ficus religiosa L. and Ficus benghalensis L. against Diarrhoeal Enterotoxigenic E. coli. Ethnobotanical leaflets, (4), 7. Babu, K., Shankar, S.G., Rai, S. 2010. Comparative pharmacognostic studies on the barks of four Ficus species. Turk. J. Bot., 34: 215224.
14. Aswar, M., Aswar, U., Watkar, B., Vyas, M., Wagh, A., & Gujar, K. N. (2008). Anthelmintic activity of Ficus benghalensis. International Journal of Green Pharmacy (IJGP), 2(3).
15. Sharma RK, Chatterji S, Rai DK, Mehta S, Rai PK, Singh RK, Watal G, Sharma B (2009). Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. J. Med. Plants Res. 3:944–948.