

Exploring the Bioactive Potential of *Ficus benghalensis* L. Leaves: Phytochemical Profiling and *In Vitro* Biological Evaluation

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Highlights

- Methanol extract had the most diverse phytochemical profile
- Methanol extract showed the highest antimicrobial, DPPH, and α -amylase activities
- Hexane and chloroform extracts showed low cytotoxicity
- *F. benghalensis* leaves represent a potential source of bioactive constituents

Abstract

Ficus benghalensis L. (Bar) is a medicinally valued plant containing a diverse range of phytochemical constituents. The present study evaluated the phytochemical profile and biological activities of hexane, chloroform, and methanol leaf extracts obtained by ultrasound-assisted extraction. Phytochemical screening revealed that the methanol extract contained a broader range of secondary metabolites, producing inhibition zones of 1.5-1.6 cm against the tested bacteria. In contrast, the hexane and chloroform extracts showed no antibacterial activity but exhibited antifungal activity. Methanol extract also showed comparatively higher DPPH radical-scavenging activity. However, for hexane and chloroform extracts, the IC_{50} value was not attained at the tested concentration range. α -Amylase inhibition was highest in methanol extract (25.8 % at 10 mg/mL), although none of the extracts achieved 50% inhibition. Brine shrimp lethality analysis revealed an LC_{50} of 429.1 ± 10.2 μ g/mL for the methanol extract, whereas the hexane and chloroform extracts showed LC_{50} values above 1000 μ g/mL, suggesting lower lethality. Overall, the extracts exhibited solvent-dependent phytochemical and biological profiles, with methanol extract showing comparatively greater activity across the assays. These findings support further investigation of *F. benghalensis* leaves to identify, isolate, and characterize the constituents responsible for the observed activities.

Keywords: *Ficus benghalensis*, phytochemicals, ultrasound-assisted extraction, antimicrobial activity

Introduction

Plants are important sources of structurally diverse bioactive compounds, particularly secondary metabolites such as alkaloids,

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flavonoids, and phenolic constituents, which are involved in plant defense and adaptation and are also associated with diverse therapeutic properties (Anulika et al., 2016; Vaishnav & Demain, 2011). Medicinal plants have been an essential component of traditional healthcare systems and continue to serve as a potential source of bioactive compounds with relevance to therapeutic development, particularly in biodiversity-rich countries including Nepal (Kunwar et al., 2010, 2013; Riaz et al., 2023). Therefore, systematic phytochemical and pharmacological investigations of plants with a history of medicinal use remain an important research priority.

Ficus benghalensis L., commonly known as “Bar”, is a medicinally and culturally important species widely distributed throughout South Asia. The plant has a long history of medicinal use, with its different parts employed in traditional remedies for a range of ailments. Phytochemical investigations have also reported several constituents, including quercetin-3-galactoside, rutin, friedelin, β -sitosterol, lupeol, 5,3-dimethyl ether of leucocyanidin 3-O- β -D galactosyl cellobioside, etc. (Ahmad et al., 2011; Chaudhary et al., 2015). Correspondingly, extracts of its leaves, bark, roots, fruits, and latex have exhibited a broad spectrum of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, immunomodulatory, wound-healing, and anticancer effects (Logesh et al., 2023; Singh et al., 2023).

However, the phytochemical composition and biological activities of medicinal plants can vary considerably with geographical location, environmental conditions, plant maturity, extraction methods, and solvent selection (Pant et al., 2021; Zhang et al., 2018). Even though *F. benghalensis* has been investigated in different regions around the globe, comprehensive studies involving the evaluation of phytochemicals along with different *in vitro* analyses are limited in the context of Nepal.

In this context, the present study seeks to bridge this research gap by investigating phytochemical profiles and different biological activities, namely antimicrobial, antioxidant, antidiabetic, and cytotoxic activities, of three extracts derived from *F. benghalensis* leaves.



Fig. 1. *F. benghalensis* plant, leaves and fruits

Materials and Methods

Solvents, including hexane, chloroform, and methanol, were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), India, while 2,2-diphenyl-1-picrylhydrazyl (DPPH), α -amylase, and 3,5-dinitrosalicylic acid (DNSA) were purchased from HiMedia Laboratories Pvt. Ltd., India. Bacterial and fungal strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*, were used for assessing antimicrobial activity. Freshly hatched *Artemia salina* (nauplii) were used for cytotoxicity assessment.

Sample collection

Leaves of *F. benghalensis* were collected from Lainchaur, Kathmandu, in September, 2024. Botanical identification was performed at the National Herbarium and Plant Laboratories (NHPL), Godawari, Lalitpur, and the specimen was deposited under voucher number ‘KATH172831’.

Extraction

Fresh leaves were thoroughly washed, shade-dried until brittle, and pulverized using an electric grinder. The resulting powder material was kept in an airtight glass vessel until extraction. Extraction was carried out using the ultrasonic-assisted extraction (UAE) technique, in which 700 g of powdered material was mixed with 2.2 L of hexane and subjected to sonication at 27 °C for 1 h. Following sonication, filtration was performed to separate the liquid extract from residual marc. The resulting filtrate was then concentrated using a rotary evaporator and subsequently evaporated in a water bath until the crude extract was obtained. The remaining marc was successively extracted with chloroform and methanol using the same procedure (Yusoff et al., 2022). Extract yields were determined using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant material}} \times 100$$

The extracts were vacuum dried to remove residual solvent and subjected to preliminary qualitative phytochemical screening following the standard protocol (Unegbu et al., 2021).

Antimicrobial activity

The extracts were tested for antimicrobial activity by employing the agar well diffusion assay in accordance with a standard protocol (Balouiri et al., 2016). The inhibitory effect of each extract was evaluated by measuring the diameter of the zone of inhibition (ZOI) formed around the respective wells. Fresh bacterial cultures were prepared in Luria-Bertani (LB) broth followed by incubation. Subsequently, 100 µL of freshly grown bacterial culture was evenly inoculated onto Mueller-Hinton Agar (MHA) plates and spread over the agar surface with a sterile cotton swab. Wells measuring 0.9 cm in diameter and 0.3 cm in depth were prepared and filled with 100 µL of each extract (100 mg/mL in DMSO). After incubation at 37 °C for 24 h, the plates were examined for inhibition zones. In the antibacterial assay, kanamycin and DMSO were included as the positive and negative controls, respectively, whereas itraconazole served as the positive control for the antifungal assay.

Antioxidant activity

The antioxidant activity of the extracts was evaluated by the DPPH assay, using ascorbic acid as the reference antioxidant, following the procedure of Sanchez-Moreno (Sanchez-Moreno, 2002). The DPPH solution was prepared in absolute ethanol, while the extracts were prepared in absolute methanol at concentrations of 20, 40, 60, 80, and 100 mg/mL. Ascorbic acid solutions were prepared at concentrations of 125, 250, 500, and 1000 µg/mL in absolute methanol. A 3 mL aliquot of DPPH solution was combined with 1 mL of each extract or standard solution. The mixtures were then kept in the dark at 30 °C for 30 min. Following incubation, absorbance was recorded at 517 nm using a spectrophotometer, and the percentage of radical scavenging activity (% RSA) was calculated.

$$\% \text{ RSA} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

Antidiabetic activity

The extracts were evaluated for their ability to inhibit α-amylase through the 3,5-dinitrosalicylic acid (DNSA) method, adapting a previously reported procedure with minor modifications (Kifle et al., 2021). Extract solutions of concentrations 2, 4, 6, 8, and 10 mg/mL were prepared and then incubated with an α-amylase enzyme solution in sodium phosphate buffer (0.02 M, pH 6.9) at 30 °C for 10 min. A 1% starch solution was subsequently introduced into the reaction mixture, followed by further incubation. Following incubation, 3,5-dinitrosalicylic acid (DNS) reagent was added, which halted the reaction, and the reaction mixture was heated in a water bath. After cooling and dilution, the reaction mixture was analyzed at 540 nm with a UV-Visible spectrophotometer, and the percentage α-amylase inhibition was obtained from the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

Brine shrimp lethality assay (cytotoxicity)

Extract-induced lethality in brine shrimp was determined following previously reported methods (Chowdhury et al., 2016; Roy,

2015). Different extract concentrations were tested against brine shrimp nauplii over a 24 h period, after which mortality was recorded. The LC_{50} values were then calculated to determine the cytotoxicity of the extracts. Mortality percentage was calculated as:

$$\% \text{ Mortality} = \frac{\text{Number of dead shrimps}}{\text{Total number of shrimps}} \times 100$$

Results and Discussion

Yield percentage and phytochemical screening

Phytochemical extraction efficiency and extract yield depend on several factors such as solvent properties, compound stability, plant particle size, solid-to-solvent ratio, extraction temperature, and duration, while advanced extraction techniques like ultrasound-assisted extraction (UAE) can improve yield, reduce extraction time and solvent consumption, and preserve sensitive phytochemicals (Khanal et al., 2025; Ojong et al., 2026; Zhang et al., 2018). Among the three extracts, methanol yielded the highest extract yield (3.7%), followed by chloroform (3.4%) and hexane (0.3%). Phytochemical screening showed greater diversity in the methanol extract with the detection of flavonoids, saponins, carbohydrates, cardiac glycosides, quinones, and alkaloids. Alkaloids were also detected in hexane and chloroform extracts, phenols in chloroform and methanol extracts, and terpenoids only in chloroform extract, while tannins and proteins were absent in all extracts. The detection of alkaloids in non-polar extracts differed from previous reports, which may be due to variations in extraction conditions, solvent polarity, and environmental factors (Nawaz et al., 2019).

Antimicrobial activity

The antimicrobial effects of the *F. benghalensis* leaf extracts were evaluated against four bacterial species, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, as well as the fungus *Candida albicans*. Kanamycin and itraconazole were employed as positive controls in the antibacterial and antifungal assays, respectively, while DMSO was included as a negative control. **Fig. 2** and **Table 1** summarize the assay findings, while the negative control, which showed no inhibition, was omitted from the table. All four bacterial strains responded to the methanol extract, which produced inhibition zones of 1.5 cm for *B. subtilis*, *S. aureus*, and *K. pneumoniae*, and 1.6 cm for *E. coli*. Kanamycin produced inhibition zones ranging from 1.8 to 2.1 cm. Hexane and chloroform extracts showed no antibacterial activity.

This result suggests that the compounds responsible for this effect are mainly present in the polar fraction of the plant extract. It is consistent with previous findings (Koonal & Rao, 2012). In the case of the antifungal test, all extracts inhibited *C. albicans*, producing a ZOI of 1.1-1.2 cm, whereas itraconazole produced a 2.0 cm ZOI. Despite showing lower inhibition than itraconazole, the clear inhibition zones demonstrate that the extracts possess appreciable antifungal potential against *C. albicans*. Earlier investigations of *F. benghalensis* leaves have documented the presence of caffeic acid, quercetin, and kaempferol in their polar fractions. (Afzal et al., 2020), and multiple studies have highlighted the antimicrobial activity of these compounds (Azeem et al., 2023; Ma et al., 2018; Periferakis et al., 2022). The observed antimicrobial activity may therefore be related to the presence of such phytochemicals, although the presence and contribution of these specific phytoconstituents were not confirmed in the present study.

Table 1. Antimicrobial test of different extracts

Strain	Reference Culture	Zone of inhibition (cm)				
		Positive Control	Negative Control	Hexane extract	Chloroform extract	Methanol extract
<i>Bacillus subtilis</i>	ATCC 6051	2.0	-	-	-	1.5
<i>Staphylococcus aureus</i>	ATCC 6538P	2.1	-	-	-	1.5
<i>Escherichia coli</i>	ATCC 8739	1.8	-	-	-	1.6
<i>Klebsiella pneumoniae</i>	ATCC 700603	1.9	-	-	-	1.5
<i>Candida albicans</i>	ATCC 2091	2.0	-	1.2	1.1	1.2

Note: Well diameter (ϕ) = 0.9 cm, Well depth = 0.3 cm

Antioxidant activity

The antioxidant potential of *F. benghalensis* leaf extracts was examined through the DPPH radical-scavenging assay, with ascorbic acid used as the reference standard. Ascorbic acid showed a concentration-dependent increase in %RSA, from 52.9% at 0.125 mg/mL to 89.7% at 2 mg/mL, with an IC_{50} of $90 \pm 20 \mu\text{g/mL}$. Among the extracts, antioxidant activity varied with solvent polarity. Hexane extract did not show detectable activity below 80 mg/mL. However, it exhibited 11.8% and 23.3% RSA at 80 and 100 mg/mL, respectively. Chloroform extract showed 6.7% RSA at 40 mg/mL, which increased to 15.1%, 20.3%, and 26.7% at 60, 80, and 100 mg/mL, respectively. IC_{50} values for both extracts were $>100 \text{ mg/mL}$. Methanol extract showed 39.1%, 51.9%, and 72.9% RSA at 10, 30, and 50 mg/mL, respectively, with an IC_{50} of $19.9 \pm 1.7 \text{ mg/mL}$ (Fig. 3). The substantially higher IC_{50} values of the extracts compared with ascorbic acid indicate considerably weaker antioxidant activity, suggesting limited DPPH radical-scavenging potential under the tested conditions. Similar findings have been reported in previous studies of *F. benghalensis* leaves, where certain solvent extracts also exhibited lower antioxidant activity than the reference standard (Bhanwase & Alagawadi, 2016; Pedgaonkar et al., 2019).

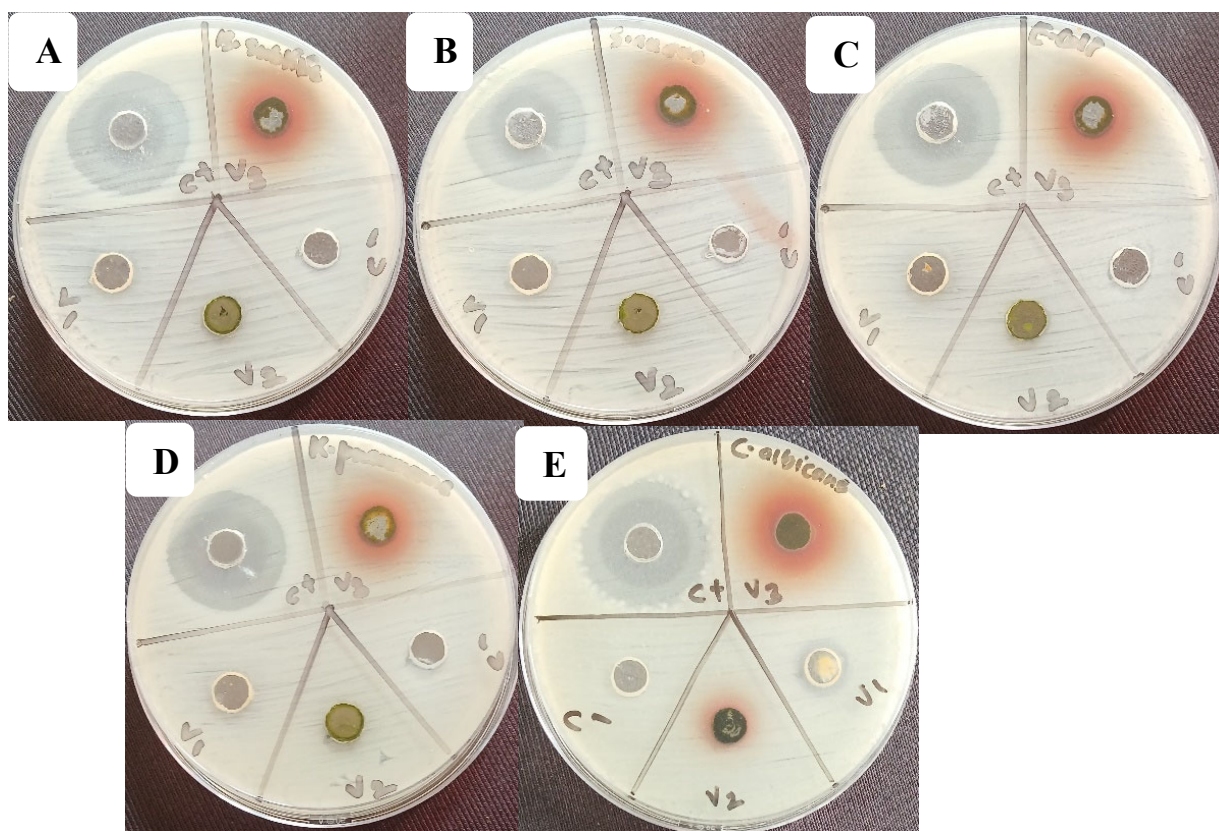


Fig. 2. ZOI exhibited by different extracts against A) *Bacillus subtilis*, B) *Staphylococcus aureus*, C) *Escherichia coli*, D) *Klebsiella pneumoniae*, E) *Candida albicans*

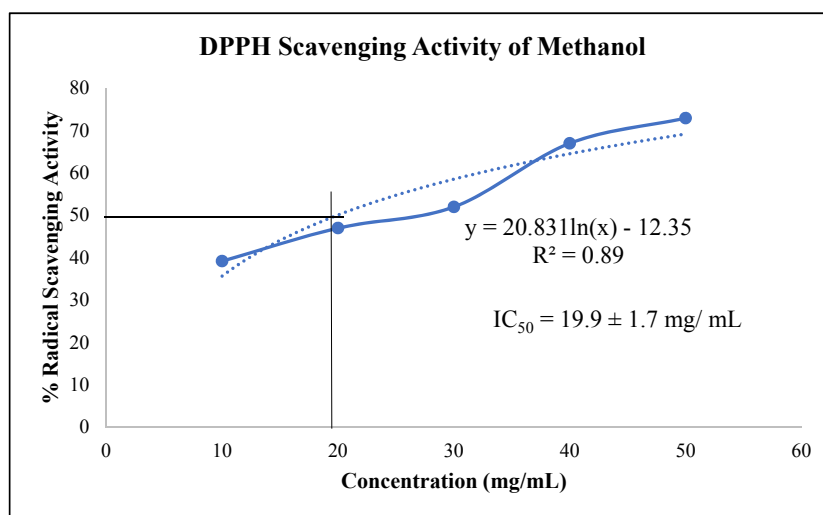


Fig. 3. IC_{50} determination of methanol extract

α -Amylase Inhibition Assay

α -Amylase inhibition by the *F. benghalensis* leaf extracts was evaluated through the DNSA assay, with acarbose serving as the reference inhibitor. Acarbose showed 27.9-65.3% inhibition at concentrations ranging from 0.5 to 2 mg/mL, giving an IC_{50} of $1030 \pm 40 \mu\text{g/mL}$. Hexane extract showed no inhibition at 2-8 mg/mL and produced 6.1% inhibition at 10 mg/mL, resulting in an $IC_{50} > 10 \text{ mg/mL}$. Similarly, chloroform and methanol extracts showed 16.2% and 25.8% inhibition, respectively, at 10 mg/mL, with an $IC_{50} > 10 \text{ mg/mL}$ for both extracts. The comparatively higher inhibitory activity of the methanol extract may be attributed to the presence of polar phytoconstituents, notably phenolic and flavonoid compounds, which are known to exhibit α -amylase inhibitory activity (de Sales et al., 2012; Lam et al., 2024). Nevertheless, all extracts showed substantially weaker activity than acarbose, indicating limited α -amylase inhibitory potential under the tested conditions.

Brine shrimp lethality assay (cytotoxicity)

The cytotoxic potential of *F. benghalensis* leaf extracts was evaluated by exposing *Artemia salina* nauplii to each extract at concentrations of 0, 10, 50, 100, 500, 800, and 1000 $\mu\text{g/mL}$ for 24 h. Increasing extract concentrations led to higher mortality, but the extent of mortality varied across the extracts. Hexane and chloroform extracts showed relatively low mortality, reaching 45% at 1000 $\mu\text{g/mL}$, with $LC_{50} > 1000 \mu\text{g/mL}$ for both extracts. In contrast, the methanol extract exhibited comparatively higher mortality, increasing from 26% at 100 $\mu\text{g/mL}$ to 73%, 93%, and 100% at 500, 800, and 1000 $\mu\text{g/mL}$, respectively, with an LC_{50} of $429.1 \pm 10.2 \mu\text{g/mL}$ (Fig. 4). Despite these differences in cytotoxicity, all three extracts were classified as non-toxic based on their LC_{50} values (Moshi et al., 2010). The dose-dependent cytotoxicity observed in the present study agrees with earlier findings (Rao et al., 2014).

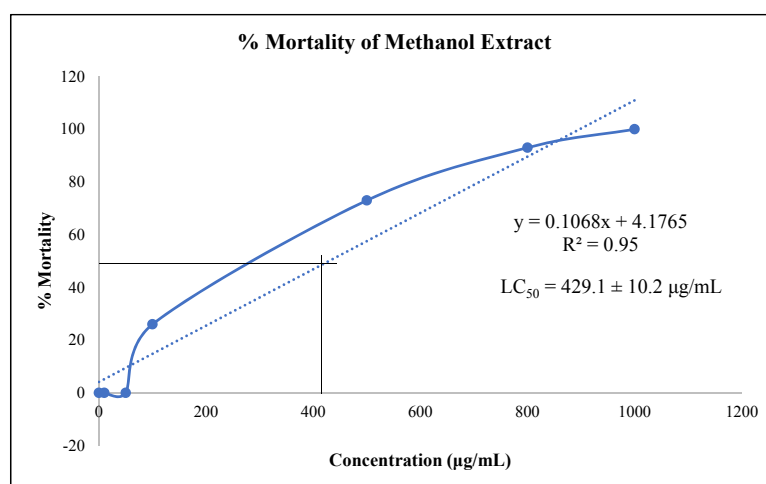


Fig. 4. LC_{50} determination of methanol extract

Conclusions

This study demonstrates that *F. benghalensis* leaves contain diverse phytoconstituents with biological activities that varied depending on the extraction solvent. Among the tested extracts, the methanol extract showed comparatively higher activity in antibacterial, DPPH radical-scavenging, and α -amylase inhibition assays than the hexane and chloroform extracts. For antifungal activity, the hexane and methanol extracts showed better activity than the chloroform extract. The brine shrimp lethality assay further indicated solvent-dependent differences in toxicity, with the methanol extract exhibiting comparatively higher lethality than hexane and chloroform extracts. However, none of the extracts were classified as toxic. Overall, these findings emphasize the role of solvent selection on phytochemical recovery and the resulting biological activities of *F. benghalensis* leaf extracts. Further studies are warranted to identify and characterize the active compounds, investigate their mechanisms of action, and determine their effects *in vivo*.

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Conflict of Interest

The authors declare no competing interests.

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