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Extraction and phytochemical analysis of secondary metabolites from *Ficus benghalensis* L: Review

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Abstract
Herbs have provided some of the most essential life-saving medications in modern medicine's arsenal. Only around 15% of the estimated 4,00,000 plant species have been explored phytochemically, and only 6% have been tested for biological activity. Any plant's pharmacological actions are determined by the existence of primary and secondary metabolites. Different plant components and extracts have different medicinal properties. *F. benghalensis* bark, leaves, and fruits were used for their antibacterial, antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties. It is used to treat dysentery, diarrhoea, asthama, ulcers, and a variety of skin disorders.

Keywords: *F. benghalensis*, phenolics, terpenoids

Introduction
Ficus benghalensis (Moraceae, Mulberry family) is called Banyan tree, Vata tree, or Vada tree in Ayurveda. Ficus has around 800 species and 2000 variations, the bulk of which are native to the old world tropics. It is used in Ayurveda to treat diarrhoea, dysentery, and piles, as well as dental problems [1]. As a diabetic, rheumatism, skin illnesses such as sores are used to improve the immune system. Plants are still viewed as important contributors to health care, despite breakthroughs in medical science [2]. Traditional medicines are used by about 80% of the population for their main health requirements, according to the World Health Organization [3]. Secondary metabolites can be synthesized by plants. Plants use aromatic molecules as defensive agents against microbes, insects, and other herbivores, such as these generated metabolites [4]. These defensive chemicals, on the other hand, offer plants their medical value, which is valued by humans.
Plants provide 11 percent of basic and essential medications, according to the WHO, and natural precursors also provide a number of synthetic drugs. Antioxidants [5, 6], antifungal [7], and antibacterial properties are all found in phytochemicals. For a range of ailments, tribal communities use a wide variety of plants as medicines. Medicinal plants are also the source of the vast majority of today's pharmaceuticals. Due to the expensive cost and potential adverse effects connected with the use of current medications, research into medicinal plants is growing by the day. Medicinal plant treatment is often less expensive and, in virtually all situations, has no side effects [8]. It is critical to look for traditional medicinal substances that are both effective and affordable. Alkaloids, polyphenols, flavonoids, glycosides, terpenes, and a few more pigments are secondary metabolites in plants that protect them against illness and stress while also assisting in their health. Plant active components have been shown to be useful to animals.
These aid in the enhancement of the digestive, nervous, respiratory, excretory, circulatory, and immune systems in both humans and animals. Plants have different amounts of active components depending on where they are found. The majority of today's pharmaceuticals are developed from ancient herbal medicines used to treat both animals and humans. Herbal medicines are utilized by over 80% of the population in Asian and African countries, according to the WHO, due to of their efficacy, low cost, and lack of side effects when compared to other treatments.
Antibacterial, antiviral, antifungal, antihelminthic, antioxidant, antiprotozoal, wound healing, anti-inflammatory, anti-tumor, anti-cancerous, and other organ-specific qualities of plants have been used for a long time. Alkaloids, flavonoids, glycosides, polyphenols, terpenes, tannins, and other plant metabolites have the ability to increase both humoral and cell-mediated immunity. [9-12] Ayurveda, Unani, and other indigenous healing practises support the use of herbal treatments. The use of plants, algae, yeast, bacteria, lichens, and a range of freshwater and marine water flora and fauna has increased widely due to their health advantages.

Introduction of *Ficus benghalensis* L. as medicinal plant
India is extremely near to the countries with the highest forest cover, having a total forest area of around 23.7 percent (26.19 percent). Worldwide, more than 12% of flowering plants are claimed to have medical use, with India accounting for more than 43% of total blooming plants. [13] *Ficus benghalensis* is a Moraceae family

member with seasonal fruity figs, aerial roots for propagation, a huge trunk with thick bark and leaves, and is mostly found in tropical and subtropical climes in Asia. Latex, a rubber-producing laticifer, and milky sap are found on the tree, which can grow to be 30 metres tall. Many Southeast Asian faiths regard a significant number of plants belonging to the same genus as sacred, and they also have ecological and medical importance.

Medicinal Properties and phytochemical constituents of *Ficus benghalensis* L.

Different plant components and extracts have many medicinal properties. F. benghalensis bark, leaves, and fruits were used for their antibacterial, antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties. It is used to treat dysentery, diarrhoea, asthama, ulcers, and a variety of skin disorders. Haemorrhages and bleeding piles have been proven to be helped by plant sap from the aerial root and leaf bud. Plant leaves are reported to help with conception, blood purification, and diarrhoea prevention. Bark extracts derived from ethanolic, methanolic, aqueous, and petroleum extractions have been demonstrated to have anti-inflammatory, anti-diabetic, hypolipidaemia, antihelminthic, anti-allergic, wound healing, and anti-stress properties. The aerial roots of *F. benghalensis* are being studied as a therapy option due to their immunomodulatory capabilities. In rural India, the aerial roots of *F. benghalensis* have been utilized to boost the immune system and for a variety of other medicinal purposes. Hair development and hair loss were also stimulated and reduced using aerial root extract (97 percent chloroform, 3 percent methanol). The chemical components found in the aerial root of *F. benghalensis* are unknown. As part of the screening method for active compounds, which included secondary metabolites, we also characterized the antibacterial, antioxidant, and cytotoxic activities of ethanol and methanol extracts of *F. benghalensis* aerial roots. The metabolites in the methanol extract were screened and identified by GC-MS analysis. [13].

The presence of antioxidants in food is thought to be a significant influence in preventing oxidation of various components such as polyunsaturated fatty acids, as well as protecting it from rapid deterioration during storage and processing. Oxidation of dietary components can induce premature ageing or cancer, whereas synthetic antioxidants like butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) are harmful to human health. As a result, both the food processing industry and individuals must investigate the potential antioxidants in food sources. In the presence of hydrogen donating antioxidants, the formation of non-radicals of DPPH, namely DPPH-H, can be used to determine the DPPH radical scavenging activity [14]. When comparing the violet hue of DPPH discolouration in the methanol and ethanol extracts of *F. benghalensis*, the methanol extract was more apparent. There was no significant difference between ascorbic acid, a naturally occurring antioxidant, and the methanol extract when used as a control. When compared to normal gallic acid, methanol extract had stronger antioxidant activity as measured by the FRAP experiment. According to the research, secondary metabolites such as phenols, flavonoids, tannins, terpenes, and other important components influence the antioxidant potential of plant extracts. Polyphenols have been proven to lower reactive oxygen species (ROS), lower lipid oxidation, and protect organisms from mutations that can cause cancer and other fatal diseases. Plant phenolic compounds are the well-studied secondary metabolites, and they can be found in every part of the plant. Flavanoids are natural phenolic chemicals with antibacterial, antiviral, antioxidation, anti-inflammatory, anti-cancer, and cardioprotective effects. Figure 1 depicts the presence of total phenolic compounds and total flavonoids in the extract [15].

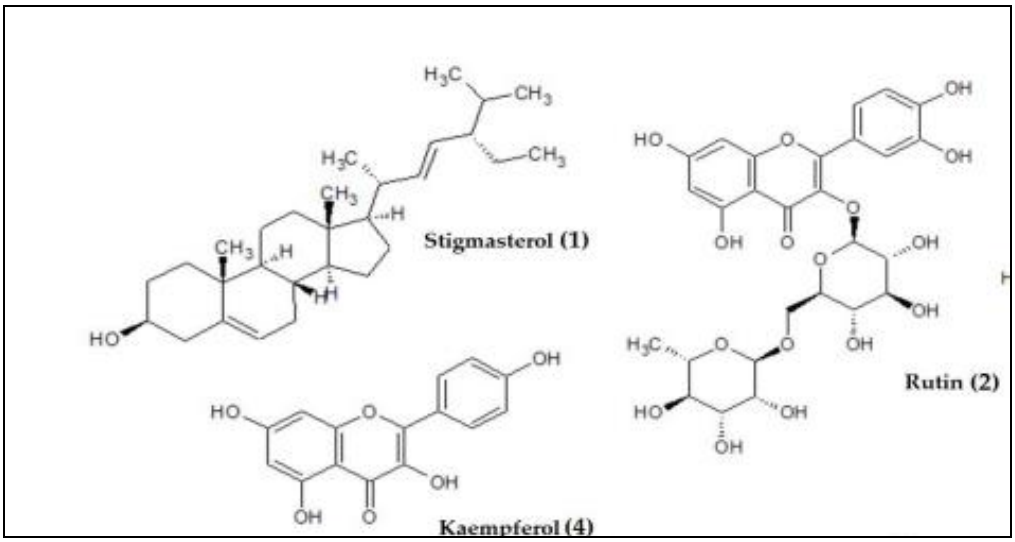

Fig 1: The chemical structure of bioactive chemicals found in *F. benghalensis* has been studied.

Total flavonoids are similar in both extracts, while total phenolic components are nearly two and a half times greater in the methanol extract than in the ethanol extract of *F. benghalensis*. The greater antioxidant activity of methanol extract may be due to higher levels of total phenolic components. When extracts from various plants and their parts are prepared with methanol as a solvent, as opposed to other solvents such as hexane, benzene,

ethyl acetate, ethanol, or water, several studies have definitely demonstrated favorable biological activities. Terpenes, also known as terpenoids, are the most common type of secondary metabolite present in all plants, and they can be used to produce a variety of terpenes at various locations within the plant for a variety of purposes. Terpenes defend plants from insects and other herbivores, despite the fact that some of them are toxic to people. Based on GC-MS study of aerial roots from *F. benghalensis*, we offer various terpenes, predominantly tri-terpenes, in this work. Tri-terpenes have been related to colon cancer, breast cancer, oral mucosa cancer, and human T-cell leukaemia. There are also analgesic, antioxidant, anti-inflammatory, antibacterial, and hepatoprotective properties. There is no record describing the different components contained in the aerial root of *F. benghalensis*, hence benghalensin is the sole relevant component documented. The aerial roots of *F. benghalensis* have immunostimulatory biological activities in two different fish species, the bony fish Channa punctata and the catfish Clariasgariepinus, according to Vipin et al. The results of the GC-MS analysis indicated a number of compounds with a variety of biological activities that could be useful in the prevention and treatment of various diseases, as well as in enhancing the health of humans and other animals. The aerial root of *F. benghalensis* methanol extract contained a variety of beneficial saturated/polyunsaturated fatty acids, terpenes, phytosterols, polyphenols, other secondary metabolites, and chemotypes with potential biological action. Antioxidant components such as quinic acid, palmitic acid, methyl ester, ergosterol acetate, and amyrenyl acetate are found in a methanol extract of *F. benghalensis*, according to GC-MS analysis. It reveals that the majority of chemicals have anti-inflammatory and anti-cancer properties. The largest quantities of lupenyl acetate and amyrenyl acetate were identified in the methanol extract of *F. benghalensis* (representing 35.4 percent and 16.34 percent, respectively). These two substances have anti-malarial, antibacterial, anti-fungal, anti-oxidant, anti-ulcer, antihyperglycemic, and anti-cancer activity, indicating that *F. benghalensis* has medicinal value. The structures of a few of the physiologically active chemicals found in large concentrations in the methanol extract of *F. benghalensis* are highlighted in Fig.2 [16].

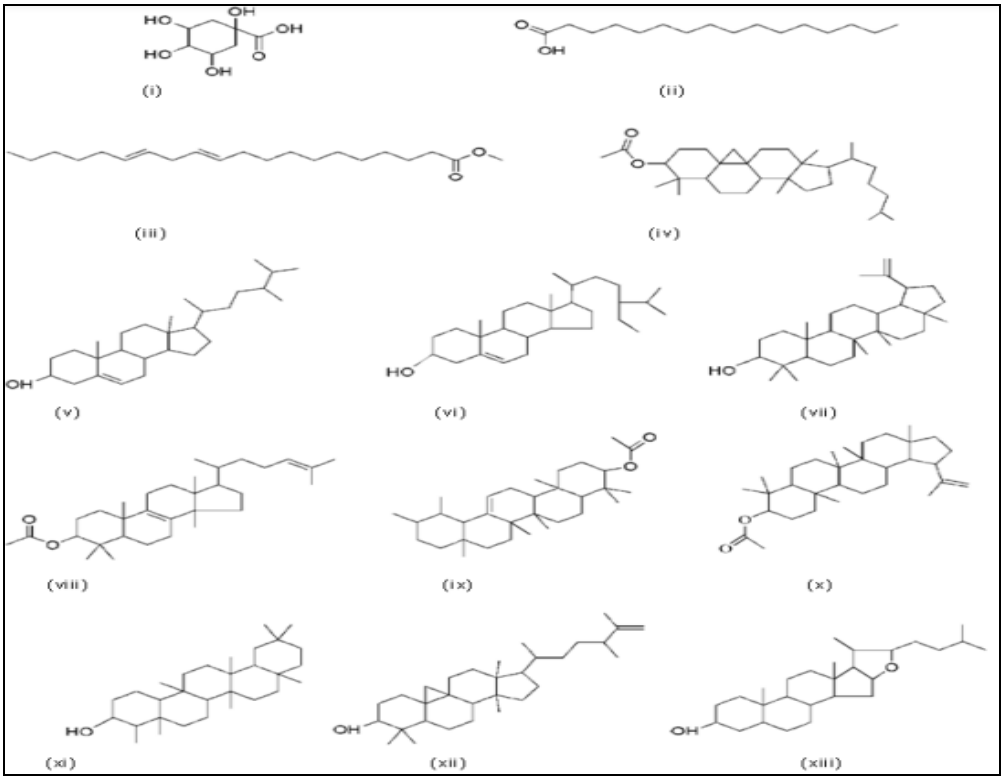

Fig 2: Chemical structure of major components in the methanol extract of *F. Benghalensis* (Aerial-Root): I Quinic acid; (ii) Palmitic acid; (iii) Eicosadinoic acid methy ester; (iv) Cycloartanyl acetate; (v) Dihy drobrassicasterol; (vi) - sitosterol; (vii) Lupeol; (viii) Lanosterol acetate; (ix) Amyrin acetate; (x) Lupeolacetate.

Materials and Methods

Extraction and pre-extraction processes were used in the research of medicinal plants. These are the most crucial processes in extracting bioactive components from plant matter. The basic stage in separating the necessary natural phytoconstituents from the plant material is extraction. Plant material is washed to remove undesirable clay particles prior to extraction. To remove moisture from the cleansed plant material, it is shade dried. The most common method is solvent extraction. The steps of the extraction process are as follows:

1. **Penetration:** the solvent penetrates into the solid matrix;
2. **Dissolvation:** the solute dissolves in the solvents;
3. **Diffusion:** the solute is diffused out of the solid matrix;
4. **Collection:** the extracted solutes are collected.

The elements that enhance extraction by increasing diffusion and solubility. The qualities of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ratio, the extraction temperature, and the extraction period all affect extraction efficiency [17]. All three solvents employed to extract fruit extracts contained phytochemical components such as terpenoids and phenol. Carbohydrate, reducing sugars, saponins, glycosides, and anthroquinones were all absolutely absent in all three solvents. Flavonoids, alkaloids, tannins, phlobatannins, and chloride were found in both aqueous and methanolic extracts. Methanolic extracts contained vitamin C and protein. Only chloroform extracts were fully devoid of steroids. Aqueous extracts contained amino acids. The majority of the components were found in a methanolic extract of *F. benghalensis* fruits. According to early qualitative phytochemical analysis of *F. benghalensis* fruit extracts, the methanolic extract contains the most secondary metabolites. Total protein, total flavonoid content, and ascorbic acid were detected in quantitative phytochemical screening of three primary secondary metabolites reported in the *F.benghalensis* methanolic fruit extract. The following chemical tests are used to detect the extracted components.

Tests for Carbohydrates using Benedict's test
The crude extract was combined with Benedict's reagent and cooked in this experiment. The presence of carbohydrates is indicated by the production of a reddish brown precipitate.

Test for reducing sugar
The extracts were filtered after being dissolved in distilled water. Fehling's solution A and B were used to boil the filtrate. The presence of reducing sugars is indicated by an orange red precipitate.

Tests for Amino Acids using Ninhydrin test
Three drops of 5% Ninhydrin solution were added to the amino acid extract. The mixture is then cooked for 10 minutes in a water bath. The presence of amino acids is indicated by the emergence of purple or bluish colour.

Tests for Proteins using
In this Biuret test, 3 ml of test solution was mixed with a few drops of 4% NaOH and 1% CuSO4 solution. The presence of violet or pink colour formation in the tubes suggests the presence of proteins.

Tests for Vitamin C
1 mL of a 2% extract solution was mixed in a test tube with 5 mL of water. 1 drop of freshly made sodium nitroprusside 5 percent w/v solution and 2 ml of diluted sodium hydroxide solution were added. Then, drop by drop, 0.6 ml of hydrochloric acid was added and stirred until the yellow colour turned blue, indicating the presence of Vitamin C.

Tests for Chloride
3 ml sample solution made in HNO_3 was added to the test tube, along with a few drops of a 10% $AgNO_3$ solution. The appearance of a white $AgCl_2$ precipitate indicates the presence of chlorine.

Tests for Tannins
Tannins were detected by adding 2-3 ml test solution to a 5 percent $FeCl_3$ solution in a test tube and looking for deep blue-black colour responses, which indicate the presence of Tannins.

Tests for Alkaloids using Wagner's test
Wagner's test consisted of separating 2-3 mL of filtrate into separate tubes. A few drops of Wagner's reagent were added to this, and a reddish brown precipitate was observed, indicating the presence of alkaloids.

Detection of flavonoids using Lead acetate test
A few drops of 10% lead acetate solution were added to the extracts. The presence of flavonoids was established by the production of yellow precipitate.

Test for phlobatannins
When a plant sample was cooked with 1 percent aqueous hydrochloric acid, a crimson precipitate formed, indicating the presence of phlobatannins.

Tests for Steroids
2 mL test solution, 2 mL acetic anhydride, and 2 mL H_2SO_4 were added to a test tube. In certain samples, the colour shifted from violet to blue or green, indicating the presence of steroids.

Detection of Terpenoids using Salkowski reaction
2 ml sample, 2 ml chloroform, and 2 ml concentrated H_2SO_4 were added, and the chloroform layer for red colour and the acid layer for fluorescence were detected. Positive results for the presence of terpenoids were shown by a reddish brown colouring of the interface.

Test for Phenolic compounds using Ferric chloride test
The extract was diluted to 5 mL with distilled water for the ferric chloride test. A few drops of a neutral 5 percent ferric chloride solution were added to the mix. The presence of phenolic compounds is indicated by a dark green colour.

Test for saponins
The fruits samples were diluted with distilled water and made into 20 ml. The suspension was shaken well in graduated cylinder for 15 minutes; 2cm layer of foam indicates the presences of saponins.

Pharmacological Actions
As mentioned below, Ficus species have been extensively investigated for a variety of pharmacological properties. All plant parts, including leaves, stem bark, root, latex, and fruits, were investigated for their potential bioactivities. Bioactivities explored include antioxidants, anti-inflammatory, anticancer, antitumor and antiproliferative, antimutagenic, antimicrobial, anti-helminthic, hepatoprotective, wound healing, anticoagulant, immunomodulatory characteristics, antistress, and toxicity. Plants are also used to make insect repellents.

Antifungal Activities
The present findings showed that a high concentration of *F. bengalensis* decoction has considerable antifungal action against Candida albicans. The high concentration of *F.bengalensis* had a more inhibitory effect than the other low concentrations, according to the findings. This suggests that the active compounds in *F. bengalensis* bark are better extracted with more water, a longer boiling time, and a higher concentration than other barks [18].

Antioxidant Activity
The usefulness of antioxidants in lowering oxidative stress has been proven in various studies, encouraging us to study the antioxidant potential of several *F. benghalensis* extracts [19]. The methanolic extract of *F. benghalensis* L latex may have good antioxidant activity, according to the results of recent antioxidant studies [22]. The methanolic extract of *F. benghalensis* leaves showed significant antioxidant activity in all of the studies, demonstrating the extract's potent antioxidant capabilities. The presence of a considerable number of polyphenolic components in *F. benghalensis* methanolic extract could explain its antioxidant properties. Antioxidant compounds such as Gallic acid, Rhein, Anthraquinone, (-) gallocatechin, Theaflavin-3,3'-digallate, and Flavone were found in the extract by HPLC, supporting the antioxidant concept.

Antibacterial Activity
Carbohydrates, reducing sugars, deoxysugars, sterols, glycosides, phenolic compounds, tannins, saponins, and flavonoids were found in the phytochemical study, showing its biological value. The antibacterial effectiveness of the plant's leaves was investigated using aqueous, methanolic, and chloroform extracts. The methanolic extract was shown to be the most effective against bacteria. The antibacterial activity of the methanolic extract against *S. aureus, S. themophillus, P. vulgaris*, and *E. coli* was determined using the agar well diffusion method. Thus, MIC of methanolic extract was performed after evaluating the antibacterial effectiveness of the methanolic extract. The methanolic extract of the plant leaves was most efficient against *S. aureus, S. themophillus, P. vulgaris, and E. coli*, according to MIC findings [20]. Both aqueous and ethanolic extracts of *F. benghalensis* roots were efficacious against Staphylococcus aureus infections, indicating that they are a promising antibacterial agent. The antibacterial action of *F. bengalensis* & *F. racemosa* roots may be due to the presence of flavonoids found through phytochemical screening [21].

Medicinal value
Traditional medicine, notably Ayurveda, uses a range of phytochemicals in *F. benghalensis* to treat kidney damage, blood clot removal, schizophrenia, heart ailment, mental difficulties, low cholesterol, and osteoarthritis. Despite the fact that a few studies have supported the use of crude extract for various disease therapies, this is the first phytochemical investigation of *F. benghalensis* aerial roots [23].

Conclusion
In this review paper, *F. bengalensis*, a medicinal plant found throughout India, was studied for its phytochemical composition and pharmacological prospects. Plants have been giving an excellent supply of medicines to humanity for generations. Ketones, flavonols, and flavonoids, terpenoids, coumarins, esters, sugars, and serine protease are among the phytochemical substances believed to be present in this plant. Some of its pharmacological qualities include antidiabetic, hypolipidemic, anthelmintic, antibacterial, immunomodulatory, antistress and antiallergic, antioxidant, anti-inflammatory, antidiarrheal, analgesic and antipyretic, antiatherogenic, wound healing, and growth promotion. As a result, the plant could be considered an important human herbal resource.

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