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PHYTOCHEMICAL SCREENING AND ANTHELMINTIC POTENTIAL OF *FICUS BENGHALENSIS* L. LEAVES AGAINST *PHERETIMA POSTHUMA*

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INTRODUCTION

Helminthiasis remains a widespread public health challenge, affecting nearly half of the global population, particularly in regions with inadequate sanitation, malnutrition, and overcrowded living conditions. This parasitic infection is primarily transmitted through contaminated soil, water, food, or direct contact with infected hosts, with insects often acting as vectors.[1,2] The major groups of helminths responsible for infections in humans and animals include trematodes (flukes), cestodes (tapeworms), and nematodes (roundworms), all of which contribute to a variety of health complications.[3,4] Helminth infections can result in immune suppression, increasing the host's susceptibility to secondary infections and life-threatening diseases such as HIV/AIDS, tuberculosis, and malaria.

Among the nematodes, species such as *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, and *Trichuris trichiura* are particularly significant as they spread infection without requiring an intermediate vector. These parasites typically infect humans through the ingestion of contaminated food or water, direct contact with contaminated soil, or skin penetration by larvae. Once inside the human body, these worms can migrate through the bloodstream and settle in organs such as the lungs and central nervous system (CNS), causing severe complications including respiratory distress, neurological disorders, and malnutrition. In contrast, trematodes such as *Schistosoma* species are primarily transmitted through contaminated water, where their larvae penetrate human skin upon contact. Chronic

schistosomiasis can result in severe organ damage, particularly affecting the liver, intestines, and urinary system, often leading to conditions such as hepatosplenomegaly, intestinal fibrosis, and bladder cancer. Cestodes, including *Taenia solium* and *Taenia saginata*, are generally transmitted through the consumption of undercooked or contaminated red meat. Once inside the host, these tapeworms can develop into cysts in various tissues, including muscles, the eyes, and the CNS, leading to a severe neurological disorder known as neurocysticercosis, which can cause seizures, chronic headaches, and cognitive dysfunction.[5, 6]

The primary treatment for helminth infections involves the use of synthetic anthelmintic drugs such as praziquantel, mebendazole, and albendazole. While these drugs are effective, they come with significant challenges, including the development of drug resistance, reduced efficacy in some cases, high costs, and potential adverse effects such as gastrointestinal discomfort, liver toxicity, and allergic reactions. Due to these limitations, there is a growing interest in exploring alternative treatment options, particularly plant-based anthelmintic therapies, which have been found to be promising due to their affordability, broad-spectrum efficacy, low toxicity, and environmental safety. Various medicinal plants have been studied for their anthelmintic properties, with bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids exhibiting strong anti-parasitic effects. These natural compounds have been shown to immobilize and kill parasites, disrupt their metabolic pathways, and prevent further infections, making them a valuable alternative for controlling helminthiasis. Furthermore, plant-based treatments are more sustainable and accessible, particularly in economically disadvantaged regions where access to healthcare and pharmaceutical drugs is limited.

In light of the increasing resistance to conventional anthelmintic drugs and the ongoing burden of helminth infections in developing countries, there is an urgent need to develop and promote plant-based treatments as viable alternatives. Extensive research and clinical trials are necessary to validate the efficacy and safety of these herbal remedies, ensuring their integration into global healthcare strategies for the prevention and treatment of helminthiasis [7-10]. By harnessing the potential of medicinal plants, researchers and healthcare professionals can work towards creating cost-effective, eco-friendly, and efficient solutions to combat helminth infections and improve public health worldwide.

Prevalence of Helminthiasis

Helminthiasis remains one of the most widespread parasitic diseases affecting humans worldwide, particularly in regions with poor sanitation, inadequate healthcare infrastructure, and limited access to clean water. According to the World Health Organization (WHO), over 2 billion people globally suffer from helminthic infections, with the majority residing in tropical and subtropical regions where environmental conditions favor parasite transmission. These infections are predominantly caused by nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms), all of which contribute to significant morbidity and mortality, particularly in low-income populations. Among these, soil-transmitted helminths, including *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale*, are the most prevalent, leading to intestinal and tissue infections that affect millions of individuals, particularly children. These parasites cause malnutrition, anemia, and stunted growth, significantly impacting cognitive development and educational performance in affected populations. The transmission of these nematodes occurs through contaminated soil, water, or food, with poor hygiene practices exacerbating their spread.

Trematode infections, particularly schistosomiasis, are another major public health concern, affecting over 250 million people worldwide, primarily in Africa, Asia, and South America. Schistosomiasis is caused by parasitic flukes of the *Schistosoma* genus, which infect humans through contact with contaminated freshwater sources. The disease is associated with severe complications, including granuloma formation, fibrosis, and enlargement of organs such as the liver and spleen. Chronic schistosomiasis can lead to hepatosplenomegaly, portal hypertension, bladder dysfunction, and, in severe cases, an increased risk of bladder cancer. The high burden of schistosomiasis in endemic areas not only affects individual health but also places a considerable strain on healthcare systems, reducing economic productivity due to long-term disability and reduced workforce participation. In endemic regions, children and agricultural workers are at the highest risk due to frequent contact with infested water bodies.

Cestode infections, caused by parasites such as *Taenia solium*, *Taenia saginata*, and *Echinococcus* species, are also significant contributors to helminthiasis-related morbidity. These parasites primarily infect the intestines when individuals consume undercooked or contaminated meat containing larval cysts. While mild cases may present with gastrointestinal discomfort, severe infections can result in the formation of cysts in various tissues, leading to life-threatening complications. One of the most severe manifestations of cestode infections is

neurocysticercosis, a condition caused by the migration of *Taenia solium* larvae to the central nervous system (CNS). Neurocysticercosis is a leading cause of adult-onset epilepsy in endemic regions and can result in seizures, chronic headaches, and even life-threatening neurological disorders. In rural areas where pig farming is common and sanitation is inadequate, the risk of cysticercosis is significantly higher.[11]

In addition to these major helminthic infections, other parasitic diseases caused by filarial worms and liver flukes further contribute to the global burden of helminthiasis. Lymphatic *filariasis*, commonly known as elephantiasis, affects millions of people worldwide, particularly in sub-Saharan Africa, South Asia, and the Pacific Islands. This disease is caused by filarial worms such as *Wuchereria bancrofti* and *Brugia malayi*, which are transmitted through mosquito bites. Chronic infections result in lymphatic dysfunction, leading to severe lymphedema, limb swelling, and disability, ultimately affecting the social and economic well-being of affected individuals. Similarly, urogenital schistosomiasis, caused by *Schistosoma haematobium*, is responsible for severe urinary tract complications, including hematuria, bladder fibrosis, and an increased risk of bladder cancer, predominantly in African countries where water sanitation is inadequate.

Other lesser-known helminthic infections include *fasciolopsiasis* and *dracunculiasis*, which, although affecting smaller populations, remain significant in certain regions. *Fasciolopsiasis* is caused by *Fasciolopsis buski*, a giant intestinal fluke that infects humans through the consumption of contaminated freshwater plants. This infection is endemic in parts of Southeast Asia and can cause severe gastrointestinal disturbances, malabsorption, and malnutrition. *Dracunculiasis*, or Guinea worm disease, caused by *Dracunculus medinensis*, was once widespread but has seen a dramatic decline due to global eradication efforts. However, sporadic cases still occur in parts of Africa, where individuals contract the disease by drinking contaminated water containing infected copepods.[12]

Overall, helminthiasis continues to be a major global health challenge, disproportionately affecting populations in low-income and rural areas. These infections not only cause significant physical suffering but also contribute to long-term economic and social burdens by reducing productivity, impairing child development, and straining healthcare resources. Effective control strategies, including improved sanitation, public health education, mass drug administration, and the development of new anthelmintic treatments, are essential for reducing the prevalence and impact of helminthic infections worldwide.[13]

Anthelmintics

Anthelmintics are a class of drugs specifically designed to eliminate parasitic worms, either by directly killing them or by expelling them from the host's body. These drugs have played a crucial role in the treatment and control of helminth infections in both human and veterinary medicine. Originally developed for livestock and domestic animals, anthelmintic treatments were later adapted for human use as helminth infections became recognized as a significant public health concern. Over time, various anthelmintic compounds have been developed, each targeting specific types of helminths, including nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms).

One of the earliest synthetic anthelmintic drugs introduced was piperazine citrate, which demonstrated high efficacy against Ascaris lumbricoides and Enterobius vermicularis infections. It worked by causing flaccid paralysis in nematodes, allowing the body to expel them naturally. Following this, more effective broad-spectrum anthelmintics were introduced, including pyrantel pamoate and levamisole, both of which are commonly used to treat hookworm infections. These drugs act by interfering with the neuromuscular function of parasitic worms, leading to their paralysis and eventual elimination. Another breakthrough in anthelmintic treatment was the development of praziquantel, which revolutionized the management of schistosomiasis and other trematode and cestode infections. Praziquantel increases the permeability of the worm's tegument, leading to calcium influx, muscle contraction, and eventual disintegration of the parasite.

Despite the effectiveness of these drugs, their widespread use has led to significant challenges, including the emergence of drug-resistant helminths. Resistance has become particularly problematic in endemic regions where mass drug administration (MDA) programs have been implemented as part of helminth control strategies. The prolonged and repeated use of anthelmintic drugs has facilitated the development of resistance in various helminth species, reducing the efficacy of these treatments over time. Furthermore, many conventional anthelmintics have a narrow spectrum of activity, requiring different drugs for different types of parasitic infections. Some drugs also cause adverse side effects such as nausea, dizziness, and gastrointestinal disturbances, further limiting their long-term usability.

To address these challenges, researchers have increasingly turned their attention to plant-based anthelmintics as a viable alternative to synthetic drugs. Medicinal plants have long been used in traditional medicine for treating parasitic infections, and modern pharmacological studies

have confirmed their efficacy against a range of helminths. Plant-derived compounds such as alkaloids, flavonoids, tannins, and terpenoids have demonstrated strong anthelmintic properties, with mechanisms of action that include disrupting parasite metabolism, impairing motility, and interfering with reproductive processes. These natural alternatives offer several advantages, including affordability, broad-spectrum activity, and lower toxicity compared to synthetic drugs. Moreover, plant-based anthelmintics are environmentally friendly and less likely to contribute to resistance development, making them a promising solution for the growing challenge of helminth infections.

As the global burden of helminthiasis continues to rise, particularly in low-income regions with poor sanitation, the development and integration of plant-based anthelmintic treatments could play a crucial role in future helminth control programs. Further research, including clinical trials and pharmacological studies, is essential to validate the efficacy and safety of these natural remedies and to develop new, sustainable strategies for combating helminth infections worldwide.[14]

Chemotherapy for Helminthiasis

Chemotherapy remains the primary approach for controlling helminthiasis, as anthelmintic drugs effectively target and eliminate parasitic worms from the host's body. These drugs work by attacking specific molecular structures unique to the parasites, which ensures selective toxicity without significantly harming the host. Due to variations in the molecular composition of different helminths, anthelmintics are classified based on their chemical structures and mechanisms of action. The major classes of anthelmintic drugs include benzimidazoles, ivermectins, piperazines, pyrazinoisoquinolines, tetrahydropyrimidines, imidazothiazoles, phenoxyalkanes, chlorinated sulfonamides, organophosphates, salicylanilides, halogenated phenols, and bisphenols.

Benzimidazoles such as albendazole, mebendazole, thiabendazole, and fenbendazole are among the most widely used anthelmintics. These compounds work by inhibiting microtubule formation in parasitic worms, leading to impaired glucose uptake, energy depletion, and eventual death of the parasite. Benzimidazoles have broad-spectrum efficacy and are particularly effective against nematodes, cestodes, and some trematodes. However, their prolonged use has resulted in the emergence of resistance in certain helminth populations, necessitating the search for alternative treatments.

Ivermectins, including ivermectin and moxidectin, are macrocyclic lactones derived from Streptomyces avermitilis. These drugs are highly effective against a range of nematodes and ectoparasites by binding to glutamate-gated chloride channels, leading to paralysis and death of the parasites. Ivermectin is widely used in mass drug administration programs for controlling onchocerciasis (river blindness) and lymphatic filariasis, two major neglected tropical diseases. Despite its efficacy, ivermectin resistance has been reported in some helminths, particularly in veterinary applications.

Piperazine derivatives, such as piperazine citrate, primarily act as neuromuscular blocking agents, causing flaccid paralysis in worms like Ascaris lumbricoides and Enterobius vermicularis. These drugs are relatively inexpensive and have been in use for decades, although they have largely been replaced by more effective alternatives.

Pyrazinoisoquinolines, represented by praziquantel, are the preferred treatment for trematode and cestode infections, including schistosomiasis and taeniasis. Praziquantel increases the permeability of the parasite's tegument to calcium ions, leading to intense muscular contractions, tegumental damage, and parasite death. Despite its widespread use, praziquantel has limited efficacy against immature schistosomes, which may contribute to reinfection in endemic areas.

Tetrahydropyrimidines, such as pyrantel pamoate, act as cholinergic agonists that cause spastic paralysis in nematodes, preventing their attachment to the intestinal wall and facilitating expulsion. Pyrantel is particularly effective against hookworms and roundworms and is commonly used in deworming programs for both humans and animals.

Imidazothiazoles, such as levamisole, function as ganglionic stimulants, inducing paralysis in nematodes by interfering with their neuromuscular signaling. Levamisole has anthelmintic as well as immunomodulatory properties, making it useful in certain therapeutic applications beyond helminth treatment.

Other classes of anthelmintic drugs, including phenoxyalkanes, chlorinated sulfonamides, organophosphates, salicylanilides, halogenated phenols, and bisphenols, have been explored for their anthelmintic properties, though they are less commonly used in human medicine due to concerns about toxicity and environmental persistence.

Plant-Based Alternatives to Synthetic Anthelmintics

With increasing concerns over drug resistance, side effects, and the environmental impact of synthetic anthelmintics, researchers have turned to plant-based alternatives as potential treatments for helminthiasis. Many medicinal plants have demonstrated anthelmintic properties, with bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids interfering with parasite metabolism, motility, and reproduction.

One such plant is *Ficus benghalensis* Linn., commonly known as the Indian Banyan or Banyan Tree. This tree is widely recognized for its medicinal properties and holds significant cultural and spiritual value in India and other parts of South Asia. Belonging to the Moraceae family, *Ficus benghalensis* is a massive evergreen tree known for its aerial roots that develop into secondary trunks, allowing it to spread across large areas and form a dense canopy. The tree is native to the Indian subcontinent, including India, Bangladesh, and Pakistan, where it thrives in warm, humid climates.

Traditionally, various parts of *Ficus benghalensis*, including its leaves, bark, roots, and latex, have been used in Ayurvedic and traditional medicine to treat a range of ailments. Recent studies have highlighted the anthelmintic potential of its bioactive compounds, which exhibit strong anti-parasitic activity against nematodes and cestodes. The latex and bark extracts contain phytochemicals that disrupt the metabolic pathways of parasites, impairing their survival and reproduction. The tannins and flavonoids present in the plant are known to inhibit helminth enzymes, weaken their structural integrity, and ultimately lead to parasite death.

Beyond its anthelmintic properties, *Ficus benghalensis* possesses antimicrobial, anti-inflammatory, anti-cancer, anti-stress and antioxidant activities, making it a valuable medicinal plant for treating a variety of diseases. Its widespread availability, affordability, and safety profile make it a promising candidate for developing natural anthelmintic therapies, particularly in regions where access to conventional pharmaceuticals is limited.

The Indian Banyan plays a pivotal ecological role, providing habitat and nourishment to a wide range of fauna. Its dark red fruits, though inedible for humans, are a rich food source for animals like monkeys and birds, enhancing biodiversity. Culturally, it is considered sacred in many Indian traditions and is often planted near temples and community spaces, symbolizing wisdom and shelter. In traditional medicine, *Ficus benghalensis* has been highly valued for its extensive therapeutic properties. Various parts of the tree, such as the bark, leaves, aerial roots, seeds,

and latex, are used to treat numerous ailments, including diabetes, dysentery, inflammation, ulcers, and arthritis. The latex is known for its aphrodisiac, tonic, and anti-inflammatory properties, while the leaves and roots are employed in remedies for hyperglycemia, fevers, and liver disorders. Modern pharmacological studies have validated many of its traditional uses, identifying bioactive compounds such as flavonoids, amino acids, and antioxidants. These constituents contribute to its hypoglycemic, anti-inflammatory, wound-healing, and immunomodulatory effects. Its diverse phytochemical profile and therapeutic potential make *Ficus benghalensis* a valuable plant in both ethnomedicine and modern pharmacology, bridging traditional wisdom with contemporary scientific inquiry. Through its cultural, ecological, and medicinal contributions, *Ficus benghalensis* Linn. continues to stand as a testament to nature's ability to sustain and heal, reinforcing its place as one of the most revered trees in the Indian subcontinent and beyond.[15-19]

REVIEW OF LITERATURE

The utilization of medicinal plants in traditional systems of medicine, particularly in South Asian countries such as India, Sri Lanka, and Nepal, has a history spanning thousands of years. Despite the rich ethnobotanical heritage and the widespread use of these plants in Ayurveda, Siddha, Unani, and other indigenous systems, a significant number of medicinal species remain underexplored from a pharmacological standpoint. Modern scientific research, aided by advances in molecular biology, pharmacognosy, and bioassay development, has enabled the evaluation of bioactive compounds from plant sources. However, these efforts tend to disproportionately focus on a small group of widely known and commercialized species, often neglecting others with equally promising traditional uses.

Ficus benghalensis (commonly known as the banyan tree), belonging to the Moraceae family, is one such species that has been frequently employed in traditional medicine for a variety of ailments including diabetes, inflammation, gastrointestinal disturbances, and infections. While scientific literature exists on its pharmacological activities, these findings are scattered, often preliminary, and sometimes not aligned with its ethnomedicinal claims. Furthermore, diverse parts of the plant — including the bark, leaves, roots, fruits, and latex — have been evaluated separately under varying conditions, making it difficult to draw conclusive evidence on the plant’s therapeutic potential as a whole.Despite the long-standing use of medicinal plants in traditional South Asian medicine, many species remain insufficiently studied concerning their pharmacological activities. While scientific advancements in recent decades have enabled

researchers to explore the detailed mechanisms of action of plant extracts and bioactive compounds, these investigations often focus on a limited number of commonly used species. In some instances, even when pharmacological activities are assessed, they do not align well with the plants' traditional applications or their chemical composition.

Our analysis highlights that *Ficus benghalensis* is one such species. Although it has been studied for various phytochemical and pharmacological properties, connecting these findings remains challenging. Different plant parts have been evaluated for diverse pharmacological activities using both in vitro and in vivo assays, yet detailed investigations are predominantly limited to its antidiabetic potential in experimental animal models.[20] The specifics of these activities are discussed in subsequent sections.

Interestingly, despite its extensive use in traditional medicine, the latex of *F. benghalensis* has received minimal attention for pharmacological evaluations. Some in vitro studies, such as those assessing free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and other reagents, have been reported but are not included in this review due to either limited clinical relevance or the nature of the journals in which they were published. Additionally, several studies suffer from methodological shortcomings, such as the absence of positive controls, lack of dose-response evaluations, and inadequate consideration of potential synergistic activities or artifacts generated during extraction, isolation, and purification of compounds.

Anti-allergic activity

Allergic reactions involve excessive immune responses, leading to inflammation and increased eosinophil and leukocyte production. In a study by Taur et al. (2007)[21], the ethyl acetate, ethanol, and aqueous bark extracts of Ficus benghalensis significantly reduced eosinophil and leukocyte levels in milk-induced allergic mice. This suggests the plant's potential to modulate immune responses and alleviate allergic inflammation.[21]

Anti-diabetic activity

The management of diabetes involves multiple mechanisms that biologically affect the pathogenesis of the disorder. These include the inhibition of glucose hydrolyzing enzymes to reduce the postprandial glucose level. An in vitro study evaluating the carbohydrate-hydrolyzing enzyme inhibition activity of *Ficus benghalensis* bark powder extract demonstrated significant potential. The aqueous extract exhibited IC_{50} values of 77 µg/mL

and 141 µg/mL against α-glucosidase and sucrose enzymes, respectively (Ahmed et al., 2011).[22]

Additionally, an *in vivo* study using ethanolic leaf extracts of *F. benghalensis* at doses of 200 mg/kg and 400 mg/kg body weight in alloxan-induced diabetic albino rats showed a notable reduction in triglyceride, cholesterol, and glucose levels, supporting the traditional use of its leaves as antidiabetic agents (Saraswathi et al., 2013).[23]

Another study reported that oral administration of *F. benghalensis* bark extract effectively lowered blood glucose levels in streptozotocin (STZ)-induced diabetic rats by stimulating insulin secretion from the beta cells of the Islets of Langerhans (Kasireddy et al., 2021).[24]

Anti-oxidant activity

The aqueous extract of *F. benghalensis* stem bark displayed a significant inhibition (IC_{50} = 80.24 µg/ml) compared to the standard reference used, tetraethoxypropane. The measurement was performed based on the thiobarbituric acid reactive substances (TBARS) value that measures the lipid peroxide generation as tested on microsomal lipid peroxidation (Satish et al., 2013).[25]

Besides that, *F. benghalensis* latex methanolic extract showed potential scavenging activity of 1-1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride (FeCl3) reducing antioxidant power (FRAP), and phosphomolybdenum (IC_{50} of 28.63, 49.82, and 31.84 µg/ml, respectively) as compared to the reference compounds (ascorbic acid and Trolox). The activity is reportedly due to abundance in flavonoids and phenolics, corresponding to their preliminary phytochemical screening (Yadav et al., 2011).[26]

Hypolipidemic activity

Leucocyanin derivatives (100 mg/kg) and quercetin (100 mg/kg) were given orally to cholesterol-fed rats for a month, while leucope largonin derivative (100 mg/kg) was given to alloxan diabetic dogs for a month. The results showed that there was a significant reduction in cholesterol levels in the blood (Daniel et al., 2003).[27]

In another study by Cherian and Augusti (1993) [28], a glycoside of leucopelargonidin discovered in the bark administered to diabetic rats revealed the hypolipidemic activity.

Anti-inflammatory activity

Ethanol extract prepared using bark (250 mg/kg) was administered orally to 2,4,6-trinitrobenzene sulfonic acid-induced inflammatory bowel disease model rats for 21 days showed anti-inflammatory effects (Patel & Patel, 2010).[29]

Orally administrating aqueous bark extract (250 mg/kg) for 21 days reduced inflammatory conditions in 2,4,6-trinitro benzene sulfonic acid-induced inflammatory bowel disease rats. Stem bark aqueous extract orally directed to acetic acid-induced vascular permeability rat (200 mg/kg) for eight days, carrageenan-induced hind paw edema in rats (100 mg/kg) for one hour, and cotton pellet-induced granuloma rat (200 mg/kg) for one hour exhibited anti-inflammatory properties (Thakare et al., 2010).[30]

The anti-Inflammatory activity targeting via Akt/PI3K Pathway and the results showed that FAG isolated from *F. benghalensis*, which is a natural EGFR inhibitor, NO-releasing, and COX-inhibiting anti-inflammatory agent via EGFR/Akt/PI3K pathway inhibition (Alaaeldin et al., 2022).[31]

Anti-microbial activity

Both ethanol and methanol extracts of prop root (25 µg/disc) revealed antibacterial activity against *Aeromonas hydrophila*, *Enterococcus faecalis*, Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio anguillarium*, and *Vibrio harveyi* (Verma et al., 2015).[32]

Likewise, another study revealed that its bark extract (1.2 mg/disc) exhibited significant against five bacterial strains (*Klebsiella pneumonia*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and two fungal strains (*Trichophyton rubrum*, *Candida albicans*). The highest zone of inhibition (ZOI) of bark extract was against S. aureus (24 mm) whereas it revealed the lowest ZOI against *P. aeruginosa* (10 mm). Besides that, *P. aeruginosa* and *K. pneumoniae* were reported to be insensitive against *F. benghalensis* root and bark extract. Also, both fungal strains were sensitive against all the leaves, stem bark, and root extract (Ogunlowo et al., 2013).[33]

Anti-diarrheal activity

The ethanol extract prepared using bark (400 mg/kg) was orally administered to castor oil-induced diarrhea and prostaglandin E2- induced entero-pooling rats individually. The results

showed ethanol extract at a dose of 400 mg/kg, p.o., as compared with the standard anti-diarrheal agent i.e. diphenoxylate, both have significantly inhibited the frequency of defecation and the wetness of the faecal droppings when compared to the control (Mukherjee et al., 1998).[34]

In another study, bark ethanol extract (400 mg/kg) was orally administered to both castor oil induced diarrhea and prostaglandin E2-induced entero-pooling rats. Inhibitory activity was observing in Prostaglandin E_2-induced enteropooling and castor oil-induced diarrhea in rats after 30 min and after 4 h, respectively (Patil et al., 2012).[35]

Anti-HIV activity

In an in vitro examination of anti-HIV effect, using PM1 (Clonal derivative of HUT 78 cells) and TZM-b1 (recombinant HeLa cells expressing high levels C CXCR4, D4 receptor, and CCR5 co-receptors) assays, aqueous leaves extract exhibited inhibition of primary isolates (IC80 of 78 µg/ml) in TZM-bl cells and laboratory-adapted HIV-1 strains (IC80 of 3.6 µg/ml) (Singh, 2020).[36]

In another study, Palshetkar and co-workers studied the anti-HIV activity of *F. benghalensis* extract against different laboratory and primary isolates strains. The results showed that aqueous extract of *F. benghalensis* inhibits the laboratory adapted HIV-1 strains with an IC_{50} value of 3.6-118 µg/ml (Palshetkar et al., 2020).[37]

Enzyme inhibitory activity

Enzyme inhibitory activity of the extract and few compounds have been performed for various diseases such as diabetes and alzheimer's etc. Aqueous bark extract inhibited human pancreatic α-amylase with the IC_{50} value of 4.4 µg/ml (Ravi Kumar et al., 2011).[38]

Two compounds isolated from the prop roots, benganoic acid and bengalensinone showed acetylcholinesterase inhibitory activity with IC_{50} values of 154.5 µM and 194.5 µM, respectively. These compounds also showed a strong butyrylcholinestarase inhibitory activity: benganoic acid (IC_{50} 120 µM) and bengalensinone (IC_{50} of 224.9 µM) (Riaz et al., 2012).[39]

Hassan et al., have studied the acetylcholinesterase activity of *F. benghalensis* leaves with an IC_{50} value of 194.6±7.9 µg ml (Hassan et al., 2020).[40]

Anti-cancer activity

In an investigation carried out by Grace et al. (2021) [41], *F. benghalensis* methanolic fruit extract was tested against the Human Papilloma Virus containing cancerous cervical cells (HeLa cell line) with the help of PCR techniques, utilizing HPV L1 primers (MY09/My011). The result suggested that treatment of extract on the cell line produced inhibition of HPV18 DNA synthesis. Also, the extract ensued significant cytotoxic activity on cancerous cells (IC50: 211.86 µg/ml).

Khanal and Patil (2020) further studied the cytotoxicity activity of the ethanolic extract of *F. benghalensis* against Chinese hamster ovary (CHO) and A549 cells and the results showed to possess anti-cancer activity with an IC_{50} value of 193.78±6.58 µg/mL.[42]

F. benghalensis latex extracted in various solvents was screened for its antiproliferative activity on multiple cell lines, including colorectal, human breast, neuroblastoma, and lymphocytes.[46] Ethanol extract has shown promising results against colorectal and neuroblastoma cells, while ethyl acetate extract against the human breast cell lines. Both the extracts were also discovered to exert lesser toxic effects on peripheral blood lymphocytes.[47] Another study investigated the ethyl acetate extract of *F. benghalensis* aerial roots to assess its anticancer activity on lung cancer (A549), breast cancer (MDA-MB-231), and cervical cancer (Hela) cell lines. The extract showed potential activity with the IC50 values of 17.81, 97.89, and 49.27 µg/mL, respectively.[48]

Mosquito larvicidal activity

The methanolic leaves extract of *F. benghalensis* was reported to show a promising larvicidal effect on three different species of a mosquito after 24 h of the extract treatment. The lethal concentration (LC_{50}) values against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*, on the early second, third, and fourth larva stages were determined to be 60.44, 76.41, and 89.55 ppm; 41.43, 58.21, and 74.32 ppm; 56.54, 70.29 & 80.85 ppm, respectively (Govindarajan and Angelina, 2010).[43]

In another similar study, its methanolic leaves extract resulted in larvicidal effect with LC_{90} and LC_{50} values of 85.84, 56.66 ppm and 159.76, 100.88 ppm in case of third instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*, respectively (Govindarajan et al., 2011).[44]

Hepatoprotective activity

F. bengalensis latex was orally administered to CCl4-induced hepatotoxicity in albino rats and paracetamol-induced hepatic damage in rats. The latex treatment showed improvement to liver function with a significant reduction in the serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and bilirubin and alkaline phosphate (ALP) levels, and improvement in the total protein level.[49] The investigation of the hepatoprotective effect of the *F. bengalensis* fruit extract on goat liver assessed through catalase activity showed that the ethanol extract of the fruit at the dose of 50 mg/kg could significantly reduce the hepatotoxicity effect against Silymarin used as standard drug.[50]

Recently, the fruit of *F. benghalensis* was evaluated for its hepatoprotective effects via in vitro assays. The fruit was extracted using different solvent systems, namely ethanol, water, chloroform, ethyl acetate, and petroleum ether. The hepatotoxicity condition was induced using carbon tetrachloride, acetaminophen, and erythromycin in the liver extracted from goat, Capra Capra, and treated with 100, 250, and 500 mg/kg of the fruit extracts. Of all the extracts tested, the ethanol extract was found to be most effective in reducing the hepatotoxic activity at the dose of 500 mg/kg of fruit extract against Silymarin, the control drug.[51-53]

This finding is supported by an in vivo study conducted recently using he fruit's ethanolic extract (500 mg/kg) against perchloromethane-induced toxic hepatitis in New Zealand albino rats, indicating the hepatoprotective action of *F. benghalensis* fruit. The elevated liver biomarkers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin, and malondialdehyde) were reduced upon administration of the fruit extract.[54]

AIMS AND OBJECTIVES

Aims:

The aim of this study is to investigate the phytochemical composition and evaluate the anthelmintic potential of *Ficus benghalensis* L. leaves against *Pheretima posthuma*.

This research seeks to explore the bioactive compounds present in the leaves and their efficacy in combating helminthic infections, thereby contributing to the development of alternative and plant-based therapeutic agents.

Objectives:

- To perform a comprehensive qualitative analysis of *Ficus benghalensis* L. leaf extracts to identify the presence of bioactive compounds such as alkaloids, flavonoids, tannins,

saponins, glycosides, and phenols.

- To evaluate the anthelmintic potential of *Ficus benghalensis* L. leaf extracts by conducting bioassays on *Pheretima posthuma* (Indian earthworm).
- To compare the anthelmintic efficacy of *Ficus benghalensis* L. leaf extracts with standard anthelmintic drugs (e.g., albendazole).

RESEARCH ENVISAGED

Helminthiasis, caused by parasitic worms such as nematodes, cestodes, and trematodes, is one of the most common infections worldwide, affecting billions of people, particularly in tropical and subtropical regions. These infections are strongly associated with poverty, poor hygiene, inadequate sanitation, and limited access to healthcare services. They are especially prevalent among children in low-income countries, where they contribute significantly to malnutrition, anemia, impaired cognitive development, and reduced school attendance and performance. Despite their widespread occurrence and their substantial public health implications, helminth infections often receive less attention in global health priorities when compared to diseases such as HIV, malaria, and tuberculosis.

To combat these infections, a range of synthetic anthelmintic drugs have been developed and deployed. These include Diethylcarbamazine, which is commonly used for filariasis; Ivermectin, widely used for both human and veterinary nematode infections; Thiabendazole, which acts on larvae and adult worms; Levamisole, known for its immunomodulatory and anthelmintic effects; Piperazine, effective against roundworms and pinworms; Niclosamide, used for tapeworm infestations; and Praziquantel, which is the drug of choice for schistosomiasis and other fluke infections. While these drugs have been effective in reducing worm burdens and disease transmission, their long-term use is fraught with several limitations.

One major concern associated with synthetic anthelmintics is the occurrence of adverse drug reactions, which range from mild to severe. Commonly reported side effects include nausea, vomiting, abdominal pain, loss of appetite, dizziness, and diarrhea. In populations with heavy worm burdens, the rapid killing of parasites can also lead to severe immune responses and inflammatory reactions, further complicating treatment. In addition to these side effects, another pressing issue is the development of drug resistance among helminths. Resistance has been well documented in veterinary helminths due to the widespread and often unregulated use of anthelmintics in livestock. Alarmingly, similar trends are now being observed in human helminths, posing a significant threat to the sustainability of current deworming programs.

The combination of toxicity, side effects, drug resistance, and high treatment costs in some regions has created an urgent need for alternative approaches to the management and treatment of helminthiasis. This has directed research attention toward the exploration of natural, plant-based remedies, many of which have been used for centuries in traditional medical systems. Medicinal plants contain a diverse array of bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolics, many of which possess proven pharmacological effects including antimicrobial, antiparasitic, and anti-inflammatory properties. These compounds often work through multiple mechanisms, reducing the likelihood of resistance development. Furthermore, plant-based remedies are often more culturally accepted, locally available, and economically accessible, especially in rural and resource- limited communities.

The traditional knowledge of plant-based medicines has accumulated over generations through observation, trial and error, and communal knowledge sharing. Many indigenous communities have long recognized specific plants that can expel intestinal worms, cleanse the digestive system, and promote overall gut health. Intriguingly, these behaviors are not limited to human societies. Ethologists have observed that wild chimpanzees, bonobos, and gorillas engage in self-medicative behavior, particularly when experiencing gastrointestinal distress. These primates have been seen chewing bitter or fibrous leaves, such as those of *Vernonia amygdalina*, and swallowing them whole to physically or chemically expel parasitic worms. Such observations suggest an evolutionary basis for the use of botanical remedies against helminths and provide valuable leads for drug discovery from nature.

Despite the promising potential of medicinal plants, research into novel anthelmintic agents derived from phytochemicals has progressed slowly over the past four decades. This slow progress is partly due to the lack of investment from pharmaceutical companies in wealthier nations, where helminth infections are rare. Consequently, there is limited commercial incentive to develop new drugs for diseases primarily affecting impoverished populations. This neglect places the responsibility for innovation in this field on researchers in endemic regions such as India, where parasitic worm infections are not only common but also a major contributor to disease burden and socioeconomic hardship.

India is uniquely positioned to contribute to this area of research due to its rich biodiversity and long-standing tradition of herbal medicine. The country is home to over 45,000 plant species, of which approximately 7,000 are known to possess medicinal properties. Several key

pharmaceutical agents have already been derived from Indian medicinal plants—Reserpine from *Rauwolfia serpentina*, Morphine from *Papaver somniferum*, and Atropine from *Atropa belladonna* are notable examples. These drugs have not only transformed modern medicine but have also served as prototypes for the synthesis of newer and more effective analogues. This historical success underscores the untapped potential of India's flora as a source of novel anthelmintic compounds.

In light of the World Health Organization's (WHO) strategic objective to reduce the prevalence of soil-transmitted helminth (STH) infections, particularly among 873 million school-aged children in endemic areas, the need for new, effective, and affordable treatments is more urgent than ever. The WHO advocates for regular mass drug administration (MDA) campaigns, yet these programs are increasingly threatened by issues of resistance, supply chain limitations, and treatment fatigue. Developing new drugs that operate through novel mechanisms of action and are affordable and accessible is critical to sustaining and advancing global deworming efforts.

As part of an extensive ethnopharmacological investigation, several Indian medicinal plants have been reviewed for their traditional and experimental anthelmintic activity. Among these, the leaves of *Ficus benghalensis* Linn., commonly referred to as the Indian banyan tree, have shown exceptional promise. Widely revered in Indian culture and Ayurveda, *Ficus benghalensis* is known for its diverse pharmacological properties, including anti-inflammatory, antioxidant, antimicrobial, wound healing, immunomodulatory, and antidiabetic activities. Preliminary studies and traditional usage suggest that its leaves also exhibit anthelmintic potential, although this aspect remains underexplored in the scientific literature.

Given its abundance, low cost, and wide traditional use, *Ficus benghalensis* presents itself as a viable candidate for the development of plant-derived anthelmintic agents. Scientific validation of its efficacy and mechanism of action could contribute significantly to the global fight against helminthiasis and provide a safer, natural alternative to synthetic drugs. Moreover, leveraging such indigenous resources aligns with the principles of sustainable healthcare, local empowerment, and bioprospecting for drug discovery.

NOVELTY OF WORK

The advancement of research in anthelmintic drugs has been relatively slow over the past forty years, despite parasitic infections being prevalent in developing and underdeveloped nations,

including India. Drug discovery for parasitic infections has been largely neglected by developed countries, highlighting the urgent need for Indian scientists to focus on this critical area.

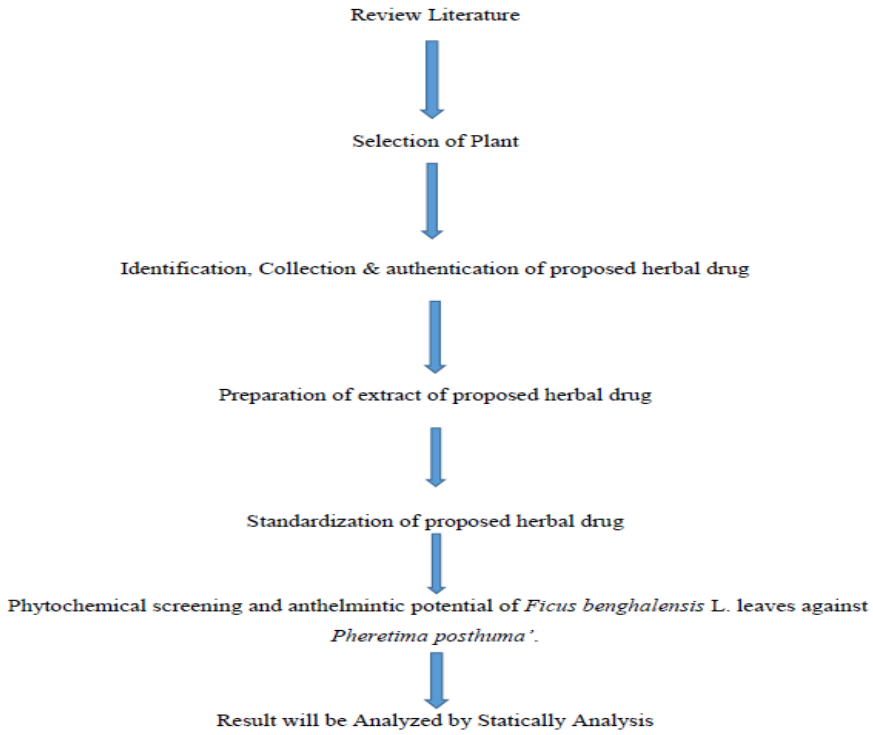
Indian medicinal plants have historically provided numerous drugs, such as reserpine, morphine, and atropine, serving as prototypes for synthetic drug development. Recognizing this potential, an innovative research program was initiated to identify more effective and affordable anthelmintic agents from Indian medicinal plants.

In alignment with the WHO's universal target to reduce soil-transmitted helminth infestations in 873 million children, especially in endemic regions, this research aims to develop novel molecules with unique mechanisms of action.

Extensive literature surveys have identified several plants with significant anthelmintic potential. Among them, the leaves of *Ficus benghalensis* Linn. emerged as a promising candidate for further investigation, showcasing the potential to contribute to affordable and effective solutions for parasitic infections.

PLAN OF WORK

Aim & Objective: - Phytochemical screening and anthelmintic potential of *Ficus benghalensis* L. leaves against *Pheretima posthuma'*



MATERIALS AND METHODS

Plant Material Collection and Authentication

The plant material utilized in this study was obtained either by local collection or from an established crude drug supplier known for providing authenticated botanical materials. In cases where the plant material was collected locally, efforts were made to identify the plant in its natural habitat to ensure correct species selection. The specific part of the plant required for the study (e.g., leaves) was carefully harvested, ensuring no damage to the remaining parts of the plant. Once collected, the botanical identity of the plant was authenticated by a certified botanist using macroscopic and microscopic evaluations, as well as comparison with herbarium specimens and existing botanical monographs. A voucher specimen of the authenticated plant material was preserved and stored in a herbarium for future reference and verification.

2. Experimental Animal Selection

The in vitro evaluation of anthelmintic activity was carried out using healthy adult earthworms, zoologically identified as *Pheretima posthuma*, which is synonymously referred to as *Metaphire posthuma*. These earthworms, ranging from 3 to 5 centimeters in length and approximately 0.1 to 0.2 centimeters in diameter, were collected locally during the monsoon season, typically between June and September. This season is considered ideal due to the abundance of earthworms and their increased surface activity. Since these organisms are invertebrates (animals without backbones), they are exempt from the regulatory oversight of the Indian Animal Ethics Committee (IAEC). Thus, no prior ethical approval was required for their use in this study. Nevertheless, all handling and experimental procedures involving earthworms were conducted with care to minimize unnecessary harm or stress.

3. Procurement and Authentication of Standard Drug

Albendazole, a broad-spectrum anthelmintic, was used as the reference or standard drug for comparing the efficacy of the plant extracts. The drug was either obtained free of cost (gratis) from a licensed pharmaceutical manufacturer or procured commercially from a pharmacy. To ensure the authenticity and chemical integrity of the standard compound, it was subjected to confirmation via spectral analysis, including UV, IR, and mass spectrometric profiling, thereby validating its identity prior to use in experimental procedures.

4. Preparation of Plant Extracts

4.1 Alcoholic Extract

Fresh leaves of the selected plant species, approximately 2 kilograms in total weight, were initially washed with distilled water to remove dirt and foreign materials, and then shade-dried for two weeks to preserve thermolabile constituents. The dried leaves were then ground into a coarse powder using a mechanical grinder and stored in an airtight, moisture-resistant container.

For the preparation of the alcoholic extract, 450 grams of this powdered material were subjected to hot continuous extraction using a Soxhlet apparatus. Ethanol (95%) was employed as the solvent due to its broad polarity range, which allows for efficient extraction of both polar and semi-polar phytochemicals. The extraction was carried out at a controlled temperature of approximately 70°C and continued until 42 siphoning cycles were completed, ensuring exhaustive extraction of constituents. The resultant alcoholic extract was filtered through Whatman filter paper No. 1 and concentrated using a rotary vacuum evaporator to remove excess ethanol. The thick concentrate was then dried under reduced pressure in a vacuum oven until a constant weight was achieved and stored in desiccators for further experimental use.

4.2 Aqueous Extract

For the aqueous extract, 200 grams of the same dried powdered leaf material were macerated with 1000 ml of chloroform water (prepared in a 1:9 ratio) for a duration of seven days at room temperature in a conical flask. The use of chloroform water served as a preservative medium to prevent microbial contamination during the maceration process. The mixture was shaken intermittently throughout the maceration period to enhance the solubility and release of phytochemicals. After seven days, the extract was filtered using a muslin cloth followed by Whatman filter paper. The filtrate was then concentrated over a water bath to remove the aqueous solvent and subsequently dried in a vacuum oven until a constant weight was attained. The dried extract was carefully stored in airtight containers until further use in the biological assays.

5. In Vitro Anthelmintic Assay

The anthelmintic activity of both the alcoholic and aqueous extracts of *Ficus benghalensis* was evaluated through an in vitro study using *Pheretima posthuma* as the model organism. Earthworms were randomly divided into eight experimental groups, with each group consisting

of six worms (n = 6). Extracts and standard drugs were freshly prepared before the initiation of each experimental batch.

Both the alcoholic and aqueous extracts were suspended in normal saline using 0.1% v/v Tween 80 as a suspending agent to aid solubility. Different concentrations—25 mg/ml, 50 mg/ml, and 100 mg/ml—of each extract were prepared in separate petri dishes with the volume adjusted to 20 ml using normal saline. A standard solution of Albendazole at 25 mg/ml was also prepared in distilled water and adjusted similarly to 20 ml with normal saline. A control group containing only normal saline (20 ml) was maintained for baseline comparison.

Before the assay, all earthworms were washed in normal saline to remove any external debris and acclimatized briefly. Each group of six worms was then introduced into the respective formulations. Observations were recorded at regular intervals to determine two primary endpoints:

- **Time to Paralysis**: Time was noted until the worms ceased to move completely and failed to recover movement even upon stimulation with saline. This marked the onset of paralysis.
- **Time to Death**: Death was confirmed when the worms lost all motility, followed by fading or discoloration of their body, indicating breakdown of cuticular integrity.

Observations were recorded for up to 4 hours post-treatment. Results were expressed in terms of mean time (in minutes) for paralysis and death. Extracts demonstrating the highest anthelmintic activity (shortest time to induce paralysis and death) were shortlisted for subsequent phytochemical analysis.

6. Preliminary Phytochemical Screening

The most active plant extracts were subjected to comprehensive phytochemical analysis to identify the classes of secondary metabolites present. The objective was to detect bioactive constituents such as alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, glycosides, and saponins, all of which are known for their pharmacological significance.

The powdered plant material was extracted with suitable solvents and subjected to the following qualitative chemical tests:

- **Alkaloids**:

Dragendorff's Test: Appearance of orange or reddish-brown precipitate.
Mayer's Test: Formation of a creamy white precipitate.

- **Flavonoids**:

Shinoda Test: Pink or red color upon addition of magnesium turnings and HCl.

Alkaline Reagent Test: Yellow color that disappears with acid.

- **Tannins & Phenols**:

Ferric Chloride Test: Blue, green, or black coloration indicating phenolic compounds.

- **Saponins**:

Foam Test: Stable frothy foam formed after vigorous shaking with water.

- **Steroids and Terpenoids**:

Salkowski Test: Formation of a red or golden yellow ring upon reaction with concentrated H_2SO_4.

- **Glycosides**:

Keller-Killiani Test: Reddish-brown ring at the interface of glacial acetic acid, $FeCl_3$, and H_2SO_4.

7. Isolation and Characterization of Bioactive Compounds

Extracts that demonstrated potent anthelmintic activity and tested positive in preliminary screening were subjected to further phytochemical investigation. Isolation of the bioactive principles was carried out using chromatographic techniques such as Thin Layer Chromatography (TLC) and preparative column chromatography. Isolated compounds were purified and characterized to confirm their chemical structures.

Spectral analyses employed included:

- **UV-Visible Spectroscopy**
- **Infrared Spectroscopy (IR)**
- **Proton Nuclear Magnetic Resonance (^{1}H-NMR)**
- **Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR)**
- **Mass Spectrometry (MS)**

These techniques helped in structural elucidation and confirmation of purity, followed by subjecting the isolated compounds to further anthelmintic screening.

RESULTS

Albendazole, a widely used anthelmintic drug, primarily exerts its effect on parasitic worms by inducing flaccid paralysis, ultimately leading to their expulsion from the host body through peristalsis. This action is achieved by increasing chloride ion conductance across the worm's muscle membrane, which results in hyperpolarization and a subsequent decrease in excitability. As a result, the muscle cells of the worm undergo relaxation, causing flaccid paralysis, which prevents the worm from maintaining its position in the host, leading to its elimination from the system.

The present study was conducted to evaluate and compare the anthelmintic efficacy of alcoholic and aqueous extracts of *F.benghalensis* Linn. leaves using the Indian earthworm (*P. posthuma*) as a surrogate biological model, as represented in **Table 1**. This model was chosen due to its significant anatomical and physiological resemblance to human intestinal helminths, particularly in its neuromuscular system and cuticular features, making it a reliable in vitro platform to assess the anthelmintic properties of both synthetic and plant-based compounds. The purpose was to determine if *F. benghalensis* extracts possess bioactive properties that could immobilize and lethally affect helminths, thereby justifying their traditional use and contributing to the development of new, accessible, and eco-compatible pharmacotherapeutic agents.

Structure of experimental design

The aqueous extract, when tested at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml, induced paralysis in the worms within 4.78 minutes, 4.26 minutes, and 3.92 minutes, respectively. The time required to cause the death of the worms at these concentrations was recorded as 15.76 minutes, 14.62 minutes, and 13.72 minutes. These results indicate a dose-dependent increase in efficacy, with higher concentrations of the extract leading to faster paralysis and death.

Similarly, the alcoholic extract at the same concentration levels exhibited even stronger anthelmintic activity. The observed paralysis times for 25 mg/ml, 50 mg/ml, and 100 mg/ml concentrations were 3.82 minutes, 2.92 minutes, and 2.12 minutes, respectively, while the corresponding times for death were 9.76 minutes, 8.04 minutes, and 7.34 minutes. Notably, the alcoholic extract at a concentration of 100 mg/ml exhibited the shortest time for inducing paralysis in the worms, suggesting that the active compounds responsible for anthelmintic activity might be more effectively extracted in alcohol than in water.

When compared to the standard drug albendazole, which at a concentration of 25 mg/ml induced paralysis at 2.36 minutes and caused death at 6.42 minutes, it is evident that the plant extracts exhibit significant anthelmintic effects, though slightly less potent than albendazole. However, the findings suggest that *Ficus benghalensis* leave extracts could be a promising natural alternative for treating parasitic worm infections.

Eight distinct groups of earthworms were prepared, each comprising six worms to ensure appropriate statistical power and reproducibility. The grouping was structured as follows:

- **Group I (Control)**: Exposed only to normal saline solution (0.9% NaCl). This group served to establish a baseline for normal motility, behavior, and survival under non-stressful conditions.
- **Group II (Standard)**: Treated with albendazole (25 mg/ml), a well-established anthelmintic drug. This group was used to provide a pharmacological benchmark.
- **Groups III–V (Alcoholic Extracts)**: Treated with ethanol-based extracts of *Ficus benghalensis* at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml, respectively.
- **Groups VI–VIII (Aqueous Extracts)**: Treated with aqueous (chloroform-water macerated) extracts of the same leaves at the same concentrations.

Each treatment group was exposed to 20 ml of the respective formulation in sterile Petri dishes. Observations were made at regular intervals to record:

- **Paralysis Time (P)** – when worms ceased all voluntary movement, even upon physical stimulation.
- **Death Time (D)** – when worms exhibited permanent flaccidity, fading of body color, and failure to respond to saline recovery.

Alcoholic extracts (Ethanolic)

The alcoholic extract of *Ficus benghalensis* leaves demonstrated strong anthelmintic activity. The results indicated a marked concentration-dependent increase in efficacy, both in terms of the rapidity of paralysis and time to death. The following effects were recorded:

Table 1 (a): Anthelmintic activity of alcoholic leaves extract of *F. benghalensis* against Indian earthworms – *Pheretima posthuma*.

Concentration	Paralysis Time (min)	Death Time (min)
25 mg/ml	3.82 ± 1.62	9.76 ± 0.84
50 mg/ml	2.92 ± 1.16	8.04 ± 1.46
100 mg/ml	2.12 ± 1.34	7.34 ± 0.32

Interpretation:

- A significant and consistent reduction in time required to paralyze and kill the worms was observed with increasing dose.
- At 100 mg/ml, the extract nearly matched the performance of albendazole, suggesting the presence of highly potent anthelmintic phytochemicals.
- The rapid neuromuscular disruption is attributed to the presence of ethanol-soluble bioactives such as alkaloids, flavonoids, terpenoids, and steroids.
  - **Alkaloids** may interfere with neurotransmission, block ion channels, or inhibit acetylcholinesterase, leading to flaccid paralysis.
  - **Flavonoids and terpenoids** likely disrupt membrane integrity or enzyme activity, accelerating worm death.
- Ethanol acts as a superior solvent for such lipophilic and semi-polar compounds, allowing deeper cellular penetration and stronger bioavailability.

Aqueous extracts

The aqueous extract showed appreciable anthelmintic activity, although its performance was somewhat subdued compared to the alcoholic extract. Nevertheless, a concentration-dependent trend was observed, confirming the extract's effectiveness.

Table 1 (b): Anthelmintic activity of aqueous leaves extract of *F. benghalensis* against Indian earthworms – *Pheretima posthuma*.

Concentration	Paralysis Time (min)	Death Time (min)
25 mg/ml	4.78 ± 0.18	15.76 ± 1.42
50 mg/ml	4.26 ± 1.04	14.62 ± 0.96
100 mg/ml	3.92 ± 1.24	13.72 ± 0.28

Interpretation:

- Although slower in action than the alcoholic extract, the aqueous extract still exhibited notable efficacy, particularly at the highest concentration.
- Its effects are likely due to hydrophilic phytochemicals, including:

Tannins – known to bind proteins and enzymes in the worm cuticle, compromising structural integrity.

Saponins– which create pore-like disruptions in the parasite's cell membranes.

Phenolic compounds– possessing free radical scavenging and enzymatic inhibitory activity.

These constituents, while effective, typically act through slower, membrane-weakening processes rather than rapid neuromuscular disruption.

Standard Drug – Albendazole (Positive control)

Table 1 (c):

Concentration	Paralysis Time (min)	Death Time (min)
25 mg/ml	2.36 ± 0.64	6.42 ± 1.92

Mechanism:

- Albendazole binds to **β-tubulin** in parasitic cells, inhibiting **microtubule polymerization**.
- This blocks glucose uptake and ATP production, resulting in **energy depletion**, **immobility**, and ultimately, **death** of the parasite.

Comparison:

- The alcoholic extract (100 mg/ml) came extremely close to albendazole, with just ~0.24 minutes' difference in paralysis time.
- Though albendazole was faster, the plant extract demonstrated near-equivalent efficacy, reinforcing its therapeutic potential.

Phytochemical-Activity Correlation

Phytochemical screening is an essential process in identifying bioactive compounds present in medicinal plants. Ficus benghalensis (Banyan tree) is widely used in traditional medicine for its therapeutic properties. This study aims to analyze the phytochemical composition of aqueous and alcoholic extracts of Ficus benghalensis leaves to identify key bioactive constituents such as alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. Standard qualitative tests were conducted to compare the presence of these compounds in both extracts shown in **table 2**.

Table 2: Phytochemical Screening Tests for Aqueous and Alcoholic Extracts of Ficus benghalensis Leaves.

Phytochemical	Aqueous Extract	Alcoholic Extract
Alkaloids	+	++
Flavonoids	++	+++
Tannins	+++	++
Saponins	++	+
Phenols	+++	++
Steroids & Terpenoids	+	++
Glycosides	++	++

Legends:

- (+++) High presence
- (++) Moderate presence
- (+) Low presence
- (-) Absence

Phytochemical screening of aqueous and alcoholic extracts of *Ficus benghalensis* leaves was conducted to identify the presence of bioactive compounds. The results revealed that both extracts contain essential phytochemicals, but their concentrations vary based on the solvent used for extraction. The alcoholic extract showed a higher presence of alkaloids, flavonoids, and steroids/terpenoids, indicating that these compounds are more soluble in organic solvents. In contrast, the aqueous extract exhibited a greater concentration of tannins, phenols, and saponins, which are typically water-soluble. Both extracts tested positive for glycosides, suggesting their potential cardiovascular and antimicrobial benefits. The presence of these phytochemicals highlights the therapeutic significance of *Ficus benghalensis*, as flavonoids and phenols contribute to antioxidant and anti-inflammatory properties, tannins possess astringent and antimicrobial effects, and steroids and terpenoids exhibit anti-inflammatory and analgesic activities. These findings support the traditional medicinal use of *Ficus benghalensis* and suggest its potential for further pharmacological research.

Biological Roles:

- **Alkaloids & flavonoids**: Disrupt nervous system signaling, block receptors, or inhibit enzymes like acetylcholinesterase.
- **Tannins & saponins**: Cause protein denaturation and lipid layer destabilization in the parasite's tegument.
- **Terpenoids & steroids**: Affect intracellular signaling and cell permeability.
- **Phenols**: Induce oxidative stress in parasites.

Thus, the **differential extraction profiles** explain the observed variance in pharmacodynamic outcomes between the two extracts.

Statistical Relevance and Reliability

- All data points were statistically expressed as Mean±Standard Error of Mean (SEM) with n=6 per group.
- The low SEM across groups indicates strong reproducibility and low intra-group variability.

- The consistent decline in both paralysis and death times with increasing concentration further validates the dose-response relationship, a hallmark of pharmacological effect.

These findings contribute substantially to the ethnopharmacological validation of *Ficus benghalensis*, a plant long revered in Ayurvedic and traditional medicine. The alcoholic extract, with its high efficacy and reproducibility, is a potential candidate for further isolation, formulation development, and preclinical toxicity testing. With the growing threat of anthelmintic resistance and limited availability of drugs in low-income settings, this study underscores the feasibility of developing locally sourced, natural deworming agents.

DISCUSSION

The findings of the present study strongly support the hypothesis that leaf extracts of *Ficus benghalensis* L. exhibit notable anthelmintic activity against *Pheretima posthuma*, a standard in vitro model for human intestinal helminths. The experimental results not only reinforce the ethnomedicinal claims associated with this sacred and widely distributed tree but also open new avenues for its potential integration into modern phytotherapeutics. Both aqueous and alcoholic extracts demonstrated significant, dose-dependent anthelmintic activity, although the alcoholic extract consistently outperformed the aqueous extract in terms of rapidity and potency.

The comparative performance of the **alcoholic extract** was particularly noteworthy. The time required to induce paralysis and death in *Pheretima posthuma* decreased significantly with increasing concentrations of the extract, revealing a consistent and reproducible pharmacological pattern. At 100 mg/ml, the alcoholic extract caused paralysis in just over two minutes and induced death in slightly over seven minutes. These results closely approach the benchmark performance of the synthetic reference drug, albendazole (25 mg/ml), which induced paralysis in just over two minutes and death in approximately six and a half minutes. This near-parallel efficacy suggests that the ethanol extract contains highly bioactive compounds with neuromuscular-disruptive or metabolically toxic effects on helminths.

The stronger performance of the alcoholic extract can be attributed to the solvent's superior ability to dissolve and extract lipophilic and semi-polar compounds. Phytochemical analysis confirmed the presence of **alkaloids, flavonoids, terpenoids, steroids**, and **glycosides** in higher concentrations in the ethanolic extract. These phytoconstituents have been widely documented for their pharmacological activities, including antiprotozoal, antiparasitic, and antimicrobial effects. For instance, **alkaloids** are known to affect neural transmission by

interacting with ion channels or neurotransmitter receptors, leading to neuromuscular paralysis in parasites. Similarly, **flavonoids** possess membrane-disruptive properties, often leading to impaired permeability and enzymatic dysfunction in helminths. **Terpenoids and steroids**, on the other hand, may integrate into lipid bilayers, compromising the structural and functional integrity of the parasite's cuticle and internal membranes.

In contrast, the **aqueous extract** also demonstrated concentration-dependent anthelmintic activity, but the onset of both paralysis and death was slower compared to the alcoholic extract. Even at the highest concentration tested (100 mg/ml), the aqueous extract induced paralysis at approximately four minutes and death at around thirteen minutes. Despite this slower response, the aqueous extract's efficacy is significant, especially given that water is a more accessible, safer, and non-toxic solvent. Its activity is likely attributed to the presence of **tannins, saponins, phenols**, and **glycosides**, all of which were present in appreciable quantities in the aqueous extract. Tannins have been reported to exert anthelmintic action by binding to glycoproteins in the cuticle and interfering with energy metabolism. **Saponins**, through their surfactant-like properties, can cause pore formation in membranes, leading to cytolysis. **Phenolic compounds** exhibit oxidative stress-inducing and enzyme-inhibiting effects that compromise helminth viability.

When compared with the standard reference drug albendazole, both plant extracts—particularly the alcoholic one—proved to be significantly effective, although not superior. Albendazole's mechanism involves binding to tubulin, thereby inhibiting microtubule assembly in helminths, leading to impaired glucose uptake, ATP depletion, and subsequent death. While the plant extracts may act through different pathways—primarily involving direct cuticular disruption, interference with neurotransmission, or inhibition of metabolic enzymes—their end result of inducing paralysis and death in helminths validates their therapeutic potential.

Another critical point of discussion is the observed **dose-response relationship** in both extracts, which is crucial for establishing the pharmacological credibility of a natural product.

The consistent decrease in both paralysis and death times with increasing concentrations of extracts confirms that the observed effects are not random but are biologically relevant and concentration-dependent. This provides a foundational rationale for the isolation of active compounds, determination of minimum inhibitory concentrations (MIC), and future dose optimization studies.

The use of *Pheretima posthuma* as a test organism also lends experimental rigor to the findings. Due to its sensitivity to both neuromuscular and metabolic insults, it serves as a robust and reproducible model for assessing anthelmintic potential in vitro. The response of the worms in this study mirrored known effects of established anthelmintic drugs, further supporting the biological activity of the plant extracts.

Moreover, this study holds substantial **ethnopharmacological significance**. *Ficus benghalensis*, traditionally regarded as sacred and medicinal in various indigenous systems, including Ayurveda and Siddha, has long been used to treat a wide range of conditions such as diarrhea, ulcers, diabetes, and parasitic infections. However, scientific validation of its anthelmintic properties has been limited. This study bridges that gap by providing empirical, laboratory-based evidence for its traditional claims, thereby promoting its legitimacy as a medicinal resource and encouraging further research and clinical evaluation.

The findings also carry implications for **rural and underserved populations**, where access to modern pharmaceuticals may be limited due to economic or geographic constraints. The use of locally available medicinal plants like *Ficus benghalensis* offers a sustainable, affordable, and culturally accepted alternative. Moreover, as drug resistance in helminths continues to rise, particularly in livestock and humans in developing regions, the discovery and utilization of plant-based anthelmintics could play a pivotal role in future integrated parasite management strategies.

SUMMARY

The present study was undertaken with the primary objective of scientifically evaluating the anthelmintic potential of *Ficus benghalensis* Linn. leaf extracts through phytochemical screening and in vitro experimentation using *Pheretima posthuma* (Indian earthworm) as a model organism. This research was motivated by the increasing interest in plant-based therapeutics, especially in regions where traditional medicine is relied upon due to limited access to modern pharmaceuticals and rising drug resistance among parasitic organisms.

Helminthic infections remain a persistent public health burden, particularly in developing countries, where they affect millions of individuals, compromising nutritional status, immunity, and overall health. The need for effective, affordable, and accessible deworming agents is urgent. While synthetic drugs like albendazole have proven to be effective, their frequent use is associated with side effects, contraindications in sensitive individuals, and the

emergence of resistance. In this context, the current research aimed to explore a sustainable herbal alternative by focusing on *Ficus benghalensis*, a plant revered in traditional medicine systems and widely recognized for its therapeutic properties.

The study began with the collection and preparation of *Ficus benghalensis* leaves, followed by their extraction using two solvents—distilled water (aqueous extract) and ethanol (alcoholic extract)—to obtain a broad range of phytochemicals with varying polarity and solubility. These extracts were then subjected to qualitative phytochemical screening to identify the presence of secondary metabolites known for their pharmacological properties.

Phytochemical analysis revealed a rich spectrum of bioactive constituents in both extracts. The alcoholic extract was particularly rich in alkaloids, flavonoids, steroids, terpenoids, and glycosides, whereas the aqueous extract contained higher levels of tannins, saponins, phenols, and also glycosides. These compounds are well documented for their anthelmintic, antimicrobial, anti-inflammatory, and antioxidant activities. Their presence in both extracts established a biochemical basis for investigating the plant's therapeutic potential against helminths.

To assess the biological activity of these extracts, in vitro anthelmintic testing was conducted using *Pheretima posthuma*. The worms were divided into eight groups, with each group receiving different treatments, including varying concentrations (25, 50, and 100 mg/ml) of aqueous and alcoholic extracts, a control group treated with saline, and a standard group treated with albendazole (25 mg/ml). The primary outcomes measured were:

1. Paralysis time – the duration taken for the worms to become completely immobile.
2. Death time – the point at which the worms exhibited flaccidity, loss of color, and failed to revive in fresh saline.

The results were significant and revealed a clear dose-dependent relationship in both extracts. The alcoholic extract, at its highest concentration (100 mg/ml), induced paralysis and death in 2.12 minutes and 7.34 minutes, respectively. This performance was very close to that of albendazole, which induced paralysis and death in 2.36 minutes and 6.42 minutes, respectively. The aqueous extract, while less potent, still demonstrated effective anthelmintic activity, with the highest dose producing paralysis in 3.92 minutes and death in 13.72 minutes.

These findings suggest that the active compounds in *Ficus benghalensis*—particularly those soluble in alcohol—are capable of interfering with the neuromuscular and metabolic systems

of parasitic worms. The alkaloids likely interfere with neural transmission, while flavonoids and terpenoids may disrupt membrane integrity and enzyme activity. The saponins and tannins in the aqueous extract likely damage the parasite's outer membrane and interfere with nutrient absorption, leading to progressive loss of function and death.

The study successfully demonstrated that *Ficus benghalensis* leaf extracts possess reliable anthelmintic activity and that their effects are comparable to those of a standard pharmaceutical drug. The results validate the traditional use of this plant in herbal medicine and establish it as a promising candidate for the development of natural anthelmintic formulations.

Importantly, this research contributes to the broader fields of pharmacognosy, phytomedicine, and ethnopharmacology by reinforcing the scientific value of traditional knowledge. It also encourages further pharmacological investigations, including:

- Isolation and structural elucidation of the active phytoconstituents.
- Toxicological profiling to assess safety in animal and human models.
- In vivo efficacy studies to validate the in vitro results in a systemic context.

In conclusion, this study not only affirms the anthelmintic potential of *Ficus benghalensis* leaf extracts but also opens a path toward the development of cost-effective, plant-based deworming agents, particularly for populations in resource-limited settings. It underscores the critical role of biodiversity and traditional medicine in modern drug discovery and lays the foundation for further explorations into the therapeutic versatility of this revered botanical species.

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