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Phytochemical Profiling and Antimicrobial Efficacy of Ficus racemosa Linn.: A Systematic Investigation Against Waterborne Pathogens

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ABSTRACT

Background: Waterborne diseases constitute a significant global health challenge, particularly in developing nations where microbial contamination of water resources remains prevalent. The emergence of antimicrobial resistance has intensified the need for alternative therapeutic strategies derived from natural sources. Ficus racemosa Linn., a medicinally important plant from the Moraceae family, has been extensively utilized in traditional medicine systems for treating infectious conditions.

Objective: This investigation aimed to systematically evaluate the phytochemical composition and antimicrobial potential of Ficus racemosa extracts against selected waterborne pathogenic microorganisms and assess the plant's role in natural water purification.

Methods: Various plant parts (roots, bark, leaves, fruits) were extracted using solvents of increasing polarity. Preliminary phytochemical screening identified major secondary metabolite classes. Antimicrobial activity was evaluated using agar diffusion and broth dilution methods against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptomyces griseus), Gram-negative bacteria (Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Corynebacterium diphtheriae), and fungal pathogens (Candida albicans, Monilinia fruticola, Auricularia polytricha). Water samples from sites with and without Ficus racemosa root growth were analyzed for microbial contamination levels.

Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, and glycosides. Methanolic and ethanolic extracts demonstrated significant antimicrobial activity in a concentration-dependent manner. Extract I (methanolic) showed zones of inhibition ranging from 13.4 to 17.4 mm against Gram-positive bacteria at 100 μg/ml concentration. Against Gram-negative bacteria, inhibition zones ranged from 14.5 to 16.3 mm. Antifungal screening revealed substantial activity, with maximum inhibition observed against Auricularia polytricha (25 mm at 1000 μg/ml). Water samples containing Ficus racemosa roots exhibited a 99.7% reduction in total bacterial count (3.6×10^5 to 1.2×10^3 CFU/ml) with complete absence of coliforms.

Conclusion: Ficus racemosa demonstrates considerable antimicrobial potential against waterborne pathogens, supporting its traditional therapeutic applications. The substantial reduction in microbial contamination in water bodies containing the plant's root system suggests promising applications in natural water purification strategies. These findings warrant further investigation into isolation and characterization of specific bioactive compounds and their mechanisms of action. ..

Keywords: Ficus racemosa, phytochemicals, antimicrobial activity, waterborne pathogens, natural water purification, medicinal plants.

1. INTRODUCTION

Waterborne diseases represent one of the most pressing public health concerns globally, affecting approximately 2 billion people annually and contributing significantly to morbidity and mortality, particularly in resource-limited settings [1]. The World Health Organization estimates that contaminated water is responsible for over 485,000 diarrheal deaths each year [2]. In India alone, waterborne illnesses impact nearly 37.7 million individuals annually, resulting in substantial economic losses estimated at 600 million USD [3].

The escalating crisis of antimicrobial resistance has further complicated the management of infectious diseases. The World Health Organization has repeatedly emphasized that resistance to antimicrobial agents poses an existential threat to modern medicine, potentially ushering in a post-antibiotic era where common infections could become life-threatening [4,5]. This alarming scenario necessitates urgent exploration of alternative antimicrobial strategies, with medicinal plants emerging as promising candidates due to their chemical diversity and multi-targeted mechanisms of action [6,7].

Medicinal plants have served as the foundation of healthcare systems throughout human civilization, with approximately 80% of the global population relying on traditional medicine for primary healthcare needs [8]. The genus Ficus, comprising over 750 species within the family Moraceae, has attracted considerable scientific attention due to extensive ethnomedicinal applications and rich phytochemical composition [9,10]. Among these species, Ficus racemosa Linn. (commonly known as Gular or Audumbar) occupies a prominent position in Ayurvedic medicine, where it is classified as a rasayana—a category of therapeutic agents believed to promote longevity and overall well-being [11,12].

Ficus racemosa is a medium to large deciduous tree widely distributed across the Indian subcontinent and tropical regions, characteristically found along riverbanks and marshy environments [13]. Traditional medicine systems have utilized various parts of this plant—including bark, leaves, fruits, roots, and latex—for treating gastrointestinal disorders, inflammatory conditions, hemorrhagic diseases, and microbial infections [14,15]. Despite its widespread traditional use, comprehensive scientific validation of its antimicrobial potential against waterborne pathogens remains limited.

1.1 Phytochemicals and Their Biological Significance

Phytochemicals, or secondary metabolites, represent biologically active compounds synthesized by plants through complex biosynthetic pathways [16]. These compounds play crucial roles in plant defense mechanisms and exhibit diverse pharmacological activities in mammalian systems [17]. Major phytochemical classes include alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds, each demonstrating distinct therapeutic properties [18,19].

Unlike synthetic antimicrobial agents that typically target singular molecular sites, plant-derived compounds often exhibit multi-targeted mechanisms, interfering with multiple cellular processes in microorganisms [20]. These mechanisms include disruption of cell membrane integrity, inhibition of essential enzymes, interference with nucleic acid synthesis, and suppression of virulence factors [21,22]. The synergistic interactions among multiple phytochemicals present in crude extracts frequently enhance therapeutic efficacy while reducing the likelihood of resistance development [23,24].

1.2 Antimicrobial Resistance: A Growing Concern

Antimicrobial resistance has emerged as a critical global health challenge, with resistant infections responsible for millions of deaths annually worldwide [25]. The development of resistance occurs through genetic mutations and horizontal gene transfer, processes accelerated by inappropriate antimicrobial use, incomplete treatment regimens, and widespread application in agriculture and animal husbandry [26,27]. In India, the high prevalence of multidrug-resistant pathogens, including methicillin-resistant Staphylococcus aureus and extended-spectrum beta-lactamase producing Enterobacteriaceae, poses significant clinical challenges [28,29].

The limited development of novel antibiotics in recent decades, combined with stringent regulatory requirements and reduced financial incentives, has resulted in a declining antimicrobial pipeline [30]. This situation underscores the urgent need for exploring alternative sources of antimicrobial agents, particularly those with novel mechanisms of action that can circumvent existing resistance mechanisms [31,32].

1.3 Rationale and Objectives

The present investigation was undertaken to systematically evaluate the phytochemical composition and antimicrobial efficacy of Ficus racemosa against selected waterborne pathogens. Additionally, this study assessed the plant's potential role in natural water purification by examining microbial contamination levels in water bodies with and without Ficus racemosa root growth. The specific objectives included:

Comprehensive phytochemical screening of various Ficus racemosa plant parts using different solvent systems

Evaluation of antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungal pathogens

Determination of minimum inhibitory concentrations and concentration-dependent effects

Assessment of microbial contamination reduction in water samples containing plant roots

Correlation of phytochemical constituents with observed antimicrobial activities

2. LITERATURE REVIEW

Extensive research on the genus Ficus has demonstrated significant pharmacological potential across multiple species. Preliminary investigations by Mandal et al. (2000) established the antibacterial efficacy of Ficus racemosa leaf extracts, with petroleum ether extracts showing superior activity comparable to chloramphenicol against both Gram-positive and Gram-negative bacteria [33]. This foundational study highlighted the importance of solvent selection in extracting bioactive antimicrobial constituents.

Li et al. (2003) demonstrated cyclooxygenase-1 inhibitory activity of Ficus racemosa bark extracts with an IC_{50} value of 100 μg/ml, supporting traditional applications in inflammatory conditions [34]. Although primarily focused on anti-inflammatory mechanisms, this finding is relevant to antimicrobial research as inflammation and infection frequently coexist in pathological states. Khan et al. (2004) investigated chemopreventive and antioxidant properties in renal carcinogenesis models, revealing significant reductions in oxidative stress markers and restoration of antioxidant enzyme levels [35].

Comparative studies within the genus Ficus by Kuete et al. (2008) examined methanolic extracts from Ficus chlamydocarpa and Ficus cordata, demonstrating broad-spectrum antimicrobial activity against fungi and both Gram-positive and Gram-negative bacteria [36]. Shaikh et al. (2010) reported strong antibacterial activity of hydro-alcoholic leaf extracts against Actinomyces viscosus with a minimum inhibitory concentration of 0.08 mg/ml, suggesting potential dental therapeutic applications [37].

Krishna Murti et al. (2011) conducted comparative analyses of Ficus racemosa and Ficus benghalensis root extracts, demonstrating superior antibacterial activity of Ficus racemosa ethanolic extracts against Staphylococcus aureus [38]. Concurrent investigations by Salman Bin Hossain et al. (2011) established correlations between antioxidant capacity and antimicrobial efficacy using DPPH assays and cytotoxicity testing [39].

Recent advances have incorporated modern analytical techniques and nanotechnology approaches. Mahesh et al. (2021) employed LC-MS analysis to confirm bioactive phytochemicals in aqueous, chloroform, methanol, and acetone extracts, with aqueous extracts demonstrating highest antibacterial activity against E. coli [40]. Maurya et al. (2021) explored green synthesis of silver nanoparticles using Ficus racemosa, achieving enhanced antibacterial efficacy and highlighting modern applications in nanomedicine [41]. Pantarak et al. (2023) demonstrated dose-dependent antibacterial activity of methanolic leaf extracts against Staphylococcus aureus, reinforcing potential applications against resistant pathogens [42].

3. MATERIALS AND METHODS

3.1 Plant Material Collection and Authentication

Fresh plant material of Ficus racemosa was collected from riverbanks in the Warud region during the appropriate harvesting season. Various plant parts including roots, bark, leaves, and fruits were gathered, cleaned thoroughly with distilled water, and subjected to shade drying for 15-20 days. Botanical authentication was performed by qualified taxonomists, and voucher specimens were preserved for future reference.

3.2 Extraction Procedure

Dried plant materials were pulverized into fine powder using a mechanical grinder and passed through appropriate mesh sieves. Successive solvent extraction was performed using petroleum ether, chloroform, ethyl acetate, methanol, and aqueous solvents in order of increasing polarity. Each extraction employed a plant material to solvent ratio of 1:10 (w/v) with continuous stirring for 48-72 hours at room temperature. Extracts were filtered through Whatman filter paper No. 1, concentrated using rotary evaporator under reduced pressure, and stored at 4°C until further analysis.

3.3 Phytochemical Screening

Preliminary qualitative phytochemical analysis was conducted using standard protocols to identify major secondary metabolite classes. Tests included Mayer's and Dragendorff's reagents for alkaloids, Shinoda test for flavonoids, ferric chloride test for phenolics and tannins, foam test for saponins, Liebermann-Burchard test for terpenoids and steroids, and Molisch's test for glycosides. Results were recorded based on characteristic color changes and precipitate formation.

3.4 Antimicrobial Activity Evaluation

Antimicrobial screening was performed using the agar well diffusion method against selected bacterial and fungal strains obtained from recognized microbial culture collections. Test organisms included Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptomyces griseus), Gram-negative bacteria (Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Corynebacterium diphtheriae), and fungal pathogens (Candida albicans, Monilinia fruticola, Auricularia polytricha).

Mueller-Hinton agar was used for bacterial cultures and Sabouraud Dextrose agar for fungal cultures. Microbial suspensions were standardized to 1.5×10^8 CFU/ml (0.5 McFarland standard) and uniformly spread using sterile cotton swabs. Wells of 7 mm diameter were created using sterile cork borers, and varying concentrations of test extracts (50, 100, 500, 1000 μg/ml) were introduced. Standard antimicrobial agents (Chloramphenicol for bacteria, Amphotericin-B for fungi) served as positive controls. Plates were incubated at 37°C for 24 hours (bacteria) or 48-72 hours (fungi), and zones of inhibition were measured in millimeters.

3.5 Water Sample Analysis

Water samples were collected aseptically from selected sites in the Warud region, comprising locations with and without Ficus racemosa root growth. Samples were transported in sterile containers under refrigerated conditions and analyzed within 6 hours of collection. Microbiological analysis included determination of total bacterial count using standard plate count method and coliform detection using membrane filtration technique following standard protocols established by the American Public Health Association.

3.6 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was determined using appropriate statistical tests, with $p < 0.05$ considered statistically significant.

4. RESULTS

4.1 Phytochemical Composition

Preliminary phytochemical screening revealed the presence of multiple classes of bioactive secondary metabolites across different extracts. Methanolic and ethanolic extracts demonstrated the highest phytochemical diversity, containing alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, and glycosides. The distribution of phytochemicals varied according to solvent polarity and plant part analyzed.

Table 1: Phytochemical Distribution in Ficus racemosa Extracts

Phytochemical Class	Petroleum Ether	Chloroform	Methanol	Aqueous
Alkaloids	-	+	++	+
Flavonoids	-	+	+++	++
Tannins	-	+	+++	++
Terpenoids	++	++	+	-
Saponins	-	-	++	+
Glycosides	-	+	++	+

Legend: (-) Absent; (+) Weakly present; (++) Moderately present; (+++) Strongly present

4.2 Antibacterial Activity Against Gram-Positive Bacteria

Methanolic extracts (Extract I) demonstrated significant antibacterial activity against all tested Gram-positive bacteria in a concentration-dependent manner. At 100 µg/ml concentration, zones of inhibition ranged from 13.4 to 17.4 mm, with Staphylococcus epidermidis showing maximum susceptibility (17.4 mm), followed by Streptomyces griseus (15.4 mm), Bacillus subtilis (10.5 mm), and Staphylococcus aureus (13.4 mm). Ethanolic extracts (Extract II) exhibited moderate activity with inhibition zones ranging from 10.3 to 16.3 mm at the same concentration.

Table 2: Antibacterial Activity Against Gram-Positive Bacteria (Zone of Inhibition in mm)

Microorganism	Extract I (100 µg/ml)	Extract II (100 µg/ml)	Standard (10 µg/ml)
Staphylococcus aureus	13.4 ± 0.3	10.3 ± 0.2	17.3 ± 0.4
Bacillus subtilis	10.5 ± 0.3	10.4 ± 0.3	14.5 ± 0.5
Staphylococcus epidermidis	17.4 ± 0.3	16.3 ± 0.3	23.6 ± 0.4
Streptomyces griseus	15.4 ± 0.4	12.3 ± 0.3	22.3 ± 0.4

Values represent mean ± standard deviation of three independent experiments

4.3 Antibacterial Activity Against Gram-Negative Bacteria

Both methanolic and ethanolic extracts exhibited noteworthy antibacterial activity against Gram-negative bacteria. Extract I demonstrated inhibition zones ranging from 14.5 to 16.3 mm at 100 µg/ml concentration, with Escherichia coli showing maximum susceptibility (16.3 mm), followed by Salmonella typhimurium (15.3 mm), Proteus vulgaris (15.5 mm), and Corynebacterium diphtheriae (14.5 mm). Extract II showed slightly reduced but significant activity with zones ranging from 12.5 to 15.2 mm.

Table 3: Antibacterial Activity Against Gram-Negative Bacteria (Zone of Inhibition in mm)

Microorganism	Extract I (100 µg/ml)	Extract II (100 µg/ml)	Standard (10 µg/ml)
Proteus vulgaris	15.5 ± 0.3	13.2 ± 0.2	15.4 ± 0.5
Escherichia coli	16.3 ± 0.3	12.5 ± 0.3	21.4 ± 0.4
Corynebacterium diphtheriae	14.5 ± 0.4	15.2 ± 0.2	22.0 ± 0.4
Salmonella typhimurium	15.3 ± 0.2	10.5 ± 0.3	23.3 ± 0.4

Values represent mean ± standard deviation of three independent experiments

4.4 Antifungal Activity

Methanolic extracts demonstrated substantial antifungal activity against all tested fungal organisms in a concentration-dependent manner. At the highest concentration (1000 µg/ml), maximum inhibition was observed against Auricularia polytricha (25 mm), followed by Monilinia fruticola (21 mm) and Candida albicans (19 mm). Activity decreased progressively with reducing concentrations, with minimal or no activity observed at 125 µg/ml and below.

Table 4: Antifungal Activity of Methanolic Extract (Zone of Inhibition in mm)

Microorganism	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	Standard
Candida albicans	19	11	8	0	0	29
Monilinia fruticola	21	14	6	0	0	25
Auricularia polytricha	25	17	4	0	0	28

Standard: Amphotericin-B (10 µg/ml)

4.5 Water Purification Potential

Comparative microbiological analysis of water samples revealed remarkable differences between sites with and without Ficus racemosa root growth. Water samples from sites lacking plant roots exhibited total bacterial counts of 3.6×10^5 CFU/ml with presence of coliform bacteria, indicating significant microbial contamination. In stark contrast, water samples from sites containing Ficus racemosa roots showed dramatically reduced bacterial counts of 1.2×10^3 CFU/ml (a 99.7% reduction) with complete absence of coliforms.

Table 5: Impact of Ficus racemosa on Water Quality Parameters

Parameter	Without Plant Roots	With F. racemosa Roots
Total Bacterial Count (CFU/ml)	3.6×10^5	1.2×10^3
Coliform Count	Present	Absent
Contamination Level	High	Significantly Reduced

Parameter	Without Plant Roots	With F. racemosa Roots
Microbial Reduction (%)	-	99.7%

Data represents average values from five sampling sites

5. DISCUSSION

The present investigation demonstrates significant antimicrobial potential of Ficus racemosa extracts against both bacterial and fungal pathogens commonly associated with waterborne diseases. The comprehensive phytochemical screening revealed the presence of multiple bioactive compound classes, including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, and glycosides, consistent with previous reports on the genus Ficus [9,10,13]. The concentration and distribution of these phytochemicals varied according to solvent polarity, with methanolic and ethanolic extracts exhibiting the richest phytochemical diversity, supporting earlier findings by Jagtap et al. (2012) and Kingsley et al. (2014) [43,44].

The observed antimicrobial activity can be attributed to the synergistic action of these phytochemicals through multiple mechanisms. Flavonoids and phenolic compounds are known to disrupt microbial cell membranes and inhibit essential enzymes [20,21]. Tannins exert antimicrobial effects through protein precipitation and complexation with microbial enzymes [22]. Terpenoids interfere with membrane integrity and cellular metabolism [23,24]. The multi-targeted nature of these compounds reduces the likelihood of resistance development, a critical advantage over single-target synthetic antimicrobials [31,32].

The concentration-dependent antimicrobial activity observed in this study aligns with fundamental pharmacological principles and corroborates findings from previous investigations on Ficus species [33,36,37]. The methanolic extract (Extract I) consistently demonstrated superior antimicrobial efficacy compared to ethanolic extract (Extract II), suggesting that methanol more effectively extracts polar bioactive compounds responsible for antimicrobial activity. This observation is consistent with reports by Mandal et al. (2000) and Mahesh et al. (2021), who identified solvent selection as a critical factor in extracting antimicrobial constituents [33,40].

Notably, Gram-positive bacteria, particularly Staphylococcus epidermidis, exhibited higher susceptibility to the extracts compared to Gram-negative organisms. This differential susceptibility can be attributed to structural differences in bacterial cell walls. Gram-negative bacteria possess an additional outer membrane containing lipopolysaccharides, which provides enhanced resistance to antimicrobial agents [45,46]. However, the extracts still demonstrated significant activity against Gram-negative pathogens, including Escherichia coli and Salmonella typhimurium, which are major causative agents of waterborne diseases [1,2].

The antifungal activity demonstrated against Candida albicans, Monilinia fruticola, and Auricularia polytricha is particularly significant given the limited availability of effective antifungal agents and the emergence of azole-resistant fungal strains [47,48]. The maximum inhibition observed against Auricularia polytricha (25 mm at 1000 µg/ml) exceeds values reported in several previous studies, suggesting the presence of potent antifungal constituents in Ficus racemosa [39,41,42].

Perhaps the most remarkable finding of this investigation is the substantial reduction in microbial contamination (99.7%) observed in water bodies containing Ficus racemosa roots. This finding has significant implications for natural water purification strategies and aligns with the traditional practice of planting Ficus species along riverbanks [13,14]. The mechanisms underlying this purification effect likely involve multiple processes, including direct antimicrobial activity of root exudates, phytoremediation, and creation of unfavorable conditions for microbial proliferation through alterations in water chemistry and oxygenation [49,50].

The complete absence of coliforms in water containing plant roots is particularly significant from a public health perspective, as coliforms serve as indicators of fecal contamination and potential presence of pathogenic microorganisms [1,3]. This finding suggests potential applications of Ficus racemosa in constructed wetlands and natural water treatment systems, particularly in rural areas lacking access to conventional water treatment infrastructure [50,51].

While standard antimicrobial agents demonstrated higher zones of inhibition compared to plant extracts, this is expected given that antibiotics are purified compounds administered at optimized concentrations [52]. The plant extracts represent crude mixtures containing varying concentrations of bioactive compounds along with inactive constituents. Bioassay-guided fractionation and isolation of specific antimicrobial compounds would likely yield preparations with enhanced potency comparable to or exceeding standard antibiotics [53,54].

The present study has several limitations that warrant consideration. The investigation was confined to in vitro antimicrobial evaluation and did not address mechanisms of action, toxicity, or pharmacokinetic parameters. Future research should focus on isolation and characterization of specific bioactive compounds, elucidation of antimicrobial mechanisms, assessment of cytotoxicity and mutagenicity, evaluation of synergistic combinations with conventional antibiotics, and in vivo efficacy studies in appropriate animal models [55,56]. Additionally, long-term studies on the sustainability and environmental impact of using Ficus racemosa for water purification are needed.

6. CONCLUSION

This systematic investigation provides comprehensive evidence supporting the antimicrobial potential of Ficus racemosa against waterborne pathogens and validates its traditional therapeutic applications. The presence of diverse phytochemical constituents correlates directly with observed antimicrobial activities against Gram-positive bacteria, Gram-negative bacteria, and fungal pathogens. The concentration-dependent antimicrobial effects suggest potential for optimization through extraction methodology refinement and bioactive compound isolation.

The remarkable 99.7% reduction in microbial contamination observed in water bodies containing Ficus racemosa roots demonstrates promising applications in natural water purification strategies. This finding has particular significance for developing regions where conventional water treatment infrastructure is limited or absent. The complete elimination of coliform bacteria in treated water samples suggests effective control of fecal contamination and associated health risks.

In the context of escalating antimicrobial resistance and declining antibiotic development, Ficus racemosa emerges as a valuable natural resource warranting further investigation. The multi-targeted mechanisms of plant-derived antimicrobials offer potential advantages over single-target synthetic agents in combating resistant pathogens. Future research should prioritize isolation and characterization of specific bioactive compounds, elucidation of antimicrobial mechanisms, toxicological evaluation, and assessment of clinical efficacy.

The findings of this investigation contribute to the scientific validation of traditional medicinal knowledge and support the integration of evidence-based herbal medicine with modern healthcare systems. The dual applications of Ficus racemosa—as a source of antimicrobial compounds and as a natural water purification agent—highlight the multifaceted value of medicinal plants in addressing contemporary health and environmental challenges..

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