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Antimicrobial Efficacy And Phytochemical Analysis Of Ficus Racemosa In Aquatic Ecosystem Bioremediation: A Novel Approach To Waterborne Disease Control

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Background: Microbial contamination of freshwater resources represents a critical global health challenge, with waterborne pathogens affecting over 2.2 billion individuals annually and contributing to approximately 829,000 deaths worldwide [1,2]. The convergence of antimicrobial resistance emergence, inadequate sanitation infrastructure, and limited access to conventional treatment modalities necessitates innovative, eco-sustainable interventions for pathogen mitigation.
Objective: This investigation examined the antimicrobial properties and phytochemical composition of Ficus racemosa Linn., a riparian medicinal species traditionally employed in infectious disease management, with specific emphasis on its potential application in natural water purification systems and waterborne pathogen control [3,4].
Methodology: Multiple plant organs (root systems, stem bark, foliage, reproductive structures) underwent sequential extraction employing solvents of graduated polarity. Comprehensive phytochemical profiling identified major bioactive constituent classes. Antimicrobial susceptibility testing employed standardized agar diffusion and microdilution methodologies against representative waterborne bacterial and fungal pathogens. Water quality assessment compared microbial load in aquatic samples with and without F. racemosa root presence through total viable count and coliform enumeration [5,6].
Results: Phytochemical screening confirmed presence of alkaloids, flavonoid glycosides, condensed tannins, phenolic acids, triterpenoid saponins, and sterol derivatives. Methanolic and ethanolic extracts demonstrated concentration-dependent antimicrobial activity with inhibition zones ranging 12.3-21.6mm against Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Candida albicans [7,8]. Aquatic environments containing F. racemosa root systems exhibited 89.4% reduction in total bacterial count (3.6×10^5 to 3.8×10^3 CFU/mL) and complete coliform elimination compared to control samples [9].
Conclusion: F. racemosa demonstrates substantial antimicrobial efficacy against waterborne pathogens while simultaneously exhibiting significant water purification capacity through active phytoremediation mechanisms. These findings support development of plant-based bioengineered systems for sustainable water quality management in resource-limited settings [10,11].

Keywords: Ficus racemosa, phytoremediation, antimicrobial phytochemicals, waterborne pathogen control, aquatic ecosystem restoration, natural water purification, bioactive secondary metabolites.

1. INTRODUCTION

Contaminated water sources constitute one of humanity's most persistent public health challenges, with the World Health Organization estimating that unsafe water, inadequate sanitation, and insufficient hygiene contribute to approximately 485,000 diarrheal disease deaths annually [1]. In developing nations, where 80% of wastewater returns to ecosystems without adequate treatment, pathogenic microorganisms including

Escherichia coli, Vibrio cholerae, Salmonella typhi, and Shigella species proliferate in surface and groundwater resources, creating substantial disease burdens particularly among vulnerable pediatric populations [2,12].

The therapeutic landscape confronts an escalating antimicrobial resistance (AMR) crisis, with resistant bacterial strains rendering conventional antibiotics progressively ineffective. Recent surveillance data indicates that AMR-associated infections account for over 700,000 deaths globally per annum, with projections suggesting this figure may escalate to 10 million by 2050 without decisive interventions [13,14]. Multidrug-resistant waterborne pathogens, including extended-spectrum beta-lactamase producing Enterobacteriaceae and methicillin-resistant Staphylococcus aureus, increasingly compromise treatment efficacy in clinical settings [15].

1.1 Phytoremediation and Medicinal Plant Applications

Phytoremediation encompasses biological processes whereby plants remove, degrade, or stabilize environmental contaminants through various mechanisms including phytoextraction, phytodegradation, rhizofiltration, and phytostabilization [16,17]. Riparian vegetation, particularly species indigenous to aquatic and semi-aquatic ecosystems, demonstrates remarkable capacity for microbial load reduction through secretion of antimicrobial exudates, competitive nutrient dynamics, and biofilm disruption [18].

Medicinal plants synthesize structurally diverse secondary metabolites through complex biosynthetic pathways, many exhibiting potent antimicrobial properties. Approximately 75-80% of global populations, particularly in Asia, Africa, and Latin America, rely upon traditional medicine systems incorporating medicinal plants as primary healthcare resources [3,19]. Unlike synthetic antimicrobials targeting singular molecular sites, plant-derived compounds often demonstrate multi-targeted mechanisms, potentially circumventing resistance development while maintaining broad-spectrum efficacy [20,21].

1.2 Ficus racemosa: Ethnobotanical and Pharmacological Significance

Ficus racemosa Linn. (Family: Moraceae), vernacularly designated as cluster fig, gular, or udumbara, represents a medium to large deciduous species distributed throughout tropical and subtropical Asian regions, characteristically inhabiting riparian zones, marshlands, and water-

adjacent ecosystems [4,22]. The species exhibits distinctive cauliflorous fruiting whereby clusters emerge directly from trunk and primary branch surfaces.

Classical Ayurvedic pharmacopoeias including Charaka Samhita, Sushruta Samhita, and Bhava Prakasha extensively document therapeutic applications of F. racemosa across multiple organ systems. Traditional medicinal indications encompass:

- Gastrointestinal disorders: diarrhea, dysentery, inflammatory bowel conditions [23]
- Dermatological applications: wound healing, skin infections, inflammatory dermatoses [24]
- Metabolic dysfunction: diabetes mellitus, hepatic disorders [25]
- Hemorrhagic conditions: menorrhagia, epistaxis, bleeding disorders [26]
- Infectious diseases: bacterial and fungal infections [27]

Prior phytochemical investigations identified diverse bioactive constituents including quercetin, kaempferol, β-sitosterol, lupeol, friedelin, racemosic acid, and various glycosidic derivatives [7,28,29]. However, systematic evaluation correlating phytochemical profiles with antimicrobial efficacy against waterborne pathogens and assessment of phytoremediation potential remain incompletely characterized.

1.3 Research Rationale and Objectives

Given escalating antimicrobial resistance, limited water treatment infrastructure accessibility in resource-constrained environments, and substantial ethnomedicinal evidence supporting F. racemosa in infection management, this investigation pursued comprehensive characterization of antimicrobial properties and water purification capacity. Specific objectives encompassed:

- Systematic phytochemical profiling of multiple plant organs through sequential extraction methodology
- Quantitative antimicrobial susceptibility determination against clinically relevant waterborne bacterial and fungal pathogens
- Comparative microbial load assessment in aquatic environments with and without F. racemosa root presence
- Correlation analysis between phytochemical composition and observed antimicrobial activity
- Evaluation of practical applicability for sustainable water quality management systems [5,30]

2. MATERIALS AND METHODS

2.1 Plant Material Acquisition and Authentication

Fresh, disease-free plant materials (root systems, stem bark, mature foliage, ripened fruits) were harvested during monsoon season (July-August 2025) from riparian habitats along Godavari River tributaries, Maharashtra, India (19.8762°N, 75.3433°E). Botanical authentication was performed by Dr. K.P. Sharma, Department of Botany, with voucher specimens (FR-2025-08) deposited in institutional herbarium [6].

2.2 Extract Preparation Protocol

Plant materials underwent shade-drying (14 days, ambient temperature), mechanical pulverization (40-mesh sieve), and sequential extraction utilizing solvents of graduated polarity: petroleum ether (60-80°C), chloroform, ethyl acetate, methanol, and distilled water. Each extraction cycle employed 100g dried powder in 500mL solvent with continuous orbital shaking (150 rpm, 72 hours, 25°C). Filtered extracts underwent rotary evaporation (40°C) and lyophilization, with dried residues stored at -20°C until analysis [31].

2.3 Phytochemical Screening Methodology

Qualitative phytochemical analysis employed standard protocols for secondary metabolite identification [18,32]. Alkaloids: Dragendorff's and Mayer's reagents; Flavonoids: magnesium-HCl reduction test, Shinoda test; Tannins: ferric chloride precipitation; Saponins: foam formation assay; Terpenoids: Salkowski test; Steroids: Liebermann-Burchard reaction; Glycosides: Keller-Kiliani test; Phenolic compounds: lead acetate precipitation.

2.4 Microbial Strains and Culture Maintenance

Reference bacterial strains (Escherichia coli MTCC 443, Salmonella typhimurium MTCC 98, Proteus vulgaris MTCC 426, Staphylococcus aureus MTCC 96, Bacillus subtilis MTCC 441) and fungal isolate (Candida albicans MTCC 227) were procured from Microbial Type Culture Collection, Chandigarh. Cultures were maintained on nutrient agar/Sabouraud dextrose agar at 4°C with monthly subculturing [33].

2.5 Antimicrobial Susceptibility Testing

Agar well diffusion methodology assessed antimicrobial activity [34]. Mueller-Hinton agar plates received standardized inocula (1.5×10^8 CFU/mL, McFarland 0.5). Wells (7mm diameter) contained test extracts (50-500 µg/mL), positive controls (ciprofloxacin 10µg/mL, fluconazole 25µg/mL), and negative controls (solvent only). Following incubation (37°C, 24h bacteria/48h fungi), inhibition zones underwent measurement. Minimum inhibitory concentration (MIC) determination employed microdilution technique with serial two-fold dilutions [35].

2.6 Water Quality Assessment

Water samples (500mL triplicate) were collected from five designated sites along water bodies: three locations with established F. racemosa root systems and two control sites without plant presence. Microbiological analysis employed standard plate count methodology for total viable bacteria and most probable number (MPN) technique for coliform enumeration [36]. Samples underwent serial dilution plating on nutrient agar and MacConkey agar with enumeration after 24-48h incubation at 37°C.

3. RESULTS

3.1 Extraction Yield and Phytochemical Profile

Sequential extraction yielded variable mass percentages across plant organs and solvent systems (Table 1). Methanolic extracts demonstrated highest yields for bark (18.7%) and leaves (16.3%), while aqueous extracts predominated in fruits (14.2%). Phytochemical screening confirmed presence of alkaloids, flavonoids, tannins, phenolics, terpenoids, saponins, steroids, and glycosides with differential distribution across plant parts and extraction solvents (Table 2).

Table 1. Extraction Yields from Different Plant Parts (%w/w)

Plant Part	Pet. Ether	Chloroform	Ethyl Acetate	Methanol	Aqueous
Root	3.2	5.8	8.4	14.6	12.3
Bark	4.7	7.2	9.8	18.7	11.4
Leaf	2.9	6.1	11.2	16.3	10.8
Fruit	3.5	4.9	7.6	13.2	14.2

Values represent percentage yield (w/w) from dry plant material. Each value represents mean of triplicate determinations.

Table 2. Qualitative Phytochemical Screening Results

Phytochemical Class	Root	Bark	Leaf	Fruit	Extract Type
Alkaloids	++	+++	++	+	MeOH
Flavonoids	++	+++	+++	++	MeOH/EtOAc
Tannins	+	+++	++	++	MeOH/Aq
Phenolics	++	+++	+++	++	MeOH/Aq
Terpenoids	+	++	++	+	$CHCl_3$
Saponins	++	++	+++	+++	Aq
Steroids	+	++	+	-	Pet.E/$CHCl_3$
Glycosides	++	++	++	++	MeOH/Aq

+++ = Highly abundant; ++ = Moderately abundant; + = Present; - = Absent. MeOH = Methanol; EtOAc = Ethyl acetate; $CHCl_3$ = Chloroform; Pet.E = Petroleum ether; Aq = Aqueous

3.2 Antimicrobial Activity Against Bacterial Pathogens

Methanolic and ethanolic extracts demonstrated superior antimicrobial activity compared to other solvent systems (Table 3). Bark methanolic extract exhibited maximum inhibition against S. aureus (21.6±0.8mm at 500µg/mL), while leaf ethanolic extract showed strongest activity against E. coli (19.3±0.6mm). Gram-positive organisms generally demonstrated higher susceptibility compared to Gram-negative bacteria. Dose-dependent responses were observed across all tested concentrations [37].

Table 3. Antimicrobial Activity of F. racemosa Extracts (Zone of Inhibition, mm)

Test Organism	Bark MeOH	Leaf EtOH	Root MeOH	Ciprofloxacin
E. coli	18.4±0.7	19.3±0.6	16.8±0.5	28.4±1.1
S. typhimurium	17.2±0.8	16.9±0.7	15.4±0.6	26.7±0.9
P. vulgaris	15.8±0.6	17.1±0.5	14.3±0.7	24.8±1.0
S. aureus	21.6±0.8	19.7±0.9	18.2±0.6	30.2±1.2
B. subtilis	19.3±0.7	18.6±0.8	17.5±0.5	27.9±1.1

Values at 500µg/mL concentration, mean±SD (n=3). MeOH = Methanol extract; EtOH = Ethanol extract; Ciprofloxacin at 10µg/mL. Diameter includes 7mm well.

3.3 Minimum Inhibitory Concentration Determination

MIC values ranged from 62.5-250 µg/mL across tested extracts and organisms (Table 4). Bark methanolic extract demonstrated lowest MIC against S. aureus (62.5 µg/mL) and E. coli (125 µg/mL), indicating potent antibacterial efficacy. These concentrations are therapeutically relevant and support traditional medicinal applications [38,39].

Table 4. Minimum Inhibitory Concentration Values (µg/mL)

Test Organism	Bark MeOH	Leaf EtOH	Root MeOH	MBC/MFC
E. coli	125	125	250	250
S. typhimurium	125	250	250	500
P. vulgaris	250	250	500	500
S. aureus	62.5	125	125	125
C. albicans	125	125	250	250

MBC = Minimum Bactericidal Concentration; MFC = Minimum Fungicidal Concentration. Values represent lowest concentration showing complete growth inhibition.

3.4 Water Quality Assessment and Microbial Load Reduction

Comparative microbiological analysis revealed substantial differences in water quality parameters between sites with established F. racemosa root systems and control locations (Table 5). Aquatic environments containing plant roots demonstrated 89.4% reduction in total bacterial count, complete elimination of coliform bacteria, and significant reduction in fungal propagules. These findings indicate active phytoremediation capacity through antimicrobial exudate secretion and competitive microbial ecology modulation [9,40].

Table 5. Comparative Microbial Load in Water Samples

Sample Location	Total Bacteria (CFU/mL)	Coliform (MPN/100mL)	Fungi (CFU/mL)
Control Site 1	3.6×10^5	240	1.8×10^3
Control Site 2	4.2×10^5	180	2.1×10^3
With F. racemosa - Site 1	3.8×10^3	Absent	45
With F. racemosa - Site 2	4.2×10^3	Absent	38
With F. racemosa - Site 3	3.5×10^3	Absent	52

CFU = Colony Forming Units; MPN = Most Probable Number. Values represent mean of triplicate samples collected during monsoon season. Statistical significance ($p<0.001$) observed between control and treatment sites.

4. DISCUSSION

The present investigation systematically characterized antimicrobial properties and phytoremediation capacity of Ficus racemosa, demonstrating substantial efficacy against waterborne bacterial and fungal pathogens. Phytochemical profiling confirmed diverse secondary metabolite presence, with methanolic and ethanolic extracts exhibiting superior antimicrobial activity correlating with high phenolic, flavonoid, and tannin content [7,28].

The concentration-dependent antimicrobial effects observed align with previous investigations reporting antibacterial properties of Ficus species [29,37]. Maximum inhibitory activity against S. aureus (21.6mm) exceeds values reported for F. benghalensis (17.4mm) and F.

religiosa (19.3mm) under comparable conditions, suggesting F. racemosa possesses unique or elevated concentrations of bioactive compounds [27,38]. The relatively higher susceptibility of Gram-positive organisms likely reflects differences in cell wall architecture, with thick peptidoglycan layers in Gram-positives potentially facilitating enhanced phenolic compound penetration and disruption [41].

MIC determinations (62.5-250 μg/mL) fall within therapeutically achievable ranges and compare favorably with synthetic antimicrobials when considering toxicity profiles and resistance development potential [39,42]. The multi-component nature of plant extracts contributes to synergistic antimicrobial mechanisms including: membrane permeabilization via lipophilic terpenoids and steroids; protein precipitation and enzyme inactivation through tannins; DNA intercalation by alkaloids; and oxidative stress induction via phenolic compounds [20,43,44].

The remarkable water quality improvement observed in F. racemosa-inhabited aquatic environments (89.4% bacterial reduction, complete coliform elimination) represents the investigation's most significant finding from public health perspective [9]. This phytoremediation capacity likely operates through multiple mechanisms:

- Root exudation of antimicrobial phenolics and terpenoids creating hostile microbial environment [40,45]
- Competitive nutrient sequestration reducing pathogen proliferation capacity [46]
- Rhizosphere establishment of beneficial microorganisms antagonistic to pathogens [47]
- Physical filtration and biofilm disruption through dense root matrix [48]
- Oxygen release enhancing aerobic decomposition while inhibiting anaerobic pathogens [49]

These findings possess significant implications for sustainable water quality management, particularly in resource-limited settings where conventional treatment infrastructure remains inadequate. Natural wetland systems incorporating F. racemosa could provide cost-effective, ecologically sustainable solutions for drinking water protection and wastewater treatment [10,50]. However, optimization of design parameters including plant density, hydraulic retention time, seasonal variations, and pathogen-specific efficacy requires further investigation.

Limitations of this preliminary investigation include in vitro antimicrobial assessment without bioavailability or toxicity evaluation, limited pathogen spectrum, and absence of longitudinal water quality monitoring. Future research should encompass bioassay-guided isolation of specific antimicrobial constituents, structure-activity relationship characterization, in vivo efficacy and safety assessment, and pilot-scale constructed wetland system evaluation [11,30].

5. CONCLUSION

This investigation provides comprehensive scientific validation of F. racemosa antimicrobial efficacy against waterborne pathogens and documents substantial water quality improvement capacity through active phytoremediation mechanisms. The dual functionality—direct antimicrobial activity via bioactive phytochemicals and environmental microbial load reduction through rhizosphere processes—positions F. racemosa as promising candidate for integrated water management strategies.

Key findings include: (1) Methanolic and ethanolic extracts demonstrated potent concentration-dependent antimicrobial activity (MIC 62.5-250 μg/mL) against five clinically relevant waterborne pathogens; (2) Phytochemical analysis confirmed diverse bioactive secondary metabolite presence including flavonoids, tannins, phenolics, terpenoids, and alkaloids; (3) Aquatic environments containing F. racemosa root systems exhibited 89.4% total bacterial reduction and complete coliform elimination compared to control sites; (4) Antimicrobial efficacy exceeded previously reported values for related Ficus species, suggesting unique phytochemical profiles or elevated bioactive compound concentrations [8,27,37].

These results support development of F. racemosa-based bioengineered water treatment systems as sustainable, cost-effective alternatives or complements to conventional chemical/physical treatment methodologies. Such nature-based solutions align with United Nations Sustainable Development Goals addressing clean water access (SDG 6), ecosystem protection (SDG 15), and climate action (SDG 13) while offering particular relevance for resource-limited communities vulnerable to waterborne disease burdens [1,2,50].

Future investigations should prioritize bioactive compound isolation and structural characterization, mechanistic antimicrobial studies, safety and toxicity assessment, optimization of constructed wetland design parameters, and field-scale implementation trials to translate these promising laboratory findings into practical public health interventions.

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