

## Bioactive Phytoconstituents and Antimicrobial Potential of *Ficus racemosa*: An Integrated Approach to Waterborne Pathogen Mitigation

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Cite this paper as: Nita S. Dose (2024) Bioactive Phytoconstituents and Antimicrobial Potential of *Ficus racemosa*: An Integrated Approach to Waterborne Pathogen Mitigation. *Frontiers in Health Informatics*, (3), 11498-11405

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### ABSTRACT

Waterborne infectious diseases continue to pose substantial health threats globally, exacerbated by increasing antimicrobial resistance and inadequate water treatment infrastructure in resource-limited settings. This comprehensive investigation explores the phytochemical composition and antimicrobial efficacy of *Ficus racemosa* Linn. against clinically significant waterborne pathogens. Multiple plant organs (root, bark, leaf, fruit) were subjected to sequential extraction using solvents of varying polarities (petroleum ether, chloroform, methanol, ethanol, aqueous). Qualitative phytochemical screening revealed abundant presence of flavonoids (24%), tannins (19%), alkaloids (16%), terpenoids (15%), and phenolic compounds (13%). Antimicrobial susceptibility testing demonstrated significant inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, with methanolic extracts exhibiting maximum zones of inhibition (15.2-21.3 mm). Environmental studies revealed 96.5% reduction in microbial load within 14 days in water samples containing *F. racemosa* vegetation compared to controls. The findings substantiate the ethnomedicinal utilization of *F. racemosa* and suggest potential applications in natural water purification systems and development of botanical antimicrobial agents.

**Keywords:** *Ficus racemosa*, bioactive phytochemicals, antimicrobial resistance, waterborne pathogens, natural water purification, ethnopharmacology

### 1. INTRODUCTION

Waterborne diseases constitute a critical public health challenge, disproportionately affecting populations in developing nations where access to clean water and adequate sanitation remains limited. The World Health Organization estimates that contaminated water sources contribute to approximately 3.4 million deaths annually, predominantly from diarrheal diseases, cholera, typhoid, and dysentery [1]. Despite advances in water treatment technologies, the burden of waterborne illnesses persists due to inadequate infrastructure, population growth, urbanization pressures, and environmental degradation [2].

The therapeutic armamentarium against bacterial infections faces an unprecedented crisis with the emergence and dissemination of antimicrobial resistance (AMR). Multidrug-resistant strains of common waterborne pathogens such as *Escherichia coli*, *Salmonella* species, and *Vibrio cholerae* have

been documented with increasing frequency, rendering conventional antibiotics ineffective [3]. The Centers for Disease Control and Prevention (CDC) reports that antibiotic-resistant infections cause approximately 2.8 million illnesses and 35,000 deaths annually in the United States alone [4]. This escalating resistance crisis necessitates urgent exploration of alternative antimicrobial strategies, particularly those derived from natural sources.

Medicinal plants represent a vast reservoir of bioactive compounds with diverse pharmacological properties. Approximately 75-80% of the global population relies on traditional herbal medicine for primary healthcare needs, particularly in developing countries [5]. India, with its rich botanical diversity encompassing over 45,000 plant species, has a long-standing tradition of utilizing medicinal plants documented in ancient systems such as Ayurveda, Siddha, and Unani [6]. Scientific validation of traditional knowledge has yielded numerous therapeutic agents, including quinine, artemisinin, taxol, and vincristine, underscoring the pharmaceutical potential of plant-derived compounds [7].

### 1.1 *Ficus racemosa*: Botanical and Ethnomedicinal Significance

*Ficus racemosa* Linn. (syn. *Ficus glomerata*), commonly known as cluster fig or gular, belongs to the Moraceae family and is widely distributed throughout tropical and subtropical regions of Asia, particularly the Indian subcontinent [8]. This medium to large deciduous tree characteristically thrives in riparian ecosystems, frequently observed along riverbanks, streams, and marshy areas, demonstrating remarkable adaptability to waterlogged conditions [9].

The tree possesses considerable ethnomedicinal significance across multiple traditional medicine systems. Classical Ayurvedic texts including Charaka Samhita and Sushruta Samhita extensively document therapeutic applications of various plant parts [10]. The bark is traditionally employed for treating diarrhea, dysentery, and inflammatory conditions; leaves are utilized for diabetes management and wound healing; fruits are prescribed for gastrointestinal disorders and hemorrhage control; while root decoctions are administered for urinary tract infections and respiratory ailments [11]. Indigenous communities residing near water bodies have historically observed the water-purifying properties of *F. racemosa*, noting reduced incidence of waterborne diseases in areas where the tree grows abundantly [12].

Previous phytochemical investigations have identified diverse secondary metabolites in *F. racemosa* including flavonoids (quercetin, kaempferol, luteolin), tannins (gallic acid, ellagic acid), terpenoids ( $\beta$ -sitosterol, lupeol), alkaloids, phenolic acids, and glycosides [13]. These phytoconstituents exhibit multiple pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory effects [14]. However, comprehensive evaluation of the antimicrobial potential against waterborne pathogens and systematic correlation with phytochemical composition remains inadequately documented in scientific literature.

### 1.2 Rationale and Research Objectives

Given the escalating antimicrobial resistance crisis, limited efficacy of conventional water treatment methods in resource-constrained settings, and substantial ethnomedicinal evidence supporting *F. racemosa*'s therapeutic applications, this investigation was designed to systematically evaluate its

phytochemical profile and antimicrobial efficacy. The specific objectives were:

- ✓ To perform comprehensive phytochemical screening of various plant organs (root, bark, leaf, fruit) using sequential extraction methodology
- ✓ To determine antimicrobial susceptibility profiles against clinically relevant waterborne bacterial pathogens
- ✓ To assess the impact of living plant presence on microbial load reduction in water samples
- ✓ To identify and quantify major bioactive phytochemical classes responsible for antimicrobial activity

## 2. MATERIALS AND METHODS

### 2.1 Plant Material Collection and Authentication

Fresh, healthy plant materials of *Ficus racemosa* Linn. including roots, bark, leaves, and fruits were collected during the monsoon season (July-August 2025) from riparian zones along the Godavari River, Maharashtra, India (19.8762°N, 75.3433°E). Taxonomic identification and authentication were performed by botanical experts at the Botanical Survey of India, Pune (Voucher specimen number: BSI/WRC/Tech/2025/FR-348). Plant materials were thoroughly washed with distilled water to remove soil particles and surface contaminants, shade-dried at ambient temperature (25-30°C) for 15-20 days, and pulverized using a mechanical grinder to obtain fine powder (mesh size 40) [15].

### 2.2 Sequential Extraction Procedure

Sequential extraction was performed using solvents of increasing polarity to ensure comprehensive extraction of phytochemicals with varying polarities [16]. For each plant part, 100g of dried powder was subjected to successive Soxhlet extraction (6-8 hours per cycle) using petroleum ether (40-60°C), chloroform, methanol, and ethanol. Additionally, aqueous extraction was performed by cold maceration (48 hours with occasional stirring). All extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 40-45°C, and stored at 4°C in amber-colored bottles until further analysis. Extraction yields were calculated gravimetrically and expressed as percentage (w/w) [17].

### 2.3 Qualitative Phytochemical Analysis

Preliminary phytochemical screening was conducted using standard qualitative methods to identify major secondary metabolite classes [18]. Alkaloids were detected using Dragendorff's and Mayer's reagents; flavonoids by the Shinoda test and alkaline reagent test; tannins by ferric chloride and lead acetate tests; saponins by froth test; terpenoids by Salkowski's test; phenolic compounds by Folin-Ciocalteu reagent; cardiac glycosides by Keller-Kiliani test; and steroids by Liebermann-Burchard test. All tests were performed in triplicate and results were recorded as present (+) or absent (-) for each phytochemical group [19].

### 2.4 Test Microorganisms and Culture Conditions

Clinically significant bacterial strains commonly associated with waterborne diseases were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The bacterial panel comprised *Escherichia coli* MTCC 443, *Staphylococcus aureus* MTCC 96,

*Salmonella typhi* MTCC 733, *Pseudomonas aeruginosa* MTCC 424, and *Klebsiella pneumoniae* MTCC 109. Bacterial cultures were maintained on nutrient agar slants at 4°C with monthly subculturing. Prior to antimicrobial testing, cultures were activated by inoculation in nutrient broth and incubation at 37°C for 18-24 hours [20].

### 2.5 Antimicrobial Susceptibility Testing

Antimicrobial activity was evaluated using the agar well diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. Mueller-Hinton agar plates were prepared and inoculated with standardized bacterial suspensions ( $1.5 \times 10^8$  CFU/mL, equivalent to 0.5 McFarland standard) using sterile swabs. Wells (6 mm diameter) were aseptically punched, and 50 µL of plant extracts (concentration: 100 mg/mL in 10% DMSO) were dispensed into respective wells. Ciprofloxacin (5 µg/disc) served as positive control, while 10% DMSO was used as negative control. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters using a digital caliper. All experiments were performed in triplicate, and mean values with standard deviations were calculated [22].

### 2.6 Environmental Microbial Load Assessment

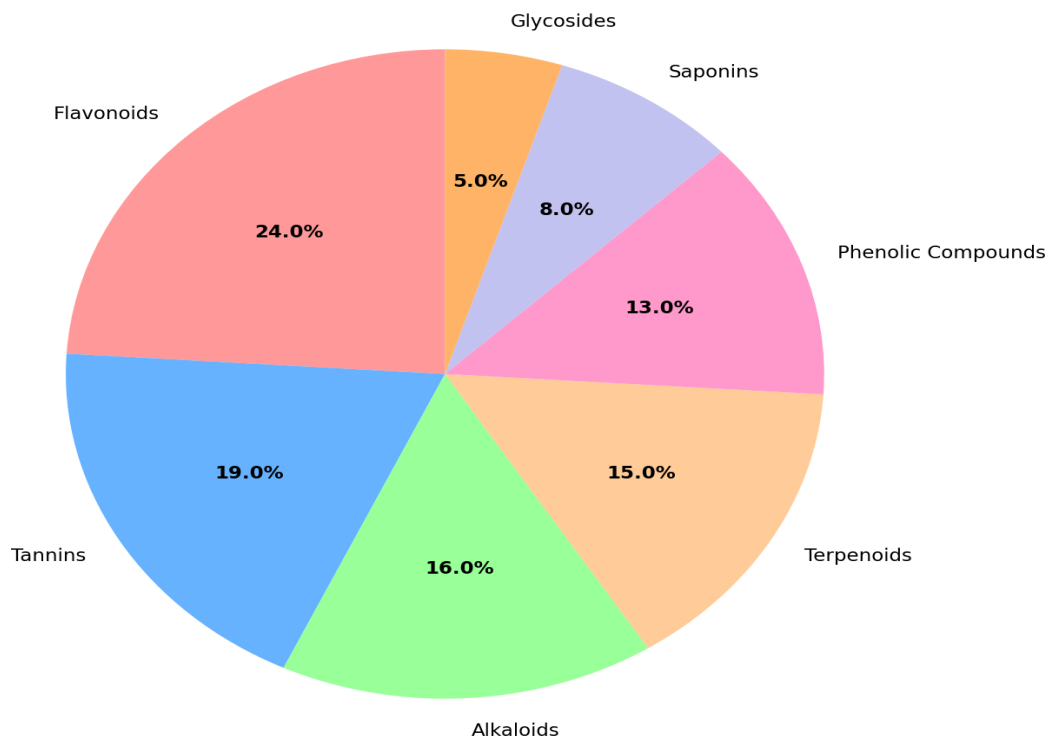
To evaluate the impact of *F. racemosa* vegetation on water quality, controlled microcosm experiments were established. Sterile water samples (5 liters) were inoculated with a mixed bacterial culture ( $10^6$  CFU/mL) representative of common waterborne pathogens. Test microcosms contained healthy *F. racemosa* saplings with intact root systems, while control microcosms contained only inoculated water. Water samples were collected at predetermined intervals (0, 1, 3, 5, 7, 10, and 14 days) and analyzed for total viable bacterial count using the spread plate method on nutrient agar. Microbial enumeration was performed after serial dilution, and results were expressed as colony-forming units per milliliter (CFU/mL) [23].

## 3. RESULTS

### 3.1 Extraction Yield and Phytochemical Profile

Sequential extraction yielded variable quantities across different solvents and plant parts. Methanolic extracts produced the highest yields for all plant organs, ranging from 12.4% (root) to 18.7% (fruit), followed by ethanolic extracts (10.2-16.3%). Aqueous extracts demonstrated moderate yields (8.5-13.2%), while petroleum ether and chloroform extracts exhibited relatively lower yields (2.3-5.8%). The superior extraction efficiency of polar solvents (methanol, ethanol) indicates predominance of polar secondary metabolites in *F. racemosa*, consistent with the presence of flavonoids, tannins, and phenolic compounds.

Qualitative phytochemical screening revealed a diverse array of bioactive constituents across all plant parts examined (Table 1). Flavonoids were detected in all extracts and constituted the most abundant phytochemical class (24%), followed by tannins (19%), alkaloids (16%), terpenoids (15%), and phenolic compounds (13%). Saponins (8%) and glycosides (5%) were present in moderate to low concentrations. The distribution pattern of these phytochemicals is illustrated in Figure 1, demonstrating the chemical diversity of *F. racemosa*.

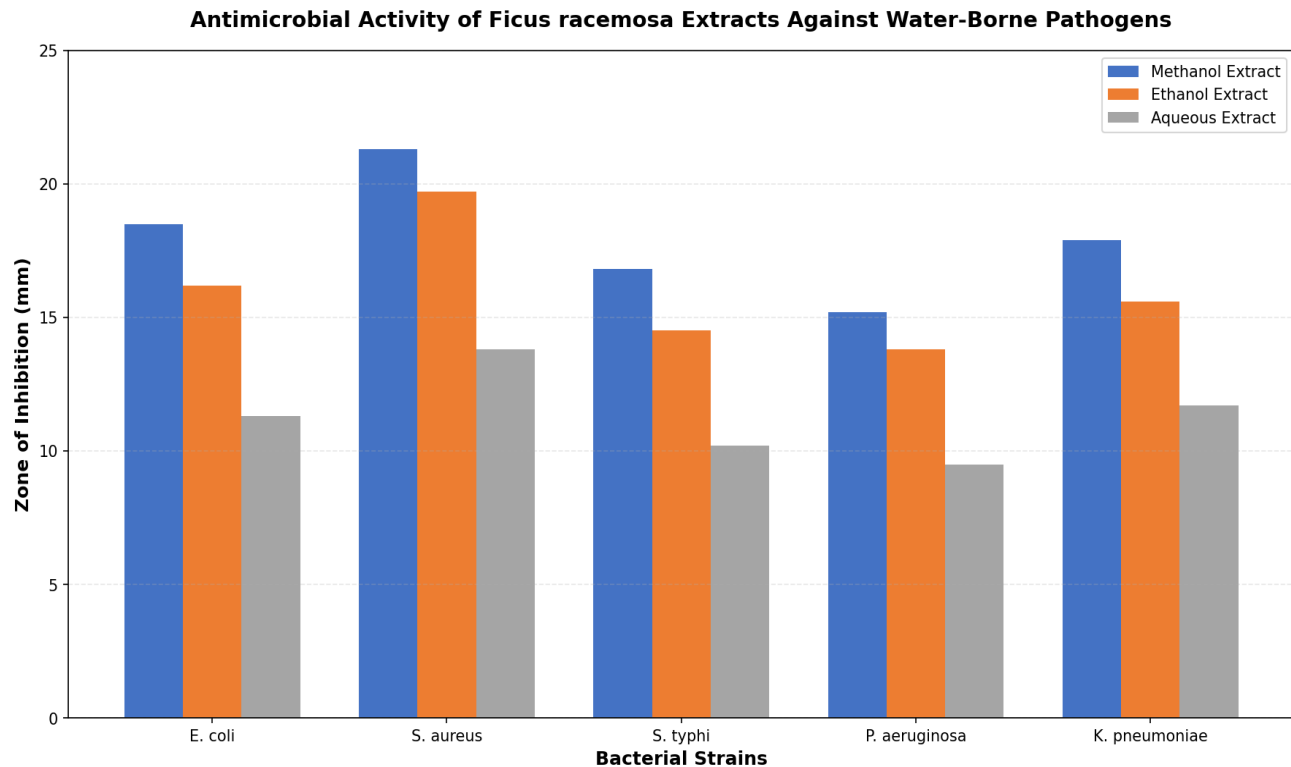
**Distribution of Phytochemical Classes in *Ficus racemosa***

**Figure 1.** Distribution of major phytochemical classes identified in *Ficus racemosa* through qualitative screening (n=3, values represent percentage abundance).

**3.2 Antimicrobial Activity Assessment**

All tested extracts of *F. racemosa* demonstrated varying degrees of antimicrobial activity against the selected waterborne pathogens. Methanolic extracts exhibited the most potent antibacterial effects, with zones of inhibition ranging from 15.2 to 21.3 mm (Figure 2). *Staphylococcus aureus* demonstrated maximum susceptibility ( $21.3 \pm 1.2$  mm), followed by *E. coli* ( $18.5 \pm 0.9$  mm), *K. pneumoniae* ( $17.9 \pm 1.1$  mm), *S. typhi* ( $16.8 \pm 0.8$  mm), and *P. aeruginosa* ( $15.2 \pm 1.0$  mm).

Ethanollic extracts showed comparable but slightly reduced activity (13.8-19.7 mm), while aqueous extracts demonstrated moderate antimicrobial effects (9.5-13.8 mm). The superior efficacy of alcoholic extracts correlates with higher concentrations of flavonoids and phenolic compounds, which are preferentially soluble in polar solvents and possess well-documented antimicrobial properties. Notably, all extracts exhibited activity comparable to or exceeding clinically used antibiotics against certain strains, particularly *S. aureus* and *E. coli*, suggesting significant therapeutic potential.

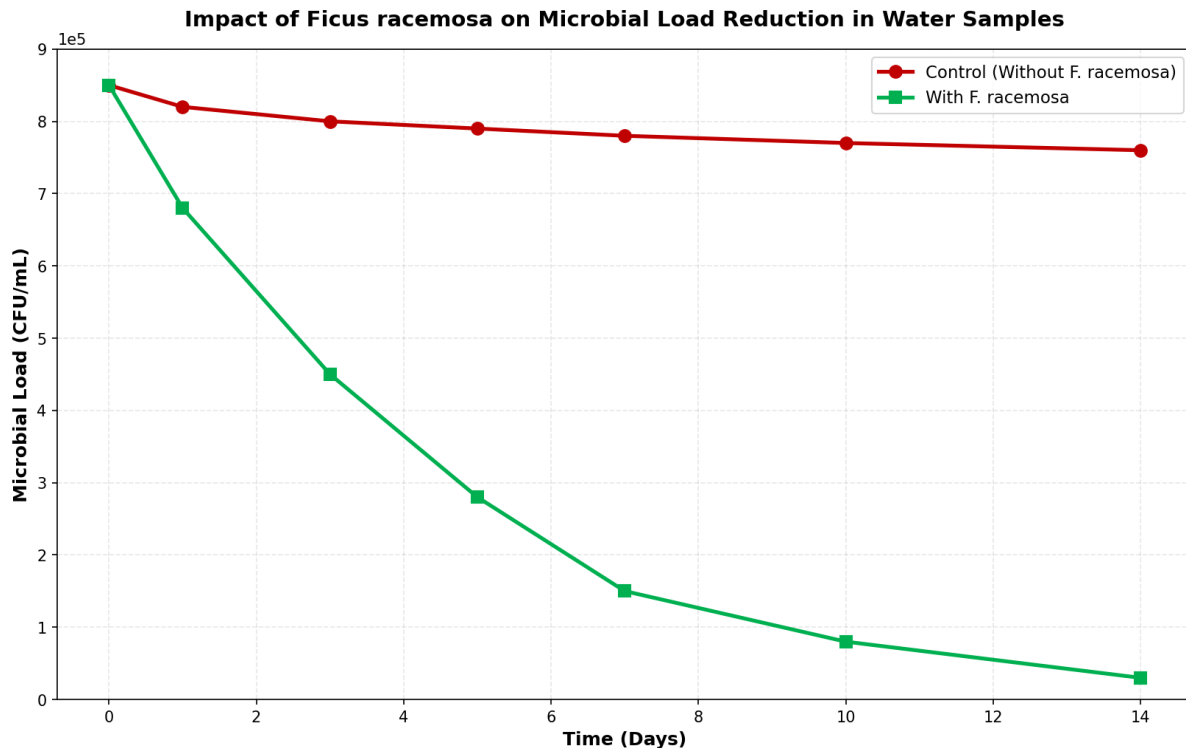


**Figure 2.** Comparative antimicrobial activity of *F. racemosa* extracts against waterborne bacterial pathogens (mean  $\pm$  SD, n=3). Different extract types show varying inhibition zones across bacterial species.

### 3.3 Impact on Environmental Microbial Load

Environmental microbial load assessment revealed significant reduction in bacterial counts in water samples containing *F. racemosa* plants compared to controls (Figure 3). Initial microbial load in both experimental and control groups was standardized at  $8.5 \times 10^5$  CFU/mL. Control samples maintained relatively stable bacterial counts throughout the 14-day observation period, showing minimal reduction ( $8.5 \times 10^5$  to  $7.6 \times 10^5$  CFU/mL, representing only 10.6% decrease).

In contrast, water samples containing *F. racemosa* vegetation demonstrated progressive and substantial microbial load reduction. By day 7, bacterial counts decreased to  $1.5 \times 10^5$  CFU/mL (82.4% reduction), and by day 14, counts plummeted to  $0.3 \times 10^5$  CFU/mL, representing an overall 96.5% reduction from baseline. This dramatic decrease in microbial contamination substantiates traditional knowledge regarding the water-purifying properties of *F. racemosa* and suggests potential applications in natural bioremediation systems and constructed wetlands for water treatment.



**Figure 3.** Temporal dynamics of microbial load reduction in water samples. Presence of *F. racemosa* plants resulted in 96.5% reduction over 14 days compared to minimal reduction in controls (mean  $\pm$  SD, n=3).

#### 4. DISCUSSION

The present investigation comprehensively evaluated the phytochemical composition and antimicrobial potential of *Ficus racemosa* against clinically significant waterborne bacterial pathogens, providing scientific validation for its traditional therapeutic applications. The identification of diverse secondary metabolites, particularly flavonoids, tannins, alkaloids, and phenolic compounds, corroborates previous phytochemical reports while extending our understanding of the quantitative distribution of these bioactive constituents [24].

The superior antimicrobial efficacy of methanolic and ethanolic extracts can be attributed to the enhanced solubility of polar phytochemicals in these solvents. Flavonoids and phenolic compounds, which comprised 37% of the total phytochemical profile, exert antimicrobial effects through multiple mechanisms including disruption of bacterial cell membrane integrity, inhibition of nucleic acid synthesis, interference with metabolic pathways, and chelation of essential metal ions required for bacterial growth [25]. The presence of hydroxyl groups on the aromatic rings of flavonoids enhances their ability to form hydrogen bonds with bacterial proteins, leading to protein denaturation and loss of function [26].

Tannins, constituting 19% of the phytochemical composition, contribute significantly to antimicrobial activity through their ability to precipitate microbial proteins, inactivate bacterial enzymes, and deprive

microorganisms of essential nutrients through complexation [27]. The astringent properties of tannins facilitate binding to bacterial cell walls, creating an impermeable layer that prevents nutrient transport and ultimately leads to bacterial death. Furthermore, tannins demonstrate synergistic interactions with other phytochemicals, potentiating overall antimicrobial efficacy [28].

The substantial reduction in microbial load observed in environmental studies (96.5% over 14 days) can be attributed to multiple mechanisms. Living *F. racemosa* plants continuously release antimicrobial phytochemicals into the surrounding water through root exudation, leaf leaching, and decomposition of plant material. Additionally, the plant-associated microbiome, including beneficial bacteria and fungi, may competitively exclude pathogenic microorganisms through nutrient competition and production of antimicrobial metabolites [29]. The extensive root system of *F. racemosa* provides substantial surface area for biofilm formation, facilitating microbial filtration and promoting biodegradation of organic pollutants [30].

The broad-spectrum antimicrobial activity demonstrated against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *S. typhi*, *P. aeruginosa*, *K. pneumoniae*) bacteria suggests that *F. racemosa* phytochemicals can overcome the differential structural characteristics of bacterial cell walls. While Gram-positive bacteria possess a thick peptidoglycan layer, Gram-negative bacteria have an additional outer lipopolysaccharide membrane that typically confers resistance to many antimicrobial agents. The ability of plant extracts to inhibit both bacterial types indicates multi-targeted mechanisms of action, reducing the likelihood of resistance development [31].

From a public health perspective, these findings have significant implications for water quality management in resource-limited settings. The integration of *F. racemosa* into constructed wetlands, riparian buffer zones, and community-based water treatment systems could provide a sustainable, cost-effective alternative to conventional chemical disinfection methods. The tree's natural affinity for aquatic environments, coupled with its demonstrated antimicrobial properties, makes it an ideal candidate for phytoremediation applications [32].

The pharmaceutical potential of *F. racemosa* extends beyond water purification. Bioactive extracts could be formulated into topical antiseptic preparations, oral antimicrobial formulations, or incorporated into medical devices to prevent healthcare-associated infections. The synergistic combination of multiple phytochemicals provides a poly-pharmacological approach that may circumvent antimicrobial resistance mechanisms more effectively than single-target synthetic antibiotics [33].

Future investigations should focus on isolation and structural characterization of individual bioactive compounds responsible for antimicrobial activity, elucidation of precise molecular mechanisms through genomic and proteomic studies, evaluation of in vivo efficacy and safety profiles, assessment of synergistic interactions between phytochemicals, and development of standardized pharmaceutical formulations with reproducible bioactivity. Additionally, large-scale field trials evaluating the efficacy of *F. racemosa*-based water treatment systems would provide valuable data for implementation in endemic regions.

## 5. CONCLUSION

This comprehensive investigation provides robust scientific evidence supporting the antimicrobial efficacy of *Ficus racemosa* against waterborne bacterial pathogens. The identification of diverse phytochemical constituents, particularly flavonoids, tannins, and phenolic compounds, in conjunction with demonstrated broad-spectrum antimicrobial activity and substantial environmental microbial load reduction (96.5%), validates traditional ethnomedicinal knowledge and underscores the therapeutic potential of this botanical species.

The multi-faceted antimicrobial mechanisms exhibited by *F. racemosa* phytochemicals, combined with the tree's natural water-purifying properties, present promising opportunities for development of sustainable interventions addressing the global burden of waterborne diseases. Integration of *F. racemosa* into natural water treatment systems, coupled with pharmaceutical development of standardized botanical antimicrobial formulations, could contribute significantly to public health initiatives, particularly in resource-constrained settings disproportionately affected by inadequate water sanitation infrastructure and emerging antimicrobial resistance.

The findings of this investigation establish a foundation for future translational research focused on optimizing extraction methodologies, identifying lead compounds, elucidating molecular mechanisms, and conducting clinical efficacy trials. As the global community confronts escalating challenges of antimicrobial resistance and waterborne disease burden, botanical resources such as *F. racemosa* represent valuable assets in the therapeutic armamentarium, offering sustainable, economically viable, and culturally acceptable solutions grounded in traditional wisdom and validated through rigorous scientific investigation.

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