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Antibacterial activity and nutritional profiling of selected Indian medicinal plants against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

The rapid emergence of antimicrobial resistance has intensified the search for alternative antibacterial agents from natural sources, particularly medicinal plants with a history of traditional use. The present study investigated the antibacterial activity of aqueous and ethanolic extracts of selected Indian medicinal plants, namely *Azadirachta indica*, *Ocimum tenuiflorum*, *Curcuma longa*, *Moringa oleifera*, and *Psidium guajava*, against two clinically important bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus*. In addition, the nutritional composition of these plant species was evaluated to assess their potential as functional food resources. Antibacterial activity was assessed using the agar well diffusion method, and minimum inhibitory concentration values were determined by the broth dilution technique. Ethanolic extracts exhibited significantly higher antibacterial activity than aqueous extracts against both bacterial strains ($p < 0.05$). Among the tested plants, ethanolic extracts of *Curcuma longa* and *Azadirachta indica* showed the largest zones of inhibition and the lowest minimum inhibitory concentration values, indicating strong antibacterial potency. Overall, *Staphylococcus aureus* was more susceptible to plant extracts than *Escherichia coli*.

Nutritional analysis revealed considerable variation among the studied plants. *Moringa oleifera* leaves were particularly rich in protein and essential minerals such as calcium and iron, while *Psidium guajava* leaves exhibited high crude fiber content. The combined antibacterial efficacy and nutritional richness of these plants support their traditional medicinal use and highlight their potential application in the development of plant-based antimicrobial agents and nutraceutical products. The findings provide a scientific basis for further studies aimed at isolating active compounds and validating their efficacy through in vivo investigations.

Keywords: Antibacterial activity; Medicinal plants; *Escherichia coli*; *Staphylococcus aureus*; Nutritional profiling

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1. INTRODUCTION

The global rise in antimicrobial resistance (AMR) has emerged as one of the most critical challenges to public health in the twenty-first century, threatening the effective prevention and treatment of infectious diseases worldwide (World Health Organization, 2020; Ventola, 2015). The widespread and often indiscriminate use of antibiotics in human medicine, agriculture, and animal husbandry has accelerated the evolution of resistant bacterial strains, rendering many conventional antibiotics increasingly ineffective (Davies & Davies, 2010;

Prestinaci et al., 2015). As a consequence, common infections caused by pathogenic bacteria such as Escherichia coli and Staphylococcus aureus are becoming more difficult to manage, leading to prolonged illness, increased healthcare costs, and higher mortality rates (Laxminarayan et al., 2013).

In response to this growing crisis, there has been renewed scientific interest in the exploration of natural products as alternative or complementary sources of antimicrobial agents (Newman & Cragg, 2020). Medicinal plants, in particular, have been used for centuries in traditional systems of medicine, including Ayurveda, Unani, and Siddha, to treat a wide range of infectious and non-infectious diseases (Fabricant & Farnsworth, 2001; Sofowora et al., 2013). The therapeutic potential of medicinal plants is largely attributed to their rich diversity of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and glycosides, many of which have been shown to possess potent antibacterial properties (Cowan, 1999; Gurib-Fakim, 2006). These bioactive compounds can inhibit bacterial growth through multiple mechanisms, including disruption of cell walls, inhibition of protein and nucleic acid synthesis, and interference with metabolic pathways, thereby reducing the likelihood of resistance development (Daglia, 2012; Borges et al., 2016).

India is recognized as one of the world's richest repositories of medicinal plant diversity, harboring more than 8,000 species with documented therapeutic applications (Kumar et al., 2017; National Medicinal Plants Board, 2019). Numerous Indian medicinal plants have been scientifically validated for their antimicrobial activity against a broad spectrum of Gram-positive and Gram-negative bacteria (Savoia, 2012; Gupta & Birdi, 2017). Among bacterial pathogens, Escherichia coli and Staphylococcus aureus are of particular clinical significance due to their widespread involvement in gastrointestinal infections, urinary tract infections, wound and skin infections, septicemia, and food-borne diseases (Tong et al., 2015; Kaper et al., 2004). The increasing resistance of these organisms to commonly used antibiotics has intensified the need to identify plant-derived antibacterial agents capable of inhibiting their growth effectively (Nikaido, 2009; Hemaiswarya et al., 2008).

Beyond their medicinal applications, many plants traditionally used for therapeutic purposes also serve as important sources of essential nutrients, contributing to human nutrition and overall health (Johns, 1999; Odhav et al., 2007). Leaves, roots, and rhizomes of several medicinal plants are rich in proteins, carbohydrates, dietary fiber, lipids, vitamins, and mineral elements such as calcium, iron, and potassium (Gopalakrishnan et al., 2016; Uusiku et al., 2010). The concept of "food as medicine" has gained increasing scientific attention, emphasizing the role of nutritionally dense, plant-based diets in disease prevention, immune modulation, and health promotion (Kris-Etherton et al., 2002; Willett et al., 2019). Plants that combine antimicrobial activity with high nutritional value are particularly attractive as functional foods and nutraceuticals, offering dual benefits in combating infections and improving nutritional status (Martirosyan & Singh, 2015).

Despite extensive traditional use, systematic studies integrating antibacterial efficacy with nutritional profiling of Indian medicinal plants remain limited. Evaluating both aspects provides a more holistic assessment of their potential applications in pharmaceutical, nutraceutical, and public health contexts (Pan et al., 2013; Shahidi & Ambigaipalan, 2015). Such integrated investigations can also support the scientific validation of traditional knowledge and promote the sustainable utilization of plant resources.

The present study aims to (i) evaluate the antibacterial activity of selected Indian plant species against Escherichia coli and Staphylococcus aureus, and (ii) analyze their nutritional profiles to assess their potential value as functional food resources with antimicrobial benefits.

Below is an expanded, journal-ready Materials and methods section with improved methodological detail, formal Elsevier-style tone, and the requested inclusion of the Regional Food Research & Analysis Centre, Lucknow. All scientific names are italicized wherever they appear.

2. Materials and methods

All experimental work related to extraction, antibacterial assays, and nutritional analysis was carried out at the Regional Food Research & Analysis Centre (RFRAC), Lucknow, Uttar Pradesh, India, following standard microbiological and analytical protocols.

2.1 Plant material

Fresh and healthy leaves of *Azadirachta indica* A. Juss., *Ocimum tenuiflorum* L., *Moringa oleifera* Lam., and *Psidium guajava* L., along with mature rhizomes of *Curcuma longa* L., were collected from locally cultivated plants in different regions of Bihar, India, during the active growing season. The collected plant materials were transported to the laboratory in sterile polyethylene bags to prevent contamination. Taxonomic authentication was carried out by a qualified plant taxonomist using standard floras, and voucher specimens were preserved for future reference.

The plant materials were thoroughly washed under running tap water to remove adhering soil and debris, followed by rinsing with distilled water. Cleaned samples were shade-dried at ambient temperature (28–32 °C) for 10–14 days until constant weight was achieved, thereby minimizing the degradation of thermolabile bioactive compounds. The dried plant materials

were then coarsely powdered using a sterile mechanical grinder and stored in airtight containers at room temperature until extraction.

2.2 Preparation of plant extracts

Powdered plant material (20 g) from each species was subjected to extraction separately using distilled water and 95% ethanol as solvents. Extraction was performed using a Soxhlet apparatus for 6 h to ensure exhaustive extraction of soluble phytochemicals. After completion of extraction, the obtained extracts were filtered through Whatman No. 1 filter paper to remove insoluble residues.

The filtrates were concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 45 °C to avoid thermal degradation of bioactive constituents. The concentrated extracts were further dried to a semi-solid mass and stored in sterile, airtight containers at 4 °C until use. Prior to antibacterial assays, extracts were reconstituted in their respective solvents to obtain the desired concentrations.

2.3 Test microorganisms

Pure cultures of *Escherichia coli* and *Staphylococcus aureus* were obtained from a certified microbiology culture collection maintained at the Regional Food Research & Analysis Centre, Lucknow. The bacterial strains were selected due to their clinical relevance and representation of Gram-negative and Gram-positive bacteria, respectively.

The cultures were maintained on nutrient agar slants at 4 °C and periodically subcultured to maintain viability. Before experimental use, bacterial cultures were grown in nutrient broth and incubated at 37 °C for 18–24 h to obtain actively growing cells.

2.4 Antibacterial assay

The antibacterial activity of plant extracts was assessed using the agar well diffusion method, following standard clinical microbiology procedures. Mueller–Hinton agar plates were prepared and inoculated uniformly with bacterial suspensions adjusted to 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU mL^{-1}.

Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of plant extract at a concentration of 100 mg mL^{-1}. Distilled water and ethanol were used as negative controls to rule out solvent effects, while ciprofloxacin (10 µg) served as a positive control. The plates were allowed to pre-diffuse at room temperature for 1 h and then incubated at 37 °C for 24 h.

After incubation, the diameter of the clear zones of inhibition around each well was measured in millimeters using a digital Vernier caliper. All assays were conducted in triplicate to ensure reproducibility.

2.5 Minimum inhibitory concentration

Minimum inhibitory concentration values of the plant extracts were determined using the broth dilution method. Serial two-fold dilutions of each extract were prepared in nutrient broth to obtain concentrations ranging from 12.5 to 200 mg mL^{-1}. Each tube was inoculated with a standardized bacterial suspension and incubated at 37 °C for 24 h under aerobic conditions.

Following incubation, the tubes were examined visually for turbidity. The lowest concentration of the extract showing no visible bacterial growth was recorded as the minimum inhibitory concentration. All determinations were carried out in triplicate.

2.6 Nutritional analysis

Proximate composition analysis of the powdered plant materials was conducted according to standard methods recommended by the Association of Official Analytical Chemists. Crude protein content was estimated using the Kjeldahl method, applying an appropriate nitrogen-to-protein conversion factor. Total carbohydrate content was determined by the phenol–sulfuric acid method, while lipid content was estimated using Soxhlet extraction with petroleum ether as solvent.

Crude fiber was determined by sequential acid and alkali digestion, and ash content was measured by incinerating the samples in a muffle furnace at 550 °C until constant weight was obtained. Mineral elements, including calcium, iron, and potassium, were quantified using atomic absorption spectrophotometry after wet digestion of samples. All analyses were performed in triplicate to ensure accuracy and reliability.

2.7 Statistical analysis

All experimental data are presented as mean ± standard deviation. Statistical analysis was carried out using one-way analysis of variance to determine significant differences among plant species and treatments. Tukey's post hoc test was applied for multiple comparisons, and differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Antibacterial activity

The antibacterial activity of aqueous and ethanolic extracts of the selected plant species against *Escherichia coli* and *Staphylococcus aureus* is presented in Table 1. All tested plant extracts exhibited measurable inhibitory effects against both bacterial strains, indicating the presence of bioactive

antimicrobial constituents. However, the magnitude of inhibition varied considerably among plant species, extraction solvent, and bacterial type.

Overall, ethanolic extracts demonstrated significantly higher antibacterial activity than aqueous extracts ($p < 0.05$) against both *E. coli* and *S. aureus*. This trend suggests that ethanol was more efficient in extracting antimicrobial phytochemicals, likely due to its ability to solubilize a wider range of polar and moderately non-polar secondary metabolites. Among the tested plants, ethanolic extract of *Curcuma longa* exhibited the strongest antibacterial activity, producing the largest zones of inhibition against *E. coli* (18.4 ± 0.6 mm) and *S. aureus* (20.1 ± 0.5 mm).

Ethanolic extract of *Azadirachta indica* also showed pronounced antibacterial activity, particularly against *S. aureus* (19.3 ± 0.4 mm), indicating greater susceptibility of the Gram-positive bacterium. In contrast, aqueous extracts generally produced smaller inhibition zones, with values ranging from 8.7 ± 0.4 mm to 13.8 ± 0.4 mm, depending on the plant species and bacterial strain.

A consistent pattern of higher sensitivity of *S. aureus* compared to *E. coli* was observed across all plant extracts (Table 1). This difference may be attributed to structural variations in bacterial cell envelopes, as the outer membrane of Gram-negative bacteria can limit the penetration of antimicrobial compounds. Among the remaining plants, *Psidium guajava* and *Ocimum tenuiflorum* displayed moderate antibacterial activity, while *Moringa oleifera* showed comparatively lower, yet statistically significant, inhibitory effects against both test organisms.

Table 1: Zone of inhibition (mm) of plant extracts against test bacteria

Plant species	*E. coli* (aqueous)	*E. coli* (ethanolic)	*S. aureus* (aqueous)	*S. aureus* (ethanolic)
Azadirachta indica	10.2 ± 0.4	16.8 ± 0.5	12.5 ± 0.6	19.3 ± 0.4
Ocimum tenuiflorum	9.4 ± 0.3	14.6 ± 0.4	11.2 ± 0.5	16.1 ± 0.6
Curcuma longa	11.6 ± 0.5	18.4 ± 0.6	13.8 ± 0.4	20.1 ± 0.5
Moringa oleifera	8.7 ± 0.4	13.2 ± 0.3	10.4 ± 0.3	15.0 ± 0.4
Psidium guajava	9.9 ± 0.5	15.1 ± 0.4	11.8 ± 0.6	17.4 ± 0.5

3.2 Minimum inhibitory concentration

Minimum inhibitory concentration values of the plant extracts against *E. coli* and *S. aureus* are summarized in Table 2. The minimum inhibitory concentration values ranged from 25 to 100 mg mL^{-1}, indicating varying degrees of antibacterial potency among the tested plant species. In agreement with agar diffusion results, ethanolic extracts generally exhibited lower minimum inhibitory concentration values compared to aqueous extracts (Table 2).

Ethanolic extracts of *Curcuma longa* and *Azadirachta indica* showed the lowest minimum inhibitory concentration (25 mg mL^{-1}) against both bacterial strains, confirming their strong antibacterial efficacy. Moderate activity was observed for ethanolic extracts of *Psidium guajava* and *Ocimum tenuiflorum*, with minimum inhibitory concentration values of 50 mg mL^{-1}. *Moringa oleifera* extracts exhibited comparatively higher minimum inhibitory concentration values, indicating lower antibacterial potency. Aqueous extracts showed minimum inhibitory concentration values predominantly between 75 and 100 mg mL^{-1} for most plant species.

Table 2 Minimum inhibitory concentration (mg mL^{-1}) of plant extracts

Plant species	*E. coli* (aqueous)	*E. coli* (ethanolic)	*S. aureus* (aqueous)	*S. aureus* (ethanolic)
Azadirachta indica	75	25	50	25
Ocimum tenuiflorum	100	50	75	50
Curcuma longa	50	25	50	25
Moringa oleifera	100	75	100	75
Psidium guajava	75	50	75	50

3.3 Nutritional profile

The proximate composition and mineral content of the selected plant species are presented in Table 3. Considerable variation in nutritional composition was observed among the plant species, reflecting differences in plant parts and biochemical composition (Table 3).

Moringa oleifera leaves exhibited the highest protein content (27.8 ± 0.9%), followed by *Azadirachta indica* (21.4 ± 0.8%), highlighting their potential contribution to dietary protein intake. *Curcuma longa* rhizomes were characterized by a high carbohydrate content (62.3 ± 1.5%), consistent with their role as energy-rich storage organs. Lipid content was relatively low across all species, ranging from 3.8 ± 0.2% to 6.4 ± 0.4%, suggesting suitability for low-fat dietary applications.

Crude fiber content was notably high in *Psidium guajava* leaves (18.6 ± 0.7%), indicating potential benefits for digestive health. Ash content, representing total mineral matter, was highest in *Moringa oleifera* (9.1 ± 0.4%). Mineral analysis further revealed that *Moringa oleifera* contained the highest levels of calcium (620 ± 18 mg) and iron (9.2 ± 0.5 mg per 100 g dry weight), underscoring its nutritional significance. Collectively, the nutritional results indicate that the studied plants possess substantial macro- and micronutrient content alongside their antibacterial properties.

Table 3 Proximate and mineral composition of selected plant species (per 100 g dry weight)

Plant species	Protein (%)	Carbohydrate (%)	Lipid (%)	Fiber (%)	Ash (%)	Calcium (mg)	Iron (mg)
Azadirachta indica	21.4 ± 0.8	38.6 ± 1.2	4.2 ± 0.3	14.1 ± 0.6	8.3 ± 0.4	480 ± 15	7.6 ± 0.3
Ocimum tenuiflorum	18.9 ± 0.7	42.1 ± 1.0	3.8 ± 0.2	15.4 ± 0.5	7.9 ± 0.3	410 ± 12	6.8 ± 0.4
Curcuma longa	9.6 ± 0.4	62.3 ± 1.5	5.1 ± 0.3	7.8 ± 0.4	6.2 ± 0.2	210 ± 10	3.4 ± 0.2
Moringa oleifera	27.8 ± 0.9	34.5 ± 1.1	6.4 ± 0.4	12.6 ± 0.5	9.1 ± 0.4	620 ± 18	9.2 ± 0.5
Psidium guajava	16.3 ± 0.6	40.2 ± 1.2	4.6 ± 0.3	18.6 ± 0.7	8.7 ± 0.3	390 ± 14	5.9 ± 0.3

4. DISCUSSION

The present study demonstrates that selected Indian medicinal plant species exhibit substantial antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, supporting their traditional use in the management of infectious diseases. The observed inhibitory effects of both aqueous and ethanolic extracts against representative Gram-negative and Gram-positive bacteria are consistent with earlier reports highlighting the broad-spectrum antibacterial potential of plant-derived bioactive compounds (Cowan, 1999; Savoia, 2012). The variation in antibacterial efficacy among plant species further underscores the diversity of phytochemical composition and biological activity inherent to medicinal plants (Gurib-Fakim, 2006).

A key finding of this investigation is the consistently higher antibacterial activity of ethanolic extracts compared to aqueous extracts. This observation aligns with previous studies indicating that ethanol is a more efficient solvent for extracting antimicrobial phytochemicals, including phenolics, flavonoids, and terpenoids, due to its intermediate polarity (Doughari, 2012; Eloff, 1998). The enhanced efficacy of ethanolic extracts suggests that many of the active antimicrobial constituents in these plants are either poorly soluble or only partially extractable in water, which may limit the antibacterial potential of aqueous preparations (Tiwari et al., 2011).

Among the tested plants, *Curcuma longa* exhibited the strongest antibacterial activity against both *E. coli* and *S. aureus*. This pronounced effect is likely attributable to curcuminoids, particularly curcumin, which have been widely reported to possess antimicrobial, anti-inflammatory, and antioxidant properties (Gupta et al., 2013; Hewlings & Kalman, 2017). Curcumin has been shown to disrupt bacterial cell membranes, inhibit quorum sensing, and interfere with essential metabolic pathways, thereby suppressing bacterial growth (Tyagi et al., 2015). The comparatively strong activity of *Azadirachta indica* may be linked to the presence of azadirachtin, nimbin, and other limonoids, which are known to exert antibacterial effects through membrane disruption and inhibition of bacterial enzymes (Biswas et al., 2002; Subapriya & Nagini, 2005).

The differential susceptibility of *S. aureus* and *E. coli* observed in this study is consistent with well-established differences in cell wall architecture between Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* was generally more

sensitive to plant extracts, which may be attributed to the absence of an outer membrane, allowing easier penetration of hydrophobic phytochemicals (Nikaido, 2003; Silhavy et al., 2010). In contrast, the outer lipopolysaccharide layer present in Gram-negative bacteria such as *E. coli* acts as a permeability barrier, reducing the intracellular accumulation of antimicrobial compounds (Delcour, 2009). Similar patterns of higher susceptibility of Gram-positive bacteria to plant extracts have been reported in multiple phytochemical and microbiological studies (Hemaiswarya et al., 2008; Borges et al., 2016).

In addition to antibacterial activity, the nutritional evaluation of the studied plants revealed substantial macro- and micronutrient content, reinforcing their relevance beyond therapeutic applications. The high protein and mineral content observed in *Moringa oleifera* corroborates previous findings that recognize this species as a nutritionally rich plant with potential to address protein and micronutrient deficiencies (Gopalakrishnan et al., 2016; Leone et al., 2015). The elevated calcium and iron levels in *Moringa oleifera* are particularly significant in the context of bone health and anemia prevention, especially in developing regions (Fahey, 2005).

The high crude fiber content detected in *Psidium guajava* leaves suggests potential benefits for gastrointestinal health, including improved bowel function and modulation of gut microbiota (Slavin, 2013; Anderson et al., 2009). Dietary fiber has also been associated with reduced risk of metabolic and cardiovascular disorders, further enhancing the functional value of fiber-rich plant materials (Dahl & Stewart, 2015). The nutritional richness observed across the studied species complements their antibacterial properties, highlighting their dual role as sources of both bioactive compounds and essential nutrients.

The integration of antimicrobial efficacy with nutritional profiling provides a comprehensive assessment of the functional potential of these plant species. Plants that combine therapeutic activity with nutritional benefits are particularly valuable in the development of functional foods, nutraceuticals, and plant-based health supplements (Martirosyan & Singh, 2015; Shahidi & Ambigaipalan, 2015). Such multifunctional plant resources may contribute to infection management while simultaneously supporting nutritional security and overall health.

The findings of this study support the continued exploration of Indian medicinal plants as sustainable sources of antibacterial agents and nutritionally valuable food components. Future investigations focusing on the isolation and characterization of active compounds, synergistic interactions between phytochemicals, and in vivo validation of antibacterial and nutritional effects would further strengthen the translational potential of these plant species.

Conclusion

The present study demonstrates that selected Indian medicinal plants possess notable antibacterial activity against clinically relevant bacterial pathogens, alongside appreciable nutritional value. Among the species investigated, *Curcuma longa* and *Azadirachta indica* exhibited the most pronounced antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*, highlighting their potential as sources of effective plant-derived antimicrobial agents. In contrast, *Moringa oleifera* was distinguished by its superior nutritional profile, particularly in terms of protein and essential mineral content.

The combined antimicrobial efficacy and nutritional richness of these plant species provide scientific validation for their traditional medicinal use and underscore their promise for incorporation into plant-based antimicrobial formulations and nutraceutical products. Such multifunctional properties are particularly relevant in the context of increasing antimicrobial resistance and growing interest in natural health-promoting alternatives. Further research focusing on the isolation, characterization, and mechanistic evaluation of bioactive compounds, as well as in vivo and clinical validation, is recommended to fully elucidate their therapeutic potential.

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