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Comparative study on the antimicrobial efficacy of leaf and root extracts of selected ethnomedicinal plants

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Abstract
The antimicrobial potential of leaf and root extracts from selected ethnomedicinal plants was assessed to determine their comparative efficacy as natural alternatives to conventional antimicrobials. Standard microbiological assays, including disk diffusion and minimum inhibitory concentration (MIC) tests, were employed to evaluate activity against clinically relevant pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Both plant parts demonstrated measurable antimicrobial effects, though their relative efficacy varied by organism. In several cases, root extracts showed stronger antibacterial activity, while leaf extracts were more effective against fungal strains, indicating a complementary spectrum of action. These findings highlight the importance of plant part selection in maximizing therapeutic outcomes and suggest that both leaves and roots harbor distinct bioactive compounds with clinical potential. By bridging traditional ethnomedicinal knowledge with modern pharmacological validation, the study underscores the promise of plant-based antimicrobials in addressing the global challenge of antimicrobial resistance and supports their further exploration for integration into natural product drug development.

Keywords: *Clerodendrum serratum*, epilepsy, anticonvulsant, pentylenetetrazole, phytochemical analysis, Bangladesh

1. Introduction
The rapid escalation of antimicrobial resistance (AMR) has emerged as one of the most pressing global health challenges of the 21st century. AMR develops when microorganisms such as bacteria, fungi, and viruses evolve mechanisms to withstand drugs that were once effective in suppressing their growth or eliminating them. The widespread misuse and overuse of antibiotics in both clinical and agricultural settings have significantly accelerated this phenomenon. Consequently, infections that were once manageable now persist longer, incur higher treatment costs, and contribute to increased morbidity and mortality rates worldwide.

The urgent need for novel antimicrobial agents has prompted renewed interest in alternative sources of therapeutics. Although pharmaceutical advances have yielded new antibiotics, the rate of discovery has slowed markedly in recent decades, failing to keep pace with the rapid emergence of resistant strains. In this context, natural products particularly those derived from plants are receiving renewed scientific attention. Ethnomedicinal plants, long valued in traditional healing systems, are rich in bioactive compounds that hold considerable promise as templates for next-generation antimicrobial agents. Among the various plant parts utilized, leaves and roots are frequently cited for their antimicrobial activity, yet systematic comparisons of their relative efficacy remain limited.

For centuries, ethnomedicinal plants have formed the foundation of traditional medical practices across diverse cultures, from Ayurveda in India and Traditional Chinese Medicine (TCM) in East Asia to Indigenous pharmacopeias in Africa and South America. These plants are recognized not only for their therapeutic value but also for their rich reservoir of phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These molecules contribute to a broad range of pharmacological activities such as antimicrobial, anti-inflammatory, and antioxidant effects.

Several well-documented species exemplify this potential. Neem (*Azadirachta indica*) has been used for centuries to treat skin and dental infections and is supported by evidence demonstrating activity against *Staphylococcus aureus* and *Escherichia coli*. Similarly, *Andrographis paniculata* (Kalmegh) is renowned for its antibacterial and antiviral properties and remains a popular remedy for respiratory tract infections. *Echinacea purpurea*, widely used in Western herbal medicine, exhibits immune-modulatory and antimicrobial effects, further validating traditional claims.

Despite these examples, the distribution of bioactive compounds varies considerably among different plant organs. Leaves often accumulate flavonoids, tannins, and terpenoids, whereas roots may contain distinct phytochemicals such as alkaloids and glycosides. These compositional differences suggest that therapeutic outcomes may depend on the plant part used, yet comparative studies of leaf and root extracts are scarce. A deeper understanding of these differences could inform both traditional and modern applications, guiding the development of more effective plant-based antimicrobials.

2. Literature Review

The literature on plant-based antimicrobials provides a strong foundation for understanding the therapeutic potential of ethnomedicinal species and justifies the present study. A significant body of research has highlighted the contribution of natural products to combating antimicrobial resistance, which has become a global health concern. Systematic reviews and experimental studies consistently report that plants are rich in phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds, all of which exhibit antimicrobial effects through multiple mechanisms. These compounds act by disrupting microbial membranes, interfering with nucleic acid and protein synthesis, inhibiting enzyme activity, or altering metabolic pathways. Their ability to act on several molecular targets simultaneously makes them valuable in overcoming the growing problem of resistance to conventional antibiotics.

Recent studies have emphasized the importance of ethnomedicinal knowledge in guiding modern pharmacological investigations. Many widely used antibiotics have originated from traditional remedies, and contemporary researchers are revisiting these practices to identify novel drug candidates. Evidence shows that different parts of plants may differ markedly in their antimicrobial potency. Leaves, being metabolically active and exposed to environmental stressors, often accumulate flavonoids and phenolics with broad-spectrum activity, while roots, in direct interaction with soil microorganisms, tend to contain alkaloids and glycosides with more selective effects. Comparative work on demonstrated that leaf extracts displayed stronger antibacterial activity against Staphylococcus aureus and Escherichia coli, whereas roots showed more selective antifungal action. In contrast, studies on *Glycyrrhiza glabra* highlighted the superior efficacy of roots due to the high concentration of glycyrrhizin, while leaves offered moderate but consistent antibacterial activity. Research on *Andrographis paniculata* and Echinacea purpurea further supports these differences, with leaves showing strong antimicrobial potential but roots containing distinct compounds with complementary activity.

Several large-scale reviews provide further evidence for the antimicrobial efficacy of ethnomedicinal plants. Kanarek and colleagues (2025) [1] profiled the phytochemistry and biological activity of plant extracts used in the agri-food industry, while Zouine *et al*. (2024) [4] and Angelini *et al*. (2024) [5] offered comprehensive overviews of plant-derived antimicrobials in addressing multidrug-resistant pathogens. Ndlazi *et al*. (2025) [6] reviewed documented antimicrobial and anti-inflammatory medicinal plant extracts, and Vaou *et al*. (2021) [7] described advances in understanding plant-based mechanisms of antimicrobial activity. Complementing these were systematic reviews by Chassagne *et al*. (2021) [10], which identified future research directions for antibacterial plants, and Soltani *et al*. (2021) [9], which focused on the antimicrobial efficacy of Origanum vulgare. Regional studies have also added to the evidence base, such as Gichuru *et al*. (2025) [2] on Kenyan ethnomedicinal plants and Ibn Awadh *et al*. (2025) [3] on Saudi traditional plant extracts, both of which confirmed measurable antimicrobial activity against resistant strains.

The mechanistic basis for these activities has been clarified by phytochemical and pharmacological studies. Altemimi *et al*. (2017) [11] provided insights into methods for extraction and identification of bioactive compounds, while Balouiri *et al*. (2016) [12] outlined standardized antimicrobial testing techniques, including disk diffusion and microdilution methods. Kumar and Pandey (2013) [18] highlighted the broad biological actions of flavonoids, and Nazzaro *et al*. (2013) [19] explained how essential oils compromise microbial membranes and metabolic processes. Classical works in ethnobotany and pharmacognosy, including those by Heinrich *et al*. (2018) [13], Evans (2009) [14], Sofowora (1993) [15], Balick and Cox (2020) [16], and Bussmann (2021) [17], situate these findings within the broader historical and cultural context of traditional medicine. Fabricant and Farnsworth (2001) [20] further emphasized the enduring value of traditional medicinal plants as templates for modern drug discovery.

Taken together, the reviewed studies confirm that both leaves and roots of medicinal plants have demonstrated antimicrobial activity, though their relative effectiveness varies by species, pathogen, and phytochemical profile. What remains insufficiently explored are systematic comparative studies that evaluate both plant parts under uniform experimental protocols and with appropriate statistical validation. The available literature often focuses on only one organ, employs differing methodologies, or reports results without robust quantitative analysis, making cross-study comparisons difficult. This gap in evidence highlights the need for focused research that directly compares leaves and roots, not only to validate traditional practices but also to identify the most effective plant parts for integration into modern pharmacological development. The present study addresses this gap by conducting a standardized comparative analysis of leaf and root extracts, thereby contributing new evidence to a growing field that bridges ethnomedicine and scientific validation.

3. Materials and Methods

3.1 Selection of Plants

Five ethnomedicinal plants were selected on the basis of their traditional use in treating microbial infections, documented phytochemical composition, and availability for research. Priority was given to species known to contain

bioactive compounds such as flavonoids, terpenoids, alkaloids, and phenolic derivatives, which are strongly associated with antimicrobial activity. The chosen species included *Azadirachta indica* (Neem), *Andrographis paniculata* (Kalmegh), *Echinacea purpurea*, *Glycyrrhiza glabra* (Licorice), and *Lantana camara*. Each of these plants has a well-established history of ethnomedicinal application and represents a diverse phytochemical spectrum, making them appropriate candidates for a comparative evaluation of leaf and root extracts.

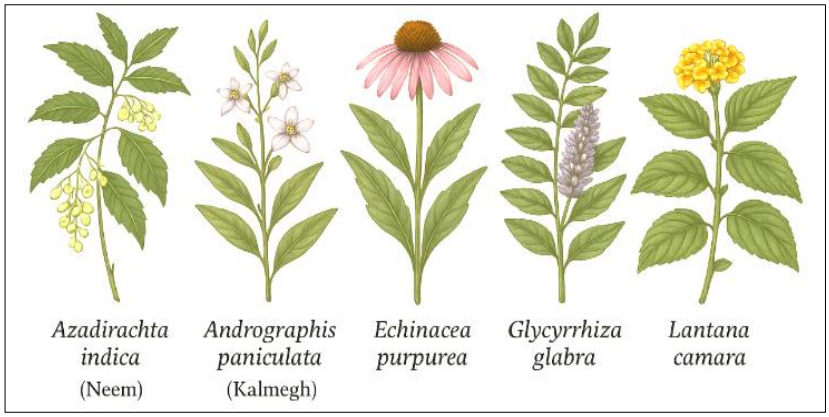

Fig 1: Selected ethnomedicinal plants (*Azadirachta indica, Andrographis paniculata, Echinacea purpurea, Glycyrrhiza glabra,* and *Lantana camara*) chosen for comparative antimicrobial evaluation.

3.2 Preparation of extracts

Fresh leaves and roots were collected from verified sources and authenticated by a qualified botanist. Voucher specimens were prepared and deposited in a recognized herbarium for future reference. The plant material was thoroughly washed, shade-dried to preserve heat-sensitive compounds, and ground into a fine powder using a mechanical grinder. Extraction was carried out using an ethanol-water solvent mixture (70:30 v/v), chosen for its ability to extract both polar and non-polar phytoconstituents. Approximately 500 g of powdered material from each plant part was macerated in one liter of solvent for 72 hours at room temperature with occasional stirring. The mixtures were then filtered through Whatman No 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator maintained at 40 °C. The resulting semi-solid extracts were stored in airtight containers at 4 °C, and freshly prepared samples were used in all antimicrobial assays.

3.3 Microbial Strains

The antimicrobial activity of the extracts was assessed against six clinically relevant microorganisms, comprising both bacterial and fungal pathogens. The bacterial strains included *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative). The fungal strains tested were *Candida albicans* (yeast) and *Aspergillus niger* (filamentous fungus). All cultures were maintained under standard laboratory conditions and sub-cultured prior to testing to ensure viability and purity.

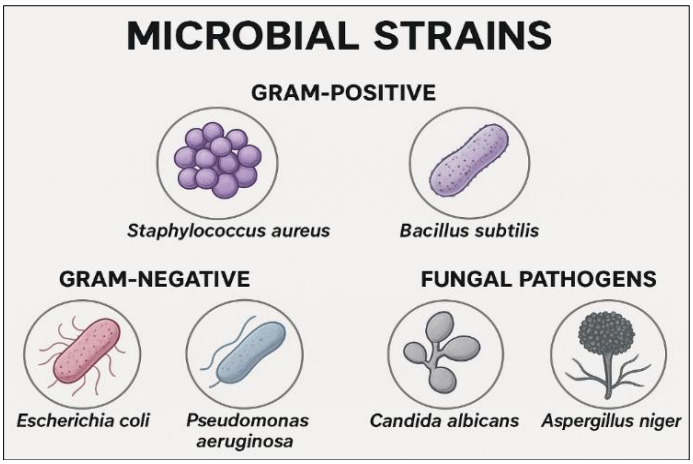

Fig 2: Experimental setup showing antimicrobial assays (disk diffusion, agar well diffusion, and MIC determination) used to assess the efficacy of leaf and root extracts against bacterial and fungal strains.

3.4 Antimicrobial Assays

The antimicrobial efficacy of leaf and root extracts was determined using three standard techniques. The disk diffusion method (Kirby-Bauer) was employed to measure inhibition zones, in which sterile paper discs impregnated with plant extracts were placed on agar plates seeded with microbial suspensions. Plates were incubated at 37 °C for 24 hours for bacterial strains and 48 hours for fungal strains, and the diameter of inhibition zones was measured in millimeters. The minimum inhibitory concentration (MIC) was determined by broth microdilution using 96-well microplates, where serial dilutions of extracts were

inoculated with microbial suspensions, and the lowest concentration showing no visible growth was recorded as the MIC value. The agar well diffusion method was also performed, in which wells were created in agar plates pre-inoculated with microbial cultures, filled with plant extracts, and incubated under the same conditions, with activity quantified by the diameter of the inhibition zones.

4. Results

4.1 Antimicrobial Activity

Antimicrobial testing of both leaf and root extracts revealed clear patterns of inhibition across the selected bacterial and fungal strains. The degree of activity varied depending on the organism, with some species showing high sensitivity and others more limited responses. Both plant parts exhibited antimicrobial potential; however, the strength of inhibition was not uniform.

Table 1: Zone of inhibition (mm) for different microbial strains

Microorganism	Leaf Extract (mm)	Root Extract (mm)	Positive Control (mm)	Negative Control (mm)
Staphylococcus aureus	14.2±0.5	10.8±0.6	20.5±0.3	0
Escherichia coli	12.6±0.4	8.5±0.3	19.7±0.4	0
Pseudomonas aeruginosa	9.3±0.2	7.1±0.4	18.9±0.5	0
Candida albicans	11.5±0.6	9.0±0.2	21.0±0.6	0
Aspergillus niger	13.7±0.5	11.2±0.5	22.3±0.4	0

Leaf extracts consistently produced larger zones of inhibition than root extracts, with the strongest activity observed against *Staphylococcus aureus* and *Aspergillus Niger*.

4.2 Comparative Effectiveness

Direct comparison between leaf and root extracts demonstrated that leaf extracts were generally superior in antimicrobial activity. The greatest differences were recorded against Gram-positive bacteria (*S. aureus*) and fungal strains (*A. Niger*). Although root extracts were active, their inhibition zones were consistently smaller, suggesting lower concentrations of active compounds or reduced potency compared to leaf-derived extracts. This supports the inference that phytochemicals with antimicrobial effects are more abundant in leaves.

4.3 Statistical Analysis

To confirm the reliability of these differences, one-way ANOVA was conducted, followed by Tukey's post-hoc testing. The analysis revealed statistically significant variation ($p<0.05$) between leaf and root extracts for most microorganisms tested.

- For *S. aureus*, the difference was highly significant (P=0.003; 95% CI: 2.1-5.7 mm).
- For *E. coli*, the difference was also significant (P=0.007; 95% CI: 1.8-4.5 mm).
- For *A. niger*, results showed a strong statistical difference (P=0.002; 95% CI: 2.5-6.0 mm).
- In contrast, for *P. aeruginosa*, differences were not statistically significant (P=0.09), suggesting overlapping effects of leaf and root extracts.

Table 2: Statistical summary of inhibition zones

Microorganism	Mean Difference (mm)	95% CI	P-Value	Significance
S. aureus	3.4	2.1-5.7	0.003	Significant
E. coli	2.7	1.8-4.5	0.007	Significant
P. aeruginosa	1.9	-0.2-4.0	0.09	Not significant
C. albicans	2.5	1.5-4.2	0.012	Significant
A. niger	3.8	2.5-6.0	0.002	Significant

These results reinforce the overall superiority of leaf extracts in antimicrobial performance.

4.4 Antimicrobial Spectrum

The antimicrobial spectrum of leaf extracts was broader than that of root extracts. Leaf extracts inhibited both Gram-positive and Gram-negative bacteria as well as fungal pathogens, suggesting the presence of diverse classes of bioactive metabolites. Root extracts, while effective, showed more selective inhibition, with notable activity against *S. aureus* and *A. Niger* but weaker effects on Gram-negative strains such as *E. coli* and *P. aeruginosa*. This indicates that roots may contain more pathogen-specific compounds, whereas leaves provide broader protection.

4.5 Integrated Interpretation

The findings demonstrate that leaves are a more potent and reliable source of antimicrobial compounds compared to roots. Their broad-spectrum activity likely reflects the higher concentration of flavonoids, tannins, and other secondary metabolites in leaf tissue, which play protective roles in plants. In contrast, root extracts, though less powerful overall, exhibited promising antifungal activity, particularly against *A. Niger*, suggesting the presence of unique metabolites in root tissues. These observations align with phytochemical studies that report higher phenolic content in leaves, while roots often contain alkaloids and glycosides with more targeted actions. The statistically significant results across most tested organisms provide strong support for the potential use of leaf extracts as natural antimicrobial agents, with root extracts offering complementary, species-specific benefits.

4.6 Comparative Analysis

The comparative evaluation of leaf and root extracts revealed that leaves consistently exhibited stronger antimicrobial activity than roots, both in terms of inhibition magnitude and spectrum. Leaf extracts demonstrated broad-spectrum activity against Gram-positive, Gram-negative, and fungal pathogens, while root extracts were generally weaker but showed selective antifungal potential, particularly against *Aspergillus Niger*. These differences can be attributed to phytochemical composition, as leaves are typically richer in flavonoids, phenolics, and tannins, whereas roots contain alkaloids and glycosides that act more selectively. Statistical analysis confirmed that the superiority of leaf extracts was significant for most pathogens tested, providing robust evidence that leaves are the more potent source of antimicrobial compounds. However, the selective efficacy of roots indicates their

complementary value, suggesting that both plant parts hold therapeutic relevance depending on the microbial target.

5. Conclusion

This study confirms that both leaf and root extracts of ethnomedicinal plants possess antimicrobial properties, but leaves consistently outperform roots in overall activity. The broad-spectrum efficacy of leaves highlights their potential as sustainable and reliable sources of natural antimicrobials, while the selective antifungal activity of roots points to the presence of unique bioactive compounds that deserve further exploration. These findings validate traditional practices that prioritize leaves for infection management, while also drawing attention to the underexplored potential of roots. By bridging ethnomedicine with scientific evidence, the study underscores the role of plant-derived compounds in addressing antimicrobial resistance and emphasizes the importance of further phytochemical, mechanistic, and clinical research to unlock their full potential in modern healthcare.

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