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Review Article

## **In vitro Antibacterial Activity of Medicinal Plants of Nepal: A Comprehensive Review**

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### ABSTRACT

Antimicrobial resistance is a growing challenge in medicine. Medicinal plants are used as antibiotic medicines for primary healthcare. This review systematically compiled and analyzed published *in vitro* studies on the antibacterial activity of medicinal plants of Nepal. A comprehensive search on PubMed, NepJOL and Google Scholar identified 27 original studies in Nepal, approximately 120 plant species from 100 genera and over 50 families. Leaves were most frequently tested (66.7%), followed by roots/rhizomes (40.7%), fruits/seeds/flowers (33.3%), bark (22.2%), and whole plant (18.5%). Methanol (51.9%) and ethanol (33.3%) were commonly used extraction solvents, with cold maceration being predominant method. Plant extracts demonstrated activity against both Gram-positive and Gram-negative bacteria, with Gram-positive strains generally more susceptible (~70% of studies). The largest ZOI reported was 31 mm for an ethyl acetate extract of *Terminalia bellirica* fruit against *Pseudomonas aeruginosa*. MIC values ranged from tens to several hundred µg/ml. However, methodological heterogeneity, limited MIC/MBC reporting and under-representation of multidrug-resistant bacteria highlight the need for standardized and reproducible studies to identify promising species for antibacterial drug development.

**Keywords:** Antibacterial activity; Antimicrobial resistance; Ethnomedicine; Medicinal plants

## Introduction

Antimicrobial resistance (AMR) poses a severe threat to global health. Resistant infections are estimated to cause thousands of deaths annually worldwide and could reach millions per year by 2050 if current trends continue (Parajuli et al., 2024). The South Asian region,

including Nepal, is particularly vulnerable due to high population density, substantial infectious disease burden and widespread antibiotics use. In Nepal, multiple interconnected factors contribute to the emergence and spread of AMR (Shrestha et al., 2010; Shakya et al., 2012; Devkota et al., 2018; Dhungana et al., 2019; Niroula et al., 2020; Shrestha et al., 2023;

Tiwari et al., 2024; Acharya et al., 2025a, 2017; Adhikari et al., 2025; Sharma et al., 2025). The country has approximately 0.17 physicians per 1,000 population, far below the World Health Organization recommendations, leading many individuals to seek antibiotics directly from pharmacies or informal drug vendors (Pokharel & Adhikari, 2020). The vendors frequently dispense antibiotics without prescription and at inappropriate doses or durations. Studies suggest that more than half of antibiotic use in Nepal occurs without medical supervision, creating strong selective pressure on bacterial populations (Rijal et al., 2021).

Nepal has recently endorsed a National Action Plan on AMR emphasizing ‘One Health’ surveillance and laboratory capacity strengthening. However, persistent community-level practices and limited healthcare resources indicate that complementary strategies are required. In this context, Nepal’s exceptional botanical diversity and long-standing ethnomedicinal traditions offer significant potential. Historically, approximately 60–80% of rural Nepalese relied on medicinal plants for primary healthcare (Chaudhary et al., 2025). Many of these plants contain bioactive secondary metabolites with documented antimicrobial properties.

While numerous *in vitro* studies have screened Nepalese medicinal plants for antibacterial activity, the findings remain dispersed across the literature. A comprehensive synthesis is therefore needed to evaluate the strength of existing evidence, identify promising plant taxa, and highlight research gaps relevant to AMR mitigation. This review aims to compile systematically and analyze critically the published *in vitro* antibacterial studies on Nepalese medicinal plants.

## Methods

### Search strategy and data sources

A comprehensive literature search was conducted to identify studies reporting the antibacterial activity of medicinal plants from Nepal. The electronic databases PubMed, NepJOL (Nepal Journals Online), and Google Scholar were systematically searched. The following combinations of keywords were used: “medicinal plants” OR “traditional medicine” AND “Nepal” AND “antibacterial” OR “antimicrobial activity” AND “*in vitro*” OR “disc diffusion” AND “zone of inhibition”.

**Inclusion criteria:** Studies were included if they-

1. used plant material collected in or traditionally used in Nepal;
2. prepared plant extracts using any solvent or method; and
3. evaluated antibacterial activity against bacterial strains using *in vitro* assays.

**Exclusion criteria:** Studies were excluded if they-

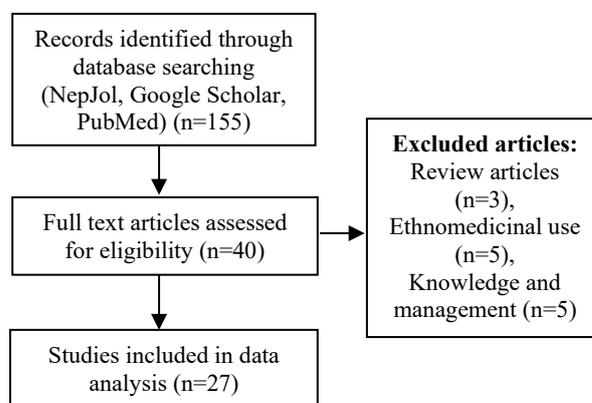
1. did not assess antibacterial activity;
2. were reviews or lacked original experimental data;
3. failed to report essential methodological details.

### Study selection

All retrieved articles were screened based on titles and abstracts for relevance. Full texts of potentially eligible studies were then assessed according to the predefined inclusion and exclusion criteria. Only studies meeting all inclusion criteria were included in the final analysis (Figure 1).

### Data extraction

Data were extracted on study details (authors, year, study type), botanical information (species, plant part, family), extraction (solvent, method, concentration), test organisms (species, strain), and quantitative results (zone of inhibition or MIC/MBC values). Commonly cited ATCC or reference strains (e.g. *E. coli* ATCC 25922, *S. aureus* ATCC 25923) were recorded when specified.



**Figure 1:** Flowchart for inclusion of published articles.

## Results and Discussion

A total of 27 original research articles evaluating the antibacterial activity of Nepalese medicinal plants were included in this review (Appendix 1). Collectively, these studies screened approximately 120 plant species representing nearly 100 genera of over 50 families, reflecting the rich ethnobotanical diversity of Nepal. The most frequently represented families were Fabaceae ( $\approx 11.7\%$ ), Asteraceae ( $\approx 10\%$ ), Lamiaceae ( $\approx 8.3\%$ ), Zingiberaceae ( $\approx 6.7\%$ ), Euphorbiaceae ( $\approx 5.8\%$ ) and Rutaceae ( $\approx 5\%$ ), collectively accounting for nearly half of all investigated species. At the genus level, most genera were represented by a single species, with only a few, including *Ocimum*, *Curcuma*, *Terminalia* and *Zingiber*, examined in multiple studies.

This indicates limited replication and suggests that the current research landscape remains largely exploratory.

### Plant parts used

Leaves were the most commonly studied plant part, reported in 66.7% of the studies, followed by roots and rhizomes (40.7%), fruits, seeds, or flowers (33.3%), bark (22.2%), and whole plant (18.5%) (Table 1). The predominance of leaves likely reflects their ease of collection, sustainability, and high concentration of bioactive secondary metabolites. Nevertheless, roots, bark and fruits often demonstrated stronger antibacterial activity, suggesting that phytochemical content and antibacterial potency can vary substantially across plant parts. The diversity of plant parts used across studies introduces variability in the observed antibacterial outcomes, complicating direct comparisons between extracts.

### Extraction methods and solvents

Cold maceration or percolation was the most frequently employed extraction method, used in 55.6% of studies. Soxhlet extraction and post-extraction fractionation were each applied in 22.2% of studies. Studies utilizing Soxhlet extraction or fractionation often reported stronger antibacterial activity, highlighting the influence of extraction efficiency on bioactive compound yield. Solvent choice also played a critical role in determining activity. Methanol was the most commonly used solvent (51.9%), followed by ethanol (33.3%). Non-polar or semi-polar solvents such as chloroform, hexane, and ethyl acetate were used in 25.9% of studies, whereas aqueous extracts were less common (14.8%) (Table 1). Extracts prepared with organic solvents consistently exhibited moderate to strong antibacterial activity, whereas aqueous extracts generally showed weak or inconsistent effects.

**Table 1:** Summary of included studies on antibacterial activity of medicinal plants.

Variables	Parameter	Number of studies (n)	Percentage (%)
<b>Plant parts used</b>	Leaves	18	66.7
	Roots/Rhizomes	11	40.7
	Bark	6	22.2
	Fruits/Seeds/Flowers	9	33.3
	Whole plant	5	18.5
<b>Extraction methods</b>	Cold maceration/percolation	15	55.6
	Soxhlet extraction	6	22.2
	Fractionation of crude extracts	6	22.2
<b>Extraction solvents</b>	Methanol	14	51.9
	Ethanol	9	33.3
	Aqueous	4	14.8
	Non-polar/semi-polar solvents*	7	25.9
<b>Bacterial spectrum tested</b>	Gram-positive only	3	11.1
	Gram-negative only	3	11.1
	Both Gram-positive and Gram-negative	15	55.6
	Not clearly specified	6	22.2
<b>Antibacterial assessment</b>	ZOI reported	27	100
	MIC and/or MBC reported	11	40.7
	ZOI only (no MIC/MBC)	16	59.3
<b>Antibacterial outcome</b>	Any antibacterial activity observed	27	100
	Strong–moderate activity with organic solvents	23	85.2
	Weak/inconsistent activity with aqueous extracts	4	14.8
<b>Susceptibility trend</b>	Gram-positive > Gram-negative bacteria	~19	~70

\*Includes chloroform, hexane, ethyl acetate, acetone and other related solvents.

Multiple responses per study led to the total percentages exceeding 100%.

### Bacterial spectrum

All included studies evaluated at least one Gram-positive and one Gram-negative bacterium (Table 1). The most frequently tested indicator organisms were

*Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), each appearing in over 70% of studies. Other commonly tested bacteria included *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* species. Gram-positive strains such as

*Bacillus subtilis* and *Streptococcus* spp. were also assessed in some studies, and a few studies included multidrug-resistant or clinical isolates. The selection of these pathogens aligns with both standard microbiological practice and the clinical relevance of these organisms in the Nepalese context.

### Antibacterial activity

The antibacterial activity of plant extracts, as measured by disc diffusion assays, varied widely (Table 1). Weak activity corresponded to inhibition zones of 7–10 mm, moderate activity to 10–20 mm, and strong activity to zones exceeding 20 mm. The largest inhibition zone reported was 31 mm, observed for an ethyl acetate extract of *Terminalia bellirica* fruit against *Pseudomonas aeruginosa*. Other extracts with notable activity included *Morus australis* root (25 mm against *K. pneumoniae*), *Glycyrrhiza glabra* root (23 mm against *S. aureus*), and *Quercus infectoria* galls (23 mm

against *S. typhi*). Overall, Gram-positive bacteria were more susceptible than Gram-negative bacteria, with approximately 70% of studies reporting higher inhibition zones against Gram-positive strains. This susceptibility pattern is consistent with structural differences in bacterial cell walls that influence permeability to plant-derived compounds.

Several plant extracts consistently exhibited strong antibacterial activity. The ethyl acetate extract of *Terminalia bellirica* fruit showed the highest inhibition (31 mm) against *P. aeruginosa*, with MIC values ranging from 200 to 800 µg/mL. *Morus australis* root methanol extract demonstrated 25 mm inhibition against *K. pneumoniae*, while *Glycyrrhiza glabra* root ethanol extract produced 23 mm inhibition against *S. aureus*. Extracts of *Quercus infectoria* galls, *Cinnamomum tamala* leaves, and *Mallotus japonicus* stems also showed notable antibacterial activity (Table 2).

**Table 2:** Extract of plant showing strong antibacterial activity (ZOI: zone of inhibition, MIC: minimum inhibitory concentration).

Plant (Family)	Part	Solvent	Target organism	ZOI (mm)	MIC (µg/ml)
<i>Terminalia bellirica</i> (Combretaceae)	Fruit	Ethyl acetate	<i>Pseudomonas aeruginosa</i>	31	200–800 (varied by strain)
<i>Morus australis</i> (Moraceae)	Root	Methanol	<i>Klebsiella pneumoniae</i>	25	100–500
<i>Glycyrrhiza glabra</i> (Fabaceae)	Root	Ethanol	<i>Staphylococcus aureus</i>	23	250
<i>Quercus infectoria</i> (Fagaceae)	Galls	Methanol	<i>Salmonella enterica</i> Typhi	23	180
<i>Cinnamomum tamala</i> (Lauraceae)	Leaf	Ethanol	<i>Escherichia coli</i>	21	–
<i>Mallotus japonicus</i> (Euphorbiaceae)	Stem	Methanol	<i>Pseudomonas aeruginosa</i>	20	320

MIC and/or MBC values were reported in 11 studies (40.7%). Potent extracts exhibited MICs in the low tens to several hundreds of micrograms per milliliter, while aqueous extracts often demonstrated MICs above 1000 µg/mL or were not reported. In general, strong disc diffusion activity corresponded to low MICs, confirming that extracts demonstrating large inhibition zones also possessed measurable bacteriostatic or bactericidal potency. Differences in methodology, such as agar dilution versus broth-based MIC determination, precluded quantitative aggregation of MIC values across studies.

This review provides a comprehensive synthesis of *in vitro* antibacterial studies on Nepalese medicinal plants, highlighting both the potential and limitations of the current evidence base. The identification of antibacterial activity in extracts from approximately 120 plant species underscores the rich ethnobotanical resources of

Nepal and supports traditional medicinal use (Ashraf et al., 2023; Joshi et al., 2019). The concentration of research on families such as Fabaceae, Asteraceae, and Lamiaceae likely reflects their known phytochemical richness, including flavonoids, alkaloids, terpenoids, and phenolics, which contribute to antibacterial activity (Ashraf et al., 2023). However, most species were assessed in only a single study, limiting reproducibility and preventing robust comparison of antibacterial potency across taxa, suggesting that medicinal plant research in Nepal remains largely exploratory.

Plant part selection emerged as a significant determinant of antibacterial outcomes. Leaves were the most frequently studied, likely due to ease of collection, sustainability, and high concentrations of bioactive metabolites (Pokharel & Adhikari, 2020). Nevertheless, roots, bark, and fruits often exhibited stronger antibacterial effects, indicating part-specific

distribution of phytochemicals (Shrestha et al., 2025). This variability complicates direct comparisons between studies and highlights the need for careful standardization of plant material in future research.

Extraction methodology and solvent choice were critical factors influencing reported activity. Studies employing polar organic solvents, particularly methanol, ethanol, and ethyl acetate, consistently reported stronger antibacterial effects compared to aqueous extracts (Basnet et al., 2020; Baral et al., 2021). This suggests that many antibacterial compounds in Nepalese medicinal plants are moderately polar and poorly extracted by water. While cold maceration was the most common extraction method, Soxhlet extraction or post-fractionation often yielded higher activity, emphasizing the importance of extraction efficiency (Shrestha et al., 2025). However, few studies systematically compared solvents or optimized extraction conditions, limiting understanding of true extract potency.

The observed variation in antibacterial potency among these extracts is fundamentally linked to their diverse mechanisms of action against bacterial cellular targets. For example, the high efficacy of *Terminalia bellirica* and *Quercus infectoria* (Table 3) is likely mediated by high concentrations of hydrolyzable tannins and phenolics. These compounds act by complexing with bacterial cell walls and inactivating membrane-bound enzymes through non-specific forces such as hydrogen bonding. In contrast, members of the Lamiaceae and Fabaceae families, rich in lipophilic terpenoids and alkaloids, may exert their effects by increasing the permeability of the cytoplasmic membrane, leading to the leakage of essential intracellular ions and ATP.

The bacterial spectrum tested primarily included standard indicator strains, with *Escherichia coli* and *Staphylococcus aureus* appearing in over 70% of studies (Pokharel et al., 2020). Gram-positive bacteria were generally more susceptible than Gram-negative bacteria, consistent with structural differences in bacterial cell walls affecting permeability to phytochemicals (Parajuli et al., 2024). While a few studies included multidrug-resistant or clinical isolates (Timilsina et al., 2024), this area remains underexplored, limiting translational relevance in the context of Nepal's growing antimicrobial resistance burden.

Methodological heterogeneity represented a major limitation. The heavy reliance on disc diffusion assays, often without standardized extract concentrations or positive controls, restricted quantitative comparison. MIC and MBC values, the most clinically relevant indicators of antibacterial potency, were underreported (Shrestha et al., 2025). Differences in plant species,

plant parts, extraction methods, and bacterial strains further constrained cross-study synthesis. Collectively, these factors indicate that while many Nepalese plants show measurable antibacterial activity, the current evidence is insufficient to support direct application for antimicrobial therapy.

Overall, these findings highlight the need for a more systematic and targeted research approach. Future studies should prioritize repeated evaluation of promising plant species, standardize extraction and assay protocols, and include both standard and clinically relevant multidrug-resistant bacterial strains. Integrating ethnobotanical knowledge with pharmacological and microbiological investigation will be essential to identify reproducible and potent antibacterial agents. With such approaches, Nepalese medicinal plants hold considerable potential as sources of new antibacterial compounds that could contribute meaningfully to combating antimicrobial resistance.

## Conclusion

Nepalese medicinal plants have considerable antibacterial potential. However, the current evidence is constrained by methodological heterogeneity and limited quantitative reporting. Addressing these limitations will be essential to harness medicinal plants as credible contributors to antibacterial discovery and AMR mitigation in Nepal.

## Recommendations

Future research on Nepalese medicinal plants should adopt standardized extraction and antibacterial testing methodologies to ensure reproducibility and comparability across studies. Quantitative measures should be prioritized to provide clinically relevant assessments. Promising plant species should be re-evaluated across multiple studies to confirm consistent activity, while investigations should also include clinically relevant and multidrug-resistant bacterial strains. Finally, an integrated approach combining ethnobotanical knowledge with microbiological and pharmacological analyses will be essential to identify bioactive compounds, optimize extraction processes, and evaluate their potential for therapeutic application.

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**Appendix 1:** Published articles included for review.

S.N.	Title	Author et al
1	Antibacterial activities of medicinal plants of Nepal	(Pokharel et al., 2008)
2	Antidiabetic and Antimicrobial Properties of Some High-Altitude Medicinal Plants of Nepal	(Regmi et al., 2019)
3	Antimicrobial Activity of Some Medicinal Plants from East and Central Part of Nepal	(Subba & Basnet, 2014)
4	Assessment of phytochemical content, antioxidant and antibacterial activities of three medicinal plants of Nepal	(Subba, B., Basnet, P., & Sharma, S, 2015)
5	Assessment of Phytochemical, Antioxidant and Antimicrobial Activities of Some Medicinal Plants from Kaski District of Nepal	(Khanal et al., 2020)
6	Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of <i>Tinospora cordifolia</i> (Gurjo)	(Shrestha & Lamichhane, 2021)
7	Phytochemical screening, Free radical scavenging, and In-vitro Anti-bacterial activity study of Chloroform, Acetone and Methanol extracts of selected medicinal plants of Nepal	(Karki et al., 2021)
8	Evaluation of Antibacterial Activities of Medicinal Plants	(Maharjan et al., 2013)
9	Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria	(Marasini et al., 2015)
10	Evaluation of Antibacterial Properties of Some Medicinal Plants Used for the Treatment of Respiratory Tract Infections in Nepal	(Kunwor & Thapa, 2013)
11	Evaluation of Antioxidant, Antimicrobial, and Cytotoxic Activities and Correlation with Phytoconstituents in Some Medicinal Plants of Nepal	(Ranabhat et al., 2022)
12	Evaluation of Phytochemical, Antioxidant and Antibacterial Activities of Selected Medicinal Plants	(Shresta et al., 2021)
13	Exploring the Potency of Medicinal Plants in Central Nepal's Highlands: A Comprehensive Analysis of Antioxidant, Antibacterial Properties, and Toxicity	(T. Shrestha et al., 2023)
14	In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria	(Manandhar et al., 2019)
15	Antimicrobial and Antioxidant Activity of Some Indigenous Plants of Nepal	(Subba & Basnet, 2014)
16	Phytochemical extraction and antimicrobial properties of different medicinal plants	(Joshi et al., 2011)
17	Phytochemical Screening and Anti-Microbial Properties of Medicinal Plants of Dhunikharka Community, Kavrepalanchowk, Nepal	(Adhikary, 2010)
18	Phytochemical Screening and Antimicrobial Activities of Some Selected Medicinal Plants of Nepal	(Paudel & Gyawali, 2010)
19	Phytochemical Screening and Biological Activity Analysis of Some Selected Medicinal Plants of Ilam District of Nepal	(Basnet, 2020)
20	Phytochemical profiling using HPLCMS and evaluation of antioxidant and antibacterial activities of Nepalese medicinal plants	(Neupane & Lamichhane, 2020)
21	Phytochemical screening, free radical scavenging and in-vitro antibacterial activity of ethanolic extracts of selected medicinal plants of Nepal and effort towards formulation of antibacterial cream from the extracts	(Tiwari et al., 2021)
22	Preliminary Study on the Antibacterial Activities and Antibacterial Guided Fractionation of Some Common Medicinal Plants Practices in Itum Bahal, Kathmandu Valley of Nepal	(Bhandari et al., 2023)
23	Screening of Antioxidant, Antibacterial, Anti-Adipogenic, and Anti-Inflammatory Activities of Five Selected Medicinal Plants of Nepal	(Lamichhane et al., 2023)
24	Study of phytochemical, anti-microbial, anti-oxidant, and anti-cancer properties of <i>Allium wallichii</i>	(Bhandari et al., 2017)
25	Study of Phytochemical, Antioxidant and Antimicrobial Activity of <i>Artocarpus heterophyllus</i>	(Thapa et al., 2016)
26	Chemical Profiling and Biological Activities on Nepalese Medicinal Plant Extracts and Isolation of Active Fraction of <i>Nyctanthes arbor-tristis</i>	(Khadka et al., 2024)
27	Antimicrobial Potential of Three Nepalese Medicinal Plants Against Multidrug Resistance <i>Escherichia coli</i> Isolates From Normal Individuals	(Timilsina et al., 2024)