

Antimicrobial Potential of Medicinal Plant Extracts Prepared by Decoction and Percolation Techniques

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Received: 15/07/2025

Accepted: 08/09/2025

Published: 15/10/2025

Abstract

Background: The growing prevalence of multidrug-resistant pathogens necessitates novel therapeutic approaches, including the use of plant-derived bioactive compounds in combination with conventional antibiotics. **Objective:** This study investigates the extractive yields, antibacterial activity, and potential of *Hibiscus sabdariffa*, *Syzygium aromaticum*, and *Thymus vulgaris* using two extraction techniques—decoction and percolation. **Material and Methods:** Extractive yields were quantified, and in vitro antimicrobial activity was evaluated against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp.) using agar well diffusion and disc diffusion methods. **Results & Discussion:** Decoction extracts showed moderate inhibition, while percolation extracts demonstrated superior activity, particularly against *B. cereus*, *S. aureus*, and *P. aeruginosa*. Studies revealed significant enhancement of antimicrobial efficacy when plant extracts were combined with Streptomycin or Fluconazole, particularly against resistant Gram-negative strains. **Conclusion:** These findings suggest that plant-antibiotic combinations represent a promising approach for combating antimicrobial resistance.

Keywords: Medicinal plants, Antimicrobial activity, Decoction, Percolation

Journal of Applied Pharmaceutical Sciences and Research, (2025);

DOI: 10.31069/japsr.v8i3.03

Introduction

The emergence of multidrug-resistant microorganisms poses a serious challenge to global health, demanding alternative therapeutic strategies. Medicinal plants, with their rich reservoir of bioactive phytochemicals, have been extensively studied for antimicrobial properties. Extraction technique plays a critical role in the recovery and preservation of active compounds.[1-4]

Traditional methods such as decoction utilize boiling water and are effective for hard plant materials, whereas percolation employs controlled solvent extraction (ethanol or methanol), which can preserve thermolabile compounds and enhance yield. Furthermore, combining plant-derived extracts with conventional antibiotics may offer effects, restoring or enhancing antimicrobial efficacy against resistant pathogens.[5-7]

This study compares decoction and percolation extracts of three medicinal plants (*Hibiscus sabdariffa*, *Syzygium aromaticum*, *Thymus vulgaris*) and evaluates their antimicrobial and potential against selected Gram-positive and Gram-negative bacteria.

Material And Methods

Chemicals, Equipment and Microbes

The study was conducted using standard microbiological and pharmacognostic equipment, including an incubator (Lab

India), oven (Tempo Instruments), laminar air flow cabinet (Labtop Horizontal), colony counter (Labcare), micropipettes (Thermo Scientific), and autoclave (Metalab). Analytical grade reagents such as methanol (Rankem), ethanol (Merck), sterile water (Merck), and in-house distilled water were used for the extraction and microbiological assays. The microbial strains employed in the study included *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

The antimicrobial efficacy of the selected plant extracts (*Hibiscus sabdariffa*, *Syzygium aromaticum*, and *Thymus vulgaris*) was certified and evaluated against a panel of standard bacterial and fungal strains obtained from the American Type Culture Collection (ATCC). The bacterial strains included *Staphylococcus aureus* (ATCC 25923), a Gram-positive pathogen commonly associated with skin, wound, and respiratory infections; *Bacillus subtilis* (ATCC 6633), a well-known model organism for Gram-positive bacteria; *Escherichia coli* (ATCC 25922), a Gram-negative intestinal pathogen widely used in antibacterial screening; *Pseudomonas aeruginosa* (ATCC 27853), an opportunistic Gram-negative pathogen frequently involved in wound and lung infections; and *Klebsiella pneumoniae* (ATCC 13883), a Gram-negative bacterium responsible for various nosocomial infections. The fungal strains employed were *Candida albicans* (ATCC 10231), a yeast species known to cause candidiasis in humans, and *Aspergillus niger* (ATCC 16404), a filamentous fungus commonly found in the environment and often used for antifungal susceptibility testing. These

Table 1: Extractive Yield from decoction method

Plant	Replicate 1	Replicate 2	Replicate 3	Average	Observation
<i>Hibiscus sabdariffa</i>	31.7 %	34.9 %	32.4 %	37.5 %	Satisfactory
<i>Syzygium aromaticum</i>	22.1 %	25.0 %	28.6 %	28.4 %	Satisfactory
<i>Thymus vulgaris</i>	44.8 %	44.8 %	42.1 %	43.4 %	Satisfactory

Table 2: Extractive Yield from percolation method

Plant	Replicate 1	Replicate 2	Replicate 3	Average	Observation
<i>Hibiscus sabdariffa</i>	34.4 %	33.5 %	32.3 %	33.4 %	Satisfactory
<i>Syzygium aromaticum</i>	33.4 %	31.5 %	34.9 %	33.2 %	Satisfactory
<i>Thymus vulgaris</i>	47.9 %	49.5 %	51.7 %	49.7 %	Satisfactory

Table 3: Antibacterial activity of different plant decoction extracts

Sr. No.	Name of Plant	Gram Positive		Gram Negative		
		<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
1.	<i>Hibiscus sabdariffa</i>	8.0	7.0	0.0	6.0	9.0
2.	<i>Syzygium aromaticum</i>	7.5	8.0	6.0	9.0	8.5
3.	<i>Thymus vulgaris</i>	6.0	8.5	7.5	0.0	6.0

Table 4: Antibacterial activity of different plant percolation extracts

Sr. No.	Name of Plant	Gram Positive		Gram Negative		
		<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
1.	<i>Hibiscus sabdariffa</i>	10.5	8.0	0.0	3.5	10.0
2.	<i>Syzygium aromaticum</i>	0.0	10.5	8.0	8.5	10.5
3.	<i>Thymus vulgaris</i>	10.5	0.0	6.0	6.5	8.5

selected microorganisms represent a broad spectrum of clinically relevant bacterial and fungal pathogens and are routinely used in antimicrobial assays to assess the inhibitory potential of plant-derived bioactive compounds.

Extraction of Plant

The extracts of selected medicinal plants were prepared using two different extraction techniques: decoction and percolation. In the decoction method, the plant material was cleaned, chopped into small pieces, and boiled in water at a ratio of one part plant to sixteen parts solvent. The mixture was simmered gently for 15–30 minutes with occasional stirring to promote efficient extraction, followed by filtration and pressing of the marc to recover additional extract. The filtrate was collected, stored in clean airtight containers, labeled, and refrigerated at 4 °C until further use. In the percolation method, dried and coarsely powdered plant material was moistened with ethanol or methanol and packed evenly in a sterilized percolator lined with filter paper. Solvent was added to the reservoir and allowed to percolate through the material under gravity at a controlled rate. The

percolate was collected until sufficient extract was obtained, filtered to remove particulates, and stored in sterile airtight containers at 4 °C.^[8-11]

Determination of in-vitro microbial activity by agar well diffusion assay

The antimicrobial activity of the extracts was determined using the agar well diffusion method. Nutrient agar plates were prepared and inoculated with microbial cultures to produce a uniform lawn. Wells were aseptically bored into the agar and filled with 100 µL of the plant extracts, while solvent alone served as the negative control. Plates were incubated at 37 °C for 16–24 hours, and the diameter of inhibition zones was measured in millimeters to assess antimicrobial efficacy.^[12-16]

Results And Discussion

Extractive Yields

The extractive yield is a vital parameter in pharmacognosy, as it provides insights into the efficiency of solvent systems and

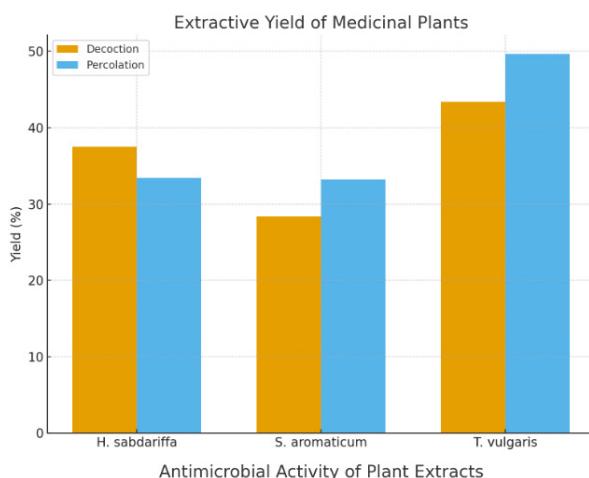


Figure 1: Extractive Yields

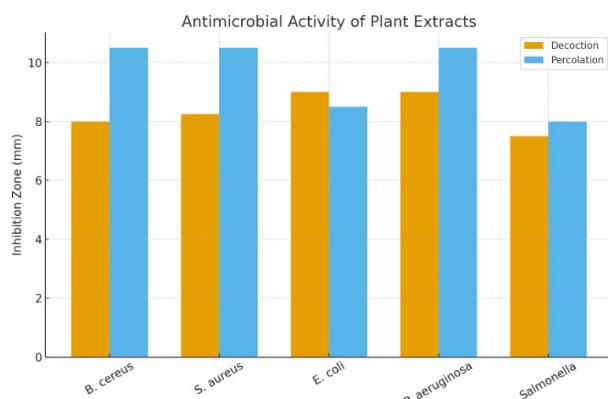


Figure 2: Antimicrobial Activity of Plant Extract

extraction techniques in recovering phytoconstituents from medicinal plants. In this study, decoction and percolation were employed to obtain extracts from *Hibiscus sabdariffa*, *Syzygium aromaticum*, and *Thymus vulgaris* (Figure 1).

The extractive yield values from the decoction method (Table 1) revealed moderate recovery across the three plants. *Hibiscus sabdariffa* produced an average yield of 37.5%, *Syzygium aromaticum* showed a lower yield of 28.4%, while *Thymus vulgaris* demonstrated the highest recovery at 43.4%. These variations reflect differences in the solubility of phytoconstituents in hot aqueous medium and the structural characteristics of plant materials. The relatively lower yield of *Syzygium aromaticum* could be attributed to the volatility or partial degradation of its essential oil constituents during heating, while the higher yield of *Thymus vulgaris* suggests the presence of water-soluble compounds that are stable at elevated temperatures.

The percolation method (Table 2) consistently resulted in higher or more stable yields compared to decoction. *Hibiscus sabdariffa* showed a yield of 33.4%, which was slightly lower than decoction, whereas *Syzygium aromaticum* exhibited a significant improvement, yielding 33.2% compared to 28.4% in decoction. The most pronounced increase was observed

for *Thymus vulgaris*, which produced a yield of 49.7%, substantially higher than the 43.4% obtained by decoction. These findings demonstrate that percolation, which employs controlled solvent penetration without prolonged heating, is more effective in preserving thermolabile compounds and extracting a broader range of bioactive constituents.

Overall, the comparative analysis highlights the impact of extraction technique on yield. Decoction, while traditional and effective for certain plant parts, may lead to the loss of volatile or heat-sensitive compounds. Percolation, by contrast, provides a gentler yet efficient means of phytochemical recovery, especially for plants rich in volatile oils and phenolic compounds such as *Syzygium aromaticum* and *Thymus vulgaris*. These results support the selection of extraction methods based on the phytochemical nature of the plant material and the desired spectrum of constituents.

In-vitro Antimicrobial Activity

The antibacterial activity of *Hibiscus sabdariffa*, *Syzygium aromaticum*, and *Thymus vulgaris* extracts varied with both extraction method and bacterial strain. Decoction extracts exhibited moderate inhibition, with *S. aromaticum* showing the most consistent broad-spectrum activity, particularly against *E. coli* (9.0 mm) and *S. aureus* (8.0 mm). *H. sabdariffa* was effective against *B. cereus* (8.0 mm) and *P. aeruginosa* (9.0 mm), while *T. vulgaris* showed activity against *S. aureus* (8.5 mm) and *Salmonella* (7.5 mm). (Table 3 and Figure 2)

Antibacterial activity of different plant decoction extracts

Percolation extracts demonstrated superior activity, reflecting better preservation of bioactive phytochemicals (Table 4). *S. aromaticum* displayed strong inhibition against *S. aureus* (10.5 mm), *E. coli* (8.5 mm), and *P. aeruginosa* (10.5 mm). *H. sabdariffa* showed enhanced inhibition of *B. cereus* (10.5 mm) and *P. aeruginosa* (10.0 mm), while *T. vulgaris* was effective against *B. cereus* (10.5 mm) and moderately active against Gram-negative bacteria.

A comparison of methods revealed that percolation consistently yielded higher inhibition zones than decoction, emphasizing its efficiency in extracting thermolabile and volatile compounds. Gram-positive bacteria (*B. cereus*, *S. aureus*) were generally more susceptible than Gram-negative strains, although notable sensitivity of *P. aeruginosa* to percolation extracts indicates the potential of certain phytochemicals to penetrate Gram-negative cell walls. Overall, *S. aromaticum* demonstrated the strongest and broadest antibacterial potential across both methods, while *H. sabdariffa* and *T. vulgaris* exhibited selective activity depending on the extraction technique and pathogen.

Conclusion

The present study highlights the influence of extraction techniques on yield and antimicrobial potential of *Hibiscus sabdariffa*, *Syzygium aromaticum*, and *Thymus vulgaris*. Percolation consistently produced higher extractive yields than decoction, particularly for *Syzygium aromaticum* and

Thymus vulgaris, reflecting its ability to preserve thermolabile and volatile phytoconstituents. Antimicrobial evaluation further revealed that percolation extracts exhibited stronger and broader activity compared to decoction, with *Syzygium aromaticum* demonstrating the most potent inhibition against both Gram-positive and Gram-negative bacteria. While Gram-positive strains were generally more susceptible, the notable sensitivity of *Pseudomonas aeruginosa* to percolation extracts underscores the therapeutic promise of these plant-derived bioactives. Overall, the findings suggest that solvent-based percolation is a superior extraction method for maximizing both yield and antimicrobial efficacy, and that these medicinal plants hold significant potential for development of alternative or complementary antimicrobial agents.

Acknowledgements

The authors thank Ozone Test House for providing excellent research facilities.

Conflict of Interest

There is no conflict of interest

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How to cite this article: Choudhary M, Gaur K, Rathore MS. Antimicrobial Potential of Medicinal Plant Extracts Prepared by Decoction and Percolation Techniques. *Journal of Applied Pharmaceutical Sciences and Research*. 2025; 8(3): 22-25 Doi : 10.31069/japsr.v8i3.03