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EVALUATION OF IN VITRO ANTIBACTERIAL ACTIVITIES OF METHANOL, ETHYL ACETATE, AND AQUEOUS EXTRACTS OF *CURCUMA LONGA*, *ALBIZIA LEBBECK*, AND *VITEX NEGUNDO* AGAINST REPRESENTATIVE BACTERIAL ISOLATES

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ABSTRACT

The emergence of antibiotic-resistant bacterial strains has necessitated the search for alternative antimicrobial agents. This study investigates the in vitro antibacterial activity of methanol, ethyl acetate, and aqueous extracts of *Curcuma longa*, *Albizia lebbeck*, and *Vitex negundo* against two clinically significant human pathogens: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The extracts were prepared using standard maceration and solvent-partitioning techniques using rotary evaporator, and the antibacterial efficacy was assessed using the broth dilution method to determine the minimum inhibitory concentrations (MICs). Results demonstrated that methanol and ethyl acetate extracts exhibited significant antibacterial activity, particularly against *S. aureus*, The methanolic extract of Curcuma longa (CL Methanol) exhibited the most significant inhibitory effect, reaching nearly 18% inhibition at 200 µg/ml. These findings support the potential use of these plant extracts as alternative therapeutic agents for bacterial infections.

KEYWORDS: *Curcuma longa*, *Albizia lebbeck*, *Vitex negundo*, antibacterial activity, *E. coli*, *S. aureus*, broth dilution, MIC.

INTRODUCTION

The alarming rise of antimicrobial resistance among pathogenic bacteria poses a significant threat to global public health, leading to increased morbidity, mortality, and healthcare costs. Escherichia coli and Staphylococcus aureus are two of the most prominent bacterial pathogens responsible for a wide array of infections, ranging from urinary tract and respiratory infections to wound and bloodstream infections. Medicinal plants have long been recognized for their rich content of bioactive compounds with potential antimicrobial properties. Ayurveda, has relied on herbal remedies for centuries to treat infections and inflammation. The present study focuses on three medicinally important plants:- Curcuma longa (turmeric), Albizia lebbeck (siris), and Vitex negundo (nirgundi), all of which are known in Ayurvedic literature for their health-promoting properties. Curcuma longa contains curcuminoids and essential oils with documented antimicrobial, anti-inflammatory, and antioxidant activities. Albizia lebbeck is valued for its tannins, flavonoids, and saponins, which are associated with antimicrobial and immunomodulatory properties. Vitex negundo is rich in flavonoids and lignans that contribute to its broad-spectrum antimicrobial efficacy. The choice of solvent plays a crucial role in the extraction of phytochemicals, as different solvents extract different classes of compounds based on polarity. Methanol and ethyl acetate are known to extract a wide range of phenolic compounds and other polar to semi-polar phytoconstituents, while aqueous extraction is more effective for hydrophilic compounds. By evaluating the extracts from these three solvents, this study aims to identify the most effective solvent system and plant species combination for antibacterial activity. This research evaluates the in vitro antibacterial potential of methanol, ethyl acetate, and aqueous extracts of Curcuma longa, Albizzia lebbeck, and Vitex negundo against Escherichia coli and Staphylococcus aureus, utilizing the broth dilution method to determine minimum inhibitory concentrations (MICs). The outcome of this study is expected to provide valuable insights into the development of plant-based antimicrobials that can serve as complementary or alternative therapies against resistant bacterial strains.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation.

Fresh rhizomes of Curcuma longa, bark of Albizia lebbeck, and roots of Vitex negundo were collected from authenticated herbal gardens and local sources. The plants were taxonomically identified and authenticated by a qualified botanist, after that each sample was Washed with

distilled water to remove dirt and Shade-dry until completely moisture-free. At last, Powdered the dried plant material using a grinder.[1]

2.2 Extraction Procedure

Approximately 50 grams of each dried and powdered plant material was weighed using an analytical balance. The powder was soaked in 200 ml of solvent methanol in clean glass stoppered conical flasks. After maceration, the mixtures were first filtered through Whatman No. 1 filter paper to obtain clear filtrates. The methanolic filtrates were subjected to solvent evaporation using a rotary evaporator under reduced pressure at 40°C, This step efficiently removed the organic solvents,[2] and for uninterrupted feed input and distillate collection the Continuous Mode Operation of Rotary Evaporator was used which lead to -yielding semi-solid concentrated methanolic crude extracts of albizzia lebbeck (sirish) and vitex negundo (nirgundi). Lyophilization was used to helps retain curcuminoids, volatile oils, and color more effectively than heat drying methods, making it suitable for high-quality turmeric extract preparations.[3,4] methanol extracts of each drug is measured by laboratory balance. 2898 mg of vitex negundo, 1049mg of curcuma longa, 3870 mg of albizzia lebbeck were procured. Transferred the methanol extract (diluted with distilled water) into a glass container, Placed the container into the ultrasonic bath. After ultrasonic cleaning, Partitioned the methanolic extracts of each sample into semi-polar (ethyl acetate fraction) and aqueous components using a separating funnel[5,6], It allows for the liquid-liquid extraction of specific compounds from mixtures and is commonly used in plant extraction protocols to isolate bioactive components. after that Used a rotary evaporator to Remove ethyl acetate under reduced pressure and Obtained the semi-polar extract (ex. rich in curcuminoids like curcumin, dimethoxy curcumin, etc.). aqueous fraction took longer than ethyl acetate, All dried extracts of each sample stored at 4°C in airtight containers.

The chemical composition

1. Harida (curcuma longa)- curcumin, turmeric oil, essential oil[7,8]
2. Sirish (albizia lebbeck)- saponins, tannins[9]
3. Sinduvara (vitex negundo)-organic acid, malic acid, traces of alkaloids, essential oil[10]

2.3. Antimicrobial Microdilution Assay Protocol Using Plant Extracts - preparation of inoculum

Luria-Bertani (LB) broth, a nutrient-rich medium, was prepared by dissolving 25 grams of LB powder in 100 ml of distilled water, followed by sterilization through autoclaving at

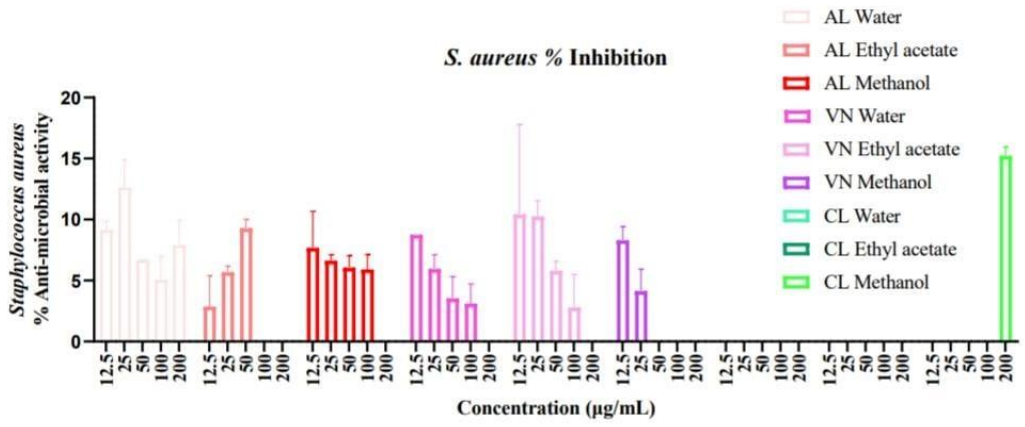
121°C for 20 minutes.[11] Once cooled, the medium was stored at 4°C until further use. For long-term bacterial preservation, glycerol stocks were prepared by mixing 10 ml of pure glycerol with 10 ml of distilled water, which was subsequently autoclaved and stored at 4°C. Overnight cultures were established by inoculating 10 ml of LB broth in 50 ml sterile falcon tubes with 100 µl of glycerol stock of either S. aureus or E. coli. The inoculated tubes were incubated at 37°C for approximately 18 hours and were then stored at 4°C. Working cultures were prepared by transferring 100 µl of the overnight bacterial culture into 10 ml of fresh LB broth.[12]

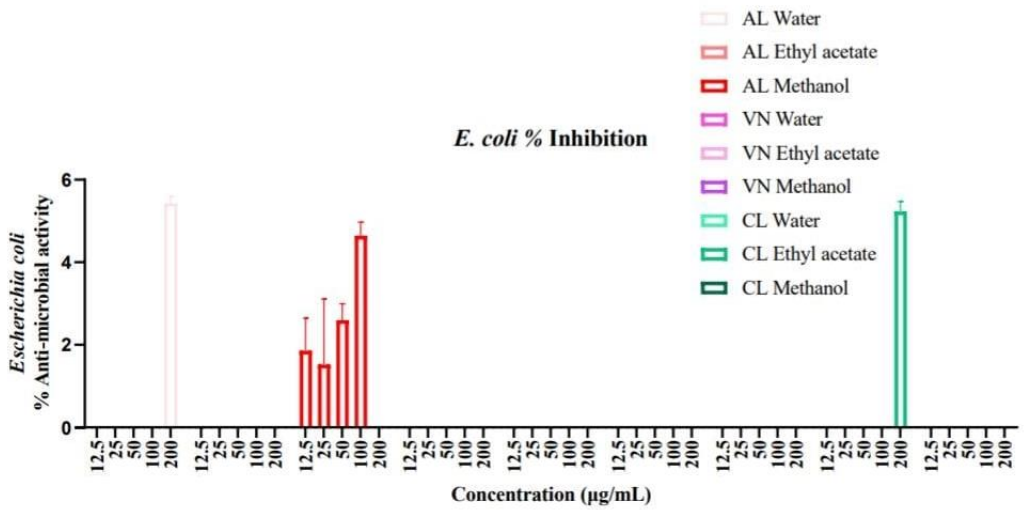
Antimicrobial screening

96-well microtiter plates were used, with each well receiving 170 µl of LB broth, 20 µl of the working culture, and 10 µl of the plant extract dissolved in 1% DMSO(dimethyl sulfoxide).[13] The plant extract stock solutions were initially prepared at 200 µg/ml and serially diluted to obtain final concentrations of 12.5, 25, 50, 100, and 200 µg/ml. To ensure accurate dosing, dilutions were adjusted to deliver a constant volume of 10 µl per well. Each assay included a blank control (200 µl LB only) and a negative control (180 µl LB with 20 µl bacterial culture, no plant extract). After setting up the plate, it was incubated at 37°C for 18 hours with shaking at 160 rpm.

Determination of minimum inhibitory concentrations

Following incubation, the optical density (OD) of each well was measured at 600 nm using a spectrophotometer. The absorbance data were used to calculate the percentage viability of bacterial cells in the presence of various concentrations of plant extracts, relative to the control. This method allowed for the determination of the Minimum Inhibitory Concentration (MIC), providing insight into the antimicrobial potential of the tested plant extracts.[14]





Graph- % inhibition of plant extracts against staphylococcus aureus and Escherichia coli.

RESULTS

Plant Extract	Solvent	Bacteria	Concentration (µg/mL)	% Inhibition (Approx.)
Albizia lebbeck	Water	*S. aureus*	12.5–200	4–8%
		E. coli	12.5–200	5% at 100 µg/mL
	Ethyl Acetate	*S. aureus*	12.5–200	4–7%
		E. coli	12.5–200	1–4%
	Methanol	*S. aureus*	12.5–200	6–9%
		E. coli	12.5–200	2–5%
Vitex negundo	Water	*S. aureus*	12.5–200	6–10%
	Ethyl Acetate	*S. aureus*	12.5–200	4–8%
	Methanol	*S. aureus*	12.5–200	5–9%
Curcuma longa	Water	*S. aureus*	12.5–200	4–7%
	Ethyl Acetate	*S. aureus*	12.5–200	6–10%
	Methanol	*S. aureus*	12.5–200	10–18% (↑ at 200 µg/mL)
		E. coli	12.5–200	5–6% at 200 µg/mL

The antimicrobial activities of the different plant extracts were quantified by assessing the percentage inhibition of Staphylococcus aureus and Escherichia coli at concentrations ranging from 12.5 to 200 µg/ml. The graphical data (Figure 1) illustrates the comparative inhibitory potential of aqueous, ethyl acetate, and methanol extracts of Albizia lebbeck (AL), Vitex negundo (VN), and Curcuma longa (CL).

Against S. aureus

The methanolic extract of Curcuma longa (CL Methanol) exhibited the most significant inhibitory effect, reaching nearly 18% inhibition at 200 µg/ml. This was followed by

Crucuma longa Ethyl Acetate and VN (vitex negundo) Methanol, both of which showed moderate inhibition. Water extracts across all three species showed comparatively weak antibacterial effects, with inhibition percentages typically under 10%.

Against E. coli

A distinct trend was observed with fewer extracts showing notable inhibition. Only AL (albizzia lebbeck) Water and CL (curcuma longa) Methanol demonstrated any appreciable antibacterial activity, with CL(curcuma longa) Methanol achieving approximately 5% inhibition at 200 µg/ml. Other extracts, especially ethyl acetate and water extracts of VN(vitex negundo) and CL (curcuma longa), had negligible effects.

DISCUSSION

The data suggests a clear solvent-dependent and plant-specific antibacterial efficacy. Methanol was the most effective extraction solvent, likely due to its ability to dissolve a wide range of bioactive compounds, including curcuminoids in Curcuma longa, flavonoids, and alkaloids in Vitex negundo, and saponins and tannins in Albizia lebbeck.

Potent Action of CL (curcuma longa) Methanol

The highest inhibition seen with Curcuma longa methanolic extract against S. aureus suggests a strong presence of curcumin and other phenolic compounds with known antibacterial activity. The polar nature of methanol allows efficient extraction of such compounds, thus enhancing antimicrobial efficacy.

Selective Efficacy

While methanolic extracts of CL (curcuma longa) and AL (albizzia lebbeck) exhibited moderate activity against E. coli, most extracts were significantly more effective against S. aureus. This can be attributed to the structural differences between Gram-positive and Gram-negative bacteria. The outer membrane in E. coli acts as a permeability barrier, reducing susceptibility to many plant-based compounds.

Aqueous Extracts

Despite being traditionally used in herbal preparations, aqueous extracts showed minimal activity in vitro, reinforcing the importance of solvent polarity in extracting antimicrobial phytochemicals.

Solvent Influence

Ethyl acetate extracts demonstrated some intermediate activity against S. aureus but negligible effect against E. coli, highlighting the moderate polarity of ethyl acetate which may extract a different profile of compounds than methanol.

CONCLUSION

This study reveals that methanol extracts, particularly from Curcuma longa, possess noteworthy antibacterial activity against S. aureus, and to a lesser extent, E. coli. The data emphasize the importance of extraction solvent in determining the antibacterial potency of phytochemicals. These findings validate the ethnopharmacological use of these herbs and suggest their potential in the development of plant-based antibacterial agents. However, the limited activity against E. coli also underscores the necessity for further compound isolation, synergistic formulation, or nano formulation approaches to enhance spectrum and potency.

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