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Antibacterial activity of Moringa oleifera against selected clinical pathogens.

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ABSTRACT

This study focus on the antibacterial potential of Moringa oleifera leaf extracts prepared using distilled water, ethanol, and chloroform against four clinically important bacterial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp. Phytochemical screening of the extracts revealed the presence of various bioactive compounds, including, flavonoids, alkaloids, saponins, tannins, and terpenoids, with their presence varying based on the solvent used. Antibacterial activity of extract was evaluated using the agar well diffusion method on Muller Hinton Agar (MHA). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined through broth dilution and sub-culturing methods. The methanol extract exhibited highest antibacterial activity, showing a 20 mm zone of inhibition against Staphylococcus aureus with MIC of 0.3 mg/ml and MBC of 1.25 mg/ml followed by aqueous and chloroform extracts with moderate activity.

Keywords: Moringa oleifera, Agar well diffusion, Muller Hinton agar..

INTRODUCTION

Historically, plants have served as fundamental sources of natural compounds for promoting health in both humans and animals. Plants contain a wide array of bioactive chemicals that offer both preventive and therapeutic health benefits (Nascimento et al., 2000). According to global estimates, approximately 80% of the population relies on traditional plant-based remedies for healthcare (Igbinosa et al., 2009). Despite advancements in pharmaceutical development, plants remain the cornerstone of natural medicine production (Mahomed et al., 2006).

Moringa oleifera, a fast-growing deciduous tree, is widely cultivated across tropical and subtropical regions in Asia, Africa, and the Middle East, where it is traditionally valued for its nutritional and medicinal properties (Lei Wang et al., 2016). Owing to the utility of its roots, leaves, flowers, seeds, and pods in nutrition, therapeutics, skincare, and water purification, M. oleifera is often regarded as a highly versatile and resourceful plant (Anwar et al., 2007). Widely known for its health and dietary significance, Moringa oleifera harbors a wealth of bioactive compounds that exhibit diverse pharmacological effects.

Traditionally, it has been employed in managing conditions such as skin diseases, high blood pressure, diabetes, parasitic infestations, and malaria (Anwar et al., 2007). Natural products like Moringa oleifera are increasingly explored as alternatives to conventional antibacterial therapies. Extracts from M. oleifera leaves have shown promise in managing bacterial infections, either as standalone treatments or synergistically with antibiotics (Mazumder et al., 2018,Momin et al., 2023)

Among its many properties, Moringa oleifera is particularly recognised for its potent antimicrobial potential. Research indicates that all plant parts—leaves, flowers, bark, roots, and seeds—possess antimicrobial actions, including antibacterial, antifungal, antiviral, and antiparasitic effects (Lei Wang et al., 2016). M. oleifera leaves exhibit a spectrum of biological activities including antimicrobial, antihypertensive, antidiabetic, anti-inflammatory, and anticancer effects. Crude extracts from different M. oleifera tissues demonstrate varying degrees of antibacterial efficacy. Several bioactive compounds with antibacterial and antioxidant attributes have been isolated from various parts of Moringa oleifera (Anwar et al., 2007). Phytochemical analysis has shown that M. oleifera leaves contain numerous antimicrobial agents such as alkaloids, flavonoids, tannins, triterpenes, polyphenols, and other secondary metabolites (Mazumder et al., 2018, Adeyinka et al., 2019).

MATERIALS AND METHODOLOGY

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Collection of plant materials:

Mature leaves of Moringa oleifera were harvested from local areas within Malappuram district, Kerala, India. The collected leaves were thoroughly rinsed with running tap water, air-dried in the shade for seven days, and then manually ground into a fine powder using a mortar and pestle. The powdered material was stored in airtight containers for subsequent use

Preparation of leaf extract:

Ten grams of dried *Moringa oleifera* leaf powder were individually combined with distilled water, chloroform, and ethanol in separate conical flasks. These mixtures were placed on a rotary shaker and agitated continuously for 24 to 48 hours to ensure optimal extraction of bioactive compounds. After 48 hours, the solutions were filtered through Whatman No.1 filter paper. The resulting filtrates were evaporated, weighed, re-dissolved in dimethyl sulfoxide (DMSO), and stored at low temperatures for later analysis.

Collection and identification of test organisms:

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* species were collected from a local hospital microbiology lab. The bacterial strains were preliminarily identified through their colony characteristics, Gram staining, and a series of biochemical assays, including IMViC reactions, catalase activity, urease test, triple sugar iron (TSI) agar reaction, and hydrogen sulfide production.

Phytochemical Analysis:

Preliminary phytochemical tests were conducted to evaluate the presence of various bioactive secondary metabolites in the leaf extracts.

Alkaloid detection (Wagner's test and Dragendorff's test):

To detect alkaloids, 1 mL of the extract was treated with a few drops of Dragendorff's reagent. The appearance of a reddish-brown precipitate indicated a positive result (Patel *et al*., 2014). Similarly, alkaloid presence was confirmed by mixing 1 mL of the extract with Wagner's reagent, which also produced a reddish-brown precipitate (Santhi *et al*., 2016).

Flavonoids detection (Alkaline reagent test)

For flavonoid detection, 1 mL of the extract was combined with sodium hydroxide solution. The development of a yellow coloration, which faded upon adding dilute hydrochloric acid, indicated the presence of flavonoids (Sudha *et al*., 2021).

Phenolics and Tannins:

To detect phenolic compounds and tannins, 1 mL of extract was reacted with 5% ferric chloride. A blue-black or greenish coloration signified a positive result. In a separate test, 1 mL of extract was treated with 1% lead acetate. The formation of a yellow precipitate confirmed the existence of tannins.

Saponins detection (Foam test):

The presence of saponins was assessed by vigorously shaking 1 mL of extract with 5 mL of distilled water. Stable foam formation lasting over ten minutes indicated a positive result (Santhi *et al*, 2016).

Terpenoids detection:

To test for terpenoids, 1 mL of extract was mixed with 2 mL of chloroform and filtered. Concentrated sulfuric acid was then added to the filtrate, with a golden-yellow coloration confirming terpenoid presence (Sudha *et al*., 2021).

Antibacterial Activity Assay

The antibacterial potential of the extracts was evaluated using the agar well diffusion method. Mueller-Hinton Agar (MHA) plates were uniformly inoculated with bacterial suspensions standardized to the 0.5 McFarland turbidity standard. Using a sterile cork borer, wells with a diameter of approximately 4 mm were punched into the agar. Each well was filled with 50 µL of the respective plant extract. The plates were then incubated at 37°C for a period of 24 hours. Following incubation, the diameters of the clear zones surrounding each well were measured in millimetres to assess the extent of bacterial inhibition.

Minimum Inhibitory Concentration (MIC)

To determine the MIC values, the extracts were subjected to serial two-fold dilutions in sterile peptone broth, producing final concentrations of 2.5 mg/mL, 1.25 mg/mL, 0.6 mg/mL, 0.3 mg/mL, and 0.15 mg/mL. Equal volumes (2 mL each) of the diluted extract and broth were dispensed into sterile test tubes. Each tube was inoculated with 0.1 mL of a bacterial suspension adjusted to match the 0.5 McFarland standard. Tubes containing only broth and bacterial inoculum served as positive controls, while those containing only broth and extract without bacteria acted as negative controls. All tubes were incubated at 37°C for 24 hours. MIC was determined by identifying the lowest concentration of the extract that showed no visible turbidity or bacterial growth (Eloff,

1998; CLSI, 2012).

Minimum Bactericidal Concentration (MBC)

After determining MIC, aliquots from the test tubes that exhibited no visible bacterial growth were streaked onto nutrient agar plates. These plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the extract at which no bacterial colonies were observed on the agar surface, indicating complete bacterial killing (Abalaka *et al.*, 2012).

Results

Phytochemical Screening

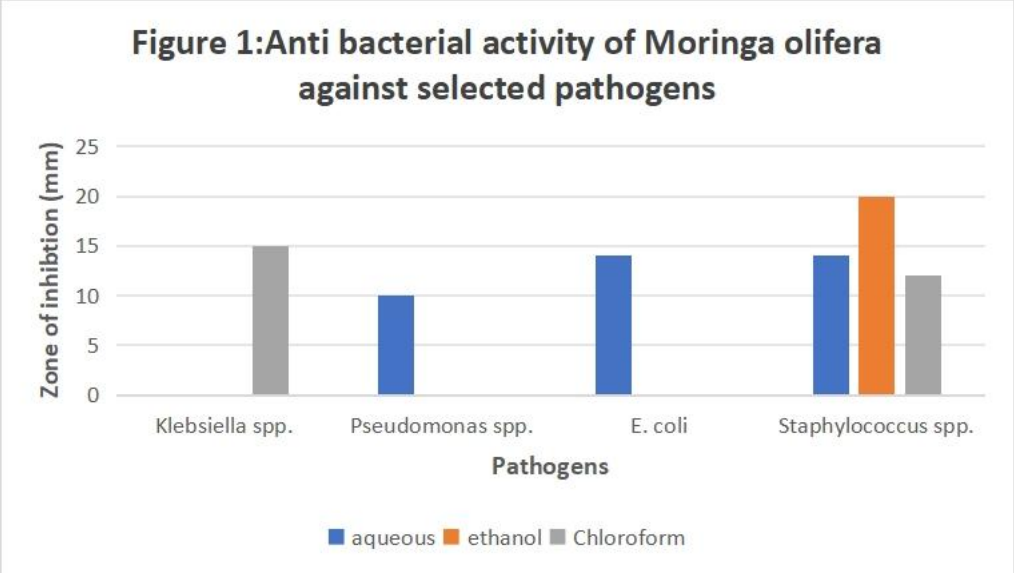
Test	Distilled Water	Chloroform	Methanol
Alkaloids	Positive	Positive	Negative
Flavonoids	Positive	Positive	Positive
Tannins	Negative	Negative	Positive
Saponins	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive

Table :1 Phytochemical results of extracts in different solvents

Antibacterial Activity

Organism	Aqueous Extract	Methanol Extract	Chloroform Extract	Positive Control (gentamycin)
Klebsiella spp.	R	R	15 mm	18mm
Pseudomonas spp.	10 mm	R	R	14mm
E. coli	14 mm	R	R	18mm
Staphylococcus spp.	14 mm	20 mm	12 mm	24mm

Table 2: Zone of inhibition shown by different extract against selected clinical pathogens



Minimum Inhibitory Concentration

Organism	Aquous extract	Chloroform extract	Methanol extract
Klebsiella spp.	-	0.6mg/ml	-
Pseudomonas spp.	0.6mg/ml	-	-
E. coli	0.3mg/ml	-	-
Staphylococcus spp.	0.6mg/ml	0.6mg/ml	0.3mg/ml

Table 3:Minimum inhibitory concentration (MIC) of different extract of *M.oleifera*

Minimal Bactericidal Concentration

Organism	Aquous extract	Chloroform extract	Ethanol extract
Klebsiella spp.	-	2.5mg/ml	-
Pseudomonas spp.	2.5mg/ml	-	-
E. coli	1.25mg/ml	-	-
Staphylococcus spp.	2.5mg/ml	2.5mg/ml	1.25mg/ml

Table 4 : Minimum bactericidal concentration of different extract of *M.oleifera*

Discussion

The analysis of *Moringa oleifera* leaf extracts revealed notable variations in phytochemical content and antibacterial efficacy depending on the extraction solvent used. Methanol proved to be the most effective medium for extracting active compounds, yielding strong antibacterial responses—particularly against *Staphylococcus aureus*, which showed a 20 mm zone of inhibition. In contrast, the chloroform extract showed moderate efficacy against *Klebsiella* spp., while the aqueous extract demonstrated limited activity against *E. coli* and *S. aureus*. Minimum inhibitory concentration (MIC) tests reinforced these findings, with the methanol extract achieving the lowest MIC of 0.3 mg/mL for both *S. aureus* and *E. coli*, indicating strong bacteriostatic potential. Additionally, the methanol extract exhibited a bactericidal effect at a minimum bactericidal concentration (MBC) of 1.25 mg/mL. Other extracts showed relatively higher MIC and MBC values, suggesting reduced potency. Overall, the data indicate that methanol is the most effective solvent for isolating bioactive antimicrobial agents from *M. oleifera* leaves, supporting its potential role in plant-based therapeutic development.

Freshly harvested *Moringa oleifera* leaves were examined for their antibacterial potential against selected clinical pathogens. Phytochemical analysis (Table 1) indicated the presence of multiple bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids. Notably, tannins were detected only in certain solvent systems, reflecting variations in their solubility profiles. Alkaloids were found in both aqueous and chloroform extracts, but were absent in the ethanol-derived sample. On the other hand, flavonoids, saponins, and terpenoids were consistently present across all solvents, while tannins appeared exclusively in the ethanol extract. These observations align with prior studies by Bhattacharjee et al. (2012) and Patel et al. (2014), which emphasized that the efficiency of compound extraction depends heavily on the polarity of the solvent used. Similar trends were reported by Jain et al. (2022), who highlighted that polar solvents like ethanol and methanol are particularly effective in isolating flavonoids and tannins.

The antimicrobial activity of the extracts (Table 2, Figure 1) evaluated through the agar well diffusion method, revealed varying levels of activity depending on both the extract type and the bacterial strain tested. Among the solvents used, methanol extract demonstrated the strongest antibacterial effect, particularly against *Staphylococcus aureus*, where it produced a 20 mm inhibition zone. Chloroform extract exhibited moderate effectiveness against *Klebsiella* spp. with a 15 mm zone, while the aqueous extract showed similar inhibition (14 mm) against *E. coli* and *S. aureus*. These findings are consistent with those of Singh et al. (2013) and Tabish et al. (2022), who observed superior antimicrobial properties in methanolic extracts of *M. oleifera* compared to other solvent types. The greater sensitivity of *S. aureus*, a Gram-positive bacterium, also supports earlier work by Khurshid et al. (2020), which suggested that the less complex cell wall structure of Gram-positive bacteria makes them more vulnerable to phytochemicals.

The strong inhibition of *S. aureus* by the methanol extract observed in this study was comparable to that of the reference antibiotic gentamycin, which recorded a 24 mm inhibition zone. This finding reinforces the extract's pharmacological relevance. The high efficacy of methanol extract is likely due to its capability to solubilize a broader spectrum of antimicrobial compounds, including flavonoids and phenolics. These compounds are known to interfere with bacterial cell processes such as DNA synthesis, membrane integrity, and enzyme function (Amaeze et al., 2022).

Broth dilution assays further substantiated the antibacterial potency of the extracts. The methanol extract had the

lowest MIC of 0.3 mg/mL against both *S. aureus* and *E. coli*, indicating strong growth-inhibitory properties. Additionally, it exhibited a minimum bactericidal concentration (MBC) of 1.25 mg/mL against *S. aureus*, confirming its bactericidal potential. These results are notably more effective than those reported by Genemo (2021), who recorded MIC values between 1.25 and 5 mg/mL for *Moringa* extracts, suggesting that the extract used in this study may have higher bioactivity, possibly due to variations in plant maturity, geography, or extraction protocols.

The chloroform extract demonstrated moderate effectiveness with MIC and MBC values (table 3 & table 4) of 0.6 mg/mL and 2.5 mg/mL respectively, particularly against *Klebsiella* spp. and *S. aureus*. Meanwhile, the aqueous extract displayed MIC values of 0.3 mg/mL against *E. coli* and 0.6 mg/mL for *S. aureus* and *Pseudomonas aeruginosa*, suggesting partial efficacy. However, its lack of effect against *Klebsiella* spp. points to a narrower antimicrobial spectrum, potentially due to limited solubility of certain active compounds in water. The relatively higher MIC and MBC values of the aqueous and chloroform extracts compared to the methanol extract highlight the importance of choosing appropriate solvents for maximizing antimicrobial extraction, as also emphasized by Parveen (2022) and Van de Berg et.al (2022).

Overall, polar solvents like methanol were found to be more efficient in extracting antibacterial constituents such as phenolics, alkaloids, and flavonoids. These molecules exert antimicrobial effects through various mechanisms, including the destabilization of bacterial membranes, interference with DNA replication, and inhibition of critical metabolic enzymes (Raibulu *et al*., 2020).

In conclusion, this study validates the significant antibacterial activity of *Moringa oleifera* leaf extracts, particularly those derived using methanol. The correlation between phytochemical composition and bioactivity underscores the therapeutic relevance of specific secondary metabolites. The pronounced effects against *S. aureus* and *E. coli*, along with favorable MIC and MBC values, suggest that methanolic extracts may serve as effective candidates for the development of alternative, plant-based antimicrobial agents. These results reinforce and expand upon previous findings in the field, indicating strong potential for further pharmaceutical exploration.

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