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Assessment of Moringa oleifera (Lam) Leaf Extracts for Their Antibacterial Efficacy Against Staphylococcus aureus Bacteria

Priyanka Tiwari, Rashmi Mishra
SAM Global University, Bhopal, India
SAM Girls College Bhopal, India

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Abstract- This study aimed to evaluate the antibacterial activity of Moringa oleifera (Lam.) leaf extract from the Moringaceae family against selected bacteria using the agar well diffusion method. Nutrient Agar (NA) media was prepared following the manufacturer's instructions. Sterile agar plates were inoculated with the test culture by surface spreading using sterile wire loops, ensuring uniform distribution. Concentrations of 30, 60, 90, and 120 mg/ml, derived from dry leaf powder, were utilized for antibacterial analysis via agar well incorporation. Plates were prepared and allowed to solidify on Petri dishes, then seeded with the test bacterium. Four holes were made in each plate using a sterile 2.0 mm diameter cork borer. Each hole was filled with a specific extract concentration and plain sterile agar. The plates were incubated at 37°C for 24 hours, and the diameters of inhibition zones were measured using a meter rule. The aqueous, ethanol, and methanol extracts of Moringa leaves exhibited inhibitory effects on the growth of tested bacteria. Specifically, the inhibitory effect on Escherichia coli was significantly higher ($P<0.05$) than Staphylococcus aureus for aqueous, ethanol, and methanol extracts. Moreover, ethanol and methanol extracts demonstrated significantly higher ($P<0.05$) inhibitory effects at 120 mg/ml concentrations. The powder derived from Moringa leaves showed promising antibacterial activity against the tested gram-positive bacterium, Staphylococcus aureus.

Keywords: Antibacterial, Moringa oleifera, Staphylococcus aureus

1. INTRODUCTION

The Moringa plant has been a staple in human diets for centuries, with its various parts utilized for their nutritional value, medicinal benefits, and culinary appeal [1]. Culturally, almost every part of the plant finds its way into recipes, offering taste, flavour, and health benefits. Particularly, the leaves of M. oleifera are versatile—they can be consumed fresh, cooked, or stored as a dried powder for extended periods without significant loss of nutritional potency [2, 3]. Research indicates that M. oleifera leaves are rich in nutrients and possess a wide array of health-promoting properties, including anti-tumour, anti-inflammatory, anti-ulcer, anti-atherosclerotic, and anti-convulsant activities [4, 5, 6]. Exploring plants' antimicrobial properties has garnered global attention, with many researchers investigating their potential as therapeutic alternatives [7]. Plants contain numerous secondary metabolites, such as alkaloids and phenolic compounds, contributing to their medicinal properties. This study explores the antibacterial activity of M. oleifera against S.

aureus, P. aeruginosa, and E. coli. The aim is to identify cost-effective and safe remedies for human health issues.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Leaves from the Moringa oleifera tree were gathered from Shivlok phase 4, Khajurikalan Road, Bhopal. Only healthy, uninfected plants were selected for the collection. The leaves were meticulously washed under running tap water to remove dust or foreign particles and then thoroughly dried. Dr. Pramod Patil conducted the botanical identification of the plant at the Botany Department of SAM Global University. Plant parts were collected for identification, including leaves, stems, flowers, seeds, fruits, and roots.

2.2 Drying and Storage of Plant Material

The collected leaves were air-dried under shade and ground into a fine powder using a pestle and mortar. The powdered leaves were then sieved and stored in polythene bags for further analysis.

2.3 Preparation and Extraction of Leaf Extracts

1. Aqueous Leaf Extract:- Fifty grams (50g) of powdered leaves were mixed with 400 ml of distilled water in a 500 ml conical flask. The mixture was allowed to stand for 12 hours with intermittent shaking every 30 minutes. The resulting extract was filtered using Whatman No.1 filter paper and then concentrated at 40°C under reduced pressure using an evaporator. The semi-solid residue obtained was stored in a refrigerator for future use.

2. Ethanol Leaf Extract:- Fifty grams (50g) of powdered leaves were combined with 200 ml of ethanol in a 500 ml conical flask. Similar to the aqueous extraction method, the mixture was left to stand for 12 hours with periodic shaking. After filtration and concentration, the resulting extract was stored for further analysis.

3. Methanol Leaf Extract:- Fifty grams (50g) of powdered leaves were mixed with 200 ml of methanol in a 500 ml conical flask. The extraction process followed the same procedure as described for the ethanol extract.

The concentrated extracts were labelled as follows: MLAE (Moringa Leaves Aqueous Extract), MLEE (Moringa Leaves Ethanol Extract), and MLME (Moringa Leaves Methanol Extract). These crude extracts were utilized for antibacterial analyses.

2.4 Preparation of Culture Media

Nutrient Agar (NA) was prepared according to the manufacturer's instructions. Thirty-five grams (35g) of media were mixed with one litre of distilled water and autoclaved at 121°C for 15 minutes. The sterile agar plates were poured and allowed to solidify. After incubating the plates for 24 hours at 37°C to confirm sterility, they were ready for use.

2.5 Antibacterial Assay

Bacterial cultures, including Staphylococcus aureus, were obtained from the Microbiology Laboratory and grown on Nutrient Agar (NA) media. The antibacterial activity of the plant extracts (aqueous, ethanolic, and methanolic) was tested using the agar well diffusion assay. Concentrations of 30, 60, 90, and 120mg/ml were prepared from the dry leaf powder for antibacterial analysis. Each plate was seeded with the test bacterium, and four holes were made using a sterile 2.0 mm diameter cork borer. The extract mixed with plain sterile agar was added to each hole. After incubation at 37°C for 24 hours, the diameters of inhibition zones were

measured, and the mean value for each organism was recorded.

2.6 Statistical Analysis of Data

Data were presented as mean ± standard deviation. Analysis of Variance (ANOVA) test was conducted to determine significant differences between extracts and the length of incubation.

3. RESULTS AND DISCUSSION

The study aimed to evaluate the antibacterial activity of aqueous, ethanol, and methanol extracts of Moringa oleifera Lam. leaves. The results demonstrated that all three types of extracts exhibited inhibitory effects on the growth of the tested bacteria. Particularly noteworthy was the significantly higher inhibitory effect observed for Staphylococcus aureus with the ethanol and methanol extracts compared to the aqueous extract ($P<0.05$). Furthermore, at a higher concentration of 120mg/ml, both ethanol and methanol extracts displayed a significantly higher inhibitory effect on the tested microorganisms compared to the aqueous extract ($P<0.05$). These findings suggest that increasing the concentration of the extracts enhances their antibacterial efficacy. In this study, the activity of the extracts was compared with the standard antibiotic septrin (Co-trimoxazole). Surprisingly, the ethanol and methanol extracts of the leaves exhibited higher antibacterial activity compared to septrin. The ethanolic extract showed the highest antibacterial activity against all the tested bacteria. The agar well diffusion method was employed to assess the antibacterial activity of the extracts. Interestingly, the powder from fresh leaves dissolved in ethanol demonstrated greater antibacterial activity compared to those dissolved in water or methanol extracts. This finding suggests that preparing an extract with an organic solvent, such as ethanol, may enhance the antibacterial activity of M. oleifera Lam. leaves. The study's findings support previous research by Nair et al. [9], which also demonstrated the superior antibacterial activity of M. oleifera extracts prepared with organic solvents. Overall, the results highlight the potential of M. oleifera Lam. leaves as a natural alternative for combating bacterial infections, particularly when prepared in ethanol extracts.

Table 1. Antibacterial activity of aqueous, ethanolic, and methanolic extracts of M. oleifera leaves

Sample (Plant Extracts)	Zone of Inhibition (mm)	
	Extract Conc. (mg/ml)	Staph.
Aqueous	30	3.67 + 0.57
	60	4.33 + 0.57
	90	6.67 + 0.57
	120	7.00 + 1.00
Ethanol	30	2.67 + 0.57
	60	4.33 + 0.57
	90	6.00 + 1.00
	120	9.67 + 0.57
Methanol	30	2.33 + 0.57
	60	4.67 + 0.57
	90	6.33 + 0.57
	120	8.33 + 0.57
Septrin (Positive control)	10	6.00 + 1.50
Water (Negative control)	30%	
Values given are mean+ standard deviation of the experiments replicated three times (where n=3), Staph. – Staphylococcus aureus, - = No activity		

CONCLUSION

In conclusion, the leaf extracts of Moringa oleifera demonstrated significant antibacterial activity against the tested bacteria, surpassing the efficacy of traditional antibiotics commonly

used to treat bacterial infections. These findings suggest the potential of Moringa oleifera as a valuable source of novel antibiotic compounds. The observed effectiveness against pathogenic bacteria responsible for severe illnesses underscores the importance of exploring natural alternatives in combating bacterial infections. Further research into the bioactive compounds in Moringa oleifera leaves could lead to the development of new therapeutic agents with enhanced antibacterial properties, offering promising prospects for addressing antibiotic resistance and improving public health outcomes.

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