



EVALUATION OF ANTIBACTERIAL POTENTIAL OF STEM BARK OF MORINGA OLEIFERA LAM

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Moringa oleifera

Antimicrobial activity

Minimum inhibitory concentration

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ABSTRACT

The present investigation was undertaken to evaluate *in vitro* antimicrobial activities of different extracts (methanol, hexane, benzene and aqueous) of stem bark extracts of *M. oleifera*. *In vitro* antimicrobial efficacy of *M. oleifera* was assessed by disc diffusion method against pathogenic bacteria such as Gram positive - *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) and Gram negative- *Proteus vulgaris* (ATCC 2027) and *Escherichia coli* (ATCC 25922). The methanol extract exhibited highest zone of inhibition against *S. aureus* (15.0 ± 0.0 mm) with low MIC values (0.78 mg/mL). However, none of activity is shown by aqueous extract against *P. vulgaris*. Gram positive bacteria showed greater susceptibility as compared to gram negative bacteria. This may attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane. Results of the present investigation indicate that *Moringa oleifera* possess compounds with antimicrobial properties and hence can be exploited for future natural plant based antimicrobial agents.

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INTRODUCTION

Plant-based drugs have been used worldwide in traditional medicines system for the treatment of various diseases. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs (Dobriyal and Narayana, 1998). Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Venkata Rao, 2000). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Bandow *et al.*, 2003).

Moringa oleifera (Moringaceae) is commonly known as Drumstick tree, found throughout India in tropical and subtropical region. Traditionally it is used against asthma, ulcers, piles and various nervous diseases (Kirtikar and Basu, 1980). The various extracts of leaves, seeds and roots have extensively been studied for its wound healing, anti-tumour, anti-hepatotoxic, anti-fertility and anti-inflammatory properties (Caceres *et al.*, 1991; Sabale *et al.*, 2008).

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Scazzocchio *et al.*, 2001). For a long period of time, plants have been used because of their antimicrobial traits, which are due to compounds known by their active substances which may represent new source of anti-microbial with stable, biologically effective components that can establish a scientific base for the use of plants in modern medicine (Kelmanson, *et al.*, 2000; Ahmad and Beg, 2001). In the present investigation, the antimicrobial potential of *M. oleifera* stem bark extracts has been evaluated against common pathogens.

MATERIALS AND METHODS

Plant material

Plants of *M. oleifera* were collected from the campus of University of Rajasthan, Jaipur. The plant was identified and voucher specimen of each of them was deposited to the Herbarium, Botany Department, University of Rajasthan, Jaipur (RUBL NO 20624). The various plant parts (stem bark) of *M. oleifera* were separated, washed with running water to remove dust and shade dried.

Preparation of extracts

The powdered stem bark (500 g) of *M. oleifera* was used to prepare extract with methanol, benzene and hexane using Soxhlet's apparatus for 12-14 hr on a water bath separately. The organic extracts were separately filtered with Whatmann No. 1 filter paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction is performed by using hot water maceration. The dried extracts were stored at 5°C in the refrigerator until used for further studies.

Antimicrobial screening

Test microorganisms

In vitro antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive - *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) Gram negative- *Escherichia coli* (ATCC 25922) and *Proteus vulgaris* (ATCC 2027). All the tested microorganisms were obtained from Batra Hospital and Medical Research Centre (BHMRC), New Delhi. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37°C for 24h.

Antimicrobial activity

Antimicrobial assay of the crude extracts was performed against ten tested pathogenic strains by disc diffusion method (Gould and Bowie, 1952). The nutrient agar plates and potato dextrose agar plates were seeded with suspension (10^6 cfu/ mL) of the bacterial and fungal strains vice-versa. The empty sterilized

Whatmann No.1 filter paper disc (6 mm) were impregnated with 1mg/ml of extracts dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre diffusion time). The standard discs (6 mm) impregnated with antibiotics *chloroamphenicol* (2µg/mL) was used as positive control. The plates were incubated at 37°C for 24 h for bacterial strains. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values (±SD) calculated for conclusion.

Determination of minimum inhibitory concentration (mic)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Basri and Fan, 2005). For broth dilution, 1ml of standardized suspension of a strain (10⁶cfu/ mL) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37°C for 24h (for bacterial strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

RESULTS AND DISCUSSION

Since ancient times, humans have derived many traditional medicines from herbs and plants. Traditional medicines are perceived as efficient, safe and cost effective. Furthermore, medicinal plants are an integral component of research and development in the pharmaceutical industry with a research focus on isolation and direct use of active medicinal constituents or on the development of semi-synthetic drugs or still again on the active screenings of natural products to yield synthetic pharmacologically active compounds. The bioactive components of plants have different solubility in different extracting solvents (Oloke and Kolawole, 1998). Several reports have shown that bioactive compounds from plants have control on the growth of pathogenic strains (Taylor *et al.*, 1996; Singh *et al.*, 2002; Kagale *et al.*, 2004; Abed, 2007).

In the present investigation, *in vitro* antimicrobial efficacy of the crude extracts of stem bark was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The methanol, benzene, hexane and aqueous extracts of *M. oleifera* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table 1 and 2). The most susceptible bacterium was *S. aureus*. The inhibition zones (IZ) were in the range of 7.0±0.0 to 15.0±0.0 mm for most of the tested strains. The MIC of crude extracts of leaves and roots was determined at the concentrations ranging from 0.078 to 0.625 mg/mL.

Crude methanol extract of *M. oleifera* stem bark parts showed more pronounced antimicrobial activity than other extracts. The methanol leaves extract exhibited highest zone of inhibition against *S. aureus* 15.0±0.0

Table 1: Antimicrobial activity of *Moringa oleifera* Lam (inhibition zone) stem bark

Tested microorganisms	Plant part assayed Stem bark			Control	
	Methanol	Benzene	Hexane	Aqueous	
<i>Bacillus subtilis</i>	9.34± 0.94	9.0±0.0	7.34± 0.47	7.34± 0.47	20.2± 0.3
<i>Staphylococcus aureus</i>	15.0±0.0	10.0± 1.41	9.34± 0.94	8.0± 0.81	12.0± 0.0
<i>Escherichia coli</i>	7.0± 0.0	8.67± 0.47	8.0± 0.81	7.0± 0.0	24.8± 0.3
<i>Proteus vulgaris</i>	8.4± 0.94	7.0± 0.0	7.0± 0.0	-	15.0± 0.0

Control: C = chloroamphenicol at 2µg/disc; Diameter of inhibition zone (mm) including the diameter of disc (6mm) values are mean (±SD)

Table 2: Antimicrobial activity of *Moringa oleifera* Lam (MIC)

Tested microorganisms	Plant part assayed Stem bark			
	Methanol	Benzene	Hexane	Aqueous
<i>Bacillus subtilis</i>	0.312	0.625	0.312	0.625
<i>Staphylococcus aureus</i>	0.078	0.312	0.312	0.625
<i>Escherichia coli</i>	0.625	0.625	0.625	0.625
<i>Proteus vulgaris</i>	0.625	0.625	0.625	-

mm with low MIC values (0.078 mg/mL for each). However, none of activity is shown by aqueous extract against *P. vulgaris*. Among bacterial pathogens, gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. This may attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane (Yao and Moellering, 1995). The findings of the present investigation suggest that *M. oleifera* is source of biologically active compounds which may potentially prove to be efficient natural antimicrobial agents.

CONCLUSION

The present investigation revealed that the various extracts from stem bark of *M. oleifera* exhibited antimicrobial properties which explain the basis for its use in traditional medicines. However, methanol extracts exhibited significant inhibitory activity against tested pathogenic microorganisms.

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