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Research Article

ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACT OF *MORINGA OLEIFERA* LEAF AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT
Leaf extracts of *Moringa oleifera* (aqueous, hexane, ethanol and methanol) were investigated for antibacterial activity and found effective against all tested strains. However leaf extracted in methanol was more effective followed by ethanol, aqueous and hexane. Methanol extract of leaf showed maximum activity against the tested bacterial strains and MIC was 2, 3 and 3 mg/ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively. Therefore this can be selected for further investigation to determine its therapeutic potential.
Keywords: Antibacterial activity, antibiotics, bacteria, *Moringa oleifera*,

INTRODUCTION

Plants are being used as a source of innovative therapeutic agents for infectious diseases, cancer, lipid disorders, and immune modulation. Natural products are being used as source of medicine for thousands of years and still continue today. Various medicinal plants have been used for years in traditional medicine in daily life to treat the different diseases and many possess antibacterial activities[1-7]. Natural products and their derivatives have traditionally been the most common source of drugs, and still represent more than 30% of the current pharmaceutical market[8].

According observations of World Health Organization (WHO), medicinal plant would be the best source for obtaining a variety of drugs [9-10]. Traditionally used medicinal plants are the major source of medicine and approximately, 80% of the world population depends on the traditional medicines, derived from medicinal plants (WHO data), of the estimated 4,00000 higher plant species in the world only about 10% have been characterized chemically to some extent[10].

Infectious diseases are big challenge for tropical countries and account for about half of the death[11]. Bacteria have genetic ability to develop resistance against antibiotics[12-14] which is a big threat to the society worldwide. Some plants have shown the ability to overcome resistance in such organisms which led the researchers' to isolate active principles and investigate mechanisms. Isolation and identification of secondary metabolites produced by plants has explored their use as active principles in medicinal preparations[15-16].

The pharmaceutical industries are looking for new chemical entities against complex diseases, especially the multi drug resistance in pathogenic bacteria, are little explored and drug developers from all over world are looking towards nature for developing front line drug to face the challenge [10].

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. A number of medicinal properties have been ascribed to various parts of this highly esteemed tree. Almost all the parts of this plant: root, bark, gum, leaf, fruit s), flowers, seed and seed oil have been used for various ailments in the indigenous medicine. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern.

Due to wide uses of *Moringa oleifera* , the present study was planned to evaluate the antibacterial potential of leaf extracts (aqueous, hexane, ethanol and methanol) against two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and one Gram positive (*Staphylococcus aureus*) bacteria.

MATERIAL AND METHODS

The leaf of *Moringa oleifera* was bought in a local herbal market in varanasi, Uttar Pradesh, India. The identity was authenticated at the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, by the expert of the medicinal plant. The leaf were initially rinsed with distilled water and dried on blotting paper in laboratory at (37 ± 1)^{0}C for 24 hours. After drying, the plant materials were ground in grinding machine (Made in India) in the laboratory. Herbarium voucher specimen reference number is 309 for selected plant (*Moringa oleifera*), preserved in our department.

Preparation of plant extract

Reported method[17] of extraction followed for aqueous and methanol extracts.

Aqueous Extract

50gm samples of *Moringa oleifera* plant leaf were weighed out and soaked separately in to 200ml distilled water in a conical flask cover with rubber cork and left undisturbed for 24 hours, then filtered off using sterile filter paper (Whatmann no.1) in to a clean conical flask and subjected to water bath evaporation , where the aqueous solvent was evaporated at its boiling temperature of 100^{0}C . The standard extracts obtained were then stored in a refrigerator at 4^{0}C for further use[18].

Methanol, hexane and ethanol extract

50gm powdered plant leaf material was mixed with 200ml of methanol. The mixtures were kept for 24 hours in tightly sealed vessels at room temperature, protected from sun light and mixed several times with a sterile glass rod. This mixture was filtered through Whattman no.1 filter paper and the residue adjusted to the required concentration (50ml of methanol for the residue of 50g of powdered plant material) with the extraction for fluid for the further extraction and it was repeated thrice and clear colourless supernatant extraction liquid was finally obtained. The extracted liquid was subjected to rotary evaporation in order to remove the methanol. The semisolid extract produced was kept in a freezer at -80^{0}C (MDFU4086S, JAPAN) overnight and then subjected to freeze drying for 24 hours at -60^{0}C in 200 ml vacuum. Then the extract was stored in a air tight container at 4^{0}C in a refrigerator for further use. Similar procedure was followed for hexane and ethanol.

All the dried extracts were exposed to UV rays (200-400 nm) for 24 hours and checked frequently for sterility by streaking on nutrient agar plates[19].

Qualitative assay

Sensitivity of bacterial test strains to the extract of *Moringa oleifera* was assayed by using the slightly modified Kirby Bauer Disk Diffusion susceptibility method[20]. The bacterial strain (4-5 colonies) to be tested was suspended in 4ml of normal saline (0.85 %) and the density of suspension adjusted to approximately 10 ^{8} CFU mL^{-1} using a 0.5 M barium sulphate suspension as the turbidity standard. The surface of the sterile 3.8% MH (Mueller Hinton) agar in Petri dishes was dried and the test strain inoculated with a sterile swab to obtain a lawn culture. 10µl of 50mg/0.1ml extracts was placed directly on the lawn. After inoculation for 18 hours at 37^{0}C the diameter of the inhibition zone was measured. DMSO was taken as control for methanol extracts. Sterile distilled water was taken a control for aqueous extracts. The dissolution of organic extracts (methanol) was aided by 1% (v/v) DMSO and that of aqueous extracts with water which did not affect the growth of microorganisms in accordance with our control experiments.

Quantitative assay

Agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC) [21]. The MIC was taken as the lowest concentration at which the test organism did not show visible growth. 1 ml extract of different concentration was added to 19 ml of MH agar in order to achieve the range of 0.1mg to 20 mg/ml. Plates were dried and divided in to sectors based on the number of organisms. Bacterial population grown overnight adjusted population density of 10^{8}CFUmL^{-1} applied to sectors (described earlier). The inocula were allowed to dry and incubated at 37^{0}C.

Test organisms

The bacterial strains used during study were obtained from the department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The bacterial strains studied are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.

RESULTS

Antibacterial activity of extracts and determination of MIC

Various solvent were used to extract the leaf of *Moringa oleifera* and different extracts were screened against test organisms to evaluate the potential of inhibition. All tested extracts like aqueous, hexane, ethanol and methanol extracts of leaf were found inhibitory against test strains. Minimum inhibitory activities were observed in hexane extract however maximum inhibitory activity were found in methanol extract followed by ethanol and aqueous extract. Extracts of Aqueous, hexane, ethanol and methanol showed 10, 8, 12 and 20 mm inhibition against Pseudomonas *aeruginosa*, 14,10, 16 and 30 mm inhibition against *Staphylococcus aureus* and 9,7, 11 and 18 mm inhibition against *Escherichia coli* respectively (Table 1). The extract (aqueous, hexane, ethanol and methanol) of *Moringa oleifera* leaf showed inhibition to all tested organism. Results revealed that leaf extract (methanol) was more effective against all the three strains. It was found that methanolic extract of leaf was more effective than those of other extract. Dimethylsulfoxide and sterile distilled water treated against test organism showed no inhibition zone in any three test organisms.

Table1. Sensitivity of bacterial strains to various extracts of leaf of *Moringa oleifera*

Diameter of inhibition zones (mm)			
Different extracts of leaf	*Pseudomonas aruginosa*	*Staphylococcus aureus*	*Escherichia coli*
Aqueous extract	10	14	9
Hexane extract	8	10	7
Ethanol extract	12	16	11
Methanol extract	20	30	18
Sterile distilled water	0	0	0
Dimethylsulphoxide	0	0	0

Various concentration of methanol extract (1 to 5 mg/ml) was used to inhibit the test organism. The lowest MIC (2 mg/ ml) was found against the *Staphylococcus aureus* and 3 mg/ ml was recorded against *Escherichia coli* and *Pseudomonas aeruginosa* (Table 2). All the values given are the mean of three sets data.

Table 2. The zone of inhibition and MICs of methanol extracts against different bacterial target

Micro organisms	Zones of inhibition (mm)	MICS (mg/ml)
Staphylococcus aureus	12	2.0
Escherichia coli	7.0	3.0
Pseudomonas aeruginosa	7.0	3.0

DISCUSSION

Antibacterial activity of leaf extract was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The results revealed that the antibacterial potential of the extracts of *Moringa oleifera*, especially the methanol leaf extract showed greater antibacterial potential than the other extracts. These findings provide an interesting view to the traditional method (decoction or boiling of the plant and plant parts) of treating bacterial infection. High polarity may provide greater extraction capacity to methanol and can produce greater number of active constituents for antibacterial activity, an observation in a agreement with reported one[22-23].

The methanol leaf extracts of *Moringa oleifera* showed considerably more effective than aqueous extracts against test organisms. The methanol extracts of *Moringa oleifera* showed maximum inhibition of Staphylococcus *aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli*. Similar trends were found in aqueous, hexane and ethanol extracts of leaf. On the contrary, the aqueous extracts of leaf were observed for maximum inhibition zone against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli*.

The methanol extract of leaf showed greater diameter of inhibition than that of plant extract of *Olax subscorpioidea* treated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*[24]. *Sapindus emarginatus* , *Hibiscus rosa sinensis*, *Mirabilis jalapa*, *Rheo discolor*, *Nyctanthes arbortristis*, *Colocasia esculata*, *Gracilaria corticata*, *Dictyota* sp. and *Pulicaria wightiana*[22]. It was also evident that methanol extract of leaf showed significant inhibition zone against Gram positive as well as Gram negative. This finding is useful because developing resistance in Gram negative bacteria creating a number of problems in treatment of infectious diseases therefore the screening of alternative drugs or natural antibacterial substances and identification of active molecules is need of the hour[25-26].

In conclusion, results from this research shows that the methanol extract of leaf have a broad spectrum against both Gram positive and Gram negative while aqueous extract has little antibacterial activity.

The quantity of active components inhibits the growth and metabolic activity of bacteria was considered as MIC to that test bacteria. The lower MIC for Staphylococcus *aureus* to that of Pseudomonas *aeruginosa* and *Escherichia coli*, suggest that Gram positive is more sensitive for test extract. The results are encouraging and suggest that further purification of extracts is needed for active principle(s) in the search of new herbal drug.

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