

Original Article

ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* (LAM) LEAVES EXTRACTS AGAINST SOME SELECTED BACTERIA

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ABSTRACT

Objective: The antibacterial activity of *Moringa oleifera* (Lam.) Leaves extract belonging to the family Moringaceae, was determined using agar well diffusion method against some selected bacteria.

Methods: Mueller Hinton Agar (MHA) (Becton Dickson M. D USA), media was prepared according to the manufacturer's instruction. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. Concentrations of 30, 60, 90 and 120mg/ml prepared from the dry leaves powder were used for antibacterial analysis using agar well incorporation methods. Plates of Mueller hinton agar were prepared and allowed to solidify on Petri dishes. Each plate was then seeded with a test bacterium. Four holes were made in each of the plate with a sterile 2.0 mm diameter cork borers. Each of the four holes was filled with a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured using a meter rule and the mean value for each organism was recorded.

Results: The aqueous, ethanol and methanol extracts of the plant leaves show an inhibitory effect on the growth of the tested bacteria. For aqueous, ethanol and methanol extracts, the inhibitory effect on *Escherichia coli* was significantly higher ($P < 0.05$) than that of *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. In addition, both ethanol and methanol extract showed a significantly higher ($P < 0.05$) inhibitory effect at higher concentration of 120mg/ml.

Conclusion: The powder from the leaves of *Moringa* show potential antibacterial activity against the tested gram positive bacteria; *Staphylococcus aureus* and gram negative bacteria i. e. *Escherichia coli* and *Pseudomonas aeruginosa*.

Keywords: Antibacterial, *Moringa oleifera*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*.

INTRODUCTION

The *Moringa* plant has been consumed by humans throughout the century in diverse culinary ways [1]. Almost all parts of the plant are used culturally for its nutritional value, medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months without any major loss of its nutritional value [2;3]. Studies have indicated that *M. oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities [4;5;6]. The antimicrobial properties of plants have been investigated by a number of workers worldwide and many of them have been used as therapeutic alternatives [7]. Plants have many secondary metabolites such as alkaloids, phenolic compounds, etc. In the present study attention has been focused on anti-bacterial activity of *M. oleifera* on *S. aureus*; *P. aeruginosa* and *E.coli*, with the broader objective of providing cheap and safe remedy for human health problems.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Moringa oleifera* tree were collected from Tudun Wada Area of Sokoto South Local Government, Sokoto State, Nigeria. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly; and dried. The plant was identified in the Botany Unit, Department of Biological Sciences Usmanu Danfodiyo University Sokoto. Parts of the plant collected for identification were: leaves, stem, flowers, seeds, fruits and roots respectively.

Drying and storage of plant material

The leaves of the plant were air dried under shed, and then grounded into powder with the aid of pestle and mortar.

The powders obtained from the leaves of the *Moringa* plant were then sieved and stored in polythene bags prior to the analysis.

Preparation and extraction of the leaf extracts

Extraction of aqueous leaf extract

Fifty grams (50g) of the powdered leaves were weighed and poured into 500 ml conical flask in which 400 ml. of distilled water was added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use.

Extraction of ethanol leaf extract

Fifty grams (50g) of the powdered leaves were weighed and poured into 500 ml conical flask in which 200 ml of ethanol was added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using rotary evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use.

Extraction of methanol leaf extract

Fifty grams (50g) of the powdered leaves were weighed and poured into 500 ml conical flask in which 200 ml of methanol were added. The mixture was kept for 12 hours with constant shaken at 30 minutes intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40°C under reduced pressure using rotary evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use.

The concentrated extracts were labelled as; MLAE (*Moringa* Leaves Aqueous Extract), MLEE (*Moringa* leaves Ethanol extract) and MLME (*Moringa* leaves Methanol extract). The extracts; crude aqueous extract, crude ethanol extract and crude methanol extract was used for antibacterial analyses.

Preparations of culture media

The media used was Mueller Hinton Agar (MHA) (Becton Dickson M. D USA), it was prepared according to the manufacturer's instruction, where 35g of media was mixed with one litre of distilled water and enclosed in a container and autoclaved at 121°C for 15 minutes. The media were later dispensed into 90 mm sterile agar plates (Oxoid, UK) and left to set. The agar plates were incubated for 24 hours at 37°C to confirm their sterility.

Absence of any kind of growth after 24 hours showed that the plates were sterile. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 holes of 2 mm diameter and 5 mm depth made into it using a sterile agar glass borer. Septrin was used as a positive control while distilled water was used as a negative control. Approximately 0.2 ml of the bioactive test compound of concentration 1g/ml was suspended in the holes and thereafter inoculated plates/culture were incubated for 24 hours at 37°C.

The plates/cultures were examined for the presence of bacterial inhibition zones around each hole. Antibacterial activity was determined from the zone of inhibition around the holes. Single readings were carried out. Non-active compounds did not show any inhibition zone. The zones of inhibition were measured using a ruler and a pair of divider (Picfare) and results were reported in millimetres (mm). All zone diameters were considered important since the extracts from the plants were still crude. A zone size interpretive chart was then drawn to show the different plant extracts and their corresponding inhibition zone diameter to the nearest millimetre.

Tests for antibacterial activity

Bacterial cultures used in this study were obtained from Microbiology Laboratory Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Bacterial cultures included in this study were; *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. All the cultures were grown in Mueller hint on agar (Media). The inoculum was used for antibacterial assay.

Antibacterial assay

The media and test bacterial cultures were poured into dishes. The test strain was inoculated into the media to inoculum size when the temperature reached 40-42°C. Care was taken to ensure proper homogenization. The plant extracts were tested for antibacterial activity in the agar well diffusion assay. The antibacterial activities of the plants extracts (aqueous, ethanolic and methanolic extracts) were tested on three bacteria species namely; *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas aeruginosa* respectively in the Microbiology Laboratory, Faculty of Veterinary Medicine of Usmanu Danfodiyo University, Sokoto, Nigeria. Concentrations of 30, 60, 90 and 120mg/ml were prepared from the dry leaves powder. These concentrations were used for antibacterial analysis using agar well incorporation methods [8]. Plates of Mueller hint on agar were prepared and allowed to solidify on Petri dishes. Each plate was then seeded with a test bacterium. Four holes were made in each of the plate with a sterile 2.0 mm diameter cork borers. Each of the four holes was filled with a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured using the meter rule and the mean value for each organism was recorded.

Statistical analysis of data

Data were expressed as mean±standard deviation. The data obtained were subjected to Analysis of Variance (ANOVA) test to determine whether there was significant difference between extract used and also between the lengths of incubation.

RESULTS AND DISCUSSION

Results obtained revealed that all the treatments viz; the aqueous, ethanol and methanol extracts of the plant leaves exhibited inhibitory effect on the growth of the tested bacteria. For aqueous, ethanol and methanol extracts, the inhibitory effect on *Escherichia coli* was significantly higher ($P < 0.05$) than that of *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively.

In addition, both ethanol and methanol extract showed a significant higher ($P < 0.05$) inhibitory effect at higher concentration of 120mg/ml on the tested microorganisms when compared to aqueous extract. In all the cases, the activity of the extracts was compared with standard antibiotic septrin (Co-trimoxazole). In this study, ethanol and methanol extracts of the leaves exhibited higher antibacterial activity compared to septrin. But only ethanolic extract exhibited the highest antibacterial activity against all the tested bacteria.

Table 1: Antibacterial activity of (aqueous, ethanolic and methanolic) extracts of *M. oleifera* leaves.

| Sample (Plant Extracts) | Extract Conc. (mg/ml) | Zone of Inhibition (mm) | | |
|-----------------------------|-----------------------|-------------------------|----------------|---------------|
| | | <i>E. coli</i> | <i>Pseudo.</i> | <i>Staph.</i> |
| Aqueous | 30 | 2.67 + 0.57 | 3.33 + 0.57 | 3.67 + 0.57 |
| | 60 | 4.67 + 0.57 | 6.67 + 0.57 | 4.33 + 0.57 |
| | 90 | 6.67 + 0.57 | 6.33 + 0.57 | 6.67 + 0.57 |
| | 120 | 7.33 + 0.57 | 7.33 + 0.57 | 7.00 + 1.00 |
| Ethanol | 30 | 3.67 + 0.57 | 6.33 + 0.57 | 2.67 + 0.57 |
| | 60 | 5.33 + 0.57 | 6.67 + 0.57 | 4.33 + 0.57 |
| | 90 | 6.00 + 1.00 | 6.67 + 0.57 | 6.00 + 1.00 |
| | 120 | 9.67 + 0.57 | 9.33 + 0.57 | 9.67 + 0.57 |
| Methanol | 30 | 4.67 + 0.57 | 6.67 + 0.57 | 2.33 + 0.57 |
| | 60 | 4.67 + 0.57 | 6.67 + 0.57 | 4.67 + 0.57 |
| | 90 | 6.33 + 0.57 | 7.33 + 0.57 | 6.33 + 0.57 |
| | 120 | 8.67 + 0.57 | 8.33 + 0.57 | 8.33 + 0.57 |
| Septtrin (Positive control) | 10 | 9.00 + 0.57 | 8.00 + 1.00 | 6.00 + 1.50 |
| Water (Negative control) | 30% | - | - | - |

Values given are mean+ standard deviation of the experiments replicated three times (where n=3), **Abb.** *E. coli* – *Escherichia coli*, *Staph.* – *Staphylococcus aureus*, *Pseudo.* – *Pseudomonas aeruginosa*, - = No activity

DISCUSSION

The present study was conducted to obtain preliminary information on the antibacterial activity of the extracts (aqueous, ethanol and

methanol) of *Moringa oleifera* Lam. Leaves. Agar well diffusion method was applied to be used in this study. The powder from fresh leaf (dissolved in ethanol) has greater antibacterial activity than that dissolved in both water and methanol extracts. The traditional

method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair *et al.*, [9]. In this investigation, highest zones of inhibition were found in powder from leaf powder dissolved in ethanol against all the bacteria tested which was more effective than known antibiotic septrin (Co-trimoxazole).

CONCLUSION

Leaf extracts of *Moringa oleifera* showed varying antibacterial activity on the tested bacteria. The extract was more effective than traditional antibiotics to combat the human pathogenic bacteria studied responsible for severe illness. The plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. This in vitro study demonstrated that traditional medicine can be as effective as modern medicine to combat human pathogenic bacteria. The use of these plants in traditional medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

Recommendations

The findings in this research work have confirmed the activity of the plant against the tested bacteria. Therefore, to take the work to beneficial level, the following recommendations were made:

1. Further research work should be carried out using chromatography techniques.
2. The extract should be explored for their medicinal applications.

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