



## Antibacterial Activity of *Moringa oleifera* Root Bark Extract against Some Pathogenic Organisms

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### Authors' contributions

This work was carried out in collaboration among all authors. Author EKA designed the study, wrote the protocol. Author UDN wrote the first draft of the manuscript. Authors HNO and CEU performed the statistical analysis. Authors CVN and CGU helped with the analyses of the work. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The study is aimed at evaluating the antibacterial activity of *Moringa oleifera* root bark extract against pathogenic organisms.

**Method:** The antibacterial activity of methanolic and aqueous extracts of *Moringa oleifera* root bark was investigated against test organisms (*Staphylococcus aureus* and *Escherichia coli*) using agar well diffusion method. Different extracts were prepared at different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml).

**Results:** The methanol extracts showed a higher zone of inhibition than the aqueous extracts at all the extract concentrations and on both test organisms. Also, the observed antibacterial activity was dose-dependent for both extract methods.

**Conclusion:** The present work showed that *Moringa oleifera* root bark has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* suggesting its potential as an antibacterial agent against infections caused by the organisms.

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## 1. INTRODUCTION

Medicinal plants contain substances that can be used for therapeutic purposes. Plants have been a valuable source of natural products for the health of human beings, and they have excellent potential for producing new drugs [1]. Therefore, such plants should be investigated for a better understanding of their properties and efficiency [2]. Antibiotic resistance is a type of drug resistance where microorganisms can survive exposure to an antibiotic. The leading causes of antibiotic resistance are genetic mutation in bacteria [3]. Inappropriate and irrational use of antimicrobial medicines provides a suitable environment for resistant microorganisms to emerge and persist. The higher the level of exposure to the antimicrobial agents, the higher the risk of the development of resistance. Irrespective of the need for the antibiotic, as resistance toward antibiotics becomes more common, a greater need for alternative treatment arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of a newly approved drugs. As regards this, there is a need for finding and investigating novel antimicrobial compounds.

While most of the antibiotics have been developed from microorganisms, there are many reports on the antibacterial effectiveness of the traditional herbs against the gram-positive and gram-negative bacteria [4]. The plant materials remain an essential resource for finding the novel antimicrobial compounds. Microbial cells are negatively affected by plant-derived substances via various mechanisms of actions, one of which is the attack of these substances on the phospholipid bilayer of the cell membrane [5]. The medicinal herbs have the bacteriostatic effects on the enzymatic activity associated with energy production, or they can cause denaturation of proteins, modifying cell wall permeability, or causing the loss of macromolecules. One such plant of medicinal value is *Moringa oleifera*, commonly known as Sahajan in Hindi; belonging to the family *Moringaceae*, a single genus family with 13 known species [6].

The different parts of the plant viz. leave, stem bark, root bark, flowers, fruits, and seeds are used in the indigenous systems of medicine for the treatment of a variety of human ailments. *M. oleifera* incredible medicinal value, which is

claimed by many cultures and communities, is based on science. *M. oleifera* has been found to contain many essential nutrients, for instance, vitamins [7,8]. Nutrition content of a plant plays an essential function in medicinal, nutritional, and therapeutic properties. *M. oleifera* leaves consist of high sources of vitamin C, calcium, and potassium as well as protein and also works as a useful source of natural antioxidants. Due to the presence of several sorts of antioxidant compounds such as flavonoids, ascorbic acid, carotenoids, and phenolics, *M. oleifera* can extend the period of food containing fats [9]. Despite the array of uses to which parts of *Moringa oleifera* trees are put to, scanty literature is available on the uses of *Moringa oleifera* bark as antimicrobial [10]. However, a critical step in the screening of plant material for antimicrobial activity is to evaluate its antibacterial activity against pathogenic microorganisms. Hence, this study evaluates the antimicrobial activity of *Moringa Oleifera* root bark extract on bacterial pathogens.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Dried samples of root barks of *M. oleifera* were collected at National Root Crop Research Institute Umudike in Ikwuano, Abia State, Nigeria. The root barks of *M. oleifera* sample was identified and authenticated at the Herbarium of the Department of Plant Sciences and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State.

### 2.2 Test Organisms

The test isolates of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922)

Used in this study were obtained from the Microbiology laboratory, Michael Okpara University of Agriculture, Umudike, and confirmed by Morphological appearance - color, shape elevation, pigmentation, opacity, and nature of edges of the colonies were observed and recorded for each isolate.

**Gram test and Motility test:** A drop of an 18-20 hours' peptone medium culture of the test organism was placed on a clean grease-free slide with the aid of Pasteur pipette. The slide was then covered with a coverslip and viewed

under the microscope using an x40 objective lens. The movement of small motile bacteria is distinguished from the on-the-spot vibratory movement (Brownian movement), which is shown by all microorganisms and particles when suspended in a fluid. True bacterial motility is the ability of an organism to move in different directions or a single direction [11].

Biochemical test –Isolated organisms were identified by standard microbiology identification techniques, including the Catalase test, Citrate utilization test, Methyl-Red test, Voges-Proskauer test, Urease test, and Indole test [11].

### 2.3 Preparation of Crude Extracts

The method of Mumtaz et al. [12] was adopted. The collected material was shade dried, made to a coarse powder, and then packed in polythene bags for further analysis. Fifty grams of the plant material was soaked in 200 ml of solvents (aqueous and methanol) and left for 24 hrs. The fraction was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 2). The crude extracts after filtration stored in a refrigerator at 4°C. All the extracts were then concentrated with a rotary vacuum evaporator at 40°C. One Gram (1 g) each of the methanol and aqueous extract was added to methanol and made up to 5 ml to give a concentration of 200 mg/ml. Other concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml were prepared by the double dilution method as described by Cheesebrough [11].

### 2.4 Antimicrobial Susceptibility Testing

The Kirby-Bauer method was applied based on zones of inhibition of agar plates [13]. Exactly 1.0 g of the methanolic and aqueous extracts were dissolved and made up to 5 ml each with Dimethyl Sulfoxide (DMSO) and water respectively to obtain 200 mg/ml of each extract. The concentration was diluted two folds to get 100 mg/ml and 50 mg/ml of each extract. Mueller-Hinton agar was prepared, and plates inoculated with the different isolates. The punched circular discs from Whatman No. 2 filter paper were impregnated with 0.1 ml of different concentrations of each extract and allowed to air-dry for a few minutes.

Each of the discs was pressed onto the surface of the inoculated medium to ensure contact with the medium. Negative control was prepared by impregnating some discs with water and DMSO,

which was transferred and pressed unto the inoculated plates while standard antibiotics discs (ciprofloxacin, tetracycline, and ampicillin) served as positive controls. All the tests were carried out in duplicates. Incubation was done at 37°C for 24 hr and plates observed for zones of inhibition. The degree of sensitivity was expressed as a measure of the diameter of zones of inhibition in millimeters (mm). A diameter of 10 mm or higher was considered as an indication of the sensitivity of the test organism to the extracts. The mean inhibition zones (mm) were calculated as the difference between the disc diameter (6 mm) and the diameter of the inhibition zones [14].

### 2.5 Determination of Minimum Inhibitory Concentration (MIC)

The inoculums of the microbial isolates used for the test were prepared by comparing with a standard of MacFaland reagent in order to reduce microbial load or count. Exactly 1.0 g each of both methanolic extracts was dissolved separately in 5 ml each of DMSO and sterile water respectively to get a concentration of 200 mg/ml and labeled as solution 1.2 ml of solution 1. This was serially diluted into 8 folds to get corresponding concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.75 mg/ml, 3.13 mg/ml, 1.56 mg/ml and 0.78 mg/ml each for both aqueous and ethanolic extract and labeled solutions 3 to 9.

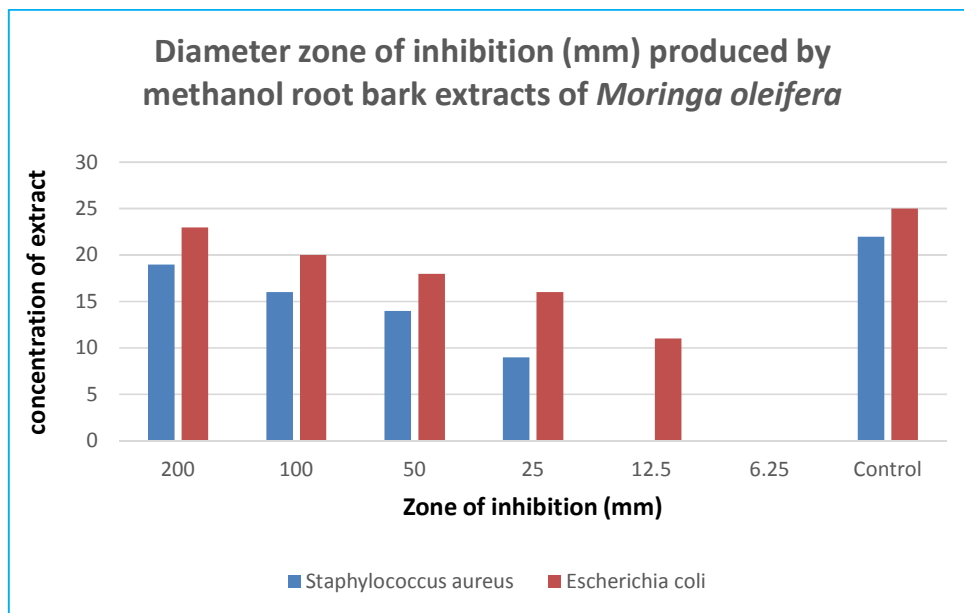
A previously prepared Mueller-Hinton broth containing various concentrations of the extract was inoculated with the standard inoculums of each test organism, followed by incubation of the tube at 37°C for 16-20 hr. Thereafter, the tubes were observed for the presence or absence of growth in each tubes determined by turbidity of the test tubes. The lowest concentration of the extracts resulting in no growth after incubation was taken as the minimum inhibitory concentration (MIC) of the melon fungus extracts [15].

### 2.6 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extract on the bacterial isolates was carried out according to Ajaiyeoba et al. [16]. One (1 ml) bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes, which did not show any growth and were sub-cultured onto nutrient agar and incubated at 37°C for 24 hours. After incubation, the concentration at which there was no single colony of bacteria was taken as MBC.

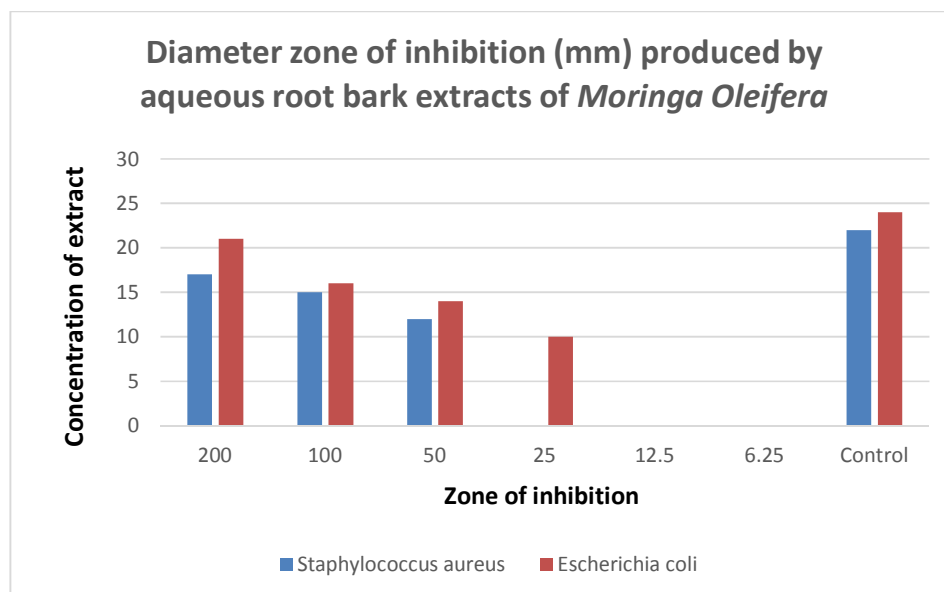
### 3. RESULTS

The root bark extracts of *Moringa oleifera* were analyzed for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.



**Fig. 1. Diameter zone of inhibition (mm) produced by methanol root bark extracts of *Moringa oleifera***

Methanol root bark extract of *Moringa Oleifera* had a concentration-dependent inhibition on both bacteria with increased activity on *E. coli* than *S. aureus*. Control drug = Erythromycin 30 mcg



**Fig. 2. Diameter zone of inhibition (mm) produced by aqueous root bark extracts of *Moringa Oleifera***

The diameter zone of inhibition (mm) produced by aqueous root bark extracts of *Moringa Oleifera*, there was a concentration-dependent inhibition on both bacteria with increased activity on *E. coli* than *S. aureus*. This activity, however, was much lower than the one exhibited by corresponding methanol extracts

**Table 1. MIC and MBC values for the methanol and aqueous extracts against susceptible organisms**

Test Organisms	Methanol extract		Aqueous extract	
	MIC	MBC	MIC	MBC
<i>Staphylococcus aureus</i>	12.5 mg/ml	25 mg/ml	25 mg/ml	50 mg/ml
<i>Escherichia coli</i>	6.25 mg/ml	12.5 mg/ml	12.5 mg/ml	25 mg/ml

Methanol extracts exhibited lower MIC and MBC on the test organisms than the aqueous extract. *Staphylococcus aureus* also required a higher concentration of extract to be inhibited than did *Escherichia coli*

Keys: MIC = Minimum Inhibitory Concentration

MBC = Minimum Bactericidal Concentration

#### 4. DISCUSSION

The place of medicinal plants as a natural remedy to diseases cannot be overemphasized [1]. While most antibiotics have been isolated from bacteria, many plants have exhibited antibacterial activities and have been used widely in the treatment of ailments caused by such organisms. Different parts of *Moringa Oleifera* have been used in the treatment of different ailments, though with a few studies evaluating its antimicrobial potentials. The study evaluated the antibacterial activity of *Moringa Oleifera* root bark extract on clinical isolates.

According to Schillinger and Lücke [17], inhibition was scored positive if the width of the clear zone around the antibacterial agent or colonies of the producer strain was 0.5 mm or larger. As the results indicate, at concentrations of 50 mg/ml and above, *Moringa Oleifera* root bark extract actually possesses inhibitory activities since the least zone of inhibition observed at these points is 12 mm.

From this investigation, across the groups, the aqueous extracts of *Moringa Oleifera* root bark showed lower antibacterial activities compared to the methanol extracts. Methanol extracts exhibited lower MIC and MBC on the test organisms. This observed difference between the extracts may be attributed to the ability of methanol to extract more of the essential secondary plant metabolites and the insolubility of active compounds in water since these compounds are believed to exert antibacterial activity on the test organisms [18]. Okigbo and Ajale [19] reported that the inactivity of plant extracts might be due to the age of the plant, extracting solvent, method of extraction, and time of harvesting of plant materials. It can be inferred that methanol is a better extracting solution for this plant and purpose than water. This difference may also be due to the inhibitory ability of the methanol even without extract,

whereas the ability of the aqueous extract to exhibit this inhibition may be primarily due to its penetrative ability. From the findings in this study, the Methanol bark extract showed promising antibacterial activity the test pathogens at most of the concentrations.

The antimicrobial activity of the leaf extracts of *Moringa Oleifera* root bark was evaluated against gram-positive and negative bacteria (*Staphylococcus aureus* and *Escherichia coli*). Data presented in Figs. 1 and 2 revealed that the leaf extracts were more effective in inhibiting *Escherichia coli* (Gram-negative) with a zone of inhibition ranging between 10 mm and 23 mm as compared to *Staphylococcus aureus* (Gram-positive) with a zone of inhibition ranging between 9 mm to 19 mm. This result is contrary to that of Agatemor [20] where it was reported that gram-negative bacteria are more resistant than gram-positive bacteria to antimicrobial plant extract.

Rao et al. [21] investigated the antibacterial activity of methanolic extract of *M. oleifera* by using well diffusion technique and reported that the most significant activity of this plant was seen against *S. aureus*. While working on the same plant species, Devi et al. [22] investigated the antibacterial activity of methanolic extract of bark by agar well diffusion method against *Bacillus spp.* And *S. aureus*. The results are also in agreement with Ahmad et al. [23], who reported methanolic leaf extract of *C. australis* had the highest activity against *S. aureus* at 200 mg/ml concentration with 10.5mm zone of inhibition.

The aqueous extract of *Moringa oleifera* bark has shown strong antibacterial activity against the test organisms. The trends in this study are contrary to the reports by Yagoub et al. [24] who, in their preliminary screening for antimicrobial activity of different plants against different organisms, methanolic extracts of *A. indica* produced zero zones of inhibition against *E. coli*.

The difference in the effect of this plant extracts within the organisms suggested that there are different antibacterial compounds in the plant extracts and that the compound that acted on one may not be the same as the one that acted on the others since antibacterial agents have different modes of action [25].

At concentrations of 200 mg/ml, the methanol extract showed an inhibitory activity (23 mm) on *E. coli* comparable to that of the standard drug Erythromycin 30 mcg (25 mm). This further suggests its potential as an antibacterial agent.

## 5. CONCLUSION

Based on these results, it could be concluded that methanol was the best extraction solvent for the antibacterial activity of *Moringa oleifera* against all the tested organisms. The activity decreased with the decrease in the concentration of the extract. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. The results of this study suggest that the *Moringa oleifera* root bark can be used as an antibacterial agent against infections caused by *Staphylococcus aureus* and *Escherichia coli*

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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