



Antimicrobial Activity of the Leaf and Seed Extracts of *Moringa Oleifera* on Some Bacteria Isolates

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ABSTRACT

This study focused on the antimicrobial activity of alcohol (ethanol) and aqueous extracts of Moringa olifera seed and leaf on Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The ethanol and aqueous extracts of the seed and leaf at concentration of 10g/100ml and the diluted ethanol and aqueous extracts at concentration of 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml were impregnated into sterilized filter paper discs. Also, ethanol alone was impregnated into sterilized filter paper discs and use as control. Agar disc diffusion method was adopted to obtain zones of inhibition. The zones of inhibition obtained for ethanol extract of Moringa olifera seed for Staphylococcus aureus was 12mm, Escherichia coli was 13mm and Pseudomonas aeruginosa was 10mm. The ethanol extract of Moringa olifera leaf gave an inhibitory zone of 11mm for Staphylococcus aureus, 10mm for Escherichia coli, 9mm for Pseudomonas aeruginosa. Different degrees of inhibition was observed for the diluted ethanol extracts of Moringa olifera seed. The diluted ethanol extracts of Moringa olifera leaf were resistant to the test organisms at concentration of 0.1g/ml, 0.6g/ml but was sensitive at concentration 0.8g/ml. The aqueous and diluted aqueous extracts of Moringa olifera seed and leaf were resistant to the test organisms. The zones of inhibition obtained for ethanol alone on the test organisms ranged between 8mm – 10mm. The degree of zone of inhibition differs, however the ethanol extracts exhibited the highest inhibitory effect than the aqueous extracts. This study revealed that Moringa olifera seed and leaf extracts are potential antimicrobial agents.

Keyword: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, microorganism, antitoxic.*

INTRODUCTION

For thousands of years, ancient people depended mostly on flowers, barks, leaves and fruits of plant for medicine. Only recently have synthetic drugs, most of which are similar to compounds identified in plants, come into use. Information on the activities and curative actions of medicinal plants were gotten from the introduction of European scientific method ^[1]. Many of the reported medicinal plants came under study, leading to extraction and characterization of their active ingredients^[2,3]. However, the mode of action of the plant extracts producing the therapeutic effect can also be better investigated, if the active ingredients are characterized and understood ^[4].

In the past twenty five years, intensive efforts have been made to discover new clinically useful antibiotics. The frequency of life-threatening infections caused by pathogenic micro-organisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries ^[5]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infection and adds urgency to the search for new infection fighting strategies ^[6,7]. For a long time, plants have been an important source of natural products of human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties^[8]. Plants have many antimicrobial

properties as secondary metabolites such as alkalioids, phenolic compounds etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to world Health organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basic for the efficacy of many plants used in folk medicine to treat infections ^[9,10].

Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs ^[11]. It is therefore very necessary that the search for newer antibiotics sources to be a continuous process. Plants are the cheapest and safer alternative source of antimicrobials ^[12,13,14].

Moringa olifera is the most widely cultivated species of monogeneric family, the *moringaceae* that is native to the sub-Himalayan tracts of Indian, Pakistan, Bangladesh and Afghanistan^[15], which is widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted disease, malnutrition and diarrhoea. *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors ^[16]. Hence, the present study was undertaken specifically to investigate the role of extracts of *Moringa oleifera* leaves as a potential antimicrobial agent against some human pathogenic bacteria.

In most parts of Nigeria, it is observed that folk medicine still abounds in medicinal plants and most tribes rely chiefly on herbal medicine, which

can be readily obtained at any time of the year; it is cheap, available, prepared and administered easily. More so, because third world countries are faced with exorbitant cost of Western drugs for treatments of ailments in the developing countries there is the need to look inward and develop possible biomarkers from local sources that could be acceptable in the world over. This study was undertaken to ascertain the antimicrobial activities of the extract of *moringa oliferea* leaf and seed, to compare the size of inhibition zone on bacteria isolates, to ascertain the different concentration of *moringa olifera* seed and leaf that shows inhibition.

MATERIALS AND METHOD

SAMPLE COLLECTION

The plant material of *Moringa oleifera* leaves and seeds used in this study was collected from Dilomat Farms and Service Limited in Rivers State University of Science and Technology, Nkpolu–Oroworuwku, Port Harcourt, Rivers State. The *Moringa oleifera* leaves was cleaned, sun-dried, pounded with a sterile mortar and pestle to a fine powder and then stored in airtight bottle. *Moringa oleifera* seeds were selected and the seed coat and wings were removed manually. The seed kernel was pounded to a fine powder using a sterile mortar and pestle and then stored in airtight bottle.

Stock cultures of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State.

SAMPLE ANALYSIS

Nutrient agar was used for the sub-culture and sensitivity testing, alcohol (ethanol) and aqueous extraction of the powder of *Moringa oleifera* seeds and leaves was done.

Preparation of Extracts:

To prepare the *Moringa* seed extract, 10g of the powder of *Moringa* seeds was weighed using a weighing balance, dissolved in 100ml of 95% ethanol in a conical flask, was mixed thoroughly by stirring with spatula and left to stand for one hour. This was then filtered using sterile muslin cloth. Also, 10g of the powdered *Moringa* seed was weighed, dissolved in 100ml of sterile distilled water in a conical flask, mixed thoroughly by stirring with a spatula and left to stand for one hour. This was then filtered using sterile muslin cloth.

To prepare *Moringa* leaf extracts, 10g of the powder of *Moringa* leaf was weighed using a weighing balance, dissolved in 100ml of 95% ethanol in a conical flask. Another 10g was weighed and dissolved in 100ml of sterilized distilled water in a conical flask. These were mixed thoroughly and left to stand for one hour and then filtered using sterile muslin cloth.

Sensitivity Test

The sensitivity test was carried out using agar disc diffusion method.

Serial dilution of ethanol and aqueous extract of both *Moringa* seed and leaf (concentration of 10g/100ml) was prepared in the order 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml

respectively in five different test tubes each, arranged in a test tube rack.

The sterilized filter paper discs were impregnated with 10 μ l of the undiluted ethanol and aqueous extracts for both seed and leaf (concentration of 10g/100ml). Also, 10 μ l of the diluted ethanol and aqueous extracts of both seed and leaf (concentration of 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml) were impregnated into sterilized filter paper discs each. 10 μ l of 95% ethanol were impregnated into sterilized filter paper discs and this served as a control for the ethanol extracts of both seed and leaf.

Thereafter, well-dried nutrient agar plates were seeded (inoculated) by streaking the test organisms (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) separately throughout the entire surface of the plates. Afterwards, discs impregnated with extracts (undiluted and diluted), and the control were transferred aseptically onto the surface of the inoculated nutrient agar plates while it was wet with the aid of sterilized forceps. The inoculated test discs in place were incubated at 37⁰ C. After 24 hours, plates having clear zone of inhibition were noted and zone diameter were measured using a ruler in millimeters.

RESULTS

The study revealed antimicrobial effect of the ethanol and aqueous extract of both *Moringa oleifera* leaf and seed on gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The inhibitory effects of the ethanol extract of *Moringa* seed (10g/100ml) on the test organisms (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) showed that *Escherichia coli* exhibited the highest level of susceptibility with an inhibitory zone of 13mm (Table 1), followed by *Staphylococcus aureus* with an inhibitory zone of 12mm, and *Pseudomonas aeruginosa* with an inhibitory zone of 10mm.

The sensitivity pattern of *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, *Escherichia coli* to the diluted ethanol extract of *Moringa* seed at different concentration showed that *Pseudomonas aeruginosa* had inhibition of 7mm, 8mm, and 9mm at concentration of 0.4g/ml, 0.6g/ml, and 0.8g/ml respectively while *Escherichia coli* at the same concentration had an inhibition of 7mm, 9mm, and 10mm. *Staphylococcus aureus* at concentration of 0.6g/ml and 0.8g/ml had an inhibition of 8mm and 9mm (Table.1).

Table 1: Comparative antimicrobial sensitivity of ethanol and ethanol extract of *Moringa oleifera* seed.

Test Organisms	Size of inhibition of zone (mm)						
	E	EE	0.1	0.2	0.4	0.6	0.8
Staphylococcus aureus	10	12	R	R	R	8	9
Escherichia Coli	10	13	R	R	R	9	10
Pseudomonas aeruginosa	8	10	R	R	7	8	9

R – Resistant

E – Ethanol

EE – Ethanol extract

Table 2 shows that the aqueous extract (10g/100ml) of the seed and dilution aqueous extract at concentration of 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml, and 0.8g/ml were resistant to the test organisms.

Table 2: Comparative antimicrobial sensitivity of aqueous extract of *Moringa oleifera* seed.

Test Organisms	Size of inhibition of zone (mm)					
	AE	0.1	0.2	0.4	0.6	0.8 (g/ml)
Staphylococcus aureus	R	R	R	R	R	R
Escherichia Coli	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R

R – Resistant

AE – Aqueous extract

The inhibitory effect of the ethanol extract of *Moringa* leaf (10g/100ml) on the test organisms show that *Staphylococcus aureus* exhibited the highest level of susceptibility with an inhibitory zone of 11mm, followed by *Escherichia coli* with an inhibitory zone of 10mm, and *Pseudomonas*

aeruginosa with an inhibitory zone of 9mm (Table 3). The diluted ethanol extract of the *Moringa* leaf at concentration of 0.8g/ml showed an inhibition zone of 8mm for *Staphylococcus aureus*, while *Escherichia coli* and *Pseudomonas aeruginosa* had an inhibition zone of 7mm (Table 3).

Table 3: Comparative antimicrobial sensitivity of ethanol and ethanol extract of *Moringa oleifera* leaf.

Test Organisms	Size of inhibition of zone (mm)						
	E	EE	0.1	0.2	0.4	0.6	0.8
Staphylococcus aureus	9	11	R	R	R	R	8
Escherichia Coli	8	10	R	R	R	R	7
Pseudomonas aeruginosa	8	10	R	R	R	R	7

R – Resistant

E – Ethanol

EE – Ethanol extract

Table 4 shows that aqueous extract of *Moringa* leaf (10g/100ml) and the dilution aqueous extract of *Moringa* seed at concentration of 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml, and 0.8g/ml were resistant to *Staphylococcus aureus* and *Escherichia coli* but *Pseudomonas aeruginosa* at concentration of 10g/100ml, 0.6g/ml, and 0.8g/ml

ml, had an inhibitory zone of 9mm, 7mm, and 8mm respectively.

95% ethanol alone showed inhibitory zone range of 8mm – 10mm on the test organisms which served as a control for the ethanol extract of *Moringa* seed and leaf (Table 1 and .3).

Table 4: Comparative antimicrobial sensitivity of aqueous extract of *Moringa oleifera* leaf.

Test Organisms	Size of inhibition of zone (mm)					
	AE	0.1	0.2	0.4	0.6	0.8 (g/ml)
Staphylococcus aureus	R	R	R	R	R	R
Escherichia Coli	R	R	R	R	R	R
Pseudomonas aeruginosa	9	R	R	R	7	8

R – Resistant

AE – Aqueous extract

DISCUSSION

In this study, ethanol extract of *Moringa oleifera* seed and leaf, aqueous extract of *Moringa oleifera* seed and leaf were prepared and tested for antimicrobial activity against bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). It was observed that ethanol extract of *Moringa* seed and leaf (at concentration of 10g/100ml) has varied antimicrobial effect on the test organisms. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* has an inhibitory zone range of 10mm – 13mm (Table 1) respectively at a concentration of 10g/100ml for ethanol extract of *Moringa* seed. This result agrees with the work done that ethanol extract of *Moringa* seed were effective against bacteria^[17].

The inhibitory effect of the ethanol extract of *Moringa* leaf (10g/100ml) on the test organisms showed that *Staphylococcus aureus* exhibited the highest level of susceptibility with an inhibitory zone of 11mm, followed by *Escherichia coli* with an inhibitory zone of 8mm and *Pseudomonas aeruginosa* has lowest inhibitory zone of 7mm (Table .3). This result is similar to the work done that ethanol extract of *Moringa oleifera* were effective against gram positive bacteria and gram negative bacteria^[18]. Ethanol (95%) was effective on the test organisms, this means that ethanol has an antimicrobial activity and ethanol helped to bring out the active component in the *Moringa* seed and leaf. Aqueous extract and diluted aqueous extracts of *Moringa* seed were resistant to the test organisms (Table.2). The aqueous extracts

were resistant because water could not extract the active component of the seed.

The aqueous extract and diluted extract of *Moringa* leaf were resistant to *Staphylococcus aureus* and *Escherichia coli* but was sensitive to *Pseudomonas aeruginosa* (Table .4). The diluted ethanol extracts of the *Moringa* seed and leaf were effective against the test organisms (Table .1 and Table .3).

The susceptibility of these organisms to this extract explains its use in native medicine for the treatment of infections caused by these test organisms. All these differences in the antimicrobial activity of the extract might be due to the chemical component of the plant, the species of the microorganism used and the method of extracts.

CONCLUSION

Moringa oleifera seed and leaf has antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Therefore, *Moringa oleifera* could be a promising natural antimicrobial agent with potential applications in pharmaceutical industry for controlling the infections caused by pathogenic bacteria used in this study. In conclusion, *Moringa* is sold commercially as powder and seed. So from this study it has been proven that *Moringa* can also be used as an antimicrobial agent for killing the most recalcitrant organism (*pseudomonas aeruginosa*). Pharmaceutical companies are advised to take advantage of this opportunity.

RECOMMENDATION

This study showed that the extracts of *Moringa oleifera* seed and leaf was effective against the test organisms (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). Therefore, *Moringa oleifera* should be used successfully for treating food poisoning, wound, diarrhea, dysentery, boil, sexually transmitted diseases and arthritis caused by the test organisms. Addition of *Moringa oleifera* to meals is highly recommended. Additionally, there is need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some plants is preserved and to provide scientific evidence for their efficacies. Also, further study will be needed to establish their exact therapeutic component and pharmacological standardization.

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