

## Antibacterial activity of ethanol extract of the defatted seed and seed coat of *Moringa oleifera*

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**Abstract:** Recent interest in infectious disease threats to clinical microbiology and public health is the emergence of pathogens with high multidrug resistance especially to available newer broad spectrum antibiotics that is cost prohibitive and are not even within the reach of the poor, making infectious diseases a global burden and therefore prompting the need for the search of potential plants in the treatment of diseases. However antibacterial activity of ethanol extract of *Moringa oleifera* defatted seed (MODS) and seed coat (MOSC) was conducted. Paper disc diffusion method was used to assess the effect of the extracts on *Pseudomonas aerogenosa*, *Escherichia coli*, *Klebsiella* sp, *Salmonella Typhi*, *Proteus mirabilis*, all the examined extract showed varying degrees of antibacterial activities against the selected pathogens. Highest inhibitory effect was observed in *Proteus mirabilis* (19mm) following *Salmonella Typhi* (16mm) with the *Moringa oleifera* seed extract, these strains *S. Typhi* and *Proteus mirabilis* were resistant to ethanol extract of *Moringa oleifera* seed coat along with their respective minimum inhibitory concentration (MIC) values ranging from extract on the test isolates ranges from a 0.1mg/ml- 100mg/ml concentration. This study has shown that the ethanol extract of *Moringa oleifera* seed possess some degree of antimicrobial activity than ethanol extract of the seed coat and could be a valuable Drug in the treatment of infections caused by the test organisms especially at high dose, therefore these findings support the traditional use of the plant in the treatment of different infections in the area.

**Key words:** *Moringa oleifera*, Test Organisms, Antibacterial Activity, Minimal Inhibitory Concentration (MIC)

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### I. INTRODUCTION

Bacterial infections are widespread throughout the world, especially in developing countries where in most cases lack of good sanitary practices are seen and primary health care programs are expensive making the fight against diseases difficult (Abdulmoneim and Zaid, 2011). For many years, medicine had depended exclusively on leaves, flowers and barks of plants. Only recently have synthetic drugs come into use and in many instances, these are replica of chemicals identified in plants. According to World Health Organisation, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Junaid *et al*, 2006). However antibacterial compounds has been studied and identified worldwide in several medicinal plants, which are important weapons in the fight against bacterial infections and have contributed greatly to the quality of human life since their introduction (Ali-Shtayeh *et al*, 1997). Many of the currently used antibacterials are associated with adverse effects such as toxicity, hypersensitivity, immunosuppression, and tissue residues posing public health menace (Gayatri *et al*, 2010). Also, the newer broad spectrum antibiotics are expensive. These disadvantages undermine the therapeutic utility of the currently available antibacterials and thus necessitating the need for finding alternative remedies for treatment of bacterial diseases (Gayatri *et al*, 2010). Innumerable antibacterial agents are currently employed in treating bacterial infections. However, the indiscriminate use of antibacterial agents have resulted in the spread of many virulent pathogenic and drug resistant bacterial species (Berkowitz, 1995). Prompting the need for safer and better therapy from medicinal plants.

*Moringa oleifera* is a well-documented world renowned plant herb known for its extraordinary nutritional and medicinal properties. It is a known anthelmintic, antibiotic, detoxifier and an outstanding immune builder used in many countries to treat malnutrition and malaria. It is also used in water purification and therefore helps in reducing the incidence of water borne diseases (Thilza *et al*, 2010). The plant *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It is a small or medium sized tree, about 10m in height with open crown of drooping fragile branches, feathery foliage of innate leaves and thick corky, whitish bark (Trapti *et al*, 2009; Roloff, 2009). The plant is variously called horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mothers best friend” (Julia, 2008). over the years significant work has demonstrated pharmacological actions of the leaves and seeds of *Moringa oleifera* but only limited work has been reported so far on antibacterial activity of *Moringa oleifera* defatted (MOD) seed and seed coat

(MOSC) on *Proteus mirabilis*, *E. coli*, *Salmonella Typhi*, *Pseudomonas mirabilis*, *Klebsiella* spp, even with reports of its high medicinal properties. Therefore, it was considered commendable to investigate the antibacterial activity of *Moringa oleifera* seed (MODS) and seed coat on diarrhoeogenic pathogens which are considered global threat to public health.

## II. MATERIALS AND METHOD

### COLLECTION OF PLANT MATERIAL

Powder from seed and seed coat of *Moringa oleifera* were obtained from Biu in Borno State from a Moringa seed oil processor through the Department of Animal Science, Faculty of Agriculture, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria.

### PREPARATION OF EXTRACT

#### *Ethanol Extract Preparation*

One hundred (100) grams of the powder for both the seed and seed coat was weighed separately using Satoric AG Gottingen Electronic weighing balance. The weighed samples were soaked in 200mls of ethanol (99.9% concentration) contained in a conical flask. The mixture was swirled. After 72h with interval stirring, the mixture was filtered using Whatman no.1 filter paper (Azoro, 2002) into a clean beaker and concentrated to dryness using a rotary evaporator at 70°C. Extracts was stored in the refrigerator at 8°C for use.

### TEST BACTERIAL ISOLATES

The test organism used in this study are strains of *Proteus mirabilis*, *E coli*, *Salmonella Typhi*, *Pseudomonas auriginosa*, *Proteus mirabilis*, isolated from stool samples of patient presenting with abdominal distress in Federal Medical Centre Yenagoa Bayelsa State Nigeria, cultured and identified using routine laboratory procedures as described by Cheesebrough (2000).

### ANTIBACTERIAL ACTIVITY TESTING FOR PLANT EXTRACTS

Sensitivity of the pure culture of bacteria isolates to ethanol extracts were determined using agar well diffusion method as reported by Kigigha and Atuzie (2012). Whatman No.1 filter paper was used. The filter paper was cut into circular disc using a perforator giving a diameter of 6mm. The disc was autoclaved at 121°C for 20min so as to denature and destroy completely the entire chemical used in its preservation as well as making it sterile before imbedding the extracts. The disc was dipped into the plant extract using a sterile forcep for 15sec and transferred aseptically into a sterile glass Petri dish and kept in the oven at 55°C until the following day.

The test organism were pre-adjusted to the 0.5 McFarland's turbidity standard in a test tube, dipped with a sterile swab stick and used to seed on a solidified oxoid sensitest agar in an inoculating chamber already set aseptically. The prepared disc was carefully transferred onto the inoculated culture plates using sterile forceps. The plates were incubated for 24h at 37°C, after which the zone of inhibition was measured and recorded.

### MINIMAL INHIBITORY CONCENTRATION

The minimum inhibitory concentration is the concentration giving the least inhibitory activity and below which there is no further inhibition. It is therefore regarded as the concentration giving the lowest possible zones of inhibition. Two fold serial dilutions were prepared to obtain a 0.1-100mg/ml concentration range. Sterile discs were immersed into the different dilutions and absorption was allowed for 30 minutes. A pair of sterile forceps was used to pick the paper discs and placed on the inoculated plates and the portions were labelled. The plates were incubated at 37°C for 24h and observed for zone of inhibition.

## III. RESULT

The antibacterial activity of ethanolic extracts of *Moringa oleifera* defatted seed (MOD) and seed coat (MOSC) was investigated using agar disc diffusion method, against the selected diarrhoeogenic pathogens such as *Escherichia Coli*, *Proteus mirabilis*, *Klebsiella* spp, *Pseudomonas aeruginosa* and *Salmonella Typhi* isolated from diarrhoeal cases. The extracts showed varying degrees of antibacterial activities against the selected pathogens. Table 1 shows that the highest inhibitory effect was observed in *Proteus mirabilis* (19mm) followed by *Salmonella Typhi* (16mm) with the *Moringa oleifera* seed extract. Some strains (*S. Typhi* and *Proteus mirabilis*) were resistant to ethanolic extracts of *Moringa oleifera* seed coat (Table 1). The seed extracts had activity against *Klebsiella* (4mm), *P. aeruginosa* (3mm) and *E. coli* (5mm). The seed coat extract was sensitive to *Klebsiella* spp (4mm), *P. aeruginosa* (4mm) and *E. coli* (2mm).

**Table 1:** In vitro antibacterial effect of ethanolic extract of *Moringa oleifera* seed and seed coat on test isolates

Antibiotics/extracts	<i>S. Typhi</i>	<i>Proteus mirabilis</i>	<i>P. auroginosa</i>	<i>E. coli</i>	<i>Klebsiella spp</i>
<i>Moringa oleifera</i> seed extract	9mm	16mm	3mm	5mm	4mm
<i>Moringa oleifera</i> seed coat extract	R	R	4mm	2mm	4mm
Gentamicin (10ug)	18mm	21mm	20mm	17mm	16mm
Blank	R	R	R	R	R

R- Resistance

Table 2 shows the result of lowest possible inhibition of *Moringa oleifera* seed extract on the test isolates on a 0.1mg/ml- 100mg/ml concentration. The results shows that *Moringa oleifera* seed extract was sensitive to *Proteus mirabilis* (5mm), *Salmonella Typhi* (1.7mm) while *Pseudomonas aeruginosa* was resistant at 50mg/ml, *Klebsiella spp*, and *E. coli* were resistant at 25mg/ml and 75mg/ml respectively.

**Table 2**

The minimum inhibitory concentration for *Moringa oleifera* seed extract

Extract /concentration	<i>S.Typhi</i>	<i>P. mirabilis</i>	<i>P. auroginosa</i>	<i>E. coli</i>	<i>Klebsiella spp</i>
0.1mg/ml	1.7mm	5mm	R	R	R
25mg/ml	3.2mm	7mm	R	R	R
50mg/ml	3.2mm	9mm	R	1mm	R
75mg/ml	3.4mm	13mm	1mm	1.7mm	1.4mm
100mg/ml	7.6mm	13.8mm	3mm	4mm	3mm

R-Resistance

Table 3, shows that at all the concentration of seed seed coat extract used in this experiment had no activity against *S. Typhi*, *Proteus mirabilis*, and *E. coli* but was sensitive against *P. auroginosa* and *klebsiella spp* at 0.1 mg/ml concentration.

**Table 3**

The minimum inhibitory concentration for *Moringa oleifera* seed coat extract

Extract /concentration	<i>S. Typhi</i>	<i>P. mirabilis</i>	<i>P. auroginosa</i>	<i>E. coli</i>	<i>Klebsiella Spp</i>
0.1mg/ml	R	R	1mm	R	1mm
25mg/ml	R	R	1.4mm	R	1.6mm
50mg/ml	R	R	3mm	R	R
75mg/ml	R	R	R	R	R
100mg/ml	R	R	R	R	R

R-Resistance

#### IV. DISCUSSION

The growing incidence of multidrug resistance of microorganisms to conventional antimicrobial agents is a source of concern to clinical microbiologists globally. As a result, efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects. The demand for more natural antimicrobials has driven scientists to examine the effectiveness of inhibitory compounds such as extracts from plants (Nasar-Abbas and Halkman, 2004.) several publications have documented plant potential antimicrobial properties (Ahmad and Aqil, 2007). Thus plant extracts are promising natural antimicrobial agents with potential applications in infectious disease for controlling pathogenic bacteria. This study reveals that all the examined extract showed varying degrees of antibacterial activities against the selected pathogens, with higher activity observed in *Moringa oleifera* seed extract, which is similar with the findings of (Thilza *et al*, 2010, Jamil *et al*, 2007; Abdulmoneim *et al*, 2011). However its activity on *Proteus mirabilis* and *Salmonella Typhi* is comparable to some commercial antibiotics. On the other hand these organisms are completely resistant to the seed coat extract in this study and therefore not an alternative therapy on ailment caused by *Salmonella* and *Proteus*.

#### V. CONCLUSION/SUGGESTION

In this study, we found out that ethanol extract of seed extracts of *M. oleifera* were active against all the bacteria tested with highest zone of inhibition observed in *Proteus mirabilis* followed by *Salmonella Typhi*. Therefore, *M. oleifera seed* could be a promising antibacterial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. On the other hand, extract from seed coat was less effective. When the obtained results were compared to commercial antibiotics used in the stud, it could be concluded that the ethanol extract of the seeds and seed coat obtained from *M. oleifera* was less effective than the standard antibiotics used as control. However, efforts should be made to conduct *in vivo* studies with the extracts on animal model so as to confirm the present *in vitro* findings as zones of inhibition is not only affected

by sensitivity of the microorganisms alone but concentration of the extract in the disc used and its rate of diffusion in the media as well. Testing the toxicity *in vivo* should also be crucial in determining the optimum dose to be administered as an effective antibiotics.

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