

ANTIMICROBIAL ACTIVITY OF *Moringa oleifera* LEAF AGAINST ISOLATES OF BEEF OFFAL

Abstract

This study was designed to evaluate the antimicrobial potentials of ethanol and aqueous extracts of *Moringa oleifera* against microorganisms isolated from beef offal. The beef offal samples (liver, kidney and intestine) were purchased from an abattoir in Port Harcourt while the *Moringa oleifera* leaves were harvested from the farm, Rivers State University of Science and Technology, Port Harcourt. Plating was done using the spread plate method while test for inhibitory potential was done using disc diffusion method. Six species of bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus spp*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) and two mould species (*Rhizopus spp* and *Mucor spp*) were identified. Antimicrobial potentials of the ethanol and aqueous extracts of *M. oleifera* on isolates were tested at various concentrations (50, 100, 200 and 400mgml⁻¹). Ethanol extract of *M. oleifera* showed strong antimicrobial activity and concentration dependent inhibitory effect on beef offal isolates while the aqueous extract was effective on test bacteria isolates except *Streptococcus spp* at 400mg/ml. At a concentration of 400 mg/ml, zones of inhibition recorded were highest with ethanol extracts for *Staphylococcus aureus* (14.00 ±0.6 mm) and *Bacillus cereus* and *Proteus vulgaris* (12.00 ±0.4mm). Minimum Inhibitory Concentration (MIC) was at 200mg ml⁻¹ for ethanol extract and 400 mg ml⁻¹ for aqueous extract. The result of this study showed that *M. oleifera* is a potential source of antimicrobial agent against some pathogenic bacteria implicated in beef offal's spoilage. The study recommends further research to be carried out on other extractive techniques since different extractive techniques may correspond to different antimicrobial effectiveness.

Key words: *Moringa oleifera*, antimicrobial, aqueous extract, ethanol extract, beef offal, Minimum Inhibitory Concentration

INTRODUCTION

There is an increasing interest in the use of natural preservatives of plant origin as antimicrobials in food [1]. This is as a result of the increased resistance in pathogenic strains against chemical food preservatives as well as declining consumer preference due to residual effect of chemical preservatives and consumer safety [2]. A lot of spices and herbs have been proven by researchers as preservatives due to the presence of phytochemicals in them [3, 4]. The use and consumption of diets prepared with spices and herbs have gained increased acceptability among consumers and the scientific community because they contain chemical compounds exhibiting antioxidant properties [5]. An important step in screening of plant materials for preservative function is evaluation of its antimicrobial activity against food borne micro-organisms [4].

42 *Moringa oleifera* commonly referred to as horse radish is the widely cultivated species of the
43 genus. It is a native to the sub- Himalayan region of India though it is now naturalized in many
44 countries in Africa, Saudi Arabia, South East Asia, the Caribbean and South America [6]. *Moringa*
45 *oleifera* has been a popular plant amongst the natives of the Northern and South–Eastern parts of
46 Nigeria, where for ages, the leaves, root, bark have been respectively exploited for food, medicines
47 and water coagulant [4]. Despite the array of uses to which parts of *Moringa* is put to, scanty
48 literature is available on the use of *M. oleifera* plant leaves as a preservative or antimicrobial agent
49 in beef offal. The increase resistance in pathogenic strains against chemical food preservatives has
50 given rise to a frantic search for the development of new and effective antimicrobial agents. The
51 evaluation of antimicrobial activity of *Moringa oleifera* leaves may contribute immensely to the
52 search. It is in view of this that the present study aims to investigate the antimicrobial activity of
53 *M. oleifera* leaves against isolates of beef offal.

54 **Materials and methods**

55 Fresh *M. oleifera* leaves were harvested from the Agricultural Farm of the Rivers state University
56 of Science and Technology, Port Harcourt. Identification and authentication of plant was done at
57 the Plant Science Laboratory, Department of Botany of the same university. Beef offal samples
58 (kidney, liver and intestine) were purchased from an abattoir in Port Harcourt metropolis. *M.*
59 *oleifera* leaves were treated according to the method described by Yadav and Singh [2]. This
60 included picking, washing under flowing tap water to remove all sand debris and oven drying at
61 45°C for 12 hours in a hot air oven. Dried leaves sample was pulverized using laboratory mortar
62 and pestle and stored at room temperature in sterile and air tight bottles until ready for use.

63 **Plant extract preparation**

64 The method of Srinivasan *et al.* [7] was used for the ethanol and aqueous extraction. 50g of the
65 finely pulverized *M. oleifera* was subjected to soxhlet extraction for 10 hours using 500ml each of
66 ethanol and distilled water (that is, 1:10 weight/volume). The extracts were recovered by filtration
67 using Whatman filter paper (No.1). The solvent extracted fractions were subjected to vacuum

desiccation at 40°C in a rotary vacuum evaporator to remove any traces of solvents and to obtain residues. The extracts were stored in sample bottles at 4°C for further analysis prior to use. The test residues were prepared as stocks using appropriate volume of distilled water (50, 100, 200, 400 mg ml⁻¹). The reconstituted extracts labeled crude aqueous and ethanol extract was used for antimicrobial analysis.

Isolation and characterization of bacteria and moulds from beef offal

Bacteria isolates were obtained from uncooked beef offal namely liver, kidney and intestine. Plating was done using the spread plate method. Nutrient agar medium (Lab M), was used for bacteria isolation and potatoes dextrose agar (Lab M) for fungi isolation. Standard microbiological techniques were used for morphological and biochemical characterization of the isolates according to the taxonomic scheme of Cowan and Steel [8]. Microscopic examination of cell shape, visual observation of colonial morphology and biochemical test were employed for characterization of fungal isolates.

Preparation of culture media

Nutrient agar and Potato dextrose agar used were obtained as commercially dehydrated powder. These were prepared according to manufacturer's instruction. The prepared culture media were sterilized at 121°C and 15psi for 20 minutes. They were cooled slowly after which a 20ml of each culture media was poured into petri dishes separately and were allowed to solidify. Nutrient broth was prepared from commercially available dehydrated powder and prepared according to manufacturer's instruction and sterilized as earlier described.

Antimicrobial activity of crude ethanol and aqueous extracts of *M. oleifera*

The antimicrobial activities of the extracts were determined by disc diffusion method as described by Gulluce *et al.* [9]. Filter paper of 6mm was prepared and sterilized in an autoclave for 15 minutes at 121°C. The filter paper was dipped in ethanol and aqueous extract of *M. oleifera* at varying concentrations of (400mg ml⁻¹, 200mg ml⁻¹, 100mg ml⁻¹ and 50mg ml⁻¹ respectively).

Using an ethanol dipped and flamed forceps, these discs were placed over nutrient agar plate seeded with the isolated organisms. All plates were incubated at 37°C for 24 hours for bacteria bioassay while for the fungi susceptibility test, the filter paper disc of 6mm was dipped in an aqueous and ethanol extract at varying concentrations (400, 200, 100 and 50mg ml⁻¹). These discs were placed over potato dextrose agar seeded with the isolated organisms. All plates were incubated at room temperature for three days. The diameter of inhibition zone appearing around the filter paper disc was measured to the nearest whole number in millimeter, using transparent ruler and the result recorded. Inhibition zones with diameter less than (<9mm) was considered inactive, diameter between 9-12mm was considered as partially active, diameter 13 -18 was considered as active and that with >18mm was considered as very active [10].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was considered as the lowest concentration of *M. oleifera* able to inhibit the growth of test micro-organisms after 24 hours. The MIC of the ethanol extract of *M. oleifera* was determined by using serially diluted solutions of the extract ranging from 50mg ml⁻¹ to 400mg ml⁻¹. The microorganism suspension of 0.2 ml was added to the broth dilutions. Plates were incubated at 37°C for 24 hours and were examined for micro-organism growth inhibition. Effects were indicated by the growth of microorganism or failure of the organisms' growth from the recovery media on the plate after incubation. Failure of micro-organism growth indicated a sensitivity effect while the plates that showed microbial growth after incubation indicated a resistant effect.

Statistical Analysis

All tests were carried out in triplicates. Data from the study was subjected to analysis of variance (ANOVA) using the SPSS software version 11. Significantly different treatment means (concentrations of 50, 100, 200, 400mg ml⁻¹) were separated using Duncan's Multiple Range Test with significance difference determined at $P < 0.05$.

Result and discussion

The microorganisms isolated from beef offal are shown in Table 1. Six species of bacteria and two species of moulds were identified. The bacteria isolates were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Streptococcus spp*, *Pseudomonas aeruginosa* and *Proteus vulgaris* whereas moulds isolated were *Rhizopus spp* and *Mucor spp*. Microbial count of the isolated microorganisms revealed *Staphylococcus aureus* and *Bacillus cereus* as the predominant bacteria isolated in all the beef offal samples. *Streptococcus faecalis*, *Escherichia coli* and *Proteus vulgaris* were also isolated alongside *Bacillus cereus* and *Staphylococcus aureus* in the intestine. The microorganisms isolated in this study are similar to the isolates reported in meat and meat products by Uzeh *et al.* [11]. Clarence *et al.* [12] also reported the presence of *Staphylococcus spp*, *Escherichia coli*, *Streptococcus spp*, *Pseudomonas aeruginosa*, and *Bacillus cereus* as isolates in meat and meat products. Similarly, Agarry *et al.* [13] isolated *Staphylococcus aureus*, *Pseudomonas spp*, *Proteus vulgaris* and *Bacillus spp* as bacteria species while *Mucor spp* and *Rhizopus spp* are fungi in beef samples. The isolation of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Streptococcus spp* in beef offal samples was expected as these organisms have their natural habitat in the intestinal tract. Besides, *Proteus vulgaris* and *Pseudomonas aeruginosa* are among the genera of bacteria most frequently found in meats and sea-foods [14, 15]. The predominance of *Staphylococcus aureus* and *Bacillus cereus* on the beef offal samples could be attributed to poor handling of meat during slaughter and display for sale. The other bacteria isolates may be as a result of poor slaughtering operations.

Table 1: Microorganisms isolated from meat (offals)

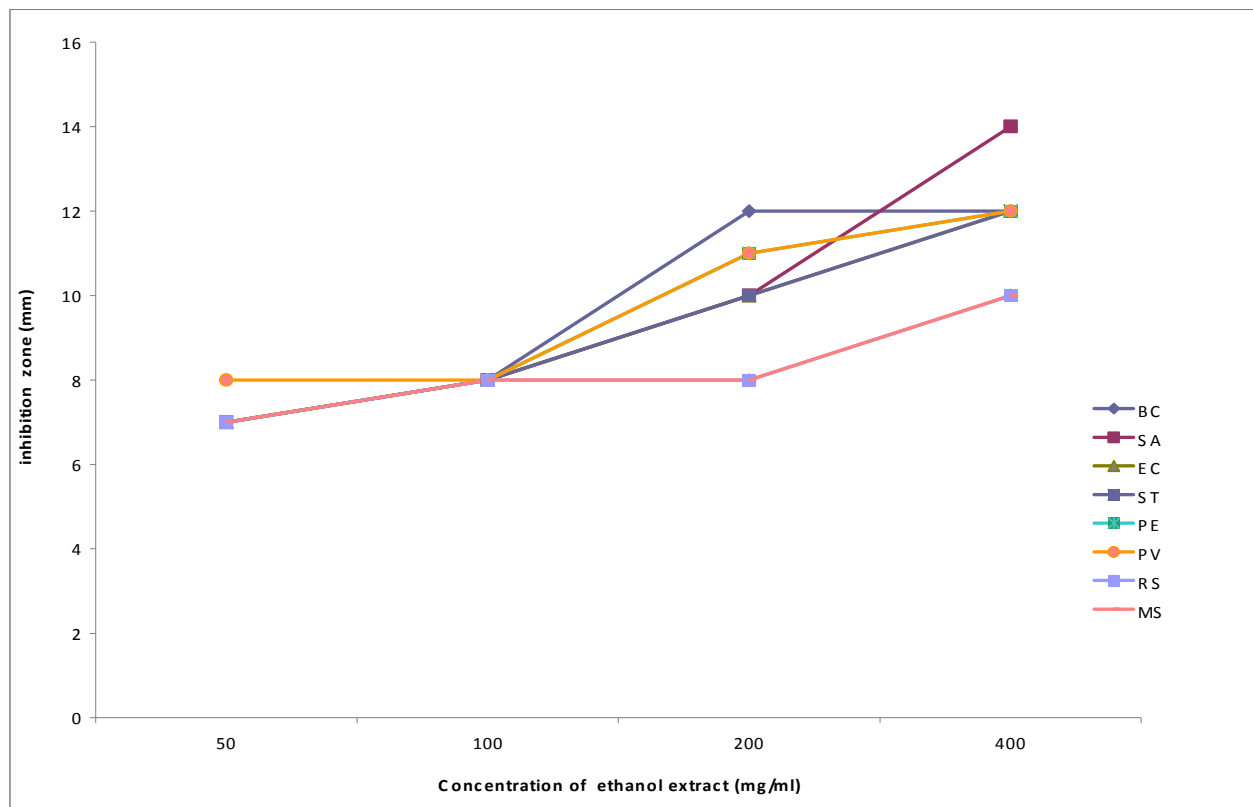
Meat offals	Bacteria Species	Fungi species
Liver	<i>Staphylococcus aureus</i> and <i>Bacillus cereus</i>	<i>Mucor spp</i>
Kidney	<i>Staphylococcus aureus</i> and <i>Bacillus cereus</i>	<i>Mucor spp</i>

Intestine	<i>Escherichia coli</i> , <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Rhizopus spp</i> <i>Streptococcus spp</i> , <i>Proteus vulgaris</i> and <i>Pseudomonas aeruginosa</i>
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139 The antimicrobial activity of ethanol and aqueous extract of *M. oleifera* against beef offal isolates
140 measured in terms of diameter of zone of inhibition showed that as the concentration of *M. oleifera*
141 increased, the diameter of zone of inhibition increased and vice versa (Fig. 1 and 2). The diameter
142 of zone of inhibition of the ethanol extract ranged from 6.0 ± 0.47 to 14.0 ± 0.6 mm for
143 *Staphylococcus aureus* while the aqueous extract inhibited the growth of microorganisms with
144 diameter zones of inhibition ranging between 6.0 ± 0.0 to 8.2 ± 0.2 mm for all tested bacteria
145 isolates. However, at ethanoic and aqueous concentration of 50 and 100 mg ml⁻¹, all isolates were
146 resistant. Sensitivity was observed for all isolates (bacteria and fungi) at ethanol concentrations of
147 200 and 400 mg ml⁻¹. There was no inhibition for aqueous extract at all concentrations for all
148 isolates. Aqueous extract did not exert antifungal effect at all concentrations against fungal
149 isolates. The result agrees with the findings of Kapoor [16] that reported increase in the diameter
150 zone of inhibition with increase in concentration of *M. oleifera*. Chloroform extracts of *M. oleifera*
151 seed has been reported to exert inhibitory effect against *E. coli* and *S. aureus* at concentrations of
152 200 mg ml⁻¹ [4]. Napoleon *et al.* [17] reported that *Enterobacter spp*, *S. aureus*, *Pseudomonas*
153 *aeruginosa*, *Staphylococcus typhi* and *Escherichia coli* were sensitive to ethanol extract of *M.*
154 *oleifera* seed at 200 mg ml⁻¹. Equally, Bako *et al.* [18] reported that the crude extract of *M. oleifera*
155 had inhibitory effects on *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Salmonella typhi*,
156 *Pseudomonas aeruginosa*, *Staphylococcus pneumonia* and *Escherichia coli*. Comparatively, the
157 ethanol extract of *M. oleifera* exhibited broad spectrum activity and showed greater potential in its
158 ability to inhibit all isolates than aqueous extract of *M. oleifera*. This may be as a result of the
159 inherent phytochemicals (alkaloids, saponins, tannins, flavonoids, steroids, phenols, phylobatannin
160 etc) as reported by Bukar *et al.* [4] and the fact that these phytochemicals readily dissolve in

161 ethanol compared to water. This agreed with the findings of Gundogan *et al.* [19] and Malu *et al.*
 162 [20] who reported that ethanol extracts of spices and herbs had maximum inhibitory effect due to
 163 better solubility of the bioactive components in organic solvents as compared to water.



164 Figure 1: Activity of varying concentrations of *M. oleifera* ethanol leaf extract on beef offal
 165 samples microbial isolates

166 Key: BC = *Bacillus cereus*, SA=*Staphylococcus*, EC=*Escherichia coli*, ST = *Streptococcus spp*, PE = *Pseudomonas aeruginosa*, PV
 167 = *Proteus vulgaris*, RS = *Rhizopus spp*, MS = *Mucor spp*
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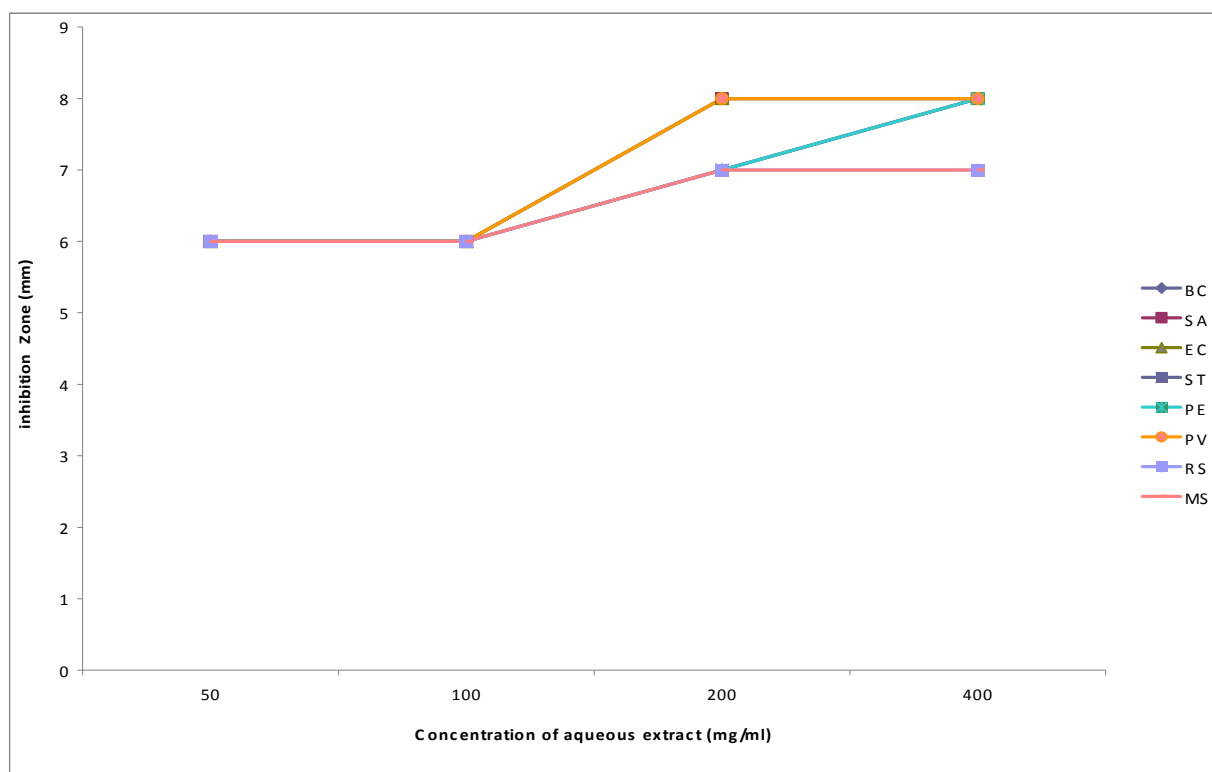


Figure 2: Activity of varying concentrations of *M. oleifera* aqueous leaf extract on beef offal samples microbial isolates.

Key: BC = *Bacillus cereus*, SA= *Staphylococcus*, EC= *Escherichia coli*, ST = *Streptococcus spp*, PE *Pseudomonas aeruginosa*, PV = *Proteus vulgaris*, RS = *Rhizopus spp*, MS = *Mucor spp*

Minimum Inhibitory Concentration (MIC) of the ethanol extract of *M. oleifera* was at 200mg ml⁻¹ (Table 2) while aqueous extract revealed inhibition at 400mg ml⁻¹. The aqueous extract of *Moringa oleifera* did not inhibit the growth of the fungal isolates at all concentrations. The result suggests that aqueous extracts require higher concentrations to inhibit growth of bacteria isolates.

Table 2: Minimum Inhibitory Concentration of ethanol and aqueous extract of *M. oleifera* on beef offal isolates

Isolates	Ethanol Concentration				Aqueous Concentration			
	50	100	200	400	50	100	200	400
<i>Bacillus Cereus</i>	NI	NI	I	I	NI	NI	NI	I
<i>Staphylococcus aureus</i>	NI	NI	I	I	NI	NI	NI	I
<i>Escherichia coli</i>	NI	NI	I	I	NI	NI	NI	I
<i>Streptococcus spp</i>	NI	NI	I	I	NI	NI	NI	NI
<i>Pseudomonas aeruginosa</i>	NI	NI	I	I	NI	NI	NI	I

189	<i>Proteus Vulgaris</i>	NI	NI	I	I	NI	NI	NI	I
190	<i>Rhizopus spp</i>	NI	NI	I	I	NI	NI	NI	NI
191	<i>Mucor spp</i>	NI	NI	I	I	NI	NI	NI	NI

192 Key: NI – No Inhibition, I- Inhibition

193 Conclusion

194 The result of the study showed that *M. oleifera* leaf extract was effective against bacteria isolates
195 of beef offals. Efficacy was however higher with ethanol extract from concentration of 200mg ml⁻¹
196 than aqueous extract of *M. oleifera* at 400mg ml⁻¹. Therefore, this potential of *Moringa oleifera*
197 leaves as a natural preservative of plant origin could be exploited by the meat industry in
198 controlling bacteria contamination though different extractive techniques correspond to different
199 antimicrobial effectiveness.

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