

Original Research Article

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 Agricultural 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', 'MIC'

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

42 materials for preservative function is evaluation of its antimicrobial activity against food
43 borne micro-organisms [4].

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

57 **Materials and methods**

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

67 **Plant extract preparation**

68 The method of Srinivasan *et al.* [7] was used for the ethanol and aqueous extraction. 50g of
 69 the finely pulverized *M. oleifera* was subjected to soxhlet extraction for 10 hours using 500ml
 70 each of ethanol and distilled water (i.e 1:10 weight/volume). The extracts were recovered by
 71 filtration using Whatman filter paper (No.1). The solvent extracted fractions were subjected
 72 to vacuum desiccation at 40°C in a rotary vacuum evaporator to remove any traces of solvents
 73 and to obtain residues. The extracts were stored in sample bottles at 4°C for further analysis
 74 prior to use. The test residues were prepared as
 75 stocks using appropriate volume of distilled water (50, 100, 200, 400 mg/ml). The
 76 reconstituted extracts labeled crude aqueous and ethanol extract was used for antimicrobial
 77 analysis.

78 **Isolation and characterization of bacteria and moulds from beef offal**

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

86 **Preparation of culture media**

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

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

94 The antimicrobial activities of the extracts were determined by disc diffusion method as
 95 described by Gulluce *et al.* [9]. Filter paper of 6mm was prepared and sterilized in an
 96 autoclave for 15 minutes at 121°C. The filter paper was dipped in an ethanol and aqueous
 97 extract of *M. oleifera* at varying concentrations of (400mg ml⁻¹, 200mg ml⁻¹, 100mg ml⁻¹ and
 98 50mg ml⁻¹ respectively). Using an ethanol dipped and flamed forceps, these discs were
 99 placed over nutrient agar plate seeded with the isolated organisms. All plates were incubated
 100 at 37°C for 24 hours for bacteria bioassay while for the fungi susceptibility test, the filter
 101 paper disc of 6mm was dipped in an aqueous and ethanol extract at varying concentrations
 102 (400, 200, 100 and 50mg ml⁻¹). These discs were placed over potato dextrose agar seeded
 103 with the isolated organisms. All plates were incubated at room temperature for three days.
 104 The diameter of inhibition zone appearing around the filter paper disc was measured to the
 105 nearest whole number in millimeter, using transparent ruler and the result recorded. Inhibition
 106 zones with diameter less than (<9mm) was considered inactive, diameter between 9-12mm
 107 was considered as
 108 partially active, diameter 13 -18 was considered as active and that with >18mm was
 109 considered as very active [10].

110 **Determination of Minimum Inhibitory Concentration (MIC)**

111 The MIC was considered as the lowest concentration of *M. oleifera* able to inhibit the growth
 112 of test micro-organisms after 24 hours. The MIC of the ethanol extract of *M. oleifera* was
 113 determined by using serially diluted solutions of the extract ranging from 50mg/ml to
 114 400mg/ml. The microorganism suspension of 0.2 ml was added to the broth dilutions. Plates
 115 were incubated at 37°C for 24 hours and were examined for micro-organism growth
 116 inhibition. Effects were indicated by the growth of microorganism or failure of the
 117 organisms' growth from the recovery media on the plate after incubation. Failure of micro-

organism growth indicated a sensitivity effect while the plates that show 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) was 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

144 poor handling of meat during slaughter and display for sale. The other bacteria isolates may
145 be as a result of poor slaughtering operations.

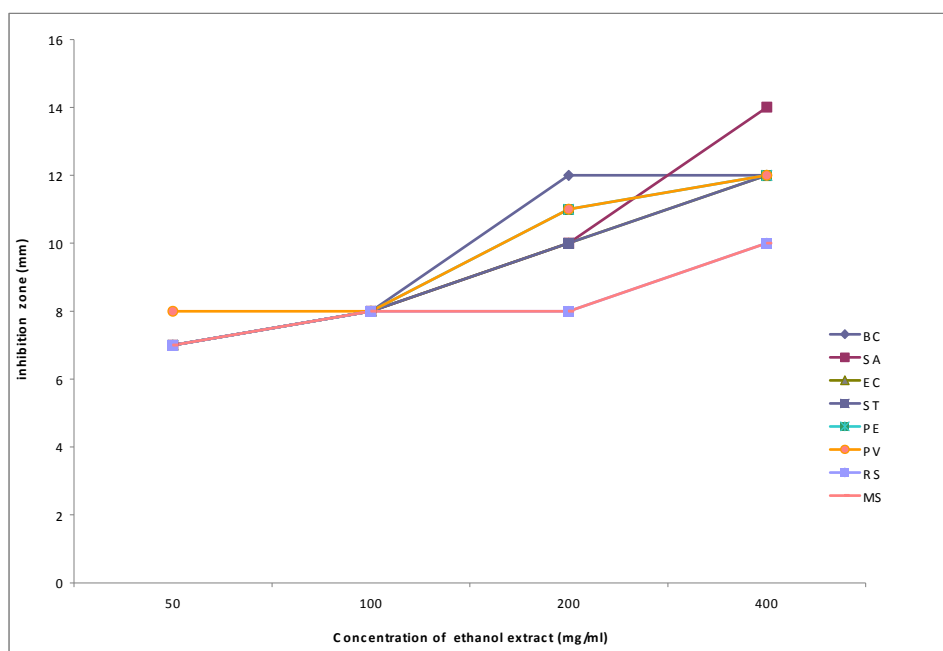
146 Table 1: Microorganisms isolated from meat (offals)

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>Streptococcus spp</i> , <i>Proteus vulgaris</i> and <i>Pseudomonas</i> <i>aeruginosa</i>	<i>Rhizopus spp</i>

147

148 The antimicrobial activity of ethanol and aqueous extract of *M. oleifera* against beef offal
149 isolates measured in terms of diameter of zone of inhibition showed that as the concentration
150 of *M. oleifera* increase, the diameter of zone of inhibition increased and vice versa (Fig. 1 and
151 2). The diameter of zone of inhibition of the ethanol extract ranged from 6.0 ± 0.47 to
152 14.0 ± 0.6 mm for *Staphylococcus aureus* while the aqueous extract inhibited the growth of
153 microorganisms with diameter zones of inhibition ranging between 6.0 ± 0.0 to 8.2 ± 0.2 mm
154 for all tested bacteria isolates. However, at ethanoic and aqueous concentration of 50 and
155 100 mg ml^{-1} , all isolates were resistant. Sensitivity was observed for all isolates (bacteria and
156 fungi) at ethanol concentrations of 200 and 400 mg ml^{-1} . There was no inhibition for aqueous
157 extract at all concentrations for all isolates. Aqueous extract did not exert antifungal effect at
158 all concentrations against fungal isolates. The result agrees with the findings of Kapoor [16]
159 that reported increase in the diameter zone of inhibition with increase in concentration of *M.*
160 *oleifera*. Chloroform extracts of *M. oleifera* seed has been reported to exert inhibitory effect
161 against *E. coli* and *S. aureus* at concentrations of 200 mg/ml [4]. Napoleon *et al.* [17] reported
162 that

163 *Enterobacter* spp, *S. aureus*, *Pseudomonas aeruginosa*, *Staphylococcus typhi* and
 164 *Escherichia coli* were sensitive to ethanol extract of *M. oleifera* seed at 200mg/ml. Equally,
 165 Bako *et al.* [18] reported that the crude extract of *M. oleifera* had inhibitory effects on
 166 *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas*
 167 *aeruginosa*, *Staphylococcus pneumonia* and *Esherichia coli*. Comparatively, the ethanol
 168 extract of *M. oleifera* exhibited broad spectrum activity and showed greater potential in its
 169 ability to inhibit all isolates than aqueous extract of *M. oleifera*. This may be as a result of the
 170 inherent phytochemicals (alkaloids, saponins, tannins, flavonoids, steroids, phenols,
 171 phylobatannin etc) as reported by Bukar *et al.* [4] and the fact that these phytochemicals
 172 readily dissolve in ethanol compared to water. This agreed with the findings of Gundogan *et*
 173 *al.* [19] and Malu *et al.* [20] who reported that ethanol extracts of spices and herbs had
 174 maximum inhibitory effect due to better solubility of the bioactive components in organic
 175 solvents as compared to water.



176
 177 Figure 1: Activity of varying concentrations of *M. oleifera* ethanol leaf extract on beef offal
 178 samples microbial isolates

179 Key: BC = *Bacillus cereus*, SA=*Staphylococcus*, EC=*Escherichia coli*, ST = *Streptococcus* spp, PE = *Pseudomonas*
 180 *aeruginosa*, PV = *Proteus vulgaris*, RS = *Rhizopus* spp, MS = *Mucor* spp
 181

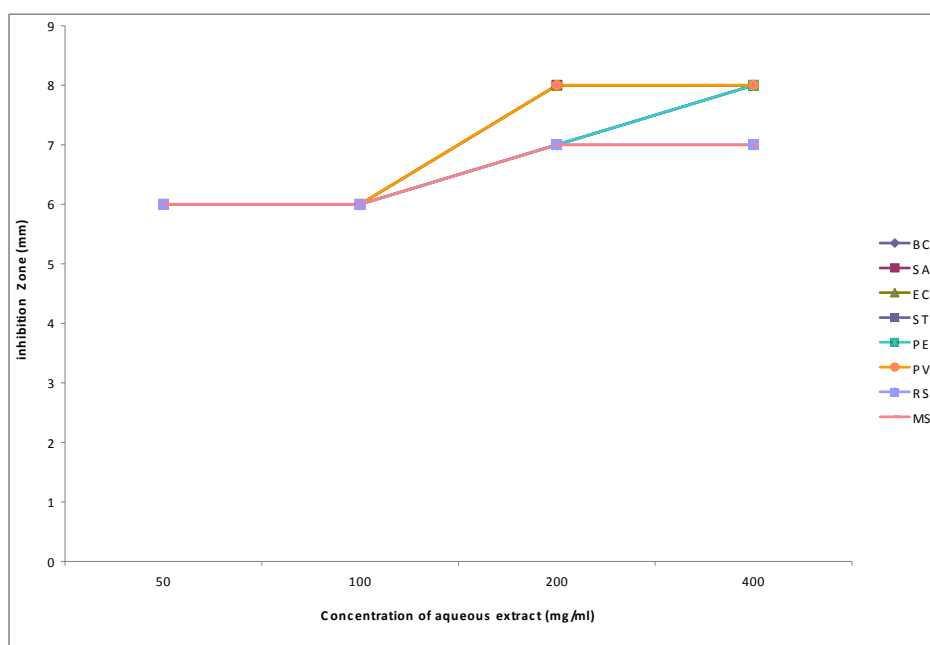


Figure 2: Activity of varying concentrations of *M. oleifera* aqueous leaf extract on beef offal samples microbial isolates.

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>Esherichia coli</i>	NI	NI	I	I	NI	NI	NI	I
<i>Streptococcus spp</i>	NI	NI	I	I	NI	NI	NI	NI
<i>Pseudomonas auruginosa</i>	NI	NI	I	I	NI	NI	NI	I
<i>Proteus Vulgaris</i>	NI	NI	I	I	NI	NI	NI	I
<i>Rhizopus spp</i>	NI	NI	I	I	NI	NI	NI	NI

204 *Mucor spp* NI NI I I NI NI NI NI

205 Key: NI – No Inhibition, I- Inhibition

206 Conclusion

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

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