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Antimicrobial Activity of *Moringa oleifera* Leaf against Isolates of Beef Offal

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Authors' contributions

This work was a collaborator study of both authors. Author CUS designed the study, performed the statistical analyses, wrote the protocol and first draft of manuscript while YEA managed literature searches, analyses of the study as well as wrote the final manuscript. Both authors read and approved the final manuscript.

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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, Streptocccus* 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 400 $mgml^{-1}$). Ethanol extract of *M. oleifera* showed strong antimicrobial activity and concentration dependent inhibitory effect on beef offal isolates while the aqueous extract was ineffective on test bacteria isolates. At a concentration of 400 mg/ml, zones of inhibition recorded were highest with ethanol extracts for *Staphylococcus aureus* (14.00±0.6 mm), *Bacillus cereus* (13.00±0.1 mm) and

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Proteus vulgaris (12.00±0.4 mm). Minimum Inhibitory Concentration (MIC) was at 200 mg 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.

Keywords: Moringa oleifera; antimicrobial; aqueous extract; ethanol extract; beef offal; Minimum Inhibitory Concentration.

1. 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].

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

2. MATERIALS AND METHODS

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

2.1 Plant Extract Preparation

The method of Srinivasan et al. [7] was used for the ethanol and aqueous extraction. 50 g of the finely pulverized *M. oleifera* was subjected to soxhlet extraction for 10 hours using 500 ml each of ethanol and distilled water (that is, 1:10 weight/volume). The extracts were recovered by filtration 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.

2.2 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.

2.3 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 15 psi for 20 minutes. They were cooled slowly after which a 20 ml 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.

2.4 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 (400 mg ml $^{-1,}$ 200 mg ml $^{-1,}$ 100 mg ml $^{-1}$ and 50 mg 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 6 mm was dipped in an aqueous and ethanol extract at varying concentrations (400, 200, 100 and 50 mg 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 (<9 mm) was considered inactive, diameter between 9-12 mm was considered as partially active, diameter 13 -18 was considered as active and that with >18 mm was considered as very active [10].

2.5 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 50 mg ml^{-1} to 400 mg 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.

2.6 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, 400 mg ml $^{-1}$) were separated using Duncan's Multiple Range Test with significance difference determined at *P< 0.05.*

3. RESULTS 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*, *Escerichia 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, Escerichia 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.

The antimicrobial activity of ethanol and aqueous extract of *M. oleifera* against beef offal isolates measured in terms of diameter of zone of inhibition showed that as the concentration of *M. oleifera* increased, the diameter of zone of inhibition increased and vice versa (Figs. 1 and 2). The diameter of zone of inhibition of the ethanol extract ranged from 6.0±0.47 to 14.0±0.6 mm for *Staphylococcus aureus* while the aqueous extract had diameter zones of inhibition ranging between 6.0±0.0 to 8.2±0.2 mm for all tested bacteria isolates. However, at ethanoic and aqueous concentration of 50 and 100 mg ml^{-1}, all isolates were resistant. Sensitivity was observed for all isolates (bacteria and fungi) at ethanol concentrations of 200 and 400 mg ml^{-1}. There was no inhibition for aqueous extract at all concentrations for all isolates. Aqueous extract did not exert antifungal effect at all concentrations against fungal isolates. The result agrees with the findings of Kapoor [16] that reported increase in the diameter zone of inhibition with increase in concentration of *M. oleifera*. Chloroform extracts of *M. oleifera* seed has been reported to exert inhibitory effect against *E. coli and S. aureus* at concentrations of 200 mg ml^{-1} [4]. Napoleon et al. [17] reported that *Enterobacter* spp*, S. aureus, Pseudomonas aeruginosa, Staphylococcus typhi and Escherichia coli* were sensitive to ethanol extract of *M. oleifera* seed at 200 mg ml^{-1}. Equally, Bako et al. [18] reported that the crude extract of *M. oleifera* had inhibitory effects on *Bacillus subtilis, Bacillus cereus, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus pneumonia and Esherichia coli*. Comparatively, the ethanol extract of *M. oleifera* exhibited broad spectrum activity and showed greater potential in its ability to inhibit all isolates than aqueous extract of *M. oleifera*. This may be as a result of the inherent phytochemicals (alkaloids, saponins, tannins, flavonoids, steroids, phenols, phylobatannin etc) as reported by Bukar et al. [4] and the fact that these phytochemicals readily dissolve in ethanol compared to water. This agreed with the findings of Gundogan et al. [19] and Malu et al. [20] who reported that ethanol extracts of spices and herbs had maximum inhibitory effect due to better solubility of the bioactive components in organic solvents as compared to water.

Minimum Inhibitory Concentration (MIC) of the ethanol extract of *M. oleifera* was at 200 mg ml^{-1} (Table 2) while aqueous extract revealed inhibition at 400 mg 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 1. Microorganisms isolated from meat (offals)

Meat offals	Bacteria species	Fungi species
Liver	*Staphylococcus aureus and Bacillus cereus*	*Mucor* spp
Kidney	*Staphylococcus aureus and Bacillus cereus*	*Mucor* spp
Intestine	*Escherichia coli, Bacillus cereus, Staphylococcus aureus, Streptococcus* spp*, Proteus vulgaris and Pseudomonas aeruginosa*	*Rhizopus* spp

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

Isolates	Ethanol concentration $mgml^{-1}$				Aqueous concentration $mgml^{-1}$			
	50	100	200	400	50	100	200	400
Bacillus cereus	NI	NI	I	I	NI	NI	NI	I
Staphylococcus aureus	NI	NI	I	I	NI	NI	NI	I
Esherichia coli	NI	NI	I	I	NI	NI	NI	I
Streptococcus spp	NI	NI	I	I	NI	NI	NI	NI
Pseudomona aeruginosa	NI	NI	I	I	NI	NI	NI	I
Proteus vulgaris	NI	NI	I	I	NI	NI	NI	I
Rhizopus spp	NI	NI	I	I	NI	NI	NI	NI
Mucor spp	NI	NI	I	I	NI	NI	NI	NI

KEY: NI – No Inhibition; I- Inhibition

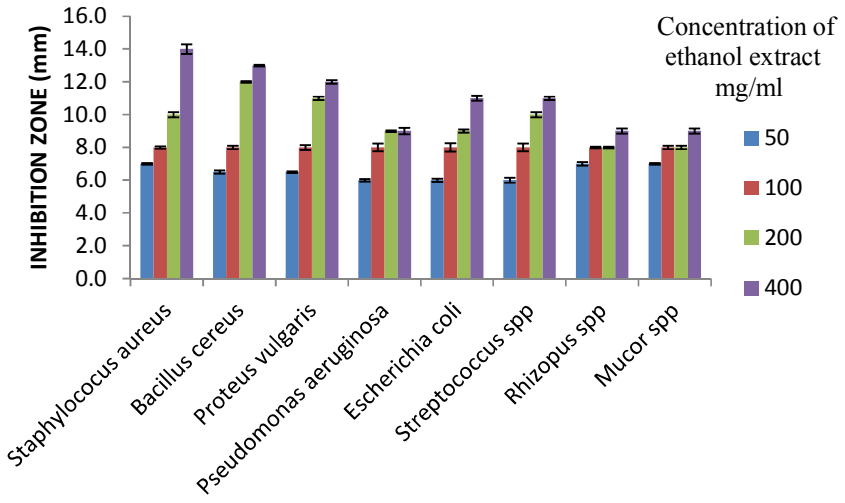

Fig. 1. Activity of varying concentrations of *M. oleifera* ethanol leaf extract on beef offal samples microbial isolates

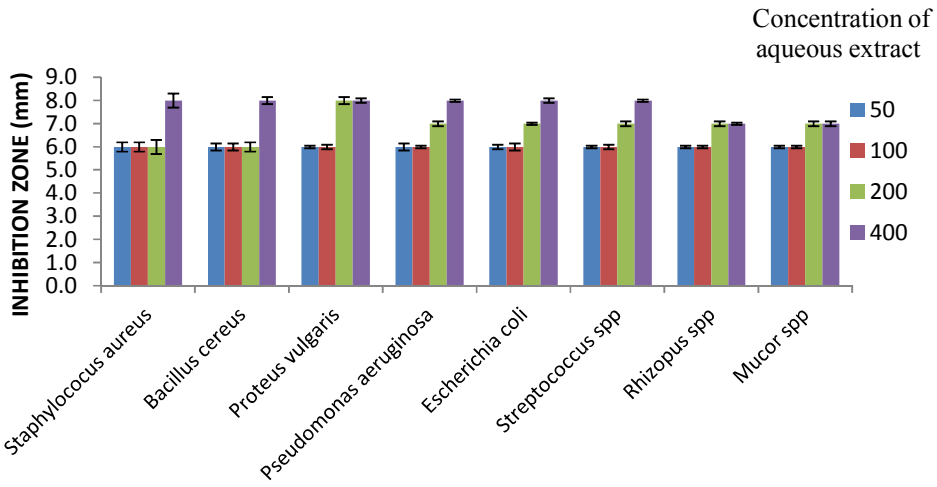

Fig. 2. Activity of varying concentrations of *M. oleifera* aqueous leaf extract on beef offal samples microbial isolates

4. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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