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ANTIMICROBIAL EVALUATION OF ETHANOLIC AND AQUEOUS LEAF EXTRACTS OF SOUTHEAST NIGERIAN GROWN *MORINGA OLEIFERA* ON SOME ENTERIC ISOLATES

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ABSTRACT

Various plants have been reported to possess therapeutic properties; and this explains the reason for their wide use in the treatment of human diseases. The extracts of the plants are known to have bioactive agents and these are found to vary with the extraction solvent used. The antimicrobial activities of the leaf extract of Southeast Nigerian grown *Moringa oleifera* on bacterial and fungal isolates were investigated using two solvents, water and ethanol, to extract the bioactive agents. The zones of inhibition were determined using different concentrations of the leaf extracts ranging from 6.25%, 12.50%, 25.0%, 50.0% and 100% by employing Kirby Bauer disc diffusion method in triplicates. It was observed that the ethanolic extracts of *M. oleifera* leaf have more appreciable antimicrobial activity than water and the bacterial pathogens were more susceptible than the fungal pathogens. In the present study, *M. oleifera* produced the highest zone of inhibition on *Staphylococcus* species from 8mm to 17mm, followed by *Escherichia coli* (7mm to 15mm) while the least was *Pseudomonas* species with zone of inhibition ranging from 7mm to 13mm. The treatment with *M. oleifera* leaf extract on *Candida* species produced a zone of inhibition ranging from 6mm to 15mm compared to Nystacin with 17mm; *Penicillium* species with 6mm to 14mm compared with Nystacin with 16mm and *Mucor* species ranging from 6mm- 12mm, compared to the control with 14mm. The Minimum inhibitory concentration (MIC) of the leaf extract on the bacterial pathogens and *Penicillium* species

was 12.5% while *Candida*, *Aspergillus* and *Mucor* species had MIC of 25%. The zones of inhibition of both ethanolic and aqueous leaf extracts were concentration dependent. The results obtained from the leaf extracts were comparable to the standard drugs used as control. This work therefore supports the use of *M. oleifera* leaf extract in drug formulation particularly antimicrobial drugs.

KEYWORD: Antimicrobial activity, *Moringa oleifera*, ethanolic extract, aqueous extract, enteric isolates.

INTRODUCTION

The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the urgency for the search of new infection-fighting strategies.[1,2] Most of the current antimicrobial agents are inefficient in controlling some microbial diseases.[3] Immense benefits have been derived by man from using medicinal herbs in disease management, because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs. They could also serve as possible source of new and cheap drugs.[4,5]

The increasing discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them. There is increased evidence to proof that medicinal plants may represent an alternative and reliable treatment for infectious diseases. The screening of plant extracts and plant products for antimicrobial activities has shown that plants represent a potential source of novel antibiotic prototypes.[6] 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.[7] The antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. These metabolites with antimicrobial properties are of great significance in therapeutic treatments.

M. oleifera Lam. is a well documented world renowned plant herb known for its extraordinary nutritional and medicinal properties. It is a highly valued plant distributed in many countries of the tropics and subtropics. It is one of the 14 species of the Family *Moringaceae*, native to western and sub-Himalayan regions, India, Pakistan, Africa, Asia Minor, Arabia, South America and the Caribbean Islands.[8] *M. Oleifera* has been used

extensively in traditional medicine for the treatment of several ailments like skin diseases, diarrhea, epilepsy, hysteria, as stimulant in paralytic afflictions and also useful in promoting digestion.[9, 10] Various parts of the plant have been reported to possess therapeutic properties.[11, 12, 13] and this explains the reason for its wide use in the treatment of human diseases. The extracts of the plant are known to have bioactive agents and these are found to vary with the extraction solvent used. The present investigation was undertaken to test the antimicrobial activity of ethanolic and aqueous leaf extract of Southeastern grown *M. oleifera* on some enteric isolates.

MATERIALS AND METHODS

Sample Collection and Preparation of Plant Materials: Fresh leaf samples of *M. oleifera* were harvested from the Botanical Garden of the Biology Department, Abia State Polytechnic, Aba. A plant taxonomist in the Department identified and authenticated the leaves as *M. oleifera* with voucher number BGBD 101. Following the procedure by Abalaka et al, [14] the plant materials were air-dried in the laboratory for two days and then grounded into powder using a food blender. 50g of each powdered plant materials were soaked in 500mL of 95% ethanol and hot distilled water respectively and allowed to stand for 48 hours for extraction of active ingredients. After 48 hours, the samples were double filtered using Whatman No 1 filter paper and porcelain cloth. The filtrates were concentrated at 40^{o}C using rotary evaporator.

Preparation of Test Organisms: The pure cultures of the organisms were obtained on the differential media, incubated and stored as stock culture for proper identification.

Media Preparation: The media were aseptically prepared according to the manufacturer's instructions. They were autoclaved at 121^{o}C for 15minutes and then poured into sterile Petri dishes and allowed to gel.

Source of Test Organisms

Pure culture of test organisms used in this study; *Staphylococcus* spp, *Salmonella* spp, *Escherichia coli*, *Pseudomonas* spp, *Candida* spp, *Aspergillus* spp, *Penicillium* spp and *Mucor* spp were obtained from the Department of Microbiology, Abia State Polytechnic, Aba, and sub-cultured in nutrient broth and sabouraud agar and incubated for 24-48 hours before use. Isolates were identified by carrying out morphological and biochemical tests

which include Gram staining, catalase, coagulase, oxidase, citrate utilization, indole and sugar fermentation.[15]

Antimicrobial Assay of the Plant Extracts: A disc diffusion technique using Kirby-Bauer method [16] was applied in testing pure cultures of the isolates for their antimicrobial sensitivities. After hot air disc sterilization at 160^{o}C, the discs (6mm diameter) were impregnated for 10 minutes with the ethanolic and aqueous leaf extracts which were dissolved separately into different concentrations- 100%, 50%, 25%, 12.5% and 6.25% according to Abalaka et al. [14] The Mueller Hinton agar plates for each test organism were inoculated with 0.1mL broth culture of the test organisms and spread with a glass rod. The discs containing the ethanolic and aqueous leaf extracts were applied on the agar in triplicates, and then the plates were incubated at 37^{o}C for 24 hours. The inhibition zones were calculated and recorded.

RESULTS

The antimicrobial effect of ethanolic and aqueous leaf extracts of *M. oleifera* on bacterial pathogens is shown in Table 1. The result showed the ethanolic leaf extract to be more effective than the aqueous extract. *Staphylococcus* spp has the highest zones of inhibition ranging from 8-17mm and closely followed by *E. coli* ranging from 7-15mm, while *Pseudomonas* spp had the least with 7-13mm. Gentamycin which servers as control has zone of inhibition ranging from 16- 19mm.

Table 1: Antimicrobial Effect of Ethanolic and Aqueous Leaf Extracts of *M. oleifera*

	Mean Zones of Inhibition of the Test Organisms (mm)				
Plant Extracts	**Concentration (%)**	***Staphylococcus* spp**	***Salmonella* spp**	***E. coli***	***Pseudomonas* spp**
Ethanolic Leaf Extract	100	17	14	15	13
	50	15	11	12	10
	25	12	10	10	8
	12.5	9	8	8	7
	6.25	0	0	0	0
Aqueous Leaf Extract	100	14	13	14	11
	50	10	10	10	9
	25	9	8	8	7
	12.5	8	0	7	0
	6.25	0	0	0	0
Control	Gentamycin	19	17	19	16

Table 2 shows the antimicrobial effect of the ethanolic and aqueous leaf extracts of *M. oleifera* on fungi. The result also showed that ethanolic leaf extract of *M. oleifera* had a better zone of inhibition on the test organisms. *Candida* spp had the highest zone of inhibition from 6-15mm; this is followed by *Penicillium* spp with 6-14mm while the least was *Mucor* spp with zone of inhibition ranging from 6-12mm. Nystacin which served as the control drug had zone of inhibition ranging from 14- 17mm.

Table 2: Antimicrobial Effect of Ethanolic and Aqueous Leaf Extracts of *M. oleifera* on Test Organisms

Mean Zones of Inhibition of the Test Organisms (mm)					
Plant Extracts	**Concentration (%)**	***Candida* spp**	***Aspergillus* spp**	***Penicillium* spp**	***Mucor* spp**
Ethanolic Leaf Extract	100	15	12.5	14	12
	50	12	6.5	10	9
	25	6.5	0	9	7
	12.5	0	0	8	0
	6.25	0	0	0	0
Aqueous Leaf Extract	100	13	10	11	10
	50	6	8	10	6
	25	0	6	6	0
	12.5	0	0	0	0
	6.25	0	0	0	0
Control	Nystacin	17	16	16	14

Tables 3 showed the minimum inhibitory concentrations of *M. oleifera* leaf extract on bacteria and fungi. The MIC occurred at 12.5% on bacteria and *Penicillum* spp while *Candida* spp, *Aspergillus* spp and *Mucor* spp have zones of inhibition at 25%.

Table 3: The Minimun Inhibitory Concentration (MIC) of *M. oleifera* leaf extracts on Test Organisms

	Minimum Inhibitory Concentration (MIC) (%)							
Plant Extracts	***Staphylococcus* spp**	***Salmonella* spp**	**E. coli**	***Pseudomonas* spp**	***Candida* spp**	***Aspergillus* spp**	***Penicillum* spp**	***Mucor* spp**
Ethanolic Extract	12.5	12.5	12.5	12.5	25.0	50.0	12.5	25.0
Aqueous Extracts	12.5	12.5	12.5	12.5	50.0	25.0	25	50

DISCUSSION

The screening of plant extracts and plant products for antimicrobial activities has shown that plants represent a potential source of novel antibiotic prototypes. The antimicrobial

properties are due to compounds synthesized in the secondary metabolism of the plant. The results in this work indicated that the extracts of *M. oleifera* leaf have varying degree of antimicrobial activities against the test organisms. The antimicrobial activities of the extracts were greater against bacteria than fungi. This is in agreement with the findings of Jacskon et al.[17] Caceres et al.[18] found *M. oleifera* leaf extracts to inhibit the growth of *S. aureus* and *P. aeruginosa*. Likewise, in a study by Valsaraj et al.[19] evaluating the antimicrobial effect of 78 plants used in India to treat infectious diseases, *P. aeruginosa* and *S. aureus* were inhibited by extracts of *Moring*a peels. The results also showed the effect of the extracts on the test organisms were concentration dependent (the test organisms were more susceptible at higher concentrations), in agreement with the findings of Ali et al.[20] and Napoleon et al.[21]

From our results on the ethanolic and aqueous extracts, *Staphylococcus* spp has zone of inhibition ranging from 8-17mm, followed by *E. coli* with 7-15mm, *Salmonella* spp with a range of 8mm-14mm while *Pseudomonas* spp has the least zone of inhibition from 7-13mm. However, the zone of inhibition of ethanolic and aqueous extracts on *Candida* spp ranged from 6-15mm, *Penicillum* spp with zone of inhibition from 6-14mm, *Aspergillus* spp from 6-12.5mm while *Mucor* spp ranged from 6-12mm. Contrary to our findings, a higher zone of inhibition was obtained by Abalaka et al.[14] and Thilza.[22] for *M. oleifera* obtained from Northern Nigeria. Higher activity was also demonstrated by the standard reference drugs used. This is because the drugs were in pure state and have been processed and refined.[16] The minimum inhibitory concentration (MIC) of *M. oleifera* leaf extracts occurred at12.5% and 12.5-50.0% for bacteria and fungi respectively.

Variations were found to exist between our results and other authors working on the same plant from different localities. These variations could be influenced by factors like the environment where the plants were collected, the season and the physiological stage of the plant when the leaves were harvested.[23] These factors affect the chemical composition and amount of bioactive compounds in these plants. In addition, extraction solvents have also been found to contribute to these variations. Extraction of bioactive agents from plants has shown different solvents to have varying extraction abilities.[24, 25, 26]. In this study, organic solvent produced a better antimicrobial activity than water which corroborates with the findings of some authors.[14, 25, 27] For example, extraction of bioactive agents from *M. oleifera* for the treatment of diabetic rats showed water to be a

better solvent than ethanol.[28, 27] The quantitative analysis by some authors revealed that the aqueous extract constituted more phytochemicals than the ethanolic extract.[30,31] and hence exhibited more hypoglycemic activity on the diabetic rats. However, several reports on antibacterial activity of *M. oleifera* have shown alcohol to be more effective than water.[24, 25, 26, 32]. The higher reducing power of the aqueous extract could be due to the better solubility of the antioxidant components in water (for diabetic treatments); whereas the predominant antibacterial activity in organic solvent extracts as compared to aqueous extracts indicated that the active components responsible for the bactericidal activity are more soluble in organic solvents.[25] This therefore suggests that treatment with *M. oleifera* for any particular ailment should consider the appropriate extraction solvent for effective result.

CONCLUSION

The result of the present work has shown the antimicrobial potentials of *M. oleifera* aqueous and ethanolic leaf extracts against some pathogenic organisms. The evaluation of the Southeast Nigerian grown *M. oleifera* leaf extract against these test organisms is an indication of the presence of broad spectrum bioactive compounds of the leaf. Our results revealed the extracts are highly comparable to the standard drugs and capable of inhibiting the growth of bacteria and fungi. Our previous work on anti-diabetic effect of the same plant showed water to be a better solvent than ethanol; however it is very interesting to find ethanolic extract of the same plant more effective for antimicrobial activities. This shows that different solvents could be used to extract the bioactive agents for different ailments. This could be verified further.

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