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Antimicrobial Activities of Seed and Leaf Extracts of *Moringa oleifera* against Common Clinical Microbial Isolates

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ABSTRACT

Seed and leaf extracts of *Moringa oleifera* were tested against selected bacterial and fungal pathogens of clinical importance comparing aqueous and ethylacetate efficacy as solvents of extraction. Agar disc and agar well diffusion methods were employed for the tested bacteria and fungi respectively. While the bacteria included *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi*, the fungal species were *Candida albicans*, *Aspergillus niger* and *A. flavus*. The *in vitro* bioassay revealed that the ethylacetate seed extracts showed better activity compared to the aqueous extracts, with the highest effectiveness observed in ethylacetate extract against *E. coli* (23mm) and lowest in aqueous extract against *A. flavus* (6 mm). Conclusively, plant parts extracted with ethylacetate proved to possess more antimicrobial activities than aqueous, thereby suggesting ethylacetate as an effective solvent in the extraction of active phytochemical compounds.

INTRODUCTION

The use of natural extracts for the control of infections has been around for centuries. However, there continues to be the need for new antimicrobial agents resulting from the continuous emergence of new antibiotic-resistant microorganisms resistant to most present-day antibiotic (Ibekwe *et al*., 2000). Antimicrobial resistance is a lingering global problem and strategies to improve the situation include search into finding new and innovative antimicrobials.

A large proportion of the population utilizes medicinal plants for the treatment of infectious diseases, and according to the World Health Organization's estimation, traditional healing provides the primary health care needs for about 80% of the population in Africa (WHO, 2003). Extracts of different plant species are being used as trado-medical remedies, and are being studied scientifically and one of such plants is *Moringa oleifera*.

Moringa oleifera is a member of the *Moringaceae* family with many nutritionally essential products of various potential use. It originated in India, Sri Lanka and can be grown up in Asia Minor and Africa as well (Caceres *et al.,* 1991). Considered one of the world's most useful trees, as almost every part of the *Moringa* tree can be used for food, or has some other beneficial property, including its use as foliage for livestock.

Moringa tree is generally a good source for Calcium and Phosphorus (Yang *et al*., 2006), while the leaves are highly nutritious sources of beta-carotene, vitamin C, protein, iron, potassium and niazirin, and also full of potential medicinal properties (Karuna *et al*., 2007).

The properties of various *M. oleifera* morphological parts health remedies have been reported. Mature and edible *Moringa* pods form a part of the traditional diets in many countries and subtropics of the world (Anhwanye *et al*., 2004), *Moringa* flowers added to drinks and taken as tea is believed to be a powerful cold remedy (Fahey, 2005), while dried *Moringa* leaves have been used to treat diarrhoea in Malawi (Sogbo, 2006).

Antimicrobial studies of Moringa plant has been made possible through the extraction of active ingredients from the different products. Extraction process, when carried out appropriately, aids the release of active ingredients which could consist of novel substances into the extracting solution.

Several studies have used different solvents in the extraction of antimicrobial substances from *M. oleifera*. Water (hot and cold), ethanol and methanol are the most commonly used solvents for extraction process. Also, Busani *et al.* (2012) employed acetone in extraction; Jabeen *et al*. (2008) employed potassium phosphate buffer solution in extraction. Also, as part of their study, Rahman *et al*. (2009) used *Moringa* powder without prior extraction while Kalpana *et al* (2013) a combination of water, ethanol, chloroform and petroleum ether as solvent. With the dearth of solvents available for possible extraction of substances with potential medicinal importance, it is imperative to look into use of other solvents.

This investigation was carried out to evaluate the antimicrobial properties of seed and leaf of *M. oleifera* extracted using ethylacetate on selected microbial pathogens of clinical importance compared with aqueous extracts.

MATERALS AND METHODS

Plant materials

Fresh leaves and seeds of *Moringa oleifera* plants were collected from Ahoyaya community, Ifelodun Local Government, Osun State, Nigeria. Plant materials were identified at the Botany section of the Department of Biological Sciences, Osun State University, Osun State, Nigeria. Plant leaves were sundried for 4-6 days at room temperature while seeds were oven-dried at 180 °C for 40 mins. Both dried plant materials were blended into powder form and passed through a mesh to obtain powder of uniform sizes. Leaf and seed samples were stored in air-tight containers until further analysis.

Test Organisms

Nine microbial test isolates used for screening in this study comprised clinical isolates of both bacteria and fungi. Two Gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia)* and four Gram negative organisms (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi)* were the bacterial species of choice. The three fungal species included a yeast (*Candida albicans*) and toxigenic mold strains of *Aspergillus niger* and *A. flavus*. All microorganisms were obtained from the Medical Microbiology Department of University College Hospital (UCH), Ibadan Oyo State, Nigeria. Appropriately identified cultures of bacteria and fungi were sub-cultured from slants onto nutrient agar medium (for bacteria) and potato dextrose agar (for fungi) respectively. Test bacteria were incubated at 37 °C for 24 hr, yeast at room temperature for 48 hr, while molds were grown for 72

hours at room temperature. Organisms were stored on slants at 4°C until needed.

Preparation of Plant Extract

Aqueous and ethylacetate were solvents used for extraction of active ingredients from plant materials. Aqueous extracts were prepared by modifying the method of Tarfa *et al*. (2010). Powdered seed and leaf (10 g) were soaked in 50 ml of de-ionised water in separate Erlenmeyer flasks for 12hours at 50 °C after which extract was collected by squeezing through sterile muslin cloth and filtering through Whatman No. 1 filter paper. Ethylacetate extract was obtained by suspending plant parts in the solvent for 24hours and filtering using Whatman No. 1 filter paper. Both aqueous and ethylacetate extracts were concentrated *in vacuo* at 40 ºC using a rotary evaporator and reconstituted in methanol for further studies, while aqueous extracts were tested directly.

Antimicrobial Assays

The antimicrobial activities of aqueous and ethylacetate extract were done as previously described by Omenka and Osuoha (2000) with disc diffusion method for bacteria and yeast, and agar well diffusion method for moulds. Overnight broth cultures (1 ml) of test organisms were spread unto plates of Muller Hinton agar (for bacteria) and PDA (for fungi) respectively. Experiments were carried out in triplicates with standard antibiotics discs of gentamycin and erythromycin used as control.

Agar disc diffusion - Sterile antibiotic discs (6.0 mm diameter) were seeded with aqueous and ethylacetate extracts and dried at 40°C for 30 minutes before use. Inoculated culture plates were seeded with disks containing *M. oleifera* extracts and incubated at 37 °C (bacteria) for 24 hours and zones of inhibition afterwards.

Agar-well diffusion – Wells were bored on PDA plates using a sterile cork borer (6 mm diameter) with plant extract (0.1 ml) dispensed accordingly and plates incubated at 30 °C (fungi) for 24hours.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of aqueous and ethylacetate extracts was determined against susceptible test isolates at varying concentrations of 2, 4, 8, 10, 12, 14, 16, 18 and 20 mg/ml. Overnight broth culture (1 ml) of test molds (10^6 spore load) and bacteria (diluted to 0.5 McFarland standard) isolates were added to PD and MH broth tubes respectively. *Moringa* extracts (0.1 ml) were added to the tubes which were incubated at 37 °C (bacteria) and 30 °C (fungi) for 24 hours. Concentration of extracts that showed no change in turbidity before and after incubation- determined using spectrophotometer- was recorded as the minimum inhibitory concentration (MIC). Broth tubes seeded with only test isolates served as control.

The MBC of the *Moringa* extract on the test isolates was determined according to Ajaiyeoba *et al*. (2003). Briefly, 1 ml of bacterial and fungal cultures obtained from MIC tubes which did not show visible growth was subcultured on MHA and PDA and incubated appropriately. After incubation, lowest concentration at which no single colony of bacteria was taken as MBC**.**

RESULTS AND DISCUSSION

The diameter of zone of inhibition of the test organism using the seed extracts shows that the ethylacetate extracts showed better activity compared to the aqueous extracts (Table 1). Highest zone of inhibition (ZI) was observed in ethylacetate extract against *E. coli* (23.0 mm) while lowest ZI was in aqueous extract against *A. flavus* (6 mm). Bacterial isolates were inhibited better than fungi isolates. However *K. pneumonia*, *S. pneumonia* and *C. albicans* showed absolute resistance against both seed extracts.

Table 1: Antimicrobial activities of *M. oleifera* seed extracts against test isolates

Test organism	**Zone of inhibition (mm) Ethylacetate**	**Aqueous**
Escherichia coli	23	15
Staphylococcus aureus	20	12
Pseudomonas aeruginosa	15	10
Salmonella typhi	12	9
Klebsiella pneumonia	-	-
Streptococcus pneumonia	-	-
Candida albicans	-	-
Aspergillus niger	15	8
Aspergillus flavus	12	6

Values of ZI for both bacteria and fungi are higher than those reported when methanol and ethanol was used as extracting solvent for *Moringa* fruit and leaves (Busani *et al*., 2012; Oludoro, 2012), while result is comparable with those reported when potassium phosphate buffer was solvent of choice (Jabeen *et al*., 2008). Results are also comparable with those of obtained for ethanolic extracts of fresh and dried *Moringa* leaves (Rahman *et al*., 2009).

Table 2 showed that antimicrobial properties of leaf extracts followed similar pattern with ethylacetate extracts showing superior antimicrobial activity. However, *Klebsiella*, *Streptococcus* and *Candida* species showed resistance to both seed and leaf extracts.

Table 2: Antimicrobial activities of *M. oleifera* leaf extracts against test isolates

Test organism	**Zone of inhibition (mm)**	
	70% Ethanol	Hot water
Escherichia coli	20	15
Staphylococcus aureus	17	12
Pseudomonas aeruginosa	14	8
Salmonella typhi	14	11
Klebsiella pneumonia	-	-
Streptococcus pneumonia	-	-
Candida albicans	-	-
Aspergillus niger	18	12
Aspergillus flavus	14	9

The MIC and MBC of *Moringa oleifera* seed and leaf extracts are described in Table 3 and 4 respectively. It was observed that ethylacetate extract showed higher activity than aqueous extract. *Escherichia coli* and *S. aureus* were inhibited by the lowest MIC values for both seed and leaf extracts, while the molds required the highest extract concentrations. No concentration of aqueous extract had MBC activity, with growth observed on sub-cultured plates while ethylacetate extracts of both seed and leaf showed MBC activity against *E. coli, S. aureus* and *P. aeruginosa*. This indicates that aqueous seed and leaf extracts are not bactericidal while extraction using ethylacetate permeated the release of substances that are both bacteriostatic and bactericidal. Also, lower MIC values for ethylacetate extracts indicates that the solvent increases the release of useful antimicrobial substances and is indicative of a better solvent.

Table 3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *moringa oleifera* seed extracts

	MIC		MBC	
	Aqueous	Ethylacetate	Aqueous	Ethylacetate
E.coli	12	6	NA	10
S. aureus	10	6	NA	12
S. Typhi	16	12	NA	NA
P. aeruginosa	12	8	NA	18
A. flavus	18	16	NA	NA
A. niger	16	16	NA	NA

Table 4: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *moringa oleifera* leaf extracts

	MIC		MBC	
	Aqueous	Ethylacetate	Aqueous	Ethylacetate
E.coli	14	10	NA	10
S. aureus	14	10	NA	12
S. Typhi	18	14	NA	NA
P. aeruginosa	12	10	NA	18
A. flavus	20	16	NA	NA
A. niger	20	16	NA	NA

CONCLUSION

Results showed that based on zone of inhibition and MIC values, *Moringa oleifera* seed and leaf extract had antimicrobial effect on both bacteria and fungi with the ethylacetate of both plant parts more effective. It was also deduced that extracting the plant parts with ethylacetate improved antimicrobial properties of *Moringa* plant parts used in the study. This therefore shows that ethylacetate is an effective solvent in the extraction of active phytochemical compounds. There is also the need to widen the scope phytochemical extraction of *M. oleifera* by applying other solvents that have not been considered from previous studies.

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