Australian Journal of Basic and Applied Sciences, 5(5): 129-134, 2011
ISSN 1991-8178

An *In vitro* Antimicrobial Activity of *Moringa oleifera* L. Seed Extracts Against Different Groups of Microorganisms

Abdulmoneim M. Saadabi and I.E.Abu Zaid

Department of Microbiology &Microbial Technology, Faculty of Science and Technology, Al-Neelain University, Khartoum, P.O. Box 12702,Sudan.

Abstract: Seed extracts of *Moringa oleifera* were evaluated for their antimicrobial activity against four types of bacteria namely *Staphylococcus aureus, Bacillus subtilis, Eschreiashia coli and Pseudomonas aeruginosa*. Two species of fungi i.e. *Aspergillus niger and Candida albicans* were also bioassayed for their response when the seed extracts were used. All of the seed extracts irrespective of their types, in different concentrations inhibited the growth of all microbes to varying degrees. Aqueous extract showed strong and superior antibacterial activity against all bacterial strains especially with regard to gram positive bacteria(*Staphylococcus aureus* and *Bacillus subtilis*) as compared to methanol or petroleum ether. Less or no activity was observed against *Aspergillus niger* and *Candida albicans.* These findings support the traditional use of the plant in the treatment of different infections in the area.

Key words: *Moringa oleifera* L., Antimicrobial activity, Bacteria, Fungi, Sudan.

INTRODUCTION

Bacterial and fungal infections are widespread throughout the world. The situation is more critical especially in the third world countries were in most cases lack of adequate sanitation and primary health care programs make it difficult and expensive to combat diseases. A number of higher plants have been used for centuries as remedies for human diseases. This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Eilert *et al.,* 1980; 1981; Omer and Elnima, 2003; Saadabi, 2006; Saadabi *et al.,* 2006; 2007; 2009). About 40% of pharmaceuticals are derived from natural sources (plants, animals, bacteria and fungi). Moreover, several natural products obtained from medicinal plants lead to the development of various pharmaceuticals and analogues or derivatives. Recently, focus on plant research has increased and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems Ernest *et al.,* (2007) *Moringa oleifera L.* vernacular name Alruwag "the tree for purifying", Family (Moringaceae) is a tropical tree with a capsulated and dehiscent fruits Njoku and Adikwu, (1997). The plant kernel contains oil rich in beta-carotene, plant sterols and lecithin. The oil contains unusual fatty acids. The significant cytotoxic activity against brine shrimp may be due to the presence of 4-alpha-L-rhamnosyloxybenzyl isothiocyanate in the seeds of *M.oleifera* Shaheen *et al.,* (1998). In Sudan, powdered seeds of *M. oleifera* have been used in water purification. Reports have been elucidated on the findings of the antibiotic principle of *M.oleifera* seeds through their purification, elucidation, and antimicrobial properties, and also on the antibiotic substance of the roots of *M. oleifera* Jamil *et al.,* (2007).

The aims and objectives of the present work are to establish a well documented information about the antimicrobial activity of *M. oleifera* seed extracts against six standard microorganisms using reference antibiotics as experimental models.

MATERIALS AND METHODS

Plant material:

The study was undertaken at the Department of Microbiology and Microbial Technology, Al-Neelain University, Khartoum, Sudan, during April to August 2009. *M. oleifera* seeds were collected from Wad madani city (Central of Sudan), identified and authenticated by the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan. A Voucher specimens were deposited at the departmental Herbarium.

Corresponding Author: Abdulmoneim M.Saadabi, Department of Microbiology & Microbial Technolology, Al-Neelain University, P.O. Box 12702, Khartoum, Sudan.
E-mail:Saadabi1@hotmail.com; Tel.+249914559607.

Preparation of extracts:

Extraction was carried out according to the method adopted by Price (2000). In accordance with this method, an amount of 80g of *M. oleifera* seeds was crashed and finely powdered using mortar and extracted successively with petroleum ether and methanol using Soxhlet apparatus for about four hours for petroleum ether and eight hours for methanol .Solvents were then evaporated under reduced pressure using rotary evaporator. Extracts were left to air till complete drying. The petroleum ether extract was dissolved in petroleum ether, and the methanol extract was dissolved in methanol.

The aqueous extract has taken another way in extraction; an amount of 100g of *M. oleifera* crashed seeds was boiled in 500 ml distilled water in a water path at 70 °C for 15 minutes and filtered. The filtrate was put in the freezer till freezing point, then extracted successfully by using Freeze drier apparatus for 96 hours. The supernatant layer was collected carefully in a clean container. Different concentrations of the all extracts were also prepared Price (2000).

Fungal species:

Two fungal species were obtained from clinical cases at Khartoum Educational Hospital, Sudan. These fungi were *Aspergillus niger and Candida albicans.* Each of the fungi was cultured on Sabouraud's dextrose agar medium (Oxoid) and incubated at 25°C for 7 days, to obtain inocula for testing.

Bacterial strains:

Four types of bacteria namely *Staphyllococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were used. They were isolated from clinical cases and provided by Khartoum educational hospital, Sudan. The bacteria were cultured on nutrient broth (Oxoid) at 37°C for 24 h.

Determination of antifungal activity:

Sterile, filter paper discs of 6 mm diameter were impregnated with 0.1ml/disc of extract which have been dissolved in dimethyl sulphoxide (DMS) and placed in duplicates onto the Sabouraud's dextrose agar plates, seeded with 0.2 ml of fungal suspension Saadabi *et al.*, (2006). The plates were then incubated at 25°C for 10 - 14 days .The zone of inhibition around each disc was measured in mm and the results are presented as means ±SD.

Antibacterial activity:

Each concentration of seed extract (water, methanol or petroleum ether) was tested against the four types of bacteria using the cup-plate agar diffusion method Groove and Randall, (1955) and the inhibition zones were measured. Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, Ofloxacin,Amikacin,Lomefloxacin,Netilmicin and Tobramycin (25 µg/disc) were used as a reference standard drugs for comparison(Table 3 &4).

RESULTS AND DISCUSSION

As a general rule, plant seed extracts are considered active against both fungi and bacteria when the zone of inhibition is greater than 6 mm Eilert *et al.,* (1981). The aqueous extract of all concentrations was found to be inhibitory to all bacterial stains, whereas the inhibition zone was increased gradually with the increase of extract concentration. In case of *Staphylococcus aureus,* it was 32,45,46 & 48 mm in 5,10,20 &40 % concentration respectively (Table 1,Figure 1 & Figure 3). Moreover, the maximum inhibition zone was observed against *Staphylococcus aureus* (45mm) followed by *Bacillus subtilis* (40mm) and (25mm) for *Pseudomonas aeruginosa*, with the same trend in the different other bacterial strains. For fungal species, extracts were slightly active against *Aspergillus niger.*

However, there is no detectable suppression in the growth of *Candida albicans* especially in 5,10 &20 % concentration, but only in 40 % concentration that reached 11 mm (Table 1). The methanolic extract of 5% concentration was found to be slight active against *Escherichia coli* and *Pseudomonas aeruginosa* (Table 2, Figure 2). Methanol extract of 10, 20 and 40% shown little inhibition to all bacterial strains. All concentrations of *M.oleifera* seed extracts in petroleum ether extract were found to be inactive against all of the tested microorganisms. Similarly, no inhibition was detected against fungal strains (Table 2).

Results obtained from *in vitro* antimicrobial activity showed that the aqueous and methanol extracts have substantial inhibitory effects against the four tested bacterial strains. Still the water extract was superior in suppressing the bacterial growth, followed by Methanol extract. The reason behind this might be due to the fact that more water dilution in 10% concentration increase hydrolysis of the active principles to work well against

the target organisms, while in 40% concentration there is less dilution and the diffusion of the active principle is limited to reach the organism. In other words, this may be due to osmotic pressure of the solutes and selective permeability. The antimicrobial activity of the extract also might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane (Boyd and Beveridge, 1979; 1981). The inactivity of petroleum ether seeds extract may be due to the fact that the active compound which posses the antimicrobial properties are polar in nature and not possibly extracted by petroleum ether.

These results are in close agreement with other findings obtained by other workers (Anwar, F. and U. Rashid, 2007; Jamil *et al.,* 2007; Kebreab *et al.,* 2005; Lockett *et al.,* 2000;Tetsuji Okuda, *et al.,* 2001).

When the obtained results were compared to antibiotics findings; it could be concluded that the methanol extract of the seeds obtained from *M.oleifera* was less effective than the standard antibiotics used. But the aqueous extract of *M. oleifera* seeds, on the other hand, was more effective. According to high antimicrobial activity of the *M.oleifera* seeds extracts which can justifies its uses in folkloric medicine, further research work should be done using this plant. More studies are needed to isolate and characterize the active compounds to be tested *in vivo* to determine the toxicity and the optimum dose to be used as effective as antibiotics.

Table 1: Antimicrobial activity of *M.oleifera* seeds aqueous extract against different species of bacteria and fungi*

	Extract concentration (%)			
Tested organisms	5	10	20	40
Staphylococcus aureus	32±0.11	45±1.12	46±0.45	48±0.31
Bacillus subtilis	31±0.90	35±0.19	37±0.37	40±0.42
Escherichia coli	18±1.96	20±0.21	22±0.89	22±0.10
Pseudomonas aeruginosa	20±0.76	22±0.16	24±0.46	25±0.54
Aspergillus niger	11±0.41	12±1.74	12±0.01	15±0.77
Candida albicans	-	-	-	11±0.80

*Data are presented as mean ±SD as measurement of inhibition zone (mm), and values are means of three replicates.

Table 2: Antimicrobial activity of *M.oleifera* seeds methanol extract against different species of bacteria and fungi*

	Extract concentration (%)			
Tested organisms	5	10	20	40
Staphylococcus aureus	-	11.00±1.98	14.25±0.12	17.75±0.41
Bacillus subtilis	-	12.95±0.99	16.95±0.76	18.00±0.27
Escherichia coli	14.28±0.75	14.00±0.22	14.00±0.11	12.00±1.94
Pseudomonas aeruginosa	12.37±0.65	12.77±0.65	12.27±1.16	13.00±1.62
Aspergillus niger	-	-	-	-
Candida albicans	-	-	-	-

*Data are presented as mean ±SD as measurement of inhibition zone (mm), and values are means of three replicates.

Table 3: Antibacterial effects of some standard antibiotics (Gram positive master multi disk) against different species of bacteria *

Antibiotics	Sensitive	*Staphylococcus aureus*	*Bacillus subtilis*
Ciprofloxacin	21	26	28
Clindamycin	21	24	23
Erythromycin	23	22	25
Gentamycin	15	25	22
Ofloxacin	16	25	20
Tobramycin	15	21	22

* = Mean measurement of inhibition zone (mm)

Table 4: Antibacterial effects of some standard antibiotics (Gram negative master multi dick) against *E.coli* and *Pseudomonas aeruginosa* *

Antibiotics	Sensitive	*Escherichia coli*	*Pseudomonas aeruginosa*
Amikacin	17	28	23
Ciprofloxacin	21	18	20
Gentamycin	15	25	13
Lomefloxacin	22	20	25
Netilmicin	15	18	10
Ofloxacin	16	20	15

* = Mean measurement of inhibition zone (mm)

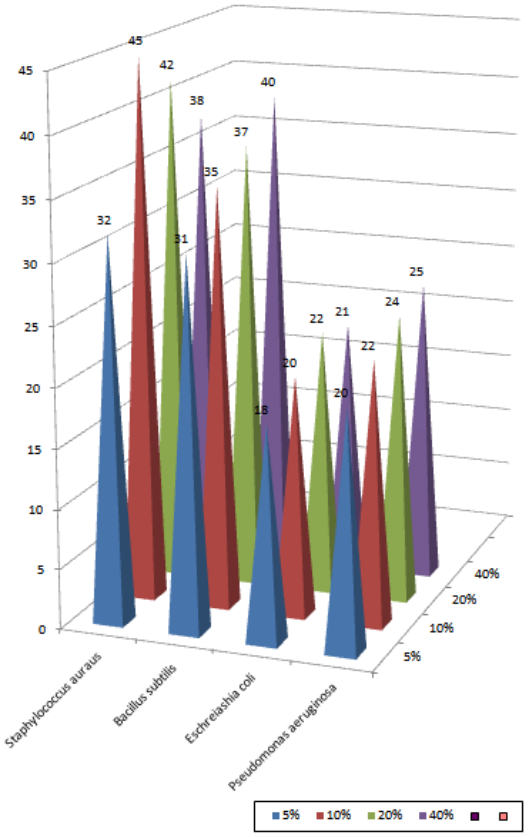

Fig. 1: Antimicrobial activity of *M.oleifera* seeds (aqueous extract) against the tested species of bacteria(Values indicate the inhibition zone in mm, while percentages expressing concentration of the plant extract).

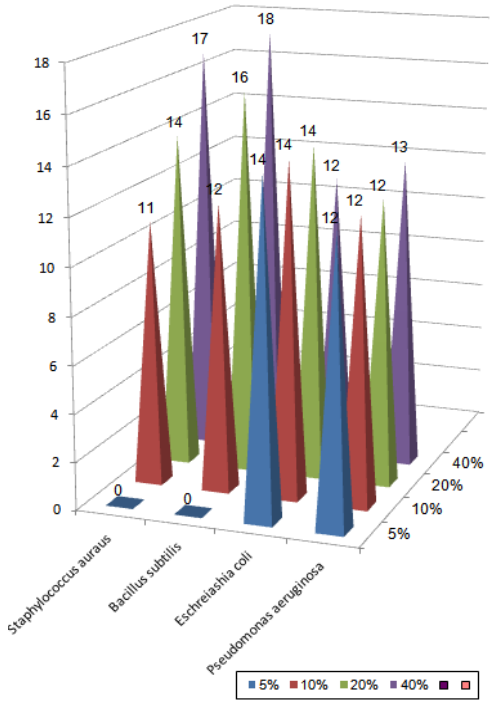

Fig. 2: Antimicrobial activity of *M.oleifera* seeds (Methanol extract) against the tested species of bacteria(Values indicate the inhibition zone in mm, while percentages expressing concentration of the plant extract).

(a) (b)

Fig. 3: Antimicrobial activity of *M.oleifera* seeds (aqueous extract) 10 and 20% concentrations against (a) *Escherichia coli* and (b) *Bacillus subtilis*

REFERENCES

Anwar, F. and U. Rashid, 2007. Physico-chemical characteristics of *Moringa Oleifera* seeds and seed oil from a wild provenance of Pakistan Pak. J. Bot., 39(5): 1443-1453.

Boyd, I. and E.G. Beveridge, 1979. Relationship between the antibacterial activity towards *E. coli* NCTC 5933 and the physicochemical properties of some esters of 3, 4, 5-trihydrobenzoic acid (gallic acid). Microbios, 24: 173-184.

Boyd, I. and E.G. Beveridge, 1981. Antimicrobial activity of some alkyl esters of gallic acid (3, 4, 5-trihydrobenzoic acid) against *E. coli* NCTC 5933 with particular reference to n-propil gallate.Microbios, 30: 73-85.

Eilert, U., B. Wolters, A. Nahrstedt, 1980. Antibiotic principles .Planta Medica, 39(3): 235.

Eilert, U., B. Wolters and A. Nahrstedt, 1981. The Antibiotic principles of seeds of *Moringa oleifera.* Planta Medica, 42(1): 55-61.

Ernest, B., M.S. Hawkins and R.Ph. Pharm, 2007. Health Education Resources; and Steven D. Ehrlich, N.M.D., private practice specializing in complementary and alternative medicine, Phoenix, AZ. Review provided by VeriMed Healthcare Network.

Groove, D.C., W.A. Randall, 1955.Assay methods of Antibiotics. Medical Encyclopoedia,New York, pp: 24-55.

Jamil, A., M. Shahid, M.M. Khan and M. Ashraf, 2007. Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pak. J. Bot., 39(1): 211-221.

Jed, W., Sc.D. Fahey, 2008. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Trees for Life Journal Part 1. http://www.tfljournal.org/article.php/20051201124931586.

Kebreab, A.G., K.R. Gunaratna, H. Henriksson, H. Brumer and G. Dalhammar, 2005. A simple purification and activity assay ofthe coagulant protein from *Moringa oleifera* seed. Water Res., 39: 2338-2344.

Lockett, C.T., C.C. Calvet and L.E. Grivetti, 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. Int. J. Food Sci. Nutr., 51(3): 195-208.

Njoku and Adikwu, 1997. Toxicological studies reveal the absence of mycotoxins in Northeastern Nigeria. Int. J. Food Sci. Nutr., 51(3): 195-208.

Omer, M.E.F.A. and E.I. Elnima, 2003. Antimicrobial Activity of *Imenia americana*. Fitoterapia, 74: 122-126.

Price, M.L, 2000. The *Moringa* Tree purification and activity assay of the coagulant protein from *Moringa oleifera* seed. Water Res., 39: 2338-2344.

Shaheen, F., B.S. Siddigui, R. Saleen, K. Aftab, F. Shaheen and A. Gilani, 1998.Hypotensive constituents from the pods of *M.oleifera* .Planta Medica,V. 64: 225-228.

Saadabi, A.M., 2006. Antifungal Activity of some Saudi Plants used in Traditional Medicine. Asian Journal of Plant Sciences, 5(5): 907-909.

Saadabi, A.M., A.G. AL-Sehemi and K.A. AL.-Zailaie, 2006.*In vitro* Antimicrobial Activity of Some Saudi Arabian Plants used in Folkloric Medicine. International Journal of Botany, 2(2): 201-204.

Saadabi, A.M, 2007. An Evaluation of *Lawsonia inermis* Linn. (Sudanese henna) Leaf Extracts as Antimicrobial Agent. Research Journal of Biological Sciences, 2(4): 419-423.

Saadabi, A.M. and S.M. H.Ayoub, 2009.Comparative bioactivity of *Hydnora abyssinica* A. Braun against different Groups of Fungi and Bacteria. Journal of Medicinal Plants Research, 3(4): 262-265.

Tetsuji Okuda, Aloysius U. Baes, Wataru Nishuima and Mitsumasa Okada, 2001. Isolation and Characterization of Coagulant Extracted from *Moringa oleifera* seed by salt solution. Water Res., 33(2): 405-410.