Bayero Journal of Pure and Applied Sciences, 3(1): 43 - 48
Received: April, 2010
Accepted: May, 2010

ANTIMICROBIAL PROFILE OF *MORINGA OLEIFERA* LAM. EXTRACTS AGAINST SOME FOOD – BORNE MICROORGANISMS

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ABSTRACT
The chloroform and ethanol extracts of seeds and leaf of Moringa oleifera were investigated for antimicrobial activity against some selected food – borne microorganisms as a first step in the screening of the extracts for preliminary sanitizing/preservative properties on foods. The preliminary phytochemical screening and antimicrobial assay were carried out using standard procedures. The results of the phytochemical analysis revealed differences in the presence of the phytochemicals among the extracts. Saponins were detected in all the extracts while tannins were only detected in Moringa oleifera leaf chloroform extract. The antibacterial assay results show that M. oleifera leaf ethanol extract exhibited broad spectrum activity against the test organisms with Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterobacter aerogenes susceptible. The MIC values ranged between 2.0 and >4.0mg/ml for all the organisms. M. oleifera seed chloroform extract was only active against E. coli and Salmonella typhimurium. The MIC values ranged between 1.0 and >4.0mg/ml for the tested organisms respectively. Antifungal activity result revealed 100% inhibition in growth of Mucor and Rhizopus species by M. oleifera seed chloroform extract at concentration of 1mg/ml. Standard Ketoconazole (control) inhibited the test organisms by 100% at 0.5mg/ml concertration. The result of this study have shown the potentials of M. oleifera extracts as sanitizers/preservatives by inhibiting the growth of the test organisms, which range from food – borne pathogens to spoilage causing organisms in foods.

Keywords: Moringa oleifera, Seed, Leaf, Antimicrobial, Phytochemistry, Sanitizer, Food

INTRODUCTION

Moringa oleifera Lam (Moringaceae), native to the western and sub – Himalayan region, India, Pakistan, Asia minor, Africa and Arabia (Somali *et al.,* 1984; Mughal *et al.,* 1999) is now distributed in the Philippines, Cambodia, Central, North and South America and the Caribbean Islands (Morton, 1991). *M. oleifera* is a tropical tree whose numerous economic applications and facility of propagation are arousing growing international interest. The *Moringa* tree is cultivated and use as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs) (Rebecca *et al.,* 2006).

Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino acids and various phenolics (Farooq *et al.,* 2007). The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour (Makonnen *et al.,* 1997), antipyretic, antiepileptic, antinflammatory, antiulcer (Pal *et al.,* 1995a). Other important medicinal properties of the plant include antispasmodic (Caceres *et al.,* 1992), diuretic (Morton, 1991), antihypertensive (Dahot, 1988), cholesterol lowering (Mehta *et al.,* 2003), antioxidant, antidiabetic, hepatoprotective (Ruckmani *et al.,* 1998), antibacterial and antifungal activities (Nickon *et al.,* 2003. *M. oleifera* parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Farooq *et al.,* 2007). In addition, *M. oleifera* seeds possess water purifying powers (Muyibi and Evison, 1995b; Kawo, 2007) by flocculating Gram – positive and Gram – negative bacterial cells (Olsen, 1987; Broin *et al.,* 2002; Kawo, 2007). *M. oleifera* seeds can also be used as a less expensive bio-absorbent for the removal of heavy metals (Sharma *et al.* 2006).

Despite this array of uses to which parts of *Moringa* tree are put to, scanty literature is available on the uses of *Moringa oleifera* plant parts as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitizing/preservative activity is to evaluate its antimicrobial activity against food – borne microorganisms (Bukar *et al.,* 2010). The determination of a plant's antimicrobial profile against food – borne microorganisms may promote the plant to further tests geared towards its evaluation as a sanitizer or preservative in foods.

It is in view of this, that the present research was set up to evaluate the phytochemical constituents and antimicrobial activity of chloroform and ethanol seed and leaf extracts of *Moringa oleifera* against some food – borne microorganisms. This is with a view to evaluating the plant's potentials for sanitation and/or preservation of foods.

MATERIALS AND METHODS

Collection, authentication and processing of plant materials

The fresh leaf and seed of *M. oleifera* were collected from Dambatta Local Government Area, Kano State, Nigeria. The plant materials were identified and authenticated by a Botanist at the Biological Science Department, Bayero University, Kano, Confirmation of taxonomic identity of the plants was achieved by comparison with voucher specimens kept at the Herbarium of the Department of Biological Sciences, BUK and use of documented literature from Dalziel (1960). The plant materials were air-dried in the laboratory for four weeks and then ground into powdered form, using a mortar and pestel, and stored for future use.

Extraction

The powdered plant material (50g) each of seed and leaf were percolated in 500mL chloroform and redistilled ethanol in separate 1L capacity conical flasks, stoppered and kept for two weeks with intermittent shaking. The percolates were filtered with Whatman's No 1 filter paper. The extracts were concentrated at 40^{o}C under reduced pressure using rotary evaporator (R110). The same quantity of plant material was again percolated with distilled water for one week and after filteration, the aqueous extract was concentrated in hot oven at 40^{o}C (Fatope *et al.,* 1993). The concentrated extracts were labeled MLE (*Moringa* ethanol leaf extract), MSE (*Moringa* seed ethanol extract), MLC (*Moringa* leaf chloroform extract) and MSC (*Moringa* leaf chloroform extract).

Phytochemical Analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was performed on the extracts as described by Trease and Evans (1989), Sofowora (1984).

Test Organisms

Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Salmonella typhimurium, Shigella spp and *Pseudomonas aeruginosa* were bacteria isolated, while *Mucor* spp and *Rhizopus* spp were fungi isolated from food samples, which included rice and beans dish, roselle calyx juice (zobo), fresh tomato, bread, lettuce, carrot and fried groundnut. This was followed by homogenizing and streaking food samples on appropriate media for isolation. Cultural and morphological identification as well as biochemical characterization of isolates using protocols described by Cheesbrough (2002) was carried out. Pure cultures of the isolates were maintained in appropriate media for future use.

Antimicrobial Disc preparation

Discs of about 6mm diameter were made from Whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121^{o}C for 15minutes. Stock solution (400mg/ml) of the plant extract was prepared by dissolving 0.8g of each extract in 2ml dimethylsulphoxide (DMSO). Serial doubling dilution was carried out by adding 1ml of DMSO at each serial dilution. Four concentrations were prepared from the stock solution such that each disc absorbed 0.01ml which was equivalent to 500 µg/disc, 1000 µg/disc, 2000 µg/disc and 4000 µg/disc respectively.

Standardization of Inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4^{o}C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002). Spore suspension for fungal bioassay was prepared according to the procedure of Murugan *et al.* (1995).

Antimicrobial Assay

Disc agar diffusion technique described by Bauer *et al.* (1966) and demonstrated by Cakir *et al.* (2004) was employed for antibacterial bioassay. For fungi susceptibility testing, the extracts were incorporated into appropriate medium and subsequently fungal spore suspension inoculated. The preparation was incubated at appropriate temperatures. After incubation, zone of inhibition (diameter) formed in the medium was measured to determine antibacterial effectiveness of the different concentrations of the extracts, while sensitivity of the fungi to the test extract was recorded as described by Murugan *et al.* (2007).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration for bacterial isolates was carried out using tube dilution technique as described by Akinyemi *et al.* (2005)**.** Stock solution of 80,000µg in 10ml sterilized distilled water was serially diluted to arrive at concentrations of 500 µg/ml, 1000 µg/ml, 2000 µg/ml and 4000 µg/ml.

RESULTS AND DISCUSSION

Moringa oleifera phytochemical screening revealed presence of flavonoids and saponins in MLE (Table 1). Tannins and saponins were detected in MSE in agreement with report by Napolean *et al.* (2009). Alkaloids were reported in the present study which was not determined by Napolean *et al.* (2009). MSC was shown to possess only saponins while MLC contained alkaloids, tannins and saponins, which were not reported by Napolean *et al.* (2009).

Farooq *et al.* (2007) reported that plants occur in varying habitats, a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of such plant is expected. Moreover, Waller and Nowacki (1978) reported that phytochemicals are produced in response to perceived threats by the plants, therefore variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant.

Table 2 presents the results of antibacterial activity of *M. oleifera* extracts on food – borne bacterial isolates. It can be deduced that *M. oleifera* leaf ethanol (MLE) extract had the broadest spectrum of activity on the test bacteria. The results show that it had activity against four bacterial isolates. *Enterobacter* spp (07mm), *S. aureus* (08), *P. aeruginosa* (07) and *E. coli* (07) were sensitive at concentration of 200mg/ml, while *Shigella* spp, *S. typhi* and *S. typhimurium* were not sensitive at all the concentrations used. Napolean *et al.* (2009) also reported *Enterobacter* spp, *S. aureus*, *P. aeruginosa, S. typi* and *E. coli* to be sensitive to MLE at concentration of 200mg/ml.

MLC was active on *E. coli* (08mm), *S. typhimurium* (10mm) and *S. typhi* (07mm) at concentration of 200mg/ml. Arzai (2008) also reported that MLE and MLC showed activity on *E. coli, P. aeruginosa, S. aureus* and *S. typhi.* From the results, MLE was observed to be more potent than MLC. This is justified by the report of Ajaiyeoba (2002) who indicated that polar solvents (ethanol and aqueous) extracts were more active than extracts of other non – polar or less polar solvents such as chloroform.

Moringa oleifera seed ethanol (MSE) extract was active against three bacterial isolates with *S. aureus* (10mm) and *E. coli* (07mm) being sensitive to the lowest concentration of 50mg/ml. *S. typhi* (10mm) was only sensitive to the extract at 200mg/ml concentration. *Enterobacter* spp, *Shigella* spp, *P. aeruginosa* and *S. typhi* were not sensitive to any of the concentrations tested.

Moringa oleifera seed chloroform (MSC) extract was active against two bacterial isolates with *E. coli* (09mm) and *S. typhimurium* (09mm) only sensitive to concentration of 100mg/ml. *Enterobacter* spp, *S. aureus, Shigella* spp, *P. aeruginosa,* and *S. typhi* were not sensitive to any of concentrations tested. The results (Table 2) show that MSC was the least extract with activity on the tested organisms. Its low antibacterial activity might be linked to its phytochemical contents as the result of phytochemical screening indicated that it possess only saponins. Possession of alkaloids, tannins and flavonoids, which were not detected in MSC extract have been shown to enhance antimicrobial property of a plant as stated by Singh and Bhat (2003) and Tschehe (1971).

The antibacterial properties of the leaf and seed of *M. oleifera* as shown in the present study corroborate the earlier claims by Aktar *et al.* (2006) and Foidl *et al.* (2001) who reported on the antibacterial properties of *M. oleifera* seed and leaf. The antibacterial activity of *Moringa oleifera* seed has been highlighted by many authors (Olsen *et al.,* 1987; Madsen *et al.,* 1987; Kawo, 2007). The antimicrobial activity of *M. oleifera* seed is due to the presence of an array of phytochemiacals, but most importantly due to the activity of a short polypeptide named 4 (ά – L – rhamnosyloxy) benzyl-isothiocyanate (Eilert *et al.,* 1981; Guevara *et al.,* 1999). The peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Silvestre *et al.,* 2000; Suarez *et al.,* 2003).

Of note is the fact that *P. aeruginosa* was only sensitive to MLE at concentration of 200mg/ml, indicating that *M. oleifera* extracts tested might have limited effect on the proliferation and activities of *P. aeruginosa* in foods. *P. aeruginosa* is well known as a hardy and difficult organism that constitutes problems to researchers (Brooks *et al.,* 2001).

Various authors have reported antimicrobial activities of plant extracts on food – borne pathogens (Afolabi, 2007; Atiqur Rahman and Sun, 2009; Moreira *et al.,* 2005; Kotzekidou *et al.,* 2007), which indicates the vigorous pursuit in the search for more candidates of plant – derived sanitizers and preservatives.

Table 3 shows the result of antifungal activities of *M. oleifera* extracts on the test fungi. MSC inhibited the growth of *Mucor* spp and *Rhizopus* spp by 100% at 1000 µg/ml while MSE inhibited growth of *Mucor* spp by 75% and *Rhizopus* spp by 50% each at 1000µg/ml concentration.

MLC inhibited the growth of *Mucor* spp and *Rhizopus* spp by 25% at 1000 µg/ml while MLE inhibited growth of *Mucor* spp by 50% and *Rhizopus* spp by 100% each at 1000µg/ml concentration. Comparatively, it could be observed that MSC proved to be the extract with the best antifungal activity on the test fungi as it prevented completely the growth of both *Mucor* spp and *Rhizopus* spp at 1000µg/ml. The standard drug, Ketoconazole inhibited growth of the test fungi at 500 µg/ml. The antifungal activities of *Moringa* seed and leaf have been reported by Nwosu and Okafor (1995), while the seed extract was shown to be active on *Rhizopus* spp (Raheela *et al.* 2008).

The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as *E. coli, S. aureus, P. aeruginosa, S. typhi, S. typhimurium* and *E. aerogenes* is encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause food – borne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically-synthesized sanitizers/preservatives with "naturally derived" ones (Jancxsen *et al.,* 2002; Lanciotti *et al.,* 2003).

Table 1: Phytochemical characteristics of the *M. oleifera* seed and leaf extracts

Phytochemicals	MSC	MSE	MLC	MLE
Alkaloids	-	+	+	-
Flavonoids	-	+	-	+
Saponins	+	+	+	+
Tannins	-	-	+	-

Key: MSC = *M. oleifera* seed chloroform extract, MSE = *M. oleifera* seed ethanol extract, MLE = *M. oleifera* leaf ethanol, MLC = *Moringa* leaf chloroform, + = present, - = absent

Table 2: Antibacterial Activities of *Moringa oleifera* seed extracts on food-borne isolates

		Zone of Inhibition (mm)						
Extract	**Conc.(mg/ml)**	**ENT**	**STA**	**SHI**	**PA**	**EC**	**STM**	**ST**
MSC	50	06	06	06	06	06	06	06
	100	06	06	06	06	09	09	06
	200	06	06	06	06	09	09	06
	400	06	06	06	06	09	10	06
	MIC (mg/ml)	>4.0	>4.0	2.0	>4.0	>4.0	1.0	2.0
		E. coli, S. typhimurium (2)						
MSE	50	06	10	06	06	07	06	06
	100	06	11	06	06	08	06	06
	200	06	11	06	06	09	10	06
	400	06	11	06	06	09	11	06
	MIC (mg/ml)	2.0	4.0	>4.0	>4.0	>4.0	>4.0	>4.0
		E. coli, S. aureus, S. typhimurium (3)						
MLC	50	06	06	06	06	06	06	06
	100	06	06	06	06	06	06	06
	200	06	06	06	06	08	10	07
	400	06	06	06	06	08	10	08
	MIC (mg/ml)	>4.0	>4.0	>4.0	>4.0	2.0	1.0	2.0
		E. coli, S. typhi, S. typhimurium (3)						
MLE	50	06	06	06	06	06	06	06
	100	06	06	06	06	06	06	06
	200	07	08	06	07	07	06	06
	400	07	09	06	08	08	06	06
	MIC (mg/ml)	2.0	2.0	2.0	>4.0	>4.0	>4.0	>4.0
		E. coli, P. aeruginosa, S. aureus, Enterobacter spp (4)						
Control	30µg	32	30	28	25	30	30	25

Key: > = greater than, MSC = Moringa seed chloroform, MSE = Moringa seed ethanol, MLC = Moringa leaf chloroform, MLE = Moringa leaf ethanol, 06mm = size of disc µg = microgramme, Control = Streptomycin, ENT = *Enterobacter aerogenes,* STA = *S. aureus*, SHI = *Shigella* spp, PA = *P. aeruginosa,* EC = *E. coli,* STM =*S. typhimurium,* ST = *S. typhi*

Table 3: Antifungal activities of different extracts of *Moringa oleifera* on food-borne isolates

Extract	Conc. (µg/ml)	*Mucor* spp	*Rhizopus* spp
MSC	**100**	+	+
	500	+	+
	1000	-	-
MSE	**100**	++++	++++
	500	++	++++
	1000	+	++
MLC	**100**	++++	++++
	500	++++	++++
	1000	+++	+++
MLE	**100**	+++	++
	500	++	++
	1000	++	-
Control	**500**	-	-

Key: MSC = *Moringa* seed chloroform, MSE = *Moringa* seed ethanol, MLC = *Moringa* leaf chloroform, MLE = *Moringa* leaf ethanol, control = Ketoconazole

++++ = normal growth, +++ = 25% inhibition, ++ = 50% inhibition, + = 75% inhibition, - = 100% inhibition

CONCLUSION AND RECOMMENDATION

The results of the present study have shown the potentials *M. oleifera* chloroform and ethanol extracts possess as sanitizers/preservatives. This is due to the fact that they were found to possess antimicrobial activities against some food – borne microorganisms often implicated in the spoilage of foods and food – borne illnesses. Further research should be conducted to test the sanitizing and preservative effect of the extracts on some foods.

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