

Full Length Research Paper

Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in water borne diseases

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The anti-bacterial activity of methanol and n-hexane extracts of *Moringa oleifera* and *Moringa stenopetala* seeds was conducted on 3 bacterial species (*Salmonella typhii*, *Vibrio cholerae* and *Escherichia coli*) which normally cause water borne diseases. The paper disc diffusion method was used with treatments arranged in a completely randomized design and replicated four times. The highest inhibitions were observed at dilutions of 20, 5 and 40% for *M. oleifera* and *M. stenopetala* methanol extracts on *E. coli*, *S. typhi* and *V. cholerae* respectively. The n-hexane extract of both *M. oleifera* and *M. stenopetala* had a higher inhibition on *S. typhii* than *V. cholerae* and *E. coli*. The results of this study showed that *M. oleifera* and *M. stenopetala* had a degree of antibacterial properties especially in low doses.

Key words: *Moringa oleifera*, *Moringa stenopetala*, methanol and n-hexane extracts, inhibition, *Salmonella typhii*, *Vibrio cholerae*, *Escherichia coli*, Kenya.

INTRODUCTION

The frequency of life threatening infections caused by consumption of untreated water has increased worldwide and is becoming an important cause of mortality in developing countries (Al-Bari et al., 2006). Microorganisms contaminating water can cause gastroenteritis or inflammation of the stomach and intestinal lining. These include typhoid caused by *Salmonella typhii*, gastroenteritis caused by *Escherichia coli* and cholera caused by *Vibrio cholerae*. Conventional water disinfectants like chlorine have been used, but due to high cost and unavailability, households in developing countries such as Kenya use unpurified water leading to increased cases of water borne diseases (Ouma et al., 2005). According to World Health Organization, microbial resistance to conventional water treatment mechanisms is on the rise and medicinal plants offer a good source of alternatives. Over 80% of the population in Kenya lives in rural areas where water purification is rarely practiced due to high cost and unavailability of conventional

purifiers. Drinking water sources include deep ground water, upland lakes and surface water which are directly consumed without boiling or treatment. The need for alternative safe and inexpensive water clarifiers cannot be gainsaid. *Moringa* species preparations can be used as a cheaper alternative to the conventional disinfectants (Gassenschmidt et al., 1995).

Moringa species are well documented plant herbs due to their extraordinary nutritional and medicinal properties. *Moringa oleifera* Lam. and *Moringa stenopetala* (Baker f.) Cufod. are the most widely cultivated species of the monogenic family, the *Moringaceae* (Fahey, 2005). They have long been known in folk medicine as having value in treating a wide variety of ailments. They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria (Thilza et al., 2010). The disinfectant efficacies of different concentrations of the seed extract of *M. oleifera* and *M. stenopetala* on the above microorganisms are not known. There is need therefore to establish the disinfectant efficacies of *Moringa* species extracts for recommendation in their processed form as an alternative to chlorine in water purification. The present study was therefore to specifically

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investigate the role of methanol and n-hexane extracts of *M. oleifera* and *M. stenopetala* seeds as potential antimicrobial agents against some human pathogenic bacteria that are known to cause water borne diseases.

MATERIALS AND METHODS

Seeds of *Moringa oleifera* were collected from a single tree grown by a farmer in Kajulu location, Kisumu East District, Kenya. Seeds of *M. stenopetala* were obtained from Kenya forestry research institute (Maseno regional centre). Botanical identification was done at Kenya national museum, Nairobi. The standard reference bacteria were obtained from Kenya Medical Research Institute (KEMRI), Kisumu. Three bacteria species, *S. typhi*, *E. coli* and *V. cholerae* were used.

The Moringa seeds were de-shelled and dried at ambient temperatures (23 to 25°C) for a period of five days before milling. The white kernels were milled into a fine powder using a Christy laboratory mill at 8000 rpm. The fine powder obtained was then sieved through a number 26 sieve. 1000 g powder of *M. oleifera* and *M. stenopetala* was soaked in 2000 ml n-hexane in a 5 litre Ehlenmeyer flask, corked and placed in a laboratory shaker for 4 h and then allowed to stay overnight. It was decanted into a one liter flask and vacuum filtered through Whatman filter paper no. 1 into a Butchner flask. The filtrate was concentrated using a rotary evaporator at 40°C water bath until the condensation of the solvent stopped dropping. The extract was then transferred into a sterilized beaker.

The solid residue obtained from powder sieving was added into two 5 litre conical flasks containing 2000 ml of methanol and placed in a laboratory shaker (UAR orbital shaker SO1) for 4 h and allowed to stay overnight in order to settle down for decantation into a one litre flask. The extract was filtered through Whatman filter paper No.1 using water suction pump and put into a Butchner flask. The filtrate was concentrated using a rotary evaporator at 40°C water bath until condensation of the solvent stopped dropping.

Preliminary screening for antibacterial activity was carried out using the disc diffusion method of Barry and Brown, (1996). Each extract was reconstituted in DMSO (dimethyl sulphoxide) at dilutions 2.5, 5, 10, 20 and 40% for n-hexane extract and 2.5, 5, 10, 20 and 40% for methanol extract, respectively to assess the antibacterial activity. McConkey agar and bacteriological peptone agar were prepared as stipulated (HI media lab PVT Ltd.). Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums. *E. coli* and *S. typhii* were inoculated into McConkey agar while *V. cholerae* was inoculated into bacteriological peptone agar. 5 mm diameter sterilized paper discs made from Whatman filter paper were impregnated with the test extracts (at concentrations 2.5, 5, 10, 20, and 40% for n-hexane and 2.5, 5, 10, 20 and 40% for methanol extract) and placed into the surface of the inoculated media. Plates set with DMSO served as controls. The experiment was set up as a complete randomized design replicated four times. The set up was allowed to stand for 48 h in an incubator at 29°C. The presence of zones of inhibition around the discs was interpreted as preliminary indication of antibacterial activity. The zones of inhibition were measured using veneer calipers in millimeters and recorded. The results were analysed using SAS GLM ANOVA.

RESULTS AND DISCUSSION

Different concentrations of *M. oleifera* and *M. stenopetala*

extracts showed significant differences ($p < 0.05$) in their efficacy against all the microorganisms tested. Methanol extracts of *M. oleifera* and *M. stenopetala* showed significantly ($p < 0.05$) higher inhibition on *E. coli* at 20% concentration (Table 2). Significantly ($p < 0.05$) the least inhibition was observed at concentration 2.5% for the two *Moringa* species. The highest inhibition values obtained in this study occurred in the range reported by Mashiar et al. (2009) for the efficacy of a powder obtained from fresh *M. oleifera* leaves extracted using ethanol. This finding suggests that extracts of *Moringa* seeds studied contain bio-compounds whose antibacterial potentials are highly comparable with that obtained from leaves. It was expected that variations in concentration would produce different results due to their levels of potency. However, there were no significant differences ($p > 0.05$) between concentrations 5% and 2.5% on their effect on *E. coli*. Similarly, no significant differences ($p > 0.05$) occurred between concentrations 10 and 40% for *M. oleifera* extracts against *E. coli*. These findings were interesting since traditional methods of treating bacterial infections rely on higher concentrations of the extract (Mashiar et al., 2009).

The n-hexane extracts showed significantly ($p < 0.05$) higher inhibitions against *E. coli* at 40% concentration and least inhibition at 5% concentration, respectively for *M. oleifera* and *M. stenopetala* (Table 1). Gauging from the area of inhibition, it was evident that n-hexane extracts of higher concentrations had numerically better efficacy on *E. coli* in comparison to extracts from methanol. However, *M. oleifera* and *M. stenopetala* extracts had low efficacy values on *E. coli* when compared to the standard antibiotics as previously reported by Mashiar et al. (2009). The magnitude of inhibition observed on *E. coli* revealed that the n-hexane extract of *Moringa* seeds has a high potency and can successfully be used for destruction of bacteria that cause water borne diseases. According to Chandarana et al. (2005) *E. coli* bacteria are known to be extremely sensitive to antibiotics such as streptomycin and a comparative study with the findings of this study is necessary. Aney et al. (2009) found an extract of *M. oleifera* seeds to be as effective as several antibiotics against *E. coli*. DMSO (control) however showed no inhibition on the microbes.

Remarkable about this investigation was that the susceptibility of *E. coli* to the *Moringa spp* n-hexane extracts decreased with increase in extract concentration (up to 10%) (Table 1) indicating that the microbe became more resistant when subjected to high concentrations. According to Shekhar et al. (2000) crude ethanol extract of *M. oleifera* tested against *E. coli*, *S. typhii*, *V. cholera*, *Shigella dysenteriae* and *Pseudomonas pyocyaneus*, showed activity against *E. coli* at reduced extract concentrations. A research conducted by Vaghasiya and Chanda (2007) showed that *M. oleifera* crude extracts had no activity against *E. coli*, showing variance with our findings. However, the purified dichloromethane extract

Table 1. Mean area of inhibition of n-hexane extract on the microbes.

Concentration (%)	<i>M. oleifera</i>			<i>M. stenopetala</i>		
	<i>E. coli</i>	<i>S. typhii</i>	<i>V. cholera</i>	<i>E. coli</i>	<i>S. typhii</i>	<i>V. cholera</i>
2.5	17.5	76.5	9.8	25.3	78.7	33.0
5	9.4	43.1	32.2	14.9	38.9	26.0
10	11.2	21.0	26.9	25.3	29.0	24.5
20	19.6	23.9	16.8	20.9	30.6	22.3
40	36.8	19.5	9.8	26.8	18.9	16.2
Mean	17.2	36.9	22.2	22.6	39.2	24.4
LSD _{0.05}			1.786			
CV%			28.61			
SEM			3.5			

Table 2. Mean area of inhibition of methanol extract on the microbes.

Concentration (%)	<i>M. oleifera</i>			<i>M. stenopetala</i>		
	<i>E. coli</i>	<i>S. typhii</i>	<i>V. cholera</i>	<i>E. coli</i>	<i>S. typhii</i>	<i>V. cholera</i>
2.5	10.4	56.0	8.6	8.6	28.9	8.6
5	11.2	67.0	9.2	11.6	26.4	11.7
10	19.5	44.7	11.7	25.3	12.3	13.2
20	36.4	41.9	14.9	32.6	13.6	18.8
40	18.2	12.4	24.5	13.0	11.7	24.5
Mean	19.2	44.4	13.8	18.2	18.6	15.4
LSD _{0.05}			1.786			
CV%			28.61			
SEM			3.5			

and isolated parts from column chromatography showed antibacterial activity against *E. coli* (Khesorn, 2009).

The results of this study demonstrate that both methanol and n-hexane extracts of *M. oleifera* and *M. stenopetala* displayed antimicrobial activity against *S. typhii* as shown in Table 1 and 2. Significantly ($p < 0.05$) the highest zones of inhibition for the methanol extract against the microbe occurred at 5% for *M. oleifera* and at 2.5% for *M. stenopetala*. The least inhibition occurred at 40% for both *M. oleifera* and *M. stenopetala*. It clearly emerged that the efficacy of the extracts against *S. typhii* was higher at low concentration levels. However, the methanol extract was numerically stronger in terms of efficacy indicating that it can be used at low concentrations to prevent the spread of typhoid. This agrees with Swanson et al. (2007) that salmonellosis can be curbed by use of plant extracts such as *Moringa* species. *M. oleifera* methanol extract exhibited higher microbial inhibition against the microbe compared to *M. stenopetala* (Table 2). Folkard and Sutherland (2005) proposed utilization of *Moringa* seeds as food since it sterilizes the food and destroys *S. typhii* which lives in the intestinal tracts of man. The antibiotic nature of *Moringa* seeds is due to an oil it contains which on consumption forms a thin film over the intestinal wall thus reducing or

preventing the pathogen (by inhibition) from penetrating the walls (Caceres and Lopez, 1991; Caceres et al., 1991; Nwosu and Okafor, 1995). Other studies have also shown that *M. oleifera* seeds produce a gum that is anti-typhoid in antibacterial activity (Fuglie, 1999, and Harristoy et al., 2005). The antibacterial activity of the plant has been demonstrated against both gram-negative and gram-positive bacteria and this is in agreement with our findings. (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007; Mashiar et al., 2009).

Table 1 and 2 show the effect of two *Moringa* species tested on *V. cholerae*. Extract concentration was found to have a direct effect on efficacy against *V. cholerae*. The methanol extract of *M. oleifera* and *M. stenopetala* gave significantly ($p < 0.05$) higher inhibitions at 40% concentration while the least significant ($p < 0.05$) inhibition occurred at 2.5%. There was no significant difference ($p > 0.05$) between the efficacy of methanol extracts of *M. oleifera* at 5% and 2.5% against *V. cholera*. Similarly, no significant differences ($p > 0.05$) occurred on extracts of *M. stenopetala* at 5 and 10% against the microbe. Unlike results on their efficacy against *E. coli* and *S. typhii*, the methanol extract had a higher efficacy at high concentration level when tested on *V. cholerae*. According to Madsen et al. (1987) *Moringa* flocculants

have been characterized as basic polypeptides which can bind suspended particles in water that contain colonies of bacteria such as *V. cholerae*. The charged protein molecules can serve as nontoxic natural polypeptides to settle mineral particles and organics in destruction of specific bacteria (Aney et al., 2009). As been reported, *Moringa* seeds show similar coagulation effects to alum. It is also reported that a recombinant protein in the seed is able to flocculate gram positive and gram negative bacterial cells. *Moringa* seeds could be used as a biosorbent for the removal of cadmium from aqueous media (Aney et al., 2009). Thus antimicrobial attributes of *Moringa* seeds are due to its properties as a coagulant, microbial binder and a biosorbant. This indicates that seed extracts of *Moringa* species can be used for low risk water treatment in rural and peri-urban areas of developing countries.

The n-hexane extracts gave significant differences ($p < 0.05$) between *M. oleifera* and *M. stenopetala* against *V. cholerae* (Tables 1 and 2). *M. stenopetala* extracts exhibited numerically higher inhibitions than *M. oleifera* extracts indicating that it could be better for reduction of incidences cholera. A concentration of 2.5% exhibited the highest potency against *V. cholerae*. The highest inhibition values reported in the present study fall within the range of 10 to 30 mm of the ethanol seed extract of *M. oleifera* reported by Mashiar et al. (2009). Ryan and Ray (2004) reported that *V. cholerae* produces an entero-toxin whose action on the mucosal epithelium lining of the small intestine is responsible for the characteristic massive diarrhea. Since *Moringa* seed extracts contain oils which adheres to human mucosal surface Nwosu and Okafor (1995) it can prevent infection when consumed as food or as a purifier. This property may be useful in water treatment through flocculation of pathogens such as *V. cholerae* thereby blocking their activity. Another important finding was that n-hexane extracts inhibition against *V. cholerae*, increased at lower concentrations. This indicated that *V. cholerae* was resistant to the extracts at higher concentration. According to Aney et al. (2009) *Moringa* seeds contain an antibiotic principle known as pterygosperrin which is responsible for destruction of micro-organisms in water.

Conclusion

This study has successfully shown that seed extracts of *M. oleifera* and *M. stenopetala* bear antimicrobial properties against *S. typhii*, *V. cholerae* and *E. coli*. These extracts could be promising natural antimicrobial agents with potential applications in controlling bacteria that cause water borne diseases. The extracts can provide a cheap and sustainable method toward disease reduction and can eventually improve the quality of life of the rural and peri-urban poor in developing countries.

However, *Moringa* extracts should not be regarded as a panacea for reducing the disease incidences since issues

of safety and toxicity need to be evaluated.

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