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Antibacterial Activity of *Moringa ovalifolia* and *Moringa oleifera* Methanol, N-Hexane and Water Seeds and Bark Extracts against Pathogens That Are Implicated in Water Borne Diseases

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Abstract

Antibacterial activity of methanol, n-hexane and water extracts of seeds and bark of M*oringa oleifera* and *Moringa ovalifolia* was conducted. The causative agents of common bacterial infections that are thought to cause water-borne diseases, namely, *Escherichia coli*, *Enterococcus faecalis*, and *Bacillus cereus* were chosen for the study. The paper-disc diffusion method was used with treatments arranged in a completely randomized design and replicated four times. The *M. oleifera* extracts showed more inhibitory effect than those of *M. ovolifolia*. The conventional antibiotic Ampicilin generally showed higher inhibitory effect than the extracts of both *M. oleifera* and *M. ovalifolia*. The inhibitory effect varied depending on the solvent used. The n-hexane extracts of both seeds and bark of *M. ovalifolia* and *M. oleifera* had almost the same inhibition activities (6 ± 1 mm mean inhibition zones) on *B. cereus*, *E. coli* and *E. faecalis*. The n-hexane extract generally gave lower antibacterial activities than those of the other solvents for seeds and back. The results of the study showed that *M. oleifera* and *M. ovalifolia* had a degree of antibacterial properties against the selected test organisms that cause water borne diseases.

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Keywords

Antibacterial Activity, *Escherichia coli*, N-Hexane, Inhibition, Methanol, *Moringa oleifera*, *Moringa ovalifolia*, *Bacillus cereus*, *Enterococcus faecalis*

1. Introduction

Treating water that contains pathogenic bacteria, viruses, protozoa, parasites and sediment is a serious challenge in both developed and developing countries. The cost, health issues and environmental side effects of the conventional water treatment chemicals are their main disadvantages. Drinking water sources include deep ground water, upland lakes and surface water which are directly consumed without boiling or treatment. A large number of people in rural areas in developing countries lack access to adequate clean water supply due to factors such as cost, unreliable or insufficient clean water supplies, long distances to clean water supply points, etc. As a result, most people in these areas use water directly from available and often contaminated sources without any treatment and therefore are exposed to many water-related diseases since polluted water is normally an important vehicle for the spread of disease. The frequency of life-threatening infections caused by consumption of untreated water has increased all over the world and become an important cause of mortality in developing countries [1] [2]. Microorganisms contaminating water can cause gastroenteritis or inflammation of the stomach and intestinal lining and these include typhoid, gastroenteritis and cholera caused by *Salmonella typhii*, *Escherichiacoli* and *Vibrio cholera*, respectively. Others include *Bacillus cereus* which produces toxins causing diarrhea whereas inflammation are caused by *Enterococcus faecalis*. The provision of portable water satisfying modern quality requirements is an enormous task of most water supply agencies all over the world. Whereas the demand for water is increasing, the costs of water supply are increasing [3] and the quality of the raw water from the various sources is decreasing due to increased industrialization [4] [5]. Moreover, the cost of treatment, especially in developing countries, is increasingly becoming beyond the reach of most water supply agencies leading to production of low-quality water [3]. In addition, the use of chemical additives for water treatment raises a lot of concerns over safety issues [6] [7]. A conventional water disinfectant like chlorine, for instance, is being widely used and however, it has a problem of decay and hence reduced concentration as the water flows. Due to unavailability and high costs of water treatment chemicals, households in developing countries, such as Namibia use unpurified water leading to increased cases of water borne diseases [8].

Innumerable antibacterial agents are currently employed in treating bacterial infections. In addition to the cost and health effects discussed above, many of the currently used antibacterial agents are associated with adverse effects such as toxicity, hypersensitivity, immunosuppression, and tissue residues posing public health menace [9]. These disadvantages undermine their therapeutic use and thus necessitating the need for finding alternative remedies for treatment of bacterial diseases. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies [10]. According to World Health Organization (WHO), microbial resistance to conventional water treatment mechanisms is on the rise. Therefore, the use of low-cost and waste materials of biological origin has shown to provide economic solutions through reduction of exorbitant costs for water treatment to this global problem and is being advocated as a sustainable technology [11]-[14].

Moringa tree could be very simple and readily available solution to water treatment problems. It is called a multipurpose tree because it has been found to have nutritional, antimicrobial, medicinal, industrial and water treatment properties [15] [16]. The seeds have been found to be effective in removing turbidity, heavy metals and bacteria from water in a sustainable and environmentally friendly way [17]. Previous studies have reported that various parts of *Moringa* roots, flowers, bark, and stem including seeds possess antimicrobial properties [18] [19]. There are 13 known species in the plant family *Moringaceae*, and of these, the *Moringa oleifera* is the most widely studied, whereas the *Moringa ovalifolia*, which is endemic to Namibia, is not reported much in literature. *M. oleifera* is the most widely cultivated species of a monogeneric family, the *Moringaceae* that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [16] which is widely used for treating bacterial infection, anti-inflammation, sexually-transmitted diseases and diarrhea. The *M. oleifera* spe-

cies is now cultivated widely in sub-Sahara Africa except Namibia as recently reported by Kwaambwa *et al*. [20] whereas *M. ovalifolia* which is endemic to Namibia and only few studies have been reported of this species to our knowledge.

As mentioned earlier, the *M. ovalifolia*, which is known to be indigenous in Namibia, has not been studied yet as much as *M. oleifera* and therefore, the present study was aimed at investigating the of both *M. oleifera* and *M. ovalifolia* seeds and bark as potential antibacterial agents against some human pathogenic bacteria that are known to cause water borne diseases. The study investigated 1) the roles of methanol, n-hexane and aqueous extracts of parts (seeds and bark) of the two *Moringa* species against water-borne diseases pathogens *Escherichia coli*, *Enterococcus feacalis* and *Bacillus cereus* and 2 the comparison of the antibacterial potentials between the two *Moringa* plant species seeds and bark against the bacterial isolates chosen. Although studies have been done on *M. oleifera*, it is important to point out that the growth conditions of the plants play of metabolomics of plants and the study further informs how the results compare to similar previous studies of *M. oleifera*.

2. Materials and Method

2.1. Sample Collection

Samples *M. ovalifolia* plant seeds and bark were collected from three different places in Namibia, namely Tsumeb, Halali and Rundu. The seeds and bark of *M. oleifera* were sourced from a neighboring country Zimbabwe.

2.2. Preparation of the Extracts

2.2.1. Seeds and Bark Extracts

Seed coats of *M. oleifera* and *M. ovalifolia* were removed and the kernels were ground into powder using an electrical grinder. Barks from the two *Moringa* species collected were dried in the shade and ground into powder in the same manner as done for seeds. Approximately 40 g powder was weighed using AG Electronic weighing balance. The weighed samples were soaked in 100 mL of n-hexane (98.9%), methanol (99.9%) and distilled water at room temperature for 72 hours in the conical flasks. The extracts were then filtered using a filter paper (Whatman size No. 1) and the filtrate were evaporated to dryness in a water rotary vapor at 60˚C. The obtained residues were then freeze-dried to obtain the crude extracts and kept in the refrigerator until further use.

2.2.2. Test Bacteria

The test organisms (indicator strains) used in the study were one Gram-negative bacteria *Escherichia coli*, and two Gram-positive bacteria *Enterococcus faecalis* and *Bacillus cereus* obtained from the Multi-Disciplinary Research Centre Laboratory of the University of Namibia.

2.2.3. Standardization of Inoculum

The inoculum was prepared from the stock culture which was maintained on nutrient agar slant at 4˚C and subcultured onto nutrient broth using a sterilized wire loop and incubated at 37˚C for three days.

2.2.4. Antibacterial Assay

Discs of about 6 mm diameter were made from Whatman No. 1 filter paper using a paper puncher and transferred into Bijou bottles and sterilized in the autoclave at 121˚C for 15 minutes. Preliminary screening for antibacterial activity was carried out using the disc diffusion method of Barry and Brown [21]. Stock solution of each plant extract was prepared by dispersing crude extract of each plant extract in less than 5 mL dimethyl sulfoxide (DMSO). Serial doubling dilution was carried out by adding 1 mL of DMSO at each dilution. The concentrations were prepared from the stock solution such that each disc absorbed about 0.01 mL equivalent to 50, 35, 20 and 15 mg/mL, respectively, but 50 mg/mL are shown graphically. Only 50, 35, 20 and 15 mg/mL due to limited material. Serial dilution was made on the bacteria that were activated for three days until the required dilution in the range 1×10^6 to 5×10^6 cells per mL. Nutrient agar was prepared and taken in plastic petri dishes, and 1 mL of each culture was spread on different plates. The extracts impregnated the discs and the reference antibiotic Ampicilin was placed on the inoculated nutrient agar of each petri dish and incubated at 37˚C. After 12 - 16 hours of incubation, the zones of inhibitions of bacterial growth around the discs were measured.

2.2.5. Determination of Minimum Inhibitory Concentrations (MIC)

MIC of six different samples of each plant, *M. oleifera* and *M. ovalifolia*, was determined using tube dilution method by Akinyemi *et al*. [22]. 1 mL of each culture was added in a test tube containing 1 mL plant extract at different concentrations and 8 mL nutrient broth. The test tube was shaken and incubated at 37˚C. After 12 - 16 hours of incubation, the lowest dilution (concentration) with no growth was taken as the minimum inhibitory concentration.

3. Results and Discussion

The antibacterial activities of water, methanol and n-hexane extracts of bark and seeds of *M. oleifera* and *M. ovalifolia* were investigated using the agar disc diffusion method against the selected pathogens *E. coli*, *E. faecalis* and *B. cereus*. **Figures 1-4** show the mean inhibition zones of the extracts of the two plants and ampicillin (reference antibiotic). The error in the determined values of the mean inhibitions was ±1 mm. The plant extracts showed varying degrees of antibacterial activities against these selected pathogens although some extracts were even comparable with each other at some points such as n-hexane and water. The results show that *M. oleifera* seeds and bark powder extracted with methanol has a greater antibacterial activity than *M. ovalifolia* seeds and bark powder extracted with the same solvent. Powder extracted with n-hexane and water was consistent but *M. oleifera* n-hexane and water extracts gave the highest inhibition zones than *M. ovalifolia* n-hexane and water extracts.

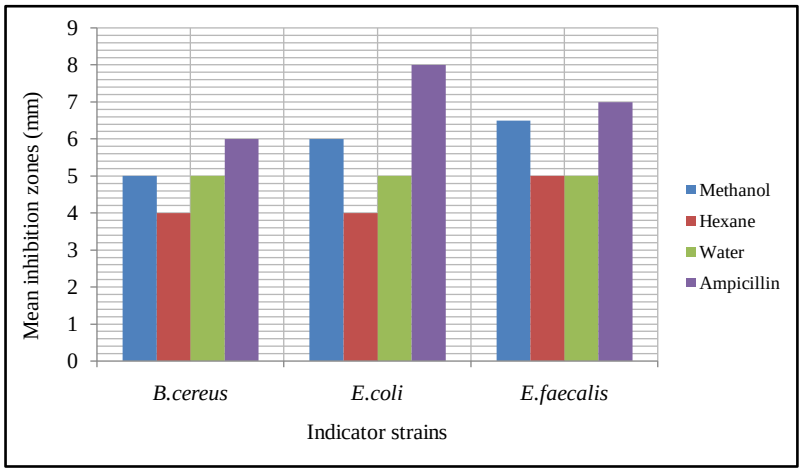

Figure 1. Comparison of the antibacterial activity of *M. ovalifolia* seed methanol, n-hexane and water against the indicator strains.

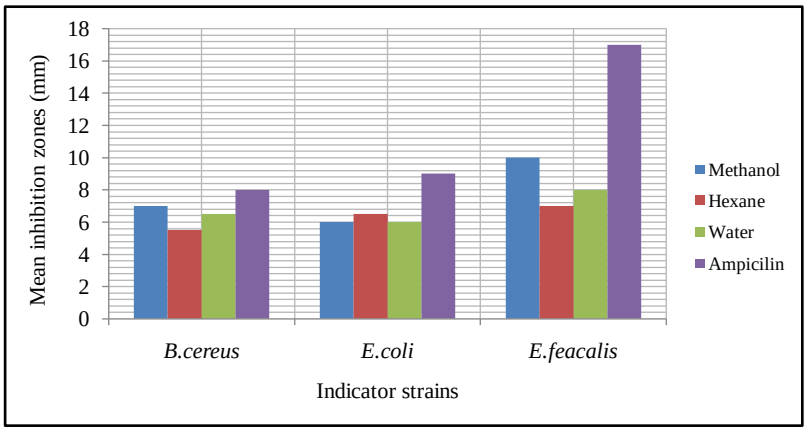

Figure 2. Comparison of the antibacterial activity of *M. oleifera* methanol, n-hexane and water seed extracts against the test organisms.

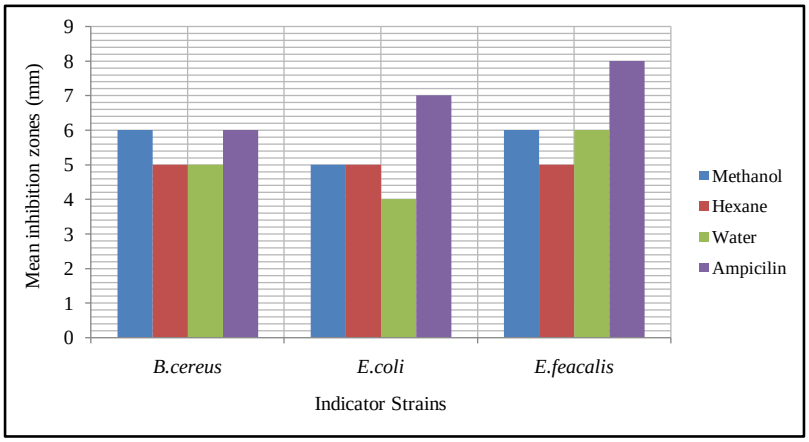

Figure 3. Comparison of the antibacterial activity of *M. ovalifolia* methanol, n-hexane and water bark extracts on the test bacteria.

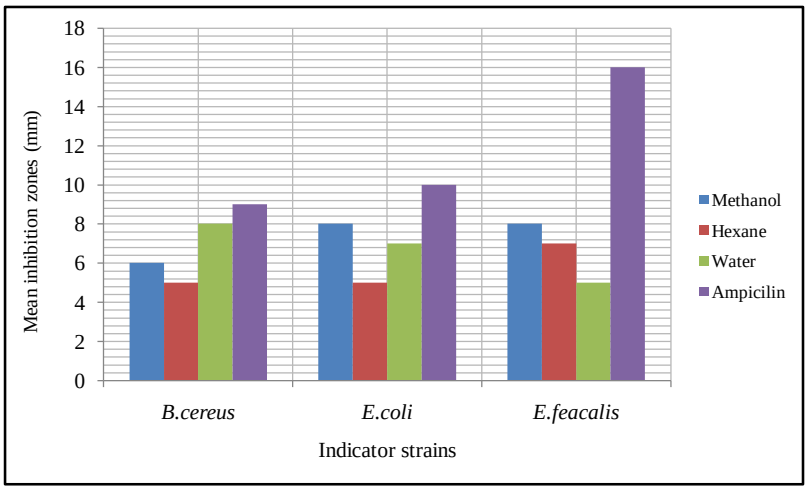

Figure 4. Comparison of antibacterial activity between *M. oleifera* methanol, n-hexane and water bark extracts on the indicator strains indicated.

The figures also compare the antibacterial potentials of seeds from *M. oleifera* and *M. ovalifolia* as well as the effectiveness of the bark from the two plants. It can be deduced that *M. oleifera* seed extracts had a broader spectrum of antibacterial activity than *M. ovalifolia* seed extracts against three bacterial isolates, *i.e. Bacillus cereus* (9 mm), *Enterococcus faecalis* (10 mm) and *Escherichia coli* (8 mm), at 50 mg/mL concentration. It can also be deduced that *M. oleifera* bark extracts had a highest antibacterial activity than the bark from *M. ovalifolia* against all three bacterial isolates with zones of inhibitions between 7 - 9 mm at a concentration of 50 mg/mL. The inhibitory effect varied depending on the solvent used. The n-hexane extracts of both seeds and bark of *M. ovalifolia and M. oleifera* had almost the same inhibition activities (6 ± 1 mm mean inhibition zones) on *B. cereus*, *E. coli* and *E. faecalis*. The n-hexane extracts were generally lower than those of the other solvents for seeds and back extracts.

MIC of four concentrations of each plant extracts was determined using tube dilution method by Akinyemi *et al*. [22]. After 24 hours of incubation at 37˚C, *M. ovalifolia* MIC from methanol seed extract was obtained at concentration 50 mg/mL for *B. cereus*, 20 mg/mL for *E. coli* and *E. faecalis* was resistant. n-Hexane seeds extracts were obtained at 50 mg/mL for *E. faecalis*, *E. coli* and *B. cereus* were resistant and for water it was obtained at 50 mg/mL for *E. faecalis* while *B. cereus* and *E. coli* were resistant. For *M. oleifera*, it was obtained at 20 mg/mL for methanol and n-hexane seed extracts, demonstrating highest antibacterial activity. All the plant extracts indicated showed either no or minimal growth at the highest concentration (50 mg/mL) and very vulnerable to the indicator strains concentrations lower than 35 mg/mL as shown in **Table 1**.

Table 1. Minimum inhibitory concentrations (MIC).

	SD																	
	OVA									OLE								
	MET			HEX			WA			MET			HEX			WAT		
	BC	EC	EF	BC	EC	EF	BC	EC	EF	BC	EC	EF	BC	EC	EF	BC	EC	EF
CON																		
15	++	++	++	++	++	++	++	++	++	+	+	+	+	++	+	++	++	++
20	+	−	+	++	++	+	++	++	+	−	+	+	++	−	+	++	++	++
35	+	+	+	+	+	+	++	+	++	+	+	+	+	+	+	+	++	+
50	−	+	+	+	+	−	+	+	−	+	−	−	−	+	+	+	++	−
	BK																	
CON																		
15	++	++	+	++	++	++	++	++	++	+	+	++	+	++	++	++	+	+
20	+	++	+	++	++	+	++	++	++	−	+	+	+	+	++	+	++	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−
50	+	+	−	+	−	+	+	+	−	−	+	+	−	+	+	+	+	+

Key: OVA = *Moringa ovalifolia*, OLE = *Moringa oleifera*, SD = Seeds, MET = Methanol extracts, HEX = n-Hexane extracts, WAT = Water extracts, BC = *Bacillus cereus*, EF = *Enterococcus faecalis*, EC= *Escherichia coli*, BK = Bark and CON = Concentration (mg/mL), ++ = More turbid, + = Less turbid, − = No growth.

In general, the antibacterial effects of all substances tested are clearly less than the effects of Ampicillin, *i.e.* the inhibition zones by *M. ovalifolia* and *M. oleifera* were less than the antibiotic Ampicillin against all the bacterial isolates. Although the results show that the conventional antibiotic resistance showed more potency than plant extracts, the use of the latter show great potential in terms of cost and health effects associated with the former. The effectiveness may be improved, for instance, by testing different extractions methods to increase the concentration of the active chemical components.

4. Conclusion

This study was conducted to demonstrate the antibacterial activity of seeds and bark of *M. ovalifolia* and *M. oleifera* which could potentially serve to treat water-borne diseases bacterial that have become more resistant to most of the common antibiotics used for treatment. The growing incidences of resistance of microorganisms to conventional antimicrobial agents are a source of concern globally. The results of the study have shown the antibacterial potential of both *M. oleifera* and *M. ovalifolia*. The results showed that the conventional antibiotic resistance has more potency than plant extracts but this, however, does not mean that the potential of natural plant shouldn't be exploited. The effectiveness of the *M. oleifera* extracts has a greater antibacterial activity than that of *M. ovalifolia*. In comparison with the previous reported studies on *M. oleifera*, the results are in good agreement in terms of antibacterial activities [9] [18] [19]. Further research should be conducted to evaluate the effectiveness of extracts, especially from *M. ovalifolia* (which hasn't been studied a lot), on some other pathogens that cause water-borne diseases. Plants like *Moringa* are available, cheap and affordable making them alternative sources for conventional antibiotics.

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