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Efficacy of *Moringa oleifera* Seed Extract on the Microflora of Surface and Underground Water

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Abstract: The efficacy of *Moringa oleifera* seed (LAM) in reducing total bacteria and coliforms in raw water was studied. Its antibacterial activity on some selected enteric bacterial pathogens was also investigated. About 88 and 97.5% of the total bacteria and coliforms,, respectively were reduced in the surface water after 24 h of treatment. Meanwhile, in the underground water sample, the seed extract reduced the total bacteria and coliforms by 88.3 and 93.3% with a precipitation rate of 7.7×10^{2} and 2.42×10^{2} cfu h^{-1}, respectively. The coagulating efficiency and zones of inhibition increased correspondingly with an increase in concentration of the seed extract. At a concentration of 30 μg mL^{-1}, there was no microbial growth recorded in the water samples even after 96 h storage. However when *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* were exposed to the seed extract, a secondary bacterial growth resulting into an increase of about 185, 189 and 198% in their concentrations, respectively was observed after 24 h. The minimum inhibitory concentration of the seed extract ranged between 20 and 50 μg mL^{-1}.

Key words: *Moringa oleifera*, bacteria, coagulation, water purification, coliforms

INTRODUCTION

The availability of portable water is an indispensable feature for preventing diseases and improving the quality of life (Aderiye *et al*., 1992). More than 80% of all human diseases are attributed to use of unsafe water (Effler *et al*., 2002; Oslen *et al*., 2002). Consequently, about 1.25 billion people in the world especially the tropics suffer from major water related diseases such as cholera, typhoid, dracunculiasis, schistosomiasis among others (Odeyemi, 1988). Most rural settings and villages in developing countries do not have access to portable water and sanitation facilities and the conventional method of water treatment is expensive (Lewis, 1985).

In Nigeria, most rural dwellers depend on water from hand-dug wells/bore holes, streams/rivers, ponds/lakes and rain when available, for domestic purpose. Most of the microorganisms isolated in water drawn from wells in Owo; running streams and even in storage tanks at Ado-Ekiti, southwest Nigeria were found to be members of the Enterobacteriaceae such as *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp. (Aderiye *et al*., 1992, 2005; Oluyege and Famurewa, 2005).

The presence of coliforms in water suggests previous human interaction and faecal pollution which may lead to serious water-borne diseases when consumed without any form of treatment. Methods usually employed for treating water for consumption include sedimentation, boiling, filtration, coagulation by addition of chemical agents such as alum and incorporation of plant parts such as *Moringa oleifera* in turbid water (Ndabigensere *et al*., 1995). Natural coagulants have been detected and extracted from seeds of seven different *Moringa* species viz., *M. stenopetala* (Kenya) *M. peregina* (Egypt), *M. drouhardii* (Madagascar), *M. longituba* (Somalia), *M. ovalifolia* (Namibia), *M. concanesis* (India and Pakistan) and *M. oleifera*

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(Sudan) (Jahn, 1988). Of all the species, the use of *M. oleifera* seed as a natural coagulant for water treatment stands out in Sudan and other parts of Africa (Eliert *et al*., 1981; Anonymous, 1987).

The plant is known to have a wide application in therapy ranging from its use as antiscorbutic and anti-irritant in Nigeria, to the treatment of diarrhea, rheumatism and goiter in Mauritius and the treatment of nervous debility and leprosy in India (Olayemi and Alabi, 1994). The seed can be consumed after drying and as condiment and garnish in food. As a multipurpose tree, it is also of great environmental interest in plantations, in private premises, around fields and on nursery plots (Madsen *et al*., 1987).

Provision of safe and clean water to rural villages remains a central objective of the World Health Organization (Pollard *et al*., 1995). A substantial research effort has focused on low cost approaches to water and waste treatment in less developed countries through the application of indigenous natural products of soil origin which offers genuine localized and appropriate solutions to water quality problem (Pollard *et al*., 1995). This study therefore examines the efficacy of *M. oleifera* seed extract treatment on the microflora of water intended for consumption.

MATERIALS AND METHODS

Source of Plant Material

Moringa oleifera seed pods were obtained from the trees planted around the Doctors' residential quarters at the State Specialist Hospital complex in Ado-Ekiti, Nigeria. The pods were broken manually to release the seeds which were later spread in the open air to dry. The seed was identified and voucher sample deposited at the herbarium unit of the Department of Plant Science, University of Ado-Ekiti, Nigeria.

Source of Microorganisms

Pure cultures of bacterial isolates obtained were identified as described by Barrow and Feltham (1993) and compared to standard stock cultures obtained from the Department of Microbiology, Obafemi Awolowo University (OAU) Ile-Ife, Nigeria. The organisms identified include *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Streptococcus faecalis*. The bacteria were maintained on nutrient and selective agar at 4°C. When needed, the organisms were cultivated on minimal broth medium and stored at 37°C for 24 h.

Source of Water

Samples of raw water were obtained aseptically from a concrete ring-walled well and a slow running stream. The well about 7 m deep is located 120 m from a seasonal water logged area along the Housing Corporation estate, Ado-Ekiti. The Elemi stream is also seasonal and situated north-east of the University of Ado-Ekiti campus, along Iworoko road, Ado-Ekiti. Water sample obtained from both the well and stream was designated as underground and surface water, respectively. Sampling was carried out daily between 7:30 and 8:00 am in July through September for three consecutive years, 2004-2006. Treatment and analyses of water samples were carried out at the Laboratory of Microbiology Department, University of Ado-Ekiti within 2 h of collection.

Preparation of *Moringa* Seed Suspension

The seed suspension was prepared as described by Olayemi and Alabi (1994) and Jahn (1988). Fifty grams of the dried seed were ground into powder and kept aseptically at room temperature until when needed. Two grams of the seed powder were soaked in 100 mL distilled water for about 1 h. Later, the suspension was thoroughly shaken and filtered through a Whatman filter paper No. 1. The filtrate served as the crude extract.

Treatment of Water Samples and Bacteriological Analysis

A known quantity (ca 100 mL) of varied concentrations (20, 40 and 60 μg L^{-1}) of *Moringa* seed extract was aseptically added to 1 L of each water sample. The untreated water samples served as control.

Water samples were cultured on the following media; nutrient agar, *Salmonella* and *Shigella* agar, Slanetz and Barthley medium and mannitol salt agar using the pour plate method (Barrow and Felthan, 1993). Bacteria counts were determined hourly for 24 h and also at 12 h interval for 96 h.

The determination of the Minimum Inhibitory Concentration (MIC) of the seed extract on the bacterial pathogens was carried out using the agar diffusion technique (Dahot, 1998). The estimation of the total bacteria and coliforms was carried out by serial dilution using the pour plate method. The inhibitory rate (cfu h^{-1}) was the difference between the microbial concentration of water sample after 24 h seed treatment and that of fresh water sample over 24 h.

Statistical Analysis

Data obtained were statistically analysed using the t-test and analysis of variance. The level of significant difference was determined at $p \leq 0.05$.

RESULTS

Twenty six percent of the total bacteria cells present in the untreated surface water sample were precipitated within 24 h (Table 1). When water was treated with 20 μg mL^{-1} *M. oleifera* seed extract, there was 93.75% reduction in total bacteria count at an inhibitory rate of 7.5×10^{2} cfu h^{-1}. About 26.67% coliforms were precipitated in the untreated surface water sample after 24 h storage while 97.4% coliforms in *Moringa* seed treated water sample were precipitated at the rate of 3.04×10^{2} cfu h^{-1}.

A 32.9% reduction in total bacteria load was observed in the fresh underground water after 24 h storage as against 89.4% in *Moringa* seed treated water precipitated at the rate of 3.17×10^{2} cfu h^{-1}. Meanwhile, the coliforms were reduced by 94.29% at the rate of 1.38×10^{2} cfu h^{-1} after 24 h treatment with the seed extract.

With 30 μg mL^{-1} seed extract treatment and 72 h storage period in surface and underground water samples, the total bacteria count reduced drastically to 1000 cells mL^{-1} (Table 2). No growth was recorded on any specialized media for the cultivation of coliforms even at concentrations as low as 10 μg mL^{-1} of the seed extract after 48 h storage.

Table 1: Effect of *Moringa oleifera*[1] seed extract on bacterial load* of surface and underground water samples

		Storage/treatment period (h)									
Water samples	Frh	1	2	3	4	5	6	7	8	9	24
Surface water											
Raw sample											
TB	1.92	1.88	1.74	1.69	1.68	1.65	1.62	1.62	1.54	1.49	1.42
CO	0.75	0.74	0.69	0.67	0.63	0.63	0.63	0.62	0.61	0.59	0.55
Treated sample											
TB	1.92	1.52	1.35	1.00	0.92	0.70	0.52	0.33	0.25	0.21	0.12
CO	0.75	0.55	0.53	0.48	0.24	0.17	0.11	0.09	0.04	0.03	0.02
Underground water											
Raw sample											
TB	0.85	0.83	0.82	0.80	0.76	0.73	0.72	0.68	0.63	0.62	0.60
CO	0.35	0.33	0.24	0.20	0.17	0.16	0.15	0.14	0.13	0.12	0.09
Treated sample											
TB	0.85	0.62	0.60	0.51	0.42	0.38	0.30	0.22	0.14	0.12	0.09
CO	0.35	0.30	0.20	0.17	0.14	0.13	0.12	0.07	0.05	0.04	0.02

[1]20 μg mL^{-1} *M. oleifera* seed extract was used; * (10^{4} cfu mL^{-1}); Frh: Fresh (untreated) water sample; TB: Total Bacterial count; CO: Coliform count

Within 6 h of seed treatment, the inoculum sizes reduced by 98.6% in *E. coli*, 97.6% in *P. aeruginosa*, 97.2% in *S. faecalis*, 94% in *S. typhi* and 36.4% in *S. dysenteriae*. The bacterial population of *P. aeruginosa* and *S. faecalis* remained constant after 7 h storage (Table 3). However there was a tremendous increase in the cell population by 98.4% in *E. coli*, 98.5% in *S. typhi* and 99.4% in *S. dysenteriae* after 24 h. There was a significant difference in the increase of the bacterial population in the different microbes at $p<0.05$.

The zones of inhibition increased correspondingly with an increase in the concentration of the seed extract (Table 4). All the pathogens except *P. aeruginosa* were less affected by the seed extract at concentrations below 40 μg mL^{-1}. It required 50 μg mL^{-1} of the seed extract as the MIC against *S. typhi*.

Table 2: Effect of *Moringa oleifera* seed extract on microbial load in surface and underground water[1]

	Sampling period (h)				
Water samples	Initial	24	48	72	96
Fresh water					
Surface					
Total bacteria count[1]	9.80	9.00	8.40	7.10	6.80
Coliform count[1]	5.20	4.80	4.10	3.50	3.10
Underground					
Total bacteria count[1]	8.80	8.60	8.20	8.10	7.30
Coliform count[1]	3.60	3.40	3.30	3.30	3.00
Seed treated water					
Total bacterial count[1]					
Concentration (μg mL^{-1})					
Underground					
10	8.80	1.20	1.10	0.90	0.30
20	8.80	1.10	0.90	0.30	NG
30	8.80	0.80	0.40	0.10	NG
Surface					
10	9.80	2.70	1.30	0.90	0.30
20	9.80	1.50	0.40	0.20	NG
30	9.80	1.10	0.70	0.10	NG
Total Coliform Count[1]					
Concentration (μg mL^{-1})					
Underground					
10	3.60	0.30	NG	NG	NG
20	3.60	0.10	NG	NG	NG
30	3.60	NG	NG	NG	NG
Surface					
10	5.20	0.70	NG	NG	NG
20	5.20	0.20	NG	NG	NG
30	5.20	NG	NG	NG	NG

[1]Count: $\times 10^4$ cfu mL^{-1}; NG: No Growth

Table 3: Antibacterial activity of *Moringa oleifera*[1] seed extract*

	Microbial count ($\times 10^6$ cfu mL^{-1})										
	Sampling period (h)										
Organisms	Fresh[a]	1	2	3	4	5	6	7	8	9	24
E. coli	2.10	1.34	1.00	0.70	0.50	0.20	0.03	0.02	0.02	0.02	3.81
P. aeruginosa	2.50	2.00	1.50	1.70	0.25	0.08	0.06	0.03	0.03	0.03	0.03
S. faecalis	2.85	1.28	1.07	0.95	0.48	0.16	0.08	0.05	0.02	0.02	0.02
S. typhi	1.90	0.18	0.10	0.10	0.10	0.09	0.02	1.29	2.12	2.80	4.20
S. dysenteriae	1.92	0.22	0.20	0.20	0.12	0.05	0.05	0.33	2.33	3.00	4.38
S. aureus	2.63	1.06	1.00	0.50	0.36	0.24	0.13	0.01	0.01	0.01	0.01

*20 μg mL^{-1} seed extract used; [1]Organisms cultivated in minimum broth medium; [a]Inoculum size of bacteria used

Table 4: Inhibitory potential and Minimum Inhibitory Concentration (MIC) of *Moringa oleifera* seed extract*

	Zone of inhibition[b] (area in mm^2) at different seed extract concentration						
	10	20	30	40	50	100	MIC
Organisms[a]	----------(μg mL^{-1})----------						(μg mL^{-1})
E. coli	+++	++	0.13	0.79	1.13	2.01	30
P. aeruginosa	+++	++	0.79	1.54	2.01	3.14	30
S. typhi	++	++	+	+	0.94	1.76	50
S. dysenteriae	++	++	+	0.50	1.32	1.76	40
S. aureus	+++	0.010	0.01	0.13	1.54	2.55	20
S. fæcalis	+++	++	0.20	0.80	1.20	1.80	30

*Seed extract incorporated into agar media; +: Poor growth, ++: Slight growth, +++: Dense growth; [a]Inoculum size seeded as in Table 3; [b]Readings taken after 24th incubation at 30°C

DISCUSSION

In this study, *M. oleifera* seed extract efficiently precipitated about 90% of the total bacteria and 99% coliforms, an indication of the seed potential to coagulate and clarify the body of water. Madsen *et al.* (1987) also reported a 90% reduction in the bacterial load of water treated with *Moringa* seed paste. Meanwhile, Sattaur (1983) and Grabow *et al.* (1985) posited that a considerable hygiene improvement amounting to a primary bacterial reduction of 90-99% or more was achieved within 1 h of *Moringa* seed treatment.

The rate at which the total bacteria and coliforms were coagulated was faster in *Moringa* seed treated surface water than in underground water; the surface water being more turbid than the underground water. This might explain in part the reason for the high efficacy of the seed in highly turbid water than in less turbid water. The bacterial regrowth observed in *S. typhi* (98.4%), *S. dysenteriae* (99.4%) and *E. coli* (98.4%) is an indication of the bacteriostatic ability of *M. oleifera* seed on these organisms. However, the seed also possessed bactericidal activity especially in water samples inoculated with *S. faecalis* and *P. aeruginosa* where there was no regrowth. It is worth noting that treated water when left to stand may deteriorate in quality than even untreated water due to bacterial regrowth. Madsen and Schlundt (1989) reported secondary increases in total bacteria and faecal coliform counts after using *Moringa* seed and filtration through Sudanese bentomite clays.

The secondary bacterial growth might be due to the presence of some bacterial cells which are partially inactivated. Experimental parameters such as time, temperature and water constituent exert a profound influence on bacterial regrowth (Madsen *et al*., 1987). Coliform regrowth conditions can be expected to occur regularly in polluted tropical waters where temperature exceeds 20°C, a feature which is reflected in real life situation by multiplication of some species of faecal bacteria in the environment under favourable conditions (Madsen *et al*., 1987). The results obtained in this study corroborate the report of Aderiye *et al*. (2005) and Oluyege and Famurewa (2005) confirming the endemic presence of these human pathogens in and around Ado-Ekiti. This may be attributed to poor environmental sanitation where wastes are disposed off indiscrimately into drainage and environ especially during the rains.

Moringa oleifera seed elicited an appreciable antimicrobial activity *in vitro* against both Gram-positive and Gram-negative bacteria. The zones of inhibition increased correspondingly with an increase in the concentration of the seed extract. The chemical structure of the bioactive components of *M. oleifera* seed may explain the exact chemical reaction on the pathogens vis-à-vis the bacteriostatic effect and eventual regrowth of these organisms. Studies on the elucidation of the structure are ongoing in our Laboratory.

REFERENCES

Aderiye, B.I., S.O. Igbedioh and A.D. Adebobuyi, 1992. Incidence of coliform in well water and outbreak of water borne diseases: Environmental consideration and empirical evidence from Owo, Nigeria. Acta Mediterr. Patalog. Infect. Tropic., 11: 1-7.

Aderiye, B.I., S.A. Laleye and T. Oke Adeoye, 2005. Evaluation of sanitary standards of *Bukaterias* at the federal polytechnic, Ado Ekiti, Nigeria. Sci. Focus, 10: 110-115.

Anonymous, 1987. Seeds Can Help to Purify Water. African Farming, New York Jan/Feb. Edn., pp: 4.

Barrow, G.I. and R.K.A. Feltham, 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edn., Cambridge University.

Dahot, M.U., 1998. Antimicrobial activity of small protein of *Moringa oleifera* leaves. J. Islamic Aca. Sci., 11: 1.

Effler, P., M. Isaacson, L. Arntzen, R. Heenar, P. Canter, T. Barret, L. Lee, C. Mambo, W. Levine, A. Zaidi and P.M. Griffin, 2002. Factors contributing to the emergence of *Escherichia coli* 0157 in Africa. Emerg. Infect. Dis., 7: 812-818.

Eliert, U., B. Wolters and W. Nahrstedt, 1981. The antibiotic principle of *Moringa oleifera* and *Moringa stenopetalla*. Plant Medica, 42: 55-61.

Grabow, W.O.K., J.L. Slabbert, W.S.G. Morgan and S.A.A. Jahn, 1985. Toxicity and mutagenicity evaluation of water coagulated with *Moringa oleifera* seed preparation using fish, protozoa, bacteria, coliphage, enzyme and Ames *Salmonella* assays. Water SA, 11: 9-14.

Jahn, S.A.A., 1988. Using *Moringa* seeds as coagulants in developing countries. J. Am. Water Works Assoc., 80: 43-50.

Lewis, W., 1985. The significance of water management in relation to public and environmental health. Soc. Applied Bacteriol. Symp., 14: 133-135.

Madsen, M., J. Schlundt and E.F.E. Omer, 1987. Effect of water coagulation by seeds of *Moringa oleifera* on bacteria contaminations. J. Trop. Med. Hyg., 90: 101-109.

Madsen, M. and J. Schlundt, 1989. Low technology water purification by bentonite clay flocculation as performed in Sudanese villages. Bacteriological examination. Water Res., 23: 873-882.

Ndabigensere, A., K.S. Narasiah and B.E. Talbot, 1995. Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. Water Res., 29: 703-710.

Odeyemi, O., 1988. Prospects and limitations of using solar energy for water disinfection. In: Solar water disinfection. Proceedings of a Workshop. Brace Research Institute, Montreal, Quebec, Canada, pp: 34-43.

Olayemi, A.B. and R.O. Alabi, 1994. Studies on traditional water purification using *Moringa oleifera* seeds. Afr. Study Monog., 15: 135-142.

Oluyege, A.O. and O. Famurewa, 2005. Microbial quality of drinking water in storage tanks in Ado-Ekiti. J. Applied Environ. Sci., 1: 30-36.

Oslen, S.J., G. Miller, T. Breuer, M. Kennedy, C. Higgins, J. Walford, G. Mckee, K. Fox, W. Bibb and P. Mead, 2002. A waterborne outbreak of *Escherichia coli* 0157: H infectious and haemolitic uremic syndrome: Implications for rural water systems. Emerg. Infect. Dis., 8: 370-375.

Pollard, S.J.T., F.E. Thompson and G.L. McConnachie, 1995. Microporous carbon from *Moringa oleifera* husks for water purification in less developed countries. Water Res., 29: 337-347.

Sattaur, O., 1983. The light of the village. New Sci., 100: 830-831.