ISSN NO 2320-5407 International Journal of Advanced Research (2013), Volume 1, Issue 4, 124-130

ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Preliminary Study on Efficacy of Leaves, Seeds and Bark Extracts of *Moringa oleifera* in Reducing Bacterial load in Water

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Manuscript Info

Manuscript History:

Received: 10 May 2013
Final Accepted: 25 May 2013
Published Online: June 2013

Key words:
Moringa oleifera,
Water treatment,
Natural Coagulants,
Moringa Extracts

Abstract

Water quality and treatment are the most important issue in everywhere, especially in the developing countries, where safe and clean water is not continuously provided. *Moringa oleifera* is one of the best natural coagulants that has effectively used in water treatments. The aqueous extract of seeds, leaves and bark of *Moringa oleifera* was evaluated for their efficacy in reducing total bacterial load, coliform count and faecal coliform counts in the treatment of drinking water. The standard pour plate method and the most probable numbers were used in the determination of bacterial count. The seed extracts showed a great effective in the reduction of total coliform count (55.9%) and faecal coliform count (92.5 %) as compared to bark (45.0 and 90.7 %) and leaf (47.1 and 88.7 %) extracts at 3g/100ml, respectively. From the results from this study, it can be concluded that the *Moringa oleifera* extracts (seed extracts) can be used as safe as non-toxic natural coagulant materials in household water treatment, especially in rural areas and small communities where no adequate and safe water supply is provided.

Introduction

Water quality is the most important things in human's life. Without water access, both in quality and safety, the human health will expose to risk due to low quality of safe drinking water, and human life can be threatened with many diseases such as diarrhoea, cholera, typhoid fever, bilharzias and dysentery (Forsythe and Hayes, 1998). Unfortunately clean and treated water is unavailable to each house in most developing countries, and poor hygienic conditions; inadequate water supply and poverty are the main factors of increase waterborne diseases among these countries (Doughari *et al.* 2007). Most of the people in developing countries struggle to obtain access to safe water, especially in remote areas, where a treated drinking water supply is not available. They receive their drinking-water by some ways such as a small tanker driven by animal or carriage by themselves from public water sources (wells; lakes, rivers, streams and catchment areas) and most of this water is collected by women and children without hygienic practices and the sources itself may be poor in cleaning, unimproved, unprotected and normally located far away from their living area (Gundry *et al.,* 2006). To overcome water contamination, aluminum salts have been used in treating of drinking water, but the excessive use of chemical coagulants increases the treatment cost and can be causing health and environment problems (Joshua and Vasu, 2013). In recent years, natural alternative methods have been applied in water treatment, especially in the rural area (Folkard *et al.*, 1993; Doer, 2005; and Onwuliri and Dawang, 2006) by using natural compounds found in plants such as Okra (Agarwal *et al.* 2001), Rice (Yarahmadi *et al.* 2009), fenugreek (Rajani and Mishra, 2008) and psyllium (Rajani *et al.* 2002) as coagulant materials to remove the contaminants from drinking water.

Moringa oleifera is one of the most widely used (leaves and seeds) in water treatment as the natural coagulants because of low cost, highly biodegradable, short shelf life, safe to human health and the environment when compared to synthetic coagulants either organic or inorganic (Muyibi *et al.* 2002; Katayon *et al.* 2005; Katayon *et al.* 2006). It can be also used all Moringa parts, including, seeds, flowers, roots, leaves and bark in medicinal treatments or in foods as a therapeutic meal (Anwar *et al.*, 2007). *Moringa oleifera* is a tropical tree belongs to Moringaceae family, originated from India, Pakistan, Bangladesh and Afghanistan (Mepba *et al.*2007; Prabhu *et al.* 2011; Abalaka *et al.* 2012; Mutiara *et al.* 2012) and is spread in many countries of South- Saharan Africa, South East Asia, Middle East, South-America, Malaysia, Philippines and Sri Lanka (Jahn, 1988; Anwar *et al.* 2006; Rahman *et al.* 2009) and has many names, including Drumstick, Sajna or Ben oil tree , Horseradish tree , Miracle Tree, Benzolive tree, Kelor, Mlonge, Mulangay, Marango, Nebeday , Saijhan, and in Sudan is known as a clarifier tree or (El Rowag tree). It has been reported that, in many countries, the seeds and leaves of *Moringa oleifera* are used in the water purification by flocculation, sedimentation and removal of suspended materials (Ndabigengesere and Narasiah, 1998; Narasiah *et al.* 2002). Reports have also described the Moringa seeds powder is most effectively used to treat, purify and contaminant removal from drinking water (Olsen, 1987; Daniyan *et al.* 2011; Prabhu *et al.* 2011). In Sudan, dried seeds of Moringa oleifera have been used by women in rural areas as a natural alternative to replace the alum (aluminum sulphate) in the removal of turbidity from Nile water (Jahn and Dirar, 1979; Muyibi and Evison, 1994). The plant has also been reported to contain flavonoids (kaempferol , rhamnatin, isoquercitrin and kampferitrin) ,alkaloids compounds (pterygospermin, moringin and moringinine) and 4-(L-ramnosyloxy) benzyl isothiocyanate (Jahn *et al.*1986; Bhoomika *et al.* 2007; Singh and Sharma 2012), which may act as antibacterial agents. So, it is important to investigate the potential power from *Moringa oleifera* parts on removal of microorganisms in water treatment. Therefore, the objective of this study is to determine the effects of aqueous extracts of seeds, leaves and bark of *Moringa oleifera* as a natural antimicrobial agent in the reduction of bacterial load in water treatment.

Material and Methods:

Sample collection:

Moringa oleifera parts, including seeds, leaves and stem bark were collected from the field of Dr. Mohamed Ismail in the faculty of Agriculture, University of Bakht Alruda, ED Dueim, Sudan. The plant parts were shade dried under aseptic condition at room temperature (28-32^{o}C) for three days, and stored for future use.

Sample treatment:

Dried parts *Moringa oleifera* were grounds using mortar and pestle and then 3g of each powder sample were extracted with deionized distilled water (3g/100ml) for 24 hours in a conical flask with hand intermittent shaking (Schwarz, 2000). After 24 hours, each of the extracts was filtered through Whatman No 1 filter paper.

Water Treatment

The extract solution (seeds, leaves and bark) was poured into one litre of the raw water placed in a sterile beaker (2 litre capacity) separately and stirred for 5 minutes and then the treated water was allowed to precipitate the suspended materials for 4 hours.

Microbial Analysis of treated water

The pour plate method was used to determine the effects of aqueous extract on reduction of the total viable counts (TVC). One ml of treated water for each extracted was a pipette into sterile Petri-dishes and molten nutrient agar (40^{o}C) was added and through and through to allow the distribution. Then the plate allowed to solidify and incubated at 37^{o}C for 24 hours, and then colonies were counted (Harrigan, 1998). For Total coliform count (TCC) and faecal coliform count (FCC), the Most Probable Number (MPN) technique was used and performed with five tube procedures (APHA, 1995).

Result and Discussion

Untreated water samples were collected from the White Nile in Ed Dueim city, Sudan. The samples were treated with the aqueous extract of *Moringa oleifera* parts (seeds, leaves and bark) to study the efficacy of these extracts in the reduction of bacterial load in drinking water.

Effect of extracts parts of *Moringa oleifera* on bacterial load:

Table 1 and Fig. 1 showed the total viable bacteria count, coliform count and faecal coliform count in water after treating with different extracts parts (3g/100ml) of *Moringa oleifera* such as seeds, leaves and bark. Nile water (untreated water) was used as a control and treated water with alum from Ed Dueim treatment station (EDTS) was used for the comparison of treatment. The initial population of the total viable bacteria count, coliform and faecal coliform count of the Nile water were 8.6 x 10^6 CFU/ml, 460 and 80 MPN/100ml, respectively.

Slight reductions (0.31, 0.57, and 0.65 log CFU/ml) on the total viable bacteria count were achieved by treating water samples with aqueous extracts of leaves, bark and seeds, respectively (Fig. 1a). This lower level in the reduction in bacterial load could be due to the conducted of an experiment in the winter season, where is the White Nile water at low turbidity level (20-25) NTU (Homaida and Goja, 2013) where *Moringa oleifera* has not possessed high sensitive to coagulate the contaminant in low turbidity level. Another factor is that larger forming of sedimentary particle (flocs) resulting from high turbidity, lead to more microorganisms attached to the solid particles, and thus leading to a significant reduction in microbial load in treating water (Joshua and Vasu, 2013). This confirmed by many Moringa workers, and they reported that the seeds of *Moringa oleifera* are more effective in reducing suspended materials from water with medium to high levels of turbidity and less effective at low levels of turbid water (Muyibi and Okuofu,1995, Paterniani *et al.* 2010; Gambhir *et al.* 2012). However, among the treatment extracts, the seeds are better than the two other (Bark and leaves) when compared drinking water samples treated with alum (1.07 log CU/ml) obtained from Ed Dueim treatment station. The results were mainly due to the presence of an active antimicrobial agent in *Moringa oleifera* seeds such as 4-(α -L-rhamnosyloxy-benzyl isothiocyanate (Vijay et al. 2012), 4-(α-l-rhamnopyranosyloxy)-benzylglucosinolate (Bennett *et al.* 2003).

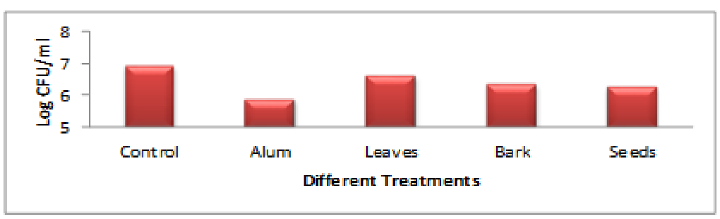
Table1. Effect of aqueous extracts of Moringa parts on bacterial loads (TVC, TCC and FCC).

Bacteria count	Before Treatment	After Treatment				Standard Guide for Drinking Water (WHO/SSMO)
	Nile water (Control)	EDTS (Alum)	*Moringa oleifera* extracts			
			Leaves	Bark	Seeds	
TVC (CFU/ml)	8.6 x 10^6	0.72 x 10^6	4.2 x 10^6	2.3 x 10^6	1.9 x 10^6	-/-
TCC (MPN/100ml)	460	5	243	253	203	(0/10)/100ml
FCC (MPN/100ml)	80	0	9	8	6	(0 /0)/100ml

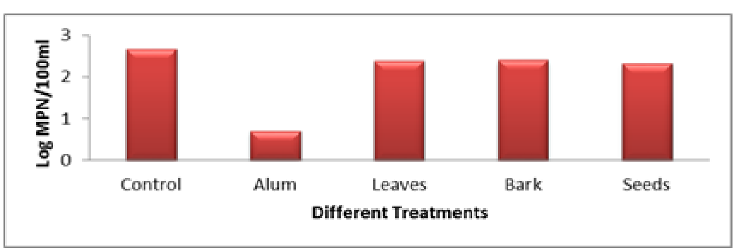
TCC= Total viable counts, TCC= Total coliform count, FCC= faecal coliform count, Alum= Alumium sulphate, EDTS= Ed Dueim Treatment Station, SSMO= Sudanese Standard Metrology Organization, (-) = Not Stablished

Fig.1. Reduced levels count of Total viable bacteria (a), Total coliform (b) and Faecal coliform (c) count in water treated with *Moringa oleifera* extracts parts and alum (aluminum sulphate).

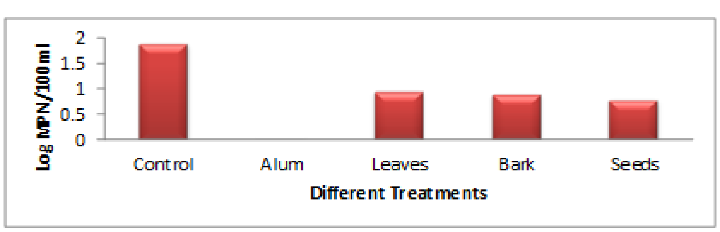
(a)



(b)



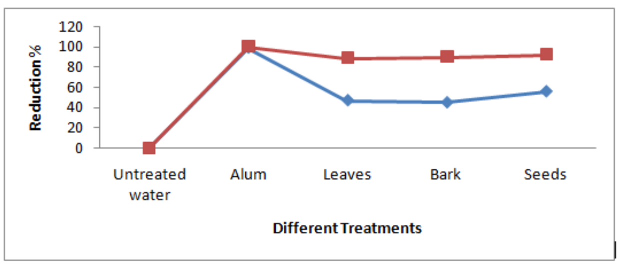
(c)



These agents may remove microorganisms by coagulation or act as inhibitors of the growth of organisms.

In the case of coliform, the water treated with alum (aluminium sulphate, a chemical synthetize coagulant) the coliform reduction was come within the acceptable level (< 10MPN/100ml or <log 1MPN/100ml) of WHO standard for drinking water. However, water treated with *Moringa oleifera* extracts (leaves, bark and seeds) do not achieve the minimum requirement of drinking water quality, and the coliform bacteria present in treated water (2.39, 2.40 and 2.31 log MPN/100ml) are higher than the WHO standard (log 1MPN/100ml) Fig.1b. The same finding was obtained in the case of faecal coliform in water treating with the same extracts, and faecal coliform bacteria were detected in all treatments (except alum treatment) and fall out of the limits given by WHO standards for drinking water (0 MPN/100ml) Fig.1c. However, on the other hand, the reduction of total coliform and faecal coliform were high when compared to the control sample (Nile water, untreated water). It was reduced to 47.1, 45.0 and 55.9 % for coliform and 88.7, 90.7 and 92.5 % for faecal coliform when treated with the extracts of leaves, bark and seeds, respectively Fig. 2.

Fig.2. The removal Percentage of coliform and faecal coliform count in water treated with *Moringa oleifera* extracts parts and alum (aluminum sulphate), ♦ = Total coliform, ■= Faecal coliform



The reduction percentages of coliform bacteria were nearly the same with the result obtained (65%) by Yahya *et al.* (2011) and differ from the results of Alo et al. (2012), who found that Moringa solution has removed about 70 - 93.3 % of the coliform bacteria. However, the reduction percentage of faecal coliform was in agreement with many workers (Vijay *et al.* 2012; Joshua and Vasu, 2013) they found that *Moringa oleifera* seed removed 90% to 99.99% of an indicator bacterial load. The reduction of coliform and faecal coliform could be attributed of possessing antimicrobial activity which is present in *Moringa oleifera* including Nitrile glycosides such as niazirin (Faizi *et al.*1995) and niazirinin (Faizi *et al.*1995 and Murakami *et al.*, 1998), pterygospermin (Anderson and Bell, 1986) and oil glycosides such as 4-[(4'-Oacetylalpha-L- rhamnosyloxy) benzyl]isothiocyanate (Abrams *et al.*, 1993), niaziminin A, and niaziminin (Faizi *et al.*1995). Generally, the study has shown the ability and effectiveness of *Moringa oleifera* extracts in reduction of the total viable count, total coliform and faecal coliform and its contribution to purify and improve water quality, although some bacteria were present in the water after treatment.

Conclusion

This study conducted as preliminary research to evaluate the efficacy of extract's parts of *Moringa oleifera* (seeds, leaves and bark) on the reduction of bacterial load in the water. The results revealed that seed extracts had more possessed antimicrobial activities against coliform and faecal coliform, and it can be used as natural coagulant agents in the reducing of suspended materials and microbial inhibition in water. This could be promising to apply these techniques as a household method, especially in rural areas to provide safe and potable drinking water.

Acknowledgement

The author would like to thank Dr. Mohamed Ismail, Department of Crop Sciences, Faculty of Agriculture and Natural Resources, University of Bakht Alruda for providing the materials for this experiment.

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