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COMPARATIVE TREATMENT OF WELL AND STREAM WAGTER FOR DRINKING USING MORINGA OLEIFERA SEEDS, ALUM, SAND FILTER BEDS AND SOLAR RADIATION

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Abstract

Objectives The objective of this research work was to compare the coagulating power of *Moringa oleifera* seeds with that of alum. Raw water samples were collected from various wells and streams in Modakeke area, Ile-Ife, Osun state, Nigeria. Results show that *Moringa oleifera* seed is a better coagulating agent than alum because it had an average coagulating power of 85.3% while that of alum was 78.3%. The filter bed had 85.7% reduction in microbial load for *Moringa oleifera* treated water while there was 79.1% reduction in microbial load for alum treated water. Also, the effectiveness of solar disinfection for the *Moringa oleifera* treated clear filtrate was 98.1% while that of alum treated water was 91.5%. Hence, *Moringa oleifera*, sand filtration and solar energy are good alternatives for water treatment especially in rural areas that can't afford sophisticated water treatment plant or chemicals.

Keywords: : Sand filter beds, *Moringa oleifera*, Solar disinfection, Alum

Introduction

The various serotype of *Eschericha coli* that causes diarrhea are classified according to their virulence determinants and these imbues the pathotypes with the capacity to cause clinical syndromes with distinctive symptoms [1]. For example enteropathogenic *E. coil (*EPEC) causes non-specific gastroenteritis especially in children in developing countries [1]. EPEC also differ from other pathotypes of *E.coli* in that it typically carries an EPEC adherence factor plasmid. These plasmids encodes bundle-forming pili (*Bfp*) which promotes bacterial adherence to epithelial cells and are an essential virulence determinant [2] and a transcriptional activator, *per* that up regulates genes within a chromosomal pathogenicity island, termed the locus of enterocyte effacement [1]. This pathogenicity island encodes a number of essential virulence proteins, including the surface protein intimin, which is required to produce the attaching-effacing lesions that are a key feature of EPEC- induced pathology. Pathogenic isolates of *E coli* have a relatively large potential for developing resistance [3] and report of multidrug resistance are not infrequent [4].

Before the advent of modern medicine of which many drugs were synthetically produced, extract of many
plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plants is *Phyllanthus niruri* L., (syn *P.*

fraternus Webster). It belongs to the Euphorbiaceae family and has been claimed to be an excellent remedy for jaundice and hepatitis [5]. Based on its long documented history of uses in the Amazonian region, the plant is considered analgesic and as aperitif, carminative, digestive, emmanagogue, laxative, stomachic tonic [6]. It is also believed to be helpful in treating edema, anorexia and diabetes [7].

Many of the active constituents found in the plant are biologically active lignands, glycosides, flavoniods, saponins, alkaloids, ellagitannins and phenylpropaniods [8], common lipid sterols and flavoniods also occur in the plant [9]. Alkaloids are organic nitrogen containing compound found in 20%-30% of vascular plants [10] and at lower doses are useful pharmacologically. Morphine, codeine, atropine and ephedrine are just a few of the plant alkaloids currently used in medicine [11]. Other alkaloids, including cocaine, nicotine and caffeine, enjoy a widespread non-medical use as stimulants or sedatives [10, 11]. Some alkaloids are medically useful for the cure of human diseases e.g. atrophies in treatment of bronchial asthma [10]; intestinal and biliary colic, and to dilate pupils of the eye [11]. The purpose of this work is to investigate the *in vivo* effectiveness of alkaloid extracted from *P.niruri;* previously discovered to be potent *in vitro* on EPEC and to study its tolerance and toxicity in rabbits infected with this bacterium.

Materials and Methods

Collection of Plant Materials

Phyllanthus niruri was collected from farmlands at Ado-Ekiti in Nigeria between the months of July and September, 2007 and identified in the herbarium of the Department of Plant Science University of Ado-Ekiti, where a voucher specimen No FPA 206 was deposited.

Group of rabbits

Thirty rabbits each divided into five groups of six were reared for five months for this purpose in the animal's house of the Science Technology Department Federal polytechnic, Ado Ekiti.

Water is very essential to life on earth and accounts for over 70% of the earth's surface. More than 80% of diseases reported to the World Health Organization are water-borne.

In developing countries, more than 25 million people; 60% of who are children die from biologically contaminated water each year (Odeyemi *et al*, 1988).Most rural villages in developing countries have poor access to a safe clean water supply and sanitation facilities (Lewis, 1985). About 75% of the people in rural areas who do not have access to treated water supplies depend mainly on untreated streams, ponds and well waters for drinking, food preparation and other domestic activities. These water sources are unfortunately the repositories of household wastes, animal manure, human faeces and community waste disposal (Odeyemi, 1987). Biological pollution of water through faecal contamination has been reported to be a basic cause of morbidity due to water-borne diseases which rank first among all other diseases in developing countries (Okoronkwo and Odeyemi, 1984). Water intended for human consumption must be free of microorganisms and concentration of chemical substances that may be hazardous to human health (WHO, 2004). Hence, this study also aimed at knowing the strength of a locally designed sand filter bed and solar

disinfection in water treatment process as an alternative to chlorination and ozonation.

Materials and methods

Sample collection and raw water coagulation:

Water samples were collected from various wells and streams with sterilized glass containers and transported immediately to the laboratory for microbial analysis and treatment. The collected water was then differently treated with granulated seeds of *Moringa oleifera* and alum. 1gram of alum and 1gram of granulated *Moringa oleifera* seeds were differently poured into 1 litre of the collected raw water and shaken properly for 10 seconds. Large flocs of particles formed in the containers once this was done. The flocculated water was then poured into the locally designed sand filter bed to get a clear filtrate that was later exposed to sunshine for some hours which ranged between 3-7 hours for disinfection.

Sand filter bed and filtration of coagulated water

The sand filter bed was constructed with a plastic 2-litre bottle whose bottom has been removed. Tiny holes were then made on the cork of the plastic bottle with a nail. A coffee filter was later secured to the neck of the bottle with a rubber band. After this, the bottle was turned upside down and secured in place using a holder.

400ml of gravel was then poured into the bottom followed by 800ml of coarse sand and later by 800ml of fine sand. A glass cup or conical flask was then placed beneath the neck of the inverted bottle to collect the water from the apparatus. The sand filter bed which now consists of an upper layer of fine sand; a middle layer of coarse sand and a bottom layer of gravels was then washed by slowly pouring 10 litres of distilled water through it. Once the cleaning of the sand filter bed was completed, the conical flask and glass cup used for collecting the filtered water was then washed; rinsed with distilled water and later returned to its place beneath the sand filter bed after sterilization in the autoclave at a temperature of 121^0C for 15 minutes. In this way, the apparatus is ready for use. The water samples coagulated differently with alum and granulated *Moringa oleifera* seeds were then introduced into the sand filter bed. The sand filter bed does a big work of trapping the flocs in the coagulated water as the sand filter bed accumulates a microbial layer when water moves down the filter by gravity.

The filter works best when larger gravels are at the bottom of the sand filter bed bottle because they plug the hole that is capped.

The sand filter bed has a height of 30cm, a width of 29.4cm, a flow rate of 0.2ml per second and a volume of 2 litres.

Solar disinfection

This is the last stage of the water treatment process in which the clear filtrate from the filter bed was poured into clean transparent bottles and exposed to sunshine for several hours to ensure complete destruction of biological contaminants like viruses which could probably pass through the sand filter bed.

Media and reagents

MacConkey broth, nutrient agar and Eosine Methylene Blue (EMB) agar were used in the course of this research work for the microbiological analysis of the raw water, filtered water and the solar disinfected water.

MacConkey broth

This medium was used for the presumptive coliform test by planting 3 portions in 3 dilutions. This presumptive test is also called Most Probable Number (MPN) technique or multiple tube test. It was used to ascertain the presence of coliform organisms per 100ml of water sample.

Nutrient agar

This was used to determine the Total Bacterial Count (TBC) in the various water samples by using the pour plate technique.

Eosine Methylene Blue (EMB) agar

This was used in the confirmed test to detect the presence of *Escherichia coli* and other faecal coliforms in the water samples by using both the pour plate and the spread plate technique.

Results and Discussion

The Total Bacterial Count (TBC) and the Most Probable Number (MPN) techniques were employed in estimating the number of coliform cells in the various water samples (untreated water from streams and wells, filtered water and solar disinfected water). The TBC technique gave the exact values of the number of coliform cells per ml in the different water samples unlike the MPN technique which gave approximate number of coliform cells per 100ml.

The results on Table1 showed that water samples from stream A and stream B had an MPN value of 1100 coliform cells per 100ml while their TBC were 280 and 295 coliform cells respectively. Table1 also showed that well A and well B had MPN values of 460 and 1100 coliform cells respectively while the values for their TBC results were 230 and 270 coliform cells respectively. The control sample was distilled water and it showed a value of zero for both the MPN and the TBC results because it was free of biotic contaminants.

Percentage microbial load reduction for Most Probable Number (MPN) technique

= **((**MPN of untreated water-MPN of treated water**)/**MPN of untreated water**)** x 100

Percentage microbial load reduction for Total Bacterial Count (TBC) technique

= **((**TBC of untreated water-TBC of treated water**)/**TBC of untreated water**)** x100

Table1. The Total Bacterial Count (TBC) and the Most Probable Number (MPN) of coliform cells in untreated water samples from streams and wells

Untreated water samples	MPN per 100ml	TBC ($1x10^2$ cfu per ml)
Stream A	1100	280
Stream B	1100	295
Well A	460	230
Well B	1100	276
Control(distilled water)	0	0

Table2. The Total Bacterial Count (TBC) and the Most Probable Number (MPN) of coliform cells in filtered *Moringa oleifera* treated water samples from streams and wells

Filtered water samples	*Moringa oleifera* treatment results			
	MPN per 100ml	Microbial load Reduction (%)	TBC ($1x10^2$ cfu/ ml)	Microbial load reduction (%)
Stream A	120	89%	40	85.7%
Stream B	150	86.4%	48	83.7%
Well A	75	83.6%	36	84.3%
Well B	160	85.6%	35	87.3%

Table3. The Total Bacterial Count (TBC) and the Most Probable Number (MPN) of coliform cells in filtered alum treated water samples from streams and wells

Filtered water samples	Alum treatment results			
	MPN per 100ml	Microbial load Reduction (%)	TBC ($1x10^2$ cfu/ ml)	Microbial load reduction (%)
Stream A	290	73.6%	75	71.7%
Stream B	75	83.7%	55	79.2%
Well A	240	78.2%	46	83.5%
Well B	210	80.9%	57	78.9%

Table4. The Total Bacterial Count (TBC) and the Most Probable Number (MPN) of coliform cells in solar treated filtrate from *Moringa oleifera* treated water.

Solar treated water samples	*Moringa oleifera* treatment results			
	MPN per 100ml	Microbial load Reduction (%)	TBC ($1x10^2$ cfu/ ml)	Microbial load reduction (%)
Stream A (3hrs at 31^0C)	3	97.5%	3	92.5%
Stream B (7hrs at 31^0C)	0	100%	0	100%
Well A (5hrs at 32^0C)	0	100%	0	100%
Well B (4hrs at 34^0C)	0	100%	0	100%

Table5. The Total Bacterial Count (TBC) and the Most Probable Number (MPN) of coliform cells in solar treated filtrate from alum treated water.

Solar treated water samples	Alum treatment results			
	MPN per 100ml	Microbial load Reduction (%)	TBC ($1x10^2$ cfu/ml)	Microbial load reduction (%)
Stream A (3hrs at 31^0C)	44	84.8%	8	89.3%
Stream B (7hrs at 31^0C)	15	80%	8	85.5%
Well A (5hrs at 32^0C)	29	87.9%	4	91.3%
Well B (4hrs at 34^0C)	0	100%	0	100%

Table2 shows the microbial load reduction in the filtered water samples when *Moringa oleifera* was used as the coagulating agent.

Table 6. Confirmation test results to ascertain the presence of *Escherichia coli* and other faecal coliforms in the water samples collected from streams and wells

Water samples	**Number of coliform cells/ml(10^2 cfu)**
Stream A	
Untreated water	34
Filtered water	0
Solar treated water	0
Control	0
Stream B	
Untreated water	28
Filtered water	0
Solar treated water	0
Control	0
Well A	
Untreated water	8
Filtered water	0
Solar treated water	0
Control	0
Well B	
Untreated water	17
Filtered water	0
Solar treated water	0
Control	0

The TBC results for the filtered water of stream A showed a coliform cell count of 40 and this is equivalent to 85.7% reduction when compared to its untreated sample which had 280 coliform cells while its MPN value had 89% reduction in microbial load. The TBC for the filtered water of stream B was 48 while that of the untreated water sample was 295 and this indicated 83.7% reduction in microbial load. Also, the MPN value for the filtered water of stream B indicated 86.4% reduction in microbial load. . The TBC results for the filtered water of well A showed a coliform cell count of 36 and this is equivalent to 84.3% reduction when compared to its untreated sample which had a value of 230 coliform cells while its MPN result indicated 83.6% reduction in microbial load.

The TBC results for the filtered water of well B showed a coliform cell count of 35 and this is equivalent to 87.3% reduction when compared with its untreated water sample which had a value of 276 coliform cells while its MPN value indicated a microbial load reduction of 85.6%. Table3 shows the microbial load reduction in the filtered water samples when alum was used as the coagulating agent. The TBC results for the filtered water of stream A showed a coliform cell count of 75 and this is equivalent to 71.7% reduction when compared to its untreated sample which had a value of 280 coliform cells while its MPN value showed 73.6% reduction in microbial load. The TBC for the filtered water of stream B was 55 while that of its untreated water sample was 295 and this indicated 79.2% reduction in microbial load while its MPN value indicated 83.7% reduction in microbial load.

The TBC results for the filtered water of well A showed a coliform cell count of 46 and this is equivalent to 83.5% reduction when compared to its untreated sample which had a value of 230 coliform cells while its MPN value showed 78.2% reduction in microbial load when compared with the value of its untreated water which has a value of 460 coliform cells per 100ml.

The TBC results for the filtered water of well B showed a coliform cell count of 57 and this is equivalent to 78.9% reduction when compared with its untreated water sample which had a value of 276 coliform cells while its MPN value indicated 80.9% reduction in microbial load.

Table 4 shows the microbial load reduction in the filtered solar treated water samples at various temperatures and hours when *Moringa oleifera* was used as the coagulating agent.

The TBC results for the filtered solar treated water from stream A showed a coliform cell count of 3 and this is equivalent to 92.5% reduction when compared to its untreated sample which had a value of 280 coliform cells while its MPN value indicated 97.5% reduction in microbial load. The filtered solar treated water of stream B had an MPN and TBC value of zero coliform cell when the filtered water was exposed to sunshine for 7 hours at a temperature of 31^0C. This result is equivalent to 100% reduction in microbial load when compared to its untreated sample which had an MPN and TBC values of 1100 and 295 coliform cells respectively.

The MPN and the TBC results on tables 2 to 6 showed that the seed of *Moringa oleifera* was in all ramification better than alum as a coagulating agent in the water treatment process. The TBC results also showed that the sand filter bed reduced the microbial load by about 83.7%-87.3% when the water was treated with *Moringa oleifera* while it was between 71.7%-83.5% when treated with Alum.

Nevertheless, viruses may possibly pass through the sand filter bed and the exposure of the filtered water to sunshine for long periods of about 5-7 hours ensures complete disinfection of the filtered water samples. In this way, the solar disinfected water is suitable and potable for drinking. This is explained by the filtered water of stream A, stream B, well A and well B. the filtered water of stream A was exposed to sunshine for 3 hours while stream B was exposed to sunshine for 7 hours (both at the same temperature of 31^0C). At the end of the exposure, stream A had 97.5% reduction in microbial load while stream B had 100% reduction in microbial load when *Moringa oleifera* was used for the MPN results.Thus, it is advisable to expose the filtered water to sunshine for about 6-7 hours to ensure the complete destruction of viruses.

The results of the effectiveness of *Moringa oleifera* seeds can be compared with the work of Madsen *et al* when they reported that the effects of water coagulation by seeds of *Moringa oleifera* was 80-99.5% reduction in microbial turbidity within one to two hours. They also reported that this reduction is proportional to 90-99.99% bacterial reduction in which the bacteria was concentrated in the coagulated sediment.

The confirmation test results in table 6 showed that all the untreated water samples collected from streams and wells contain coliform bacteria cells which ranged between 8 and 34 with characteristic pink, purple and green metallic sheen of *Escherichia coli* when plated on EMB agar. This result showed that some wells and streams in Modakeke area are polluted and unsafe to consume without any form of treatment. All the filtered water samples showed no growth of *Escherichia coli* when plated on the EMB agar and this shows that the sand filter bed trapped all the pathogenic bacterial coliforms and a larger percentage of the coagulated bacteria. Also, solar treated water does not contain any *Escherichia coli* cell because the sand filter bed did the excellent job of removing them.

Conclusion

Moringa oleifera seed is a better coagulating agent than alum and is more effective in water treatment processes because *Moringa oleifera* had an average coagulating power of 85.3% while that of alum was 78.3%.

Solar radiation targets and eliminates the biotic contaminants in water and the results of this research work showed that the solar disinfected water and the filtered water conforms with the standard recommended by the World Health Organization (WHO) that no *Escherichia coli* cell should be found in potable drinking water per 100ml.

The combined action of *Moringa oleifera* seeds, sand filter bed and the use of sunshine will make water potable for drinking especially for rural dwellers and people living in developing countries that can't afford sophisticated water treatment materials.

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