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WATER PURIFICATION AND ANTIMICROBIAL EFFICACY OF *Moringa Oleifera* DRIED AND MACERATED SEED

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ABSTRACT

Moringa oleifera is an economically important plant with multipurpose benefits. The seed of moringa plant has been known as a coagulant and an antimicrobial agent in water treatment. Availability of clean drinking water is still a major concern in most developing countries especially in rural areas due to high cost. A simple and cost-effective method for water purification is required for rural communities. Dried and macerated moringa seed were used to purify borehole and well water in Osogbo, Nigeria. Experiments in this study are conducted using jar test . The water samples were treated at varying duations and concentrations. Treated water samples were subjected to bacteriological analysis using most probable number technique. The total mesophilic bacteria, total mesophilic fungi, and total coliforms of borehole water enumerated after treatment with moringa seed were 190×10^1 cfu/ml, 6×10^1 cfu/ml, and 2 MPN/ml respectively, while the total mesophilic bacteria, total mesophilic fungi, and total coliforms of well water after treatment is 210×10^1 cfu/ml, 10×10^1 cfu/ml and 425 MPN/ml respectively. The pH and the temperature of the treated water fall between WHO standards. Therefore, this study underscores the efficacy of dried and macerated seeds of *Moringa oleifera* as antimicrobial agents in water treatment and purification.

Keywords: *Moringa*, Antimicrobial agent, Mesophilic bacteria, Water treatment, Water purificaion

INTRODUCTION

Water is one of the most essential necessities of human life. Unavailability of clean water contributes to several human diseases and morbidity (Rashid *et al*., 2018). Most rural communities especially in developing countries such as Nigeria lack access to safe drinking water, and globally, at least 2 billion people use contaminated drinking water sources (WHO, 2019). This situation has resulted in health hazards, especially the transmission of diseases such as diarrhea, cholera, dysentery, typhoid, and polio which cause considerable mortality (WHO, 2019). The United Nations (UN, 2018), reported that 2.1 billion people live today without access to safe drinking water at home. World Health Organization (WHO, 2017) reported that almost 159 million people depend on surface water, and universally, at least 2 billion people use drinking water contaminated with faeces. Researchers show that progress in the population's way of life in terms of production and consumption has resulted in an exponential increase in household waste (Chaouki *et al*., 2021).

Conventional drinking water treatment comprises coagulation, flocculation, sedimentation, filtration, and disinfection processes (Abiyu *et al*., 2018). Researchers have discovered that physicochemical processes such as coagulation and flocculation can minimize pollution and provide clean water (Manhokwe and Zvidzai, 2019). Coagulation-flocculation is commonly included either as a pre-treatment phase or as a post-treatment step depending on the types of water and the overall treatment plan used. Coagulation is the mechanism by which a given colloidal suspension or solution is destabilized while flocculation refers to the incorporation of destabilizing particles to come together and thus form large agglomerates, which can normally separate by settling gravity. Coagulation frequently takes 10 seconds but flocculation takes 20 – 45 min. The resulting destabilized particles are sufficiently stuck and settle in the following treatment unit (Narmatha *et al*., 2017).

Moringa oleifera seed, a natural coagulant is one of the most important ingredients in water purification. *Moringa oleifera* belongs to the Moringaceae family and is widely spread throughout tropical countries. It is a non-toxic and drought-tolerant tree that possesses medicinal and nutritional properties, including water-purification properties (Hellsing *et al*. 2014). From existing reports, there were allegations that the powder of *Moringa oleifera* seeds has antimicrobial properties and acts as an agent against microorganisms that are present in drinking water and decrease the number of bacteria (Mangale *et al*., 2012). The advantages of natural coagulants include being environmentally sustainable, environmentally friendly, inexpensive, and a simple process for developing countries. The performance is independent of raw water pH safe for human health, and antibiotic impact on various bacteria and fungi. The alkalinity of the water can be significantly reduced, compared to chemical coagulants. Natural coagulants are cost-effective. According to Vigneshwaran *et al*., (2020), the widespread use of aluminum-based chemical coagulants causes a variety of neurological problems, whereas bio-coagulants have natural properties that make them toxic to aquatic life. Different researchers have studied natural coagulants such as biowastes (Atchudan *et al*., 2020), *Moringa oleifera* (Rocha *et al*., 2020), *Moringa* oil (Chitra and Muruganandam, 2019), Microbial polysaccharides (Saleem and Bachmann, 2019). In less developed populations, natural coagulants are commonly used as a point-of-use product, as they are fairly cost-effective compared to chemical coagulants. It is not difficult to use the seeds as a natural coagulant because the use of natural coagulants in the water treatment process is expected to provide more advantages than the use of synthetic materials as they are natural and reported safe to be consumed (Hendrawati *et al.*, 2016). Therefore, the aim of this study is to determine the efficacy of Indigenous *Moringa* seed as an antimicrobial agent against some potable water around the Oke-Baale axis of Osogbo, Osun State, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted at Osun State University, Osogbo, Osun State, Nigeria. The site is located in Southwest Nigeria on latitude 7.5876°N and longitude 4.5624°E.

Sample collection

Raw water samples from wells and boreholes were collected from three different locations within the university campus. All samples were aseptically and transported to the Microbiology laboratory, Osun State University, Nigeria.

Collection and identification of *Moringa* seeds

Seeds of *Moringa oleifera* were collected from a *Moringa* tree at Modakeke town in Osun State, Nigeria. The seeds were identified and authenticated at the Department of Plant Biology, Osun State University, Osogbo, Nigeria.

Preparation of *M. oleifera* seeds for extraction

The seeds were air-dried and ground to powder form using a sterile laboratory mortar and pestle. The powder was stored in an air-tight container for further extraction and analysis.

Extraction of active compounds from *M. oleifera* seeds

Aqueous extraction

With a slight modification to the method of Lar *et al*. (2011), forty grams (40g) of the powdered seed was weighed and dissolved in 320 mL of distilled water. The mixture was heated at 60ºC in a water bath and filtered while hot. Hot water was continuously added to the residue and subsequently filtered. The procedure was repeated three times and the filtrate was evaporated to dryness at 60 ºC.

Ethanol extraction

According to the method of Lar *et al*., (2011), forty grams (40g) of the seed powder was dissolved in 320 mL of ethanol and allowed to stand for 48 hrs. The residue was then transferred into a soxhlet apparatus with ethanol for 48hrs and then evaporated to dryness at 60 ºC.

Water treatment with dried and macerated *Moringa* seed

Dried and macerated *Moringa* seed in concentration of 0.001, 0.002, 0.004, and 0.008 g/mL were transferred into the well water, borehole, and bottled water samples respectively. The mixture was allowed to stay at room temperature for 72 hours and the experiment was carried out in duplicate. Chlorine (5 mg/mL) was used as a positive control for the experiment and the raw water samples with no addition dried and macerated *Moringa* seed were used as a negative control (Amadi and Ayogu, 2005).

Water treatment with *Moringa* seed extracts:

Different concentrations of aqueous *Moringa* extracts and ethanol extract with the range 0.04 g/mL, 0.02 g/mL, 0.01 g/mL, and 0.005 g/mL were transferred into the well water, borehole, and bottled water samples respectively. The mixture was allowed to stay at room temperature for 72 hours and the experiment was carried out in duplicate. Chlorine (5 mg/mL) was used as a positive control for the experiment and the raw water samples with no addition of extract were used as a negative control. The treated water was filtered into a sterile flask with sterile Whatman filter No. 4.

Determination of microbiological quality of the water

The microbial analysis was performed to determine the total mesophilic bacteria count, total mesophilic fungal count, and estimation of the most probable number (MPN) of faecal coliform bacteria in before and after treatment. With modification to the method of Amadi and Ayogu, (2005), for the total mesophilic bacterial counts, 100 µL of the treated and untreated water sample was spread on nutrient agar plates and incubated at 37ºC for 24 hours. For total mesophilic fungal counts, 100 µL of the treated and untreated water sample was spread on potato dextrose agar plates and incubated at 25ºC for 72 hours. Colonies observed on each plates were counted and recorded.

Determination of most probable number (MPN)

The water sample was thoroughly mixed. Using a 10 ml sterile syringe, 10 ml of water was added to each of the five bottles containing 10 ml of lactose broth (double strength), 1 ml of the water sample was then added into each of the 5 bottles containing 10 ml of lactose broth (single strength) and 0.1ml of water sample then was added into each of the five bottles containing 10ml of lactose broth (single strength). Durham tube was added to each bottle. The inoculated broths were incubated at 37ºC for 24 hours with the bottles loosely capped. Following incubation, the positive tubes were streaked on already prepared Eosin methylene blue agar for a confirmatory test. The plates were checked for greenish metallic sheen; the results were observed and recorded using Cheesbrough (2000) standards.

Physicochemical analysis of the water sample

The physicochemical parameters of the water samples were determined before and after treatment with *M. oleifera*. The pH, temperature, total dissolved solids (TDS), and conductivity of the water were determined using a multimeter analyzer (HACH, Cloverland), and the values for each of the parameters were recorded (Alo *et al.*, 2012).

Phytochemical analysis of *Moringa* seed extracts

With slight modification to the method of Odeja *et al.*, (2015), the phytochemical contents of the extract were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, and phenol.

RESULTS

Water treatment with dried and macerated seed

The result of the total mesophilic bacteria, total mesophilic fungi, and total coliforms present in untreated borehole and well water is shown in Figures 1 and 2. It was observed that total mesophilic bacteria 190×10^1 cfu/mL, total mesophilic fungi 6×10^1 cfu/mL, and total coliforms 2 MPN/mL were recorded in the borehole water while the total mesophilic bacteria 210×10^1 cfu/mL, total mesophilic fungi 10×10^1 cfu/mL, and total coliforms 425 MPN/mL were present in the well water. However, no colony was seen in the untreated bottled water.

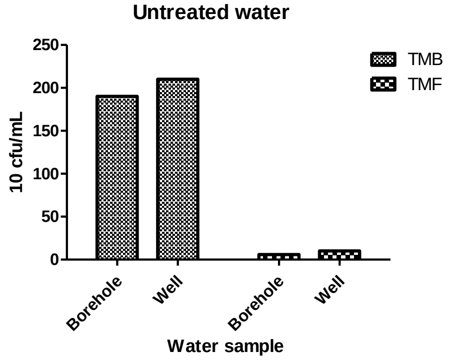

Figure 1: Total mesophilic bacteria and total mesophilic fungi present in untreated water samples

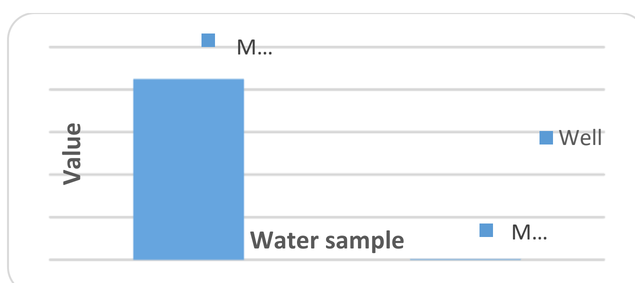

Figure 2: Total coliforms present in untreated water samples

The treatment with different concentrations of dried and macerated *Moringa* seed is shown in Table 1. It was observed that as the concentration of the seed increased in borehole water, there was an increase in the microbial load of the treated water with time. However, as the concentration of the seed increased in well water, there was variation in the microbial load of the treated well water with time Figure 3.

Table 1: Total mesophilic bacteria (TMB) for borehole water after treatment with *Moringa* dried and macerated seed

Concentration (g/ml)	Treatment					
	Dried seed (10cfu/mL)			Macerated seed (10cfu/mL)		
	Time					
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
0.001	200	NTC	NTC	120	NTC	NTC
0.002	240	NTC	NTC	200	NTC	NTC
0.004	NTC	NTC	NTC	NTC	NTC	NTC
0.008	NTC	NTC	NTC	NTC	NTC	NTC

Key: Numerous to count (NTC), colony forming unit (cfu)

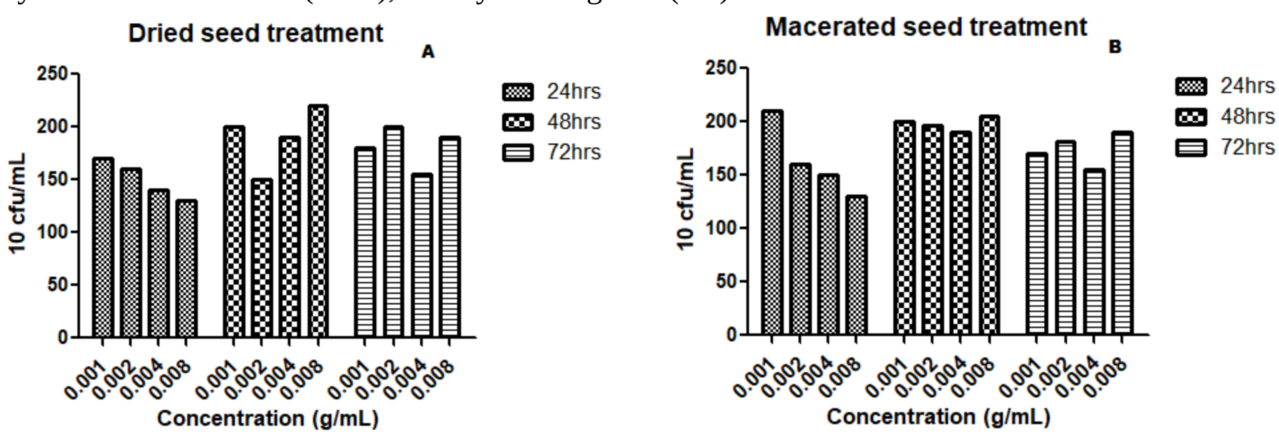

Figure 3: Total mesophilic bacteria (TMB) for well water after treatment with *Moringa* (A) dried seed (B) macerated seed.

The total mesophilic fungi present in the treated borehole water is shown in Table 2. It was observed that as the concentration of the *Moringa* dried and macerated seed increased there was an increase in the fungi load while an increase in the treatment time led to a decrease in the fungi load. In addition, for well water treatment, as the concentration of *Moringa* dried and macerated seed and treatment time increased, there was an increase in the fungi load Table 3. The total mesophilic bacteria for borehole and well water treated with aqueous and ethanol seed extracts is presented in Table 4 and 5. The table shows that as treatment concentration and time increase, there is an increase in the microbial load. Chlorine was used as a control and it reduced the microbial load to zero. The result of the total mesophilic fungi for water treated with aqueous and ethanol extract is shown in Table 6 and 7. It was observed that as the concentration and time of treatment increased, there was an increase in the microbial load in the treated borehole and well water. The result of the most probable number (MPN) of the treated water is shown in Table 8. No MPN value was recorded in borehole water with all the treatments and in well water with aqueous and ethanol extract of *Moringa* seed. A reduction in the MPN of dried seed treatment 4 MPN/mL and macerated seed treatment 2

MPN/mL was recorded in the well water. The physicochemical analysis of the untreated and treated water samples is presented in Table 9. The pH and temperature reduced after treatment while the conductivity and total dissolved solids (TDS) increased with dried and macerated treatment but reduced in the seed extracts treatment. The qualitative phytochemical assay of ethanol and aqueous seed extracts is shown in Table 10. The table shows that ethanol seed extract contains saponins and steroids while the aqueous seed extract contains steroids, flavonoids, alkaloids, and anthraquinones.

Table 2: Total mesophilic fungi (TMF) for borehole water after treatment with *Moringa* dried and macerated seed

Concentration (g/mL)	Treatment: Dried seed (10cfu/mL)			Macerated seed (10cfu/mL)		
	TIME					
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
0.001	8	0	0	46	0	0
0.002	1	1	0	77	0	1
0.004	0	1	0	27	0	0
0.008	42	1	2	38	0	0

Key: Colony forming unit (cfu)

Table 3: Total mesophilic fungi (TMF) for well water after treatment with *Moringa* dried and macerated seed

Concentration (g/mL)	Treatment: Dried seed (10cfu/mL)			Macerated seed (10cfu/mL)		
	TIME					
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
0.001	0	0	1	1	17	1
0.002	1	0	1	1	2	1
0.004	1	1	3	1	2	1
0.008	1	5	1	1	5	19

Key: Colony forming unit (cfu)

Table 4: Total mesophilic bacteria (TMB) after treatments of borehole water with aqueous and ethanol seed extracts.

Concentration (g/ml)	Treatment: Aqueous Extract (10cfu/mL)			Ethanol Extract (10cfu/mL)			Control (10cfu/mL)
	TIME						
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	
0.05	NTC	NTC	NTC	NTC	NTC	NTC	No growth
0.1	NTC	NTC	NTC	NTC	NTC	NTC	
0.2	NTC	NTC	NTC	NTC	NTC	NTC	
0.4	NTC	NTC	NTC	NTC	NTC	NTC	

Key: Numerous to count (NTC), colony forming unit (cfu)

Table 5: Total mesophilic bacteria (TMB) after treatments of well water with aqueous and ethanol seed extracts.

Concentration (g/ml)	Treatment: Aqueous Extract (10cfu/mL)			Ethanol Extract (10cfu/mL)			Control (10cfu/mL)
	TIME						
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	
0.05	NTC	NTC	NTC	NTC	NTC	NTC	No growth
0.1	NTC	NTC	NTC	NTC	NTC	NTC	
0.2	NTC	NTC	NTC	NTC	NTC	NTC	
0.4	NTC	NTC	NTC	NTC	NTC	NTC	

Key: Numerous to count (NTC), colony forming unit (cfu)

Table 6: Total mesophilic fungi (TMF) for borehole water after treatment with aqueous and ethanol seed extracts

Concentration (g/ml)	Treatment						Control
	Aqueous Extract (10cfu/ml)			Ethanol Extract (10cfu/ml)			
	TIME						
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	
0.05	2	0	0	0	3	0	No growth
0.1	2	1	0	1	2	3	
0.2	11	3	0	1	11	1	
0.4	30	60	10	14	NTC	0	

Table 7: Total mesophilic fungi (TMF) for well water after treatment with aqueous and ethanol seed extracts

Concentration (g/ml)	Treatment						Control
	Aqueous Extract (10cfu/ml)			Ethanol Extract (10cfu/ml)			
	TIME						
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	
0.05	0	4	10	1	3	1	No growth
0.1	7	5	11	1	16	6	
0.2	34	47	70	0	18	25	
0.4	52	61	61	36	100	51	

Table 8: Most probable number for borehole and well water after treatment with Moringa seed.

	Borehole		Well water	
	Treated	Control	Treated	Control
Moringa oleifera	**MPN**	**MPN**	**MPN**	**MPN**
Dried seed	0	0	4	0
Macerated seed	0	0	2	0
Aqueous extract	0	0	0	0
Ethanol extract	0	0	0	0

Key: Most probable number (MPN)

Table 9: Physicochemical analysis of water samples before and after treatment with *Moringa* seed and extracts

	Untreated			Treated									
				Dried seed		Macerated Seed		Aqueous Extract		Ethanol Extract		Chlorine (control)	
Parameters	Bottled water	Borehole	Well water	Borehole	Well water	Borehole	Well water	Borehole	Well water	Borehole	Well water	Borehole	Well water
pH	6.67	7.25	6.91	6.55	5.46	6.42	4.64	7.25	6.6	6.91	6.62	7.44	6.87
Temperature (°C)	26.7	26.9	25.9	25.9	25.2	25.7	25.4	25.1	25.6	25.4	25.7	25.4	25.3
Conductivity (µS)	0.08	0.3	0.11	0.33	0.12	0.38	0.47	0.26	0.08	0.26	0.08	0.33	0.1
Total dissolved solid (ppm)	0.04	0.15	0.05	0.17	0.06	0.19	0.24	0.13	0.04	0.13	0.04	0.17	0.05

Table 10: Qualitative phytochemical assay of ethanol and aqueous seed extracts of *Moringa oleifera*

S/N	Phytochemicals	Ethanol extract	Aqueous extract
1	Alkaloids	−	+
2	Steroid	+	+
3	Flavonoids	−	+
4	Saponins	+	−
5	Tannins	−	−
6	Anthraquinones	−	+
7	Phenols	−	−

DISCUSSION

The result of this study indicated that bottled water is free from microorganisms. Therefore, there is no need for further treatment. The borehole and well (groundwater) water contain microorganisms especially coliforms which makes it unsafe for human consumption and other domestic uses as it could cause gastrointestinal infections and diseases. This result is in agreement with the report of Lar *et al.*, 2011 who reported the presence of some gram-negative bacteria causing gastrointestinal infections.

However, treatment of water with *Moringa* dried, macerated, aqueous, and ethanol extracts (Tables 1, 2, 4, and 5) shows that the total mesophilic bacteria (Tables 1 and 4) of borehole water increased with an increase in the concentration of seed and treatment time while the total mesophilic bacteria for well water after treatment with *Moringa* dried and macerated seed varies with an increase in concentration and treatment time but without any significant changes. The total mesophilic fungi (Tables 2 and 5) of the borehole and well water were reduced but varied with increasing concentration and treatment time. This result is consistent with the report by Delelegn *et al.*, 2018 who reported that increase in the concentration of the seed leads to an increase in the turbidity of the water. The total fecal coliform (Table 3) after treatment was reduced with borehole water being reduced to zero while well water treated with dried and macerated seed was reduced to 4 MPN/ml and 2 MPN/ml respectively. Seed extracts reduced the total fecal coliform to zero. This result is in agreement with the study by Delelegn *et al.*, 2018 who reported the reduction in the total faecal coliforms using *Moringa* seed. The results presented in this study suggested that *Moringa* seed serves as nutrients for the growth of some bacteria species which increases the microbial load but inhibit the growth of fecal coliforms and fungi. A recent report by Chandrashekar *et al.* (2020) indicated that crude extract and coagulant protein from Moringa seeds exhibited cell aggregation and growth inhibition of six different pathogenic microorganisms including E. coli and Salmonella Paratyphi B and they attributed this to the presence of peptides with antibacterial property in the Moringa seeds. The antibacterial effects of the Moringa seed extract observed in the present study could be attributed to its possession of antibacterial proteins or the presence of various phytochemicals with antimicrobial properties in the seeds as reported by Begum et al. (2021). The results from this work indicated that *Moringa* seed solution showed antimicrobial efficacy against coliform bacteria, and fungi count but not for mesophilic bacteria which is in contrast with Alo *et al*., 2012 which reported that *Moringa* seed extracts reduced the total mesophilic bacteria present in the water sample. Also, the results for total coliforms were in agreement with the report of Babu and Chaudhuri (2015) and fall into the range given by Cheesbrough, 2000 for WHO standards for drinking water. Chlorine used as a control reduces the total mesophilic bacteria, total mesophilic fungi, and total fecal coliforms to zero.

The physicochemical analysis of the treated water (Table 6) shows that there is a reduction in the pH and temperature. Although there is an increase in the conductivity and total dissolved solids but on treatment with seed extracts, there was a reduction in the values. This observation is in agreement with the findings of Shan et al. (2017) who reported an increase in TDS content of water with increasing concentration of Moringa seed. Kitheka et al. (2022) reported a significant increase in TDS of water with increased concentration of Moringa seed powder and showed the existence of a strong positive relationship between Moringa seed coagulant concentration and TDS. The slight decrease in the pH following treatment with *Moringa* seed solution may be due to hydrogen ions of the weak acidity of the *M. oleifera* solution. These results are in agreement with the report of Alo *et al*., 2012 that the pH is in line with the standard pH for portable water (6- 8.5).

The phytochemical analysis (Table 7) of the extracts shows that ethanol extracts contain saponin and steroids while aqueous extract contains steroids, flavonoids, alkaloids, and anthraquinones which is in agreement with Fahey, 2005, which reported that alkaloids, flavonoids, saponins were present in the extracts of *M. oleifera* seed. The phytochemicals in the extracts are known to have some antimicrobial properties (Fahey, 2005).

CONCLUSION

This study has successfully revealed that dried, macerated and seed extracts of *M. oleifera* seed possess antimicrobial properties against fecal coliforms and mesophilic fungi though may serve as nutrients for mesophilic bacteria. These extracts could serve as promising natural antimicrobial agents with potential applications in controlling coliform bacteria which are indicators of pathogenic organisms in water samples. The technique is simple and can be used anywhere without high technical assistance. While water purification using chlorine can be very dangerous for human consumption because of its corrosiveness, *M. oleifera* can be cultivated very cheaply at the household level or in small communal nurseries which is to be encouraged in the rural population.

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