

Evaluation of surface water by seed extract *M. Stenopetala* plant: water efficacy for drinking water purposes

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Tareegn MM, Salilih FZ, Kidus B. Evaluation of surface water by seed extract *M. Stenopetala* plant: water efficacy for drinking water purposes. *AGBIR*.2022; 38(6): 386-391.

Herbs are enriched in bioactive compounds and utilized to treat a variety of ailments in the indigenous medicinal system. This research looks at the water-cleaning potential of *Moringa stenopetal* seed extract. Water samples were mainly collected in the Wuseta and Teklehaimanot rivers of Debre Markos, Ethiopia. Both samples of water have been purified and then treated using dried seed extract. The treated samples collected were analyzed using bacterial study via utmost of the possible techniques. The introduction of aluminium sulfate as a flocculant reduced the pH of the water from 7.32 to 3.95, but the pH of the seed extract water remained constant. Usage of 0.036 g/L of *M. stenopetal* reduced water turbidity from 131.11 NTU to

16.82 NTU (87.30%) and even from 210 NTU to 33.68 NTU (84.25%), respectively, in the Weseta and Tekelehymanot samples from the river. The acetone extraction had the highest antibacterial activity against *Salmonella enterica* with 19.20 mm, whereas *E. coli* (clinical isolate) had the lowest value, at 8.75 mm on the methanol source. Acetone extract's most common MIC value was 7.50 mg/mL. At extremely low concentrations, the acetone extract is the most efficient in inhibiting and killing *Salmonella typhi* (clinical isolate). The seed acetone extracts showed significant antibacterial action. It determines that the seed extracts can manage and diminish waterborne bacterial infections. The research assists people in Ethiopia and throughout the world who can still not acquire or do not have clean drinking water available to them.

Key Words: *M. stenopetal seed*; Water; Microbes; MIC; MBC

INTRODUCTION

One of the most fundamental human rights is the right to clean drinking water and water supplies that are adequate, harmless, actually reachable, and private for everybody. However, unclean water supply seems to be a serious problem in third-world countries, where polluted water causes 75% of all infections. One-third of Africa's population lacks reliable access to clean water, besides nearly all suffer from a waterborne disease that leads to productive water shortages [1,2].

Frequent water-borne diseases in the countryside and municipal zones are a main problem in water quality. It is also stated that many people in Ethiopia were at risk of cholera in 2009 due to overcrowding and unsanitary conditions in urban and rural areas [3]. Even though tap water is a long-term solution for the supply of clean water, it is costly and difficult to introduce in rural regions of developing countries. Providing safe water is very expensive and difficult to implement in developing regions of the world. As a result, improving the level of water quality in the home is thought to be effective in combating contagious diarrhea [4-6]. The residential water care is also said to decrease diarrhoea by 71% [7].

In underdeveloped nations such as Ethiopia, the much more popular home treatment methods are filtration (ceramic and biosand), chlorination, and Solar water Disinfection (SODIS) [8]. In regards to effectiveness, accessibility, and opportunities for plant water treatment, chlorine is one of the most promising [9]. There is currently no standard measurement for chlorine dosage at home treatment for water, though 1.875-3.75 mg/L is recommended for low-and high-pollution water [10].

Until recently, chlorine use was thought to be safe and effective in drinking water only for the removal of pathogens. But chlorine was used to reduce exposure to drinking water and, in combination with drinking water, plays a major role in the risk of cancer by-products. Sadiq and Rodriguez said that over 250 diverse kinds of DBPs have been known. Aluminium sulfate (Alum), an Alzheimer's chemical commonly used for coagulation and inflammation, is said to have nervous system effects. Many diseases,

including Parkinson's and Alzheimer's, cause bone problems [11,12]. Alum changes the natural alkalinity of water [13]. Consequently, because of the above-mentioned side effects, it is important to explore other options such as chemical coagulants, such as cost-effective, secure, reliable, durable, and environmentally sustainable coagulants to counterbalance the aforesaid complications.

The seeds of *M. oeilfera* Lam., *M. subcordata*, and as well as *M. stenopetala* are intensively researched natural coagulants and are mentioned as the most efficient water purification entity in trying to treat low to high-turbidity levels of surface and ground water [14-16]. Numerous natural compounds have inherent antibacterial activity that should be used for clinical applications [17].

Aside from the well-studied *M. stenopetala*, numerous other plant materials have been discovered to be capable of coagulation and disinfection [15,16]. For example, Pritchard et al. investigated the ability of *Jatropha curcas* and Guar gum to coagulate and thoroughly clean well water [18]. The plants have shown a problem-solving efficiency of up to 71% at different well water levels found in Malawi. The number of faecal coliforms treated in both plants also decreased dramatically [19]. *Jatropha curcas*, however, conducted a similar study on the surface of the water, with the plant growing to about 60 to 90% of the cases blocked in the samples. On the other hand, of the above-mentioned organic coagulants, current research has shown the great promise of *Brassica napus*, *Cocosnucifera*, *Oryza sativa*, *Plantagoovata* and *Viciafaba* [20-24].

The accessibility among those plant species varies in parts of the world, so it is beneficial to investigate new biocoagulant nominees that are plentiful in a specific nation [25]. It has a lot of benefits and reduces the cost of transporting plant-based coagulants that originate in a specific district. Indigenous peoples all over the world utilize herbal-based coagulants to cure turbid water in their homes. Tanzanian farmers, for example, consume the seeds of *Vignaunguiculata* and *Parkinsonia aculeata* [26]. *M. stenopetala* is used as a source of nutrients and has water clarifying properties. It is also used in moringa-based agroforestry systems to improve soil quality [27,28]. Local

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Received: 02-Nov-2022, Manuscript No. AGBIR-22-78889; **Editor assigned:** 07-Nov-2022, Pre QC No. AGBIR-22-78889 (PQ); **Reviewed:** 21-Nov-2022, QC No. AGBIR-22-78889; **Revised:** 25-Nov-2022, Manuscript No. AGBIR-22-78889 (R); **Published:** 02-Dec-2022, DOI:10.35248/0970.1907.22.38.386-391



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Ethiopian societies, like those in other countries, use natural coagulants to handle turbid water on a household scale for drinking and domestic uses. In Ethiopia, plant species such as *M. oleifera*, *Maerua subcordata*, *Sansevieria ehrenbergii*, and *Sansevieria forskaoliana* were used for water purification. Among these are *M. subcordata* (Gilg.) DeWolf tubers and *M. stenopetala* seeds (Baker f.), which appear to be effective in contaminating low to high turbidity surfaces and groundwater [6]. Even though researchers have described the application of leaf and/or bark extracts all over the world, the scholarly and efficient need for indigenous plants (*M. stenopetala*) dried seed powder for purifying water, coagulation, and disinfection potential in the Debre Markos, Ethiopia, agro-climatic zone has yet to be reported. The current study was then planned. The potential of *M. stenopetala* seed extract was investigated in this study for the Wuseta and Teklehaimanot rivers.

MATERIALS AND METHODS

Coagulant preparation and study area

The research was carried out at Debre Markos University's Biotechnology and Microbiology Laboratory, Department of Biotechnology and Biology. In this work, two forms of water purification were used: a naturally occurring coagulant, *M. stenopetala*, and a synthetic coagulant, aluminium sulphate. Pods of *M. stenopetala* were originally gathered in Arbaminch in southern Ethiopia. Dried fruits were used to select mature *M. stenopetal* seeds. After soaking and having to wash in deionized water and drying in an oven at 105°C for 1 hour, the seeds were manually removed from the plant. The oven-dried seeds were ground into a fine powder with a laboratory mortar and pestle and mixed thoroughly with a plant mixer with a pore size of 2.5 mm².

After that, the solution was then created by diluting 5 g of seed products in distilled water containing 100 ml. Aluminum sulfate, which acts as a synthetic coagulant, was obtained from the Debre Markos town water treatment facility and was utilized to compare treatment efficiency with *M. stenopetal*. For water filtration, several amounts of seed products made from *M. stenopetal* seeds were used, as well as a positive and negative control (aluminium sulfate and no seed extracts, respectively). Subsequently, various amounts of extracts from *M. stenopetal* seeds were carried out using different solvents, and the bactericidal test was done by using a positive control (ciprofloxacin). The turbidity removal test employed a solution made up of 5 g of seed products and 100 ml of distilled water.

Extraction of *M. stenopetala* seed products and antibacterial test organisms

The mature seeds of *M. stenopetal* were selected from dry ruptured fruits. The fruits were broken down to extract the seeds, which were then air-dried for two days. The parts encompassing the seed kernels were excluded using a laboratory mortar and pestle and a sieve with a pore size of 2.5 mm², and the kernels were crushed and sieved to obtain a powder form. The powder was stored at room temperature in a sterile container in the dark. The powdered sample was extracted with methanol and acetone in increasing polarity. In this procedure, 50 g of pulverized *M. stenopetal* seeds were soaked in 250 mL each of the two solvents, acetone and methanol, in equal amounts.

They have been shaken for three days on a horizontal shaker. After that, the samples were separated and filtered using Whatman No. 1 filter paper. The filtrates were then centrifuged at 5000 rpm for about fifteen minutes. The supernatant of each extract was evaporated using rota vapour (Laboratory 4000-efficient, Germany). The crude extracts were kept at 4 degrees Celsius. The yields of acetone and methanol extracts were 16 and 15%, respectively. Each test was repeated three times [29]. Crude extracts were tested for antibacterial activity against various bacteria. Isolated colonies of *E. coli* (ATCC 2592) were obtained from the Department of Biology's microbiology laboratory, whereas clinical isolates of *E. coli*, *Salmonella typhii*, and *Salmonella enterica* have been collected from the Debre Markos referral hospital in Ethiopia.

Preparation of samples and laboratory processing

Raw water samples of 30 liters were collected from the Wuseta and Teklehaimanot rivers. Weighing 2.0, 4.0, 6.0, 8.0, and 10.0 g of *M. stenopetala* seed extract and aluminum sulfate independently into a beaker containing 500 ml of distilled water yielded 5 distinct levels of the loading dose stock solution. To create a clear solution, the liquids in the beakers were agitated with a glass rod. As the negative control, 500 ml of distilled water with no

M. stenopetala seed extract product was used [30]. Two milliliters of each concentration, with the controller of all loading doses created, were evaluated in a beaker holding 500 milliliters of tested river water. The treatments were quickly combined for 2 minutes, and then gently mixed for 10 minutes using a sterile glass rod to help in coagulant production. Because there is no benchmark method for performing the jar test [6], the suspensions were left alone. The generated supernatants were collected and tested for the total coliform count, pH, and turbidity. The total was coliform utilizing the most likely number of tests. The multiple tube fermentation technique was used to determine the MPN of coliforms present in each of the treated water samples.

The samples were incubated in LTB tubes (Lauryl Tryptose Broth). Two types of LTB tubes were made. A Single-Strength Lauryl Tryptose Broth (SSLTB) and a Double-Strength Lauryl Tryptose Broth (DSLTB) were used (DSLTB). 6.5 g of LTB powder was weighed and dissolved in 500 ml of distilled water for the single strength. As with the single-strength LTB preparation, the double-strength was trained using accurately twice the weights of the reagents that were used, gently stirred for 10 minutes [31]. The test tubes were then incubated at 37°C for 24 to 48 hours. For the positive test, three loopfuls of samples were relocated from each positive presumptive test. The tube was transferred to test tubes containing Brilliant Green Lactose Bile Broth (BGLB), incubated at 37°C for 24 hours, and the presence of gas was checked. The number of tubes that produced gas was counted and compared to a table developed by the American Public Health Association (Lea) [32]. The figure represented the coliform MPN per 100 ml of sample [33].

pH and turbidity measurements

The turbidity of samples collected from the jar tests was tested using a turbid meter and the nephelometric protocol. The turbidity of the water samples was evaluated with a turbidity meter prior to and after treatment with various doses of *M. stenopetala* seed extract and aluminum sulfate. With the sample, an assessment was added to the 10 ml mark, and a turbidity meter evaluation was obtained with an empty tube. The turbidity meter readout was directly measured, and the outcomes were published as Nephelometric Turbidity Units (NTU) [34].

Anti-microbial tests using various methods

According to the assembly specifications, the isolates were retained on a nutrient agar plate and incubated for 24 hours at 37°C. The isolates have been kept at 4°C on an agar medium until next use. The bacterial isolates were plated into sterile normal saline in a loop. The bacterial suspension was especially in comparison to the McFarland standard of 0.5 [35]. The antimicrobial properties assay was performed using the diffusion on agar wells procedure, and the MIC and MBC were calculated.

Diffusion of agar wells

Bacterial broth culture was planned at a density of 108 cells per milliliter (mL) of 1 of 0.5 McFarland standards. This used a sterilized cotton swab; the aliquot was uniformly distributed onto Mueller-Hinton agar. The plated medium was also set aside to dry for 30 minutes at room temperature [36]. A uniformly spaced well was planned on each plate using a 6 mm diameter disinfected cork borer 2 mm from the plate's edge. Each extract (50 mg/mL) was presented aseptically in a separate agar well in 50 microliters. Ciprofloxacin (25 g/mL) served as the standard (positive) control, while sample-free solutions served as the blank control. The nutrient agar was again left on the bench for 40 minutes to pre-diffusion before being incubated at 37°C for 24–48 hours. The formation of a clear inhibition zone of 7 mm in diameter around the wells was regarded as evidence of the organisms' sensitivity to the extract [37]. The test was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was calculated for extracts with a 7 mm diameter growth inhibition zone. The agar dilution procedure was used for the test. A series of twofold dilutions of the extract were prepared in Mueller-Hinton agar using this method. The standardized bacterial isolated colony according to the McFarland standard was plated on the agar surface. The crude extract (50 mg/mL) was serially diluted as 1:2, 1:4, and 1:8 to achieve concentrations of 25, 12.5, and 6.25 mg/mL, respectively. The samples were once again initiated under aseptic conditions, as previously explained. After 24 hours of incubation at 37°C, the extract's MIC value was determined by calculating the minimum concentration that inhibited growth.

Minimum Bactericidal Concentration determination (MBC)

The MBC of the plant extracts was ascertained using an altered Spencer and Spencer method [38]. Plates that displayed no growth after 24 hours of incubation on MIC were subcultured on Mueller-Hinton agar plates and incubated at 37°C. The MBC was determined to be the highest dilution (least concentration) that produced no single bacterial colony and had shown no growth of bacteria after incubation.

Statistical analysis

SPSS (version 16) statistical software was used to analyze the data. The average coliform and turbidity reductions were computed. One-way ANOVA has been used to determine whether there were statistically significant differences between mean zones of inhibition. A p value of less than 0.05 was chosen as the level of significance.

RESULTS

Purification of water

The two water source samples investigated in this study are commonly used by the inhabitants in and around Debre Markos Town. The turbidity of the Wuseta and Teklehaimanot samples of water found in the river was 131.10 NTU and 210 NTU, respectively. The findings demonstrate that the lowest

value of aluminum sulfate reduction turbidity is the most important one. After that hour, constant aluminum sulfate at 0.036 g/L decreased turbidity from 131.10 NTU to 2.30 NTU (99.2%) in the Wuseta sample of water, whereas the similar concentration was determined to be 210 NTU to 1.88 NTU (99.56%) in the Teklehaimanot sample of water. Furthermore, at 0.036 g/L, *M. stenopetala* seed product reduced turbidity from 131.10 NTU to 16.82 NTU (87.30%) in a Wuseta sample of river water and from 210 NTU to 33.68 NTU (84.25%) in a Teklehaimanot sample of water.

Excessive amounts of *M. stenopetala* seed products, on the other hand, increased water turbidity. Before treatment, the pH of a sample of river water was found to be 6.65 for Wuseta and 7.32 for the Teklehaimanot River. At 0.084 g/L of aluminium sulfate, the pH was reduced to 3.81 (44%) and 3.95 (45.45%) in the Wuseta and Teklehaimanot samples of river water, respectively.

In contrast to aluminium sulfate, it had no measurable influence on the pH of both water samples noted after treatment with various concentrations of *M. stenopetala*, except for a corresponding decline from 6.65 to 6.45 (4.05%) in Wuseta at a concentration of 0.036 g/L and a decrease from 7.32 to 6.92 (2.4%) in the Teklehaimanot river detected at a concentration of 0.068 g/L (Table 1).

TABLE 1

Shows the turbidity and pH of two water samples from Weseta and Teklehaimanot treated with *Moringa oleifera* and aluminum sulfate after 1 hour of settling time.

Treatments	Water source	Treatment concentration (g/L)	pH		Turbidity (NTU)	
			Mean Reduction ± SD	% reduction	Mean reduction (NTU) ± SD	% reduction
<i>M. stenopetala</i> seed powder	Wuseta	Control	6.66 ± 0.00	0	131.11	0
		0.036	6.45 ± 0.47	4.05	16.82 ± 0.12	87.3
		0.052	6.62 ± 0.45	1.51	33.58 ± 1.02	77
		0.068	6.55 ± 0.59	2.56	31.45 ± 0.49	75.74
		0.084	6.73 ± 0.02	0	29.08 ± 0.58	77.45
	Teklehaymanot	Control	7.32 ± 0.00	0	210 ± 0.00	0
		0.036	7.33 ± 0.12	5.2/4	33.68 ± 0.82	84.25
		0.052	6.88 ± 0.59	4.74	43.22 ± 1.26	80.1
		0.068	6.92 ± 0.27	2.49	44.80 ± 0.46	77.05
		0.084	7.02 ± 0.10	4.41	49.80 ± 0.89	79.2
Aluminum sulfate	Wuseta	Control	6.65 ± 0.00	0	210	0
		0.036	4.50 ± 0.25	34.83	2.30 ± 0.30	99.2
		0.052	4.47 ± 0.20	39.45	2.55 ± 0.29	98.05
		0.068	4.53 ± 0.25	39.25	2.73 ± 0.17	97.99
		0.084	3.81 ± 0.10	44	3.61 ± 0.00	97.51
	Teklehaymanot	Control	7.32 ± 0.00	0	210	0
		0.036	4.68 ± 0.07	38.08	1.88 ± 0.059	99.56
		0.052	4.85 ± 0.07	32.94	2.2 ± 0.22	99.25
		0.068	4.25 ± 0.27	41.27	2.39 ± 0.07	98.25
		0.084	3.95 ± 0.17	45.45	2.89 ± 0.59	98.69

Table 2 shows the coliform count results represented as the most probable number (MPN) for both raw and treated water samples. The coliform levels for the Wuseta and Teklehaimanot differ significantly between treated and untreated river water. Coliform levels were significantly reduced in the treated water samples. *M. stenopetala* seed products were used in the MPN assessment and uncontrolled water samples were supposed to contain more than 2600 coliforms/100 mL. The MPN for the Wuseta and Teklehaimanot river waters was 600 coliforms/100 mL and 850 coliforms/100 mL, respectively, at a minimum concentration of 0.036 mg/L. Meanwhile, at the maximum concentration (0.08 mg/L), the MPN for the Wuseta and Teklehaimanot river waters was 90 coliforms/100 mL and 100 coliforms/100 mL, respectively. In addition to this, Table 2 shows the percentage difference for both sources of water (Table 2).

TABLE 2

The most likely number (MPN) test was performed with different concentrations of *M. stenopetala* seed extract

Treatments	Source of water	Concentration g/l	Coliform counts/100 mL
Seed powder	Wuseta	0	>2600
		0.036	600
		0.052	250
		0.068	180
		0.084	90
Seed powder	Teklehaymanot	0	>2600
		0.036	850
		0.052	400
		0.068	150
		0.084	100

Antibacterial activity

According to the agar well diffusion results, *E. coli* (ATCC 2592) was considerably sensitive to among all concentrates ($p=0.028$). The acetone products showed the highest antibacterial activity (14.48) against *E. coli* (ATCC 2592), while the sensitivity of *E. coli* (ATCC 2592) to the methanol products (8.75) differed considerably from the aqueous products. The reaction of *E. coli* (clinical isolate) differed substantially across the extracts examined ($p=0.028$). Compared to the other extracts, the acetone extract demonstrated the highest antibacterial activity (19.20) against *Salmonella enterica* (clinical isolate). *Salmonella typhi* (clinical isolate) was somewhat sensitive to the methanol extract (14.00). The clinical isolate of *Staphylococcus aureus* was likewise considerably sensitive to all products ($p=0.002$). Amid the *M. stenopetala* seed products tested, acetone had the highest antibacterial activity (19.20) against *Salmonella enterica* (clinical isolate); second was methanol products (17.00). Furthermore, for *Salmonella enterica* (clinical isolate), the acetone products showed the most significant change when compared to the other products. As a result, acetone had the highest antibacterial activity (19.20) against *Salmonella enterica* (clinical isolate), followed by methanol (17.00). As a result, when compared to the *M. stenopetala* seed products used, ciprofloxacin was regarded as a standard medicine and demonstrated full support against all tests performed for the growth of bacteria (Table 3).

TABLE 3

Bacterial growth inhibition zones were created using the agar well diffusion method and *M. stenopetala* seed products containing 50 mg/mL methanol and acetone.

Organisms	Zone of inhibition (mm) \pm standard deviation		Positive control (ciprofloxacin)	p-value using LSD
	Seeds products			
	Methanol	Acetone		
<i>E. coli</i> (ATCC 2592)	8.75 \pm 0.59 ^b	14.48 \pm 0.58 ^a	29.59 \pm 0.62	0.028
<i>E. coli</i> (clinical isolate)	16.16 \pm 0.61 ^b	17.50 \pm 0.02 ^a	27.50 \pm 1.50	0.035
<i>Salmonella typhi</i> (clinical isolate)	14.00 \pm 0.40 ^b	18.25 \pm 0.00 ^a	25.02 \pm 0.35	0.024
<i>Salmonella enterica</i> (clinical isolate)	17.00 \pm 0.00 ^b	19.20 \pm 0.57 ^a	26.00 \pm 0.00	0.003

<i>Staphylococcus aureus</i> (clinical isolate)	15.50 \pm 0.00 ^b	17.65 \pm 0.61 ^a	23.40 \pm 0.01	0.002
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Note: a=Highest antibacterial activity; b=Lowest antibacterial activity

Seed products MIC and MBC

M. stenopetala seed products had MIC values ranging from 7.25 to 13.90 mg/mL against the bacteria tested. The methanol products had a similar reaction (7.25 mg/mL) for clinical isolates of *E. coli* and *Salmonella enterica*, whereas the MIC values for *E. coli* (ATCC 2592) and *Salmonella enterica* were 13.25 and 7.25 mg/mL, respectively. Acetone products produced similar results (7.50 mg/mL) for clinical isolates of *E. coli* (ATCC 2592) and *E. coli*. Because of the acetone products, the MIC values for *Salmonella typhi* (clinical isolate) and *E. coli* (clinical isolate) were observed to be comparable (7.50 mg/mL). For clinical isolates of *Salmonella typhi* and *Staphylococcus aureus* (clinical isolate) were the methanol product produced a comparable response (11.50 mg/mL). Ultimately, the methanol product had the highest MIC for *E. coli* (ATCC 2592) (13.25 mg/mL) (Table 4).

The MBC values over several solvent extract products of *M. stenopetala* seed are shown in Table 4. The maximum MBC value for *E. coli* (clinical isolate) in the acetone extract was 13.90 mg/mL. The acetone extract had the highest response (13.90 mg/mL) for *E. coli* (clinical isolate) and *Salmonella enterica* (clinical isolate) (11.25), whereas *Salmonella typhi* (clinical isolate) extracts had the lowest MBC value (10.00) (Table 4).

TABLE 4

***M. stenopetala* seed products' antimicrobial properties (MIC) and minimum inhibitory concentration (MBC) values are shown.**

Organisms	Seed products			
	Methanol		Acetone	
	MIC	MBC	MIC	MBC
<i>E. coli</i> (ATCC 2592)	13.25	12.95	7.5	12.5
<i>E. coli</i> (clinical isolate)	7.25	13.65	7.5	13.9
<i>Salmonella typhi</i> (clinical isolate)	11.5	13	7.5	10
<i>Salmonella enterica</i> (clinical isolate)	7.25	12.5	8.9	11.25
<i>Staphylococcus aureus</i> (clinical isolate)	11.5	12.25	11.5	13.5

DISCUSSION

The two sources of water samples that were examined are commonly utilized by the residents of Debre Markos Municipality. The turbidity of the Wuseta (131.10 NTU) and Teklehaimanot (210.00 NTU) rivers was found to be different. The pH of drinking water recommended by WHO [39] is between 6.5 and 8.5. The pH of the Wuseta and Teklehaimanot rivers was within acceptable limits. The inclusion of 0.036 g/L aluminum sulfate lowered the pH of the Teklehaimanot and Wuseta river water samples from 6.65 and 7.2 to 3.95 and 3.81, respectively. It is typical practice in municipal water treatment plants to add lime or soda ash to elevate the pH to reasonable bounds. *M. oleifera* seed powders, on the other hand, had no discernible effect on the pH of the water. This observation was congruent with that of Arama et al. [40]. In research, Pal et al. discovered that *M. oleifera* seed extract and leaf extracts exhibit antiulcer and antagastric efficacy in research [41]. Water treatment with *M. stenopetala* was found to be more effective for water purification than treatment with *M. oleifera* seed [15]. As a result of these qualities of *M. stenopetala* seed, as well as its inexpensive cost, *M. stenopetala* is a superior choice for water purification, and it minimizes the possibility of forming or speeding up ulcers in people. According to WHO guidelines, the turbidity of safe drinking water should be less than 5 NTU.

When compared to the negative control, *M. stenopetala* seed powder at a concentration of 0.036 g/L reduced turbidity in both water samples (131.10 NTU to 16.82 NTU (87.3%) and from 210.00 NTU to 33.68 NTU (84.25%) for the Wuseta and Teklehaimanot river water samples, respectively). However, aluminium sulfate outperformed *M. stenopetala* in terms of turbidity reduction, with a concentration of 0.036 g/L (from 131.10 NTU to 2.30 NTU (99.20%) for the Wuseta water sample and from 210.00 NTU to 1.88 NTU (99.56%) for the Teklehaimanot water sample).

Previous research found that whole crushed *M. stenopetala* seeds were successful in removing turbidity from waters that had high initial turbidity, and bacterial contamination was significantly lowered by 90 to 99.9% [15]. *M. oleifera* coagulant protein drastically decreased turbidity (p 0.05) and organic load, contributing to a 58.18% reduction in total coliform of treated water. Also, it incited hopeful antimicrobial activity against bacterial isolates in Opa reservoir water [42].

Likewise, Seifu mentioned that 100-50 mg/L of *M. stenopetala* was as impactful as 200 mg/L of *M. oleifera* in water clarity, indicating that *M. stenopetala* is more efficient than *M. oleifera* [43]. Turbidity increased further in this investigation when the *M. stenopetala* seed powder concentration was boosted from 0.036 to 0.084 g/L. This could be credited to the flocculants' free positively charged molecules designed to resist each other, likely to result in flocs floating or suspended within the water [22,44]. These floating flocs might be filtered to reduce turbidity. According to studies, the maximum reduction in the coliform count was observed at 0.084 g/L concentration of *M. stenopetala* seed product from >2600 to 90 CFU/100 ml (97.51%) for the Wuseta water sample >2600 to 100 CFU/100 ml (98.69%) for the five sample of Teklehaimanot using the most probable number method.

M. Stenocephala seed products coagulate 90.0-99.9% turbidity and color, resulting in a visually clean supernatant [15]. This was supported by a 90.00–100% reduction in bacterial load (fecal coliforms), with bacteria focusing attention on the sediment sludge. Adejumo et al. found no significant difference in the coliform count after treating water samples with varying amounts of *M. oleifera* leaf powder, contradicting this decision [45]. Perhaps this is due to differences in the amount of the substance that aids in the decline of coliforms in different parts of the tree (seed vs. leaf).

The acetone extract generated from *M. stenopetala* seed displayed the greatest antibacterial efficacy against all of the experimental species, whereas the methanol extracts demonstrated the smallest zone of inhibition. The lower activity of water extract against microorganisms tested in this investigation is consistent with earlier findings that aqueous extracts of plants often demonstrated little or no antibacterial activity [46-48].

The seeds of *M. stenopetala* have natural flocculating and antimicrobial properties, and active coagulating substances are found in the cotyledons of the seeds [49,50]. The proteins that cause coagulation have been found to improve the quality of water treated with *M. stenopetala* seed flour, and coagulant proteins have been shown to reduce the density of *E. coli*, *Bacillus thuringiensis*, and *Pseudomonas aeruginosa* populations [51,52]. *M. stenopetala* seed extracts had MIC values ranging from 7.25 mg/mL acetone extract on all tested species to 13.25 mg/mL methanol extract on *E. coli* and *Salmonella typhi* clinical isolate and *E. coli*—ATCC2592).

The acetone extract has the highest MIC value at 7.50 mg/mL. This conclusion is analogous to that of Moyo et al., who discovered a MIC value of 5.00 mg/mL of acetone leaf extract of *M. oleifera* for *E. coli*. The MIC value of the extracts was comparable (7.50 mg/mL) for all experimental species except *Salmonella enterica* and *Staphylococcus aureus* for the acetone extract of *M. stenopetala*. In comparison, an acetone extract of *M. oleifera* leaf demonstrated a comparable MIC and MBC against *E. coli* at a 5.00 mg/mL concentration. It should be mentioned that there is no standard concentration as a model measure for assessing antibacterial activity in plant materials. However, Moyo et al. used 5.00 mg/mL as their maximum concentration threshold [53].

CONCLUSION

M. stenopetala seed powder showed a considerable reduction in turbidity and coliform count when it was applied in smaller quantities without altering the pH of the water. Furthermore, extracts of the seed in various solvents demonstrated antibacterial activity against all five test species, namely *Escherichia coli* (ATCC2592), *E. coli* (clinical isolate), *Salmonella enterica*, *Salmonella typhi* (clinical isolate), and *Staphylococcus aureus* (clinical isolate). At extremely low concentrations, the acetone extract is the most efficient in inhibiting and killing the test organisms for *E. coli* (ATCC 2592), *E. coli* (clinical isolate), and *Salmonella typhi* (clinical isolate). MIC= (7.50 mg/mL) and MBC (10.0 mg/mL) for *Salmonella typhi* (clinical isolate). The current study suggests that acetone extracts of *M. stenopetala* seeds have antibacterial efficacy against pathogens, and their capacity to either block or circumvent resistance pathways might enhance microbial strain treatment and eradication. Plant seed extracts might thus be utilized to treat

infectious disorders caused by microorganisms. These extracts are likely to be promising natural antibacterial agents with applications in the control of bacteria that cause waterborne bacterial illnesses. Because the acetone extract of the seeds demonstrated superior bactericidal action, more research into the separation of active components is required. This investigation helps those in Ethiopia and elsewhere who cannot afford or do not have access to safe drinking water. Additionally, research is also required to optimize the suitable concentration needed to bring about the reduction in turbidity and coliform count to WHO's requirement for efficient application of the seed for water purification. *M. stenopetala* was selected in this study to pay greater attention to a green and eco-friendly method that prevents environmental damage and/or contamination.

DECLARATIONS

Molaligne Medfu carried out the experiments; analyzed and interpreted the data; provided reagents, materials, analysis tools or data; and wrote the paper.

FUNDING STATEMENT

This research did not receive any specific funding from funding agencies.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The author thanks Debre Markos University, department of biology and biotechnology for the necessary guidance and use of facilities in his laboratory for the water analysis.

REFERENCES

- Salazar Gamez LL, Luna-delRisco M, Salazar Cano R. Effect of storage and preparation methods of *Moringa oleifera* seeds during the coagulation process. *Desalin Water Treat.* 2016;57(35):16376-16383.
- WHO, UNICEF (2012). Progress on sanitation and drinking water update. World Health Organization, Geneva
- WHO, (2011). Guideline for drinking-water quality: incorporating first addendum recommendations, 3rd edn. World Health Organization, Geneva
- Clasen TF, Brown J, Collin SM. Preventing diarrhoea with household ceramic water filters: assessment of a pilot project in Bolivia. *Int J Environ Health Res.* 2006;16(03):231-239.
- Hutton G, Haller L, Bartram J. Global cost-benefit analysis of water supply and sanitation interventions. *J Water Health.* 2007;5(4):481-502.
- Megersa M, Beyene A, Ambelu A, et al. A preliminary evaluation of locally used plant coagulants for household water treatment. *Water Conserv Sci Eng.* 2016;1(2):95-102.
- Arnold BF, Colford JM. Treating water with chlorine at point-of-use to improve water quality and reduce child diarrhea in developing countries: a systematic review and meta-analysis. *Am J Trop Med Hyg.* 2007;76(2):354-364.
- Sobsey MD, Stauber CE, Casanova LM, et al. Point of use household drinking water filtration: a practical, effective solution for providing sustained access to safe drinking water in the developing world. *Environ Sci Technol.* 2008;42(12):4261-4267.
- Hutton G, Haller L, Water S, World Health Organization. Evaluation of the costs and benefits of water and sanitation improvements at the global level. World Health Organization; 2004.
- Lantagne D, Preston K, Blanton E, et al. Hypochlorite solution expiration and stability in household water treatment in developing countries. *J Environ Eng.* 2011;137(2):131-136.
- Sadiq R, Rodriguez MJ. Disinfection by-products (DBPs) in drinking water and predictive models for their occurrence: a review. *Sci Total Environ.* 2004;321(1-3):21-46.
- Flaten TP. Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Res. Bull.* 2001;55(2):187-196.
- Ndabigengesere A, Narasiah KS. Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water research.* 1998;32(3):781-791.

14. Alam MW, Pandey P, Khan F, et al. Study to investigate the potential of combined extract of leaves and seeds of *Moringa oleifera* in groundwater purification. *International J. Environ Res Public Health*. 2020;17(20):7468.
15. Abiyu A, Yan D, Girma A, et al. Wastewater treatment potential of *Moringa stenopetala* over *Moringa olifera* as a natural coagulant, antimicrobial agent and heavy metal removals. *Cogent Environ Sci*. 2018;4(1):1433507.
16. Megersa M, Beyene A, Ambelu A, et al. A preliminary evaluation of locally used plant coagulants for household water treatment. *Water Conserv Sci Eng*. 2016;1(2):95-102.
17. Alrajhi M, AL-Rasheedi M, Eltom SE, et al. Antibacterial activity of date palm cake extracts (*Phoenix dactylifera*). *Cogent food agric*. 2019;5(1):1625479.
18. Pritchard M, Mkandawire T, Edmondson A, et al. Potential of using plant extracts for purification of shallow well water in Malawi. *Phys Chem Earth*. 2009;34(13-16):799-805.
19. Yongabi KA, Lewis DM, Harris PL. Indigenous plant based coagulants/disinfectants and sand filter media for surface water treatment in Bamenda, Cameroon. *African Journal of Biotechnology*. 2011;10(43):8625-9.
20. Tassinari B, Doherty S, Marison IW. Submicron capsules extracted from rapeseed as novel flocculant agents for the treatment of turbid water. *Water research*. 2013;47(14):4957-4965.
21. Fatombi JK, Lartiges B, Aminou T, et al. A natural coagulant protein from copra (*Cocos nucifera*): Isolation, characterization, and potential for water purification. *Sep Purif Technol*. 2013;116:35-40.
22. Adams FV, Mulaba-Bafubiandi AF. Application of rice hull ash for turbidity removal from water. *Physics and Chemistry of the Earth, Parts A/B/C*. 2014;72:73-76.
23. Ramavandi B. Treatment of water turbidity and bacteria by using a coagulant extracted from *Plantago ovata*. *Water Resour Ind*. 2014;6:36-50.
24. Kukic DV, Šćiban MB, Prodanović JM, et al. Extracts of fava bean (*Vicia faba L.*) seeds as natural coagulants. *Ecol Eng*. 2015;84:229-232.
25. Sciban MB, Antov MG, Klačnja MT. Extraction and partial purification of coagulation active components from common bean seed. *Acta Period Technol*. 2006(37):37-43.
26. Marobhe NJ, Dalhammar G, Gunaratna KR. Simple and rapid methods for purification and characterization of active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeata*. *Environ Technol*. 200;28(6):671-681.
27. Tafesse A, Goshu D, Gelaw F, et al. Food and nutrition security impacts of *Moringa*: Evidence from Southern Ethiopia. *Cogent food agric*. 2020;6(1):1733330.
28. Devkota S, Bhusal KK. *Moringa oleifera*: a miracle multipurpose tree for agroforestry and climate change mitigation from the Himalayas—a review. *Cogent food agric*. 2020 ;6(1):1805951.
29. Sciban MB, Antov MG, Klačnja MT. Extraction and partial purification of coagulation active components from common bean seed. *Acta Period Technol*. 2006(37):37-43.
30. Marobhe NJ, Dalhammar G, Gunaratna KR. Simple and rapid methods for purification and characterization of active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeata*. *Environ Technol*. 200;28(6):671-681.
31. Akinyemi KO, Oladapo O, Okwara CE, et al. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Me*. 2005;5(1):1-7.
32. Lea M. Bioremediation of turbid surface water using seed extract from *Moringa oleifera Lam.*(drumstick) tree. *Curr Protoc microbiol*. 2010;16(1):1G-2.
33. Ndabigengesere A, Narasiah KS, Talbot BG. Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water research*. 1995;29(2):703-710.
34. Porter WM. The 'Most Probable Number' method for enumerating infective propagules of vesicular *arbuscular mycorrhizal* fungi in soil. *Soil Research*. 1979;17(3):515-9.
35. Shahidi GA. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchiseptica*. *Asian J Plant Sci*. 82-86
36. Lopez V, Jäger AK, Akerrera S, et al. Pharmacological properties of *Anagallis arvensis L.* ("scarlet pimpernel") and *Anagallis foemina Mill.* ("blue pimpernel") traditionally used as wound healing remedies in Navarra (Spain). *J Ethnopharmacol*. 2011;134(3):1014-1017.
37. Okwori AE, Dina CO, Junaid S, et al. Antibacterial activities of *Ageratum conyzoides* extracts on selected bacterial pathogens. *Internet J Microbiol*. 2007;4(1):34-56.
38. Spencer JF, de Spencer AL, editors. *Public health microbiology: methods and protocols*. Springer Science & Business Media; 2004.
39. WHO. (2008). *Guidelines for drinking water quality*. 3rd Ed, Incorporating 1st and 2nd addenda 1: 668.
40. Wagai S, Arama P, Ogur JA, et al. Harvesting surface rainwater-purification using *Moringa oleifera* seed extracts and aluminum sulfate.
41. Pal SK, Mukherjee PK, Saha K, et al. Antimicrobial action of the leaf extract of *moringa oleifera lam*. *Anc Sci Life*. 1995;14(3):197.
42. Taiwo AS, Adenike K, Aderonke O. Efficacy of a natural coagulant protein from *Moringa oleifera* (Lam) seeds in treatment of Opa reservoir water, Ile-Ife, Nigeria. *Heliyon*. 2020;6(1):e03335.
43. Seifu E. Actual and potential applications of *Moringa stenopetala*, underutilized indigenous vegetable of Southern Ethiopia: a review. *Int J Agric Food Res*. 2015;3(4).
44. Sutherland JP, Folkard GK, Mtawali MA, et al. *Moringa oleifera* at pilot/full scale.
45. Adejumo O, Chukwujekw C, Kolapo A, et al. Chemical analysis and investigative study on water disinfecting properties of *Moringa oleifera* (*Moringaceae*) leaf. *Pharmacologia*. 2012;3(10):530-534.
46. Aiyegoro OA, Akinpelu DA, Afolayan AJ, et al. Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Baill. *J Biol Sci*. 2008;8(2):356-361.
47. Ashafa AO, Afolayan AJ. Screening the root extracts from *Biden pilosa L. var. radiata* (Asteraceae) for antimicrobial potentials. *J Med Plant Res*. 2009;3(8):568-572.
48. Merawie Y, Sahile S, Moges F, et al. Antimicrobial activity of crude and semi-purified fractions of *Warburgia ugandensis* against some pathogens. *J Coast Life Med*. 2013;1(3):184-191.
49. ICRAF. (2006a). *Moringa stenopetala*. Retrieved
50. Peter A, Walter A, Wagai S, et al. Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *Afr J Microbiol Res*. 5(2):153-157.
51. Hellsing MS, Kwaambwa HM, Nermark FM, et al. Structure of flocs of latex particles formed by addition of protein from *Moringa* seeds. *Colloids Surf A Physicochem Eng Asp*. 2014;460:460-467.
52. Ghebremichael KA, Gunaratna KR, Henriksson H, et al. A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water research*. 2005;39(11):2338-2344.
53. Moyo B, Masika PJ, Muchenje V. Antimicrobial activities of *Moringa oleifera Lam* leaf extracts. *African Journal of Biotechnology*. 2012;11(11):2797-2802.