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Sustainable Green Treatment of Potable Water Utilizing Micronized Moringa oleifera Seed Powder

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Abstract

This study investigated the natural coagulation and flocculation process and evaluated the effectiveness of Moringa oleifera seeds as a bio-product used as an environmentally. A 0.018 µg/L dosage of Moringa oleifera significantly improved water clarity, reducing turbidity in the Tigris River from 220.4 NTU to 52.65 NTU (82.84%) and in the Euphrates River from 141 NTU to 19.4 NTU (85.98%) while the pH of the water treated with the seed extract did not change significantly. At 96.15% and 96.45%, respectively, measurements of the microbial load in the Tigris and Euphrates rivers showed the biggest decrease. Among the plant extracts examined, acetone extract demonstrated the strongest antibacterial action, forming an inhibitory zone against clinically isolated Salmonella typhi with a diameter of 18.00 mm. Conversely, a clinical isolate of Shigella dysenteries showed a diminished response. with an inhibitory zone of only 7.46 mm when exposed to the aqueous extract. The most often recorded minimum inhibitory concentration (MIC) was 6.35 µg/L, with 13.4 µg/L coming in second. The acetone extract was the most effective overall because it exhibited both inhibitory and bactericidal efficacy against Shigella typhi even at relatively low doses. Overall, the seed powder demonstrated an unexpected ability to reduce turbidity and coliform, indicating its potential as a natural water purification agent. Furthermore, the acetone seed extract exhibited potent antibacterial activity, suggesting that the seed powder and its extracts can control and reduce bacterial infections in water.

Keyword: Moringa oleifera, Green Treatment, Seed Powder, MBC, MIC

I. INTRODUCTION

Since many people died from cholera, typhoid, and amoebic dysentery brought on by tainted drinking water in the 1920s and 1930s, water pollution and scarcity can result in the extinction of life (Shah, 2023) In small towns and remote areas without purification facilities, there is an urgent demand for water filtering in researched methods employing natural materials at a low cost (O. Mitiku,2020). A basic requirement for human safety and well-being is clean water, Yamaguchi. Due to source pollution brought on by population growth, industrialization, and agricultural waste, millions of people are currently exposed to hazardous levels of chemical and biological contaminants in drinking water (Singh, 2022). The quality of the drinking water near the main source has been negatively impacted by ion pollution. For instance, harmful ions including phosphorus, fluoride, iron, and turbidity pose a major threat to drinking water quality (O. Mitiku,2020). Aluminium sulphate (Al2(SO4)3·18H2O) is the most widely used synthetic coagulant for treating water from public sources. It has a fast rate of aggregation, produces heavy flocs (Maroušek, 2022), is more efficient than other coagulants at eliminating color and turbidity, and has less pH dependence (Dayarathne,2022). Despite its well-known effectiveness, one of its main problems is the significant amount of residual aluminium in water after treatment, which has been connected to an acceleration of Alzheimer's disease worsening processes (Maroušek,2022). Therefore, it is interesting to propose options to reduce the amount of synthetic coagulant, such as using natural coagulants in water treatment. It is believed that natural

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coagulants are safe, biodegradable, and have low levels of toxicity and residual sludge production (O.Mitiku, 2020). We proclaim that water is the primary source of human drinking water and that it requires a variety of treatments. It can come from the soil, such as springs or wells, or it can be found naturally on the surface of the earth, such as rivers or rainwater. There are international regulations, most of which adhere to World Health Organization regulations, regardless of the source of such water Yamaguchi et al., (2020). Globally, there is a problem with providing communities with enough clean water, especially in developing countries where rural inhabitants rely on water from rivers, dams, and streams for domestic use, which may contain dangerous substances and viruses. Drinking such contaminated water can lead to serious health problems for the community in areas where water is scarce (UN, 2003).

The Moringa tree, scientifically known as Moringa oleifera, is a member of the Moringaceae family of grasses. Other names for it include dairy, rural horseradish, drum stick, and well tree oil .Due to their aqueous solubility, the seeds of this tropical plant exhibit both coagulation activity and antibacterial efficacy. The findings show that the proteins can both directly suppress the growth of the microorganisms and eradicate them from the coagulant. It is acknowledged that moringa would eliminate 90–99.9% of contaminants in water, despite ongoing research on the makeup and potential of these compounds Nhantumbo et al., (2020). Due to their aqueous solubility, the seeds of this tropical plant exhibit both coagulation activity and antibacterial efficacy. The findings show that the proteins can both directly suppress the growth of the microorganisms and eradicate them from the coagulant. It is acknowledged that moringa would eliminate 90–99.9% of contaminants in water, despite ongoing research on the makeup and potential of these compounds Nhantumbo et al., (2020). One of the most significant factors influencing the quality of drinking water for human use is the removal of raw water recession and its pollutants, which is one of the main objectives of water filtration facilities worldwide (9). Because microscopic particles include bacteria, illnesses, and dangerous toxins, it is unacceptable to have them suspended in the water. These particles surround the suspended particles, protecting them from disinfectants Owodunni et al., (2023). Furthermore, worry keeps the water from being disinfected by the visible UV spectrum. Organic compounds, metal oxides, and other insoluble materials of different sizes make up the majority of the Acorus's contents, which are quiescent and affinity-loving substances.

Because of the incompatible interactions between the electrical charges in their outer shells, these compounds are diffused and stable in water. Following the addition of Vega et al., (2021). seeds using the jar approach, the samples exhibit varying degrees of disruption. Following the addition of the entire oval seeds, it was found that the samples' degree of disturbance varied, as shown in Figure (1). When chlorine is used to sterilize water, it may react with organic materials to produce adverse consequences that are very dangerous to human health. For example, compounds (three hills) are substances that result from the interaction of chlorine with organic materials known as trihalomethanes, and a shortcut known as (Thms) are organic compounds made from vinegar acid and methane that induce Hazer (Haas) (Bazaanah, 2024). The composition and properties of these constituents have been studied since 1970 in order to assess the efficacy of Moringa seeds in water treatment (Bhalla, 2021). While there is continuous study working on the nature and characteristics of these substances. Chemicals controlling the growth of bacteria identified in Moringa seeds, which are various substances, including: Glycosides (alpha - 4 - l - ahamno syloxy) benzliso 4 - l - (rhamnsyloxy) - phenyl - lacato nitrite. These chemicals had a fatal effect on a group of bacteria such as Bacillus subtilus, Mycobacterium phei, Serratia Pseudomonas aeruginosa, E. coli– Streptococcus -shigella. It was found that Moringa seeds contain 40% protein, 18.8% fat, 18.8% starch, 6.02% and soluble sugars (Aderinola, 2018).

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Figure .1 Moringa oleifera seeds

This study intends to conduct a laboratory study of water specifications before and after purification with Moringa tree seeds and to develop water treatment unification with Moringa tree seed powder in order to achieve effective drinking water purification from various sources in small mm units and remote areas without adverse effects and harm to human health, using natural materials and at a cost. The feasibility of doing it out in a reasonable amount of time and at a reasonable cost (Figure 1).

II. Materials and Methods

The experiment was carried out at the Scientific Research Authority's Agricultural Research Center in Zaafaraniya between October 2025 and May 2026. Two water samples were taken from the Euphrates River in the Iskandariya region and the Tigris River in the Jadriya region. In Baghdad, moringa seeds were gathered from trees in nurseries. From dry, broken fruits, mature Moringa oleifera seeds were chosen. To remove the seeds, the fruits were opened. They were thereafter allowed to air dry for two days. Before being processed, the seeds were gathered, cleaned, hulled, and dried for five days at temperatures between 22 and 25°C. After being pounded into a fine powder, the white seeds were passed through a fine sieve. For the experiment, the powder was gathered in a sterile vial. The two water samples and the prepared solutions were combined for 45 minutes at 120 rpm in a mixer. Depending on the turbidity of the various water samples, the sedimentation process took one to two hours. An equivalent volume of water was collected for testing following precipitation. Methanol, acetone, and aqueous solutions in progressively higher polarity ratios were used to extract the powdered material. This procedure involved soaking 50 micrograms of powdered Moringa oleifera seed in 250 milliliters of each solvent (acetone, methanol, and aqueous solution), with equal amounts of solvent used in each instance. For three days, the seeds were allowed to vibrate on a horizontal shaker. Whatman No. 1 filter paper was then used to filter each extract separately.

Forty liters of untreated water samples were obtained from the Tigris and Euphrates rivers. After that, these were split up among six beakers containing 500 mL of aluminum sulfate and seed extract from Sage oleifera. Five concentrations of the loading dose base solution, each weighing 3.0, 6.0, 9.0, 12.0, and 10.0 µg of Sage oleifera seed powder and aluminum sulfate individually, were added to a beaker filled with 500 mL of distilled water. The solutions in the beakers were stirred with a glass rod to produce a clear solution. 500 milliliters of distilled water without any M. oleifera seed powder extract served as the negative control (Rifi, 2022). Two milliliters of each of the various concentrations, including the control of all the loading dosages created, were added to a beaker containing 500 milliliters of the sample river water. The solutions were swiftly mixed for two minutes and then gently mixed with a sterile glass rod for ten minutes to aid in the synthesis of coagulant. uninterrupted for an hour. This method was selected because the jar test lacks a standard protocol (APHA,2023). The supernatants were tested for turbidity, pH, and total count after being recovered.

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Figure .2 The difference in turbidity before and after treatment with seed powder

A. Detection of active compounds

FTIR analysis revealed the active compounds in ground moringa seeds using infrared rays, since these substances are essential to the treatment and purification of water, as Figure (2) illustrates with the presence of standard peaks. A wavelength of 2400 was found to be the peak for the effective group O-H and hydrogen bond. At a wavelength of 1650 cm-1, the effective group c=o. Additionally, C-H and C-C bonds were detected at cm-1 (4000-500), c-o, and C-N wavelengths within a range of 1000 cm-1. This validates the existence of OH connections and shows water molecules connected to the Na and Ca peaks on the zeolite structure. For M. Oleifera seed powder, the absorption peak at 11059 cm-1 demonstrated that the effective groups from the ATR-FTIR analysis were comparable to those discovered by (Moyo, 2012). which reflects six peaks of bioactive compounds. There are a few major compounds present with a high peak area, namely (Desta,2021), reflecting six peaks of bioactive compounds. A few major compounds present with high peak areas are carbonic acid, 2-butyl-2-pentyl ester, 2-isopropoxyethyl propionate (16.8%), 4 H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-8.98, and -Dioxolan-2-one, 4,5-dimethyl-) Additional compounds with a reasonable peak area are Tetra acetyl-d-xylonic nitrile , Azetidin-2-one 3,3-dimethyl-4-, 1-amino ethyl) - (-dihydroxyacetone, Alpha-D-Glucose), and butanediol acid, 2hydroxy-2-methyl-, which are rarely detected in plant extracts (Kumar,2010) (Figure 3).

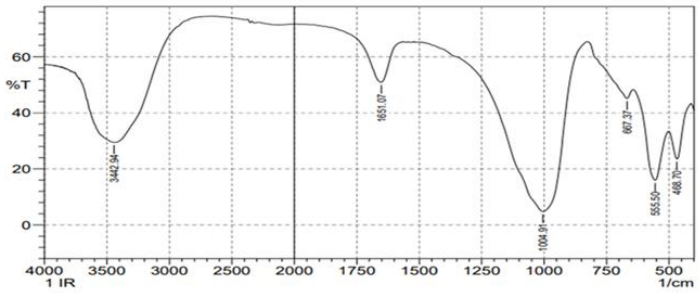

Figure 3. ATR-FTIR infrared spectrum for detecting the active chemicals in moringa seed extract

After that, the filtrates were centrifuged for 15 minutes at 5000 rpm. Rota vapor (Laboratory 4000-efficient, Heidolph, Germany) was used to evaporate each extract's supernatant. The crude extracts were kept in storage at 4 °C. Acetone, methanol, and water extract yields were 16, 15, and 13%, respectively. Every test was conducted three times (Akinyemi,2005). Cultures that are pure First, a jar device was used to combine the prepared solutions with two water samples for 45 minutes at 120 rpm. Depending on the turbidity of the various water samples, the settling period was one to two hours. The same amount of water was obtained for testing after it had settled. The amount of seed powder was measured, and the water quality parameters were examined both before and after treatment. To ascertain whether the water's characteristics had changed, the experiment findings were compared to Iraqi drinking water standards and a control alum sample. concentrations of 1%, 1.5%, and 2% in succession (Al-Jadabi,2023).

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To extract and activate the antibacterial and thrombosis proteins in the seed powder, the solution was filtered for one minute. One liter of raw water for each concentration was added to a royal (2 liter), and the mixture was stirred for sixty seconds. After that, let the treated water settle for six hours without moving. Following that, 100 cc of water was gathered for post-treatment analysis. Three repetitions for each transaction were used to calculate the percentage of turbidity elimination using the formula given by (Kenea, 2023).

B. Turbidity and pH measurement

The nephelometric method was employed to assess turbidity using a turbidimeter on water samples from the jar tests. Using a turbidity meter, the water samples' turbidity levels were measured both before and after they were treated with varying amounts of aluminum sulfate and M. oleifera seed powder. The turbidity meter reading was compared to the blank tube after the sample was introduced to a test tube until it reached the 10 ml level. The results were collected directly from the turbidity meter display and reported in nephelometric turbidity units (NTU). The hydrogen ion content of the water was measured both before and after it was treated with aluminum sulfate and powdered moringa seed using a buffer solution calibrated pH meter. Consequently, the display was used to read the outcome (*APHA*,2023).

Turbidity removal (%) = Initial turbidity – Residual turbidity in sample / Initial turbidity x 100

Antimicrobial tests with various techniques, the isolates were maintained on nutrient agar plates and incubated at 37 °C for a full day in accordance with the manufacturer's instructions. The isolates were maintained on agar slant and stored at 4 °C until further use. Sterile normal saline was mixed with a loopful of the test organisms. The 0.5 McFarland standards were used to compare the bacterial solution. For the antibacterial test, MIC and MBC were determined using the agar well diffusion technique (APHA, 2023).

C. Diffusion of Agar wells

The bacterial broth culture had a density of 108 cells mL−1 of 0.5 McFarland standards. The aliquot was evenly distributed over Mueller-Hinton agar using a sterile cotton swab. After that, the plated media was given 30 minutes to cure at room temperature (WHO,2023). A 6-mm-diameter, sterile cork borer was used to create equally spaced wells on each plate, two millimeters from the plate's edge. Each extract was aseptically injected into a corresponding agar well in 50 microliters (50 µg/mL). The standard (positive) control was ciprofloxacin (25 µg/mL), while the blank control was sample-free solutions. The agar plate was then allowed to pre-diffuse for 40 minutes on the bench before being incubated for 24 to 48 hours at 37 °C. The formation of a clear inhibitory zone with a width of at least 7 mm surrounding the wells was thought to indicate the organisms' significant susceptibility to the extract (Moyo, 2012). Three replications of the experiment were conducted.

D. Calculating the minimal inhibitory concentration (MIC)

The MIC was determined for extracts that had a growth inhibition zone that was at least 7 mm in diameter. The agar dilution technique was used to conduct the test. Using this method, the extract was serially diluted twice in Mueller-Hinton agar. The bacterial inoculum, which was standardized using the McFarland standard, was applied to the agar's surface. The extract solution (50 µg /mL) should bring 50, 100 and 150 µg /mL concentrations, correspondingly (NCCLS, 2003). As mentioned above, the extracts were then introduced aseptically. The extract's minimum inhibitory concentration (MIC) was established and the growth inhibition was assessed following a 24-hour incubation period at 37 °C.

E. Finding the minimum bactericidal concentration (MBC)

The MBC of the plant extracts was determined using the modified Spencer and Pencer method (Moyo, 2012). After the batch of agar plates on MIC was incubated, the plates that did not grow were sub cultured on Mueller-Hinton agar plates and incubated for 24 hours at 37 °C. The MBC was determined by taking the highest dilution (lowest concentration) that produced no single bacterial colony and showed no discernible development throughout incubation.

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F. Analysis of statistics

The statistical program SPSS (version 16) was used to examine the data. The mean decrease in turbidity and coliform was computed. The presence of a statistically significant difference between mean zones of inhibition was investigated using one-way ANOVA. A significance criterion of $p < 0.05$ was established.

III. 111. Results and Discussion

A. Purification of water

The raw water turbidity of the Tigris and Euphrates rivers was found to be 130 NTU and 212.30 NTU, respectively. The greatest reduction in turbidity was observed at the lowest quantity of aluminum sulfate, according to the results. After an hour of settling time, aluminum sulfate at 0.018 µg/mL decreased turbidity from 130 NTU to 2.2 NTU (97.38%) for the Tigris water sample and from 209.35 NTU to 1.89 NTU (98.78%) for the Euphrates water sample Furthermore, M. oleifera seed powder at 0.018 µg/mL reduced turbidity in the Euphrates River water from 212.31 NTU to 34.25 NTU (83.84%) and in the Tigris River water from 130.00 NTU to 18.20 NTU (87.96%). Turbidities of 130 BTU and 212.31 BTU were discovered in the raw waters of the Tigris and Euphrates rivers, respectively. The findings demonstrated that the largest decrease in turbidity was achieved at the lowest aluminum sulfate content. Aluminum sulfate at a concentration of 0.018 µg/mL reduced the turbidity in the Euphrates River from 212.31 BTU to 1.91 BTU (98.91%) and the Tigris River from 130 BTU to 1.91 BTU (97.38%) an hour after precipitation, Figures (3) and (4).

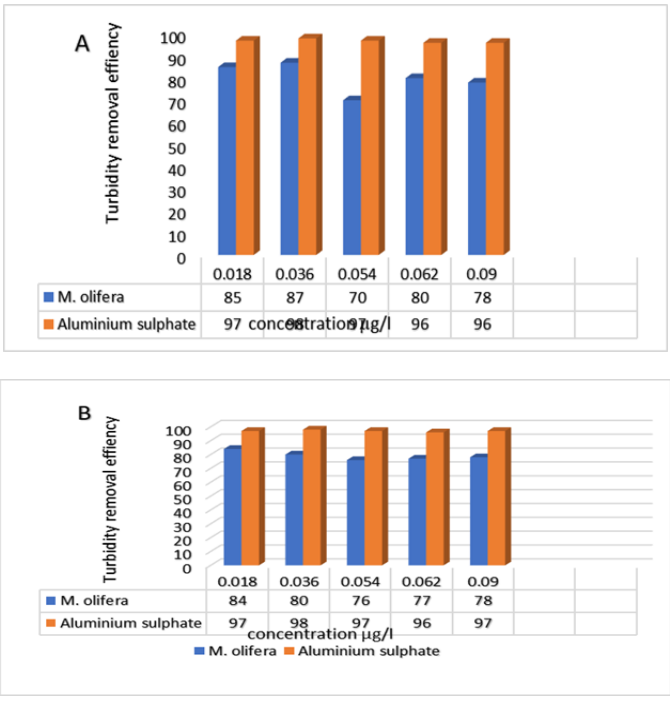

Figure. (3,4) percentage difference from the control, as seen in the turbidity decrease following treatment of water samples from the Tigris and Euphrates rivers with different amounts of aluminum sulfate and Moringa oleifera

Additionally, adding M. oleifera seed powder at a concentration of 0.018 µg/mL reduced the turbidity in the Tigris River water from 130.00 BTU to 16.8 BTU (86.96%) and in the Euphrates River water from 212.35 BTU to 34.25 BTU (84.82%). With the exception of a slight drop in pH from 6.75 to 6.46 (4.03%) for the Tigris River water samples at a concentration of 0.018 µg/mL and a drop from 7.2 to 6.9 (4.18%) for the

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Euphrates River water samples at a concentration of 0.036 µg/L, there was no discernible impact on the pH of any water sample following treatment with varying doses of M. oleifera, unlike aluminum sulfate. The coliform count data for both raw and treated water samples are shown in Table 2 as the most probable number (MPN). For both treated and untreated water samples, the coliform counts for the Tigris and Euphrates rivers exhibit significant variation. The treated water samples had much lower amounts of coliform. M. oleifera seed powder was used for the MPN testing.

C. was observed that both untreated water samples contained

over 2500 coliforms for every 100 milliliters. At the lowest concentration (0.018 µg/mL), the average bacterial growth rates (MPNs) in the Tigris and Euphrates rivers were 450 coliforms/100 ml and 800 coliforms/100 ml, respectively. The MPN was found to be 75 coliforms/100 ml and 65 coliforms/100 ml in the Tigris and Euphrates rivers, respectively, at the maximum quantity (0.09 µg/mL). Additionally, the percentage change in both water sources is shown in Table 2.

C. Antibacterial activity.

E. Coli (ATCC 2592) was significantly susceptible to each extract, according to the agar well diffusion result ($p = 0.008$). However, the acetone extract demonstrated the maximum antibacterial activity (14.34) against E. coli (ATCC 2592), whereas the aqueous extract showed the lowest antibacterial activity (8.44). E. Coli (ATCC 592) was much less sensitive to the methanol extracts (9.77) compared to the aqueous extract. Additionally, there was a substantial variation in the E. Coli (clinical isolate) reactivity amongst the examined extracts ($p = 0.05$).

The acetone extract showed the strongest antibacterial activity (19.00) against the clinical isolate E. coli when compared to the other extracts. The methanol extract had the highest sensitivity (16.76) for the clinical isolate E. coli, whereas the aqueous extract had the lowest sensitivity (8.44). The clinical isolate Salmonella typhi also showed significant sensitivity to all extracts ($p = 0.025$). Among the M. oleifera seed extracts examined, acetone has shown the best antibacterial activity (18.00) against Salmonella typhi (clinical isolate), followed by methanol (16.44) and aqueous (7.88). Additionally, compared to the other extracts, the acetone extract has shown a maximum and significant variance for the clinical isolate Shigella dysenteriae. As a result, the acetone exhibited the highest antibacterial activity (18.77) against the clinical isolate Salmonella typhi, followed by the methanol (18.00) and the aqueous extract (7.88). Nevertheless, ciprofloxacin, which was considered the standard drug, was discovered to be more effective than the M. oleifera seed extracts utilized, exhibiting highest activity against all evaluated bacterial growths (Table 3). The MIC and MBC values of the seed extracts ranged from 6.30 to 26.0 µg/mL. Clinical isolates of Shigella dysenteriae and E. coli responded similarly to the methanol extract (6.30 µg/mL), whereas the MIC value for E. coli (ATCC 592) and Salmonella typhi was 12.60 µg/mL. Clinical isolates of Salmonella typhi and E. coli responded similarly to the acetone extract (6.30 µg/mL).

Although the MIC values for Salmonella typhi and E. coli (ATCC 2592) were 6.30 and 26.00 µg/mL, respectively, the acetone extract for clinical isolates of Shigella dysenteriae and E. coli demonstrated a similar reaction (12.60 µg/mL). For Salmonella typhi, the aqueous extract has shown the highest MIC (26.00 µg/mL) overall (Table 5). Table 4 displays the MBC values for a number of M. oleifera seed solvent extractsThe clinical isolate of Shigella dysenteriae had the lowest MBC value (6.30 µg/mL) under the ethanol extract, while E. coli (ATCC 2592) and clinical isolates of E. coli and Salmonella typhi had the greatest MBC value (12.60 µg/mL). For E. coli (ATCC 2592) and clinical isolates of E. coli and Shigella typhi, the acetone extract showed a similar and greatest reaction (12.60 µg/mL), but for Salmonella typhi (clinical isolate), it showed the lowest reaction (6.30 mg/mL). The aqueous extract showed a maximum and similar reactivity (26.00 µg/mL) for clinical isolates of Shigella dysenteriae, Salmonella typhi, and E. Coli; however, the MBC value was found to be the lowest (12.60). for E. Coli (ATCC 2592) (Table 4).

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Table 1. After one hour of settling, the turbidity and pH of two water samples (from the Tigris and Euphrates rivers) treated with aluminum sulfate and Moringa oleifera

Treatments	Water source	Treatment (µg/l)	pH		Turbidity (NTU)	
			Mean reduction ± SD	% Reduction	Mean reduction (NTU) ± SD	% Reduction
Moringa oleifera seed powder	Tigris	Control	6.75 ± 0.00	0	130.00 ± 0.00	0
		0.018	6.45 ± 0.00	4.11	130.00 ± 0.00	87.96
		0.036	6.65 ± 0.00	1.50	18.20 ± 0.11	74.96
		0.054	6.60 ± 0.00	2.55	34.40 ± 0.00	76.78
		0.072	6.75 ± 0.00	0	31.36 ± 0.48	78.56
		0.09	6.95± 0.00	-3.01	30.12 ± 2.57	74.17
	Euphrates	Control	7.25 ± 0.00	0	212.31 ± 0.00	0
		0.018	7.35 ± 0.00	5	34.25 ± 0.90	83.84
		0.036	6.91 ± 0.00	4.73	48.70 ± 1.25	80.50
		0.054	7.08 ± 0.00	2.40	44.91 ± 0.44	76.68
		0.072	6.95 ± 0.00	4.18	42.03± 0.87	77.50
		0.09	7.28 ± 0.00	-0.44	130.00 ± 0.00	78.72
Aluminum sulfate	Tigris	Control	6.75± 0.00	0	2.20 ± 0.11	0
		0.018	4.45 ± 0.00	34.36	2.60 ± 0.11	97.38
		0.036	4.15± 0.00	38.83	2.56 ± 0.11	97.08
		0.054	4.05± 0.00	40.33	2.70 ± 0.058	96.88
		0.072	3.78 ± 0.00	43.89	3.64 ± 0.00	96.28
		0.09	3.98 ± 0.00	41.26	2.94 ± 0.00	97.78
	Euphrates	Control	7.25 ± 0.00	0	212.35 ± 0.00	0
		0.018	4.49 ± 0.00	39.07	1.91 ± 0.00	98.91
		0.036	4.88 ± 0.00	32.93	2.25 ± 0.00	98.96
		0.054	4.28 ± 0.00	41.26	2.38 ± 0.00	98.88
		0.072	3.71 ± 0.00	48.13	2.31 ± 0.00	98.95
		0.09	3.98 ± 0.00	45.43	2.90± 0.00	97.65

Communities residing in and around Baghdad often use the two water source samples examined in this investigation. The turbidity levels of the Tigris (130 NTU) and Shinta (212.31 NTU) river streams varied. The WHO recommends a pH of 6.5 to 8.5 for drinking water (H de O. Gomes, 2022). The Tigris and Euphrates rivers' water pH levels were within permissible limits. After adding 0.018 µg/mL of aluminum sulfate, the pH of the water samples from the Tigris and Euphrates rivers dropped from 6.7 and 7.2 to 3.76 and 3.71, respectively. In municipal water treatment facilities, M. oleifera seed powder had no appreciable impact on the pH of the water, despite the common practice of adding lime or soda ash to bring the pH up to acceptable values. This outcome was in line with the findings of (H de O. Gomes, 2022). According to a study, M. oleifera leaf extracts and seed powder have anti-ulcer and anti-gastritis properties. Consequently, these characteristics of M. oleifera seed, along with its low cost, make it an excellent substitute for purifying water and reduce the risk of developing or accelerating ulcers in people According to WHO criteria, safe drinking water has a turbidity of less than 5 NTU. In comparison to the negative control, M. oleifera seed powder at a concentration of 0.018 µg/mL significantly reduced the turbidity in the Tigris and Euphrates River water samples, respectively, from 130 NTU to 16.8 NTU (86.98%) and from 212.3 NTU to 34.25 NTU

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(84.82%). However, aluminum sulfate outperformed M. oleifera as a water purifier since it showed a greater reduction in turbidity, which complies with WHO guidelines.

Table 2. Test for most probable number (MPN) using different amounts of powdered Moringa oleifera seed.

Treatment	Water source	Concentration µg /mL	Combination of the positives	Coliform counts/100 mL	% Reduction
Seed powder	Tigris	Control	5-5-5	> 2500	0
		0.018	5-5-3	450	80.17
		0.036	5-4-0	250	95
		0.054	5-3-3	150	95.18
		0.072	5-3-1	85	97.70
		0.09	5-2-1	75	98.09
	Euphrates	Control	5-5-5	> 2500	0
		0.018	5-4-3	800	68.09
		0.036	5-3-0	370	90.32
		0.054	5-4-1	140	96.59
		0.072	5-2-1	95	97.27

Table 3. Agar well diffusion technique bacterial growth suppression zones treated with 50 mg/mL of methanol, acetone, and Moringa oleifera seed aqueous extract

Organisms	Zone of inhibition (mm) ± standard deviation			Positive control ciprofloxacin	p value using LSD
	Seeds extract				
	Methanol	Acetone	Aqueous		
E. coli (ATCC 2592)	9.77 ± 0.58^{b}	14.34 ± 0.58^{c}	8.44 ± 0.58^{a}	34.34 ± 0.58^{d}	0.009
E. coli (clinical isolate)	16.67 ± 0.58^{b}	$0.00^{c}\pm19.00$	8.44 ± 0.58^{a}	31.77 ± 2.4^{d}	0.05
Salmonella typhi (clinical isolate)	$0.58^{b}\pm16.44$	18.00 ± 0.00^{c}	7.88 ± 0.57^{a}	$0.58^{d}\pm30.22$	0.025
Shigella dysenteriae (clinical isolate)	18.00 ± 0.00^{b}	18.77 ± 0.58^{c}	7.88 ± 1.17^{a}	30.00±0.00	0.003

Means followed by the same letter in a row, are not significantly different at p = 0.05, using LSD

Table 4. Moringa oleifera seed extracts' minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values

Organism	Seed extracts					
	Methanol		Acetone		Aqueous	
	MIC and MBC µg /mL					
	MBC	MIC	MIC	MBC	MIC	MBC
E. coli (ATCC 2592)	12.6	12.6	12.6	12.6	6.30	12.60
E. coli (clinical isolate)	6.26	12.6	6.30	12.6	12.6	26.0
Salmonella typhi (clinical isolate)	12.6	12.6	6.30	6.30	26.0	26.0
Shigella dysenteriae (clinical isolate)	6.26	6.30	12.6	12.6	12.6	26.0

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For the Tigris water sample, the concentration of 0.018 µg/mL varied from 130 NTU to 2.1 NTU (98.37%) and for the Euphrates water sample, from 212.3 NTU to 1.91 NTU (98.18%) (Zakaria, 2022). also found that M. oleifera seed extract reduced the number of fecal coliforms and turbidity in water samples from shallow wells. In a similar vein, turbidity was reduced at the lowest dosage of M. oleifera seed powder treatment, according to Al-Kindi et al. (2021). In this experiment, turbidity increased from 0.018 to 0.09 µg/mL of M. oleifera seed powder. The reason the flocs float or suspend in the water could be explained by the free positively charged molecules of the flocculants rejecting one another (Okafor ,2024)

Such floating flocs could be made less turbid by filtering them. According to studies, the greatest decrease in coliform count, as determined by the most probable number method, was found at a concentration of 0.09 µg/mL of M. oleifera seed powder from > 2500 to 75 CFU/100 ml (98.09%) for the Tigris water sample and > 2500 to 65 CFU/100 ml (98.6%) for the Euphrates River sample. Madsen and colleagues (Desta, 2021). have demonstrated that M. oleifera seeds successfully coagulate 80.0–99.5% turbidity (a stand-in for suspended fine particles) and color (a stand-in for natural organic material) to generate an aesthetically clean supernatant. Concurrently, the bacterial burden (fecal coliforms), a safer indicator, decreased by 90.00–99.99%, with germs concentrated in the sediment sludge. On the other hand, a study by (Desta, 2021). discovered that adding different amounts of M. oleifera leaf powder to a water sample did not significantly change the coliform count. This may be due to the fact that different parts of the tree contain variable quantities of the substance that reduces coliforms (leaf vs. seed). The aqueous extracts showed the least zone of inhibition, whereas the acetone extract derived from M. oleifera seed showed superior antibacterial activity against all experimental species, followed by the methanol extract. Previous studies that demonstrated that plant aqueous extracts generally showed little to no antibacterial activity are consistent with the reduced efficacy of water extract against the pathogens investigated in this study (Adejumo,2012). Masika and Afolayan claim that gram-negative bacteria are more resistant to water extracts (Ashafa, 2009). Additionally, the majority of researchers have noted that plant aqueous extracts have little effect on bacteria (Desta, 2021). It is possible that water extracts differ from other solvents, which contain a variety of components that could interact negatively in their overall activities. Additionally, it is challenging to extract the active chemicals from plant materials in water. M. oleifera contains substances including tannins and polyphenols that are soluble in acetone (Okafor, 2024) and have been shown to have antimicrobial properties (Adejumo,2012). It has also been reported that the quality of water treated with M. oleifera seed flour is improved through the proteins that promote coagulation (Okafor, 2024), and coagulant proteins also demonstrated an ability to reduce the density of E. coli, Bacillus thuringiensis and Pseudomonas aeruginosa populations (Nhantumbo,2023).

The MIC value of M. oleifera seed extracts against the tested bacteria ranged from 6.30 26.00 mg/mL (aqueous extract of M. oleifera on Salmonella typhi—clinical isolate) to µg/mL (acetone extract to all examined organisms and methanol extract of M. oleifera on Shigella dysenteries—clinical isolate and E. coli—ATCC2592). For the acetone extract, the most common MIC value was 6.30 µg/mL. This discovery is. The most typical MIC value for the acetone extract was 6.30 µg/mL. This result is in line with the research conducted by. (Nhantumbo,2023), which revealed that the MIC value of M. oleifera acetone leaf extract for E. coli was 5.00 µg/mL. Except for the clinical isolate of E. coli, the MIC value of the aqueous extract was higher than that of the methanol extract every experimental species had the same MBC value (12.60 µg/mL) for the acetone extract of M oleifera, with the exception of Salmonella typhi, which displayed MBC and MIC values of 6.30 µg/mL .

It should be mentioned that the antibacterial activity of plant materials cannot be measured using a standard concentration as a model measure. On the other hand, M. oleifera leaf acetone extract demonstrated the same MIC and MBC against E. coli at a concentration of 5.00 µg/mL (Okafor,2024). The corresponding minimum inhibitory concentration (MIC) of its aqueous extract varied from 12.60 µg/mL against E. coli (ATCC2592), E. coli (clinical isolate), and Shigella dysenteries (clinical isolate) to 26 mg/mL against Salmonella typhi (clinical isolate). The MBC of an aqueous extract was 26 µg/mL for all four organisms, with the exception of Salmonella typhi (12.60 µg/mL). To sum up Moringa oleifera seed powder has shown a significant reduction in turbidity and coliform count when administered at lower doses without altering the pH of the water. Additionally, all four test species—Escherichia coli (ATCC2592), E. coli (clinical isolate), Salmonella

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typhi (clinical isolate), and Shigella dysenteries (clinical isolate)—showed antibacterial activity when the seed was extracted using various solvents. At a very low dosage (MIC and MBC = 6.30 µg /mL) for Salmonella typhi (clinical isolate), the acetone extract is the most effective at inhibiting and killing the test organisms.

IV. V1. Conclusions

According to the study, an acetone-based extract of Moringa oleifera seeds has antibacterial properties and could be useful in the fight against waterborne infections. It may also be utilized as an eco-friendly and natural method of purifying water, although more investigation is required to determine the active ingredients and the right concentration to satisfy WHO regulations.

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