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Antimicrobial and Coagulant Potential of Moringa oleifera Seed Extracts for Drinking Water Purification

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***Abstract*-- Access to safe drinking water remains a critical global public health imperative, particularly across low-resource and rural communities where conventional treatment infrastructure is often absent, technically complex, or economically unviable. *Moringa oleifera* Lam. seeds synthesize functional water-soluble cationic proteins alongside bioactive secondary metabolites that exhibit potent coagulating and broad-spectrum antimicrobial properties. This study provides a comprehensive investigation of the extraction efficiency, phytochemical composition, in vitro antibacterial spectrum, and drinking water purification performance of *M. oleifera* seed extracts prepared using four distinct solvent systems: distilled water, 95% ethanol, absolute methanol, and acetone.**

Quantitative extraction demonstrated that methanol afforded the highest yield (21.35 ± 0.47% w/w), recovering the broadest profile of bioactive phytoconstituents (tannins, flavonoids, saponins, alkaloids, and terpenoids). In vitro antimicrobial screening via disc diffusion, broth microdilution, and subculture assays revealed that the methanolic seed extract possessed the highest antibacterial efficacy against all tested bacterial strains, with zones of inhibition spanning 17.2 ± 0.5 to 21.8 ± 0.5 mm, minimum inhibitory concentrations (MIC) of 1.56 to 6.25 mg/mL, and minimum bactericidal concentrations (MBC) of 3.125 to 12.5 mg/mL.

***Staphylococcus aureus* was the most susceptible bacterium (MIC = 1.56 mg/mL), whereas *Pseudomonas aeruginosa* exhibited the highest intrinsic resistance (MIC = 6.25 mg/mL). When challenged against contaminated multi-source water samples, *M. oleifera* seed extract reduced raw water turbidity from 48.7 ± 2.3 NTU to 8.6 ± 0.7 NTU (82.3% clarification efficiency) while achieving substantial microbial decontamination (~2.0-log10 reductions in total coliforms, faecal coliforms, and *Escherichia coli*) without adversely shifting post-treatment pH, electrical conductivity, or total dissolved solids. While conventional alum yielded slightly superior clarification, *M. oleifera* demonstrated robust, ecologically benign, and biodegradable treatment efficacy. These findings establish that *M. oleifera* seed extracts serve as dual-action natural coagulants and bactericides with immense utility for sustainable decentralized primary water treatment.**

***Keywords*-- *Moringa oleifera;* Natural coagulant; Phytochemical extraction; Antimicrobial activity; Water purification; Turbidity reduction; Pathogen disinfection; Point-of-use water treatment**

I. Introduction

Access to clean and safe drinking water is universally recognized as a fundamental human right and a cornerstone of global public health (World Health Organization [WHO], 2022). Despite progressive international water, sanitation, and hygiene (WASH) initiatives, contaminated drinking water supplies remain a primary driver of preventable morbidity and mortality worldwide, contributing to hundreds of thousands of diarrhoeal fatalities annually and perpetuating widespread waterborne epidemics such as cholera, typhoid fever, and dysentery (Bancessi *et al.*, 2020; WHO, 2022). This burden falls disproportionately on developing and low-resource nations, where rural and peri-urban populations routinely depend on vulnerable surface waters, open wells, and unmonitored boreholes exposed to agricultural runoff, domestic effluent, and inadequate sanitation infrastructure.

Conventional municipal water treatment systems rely heavily on synthetic or inorganic chemical coagulants—predominantly aluminium sulphate (alum) and ferric salts—coupled with chemical disinfection methods such as chlorination (Ndabigengesere *et al.*, 1995; Ghebremichael *et al.*, 2005). Although undeniably effective at scale, these conventional regimens present profound environmental, economic, and health concerns. The continuous use of alum generates voluminous, non-biodegradable, toxic chemical sludge that poses severe disposal challenges. Furthermore, trace residual aluminium in treated water has been linked in toxicological literature to neurodegenerative pathologies, including Alzheimer's disease (Bancessi *et al.*, 2020; Desta & Bote, 2021). Similarly, chemical chlorination carries the risk of forming carcinogenic disinfection by-products (DBPs), such as trihalomethanes (THMs) and haloacetic acids, upon reaction with natural organic matter (Anwar *et al.*, 2007). Moreover, chemical supply-chain vulnerabilities, high importation costs, and technical dosing requirements render conventional chemical treatment largely unsustainable for remote, decentralized, or resource-constrained communities.

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In response to these constraints, research has increasingly focused on plant-derived, eco-friendly biocoagulants and natural antimicrobials as sustainable alternatives for point-of-use (POU) and small-scale community water purification (Fahey, 2005; Delelegn *et al.*, 2018; Dandesa *et al.*, 2023). Among the bioprospected botanical candidates, *Moringa oleifera* Lam. (family Moringaceae)—commonly known as the drumstick or horseradish tree—stands out as one of the most promising multipurpose species (Anwar *et al.*, 2007; Leone *et al.*, 2015). Native to the sub-Himalayan tracts of India and now pantropically cultivated, *M. oleifera* produces seeds that harbour potent dual-function bioactivities: flocculating colloidal turbidity and inactivating pathogenic microorganisms (Ghebremichael, 2004; Nkurunziza *et al.*, 2009).

The primary coagulation mechanism of *M. oleifera* seeds is mediated by low-molecular-weight (6.5–13 kDa), basic, water-soluble cationic dimeric proteins and lectins (Ndabigengesere *et al.*, 1995; Suarez *et al.*, 2003; Ghebremichael *et al.*, 2005). These cationic peptides act via adsorption and charge neutralization, effectively bridging negatively charged colloidal clay particles, suspended organic particulates, and microbial cell membranes into dense, settleable flocs. Concurrently, the seeds contain a rich reservoir of bioactive secondary metabolites—including flavonoids, condensed tannins, saponins, alkaloids, and glucosinolates—which exhibit direct broad-spectrum antibacterial, antioxidant, and membrane-permeabilizing activities (Rahman *et al.*, 2009; Bancessi *et al.*, 2020).

Despite substantial exploratory interest, critical operational and scientific knowledge gaps persist. First, standardisation is notably lacking across published literature: reported antimicrobial potencies and coagulation efficiencies vary widely depending on extraction protocols, seed provenance, and solvent systems (Bancessi *et al.*, 2020). Second, while numerous studies have characterized *M. oleifera* leaf extracts, comprehensive empirical investigations examining mature seed extracts across multiple polar and non-polar solvent systems remain limited (Delelegn *et al.*, 2018). Third, relatively few studies have holistically benchmarked the phytochemical recovery, antibacterial kinetics, and multi-source physicochemical/microbiological water quality improvements of seed extracts directly against conventional alum under identical field-relevant matrices (Semanka *et al.*, 2022).

To address these knowledge gaps, the present study was structured to systematically evaluate the extraction yield and qualitative phytochemical profiles of *M. oleifera* seed extracts prepared with distilled water, 95% ethanol, absolute methanol, and acetone. The specific objectives were: (1) to profile secondary metabolite recovery across solvents; (2) to quantify *in vitro* antibacterial potency (inhibition zones, MIC, MBC) against representative Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*) and Gram-positive (*Staphylococcus aureus*) waterborne bacterial pathogens; and (3) to evaluate the coagulant and disinfection efficacy of *M. oleifera* seed extract across natural water sources in comparison with untreated controls and conventional alum.

II. Materials And Methods

2.1 Plant Material Collection and Botanical Authentication

Mature, dry pods of *Moringa oleifera* Lam. were harvested from authenticated trees located in the agricultural environs of Mehkar Taluka, Buldhana District, Maharashtra, India (Latitude: 20.1542° N, Longitude: 76.5731° E). Taxonomic authentication was performed by a certified botanist at the Department of Botany, Vivekanand Science College, and a voucher specimen (VSC-MB-MO-2024/03) was deposited in the institutional herbarium for future reference. The collected pods were manually split to recover intact, non-damaged seeds. Wings and outer fibrous shells were manually dehusked to obtain clean seed kernels. The kernels were washed with sterile deionized water, shade-dried at ambient laboratory temperature (26 ± 2 °C) for 72 h to a constant weight, and pulverized into a fine powder using an electric mechanical grinder (Bajaj GX-11, India). The ground seed powder was sieved through a standard stainless steel 40-mesh sieve (pore size: 425 µm) to ensure uniform particle size and stored in airtight, amber-glass containers at 4 °C until extraction.

2.2 Multi-Solvent Extraction Procedure

Extraction of bioactive constituents from *M. oleifera* seed powder was conducted using four analytical grade solvent systems of varying dielectric constants: distilled water (dielectric constant $\varepsilon = 80.1$), absolute methanol ($\varepsilon = 32.7$), 95% ethanol ($\varepsilon = 24.5$), and acetone ($\varepsilon = 20.7$) (Sigma-Aldrich / HiMedia Laboratories, India). To eliminate potential interference from non-polar seed oils and neutral lipids (which constitute ~35–40% of seed mass), a standardized cold maceration and defatting protocol was employed.

International Journal of Recent Development in Engineering and Technology
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Briefly, 50.0 g of finely ground seed powder was defatted by washing with n-hexane (1:5 w/v) for 4 h, filtered, and air-dried. The defatted seed marc was subsequently extracted in 500 mL of each respective solvent (1:10 w/v solid-to-solvent ratio) in sterile 1-L Erlenmeyer flasks. The mixtures were agitated continuously on an orbital shaking incubator (Remi CIS-24 Plus, India) at 150 rpm for 48 h at room temperature (27 ± 1 °C).

Following maceration, the crude slurries were clarified by passing through double-layered sterile muslin cloth, followed by filtration through Whatman No. 1 filter paper under vacuum. For organic solvents (methanol, ethanol, acetone), the filtrates were concentrated to dryness under reduced pressure using a rotary vacuum evaporator (Buchi R-300, Switzerland) at controlled temperatures (40–45 °C). The aqueous extract was frozen at -80 °C and lyophilized in a benchtop freeze dryer (Labconco FreeZone 4.5, USA) to yield dry crystalline powders. The extraction yield for each solvent was calculated on a dry weight basis according to Eq. (1):

[Equation 1] Extraction Yield (%) = (Dry Mass of Extract (g) / Initial Dry Mass of Seed Powder (g)) × 100

The dried extracts were packaged in sterile amber glass vials, sealed under nitrogen flush, and stored at -20 °C until required for phytochemical, microbiological, and coagulation assays.

2.3 Qualitative Phytochemical Profiling

Standard qualitative colorimetric and precipitation screening assays were performed on each reconstituted *M. oleifera* extract (10 mg/mL) to detect the presence of major secondary metabolite classes according to standard phytochemistry protocols (Harborne, 1998; Evans, 2009):

- *Tannins (Ferric Chloride & Gelatin Tests):* 2 mL of extract was treated with 3 drops of 1% aqueous FeCl3; formation of a brownish-green or blue-black precipitate indicated the presence of gallic or catechol tannins. Confirmatory gelatin testing was conducted with 1% gelatin solution containing 10% NaCl.
- *Flavonoids (Shinoda & Alkaline Reagent Tests):* A piece of magnesium ribbon and 5 drops of concentrated HCl were added to 2 mL of extract; development of a crimson-red coloration confirmed flavonols. In alkaline testing, 2 mL of 2% NaOH was added, producing an intense yellow solution that turned colorless upon addition of dilute HCl.
- *Saponins (Frothing Test):* 5 mL of extract was vigorously shaken with 5 mL of distilled water in a graduated cylinder for 15 min; persistent honeycomb froth (>1 cm height) remaining stable for >15 min confirmed saponins.
- *Alkaloids (Mayer's & Dragendorff's Tests):* 2 mL of extract was acidified with 1% HCl and filtered. Aliquots were treated with Mayer's reagent (potassium mercuric iodide, yielding a cream precipitate) and Dragendorff's reagent (potassium bismuth iodide, yielding an orange-red precipitate).
- *Terpenoids and Steroids (Salkowski Test):* 2 mL of extract was dissolved in 2 mL of chloroform and treated with 3 mL of concentrated H2SO4; the appearance of a reddish-brown interfacial ring confirmed the presence of terpenoid aglycones.

2.4 Bacterial Strains, Inoculum Preparation, and Maintenance

Antimicrobial susceptibility assays were conducted against four clinically and environmentally significant reference bacterial strains obtained from the Microbial Type Culture Collection and Gene Bank (MTCC / ATCC): *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), Salmonella enterica serovar Typhi (ATCC 14028), and *Staphylococcus aureus* (ATCC 25923). Bacterial cultures were routinely maintained on Mueller-Hinton Agar (MHA; HiMedia, India) slants at 4 °C and subcultured every two weeks. Working inocula were prepared by transferring 3–4 isolated colonies into sterile Mueller-Hinton Broth (MHB) and incubating at 37 °C for 4–6 h until log-phase growth was reached. Cell suspensions were adjusted with sterile 0.85% physiological saline to match the turbidity of a 0.5 McFarland standard (absorbance of 0.08–0.10 at λ = 625 nm, corresponding to approximately 1.5 × 10^8 CFU/mL).

2.5 In Vitro Antimicrobial Assays

2.5.1 Agar Disc Diffusion Method

In vitro antibacterial activity was quantified using the Kirby-Bauer agar disc diffusion method in compliance with Clinical and Laboratory Standards Institute (CLSI M02-A12) guidelines. Sterile MHA plates (90 mm diameter, 4 mm agar depth) were seeded uniformly with 100 µL of standardised bacterial suspension using sterile cotton swabs. Sterile Whatman No. 1 filter-paper discs (6.0 mm diameter) were impregnated with 20 µL of reconstituted seed extracts (final concentration: 100 mg/mL in 5% DMSO; 2 mg extract/disc).

International Journal of Recent Development in Engineering and Technology
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Discs impregnated with 20 µL of 5% DMSO served as negative controls, while Ciprofloxacin discs (10 µg/disc; HiMedia, India) served as positive reference controls. The plates were allowed to pre-diffuse at 4 °C for 1 h, then inverted and incubated at 37 ± 1 °C for 24 h. Diameters of the clear zones of inhibition (ZOI) were measured in millimetres using a digital vernier calliper (Mitutoyo, Japan). Assays were conducted in biological triplicate.

2.5.2 Minimum Inhibitory Concentration (MIC) Determination

Broth microdilution assays were carried out in sterile 96-well polystyrene microtiter plates (Tarson, India) in accordance with CLSI (M07-A10) protocols. Stock solutions of each extract were prepared in MHB supplemented with 2.5% DMSO to enhance solubility. Two-fold serial dilutions were performed across the plates to yield final extract concentrations ranging from 0.78 mg/mL to 100.0 mg/mL. Each well was inoculated with 10 µL of the standardized bacterial suspension (final well inoculum ~ 5 × 10^5 CFU/mL) in a final volume of 200 µL per well. Growth controls (broth + inoculum), sterility controls (uninoculated broth), and solvent controls (broth + 2.5% DMSO + inoculum) were maintained on each plate. Microplates were incubated at 37 °C for 24 h. Microbial growth inhibition was verified visually and confirmed by adding 30 µL of 0.02% aqueous resazurin sodium dye (Sigma-Aldrich, USA) followed by incubation for 2 h. The lowest extract concentration that prevented a color change from blue (oxidized, no growth) to pink (reduced, viable bacteria) was recorded as the MIC.

2.5.3 Minimum Bactericidal Concentration (MBC) Determination

To establish the minimum bactericidal concentration, a 10 µL aliquot from each microplate well showing no visible bacterial growth or resazurin reduction in the MIC assay was subcultured onto fresh, antibiotic-free MHA plates. The plates were incubated at 37 °C for 24–48 h. The MBC was defined as the lowest extract concentration that killed ≥ 99.9% of the original bacterial inoculum, evidenced by the total absence of colony-forming units on the agar surface.

2.6 Environmental Water Sampling and Source Characterization

To evaluate the field efficacy of *M. oleifera* seed extract under natural matrix conditions, raw water samples (5 L each) were collected from four strategically selected drinking water sources across Mehkar Taluka, Buldhana District, Maharashtra, India:

- Site 1 (Protected Borewell, 20.1511° N, 76.5702° E): Deep groundwater borehole supplying community drinking water.
- Site 2 (Open Dug Well, 20.1584° N, 76.5689° E): Shallow unsealed domestic well subject to local surface infiltration.
- Site 3 (Surface River/Pond, 20.1630° N, 76.5821° E): Surface waterbody heavily influenced by agricultural runoff and livestock activity.
- Site 4 (Community Storage Tank, 20.1495° N, 76.5776° E): Overhead distribution reservoir storing municipal water.

Sampling was executed using pre-sterilized polypropylene carboys in accordance with APHA Standard Methods (2017). Samples were stored in dark insulated cooler boxes at 4 ± 1 °C during transit and processed in the laboratory within 4 h of collection.

2.7 Water Treatment Coagulation and Disinfection Protocol

Coagulation and flocculation trials were executed using a standard laboratory jar-test apparatus (Phipps & Bird / Remi Jar Tester, 6-paddle). Raw water samples were homogenized, and 1000-mL aliquots were distributed into 2-L glass beakers. Treatment regimens comprised:

1. *Untreated Control:* Raw sample stirred without coagulant addition.
2. *Moringa oleifera Treatment:* Dosed with freshly prepared aqueous seed extract (100 mg/L optimized coagulant dosage).
3. *Conventional Alum Treatment:* Dosed with analytical grade aluminium sulphate [Al2(SO4)3·18H2O] (50 mg/L benchmark dosage).

The jar-test protocol consisted of rapid flash mixing at 120 rpm for 2 min (to facilitate uniform coagulant dispersion and colloidal destabilization), followed by gentle slow flocculation mixing at 30 rpm for 20 min (to promote inter-particle bridge formation), and a quiescent gravitational settling period of 60 min. Supernatant samples were carefully siphoned from 2 cm below the water surface for immediate physicochemical and microbiological analysis.

2.8 Physicochemical and Microbiological Water Quality Testing

Water quality testing was conducted in triplicate before and after treatment according to standard APHA (2017) protocols:

International Journal of Recent Development in Engineering and Technology
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- Turbidity was determined using a calibrated digital Nephelometric Turbidimeter (Model HI-93703, Hanna Instruments, USA) and expressed in Nephelometric Turbidity Units (NTU).
- pH was determined using a benchtop glass-electrode pH meter (Model pH-510, Eutech Instruments, Singapore) calibrated against standard buffer solutions (pH 4.01, 7.00, and 10.01).
- Electrical Conductivity (EC, µS/cm) and Total Dissolved Solids (TDS, mg/L) were measured using a multi-parameter probe (Thermo Scientific Orion Star A212, USA).
- Microbiological indicator enumeration (Total Coliforms, Faecal Coliforms, and *E. coli*) was performed via the membrane filtration technique. Aliquots of 100 mL of water (diluted appropriately where necessary) were filtered through sterile 0.45-µm cellulose nitrate membrane filters (Millipore, USA). Total coliforms were cultured on M-Endo Agar LES at 35 ± 0.5 °C for 24 h; faecal coliforms were cultured on m-FC Agar at 44.5 ± 0.2 °C for 24 h; and *E. coli* was enumerated on HiCrome *E. coli* Agar at 37 °C for 24 h. Colonies were counted using a digital colony counter and expressed as CFU/100 mL. Log10 reduction factors were computed via Eq. (2):

[Equation 2] Log10 Reduction = Log10 (N_untreated) - Log10 (N_treated)

2.9 Statistical Analysis

All experimental determinations were performed in triplicates (n = 3 independent batches/replicates), and data are presented as Mean ± Standard Deviation (SD). Statistical significance was evaluated using One-Way Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for multiple pairwise comparisons using IBM SPSS Statistics (Version 26.0) and GraphPad Prism (Version 9.0). Differences were considered statistically significant at $p < 0.05$.

III. Results

3.1 Extraction Yield and Phytochemical Characterization

The extraction yield of *Moringa oleifera* seed constituents was profoundly influenced by the polarity of the extracting solvent (Table 1). Absolute methanol demonstrated the highest extraction efficiency, yielding 21.35 ± 0.47% w/w of crude extractable solids, followed in decreasing order by 95% ethanol (18.76 ± 0.58% w/w), acetone (15.62 ± 0.39% w/w), and distilled water (12.84 ± 0.42% w/w).

The extraction yield of methanol was significantly higher ($p < 0.01$) than all other evaluated solvent systems.

Table 1. Extraction yield of Moringa oleifera seed extracts obtained using different solvent systems (Mean ± SD, n = 3 independent batches).

Extraction Solvent	Dielectric Constant (ε)	Extraction Yield (% w/w, Mean ± SD)
Distilled Water	80.1	12.84 ± 0.42
Ethanol (95%)	24.5	18.76 ± 0.58
Methanol (Absolute)	32.7	21.35 ± 0.47
Acetone	20.7	15.62 ± 0.39

Qualitative phytochemical screening (Table 2) revealed distinct solubility patterns among secondary metabolite classes across the four solvents. Methanol and 95% ethanol successfully solubilized all five targeted phytochemical classes: tannins, flavonoids, saponins, alkaloids, and terpenoids. The aqueous extract tested positive for tannins, flavonoids, and saponins, but failed to extract detectable quantities of alkaloids and terpenoids. Conversely, acetone yielded positive tests for tannins, flavonoids, alkaloids, and terpenoids, but was incapable of extracting saponins.

Table 2. Qualitative phytochemical screening of Moringa oleifera seed extracts across different solvents.

Phytochemical Class	Distilled Water	Ethanol (95%)	Methanol	Acetone
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	-
Alkaloids	-	+	+	+
Terpenoids & Steroids	-	+	+	+

Note: (+) indicates presence; (-) indicates absence or below analytical detection limit.

International Journal of Recent Development in Engineering and Technology
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3.2 In Vitro Antibacterial Efficacy

The antibacterial activity of *M. oleifera* seed extracts varied significantly across solvents and bacterial test species ($p < 0.05$), as detailed in Table 3. Methanol extract demonstrated the most potent growth inhibition against all tested pathogens, generating clear inhibition zone diameters of 21.8 ± 0.5 mm for *S. aureus*, 20.4 ± 0.7 mm for *S. typhi*, 19.6 ± 0.6 mm for *E. coli*, and 17.2 ± 0.5 mm for *P. aeruginosa*. Ethanol (95%) exhibited the second highest potency (ZOI: 14.8–18.5 mm), followed by acetone (ZOI: 13.5–17.1 mm) and aqueous extract (ZOI: 8.9–12.6 mm). The Gram-positive bacterium *S. aureus* showed the greatest sensitivity across all solvent types, whereas the opportunistic Gram-negative pathogen *P. aeruginosa* exhibited the lowest susceptibility. Negative control discs (5% DMSO) produced zero inhibition zones (0.0 mm), confirming that the carrier solvent contributed no background antibacterial activity.

Table 3. Zone of inhibition (mm) of Moringa oleifera seed extracts against test bacterial pathogens via disc diffusion assay (Mean ± SD, n = 3).

Bacterial Strain	Distilled Water	Ethanol (95%)	Methanol	Acetone	Ciprofloxacin*
Escherichia coli (ATCC 25922)	10.8 ± 0.5	16.9 ± 0.7	19.6 ± 0.6	15.2 ± 0.5	27.8 ± 0.4
Staphylococcus aureus (ATCC	12.6 ± 0.4	18.5 ± 0.5	21.8 ± 0.5	17.1 ± 0.6	30.2 ± 0.5
Pseudomonas aeruginosa (ATCC	8.9 ± 0.4	14.8 ± 0.6	17.2 ± 0.5	13.5 ± 0.5	25.4 ± 0.4
Salmonella typhi (ATCC 14028)	11.3 ± 0.5	17.6 ± 0.6	20.4 ± 0.7	16.3 ± 0.5	28.6 ± 0.5

**Positive control: Ciprofloxacin (10 µg/disc). Extract disc load: 2.0 mg/disc. Negative control (5% DMSO) exhibited 0.0 mm zone.*

Quantitative determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) via broth microdilution corroborated the agar disc diffusion patterns (Table 4).

Methanolic extract exhibited the lowest MIC values (1.56–6.25 mg/mL) and MBC values (3.125–12.5 mg/mL), demonstrating that significantly smaller concentrations were required to achieve bacteriostatic and bactericidal endpoints. In comparison, the aqueous extract required considerably higher concentrations (MIC: 12.5–50.0 mg/mL; MBC: 25.0–100.0 mg/mL). Across all extracts, the MBC/MIC ratios ranged between 2.0 and 4.0, indicating definitive bactericidal rather than solely bacteriostatic mode of action.

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of M. oleifera seed extracts (mg/mL).

Extraction Solvent	Bacterial Pathogen	MIC (mg/mL)	MBC (mg/mL)
Distilled Water	*Escherichia coli*	25.00	50.00
Distilled Water	*Staphylococcus aureus*	12.50	25.00
Distilled Water	*Pseudomonas aeruginosa*	50.00	100.00
Distilled Water	*Salmonella typhi*	25.00	50.00
Ethanol (95%)	*Escherichia coli*	6.25	12.50
Ethanol (95%)	*Staphylococcus aureus*	3.125	6.25
Ethanol (95%)	*Pseudomonas aeruginosa*	12.50	25.00
Ethanol (95%)	*Salmonella typhi*	6.25	12.50
Methanol	*Escherichia coli*	3.125	6.25
Methanol	*Staphylococcus aureus*	1.56	3.125
Methanol	*Pseudomonas aeruginosa*	6.25	12.50
Methanol	*Salmonella typhi*	3.125	6.25
Acetone	*Escherichia coli*	12.50	25.00
Acetone	*Staphylococcus aureus*	6.25	12.50
Acetone	*Pseudomonas aeruginosa*	25.00	50.00
Acetone	*Salmonella typhi*	12.50	25.00

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3.3 Physicochemical Water Quality Transformation

Application of *M. oleifera* seed extract to raw environmental water samples achieved marked improvements in water clarity and quality (Table 5). Raw untreated water exhibited an average baseline turbidity of 48.7 ± 2.3 NTU across the four sampling sites. Treatment with *M. oleifera* coagulant reduced turbidity sharply to 8.6 ± 0.7 NTU, corresponding to an 82.3% clarification efficiency. Conventional alum treatment produced a slightly greater clarification, reducing turbidity to 4.3 ± 0.5 NTU (91.2% removal).

Importantly, *M. oleifera* treatment maintained a near-neutral, potable pH (7.35 ± 0.08 compared to 7.82 ± 0.09 in untreated water), whereas alum treatment caused a statistically significant drop in pH to 6.94 ± 0.07 due to the acidic hydrolysis of aluminium sulphate. Minor, non-significant reductions were observed in electrical conductivity (from 612 ± 15 to 586 ± 12 µS/cm) and total dissolved solids (from 384 ± 11 to 366 ± 9 mg/L) following Moringa treatment, confirming that the biocoagulant did not introduce excessive soluble ionic loads into the treated water.

Table 5. Physicochemical parameters of water samples before and after coagulation treatment (Mean ± SD, pooled across 4 sampling sites).

Water Quality Parameter	Raw (Untreated)	Moringa-Treated	Alum-Treated
Turbidity (NTU)	48.7 ± 2.3	8.6 ± 0.7	4.3 ± 0.5
pH	7.82 ± 0.09	7.35 ± 0.08	6.94 ± 0.07
Electrical Conductivity	612 ± 15	586 ± 12	579 ± 10
Total Dissolved Solids (mg/L)	384 ± 11	366 ± 9	359 ± 8

3.4 Microbiological Decontamination and Indicator Log Reduction

Microbiological examination demonstrated robust pathogen inactivation and removal following *M. oleifera* treatment (Table 6).

Raw water harboured substantial microbial contamination: Total Coliforms (3.8 × 10^4 ± 2.1 × 10^3 CFU/100 mL), Faecal Coliforms (1.6 × 10^4 ± 1.3 × 10^3 CFU/100 mL), and *E. coli* (9.5 × 10^3 ± 8.6 × 10^2 CFU/100 mL).

Following coagulation-flocculation and settling with *M. oleifera* seed extract, bacterial counts plummeted to 4.5 × 10^2 ± 3.8 × 10^1 CFU/100 mL for total coliforms (1.93-log10 reduction; 98.8% inactivation), 1.8 × 10^2 ± 2.2 × 10^1 CFU/100 mL for faecal coliforms (1.95-log10 reduction; 98.9% inactivation), and 8.7 × 10^1 ± 1.6 × 10^1 CFU/100 mL for *E. coli* (2.04-log10 reduction; 99.1% inactivation). Although alum treatment achieved marginally higher log-reductions (2.22 to 2.36-log10), *M. oleifera* provided highly comparable decontamination without introducing synthetic chemical residuals.

Table 6. Microbiological quality and indicator log10 reduction in water samples pre- and post-treatment (Mean ± SD, CFU/100 mL).

Microbial Indicator	Raw Water (Untreated)	Moringa-Treated	Alum-Treated	Log10 Reduction (Moringa)
Total Coliforms	$3.8 \times 10^4 \pm 2.1 \times 10^3$	$4.5 \times 10^2 \pm 3.8 \times 10^1$	$2.3 \times 10^2 \pm 2.5 \times 10^1$	1.93
Faecal Coliforms	$1.6 \times 10^4 \pm 1.3 \times 10^3$	$1.8 \times 10^2 \pm 2.2 \times 10^1$	$8.5 \times 10^1 \pm 1.4 \times 10^1$	1.95
Escherichia coli	$9.5 \times 10^3 \pm 8.6 \times 10^2$	$8.7 \times 10^1 \pm 1.6 \times 10^1$	$4.2 \times 10^1 \pm 9.0$	2.04

IV. Discussion

The escalating global water crisis necessitates decentralized, cost-effective, and ecologically benign water purification modalities. The present findings provide compelling empirical evidence that *Moringa oleifera* seed extracts function as exceptionally effective dual-action biocoagulants and natural bactericides. Our investigation systematically connects the solvent-dependent recovery of phytochemicals to *in vitro* antibacterial potency and real-world water matrix clarification.

Solvent polarity played a decisive role in determining extraction yield and the qualitative distribution of secondary metabolites. Absolute methanol yielded the highest mass recovery (21.35 ± 0.47%), outperforming ethanol, acetone, and water.

This is consistent with fundamental principles of solvent-solute thermodynamics: methanol possesses both polar hydrophilic hydroxyl functionality and a small lipophilic methyl group, facilitating the simultaneous dissolution of polar polyphenols and moderately hydrophobic aglycones (Delelegn *et al.*, 2018). Qualitative profiling confirmed that methanolic and ethanolic extracts retained a comprehensive phytoconstituent matrix, whereas aqueous extraction failed to isolate hydrophobic alkaloids and terpenoids, and acetone failed to extract hydrophilic saponins.

The *in vitro* antibacterial data established that *M. oleifera* seed extracts exert pronounced, broad-spectrum growth inhibition against both Gram-negative (*E. coli*, *S. typhi*, *P. aeruginosa*) and Gram-positive (*S. aureus*) pathogens. Methanol extracts generated the largest inhibition zones (up to 21.8 mm) and the lowest MIC/MBC values (1.56–6.25 mg/mL), directly reflecting the synergistic richness of its extracted phytochemical pool. *S. aureus* displayed the highest vulnerability, which aligns with classical microbial architecture: Gram-positive bacteria lack the protective lipopolysaccharide (LPS) outer membrane barrier characteristic of Gram-negative organisms, rendering their peptidoglycan cell walls readily permeable to cationic peptides and phenolic disruptors (Fahey, 2005; Rahman *et al.*, 2009). Conversely, *P. aeruginosa* demonstrated greater intrinsic tolerance, attributable to its outer membrane porin restrictions and active multidrug efflux pump systems (Bancessi *et al.*, 2020).

Mechanistically, the water purification performance of *M. oleifera* operates via a dual, complementary pathway: physicochemical particle bridging and direct membrane-targeted bactericidal action. During the coagulation phase, low-molecular-weight cationic dimeric proteins (such as MO2.1 and MOCP) adsorb onto the electronegative surfaces of suspended colloidal clays, mineral particles, and bacterial cells via charge neutralization (Ndabigengesere *et al.*, 1995; Ghebremichael *et al.*, 2005). The neutralization of repulsive zeta potentials enables rapid polymer bridging, forming dense macromolecular flocs that enmesh planktonic bacteria and settle out by gravity. Concurrently, bioactive tannins, flavonoids, and cationic lectins disrupt bacterial membrane integrity, inducing intracellular leakages of essential electrolytes, nucleotides, and potassium ions, leading to irreversible cell lysis (Suarez *et al.*, 2003; Delelegn *et al.*, 2018).

A pivotal operational advantage demonstrated in this study is the buffering stability of *M. oleifera*. Unlike conventional alum—which hydro-chemically dissociates to release sulphuric acid and lowers water pH toward acidic levels (6.94 ± 0.07)—Moringa-treated water maintained an optimal, stable pH of 7.35 ± 0.08. This eliminates the need for post-treatment chemical pH re-adjusters (e.g., lime or sodium carbonate), substantially reducing operating expenses and technical complexity in rural installations (Nkurunziza *et al.*, 2009; Semanka *et al.*, 2022). Furthermore, *M. oleifera* coagulant does not impart toxic metallic residues or generate hazardous non-biodegradable sludge, establishing it as a truly sustainable, zero-waste water purification biotechnology.

V. Conclusion

In conclusion, this study demonstrates that *Moringa oleifera* seed extracts represent an exceptional, eco-friendly, and highly effective natural coagulant and broad-spectrum antimicrobial agent for drinking water purification. Methanol proved to be the superior solvent for extracting bioactive secondary metabolites, yielding the highest extract mass (21.35%) and demonstrating superior *in vitro* antibacterial potency with minimum inhibitory concentrations down to 1.56 mg/mL. When applied to contaminated multi-source water, *M. oleifera* seed extract reduced raw water turbidity by 82.3% and achieved approximately 2.0-log10 reductions in total coliforms, faecal coliforms, and *Escherichia coli*, while preserving potable pH and electrical conductivity. These findings substantiate *M. oleifera* as a viable, low-cost, biodegradable alternative to conventional aluminium sulphate coagulants, offering transformative potential for decentralized household and community-level primary water treatment in resource-limited regions.

VI. Limitations Of The Study

Several methodological and operational limitations should be acknowledged:

1. *In Vitro Laboratory Testing:* Antibacterial evaluations were performed under controlled laboratory conditions utilizing standard ATCC reference strains, which may not fully reflect the diverse physiological states, biofilm dynamics, or phenotypic resistance profiles of wild environmental strains.

2. *Extract Shelf-Life and Storage Stability:* The study evaluated freshly prepared extracts; further data are required regarding the long-term degradation kinetics and thermal stability of active cationic coagulant proteins during prolonged storage in tropical climates.
3. *Sourcing and Seasonal Variability:* Seed batches were collected from a single agro-climatic zone during one harvest cycle; geographic, soil, and seasonal variations in seed phytochemistry warrant broader multi-origin sampling.
4. *Toxicological and Residual Solvent Considerations:* While aqueous extraction is immediately deployable, the practical drinking water use of organic solvent extracts (methanol, ethanol, acetone) requires extensive residual solvent stripping, cytotoxicity profiling, and safety validation before full-scale human consumption.

VII. Recommendations And Future Scope

Based on the experimental outcomes, the following research directions and field applications are recommended:

1. *Combined Point-of-Use Multi-Barrier Systems:* Integrate *M. oleifera* biocoagulation as a pretreatment stage coupled with slow sand, biochar, or volcanic scoria filtration to achieve complete (>4-log10) pathogen elimination and satisfy stringent WHO drinking water standards.
2. *Active Principle Isolation:* Conduct chromatographic purification (e.g., bioassay-guided fractionation, HPLC-MS) to isolate, sequence, and structurally characterize the specific cationic peptides responsible for enhanced coagulation and bactericidal synergy.
3. *Pilot Community-Scale Deployments:* Establish continuous-flow pilot water treatment units in rural WASH-deprived settlements to assess operational robustness across monsoon and dry seasons.
4. *Eco-toxicological Safety Profiling:* Perform standard mammalian cytotoxicity, sub-chronic oral toxicity, and genotoxicity evaluations on concentrated purified seed fractions.

VIII. Declarations

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Author Contributions (CRediT): S.A.A. conceived and designed the study, performed all laboratory extractions, microbiological assays, and water treatment experiments, analyzed the data, and authored the manuscript.

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