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Eco-Friendly Water Treatment: Antibacterial & Coagulation Potential of *Moringa oleifera* Seeds from Bundelkhand, India

Dr.Amit Kumar Tiwari[1],Shahnaz Bano[2], Shweta Jaiswal[3],Ganga Gupta[4]
[1]*Assistant Professor, Institute of Basic Science, Department of Botany, Bundelkhand University, Jhansi, Uttar Pradesh, India*
[2,3,4]*Research Scholars,Institute of Basic Science, Department of Botany, Bundelkhand University, Jhansi, Uttar Pradesh, India*
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Abstract-Safe drinking water remains a challenge in many rural and drought-prone regions. This study explores the dual role of *Moringa oleifera* seed powder and its solvent extracts — as a natural coagulant for water purification and as an antibacterial agent against waterborne pathogens. Water samples from various sources (wells, hand-pumps, ponds, rivers) across the Bundelkhand region were subjected to treatment with seed powder; concomitantly, methanol, acetone, and aqueous extracts of the seeds were tested against *Salmonella typhii* (MTCC 98) and *Shigella dysenteriae* (ATCC 23513). The acetone extract exhibited the strongest antibacterial activity (inhibition zone 18.0 mm for S. typhii) and lowest MIC (5.8–6.0 mg/mL). Additionally, treatment with seed powder resulted in significant turbidity reduction and dramatic decrease in coliform and total bacterial counts. The results underscore that *M. oleifera* seeds offer an affordable, eco-friendly, and locally accessible solution for improving potable water quality and combating waterborne microbial diseases an approach especially relevant for low-income and resource-limited settings.

Index Terms: *Moringa oleifera*, natural coagulant, antibacterial activity, water purification, seed extract, MIC, turbidity reduction, waterborne pathogens.

I. INTRODUCTION

Access to safe and clean drinking water is a major public health concern worldwide, particularly in developing and drought-prone regions where conventional water treatment infrastructure may be inadequate. Chemical coagulants such as alum and synthetic polymers are widely used but pose risks including harmful by-products, toxicity, and disposal issues.

As an alternative, natural coagulants derived from plants have gained attention due to their biodegradability, low cost, eco-friendliness, and local availability. Among these, *Moringa oleifera* has shown significant promise: its seeds contain water-soluble cationic proteins and phytochemicals capable of flocculation, turbidity reduction, and antimicrobial activity (1)

Previous studies have demonstrated that crushed or powdered *M. oleifera* seeds substantially reduce turbidity, suspended solids, and microbial load in raw waters often with removal efficiencies exceeding 80-90%. Further, isolated seed proteins such as the low-molecular weight (~6–7 kDa) cationic peptides have shown flocculant and bactericidal activity under simulated water treatment conditions(2&3).

Given the widespread cultivation and adaptability of *M. oleifera* in semi-arid and arid regions, including Bundelkhand, its use as a natural water treatment agent holds substantial potential for improving public health and water security in resource-limited rural settings. This study aims to evaluate both 1. the water-purifying (coagulation disinfection) efficacy of locally obtained *M. oleifera* seed powder on varied water sources, and 2. The antibacterial potency of different solvent extracts of seed powder against two common waterborne pathogens.

II. MATERIALS AND METHODS

2.1 Study Area and Water Sampling

Water samples were collected between JuneSeptember 2022-2023 from ten districts of Bundelkhand: Jhansi, Lalitpur, Mahoba, Banda, Chitrakoot, Hamirpur, Tikamgarh, Datia, Panna, and Sagar. Sources included wells, hand-pumps, ponds, and rivers representative of typical rural water sources often used for drinking and household needs.

2.2 Seed Collection and Preparation

2.3 Preparation of Solvent Extracts

50 g of seed powder was sequentially extracted with 250 mL of methanol, acetone, and distilled water (aqueous), representing increasing solvent polarity. For each solvent: the mixture was shaken for 72 hours, filtered (Whatman No. 1), centrifuged at 5000 rpm for 15 min, and the supernatant evaporated under reduced pressure using a rotary evaporator. The dried extracts were weighed and reconstituted to defined concentrations for antibacterial assays.

2.4 Water Purification Treatment

Raw water samples were treated with varying concentrations of seed powder (experimentally optimized based on turbidity and microbial load assays). Treated samples were allowed to settle (or flocculate) for a fixed time; thereafter, supernatants were analyzed for turbidity, coliform count, total bacterial count, and relevant physico-chemical parameters (pH, conductivity, TDS).As a control, comparative treatments using conventional alum (aluminum sulfate) were also performed, where necessary.

2.5 Antibacterial Assays

2.5.1 Test Organisms

- *Salmonella typhii* (MTCC 98)
- *Shigella dysenteriae* (ATCC 23513)

2.5.2 Agar Well Diffusion

Bacterial suspensions were adjusted to ~10^8 cells/mL (0.5 McFarland standard). Mueller–Hinton agar plates were swabbed uniformly, wells (6 mm diameter) created, and 50 µL of each extract (50 mg/mL) added. A standard antibiotic (Amoxicillin, 25 µg/mL) was used as positive control. Plates were incubated at 37 °C for 24–48 h, and zones of inhibition (ZOI) measured. ZOI ≥ 7 mm was considered indicative of susceptibility.

2.5.3 Minimum Inhibitory Concentration (MIC)

For extracts showing significant inhibition, MICs were determined by serial two-fold dilutions in appropriate broth, incubated for 24 h, and the lowest concentration that prevented visible growth recorded as MIC

III. RESULTS

3.1 Water Purification Efficacy

Treatment with M. oleifera seed powder led to a marked reduction in turbidity, coliform count, and total bacterial load across various water sources. In certain cases, total coliforms and fecal coliforms were reduced by more than 90%, comparable to traditional chemical coagulants, without significant change in pH. These findings align with earlier reports where seed-derived coagulant significantly reduced turbidity (up to ~84–98%) and microbial loads in raw water samples(4).

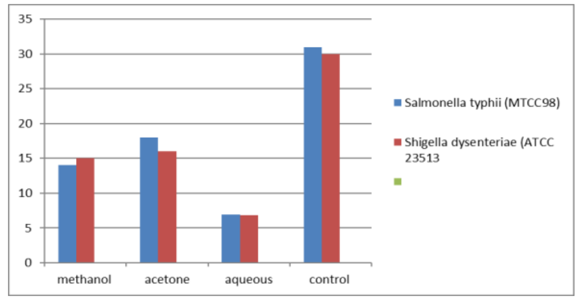
3.2 Antibacterial Activity of Seed Extracts

Table1: Bacterial growth inhibition zones in agar well diffusion method treated with 50 mg/mL of methanol, acetone and aqueous extract of *Moringa oleifera* seed and control

Organism	Methanol Extract (ZOI mm)	Acetone Extract (ZOI mm)	Aqueous Extract (ZOI mm)	Positive Control (Amoxicillin)	MIC for Acetone (mg/mL)
Salmonella typhii	14 ±1	18 ±1	6.9 ±1	31 ±1	5.8–6.0
Shigella	15 ±1	16 ±1	6.8 ±1	30 ±1	5.8–6.0

Organism	Methanol Extract (ZOI mm)	Acetone Extract (ZOI mm)	Aqueous Extract (ZOI mm)	Positive Control (Amoxicillin)	MIC for Acetone (mg/mL)
dysenteriae					

Acetone extract exhibited the highest antibacterial activity, with a clear inhibition zone and low MIC values, indicating strong potency at relatively low concentrations. Methanol extract also showed moderate activity, while aqueous extract was least effective. These results confirm prior findings that non-polar or intermediate polarity solvents (like acetone) better extract bioactive compounds such as polyphenols, flavonoids, and possibly cationic peptides which contribute to antibacterial activity(2)



Graph 1: Comparison of bacterial growth inhibition zones obtained by agar well diffusion method using 50 mg/mL methanol, acetone, and aqueous extracts of *Moringa oleifera* seeds versus control.

Table2:Minimum inhibitory concentration (MIC) of seed extracts of Moringa oleifera

Organism		Seed extract MIC and MBC mg/ml		
	Methanol	Acetone	Aqueous	Positive control (Amoxicillin)
Salmonella typhii (MTCC98)	13-14 mg/mL	5.8-6 mg/mL	25.8-26 mg/mL	0.0625 - 0.25 mg/ml.
Shigella dysenteriae (ATCC 23513)	6-6.50 mg/mL	5.8 -6mg/mL	25 -27mg/mL	0.625 - 0.25 mg/ml

IV. DISCUSSION

4.1 Mechanism: Coagulation, Flocculation & Antimicrobial Action

The effectiveness of *M. oleifera* seed powder in water clarification is widely attributed to water-soluble cationic proteins (coagulant proteins) that neutralize negatively charged particles (clay, silt, bacteria) causing floc formation, which then settle or can be filtered.(1)

Specifically, a low-molecular-weight (~6–7 kDa) cationic peptide often referred to as MO_2.1 has been characterized, showing both coagulation and antibacterial activity, even maintaining stability at high temperature (95 °C for 5 h).(3) Such proteins, along with phytochemicals (tannins, flavonoids, saponins), likely act synergistically: the proteins cause flocculation and physical removal, while phytochemicals exert bactericidal or bacteriostatic effects.

4.2 Advantages over Conventional Chemicals

- Eco-friendly & non-toxic: Unlike alum or synthetic polymers, *M. oleifera* seeds do not significantly alter water pH or leave harmful residuals(3)
- Locally available and low-cost: In regions like Bundelkhand, where Moringa trees grow easily on marginal soils, seeds can be harvested locally makingthis approach sustainable and community-driven.
- Dual benefit: Simultaneous coagulation (turbidity/coliform reduction) and disinfection (antibacterial effect) reduces reliance on multiple treatment steps or chemicals.

4.3 Limitations and Considerations

- Extract variability: Solvent choice significantly affects yield and potency of bioactive compounds. Aqueous extracts often show lower antimicrobial activity, suggesting water alone may not extract responsible compounds efficiently.

- Sludge generation: As with any coagulant, flocculation leads to sludge formation, which must be safely disposed of or managed. Some studies note that seed-based flocculation produces more organic sludge compared to alum.
- Incomplete removal of chemical pollutants: While effective at removing turbidity, bacteria, and some pathogens, seed treatment may not significantly reduce other contaminants (e.g., heavy metals, nitrates, some dissolved organic compounds) hence, may need to be combined with additional treatment steps depending on water quality. Studies show limited effect on nutrients (e.g., phosphates, nitrates) post-treatment(4)
- Standardization and scalability: For large-scale or community-level use, standard dosage, extraction method, and treatment protocols need optimization.

4.4 Broader Evidence from Recent Literature

- A recent wastewater-treatment study demonstrated that *M. oleifera* seed aqueous extract (MOS) achieved >99% removal in bacterial load, turbidity, and color in tertiary domestic wastewater treatment, with lower cytotoxicity and less bulky sludge compared to alum though removal of nutrients remained modes.
- Another recent transdisciplinary investigation (2024) showed that MOS treatment of domestic wastewater significantly reduced total bacterial load and even lowered antibiotic-resistant bacteria and antibiotic-resistance-genes (ARGs), highlighting its potential role in limiting spread of antimicrobial resistance (4 &6).
- An integrative review summarizing globally published work confirms that M. oleifera seeds are among the most studied and effective natural coagulants, capable of reducing turbidity, color, and microbial contamination often without pH alteration (5,6&7) These findings strengthen the conclusion that *M. oleifera* seed-based treatment is a viable, sustainable alternative especially for rural and low-resource communities.

V. CONCLUSION & RECOMMENDATIONS

This study provides further evidence that Moringa oleifera seeds a locally available and affordable resource can act as an effective, eco-friendly dual-function agent, both clarifying water (coagulation + flocculation) and reducing bacterial contamination. In particular, acetone extracts show strong in-vitro antibacterial activity against common waterborne pathogens at relatively low MIC values.

Given the benefits and limitations, we recommend:

1. Community-levelimplementation trials deploying seed-powder treatment in rural drinking water systems (wells, ponds, hand-pumps) to assess real-world efficacy, user acceptability, and safety. This study has successfully tested the use of Moringa seed powder for purifying drinking water from wells, ponds, and hand-pumps in Bundelkhand villages and Jhansi city. Further implementation can be done in collaboration with Jal Sansthan and Jhansi Municipal Corporation to benefit local communities
2. Standardization of protocols optimize seed dosage, extraction/treatment method, settling/filtration time, and sludge handling to ensure consistent water quality.
3. Combined treatment strategies integrate seed-based coagulation with other treatment steps (e.g., filtration, disinfection) when chemical or biological contaminants (heavy metals, pesticides, antibiotic-resistant bacteria) are present.
4. Further research on active compounds Further research will focus on separating and identifying the main active compounds in Moringa seeds, especially the protein and peptide molecules that help clean water. By purifying these compounds, we can develop a stable and concentrated product for water purification that works better and can be used safely on a larger scale.
5. Monitoring long-term health and environmental safety evaluate potential long-term effects of seed-derived coagulant use on water chemistry,

residual organic matter, microbial ecology, and sludge disposal.

This study shows that Moringa seed powder can safely and effectively purify drinking water from wells, ponds, and hand-pumps in the Bundelkhand region and Jhansi city. Future work should monitor long-term health and environmental safety, including changes in water chemistry, remaining organic matter, microbial balance, sludge disposal, and overall ecological impact.

Industrial expansion in urban areas [e.g., Bundelkhand Industrial Development Authority, (BIDA] may lead to loss of plant species, so sustainable botanical practices are needed. The study recommends that Uttar Pradesh State Government and Central Governmentpromote Moringa plantation in urban green spaces and community areas. This can support biodiversity, provide raw material for water treatment, and improve public participation and environmental awareness. Collaboration with Jal Sansthan and Jhansi Municipal Corporation can help bring these solutions to local communities.

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REFERENCES

[1] Ferreira RS, Napoleão TH, Santos AF, Sá RA, Carneiro-da-Cunha MG, Morais MM, Silva-Lucca RA, Oliva ML, Coelho LC, Paiva PM. Coagulant and antibacterial activities of the water-soluble seed lectin from *Moringa oleifera*. Lett Appl Microbiol. 2011 Aug;53(2):186-92. doi: 10.1111/j.1472-765X.2011.03089.x.Epub 2011 Jun 10. PMID: 21605145.

[2] Delelegn, A., Sahile, S. & Husen, A. Water purification and antibacterial efficacy of *Moringa oleifera* Lam. Agric & Food Secur 7, 25 (2018). https://doi.org/10.1186/s40066-018-0177-1

[3] Ghebremichael KA, Gunaratna KR, Henriksson H, Brumer H, Dalhammar G. A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. Water Res. 2005Jun;39(11):233844.doi:10.1016/j.watres.2005.04.012. PMID: 15921719.

[4] Sané, N., Sivalingam, P., Koželuh, M. et al. Effect of *Moringa oleifera* seeds on the removal of pathogens and pharmaceutical residues in a domestic wastewater treatment plant by an interdisciplinary approach. Environ Sci Pollut Res 31, 65123–65136 (2024). https://doi.org/10.1007/s11356-024-35362-8

[5] Maria Thalillian Santos Figueiredo,Claudimary Bispo dos Santo. et al. "Water Treatment Using *Moringa Oleifera* Seed Extract: An Integrative Review". 2022. Research, Society and Development 11 (2): e41411225889. https://doi.org/10.33448/rsd-v11i2.25889.

[6] Abu-Gharbia, M. A., Farghaly, M. M., &Haridi, A. M. (2024). *Moringa oleifera* Seed Extracts as a Natural Coagulant for Raw Water Treatment and Improving its Quality. Sohag Journal of Sciences, 9(2), 114–120.

[7] Figueiredo, M. T. S., dos Santos, C. B., dos Santos, M. H., da Silva, D. K., & Oliveira, T. L. R. (2023). Water treatment using *Moringa oleifera* seed extract: An integrative review. Research, Society and Development.