JOURNAL OF INTERDISCIPLINARY PERSPECTIVES

ISSN Print: 2984-8288, ISSN Online: 2984-8385
Vol. 4 No. 4, pp. 304-310, April 2026

Original Article

Efficacy of Crude Extract from *Moringa oleifera* Leaves as an Alternative Bactericide for *Escherichia coli* in Wastewater

Mark P. Vacunawa, Alfred John D. Sy, Vhon Daryl A. Creencia, Alexander Gregg M. Atorda

Author Information:

St. Dominic College of Asia, Bacoor City, Cavite, Philippines

Correspondence:
markpvacunawa@gmail.com

Article History:

Date received: February 19, 2026
Date revised: March 25, 2026
Date accepted: April 1, 2026

Recommended citation:

Vacunawa, M., Sy, A.J., Creencia, V.D., & Atorda, A.G. (2026). Efficacy of crude extract from *Moringa oleifera* leaves as an alternative bactericide for *Escherichia coli* in wastewater. *Journal of Interdisciplinary Perspectives*, 4(4), 304-310. https://doi.org/10.69569/jip.2026.082

Abstract. This study examined the antibacterial effect of crude extract from *Moringa oleifera* leaves against *Escherichia coli* in wastewater from the Imus River. To address limited evidence on the use of macerated moringa extract for river water disinfection, three extract concentrations (5 mL, 15 mL, and 30 mL) were tested under a fixed wastewater volume and 10-minute contact time. Fresh moringa leaves were air-dried, milled, and macerated in 95% ethanol for 48 hours, then concentrated to obtain a crude extract. *E. coli* in wastewater samples was confirmed through colonial morphology on Eosin Methylene Blue agar and Gram staining. Samples were treated in three trials per concentration, with untreated controls. After 24 hours of incubation, colony-forming units were counted and analyzed using one-way ANOVA. Results showed a dose-dependent reduction in *E. coli* counts, with mean colony counts of 543.33 in the control, 383.33 at 5 mL, 159.33 at 15 mL, and 63.33 at 30 mL. ANOVA revealed a significant difference among groups ($F = 18.07$, $p < .001$). These findings indicate that crude *Moringa oleifera* leaf extract significantly reduced *E. coli* in wastewater and may serve as a low-cost natural bactericide for preliminary disinfection. Further studies should improve extraction methods and compare their efficacy with standard disinfectants.

Keywords: *Alternative bactericide; Colonial morphology; Crude extract; Escherichia coli; Wastewater treatment.*

Water is a vital resource for all living organisms, including humans and ecosystems. Water contamination has adversely affected the ecosystem and human health, exacerbated by urban and industrial growth (El-Azazy et al., 2019). Recent studies have demonstrated the worldwide prevalence of *Escherichia coli* (*E. coli*) discharges from wastewater treatment facilities, highlighting the imperative for improved microbiological water quality management to mitigate health risks associated with contaminated effluents (Li, 2025). One of the major concerns is the existence of coliform bacteria, especially *E. coli*, in water systems. This occurs when residents near bodies of water disregard regulations for proper waste disposal (De Castro, 2022). E. coli and other pollutants, such as heavy metals and nutrients, have been found to persist in urban streams and wastewater treatment plant water. This demonstrates how risky conventional therapeutic approaches might be in crowded settings (Tasselli et al., 2025).

Contaminated water is a serious health risk, so this problem needs to be fixed right now. Enteric bacteria like *E. coli* may make aquatic diseases more common. Kunz et al. (2024) observed that this occurred sporadically from 2015 to 2020 due to inadequate treatment of potable water. The gathered data supported this claim. Chemical and physical treatment methods are widely used on water and wastewater. However, these things are expensive and harm both people and the environment. Researchers are presently examining the effectiveness of plant-derived disinfectants, adsorbents, and coagulants (Kumar et al., 2024). Recent research suggests that plant-derived materials can serve as long-lasting substitutes. They can reduce silt and chemical pollutants and remove turbidity, pathogens, and heavy metals (Kumar et al., 2024; Lwasa et al., 2024).

Moringa oleifera, often known as Malunggay, is valued for its nutritional properties and health benefits, particularly its ability to prevent infections (Pelegrino, 2022). Methanol, n-hexane, and ethyl acetate are some of the solvent extracts found in the seeds and leaves of *Moringa oleifera*. Other compounds include flavonoids, phenols, saponins, alkaloids, tannins, and steroids. These medications alleviate specific adverse symptoms related to bacterial gastroenteritis and target infections (Adji et al., 2022). Studies show that moringa leaf extracts exhibit considerable antibacterial properties against *E. coli* (Raheem et al., 2015; Abbas & Elsharbasy, 2018). In addition, Wizrah et al. (2025) emphasized that it is also effective at killing Gram-negative bacteria such as *E. coli,* as supported by the study of El-Sherbiny et al. (2024), which found that ethanol extracts exhibit inhibition zones of up to 23 mm and minimum inhibitory concentrations as low as 50 µg/mL, due to bioactive compounds that disrupt bacterial cell membranes. Transmission electron microscopy has shown that they break down cell walls, which causes cytoplasmic leakage (Ali et al., 2025; Selmi et al., 2025). Miller et al. (2024) assert that aqueous seed extracts exhibit antibacterial properties, particularly against Gram-positive bacteria and *E. coli*.

Moringa oleifera can reduce *E. coli* counts by 1 to 3 logs and lower turbidity in drinking water by more than 90% when applied appropriately, indicating the need for further toxicological and scalability evaluations (Shah et al., 2025; Sané et al., 2024). This current study aims to evaluate the effectiveness of a crude moringa leaf extract as an alternative bactericide for *E. coli* in effluent. This will utilize a maceration extraction approach to do a comparative examination of the antibacterial characteristics of moringa and traditional chemical treatments, aiming to fill the research gap and offer effective solutions for wastewater management. Recent evaluations have revealed efficacy rates exceeding 99%, highlighting *Moringa oleifera*'s dual role as a coagulant and an antibacterial agent, thereby decreasing coliform levels (Al-Jadabi et al., 2023).

This discovery is significant because it will encourage the sustainable use of natural resources and the potential to reduce *E. coli* levels in wastewater. Phytoremediation methods leverage the antiviral and antibacterial properties of plants like Moringa to offer environmentally sustainable and cost-effective alternatives to energy-intensive cleanup processes. In hybrid systems, these solutions can simultaneously reduce infections by 3–7 logs while enhancing biodiversity and population health in resource-limited regions (Zure et al., 2026).

Methodology

Research Design

This study employed a true experimental design to evaluate the cause-and-effect relationship between concentrations of crude moringa extract and the reduction of *Escherichia coli* (*E. coli*) in wastewater. This design was selected for its ability to test the efficacy of the extract as an alternative bactericide, allowing controlled manipulation of variables to address the research question on mitigating water pollution.

Research Instrument

The primary instruments included Eosin Methylene Blue (EMB) agar plates for cultivating and identifying *E. coli* colonies, adapted from standard microbiological protocols (Tille, 2017). Gram staining reagents (crystal violet, iodine solution, 95% ethanol, safranin) were used to differentiate Gram-negative bacteria according to established methods (Kaiser, 2009). Validity was ensured through pilot testing of staining and colony count, confirming the reliability of detecting *E. coli* via green metallic sheen. Additional tools included a manual colony counter for CFU enumeration, a compound microscope for morphological analysis, and standard lab apparatus (e.g., water bath, Thomas Wiley mill, volumetric flasks) for extract preparation and measurements.

Data Gathering Procedure

Moringa leaves were harvested from Bacoor, Cavite, washed, air-dried, separated from stems, sun-dried, and ground into powder using a Thomas Wiley mill. Crude extract was obtained via maceration: 475 g of powdered leaves were soaked in 95% ethanol for 48 hours with occasional stirring, filtered, and concentrated in a water bath at 60°C to yield a semi-solid extract, which was stored at 2-4°C (adapted from Liberal et al., 2022). Wastewater samples were collected from the Imus River (Salinas 1, Pinyahan Street), filtered to remove solids, and tested for *E. coli* presence by Gram staining and by colony morphology on EMB agar.

Rationale of Extraction Method and Comparison to Green Alternatives

95% ethanol maceration was selected for its simplicity, minimal equipment needs, and proven high yield of antibacterial flavonoids and phenols from *M. oleifera* leaves. Comparative studies show that maceration with 70–80% ethanol achieves phenolic and antimicrobial efficacy comparable to ultrasound-assisted extraction (UAE) while requiring no specialized sonicator or high-energy input (Liga et al., 2025). Although UAE or vacuum-assisted methods can increase total phenolic recovery by 37–48% and shorten processing time (Gomes et al., 2025; Sandeep et al., 2023), they require costly equipment that is often unavailable in many Philippine laboratory or community settings. Aqueous extraction yields lower non-polar bioactives and weaker antibacterial activity against *E. coli* than ethanol (Deepali et al., 2025). Thus, conventional maceration remains the most accessible, low-cost, and sustainable option for preliminary wastewater disinfection in resource-limited tropical environments, aligning with our study objectives.

The experiment used three concentration set-ups, each with three trials, conducted face-to-face in the laboratory over 10 minutes at room temperature: (a) 5 mL extract in 0.095 L wastewater; (b) 15 mL in 0.085 L; (c) 30 mL in 0.070 L. The total treatment volume was deliberately standardized to 0.1 L (100 mL) across all setups by adjusting the wastewater volume accordingly. This volumetric standardization isolates the dose-dependent effect of extract concentration on *E. coli* reduction and eliminates dilution ratio as a confounding variable, following standard antimicrobial dose-response protocols (Raheem et al., 2015; Peixoto et al., 2011). Treated samples were streaked onto EMB agar plates using a sterilized inoculating loop in a whole-quadrant pattern, incubated for 24 hours, and colonies counted manually. Safety measures included autoclaving glassware and using personal protective equipment.

Although a direct experimental positive control using a standard chemical disinfectant (e.g., free chlorine) was not performed due to laboratory resource constraints typical of student-led research in resource-limited settings, a literature-based benchmark comparison with conventional chlorine disinfection is provided in the Discussion to enable evaluation of engineering-scale efficacy.

Data Analysis Procedure

Quantitative data on *E. coli* colony counts (CFU) were recorded for each trial, and means were calculated for each setup. One-way ANOVA was applied to assess significant differences across four groups (control, 5 mL, 15 mL, and 30 mL extract. When the ANOVA was significant, Turkey's Honestly Significant Difference (HSD) post-hoc test was performed to determine which specific concentration levels differed from one another, with $\alpha = 0.05$.

Ethical Considerations

As this study involved no human or animal participants, ethical considerations focused on environmental responsibility, including proper wastewater and chemical disposal to prevent contamination, and on adherence to laboratory safety protocols.

Results and Discussion

This study evaluated the antibacterial efficacy of *Moringa oleifera* leaf crude extract against *Escherichia coli* in wastewater samples from the Imus River, using varying concentrations (5 mL, 15 mL, 30 mL) in experimental setups, compared with an untreated control. Results demonstrate a dose-dependent reduction in E. coli colony-forming units (CFU), as supported by observations of colonial morphology and statistical analysis. Colony counts were enumerated manually on Eosin Methylene Blue (EMB) agar plates after 24-hour incubation, revealing a progressive decline in *E. coli* growth with increasing extract concentrations. In the control group (untreated wastewater), the mean CFU was 543.33 (SD = 133.03), with individual trials yielding 694, 494, and 442 colonies (Table 1). This baseline indicates high bacterial contamination typical of polluted river systems, consistent with reports of pathogen contamination in the Imus River due to sewage inputs.

Table 1. *Controlled set-ups on the effects of alternative bactericides on the number of bacterial colonies*

Controlled Set-Up	Number of Bacterial Colonies
WW-1	694
WW-2	494
WW-3	442
Mean	**543.33**

Table 2. *First concentration set-up*

Concentration Set-Ups	Number of Bacterial Colonies
1-1	304
1-2	511
1-3	335
Mean	**383.33**

Table 3. *Second concentration set-up*

Concentration Set-Ups	Number of Bacterial Colonies
2-1	129
2-2	159
2-3	190
Mean	**159.33**

Table 4. *Third concentration set-up*

Concentration Set-Ups	Number of Bacterial Colonies
3-1	78
3-2	60
3-3	52
Mean	**63.33**

For Treatment 1 (5 mL extract in 0.095 L wastewater), the mean CFU decreased to 383.33 (SD = 111.65), with counts of 304, 511, and 335 (Table 2). This reduction suggests initial inhibitory effects, consistent with moringa's bioactive compounds, such as isothiocyanates, disrupting bacterial cell membranes (Raheem et al., 2015). Treatment 2 (15 mL extract in 0.085 L wastewater) further lowered the mean to 159.33 (SD = 30.51), with 129, 159, and 190 colonies (Table 3), indicating enhanced bactericidal activity at moderate concentrations. The lowest counts occurred in Treatment 3 (30 mL extract in 0.070 L wastewater), with a mean of 63.33 (SD = 13.32) and individual values of 78, 60, and 52 (Table 4). This trend underscores a dose-dependent response, in which higher extract concentrations likely increase the levels of antimicrobial peptides and flavonoids, thereby inhibiting *E. coli* proliferation (Abbas & Eisharbasy, 2018; Bukar et al., 2010). Because total volume was held constant at 0.1 L across all groups, the progressive decline in mean CFU (543.33 → 63.33) can be confidently attributed to increasing bioactive-compound concentration rather than differential dilution. Colonies on EMB agar exhibited the characteristic green metallic sheen of *E. coli* in all groups. However, colonies in treated samples appeared smaller and fewer, providing visual evidence of growth suppression without requiring repeated quantification.

One-way ANOVA confirmed significant differences in mean CFU across groups ($F(3,8) = 18.07$, $p = 0.0006 < 0.05$), rejecting the null hypothesis of no effect (Table 6). Results from Tukey's HSD post-hoc test revealed that the 30 mL extract concentration produced a significantly lower mean CFU count than the control (mean difference = 480, $p = 0.0007$) and the 5 mL concentration (mean difference = 320, $p = 0.0094$). The 15 mL concentration also differed significantly from the control (mean difference = 384, $p = 0.0032$). However, no significant difference was found between the 15 mL and 30 mL groups ($p = 0.5713$) or between the 5 mL and control groups ($p = 0.1984$). These findings confirm a clear dose-dependent antibacterial effect, with the highest concentration (30 mL under a standardized 0.1 L total volume) providing the strongest reduction.

Table 5. *Tukey's HSD Post-hoc Test Results for E. coli Colony Counts Across Extract Concentrations*

Group 1	Group 2	Mean Difference	*p*-value	Significant (p<0.05)
Control	5 mL	160.00	0.1984	No
Control	15 mL	384.00	0.0032	Yes
Control	30 mL	480.00	0.0007	Yes
5 mL	15 mL	224.00	0.0575	No
5 mL	30 mL	320.00	0.0094	Yes
15 mL	30 mL	96.00	0.5713	No

The between-groups sum of squares (423,936) far exceeded the within-groups sum of squares (62,546.67), indicating that extract concentration accounted for substantial variance. This quantitative support aligns with prior studies on moringa's ethanolic extracts against *E. coli*, in which similar dose-response patterns were observed (Raheem et al., 2015; Piexoto et al., 2011; Rahman et al., 2009). The low p-value suggests practical implications for organic wastewater treatment, offering a cost-effective alternative to chemical bactericides in polluted systems like the Imus River, though further comparisons with commercial agents could address literature gaps (Liberal et al., 2022). The chosen maceration method supports scalability claims, as it avoids the higher capital costs of UAE while delivering statistically significant ($p = 0.0006$) *E. coli* reduction, comparable to that of more advanced techniques reported in recent literature.

Comparative Analysis with Conventional Chlorine Disinfection (Positive Control Benchmark)
To benchmark the efficacy of the crude *Moringa oleifera* extract against established chemical treatments, literature data on chlorine disinfection of wastewater *E. coli* under comparable contact times (10–30 min) were reviewed. Standard free chlorine doses of 0.5–1.5 mg/L routinely achieve 3.9–7.3 log reductions of *E. coli* in secondary wastewater effluents within 30 min (Owoseni et al., 2017), with >4 log inactivation often reported at 1 mg/L even after only 10–15 min when residuals reach 0.2–0.5 mg/L (Tree et al., 2003; Wang et al., 2022). In contrast, the highest dose in the present study (30 mL extract under a standardized total volume of 0.1 L) produced an approximately 0.93 log reduction (mean CFU from 543.33 to 63.33). This demonstrates that while the crude moringa extract provides a statistically significant dose-dependent antibacterial effect suitable for preliminary or low-cost disinfection in tropical developing contexts, it does not yet match the rapid, high-log inactivation achieved by chlorine. However, moringa avoids harmful disinfection by-products, high operational costs, and equipment needs associated with chlorination, making it a complementary organic alternative for initial treatment stages in resource-constrained Philippine settings such as the Imus River catchment.

Table 6. *Summary of the concentration set-up treatment of the alternative bactericide*

Groups	Count	Sum	Mean	Variance
Controlled	3	1630	543.33	17701.33
Treatment 1	3	1150	383.33	12464.33
Treatment 2	3	478	159.33	930.33
Treatment 3	3	190	63.33	177.33

Table 7. *One-way ANOVA analysis of different concentration set-ups to E. coli colony units*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	423936	3	141312	18.07	<.001	4.0661
Within Groups	62546.66	8	7818.33			
	86482	11				

Table 8. *Log Reduction Comparison: Moringa Crude Extract vs. Literature-Based Chlorine Positive Control*

Treatment	Contact Time	Mean Log Reduction	Source
30 mL Moringa extract	10 mins	0.93	Present Study
0.5 mg/L free chlorine	30 mins	3.9-6.0	Owoseni et al. (2017)
1.5 mg/L free chlorine	30 min	>7.3	Owoseni et al. (2017)
1 mg/L free chlorine	10-15 min	> 4	Tree et al. (2003)

Conclusion

It is demonstrated that the antibacterial efficacy of a crude *Moringa oleifera* leaf extract against *Escherichia coli* in wastewater from the Imus River is dose-dependent, with experimental treatments reducing bacterial colony counts. The essential contributions lie in establishing moringa extract as a viable, organic bactericide, thereby affirming its potential to mitigate coliform contamination cost-effectively without synthetic chemicals. Post-hoc analysis further confirmed that the 30 mL dose produced significantly greater reductions than both the control and the lowest concentration, reinforcing the dose-dependent efficacy of the crude extract.

These findings have broad implications for environmental practice, offering a sustainable wastewater treatment option that, while achieving moderate log reduction compared with chlorine (0.93 vs. 4–7 log), reduces reliance on chlorine and similar agents, thereby minimizing ecological harm and supporting biodiversity conservation. In policy terms, they advocate integrating natural extracts into water management regulations, particularly in tropical regions such as the Philippines, where moringa is abundant and locally cultivable, thereby fostering economic benefits through reduced import dependence and enhanced community health by lowering *E. coli*-

related disease risks. For educational purposes, the research promotes awareness among students and communities of pollution mitigation, thereby encouraging interdisciplinary approaches in environmental science curricula. In research, it bridges gaps in extraction methods by employing maceration, highlighting opportunities for scalable, regenerative resource use.

Future work could explore optimized conditions, such as temperature and contact time, as suggested by ongoing studies on moringa's bioactive compounds (e.g., chlorogenic acid and flavonoids), or compare efficacy against other pathogens in aquaculture settings to expand its applications. Future studies should concentrate on optimizing the extraction technique (e.g., testing UAE for higher yield) to improve reproducibility and sustainability, while retaining the low-cost maceration protocol validated here.

Contributions of Authors

Author 1: oversees laboratory procedure, manuscript writing, adviser
Author 2: led laboratory procedures, handled bacterial culture identification, manuscript writing
Author 3: conducted literature review, managed data collection, prepared methodological framework
Author 4: coordinated wastewater sampling, performed statistical analysis

Funding

This research was entirely self-funded, with all expenses personally shouldered and equally shared among the researchers.

Conflict of Interests

The authors declare that there is no conflict of interest in the conduct and publication of this study.

Acknowledgment

The researchers express profound gratitude to all individuals and institutions for their steadfast assistance in facilitating the study. They express gratitude to the Almighty Father for bestowing insight, courage, strength, and blessings that directed them with dedication and integrity during the research. Gratitude is expressed to their parents for their moral, material, and spiritual support that facilitated the surmounting of adversities. Gratitude is extended to research adviser Mr. Mark P. Vacunawa for his invaluable guidance, revisions, and expertise; to St. Dominic College of Asia educators for their support in progress, data analysis, and revisions; and to the research personnel from PS-DOST in Taguig and Adamson University in Manila for their assistance in material extraction and preparation, along with all other contributors for their commitment.

References

Abbas, R., & Elsharbasy, F.S. (2019). Antibacterial activity of *Moringa oleifera* against pathogenic. International Journal of Current Research, 11(1), 27–30.
Adji A.S., Atika N., Kusbijantoro Y.B., Billah A., Putri, A. & Handajani F. A. (2024). A review of leaves and seeds *Moringa oleifera* extract: The potential *Moringa oleifera* as antibacterial, anti-inflammatory, antidiarrhoeal, and antiulcer approaches to bacterial gastroenteritis. Open Access Macedonian Journal of Medical Sciences, 10(F), 301–315.
ABS-CBN News. (2010). The many benefits of malunggay. ABS-CBN News. https://tinyurl.com/fu45b4ks
Ali, M., Chand, N., Khan, S., Khan, R.U., Maqbool, B., Naz, S., Abudabos, A., Hafeez, A., & Alhidary, I. (2025). In vitro screening of antibacterial efficacy of *Moringa oleifera* and *Thymus vulgaris* methanolic extracts against different *Escherichia coli* strains and their in vivo effects against *E. coli*-induced infection in broiler chickens. Veterinary Sciences, 12(10), 957. https://doi.org/10.3390/vetsci12100957
Al-Jadabi, N., Laaouan, M., El Hajjaji, S., Mabrouki, J., Benbouzid, M., & Dhiba, D. (2023). The dual performance of *Moringa oleifera* seeds as eco-friendly natural coagulant and as an antimicrobial for wastewater treatment: A review. Sustainability, 15(5), 4280. https://doi.org/10.3390/su15054280
Bibbal, D., Um, M.M., Diallo, A., Kérourédan, M., Dupouy, V., Toutain, P., Bousquet-Mélou, A., Oswald, E., & Brugère, H. (2017). Mixing of Shiga toxin-producing and enteropathogenic *Escherichia coli* in a wastewater treatment plant receiving city and slaughterhouse wastewater. International Journal of Hygiene and Environmental Health, 221(2), 355–363. https://doi.org/10.1016/j.ijheh.2017.12.009
Bukar, A., Uba, A., & Oyeyi, T. (2010). Antimicrobial profile of *Moringa oleifera* extracts against some food-borne microorganisms. Bayero Journal of Pure and Applied Sciences, 3(1), 43 - 48. http://dx.doi.org/10.4314/bajopas.v3i1.58706
Creencia, G.B., Sedigo, N., Dizon, J.C., & Mojica, D. (2022). Mapping and characterization of Imus River watershed using geographic information system and remote sensing technology. ResearchGate. https://www.researchgate.net/publication/360737969
dela Peña, L.B.R., Labrador, K., Nacario, M.A., Bolo, N., & Rivera, W. (2021). Microbial source tracking of fecal contamination in Laguna Lake, Philippines using the library-dependent method, rep-PCR. Journal of Water and Health, 19(5), 762–774. https://doi.org/10.2166/wh.2021.119
Deepali, D., Gulia, V., Dhull, S.S., et al. (2025). Unveiling *Moringa oleifera*: Potent source of antioxidant and antibacterial properties. Discover Applied Sciences, 7(381). https://doi.org/10.1007/s42452-025-06836-2
El-Azazy, M., El-Shafie, A., Issa, A., Al-Sulati, M., Al-Yafie, J., Shomar, B., & Al-Saad, K. (2019). Potato peels as an adsorbent for heavy metals from aqueous solutions: Eco-structuring of a green adsorbent operating Plackett-Burman design. Journal of Chemistry, 2019, 1-14. https://doi.org/10.1155/2019/4926240
El-Shirbiny, G., Allugmani, A., Elsehemy, I., & Kalaba, M. (2024). Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of *Moringa oleifera* leaves. Scientific Reports, 14:30485. https://doi.org/10.1038/s41598-024-80700-y
Ervianingsih, Mursyid, M., Annisa, R.N., Zahran, I., Langkong, J., & Kamaruddin, I. (2019). Antimicrobial activity of moringa leaf (*Moringa oleifera* L.) extract against the growth of *Staphylococcus epidermidis*. IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/343/1/012145
Gomes, O., Leitão, A., de Sousa, H., Gando-Ferreira, L., & Braga, M. (2025). Bioactive compounds extraction from *Moringa oleifera* leaves: A comparative study of vacuum-assisted and bed-stirred extractions. Journal of Food Science, 90(11), e70700. https://doi.org/10.1111/1750-3841.70700
Kaiser, G.E. (2009). *E. Coli* under the microscope -Types, techniques, Gram stain, hanging drop method. MicroscopeMaster. https://tinyurl.com/y6nmj8uk
Kumar, J., Choudhary, M., Dikshit, P., & Kumar, S. (2024). Recent advancements in utilizing plant-based approaches for water and wastewater treatment technologies. Clean Water, 2, 100030. https://doi.org/10.1016/j.clwat.2024.100030
Kunz, J., Lawinger, H., Miko, S., Gerdes, M., Thuneibat, M., Hannapel, E., & Roberts, V. (2024). Surveillance of waterborne disease outbreaks associated with drinking water — United States, 2015–2020. US Center for Disease Control. https://www.cdc.gov/mmwr/volumes/73/ss/ss7301a1.htm
Li, W., Yuan, Q., Wang, X., Wang, W., Wu, X., Zhang, Y., Zhao, X., Luo, Y., & Wu, F. (2026). Integrated global assessment of *Escherichia coli* emissions from wastewater treatment plants. Eco- Environment & Health, 5(1). https://doi.org/10.1016/j.eehl.2025.100209
Liberal, A., Molina, A., Pereira, C., Dias, M.I., Ferreira, I., & Barros, L. (2022). Solid-liquid extraction of polyphenols. Elsevier eBooks, 73–112. https://doi.org/10.1016/b978-0-323-85273-9.00004-1
Liga, S., Magyari-Pavel, I.Z., Avram, Ş., Minda, D.I., Vlase, A.-M., Muntean, D., Vlase, L., Moacă, E.-A., & Danciu, C. (2025). Comparative analysis of *Moringa oleifera* Lam. leaves ethanolic extracts: Effects of extraction methods on phytochemicals, antioxidant, antimicrobial, and in ovo profile. Plants (Basel, Switzerland), 14(11), 1653. https://doi.org/10.3390/plants14111653
Lwasa, A., Mdee, O.J., Ntalikwa, J.W., & Sadiki, N. (2024). Performance analysis of plant-based coagulants in water purification: A review. Discover Water 4, 108 https://doi.org/10.1007/s43832-024-00171-0
Mohan, G., & Lyons, S. (2022). The association between *E. coli* exceedances in drinking water supplies and healthcare utilisation of older people. PLOS One, 17(9), e0273870. https://doi.org/10.1371/journal.pone.0273870
Pelegrino, E.N. (2022). Amazing health benefits of malunggay leaves. National Nutrition Council. https://tinyurl.com/ntrbrtbv
Peixoto, J.R., Silva, G.C., Costa, R.A., de Sousa Fontenelle, J.r.L., Vieira, G.H., Filho, A.A., & dos Fernandes Vieira, R.H. (2011). In vitro antibacterial effect of aqueous and ethanolic moringa leaf extracts. Asian Pacific Journal of Tropical Medicine, 4(3), 201–204. https://doi.org/10.1016/s1995-7645(11)60069-2

Raheem, R., Mk, B., Njan, A.A., & Oe, O. (2015). Antibacterial activity of the crude extracts of *Moringa oleifera* leaf (Moringaceae). Nigerian Journal of Pharmaceutical Sciences, 14(1).

Rahman, M.M., Sheik, M.M.I., Sharmin, S.A., & Alam, M. (2009). Antibacterial activity of leaves juice and extracts of *Moringa oleifera* Lam. (2n = 28) against some human pathogenic bacteria. ResearchGate. https://www.researchgate.net/publication/216564899

Sandeep, G., Arumugam, T., Janavi, G.J., Anitha, T., Senthil, K., & Lakshaman, A. (2023). A comparative study on conventional and non-conventional extraction methodologies for extraction yield, quality and antibacterial properties of Moringa (*Moringa oleifera* Lam.). Journal of Applied Horticulture, 25(1), 17–24. https://doi.org/10.37855/jah.2023.v25i01.03

Sané, N., Sivalingam, P., Koželuh, M., Mbengue, M., Stoll, S., Poté, J., & Le Coustumer, P. (2024). Effect of *Moringa oleifera* seeds on the removal of pathogens and pharmaceutical residues in a domestic wastewater treatment plant by an interdisciplinary approach. Environmental Science and Pollution Research, 31(56), 65123–65136. https://doi.org/10.1007/s11356-024-35362-8

Selmi, S., Taamalli, W., Mhimdi, M., Hraoui, M., Jridi, M., & Sebai, H. (2025). Antibacterial properties *of Moringa oleifera*, *Cynara scolymus* and *Allium fistulosum* leaf extracts: Molecular docking insights into quercetin's mechanism of action. Journal of the Science of Food and Agriculture, 105: 5090–5101. https://doi.org/10.1002/jsfa.14235

Shah, A., Arjunan, A., Batool, M., Chike, O., Dhir, A., Arafat, A., Manning, G., Zakharova, J., Abbas, N., Mehran, M.T., Rubab, R., & Husnain, M. (2026). Green yet constrained: Reassessing *Moringa oleifera*'s role in drinking water and wastewater treatment. Cleaner Water, 5, 100202. https://doi.org/10.1016/j.clwat.2025.100202

Talreja, T. (2010). Screening of crude extract of flavonoids of *Moringa oleifera* against bacteria and fungal pathogen. Journal of Phytology, 2(11). https://tinyurl.com/yw82p7m2

Tasselli, S., Marziali, L., Guzzella, L., Valsecchi, L., Palumbo, M.T., Salerno, F., & Copetti, D. (2025). Impact of wastewater treatment plant discharge on water quality of a heavily urbanized river in Milan metropolitan area: Traditional and emerging contaminant analysis. Water, 17(22), 3276. https://doi.org/10.3390/w17223276

Tille, P. (2017). Bailey & Scott's diagnostic microbiology (Fourteenth Edition). Elsevier.

Tree, J., Adams, M., & Lees, D. (2003). Chlorination of indicator bacteria and viruses in primary sewage effluent. Applied and Environmental Microbiology, 69(4), 2038–2043. https://doi.org/10.1128/aem.69.4.2038-2043.2003

US EPA. (2024). Basics of green chemistry. US EPA. https://www.epa.gov/greenchemistry/basics-green-chemistry

Wizrah, M., Aldwsari, N., & Yahia, Z. (2026). Antibacterial activity of moringa leaf extracts against Gram-negative bacteria from Wadi Ad-Dawasir, Saudi Arabia. Frontiers in Microbiology, 16, 1568105. https://doi.org/10.3389/fmicb.2025.1568105

Zure, D., Drizo, A., Sung, M.-H., Mehari, A., Maiguo, E., & Kuo, D.H.-W. (2026). Antiviral phytoremediation for sustainable wastewater treatment. Sustainability, 18(1), 523. https://doi.org/10.3390/su18010523