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COMPARATIVE ANTIBACTERIAL EFFECTIVENESS OF SELECTED SEED POWDERS AND GREEN-SYNTHESIZED MAGNESIUM OXIDE NANOPARTICLES IN BACTERIALLY CONTAMINATED WATER.

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Abstract

Bacterial contamination of water presents a serious public-health risk and creates a need for effective and affordable treatment methods. This study evaluated the antibacterial effectiveness of moringa, pawpaw and orange seed powders, green-synthesized magnesium oxide nanoparticles (MgONPs), and their combinations in experimentally contaminated water. Antibacterial performance was assessed using total viable count (TVC), total coliform count (TCC), bacterial occurrence, inhibition zones, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The untreated contaminated water recorded TVC and TCC values of 257.33 ± 3.21 and 120.33 ± 4.04 CFU/mL, respectively, and contained *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Klebsiella pneumoniae*. Orange seed powder produced the greatest reduction among the individual seed treatments, reducing TVC and TCC by 35.23% and 72.30%, respectively, while MgONPs produced larger inhibition zones than the individual seed powders. Selected multi-component treatments demonstrated greater antibacterial effectiveness. None of the five target bacteria was recovered following MgONP–pawpaw, moringa-MgONP-pawpaw, pawpaw-MgONP-orange or complete moringa-pawpaw-MgONP-orange treatment. The complete treatment produced the lowest TVC and TCC values of 2.67 ± 0.58 and 1.00 ± 1.00 CFU/mL, corresponding to reductions of 98.96% and 99.17%, respectively. It also produced large inhibition zones and low MIC and MBC values. The findings indicate that selected seed powder-MgONP combinations are promising treatment or pretreatment materials for bacterially contaminated water. Further optimizations and safety assessment are required before practical application.

Keywords: antibacterial activity; contaminated water; magnesium oxide nanoparticles; moringa seeds; orange seeds; pawpaw seeds

Introduction

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Microbial contamination remains a major threat to drinking-water safety, particularly in communities with inadequate sanitation and limited access to centralized water-treatment facilities (WHO, 2023). Contaminated water can transmit cholera, diarrhoea, dysentery, typhoid and other infections caused by pathogenic microorganisms (WHO, 2023). Although chlorination and other conventional disinfection methods are effective, their application may be constrained by cost, technical requirements, chemical residues and the possible formation of harmful disinfection by-products (Kali *et al.*, 2021). These limitations have encouraged the investigation of affordable biological materials for improving the microbiological quality of water (Adeeyo *et al.,* 2025).

Plant seeds contain proteins and bioactive compounds that may remove microorganisms through coagulation, adsorption and direct antibacterial action (Al-Jadabi *et al.,* 2023). *Moringa oleifera* seeds contain positively charged, water-soluble proteins that neutralize suspended particles and promote the entrapment and sedimentation of bacterial cells (Mohamed, 2025). Extracts obtained from moringa seeds have also reduced coliforms and other bacteria in contaminated water (Sané *et al.,* 2024). *Carica papaya* seeds contain alkaloids, flavonoids, saponins and terpenoids with reported antibacterial activity against organisms such as *Escherichia coli* and *Salmonella Typhi* (Irmawati *et al.,* 2024). Papaya seeds also possess functional groups and proteins capable of supporting coagulation and pollutant removal from water (Amran *et al.,* 2021). Orange seeds are an abundant agro-waste material containing proteins, phenolic compounds and surface functional groups that may contribute to microbial adsorption and inhibition, although their application in microbiological water treatment remains comparatively underexplored (Flores Alarcón *et al.,* 2022).

Magnesium oxide nanoparticles offer an additional treatment option because of their high surface area, adsorptive capacity and broad antibacterial activity (Saberi *et al.,* 2024). Their antibacterial effects have been associated with

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membrane damage, alkaline conditions, oxidative stress and interactions between the nanoparticles and bacterial cell surfaces (Manaa *et al.,* 2025). Green synthesis using plant extracts can reduce the use of hazardous chemicals while providing natural compounds that assist nanoparticle formation and stabilization (Rotti *et al.,* 2023). However, limited information is available on the comparative antibacterial performance of moringa, pawpaw and orange seed extracts or on their combined application with green-synthesized MgONPs. This study therefore evaluated the antibacterial effectiveness of the selected seed powders and green-synthesized MgONPs, applied individually and in combination, in the treatment of contaminated water.

Materials and Methods

Study area and water-sample collection

The study was conducted at Joseph Sarwuan Tarka University, Makurdi (JOSTUM), Benue State, Nigeria. The university is located in the northern part of Makurdi Local Government Area at latitude 07°45.53′ N and longitude 008°37.41′ E. It lies approximately 8 km from the federal trunk road along Gbajimba Road and falls within the Southern Guinea Savanna agroecological zone. The area is characterized by an undulating terrain, with an elevation of approximately 83-167 m above sea level. Its drainage system comprises seasonal streams, including the Ambikapa, Baa and Najime streams, which are tributaries of the River Benue (Idris *et al.,* 2025).

Water samples were collected from the Waterworks Unit of JOSTUM, which supplies water for domestic and other uses within the university. The water was collected in ten clean, covered plastic containers, each with a capacity of 25 L. The containers were transported to the Advanced Biology Laboratory (B41), JOSTUM, for experimental contamination, treatment and microbiological analysis.

Collection and preparation of seed materials

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Seeds of *Moringa oleifera*, *Carica papaya* and *Citrus sinensis* were obtained from mature fruits purchased from Railway Market, Makurdi. The plant materials were authenticated in the Department of Plant Science and Biotechnology, Joseph Sarwuan Tarka University, Makurdi.

Approximately 5 kg of each seed type was washed with clean water, sun-dried and pulverized using a clean mortar and pestle (Keneni *et al.,* 2021). The powdered materials were stored separately in sterile, sealed bags until required. The moringa, pawpaw and orange seed preparations were coded M, P and O, respectively.

Test bacteria and molecular confirmation

Pure cultures belonging to the genera *Escherichia*, *Proteus*, *Salmonella*, *Shigella* and *Klebsiella* were obtained from the New Biology Laboratory of Joseph Sarwuan Tarka University, Makurdi. The isolates were subcultured on Luria-Bertani medium and incubated at 37°C overnight before broth cultures were prepared.

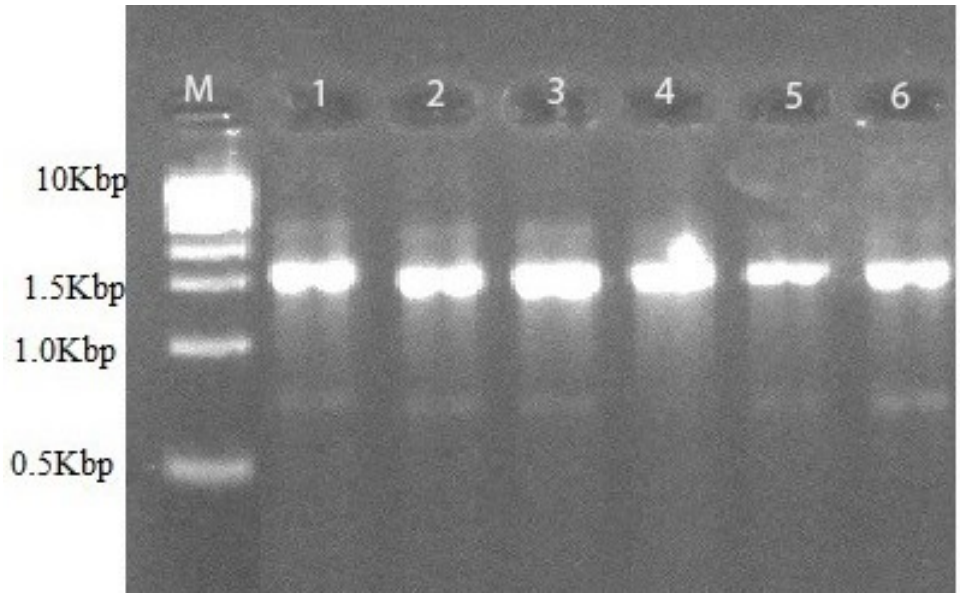

Plate 1: Gel Image of Amplified 16SrRNA gene (1500bp)

1= *Salmonella*

2= *Shigella*

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3= *Escherichia coli*
4= *Proteus*
5= *Klebsiella*
6= Reference bacterium
M= 1Kbp DNA ladder

The identities of the test bacteria were confirmed through 16S rRNA gene amplification and sequencing. Genomic DNA was extracted from the broth cultures using the boiling method. The 16S rRNA gene was amplified using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCCGCA-3′). Amplification was performed for 35 cycles, comprising initial denaturation at 95°C for 5 min, denaturation at 95°C for 2 min, annealing at 55°C for 30 s, extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR products were sequenced using the Sanger method, and the resulting sequences were compared with sequences in the National Center for Biotechnology Information database using BLASTn.

The test organisms were identified as *Escherichia coli* strain G1F12, *Proteus vulgaris* strain X17-1, *Salmonella typhimurium* strain ST313, *Shigella dysenteriae* strain Sd197 and *Klebsiella pneumoniae* strain ST147.

Green synthesis of magnesium oxide nanoparticles

Fresh leaves of *Jatropha tajorensis* were collected from a farm in Tarka Local Government Area, Benue State, and authenticated in the Department of Plant Science and Biotechnology, Joseph Sarwuan Tarka University, Makurdi. The leaves were washed with deionized water, air-dried and pulverized. Six grams of the powdered leaves was added to 100 mL of deionized water and heated at 80°C for 20-30 min. The powder was filtered through Whatman No. 1 filter paper and stored at 4°C until required (Essien *et al.,* 2020).

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For nanoparticle synthesis, 20 mL of the leaf powder was heated at 60°C with continuous stirring, after which 80 mL of 0.2 M magnesium nitrate solution, prepared using 5 g of magnesium nitrate hexahydrate, was added dropwise. A few drops of 1 M sodium hydroxide were subsequently added, and the mixture was continuously stirred and heated at 80°C for 4 h. The resulting precipitate was recovered by centrifugation, washed repeatedly with ethanol and oven-dried at 70°C for 3 hours. The dried material was calcined at 400°C for 2 h to obtain magnesium oxide nanoparticles, coded N (Essien *et al.,* 2020; Zhao *et al.*, 2019).

The formation and properties of the MgONPs were verified using UV-visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy coupled with energy-dispersive X-ray analysis.

Experimental design and treatments

The water-treatment experiment was arranged in a completely randomized design with three replicates per treatment. The treatments consisted of individual seed powders, MgONPs, selected two- and three-component combinations, a complete four-component combination, and positive and negative controls. Chlorinated commercial Water Guard Plus containing sodium dichloroisocyanurate was used as the positive control, whereas contaminated water that received no treatment served as the negative control.

The treatments consisted of individual seed powders, MgONPs, selected two- and three-component combinations, a complete four-component combination, and positive and negative controls.

For the water-treatment experiment, each seed component was applied at 5 g per 2.5 L of water, equivalent to 2 g/L, while MgONPs were applied at 200 mg/L. Water Guard Plus was applied according to the manufacturer’s specification of 5 g per 25 L, equivalent to 0.5 g per 2.5 L.

Table 1: Experimental treatments used in the study

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Code	Treatment components	Applied quantity in 2.5 L
M	Moringa seed powder	5 g
P	Pawpaw seed powder	5 g
O	Orange seed powder	5 g
N	MgONPs	200 mg/L
M+N	Moringa + MgONPs	5 g M + 200 mg/L N
M+O	Moringa + orange	5 g of each seed powder
M+P	Moringa + pawpaw	5 g of each seed powder
N+O	MgONPs + orange	200 mg/L N + 5 g O
N+P	MgONPs + pawpaw	200 mg/L N + 5 g P
M+N+P	Moringa + MgONPs + pawpaw	5 g M + 200 mg/L N + 5 g P
P+N+O	Pawpaw + MgONPs + orange	5 g P + 200 mg/L N + 5 g O
P+M+O	Pawpaw + moringa + orange	5 g of each seed powder
M+N+P+O	Moringa + MgONPs + pawpaw + orange	5 g of each seed powder + 200 mg/L N
Positive control	WaterGuard Plus	0.5 g
Negative control	Contaminated water without treatment	Not applicable

Preparation and treatment of bacterially contaminated water

Each experimental container was filled with 2.5 L of water. Broth cultures of *E. coli, P. vulgaris, S. typhimurium, S. dysenteriae* and *K. pneumoniae* were

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prepared, and an inoculum of each organism at approximately 10^6 cfu/ml, was introduced into the water assigned to the biological-contamination experiment. The containers were covered, sealed and maintained for two weeks before baseline microbiological assessment.

Following the baseline assessment, the seed powders and MgONPs were added individually or in combinations according to the treatment design. The experiment continued from the second to the tenth week, with microbiological assessments conducted at two-week intervals. The contaminated but untreated samples and the WaterGuard-treated samples were maintained as negative and positive controls, respectively.

Microbiological analysis

Total viable and total coliform counts

Total viable count and total coliform count were determined using the pour-plate method (Li *et al.*, 2022). One millilitre of each water sample was transferred into 9 mL of sterile normal saline and serially diluted tenfold to 10-5 in order to have countable colonies. One millilitre of an appropriate dilution was inoculated into sterile Petri dishes containing nutrient agar for total viable count and MacConkey agar for total coliform count.

The inoculated plates were allowed to solidify and incubated at 35–37°C for 24 hours. Colonies were counted after incubation, and the bacterial loads were expressed as colony-forming units per millilitre of water.

Occurrence of test bacteria after treatment

Selective culture media were used to determine the presence or absence of each of the five introduced bacterial species in the treated water samples (Uzeh and Imafidon, 2022). Bacterial occurrence was calculated from the number of the five test organisms recovered from each treatment:

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$$\text{Bacterial occurrence (\%)} = \frac{\text{Number of bacterial species recovered}}{\text{Total number of species examined}} \times 100$$

The absence of recoverable growth was interpreted as the elimination of the organism under the culture conditions used.

Zone-of-inhibition assay

The antibacterial activities of the individual and combined treatments were evaluated against the five test bacteria using an agar-diffusion procedure. Nutrient agar was prepared, sterilized and poured into sterile Petri dishes. Each test organism was inoculated onto the agar surface and spread uniformly. The treatment preparations were tested at 2.5 and 5.0 mg/mL in duplicate.

After incubation at 37°C for 24 hours, the diameter of the clear zone surrounding each treatment well was measured in millimetres. The mean zone of inhibition was used to compare the antibacterial activity of the treatments against each test organism (Uzeh and Imafidon, 2022).

Minimum inhibitory concentration

The minimum inhibitory concentration was determined using the broth-dilution method described by Mofolorunsho *et al.* (2021), with modifications. Serial dilutions of each treatment were prepared to obtain concentrations of 50.00, 25.00, 12.50, 6.25 and 3.12 mg/mL. The tubes were inoculated with the respective test bacteria and incubated at 37°C for 24 hours. The lowest treatment concentration that produced no visible bacterial growth or turbidity was recorded as the MIC.

Minimum bactericidal concentration

The minimum bactericidal concentration was determined by subculturing samples from the MIC tubes that showed no visible growth. Mueller-Hinton

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agar was poured into sterile Petri dishes, allowed to solidify and divided into appropriately labelled sections. A sterile inoculating loop was used to transfer material from each clear MIC tube onto the corresponding section of the agar. The plates were incubated at 37°C for 24 hours. The lowest treatment concentration from which no bacterial growth was recovered was recorded as the MBC.

Statistical analysis

Data were analyzed using Minitab version 17.0. Total viable count and total coliform count measurements collected at two-week intervals were analyzed using repeated-measures analysis of variance, with treatment and sampling time as factors. The treatment-by-time interaction was also assessed. Where significant differences occurred, Fisher's least significant difference test was used to separate the means at $p \leq 0.05$. Quantitative results are presented as means ± standard deviations, while bacterial occurrence is presented as frequencies and percentages.

Results

Characterization of Green-Synthesized MgONPs

The characterization results confirmed the successful formation of magnesium oxide nanoparticles. UV-VIS analysis showed a prominent absorption peak at approximately 280 nm, while FTIR revealed O-H stretching around 3400 cm^{-1} and Mg-O vibrations at approximately 1000 cm^{-1} and within 800-500 cm^{-1}. SEM analysis showed relatively uniform, spherical nanoparticles with limited agglomeration, while EDX confirmed magnesium as the predominant element, with atomic and weight concentrations of 46.51% and 46.46%, respectively. XRD peaks occurred between 8.64° and 67.31°, with an average crystallite size of 22.33 nm, confirming the nanocrystalline nature of the synthesized MgONPs.

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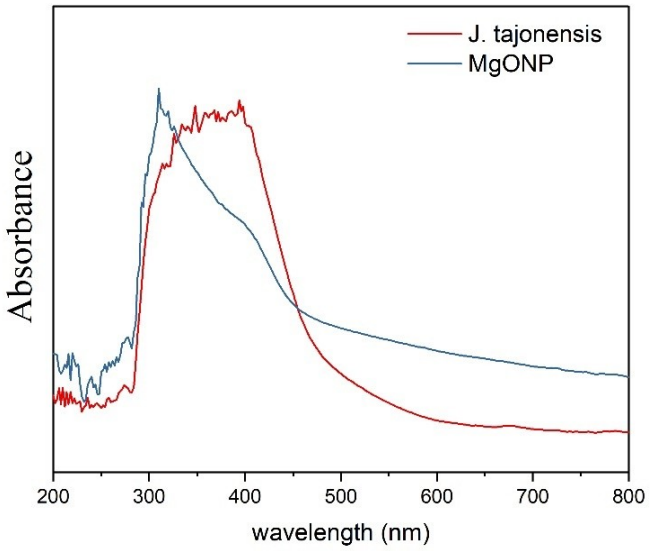

Figure 1: UV-VIS Spectra of *J. tajorensis* Leaf Extract and Magnesium Oxide Nanoparticles

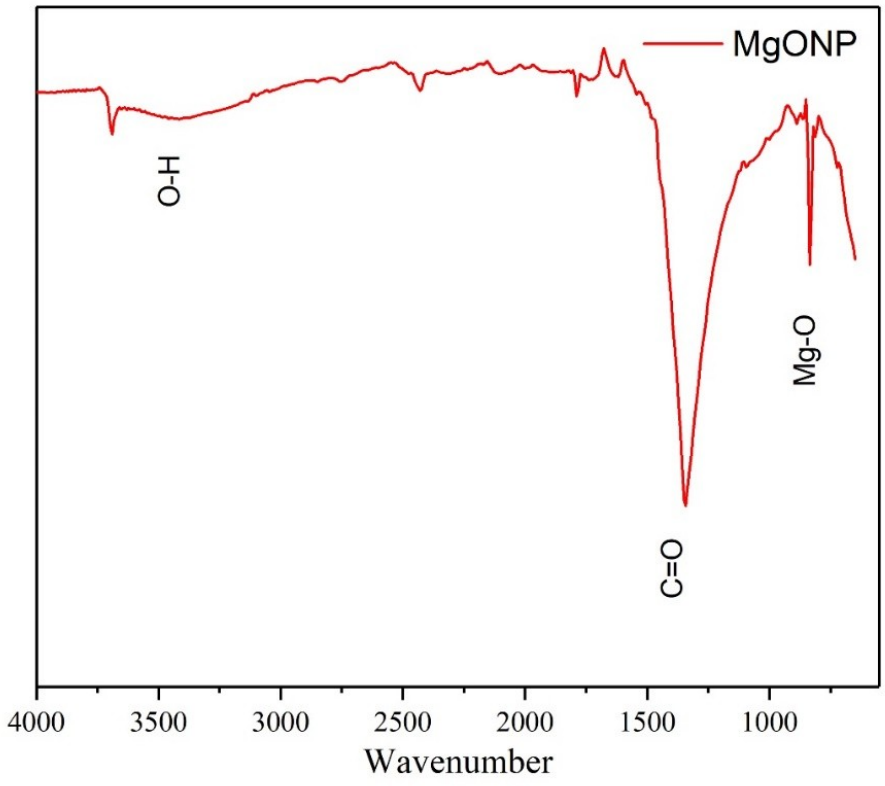

Figure 2: FTIR Spectrum of the synthesized Magnesium Oxide Nanoparticles

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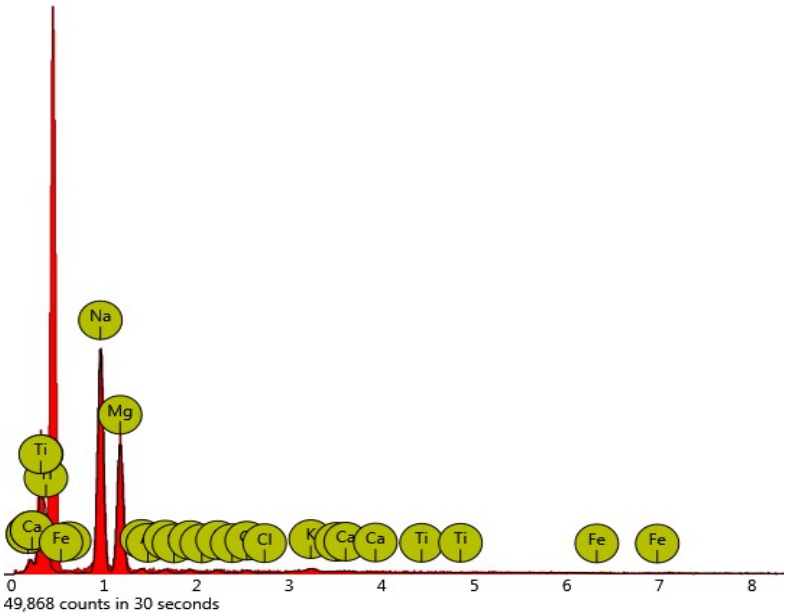

Figure 3: EDX Spectra of the Magnesium Oxide Nanoparticles

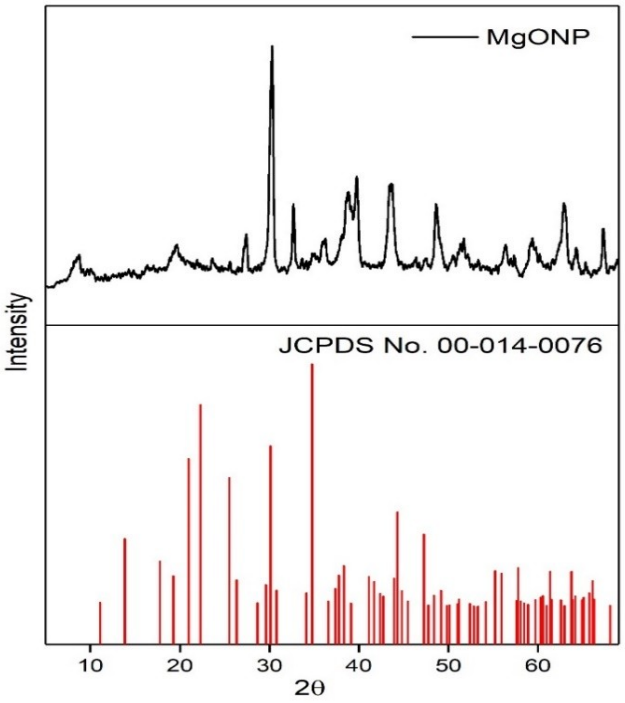

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Figure 4: XRD Pattern of Magnesium Oxide Nanoparticles Derived from *Jatropha tajorensis*

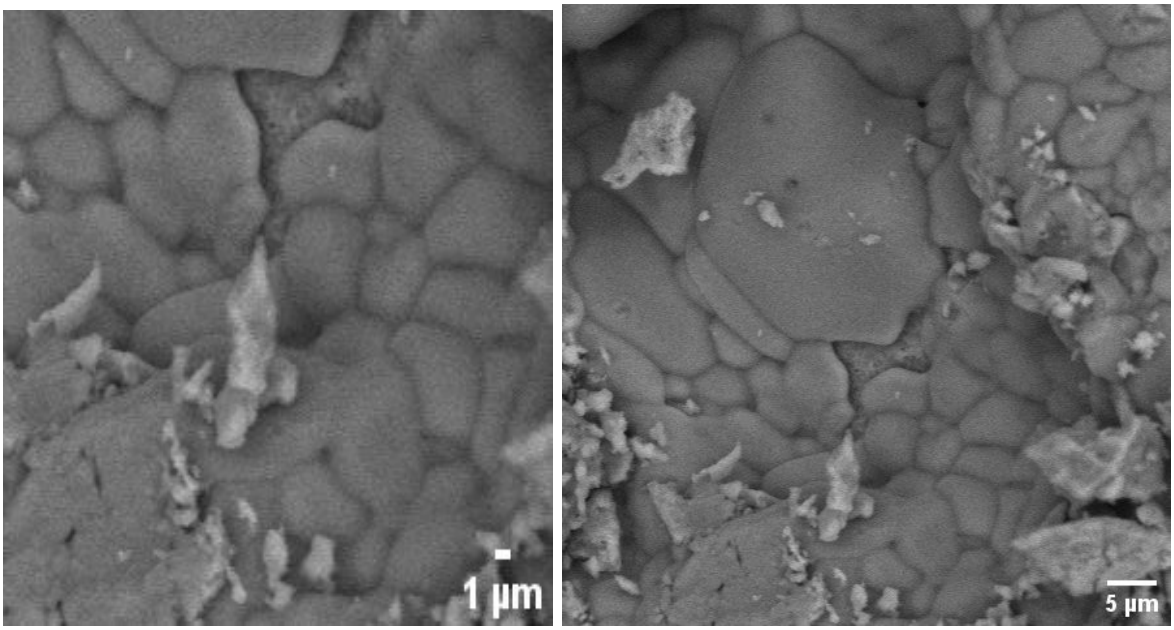

Plate 2: SEM Images of the Synthesized Magnesium Oxide Nanoparticles

Bacterial quality of untreated contaminated water

The untreated contaminated water recorded a total viable count (TVC) of 257.33 ± 3.21 CFU/mL and a total coliform count (TCC) of 120.33 ± 4.04

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CFU/mL (Table 2). Five bacterial species were recovered: *Escherichia coli* strain G1F12, *Proteus vulgaris* strain X17-1, *Salmonella typhimurium* strain ST313, *Shigella dysenteriae* strain Sd197 and *Klebsiella pneumoniae* strain ST147. All five species occurred in the untreated control, representing 100% bacterial occurrence (Table 3).

Effects of individual treatments

The individual treatments significantly affected TVC and TCC ($p < 0.001$; Table 2). Orange seed powder reduced TVC and TCC to 166.67 ± 5.13 and 33.33 ± 2.31 CFU/mL, respectively. These values represented reductions of 35.23% in TVC and 72.30% in TCC relative to the untreated control. Orange seed powder therefore produced the greatest reduction in microbial counts among the three individual seed treatments.

Pawpaw seed powder reduced TVC to 185.67 ± 4.04 CFU/mL and TCC to 52.67 ± 4.16 CFU/mL, corresponding to reductions of 27.85% and 56.23%, respectively. Moringa seed powder produced TVC and TCC values of 193.33 ± 5.13 and 58.00 ± 0.00 CFU/mL, representing respective reductions of 24.87% and 51.80%.

Treatment with MgONPs reduced TVC to 172.33 ± 3.51 CFU/mL and TCC to 45.33 ± 1.16 CFU/mL. These values corresponded to reductions of 33.03% and 62.33%, respectively. MgONPs produced greater reductions in microbial counts than pawpaw and moringa seed powders but were less effective than orange seed powder.

Table 2: Effects of Single and Combined Treatments on Microbial Count of Water Samples

Treatments	TVC	TCC
O	166.67 ± 5.13^{de}	33.33 ± 2.31^{g}
P	185.67 ± 4.04^{bcd}	52.67 ± 4.16^{e}
M	193.33 ± 5.13^{bcd}	58.00 ± 0.00^{d}
N	172.33 ± 3.51^{de}	45.33 ± 1.16^{f}

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M+N	155.67±2.08^{ef}	51.33±2.52^{e}
M+O	126.67±2.31^{ef}	56.67±0.58^{d}
M+P	205.33±2.31^{bc}	76.33±2.08^{c}
N+O	214.67±2.31^{b}	77.33±1.16^{c}
N+P	181.30±67.1^{cde}	81.00±2.00^{b}
M+N+P	15.00±1.00^{gh}	4.00±0.00^{i}
P+N+O	11.33±0.58^{h}	3.00±1.00^{i}
P+M+O	9.67±1.16^{h}	3.33±1.16^{i}
M+P+N+O	2.67±0.58^{h}	1.00±1.00^{i}
P-CTRL	44.00±1.00^{g}	11.33±0.58^{h}
N-CTRL	257.33±3.21^{a}	120.33±4.04^{a}
F-VALUE	75.18	947.72
P-VALUE	p < 0.001	p < 0.001

Legend: O = orange, P= pawpaw, M = moringa, N = nanoparticle, CTRL = control
TVC = total viable count; TCC = total coliform count.

None of the individual treatments completely eliminated the target bacteria (Table 3). All five bacterial species were recovered following treatment with orange and moringa seed powders, representing 100% bacterial occurrence. Four species were recovered following pawpaw treatment, giving 80% occurrence, with *S. dysenteriae* not detected. MgONP treatment reduced bacterial occurrence to 100%, but the target organisms were not completely eliminated.

The inhibition zones differed significantly among the individual treatments and bacterial species ($p < 0.001$; Table 4). Orange seed powder produced inhibition zones ranging from 12.33 ± 0.58 mm against *S. typhimurium* to 15.67 ± 0.58 mm against *K. pneumoniae*. Pawpaw seed powder produced inhibition zones ranging from 11.67 to 15.67 mm, while moringa seed powder produced values ranging from 11.67 to 16.33 mm. MgONPs produced larger inhibition zones than the individual seed powder, with values ranging from 16.67 ± 0.58 to 18.33 ± 0.58 mm.

4.3 Effects of combined treatments

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The combined treatments produced varying effects on the microbial counts (Table 2). Among the two-component treatments, moringa–orange produced the lowest TVC of 126.67 ± 2.31 CFU/mL, representing a reduction of 50.78% relative to the untreated control. Moringa-MgONP produced a TVC of 155.67 ± 2.08 CFU/mL and a TCC of 51.33 ± 2.52 CFU/mL, corresponding to reductions of 39.51% and 57.34%, respectively.

Moringa-pawpaw, MgONP-orange and MgONP-pawpaw produced TVCs of 205.33 ± 2.31, 214.67 ± 2.31 and 181.30 ± 67.10 CFU/mL, respectively. Their corresponding TCCs ranged from 76.33 to 81.00 CFU/mL.

The three-component treatments produced greater reductions in microbial counts. Moringa-MgONP-pawpaw reduced TVC and TCC to 15.00 ± 1.00 and 4.00 ± 0.00 CFU/mL, respectively. Pawpaw-MgONP-orange produced a TVC of 11.33 ± 0.58 CFU/mL and a TCC of 3.00 ± 1.00 CFU/mL. The combination of the three seed powders, pawpaw-moringa-orange, produced TVC and TCC values of 9.67 ± 1.16 and 3.33 ± 1.16 CFU/mL, respectively.

The complete moringa-pawpaw-MgONP-orange treatment produced the lowest microbial counts, with a TVC of 2.67 ± 0.58 CFU/mL and a TCC of 1.00 ± 1.00 CFU/mL. These values represented reductions of 98.96% and 99.17%, respectively, relative to the untreated control.

Bacterial occurrence also varied among the combined treatments (Table 3). *Escherichia coli* and *S. typhimurium* were recovered following moringa-MgONP treatment, resulting in 40% bacterial occurrence. Moringa-orange treatment retained *E. coli*, *S. typhimurium* and *K. pneumoniae*, giving 60% occurrence. Four of the five target species persisted following moringa-pawpaw treatment, representing 80% occurrence.

Only *P. vulgaris* was recovered following MgONP-orange treatment, corresponding to 20% bacterial occurrence. The combination of the three seed powder retained *P. vulgaris*, *S. dysenteriae* and *K. pneumoniae*, giving 60% occurrence. In contrast, none of the five target bacterial species was

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recovered following treatment with MgONP-pawpaw, moringa-MgONP-pawpaw, pawpaw-MgONP-orange or the complete moringa-pawpaw-MgONP-orange combination.

Table 3: Effects of Single and Combined Treatments on Occurrence of Bacterial Species in Water Samples

Treatment	*Escherichia coli* strain G1F12	*Proteus vulgaris* strain X17-1	*Salmonella typhimurium* strain ST313	*Shigella dysenteriae* strain Sd197	*Klebsiella pneumoniae* strain ST147	Frequency	Bacteria occurrence (%) microbes
O	+	+	+	+	+	5	100%
P	+	+	+	-	+	4	80%
M	+	+	+	+	+	5	100%
N	+	+	+	+	+	5	100%
M+N	+	-	+	-	-	2	40%
M+O	+	-	+	-	+	3	60%
M+P	+	+	+	-	+	4	80%
N+O	-	+	-	-	-	1	20%
N+P	-	-	-	-	-	0	0%
M+N+P	-	-	-	-	-	0	0%
P+N+O	-	-	-	-	-	0	0%
P+M+O	-	+	-	+	+	3	60%
M+P+N+O	-	-	-	-	-	0	0%
P-CTRL	-	-	+	-	-	1	20%
N-CTRL	+	+	+	+	+	5	100%

Legend: O = orange, P= pawpaw, M = moringa, N = nanoparticle, N-CTRL = negative control, "+/-" = presence or absence of bacterium

The combined treatments generally produced larger inhibition zones than the individual treatments (Table 4). The complete moringa-pawpaw-MgONP-orange treatment produced inhibition zones of 32.67 ± 1.16, 28.67 ± 0.58, 31.33 ± 0.58 and 32.67 ± 0.58 mm against *P. vulgaris*, *S. typhimurium*, *S. dysenteriae* and *K. pneumoniae*, respectively. Pawpaw-MgONP-orange produced the largest inhibition zone against *E. coli*, with a value of 28.33 ± 0.58 mm.

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Table 4: Zone of Inhibition of Test Bacteria by Single and Combined Treatments in Petri Dishes

Treatment	*Escherichia coli* strain G1F12	*Proteus vulgaris* strain X17-1	*Salmonella typhimurium* strain ST313	*Shigella dysenteriae* strain Sd197	*Klebsiella pneumoniae* strain ST147
O	13.33±0.58^{f}	15.00±0.00^{i}	12.33±0.58^{j}	13.33±1.16^{h}	15.67±0.58^{i}
P	11.67±1.16^{g}	15.67±0.58^{hi}	12.00±1.00^{j}	13.33±0.58^{h}	12.33±0.58^{j}
M	16.33±0.58^{e}	12.33±0.58^{j}	14.00±0.00^{i}	15.00±0.00^{g}	11.67±1.16^{j}
N	17.67±0.58^{e}	16.67±0.58^{gh}	17.67±1.16^{gh}	18.33±0.58^{f}	16.67±0.58^{hi}
M+N	21.00±1.00^{d}	18.67±0.58^{f}	20.67±0.58^{e}	22.67±0.58^{e}	24.00±1.00^{e}
M+O	16.33±0.58^{e}	15.00±1.00^{i}	17.33±0.58^{h}	18.00±1.00^{f}	17.67±1.16^{gh}
M+P	17.67±0.58^{e}	17.67±0.58^{fg}	19.00±1.00f	18.67±0.58^{f}	18.67±0.57^{g}
N+O	21.67±0.58^{d}	23.67±1.16^{e}	23.00±0.00^{d}	22.00±1.00^{e}	20.67±0.58^{f}
N+P	23.67±1.16^{c}	23.33±0.58^{e}	25.33±0.58^{c}	24.33±0.58^{d}	26.00±0.00^{d}
M+N+P	26.67±0.58^{b}	28.67±0.58^{c}	27.33±0.58^{b}	28.67±0.58^{b}	28.67±0.58^{c}
P+N+O	28.33±0.58^{a}	30.67±0.58^{b}	28.33±0.58^{ab}	27.67±0.58^{b}	30.67±0.58^{b}
P+M+O	22.33±0.58^{cd}	26.00±1.00^{d}	24.33±0.58^{c}	25.67±0.58^{c}	27.00±1.00^{d}
M+P+N+O	28.00±1.73^{ab}	32.67±1.16^{a}	28.67±0.58^{a}	31.33±0.58^{a}	32.67±0.58^{a}
P-CONTROL	21.67±0.58^{d}	26.00±0.00^{d}	18.67±0.58^{fg}	22.33±0.58^{e}	26.67±0.58^{d}
F-value	113.77	245.48	216.16	203.40	253.05
P-value	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Legend: O = orange, P= pawpaw, M = moringa, N = nanoparticle

Comparative antibacterial effectiveness

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The individual treatments reduced the microbial counts but were less effective than the best three- and four-component treatments. Orange seed powder produced the greatest reduction in TVC and TCC among the individual seed treatments, although all five target bacterial species remained detectable. All five species also persisted following treatment with moringa seed powder. Pawpaw seed powder and MgONPs reduced bacterial occurrence but did not completely eliminate the target organisms when applied individually.

Bacterial persistence depended on the treatment applied. Four species; *E. coli*, *P. vulgaris*, *S. typhimurium* and *K. pneumoniae,* persisted following pawpaw treatment. Among the combined treatments, *E. coli* and *S. typhimurium* persisted following moringa, MgONP treatment, while *E. coli*, *S. typhimurium* and *K. pneumoniae* remained detectable following moringa, orange treatment. Moringa-pawpaw failed to eliminate *E. coli*, *P. vulgaris*, *S. typhimurium* and *K. pneumoniae*. MgONP-orange eliminated four of the five target species, with only *P. vulgaris* remaining detectable.

The combination of the three seed powders eliminated *E. coli* and *S. typhimurium* but retained *P. vulgaris*, *S. dysenteriae* and *K. pneumoniae*. No target bacterial species was detected following MgONP-pawpaw, moringa-MgONP-pawpaw, pawpaw-MgONP-orange or complete moringa-pawpaw-MgONP-orange treatment.

The complete treatment demonstrated the greatest overall antibacterial effectiveness because it combined the absence of the five target species with the lowest TVC and TCC values and the largest inhibition zones against most of the bacterial species. Pawpaw-MgONP-orange produced inhibition zones of 28.33 ± 0.58, 30.67 ± 0.58, 28.33 ± 0.58, 27.67 ± 0.58 and 30.67 ± 0.58 mm against *E. coli*, *P. vulgaris*, *S. typhimurium*, *S. dysenteriae* and *K. pneumoniae*, respectively. Moringa-MgONP-pawpaw produced inhibition zones ranging from 26.67 to 28.67 mm. These values were generally larger than those produced by the individual seed powders and MgONPs.

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The lowest minimum inhibitory concentrations were recorded for moringa-pawpaw-MgONP-orange, pawpaw-MgONP-orange and moringa-MgONP-pawpaw, which produced MIC values below 5 mg/mL against the five target bacteria. Moringa-MgONP, MgONP-orange, MgONP-pawpaw and pawpaw-moringa-orange produced MIC values of 5 mg/mL, whereas the individual treatments generally required higher concentrations.

The lowest minimum bactericidal concentration of 5 mg/mL was recorded for moringa-pawpaw-MgONP-orange, pawpaw-MgONP-orange, moringa-MgONPs-pawpaw and MgONPs-pawpaw. Moringa-MgONP, MgONP-orange and pawpaw-moringa-orange produced MBC values of 10 mg/mL, while the individual treatments generally produced MBC values ranging from 20 to 50 mg/mL.

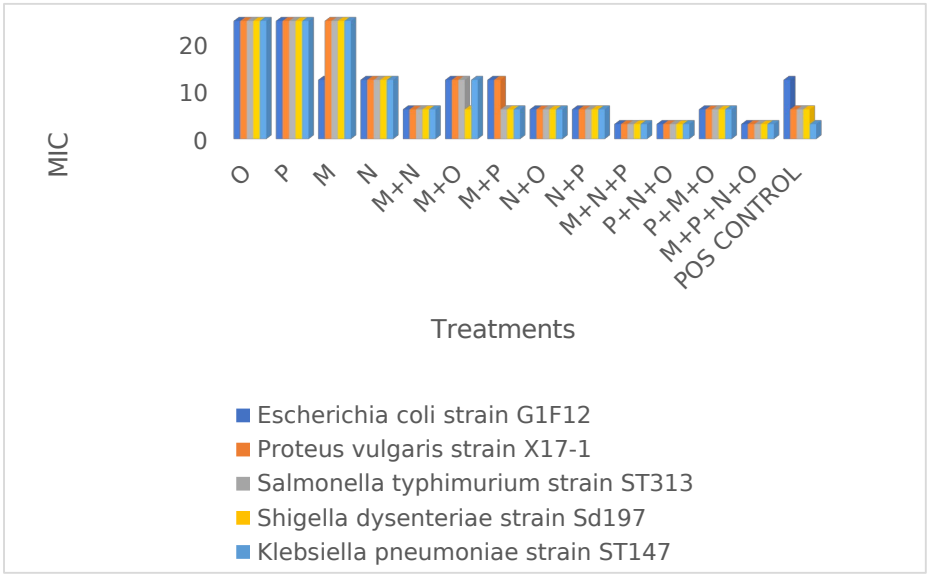

Figure 5: Minimum Inhibitory Concentration (MIC; mg/mL) of Single and Combined Treatments Against Test Bacteria
Legend: O = orange, P= pawpaw, M = moringa, N = nanoparticle, MIC = minimum inhibitory concentration

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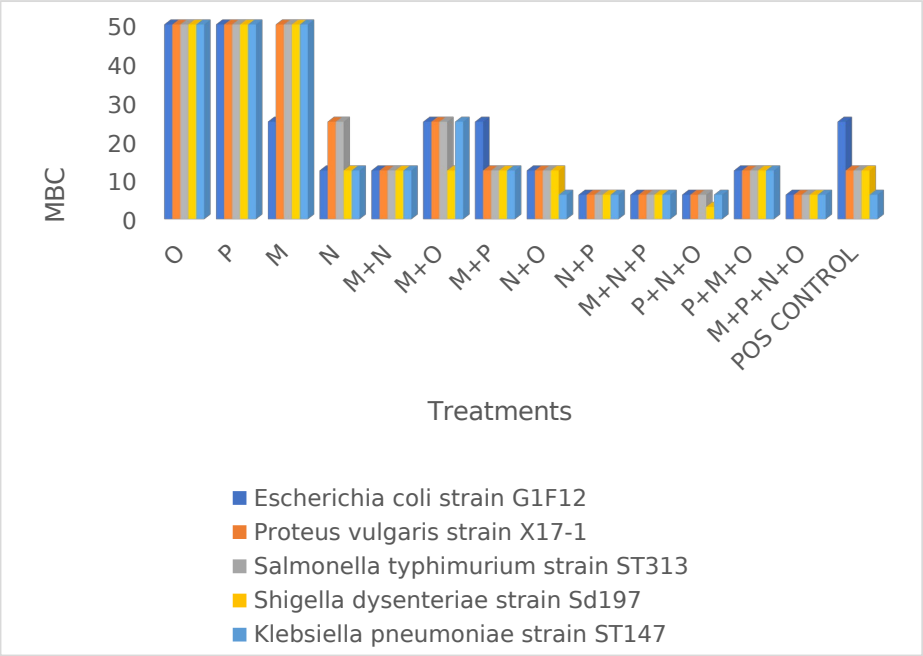

Figure 6: Minimum Bactericidal Concentration (MBC; mg/mL) of Single and Combined Treatments Against Test Bacteria

Legend: O = orange, P= pawpaw, M = moringa, N = nanoparticle, MBC = minimum bactericidal concentration

GCMS Analysis of Seed Powders

Table 5: Summary GCMS Profile of Active Ingredients Present in Seed Extracts

Moringa seed	Pawpaw seed	Orange seed
Quinoxaline, 2,3-diphenyl	Quinoxaline, 2,3-diphenyl	Quinoxaline, 2,3-diphenyl
Linoleic acid ethyl ester	Linoleic acid ethyl ester	Linoleic acid ethyl ester
Oleic acid	Oleic acid	Isopropyl linoleate (linoleic acid)
Glucosinolates	Glucosinolates	
Benzyl isothiocyanate (BITC)	Benzyl isothiocyanate (BITC)	Chalcone
Vitamin E (tocopherol)	Vitamin E (tocopherol)	Vitamin E (tocopherol)

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Di-alpha-Tocopherol Gamma silosterol	Di-alpha-Tocopherol	Di-alpha-Tocopherol
Behenic acid	Palmitic acid	Palmitic acid
9-Octadecenoic acid (Z)-methyl ester	9-Octadecenoic acid (Z)-methyl ester	Limonin
9-Octadecenoic acid (Z)- 2,3-dihydroxypropyl ester	9-Octadecenoic acid (Z)- 2,3-dihydroxypropyl ester	Terpenoids
9,12-Octadecadienoic acid	9,12-Octadecadienoic acid	Terpeines
4,4-isopropylidene bis)2-chlorophenol)	1, (4-Nitrophenyl) piperazine	Pentasiloxane, dodecamethyl
Furanone	Diosgenin	Quinoxaline, 2,3-diphenyl
Methylthiolane	1, 2-Benzenediol, 3,5 bis (1,1-dimethylethyl)	Alpha- Tocopheryl acetate
cis vaccenic acid	Carpaine	1-H-Inden-1-one, 2,3-diphenyl

Discussion

The high total viable count (TVC), total coliform count (TCC) and recovery of all five target bacterial species from the untreated contaminated water confirmed its poor microbiological quality and provided an appropriate baseline for comparing the treatments. The detection of *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Klebsiella pneumoniae* is important because these organisms include enteric and opportunistic pathogens associated with waterborne infections. However, as the water was experimentally inoculated, the findings reflect

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treatment performance under controlled conditions rather than the natural microbiological status of the original water source.

The individual seed powders significantly reduced TVC and TCC but did not eliminate all the target bacteria. Orange seed powder produced the greatest reduction among the individual seed treatments, whereas pawpaw and moringa seed powders showed comparatively lower activity. The greater reduction in TCC than TVC suggests that the treatments were more effective against coliforms than against the broader culturable bacterial population. Nevertheless, all five target species remained detectable after orange and moringa treatments, while four persisted after pawpaw treatment. Thus, reduced microbial counts did not necessarily indicate complete removal of the selected organisms.

The antibacterial effects of the seed powders may be associated with proteins, fatty acids, isothiocyanates and other constituents detected by GC-MS. Seed-derived proteins may promote the aggregation and settling of bacterial cells, while bioactive compounds may contribute to growth inhibition. Recent studies have demonstrated the coagulating and antibacterial potential of moringa seed powder and shown that its performance is affected by particle size, dosage, processing method and water characteristics (Aboagye *et al.*, 2021; Chales *et al.*, 2022; Shah *et al.*, 2024). Papaya seed-derived materials have similarly demonstrated potential as natural coagulants in water treatment (Cadena-Álava *et al.*, 2024). However, coagulation, bacterial-cell aggregation and the activities of individual GC–MS compounds were not measured directly in the present study. These processes therefore remain possible explanations rather than confirmed mechanisms.

The spectroscopic and microscopic analyses supported the successful formation of MgONPs. The UV–visible absorption peak, Mg-O-associated FTIR bands, predominantly magnesium EDX profile, approximately spherical morphology and XRD-derived crystallite size of 22.33 nm was consistent with

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recent characterizations of green-synthesized MgONPs (Muhaymin *et al.*, 2024). MgONPs produced larger inhibition zones than the individual seed powders, indicating stronger direct antibacterial activity under the agar-diffusion conditions. However, orange seed powder produced a slightly greater reduction in microbial counts in treated water. This difference may reflect the distinct principles of the assays: inhibition zones depend partly on diffusion through agar, whereas TVC and TCC measure surviving culturable organisms within the treated water.

The antibacterial activity of MgONPs has been associated with interactions with bacterial cell surfaces, changes in membrane permeability and oxidative stress (Rotti *et al.*, 2023). However, membrane integrity, reactive oxygen species and intracellular leakage were not evaluated in this study. These mechanisms cannot therefore be presented as experimentally confirmed explanations for the observed activity. Moreover, MgONPs alone did not eliminate all five target organisms, showing that their antibacterial activity was insufficient for complete treatment under the conditions examined.

Treatment performance improved markedly in selected multi-component combinations. The three-component treatments produced substantially lower TVC and TCC than the individual and most two-component treatments, while the complete moringa-pawpaw-MgONP-orange treatment produced the greatest overall reduction. No target species was recovered following MgONP-pawpaw, moringa-MgONP-pawpaw, pawpaw-MgONP-orange or the complete treatment. The complete treatment also produced the lowest TVC and TCC, large inhibition zones against most of the test organisms and low MIC and MBC values. Taken together, these indicators demonstrate that it was the most effective treatment under the experimental conditions.

Nevertheless, the results also show that the absence of the five target species did not always correspond to low overall microbial counts. For example, MgONP-pawpaw prevented the recovery of the selected organisms but retained comparatively high TVC and TCC values. This suggests that other

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culturable bacteria remained after treatment. Conversely, some treatments substantially reduced microbial counts while one or more target species remained detectable. Treatment effectiveness should therefore be interpreted using TVC, TCC, bacterial occurrence, inhibition zones, MIC and MBC collectively rather than relying on a single measurement.

The lower MIC and MBC values recorded for the most effective combinations confirm that smaller treatment concentrations were required to inhibit or kill the target bacteria than were required for the individual treatments. However, these findings should not be described as definitive evidence of synergy. Formal synergy assessment requires comparison of individual and combined MIC values using methods such as checkerboard assays and fractional inhibitory concentration indices (Bellio *et al.*, 2021). Because such analyses were not conducted, the results demonstrate enhanced antibacterial activity of selected combinations but do not establish whether the interactions were synergistic or additive. Similarly, the weaker performance of some two-component treatments should not be described as antagonism without appropriate interaction testing.

Differences in bacterial persistence may reflect variations in cell-envelope structure, physiological state and susceptibility to the treatment materials. These differences should not be interpreted as genetic resistance because resistance genes and molecular responses were not investigated. The contribution of pawpaw to all four treatments in which no target species was detected is notable; however, pawpaw alone was considerably less effective. Its presence may therefore have complemented the activities of MgONPs and the other seed powders, although the nature of this interaction requires further investigation.

The findings demonstrate that selected seed powder-MgONP combinations produced greater antibacterial effects than the individual treatments. The complete treatment showed the strongest performance across microbial counts, bacterial occurrence, inhibition zones, MIC and MBC. Nevertheless,

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the persistence of non-target culturable bacteria, together with the absence of toxicity, nanoparticle-residue and microbial-regrowth assessments, means that the treated water cannot yet be considered safe for drinking. The materials should presently be regarded as promising treatment or pretreatment agents requiring further optimization, filtration and safety evaluation before practical application.

Conclusion

The individual seed powders and green-synthesized MgONPs demonstrated antibacterial activity against *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Klebsiella pneumoniae*, but none completely eliminated the five target bacteria. Orange seed powder produced the greatest reduction in TVC and TCC among the individual seed treatments, whereas MgONPs produced the largest inhibition zones among the individual treatments. Selected multi-component treatments showed greater antibacterial effectiveness, although performance varied with treatment composition and bacterial species. No target bacterium was recovered following treatment with MgONP-pawpaw, moringa-MgONP-pawpaw, pawpaw-MgONP-orange or the complete moringa-pawpaw-MgONP-orange combination. The complete treatment demonstrated the strongest overall performance, producing the lowest TVC and TCC, large inhibition zones and low MIC and MBC values. These findings indicate that selected seed powder–MgONP combinations are promising treatment or pretreatment materials for bacterially contaminated water. However, the results do not independently confirm that the treated water is safe for direct consumption.

Recommendations

Further studies should optimize the concentrations, component ratios and contact times of the most effective treatments. The complete moringa-pawpaw-MgONP-orange treatment and the MgONP-pawpaw, moringa-MgONP-pawpaw and pawpaw-MgONP-orange combinations should be

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evaluated using larger water volumes, naturally contaminated water and field-scale treatment conditions. Future investigations should assess nanoparticle residues, microbial regrowth, storage stability and toxicological and environmental safety. Formal checkerboard or fractional inhibitory concentration assays should also be conducted to determine whether the enhanced activity of the combinations represents synergistic or additive interactions. Before household or community application, the treatment process should be standardized, its cost-effectiveness established and the treated water confirmed to meet applicable microbiological and chemical drinking-water standards.

Declarations

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Conflict of interest

The authors declare that they have no competing interests.

Data-availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical and environmental compliance

The study did not involve human participants or experimental animals. Laboratory procedures were conducted in accordance with applicable institutional safety and environmental requirements.

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