Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2024, Volume 15, Issue 9, Page 1233-1249

http://www.jmaterenvironsci.com

Evaluation of the Water Purification/Disinfection Efficacy of Neem and Basil Leaves for Reducing Pathogens in Drinking Water

[1]Isa L. I., [1]Ogofure A. G*., [1]Isichei-Ukah O. B., [2]Ehigiegba A. T., [1]Oviasogie F. E.
[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, P.M.B.1154, Nigeria
[2]Energy Commission of Nigeria, National Centre for Energy and Environment, University of Benin.
**Corresponding author, Email address: abraham.ogofure@uniben.edu*

Received *03 July 2024,*
Revised *05 Sept 2024,*
Accepted *05 Sept 2024*

Keywords:
- ✓ *Solar disinfection;*
- ✓ *Neem Leaves;*
- ✓ *Basil Leaves;*
- ✓ *Water Purification;*
- ✓ *Drinking water*

Citation*: Isa L.I., Ogofure, A. G., Isichei-Ukah, O. B., Ehigiegba, A. T. and Oviasogie F. E. (2024) Evaluation of the Water Purification/Disinfection Efficacy of Neem and Basil Leaves for Reducing Pathogens in Drinking Water. J. Mater. Environ. Sci., 15(9), 1233-1249*

Abstract: This study evaluated the water purification efficacy of Neem, and Basil leaves using two separate experimental processes with four treatments each to reduce pathogens in river water samples. The samples' physicochemical parameters (pH, electrical conductivity, turbidity, nitrate, sulphate, and dissolved oxygen) were assessed using standard analytical methods, and heterotrophic plate count was used for bacterial analysis. Bacteria were identified using biochemical and molecular techniques, with phenotypic virulence properties (hemolysin, DNase, gelatinase, and lipase) and antibiotic susceptibility tested via Kirby-Bauer disc diffusion. The purification efficacy was assessed using a log reduction model, with statistical analysis by Student's t-tests. Results showed pH ranged from 6.63±0.12 (control) to 7.20±0.10 (Basil leaves), temperature from 26.00±1.00°C (control) to 27.00±1.50°C (neem leaves), and turbidity from 0.30±0.10 NTU (control) to 0.17±0.06 NTU (neem leaves). Heterotrophic bacterial counts (log_{10} CFU/ml) before treatment ranged from 2.22±0.03 (control) to 2.29±0.02 (water + neem + Basil) and from 1.50±0.04 (control) to 1.32±0.03 (water + neem + Basil) after treatment. Neem leaves showed an 80.85% bacterial reduction in control samples and 90.2% in neem-treated samples. *E. coli* reduction was 90.35% with neem leaves, compared to 78.65% in the control. Identified bacteria included E. coli, Enterobacter cloacae, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Proteus mirabilis*. The isolates showed susceptibility to meropenem, gentamicin, amoxicillin/clavulanic acid, and ciprofloxacin but resistance to sulfamethoxazole, erythromycin, and tetracyclines

1. Introduction

Water is essential for all living organisms, supporting numerous physiological and ecological functions. However, in many parts of the world, including Nigeria, access to potable water remains a significant challenge. Potable water, necessary for drinking, bathing, washing, and cooking, is primarily supplied by the government in Nigeria. Yet, due to the growing population and expanding settlements, this supply often falls short, prompting communities to rely on alternative sources such as rivers, streams, boreholes, and wells. According to a report by Premium Times, over 26.5 million children in Nigeria are vulnerable to water-related diseases, as estimated by UNICEF (Adebowale-Tambe, 2021). The use of contaminated water and poor sanitation practices significantly increase the risk of waterborne diseases, with only 26.5% of the population having access to improved drinking water sources and sanitation facilities (FMWR, NBS, and UNICEF, 2020). Achieving Sustainable

Development Goal 6 (SDG 6), which calls for universal access to safe and affordable drinking water and sanitation by 2030, requires extraordinary efforts.

Diarrheal diseases claim the lives of over 6 million children annually in developing countries (Ali *et al.*, 2010). The World Health Organization (2023) notes that waterborne diseases cause four-fifths of illnesses, leading to dehydration and being the primary cause of childhood death in these regions. Contaminated water is responsible for millions of gastrointestinal illness cases each year, particularly in rural communities with inadequate sanitation (Caslake *et al*., 2004). Common pathogens in contaminated water include fecal coliforms and opportunistic bacteria such as *Klebsiella oxytoca*, *Escherichia coli*, *Enterobacter cloacae*, *Salmonella enterica*, *Vibrio cholerae*, and *Shigella* spp. (Willey *et al.*, 2008; Gupta *et al.*, 2015). These pathogens have been linked to outbreaks of gastrointestinal diseases worldwide (Omole *et al*., 2015; Kotloff, 2017; Tarr *et al.*, 2018).

Contamination of drinking water can occur at collection points or during storage, necessitating strategies to protect water quality before consumption (Brahimi *et al.*, 2015). In rural areas, common water sources include unprotected wells, streams, ponds, and harvested rainwater, posing significant health risks. Consequently, effective water treatment methods are crucial to reduce pathogen loads and ensure safe drinking water. Various treatment processes, such as coagulation/flocculation, sedimentation, filtration, and disinfection (typically using chlorine), are standard in the industry (Ali *et al.*, 2010). Advanced technologies involve using chemicals like aluminum sulfate and plant-based coagulants to enhance purification and reduce pathogenic bacteria (Joshi and Jani, 2016; Tunggolou and Payus, 2017). However, these methods are often expensive and impractical for poor rural communities. Limited research exists on the use of plant leaves combined with solar disinfection for water purification, with most studies focusing on either solar disinfection or plant parts alone (Dhruva and Suresh, 2016; Tunggolou and Payus, 2017; Geng *et al.*, 2019; Hamed *et al.*, 2019; Okuda and Ali, 2019). Preliminary studies indicate that phyto-disinfectants could be a cost-effective and ecological alternative for water purification.

This study aimed to evaluate the purification efficacy of neem and Basil leaves on contaminated water samples. Specifically, it sought to determine the physicochemical properties of water samples before and after treatment, evaluate the heterotrophic bacterial burden before and after treatment, and assess the purification efficacy of neem and Basil leaves through log and percentage bacterial reduction. Additionally, the study aimed to identify bacterial isolates in the water samples at the molecular level, evaluate the phenotypic virulence of these bacterial isolates, and assess their antimicrobial susceptibility and multiple antibiotic resistance index.

2. Methodology

2.1 Study Design

The study was carried out to evaluate the water purification capacity of neem and basil leaves. Eight different independent treatments (T1 -T8) were employed in this study. The treatments were divided into two experiments, with the first four treatments containing the river water sample only (T1), water sample + neem leaves (T2), Water + Basil leaves (T3), and water sample + Neem and Basil leaves (T4). The second set included the use of standardized density of bacterium (E. coli) + water sample only (T1), water + E. coli + Neem leaves (T2), water + E. coli + Basil leaves (T3), and water + E. coli + Neem and Basil leaves (T4). A 500 ml volume of Water was used constantly across all treatments. The plastic pet bottles had a capacity of 1000 ml (1 L), and an equal number of leaves (30) were added to each bottle in the treatment. The experiment was carried out in three independent replicates.

2.2 Sample Collection and Preparation

Fresh leaves (Neem and Basil) were obtained from the market and identified by a taxonomist in the Department of Plant Biology and Biotechnology, UNIBEN. The leaves were washed with sterile water and surface sterilized before rinsing again with cool sterile water and placed into the well-labelled plastic containers before adding water samples (and standardized bacterium + water sample for experiment 2). All treatments were left to stand for 4 hours under the sun, and microbiological analyses were carried out for the isolates before and after treatments (4-hour exposure in the sun)

2.3 Enumeration and Isolation of Total Heterotrophic Bacteria

The method employed was the standard plate count, where 0.1 ml of the samples were cultured in triplicates onto nutrient agar plates for heterotrophic bacteria count using the pour-plate technique. The plates were incubated at 37^0C for the total bacterial count. Colonies were counted after 24 hours of incubation, and the count in colony-forming units per ml was calculated using the formula below.

$$\frac{cfu}{ml} = \frac{number\ of\ colonies}{volume\ of\ inoculum} \qquad (1)$$

Log reduction for the counts obtained was evaluated using the formula below.

$$LR = log_{10}(A) - log_{10}(B)$$

Where: A is the number of organisms before treatment, and B is the amount after treatment
For percentage bacteria reduction, the following mathematical formula was employed

$$P = (1 - 10^{-L})\ x\ 100$$

$$where\ P = percentage\ microbial\ reduction, L = \log reduction$$

(Willey *et al*., 2008; Ogofure and Ologbosere, 2023).

2.3 Enumeration and Isolation of Total Heterotrophic Bacteria

Pure cultures of the bacterial isolates were obtained from the subculture of a single colony from the successful pour plate technique and were characterized using cultural, morphological and biochemical methods. Several tests, such as Gram reaction, catalase, urease, indole, oxidase, citrate utilization and respective reactions of bacteria on triple sugar iron agar, were carried out to identify bacterial isolates presumptively (Holt *et al*., 1994).

2.4 Molecular Analysis and Sequencing of Bacterial DNA

The methodology for this study involved a detailed process of DNA extraction, PCR amplification, and subsequent phylogenetic analysis to characterize and analyze bacterial DNA. DNA extraction was performed using the boiling method by Chakravorty *et al*. (2007), where a 2 mL nutrient broth culture of the bacterium was centrifuged, and the supernatant was discarded. The pellets were mixed with sterile distilled water, vortexed, heated, and centrifuged again, yielding the supernatant as pure DNA for further analysis. Ten microliters of this DNA were utilized for gene amplification through PCR. For the PCR, a reaction mixture was prepared, comprising various components such as GoTaq® buffer, MgCl2, dNTPs mix, specific primers, Taq DNA polymerase, and the DNA template, adjusted to a total

volume of 42 µL. The PCR process was executed using a GeneAmp 9700 thermal cycler, with an initial denaturation step followed by 30 cycles of denaturation, annealing, and extension, concluding with a final extension step. The amplified PCR products were stored at 4°C until further use. The integrity of the amplified DNA fragment was confirmed using 1% agarose gel electrophoresis. The gel was prepared with TAE buffer and ethidium bromide, solidified, and submerged in a gel tank. Samples mixed with a loading dye were added to the wells, and electrophoresis was carried out at 120V for 45 minutes. The gel was then visualized under UV light, and the size of the PCR products was compared with a molecular weight ladder. To purify the amplified DNA fragments, ethanol was used to eliminate PCR reagents. The PCR product was mixed with ethanol and sodium acetate, vortexed, and stored at -20°C. The mixture was centrifuged, the supernatant discarded, and the pellet washed with ethanol, centrifuged again, dried, and resuspended in sterile distilled water. The purity was verified using agarose gel electrophoresis and a Nano-Drop spectrophotometer.

Sequencing of the amplified DNA was conducted using the Applied Biosystems™ Genetic Analyzer and the BigDye™ terminator kit, following the manufacturer's guidelines. The resulting sequences were analyzed using the NCBI BLAST tool, and phylogenetic analysis was performed using PhloT software and the Itol platform, providing a comprehensive molecular characterization of the bacterial DNA (Ogofure and Ologbosere, 2023).

2.5 Phenotypic Virulence Tests for Bacterial Isolates

To characterize the phenotypic virulence factors of bacterial isolates, several tests were conducted, including the nutrient gelatin test, DNase test, spirit blue agar test, and hemolysis on blood agar base. Each test aimed to identify specific enzymatic activities that contribute to the pathogenicity of the bacteria.

The nutrient gelatin test was employed to differentiate gelatinase-producing bacteria based on their ability to hydrolyze gelatin. Nutrient gelatin was prepared, dispensed into test tubes, and autoclaved. After cooling, the tubes were inoculated with pure bacterial isolates and incubated at 37°C for up to 48 hours. Post-incubation, the tubes were placed in an ice bath for 30 minutes to verify gelatin liquefaction. Gelatinase-positive bacteria liquefied the medium, whereas gelatinase-negative bacteria left it solidified (Duarte *et al*., 2016).

For the DNase test, DNase agar was prepared, autoclaved, cooled, and poured into sterile petri plates. The plates were inoculated with pure isolates and incubated at 37°C for 24 hours. DNase-positive strains produced a clear zone around the colonies, indicating DNA hydrolysis, while DNase-negative strains showed no such zone (Sharma *et al*., 2019).

The spirit blue agar test identified lipolytic microorganisms capable of producing lipase, which hydrolyzes triglycerides into glycerol and fatty acids. Spirit blue medium was prepared, autoclaved, and mixed with 5% sterile olive oil. After cooling, the medium was poured into Petri dishes, inoculated with pure isolates, and incubated at 37°C for 24 hours. Lipase-producing organisms developed a clear zone and/or deep blue coloration around the colonies (Pascoal *et al*., 2018).

The hemolysis test on sheep blood agar was used to observe hemolytic reactions. Sheep blood agar was prepared, sterilized, enriched with 1% sterile sheep blood, and poured into Petri dishes. After inoculation with pure isolates, the plates were incubated at 37°C for 24 hours. Hemolysis was categorized as alpha (partial hemolysis with greenish discoloration), beta (complete hemolysis with clear zones), or gamma (no hemolysis with no medium change) (Sarowska *et al*., 2019). These phenotypic tests provided essential insights into the virulence characteristics of the bacterial isolates, aiding in their identification and understanding of their pathogenic potential.

2.6 Antibiotic Sensitivity Testing and MAR Index Evaluation

The antimicrobial susceptibility testing and MAR index evaluation provided a comprehensive understanding of the resistance profiles of the bacterial isolates, highlighting the importance of continuous monitoring and the need for effective antibiotic stewardship. The antimicrobial susceptibility of bacterial isolates was assessed using the Kirby-Bauer disk diffusion method. This test followed the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020). Eight antibiotic discs representing drugs commonly used in treating human and animal infections were tested against the bacterial isolates on Mueller-Hinton agar. The antibiotics tested included Ciprofloxacin (5µg), Ceftriaxone (30 µg), Sulfamethoxazole (1.25/23.75 µg), Erythromycin (15 µg), Tetracycline (30 µg), Meropenem (10 µg), Amoxicillin/clavulanic acid (20/10 µg), and Gentamicin (10 µg). The bacterial cells were standardized to a 0.5 McFarland turbidity standard, and 0.1 ml of this standardized bacterial suspension was spread evenly on Mueller-Hinton agar plates using sterile swabs. Once the plates were dry, antibiotic discs were aseptically placed onto the surface. The plates were then incubated at 37°C for 24 hours. Post-incubation, the zones of growth inhibition around the antibiotic discs were measured. The sizes of these zones were evaluated according to the CLSI standard guidelines to determine the susceptibility of the isolates to each antibiotic. The Multiple antibiotic resistance (MAR) index was determined for $MAR\ index = \frac{y}{nx}$. Where y = number of resistances scored, n = number of isolates, x = number of antibiotics. A MAR index above the standard permissible limit of 0.2 indicates significant resistance, suggesting potential health implications. This evaluation was crucial for understanding the phenotypic drug resistance patterns among the bacterial isolates, providing insights into their resistance levels and the potential challenges in treating infections (Chitanand *et al*., 2010; Davis and Brown, 2016; Ogofure *et al*., 2022).

2.7 Physicochemical Properties of Water Samples

The pH of water samples was determined using a Hanna® pH meter. Calibration was done with a bicarbonate buffer at 7.0 before measuring 20mL of the samples in rinsed beakers. Salinity was measured in situ using a Water-Testing Salinity meter, immersing the device in the water samples for five minutes to equilibrate. Temperature readings were obtained using a Hanna® thermometer, immersing it for three minutes. Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were measured with a WTW Series Cond 730 conductivity meter. Calibration was done with potassium chloride, and 100mL of water samples were analyzed. Total Suspended Solids (TSS) and turbidity were determined using a HACH® colorimeter (DR/890). For TSS, the colorimeter was programmed and zeroed with distilled water before measuring the samples. Turbidity was measured in Formazin Attenuation Units (FAU). Nitrate and phosphate concentrations were analyzed with the same colorimeter, adding specific reagents and measuring the resulting concentrations. Sulphate was analyzed similarly with the colorimeter, using Sulfa Ver 4 reagent. Biological Oxygen Demand (BOD) was calculated by incubating water samples in airtight bottles and measuring dissolved oxygen initially and after five days. Chemical Oxygen Demand (COD) was determined by refluxing the sample with potassium dichromate and titrating with ferrous ammonium sulphate. Heavy metals, including lead and cadmium, were measured using Atomic Absorption Spectrometry (AAS), with wavelengths of 217nm and 228.8nm, respectively. Zinc concentration was determined using the colorimeter, adding Zinco Ver 5 powder and measuring the resulting orange color (APHA, 1995).

3. Results and Discussion

3.1 Bacteriological Evaluation of Water Samples

The evaluation and purification efficacy of Neem and Basil leaves on contaminated water samples were assessed in this chapter. The heterotrophic bacterial counts (log_{10} CFU/ml) before treatment ranged from 2.22±0.03 (control or river water only) – 2.29±0.02 (Water + Neem + Basil Leaves) and from 1.50±0.04 (control) – 1.32±0.03 (Water + Neem + Basil leaves) after treatment. A similar trend was also observed for heterotrophic bacterial counts of water samples before treatment for other matrixes/regimes (water + neem leaves and water + neem + Basil leaves) and after treatment. Generally, it was observed that there was a reduction in the bacteria density/counts after treatment using the plant leaves. Furthermore, the control sample/regime also had a reduced bacteria density, suggesting that the reduction could be due to the effect of solar disinfection. There was a significant difference ($p = 0.044$) in the bacteria density of the water samples in the control regime (Water only). There was also no significant difference ($p = 0.061$) in the bacteria density for the regime containing water + basil leaves. Aside from the regime mentioned above, with no significant difference in the bacterial enumeration before and after treatment, other regimes such as water + neem leaves ($p= 0.034$) and water + neem+ basil leaves ($p=0.023$) had significant differences in bacterial counts before and after treatment. Aside from the statistically insignificant regime (water + basil leaves), other regimes, including the control, had significant differences in the bacteria population of the water samples.

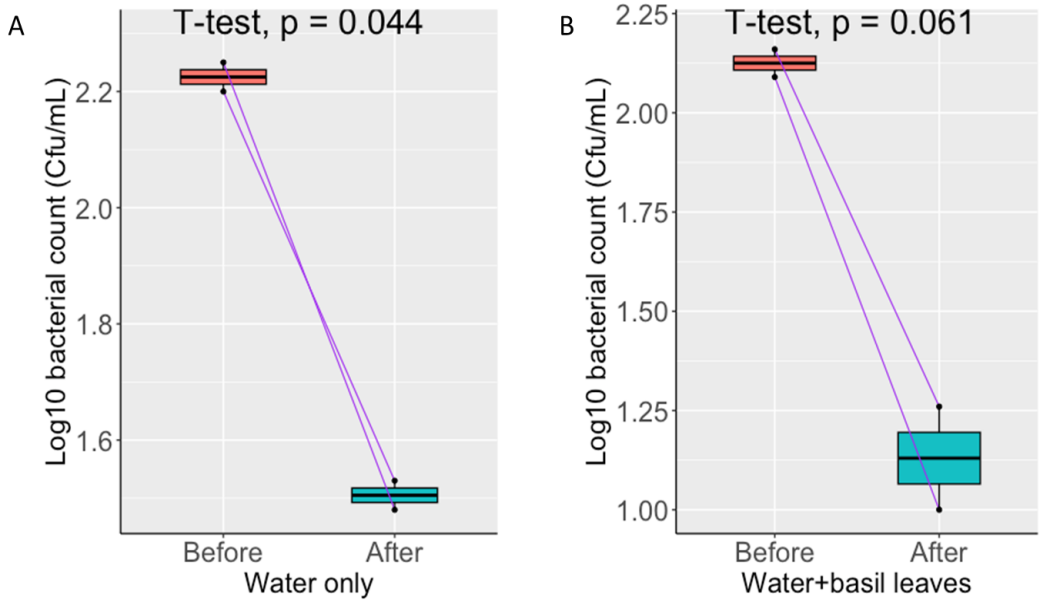

Figure 1: Boxplot of heterotrophic bacterial counts before and after 4 hours of solar treatment for experiments 1 matrix in (A) River water only (B Water+basil leaves. Data were analysed in R studio using paired T-test. *p* values > 0.05 were considered statistically insignificant, while *p* values < 0.05 are significant

Following 4 hours of exposure to the respective regimes in the sun, the bacteria counts were evaluated, and it was revealed that treatments containing water + basil leaves, water + neem leaves and a consortium of both leaves were found to be within the ground mean value while the control was found to be above the said value. Evaluating the statistical differences between treatments, it was evident that no significant difference ($p=0.08$) or an insignificant difference exists between control and treatment regimes (**Figure 3**).

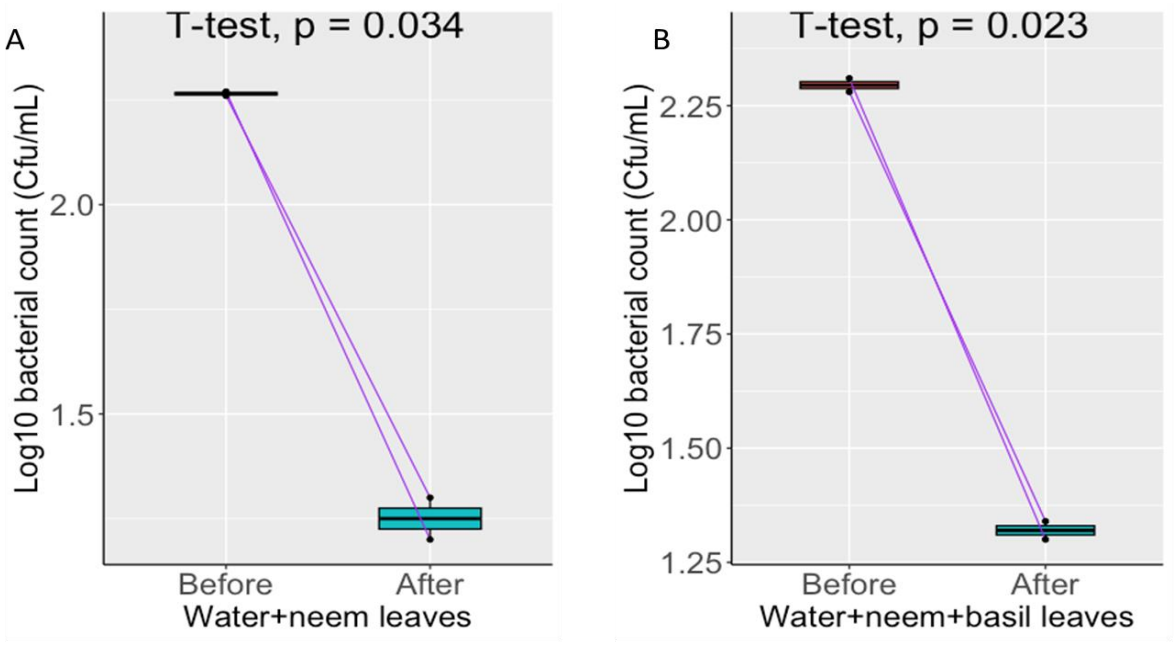

Figure 2: Boxplot of heterotrophic bacterial counts before and after 4 hours of solar treatment for experiments 1 matrix in (A) Water + Neem leaves (B) Water + Neem+ Basil leaves. Data were analysed in R studio using paired T-test. *p* values>0.05 were considered statistically insignificant, while *p* values < 0.05 are significant

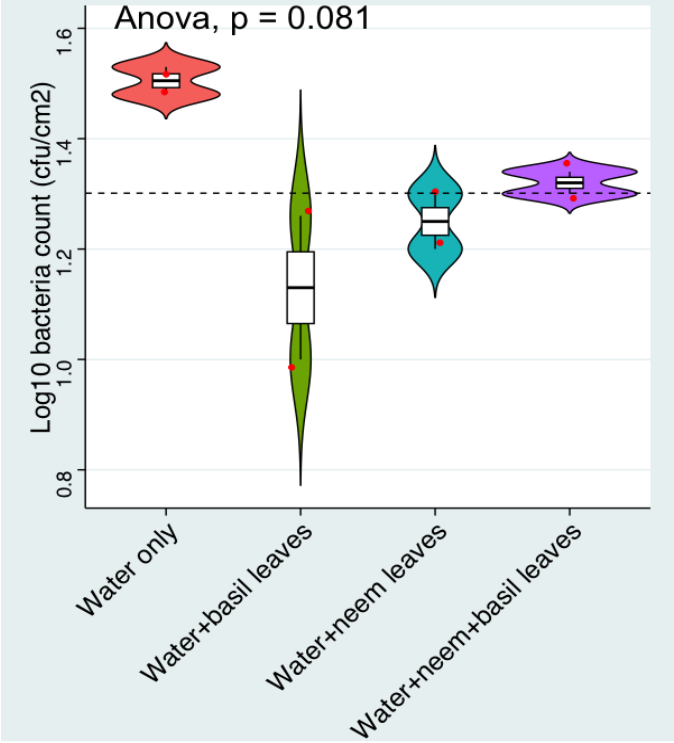

Figure 3: Violin plot of heterotrophic bacteria counts after 4 hours of solar treatment for experimental matrix. The dotted horizontal line represents the grand mean. Data were analysed in R studio using one-way ANOVA. *p* values > 0.05 were considered statistically insignificant.

The efficacy of the treatment as represented by their respective percentage bacteria reduction revealed that there was a 90.28% reduction of bacteria population in the water + neem leaves regime while 89.96 % and 89.35% reduction was observed for water + basil leaves and water + neem + basil leaves. These values were higher than the percentage bacteria reduction observed for the control samples containing

river water only. These percentage reductions observed in the treatments correspond to the bacteria log reduction observed before and after treatment for the respective regimes employed in the study. A 1 log reduction was observed for wter + neem leaves and water + basil leaves while a 0.97 log difference was observed for water + neem + basil leaves. The purification efficacy of the leaves after 4 hours of exposure in sunlight showed that there was 80.85 % bacterial reduction in control samples (river water), while a 90.2 % bacterial reduction was obtained in the treatment containing Neem leaves + River water samples. Generally, other treatments compared favorably with the control and this further describes the efficacy of using neem and Basil leaves for water purification purposes.

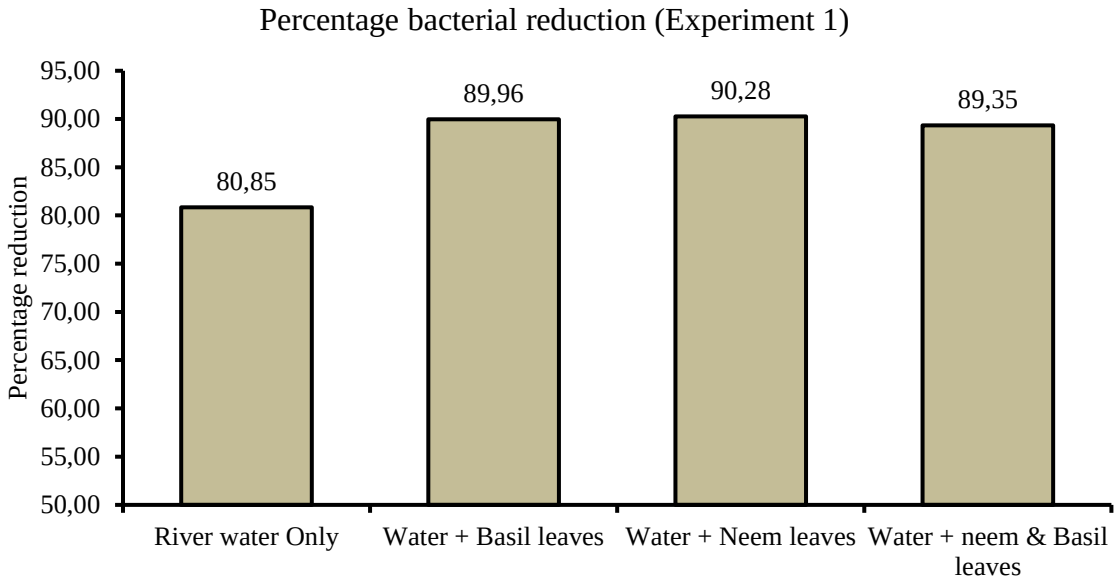

Figure 4: Bacterial log reduction and efficacy of solar treatment for experiments one matrix after 4 hours

When *E. coli* was used in another experimental setup (experiment 2) with the same variables, the *E. coli* counts before treatment ranged from 3.00±0.01 (control) – 3.06±0.02 (Water + E. coli + Neem and Basil leaves). Concerning the counts of the bacterium in the treatment regimes, it was observed that (similar to the trend in experiment 1) *E. coli* counts in water samples before and after treatment in all matrixes (water + neem leaves, water + neem + Basil leaves, and control) had a reduced population or density. More so, the reduction of the bacterium before and after treatment in all the regimes was found to be statistically significant, with the control regime (Water + *E. coli*) having a probability value of *0.023* (*p=0.023*) and the regimes containing water + *E. coli* + neem + Basil leaves having a probability value of 0.037 (*p=0.037*). More so, it was observed that regimes containing the consortium of leaves and that of water + neem leaves were found to have values below the grand mean of $\log_{10}$ of 2.0. This further suggests that the regimes containing neem leaves and the consortium (Basil + neem leaves) were more effective in reducing the density/contamination of the bacterium in the water samples than the other treatments employed in the study. More importantly, only the two treatments mentioned above had a 1 log reduction, which culminates to greater than 90% of *E. coli* reduction in water samples. In this particular experimental set up, it was observed that the percentage bacteria reduction in the control was least (78.65%) while it was highest in the consortium containing water + *E. coli* + neem + Basil leaves (95.4%). There was also a favourable decrease in the burden when *E. coli* was used as the test bacterium with a percentage reduction of 90.35 % for the treatment containing water + *E. coli* + Neem leaves compared to control (*E. coli* + water) with 78.65 % bacterial reduction.

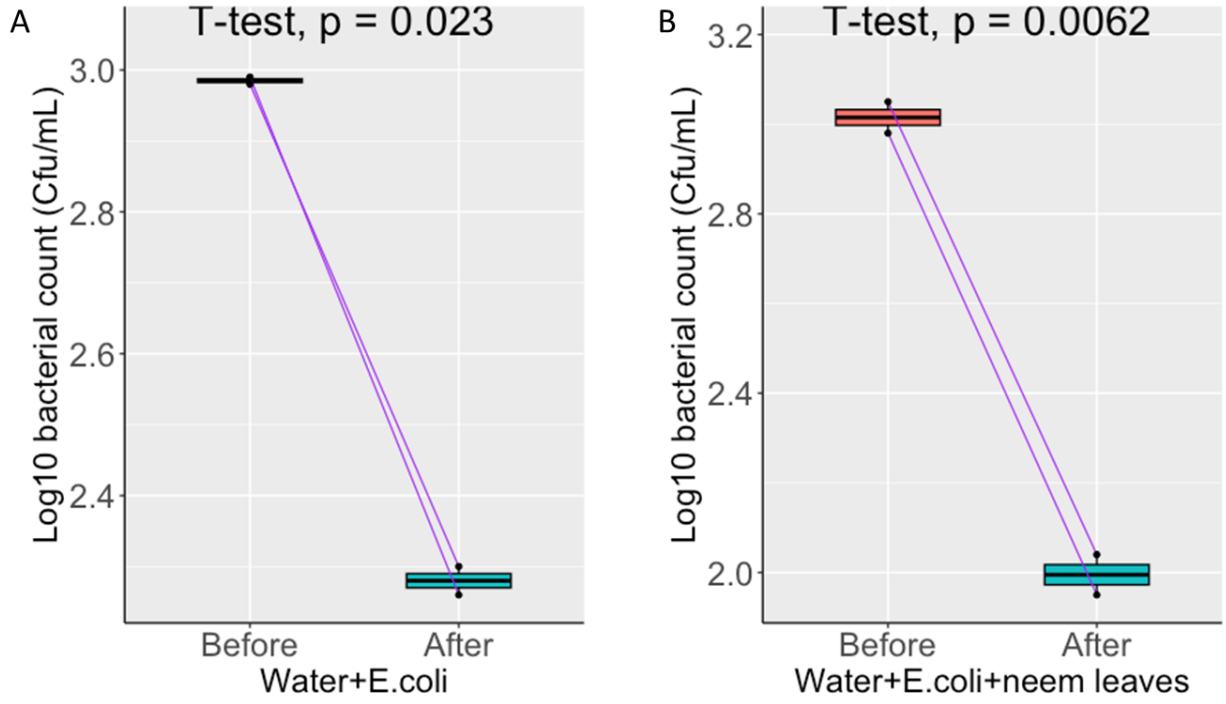

Figure 5: Boxplot of heterotrophic bacterial counts before and after 4 hours of solar treatment for experiment 2 matrixes using 0.5 McFarland E. coli in (A) Water + E. coli (B) Water + E. coli + Neem+ Basil leaves. Data were analysed in R studio using paired T-test. *p* values > 0.05 were considered statistically insignificant, while *p* values < 0.05 are significant.

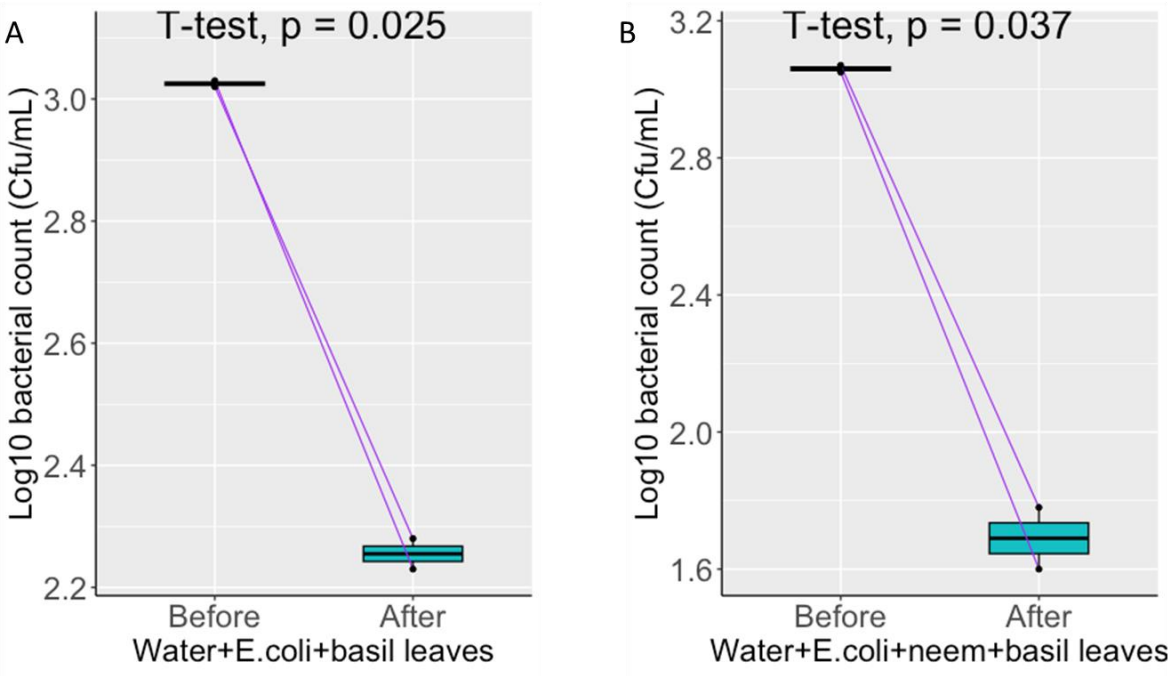

Figure 6: Boxplot of heterotrophic bacterial counts before and after 4 hours of solar treatment for experiment 2 matrixes using 0.5 McFarland *E. coli* in 2 in (A) Water + *E. coli* + Basil leaves (B) Water + *E. coli* + Neem + Basil leaves. Data were analysed in R studio using paired T-test. *p* values > 0.05 were considered statistically insignificant, while *p* values < 0.05 are significant

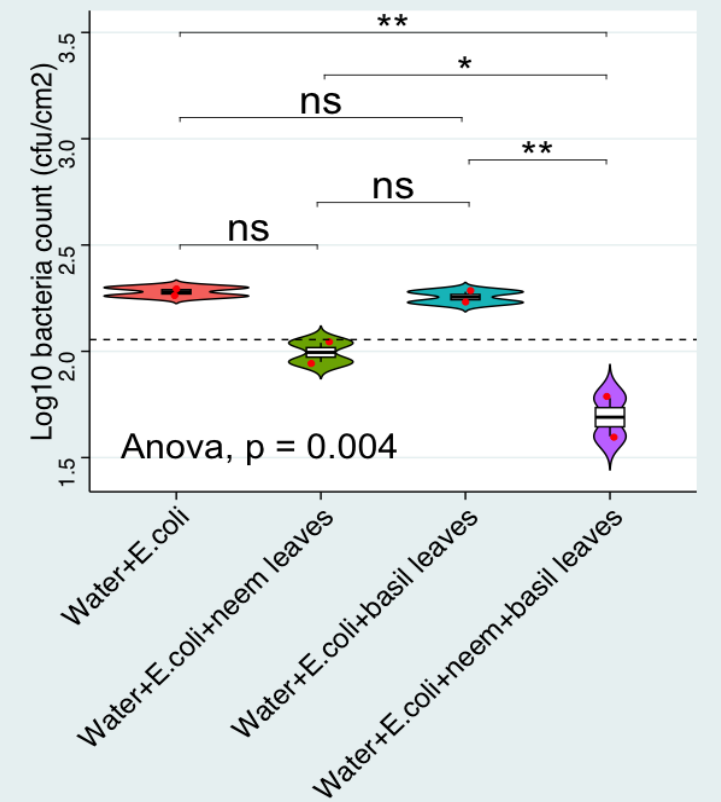

Figure 7: (A) Violin plot of heterotrophic bacteria counts after 4 hours of solar treatment for experiments two matrix. The dotted horizontal line represents the grand mean. Data were analysed in R studio using one-way ANOVA. nsp >0.05; *p < 0.05; **p < 0.01

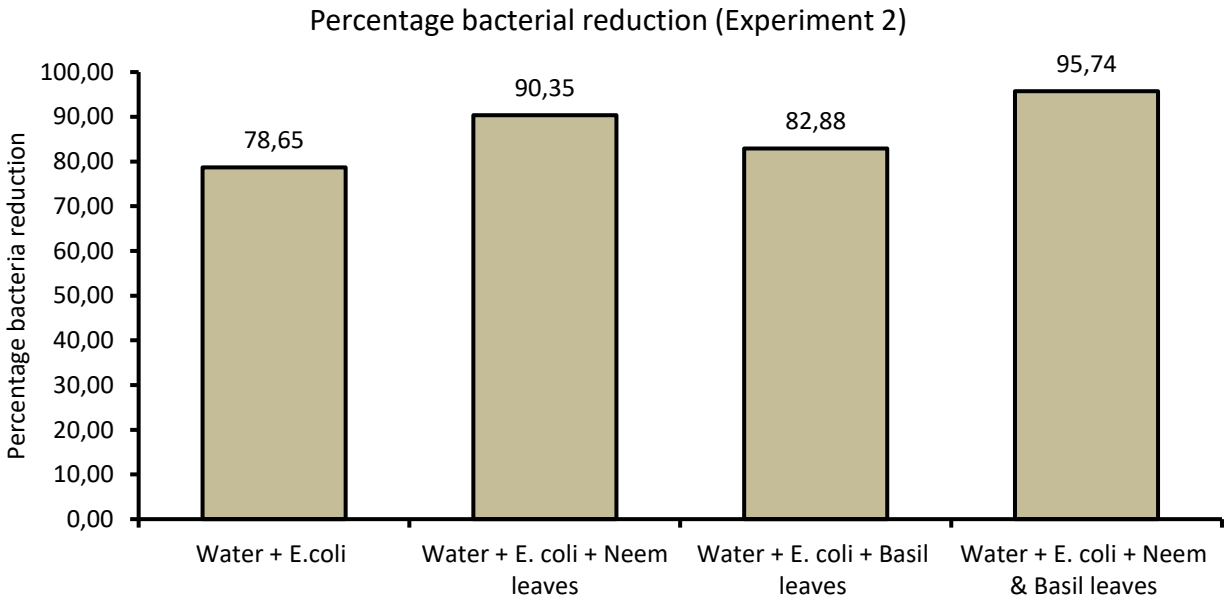

Figure 8: Bacterial log reduction of solar treatment for experiments two matrix after 4 hours.

The microbiological evaluation (before and after treatment) of the regimens using leaves of neem, basil and a consortium of both revealed that the heterotrophic bacterial counts (log10 CFU/ml) before treatment ranged from 2.22±0.03 (control or river water only) – 2.29±0.02 (Water + Neem + Basil Leaves) and from 1.50±0.04 (control) – 1.32±0.03 (Water + Neem + Basil leaves) after treatment. The general bacteria reduction observed across the regimens employed in the study was consistent with the

reports in the literature concerning the use of the plant for decontamination and purification of water, as reported by Asemave and Ayom (2021) who employed the use of neem leaves powder as an alternative anticoagulant for water purification. Desu (2022) also revealed that neem leaves have been used for decontamination and purification of water. Notably, the methods used for decontaminating water using the plants were somewhat different than what was obtained in this study. Neem leaves powder was employed as a decontaminant and a water purifier in the study of Asemave and Ayom (2021), while the fresh leaves in this study were added to the contaminated water and the microbial reduction was evaluated before and after treatment. Regardless, it has been found that plant parts have been used over time for the treatment of wastewater (Achupriya, 2022) and for decontamination of decontaminated water (Chandel *et al.*, 2022). In this study, the purification efficacy of the leaves after 4 hours of exposure to sunlight showed an 80.85 % bacterial reduction in control samples (river water), while a 90.2 % bacterial reduction was obtained in the treatment containing Neem leaves + River water samples. Generally, other treatments are compared favourably with the control, and this further describes the efficacy of using neem and basil leaves for water purification purposes. There was also a favourable decrease in the burden when E. coli was used as the test bacterium, with a percentage reduction of 90.35 % for the treatment containing water + E. coli + Neem leaves compared to the control (E. coli + water) with 78.65 % bacterial reduction.

The results obtained in this study were also consistent with the findings of Asemave and Ayom (2021), who reported a 2 log decrease in the after-effect of using dried powdered herbs such as neem and moringa leaves powder to decontaminate and treat wastewater. Similar to the report by Asemave and Ayom (2021), and Chandel *et al*. (2022) evaluated the efficacy of some herbs to decontaminate and treat water samples with interesting results similar to the findings obtained in this study.

3.2 Isolation of Bacteria, Phenotypic Virulence and Antibacterial Sensitivity

The identified bacterial isolates obtained from the river water samples evaluated in the study include *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa, Salmonella enterica* and *Proteus mirabilis*. The phylogenetic relatedness of the isolates revealed a close relationship amongst the all bacteria except for *P. aeruginosa*. One of the isolates specifically the bacterium (*E. coli*) used in experiment 2 was obtained from bacteriology laboratory of University of Benin Teaching Hospital (UBTH). The isolates (*Pseudomonas* and *Proteus*) were found to possess certain phenotypic virulence properties such as DNase, gelatinase and lipase. This further suggests that all isolates were potential pathogens of public health significance and they are capable of causing diseases of varying severity in the community. The bacterial isolates were found to be susceptible to some of the antibiotics from different classes used in the study, such as meropenem, gentamicin, amoxicillin/clavulanic acid and ciprofloxacin, while resistance was observed most for sulfamethoxazole, erythromycin and tetracyclines. The isolates obtained from the water samples were found to be of public health importance because they had multiple antibiotic resistance index greater than 0.2 which is regarded as the permissible limit for any bacterium that is not termed a multidrug resistant bacterium (Table 1). All the isolates obtained in the study were multi resistant and there were of public health importance or consequence.

The identified bacterial isolates in the water samples evaluated in this study include E. coli, Enterobacter cloacae, Pseudomonas aeruginosa, Salmonella enterica and Proteus mirabilis. The results obtained in this study were similar to reports by Ekhaise and Anyasi (2005), Ololade and Ajayi (2009), Aliyu *et al*. (2016), as well as Akinbile and Omoniyi (2018), who analyzed different river water samples in Benin City, Edo State. The isolates (*Pseudomonas* and *Proteus*) were found to possess

certain phenotypic virulence properties such as DNase, gelatinase and lipase, which further suggest that the water samples were contaminated with pathogens that are of public health importance. These bacterial isolates were found to be susceptible to some of the antibiotics from different classes used in the study, such as meropenem, gentamicin, amoxicillin/clavulanic acid and ciprofloxacin, while resistance was observed most for sulfamethoxazole, erythromycin and tetracyclines. Overall, the isolates were multi-drug resistant as they were all above the permissible limit of 0.2 (in terms of multiple antibiotic resistance index) and thus are of public health significance (Chitanald *et al*., 2010; Davis and Brown, 2016; Ogofure and Igbinosa, 2021; Ogofure *et al*., 2022).

Table 1. Molecular identification of bacterial isolates from water samples

Sample Code	Bacterial identity	Query cover (%)	Identity (%)	Accession No.
UBTH	*Escherichia coli**	90.00	100.00	MN107512.1
RwS1	*Enterobacter cloacae*	99.00	99.87	NR118011.1
RwS2	*Pseudomonas aeruginosa*	99.00	99.41	MN490072.1
RwS1	*Escherichia coli*	90.00	100.00	MN107512.1
RWS6	*Salmonella enterica*	99.00	100.00	MN129064.1
RwS3	*Proteus mirabilis*	99.00	99.79	MN046429.1

Table 2. Phenotypic virulence determinants of bacterial isolates

Isolates	Hemolysin	DNAse	Gelatinase	Lipase
*Escherichia coli**	β 0(0)	0(0)	0(0)	0(0)
Enterobacter cloacae	β (0)	0(0)	0(0)	0(0)
Pseudomonas aeruginosa	β 4(100)	4(100)	4(100)	4(100)
Salmonella enterica	β 0(0)	0(0)	0(0)	2(100)
Escherichia coli	β 0(0)	0(0)	0(0)	0(0)
Proteus mirabilis	β 0(0)	4(100)	4(100)	4(100)

Table 3. Antibiotic susceptibility of bacterial isolates from river water samples used for different treatments

Isolates	No.	CIP	CRO	RL	E	TET	MEM	AMC	CN
E. coli	5	4(80)	3(60)	0(0)	1(20)	1(20)	5(100)	4(80)	4(80)
E. cloacae	4	4(100)	3(75)	1(25)	2(50)	1(25)	4(100)	3(75)	3(75)
S. enterica	2	2(100)	1(50)	0(0)	0(0)	0(0)	2(100)	2(100)	1(50)
P. aeruginosa	4	4(100)	3(75)	2(50)	1(25)	0(0)	4(100)	4(100)	4(100)
P. mirabilis	4	4(100)	4(100)	1(25)	1(25)	2(50)	4(100)	3(75)	3(75)

Legend: CIP; Ciprofloxacin (5µg), CRO; Ceftriaxone (30 µg), RL; Sulfamethoxazole (1.25/23.75 µg), E; Erythromycin (15 µg), TET; tetracycline (30 µg), MEM; Meropenem (10 µg), AMC; Amoxicillin/clavulanic acid (20/10 µg), CN; Gentamicin (10 µg).

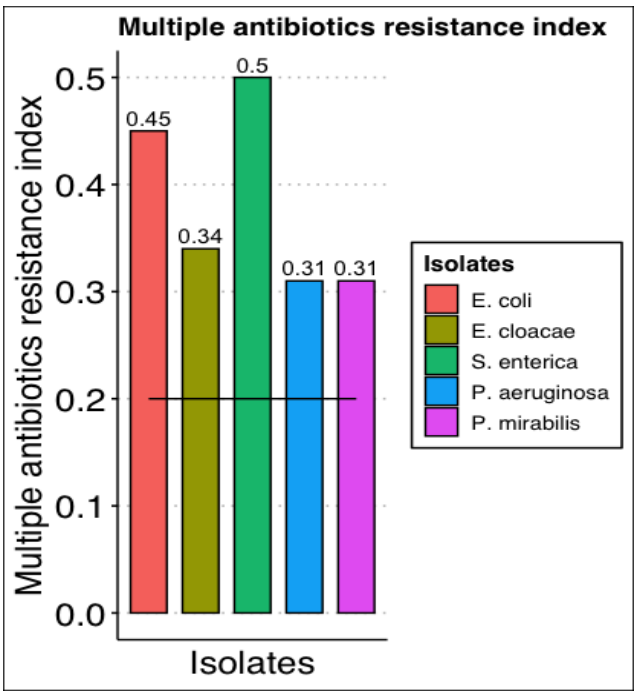

Figure 9: MAR index of bacterial isolates from water samples used in the study. The black horizontal line represents the permissible limit.

3.3 Physicochemical Properties

The physicochemical analysis of the water samples showed that pH ranged from 6.63±0.12 (control) - 7.20±0.10 (Basil leaves). At the same time, the temperature ranged from 26.00±1.00 ^{0}C (control) to 27.00±1.50 ^{0}C (Neem leaves). The turbidity of the samples ranged from 0.30±0.10 NTU (control) to 0.17±0.06 NTU (Neem leaves). Most parameters were within the NSDWQ stipulated guidelines for drinking water quality. More so, there was no significant difference ($p>0.05$) between the control samples and the treatment regimes in most of the physicochemical parameters evaluated in the study. This further suggests that using neem or Basil leaves as water-purifying agents does not significantly alter the physicochemical parameters of the water samples.

Table 4. Physicochemical properties of water samples from different treatments

Parameters		Control	Basil leaves	Neem leaves	WHO/NSDWQ
Temperature (^{0}C)		26.00±1.00^a	27.00±1.00^a	27.00±1.50^a	25-30
pH		6.63±0.12^a	6.82±0.10^b	6.70±0.00^a	6.5-8.5
Turbidity (NTU)		0.30±0.10^a	0.20±0.20^{ab}	0.17±0.06^b	5
Suspended solids		0.33±0.12^a	0.43±0.15^a	0.47±0.15^a	3
Conductivity		48.00±3.00^a	224.07±166.53^a	199.33±1.15^a	1000
Alkalinity		15.00±3.00^{ab}	11.33±1.53^a	17.33±1.15^b	100
Nitrate		1.18±0.10^a	1.36±0.54^a	1.40±0.10^a	50
Phosphate		0.48±0.06^a	0.42±0.04^a	0.44±0.04^a	-
Sulphate	mg/l	4.00±0.00^a	3.67±1.15^a	3.67±1.15^a	100
BOD		1.37±0.21^a	1.40±0.36^a	1.73±0.15^a	-
COD		31.33±3.06^a	34.67±10.69^a	45.00±3.00^a	-
Lead		0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.01
Cadmium		0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.003
Zinc		0.33±0.05^a	0.33±0.10^a	0.33±0.03^a	3
Chromium		0.06±0.01^a	0.03±0.00^a	0.07±0.02^a	-

The physicochemical parameters of the water samples in the respective treatment regimens containing neem and Basil leaves (aside from the control) reduced certain parameters such as nitrate and sulphate contents and turbidity. Low turbidity in drinking water is a proven indicator of pathogen removal, thus enhancing drinking water's safety (WHO, 2017). The reports of elevated turbidity in drinking water have been associated with numerous outbreaks of waterborne diseases, according to Mann *et al*. (2007). Several of the evaluated physicochemical parameters, such as pH, temperature, turbidity, and alkalinity, were within acceptable limits for portable water standards stipulated by WHO (2017) and also similar to reports by Ekhaise and Anyasi (2005); Ololade and Ajayi (2009), Aliyu *et al*. (2016), as well as Akinbile and Omoniyi (2018) who analyzed different river water samples in Benin City, Edo State. These authors evaluated the bacteriological quality of Ikpoba, Oluwa, Ogbese, Osa, Owena, River Lavun and Ogbese Rivers. All water samples and treatment regimes employed were observed to be close to neutrality on the pH scale. They, however, were lower than the benchmark stipulated by WHO (2017). The results obtained in this study were found to agree with the reports of Shittu *et al*. (2008), who opined that the pH of river water samples was within the pH range of 6.65 – 6.85. WHO (2017) reported that health effects are more pronounced in pH extremes, and drinking water above 11 can cause skin, eye and mucous membrane irritation. On the opposite end of the scale, pH values below four can also irritate due to the corrosive effect of the low pH levels (Engwa *et al*., 2015). It was evident that certain physicochemical parameters of the treatment regimes were not significantly different from the control. This further suggests that the addition or the use of leaves (Neem and Basil) for the purification of water samples has no adverse effect on the physicochemical properties of the water samples. Hence, the use of plants as natural anticoagulants for water treatment and purification is reported in the study of Achupriya *et al*. (2022). Also consistent with the results obtained in this study were the reports of Chandel *et al*. (2022), who evaluated the use of low-cost herbs against microbial contaminants of water and their public health consequences. The study employed Azadiractha (neem), Ocimum (Basil), and Pinus species extracts to purify or decontaminate wastewater. Little wonder Desu (2022) reviewed the use of neem and tulsi leaves for purification and decontamination of water in India and found that the leaves can serve to decontaminate the water and also confer health benefits without any adverse effect on the physicochemical properties of the water.

Conclusion

The use of plant leaves for purification and decontamination of water has been established in this study. Previous studies employed using the powder of plant leaves for decontamination and purification. However, this study used whole leaves of neem and basil in addition to solar treatment for 4 hours to evaluate their efficacy in water purification and successfully reduced pathogenic bacteria by 90%, as shown in a few of the treatment regimens. Neem leaves and a consortium of neem and basil leaves showed the best water purification efficacy in contrast to basil leaves alone, especially regarding the reduction of pathogenic E. coli isolates in water. Thus, more research into easy, quick and effective means of water decontamination is essential so that the millions of Nigerians without access to drinking water can effectively carry out decontamination for their public health and safety.

Acknowledgement: We appreciate Prof. E.O. Igbinosa and the team of NCCE for always availiang laboratory space and materials whenever needed.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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