Faculty of Natural and Applied Sciences Journal of Scientific Innovations
Print ISSN: 2814-0877 e-ISSN: 2814-0923
www.fnasjournals.com
Volume 4; Issue 2; October 2023; Page No. 57-62.

EFFECT OF *Moringa oleifera* SEED POWDER ON BACTERIA ISOLATED FROM WELL WATER OF RUMUOLUMENI COMMUNITY, PORT HARCOURT

***Tom, M.U., Amadi-Ikpa, C.N., & Ogbonda, K.H.**
Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Nigeria

***Corresponding author email**: mondaytom60@gmail.com.

Abstract
The effect of *Moringa oleifera* seed powder on bacteria isolated from the well water of Rumuolumeni Community of Port Harcourt was investigated. Hence, standard microbiological procedures were adopted in the preparation of the *Moringa oleifera* seed powder, collection of the water samples, and determination of the bacterial load vis-à-vis the effect of the *Moringa oleifera* seed powder on the bacteria isolated. Results obtained showed a mean heterotrophic bacteria load of 3.8 x 10^5 and 3.0 x 10^5 cfu/ml for water analyzed before and after treatment with *Moringa oleifera* seed powder, respectively. Three bacteria species were recovered and characterized biochemically: *Enterobacter aerogens*, *Escherichia coli*, and *Streptococcus feacalis*. The result showed that at varying concentrations of the seed powder, the inhibition zone sizes of the bacteria increased. At 10 and 20mg concentrations, *S. faecalis* was greatly inhibited in comparison to *E. coli* and *E. aerogens*, respectively. The 30mg concentration showed a higher inhibitory effect on *E. coli* compared to *E. aerogens* and *S. faecalis.* The sensitivity and resistivity of the bacteria to *Moringa oleifera* seed powder showed a dose-dependent response or control on the bacteria. Hence, the study recommends an increased dosage of Moringa seed powder in the purification of well water to be adopted.

Keywords: *Moringa oleifera* Plant, Bacteria, River, Seed Powder, Rumuolumeni Community

Introduction
The use of eco-safety plants such as *Moringa oleifera* to control some diseases has been reported by Ramo-Tejada et al. (2002). *Moringa oleifera* plants have been used as coagulants and bio-absorbents in water treatment (Shannon et al., 2007), with an impressive array of medicinal and nutritional properties (Tamanna, 2010). In addition, *Moringa oleifera* seed powder has shown broad applicability in the control of diseases (Amal & Nashwa, 2017). Yang et al. (2006), in their study, pointed out that the inclusion of *Moringa oleifera* leaf diet in broiler feed reduced *Escherichia coli* counts in the intestines of birds. Also, *Moringa oleifera* leaf extract expressed antimicrobial features under the control of *Staphylococcus aureus* (Amal & Nashwa, 2017). The performance of ethanolic extracts of *Moringa oleifera* as recorded by Chinwe et al. (2016), demonstrated a high susceptibility effect against most bacterial isolates when compared with established commercial antibiotics. Consequently, water purification has become necessary due to the contamination or pollution of water by man and his activities; thus, purification is aimed at removing foreign substances or any impurities and thereby bringing the water fit for use once again (Shannon et al., 2007). According to Chinwe et al. (2016), decontamination of water is adopted to decrease the concentration of particulate matter such as suspended particles and dissolved solids and also remove organic matter. Several methods of purifying water have been noted; they include granular activated carbon filtering, reverse osmosis, direct contact membrane distillation, boiling, distillation, and desalination, amongst many others (Shannon et al., 2007). According to Shannon et al. (2007), the water after purification is used; however, the level of purity is questionable concerning the health of the consumer (WHO, 2014). Most illnesses and deaths among children in the world today are caused by microorganisms, in particular bacteria (WHO, 2014). The bacteria that get into the mouth via water are estimated to cause 80% of diseases and illnesses in the world, according to the WHO (2014). These diseases, according to WHO (2014), are acquired from the discharge of sewage and industrial and agricultural wastes into the river. Hence,

Cite this article as:
Tom, M.U., Amadi-Ikpa, C.N., & Ogbonda, K.H. (2023). Effect of *moringa oleifera* seed powder on bacteria isolated from well water of Rumuolumeni Community, Port Harcourt. *FNAS Journal of Scientific Innovations*,*4*(2), 57-62.

the need for an alternative solution to water treatment necessitated this work, which determined the effect of *Moringa oleifera* seed powder on bacteria isolated from well water in the Rumuolumeni Community of Port Harcourt. The study will solve the challenges of water treatment with chemicals that are harmful to the health of the Rumuolumeni community.

Materials and Methods

The study zone, Rumuolumeni Community, is one of the several districts that make up the Port Harcourt metropolis. The community, therefore, is surrounded by a river that cuts across several neighbouring communities and empties into the Atlantic Ocean at Bonny. The well water is sourced for drinking and is sometimes observed to be polluted; hence, it cannot be consumed except after treatment. Thus, the well water pollution resulted from runoff during rainfall and flooding of the area. Ten water wells were selected for this study based on public accessibility. The wells were noted to be open and not covered by the atmosphere; thus, the study involved screening for heterotrophic bacteria in water treated with Moringa and non-Moringa plants.

In the collection of *moringa oleifera* seed and water samples, *Moringa oleifera* seeds were purchased while water samples were collected aseptically from the wells of the Rumuolumeni community with a sterile cock screw container and put in the ice-block cooler. The water samples were then transferred to the Biology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, for microbiological analysis.

The preparation of the *Moringa oleifera* seed powder was carried out as adopted by Nweze and Nwafor (2014). *Moringa oleifera* plant seed powder was prepared by manually removing the dry seeds from the shells and then grinding them with a blender. The blended content was then socked in water and sieved using Whitman filter paper to get the residue (Nweze & Nwafor, 2014). The residue was dried in an oven at 120^{o}C to get the powdery substance.

Determination of the Bacterial Load in the Water Samples Before and After Treatment with *Moringa oleifera* Plant Seed Powder. Standard bacteriological techniques, as adopted by Eaton et al. (1995) and modified by Amadi-Ikpa et al. (2020) were used to determine the bacterial population in the water samples before and after treatment with Moringa seed powder (10g in 100 ml of water). Before the investigation, the water samples were serially diluted to a factor of 10^{-3}. One millilitre (1ml) of the final dilution 10^{-3} was pipetted into a plate-count nutrient agar medium and incubated at room temperature (approximately 27 ^{o}C) foC growth to occur within 24 hours. The number of colonies was recorded as colony-forming units per mL.

The morphological characterization of the isolates involved the Gram staining procedure to determine the shapes of the bacteria cells with the use of a microscope and macroscopic visualization of the colony appearance in terms of colour, size, and elevation. edge, opacity, shape etc. (Franco-Duarte et al., 2019). The procedure was carried out to classify the isolates into Gram-positive and Gram-negative bacteria based on the composition of their cell walls. Consequently, the process involved picking and making a smear of a 24-hour loopful culture on a clean, grease-free slide. The smear was heat-fixed by passing over a flame, and then the slide was flooded with crystal violet for 1 minute and rinsed with water. The smeared slide was again flooded with Gram's iodine for 1 minute and rinsed with water. Thereafter, the smeared slide was decolourized using 95% alcohol for 30 minutes. Furthermore, the slide was counter-stained with safranin for 30 seconds, rinsed with water, allowed to air-dry, and viewed under an oil immersion objective lens (light microscope). Cells that appeared purple were recorded as gram-positive cells, while cells that appeared pink were gram-negative cells. further morphological characterization.

The microscopic characterization of the isolates involved: the motility test, the IMViC test (Indole, Methyl Red, Voges Proskauer and Citrate), the sugar test, and the catalase assay (Chakraborty & Pal, 2008).

The motility test was done to spot the presence of flagella in the unknown bacteria colony. In carrying out the text, a semi-solid nutrient agar was freshly prepared and dispensed into a test tube where it was sterilized. Following sterilization of the media, a sterile wire was used to transfer the unknown isolate by stabbing it into the freshly prepared nutrient medium. The medium was then incubated at 37^{0}C for 24 hours. A diffused hazy growth on the inoculating line in the medium showed the unknown bacteria possessed flagella, while an absence indicated the unknown bacteria do not possess flagella.

Cite this article as:
Tom, M.U., Amadi-Ikpa, C.N., & Ogbonda, K.H. (2023). Effect of *moringa oleifera* seed powder on bacteria isolated from well water of Rumuolumeni Community, Port Harcourt. *FNAS Journal of Scientific Innovations*,*4*(2), 57-62.

The Methyl Red and Voges Proskauer tests were done to observe the metabolic features of the unknown isolate in expressing acid and acetone products respectively from the utilization or breakdown of glucose phosphate broth. The test involved inoculating the unknown bacteria into a freshly prepared glucose phosphate broth and the broth incubated for 2-5 days at a temperature of 37^0C. Thereafter, the broth was divided into two parts, parts A and B. To the various parts, 5 drops of methyl red and naphthol solutions were added to them respectively. A change in colour from red to yellow indicated, that metabolic activity took place while no change indicated no metabolic activity took place.

The citrate test was used to determine the ability of the unknown bacteria to degrade sodium citrate. Following the mechanism of the test, the test bacteria were introduced into a sodium citrate medium, and thereafter, the medium was incubated for 24 hours at a temperature of 37 °C. A change in colour from green to blue of the medium precisely indicated the unknown bacteria broke down the sodium citrate, whereas no change in colour indicated the unknown bacteria failed to break down the sodium citrate.

The indole test sought to observe the ability of the unknown bacteria to break down the amino acid tryptophan (peptone water) medium. In carrying out the test, the unknown bacteria isolate was inoculated into the medium, and the medium was incubated at 37^0 °C for 48 hours. Thereafter, Kovac's reagent was added. A red ring spot observed on the medium indicated that the unknown bacteria successfully broke down the amino acid tryptophan (peptone water), while the inability to break down tryptophan showed no red ring spot.

The sugar test was used to determine the ability of the isolates to ferment glucose, lactose, and sucrose. In preparing the broth, 1% of each of the various sugars and peptone agar with a litmus red indicator reagent was constituted, and a Durham tube placed in an inverted position inside a test tube was constituted with the broth. After sterilization, a loopful of the isolate was then incubated in the broth at 37^0C for 18–24 hours. A change in colour of the broth culture from red to yellow and gas inside Durham's tube indicated a positive sugar test.

The catalase test was employed to investigate the ability of the isolates to degrade hydrogen peroxide into oxygen and water. The text involved the introduction of a 3% hydrogen peroxide solution to the unidentified bacteria placed on a clean microscopic slide. An increased effervescence indicated the enzyme catalase was expressed in the breakdown, while the absence of catalase was indicative of weak effervescence.

The sensitivity and resistivity of the isolated bacteria were measured to determine the effectiveness of the *Moringa oleifera* seed powder on the bacteria. In achieving this test, a disc diffusion method was employed, as described by Wise (2002). The bacteria were inoculated into a freshly prepared nutrient medium, and the bacteria spread evenly over the surface using a glass spreader. Following this, with the use of a cork borer, 6mm in diameter was punched out, and the seed powder was introduced in 10, 20, and 30 mg concentrations, incubated for 18-24 hours at a temperature $^{of\ 37}$ ^{o}C. Zones of inhibition were observed and measured as recommended by Wise (2002). Following all these processes, the test was done in triplicate, and the result was reported as resistant, moderate, and sensitive.

Results

Bacteria Load in Water Samples

Table 1: showed the population of heterotrophic bacteria at 3.8 x 10^5 and 3.8 x 10^5cfu/ ml in the water samples before and after treatment with *Moringa oleifera* seed powder respectively. The water sample before treatment had higher heterotrophic bacteria loads than the treated water samples, although the high counts obtained before treatment were not significantly different from the counts obtained after treatment with seed powder at a probability less than 0.05.

Table 1: Mean Heterotrophic Bacteria Count in Water Samples

Parameter	Heterotrophic Bacteria Counts (cfu/ml)
Water Before Treatment with Seed Powder	3.8 x 10^5
Water After Treatment with Seed Powder	3.0 x 10^5
T-test	$P < 0.05$

Cite this article as:
Tom, M.U., Amadi-Ikpa, C.N., & Ogbonda, K.H. (2023). Effect of *moringa oleifera* seed powder on bacteria isolated from well water of Rumuolumeni Community, Port Harcourt. *FNAS Journal of Scientific Innovations*,*4*(2), 57-62.

The Colonial and Morphological Characterization of the Isolates

Table 2 shows the colonial and morphological characterization of the isolates. The colouration of the colonies on culture plates revealed white and grey colours with small-sized colonies that had low elevation. The edges of the colonies were curved and they appeared opaque under the microscope. Gram reaction presumed a bacilli-shaped Gram-positive rod.

Table: 2 Colonial and Morphological Characterization of the Isolates

Isolates	Colour	Size	Elevate.	Edge	Opacity	Shape	Gram React.
Escherichia coli	Green	Small	Low	Curve	Opaque	Round	-
Streptococcus faecalis	Grey	Small	Low	Curve	Opaque	Rod	+
Enterobacter aerogenes	White	Small	Low	Curve	Opaque	Bacilli	-

Note; Gram Reaction, (+) = Positive, (-) =Negative

Biochemical Reaction of the Isolates

Table 3 shows the biochemical reaction of the isolates recovered, the Gram-negative bacteria were distinguished with a positive lactose and motility test, which indicated *Escherichia coli*. The other Gram-negative isolate showed negative lactose and citrate expression, thus indicating *Enterobacter aerogens*. *Enterobacter aerogens* were distinguished as positive for motility and citrate expressions. The Gram-positive isolate reacted negatively, to Methyl red, citrate, indole, catalase and lactose reagents. However, Voges Proskauer reagent reacted positively to the isolate, *Streptococcus faecalis*.

Table 3: Biochemical Characterization of the Isolates

Motility	Cat	Glu	Indol	Cit	MR	VP	Lac	Sucr	Gram React.	Bacteria
+	+	+	+	-	+	-	+	+	-	*E.coli*
+	+	-	+	+	+	+	-	-	-	*E. aerogens*
-	-	-	-	-	-	+	-	-	+	*S. faecalis*

Note: Cat = Catalase, Glu = Glucose, Indol=Indole, Cit= Citrate MR= Methyl Red, VP= Voges Proskaurer, Lac = Lactose, Sucr = Sucrose, Gram React. =Gram Reaction

Zones of Bacterial Inhibition at 10mg, 20mg and 30mg Concentrations of *Moringa oleifera* Seed Powder on the Bacteria

Zones of inhibition of the seed powder of *Moringa oleifera* on the bacteria are shown in Table 4. The result showed a varying concentration of the seed powder on the bacteria. At 10,20 and 30mg concentrations, *Escherichia coli* were inhibited at a zone size of 12.5 ± 2.12, 20.0 ± 0.00 and 34.0 ± 1.41 mm respectively. Similarly, *E. aerogens* were inhibited at 13.5 ± 0.70, 17.5 ± 2.12 and 26.0 ± 0.00 mm sizes at 10, 20 and 30 mg concentrations of Moringa seed powder respectively. *S. faecalis* were observed inhibited with zone sizes of 17.0 ± 0.00, 24.0 ± 0.00 and 26.0 ± 0.00 mm at 10, 20 and 30 mg concentrations of Moringa seed powder respectively. The effect of concentrations of the seed powder on the bacteria was significantly different at $P<0.05$.

Table 4: Mean Zones of Bacterial Inhibition at 10mg, 20mg and 30mg Concentration of *Moringa oleifera* Seed Powder on the Bacteria

Parameters	*E.coli* (Mean±SD)mm	*E. aerogens* (Mean±SD)mm	*S. faecalis* (Mean±SD)mm
Seed Powder at 10mg	12.5 ± 2.12	13.5 ± 0.70	17.0 ± 0.00
Seed Powder at 20mg	20.0 ± 0.00	17.5 ± 2.12	24.0 ± 0.00
Seed Powder at 30mg	34.0 ± 1.41	26.0 ± 0.00	26.0 ± 0.00

Cite this article as:
Tom, M.U., Amadi-Ikpa, C.N., & Ogbonda, K.H. (2023). Effect of *moringa oleifera* seed powder on bacteria isolated from well water of Rumuolumeni Community, Port Harcourt. *FNAS Journal of Scientific Innovations*,*4*(2), 57-62.

Note: (Mean±SD)mm : Mean±Standard Deviation , ml millimeters, mg = milligram

Discussion

The population of bacteria in the well water samples differed with a decrease in heterotrophic bacteria load as a result of the treatment with *Moringa oleifera*. This result was also observed in studies carried out by Delelegn et al. (2018). Delelegn et al. (2018) employed aqueous and ethanolic extracts of *Moringa oleifera* leaves on polluted water and noted the reduction of 66% of heterotrophic bacteria. The reduction in heterotrophic bacteria load in the water samples after treatment with *Moringa* seed powder also agreed with studies carried out by Nascimento et al. (2020). Nascimento et al. (2020) reported a positive antimicrobial property of *the Moringa oleifera* plant concerning water purification from biological agents. However, the presence of heterotrophic bacteria after treatment may be a result of the isolate's dispersal and adherence properties (Penna et al., 2002). The dispersal and adherence properties enabled the isolate to move to surfaces where they formed a biofilm, which more or less inhibited the effect of the Moringa treatment as seen also in studies by Penna et al. (2002). Penna et al. (2002) noted and reported the isolation of some Gram-negative, non-fermentative bacteria after purification/disinfection of water. The sanitary quality of the well water sample showed traditionally, morphological bacteria such as *Streptococcus feacalis, Enterobacter aerogens* and *Escherichia coli.* These bacteria isolated are amongst the indicator bacteria of water quality, and their presence suggests the water is contaminated by human or animal faecal matter (Clasen et al., 2006). Hence, bacteria-contaminated water is capable of causing diseases such as diarrhoea, dysentery and many more ill-health to consumers (Gupta & Quick, 2006). The *Moringa oleifera* seed powder's ability to control *Enterobacter aerogens*, *Escherichia coli* and *Streptococcus feacalis* showed the capacity of the plant as an alternative to antibiotics or any other chemical (Ilanko et al., 2019). At a higher (30mg) concentration of the seed powder, *Escherichia coli* were seen to be controlled effectively whereas, in a study by Jabeen et al. (2018), the seed powder was more effective against *Pasteurella multocida* and *Bacillus subtilis* only. In all responses of the bacteria to *Moringa oleifera* seed powder, a dose-dependent response was observed. This report was also noted by Rani et al. (2018), who in their study employed the extract of Hexane, ethyl acetate, methanol and chloroform extracts on bacteria such as *Serratia marcescens, Shigella dysenteriae*, *Enterobacter* sp., *Escherichia coli, Klebsiella pneumonia*.

Conclusion

The study thus concludes that *Moringa oliefera* seed powder can control heterotrophic bacteria (THB) in the water samples and at a higher concentration the growth or presence of *Enterobacter aerogens*, *Escherichia coli* and *Streptococcus feacalis* could be inhibited effectively. Following this, therefore, *the* Moringa plant has established itself successfully.

Recommendation

The study recommends that due to the success recorded in controlling bacterially contaminated water, increased dosage/application of the plant seed should be adopted.

References

Amadi-Ikpa, C. N., Akani, N.P., Wemedo, S. A. & Williams, J. O. (2020). Biofilm Formation and Virulent Properties of Bacterial Isolates in Stored Drinking Water of Some Homes. *International Journal of Research and Innovation in Applied Science, 5*(8), 2454-619.

Amal, S.O., & Nashwa, A.A. (2017). Antibacterial Effect of the Ethanol Leaves Extract of Moringa oleifera and Camellia sinensis against Multi-Drug Resistant Bacteria. *International Journal of Pharmacology*, *13*(2), 156-165.

Chakraborty P.S. & Nishith, K.L. (2008). *Manual of Practical Microbiology and Parasitology* (Fifth Edition) Kolkarta India: New Central Book Agency.

Chinwe, C.I., Isaiah, I. & Olayinka, J. N. (2016). Antibacterial Activity of *Moringa oleifera* Lam Leaves on Enterica Human Pathogens, *Indian Journal of Applied Research* 6(8), 55-59.

Clasen, T., Smith, L., Albert, J., Bastable, A., & Fesselet, J. (2006). The Drinking Water Response to the Indian Occean Tsunami, Including the Role of Household Water Treatment. *Disaster Prev. Manag, 15*, 213-215

Delelegn, A., Sahile, S. & Husein, A. (2018). Water Purification and Antibacterial Efficacy of *Moringa oleifera* Lam. *Agriculture and Food Safety, 7*,25-34.

Eaton, A.D., Clesceri, L.S. & Greanberg, A. E. (1995). *Standard Methods for Examination of Water and Waste Water* (19th Edition) American Public Health Association.

Franco-Duarte, R., Cernakova, L. & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms from past to present. *Multidisciplinary Digital Publishing Institute, 7*(5), 130.

Gupta, S.K. & Quick, R. (2006). Inadequate Drinking Water Quality from Tanker Trucks Following a Tsunami Disaster, Aceh, Indonesia. *Disaster Prev. Manag., 15*, 213-215.

Ilanko, P., McDonnell, P.A., Vunren, S. & Cock, I. E. (2019). Interactive Antibacterial Profile of *Moringa oleifera* Lam Extracts and Conventional Antibiotics Against Bacterial Triggers of Some Autoimmune Inflammatory Diseases, *South African Journal of Botany, 124*, 420-435.

Jabeen, J.C. (2017). Traditional Uses, Phytochemistry and Pharmacology Properties of *Moringa oleifera* Plant: an Overview, *Pharmacia Lettre, 3*(2), 141-164.

Nascimento, A.C.S., Oliveira, A.F., Lavor, M.B. & Pereira,E.C (2020). Low Turbidity Water Treated with Seeds of *Moringa oleifera* Lam. *Environmental Management,45*(7), 223-229.

Nweze, N.O., & Nwafor, F. I. (2014). Phytochemical, Mineral and Proximate Composition of the Leaf Extract of *Moringa oliefera* Lam, from Nsuka, South Eastern Nigeria. *IOSR J of Pharmacy and Biological Science, 9*(1), 99-103.

Penna, V.T.C., Martins,S.A.M. & Mazzola, P.G. (2002). Identification of Bacteria in Drinking and Purified Water During the Monitoring of a Typical Water Purification System, *BioMedCenter Public Health, 2,* (13)

Ramo-Tejada, D.J., Ontiveros-ortega, A., Esinosa-Jimnez, M., Perea-Carpio, R. & Chibowski, E.(2002).Investigation of Alumina, Catechin System Properties. Part I: A Study of the System by FTIR-Vis Spectroscopy. Colloids and Surfaces B. *Biointerface*, *24*, 297-308.

Rani, N.Z.A., Husain, K. & Kumolosasi, E. (2018). *Moringa* Genius: *A Review of Phytochemistry and Pharmacology Frontiers in Pharmacology, 4*(8), 234-245.

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiasi, J.G., Marinas, B. J., & Mayes, A.M. (2007). Science and Technology for Water Purification in the Coming Decades. *Nature, 4*, 201-310.

Tamanna, A. W. (2010). Antimicrobial Activity of Small Protein of *Moringa oleifera* Leaves. *Journal of Islam Academic Science, 11*(1), 27-32.

WHO (2014). *Guideline for drinking water quality incorporation*, Water Quality and Health: First addendum, Volume 1. http://www.waterandhealth.org/drinkingwater/wp.html

Wise, R. (2002). Antimicrobial Resistance Priorities for Action; *Journal of Antimicrobial Chemotherapy*, 49: 585 – 586.

Yang, R., Chang, L., Hsu, J., Weng, B.B.C., Palada, C., Chadha, M.L., & Levasseur, V. (2006). Nutritional and Functional Properties of Moringa Leaves from Germplasm to Plant to food to Health. Moringa and Other Highly Nutritious Plant Resources; Strategies, Standards and Markets for a Better on Nutrition in Africa, Accra, Ghana,*4*, 16-18.