

# Disinfection Effect of *Moringa Oleifera* Seed Extracts Against Bacteria Isolated from River Water

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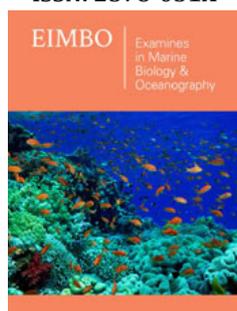
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## Abstract

The traditional use of the *Moringa Oleifera* seeds for domestic household water treatment has been well known in some part of the world. The study was conducted to evaluate the disinfection effects of *Moringa* seeds extract against bacteria isolated from River Wudil, Kano State Nigeria. Two hundred and fifty millilitres (250mL) of water sample was collected in a sterile bottle from the bank of river. The bacteria were filtered from the water sample using Membrane filters (MF) method and inoculated onto the surface of nutrient agar. Identification of bacterial isolates was done by Gram staining, microbiological analysis and biochemical test. The *Moringa* seeds extract was screened for phytochemical screening and test for disinfection efficacy of the extract against bacteria isolated from river Wudil. The result of the study showed a total of 5 isolates obtained namely; *Escherichia coli*, *Salmonella typhi*, *Enterobacter*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The results of phytochemical screening of the extract indicated the presence of saponins, flavonoids, glycoside, anthraquinones, tannin and terpenoids. The finding of the study on the disinfection property of the extract demonstrated that the extract possessed disinfection effect against the isolate and the activity of the extract is dose dependent wise. Higher activity was shown by 100mg/L. The sensitivity of the isolate to the extract also differs. Statistical analysis of the result showed that *Proteus mirabilis* is more sensitive to the extract with average zone of inhibition of 13.75mm while *Escherichia coli* is the less sensitive (10.37mm) among the isolates. However, there is no statistical difference on the sensitivity of the isolates to the extract. Based on the finding of the study, *Moringa* seeds extract can be applied in water treatment as disinfection agent.

**Keywords:** *Moringa oleifera*; Disinfection; Bacteria; Seeds extract; River water

## Introduction

Drinking water has always been a major issue in many countries, especially developing countries such as Nigeria. In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river water for domestic use [1]. Contaminated water sources are vehicles for the transmission of waterborne diseases such as cholera, shigellosis, and *Campylobacter*'s [2]. The World Health Organization (WHO) estimated that about 1.1 billion people globally drink unsafe water and most diarrheal diseases in the world (88%) are attributable to unsafe water, sanitation and hygiene. Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden world-wide (54.2 million) are attributable to unsafe water, sanitation and hygiene [3]. Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms are the most commonly used bacterial indicator of faecal pollution [4]. They are found in water that is contaminated with faecal wastes of human and animal origin. Nowadays, numerous drug resistance has developed due to the indiscriminate use of synthetic antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem antibiotics are sometimes associated

with adverse effects on the host including immune suppression, hypersensitivity and allergic reactions [5]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [5].

One of the greatest dangers to which ground water can be exposed to is contamination by pathogenic organisms. Disinfection is a chemical process for eliminating pathogenic microbes from an environment using chemical agents such as halogens, phenols, alcohols, heavy metals, dyes, soap and detergents, ammonia compounds, hydrogen peroxide, and various alkalis and acids [6]. Most of the chemicals have the potential of forming carcinogenic and mutagenic disinfection by-products (DBPs) [7]. Disinfectants and their by-products may also be associated with increased risks of cardiovascular diseases, cancers, and birth defects [8]. These, and the high cost of chemicals, especially in developing countries where it needs to be imported, makes it imperative to look for cheaper alternatives that are also environmentally friendly. The traditional use of the *M. oleifera* seeds for domestic household water treatment has been well known to certain rural areas in Africa [9]. In the West Asia, one of the best-known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water [10]. The seeds of *Moringa* also exhibit antimicrobial properties [10,11]. It purifies water by removing microbes as a result of settling same as the colloids in properly coagulated and flocculated water is processed [12]. Additionally, these seeds are also expected to perform their actions directly on microorganisms and cause growth inhibition. The action of antimicrobial peptides produced by this plant parts are supposed to disrupt the cell membrane or inhibit necessary enzymes [13]. Studies by [14-17] identified the presence of an active antimicrobial agent in *Moringa Oleifera* seeds. The study was conducted to identify bacteria in River Wudil and test for disinfection effect of *Moringa* seeds extract against the faecal coliforms.

## Materials and Methods

### Study site

Wudil is one of the Local Government Council in Kano State, Nigeria. It is geographically located in the south-eastern part of Kano state along Maiduguri-Kano way with about 41 Kilometres from the State capital. It is located at Latitude 11° 49' 0" N and Longitude 8° 51' 0" E. It covers an area of about 362Km<sup>2</sup> of land and population of about 185,189 according to 2006 census [18,19]. Wudil Local Government shares common boundaries with Warawa (North), Dawakin-kudu (West), Garko (South) and Gaya Local Government (East).

### Collection of water sample

Two hundred and fifty millilitre (250mL) of water sample was collected in a sterile bottle from the bank of river Wudil in Wudil Local Government Area Kano State, Nigeria. The water sample

was transported as soon as possible to Laboratory of Microbiology Department, Kano State University of Science and Technology Wudil for bacteriological analysis of the water.

### Microbiological analysis of the water

Membrane filters (MF) method as described by Anas and Ali, [20] was adopted. During the process, 100mL of the water sample was filtered through sterile membrane which retained the bacteria on its surface. The membrane was removed aseptically and placed on a Nutrient agar (NA) as a basal medium and MacConkey agar as a differential medium to determine bacteria isolates. The plates were incubated at 37°C for 24 hours. Each colony was isolated in a pure form by sub culturing in fresh MacConkey agar plates for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed.

### Characterization of bacteria

Isolated colonies were confirmed by Gram staining and Biochemical (Indole, Methyl-red, Voges Proskauer and Citrate utilization) tests and each plate were graded as positive or negative for the test as according to Bergy's manual of systemic determinative Bacteriology by Holt et al. [21]

### Collection and identification of moringa oleifera seeds

*Moringa Oleifera* seeds used were purchased from Kano central market on 23<sup>th</sup> October 2018. Identification and authentication of the seeds was done at Herbarium in the Department of Plant Science, Bayero University Kano with the following voucher number BUKHAN 11 and voucher specimen were deposited there for reference. The seed wings were removed, and the kernels were air dried for two weeks, then grounded into a fine powder using sterile pestle and mortar under laboratory condition and stored in air tight container for further use.

### Preparation of moringa oleifera seeds extract

Fifty grams (50g) of the seeds powder were extracted using 1000mL of 1 molar solution of sodium chloride by maceration for 2 days [22]. The resulting suspension was filtered through a muslin cloth and then using Whatman filter paper. The filtrate was concentrated in a water bath until a solid residue was obtained. The solid residue was diluted using 30% Dimethyl-sulphoxide (DMSO) to produce a stock solution of 100mL, and from the stock solution, different concentrations (25,50 & 75mg/L) were prepared and stored further use.

### Phytochemical screening of moringa oleifera seeds extract

Phytochemical screening of the seeds extract was conducted to determine the presence of bioactive components in the seeds extract of *Moringa oleifera*. Presence of alkaloids, saponin, glycoside, tannin, flavonoids, steroids, terpenoids, anthraquinones and phenols was determined using procedures described by [23,24].

### Antibacterial activity of the extracts

The antibacterial activity of the extract against the isolates was determined using agar well diffusion method as adopted by Gambo et al. [25] with little modifications. During the process, 0.1ml of standardize organism equivalent to 0.5 MacFarland standard, were introduced onto the surface of newly prepared Mueller Hinton agar in a sterile Petri dish and labelled accordingly. A sterile cork-borer 6mm was used to produce five wells at equal distance in the inoculated agar. The wells were filled with 4 different concentrations of the extracts accordingly as 25,50,75 and 100mg/L. 5<sup>th</sup> well was filled with 50mg/L of the while the last well contain 50mg/mL of Amoxicillin (Chi pharmaceuticals) as positive control in the study. The inoculated agar plates could diffuse for a period of hour and incubated at 37°C for 24hours. After then, the diameter of the zones of inhibition around each well was measured to the nearest millimeter (mm) [26]. The experiment was conducted in triplicate for each organism.

### Statistical analysis

The data of average zone of inhibition produced by the isolates against the seeds extract for each organism was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). Significance level for the differences was set at  $p < 0.05$ .

## Results

### Identification of bacteria

The biochemical characterization of the bacteria isolated from River Wudil is presented in Table below (Table 1). The results showed a total of 5 isolates obtained namely; *Escherichia coli*, *Salmonella typhi*, *Enterobacter*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Isolates were tested based on Gram staining, Lactose fermentation using MacConkey agar, Indole, Methyl red, Voges Proskauer and Citrate utilization test.

**Table 1:** Biochemical Characterization of the Bacteria Isolated from River Wudil.

Isolates	GS	IN	MR	VP	CU	LF	Identified isolate
A	-	+	+	-	-	+	<i>Escherichia coli</i>
B	-	-	+	-	-	-	<i>Salmonella typhi</i>
C	-	-	-	+	+	+	<i>Enterobacter</i>
D	-	-	-	+	+	+	<i>Klebsiella pneumoniae</i>
E	-	-	+	-	+	-	<i>Proteus mirabilis</i>

### Phytochemical screening

The phytochemical constituent of *Moringa* seeds extracts is

presented in Table 2. The results indicate the presence of saponins, flavonoids, glycoside, anthraquinones, tannin and terpenoids.

**Table 2:** Phytochemical constituents of *Moringa oleifera* seed extract.

S/N	Phytochemicals	Test	Inference
1	Alkaloid	Wagner's test	-
2	Saponin	Foam test	+
3	Phenol	Ferric chloride test	-
4	Flavonoid	Lead acetate test	+
5	Tannin	Gelatin test	+
6	Reducing sugar	Fehling test	+
7	Anthraquinone	Benzene test	+
8	Steroid	Acetic test	-
9	Terpenoid	Salkowski test	+

Key: +=Presence of phytochemical & -=Absence of phytochemical.

### Antibacterial activity of the extracts

The antibacterial activity of different concentration of *Moringa* seeds extract against the isolates is presented in Table 3. The result showed that the extract is active against the isolates. However, the activity of the extract is dose dependant wise. Higher activity was

shown by 100mg/L. The sensitivity of the isolate to the extract also differs. *Proteus* is more sensitive to the extract with average zone of inhibition of 13.75mm while *E. coli* is the less sensitive among the isolates with average zone of inhibition of 10.37mm. Zones of inhibition shown by the control ranged from 16.2-21.6mm.

**Table 3:** Antibacterial Activity of *Moringa* seeds Extract against bacteria isolated from River Wudil Concentration (mg/mL)/ Zone of inhibition (mm)

Organisms	25	50	75	100	Control
<i>E. coli</i>	8.3±0.27a	9.3±0.27a	11.6±0.23b	12.3±0.27b	16.2±0.23
<i>S. typhi</i>	8.6±0.23a	10.6±0.23a	14.6±0.23b	16.6±0.23b	20.4±0.23
<i>Enterobacter</i>	10.6±0.23a	11.3±0.27a	15.3±0.27b	17.3±0.27b	21.6±0.27
<i>Klebsiella</i>	9.3±0.27a	12.3±0.27b	14.6±0.23b	16.2±0.26c	20.2±0.26
<i>P. mirabilis</i>	10.6±0.23a	11.3±0.27a	15.3±0.27b	17.8±0.27b	21.0±0.23

Key: Values having different superscript in the same row are considered significantly different at probability level of  $p < 0.05$ .

## Discussion

Bacteria such as coliforms are the most frequent bacteria in water responsible for water borne diseases such as cholera, dysentery, diarrhea, typhoid fever which is responsible for mortality across the world especially in Africa [27,28]. The finding of the present study revealed the presence of *E. coli*, *Salmonella typhi*, *Proteus mirabilis*, *Enterobacter* sp and *Klebsiella pneumoniae* in River Wudil. Presence of fecal coliforms such as *Escherichia coli*, *Enterobacter* sp and *Klebsiella pneumoniae* indicates high pollution of the River. The poor microbiological quality might be due to contamination caused by human activities and livestock [29]. It is a common practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human wastes into rivers. In addition to using the river as a source of drinking water people use the source for bathing, washing of clothes and for recreational purposes such as swimming [30]. In addition to that wild and domestic animals seeking drinking water can also contaminate the water through direct defecation and urination [31]. The result of this study was in conformity with several results of similar findings which showed the presence of coliforms such as *E. coli*, *Salmonella typhi*, *Proteus mirabilis*, *Enterobacter* sp and *Klebsiella pneumoniae* in most water sources [32-34]. The finding of this study supported that of Olorode et al. [35] who study the physicochemical and microbial analysis of some rivers in Rivers State, Niger-delta in Nigeria, and found the presence of bacteria such as *Escherichia coli*, *Campylobacter*, *Pseudomonas*, *Salmonella*, *Vibrio*, *Proteus*, *Shigella*, *Enterococcus* sp. A study on Physico-Chemical and Microbiological Analysis of Well Water Samples in Settlements around Akperan Orshi College of Agriculture, Yandev in Benue State, Nigeria was conducted by Mwekaven et al. [36]. The results of microbiological analysis of the samples indicates that most of the wells were grossly contaminated with bacteria pathogens especially, *Escherichia coli* (100%), *Proteus* species (47%) and *salmonella* specie (7%). This result supported the present study. The phytochemical screening of *Moringa* seeds extracts indicates the presence of saponins, flavonoids, reducing sugar, glycoside, anthraquinones, tannin and terpenoids. Several studies conducted on phytochemical screening of *Moringa oleifera* seed extract. Study conducted by Fahey [37] revealed that the *Moringa oleifera* seed contain Alkaloids, Glycoside, Flavonoids and Saponins were present in the extract of *Moringa oleifera* seed.

This finding supported the present study. The result of disinfection effect of *Moringa* seeds extract against the isolates showed that the extract possessed antibacterial agent. This is due to the presence of bioactive ingredients presence in the extract. Flavonoids have been shown to have antimicrobial activity *in vitro* [38]. Terpenoids are also known to possess antimicrobial [39]. Studies by [14-18] identified the presence of an active antimicrobial agent in seeds. Eilert et al [14] identified 4 $\alpha$ -4-rhamnouslyloxy-benzyf-isothiocynate as an active antimicrobial agent in *M. oleifera*. This is readily soluble to water at 1.3 $\mu$ mol/l and is non-volatile. In a study using pure 4 $\alpha$ -4- rhamnotyloxy-benzylisothiocynate isolated from defatted *M. oleifera* seeds, the antimicrobial action of *M. oleifera* was investigated on three bacteria species -*Bacillus Subtilis*, *Serratia Marcescens* and *Mycobacterium Pheli*. The result showed that *B. Subtilis* was completely inhibited by 56 $\mu$ mol/l and *M. Pheli* by 40 $\mu$ mol/l. the finding of this study was in conformity with that of Bukar et al. [18] who studied the antimicrobial activities of *Moringa* Seed Chloroform extract and *Moringa* Seed Ethanol extract. They found both to have inhibitory effects on the growth of *E. coli* and determined the Minimum Inhibitory Concentration (MIC) to be >4mg/ml. Thilza et al. [17] using extract from *Moringa* leaf stalk, found that at dilutions of 1000mg/ml, 700mg/ml, 400mg/ml, and 200mg/ml, only mild activity against *E. coli* and *Enterobacter* was noticed. They also found that the highest activity was produced by *E. coli* at 1000mg/ml which comparatively was less than that of the standard drug tetracycline (250mg/ml). Suarez et al. [15] had reported that *Moringa* seeds protein may be a viable alternative to chemicals commonly used as food preservatives or for water disinfection. The mode of attack of the *Moringa* seeds extract on the bacteria cell was explained as by rupturing the cell and damaging the intercellular components, when water dips in to the cell which causes it to swell more and burst leading to death [40].

## Conclusion

The findings of the present study revealed the presence of *E. coli*, *Salmonella typhi*, *Proteus mirabilis*, *Enterobacter* sp and *Klebsiella pneumoniae* in River Wudil which indicates high pollution of the River. The phytochemical screening of *Moringa* seeds extracts indicates the presence of saponins, flavonoids, reducing sugar, glycoside, anthraquinones, tannin and terpenoids. The result of disinfection effect of *Moringa* seeds extract against the isolates showed that the extract possessed antibacterial agent. This is due

to the presence of bioactive ingredients such as alkaloid, flavonoids and terpenoids presence in the extract. *Moringa* seeds extract can be applied in water treatment as disinfection agent.

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