



Antibacterial Effect of *Nymphaea lotus* (Linn) Extracts on Enteric Bacteria Isolated from River Ogbese, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2021/v21i1130402

Editor(s):

(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Nasrin Habib, Quest International University, Malaysia.

(2) Tasnia Ahmed, Stamford University Bangladesh, Bangladesh.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/72660>

Original Research Article

Received 10 June 2021
Accepted 20 August 2021
Published 04 November 2021

ABSTRACT

The increase in multidrug resistance pathogenic bacteria and decrease in efficiency of existing antibiotics is a serious global health concern which call for development of new alternative medicine and continuous research for new classes of antimicrobial agents that can be effective in destroying these multidrug resistant pathogens without or with minimal side effect and at an affordable cost. *N. lotus* has been reported to possess some amount of phytochemicals such as phenols, tannins, saponins, steroids, proanthocyanidins and flavanols which may serve as an effective antimicrobial agent. This study was aimed at determining the antibacterial effect of *Nymphaea lotus* (Linn) extracts on enteric bacteria isolated from River Ogbese, Nigeria. Water samples were collected every week for a period of sixteen (16) weeks aseptically and transported to the laboratory for microbiological and physicochemical examinations; enteric bacteria were isolated from the water samples using membrane filtration method. Bioactive components of *N. lotus* were extracted using Ethanol, water and N-Hexane solvents. *In-vitro* antibacterial effect of *N. lotus* extracts was assayed using agar well diffusion technique. Results showed *Shigella* and faecal coliforms had the highest occurrence in the water samples at (30.19%) followed by *Salmonella* (20.76%) and *Escherichia coli* (18.87%). Physicochemical characteristics of water samples from River Ogbese showed that water temperature ranged from 22.00 to 28.10 °C, turbidity ranged from 2.00 to 33.80 NTU and phosphate ranged from 5.45 to 68.57 mg/L respectively. Whilst the isolates had the highest total percentage

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resistance to Augmentin at 20.03 %, ethanol extract of *N. lotus* exhibited the highest mean zone of inhibition of 24.67 ± 0.67 mm against the isolates at 100 mg/mL respectively. The findings from this study suggest *N. lotus* extracts to be effective in the treatment of enteric infections that may occur as a result of consumption or contact with faecal impacted water.

Keywords: *Antibacterial; Nymphaea lotus (Linn); enteric bacteria; River Ogbese; physicochemical; membrane filtration; In-vitro; faecal coliforms.*

1. INTRODUCTION

Water is vital for all known forms of life and safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients [1]. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion people lack access to adequate sanitation [2]. Rivers are part of the hydrological cycle [3] and have been used as a source of water, for obtaining food, for transport, as a defensive measure, as a source of hydropower to drive machinery, for bathing, and as a means of disposing waste [4]. They are often a rich source of fish and other edible aquatic life, and are a major source of fresh water, which can be used for drinking and irrigation. Water quality is commonly defined by its physical, Chemical, biological and aesthetic (appearance and smell) characteristics [5].

Waterborne diseases are a major cause of morbidity and mortality [6,7]. It is estimated globally that approximately 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters and seafood [7]. Polluted surface waters may contain a large variety of pathogenic microorganisms including viruses, bacteria and protozoa [8]. These pathogens, often of faecal source, may be from point sources such as municipal wastewater treatment plants [9,10] and drainage from areas where livestock are handled [11] or from non-point sources such as domestic and wild animal defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff [12,13].

Antimicrobial resistance (AMR) has emerged as one of the principal public health challenges of the 21st century that threatens the effective prevention and treatment of increasing range of infections caused by bacteria, parasites, viruses and fungi [14]. The problem of AMR is especially urgent regarding antibiotic resistance in bacteria. Over several decades, to varying degrees,

bacteria causing common or severe infections have developed resistance to each new antibiotic. Faced with this reality, the need for action to avert a developing global crisis in health care is imperative. The World Health Organization [15] has long recognised the need for an improved and coordinated global effort to contain AMR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance provided a framework of interventions to slow the emergence and reduce the spread of antimicrobial-resistant microorganisms [14]. In 2012, WHO published The Evolving Threat of Antimicrobial Resistance – Options for Action [16], proposing a combination of interventions that include strengthening health systems and surveillance; improving use of antimicrobials in hospitals and in community; infection prevention and control; encouraging the development of appropriate new drugs and vaccines; and political commitment [14].

Waterborne diseases are a major cause of morbidity and mortality [6,15]. Pathogenic bacteria responsible for common or severe waterborne infections that have developed resistance to antibiotics remain a major concern to public health [16].

Nymphaea lotus (Linn) is an important component of the Egyptian vascular aquatic plants, and more especially encountered in the irrigation and drainage canals in the Nile delta. It has been receiving much attention from the ecological, medicinal and environmental points of view, in particular due to its ability to absorb and accumulate heavy metals from polluted water [17,18].

The syrup of the roots was used as an anti-inflammatory, and in fever, and the seeds were used for hemorrhoids [18]. Nutritionally, the tuberous rhizomes and seeds of the plant could be eaten, the first either boiled or roasted, and the latter in bakery [19]. The aim of this study was to determine the antibacterial effect of *Nymphaea lotus* (linn) extracts on *E. coli* and *Salmonella* and *Shigella* isolated from river Ogbese, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of *Nymphae lotus*

Water lilies (*Nymphae lotus*) were collected from stagnant water at Okitipupa Local Government Area of Ondo State, Nigeria. The plant was identified and authenticated at the Crop, Soil and Pest Department, Federal University of Technology, Akure, Nigeria. The leaves, stems and roots were separated from each other into sterile containers, washed with running clean tap water and dried at room temperature. The dry leaves, stems and roots were milled separately to fine powder, and stored in airtight containers at room temperature until when required.

2.2 Preparation and Storage of Extracts from *N. lotus*

Phytochemicals were extracted from the plant's leaves, stems and roots with water, ethanol and N-hexane using the method described by Olukunle and Adenola [20]. Three equal weight of finely grounded dried *N. lotus* leaves, *N. lotus* stems and *N. lotus* roots were measured into 9 sterile plastic containers respectively. Each part of the finely grounded plants parts was homogenized in sterile distilled water, ethanol and N-hexane at 200 g to 1litre of solvent respectively. The homogenates were kept in covered sterile container for three days. Sterile muslin cloth was used to remove the large particles from the homogenate and then filtered using Whatman No. 1 filter paper. Extracts obtained were then concentrated in vacuum using rotary evaporator to remove the solvents [21]. The extraction efficiency was quantified by determining and comparing the weight of each of the extracts yield.

The 100% stock concentration of the extracts *N. lotus* was obtained and stored at 4°C in well corked universal bottle. It was reconstituted with DMSO to a required concentration at each use [20].

2.3 Description of the study area

River Ogbese is located at Ogbese village few kilometers away from Akure, Ondo State, Nigeria. It has an annual rainfall of about 1600 mm to 2100 mm, which covers the month of April to October and drainage area of 2039 km² [22]. Samples are taking for the period of sixteen weeks at GPS coordinate 7°15'34.8"N 5°22'45.3"E (Fig. 1). River Ogbese is one of the most important rivers in both the central and

northern zones of Ondo State, Nigeria [23]. Due to its strategic location within the two zones and availability for agricultural purposes, industrial and commercial consumption, the river has witnessed tremendous change in quality as a result of both human and industrial wastes.

2.4 Collection of Water Samples from River Ogbese

Water samples were collected from River Ogbese weekly during morning hours in between 8.00 to 9.00 a.m for a period of 16 weeks using clean and sterilized polypropylene one litre plastic containers as described by Apeh and Ekenta [24]. One litre of the river water was collected at about 40-50 cm depth at a representative monitoring point and the water samples were transported in an ice-packed bag to the laboratory and analyzed within an hour as described by Apeh and Ekenta [24].

2.5 Enumeration of *E. coli*, Faecal coliforms, *Shigella* and *Salmonella* in Water Samples from River Ogbese

The concentrations of *E. coli* in river water samples were determined using the membrane filtration method [25,26]. The membrane filter was placed on membrane setup and about 100 ml of water sample was filtered through. The membrane filters were placed on freshly prepared selective media; membrane lauryl sulphate agar (MLSA) and were incubated at 37°C for 24 h (MLSA) and colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml⁻¹ of water.

2.5.1 Confirmatory test for *E. coli*

The yellow colonies on MLSA were picked by sterile inoculating loop and streaked on freshly prepared eosin methylene blue agar (EMB) and incubated at 37 °C for 24 h. Green metallic sheen confirmed the presence of *E. coli* [27].

The concentrations of faecal coliforms in river water samples were determined using the membrane filtration method [25,26]. The membrane filter was placed on membrane setup and about 100 ml of water sample were filtered through. The membrane filters were placed on freshly prepared selective media; membrane faecal coliform agar (MFCA) and inoculate were incubated at 44°C for 24 h (m-FC) and colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml⁻¹ of water [25].

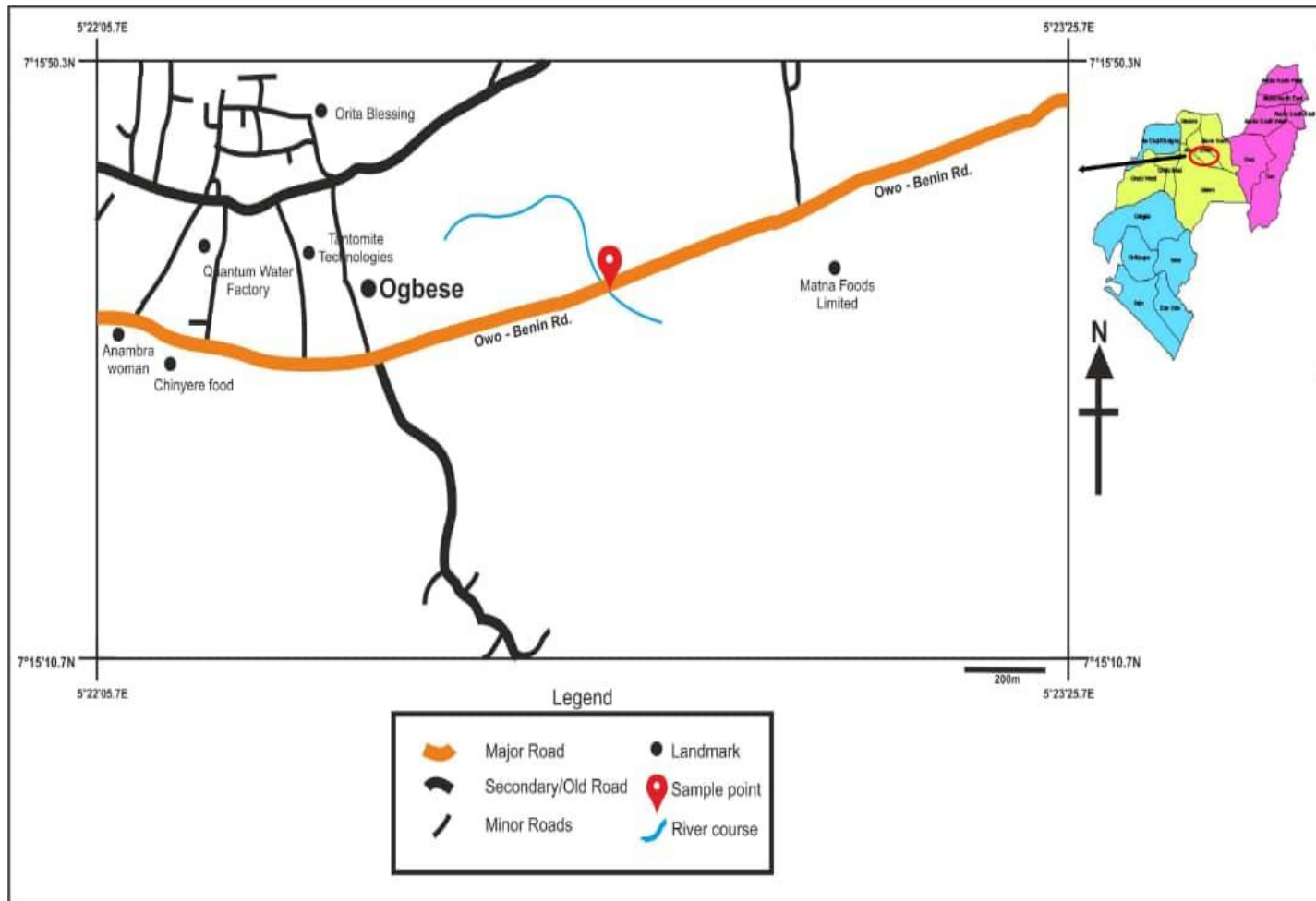


Fig. 1. Map showing the sampling point along River Ogbese, Ondo State

The concentrations of *Shigella* in river water samples were determined using the membrane filtration method. The membrane filter was placed on membrane setup and about 100 ml of water sample was filtered through. The membrane filters were placed on freshly prepared selective media; salmonella shigella agar (SSA) and inoculates were incubated at 37°C for 24 h (SSA) and white colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml⁻¹ of water [25].

The concentrations of *Salmonella* in river water samples were determined using the membrane filtration method. The membrane filter was placed on membrane setup and about 100 ml of water sample was filtered through. The membrane filters were placed on freshly prepared selective media; salmonella shigella agar (SSA) and innoculates were incubated at 37°C for 24 h (SSA) and black colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml⁻¹ of water [25].

2.6 Qualitative and Quantitative Determination of Phytochemicals in the Extracts of *N. lotus* (Water lily)

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients [28]. They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [29]. Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed [29]. Many phytochemicals, particularly the pigment molecules like anthocyanines and flavonoids, are often concentrated in the outer layers of the various plant parts like leaves and fruits of vegetables. However, the levels of these phytochemicals vary from plant to plant depending upon the variety, climatic growing conditions [30].

The Qualitative and quantitative phytochemical properties of *N. lotus* leave, stems and roots extracts was determined using method described by [31]. The extracts were examined for the presence of different phytoconstituents like alkaloids, saponin, tannin, phlobatanin, flavonoid, phenol, steroids, terpenoid and cardiac glycosides.

2.7 Mc Farland Turbidity Standard for Test Inoculums

The method modified by Cheesbrough [32] was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of bacterial cells. In this method, fifty milliliter (50 ml) of a 1.175% (wt/vol) dehydrate Barium chloride (BaCl₂.2H₂O) solution was added to 99.4 ml of 1% (vol/vol) sulphuric acid. McFarland standard tube was then sealed with paraffin to prevent evaporation and stored in the dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1 cm light path. The 0.5 McFarland standards were vigorously agitated on a vortex mixer before use.

2.8 Sensitivity Pattern of Enteric Bacterial Isolates to Commercial Antibiotics

Antibiotic susceptibility patterns of the enteric bacterial isolates were determined by disc diffusion method as described by Cheesbrough [32]. Standard inoculum of 18 hours broth was spread on Muller Hinton agar using sterile swab in triplicate. The antibiotic discs were placed on the plate at equidistance. The plates were then incubated for 18 hours at 37°C and diameter of zone of inhibition were measured and recorded in millimeters. The commercial antibiotics discs (Fonoz Laboratories Ltd, Nigeria) used were; Tarivid Ofloxacin (30 µg), Gentamicin (20 µg), Chloramphenicol (30 µg), Augmentin (30 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Streptomycin (10 µg), Sparfloxacin (10 µg), Septrin (30 µg), and Pefloxacin (10 µg).

2.9 Sensitivity Pattern of Enteric Bacterial Isolates to Extracts of *N. lotus*

Each of the plant extracts was screened for antimicrobial activity on the isolated enteric bacteria and pathogens by performing agar well diffusion assay as described by Olukunle and Adenola [20]. The plant extracts were being reconstituted using 30% v/v Dimethyl sulfoxide and sterilized (by filtration) using sterile injection filters of 0.22 µm pore size. The various plant extracts to be screened were reconstituted to concentration of 100, 50, 25 and 12.50 mg/ml respectively and about 0.5 ml each was introduced to the agar wells in each of the test bacterial seeded agar. The negative control for the experiment was 30% aqueous DMSO while Ciprofloxacin (0.63 mg/ml) was used as the positive control. All the plates were incubated at

37 °C for 24 hours after which the zones of inhibitions were measured.

2.10 Minimum Inhibitory and Bactericidal Concentrations of extracts of *N. lotus*

The Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using the broth (tube) dilution technique [33]. Dilutions of the extract in Mueller Hinton broth were prepared in tubes. The concentration of inoculum was also standardized to 0.5 McFarland's turbidity, The Mueller Hinton broth in tubes containing the different concentration of plant extract, 2.5 mg/ml, 5mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml were then inoculated with 0.5 ml of the standardized culture. The tubes were then incubated at 37°C for 24 hours. MIC and MBC values were recorded.

2.11 Molecular Identification of Resistant Enteric Bacterial Isolates to Antibiotics and Extracts of *N. lotus*

Enteric Bacterial isolates that exhibited significant resistance to commercial antibiotics/extracts of *N. lotus* were preserved in 15% glycerol at 0°C for molecular analysis.

2.11.1 Isolation of genomic DNA from resistant enteric bacterial isolates

The method of Nicole *et al.* [34] was adopted; 1.5 ml of multidrug resistant enteric bacteria broth culture was taken in centrifuge tube. Centrifuged at 10,000 rpm for 2 minutes and supernatant was discarded. To the pellet 1 ml of distilled water was added and dissolved the pellet completely. The dissolved pellet was then centrifuged at 10,000 rpm for 2 min. The procedure was repeated twice. The supernatant was discarded then 100 µl of Tris EDTA buffer was added to dissolve the pellet completely in buffer. The supernatant containing the DNA was transferred to another tube and stored at -20°C. The concentration and purity of the extracted DNA was estimated using a Nanodrop spectrophotometer (Model: 752).

2.11.2 DNA electrophoresis of resistant enteric bacterial isolates

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gel. Agarose

gels were prepared by dissolving and boiling 1.0 g agarose in 100 ml 0.5 X TBE buffer solution. The gels were allowed to cool down to about 45°C and 10 µl of 5 mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µl of the DNA with 5 µl sterile distilled water and 2 µl of 6 X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source [34].

2.11.3 Polymerase Chain Reaction (PCR) to identify the resistant enteric bacterial isolates

PCR master mix (2X) which contains components required for PCR amplification including *Taq* DNA polymerase, dNTPs, reaction buffer, MgCl₂, KCl and a PCR enhancer/stabilizer with catalogue number: 28009, was used for the PCR reaction. Water and primer set were added to set up the PCR reaction.

Twenty-five microliter of 2X PCR master mix was dispensed into a PCR tube, the template DNA (1 ml), forward and reverse general-purpose primers (Pfast bact. 1 and 2) 2.5 Mm of each were added to the PCR tube, water was added to bring the total volume to 50 ml. The PCR mixture was thoroughly mixed using a rotax and the PCR tubes were carefully placed into the thermocycler and the process was carried out. Initial denaturation was carried out at 95°C for 2 mins in one cycle. Final denaturation was done at 95°C for 20 secs in 40 cycles. Annealing temperature was set at 60°C for 30 secs in 40 cycles. Extension was done at 72°C for 1 min in 40 cycles and during the final extension, temperature was set at 72°C for 5 mins in 1 cycle. At the end of the process, 10 ml aliquot of the reaction was mixed with 2 ml of loading dye (6X) and loaded onto agarose gel for visual analysis of the DNA bands [35]. Sequencing of microbial isolates was done using DNA sequencer and Blasting process was carried out in NCBI Gene Bank (FASTAC format), BLAST (Basic Local Alignment Search Tool) to compare the primary biological sequence information of the isolates [35].

2.12 Statistical Analysis

Data obtained were expressed as mean ± Standard Error of Mean and were statistically analysed using One Way Analysis of Variance

(ANOVA). The new Duncan Multiple Range test was used to separate and compare means of different groups. A *P*-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1 Detection of *E. coli* in Water Samples from River Ogbese

The bacterial colony counts in water samples from River Ogbese determined over a period of sixteen weeks revealed that *E. coli* ranged from zero to 3.2×10^3 cfu/100 ml. The highest count of *E. coli* was recorded in sixth week 3.2×10^3 cfu/100 ml whereas the level of *E. coli* was below detection limit of 1 cfu/100 ml on six sampling occasions i.e., week seven, nine, ten, eleven, twelve and sixteen respectively (Fig. 2).

3.2 Detection of *Salmonella* in Water Samples from River Ogbese

The bacterial colony counts in water samples from River Ogbese determined over a period of sixteen weeks revealed that *Salmonella* ranged from zero to 2.0×10^3 cfu/100 ml. The highest *Salmonella* count was recorded in fourth week 2.0×10^3 cfu/100 ml, while zero count was recorded in week eight, nine, ten, thirteen and fourteen respectively (Fig. 3).

3.3 Detection of Faecal Coliforms in Water Samples from River Ogbese

The bacterial colony counts in water samples from River Ogbese determined over a period of sixteen weeks revealed that faecal coliforms ranged from 5.4×10^2 to 4.5×10^4 cfu/100 ml. The highest faecal coliforms count was recorded on seventh week 4.5×10^4 cfu/100 ml and the least on first week 5.4×10^2 cfu/100 ml (Fig. 4).

3.4 Detection of *Shigella* in Water Samples from River Ogbese

The bacterial colony counts in water samples from River Ogbese determined over a period of sixteen weeks revealed that *Shigella* ranged from 1.5×10^2 to 4.1×10^3 cfu/100 ml as shown in Figure 5. The highest *Shigella* count was recorded in week seven (4.1×10^3 cfu/100 ml) and the least was recorded in week one (1.5×10^2 cfu/100 ml).

3.5 Percentage Occurrence of Enteric Bacteria in Water Samples from River Ogbese

Shigella and faecal coliforms had the highest occurrence (30.19%) followed by *Salmonella* (20.76%), and *Escherichia coli* (18.87%) had the least occurrence as represented in Table 1.

3.6 Seasonality of Bacterial Isolates in Water Samples from River Ogbese

The seasonal occurrence of bacterial isolates in water samples from River Ogbese indicated that *E. coli* had higher mean colony count in wet season 9.71×10^3 cfu/100 ml compared to dry season (6.40×10^3 cfu/100 ml) while *Salmonella* and *Shigella* had higher mean colony count in dry season (4.36×10^3 and 7.46×10^4 cfu/100 ml) compared to wet season (1.40×10^3 and 4.60×10^4 cfu/100 ml respectively). Faecal coliforms had higher mean colony count in wet season (2.49×10^5 cfu/100 ml) compared to dry season (1.03×10^5 cfu/100 ml) as show in Fig. 6.

3.7 Physicochemical Characteristics of Water Samples from River Ogbese

Physicochemical characteristics of water samples from River Ogbese showed that water temperature ranged from 22.00 to 28.10 °C, turbidity ranged from 2.00 to 33.80 NTU, salinity ranged from 32.93 to 113.94 mg/l, dissolved oxygen ranged from 5.00 to 6.82 mg/l, total dissolved solids ranged from 106.88 to 139.10 mg/l, phosphate ranged from 5.45 to 68.57 mg/l, nitrate ranged from 0.02 to 3.42 mg/l (Table 2).

3.8 Percentage Yield of Extracts of *N. lotus*

Water extract of *N. lotus* Stem had the highest percentage yield (16.98%), while N-hexane extract of *N. lotus* root had the least percentage yield (1.70%) for all *N. lotus* extracts as shown in Table 3.

3.9 Qualitative phytochemicals in the extracts of *N. Lotus*

Results revealed that all *N. lotus* extracts possess some amount of phytochemicals such as Saponin, Tannin, Flavonoid, Phenol, Steroid, Terpenoid and Glycoside while Phlobatannin and Alkaloid were absent in all the extracts (Table 4).

Table 1. Percentage occurrence of enteric bacteria in water samples from River Ogbese

Isolates	Sampling weeks																Total (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
<i>Escherichia coli</i>	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	-	10 (18.87)
<i>Salmonella</i>	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+	11 (20.76)
<i>Shigella</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 (30.19)
Faecal coliforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 (30.19)
Total	4	4	4	4	4	4	3	3	2	2	3	3	3	3	4	3	53 (100)

KEY: + (present), - (absent)

Table 2. Physicochemical characteristics of water samples from River Ogbese, Nigeria for the period of sixteen weeks

Parameters	Mean± std. Error	Minimum	Maximum
Temperature (°C)	26.50±0.26	22.00	28.10
pH	7.70±0.03	7.29	8.12
Conductivity (µS/cm)	244.03±3.26	212.00	278.00
Turbidity (NTU)	14.97±1.13	2.00	33.80
Salinity (mg/L)	53.67±2.96	32.93	113.94
DO (mg/L)	6.06±0.06	5.00	6.82
BOD (mg/L)	2.33±0.07	1.20	3.18
Hardness (mg/L)	98.20±3.22	54.60	128.20
Phosphate (mg/L)	28.33±2.60	5.45	68.57
Nitrate (mg/L)	1.32±0.18	0.02	3.42
Chloride (mg/L)	29.80±1.63	18.29	63.30
TDS (mg/L)	122.90±1.68	106.88	139.10

KEY- BOD- Biological Oxygen Demands, DO- Dissolved Oxygen, and TDS- Total dissolved solid

Table 3. Percentage yield of *N. lotus* extracts

Solvents	<i>N. lotus</i> Root (%)	<i>N. lotus</i> Leaves (%)	<i>N. lotus</i> Stem (%)
ETHANOL	6.24	9.23	7.01
WATER	9.74	14.13	16.98
N- HEXANE	1.70	2.80	2.20

Table 4. Qualitative phytochemicals in the extracts of *N. lotus*

Phytochemical	A	B	C	D	E	F	G	H	I
Saponin	+	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+
Phlobatannin	-	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+
Steroid	-	+	+	-	+	+	-	+	+
Terpenoid	+	-	-	-	+	+	+	+	+
Alkaloid	-	-	-	-	-	-	-	-	-
Keller kiliani test	+	+	+	+	+	+	+	+	+
Salkowski test	+	-	-	-	+	+	+	+	+
Lieberman test	-	+	+	-	+	+	-	+	+

Key : A- Water extract of *N. lotus* Leaves, B- Ethanol extract of *N. lotus* Leaves, C- N-hexane extract of *N. lotus* Leaves, D- Water extract of *N. lotus* Stem, E- Ethanol extract of *N. lotus* Stem, F- N-hexane extract of *N. lotus* Stem, G- Water extract of *N. lotus* Root, H- Ethanol extract of *N. lotus* Root, I- N-hexane extract of *N. lotus* Root

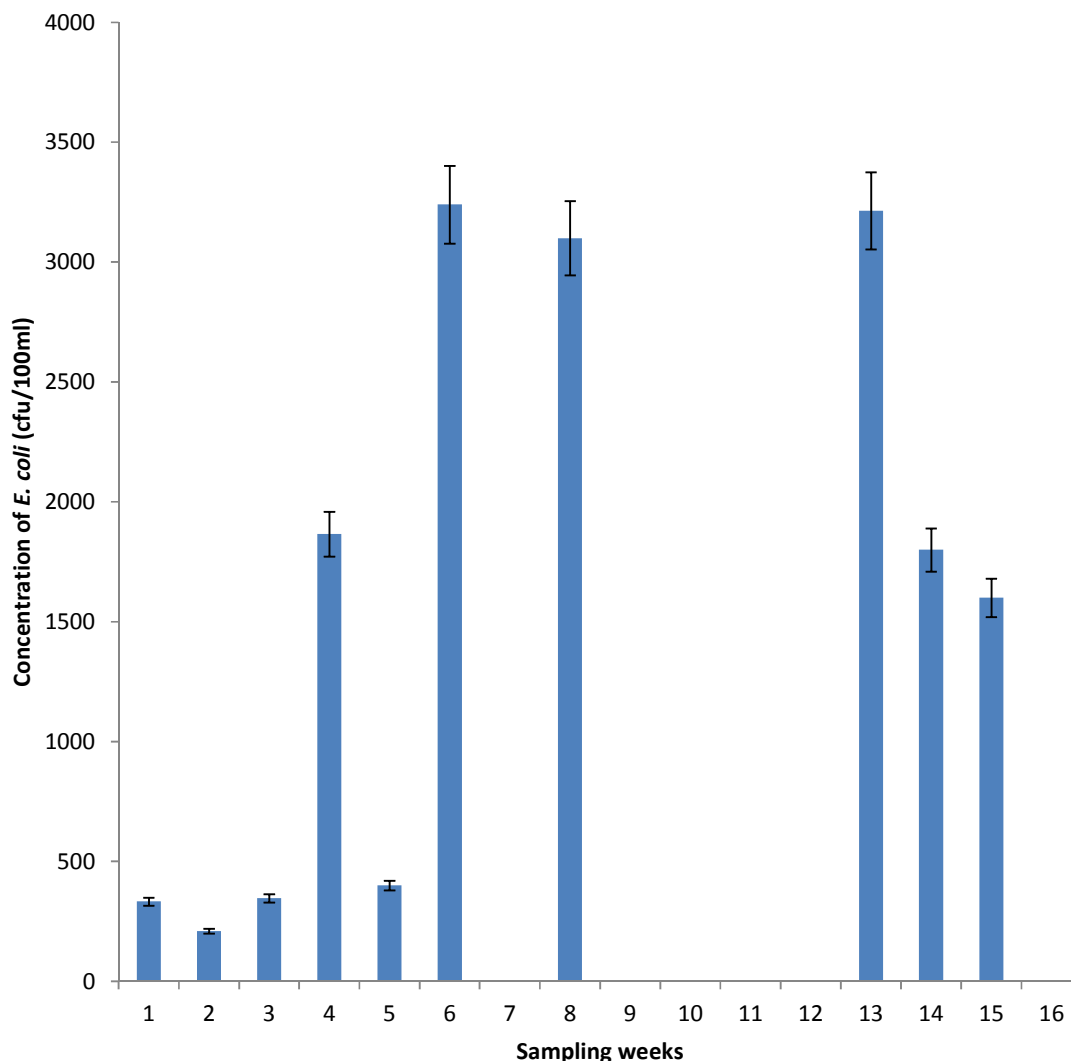


Fig. 2. Load of *Escherichia coli* in water samples from River Ogbese

3.10 Quantitative phytochemicals in the extracts of *N. Lotus*

Ethanol extract of *N. lotus* Root had the highest quantity of Saponin 19.15 ± 0.12 , Steroid 21.99 ± 0.01 , Terpenoid 40.06 ± 0.02 , Tannin 8.85 ± 0.00 , Flavonoid 5.44 ± 0.00 , Glycoside 49.18 ± 0.02 and Phenol 1.55 ± 0.00 . While N-hexane extract of *N. lotus* Stem had the lowest quantity of Saponin 3.70 ± 0.12 , Steroid 4.35 ± 0.01 , Terpenoid 3.89 ± 0.02 , Tannin 1.49 ± 0.00 , Flavonoid 0.21 ± 0.00 , Glycoside 5.45 ± 0.02 and Phenol 0.19 ± 0.00 among *N. lotus* extracts. Meanwhile 0.00 ± 0.00 quantity of steroid was reported for water extract of *N. lotus* Leaves and Water extract of *N. lotus* Root and 0.00 ± 0.00

quantity of Terpenoid is reported for Ethanol extract of *N. lotus* Leaves, N-hexane extract of *N. lotus* Leaves and Water extract of *N. lotus* Stem (Table 5).

3.11 Sensitivity Pattern of Enteric Bacterial Isolates to Commercial Antibiotics

The antibiotic sensitivity patterns of enteric bacteria isolated from water samples from River Ogbese is shown in Table 6. The result showed that the diameter of mean zones of inhibition of all antibiotics used against the isolated enteric bacteria ranged from 5.44 ± 1.22 to

28.75±0.37 mm. Ciprofloxacin had highest mean zone of inhibition 28.75±0.37 mm against *Shigella*. The least zone of inhibition was observed in Augmentin (5.44±1.22 mm) against *Salmonella*.

3.11.1 Classification of sensitivity pattern of enteric bacterial isolates to commercial antibiotics into resistant, susceptible or intermediate

Classification of mean zone of inhibition of commercial antibiotics to bacterial isolates into resistant, susceptible or intermediate using Clinical and Laboratory Standards Institute guidelines shows that out of ten commercial antibiotics used for test the highest number of

resistance [6] was recorded for *E. coli* and *Salmonella*, the same number of intermediate [2] was recorded for all enteric bacteria and the highest number of susceptibility [5] was recorded for *Shigella* (Table 7).

3.11.2 Antibiotic resistance pattern of the isolated enteric bacteria

The antibiotics resistance pattern of the bacteria isolates is shown in Table 8. Significance is taking as P< 0.05. Augmentin had the highest total percentage resistance to the enteric bacteria at 20.03%. *Shigella* was the only bacteria that had resistance to all the antibiotics used in this study.

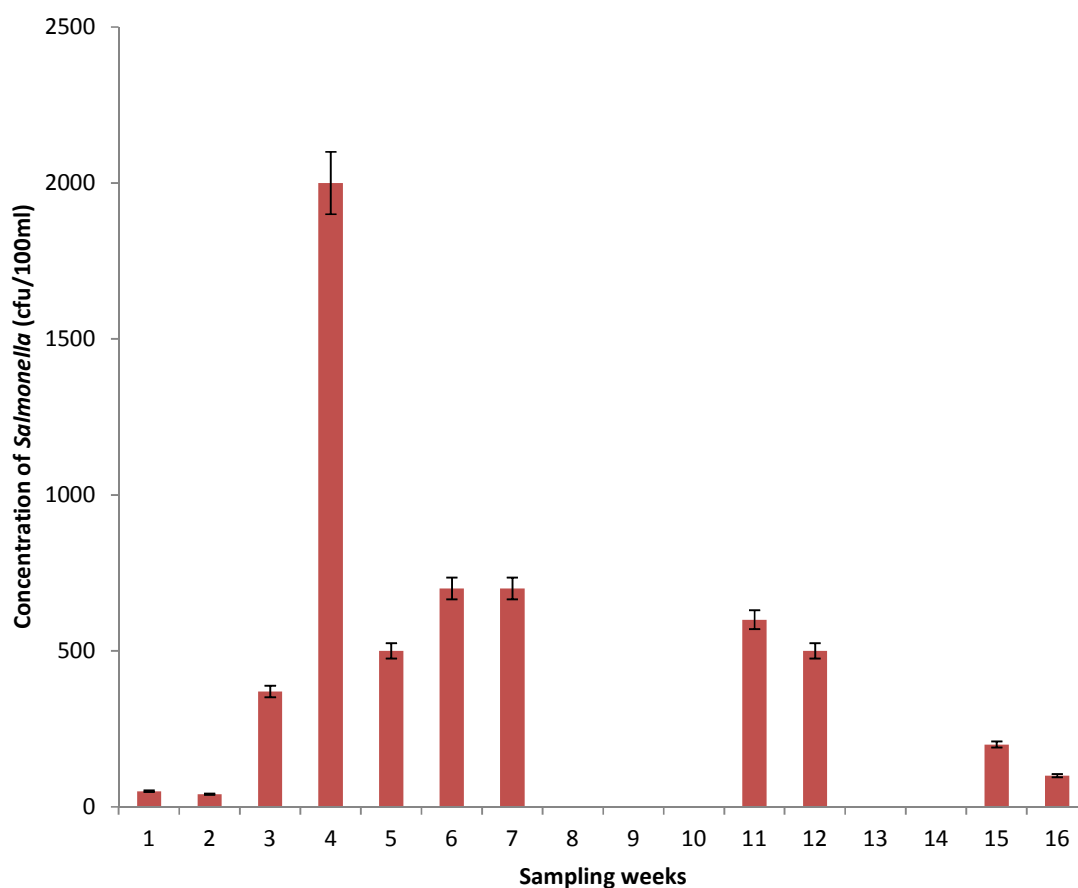


Fig. 3. Load of *Salmonella* in water samples from River Ogbese

3.12 Sensitivity pattern of enteric bacterial isolates to extracts of *N. lotus*

The antimicrobial susceptibility patterns of extracts of *N. lotus* to resistant enteric bacteria isolates Table 9.1 to 9.3. Extracts of *N. lotus* except for N-hexane extract of *N. lotus* Leaves inhibited the isolates of *Escherichia coli*, *Salmonella* and *Shigella* at lower concentration of 12.50 mg/ml (Table 9.1 to 9.3.). Ethanol extract of *N. lotus* root had the highest mean zone of inhibition (24.67 ± 0.67 mm) against the bacteria isolates at concentration of 100 mg/ml, while N-hexane extract of *N. lotus* Leaves had

the least zone of inhibition 0.00 ± 0.00 mm at concentration of 25 mg/ml and 12.5 mg/ml respectively.

3.13 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *N. lotus* extracts to enteric bacteria isolates

The Minimum Inhibitory Concentration (MIC) of *N. lotus* extracts to resistant enteric bacteria isolates ranges from 10mg/ml to 40mg/ml as seen in Table 10, while the Minimum Bactericidal Concentration (MBC) of *N. lotus* extract to resistant enteric bacteria isolates ranges from 20mg/ml to 80mg/ml (Table 11).

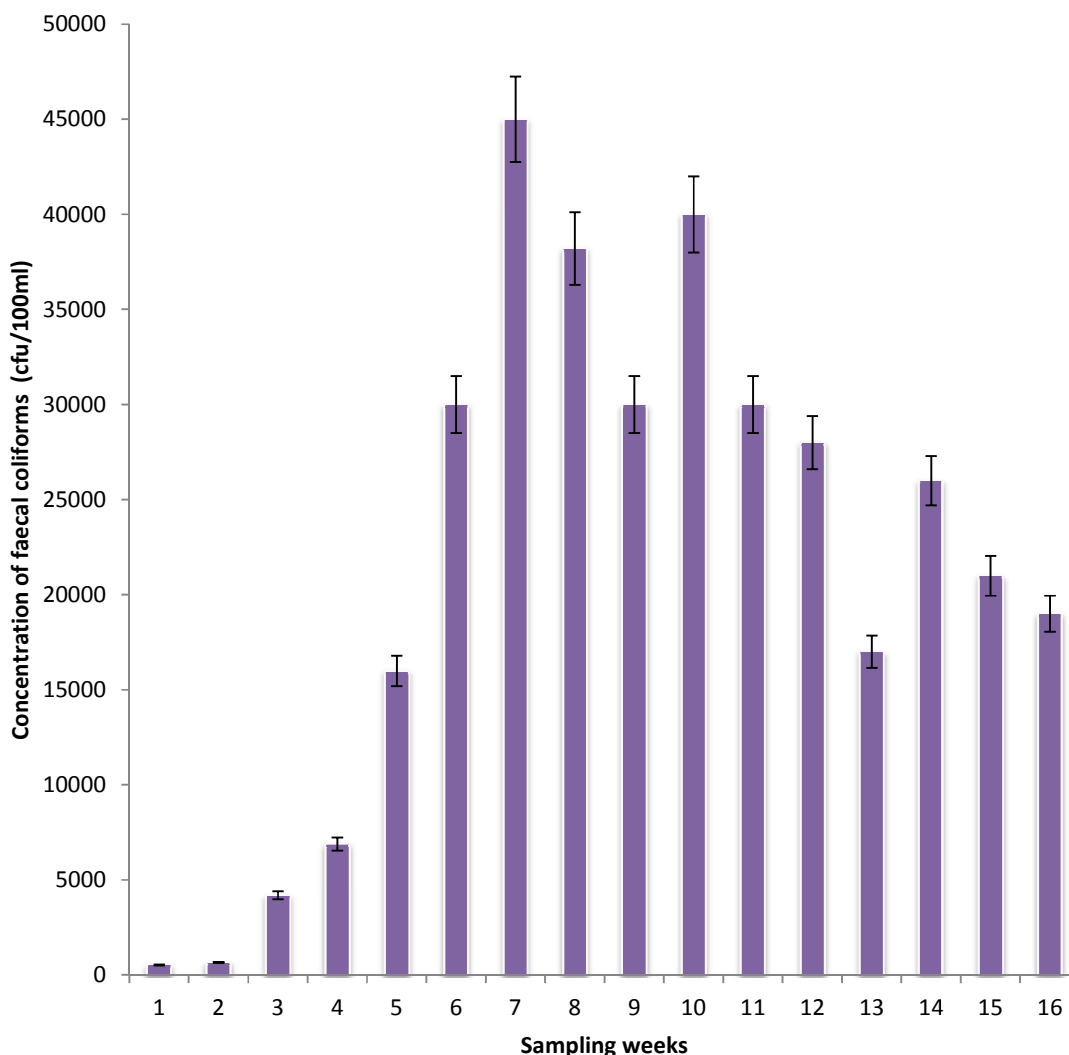


Fig. 4. Load of faecal coliforms in water samples from River Ogbese

3.14 Molecular identification of resistant enteric bacterial isolates from water samples from River Ogbese

The molecular identity of a resistant enteric bacterial isolates from water samples from River Ogbese is represented in Table 12. It was observed that the identified isolate showed 95.27% to 98.48% similarities with what has been deposited in gene data base.

4. DISCUSSION

The high faecal coliform count of Ogbese River may be due to human activities in the river such as spiritual bathing, washing, defecation, swimming, industrial waste runoff, domestic waste runoff, farm waste run off and disposal of

waste into the river. This makes this water not suitable for drinking and domestic use. Such gross contamination of surface water was also reported by Abok *et al.* [36], in which they reported bacteria and cyst contamination of surface which carries these faecal coliforms on lands, roofs and other surfaces during the dry season that have just been washed into the river at the onset of the early rain. This is in accordance with the report of Elisante and Muzuka [37], in which they reported highest number of faecal coliforms during wet than the dry season owing to rising of water table and leaching during raining season in their work “sources and seasonal variation of coliform bacteria abundance in ground I colony count on week thirteenth (wet season), four (dry season) and seven (wet season) respectively.

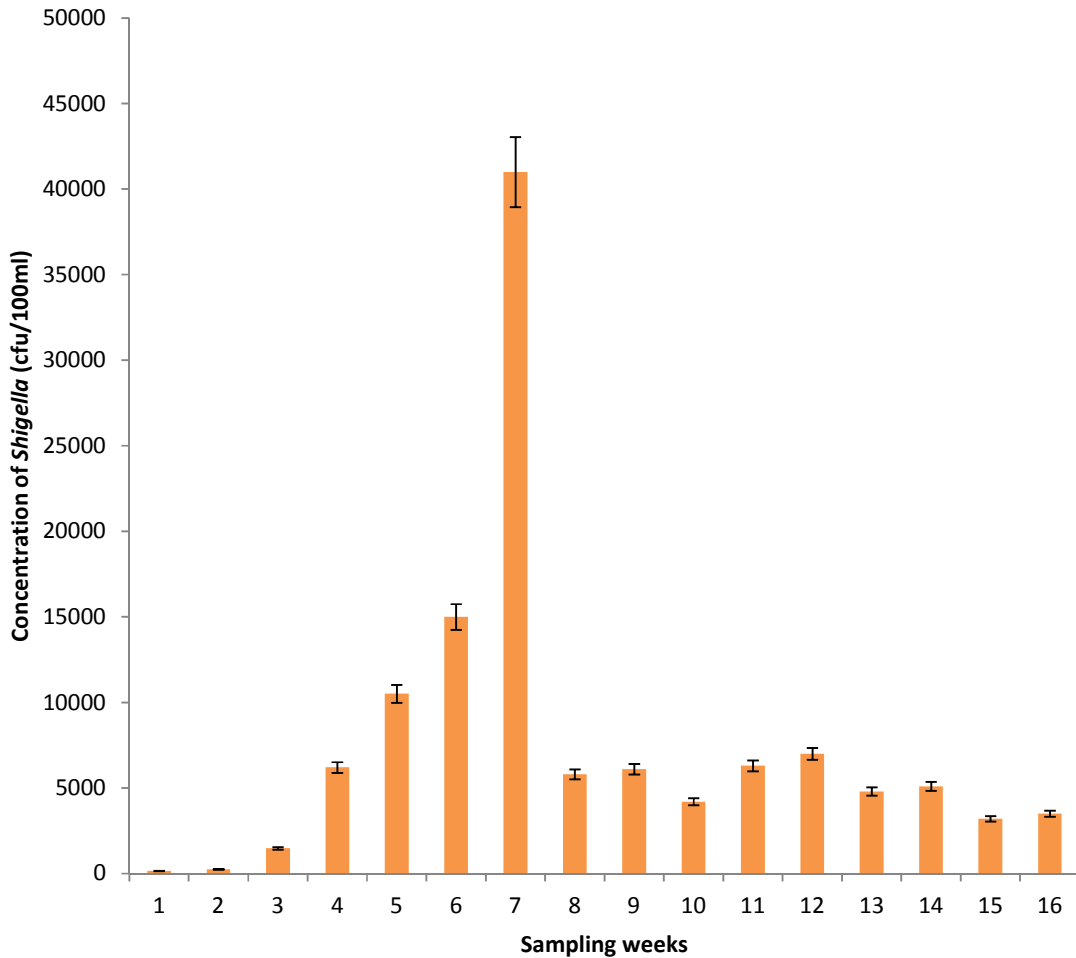


Fig. 5. Load of *Shigella* in water samples from River Ogbese

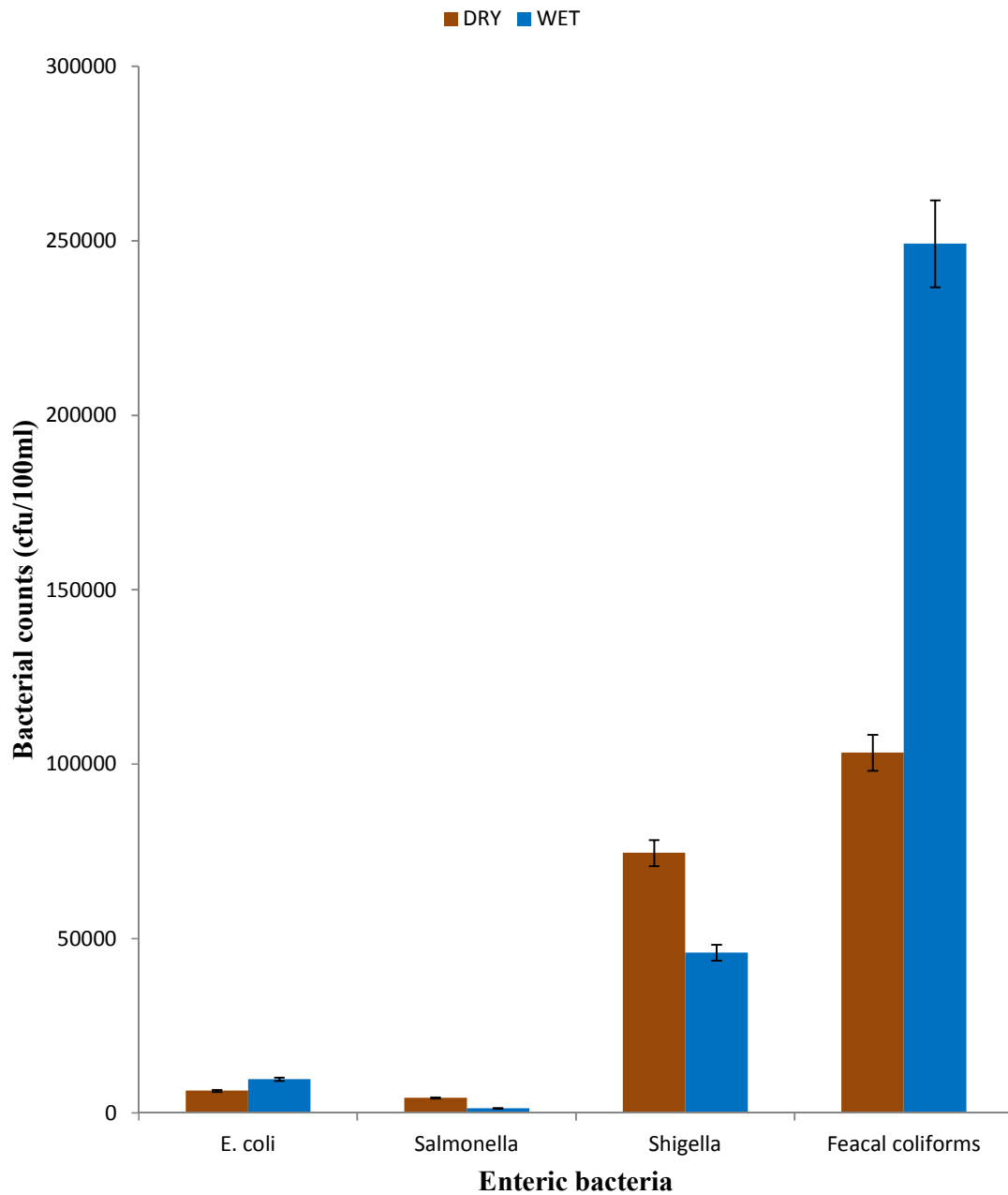


Fig. 6. Seasonality of bacterial isolates in water samples from River Ogbese

The high colony counts of *Escherichia coli* and *Shigella* recorded during wet season may be due to the activities of households, industries, fish farmers and local waste managers in the location of the river which include disposal of domestic and farm wastes into overland flows, canals and drainages during rain falls. Large portion of this waste are faecal waste and *Escherichia coli* and *Shigella* are of faecal origin. High colony of

Shigella during wet season may also be attributed to high temperature and wet environment which supports the growth of bacteria [38]. This is related to findings of Kumarasamy *et al.* [39] where the authors isolated the highest number of pathogenic bacteria indicators from Cauvery River, South India.

Table 5. Quantitative phytochemicals in the extracts of *N. lotus*

Plant solvent	extract/	SAPONIN mg/g	STERIOD mg/g	TERPENOID mg/g	Tannin mg/g	Flavonoid mg/g	Glycosides mg/g	Phenol mg/g
A		7.70±0.12 ^e	0.00±0.00 ^a	13.25±0.02 ^f	3.40±0.00 ^f	1.56±0.00 ^f	16.76±0.02 ^f	0.54±0.00 ^f
B		15.15±0.12 ^l	17.42±0.01 ^l	0.00±0.00 ^a	6.95±0.00 ^k	4.09±0.00 ^k	37.86±0.02 ^k	1.19±0.00 ^k
C		6.55±0.18 ^d	7.26±0.01 ^e	0.00±0.00 ^a	2.71±0.00 ^d	1.07±0.00 ^d	12.65±0.02 ^d	0.41±0.00 ^d
D		8.79±0.12 ^f	0.00±0.00 ^a	0.00±0.00 ^a	3.92±0.00 ^g	1.93±0.00 ^g	19.85±0.02 ^g	0.63±0.00 ^g
E		12.79±0.12 ^l	14.73±0.01 ^h	25.17±0.02 ^l	5.82±0.00 ^l	3.29±0.00 ^l	31.17±0.02 ^l	0.99±0.00 ^l
F		3.70±0.12 ^c	4.35±0.01 ^d	3.89±0.02 ^d	1.49±0.00 ^c	0.21±0.00 ^a	5.45±0.02 ^c	0.19±0.00 ^a
G		10.42±0.12 ^g	0.00±0.00 ^a	19.64±0.02 ^g	4.70±0.00 ^h	2.49±0.00 ^h	24.48±0.02 ^h	0.78±0.00 ^h
H		19.15±0.12 ^k	21.99±0.01 ^l	40.06±0.02 ^l	8.85±0.00 ^l	5.44±0.00 ^l	49.18±0.02 ^l	1.55±0.00 ^l
I		7.52±0.12 ^e	8.71±0.01 ^f	12.83±0.02 ^e	3.31±0.00 ^e	1.50±0.00 ^e	16.25±0.02 ^e	0.52±0.00 ^e

Key: A- Water extract of *N. lotus* Leaves, B- Ethanol extract of *N. lotus* Leaves, C- N-hexane extract of *N. lotus* Leaves, D- Water extract of *N. lotus* Stem, E- Ethanol extract of *N. lotus* Stem, F- N-hexane extract of *N. lotus* Stem, G- Water extract of *N. lotus* Root, H- Ethanol extract of *N. lotus* Root, I- N-hexane extract of *N. lotus* Root
 Values are means ± SEM (Standard error of mean) of triplicates, values in the same column carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$

Table 6. Sensitivity pattern of enteric bacterial isolates to commercial antibiotics

Isolates	S	OFX	PEF	CN	AU	AM	CPX	SP	CH	SXT
<i>Escherichia coli</i>	11.03±1.22 ^b	20.37±0.98 ^{ab}	20.17±0.95 ^a	16.83±0.67 ^c	9.40±1.12 ^{abc}	12.27±0.97 ^{ab}	25.33±0.80 ^{abc}	17.87±0.76 ^{bc}	11.40±1.50 ^a	8.87±1.15 ^b
<i>Salmonella</i>	12.48±1.50 ^{bc}	20.67±0.98 ^{ab}	22.63±0.75 ^{abc}	18.70±0.70 ^{cd}	5.44±1.22^a	9.04±1.31 ^a	27.07±0.57 ^{bcd}	17.33±0.68 ^{bc}	10.26±1.82 ^a	10.93±1.60 ^b
<i>Shigella</i>	14.83±0.44 ^{bcd}	17.75±3.10 ^a	24.42±0.70 ^{bc}	16.50±1.35 ^c	9.33±2.15 ^{abc}	10.58±2.36 ^{ab}	28.75±0.37^d	19.17±0.94 ^{cd}	16.25±1.36 ^a	9.42±2.04 ^b

KEY: S-Streptomycin, OFX-Ofloxacin, PEF-Pefloxacin, CN-Gentamycin, AU-Augmentin, AM-Amoxicillin, CPX-Ciprofloxacin, SP-Sparfloxacin, CH-Chloramphenicol, SXT-Septrin
 Value in bold – CPX (28.75±0.37^d mm) showing the highest mean zone of inhibition. Values are expressed as mean ± standard error (n = 3)

Values are means ± SEM (Standard error of mean) of triplicates, values in the same row carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$

Table 7. Classification of Sensitivity pattern of bacterial isolates to commercial antibiotics into resistant (R), susceptible (S) or intermediate (I)

Isolates	S	OFX	PEF	CN	AU	AM	CPX	SP	CH	SXT
<i>Escherichia coli</i>	R	S	R	S	R	R	I	I	R	R
<i>Salmonella</i>	R	S	R	S	R	R	I	I	R	R
<i>Shigella</i>	I	S	S	S	R	R	S	S	I	R

KEY: S-Streptomycin, OFX-Ofloxacin, PEF-Pefloxacin, CN-Gentamycin, AU-Augmentin, AM-Amoxicillin, CPX-Ciprofloxacin, SP-Sparfloxacin, CH-Chloramphenicol, SXT-Septrin

Table 8. Antibiotics resistance pattern of the isolated enteric bacteria

BACTERIA	S %	OFX %	PEF %	CN %	AU %	AM %	CPX %	SP %	CH %	SXT %	TOTAL
<i>Escherichia coli</i>	3(12)	2(8)	3(12)	1(4)	5(20)	3(12)	0(0)	1(4)	3(12)	4(16)	25
<i>Salmonella</i>	3(12)	2(8)	0(0)	0(0)	6(24)	5(20)	0(0)	0(0)	4(16)	5(20)	25
<i>Shigella</i>	1(2.78)	4(11.11)	4(11.11)	2(5.56)	8(22.22)	6(16.67)	1(2.78)	2(5.56)	4(11.11)	4(11.11)	36
Total	7(8.14)	8(9.30)	7(8.14)	3(3.49)	19(20.03)	14(16.28)	1(1.16)	3(3.49)	11(12.79)	13(15.12)	86

KEY: S-Streptomycin, OFX-Ofloxacin, PEF-Pefloxacin, CN-Gentamycin, AU-Augmenthin, AM-Amoxicillin, CPX-Ciprofloxacin, SP- Septrin, CH-Chloramphenicol, SXT-Septrin

Table 9.1. Sensitivity pattern of bacterial isolates to extracts of *N. lotus* root

Extracts	Concentration	Mean zone of inhibition (mm)		
		<i>E. coli</i>	<i>Salmonella</i>	<i>Shigella</i>
Ethanol extract of <i>N. lotus</i> Root	100 mg/ml	24.67±0.67 ^d	23.00±0.58 ^c	19.00±0.58 ^c
	50 mg/ml	21.00±0.58 ^c	16.67±3.38 ^{ab}	19.33±0.88 ^c
	25 mg/ml	20.00±0.58 ^c	17.67±0.88 ^b	15.00±0.58 ^b
	12.50 mg/ml	14.00±1.15 ^a	11.33±0.67 ^a	12.67±0.67 ^a
	Control	17.67±0.33 ^b	16.67±0.88 ^{ab}	16.33±0.88 ^b
Water extract of <i>N. lotus</i> Root	100 mg/ml	18.67±0.67 ^d	19.33±0.88 ^c	16.33±0.88 ^b
	50 mg/ml	16.33±0.88 ^c	15.33±0.33 ^b	14.00±0.58 ^{ab}
	25 mg/ml	13.33±0.67 ^b	15.00±0.58 ^{ab}	12.67±0.67 ^a
	12.50 mg/ml	10.67±0.67 ^a	13.00±0.58 ^a	12.00±0.58 ^a
	Control	17.67±0.33 ^{cd}	16.67±0.88 ^b	16.33±0.88 ^b
N-hexane extract of <i>N. lotus</i> Root	100 mg/ml	15.00±0.58 ^c	18.33±0.33 ^d	18.00±1.16 ^d
	50 mg/ml	14.67±0.67 ^c	14.33±0.33 ^c	15.00±0.58 ^{bc}
	25 mg/ml	11.33±0.33 ^b	9.67±0.33 ^b	13.00±1.00 ^b
	12.50 mg/ml	9.00±0.58 ^a	6.00±0.58^a	7.33±0.33 ^a
	Control	17.67±0.33 ^d	16.67±0.88 ^d	16.33±0.88 ^c

Key: Positive control- ciprofloxacin (0.63 mg/ml)

Values are means ± SEM (Standard error of mean) of triplicates, values in the same row carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$

Table 9.2. Sensitivity pattern of bacterial isolates to extracts of *N. lotus* leaves

Extracts	Concentration	Mean zone of inhibition (mm)		
		<i>E. coli</i>	<i>Salmonella</i>	<i>Shigella</i>
Ethanol extract of <i>N. lotus</i> Leaves	100 mg/ml	23.00±0.58 ^d	21.00±0.58 ^d	16.67±0.88 ^c
	50 mg/ml	22.00±1.16 ^c	15.33±0.33 ^{bc}	12.00±1.16 ^b
	25 mg/ml	16.00±0.58 ^{ab}	13.67±0.33 ^b	10.67±0.67 ^{ab}
	12.50 mg/ml	14.00±1.16 ^a	6.33±0.88 ^a	8.67±0.88 ^a
	Control	17.67±0.33 ^b	16.67±0.88 ^c	16.33±0.88 ^c
Water extract of <i>N. lotus</i> Leaves	100 mg/ml	9.33±0.33 ^{ab}	20.00±0.58 ^d	15.33±0.67 ^c
	50 mg/ml	9.00±0.58 ^{ab}	15.00±0.58 ^{bc}	10.67±0.67 ^b
	25 mg/ml	8.00±0.58 ^a	13.00±0.58 ^b	9.00±0.58 ^{ab}
	12.50 mg/ml	10.67±0.67 ^b	8.00±0.58 ^a	8.00±0.58 ^a
	Control	17.67±0.33 ^c	16.67±0.88 ^c	16.33±0.88 ^c
N-hexane extract of <i>N. lotus</i> Leaves	100 mg/ml	9.00±0.58 ^b	14.67±0.67 ^c	14.00±0.58 ^d
	50 mg/ml	8.00±1.16 ^b	9.67±0.33 ^b	11.33±0.33 ^c
	25 mg/ml	0.00±0.00 ^a	8.00±0.58 ^b	8.33±0.88 ^b
	12.50 mg/ml	0.00±0.00 ^a	6.00±0.58 ^a	0.00±0.00 ^a
	Control	17.67±0.33 ^c	16.67±0.88 ^d	16.33±0.88 ^e

Key: Positive control- ciprofloxacin (0.63 mg/ml)

Values are means ± SEM (Standard error of mean) of triplicates, values in the same row carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$

Table 9.3. Sensitivity pattern of bacterial isolates to extracts of *N. lotus* stem

Extracts	Concentration	Mean zone of inhibition (mm)	
		<i>E. coli</i>	<i>Salmonella</i>
Ethanol extract of <i>N. lotus</i> Stem	100 mg/ml	21.00±0.58 ^d	20.00±1.16 ^d
	50 mg/ml	19.00±0.58 ^c	16.67±0.67 ^c
	25 mg/ml	14.33±0.33 ^b	13.00±0.58 ^b
	12.50 mg/ml	11.00±0.58 ^a	9.00±0.58 ^a
	Control	17.67±0.33 ^c	16.67±0.88 ^c
Water extract of <i>N. lotus</i> Stem	100 mg/ml	9.00±0.58 ^b	15.00±0.58 ^c
	50 mg/ml	10.00±1.16 ^b	9.67±0.33 ^b
	25 mg/ml	8.00±0.58 ^{ab}	8.00±0.58 ^{ab}
	12.50 mg/ml	6.00±0.58 ^a	6.33±0.88 ^a
	Control	17.67±0.33 ^c	16.67±0.88 ^c
N-hexane extract of <i>N. lotus</i> Stem	100 mg/ml	7.00±0.58 ^b	12.67±0.67 ^c
	50 mg/ml	5.00±0.58 ^a	10.00±0.58 ^b
	25 mg/ml	5.00±0.00 ^a	10.67±0.88 ^{bc}
	12.50 mg/ml	5.33±0.33 ^a	7.33±0.67 ^a
	Control	17.67±0.33 ^c	16.67±0.88 ^d

Key: Positive control- ciprofloxacin (0.63 mg/ml)

Values are means ± SEM (Standard error of mean) of triplicates, values in the same row carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$

Table 10. Minimum Inhibitory Concentration (MIC) of ethanol extracts of *N lotus* root and water extracts of *A sativum* to enteric bacteria isolates

Isolates	RE (mg/ml)	RW (mg/ml)	RN (mg/ml)	LE (mg/ml)	LW (mg/ml)	LN (mg/ml)	SE (mg/ml)	SW (mg/ml)	SN (mg/ml)	
<i>Escherichia coli</i>	10	10	10	10	10	10	10	20	20	40
<i>Salmonella</i>	10	10	10	10	10	10	10	10	20	20
<i>Shigella</i>	10	10	10	10	10	10	10	20	20	20

Key: RE- Ethanol extract of *N. lotus* Root, RW- Water extract of *N. lotus* Root, RN- N-hexane extract of *N. lotus* Root, LE- Ethanol extract of *N. lotus* Leaves, LW- Water extract of *N. lotus* Leaves, LN- N-hexane extract of *N. lotus* Leaves, SE- Ethanol extract of *N. lotus* Stem, SW- Water extract of *N. lotus* Stem and SN- N-hexane extract of *N. lotus* Stem

Table 11. Minimum Bactericidal Concentration (MBC) of ethanol extracts of *N. lotus* root to enteric bacteria isolates

Isolates	RE (mg/ml)	RW (mg/ml)	RN (mg/ml)	LE (mg/ml)	LW (mg/ml)	LN (mg/ml)	SE (mg/ml)	SW (mg/ml)	SN (mg/ml)
<i>Escherichia coli</i>	20	20	20	20	20	20	40	40	80
<i>Salmonella</i>	20	20	20	20	20	40	40	40	40
<i>Shigella</i>	20	20	20	20	20	20	40	40	80

Key : RE- Ethanol extract of *N. lotus* Root, RW- Water extract of *N. lotus* Root, RN- N-hexane extract of *N. lotus* Root, LE- Ethanol extract of *N. lotus* Leaves, LW- Water extract of *N. lotus* Leaves, LN- N-hexane extract of *N. lotus* Leaves, SE- Ethanol extract of *N. lotus* Stem, SW- Water extract of *N. lotus* Stem and SN- N-hexane extract of *N. lotus* Stem

Table 12. Molecular identification of resistant enteric bacteria isolates from Ogbese river water samples

Description of isolates	Max Score	Total Score	Query Cover	% Identity	Accession Number
<i>Salmonella enterica</i> subsp. <i>enterica</i> strain Ty2	1792	1792	99%	95.75%	NR_074799.1

The high colony counts of *Salmonella* recorded in week four which is during dry season may be due to activity of cattle and their herdsmen in the river during dry season in search for water. It was noted during this study that the cattle defecated into the river as they went in search of water and it has been reported that cow faeces is a reservoir for *Salmonella* [40] This is in agreement with the findings of Liu et al. [41] in which they reported the presence and persistence of salmonella in water.

The high occurrence of *Shigella* in water samples from River Ogbese may be due to *Shigella* ability to grow with or without oxygen which make them survive all seasons [42]. This is related to the findings of Franca et al. [43] in which they reported the high incidence of Shigellosis among Peruvian soldiers deployed in the Amazon River basin.

High level of turbidity and phosphate of water samples from River Ogbese makes the river water not suitable for drinking and domestic use [44]. Choi and Kweon [45] reported similar findings in which they related the impacts of highly turbid water on microfiltration with coagulation pretreatment. The high load of enteric bacteria in the water samples may be attributed to phosphate level of the river water samples which has the ability to stimulate the growth of pathogenic bacteria [46]. The highest value of turbidity was recorded within week five to sixteen (wet season), this may be due to farm land, fish farm, roof surface, land surface, domestic and industrial runoff into the river at the beginning of the year's rainfall. This is in accordance with Ajibade et al. [47] findings of water quality parameters in the major rivers of Kainji Lake National Park, Nigeria in which they recorded highest value for turbidity and nitrate in wet season.

Water extract of *N. lotus* stem having the highest percentage yield of *N. lotus* extracts is in contrast with the findings of Wankupar et al. [48] in which methanol extracts of *Scoparia dulcis* had the highest percentage yield.

The presence of phytochemical compounds in *N. lotus* extracts is related to the findings of Toryali et al. [49] and Adalakun et al. [50] in which they reported the presence of phytochemical compounds in *N. lotus* extracts. The absence of phlobatannin and alkaloid in all the extracts is in accordance with the findings of Toryali et al. [49] in which the absence of Phlobatannin. This is

related to Adalakun et al. [50] findings in which they reported the absence of Phlobatannin.

The high mean zone of inhibition of Ciprofloxacin recorded against most of the bacteria isolates is related to the findings of Waleed et al. [51] in which ciprofloxacin had the highest zone of inhibition and inhibited most of the bacteria pathogens recovered from the hands and mobile phones of university students. The isolated bacteria having the highest total percentage resistance antibiotics resistance pattern to Augmentin maybe caused due to initial exposure of the bacteria to these antibiotics due to self-medication i.e abuse of well-known antibiotics such as augmentin, chloramphenicol and amoxicillin which act is common among people living in rural communities. This is also related with the findings of Waleed et al. [51] in which they reported resistance pattern of some gram negative bacteria to chloramphenicol and amoxicillin.

The antimicrobial susceptibility patterns of extracts of *N. lotus* to the isolated enteric bacteria shows that ethanol extract of *N. lotus* root inhibited all isolates at a reasonable length of zone of inhibition invitro. Ethanol extract of *N. lotus* root was recorded to have the highest mean zone of inhibition. This is related to Akinjogunla et al. [52] findings in which they reported that *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were highly susceptible to ethanol extract of *N. lotus* leaf compare with commercially available antibiotics *in vitro*. *N. lotus* extracts had a lower Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) to the isolated bacteria which indicates these extracts can inhibit the growth of enteric bacteria at a lower concentration. This is related to Akinjogunla et al. [52] findings in which they reported a lower MIC and MBC of *N. lotus* extract at 10-30 mg/ml against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively.

5. CONCLUSION

This study revealed that the coliform counts in water samples from River Ogbese is higher than the recommended number of coliforms in table water by World Health Organization in WHO Guidelines for drinking-water quality, which

makes the river not suitable for drinking and domestic use. The physicochemical characteristics of the river water samples revealed the river has turbidity which is not safe for drinking or domestic use.

The findings from this study showed that N-hexane solvent had the highest yield of *N. lotus* extracts. The phytochemical study reveals *N. lotus* extracts had numerous phytochemicals that can serve as antimicrobial agent.

Commercially available antibiotics were not able to inhibit some of the isolated enteric bacteria from the river *in vitro*. The extracts of *N. lotus* were able to inhibit these enteric bacteria effectively. Ethanol extract of *N. lotus* had the highest zone of inhibition *in vitro*. *N. lotus* root extracts recorded a low Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the resistant enteric bacterial isolates.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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