



Phytochemical and Antibacterial Properties of *Andrographis paniculata* (Burn.F.) on Enteric Bacteria Isolated from a Surface Water in Owena, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Adewale Olalemi and Ayodele Ogundare managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at determining the antibacterial activities of *Andrographis paniculata* on methicillin and vancomycin resistant enteric bacteria isolated from River Owena, Owena, Nigeria. Water samples were collected weekly from River Owena over a period of six weeks (n = 6). The bacteriological evaluation of the water samples was carried out using standard microbiological method. *A. paniculata* leaf and stem extracts were prepared using the polar (i.e., methanol) and nonpolar (i.e., n-hexane) solvents. The phytochemical constituents and antibacterial activity of all the extracts of *A. paniculata* leaf and stem were investigated using standard methods. Results revealed that methanol extracts from *A. paniculata* contained the maximum amount of phytochemicals when compared to that of the n-hexane extracts. *Salmonella* and *Shigella* were both resistant to vancomycin, while they were only susceptible to methicillin once throughout the sampling week. The methanol extracts from *A. paniculata* showed higher antibacterial activity

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against the targeted bacterial isolates of enteric origin which include *E. coli*, faecal coliforms, *Shigella*, *Salmonella* and intestinal enterococci. The methanol leaf extract of *A. paniculata* showed the maximum antibacterial activity against *E. coli*, *Salmonella*, *Shigella*, intestinal enterococci and faecal coliform with zones of inhibition 29.0 mm, 26.00 mm, 21.3 mm, 21 mm and 19 mm respectively. The methanolic stem extract of *A. paniculata* showed a maximum antibacterial activity against *Salmonella* with zone of inhibition of 25 mm while n-hexane stem extract exhibited a maximum zone inhibition of 17.6 mm against *Salmonella*. The minimum inhibitory concentration (MIC) value for all the *A. paniculata* extracts was at a concentration of 12.5 – 50 ml. The pH of the water ranged from 6.50 to 6.90, temperature ranged from 26 to 31°C. The findings from this study demonstrated that River Owena is highly contaminated with faecal materials and that leaf and stem extracts of *A. paniculata* may be used as antibacterial agents against methicillin and vancomycin resistant bacteria of enteric origin.

Keywords: *Phytochemical properties; antibacterial properties; Andrographis paniculata; enteric bacteria; surface water.*

1. INTRODUCTION

Plants remain the natural and undisputable reservoir of antibacterial agents, the quest for scientific validation and development of new drugs or therapeutic combinations from yet unexplored plants used in traditional pharmacopoeia remains very imperative due to resurgent problems of resistance, affordability, and efficacy [1]. The drugs already in use to treat infectious bacterial diseases are of concern because drug safety remains a huge global issue. Almost all synthetic drugs have side effects and microbes are also developing resistance to these synthetic drugs. To alleviate this problem, antimicrobial compounds from potential plants are constantly being investigated [1]. The drugs from plants have fewer side effects, less toxic, scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [1]. Plant initially produces their phytochemical compounds to protect themselves from pathogens and predator [2]. However, studies have demonstrated that these phytochemical constituents produced by plant are able to exhibit some biological activities such as, antiperiodic, antibacterial, antitumor, antidiabetic, antithrombotic, anti-inflammatory, antifeedant and antiviral [3]. Antimicrobial resistance (AMR), especially multidrug-resistance, is posing a great threat to public health [4]. Every year, around 700,000 people die from AMR infections globally and this number is estimated to reach 10 million by 2050 [5]. The misuse and overuse of antimicrobial agents in humans and animals provide favourable conditions for the acceleration of the selection, spread and persistence of AMR bacteria [6]. The

increase of bacterial resistance to the common use of antibiotic increases the demand to search for new, potential and alternative active compound in plants to treat bacterial infection caused by antibiotic resistant bacteria [7]. One of the famous medicinal plant is *Andrographis paniculata* which is called "Hempedu bumi" in Malaysia. Many researchers in India, Thailand and other countries are keen to study the antibacterial and antioxidant activities of *A. paniculata*. However, their results are not confidently consistent because the phytochemical compounds present in the plant may change as a result of geographical variation and growth environment of *A. paniculata* [8].

The disease burden from poor water quality, sanitation, and hygiene is estimated to be responsible for up to 4% of all deaths worldwide [9,10]. Cultivation of standard faecal indicator bacteria, such as *E. coli* and enterococci, are typically used according to certified standard procedures [11,12].

Several terms have been used to describe the organisms used in monitoring the microbiological quality of water, including 'microbial indicator', 'index organism', 'faecal indicator' and 'surrogate organism' [13,14]. *Escherichia coli*, faecal coliforms and intestinal enterococci represent the classic bacterial indicators of the presence of faecal pollution (faecal indicator bacteria) in aquatic environment. Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes [15]. However, inadequate sanitation and

persistent faecal contamination of water sources is responsible for a large percentage of people in low- and middle-income countries not having access to microbiologically safe drinking water and at risk of diarrhoeal diseases [16,17]. Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation [18-21].

Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption. In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes. These unprotected water sources may be contaminated with pathogens through run-offs after rain or storm events, agricultural inputs, sewage effluents and faeces from wild life that may render the water sources unacceptable for human consumption [22,23].

This study was aimed at determining the phytochemical and antibacterial properties of *Andrographis Paniculata* (Burn. F.) on enteric bacteria isolated from River Owena, Owena, Nigeria. The objectives of this study were to investigate the occurrence and distribution pattern of indicator bacteria and pathogens of enteric origin in River Owena; determine the physicochemical properties of the water sample from River Owena; examine the methicillin and vancomycin resistance profile of the isolated organisms; assess the antibacterial potency of the leaf and stem extracts of *Andrographis paniculata* against resistant bacteria isolates; and determine the phytochemical constituents of the leaf and stem extracts of *Andrographis paniculata*.

2. MATERIALS AND METHODS

2.1 Sampling Site and Collection of Samples

River Owena is located about four kilometers from Joseph Ayo Babalola University Ikeji Arakeji along Ilesha-Akure express way in Oriade Local Government Area of Osun State, Nigeria on latitude N 7.403135 and longitude E 5.014589 (Fig. 1). It is a fresh water and free-flowing during raining season but slow-moving at the onset of dry season. Water from River Owena is used for domestic purposes especially by the people

living near and close to the river. Human activities on the river include the use of the water for cement molding, car wash, and commercial water supply to people. Along the bank of the river are farms with crops such as maize, sugar cane and vegetables such as spinach and pepper plantation. The river also serves as recreational swimming pool for small children from nearby primary and secondary schools. Various types of birds are always around the river.

Water samples were collected from River Owena for a period of 6 weeks from April to May, 2018. On each sampling occasion, water samples were collected aseptically in sterile 500 ml Duran Schott glass bottles from two different sampling points by directly dipping the bottles into the water at about 10 – 12 cm depth. The samples were labelled properly and transported to the laboratory inside an ice pack for analysis within one hour.

2.2 Isolation and Enumeration of Enteric Bacteria in Water Samples from River Owena

Aliquots of the water samples were used for selective isolation of enteric bacteria using standard microbiological procedures. The concentrations of *E. coli*, faecal coliforms, *Salmonella*, *Shigella* and intestinal enterococci in waters samples from River Owena were determined using the membrane filtration method [24,25]. The membrane filters were placed on freshly prepared selective media (m-FC, m-Ent, MLSA and SSA). Agar plates were incubated at 37°C for 48 h (m-Ent), 37°C for 24 h (MLSA) and 44°C for 24 h (m-FC, SSA), and colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml of water.

Serial dilution of each of the collected water sample was carried out to 5 dilution factor. These were then aseptically placed on plates with appropriate selective media ensuring that no air bubbles were trapped. The selective media used was *Salmonella – Shigella* agar and Eosin Methylene Blue agar. All the media was prepared according to the manufacturers' instructions. Each sample was analysed in triplicate. Water from River Owena was serially diluted and 1 ml of the 5 fold serial dilutions was poured into a sterile petri dish. The plates were allowed to set and were incubated in an inverted position at appropriate temperature and duration. The direct count of colonies was carried out and result were

expressed as colony forming unit (CFU) per 100 ml. Agar slant technique was employed in preservation of the culture by preparing double strength Nutrient agar in McCartney bottles, allowed to cool and set in sloping position. An inoculating loop was sterilized and used to transfer a loopful of the colony to the surface of the slope agar slant. This was then incubated at 37°C for 24 hours after which it was refrigerated at 4°C until when required [26].

2.3 Collection, Processing and Preparation of Leaf and Stem Extracts from *Andrographis paniculata*

Fresh leaves and stem of *Andrographis paniculata* were collected from a farm from Akure, Ondo State, Nigeria where they were growing naturally. The plant material was identified and authenticated at the Crop, Soil and Pest Department, Federal University of Technology, Akure, Nigeria. The method of Leonard et al. [27] was employed. The fresh leaf and stem of *Andrographis paniculata* were air dried for four weeks until fully crispy before crushing it with hands. The crushed leaf and stem were macerated using clean mortar and pestle, then pulverized into fine powder by blending machine. They were separately kept in an airtight container to avoid the absorption of moisture. The powdered samples were soaked in 70% methanol, n-hexane in the ratio 1:10 each (250 g of the powdered sample in 2500 millimeter of 70% methanol and N-hexane respectively) as solvents to extract the bioactive compounds. Each container was labeled appropriately and left for 72 hours (3 days) at room temperature with agitations at intervals.

The sieving of the mixture was carried using muslin cloth, then filtered through a Whatman (No. 1) filter paper and was concentrated *en vacuo* using rotary evaporator. The methanolic, N-hexane extract was preserved in a sterile bottle at 4°C ready for use.

2.4 Determination of the Physicochemical Characteristics of Water Samples from River Owena

The physicochemical properties of the water from River Owena were measured using standard methods [28]. These include temperature (Celsius), pH, electrical conductivity (micro Siemens per centimetre), alkalinity (milligrams per litre), turbidity (nephelometric turbidity units),

total dissolved solids (milligrams per litre), dissolved oxygen (milligrams per litre) and salinity (parts per thousand).

2.5 Phytochemical Components of Stem and Leaf Extracts of *Andrographis paniculata*

The phytochemical components of stem and leaf extracts of *Andrographis paniculata* were determined quantitatively and qualitative using standard methods.

2.5.1 Qualitative determination of phytochemical components of *A. paniculata*

The leaf and stem extract of *A. paniculata* were tested for the presence of different phytochemicals such as alkaloids, saponin, tannin, phlobatanin, anthraquinone, flavonoid, steroids, terpenoid and cardiac glycosides using standard methods [29].

2.5.2 Quantitative determination of phytochemical components of *A. paniculata*

The leaf and stem extracts of *A. paniculata* were tested for the amount of different phytochemicals such as alkaloids, saponin, tannin, phlobatanin, anthraquinone, flavonoid, steroids, terpenoid and cardiac glycosides using standard methods [30].

2.6 Antibiotics Sensitivity Test using Methicillin and Vancomycin

Antibiotics sensitivity tests was performed on the isolates using discs diffusion technique as described by Clinical Laboratory Standard Institute [31]. The antibiotics used in this study were methicillin and vancomycin. These antibiotics were selected because they are used in both human medicine and animal veterinary practices and studies have demonstrated that some enteric bacteria exhibit resistance to methicillin and vancomycin [32].

2.7 Determination of Antibacterial Potency of the Leaf and Stem Extracts of *Andrographis paniculata* Against Bacterial Isolates

Each of the extracts (methanol and N-hexane) was reconstituted using 0.01% Tween 20 as described by Onifade [33]. This was done by dissolving 0.5 g of the extract in 10 ml 0.01%

Tween 20. The resultant solution was filtered using sterile Millipore membrane filter (0.45 µm). The method described by Chukwuka and Uka [34] was employed in preparing the standard inoculums of the clinical isolates for *in vitro* assay. Overnight colonies were transferred to a tube of sterile saline. The bacterial suspension was compared to the 0.5 McFarland standards against a sheet of white paper on which black lines were drawn. The bacterial suspension was adjusted to the proper density as the 0.5 McFarland by adding sterile saline or more bacterial growth. Then bacterial suspension was diluted to obtain 10⁶ cfu/ml.

To determine the sensitivity of the bacterial isolates to the leaf extracts of *Andrographis paniculata* the method of NCCLS [35] was adopted. About 0.1 ml of the bacterial suspension was drawn out with the aid of a sterile pipette and was aseptically introduced into sterile petri dishes. Sterilized Mueller Hinton Agar (MHA, Difco, USA) that had been cooled to about 45°C was aseptically poured into the petri dishes containing 0.1 ml of the bacterial isolates; each petri dish was gently swirled in a clockwise direction in order to ensure that the bacterium is homogeneously distributed with MHA. The plates were then allowed to stand for 40 minutes for the inoculated bacteria to be established in the medium. After 40 min, four wells each for clinical isolates were aseptically bored on each agar plate using a sterile cork borer (6 mm) at allowance of 30 mm between opposite wells and

the edges of the petri dishes. 0.15 ml of each reconstituted the extracts was then introduced into each well in the plates using sterile pipette. One out of the four wells bored was used for negative control, 0.1 ml of the reconstituting agent (Tween 20) was used. The plates were incubated at 37°C for 24 hours. The resulting zones of inhibition were measured using a transparent meter rule. The experiment was done in triplicates and the average reading was taken to be the zone of inhibition of the test bacteria.

2.7.1 Determination of Minimum Inhibitory Concentration (MIC) of leaf and stem extracts of *Andrographis paniculata*

The minimum inhibitory concentration of an antimicrobial agent is the least concentration that will inhibit the growth of a particular organism [36]. The test organisms were inoculated by pour plating. Exactly 1 ml of a 3-5 hours broth culture was poured into a sterile petri dishes; after which 15 ml of the prepared agar was poured on the plate containing the culture and allowed to set. Four holes were made on each plate aseptically using 10 mm diameter sterile cork bore. Extract concentration of 25 mg/ml were poured carefully into the labeled holes. A 30% DMSO was filled into the fourth hole serving as a control. All the plates were incubated into the incubator at 37°C for 24 hours. After incubation; zone of inhibition were observed and the lowest concentration that could inhibit the growth of the test organism

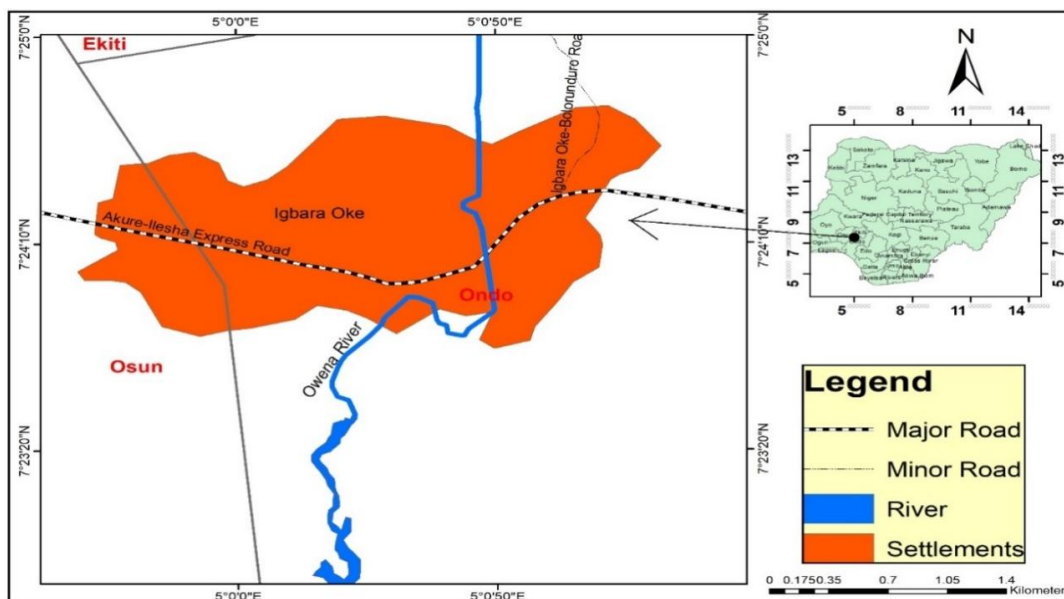


Fig. 1. Location of River Owena along Ilesha-Akure express way in Oriade Local Government Area of Osun State, Nigeria

indicates the minimum inhibitory concentration (MIC).

2.8 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) was carried out to determine whether there were significant differences in the treatments. The treatments were separated by least significance difference (LSD) at $P \geq 0.05$ level.

3. RESULTS

3.1 Detection of the Enteric Bacteria in Water Samples from River Owena

The concentration of intestinal enterococci in River Owena over the study period ranged from 6.1×10^2 to 1.5×10^4 cfu/100 ml. The lowest count occurred in sample collected in the sixth week while the highest count was on the first week. The mean concentration was 8683.33 cfu/100 ml. The concentration of *Salmonella* in Owena river over the study period ranged from 2×10^3 to 4×10^4 cfu/100 ml. The lowest count occurred in sample collected in the sixth week while the highest count was on the first week. The mean concentration was 8450 cfu/100 ml. The concentration of *Shigella* in Owena river over the study period ranged from 2×10^4 to 5.4×10^4 cfu/100 ml. The lowest count occurred in sample collected in the third week while the highest count was on the fourth week. The mean concentration was 37000 cfu/100 ml. The concentration of *E. coli* in Owena river over the study period ranged from 2×10^3 to 4×10^4 cfu/100 ml. The lowest count occurred in sample collected in the first week while the highest count was on the second week. The mean concentration was 12600 cfu/100 ml. The concentration of faecal coliform in Owena river over the study period ranged from 2.1×10^2 to 6×10^4 cfu/100 ml. The lowest count occurred in sample collected in the fifth and sixth week while the highest count was on the second week. The mean concentration was 33050 cfu/100 ml (Fig. 2).

3.2 Physicochemical Characteristics of Water Samples from River Owena

The pH value ranged from 6.5 to 6.9, with the least acidic recorded on the third week and most acidic on the first week. The turbidity level of the water sample ranged from 2.60 to 7.70 (NTU),

the water is less turbid on the second week and most turbid on the first week. Temperature value of the water samples ranged from 26°C to 31°C, with the lowest value recorded on the fifth week and the highest value at week two. The salt level of the water sample ranged from 9.57 to 15.95 mg/l, the salt level of the water is highest on the fifth week and lesser on the second week. The conductivity of the water sample ranged from 96.00 to 165.00 $\mu\text{s}/\text{cm}$, the water showed the highest level of conductivity on the fifth week and lesser on the first week. The dissolved oxygen of the water sample ranged from 6.10 to 6.84 mg/l, the water showed the highest level of dissolved oxygen on the first week and lesser on the second week. The total dissolved solid of the water sample ranged from 48.00 to 82.50 mg/l, the TDS of the water was highest on the fifth week and lesser on the first week (Table 1).

3.3 Phytochemical Constituents of Leaf and Stem Extracts of *Andrographis paniculata*

The yield of the extracts of *A. paniculata* showed that methanol extracts of the leaf had the highest weight (Table 2). Qualitative determination of the phytochemical constituents of the leaf extract revealed the presence of alkaloids, saponins, tannins, flavonoid, steroid, terpenoid and cardiac glycosides. Most of the constituents tested positive (+) i.e., were present in the methanol leaf and stem extracts, except anthraquinone and phlobatanin which recorded negative (-ve) (i.e., absent in the extracts) while most were absent in the n-hexane leaf extract except flavonoid. Most of the constituents of cardiac glycosides were present in all the extracts except liberman which was absent (Table 3). The methanol extract has the highest quantity of glycoside at 11.83 mg/ml, while flavonoid has the least quantity of 6.51 mg/ml (Table 4).

Qualitative determination of the phytochemical constituents of the stem extract revealed the presence of alkaloids, saponins, tannins, flavonoid, steroid, terpenoid and cardiac glycosides. Most of the constituents tested positive (+) i.e were present in the methanol leaf extract, except anthraquinone and phlobatanin which recorded negative (-ve)(i.e absent in the extracts) while most were absent in the n-hexane stem extract except flavonoid and glycosides. Most of the constituents of cardiac glycosides were present in all the extracts except liberman which was absent in the methanol stem extract (Table 5). The methanol extract has the highest quantity of glycoside of 11.88 mg/ml, while

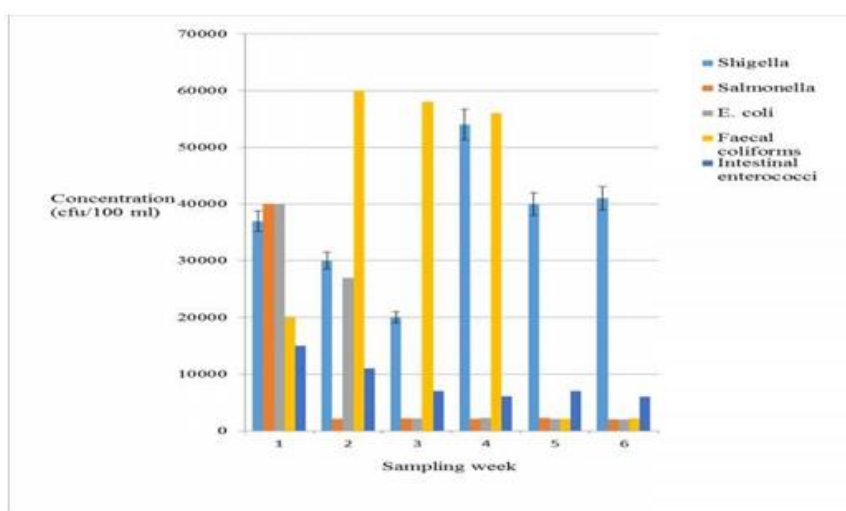


Fig. 2. The concentration of the bacteria sample throughout the sample week

Table 1. Physicochemical characteristics of water samples from River Owena

Physicochemical parameter	Mean ± standard deviation	Range (minimum- maximum)
pH	6.72±0.1	6.5 - 6.9
Turbidity (NTU)	5.95±2.4	2.60 - 7.70
Temperature (°C)	29±1.8	26 - 31
Salinity (mg/l)	12.22±2.2	9.57 - 15.95
Electrical conductivity (µs/cm)	119.83±24.1	96 – 165
Dissolved oxygen (mg/ml)	6.44±0.3	6.10 – 6.84
Total dissolved solids (mg/l)	64±13.9	48.00 – 82.50

¹ Key: n (total number of samples) = 6

glycoside has the least quantity of 6.51 mg/ml using n- hexane as solvent (Table 6).

3.4 Antibacterial Effect of Methicillin and Vancomycin on Enteric Bacteria Isolated from River Owena

The result of methicillin and vancomycin activities on enteric bacteria isolated from River Owena shows that intestinal enterococci, *E. coli* and faecal coliforms were susceptible to vancomycin with a zone of inhibition ranging from 16 mm to 40 mm, with faecal coliforms having the highest zone of inhibition. While *Shigella* and *Salmonella* were resistant to vancomycin. However, all of the organisms were susceptible to methicillin, with faecal coliforms showing the highest zone of inhibition of 36 mm and *Shigella* had the lowest zone of inhibition of 10 mm. (Fig. 3a and b).

3.5 The Result of the Antibacterial Activity of the Leaf and Stem Extracts of *A. paniculata* on Bacterial Isolates using n-hexane

The antibacterial activity of the leaf and stem extract of *A. paniculata* using n-hexane as

solvent against intestinal enterococci showed that the leaf extract has the highest zone of inhibition of 14 mm and stem extract was 13 mm (Fig. 4a & b). Fig. 5a & b in the same way shows the antibacterial activity of the leaf and stem extract of *A. paniculata* using n-hexane as solvent against *Shigella*. The stem has the highest zone of inhibition of 16 mm and leaf shows the lowest at 15 mm.

The antibacterial activity of the leaf and stem extract of *A. paniculata* using n-hexane as solvent against faecal coliform revealed that the leaf has the highest zone of inhibition of 12 mm and stem shows the lowest at 11 mm (Fig. 6a & b). Fig. 7a & b in the same way shows the antibacterial activity of the leaf and stem extract of *A. paniculata* using n-hexane as solvent against *E. coli*. The stem has the highest zone of inhibition of 18 mm and leaf shows the lowest at 15.2 mm. The antibacterial activity of the leaf and stem extract of *A. paniculata* using n-hexane as solvent against *Salmonella* showed that the stem has the highest zone of inhibition of 17 mm and leaf shows the lowest at 14 mm (Fig. 8a & b).

Table 2. The yield of the leaf and stem extract of *A. paniculata*

Solvent	Original weight(g)	Extracted weight(g)
A. Methanol	250	20.6
I. Methanol	250	19.3
B. N-hexane	250	4.0
II. N-hexane	250	3.6

Legends: A, B = *Andrographis paniculata* leaf extracts, and I, II = *Andrographis paniculata* stem extracts, WA = weight of extracts after extraction, IW = Original weight of plant in powder

Table 3. Qualitative phytochemical constituents of leaf extract of *A. paniculata* (Positive '+' Negative '-')

Phytochemicals	Methanol	N-hexane
Saponin	+	-
Tannin	++	-
Phlobatannin	-	-
Flavonoid	++	-
Steroid	++	-
Terpenoid	+	-
Alkaloid	++	-
Anthraquinone	-	-
Starch	+++	-
Cardiac glycoside		
Legal test	+	+
Keller kilian test	+	+
Salkowski test	+	+
Lieberman test	-	-

3.5.1 The result of the antibacterial activity of leaf and stem extracts of *A. paniculata* against all the enteric bacteria isolated from River Owena using methanol

The antibacterial activity of the leaf and stem extract of *A. paniculata* using methanol as solvent against intestinal enterococci showed that the leaf extract has the highest zone of inhibition of 22 mm and stem extract was 17.5 mm (Fig. 9a & b). The antibacterial activity of the stem and leaf extract of *A. paniculata* using methanol as solvent against *E.coli* revealed that the leaf extract has the highest zone of inhibition

of 29 mm while the stem has the lowest zone of inhibition of 20 mm (Fig. 10a & b). This result revealed that the leaf and stem extract of *A. paniculata* using methanol as solvent against *Salmonella* leaf extract has the highest zone of inhibition of 27 mm and the stem was 25 mm (Fig. 11a & b).

The antibacterial activity of the leaf and stem extract of *A. paniculata* using methanol as solvent against faecal coliforms showed that the leaf extract has the highest zone of inhibition of 19 mm while the stem extract has the lowest zone of 17 mm (Figure 12a & b). Figure 13 a & b shows the antibacterial activity of the leaf and stem extract of *A. paniculata* using methanol as solvent against *Shigella*. The leaf extract has the highest zone of inhibition of 21 mm while the stem was 19 mm.

3.6 Minimum Inhibitory Concentrations (mg/ml) of Methanol and n-hexane Leaf and Stem Extracts of *A. paniculata* on Enteric Bacteria

The minimum inhibitory concentration (MIC) of the leaf extract on the organisms as shown on Table 7 revealed that MIC of methanolic extract ranged from 12.5 mg/ml to 25 mg/ml. The lowest value of 12.5 mg/ml was recorded against *E. coli*, *Shigella*, faecal coliforms and *Salmonella*. Followed by intestinal enterococci (12.5 mg/ml). MIC of n-hexane extract ranged from (25 mg/ml to 50 mg/ml). Intestinal enterococci, faecal coliforms and *E. coli* has the lowest MIC (6.25mg/ml), followed by *Shigella* and *Salmonella* to which the highest MIC (25 mg/ml) was recorded against.

The minimum inhibitory concentration (MIC) of the stem extract on the organisms as shown on Table 8 revealed that MIC of methanolic extract ranged from 12.5 mg/ml to 25 mg/ml. The lowest value of 12.5 mg/ml was recorded against *Salmonella*, followed by intestinal enterococci, *E. coli*, *Shigella*, faecal coliforms (25 mg/ml). The MIC of n-hexane stem extract ranged from (25 mg/ml to 50 mg/ml). Intestinal enterococci,

Table 4. Quantitative phytochemical constituent (mg/ml) of leaf extract of *A. paniculata*

Phytochemicals (mg/ml)	N-hexane		Methanol	
Tanin	-----	-----	9.58304	9.58304
Flavonoid	9.61988	9.27867	6.51180	7.09627
Terpenoid	-----	-----	14.01596	13.96276
Glycosides	8.21809	-----	11.83511	11.88833
Saponin	-----	-----	11.63636	11.88834

and *E. coli* has the lowest MIC (25 mg/ml), followed by *Shigella*, *E.coli* and *Salmonella* to which the highest MIC (50 mg/ml) was recorded against.

Table 5. Qualitative phytochemical constituent of stem extract of *A. paniculata* (Positive '+' Negative '-')

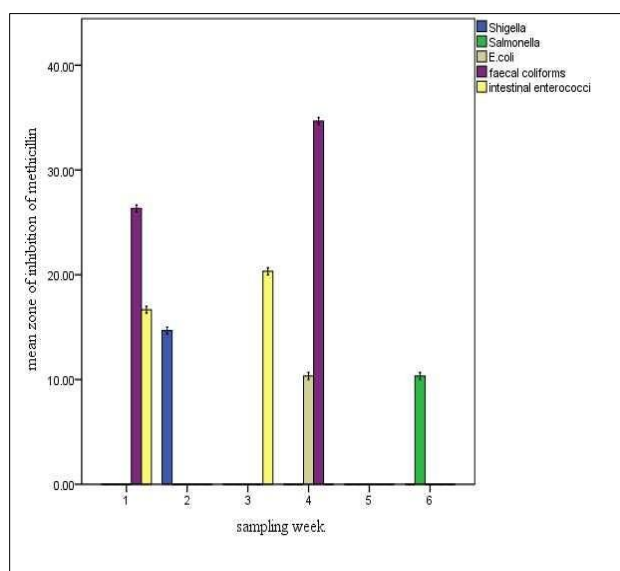
Phytochemicals	Methanol	N-hexane
Saponin	+	-
Tannin	++	-
Phlobatannin	-	-
Flavonoid	+	+
Steroid	+	-
Terpenoid	+	-
Alkaloid	++	-
Anthraquinone	-	-
Cardiac glycoside		
Legal test	+	+
Keller kilian test	+	+
Salkowski test	+	+
Lieberman test	-	+

4. DISCUSSION

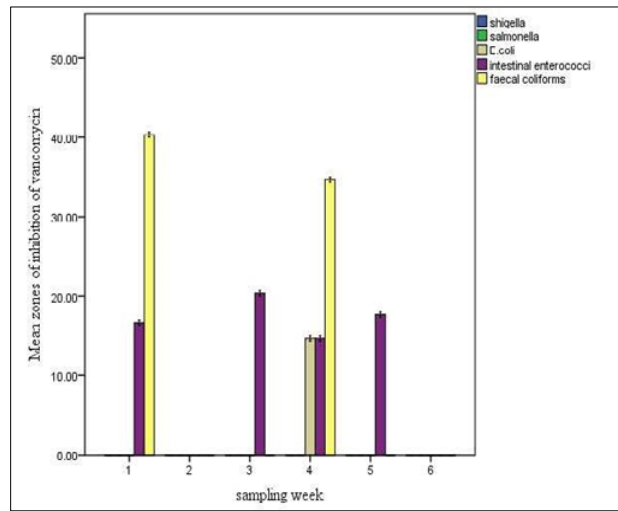
4.1 Detection of the Enteric Bacteria in Water Samples from River Owena

The high concentration of intestinal enterococci may be as a result of many locals defecating into the water and they are often seen bathing in the River. There are also farming activities going on in the area surrounding the sampling site. Organic and inorganic fertilizers are freely used.

The local people are frequently cooking, washing cooking utensils and plates in the sampling site. All these human activities may have contributed to the high concentration of intestinal enterococci in River Owena. This study demonstrates the influence of human activities on the microbial profile of a water body and it is accordance with the findings of Showell et al. [37] who stated that human activities tends to be more during wet season. Chicken is a major vehicle for the transmission of *Salmonella* and it is considered a major problem in the poultry industry [38]. The chicken breeding is a common practice around Owena River. Results from this study showed that there were high levels of *Salmonella* in the water samples from River Owena. These findings are in agreement with those reported by [39] in Gudu stream Abuja, Nigeria, where the authors observed that *Salmonella typhi* and *paratyphi* were among the prevalent bacteria isolated at the sampling site and [40] in Nairobi where the authors observed that *Salmonella* spp are also present in surface waters of Nairobi River respectively. This study reveals that contamination of River Owena is by humans and other animal sources through activities like bathing, farming, grazing and washing. This is not surprising because some members of the community carryout enormous farming activities near the sampling site; some of the farmers and residents defecate along the farm lands and they also use bare hands to pack animal manures used for fertilizer on the farm lands. There is high tendency that both the faecal matter from human and animals may be washed into the river during storm and flood events.



(a)



(b)

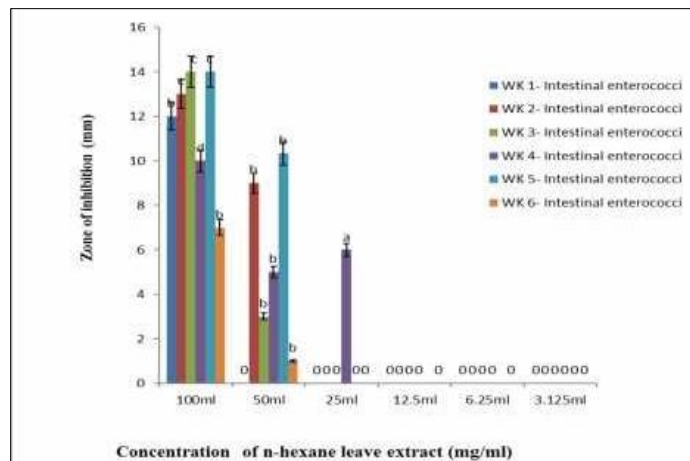
Fig. 3. (a)&(b) depicts the methicillin activities on enteric bacteria isolated from River Owena and vancomycin activities on enteric bacteria isolated from River Owena

Table 6. Quantitative phytochemical constituent (mg/ml) of stem extract of *A. paniculata*

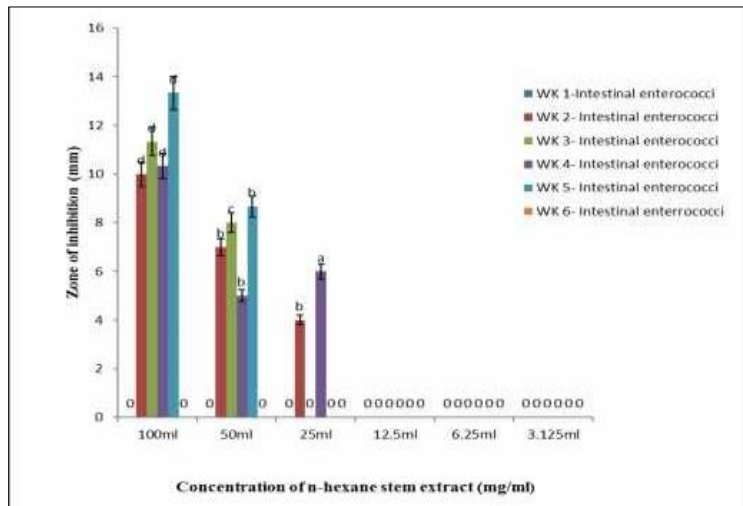
Phytochemicals (mg/ml)	N-hexane		Methanol	
Glycosides	6.67643	6.64332	11.83511	11.88833
Saponin	----	---	11.63636	11.63636
Tanin	----	-----	9.58304	9.58304
Flavonoid	11.54345	11.56432	7.11180	7.09627
Terpenoid	-----	-----	14.01595	13.96276

Table 7. The minimum inhibitory concentrations (mg/ml) of methanol and n-hexane leave extract of *A. paniculata* on test bacteria

Isolates	Methanol (mg/ml)	Hexane (mg/ml)
<i>Shigella</i>	12.5	50
Intestinal enterococci	25	25
<i>Escherichia coli</i>	12.5	25
<i>Salmonella</i>	12.5	50
Faecal coliforms	12.5	25



(a)

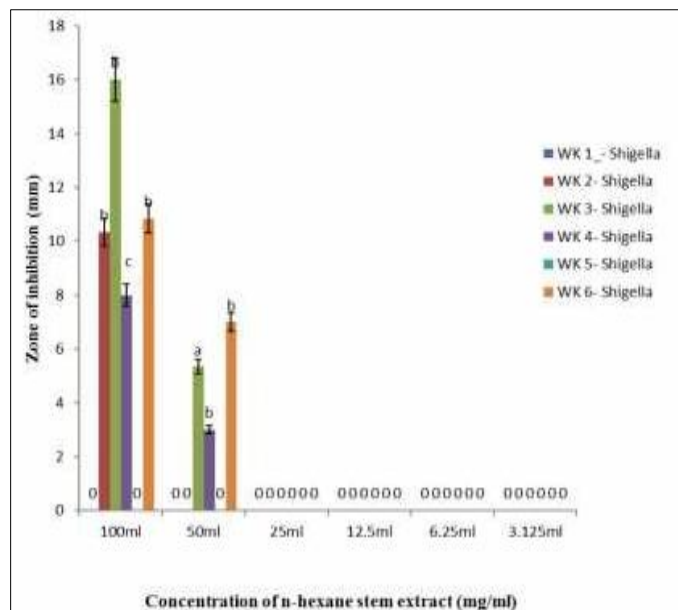


(b)

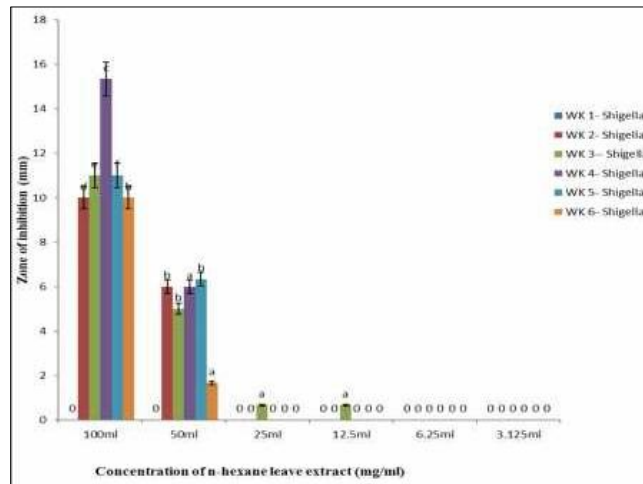
Fig. 4. (a)&(b) depicts the antibacterial activity of hexane leave extracts against intestinal enterococci and antibacterial activity of hexane stem extracts against intestinal enterococci
Key: Wk = Week

Table 8. The minimum inhibitory concentrations (mg/ml) of methanol and n-hexane stem extract of *A. paniculata* on test bacteria

Isolates	Methanol (mg/ml)	N-hexane (mg/ml)
<i>Shigella</i>	25	50
Intestinal enterococci	25	25
<i>Escherichia coli</i>	25	25
<i>Salmonella</i>	12.5	50
Faecal coliforms	25	50



(a)



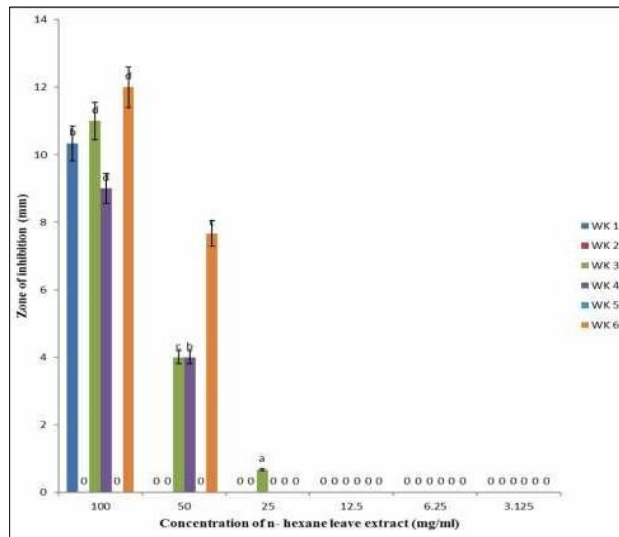
(b)

Fig. 5. (a)&(b) depicts the antibacterial activity of hexane stem extracts against *Shigella* and antibacterial activity of hexane leaf extracts against *Shigella*

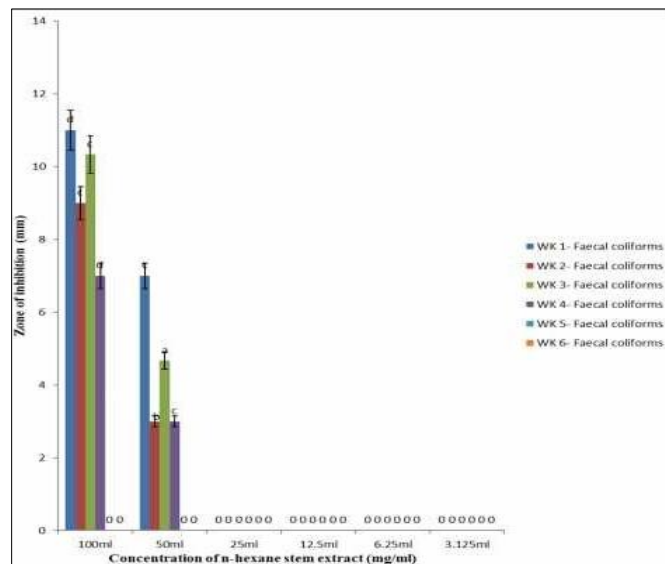
Key: *Wk* = Week

The presence of *Shigella* spp in these studies is high and most were resistant to vancomycin and methicillin this agrees with the study of [41]. According to their findings, *S. sonnei* isolated in stool and water samples has the same antibiotic-resistance profile in all specimens examined. The study also revealed that the outbreak of *Shigella* also took place in largely rural communities of more than 1000 inhabitant. Also there are seasonal variations in their appearance. In outbreak II, heavy rainfall was reported a few days before the outbreak and at the same time the work was being performed on water distribution system. Thus, a possible contamination of the network system from sewage or ground surface material was

suspected. The level of *E. coli* observed in this study are consistent with previous reports on the water quality of the River Owena [42], high *E. coli* counts were detected in the present study, which is evidence for widespread faecal contamination within the watershed. The *E. coli* counts of the water samples exceeded the EPA threshold for recreational activities such as swimming which is 235 cfu/100 ml based on one-time measurement [43]. Multidrug resistance patterns have been also detected in *E. coli* isolates isolated from river water in Osun State, Nigeria [44] and from the holy city of Mathura, India [45]. In this study it was noted that *E. coli* showed a lot of resistance to methicillin and vancomycin except for the sample collected on the fourth week.



(a)



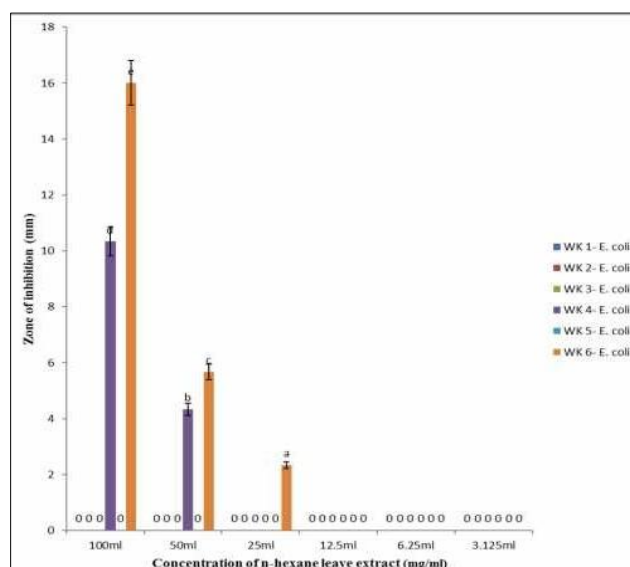
(b)

Fig. 6. (a)&(b) depicts the antibacterial activity of hexane leaf extracts against faecal coliforms

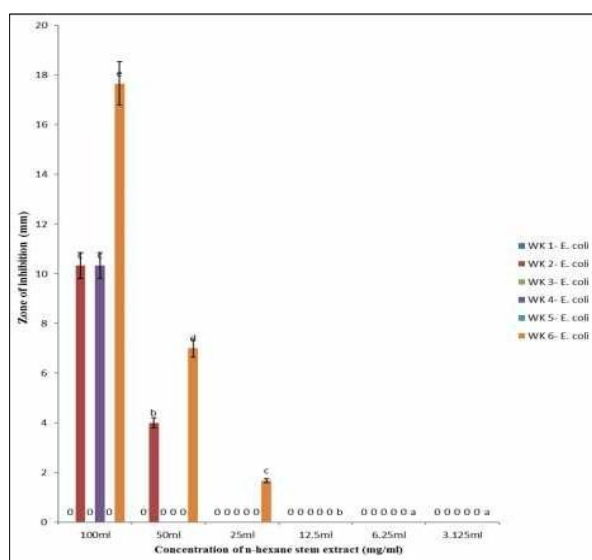
antibacterial activity of hexane stem extracts against faecal coliforms
Key: Wk = Week

The alarming high number of faecal coliforms per 100 mL obtained from the water samples, which exceeds at least ten times the recommended limit, indicates high level of faecal pollution of the river water which potentially poses a high health risk for recreational purposes, let alone for drinking purpose. The occurrence of irregular variations in coliform bacteria was congruent with the findings of Chandra et al. [46], they observed that bacteria, especially faecal coliform count increases in wet

season compared to that of dry season. Also, [47] investigated seasonal variation in the bacteriological quality of Ebutte River in Edo State, Southern Nigeria and reported that bacterial counts were highest in the wet season and the least total viable count were recorded in the dry season month of January. Human activities in the sampling site in River Owena as hitherto described may also have played a significant role in the higher number of faecal coliforms at week two.



(a)



(b)

Fig. 7. Figure 7(a)&(b) depicts the antibacterial activity of hexane leaf extracts against *E. coli* antibacterial activity of hexane stem extracts against *E. coli*

Key: Wk = Week

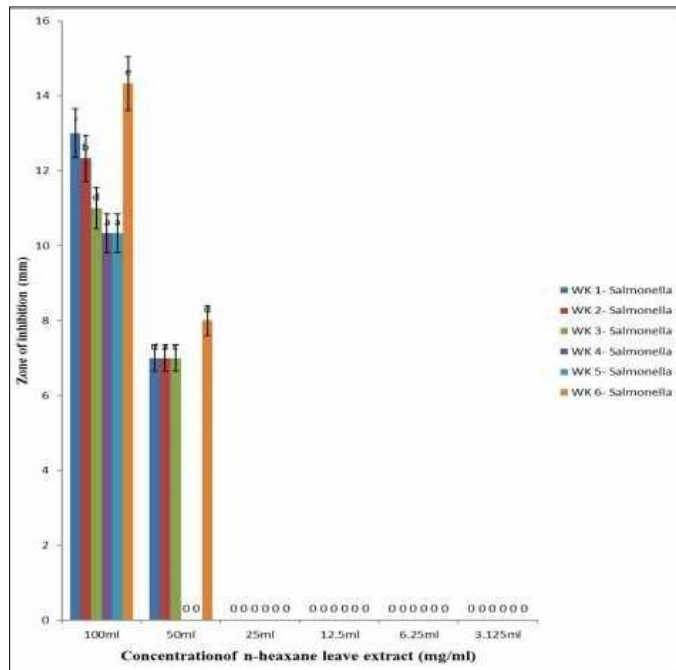
4.2 Physicochemical Characteristics of Water Sample from River Owena

In this study, the pH of water samples collected in time and space from the river section was slightly below neutral and these values fall within the accepted range of 6.0–8.5, thus indicating corrosiveness. The result recorded is consistent with results obtained by Omoigberale et al. [48]. The mean temperature values of the water samples obtained from the study were between the range of 26°C and 31°C [49] does not fall within the normal temperature range supportive of good surface water quality which is 0°C to 30°C [50]. The range of water temperature observed in this study is similar to those obtained by WQA [51] where the authors reported the same range of temperature in his study of Omi water body of Ago Iwoye, Nigeria. Hence, the temperature of the water from River Owena influenced the observed variations in the bacterial population as well as in other physicochemical parameters, since high temperature negatively impact water quality by enhancing the growth of microorganisms which may increase taste, odour, colour and corrosion problems [52]. Temperature affects physical activities in the water [53]. The values of total dissolved solids (TDS) in water samples were not implicative of a high level of pollution River Owena when compared to the WHO standard limit for good water quality which is 1,000 mg/L

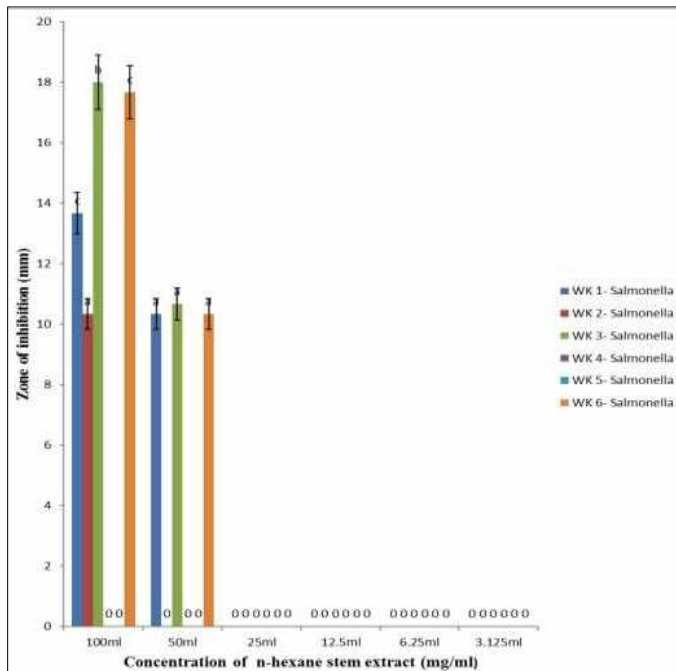
for TDS [54]. TDS is indicative of materials carried in suspension and solid respectively [55]. Low TDS is said to be a characteristics of hills and upland areas that represent areas of recharge according to [56].

Dissolved oxygen is the level of free, non-compound oxygen in water or other liquids. It is one of the most important parameters in aquatic systems [57]. The level of dissolved oxygen obtained in this study is within the maximum permissible limit of 1-18 mg/l [58]. The values of electrical conductivity obtained in this study suggest that solute dissolution was minimal in the water samples, rapid ion-exchange between the soil and water, or basically a poor and rather insoluble geologic rock and mineral types. This is in line with [59-60] where the authors observed that a higher mean value of electrical conductivity was recorded during wet season ($297.09 \pm 36.91 \mu\text{S cm}^{-1}$), also the study of Abowei [61] which recorded a value of 8.25-14.46 $\mu\text{S cm}^{-1}$. The result of conductivity in this study is still within the maximum permissible range of electrical conductivity which is 0-50000 $\mu\text{S cm}^{-1}$ [62]. The salinity values ranging from 9.57 to 15.95 gotten from this study during the wet season does not agree with the findings of WHO [63] who had a higher salinity value ($11.67 \pm 0.517\%$) recorded during the dry season (November to March) than the wet season ($6.98 \pm 0.701\%$) April to October. He stated that the months of April to October in

West Africa usually coincide with the rainy season when high volumes of freshwater are discharged into coastal or estuarine waters that lower or dilute the water.

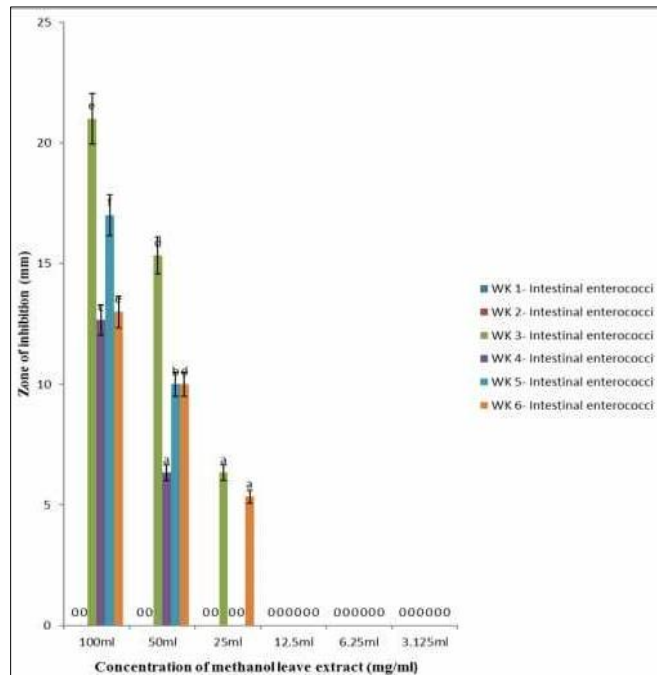


(a)

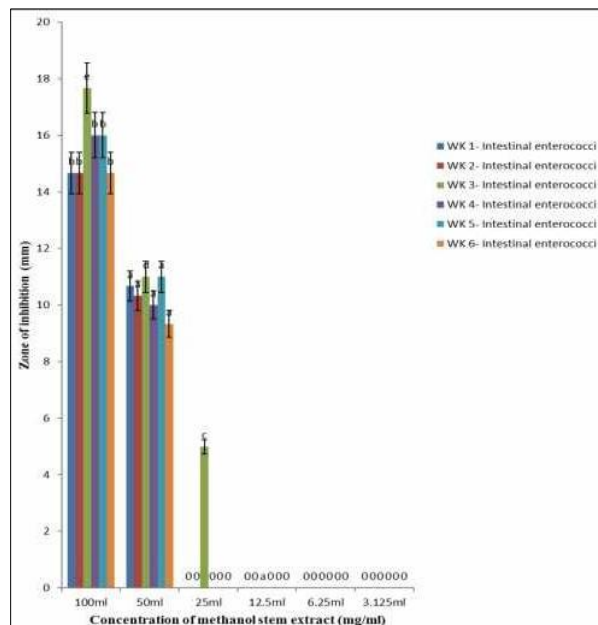


(b)

Fig. 8. (a)&(b) depicts the antibacterial activity of hexane leaf extracts against *Salmonella* antibacterial activity of hexane stem extracts against *Salmonella*
Key: Wk = Week



(a)



(b)

Fig. 9. 9(a)&9(b) depicts the antibacterial activity of hexane leaf extracts against intestinal enterococci and antibacterial activity of hexane stem extracts against intestinal enterococci
Key: Wk = Week

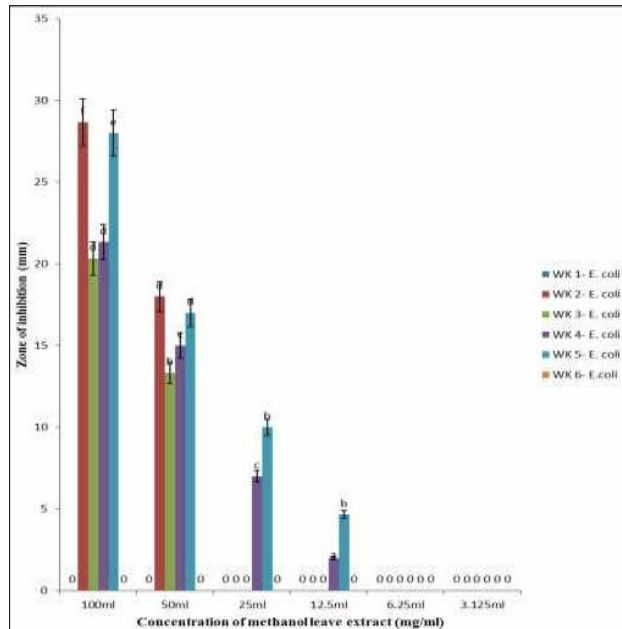
4.3 Percentages yield of Methanolic and Hexane Extract of *Andrographis paniculata*

The methanol extract obtained the highest yield of extraction for both leaf and stem (20.6 g and

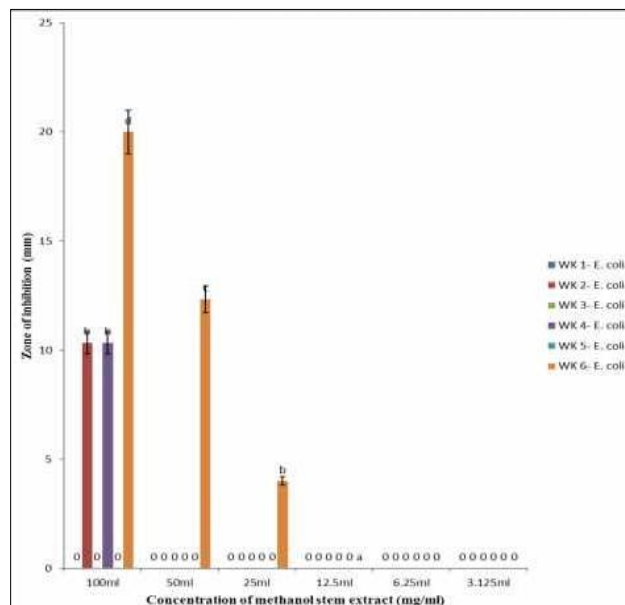
19.3 g). Meanwhile, the hexane extract obtained the lowest yield of extraction for leaf and stem 4.0 and 3.6 g. This result is compatible to [64] who observed that methanol gave the highest percentage yield of extraction from *A. paniculata* leaves. This can be implied that the

phytochemical compounds in *A. paniculata* dissolved better in methanol solvent. However, high percentage yield of plant extraction does not indicate that the solvent extract will exhibit high antibacterial activity [65]. According to the result the methanol extract contained the most of the

phytochemicals compounds which are terpenoids, cardiac glycosides, alkaloids, flavonoids, saponins and steroids. This observation may be as a result of the solvent used for the extracts. The result of this study agrees with the study of [66].



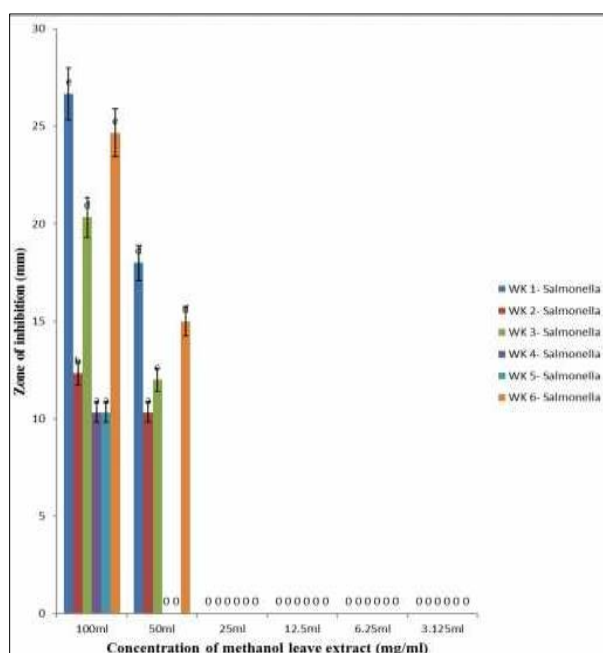
(a)



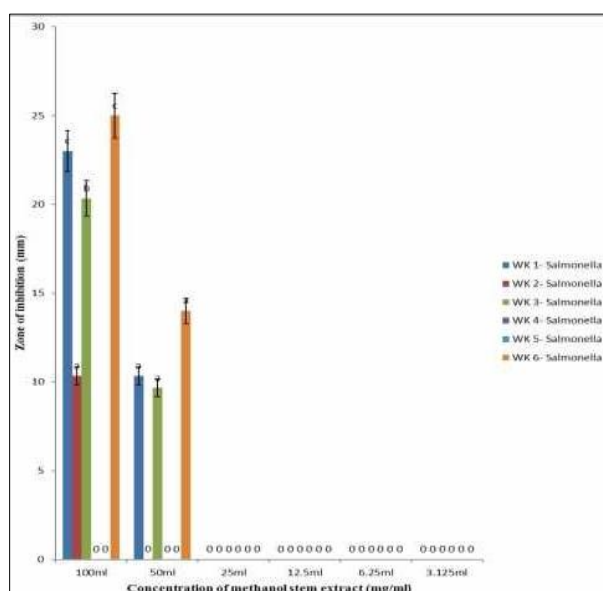
(b)

Fig. 10. 10(a)&(b) depicts the antibacterial activity of hexane leaf extracts against *E. coli* antibacterial activity of hexane stem extracts against *E. coli*

Key: *Wk* = *Week*



(a)



(b)

Fig. 11. 11(a)&(b) depicts the antibacterial activity of hexane leaf extracts against *Salmonella* (b) antibacterial activity of hexane stem extracts against *Salmonella*

Key: Wk = Week

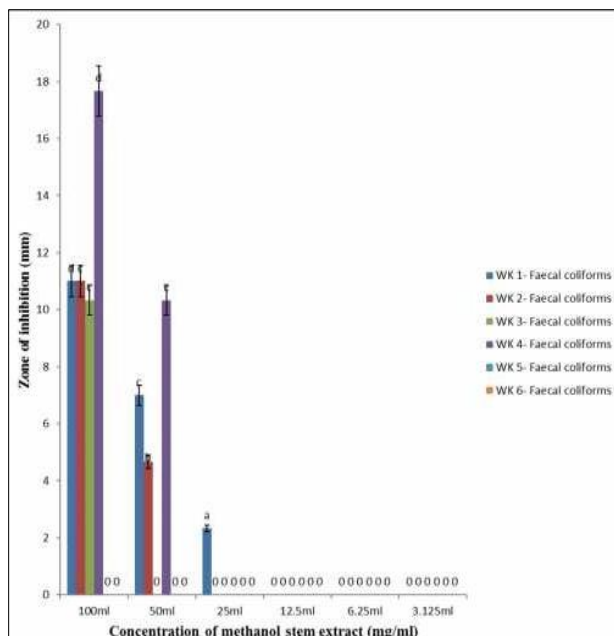
4.4 Phytochemicals Constituents of Leaf and Stem Extract of *A. paniculata*

In this study, ten phytochemical screening tests that were carried out included terpenoids, cardiac glycosides, phenols, alkaloids, flavonoids,

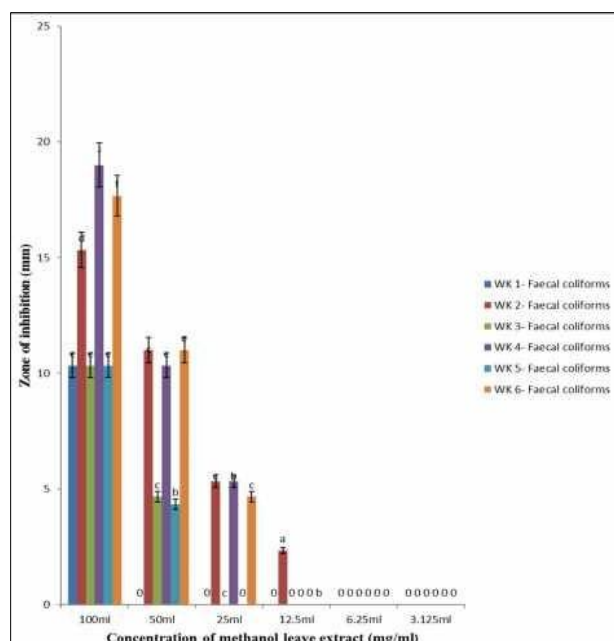
saponins, steroids, anthraquinones, reducing sugars and tannins tests. The findings of this study suggests terpenoids, cardiac glycosides, phenols, alkaloids, flavonoids, saponins, steroids were present in more than one solvent extracts of *A. paniculata*. In contrast, anthraquinones, and

phlobatannin that failed to show positive results in all solvent extracts may be as a result of the solvents used for the extraction. According to the result the methanol extract contained the most of the phytochemicals compounds which are terpenoids, cardiac glycosides, alkaloids,

flavonoids, saponins and steroids. This observation may be as a result of the solvent used for the extracts. The result of this study agrees with the study of Armando et al. [66]. The antimicrobial activity of hexane extracts was found due to the presence of glycosides [67].



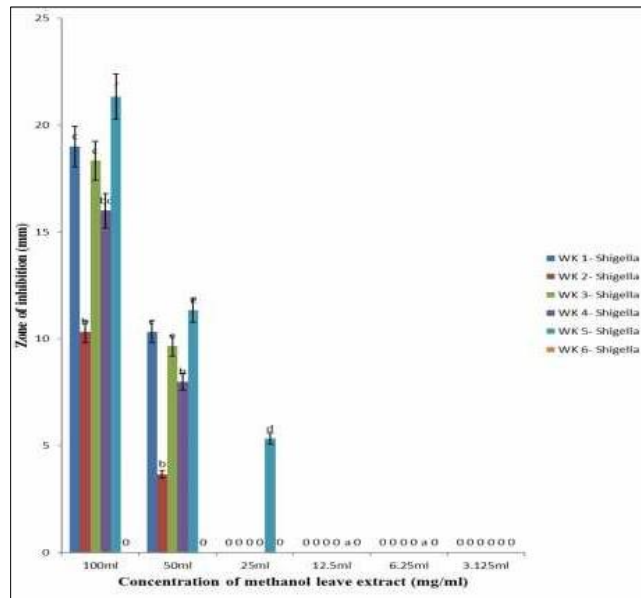
(a)



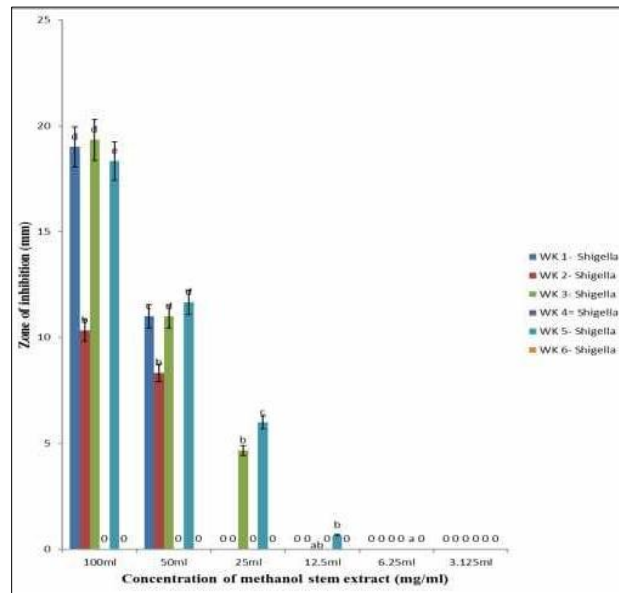
(b)

Fig. 12. (a)&(b) depicts the antibacterial activity of hexane stem extracts against faecal coliforms antibacterial activity of hexane leaf extracts against faecal coliforms

Key: Wk = Week



(a)



(b)

Fig. 13. (a)&(b) depicts the antibacterial activity of hexane stem extracts against *Shigella* antibacterial activity of hexane leaf extracts against *Shigella*

Key: Wk = Week

Terpenoids were found to be the most abundant compound in *A. paniculata* leaves. It has been observed in previous studies in hexane and methanol extracts [68]. Terpenoids have been reported that it had antibacterial, antiviral, antiarrhoeal and antineoplastic effect [68]. The terpenoids exhibit antibacterial activity through the mechanism of membrane disruption by its lipophilic compounds [68,69]. The most common

terpenoid compounds isolated from *A. paniculata* are diterpenoids lactones. One of the example of diterpenoids lactone is the andrographolide which has bitter taste, colourless and in crystal form [70].

Flavonoids are hydroxylated phenolic compounds that have antimicrobial activity against a wide range of microorganisms [71]. In

addition, they are potential antioxidant agents that act as free radical scavenger. The example of flavonoids that have antioxidant properties are flavonols, flavones, anthocyanins, isoflavonoids, flavanols and flavanones [72]. The common flavonoids that have been isolated from *A. paniculata* [73]. Besides, flavonoids also exhibit the property of anti-allergic, antifungal, antiviral, anticancer and anti-inflammatory properties [74,75]. Majority of phytochemical compounds namely terpenoids, alkaloids, saponin, steroids and anthraquinones are mainly involved in antimicrobial activity. Meanwhile, phenolic and flavonoids compounds are found to have antioxidant property [76].

4.5 Effect of Methicillin and Vancomycin on Enteric Bacteria Isolated from River Owena

The result of methicillin and vancomycin activities on enteric bacteria isolated from River Owena showed that intestinal enterococci, *E. coli* and faecal coliforms were susceptible to vancomycin with zone of inhibition ranging from 16.00 mm to 40.00 mm, with faecal coliforms having the highest zone of inhibition. While *Shigella* and *Salmonella* were resistant to vancomycin. Studies have demonstrated that most species of *Salmonella* and *Shigella* exhibit multi-drug resistance [77,78]. However, all the enteric bacteria in this study were susceptible to methicillin, with faecal coliforms showing the highest zone of inhibition of 36 mm and *Shigella* had the smallest zone of inhibition of 10 mm. This observation may be as a result of the potency of methicillin or the fact that the enteric bacteria isolated have not grown any resistance to the drug.

4.6 Antibacterial Activity of *A. paniculata* against the Enteric Bacteria Isolated from River Owena

The results from this study showed that the stem and leaf of *A. paniculata* possesses antibacterial properties. Of all the extracts tested methanol leaf extracts proved to be the best for *E. coli* with 29.0 mm zone of inhibition while stem extract has the lowest zone of inhibition of 17mm on *Intestinal enterococcus*. However, these results are in accordance with studies that had reported that the methanol extract of *A. paniculata* showed antibacterial activity against *E. coli* [79,80]. Also in another study by Sharma et al. [81], it was found that the MIC of *Camellia sinensis* and *Andrographis paniculata* extract

was found to be 200 µg/mL against exponentially growing VRE. The anti-bacterial activity of both the extracts was found to be comparable with respect to reported values for standard antibiotic i.e. Gentamycin and Ampicillin with respective MICs as 103 µg/mL and 160 µg/mL [82,83]. Premnath et al. [84] had also reported that the hexane, chloroform and methanol extracts of *A. paniculata* showed antibacterial activity against *E. coli* through agar disc diffusion method. The hexane stem extract showed an inhibitory value ranging between 11-18 mm with *Salmonella* and *E. coli* having the highest zone of inhibition and faecal coliforms had the lowest. The hexane leaves extract showed inhibitory value ranging between 12 mm – 16 mm with *E. coli* having the highest and faecal coliforms the lowest.

The hexane extract showed the least antibacterial activity as it is a non-polar solvent which commonly used to extract essential oils and lipids. Suggesting that the antibacterial compounds *A. paniculata* are lipid soluble compounds [85]. The low antibacterial activity of hexane extract might be due to their bioactive phytochemicals that are present in low concentration. This low antibacterial activity may seem to be insignificant if hexane extract of *A. paniculata* were subjected to further purification, the antibacterial activity may increase. The low levels of antibacterial activity recorded in this study against the enteric bacteria by the hexane extracts of both the leaf and stem extracts of *A. paniculata* may be because of the presence of little amount of glycosides (+) and the absence of excellent antibacterial agents such as tannin, and saponin.

The methanolic extract of *A. paniculata* exhibited the lowest MIC value at 12.5 mg/ml against *Salmonella*, *Escherichia coli*, faecal coliforms and *Shigella*. The MIC value of the stem extract of *A. paniculata* with hexane was at 50 mg/ml against *Shigella* and *Salmonella* and faecal coliforms.

The ability of the leaves and stem extracts of *A. paniculata* to inhibit both gram positive and gram negative bacteria suggests that the extracts have broad spectrum activity. Extracts of *A. paniculata* have been shown to have significant effects against the diarrhea associated with *E. coli* bacterial infection [86]. The *A. paniculata* components, andrographolide and neo-andrographolide have been demonstrated to have similar activity to loperamide (imodium) the most common antidiarrheal drug [87]. In another study, *A. paniculata* was used to treat 1,611

cases of bacterial dysentery and 955 cases of diarrhea with overall effectiveness of 91.3% [88,89].

It was also reported that methanol is the best solvent for exhaustive extraction of andrographolide and its derivatives. The enhanced antibacterial activity of methanolic extracts observed in this study may be due to the presence of these compounds which are known to exhibit antibacterial activity [90].

5. CONCLUSIONS

The results obtained from this study demonstrated that the extracts of *A. paniculata* have high inhibitory potential against enteric bacteria isolated from River Owena that were resistant to vancomycin and methicillin. The findings of this study suggest that the bioactive compounds in the leaf and stem extracts of *A. paniculata* and these were most probably responsible for its antibacterial activities. The inhibitory activities of the extracts of *A. paniculata* on vancomycin and methicillin resistant bacteria support the use of this plant in modern medicine and that the origin of the enteric bacterial isolates in water samples from River Owena is majorly from human sources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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