

EFFECTS OF METHANOLIC TURMERIC EXTRACT, ALUM ON *E. COLI* IN BOREHOLE & SACHET WATER PH.

ABSTRACT

The aim of this research were to determine the antibiogram and effects of methanolic turmeric extract and alum on *Escherichia coli* in some borehole and sachet water in Rivers State respectively. The various concentrations (100mg/ml,50mg/ml,25mg/ml and 12.5mg/ml) of these combinations, methanolic turmeric extract, Alum, MTE+ Alum were tested respectively for antibacterial activity on some strains of *E. coli* isolated from borehole and sachet water samples. These were compared with an antibiotic standard, using chloramphenicol as a positive control. Antibacterial activity was assessed using disc diffusion method (DDM) respectively. Of these combinations, methanolic turmeric extracts in synergism with Alum (MTE+Alum) exhibited the lowest mean diameter of inhibitory zone (DIZ) values of 5.0-20.0mm and Alum exhibited the largest mean of inhibitory zone (DIZ) values of 8.0-20.0mm on the test organism using disc diffusion method (DDM) respectively. There was no inhibitory zone with MTE+Alum at 12.5 concentration whereas (DIZ) values for methanolic turmeric extract ranged from 8.0-16.0mm by DDM. The susceptibility of combinations were more pronounced against CP064383 *E. coli* (20.0mm), CP089272 *E. coli* (14.0mm), AF099077 *E. coli* (14.0mm) and LG142154 *E. coli* (18.0mm) respectively. This results revealed that chloramphenicol inhibited the largest DIZ values. However, the activity of chloramphenicol revealed that purified and tested antibiotic should be used as the drug of choice for treatments. And the synergistic effects of methanolic turmeric extract plus Alum was effective against all strains of *E. coli* with concentration of 100mg/ml and can be used as second line of treatment against infections caused by *E. coli*. *E. coli* isolates with a prevalence of 100% were obtained as the test organisms. Susceptibility pattern of the test isolates showed 100% susceptibility to Gentamycin,75% by chloramphenicol, Ciprofloxacin and Vancomycin. They were resistant to Cefotaxime (75%) and Cotriazole (25%) respectively. Multiple antibiotic resistance index for test isolates revealed 3(75%) of four *E. coli* strains was >0.2..The results for total heterotrophic bacterial count showed that there is no significant difference($p>0.05$) in the mean counts sampling locations Ikwerre, Obio/Akpor and Phalga. Faecal Coliform Count revealed that there was no significant difference($p>0.05$) in the mean counts with sachet water having the highest values of (2.77 ± 0.14 , 2.76 ± 0.09 and 2.77 ± 0.17) $\text{Log}_{10}\text{CFU/ml}$ while borehole water revealed least counts of (1.46 ± 1.29 , 0.79 ± 1.18 and 1.86 ± 1.44) $\text{Log}_{10}\text{CFU/ml}$. In conclusion, this study demonstrated the potentials of Alum, MTE in synergism with alum as choice of treatment caused by *E. coli* in water samples from the study area.

Keyword: Antibacterial activity, Turmeric extracts, *E. coli*, Disc diffusion method, Alum, Borehole (BWS) and Sachet (SWS) water samples.

Antimicrobial Residues (AMR)

Introduction

Turmeric

Turmeric is a native antimicrobial plant with the scientific name *Curcuma longa* L., turmeric is a member of the ginger family, or Zingiberaceae. It originated in India and is now widely cultivated throughout South and Southeast Asia's tropical and subtropical regions, including China, India, and some tropical regions of Africa. The word "curcuma" is derived from the Arabic word "kurkum" or the Hebrew word "karkom," both of which imply "yellow."

The term "longa" refers to the underground stem's elongated shape. The rhizome, which has a distinctive deep orange-yellow colour, is a crucial component in curry and is frequently used as a flavouring and colouring agent {1}. For the treatment of a number of illnesses, including cough, diabetic wounds, hepatic disorders, and cardiovascular disease, turmeric was also a crucial herb in traditional Chinese and Indian medical systems. 2012 {2}. In particular, turmeric has proven to be an effective anticancer drug, possessing anti-inflammatory, anti-Alzheimer's, and anticancer effects in both pre-clinical and clinical investigations. Turmeric also contains cardioprotective, hypoglycemic, anti-rheumatic, and anti-diabetic properties. There are several examples that highlight the significance of medicinal plants as potential drug sources, such as aspirin, which has been used for thousands of years to alleviate pain and fever and whose active metabolite, salicylic acid, was first isolated from the willow tree's bark {3}. The therapeutic benefits of specific medicinal plants have been discovered, recognised, and passed down to succeeding generations during every phase of humankind's progress into complex civilizations.

The breakdown of tissue homeostasis is thought to be the primary mediator of inflammation, which is recognized as a complex biological process. Inflammation can be classified as acute or chronic based on the type of stimuli and is brought on by the presence of various biological, chemical, or physical agents.

Escherichia coli

Due to its potential to carry virulence and antibiotic resistance genes, *E. coli* O157:H7 strain has been recognised as a significant environmental and clinical pathogen {4}. Because antibiotics may encourage the formation of Shiga toxins, their use in infections brought on by this disease is debatable. Nevertheless, the alignment of the proper sets *E. coli* O157:H7 strain has been acknowledged as a significant environmental and clinical pathogen due to its capacity to carry virulence and antibiotic resistance genes {4}. *E. coli* O157:H7 strain has been identified as an important environmental, clinical pathogen because it can harbor virulence and antibiotic resistance genes {4}.

Because antibiotics may encourage the formation of Shiga toxins, their use in infections brought on by this disease is debatable. Nevertheless, the alignment of the proper sets by reducing the severity of the condition and improving the overall outcome, the use of antibiotics and supportive therapies, such as rehydration, can be utilised to combat the infection caused by this pathogen {5}. According to studies, *E. coli* O157:H7, a bacterium that is multidrug resistant (MDR) to routinely used antibiotics, is collected from the environment, animals, and humans {6}.

This is probably because antibiotics are continuously injected into the main reservoir to increase output. Antimicrobial residues (AMR) strains and their determinants eventually arise and disseminate in the environment as a result of this {7}. Antimicrobial resistance indicators may

include *E. coli O157:H7*. For the monitoring and tracking of antimicrobial residues (AMR) in the environment, livestock, people, and the food chain, they are crucial.

The presence of multidrug-resistant *E. coli O157:H7* in irrigation water and agricultural soil, which facilitate the transfer of enteric bacteria from the farm to fresh product, is documented in small levels.

Potassium Aluminium Sulfate (Alum)

The chemical compound $KAL(SO_4)_2$ is potassium aluminium sulphate, generally known as alum. It crystallises in an octahedral structure in a natural solution and in a cubic structure in an alkali solution. Alum is bacteriostatic which inhibits the growth of microorganisms in water. The aluminium present in alum helps to coagulate the particles, and as a result of high charge on aluminium ion with (AL^{3+}).

In addition, it can be used as a deodorant, aftershave, and to treat minor cuts from shaving and nosebleeds, potassium aluminium sulphate (Alum) is also frequently used to purify water {8}.

According to {9}, alum and *Gongronema latifolium* work together to kill some clinical microorganisms.

Materials and Methods

Sample Collection

The water samples were collected from Marine Base Market, Creek Road Market, New Layout Market, Mile 1 Market, Mile 3 Market (Phalga), Wimpy Market (Obio/Akpor), Igwuruta Market, Omagwa Market, and Ozuoha Market (Ikwerre) all in Rivers State. The samples were transported to Rivers State University's Microbiology laboratory for analysis. Thereafter, a rubber of turmeric was purchased from the fruit garden market in July and August 2022, using a sterile polyethylene bag during the rainy season. The turmeric were delivered to the microbiology department for identification and extraction.

Preparation of Turmeric Plant Extracts

Before extraction, the turmeric were washed in clean water (tap) to eliminate foreign objects, then they were sliced and stored in an oven set at $40^{\circ}C$ for three (3) to five (5) days before being ground using a sterile hand homogenizer. The graded powder of 100g of pulverised turmeric was dissolved in 200 mill of methanolic solvent. Thereafter, the mixture was covered with aluminium foil and left in the dark for two (2) hours at room temperature to prevent exposure and evaporation. The mixture was filtered into another sterile beaker of 250 ml using standard Whatman No. 1 filter paper and a sterile hand glove and the filtrate was then maintained in an oven at $44.5^{\circ}C$ to guarantee complete evaporation in the refrigerator.

Test bacterial pathogens

The test bacterial pathogens, *Escherichia coli* was isolated from sachet and borehole water samples obtained from Ikwerre, Obio/Akpor and Port Harcourt (Phalga) Local Government Area, Rivers State Nigeria.

The strains of *Escherichia coli* were recovered from sachets and boreholes water samples in Ikwerre, Obio/Akpor and Phalga LGA, Rivers State Nigerian.

Preparation for Turmeric Extract (TE) and Alum

Turmeric extract (TE) and Alum {10} were manufactured in various concentrations to produce concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively. Chloramphenicol was used as the control to compare the potency of the extract.

Microbiological Analysis

Enumeration and Isolation of Total Heterotrophic Bacterial Counts.

The count were carried out using the spread plate method, total heterotrophic bacteria (THB) was counted and isolated. Ten (10ml) of the principal sample was measured into a sterile beaker, thereafter one (1ml) of the water sample was measured from each sample and aseptically transferred into test tubes containing 9ml of normal saline. This was serially diluted to 10^{-1} , 10^{-4} and 10^{-6} for borehole water samples and 10^{-1} , 10^{-3} , 10^{-4} for sachet water samples, and direct inoculation was done for all the samples, then an aliquot (0.1ml) with a sterile 1ml pipette was inoculated unto the surface of nutrient agar plates, Eosine methylene blue (EMB) plates, and the inoculated plates were incubated at temperature of 37°C for 24-48 hours {11}. After incubation, the plate counts were recorded and the values obtained was expressed as colony forming units per mill (CFU/ml) of the cells present in 0.1ml of the broth which served as the standard structure. The discrete colonies were sub cultured using a freshly prepared nutrient agar plate and were stored in 10% frozen glycerol {11}.

Characterization and Identification of bacterial Isolates

According to {12}, the biochemical tests catalase, oxidase, mortality, fermentation of certain sugars, methyl red, indole, and citrate were used to characterise the bacterial isolates. And discrete colonies were picked based on their morphology, microscopic and macroscopic examination. The isolates were sub-cultured on nutrient agar (NA) and Eoisine methylene blue (EMB). And identification of the bacteria isolate was carried out {11}.

Determiation of Antibiotic Susceptibility Test for Methanolic Turmeric Extract and Alum

The antibacterial susceptibility test was performed by the disc diffusion method (DDM) using {12}. Each of the bacterial suspensions from the overnight culture, following adjustment to 0.5 McFarland turbidity standards were spread on Muller Hinton Agar, using a swab stick and allowed to dry for 2 to 5 minutes as described by {9}. Thereafter, filter paper discs, made as described by {13} were impregnated with Alum and methanolic turmeric extract (MTE) and were dissolved in 10ml of dimethyl sulfoxide (DMSO) to give 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentration respectively. Commercially supplied antibiotic such as chloramphenicol (CH 30µg as a positive control) was placed on the surface-dried inoculated Mueller Hinton Agar (MHA) using sterile forceps. {14}. For disc diffusion method, various concentrations were impregnated into the filter paper and the bacterial suspension from the overnight culture was spread onto surface-dried MHA. Thereafter, the filter papers with different concentrations of methanolic turmeric extracts and Alum were placed into the agar plates containing the organism of interest using sterile forceps with the chloramphenicol which served as positive control respectively. The plates were incubated at 37°C for 24 hours, and the diameter of inhibitory zones (DIZ) were measured using a transparent meter rule which is expressed in millimeters (mm).

The diameter inhibition zone of different concentrations of extracts were compared with chloramphenicol as the control as to check the potency of the extracts. The interpretation of results was done based on the zone of inhibition, resistant (<14), intermediate (<20) and susceptibility (>20) {15}.

Prevalence of Bacterial Isolates in all Samples

Four (4) *E. coli* isolates were found in the water samples from the three distinct locations as test organisms, out of a total of forty (40) isolates obtained from the water samples. The biochemical characterization showed that the isolates present were *Enterobacter asburiae*, *Serratia fonticola*, *Citrobacter freundii*, *Klebsiella singaporensis*, *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Enterobacter cloacae* and *Escherichia spp.* The prevalence of *E. coli* in this study was high across all, at 75%. Similar results were observed {16} who also discovered a significant prevalence of *E. coli* at 74%.

In this study, *E. coli* O157:H7 was identified among the other *E. coli* strains. This result is in line with {17} hypothesis that the high incidence of *E. coli* O157:H7 is a reflection of the unclean settings in which animals are slaughtered and sold close to water environments. The high frequency of *Enterohemorrhagic Escherichia coli* (EHEC) in this study is caused by unhygienic settings, cross contamination, the areas with high population density, anthropogenic activities, and the discharge of feces into the water body {18}.

Susceptibility of *E. coli* to Antibiotics

Worldwide, antibiotic resistance in bacteria, especially *E. coli* linked to water, has been a worry. Its susceptibility patterns revealed great diversity as well as variations in population and environment. It is now commonly acknowledged that the development of resistance and antimicrobial drugs are related. The antibiotic sensitivity pattern of the *E. coli* found in this study has a significant impact on the public health implications of these organisms because it affects the clinical treatment option(s) that are accessible for therapy. As a result, the antimicrobials activity put organisms under selective pressure, which is a major problem in epidemiological investigations. The results of the antibiotic sensitivity patterns, as interpreted by the Clinical Laboratory Standard Institute Guideline (2020), revealed that a significant number of the test isolates was 100% susceptible to gentamycin, followed by chloramphenicol, ciprofloxacin, amikacin, and vancomycin, demonstrating that they are the most sensitive and effective medications on *E. coli* from this research work while showing resistance to cefotaxime and co-triazole and it is consistent with the results of {19}, which showed that *E. coli* is most sensitive to Gentamycin, Ciprofloxacin and Chloramphenicol isolated from clinical samples.

Antibiotic Susceptibility Test of Turmeric Extracts

Plants have created new compounds that have major human benefits. Numerous attempts have been made to identify the biological principles of nature in plants and one of these resources is public health, and countless studies have demonstrated that natural compounds have antibacterial effect against pathogenic organisms {20}. A thorough examination of public health resources may reveal this conduct. As the spectrum of pathogenic drug resistance, researchers are looking for natural extracts with anti-virulence characteristics. Findings in this study showed that antibiotic of alum inhibited at 100mg/ml and 50mg/ml while alum in synergism with methanolic turmeric extracts inhibited their growth effectively at 100mg/ml only. The antibiotic turmeric extract of LG142154 *Enterohemorrhagic Escherichia coli* revealed that methanolic turmeric extracts inhibited their growth at concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml; alum inhibited at 100mg/ml, 50mg/ml and 25mg/ml while alum in synergism with methanolic extracts inhibited their growth effectively at 100mg/ml. The antibiotic turmeric extract of AF099077 *Escherichia coli* revealed that methanolic turmeric extracts inhibited their growth at concentrations of 100mg/ml, 50mg/ml, and 12.5mg/ml; alum inhibited growth effectively at 100mg/ml, 50mg/ml and 25mg/ml while alum in synergism with methanolic turmeric extracts inhibited their growth effectively at 100mg/ml. The susceptibility test of CP089272 *Escherichia coli* 0157:H7 revealed that methanolic turmeric extracts inhibited their growth at a concentration of 100mg/ml, 50mg/ml, and 25mg/ml; while alum in synergism with methanolic extracts inhibited their growth effectively at 100mg/ml, 50mg/ml and 25mg/ml respectively. The antibiotic turmeric extract of CP064383 *Escherichia coli* 0157:H7 revealed that methanolic turmeric extracts and alum inhibited their growth at a concentration of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively; alum in synergism with methanolic extracts inhibited their growth effectively at 100mg/ml and 50mg/ml and 25mg/ml. According to the Council for Innovation, all isolates were inhibited by both extracts up to 50% concentration, which is consistent with the results of this study.

Multiple Antibiotic Resistance (MAR) Index of Test Isolates

The emergence of new antibiotic resistance mechanisms in *E. coli* is a serious public health problem. *E. coli* is evolving these novel strategies, which are costly and limited in terms of treatment alternatives, as seen by the development of multi-drug resistant bacteria. As a result, the Multiple Antibiotic Resistance (MAR) index score of 50% of the *E. coli* isolates used in this experiment was less than 0.2. It is crucial to comprehend that contamination sites have MAR index values greater than 0.2 when antibiotics are often used {21}. However, a sizable fraction of the MAR indices of *E. coli* found in this analysis showed diverse antibiotic resistances and indiscriminate usage of these treatments for water infections {21}.

Statistical Analysis

A p-value of 0.05 was taken into account at the 95% level of significance to determine whether there was a significant difference between the data presented as means with standard deviations and plotted graphically using Microsoft Excel 2016 and to all data gathered during the study using the IBM SPSS (Statistical Package for the Social Sciences) software.

RESULTS AND DISCUSSION

Table 1: Antibacterial Activity of Methanolic Turmeric (*Curcuma longa*L) Extracts

Bacterium	Diameter of inhibitory zone (DIZmm)				
	Concentration of MTE (%)				
Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	30µg/ml (CHL)
<i>LG142154Enterohemorrhagic Escherichia coli</i>	15.0	10.0	10.0	8.0	28.0
<i>AF099077Escherichia coli</i>	14.0	8.0	8.0	0	29.0
<i>CP089272Escherichia coli 0157:H7</i>	16.0	8.0	6.0	0	43.0
<i>CP064383Escherichia coli 0157:H7</i>	13.0	13.0	11.0	10.0	30.0

Legend: MTE = Methanolic Turmeric Extract, CH= Chloramphenicol, DIZ= Diameter of Inhibitory Zone

Table 2: Antibacterial Activity of Alum Turmeric Extracts

Bacterium	Diameter of Inhibitory Zone (mm)				
	Concentration of ALTE (%)				
Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	30µg/ml (CHL)
<i>LG142154Enterohemorrhagic Escherichia coli</i>	8.0	8.0	8.0	0	25.0
<i>AF099077Escherichia coli</i>	12.0	9.0	8.0	0	0
<i>CP089272Escherichia coli 0157:H7</i>	12.0	10.0	0	0	32.0
<i>CP064383Escherichia coli 0157:H7</i>	20.0	10.0	8.0	8.0	30.0

Table 3: Antibacterial Activity of Alum and Methanolic Turmeric Extracts

Bacterium	Diameter of Inhibitory Zone (mm)				
	Concentration of AL+MTE (%)				
Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	30µg/ml (CHL)
LG142154 <i>Enterohemorrhagic Escherichia coli</i>	18.0	10.0	5.0	0	25.0
AF099077 <i>Escherichia coli</i>	14.0	12.0	0	0	0
CP089272 <i>Escherichia coli</i> 0157:H7	14.0	12.0	8.0	0	34.0
CP064383 <i>Escherichia coli</i> 0157:H7	20.0	18.0	10.0	0	30.0

Legend: MTE = Methanolic Turmeric Extract, CH= Chloramphenicol, DIZ= Diameter of Inhibitory Zone.

Table 4: Antibiotics Inhibitory Zone of Gram Negative Isolates in Borehole and Sachet water Samples.

S/N	Organism	CTR	CTX	CPZ	TET	COT	GEN	CRX	CHL	MEM	CIP	AMK	VAN
1	CP064383 <i>Enterohemorrhagic E. coli</i>	-	-	-	15	16	25	-	25	13	26	22	21
2	CP089272 <i>E. coli</i>	22	-	-	-	-	20	-	20	21	26	18	20
3	AF099077 <i>E. coli</i> 0157:H7	16	15	20	17	12	20	18	15	10	14	13	10
4	LG142134 <i>E. coli</i> 0157:H7	20	18	16	20	18	20	21	23	20	25	24	20

KEY: CTR = (Ceftriaxone), CTX = (Cefotaxime), CPZ = (Chlorpromazine), TET = (Tetracycline), COT = (Cotrimoxazole), GEN = (Gentamycin), CRX = (Cefuroxime), CHL = (Chloramphenicol), MEM= (Meropenem), CIP = (Ciprofloxacin), AMK = (Amikacin), VAN = (Vancomycin).

List 1: Antibiotics Susceptibility Test Interpretive Criteria (CLSI,2010)

Antibiotics	Conc (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
CTR	30	<13	14-20	>21
CTX	30	<15	16-22	>23
CPZ	30	<13	14-20	>21
TET	30	<15	16-18	>19
COT	30	>10	11-15	>16
GEN	10	<12	13-14	>15
CRX	30	<14	15-22	>2
CHL	30	<13	14-17	>18
MEM	10	<14	15-17	>18
CIP	5	<15	16-20	>21
AMK	30	<14	15-16	>17
VAN	30	<14	15-16	>17

List 2: Antibiotics Susceptibility Profile of Gram Negative Isolates. Borehole and Sachet water Samples.

S/N	Organism	CTR	CTX	CPZ	TET	COT	GEN	CRX	CHL	MEM	CIP	AMK	VAN
1	CP064383 <i>Enterohemorrhagic E. coli</i>	R	R	R	R	S	S	R	S	R	S	S	S
2	CP089272 <i>E. coli</i>	S	R	R	R	R	S	R	S	S	S	S	S
3	AF099077 <i>E. coli</i> 0157:H7	I	R	I	I	I	S	I	I	R	R	R	R
4	LG142154 <i>E. coli</i> 0157:H7	I	I	I	S	S	S	I	S	S	S	S	S

KEY: CTR = (Ceftriaxone), CTX = (Cefotaxime), CPZ = (Chlorpromazine), TET = (Tetracycline), COT = (Cotrimoxazole), GEN = (Gentamycin), CRX = (Cefuroxime), CHL = (Chloramphenicol), MEM = (Meropenem), CIP = (Ciprofloxacin), AMK = (Amikacin), VAN = (Vancomycin).

Table 5: Susceptibility Pattern of Bacterium Isolated from Borehole(BWS) and Sachet Water(SWS) Samples

Antibiotics	Conc. (μg)	Resistant(R)	Intermediate(I)	Susceptible(S)
		n (%)	n (%)	n (%)
CTR	30	1(25.0)	2(50.0)	1(25.0)
CTX	30	3(75.0)	1(25.0)	0(0.00)
CPZ	30	2(50.0)	2(50.0)	0(0.00)
TET	30	2(50.0)	1(25.0)	1(25.0)
COT	30	1(25.0)	1(25.0)	2(50.0)
GEN	10	0(0.00)	0(0.00)	4(100.0)
CRX	30	2(50.0)	2(50.0)	0(0.00)
CHL	30	0(0.00)	1(25.0)	3(75.0)
MEM	10	2(50.0)	0(00.0)	2(50.0)
CIP	5	1(25.0)	0(00.0)	3(75.0)
AMK	30	1(25.0)	0(00.0)	3(75.0)
VAN	30	1(25.0)	0(0.00)	3(75.0)

KEY: CTR (Ceftriaxone), CTX (Cefotaxime), CPZ (Ceftazidime), TET (Tetracycline), COT (Co-triazole), GEN (Gentamycin), CRX (Cefuroxime), CHL (Chloramphenicol), MEM (Meropenem), CIP (Ciprofloxacin), AMK (Amikacin), VAN (Vancomycin)

Table 6: Comparative of microbial count (Log₁₀CFU/ml) of Sachet and Borehole water according to their Different Locations

Local Government Area	Source of water	THBC	Faecal Coliform Count
Ikwerre	Borehole	3.06±0.21 ^a	1.46±1.29 ^a
	Sachet	2.82±0.14 ^a	2.77±0.14 ^a
Obio/Akpor	Borehole	2.88±0.18 ^{ab}	0.79±1.18 ^a
	Sachet	2.85±0.18 ^a	2.76±0.09 ^a
Port Harcourt	Borehole	2.75±0.26 ^b	1.86±1.44 ^a
	Sachet	2.77±0.17 ^a	2.77±0.17 ^a

Total Heterotrophic Bacterial Count (THBC)

The result for Total Heterotrophic Bacterial Count (THBC) revealed that there was no significant difference ($p>0.05$) in the mean counts for the three sampling local government areas (Ikwerre, Obio/Akpor and PHALGA) with borehole water having the highest mean values of (3.06±0.21, 2.88±0.18 and 2.75±0.26) Log₁₀CFU/ml while sachet water revealed least mean counts of (2.82±0.21, 2.85±0.18 and 2.77±0.17) Log₁₀CFU/ml respectively.

Faecal Coliform Count (TFC)

The result for Faecal Coliform Count (FCC) revealed that there was no significant difference ($p>0.05$) in the mean counts for the three sampling local government areas (Ikwerre, Obio/Akpor and PHALGA) with sachet water having the highest mean values of (2.77±0.14, 2.76±0.09 and 2.77±0.17) Log₁₀CFU/ml while borehole water revealed least mean counts of (1.46±1.29, 0.79±1.18 and 1.86±1.44) Log₁₀CFU/ml respectively.

Discussion

The diameter of inhibitory zone (DIZ) values increased with increasing concentrations of methanolic turmeric extract (MTE), with the highest mean inhibitory zone of 16.0mm against CP089272 *E. coli* and least mean inhibitory zone of 13.0mm against CP064383 *E. coli* at 100mg/ml concentration respectively. The least zone of inhibition 6.0mm against test bacterium occurred at 25mg/ml concentration on CP089272. Similarly, the inhibition of *E. coli* with MTE using disc diffusion method (DDM) has been reported {23}. Chloramphenicol showed the highest mean DIZ values on CP089272 against all the test bacterium (Table 1). This confirms the fact that standard antibiotics are purified compounds with active antibacterial agents whereas the activity of turmeric extracts may be attributed to the fact that they are crude. Generally, the antibacterial activity was slightly higher on gram negative bacterium with Alum {9}. Similar trends in bacterium activity were observed with increasing concentrations of Alum but with higher inhibitory effects (Table 2) indicating Alum to be more beneficial than methanolic turmeric extract in terms of potency against CP064383 *E. coli* at 100mg/ml concentrations. (Table 3) revealed the ability of these combinations to inhibit the growth of gram negative organisms which demonstrates broad spectrum activity. Consequently, the inhibitory effects of MTE+ALUM on *E. coli* at 100mg/ml concentrations revealed increase in DIZ values of 20.0mm in CP064383 using disc diffusion methods (DDM) whereas MTE+ALUM at 12.5mg/ml concentration displayed no antibacterial activity. In contrast, the DIZ of the commercial antibiotic standard decreased (Table 2 and 3) but increased (28-43mm) using DDM (Table 1). The study found that out of the 36 water samples that were collected, there was higher total heterotrophic bacterial counts in the samples, with no significant difference ($p>0.05$) in the bacterial load analysed. High mean counts were observed in borehole water samples from the three sampling locations in (Ikwerre, Obio/Akpor, and PHALGA), LGAs in Rivers State, while sachet water samples had the lowest mean counts and were probably caused by environmental

factors. The overall heterotrophic bacterial count investigation was also found to be greater than the World Health Organization's (WHO) suggested limit {24}. Higher faecal coliform counts were also obtained in these water samples, showing no significant difference ($p>0.05$) in the bacterial load analysis where high mean counts were observed in sachet water samples while borehole water samples had the lowest mean counts and probably related to storage conditions of water during processing, packaging and production.

Conclusion

The present study demonstrated the antibacterial potentials of MTE+Alum and Alum respectively. The results indicate that MTE+Alum concentrations using DDM, inhibited all test bacterium at 100mg/ml and 50mg/ml, while MTE concentrations using DDM, inhibited all the bacterium at 100mg/ml, 50mg/ml and 25mg/ml with DIZ values almost comparable with the standard antibiotic control. Furthermore, the MTE and Alum+ MTE concentrations showed more beneficial antibacterial activity than Alum concentrations. The MTE+Alum concentrations against all test bacterium which suggests synergism in antibacterial activity, almost comparable to the commercial antibiotic standard (Table 3). The study also indicated that Physicochemical Parameters such as pH values and Temperature were within World Health Organization (WHO) and Nigerian Standard of Drinking Water Quality (NSDWQ) acceptable limit. Biochemical characterization confirmed the presence of *Enterobacter asburiae*, *Serratia fonticola*, *Citrobacter freundii*, *Klebsiella singaporensis*, *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Enterobacter cloacae* and *Escherichia spp.* This study has shown that there is significant potential for treating infections caused by *E. coli* strains using the extracts of methanolic turmeric and alum synergism. Alum can be used as an inhibitor and treatment of bacteria growth in water, especially for *Escherichia coli O157:H7*.

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Figure 1: **Image of Methanolic Turmeric Extract Inhibitory Zone.**



Figure 2: **Image of Alum, Methanolic + Alum Inhibitory Zone**



Figure 3: **Image of Turmeric Plant**