Original Research Article

ANTIBIOGRAM AND EFFECT OF METHANOLIC TURMERIC EXTRACT AND ALUM ON *ESCHERICHIACOLI* IN BOREHOLE AND SACHET WATER IN PORT HARCOURT.

Comment [s1]: Make it within 12 words

ABSTRACT

The study objective were to investigate the antibiogram and effects of methanolic turmeric extract and alum on different strains of *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. In contrast, there was no inhibitory zone with MTE+Aum at 12.5 concentration whereas diameter inhibitory zone (DIZ) values for methanolic turmeric extract ranged from 8.0-16.0mm by DDM. The susceptibility of these bacterium to the 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 exhibited the overall largest mean DIZ values. However, the activity of chloramphenicol which 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 the most 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*. Four (4) *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, Amikacin and Vancomycin respective. They were resistant to Cefotaxime (75%) while (50%) by Cefuroxime, Meropenem, Chlorpromazine, Tetracycline and Cotriazole (25%) only. Multiple antibiotic resistance index for test isolates revealed 3(75%) of the four (4) *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 for the three sampling locations Ikwerre, Obio/Akpor and Phalga.Faecal Coliform Count revealed that there was no significant difference($p>0.05$) in their mean counts with sachet water having the highest mean values of (2.77±0.14, 2.76±0.09 and 2.77±0.17) Log_{10}CFU/ml while borehole water revealed least mean counts of (1.46±1.29, 0.79±1.18 and 1.86±1.44) Log_{10}CFU/ml. Results of most probable number (MPN) for sachet water samples revealed that PHALGA has MPN Index of (2, 33, 2) per 100ml, Ikwerre (23, 7, <2) per 100ml and Obio/Akpor (2, 17, 2) per 100ml while for borehole water samples revealed that PHALGA has MPN Index of (33, <2, <2) per 100ml, Ikwerre (13, 23, <2) per 100ml and Obio/Akpor (<2, 17, 17) per 100ml. Results of pH and temperature values of the water samples in three sampling locations revealed moderate values within World Health Organization (WHO) and Nigerian Standard For Drinking Water Quality (NSDWQ) acceptable limit. Four (4) *E. coli* isolates with a prevalence of 100% was obtained as the test organisms. In conclusion, this study demonstrated the antibacterial potentials of MTE + Alum and Alum, which revealed significant prevalence of *E. coli* and unacceptable levels of *EnterohemorrhagicEscherichiacoli* (EHEC) in water samples from the study area, and can constitute a serious risk to public health.

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

Introduction

Turmeric

Comment [s2]: Please write AMR first then reasons followed by remedies

A native antimicrobial plant with the scientific name Curcuma longaL, 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 (Kress et al., 2012). 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 (Petrovska et al.). 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 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 (Lichterman et al., 2004).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.

Potassium Aluminium Sulfate (Alum)

The chemical compound KAL(S04)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 coagulates the particles, and as a result of high charge on aluminium ion with (AL3+).

In addition to being 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. In 2001, Chaudhury *et al.*

According to (Amadi et al., 2018), alum and Gongronema latifolium work together to kill some clinical microorganisms.

Escherichia coli

Due to its potential to carry virulence and antibiotic resistance genes, *E. coli O157:H7* stain has been recognised as a significant environmental and clinical pathogen (Bolukaoto *et al.*, 2019). 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*

stain has been acknowledged as a significant environmental and clinical pathogen due to its capacity to carry virulence and antibiotic resistance genes (Bolukaoto *et al.,* 2019). coli O157:H7 stain has been identified as an important environmental, clinical pathogen because it can harbor virulence and antibiotic resistance genes (Bolukaoto *et al.*, 2019).

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 (Lupindu et al., 2018). 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 (Adefisoye et al., 2016).

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 (Iwu et al., 2020). 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.

Materials and Methods

Sample Collection

The water samples were collected from the markets in the following local government areas: 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). They were then transported to Rivers State University's microbiology lab for analysis. Using a sterile plastic bag, a rubber of turmeric plant was procured from the fruit garden market in July and August of 2022, during the rainy season. The plants were delivered to the microbiology department for identification and extraction.

Preparation of Plant Extracts

Before extraction, the turmeric plants were washed in clean water (tap) to eliminate foreign objects, then they were stored in an oven set at 40°C for three to five days before being ground using a sterile hand homogenizer. The graded powder of 100g of pulverised turmeric was dissolved in 200 millilitres of methanolic solvent.

The mixture was covered in aluminium foil and left in the dark for two hours at room temperature to prevent exposure and evaporation. Using standard Whatman No. 1 filter paper and a sterile hand glove, the mixture was filtered into another sterile beaker of 250 ml, and the filtrate was then maintained in an oven at 44.5^{oC} to guarantee complete evaporation in the refrigerator.

Test bacterial pathogens

The test bacterial pathogens; such as strains of *Escherichia coli* were isolated from sachet and borehole water samples obtained from Ikwerre, Obio/Akpor and Phalga Local Government Area, Rivers State Nigeria.

The test bacterial pathogens, including strains of *Escherichia coli* were recovered from water samples taken from sachets and boreholes in Rivers State Nigerian, local governments of Ikwerre, Obio/Akpor and Port Harcourt.

Preparation for Combination of Turmeric Plant Extract (TPE) and Alum

Turmeric plant extract(TPE) and Alum (Vickers Laboratories Ltd.: England) 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 combination's potency.

Microbiological Analysis

Enumeration and Isolation of Total Heterotrophic Bacterial Counts. Using the spread plate method, the total heterotrophic bacteria was counted and isolated. One (1ml) of the water sample was extracted from each sample and aseptically transferred into test tubes containing 9ml of normal saline. Therafter, ten (10ml) of the principal sample was measured into a sterile beaker. This was serially diluted to 10^{-1}, 10^{-4} and 10^{-6} for borehole, 10^{-1} $10^{-3,}$ 10^{-4} for sachet water and direct inoculation was done also for all the samples, and an aliquot (0.1ml) with the aid of a sterile 1ml pipette was inoculated unto the surface of nutrient agar plates in duplicates. Same procedure was applicable to Eosine methylene blue (EMB), and the inoculated plates were incubated at temperature of 37℃ for 24-48 hours (Chesbrough *et., al* 2005). 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. (Cheesbrough *et al.*, 2005). **Characterization and Identification of bacterial Isolates**

According to (Bergy; et al., 2019), 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 subcultured on solid nutrient agar (NA) and eoisine methylene blue (EMB) and subsequently on slants of the respective agar media as mentioned above and persevered at refrigeration temperature. Identification of the isolates as bacteria was carried out as described in Cheesbrough, (2005).

Determination of Antibiotic Susceptibility Test for Methanolic Turmeric Extract and Alum

The antibacterial susceptibility test was performed by the disc diffusion method (DDM) using Bauer *et al*., 2019). 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 (Amadi *et al*., 2018). Thereafter, filter paper discs, made as described by Ochie and Kolhatker (2008) 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. And commercially supplied antibiotic chloramphenicol (CH 30µg as a positive control) which was placed on the surface-dried inoculated Mueller Hinton Agar (MHA) using sterile forceps. (Ablek Biologicals Ltd., UK). For disk 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 was placed into the agar plates of organism of interest using sterile forceps with the chloramphenicol which served as positive control respectively. The plates were incubated at 37ºc for 24 hours, after that the diameter of inhibition zones (DIZ) were measured using a transparent meter rule which is expressed in millimeters (mm). The diameter inhibition zone of different extracts was 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) CLSI, (2013).

Prevalence of Bacterial Isolates in all Samples

Four (4) *E. coli* isolates were found in the water samples taken 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, Serratiafonticola, Citrobacterfreundii, Klebsiellasingaporensis, Klebsiellapneumoniae, Pseudomonas sp., Staphylococcus sp., Bacillus sp., Enterobactercloacae* and *Escherichia spp.*The prevalence of test organisms *E. coli* in this study was high across all samples, at 75%. Similar results were observed by Azuonwu et al. (2019), 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 Nester's (2004) 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 *EnterohemorrhagicEscherichiacoli* (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 (Wemedo *et al.*, 2022).

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 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 effective medications on *E. coli* from this work while showing resistance to cefotaxime and co-triazole

and it is consistent with the results of the Kibret and Abera (2011), which showed that *E. coli* is most sensitive to Gentamycin, Ciprofloxacin and Chloramphenicol isolated from clinical samples.

Antibiotic Susceptibility Test of Plant 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. One of these resources is public health, and countless studies have demonstrated that natural compounds have antibacterial effect against pathogenic or spoilage organisms (El-Azzouny et al.,2018) A thorough examination of public health resources may reveal this conduct. As the spectrum of pathogenic drug resistance widens, 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 extracts inhibited their growth effectively at 100mg/ml. The susceptibility test of CP089272*Escherichia coli0157: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 and 50mg/ml and 25mg/ml respectively. The antibiotic turmeric extract of CP64383*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. Saeed (2014) described the antiseptic effect of turmeric (Curcuma longaL) extraction on the Bacterial Growth *Escherichia coli* that cause food poisoning. 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 (Davis and Brown, 2016; Krumperman, 2016). 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 (Davis and Brown, 2016).

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.

Table 1: Antibacterial Activity of Methanolic Turmeric (Curcuma longaL) Extracts

Bacterium **Diameter of inhibitory zone (DIZmm)**

Concentration of MTE (%)

Comment [s3]: Attach images of antibiotics sensitivity tests

Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	30µg/ml (CHL)
LG142154Enterohemorrhagic Escherichia coli	15.0	10.0	10.0	8.0	28.0
AF099077Escherichia coli	14.0	8.0	8.0	0	29.0
CP089272Escherichia coli 0157:H7	16.0	8.0	6.0	0	43.0
CP064383Escherichia coli 0157:H7	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)
LG142154Enterohemorrhagic Escherichia coli	8.0	8.0	8.0	0	25.0
AF099077Escherichia coli	12.0	9.0	8.0	0	0
CP089272Escherichia coli 0157:H7	12.0	10.0	0	0	32.0
CP064383Escherichia coli 0157:H7	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)**
LG142154Enterohemorrhagic Escherichia coli	18.0	10.0	5.0	0	25.0
AF099077Escherichia coli	14.0	12.0	0	0	0
CP089272Escherichia coli 0157:H7	14.0	12.0	8.0	0	34.0
CP064383Escherichia coli 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	*CP064383Enterohemorrhagic E. coli*	-	-	-	15	16	25	-	25	13	26	22	21
2	*CP089272E. coli*	22	-	-	-	-	20	-	20	21	26	18	20
3	*AF099077E. coli 0157:H7*	16	15	20	17	12	20	18	15	10	14	13	10
4	*LG142134E. coli 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	*CP064383Enterohemorrhagic E. coli*	R	R	R	R	S	S	R	S	R	S	S	S
2	*CP089272E. coli*	S	R	R	R	R	S	R	S	S	S	S	S
3	*AF099077E. coli 0157:H7*	I	R	I	I	I	S	I	I	R	R	R	R
4	*LG142154E. coli 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).

RESULTS AND DISCUSSION

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

Antibiotics	Conc. (µg)	Resistant(R) n (%)	Intermediate(I) n (%)	Susceptible(S) 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(250)	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 (Log_{10}CFU/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) Log_{10}CFU/ml while sachet water revealed least mean counts of (2.82±0.21, 2.85±0.18 and 2.77±0.17) Log_{10}CFU/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) Log_{10}CFU/ml while borehole water revealed least mean counts of (1.46±1.29, 0.79±1.18 and 1.86±1.44) Log_{10}CFU/ml respectively.

Table 7: Temperature and pH values of the sachet package water samples.

SAMPLE	TEMPERATURE (ºC)	pH
OPT A	29	7.3
WIN B	27	7.2
ND&S C	28	7.5
TSM D	27	6.9
REN E	27	5.9
GHP F	29	6.6
MAG G	28	6.8
HEM H	25	6.8
NAV I	25	4.4

Borehole samples

List 3: Temperature and pH values of the borehole water samples

SAMPLE	TEMPERATURE (ºC)	pH
MILL A	27.2	6.4
AC B	27.2	6.0
IK C	27.3	6.5
GH D	27.6	6.3
FH E	27.6	6.6
GW F	28.1	6.3
YC G	27.3	6.4
RC H	28.1	6.5
MILE 3	27.2	6.4

Table 8: Complete Test for MPN Results of Sachet Water

Water Samples	Location	Presumptive Test	Confirmatory Test	Complete Test (EMB)	Temperature (37^0C)	(45^0C)
GHP	**PHALGA**	1	1	2	1	1
REN		5	4	4	3	1
TSM		1	1	2	1	1
NAV	**IKWERRE**	5	2	4	2	2
MAG		3	3	5	2	3
HEM		0	0	0	0	0
OPT	**OBIO/AKPOR**	1	1	2	1	1
ND&S		6	6	12	6	6
WIN		0	0	0	0	0

List 4 : Complete Test for MPN Results of Borehole Water

Water Samples	Location	Presumptive Test	Confirmatory Test	Complete Test (EMB)	Temperature (37^0C)	(45^0C)
GEWC	**PHALGA**	5	3	1	0	1
RCCG		0	0	0	0	0
YORUBA CHURCH		0	0	0	0	0
IK OIL MIL	**IKWERRE**	5	2	4	2	2
STEPHEN		5	4	2	1	1
IK HOUSE		4	4	2	1	1
FELIX HOUSE	**OBIO/AKPOR**	0	0	0	0	0
MILE 3		4	2	2	1	1
GOLD HOUSE		4	3	1	0	1

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 Kim et al.(2005). Chloramphenicol showed the highest mean DIZ values on CP089272 against all the test bacterium (Table 1). This confirms the fact that standard antibiotics are puried 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 (Amadi et al., 2018). 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 in Fakeye et al.'s (2018) investigation was also found to be greater than the World Health Organization's (WHO) suggested limit. 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. The detection of higher Most Probable Number (MPN) indices on the sachet and borehole water samples observed during this study's examination revealed that they were within World Health Organization (WHO) permissible limits on water quality of 2.2 MPN total coliform per 100ml which indicates the water sources should be treated (WHO, 2012). The technique was used to

estimate microbial populations in the water samples that contain particulate materials which interferes with plate count enumeration methods used above (Cappuccino and Sherman, 2008).

Conclusion

The present study demonstrated the antibacterial potentials of MTE+Alum and Alum respectively. The results indicate that MTE+Aum 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, Serratiafonticola, Citrobacterfreundii, Klebsiellasingaporensis, Klebsiellapneumoniae, Pseudomonas sp., Staphylococcus sp., Bacillus sp., Enterobactercloacae* 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 ca be used as an inhibitor and treatment of bacteria growth in water, especially for *Escherichia coli O157:H7.*

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