International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Antibacterial Efficacy of Some Generic Antibacterial Agents and Extracts of *Occimum Gratissimum* on Selected Enteric Pathogens

***Godwin Edo Ochiheche* [a], *Ibiam Terkaa Philemond* [b]**

[a] *Department of Science Laboratory Technology, Fidei Polytechnic Gboko, Benue State*
[b] *Health Informatics Unit, APIN Public Health Initiative*
E-mail: ochihechegodwin@yahoo.com, +2347060646709

ABSTRACT

The antibacterial efficacy of selected generic antibacterial agents (ofloxacin, nitrofurantoin, cefprozil, gentamicin, cefuroxime and cefixime) and that of ethanol and aqueous extracts of *Ocimum gratissimum* leaves were measured using the Kirby-Bauer Disk Diffusion Assay method, and the efficacies compared in light of their efficacies on *Escherichia coli* and *Salmonella typhi*. Phytochemical analysis of the ethanol and aqueous extracts of *Occimum gratissimum* revealed the presence of alkaloid, flavonoid, saponin, tannin, steroid, glycoside and reducing sugar. Saponins were the highest in concentration among the phytochemicals, with mean concentrations of 6.81±0.01. The least phytochemical was tannin with 0.00±0.00. The ethanol and aqueous extracts of *Occimum gratissimum* all inhibited isolates of *E. coli* and *S. typhi*, with extracts of Occimum gratissimum showing significant efficacy against both isolates. All the generic antimicrobials, except cefuroxime and cefixime, inhibited the isolates, with ofloxacine proving to be the most efficient. It was observed that the generic antimicrobials were slightly significantly more reactive than the *O. gratissimum* extracts on *E. coli* and *S. typhi* ($P<0.05$). This slight difference in efficacy between the plant extracts and the generic antimicrobials could have been as a result of the varying concentration types or level applied in the study, compared to that already formulated for the generic antimicrobials used. The observed high antimicrobial activity on *S. typhi* shows that *Occimum gratissimum* could be used as a potent substitute in the treatment of typhoid fever. The minimum inhibitory concentration (MIC) of the extracts of *Occimum gratissimum* on *E. coli* and *S. typhi* showed that all the extracts presented low MIC (1.33x103 mg/ml: 50% concentration of extracts) across both isolates. The low MIC values observed on *E. coli* and *S. typhi* shows that lesser concentrations of the extracts would still be very effective against *E. coli* and *S. typhi*. The observed high antimicrobial activity of the extracts on *S. typhi* shows that *Occimum gratissimum* could be used as a potent combination in the treatment of typhoid fever.

Key Words: Generic Antibacterials, Enteric Pathogens, Phytochemicals

1.1 Introduction

For more than 60 years, antibacterial drugs have been regarded as the panacea for infections, whether or not their use is appropriate, and whether the infection is acquired in the community or in the hospital setting (WHO, 2014). Herbal medicine has its root in prehistory making every bit as ancient tradition as farming or cooking. In the Graeco-Roman era, Hippocrates (father of medicine), Theophrastus (father of botany), Galen (originator of pharmaceutical galenicals) and Dioscoroides were all herbalists (Frey and Meyers, 2010).

There is this widely held view that over 80% of people in developing countries use herbal medicines as their first line of choice in the treatment of diseases (Osemene *et al*., 2011).

It is also noted that a high percentage of the rural populace patronize traditional midwifery for their maternal and neonatal health problems. Traditional Birth Attendants (TBA5) assists in majority of birth delivery of pregnant women in most Nigerian villages and communities (Adesina, 2013).

There are myriads of serious challenges militating against the progress and development of Traditional Medical practice in Nigeria. Although a lot of progress has been made in implementing the regional strategy on promoting the role of traditional medicine in Health Care System (Anna, 2013), the Nigerian state has continued to face some challenges that hamper the institutionalization of traditional medicine into our National Health Care Systems (Ekeopara and Ugoha, 2017).

Unlike generic antibacterial agents, traditional antibacterial agents in the African setting are generally employed to remedy disrupted physiological processes in order to restore homoeostasis rather than meet disease head on. By enhancing the body's own healing mechanisms, disease may be eliminated in a process that is usually slow, requiring the patients to be very patient. The plants used in herbal medicine carry their own in-built safety mechanisms. Furthermore, they are ideal tools to restore damaged physiological processes since they consist of a multiplicity of chemical components which act

synergistically to make active bio-constituents available or to buffer the otherwise potentially powerful active principles thus preventing harmful side effects.

2.0 Methodology

2.1 Sample Collection

2.1.1 Plant Materials

Fresh *Occimum gratissium* (commonly called *Kungureku* in Tiv) were bought from the Wurukum Market in Makurdi, on the advice of traditional medicine practitioners in Makurdi, The collected plants were identified and authenticated with the aid of literatures by Shomkegh *et al*. (2016), under the guidance of Gemanen Iormanger, a botanist and Divisional Forestry Officer with the Benue State Ministry of Water Resources and Environment.

2.1.2 Organisms

Isolates of *Escherichia coli* and *Salmonella typhi,* were obtained from the microbiology laboratory of theBenue State University Teaching Hospital Makurdi, Benue State, Nigeria.

2.2 Sample treatment and preparation

2.2.1 Processing of the plant parts

Occimum gratissium (leaves) were cleaned of debris and air-dried at room temperature for 14 days. The dried leaves were then pulverized into fine powder. The fine plant parts' powders were measured and weighed. After weighing, *Occimum gratissium* leaves weighed 200g. Powdered samples were subsequently subjected to ethanol and aqueous extractions using the soxhlet extraction technique.

2.2.1.1 Ethanol Extract

The plant materials were subjected to extraction in a soxhlet extractor, as described by Yeh *et al*. (2014) using 25ml of 40% ethanol in a process that lasted for 24 hours. The wet extracts were filtered using Whattmanno.1 filter paper and the filtrates were then evaporated to dryness at 60ºC in a regulated water bath. The resulting extracts of *Occimum gratissium* (leaves) after weighing was 0.3g.

2.2.1.2 Aqueous Extract

The pulverized dried extracts of *Occimum gratissium* (leaves) were soaked separately in sterile distilled water at different concentrations, each in a ratio of 1:1; 2:1; 3:1; W/V. The resulting suspensions were boiled for 40 minutes and filtered through a Whatman no.1 filter paper into a glass funnel. The resulting filtrates were evaporated to dryness at 60°C in a regulated water bath (Yeh *et al*., 2014). The resulting extracts were weighed to produce 3.2g of *Occimum gratissium* (leaves) respectively.

2.2.1.3 Sterility testing of the extracts

The aqueous and ethanol extracts of the plants were tested for sterility using the method of Dalitha (2008). One milliliter (1ml) of each extracts was added to test tubes containing 5ml of sterile nutrient broth. They were then incubated at 37°C for 24hrs. The presence of growth, which was determined by the observation of visible microbial colonies, or absence of growth signified contamination or sterility of the extract respectively. Sterile extracts were subsequently used for further microbial analysis in the work, while contaminated extracts were discarded and new extraction carried out.

2.3 Phytochemical analysis of Occimum gratissium extracts

The extracts were subjected to qualitative and quantitative phytochemical tests for alkaloids, saponins, flavonoids, cardiac glycosides, steroids, reducing sugars and tannins.

2.3.1 Qualitative Phytochemical Screening

i. Test for tannins (Ferric Chloride Test)

To about 5ml of the aqueous extract in the test tube, 3-5 drops of 0.1% ferric chloride was added and observed for brownish green, dirty green, or a blue-black precipitate showed the presence of tannins (Okerulu and Ani, 2001).

ii. Test for Flavonoids (Shinoda Test)

Magnesium sulphate powder (0.5g) and 2-3 drops of concentrated HCl was added to 3ml of each water extract. A red coloration indicated the presence of flavonoids (Hassan *et al.,* 2004).

iii. Test for Saponins (Frothing Test)

Ten millimiter of the aqueous extract was vigorously shaken with water in a test tube. Frothing, which persists on warming was taken as a preliminary evidence for the presence of saponins (Hassan *et al.,* 2004).

iv. Test for Alkaloids

A quantity ($1cm^3$) of 1% aqueousHCl was added to $3cm^3$ of each extract in a test-tube and the mixture heated for 20min, cooled and filtered. $1cm^3$ portion of the filtrate was treated with two drops of Wagner's reagent. Formation of cream or brown precipitate respectively indicated the presence of alkaloids (Sofowora, 1993).

v. **Test for Steroids (Lieberman-Burchand Reaction)**

One millimiter of concentrated $H_2SO_4$was added to 1ml of each extract. A red colouration indicated the presence of steroids (Hassan *et al.,* 2004).

vi. Test for Cardiac Glycosides

Ten millimeter of 50% H_2SO_4 was added to 1ml of the extracts of plants in a test tube. The mixture was heated in boiling water for 15 minutes. 2ml of Fehling's solution was added and the mixture boiled. A brick-red precipitate indicated the presence of glycosides (Okerulu and Ani, 2001).

vii. Test Reducing Sugars

Three drops of Fehling's solution I and II were added to 5ml of the filtrate and heated over a water bath to boil. A red precipitate indicates the presence of reducing sugars.

2.3.2 Quantitative Phytochemical Screening

i. Total Tannins Content Determination:

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 395 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract (Prabhavathi *et al.,* 2016).

ii. Total Flavonoids Content Determination:

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Edeoga *et al.,* 2005).

iii. Total Saponin Content Determination:

The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven at 70ºC to a constant weight; the saponin content was calculated as percentage (Obadoni and Ochuko, 2001).

i. Total Alkaloid Content Determination:

Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Edeoga *et al.,* 2005).

ii. Total Steroid Content Determination:

One milliliter (1ml) of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±2ºC for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Madhu *et al.,* 2016).

iii. Total Cardiac Glycosides Content Determination:

Eight millilitres (8ml) of plant extract was transferred to a 100ml volumetric flask and 60ml of H_2O and 8ml of 12.5% lead acetate were added, mixed and filtered.50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO4 were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. 10ml of purified filtrate was transferred into clean Erlyn – Meyer flask and treated with 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495nm (El-Olemy*et al.,* 1994).

vii. Total Reducing Sugar Content Determination:

To 1ml of sample and standard solutions, 1ml of water was added and mixed thoroughly. 3ml of DNSA reagent was then added to the mixture. The tubes were heated to boiling in a water bath for 10 minutes. The tubes were taken out of the water bath and immediately 5 ml of water was added to stop the reaction. After mixing properly, the extent of reducing sugar in the sample was measured at an absorbance of 595 nm.

2.4 Culture media used

All media used were prepared according to manufacturers' specifications. Nutrient broth, MacConkey agar and Mueller Hilton agar were prepared and sterilized at 121ºC for 15 minutes.

2.5 Isolation and identification of test organisms

Colonies of *Escherichia coli* and *Salmonella typhi* collected from the Microbiology Laboratory of the Benue State University Teaching Hospital were subjected to cultural, morphological and biochemical characteristics as described by Cheesbrough (2000) and compared with criteria in the Bergey's Manual of Determinative Bacteriology (1993) to confirm the isolates.

2.6 Antibacterial susceptibility testing

2.6.1 Kirby-Bauer Disk Diffusion Assay:

Sterile circular 20mm discs made out of whattman No.1 filter paper were soaked in the various aqueous and ethanol plant extracts (leaves of *Occimum gratissimum*), and placed on freshly prepared Mueller Hilton agar already inoculated with *Escherichia coli* and *Salmonella typhi.* This was carried out for various concentrations of each plant extract, 100% (2000mg/ml), 75% (1666.67mg/ml) and 50% (1333.33mg/ml). The various generic antibacterials (ofloxacin, nitrofurantoin, cefprozil, gentamicin, cefuroxime and cefixime) were also analysed for their respective antimicrobial efficacies each on *Escherichia coli* and *Salmonella typhi* isolates. The plates were incubated aerobically at 37ºC and examined for zone of inhibition after 24 hours. Each zone of inhibition was measured with a ruler in millimeter. Visible and measurable zones of inhibition represented a positive antibacterial activity of the extracts on the enteric isolates, while a lack of visible inhibition zone represented absence of antibacterial activity.

2.7 Determination of Minimum Inhibitory Concentration (MIC) of Extracts

Five milliliters (5ml) of nutrient broth was dispensed into test tubes and sterilized by autoclaving at 121ºC for 15 minutes. A loop full of isolates of *Escherichia coli* and *Salmonella typhi* were suspended in five millilitres (5 ml) of sterile distilled water to form an isolate solution for each of *Escherichia coli* and *Salmonella typhi.*One milliliter (1ml) of the solution was inoculated into the tubes of the nutrient broth. The MIC of the extracts was determined for the test organisms in triplicates at the varying concentrations (100%, 75% and 50%). The mixture of the inoculated nutrient broth and the various concentrations of the plant extracts were incubated at 37ºC for 24hours. After incubation, the presence or absence of growth on each tube was rated.

2.8 Statistical Analysis

All value for the zone of inhibition diameters were expressed as means. The data obtained for the sensitivity of the test organisms to the plant extracts and generic antibacterials, as well as duplicate quantities of phytochemicals in each of the plant extracts, were analyzed using two-way analysis of variance (ANOVA). The differences among group means were analyzed using the Dunnett's multiple comparison test. P value < 0.05 was considered as significant.

3.0 Results

3.1 Percentage yield of extracts

The solvent extraction process employed for the three plant parts were ethanolic sohxlet extraction and aqueous extraction. The aqueous extraction process yielded a relatively higher amount of *Occimum gratissium* leaves extract, in grams, than the ethanol extraction process; as seen with 1.60g. The ethanol-based soxhlet extraction method, as conversely, yielded 0.15g of extracts. The results of the extraction are summarized in Table 1 below. The results indicated the weight of the extract from both extraction processes as well as the percentage yield of the extracts, amongst others.

Table 1: Yield of Plant Extracts

Plant Material (Part)	Extraction Solvent	Weight of Plant Material (g)	Weight of Extract (g)
***O. gratissimum* leaf**	Aqueous	200.00	1.60
	Ethanol	200.00	0.15

3.2 Phytochemical contents of plant extracts

The result of the phytochemical screening of aqueous extracts of *Ocimum gratissimum* are as presented in Table 2 and 3. Saponin had the highest concentration among the phytochemicals, with mean concentrations of 6.81±0.01. The least phytochemical was tannin with no yield in *O. gratissimum*.

Table 4 presents phytochemical contents of *O. gratissimum* extracts. Saponin had the highest concentration with mean values of 4.04±0.01. Steroid had the least concentration among the phytochemicals with mean values of 0.24±0.01, 0.66±0.01 and 0.99±0.01 respectively.

Table 2: Qualitative phytochemical constituents of *O. gratissimum* extracts

Phytochemical	Extraction reagent/Test	*O. gratissimum*
Alkaloid	wagner's reagent	+
Flavonoid	Shinoda	+
Saponin	Frothing	+
Tannin	Ferric chloride	-
Steroid	Lieberman-Burchard	-
Glycoside	Fehling's solution	-
Reducing sugar	Fehling's solution	+

+ = Presence

- = Absence

Table 3: Phytochemical Contents of Aqueous *O. gratissimum* Extracts (g/100g)

Phytochemical	*O. gratissimum*
Glycoside	1.33 ± 0.01^{ab}
Tannin	0.00 ± 0.00^{a}
Saponin	6.81 ± 0.01^{ef}
Flavonoid	0.64 ± 0.01^{a}
Steroid	1.25 ± 0.01^{ab}
Reducing sugar	1.66 ± 0.03^{ab}
Alkaloid	1.88 ± 0.02^{ab}

Values are means of duplicates.

Means with different superscripts on the same row differ significantly (P<0.05)\

Table 4: Phytochemical Contents of Ethanol *O. gratissimum* Extracts (g/100g)

Phytochemical	*O. gratissimum*
Glycoside	0.77±0.01[a]
Tannin	0.00±0.00[a]
Saponin	4.04±0.01[cd]
Flavonoid	2.03±0.04[ab]
Steroid	0.99±0.01[ab]
Reducing sugar	2.01±0.01[c]
Alkaloid	2.86±0.01[c]

Values are means of duplicates.

Means with different superscripts on the same row differ significantly (P<0.05)

3.3 Antibacterial zone of inhibition of plant extracts and generic antibiotics

3.3.1 Antibacterial efficacy of O. gratissimum bulb extract

Table 5 presents the zone of inhibition (mm) of *O. gratissimum* leaf extracts and generic antimicrobials on *E. coli* and *S. typhi*. *E. coli* showed OFL with the highest mean zone of inhibition of 8.00±2.00 and CRX with no visible inhibition zone than 50% of the generic drugs (CRX 0.00±0.00, GEN 3.67±0.58, and CXM 1.00±0.00), with a mean zone of 3.89±2.70. On the other hand, *S. typhi* had CPR with the highest inhibition zones. Comparatively, the aqueous *O. gratissimum* extract had a significantly higher (P<0.05) inhibition zone (6.61±2.07) than all the generic antimicrobials except CPR which had a slightly higher zone of inhibition.

Table 5: Activity of *O. Gratissimum* Leaf Extract and Generic Antimicrobials on Test Organisms

Antimicrobial	Zones of Inhibition (mm)	
	Escherichia coli	*Salmonella typhi*
Ethanol Extract	2.00±1.00[c]	4.00±1.00[c]
Aqueous Extract	3.89±2.70[cd]	6.61±2.07[de]
OFL	8.00±2.00[f]	6.00±1.00[cd]
NIT	4.67±1.16[d]	1.67±0.58[a]
CPR	5.33±0.58[d]	7.67±1.53[e]
CRX	-	-
GEN	3.67±0.58[cd]	4.33±1.53[c]
CXM	1.00±0.00[ab]	-

Key: OFL(Ofloxacin); NIT(Nitrofurantoin); CPR(Cefprozil); CRX(Cefuroxime); GEN(Gentamicin); CXM(Cefixime).

Values are means of duplicates.

Means with different superscripts on the same row differ significantly (P<0.05)

3.3.1.1 Antibacterial efficacy of ethanol plant extracts and generic antibacterials

Table 6 presents the zone of inhibition of ethanol plant extracts and generic antimicrobials on *E. coli* and *S. typhi*. OFL had the highest mean inhibition zone against *E. coli* (8.00±2.00) CRX showed no visible inhibition zone. CPR and NIT had significantly higher inhibition zones of 5.33±0.58 and 4.67±1.16 against *E. coli* respectively. Comparatively, there was a significant difference (P<0.05) between the generic antimicrobials and the ethanol

plant extracts, to the favour of the generic antimicrobials. On the other hand, *S. typhi* had CPR with the highest inhibition zone of 7.67±1.53, followed by OFL (6.00±1.00). There was a significant difference (P<0.05) between the inhibition zones of the antimicrobials against *S. typhi* isolates.

Table 6: Activity of Ethanol Plant Extracts and Generic Antimicrobials on Test Organisms

Antimicrobial	**Zones of Inhibition (mm)**	
	Escherichia coli	***Salmonella typhi***
***O. gratissimum* (Ethanol)**	$2.00±1.00^{b}$	$4.00±1.00^{cd}$
OFL	$8.00±2.00^{ef}$	$6.00±1.00^{cde}$
NIT	$4.67±1.16^{d}$	$1.67±0.58^{ab}$
CPR	$5.33±0.58^{d}$	$7.67±1.53^{ef}$
CRX	$0.00±0.00^{a}$	$0.00±0.00^{a}$
GEN	$3.67±0.58^{cd}$	$4.33±1.53^{cd}$
CXM	$1.00±0.00^{a}$	$0.00±0.00^{a}$
LSD	1.54^{a}	1.95^{a}

Key: OFL(Ofloxacin); NIT(Nitrofurantoin); CPR(Cefprozil); CRX(Cefuroxime); GEN(Gentamicin); CXM(Cefixime); LSD(Least Significant Difference)

Values are means of duplicates.

Means with different superscripts on the same row differ significantly (P< 0.05)

3.3.1.2 Antibacterial efficacy of aqueous plant extracts and generic antibacterials

Table 7 presents the inhibition zones (mm) of aqueous plant extracts and generic antimicrobials on *E. coli* and *S. typhi. E. coli* showed OFL with the highest mean inhibition zone of 8.00±2.00, while the least was CRX with no visible inhibition zone. Aqueous extracts of *O. gratissimum*, NIT, CPR and GEN also presented significantly higher mean zones of 3.89±2.70, 4.67±1.16, 5.33±0.58 and 3.67±0.58 respectively. There was a statistically significant difference (P<0.05) between the antimicrobials as seen against *S. typhi*.

Table 7: Activity of AqueousPlant Extractsand Generic Antimicrobials on Test Organisms

Antimicrobial	**Zones of Inhibition (mm)**	
	Escherichia coli	***Salmonella typhi***
***O. gratissimum* (Aqueous)**	$3.89±2.70^{cd}$	$6.61±2.07^{de}$
OFL	$8.00±2.00^{e}$	$6.00±1.00^{cde}$
NIT	$4.67±1.16^{d}$	$1.67±0.58^{ab}$
CPR	$5.33±0.58^{d}$	$7.67±1.53^{ef}$
CRX	$0.00±0.00^{a}$	$0.00±0.00^{a}$
GEN	$3.67±0.58^{cd}$	$4.33±1.53^{cd}$
CXM	$1.00±0.00^{a}$	$0.00±0.00^{a}$
LSD	2.12^{a}	1.84^{ab}

Key: OFL(Ofloxacin); NIT(Nitrofurantoin); CPR(Cefprozil); CRX(Cefuroxime); GEN(Gentamicin); CXM(Cefixime); LSD(Least Significant Difference)

Values are means of duplicates.

Means with different superscripts on the same row differ significantly (P<0.05)

3.4 Minimum inhibitory concentration of plant extracts on test organisms

Table 8 presents the minimum inhibitory concentrations (mm) of *O. gratissimum* on *E. coli* and *S. typhi* respectively.

On *Escherichia coli*, both ethanol and aqueous extracts of *O. gratissimum* leaves all had a minimum inhibitory concentration of 1.33×10^{3} mg/ml (50% concentration of extracts). Aqueous extracts of *O. gratissimum* had an MIC of 1.33×10^{3} mg/ml.

Table 8: Minimum Inhibitory Concentration (MIC) of Plant Extracts

Plant extract (extraction solvent)	Minimum Inhibitory Concentration (MIC) (mg/ml)	
	Escherichia coli	***Salmonella typhi***
***O. gratissimum* (Ethanol)**	1.33×10^3	1.33×10^3
***O. gratissimum* (Aqueous)**	1.33×10^3	1.33×10^3

4.0 Discussion of findings

The phytochemical analysis of the *Ocimum gratissimum* extracts revealed the presence of alkaloid, flavonoid, saponin, and reducing sugar were found to be present in *Ocimum gratissimum*. The presence of these phytochemicals reiterates the importance of this plant in the field of traditional medicine practice.

The presence of these phytochemicals in the plant extracts would definitely have accounted for the visible antimicrobial activities observe in *O. gratissimum* against *E. coli* and *S. typhi*. The prevalence on *Escherichia coli* agrees the work by Okigbo and Igwe (2007) who stated that the antidiarrhoeal activity of many plants have been found to be due to the presence of tannins, alkaloids, saponins, flavonoids and steroids. All these phytochemicals were prevalent in the extracts of *O. gratissimum.*

The antimicrobial activity of an extract can be due to the synergistic interactions of several secondary metabolites, which cannot be detected when single compounds alone are evaluated (Mulyanigsih *et al.,* 2010). These extracts are often used to treat a broad spectrum of health disorders (Wink, 2015).

Alkaloid is an antibacterial and antifungal compound which primarily inhibits microorganisms and reduces the risk of fungal infection (Saxena *et al*., 2013). Flavonoids are polyphenolic compounds with biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities, and most notably, their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Saxena *et al*. 2013). Tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors (De Bruyne *et al*., 1999), and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al*., 2005). Saponins have been observed to kill protozoans and mollusks, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals, and to act as antifungal and antiviral (Morrisey and Osbourn, 1999; Traore *et al*., 2000).

The results of the test of the antimicrobial efficacy of all the extracts showed *Ocimum gratissimum* all inhibited isolates of *Escherichia coli* and *Salmonella typhi* at varying levels as shown by their respective inhibition zones. On the other hand, all the generic antimicrobials used (Ofloxacin, Nitrofurantoin, Cefprozil, Cefuroxime, Gentamicin and Cefixime) were effective on *E. coli* and *S. typhi*, except cefuroxime (inactive against *E. coli* and *S. typhi*) and cefixime (inactive against *S. typhi*).

The mean zones of inhibition shown by generic antimicrobials on the *E. coli* and *S. typhi* isolates were slightly significantly higher than the zones showed by the extracts of *O. gratissimum*. This difference could have been due to the the fact that generic antibacterials contain certain additives or adjuncts that could also facilitate their antibacterial efficacies, as against the pure plant extracts used in this study.

O. gratissimum extracts (ethanol and aqueous) had significantly high ($P<0.05$) mean zones of inhibition against *E. coli* and *S. typhi*. This is in agreement with previous works by Adebolu and Salau (2005) which resolved that steam distillation extracts of *O. gratissimum* had inhibitory effects on *E. coli* and *S. typhi*; as well as Begum *et al*. (1993), Nwosu and Okafor (1995), Akinyemi *et al*, (2004), Janine de Aquino Lemos *et al*. (2005) and Lopez *et al*. (2005) who all stated that the antimicrobial efficacy of *O. gratissimum* could have been due to the presence of alkaloids, flavonoids, reducing sugars. Okigbo and Igwe (2007) also stated the presence of phenols, essential oils and peptides in *O. gratissimum*could also make it antimicrobial.

The observed antibacterial properties of the ethanolic and aqueous extracts of *O. gratissimum* compared to those observed in the generic antibaterials used suggests that the plant parts could very favourably be employed in the treatment of infections caused by *E. coli* and *S. typhi* but all more efficiently against *S. typhi*.

The result of the minimum inhibitory concentration (MIC) of the extracts of *O. gratissimum* on *E. coli* and *S. typhi* interestingly showed that all the extracts had low MIC across both isolates.

The MIC results agrees with findings by Adebolu and Salau (2005) who stated that low MIC for *O. gratissimum* against *E. coli* .

5.0 Conclusion

The presence of relevant phytochemicals in extracts of *O. gratissimum* proves that, as well as support previous findings, that these plants have antibacterial properties, adding to their other medicinal properties, making them a huge resource in the field of medicine.

The extracts of *O. gratissimum* leaves showed considerable inhibitory activity against *E. coli* and *S. typhi*, but mostly on *S. typhi*. This high efficacy could have been as a result of the presence of phytochemicals in the extracts. The observed high antimicrobial activity of the extracts on *S. typhi* shows that *O. gratissimum* could be used as a potent combination in the treatment of typhoid fever.

On the other hand, all the generic antimicrobials used (Ofloxacin, Nitrofurantoin, Cefprozil, Cefuroxime, Gentamicin and Cefixime) were effective on *E. coli* and *S. typhi*, except cefuroxime (inactive against *E. coli* and *S. typhi*) and cefixime (inactive against *S. typhi*).

The low MIC values observed across the plant extracts on *E. coli* and *S. typhi* shows that lesser concentrations of the extracts would still be very effective against *E. coli* and *S. typhi*. This low MIC could be as a result of the ability of the reactive phytochemicals in the extracts to be more reactive at lower concentrations.

Importantly, it was observed that the generic antimicrobials were relatively more reactive than the *O. gratissimum* extracts on *E. coli* and *S. typhi*. This slight difference between the plant extracts and the generic antimicrobials could have been as a result of the varying concentration types or level applied in the study, compared to that already formulated for the generic antimicrobials used. This difference could also have been due to the fact that only pure extracts of *O. gratissimum* were used in the study, as against the generic antimicrobials with other reactive additives added. This reactivity of the generic antimicrobials over the plant extracts was expected prior to the study, but the closeness of the reactivity of both sets of antibacterials was not expected as the plant extracts showed reasonable effects.

A better assessment of the comparison between the generic antimicrobials and extracts of *O. gratissimum* would have been more complete and more informative had an *in vitro* comparative study was carried out, which this work did not satisfy.

6.0 Recommendations

Based on the findings of this work, the reasonable antimicrobial activity *O. gratissimum* should pave way for a more comprehensive *in vitro* comparative study of both antimicrobials.

The use of extracts of *O. gratissimum* in the treatment of gastroenteritis and typhoid fever is highly recommended, especially a combination of the three plant parts in the local herb formulation.

REFERENCES

Adesina.S.K. (2013).Traditional Medical Healthcare in Nigeria.http: /www.google.com/search?hl=enGB&redir.. .es...

Bergey, D. H., & Holt, J. G. (1993). *Bergey's manual of determinative bacteriology. Baltimore*: Williams & Wilkins. 48

Cheesbrough, M. (2000). Microbiological test: District Laboratory Practice in Tropical Countries. In: Cremer, A. and Evan, G. (eds). Cambridge University Press, UK. 36-62, 132-143

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7): 685-688.

Ekeopara, Chike & M.I Rev. Ugoha, Azubuike. (2017). The Contributions of African Traditional Medicine to Nigeria's Health Care Delivery System..*IOSR Journal of Humanities and Social Science*. 22. 32-43. 10.9790/0837-2205043243.

El- Olemy M. M. Farid J. A. and Abdel- Fattah A. A. (1994): Ethanol Extract of P. stratiotes; *NISEB Journal* 1(1) 51-59.

Frey, F.M. and Meyers, R. (2010) Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York. *BMC Complementary and Alternative Medicine*, 10, 64-68. Hdoi:10.1186/1472-6882-10-64.

Hassan, M.M., A.O. Oyewale, J.O. Amupitan, M.S. Abdullahi and B. Okonkwo, (2004). Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of Detarium microcapum. *Journal of Chemical Society ofNigeria*., 29: 26-29.

Madhu, M; Sailaja, V; Satyadev, TNVSS; Satyanarayana;MV (2016).Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents.*Journal of Pharmacognosy and Phytochemistry*; 5(2): 25-29.

Obadoni, B.O. and Ochuko, P.O. (2001) Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*. 8, 203-208.

Okerulu, Isaac & Ani, C.J.. (2001). The phytochemical analysis and antibacterial screening of extracts of *Tetracarpidium conophorum*. *Journal of the Chemical Society of Nigeria*.. 26. 223-228.

Osemene, K.P.Elujoba A.A. and Ilori M.O. (2011). A Comparative Assessment of Herbal and Orthodox Medicines in Nigeria.*Research Journal of Medical Sciences*(5):5 :280-285.

Prabhavathi R. M., Prasad M. P. and Jayaramu M. (2016).Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis.*Journal Advanced and Applied Scientific Research*. 7(4):11-17.

Shomkegh, S.A; Mbakwe, R; Dagba, B. I. (2016). Utilization of wild plants for medicinal purposes in selected Tiv communities of Benue State, Nigeria: An Ethnobotanical approach. *European Journal of Medicinal Plants*. 14(4): 1-14. ISSN: 2231-0894.

Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria.191-289.

World Health Organization (2014). Antimicrobial resistance: global report on surveillance. *WHO Library Cataloguing-in-Publication* Data. WHO Press, Geneva. ISBN 978 92 4 156474 8.Page 1.

Yeh, H.Y; Chuang, H.C; Chen, C.J; Wan, T.L; Lin, L.Y. (2014). Bioactive components analysis of two various gingers (Zingiber officinale Roscoe) and antioxidant effect of ginger extracts. *LWT-Food Science and Technology*.67.