

RESEARCH PAPER

**ANTIBACTERIAL EFFECT OF *OCIMUM GRATISSIMUM*
AGAINST BACTERIA FROM PAEDIATRIC DIARRHOEAL
STOOL SAMPLES IN THE TROPICS**

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ABSTRACT

The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multidrug resistant bacteria has spurred scientists on the research for plant based antimicrobial of therapeutic potentials. Ocimum gratissimum presents such potential of high medicinal value. This plant is used in Nigeria traditionally as condiments and for the treatment of various ailments such as pyorrhoea, dysentery and bronchitis. Aqueous and ethanolic leaf extracts of O. gratissimum was screened for antibacterial activity against bacteria recovered from paediatric diarrhoea stool samples. Both extracts were found to exhibit selective inhibition against the isolates. The diameter zones of inhibition exhibited by the extracts were between $2.50 \pm 0.50\text{mm}$ – $26.00 \pm 1.00\text{mm}$. The ethanolic and aqueous extracts had minimum inhibitory concentrations that ranged between 15 and 25mg/ml, the extracts were bacteriostatic. Ethanolic extract showed more inhibitory effects compared to the aqueous extracts. The phytochemical analysis of both extracts revealed the presence of saponins, alkaloids, flavonoids, phenolics, tannins and glycosides, volatile oils, proteins and amino acids and terpenoids. In view of the efficacy of these extracts in inhibiting the growth of diarrhoea- causing organisms in paediatric stool samples, the utilization of the extracts in the formulation of new antibacterial drugs for the treatment of gastroenteritis caused by these organisms is strongly recommended especially when the availability and low cost of this medicinal plant are put into strong consideration.

Keywords: *Ocimum, diarrhoea, paediatric, stool, antibacterial*

INTRODUCTION

There are about two billion cases of diarrhoeal disease worldwide every year, and 1.9 million children younger than 5 years of age perish from diarrhoea each year, mostly in developing countries. This amounts to 18% of all the

deaths of children under the age of five and means that more than 5000 children are dying every day as a result of diarrhoeal diseases. Of all child deaths from diarrhoea, 78% occur in the African and South-East Asian regions (WHO, 2014).

Diarrhoea is the condition of having at least three loose or liquid bowel movements each day which often lasts for a few days and can result in dehydration due to fluid loss. Signs of dehydration often begins with loss of the normal stretchiness of the skin and changes in personality. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decrease in responsiveness as it becomes more severe (WHO, 2014).

Each child under 5 years of age experiences an average of three annual episodes of acute diarrhoea. Globally in this age group, acute diarrhoea is the second leading cause of death (after pneumonia), and both the incidence and the risk of mortality from diarrhoeal diseases are greatest among children in this age group, particularly during infancy – thereafter, rates decline incrementally (WHO, 2010).

In developing countries, enteric bacteria and parasites causing diarrhoea are more prevalent than viruses and typically peak during the summer months. The bacteria species involved include: *Campylobacter* species, *Salmonella* species, *Shigella* species, *E. coli* VTEC, Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC) and *Vibrio* species. Several other bacteria species have been found out to cause diarrhoea (Kent and Banks, 2009).

Ocimum gratissimum (Lamiaceae), commonly known as “alfavaca” is naturally used in the treatment of diarrhoea, headache, fever, ophthalmic, skin disease and pneumonia. The *Ocimum* oil is also active against several species of bacteria, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella*, *Salmonella* and *Proteus* (Janine *et al.*, 2005). In Nigeria, it is found in the Savannah and coastal areas, the plant is called “effinrinla” by the Yoruba speaking tribe, “Ahuji” by the Igbos, and the Hausas call it Dai-doya” (Effraim *et al.*, 2003). The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion

(Rabelo *et al.*, 2003).

The tribes of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids (Kabir *et al.*, 2005). Various species of *Ocimum gratissimum*, for example *O. viride* Linn, *O. suave* Linn, *O. basilicum* Linn and *O. canum* Sims have been reported for their numerous medical uses (Mshana *et al.*, 2000). Studies have proved *Ocimum gratissimum* to be a useful medication for diarrhoea in people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus AIDs (Elujoba, 2000).

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature, commonly known as basilic oils. Janine *et al.*, (2005) reported some chemical compounds and active ingredients found in these plants such as; eugenol, linalol, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from *Ocimum gratissimum* presented antimicrobial, antihelminthic, nematocidal activities or fungistatic properties (Pessoa *et al.*, 2005). The objectives of this study were to ascertain the activity of aqueous and ethanolic leaf extracts of *Ocimum gratissimum* against bacteria isolated from paediatric diarrhoeal stool samples; determine the minimum concentration of the extracts required to inhibit the growth of the isolates by both plate and broth method (MIC); determine the minimum concentration of the extracts required to kill the isolates (MBC) and compare the activity of the plant extracts to that of standard antibiotics.

MATERIALS AND METHODS

Bacterial cultures

A total of 36 samples from patients suffering from diarrhoea were collected from the “Center Igboro Pediatric Hospital”, Ilorin, Kwara State, Nigeria. Culture media which included Eosin Methylene Blue Agar and Salmonella Shigella Agar and MacConkey Agar were used for the

isolation of the organisms.

Plant identification

Fresh leaves of *Ocimum gratissimum* were collected from Ilorin, Kwara State of Nigeria. They were authenticated and identified at the herbarium of the University of Ilorin, Ilorin, Kwara State, Nigeria and given voucher number: UIH001/984.

Ethical issue

Ethical approval was given for this study by Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

Preparation and extraction of crude extracts

The leaves of *Ocimum gratissimum* were screened manually. The materials were cleaned with sterile distilled water; air dried and pounded. Five hundred grams of the pounded *Ocimum gratissimum* leaves were soaked in 70% ethanol for 72hrs. The extracts were filtered and ethanol extracts were evaporated using a rotary evaporator at 40°C and concentrated further using a water bath at temperature 50°C. The residues obtained were dissolved in 1% dimethyl sulphoxide (DMSO). The weight of the extract was determined and stored below ambient temperature (Mbata and Saikia, 2012). The same procedure was used for the aqueous extraction using sterile distilled water as the solvent and 1000ml of water was used to soak 90g of *Ocimum gratissimum* leaf.

Analysis of phytochemicals in the plant extracts

The photochemical screening was carried out using the standard procedures of analysis as described by Trease and Evans (2002).

Saponins

5ml of distilled water was added to 0.5g of the plant extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth or foam at the top. It was then allowed to stand for about 10 minutes; persistence of the foam after 10mins shows positive result.

Tannins

About 0.5g of the plant extract was boiled in 10ml of water in a test tube and then filtered. A few drop of 0.1% ferric chloride was added and observed for brownish green or blue or blue green coloration.

Alkaloids

The test for alkaloids included diluting 0.5g of the plant extract with 10ml of acid alcohol, boiling and filtering it. 2ml of dilute ammonia was added to 5ml of the filtrate; 5ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. Meyer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Meyer's reagent) or reddish brown precipitate (Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Flavonoids

About 5ml dilute ammonia was added to a portion of the plant extract, followed by addition of 1ml of concentrated sulphuric acid. A yellow coloration that disappears on staining indicated the presence of flavonoid.

Carbohydrates

Sulphuric acid was added to 1ml of the plant extract in a test tube. The mixture was heated in a water bath for 15minutes and Fehling solution added to the mixture. A brick red precipitate indicating the presence of carbohydrate was formed .

Terpenoids

2ml of chloroform was added to 0.5g of the extract. Concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed indicating the presence of terpenoids.

Phytosterols

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of

brown ring at the junction indicated the presence of phytosterols.

Phenols

Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Protein and amino acid

The extracts were treated with few drops of concentrated nitric acid. Formation of yellow colour indicated the presence of proteins.

Glycoside

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer indicated the presence of anthranol glycoside.

Volatile oil

Extracts were treated with 0.1ml dilute sodium hydroxide and dilute hydrochloric acid. Formation of white precipitate indicated the presence of volatile oil.

Antibacterial sensitivity test

A stock preparation of the extract was got by dissolving 2g of crude extract in 5ml of dimethylsulphoxide (DMSO) at concentration of 400mg/ml. Concentration of 300, 200 and 100mg/ml were then prepared from the stock (400mg/ml) concentration (Akujobi *et al.*, 2004).

The antibacterial tests of the plant extracts were done on the test isolate using the agar-gel diffusion inhibition test as described by Opara and Anasa (1993). The organisms were inoculated into 9ml nutrient broth and incubated till turbidity was noticed. It was then aseptically introduced and evenly spread on the surface of gelled sterile Mueller-Hinton agar plates using a sterile swab stick. Four wells of about 6.0 mm diameter were aseptically punched on each

agar plate using a sterile cork borer, allowing at least 30mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes of the extract were then introduced into the wells in the plates. A control well was in the center with the extracting solvent. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur and then incubated at 37°C for 24 hours. The resulting zones of inhibition were measured using a ruler calibrated in millimeters (Pessoa *et al.*, 2005). Assays were performed in triplicate and the data are shown as the mean \pm standard deviation (SD).

Determination of minimum inhibitory concentration (MIC)

The MIC of the potent extract was determined using the agar plate technique. Lower concentrations such as: 90, 70, 50, 45, 40, 30, 25, 20, 15 and 10mg/ml were used following the normal testing procedure. The MICs were read as the least concentration that inhibited the growth of the test organisms. The same procedure was followed for determining the MIC for the aqueous extract.

Determination of MIC using McFarland's standard

Nutrients broths were prepared and autoclaved then dispensed into test tubes accordingly. The broths were prepared for the treatment, control for organism and controls for extract. The treatment test tubes contained 9.7ml of sterile nutrient broth, 0.2ml of the extract, 0.1ml of the organism and it was incubated at 37°C for 18hrs. The organism control test tubes (9.9ml sterile nutrient broth and 0.1ml organism) were also incubated at 37°C for 18hrs. The incubated organism was standardized with the McFarlands solution and used for the inoculation. The extract control test tubes contained 9.8ml of sterile nutrient broth and 0.2ml of extract. The turbidity of the different preparations was then checked with a spectrophotometer and the result analyzed.

Minimum bactericidal concentration (MBC)

The MBCs were determined by selecting the least concentration that inhibited the growth of the test organisms. The organism was inoculated into the concentration preparation and incubated at 37°C for 18hrs and 0.1ml from it was inoculated into nutrient agar using pour plate method. The plate was then incubated for further 24 hours at 37°C. Absence of microbial growth at this concentration was taken as the MBC.

Standard antibiotics sensitivity test

Mueller Hilton agar was prepared according to the manufacturer's instruction, autoclaved and poured into Petridishes. It was allowed to solidify and the test organisms were spread on the medium using a sterile swab stick. The antibiotic disc was then placed on it and incubated at 37°C for 18hours. Zones of inhibition after incubation were measured in millimeters. The interpretations of the measurements as sensitive, intermediate and resistant were made according to the manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data.

RESULTS

Table 1 shows the phytochemicals of the leaf extracts of *Ocimum gratissimum*. Result shows the presence of carbohydrates, saponins, phenol, flavonoids proteins, volatile oil, terpenoids and tannins in both aqueous and ethanolic extract while alkaloids was only present in the aqueous extract. Volatile oil and glycosides were present in the ethanolic extract but absent in the aqueous extract.

Table 2 shows the antimicrobial activity of ethanolic leaf extract with the highest diameter zone of inhibition against *Salmonella enterica* (26.00±1.00mm) and the lowest against *Shigella sonnei* (10.00±1.00mm) and for the aqueous leaf extract, *Klebsiella pneumoniae* showed the highest susceptibility with diameter zone of inhibition of 19.00±1.00mm while *Bacillus cereus* showed the lowest susceptibility with diameter zone of inhibition of 2.50±0.50mm.

Each value is the mean ± standard deviation of the zones of inhibition in triplicate (n=3) readings. The letters a, b c and d represent ranking of the means across each group of isolate. Values not sharing the same superscript differ

Table 1: Phytochemicals detected in the aqueous and ethanolic leaf extracts of *Ocimum gratissimum*

S/No.	Phytochemicals	Aqueous	Ethanolic
1.	Saponins	+	+
2.	Alkaloids	+	-
3.	Carbohydrates	-	+
4.	Phytosterols	-	-
5.	Phenol	+	+
6.	Flavonoids	+	+
7.	Glycosides	-	+
8.	Volatile oil	-	-
9.	Terpenoids	+	+
10.	Tannins	+	+

KEY: + Positive; - Negative

Table 2: Antimicrobial activities of aqueous and ethanolic leaf extracts of *Ocimum gratissimum* at different concentrations on bacterial isolates

		SAL	SHI	ESC	KLE	BAC
Aqueous	400	18.00±1.00 ^d	16.50±0.50 ^d	17.00±1.00 ^d	19.00±1.00 ^c	8.50±0.50 ^d
	300	14.00±1.00 ^c	14.50±0.50 ^c	14.00±1.00 ^c	17.00±1.00 ^b	5.50±0.50 ^c
	200	13.50±0.50 ^b	11.50±0.50 ^a	11.50±0.50 ^a	17.00±1.00 ^b	3.00±1.00 ^b
	100	12.00±1.00 ^a	12.00±1.00 ^b	11.00±1.00 ^b	16.50±0.50 ^a	2.50±0.50 ^a
Ethanolic	400	26.00±1.00 ^d	11.00±1.00 ^d	18.50±0.50 ^d	16.50±0.50 ^d	19.00±1.00 ^c
	300	23.83±0.76 ^c	10.50±0.50 ^a	15.50±0.50 ^c	16.00±1.00 ^b	17.00±1.00 ^b
	200	21.50±0.50 ^b	10.50±0.50 ^a	14.00±1.00 ^b	16.00±1.00 ^b	17.00±1.00 ^b
	100	14.00±1.00 ^a	10.00±1.00 ^b	10.50±0.50 ^a	12.50±0.50 ^a	15.00±1.00 ^a

KEY: SAL=*Salmonella enterica*; SHI=*Shigella sonnei*; ESC=*Escherichia coli*; KLE=*Klebsiella pneumoniae*; BAC=*Bacillus cereus*

Table 3: Minimum inhibitory concentration of the aqueous leaf extract of *Ocimum gratissimum*

Test organisms	Minimum inhibitory concentrations (MIC) mg/ml	
	Aqueous	Ethanolic
<i>Salmonella enterica</i>	25	15
<i>Shigella sonnei</i>	25	15
<i>Escherichia coli</i>	25	25
<i>Klebsiella pneumoniae</i>	25	15
<i>Bacillus cereus</i>	25	20

significantly at $p < 0.05$.

Table 3 shows the minimum inhibitory concentration (MIC) of the aqueous and extracts on the five bacterial isolates as 25mg/ml. The MIC for *S. enterica* and *S. sonnei* is 15mg/ml, that of *E. coli* is 25mg/ml while that of *K. pneumoniae* and *B. cereus* is 15mg/ml and 20mg/ml respectively.

Table 4 shows that both the aqueous and ethanolic extract had no bactericidal activity

against the bacterial isolates.

Table 5 shows the antimicrobial susceptibility to standard antibiotics. The antibiotics show higher antimicrobial activity than that of the leaf extract even at lower concentrations. *Salmonella enterica* has the highest susceptibility to ciprofloxacin at concentration five.

DISCUSSION

Several authors have described diarrhoea infections as the most common cause of morbidity

Table 4: Minimum bactericidal concentration of the aqueous and ethanolic leaf extracts of *Ocimum gratissimum* against the bacterial isolates

Isolates	Minimum Bacterial Concentration (MBC), mg/ml.	
	Aqueous extracts	Ethanolic extract
<i>Salmonella enterica</i>	-	-
<i>Shigella sonnei</i>	-	-
<i>Escherichia coli</i>	-	-
<i>Klebsiella pneumoniae</i>	-	-
<i>Bacillus cereus</i>	-	-

KEY: No bactericidal activity

Table 5: Antibacterial susceptibility of the bacterial isolates to standard antibiotics

Antibiotics used	Mean values of zone of inhibition (mm)				
	<i>S. enterica</i>	<i>S. sonnei</i>	<i>E.coli</i>	<i>K. pneumoniae</i>	<i>B. cereus</i>
CIP	28 [S]	20 [S]	20 [S]	21.5 [S]	20 [S]
LEV	25.5 [S]	19.5 [S]	20 [S]	20.5 [S]	24 [S]
GEN	16.5 [S]	15 [S]	17 [S]	20 [S]	15.5[S]
AMK	25 [S]	20.5 [S]	20 [S]	20 [S]	0 [R]
MEM	0 [R]	0 [R]	20 [S]	0 [R]	0 [R]
CTX	0 [R]	0 [R]	0 [R]	23 [S]	0 [R]
AMX	0 [R]	0 [R]	0 [R]	0 [R]	0 [R]
TET	17 [I]	0 [R]	20 [S]	0 [R]	0 [R]
COT	0 [R]	0 [R]	0 [R]	0 [R]	0 [R]
CRX	0 [R]	0 [R]	0 [R]	0 [R]	13.5[R]
CN	0 [R]	0 [R]	0 [R]	0 [R]	13.5[R]
CTR	15 [I]	19 [I]	17.5 [I]	20.5 [I]	0 [R]
VAN	0 [R]	0 [R]	16.5 [S]	0 [R]	19 [S]
AUG	0 [R]	0 [R]	0 [R]	0 [R]	0 [R]
ERY	0 [R]	0 [R]	0 [R]	0 [R]	0 [R]
AMP	0 [R]	0 [R]	0 [R]	0 [R]	13 [R]

KEY: CIP Ciprofloxacin, LEV Levofloxacin, GEN Gentamicin, AMK Amikacin, MEM Meropenem, CTX Cefotaxime, AMX Amoxicillin TET Tetracyclin, COT Cotrimoxazole, CRX Cifuroxime, CN Cephalexin, CTR Ceftriaxone, VAN Vancomycin, AUG Augmentin, ERY Erythromycin, AMP Ampicillin, S-Susceptible, I- Intermediate, R-Resistant

and mortality in children worldwide (Bente *et al.*, 2005; WHO, 2005). Five bacteria isolated from the samples included *Salmonella enterica*, *Shigella sonnei*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*, and these were similar to what was obtained by Ani *et al.*

(2003) from stool samples of children. The high occurrence of these organisms could be attributed to poor sanitation on the part of the parents or wards of such infants.

As shown in Table 1, the antibacterial activities

of the aqueous and ethanolic extracts of this plant could be attributed to the presence of the active ingredients and secondary metabolites. The mechanism of action by which the phytochemical constituents of *Ocimum gratissimum* exert its antibacterial activity might be attributed to bacterial enzyme inhibition (Janine *et al.*, 2005).

Surh (2003) opined that phytochemicals may act by inhibiting microbial growth, inducing cellular membrane perturbations, interference with certain microbial metabolic processes, modulation of signal transduction or gene expression pathways.

Saponins obtained from the plant extract were also obtained by Moyo *et al.* (2012) which is believed to act by altering the permeability of cell walls and hence exert toxicity on all organized tissues. Saponins exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis.

The result of this study as stated in Table 2 showed that aqueous and ethanolic extracts *Ocimum gratissimum* showed varying degrees of inhibitory effects on the growth of the five organisms but the highest inhibition was at 400mg/ml and the lowest was at 100mg/ml.

The result of the preliminary phytochemical screening revealed the presence of tannins, phenolic compounds, terpenoids, sterols, saponins and alkaloids in *O. gratissimum*. Ali *et al.* (2001) also obtained alkaloids, flavonoids, saponins, sterols and tannins and opined that the associated activity might be correlated to the presence of the individual fraction. The antimicrobial activity of flavonoids may be due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall; thereby disrupting their membrane integrity (Tsuchiya *et al.*, 1996). It is noteworthy that phytochemicals are the most important antioxidants in dietary.

Tannins have been reported to hinder the development of micro-organisms by their ability to precipitate and inactivate microbial adhesions enzymes and cell envelope proteins. The significant activity observed in this study could thus be attributed to the interaction of one or more of the identified metabolites against the bacterial isolates.

The ethanolic and aqueous extracts exhibited broad-spectrum antibacterial activity against the isolates at concentrations of 400 mg/ml and below are considered as promising bioactive agents (Mitscher *et al.*, 1999). Falodun *et al.* (2006) reported that polar solvents like ethanol diffuse faster into the solid plant tissues and solubilize compounds faster than water, which is a non-polar solvent. This justifies the results in Table 2 that showed that the ethanolic extract has the higher zone of inhibition at 400mg/ml compared to that of the aqueous extracts.

The result in Table 2 concluded that the ethanolic leaf extract showed broader and greater spectra of activity against the tested organisms than the aqueous leaf extracts. The results obtained also showed that the activity of all the extracts were concentration dependent as the zone of inhibition decreases with decreasing concentrations and similar results have been reported by several researchers (El-Mahmood *et al.*, 2008).

Demonstration of low MIC values is an indication that the phytoconstituents of the plant have high therapeutic properties and therefore justifies its common usage in traditional medicinal uses though the therapeutic properties may even be more greatly increased if used in a refined form. The aqueous extract has a uniform MIC value of 25mg/ml for all the five bacterial isolates (Table 3) but the MIC for the ethanolic extract differs for some of the bacterial isolates, *Salmonella enterica*, *Shigella sonnei* and *Klebsiella pneumoniae* have percentage MIC values of 15mg/ml while *Escherichia coli* had 25mg/ml and *Bacillus cereus* had 20mg/ml. However when both extract were tested for MBC they

were bacteriostatic and this may be due to contamination of the leaf extracts or the evaporation of some essential oils during concentration as heat was applied to aid easier evaporation of the extracting solvent.

All the five bacterial isolates were tested against standard antibiotics like Ciprofloxacin, Levofloxacin, Gentamicin, Amikacin, Meropenem, Cefotaxime, Amoxicillin Tetracycline, Cotrimoxazole, Cefuroxime, Cephalexin, Ceftriaxone, Vancomycin, Augmentin, Erythromycin, and Ampicillin. The result in Table 5 showed that the bacterial isolates showed resistance to most of the standard antibiotics but most of the bacterial isolates were susceptible to Ciprofloxacin, Levofloxacin and Gentamicin. This may be due to the fact that the antibiotics are in pure state and has refined processes that have established it as a standard antibiotic. The result when compared with the diameter zone of inhibition of the aqueous and ethanolic leaf extracts showed that the leaf extracts competed fairly with the standard antibiotics.

This research work established the fact that *Ocimum gratissimum* leaf extract could be used in the treatment of dysentery or diarrhoea infection in children associated with *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Shigella sonnei* and even *Bacillus cereus*. Recently, there has been an increase in the number of people in Nigeria depending on herbal drugs. Herbal drugs are cheap, rapidly available and unadulterated. Their antibacterial activity could be increased by purification and subsequent concentration of the active ingredients.

The 'Green' movement in Western society has changed attitudes of the general public who now view naturally derived substances and extracts as being inherently safer and more desirable than synthetic chemical products thereby leading to the net increase in sales of herbal preparations. Therefore, 80% of people in the developing world rely on natural prod-

ucts for primary healthcare for man (WHO, 2002).

CONCLUSION

Aqueous and ethanolic extract of *Ocimum gratissimum* at different concentrations exhibited antimicrobial activity on bacterial isolated from paediatric diarrhoeal stool, the bacterial isolates included *Salmonella enterica*, *Shigella sonnei*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*. This may be attributed to the presence of phytochemical compounds identified in this study. The lower inhibitory properties may be due to over exposure of the plant extract to heat source, air, sunlight and the unit operations during the production of the paste from the leaves which might have influenced their activity as some of the active ingredients may be volatile in nature. This study indicates clearly that *Ocimum gratissimum* possesses a valuable but yet to be tapped potential which if exploited will benefit the pharmaceutical industries and the nation as a whole.

However the findings from this study could be of interest and bring about the need for further investigations in terms of toxicological studies and purification of active substances with the plant extract in drug development.

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