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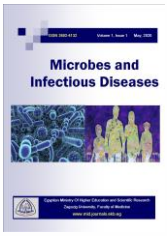

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Original article

GC-MS profiling and antibacterial efficacy of *Ocimum gratissimum* (Linn.) against bacteria associated with gastroenteritis

Risikat Idowu Ogundoju [1*], Muftau Kolawole Oladunmoye [1], Olamide Jos Babatunde [2]

1- Department of Microbiology, Federal University of Technology, Akure
2- Department of Microbiology, Federal University of Technology, Akure, Nigeria

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ABSTRACT

Background and rationale: The development of antibiotic resistance as well as the present adverse side effects of several of the commercially available antibiotics have necessitated the screening of plant extracts in quest of novel medications. This study evaluated Ocimum gratissimum (O. gratissimum) (Linn.) antibacterial potency against bacteria associated with gastroenteritis. **Methods:** The collection and preparation of plant material, phytochemical analysis, MIC and MBC calculations, bacterial isolation and susceptibility testing, gas chromatography-mass spectroscopy (GC-MS) analysis, and bacteria susceptibility testing were all carried out in accordance with the standard protocol. Antibiotic sensitivity patterns and antibacterial susceptibility tests were studied using the disc diffusion method and the agar well diffusion method, respectively. **Results:** In the GC-MS study, three important bioactive compounds were identified: N-hexadecanoic acid, 6 octadecenoic acid, and 9, 12 octadecadienoic acid (14.98, 8.05, and 13.15 percentage composition, respectively). The remaining compounds were minor in nature. Ethanol extract showed the highest zone of inhibition of 29.67±0.33mm against *Escherichia coli (E. coli*) and the least antibacterial activity against *Shigella flexneri* with 13.67±0.33 mm diameter zone of inhibition at 100mg/ml. N-hexane extract lacked any antibacterial effects at 100mg/ml. The MBC values ranged between 50 and 100 mg/ml, while the MIC values ranged from 12.5 to 100mg/ml. **Conclusion:** From this study, O. gratissimum ethanol extracts have the strongest antibacterial activity against *E. coli* when compared to other extracts in the treatment of bacteria associated with gastroenteritis. N-hexadecanoic acid, 6 octadecenoic acid, and 9, 12 octadecadienoic acid all have significant biological properties that can be used to combat gastroenteritis-causing bacteria.

Introduction

The clinical condition known as gastroenteritis is defined by an inflammation of the gastrointestinal tract that affects the stomach and small intestines and can cause diarrhea, vomiting, and abdominal pain [1,2]. One of the main health burdens associated with infectious diseases around the world is gastroenteritis. In low- and middle-income nations, it is one of the most prevalent infectious diseases affecting people and a leading cause of death. Since gastroenteritis is an infectious disease with a high fatality rate, it is extremely

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* *Corresponding author:* Risikat Idowu Ogundoju
E-mail address: *idowu.ogundoju@gmail.com*

important for public health [3]. Due to a lack of potable water, hunger, and inadequate sanitation, the burden of this infection is particularly severe in poorer nations. There are bacterial, viral, fungal, and parasite causes of gastroenteritis; however, the focus of this study will be on bacterial causes. Infectious diarrhea has a variety of geographic origins, from urban to rural places, and these origins rely on co-morbidities and the host's immune system. However, viruses (including norovirus, rotavirus, adenovirus, and others) are the most frequent cause of acute infectious diarrhea. More severe cases of infectious diarrhea are caused by bacterial causes than by other infectious aetiologies [4,5]. Diarrheal disease is the second leading cause of infectious disease death in children under the age of five [6].

Antimicrobial resistance has become a serious issue that threatens the very survival of the human species over time [7]. Since the 19th century, when antibiotics were first discovered, bacteria have developed ways to evade, avoid, or continue to be resistant to all kinds of antibiotics [8]. With the largest impact in sub-Saharan Africa, the incidence of this resistant bacterium has rapidly expanded, particularly in the last four decades [9].

One of the recently found medicinal plants with the potential to be used as an alternative therapy for the treatment of various illnesses or as a source of novel pharmaceuticals is Ocimum gratissimum L., also known as scent leaf. It is a common perennial herbaceous plant with a potent aroma that is also commercially viable. It can be found in Africa, Asia, and South America and is a member of the Lamiaceae family [10]. Ocimum gratissimum is a member of the botanical family known as spices. In Nigeria, it is Diadoyal in Hausa, Nchuanwu in Igbo, and Efinrin in Yoruba [11]. It is employed for numerous purposes. It is utilized in different dishes like salads, soups, pastas, vinegars, and jellies. The leaves have been administered topically to cure skin infections and ingested to treat bronchitis in traditional medicine. The leaves have also been used as a general tonic, an anti-diarrheal, and to treat conjunctivitis by injecting directly into the eyes. Many nations' traditional medical systems have made substantial use of O. gratissimum leaf extract. The goal of the current study was to look into the phytochemical, bioactive, and antibacterial properties of O. gratissimum leaf extracts. The chemical components of the plant that give it its therapeutic effects - in the fight against MDR phenotypes- were profiled using a GC-MS analysis of the ethanol extract of this medicinal plant.

Materials and methods

Collection of plant material

The healthy and fresh leaf materials were harvested in Akure metropolis and identified at the Crop, Soil and Pest Management Department (CSP) of the Federal University of Technology, Akure. The fresh leaves were allowed to air dry at room temperature. Cold and hot aqueous extraction as well as ethanolic and N hexane extraction was carried out as described by **Asoso et al.** [12]. The fresh leaves of Ocimum gratissimum were rinsed in tap water and allowed to dry at room temperature until they were completely dried. The dried leaves were grounded using an electric blender (Binatone BLG 621).

Preparation of extracts

One hundred grams (100 g) of the ground leaves were weighed into four airtight containers, to which solvents were added. The solvents used were ethanol, N hexane, cold and hot water. The mixtures were left for 72 hrs for N-hexane and ethanol, 48 hrs for the aqueous extraction with frequent stirring, after which each mixture was sieved using threefold sterile muslin cloth and filtered further using sterile No 1 Whatman filter paper. The filtrate was collected into a sterile beaker and concentrated in vacuo using rotary evaporator (Resona, Germany). The weight of the dried extract was measured and recorded as percentage recovery. The extracts were reconstituted with 30% Dimethyl sulphoxide (DMSO) and sterilized by filtration using Millipore membrane filter (0.45 µm).

Phytochemical analysis

The leaf extracts of O. gratissimum were analysed for the presence or absence of the following phytochemicals: alkaloids, terpenoids, tannins, saponins, and flavonoids, using standard methods described by the AOAC [13].

GC-MS analysis

The ethanol extract of O. gratissimum was analysed using a GC-MS analytical tool. The method of **Olusola-Makinde et al.** [14] was used for the evaluation of the chemical components using Varian GC-MS equipment (Varian 4000 mass spectrometer, USA) alongside a mass spectrometer (MS) 3000 equipped with an Agilient MS capillary column (30 m × 0.25 mm, i.e., film thickness).

Isolation and identification of bacteria

The media used in this study were MacConkey agar, eosin methylene blue (EMB), Salmonella shigella agar (SSA), and nutrient agar. They were all prepared according to the manufacturers' instructions, and the isolation of bacteria from stool samples was carried out as described by **Onifade et al.** [15]. Inoculum was standardised using 0.5 McFarland's standard as described by **Isunu et al.** [16]. Bacterial isolates were identified using both cultural and molecular methods, as described by **Olutiola et al.** [17] and **Cosa et al.** [18], respectively.

Antibiotic susceptibility testing

The method of Cheesbrough [19] was adopted. Pure cultures of the bacterial isolates were subjected to antibacterial agents using Mueller-Hinton agar plates. Each antibacterial disc was aseptically picked using sterile forceps and placed in the centre of an already prepared Mueller-Hinton agar plate. The plates were incubated at 37 °C for 24 hours. The antibiotics on the discs include augmentin (30µg), gentamycin (30µg), amoxicillin (30µg), ciprofloxacin (10µg), chloramphenicol (10µg), streptomycin (30µg) and ofloxacin (30µg).

Antibacterial assay of Ocimum gratissimum extracts

The assay for the antibacterial activity of O. gratissimum extracts was carried out using the agar well diffusion method as described by **Okonkwo et al.** [20]. The standardised bacterial isolates were aseptically inoculated on the surface of sterile Mueller-Hinton agar (MHA) plates with the aid of a sterile swab stick by the spread method. Four wells of 8 mm in diameter were punctured in the culture medium with a sterile cork borer. Half a millilitre of the extracts was used to fill the wells, with ciprofloxacin as the positive control and water as the negative control. The plates were allowed to stand on the laboratory bench before incubation at 37°C for 24 hours. The production of zones of inhibition was observed, measured with a metre rule, and recorded appropriately.

Results

Phytochemical composition of O. gratissimum

The phytochemical constituents of O. gratissimum as shown in **tables (1, 2)** include flavonoids, tannins, phenolic amino acids, saponins, glycosides, steroids, alkaloids, terpenoids, and phlobatanin. Flavonoids and glycosides are present in all the four extracts. Steroids are absent in the hot and cold-water extracts but present in ethanol and N-hexane extracts. Ocimum ethanol contains all the phytochemicals while Ocimum n hexane has the least phytochemicals. **Table 2** shows the quantity of phytochemicals present in O. gratissimum leaves. Ocimum ethanol has the highest quantity of flavonoids and tannin (24.05±0.75) and (1.37±0.2) respectively. Likewise, Ocimum cold has the highest quantity of saponin (40.44±0.02) but is absent in n-hexane extract. Ocimum hot also has the highest amount of glycosides (40.31±0.01) followed by Ocimum ethanol (32.22±0.02).

Compounds detected in the ethanol leaf extract of O. gratissimum

The GC-MS analysis of O. gratissimum ethanolic leaf extract reflected 21 compounds. The names of the compounds, retention time, molecular weights and peak values are listed in **table (3).** The peak values and structural elucidation of each compound was depicted in **figure (1).** The major compounds detected in this study are N-hexadecanoic acid, 6 octadecenoic acid, 9,12 octadecadienoic acid (1, 12.26, 12.63 respectively) and the remaining are minor compounds. **Table 3** Shows the compounds detected in the gas chromatography - mass spectrometry of O. gratissimum ethanol extract.

Bacterial identification

The following bacteria were isolated from stool samples of patients suffering from gastroenteritis: *Escherichia coli, Salmonella typhi, Proteus vulgari, Shigella flexneri, Citrobacter freundi, Morganella morganii.* **Table 5** reveals the biochemical characterization of the bacterial isolates while **Table 4** shows the molecular identity of the isolates with their respective percentage similarity, strain no and ascension no.

Antibiotic susceptibility pattern of clinical isolates

Morganella morganii and *Proteus vulgaris* isolated from stool samples were susceptible to all the tested conventional antibiotics while *E. coli, Salmonella typhi, Shigella flexneri* and *Citrobacter freundii* from this study showed resistance to gentamicin, augmentin, amoxicillin and chloramphenicol while all the bacteria isolated were susceptible to ciprofloxacin.

Antibacterial activity of O. gratissimum leaf extracts against bacteria isolated from stool samples

Aqueous, ethanol, and N-hexane extracts of O. gratissimum were screened for antibacterial activity

against bacteria isolated from patients suffering from gastroenteritis. Both aqueous and ethanol leaf extracts were found to exhibit antibacterial activity against the isolates. Ethanol extract showed the highest zone of inhibition of 29.67 ± 0.33 mm against *E. coli* and the least antibacterial activity against *Shigella flexneri* with 13.67 ± 0.33 mm diameter zone of inhibition as shown in **table (7).**

The minimum inhibitory concentration of O. gratissimum leaf extracts

The minimum inhibitory concentration (MIC) of leaf extracts of Ocimum gratissimum on the bacteria isolates is found in **table (8).** The lowest MIC of 12.5mg/ml and 50mg/ml is recorded in ethanol extracts. N-hexane extracts show the highest MIC of 100mg/ml against all the tested isolates.

The minimum bactericidal concentration of O. gratissimum leaf extracts

The Minimum Bactericidal Concentration (MBC) of leaf extracts of O. gratissimum in ethanol is between 50mg/ml and 25mg/ml while N-hexane extracts showed highest concentration of 100mg/ml. The results are presented in **table (9).**

Table 1. Qualitative phytochemical profile of O. gratissimum leaf extracts.

Extract	Saponin	Flavonoid	Tannin	Glycoside	Steroids	Philobatanin	Alkaloids	Terpenoid	Phenol
Ocimum cold	+ +	+	+	+ +	–	+	–	+	–
Ocimum hot	+	+ +	+ +	+	–	+	–	+	+ +
Ocimum ethanol	+ +	+ + +	+ +	+ +	+	+	+ +	+	+ +
Ocimum N-hexane	–	+	–	+	+	–	–	–	–

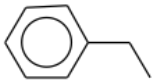
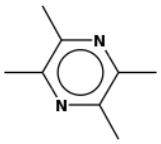
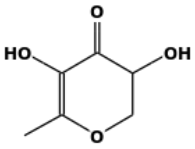
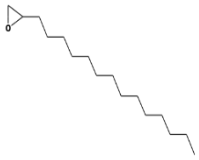
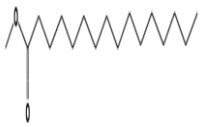
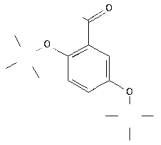
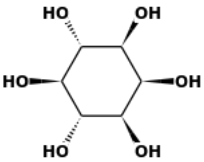
Key; - Absent, + Present

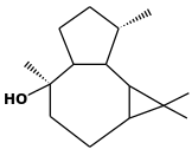
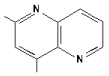
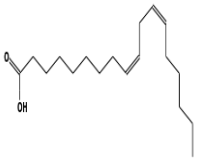
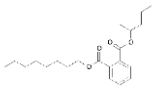
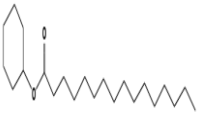
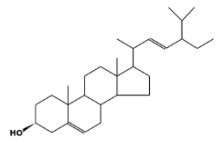
Table 2. Quantitative phytochemical profile of O. gratissimum leaf extracts.

S/N	Constituents	Ocimum cold	Ocimum hot	Ocimum ethanol	Ocimum N-hexane
1	Alkaloids	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	13.5 ± 0.11^{b}	0.00 ± 0.0^{a}
2	Saponin	40.44 ± 0.02^{c}	30.3 ± 0.1^{b}	27.5 ± 0.0^{b}	0.00 ± 0.0^{a}
3	Steroids	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	3.09 ± 0.03^{b}	8.60 ± 0.2^{c}
4	Terpenoids	15.3 ± 0.1^{c}	21.05 ± 1.05^{d}	9.10 ± 0.03^{b}	0.00 ± 0.0^{a}
5	Phenol	0.00 ± 0.0^{a}	20.3 ± 0.0^{b}	18.37 ± 0.05^{b}	0.00 ± 0.0^{a}
6	Flavonoid	1.11 ± 0.01^{a}	3.41 ± 0.01^{a}	24.05 ± 0.75^{b}	21.95 ± 0.45^{b}
7	Glycoside	12.26 ± 0.05^{a}	40.31 ± 0.01^{d}	32.22 ± 0.02^{c}	21.7 ± 0.1^{b}
8	Tannin	0.53 ± 0.1^{b}	0.83 ± 0.2^{b}	1.37 ± 0.2^{c}	0.00 ± 0.0^{a}

Data are presented as Mean ± S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different ($P < 0.05$).

Table 3. Compounds identified in GC-MS analysis of O. gratissimum ethanol extracts.

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	m/z	Structures
1	6.00	Ethylbenzene	C_8H_{10}	106	2.97	51, 91, 106	
2	6.50	Pyrazine, tetramethyl-	$C_8H_{12}N_2$	136	3.37	42, 54, 136	
3	7.50	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	2.12	43, 44, 144	
4	11.00	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	2.47	60, 73, 240	
5	13.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.67	74, 87, 270	
6	14.21	Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]-	$C_{13}H_{22}O_3Si_2$	282	4.46	73, 267, 282	
7	17.85	Myo-Inositol	$C_6H_{12}O_6$	180	2.60	60, 73, 180	
8	20.00	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280	2.23	42, 58, 280	
9	21.00	Phytol	$C_{20}H_{40}O$	296	1.67	43, 71, 296	

10	23.50	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4β,4aβ,7α,7aβ,7bα)]-	$C_{15}H_{26}O$	222	2.16	43, 109, 222	
11	26.50	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.75	43, 81, 296	
12	28.77	Octadecanamide	$C_{18}H_{37}NO$	283	5.20	43, 59, 283	
13	30.58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.61	43, 73, 256	
14	33.17	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	12.26	41, 55, 282	
15	34.48	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	12.63	43, 81, 280	
16	34.50	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	4.53	43, 73, 282	
17	36.26	Phthalic acid, octyl 2-pentyl ester	$C_{21}H_{32}O_4$	348	5.28	149, 167, 348	
18	37.48	Hexadecanoic acid, cyclohexyl ester	$C_{22}H_{42}O_2$	338	9.29	67, 79, 304	
19	37.75	Stigmasterol	$C_{29}H_{48}O$	412	3.72	55, 83, 412	

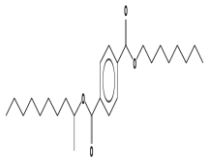
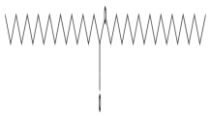
20	40.25	Terephthalic acid, 2-decyl octyl ester	$C_{26}H_{42}O_4$	418	1.41	43, 57, 418	
21	43.00	Octadecanoic acid, octadecyl ester	$C_{36}H_{72}O_2$	536	4.31	43, 57, 536	

Table 4. Molecular identification of resistant bacterial isolates.

S/N	Biochemical identity	Molecular identification	Percentage similarity	Strain No1	Accession No
1	*C. freundi*	*C. freundi*	99.67	ATCC 8090	NR 028894.1
2	*S flexneri*	*S flexneri*	95.17	ATCC 29903	NR 026331.1
3	*S typhi*	*S typhi*	99.00	ATCC 1377	Z47544
4	*E. coli*	*E. coli*	94.87	NBRC 102203	NR 114042.1

Table 5. Biochemical characteristics of bacteria isolated from stool samples.

S/N	Gram's Reaction	Catalase	Coagulase	Oxidase	Citrate	Motility	Urea	Indole	H2S	Gas	Lactose	Sucrose	Glucose	Mannitol	Maltose	Suspected Organisms
1	GNB	+	ND	-	-	+	-	+	-	+	+	+	+	+	+	*Escherichia coli*
2	GNB	+	ND	-	-	+	-	-	+	-	-	-	+	+	+	*Salmonella typhi*
3	GNB	+	ND		+	+	+	-	+	-	-	-	+	-	+	*Proteus vulgaris*
4	GNB	+	ND	-	-	-	-	-	-	-	-	-	+	+	+	*Shigella flexneri*
5	GNB	+	ND	-	+	+	V	+	+	+	+	+	+	+	+	*Citrobacter freundii*
6	GNB	+	ND	-	-	+	+	-	-	+	-	-	+	-	-	*Morganella morganii*

Key: GNB = Gram negative bacilli, ND= Not determined, Y= Yellow, R= Red, - = Absent, + = Present, V = Variable, H2S = Hydrogen Sulphide.

Table 6. Antibiotic susceptibility pattern of clinical isolates associated with gastroenteritis.

Isolates	AU	CN	AM	CPX	CHL	S	OFX
E. coli	13.00 ± 1.0^{b}	0.0 ± 0.0^{a}	10.0 ± 0.0^{b}	19.0 ± 1.0^{b}	10.5 ± 0.5^{a}	10.0 ± 0.0^{a}	12.5 ± 0.5^{a}
S. typhi	12.00 ± 0.0^{ab}	13.5 ± 0.5^{b}	0.0 ± 0.0^{a}	25.5 ± 0.5^{c}	10.0 ± 0.0^{a}	11.0 ± 1.0^{a}	17.0 ± 1.0^{bc}
C. freundi	17.00 ± 0.0^{c}	0.0 ± 0.0^{a}	10.0 ± 0.0^{b}	19.0 ± 1.0^{b}	10.0 ± 0.0^{a}	15.0 ± 1.0^{b}	17.0 ± 0.0^{bc}
S. flexneri	10.0 ± 0.0^{a}	11.5 ± 0.5^{b}	12.5 ± 0.5^{c}	19.0 ± 1.0^{b}	13.0 ± 1.0^{b}	15.5 ± 0.5^{b}	17.5 ± 0.5^{bc}
M. morganii	21.0 ± 1.0^{d}	20.5 ± 0.5^{c}	18.00 ± 0.0^{d}	22.5 ± 0.5^{c}	19.5 ± 0.5^{c}	19.5 ± 0.5^{c}	20.5 ± 0.5^{d}
P. Vulgaris	21.5 ± 0.5^{d}	20.5 ± 0.5^{c}	19.5 ± 0.5^{d}	10.5 ± 0.5^{a}	15.0 ± 1.0^{bc}	18.5 ± 0.5^{c}	19.0 ± 1.0^{c}

Data are represented as mean ± standard error (n=3) with the same superscript across the column are not significantly different ($p<0.05$).
Keys: AU= Augmentin (30ug), CN= Gentamycin (30ug), AM= Amoxicillin (30ug) CPX= Ciprofloxacin (10ug), CHL= Chloramphenicol (10ug), S= Streptomycin (30ug), OFX= Ofloxacin (30ug).

Table 7. Antibacterial susceptibility pattern of clinical isolates to leaf extracts of O. gratissimum at 100mg/ml concentration.

S/N	Isolates	OC	OH	OE	ON	N	P
1	*E. coli*	10.33 ± 0.33^{b}	10.67 ± 0.67^{b}	29.67 ± 0.33^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	32.0 ± 0.5^{c}
2	*S. flexneri*	0.00 ± 0.0^{a}	0.00 ± 00^{a}	14.33 ± 0.33^{b}	0.0 ± 0^{a}	0.0 ± 0.0^{a}	30.0 ± 0.5^{c}
3	*C. freundii*	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	13.67 ± 0.33^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	31.0 ± 0.5^{c}
4	*S. typhi*	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	15.67 ± 0.33^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	25.0 ± 0.5^{c}

KEY: OC= Ocimum cold, OH= Ocimum hot, OE= Ocimum ethanol, ON= Ocimum N hexane, N= negative control, P= positive control.

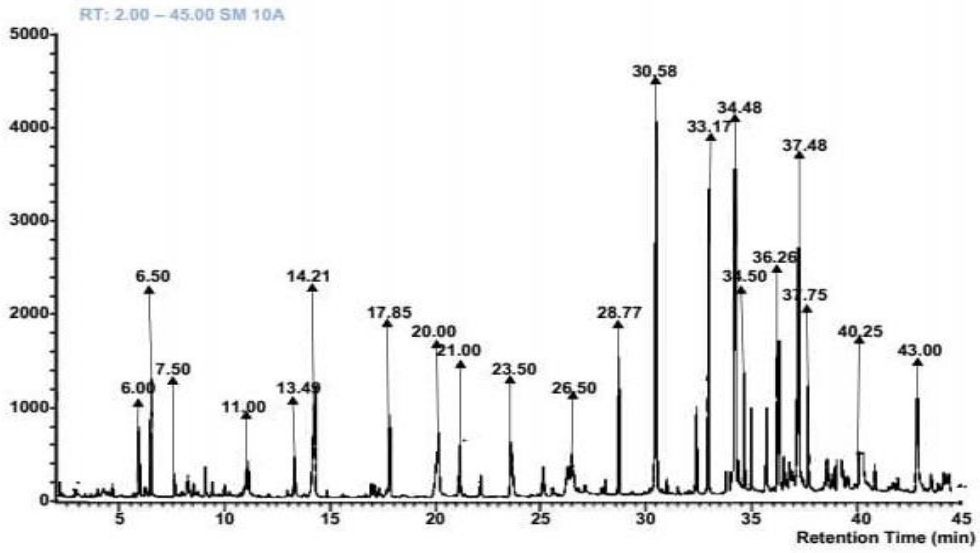
Table 8. Minimum inhibitory concentration (MIC) of O. gratissimum leaf extract.

SN	Bacteria isolates	Cold water (mg/ml)	Hot water (mg/ml)	Ethanol (mg/ml)	N-hexane (mg/ml)
1	*Shigella flexneri*	50	50	12.5	100
2	*Citrobacter freundii*	100	100	25	100
3	*Salmonella typhi*	50	100	50	100
4	*Staph aureus*	50	50	12.5	100
5	*Eschericial coli*	100	50	12.5	100

Table 9. Minimum bactericidal concentration of O. gratissimum leaf extracts.

SN	Bacteria isolates	Cold water (mg/ml)	Hot water (mg/ml)	Ethanol (mg/ml)	N-hexane (mg/ml)
1	*Shigella flexneri*	100	100	25	100
2	*Citrobacter freundii*	100	100	25	100
3	*Salmonella typhi*	100	100	50	100
4	*Staph aureus*	100	100	25	100
5	*Escherichia coli*	100	50	25	100

Figure 1. GC-MS spectrum of ethanol leaf extract of O. gratissimum.



Discussion

Gastroenteritis is a disease that causes inflammation of the stomach and small intestine, which can cause diarrhea, vomiting, abdominal pain, and cramping [21]. One of the main health burdens of infectious diseases in the world is gastroenteritis. It is one of the most widespread infectious diseases affecting people and a leading cause of death in low- and middle-income nations [22].

The results of the qualitative phytochemical screening of the O. gratissimum leaf extract in this study showed the presence of tannins, saponins, glycosides, alkaloids, steroids, philobatanin, terpenoids, and phenol. This result is consistent with the study of **Odebisi et al.** [23] whose phytochemical investigation of O. gratissimum revealed tannins, saponins, alkaloids, steroids, phlobatanin and phenol, glycosides, and terpenoids. Saponin has the largest number of phytochemicals (40.45 mg/g), according to quantitative phytochemical analysis in this study. In a similar vein, the quantitative phytochemical investigation carried out by **Odebisi et al.** [23] on five classes of discovered metabolites revealed that saponin had the highest phytochemical constituent (52.48 mg/g). In the present study, GC-MS analysis of an ethanol extract of O. gratissimum revealed the presence of some significant chemical components, including N-hexadecanoic acid, 6 octadecenoic acid, and 9,12 octadecanoic acid, which are known to have many biologically significant properties, including antimicrobial, antioxidant, and anti-inflammatory properties. **Haruna et al.** [24] reported the presence of N -hexadecanoic acid and 9, 12 octadecenoic acid in O. gratissimum leaf extract. **Baba et al.** [25] reported major chemical constituent in Ocimum ethanol extracts by GC-MS as Phenol; 23.75, Phytol; 12.45, fatty acid and steroids 6.56. **Yuvarajan et al.** [26] identified 45 compounds by GC-MS profiling out of which three compound reflect the highest peak values which are; phenol, 2- methoxy -3 (-2- propenyl), Estrogole, Squalene (peak values are 59.3316, 6.8218 and 4.7581 respectively) and rest of them are minor compounds. All the afore mentioned bioactive compounds have significant biological functions such as antimicrobial, antioxidant and anti-inflammatory properties.

In this study, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus. Proteus vulgaris*, *Citrobacter freundi*, *Morganella morganii and Shigella flexineri* were isolated from stool samples of patients suffering from gastroenteritis and were presumptively identified. Four out of the six isolates were found to be multidrug resistant to conventional antibiotics and were identified at the molecular level which are: *E. coli*, *Shigella flexneri*, *Citrobacter freundi*, *Shigella flexneri*. This work is in agreement with the work of **Adeh et al.** [27] that isolated the following bacteria from stool samples: *Salmonella typhi*, *E. coli*, *Shigella flexneri*, *Proteus vulgaris*, *Citrobacter*, *Morganella morganii* (a study conducted in Kaduna Nigeria metropolis).

Escherichia coli, Salmonella typhi, Shigella flexneri, and Citrobacter freundii, were found to be multidrug resistant to standard antibiotics in this investigation. This was in line with a report by **Andrea et al.** [28] that found intestinal bacteria from children in Urban Northern Ghana were extremely resistant to the antibiotics used there. It is significant to emphasize that the establishment of antimicrobial resistance is mostly due to the surge in the use of antibiotics indiscriminately for symptomatic relief [29].

Bacteria isolated from this study showed resistance to gentamycin, augmentin, amoxicillin and chloramphenicol while all the bacteria isolated were susceptible to ciprofloxacin. This is in agreement with the report of **Clarence et al.** [30] where most of the enteric bacteria were resistance to gentamycin, streptomycin and chloramphenicol. **Getie et al.** [31] reported all enteric bacteria worked on were susceptible to ciprofloxacin but a substantial number of isolates were resistant to commonly prescribed antibiotics and the prevalence of multidrug resistance was high.

Aqueous, ethanol, and N-hexane extracts of O. gratissimum was screened for antibacterial activity against bacteria isolated from patients suffering from gastroenteritis. Both aqueous and ethanol leaf extracts were found to exhibit antibacterial activity against the isolates. The diameter zone of inhibition demonstrated by the extract is between 13.67±0.33 mm to 29.67±0.33 mm. Ethanol extract showed the highest zone of inhibition of 29.67±0.33 mm against *E. coli* and the least antibacterial activity against *Citrobacter freundi* with 13.67±0.33 mm diameter zone of inhibition. The work is similar to the work of **Odebisi et al.** [23] who carried out antibacterial activity of O. gratissimum aqueous and ethanol leaf extract against bacteria isolated from diarrheic stool with ethanol extract with highest zone of inhibition of 2.50-0.5 and 26.00-0.00mm. N-hexane extract did not show any antibacterial activity against all the isolates, this might be due to the high nonpolar nature of the solvent. The Polarity of solvent and its capacity to dissolve desired active component plays a very important factor in selecting a good extraction solvent.

Conclusion

The overall result of this investigation shows that the plant's ethanol extract exhibited the strongest antibacterial activity against *E. coli.* Ocimum gratissimum ethanoic extracts revealed a variety of medicinally useful active ingredients through GC-MS analysis. The widespread use of this medicinal plant to treat and manage diseases associated with gastroenteritis in many rural and even urban populations in Nigeria may be explained by the existence of these various multifunctional chemicals. Because of its effectiveness and low risk of an allergic reaction, O. gratissimum may therefore be a significant alternative medicine in the treatment of bacterial infections associated with gastroenteritis.

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Author's contributions

Author Oladunmoye, K. M. designed the study. Author Ogundoju, R. I. developed the methodology, acquired the data, analysed and interpreted the data. Author Babatunde, O. J. wrote the first draft of the manuscript. All authors read and approved the final draft of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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