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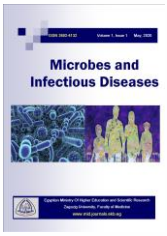

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Original article

Biological activities of *Ocimum gratissimum* (Linn) ethanol extracts on bacteria associated with surface waters Akure, Nigeria

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ABSTRACT

Background: There has been increasing antibiotic resistance by waterborne diseases related bacteria. Despite the use of various antibiotics, there is still a threat in the treatment of water-borne diseases. This study evaluated the antibacterial potentials of *Ocimum gratissimum (O. gratissimum)* on faecal bacteria associated with surface water. **Methods:** The collection, extraction, Gas chromatography, and mass spectroscopy (GC-MS) of *O. gratissimum* leaves and enumeration of bacterial derivatives from surface water samples were conducted per specified protocols. Antibacterial susceptibility test, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of *O. gratissimum* extracts on bacteria isolates were conducted via agar well diffusion and tube dilution respectively. **Results:** The GC-MS revealed the presence of eugenol with the highest percentage composition of 33.0 % in ethanol extract, while γ-Terpinene had the highest percentage composition of 11.88% in aqueous extract. *Escherichia coli* and *Enterococcus faecalis* were the faecal bacteria observed. *Escherichia coli* had an inhibition zone of 23.67 mm and 30.67 mm at 50 mg/ml and 100 mg/ml on *O. gratissimum* aqueous extract respectively, while ethanol extracts were inhibited mostly with an inhibition range of 26.67 mm - 30.32 mm. The MIC obtained varied from 25 mg/ml to 50 mg/ml, the MBC was constant at 100 mg/ml for all bacterial isolates. **Conclusion:** Findings revealed that the ethanol extract of *O. gratissimum* leaf can be employed in the management of the faecal indicator bacteria observed in this study. Eugenol could be used explored for contemporary antibacterial studies to combat water-borne bacteria.

Introduction

Surface water refers to water on the surface of the planet occurring in lakes, rivers, streams, or other freshwater sources used for drinking water supplies [1]. Lakes, rivers, and streams have important multi-usage components, such as sources of drinking water, irrigation, fishery, and energy production [2]. The impact of these anthropogenic activities has been so extensive that the water bodies have lost their self-purification capacity to a large extent [3]. Contaminated fresh water is a major cause of waterborne diseases and when used in the preparation of food, can be the source of food-borne diseases [4]. Waterborne diseases emerge from water contaminated either by pathogenic viruses, bacteria, protozoa, or by chemical substances [5].

There is a growing interest in exploiting plants for medicinal purposes especially in Africa

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[6]. This stems from the fact that microorganisms are developing resistance to many drugs and as such created a situation where some of the common and less expensive antimicrobial agents are losing effectiveness [6]. Herbal medicine which uses medicinal plants primarily presents an alternative to such a situation [7]. These medicinal plants have immensely contributed to the development of human health and welfare. Concurrently, there is an increase in data and huge patronage to herbal products around the world [8].

Medicinal plants like *Ocimum gratissimum* (*O. gratissimum)* (scent leaf) have been asserted to provide various culinary and medicinal properties [9]. These medicinal properties exert bacteriostatic and bactericidal effects on some bacteria [9]. These effects have been attributed to the peptides, alkaloids, essential oils, phenols, and flavonoids which are phytochemical components in these plants [9]. *Ocimum gratisssimum* belongs to the family *leguminocaeae*, commonly known as "aflavaca". It is naturally used in the treatment of different diseases which include: upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin disease, pneumonia, fever and mosquito repellents [10-12].

Ocimum gratissimum is found in tropical and warm temperature regions such as India and Nigeria [13]. Some of the vernacular names in Nigeria include Igbo (Ncho-anwu, Ahuji), Yoruba (Efinrin), Edo (Aramagbo), and Hausa (Daidoya). [14]. *Ocimum gratissimum* has been described to have other species in the flora of tropical West Africa. These include *Ocimum viride* Linn, *Ocimum canum* Sims, *Ocimum suave* Linn, and *Ocimum basilicum* Linn [14]. The Ocimum oil has been described to be active against several species of bacteria and fungi. These include: *Listeria monocytogenes, Shigella, Salmonella* and *Proteus, Bacillus* and *Alcaligenes faecalis* [15-17]. **Oboh,** [18] reported the antioxidant and antimicrobial properties of *O. gratissimum.*

There has been increasing antibiotic resistance by faecal bacteria associated with contaminated surface water and a consequence of immortality to humanity. Despite the use of various antibiotics, there is still a threat in the treatment of water-borne related faecal bacteria. Hence, a necessity for the search for an alternative drug from natural products is important. This study evaluates the antibacterial potential of *O.gratissimum* leaf extracts against bacteria associated with contaminated surface water. The aim of this study is to access the bioactive compounds of *O. gratissimum* extracts and their antibacterial potential on water-related bacteria.

Materials and Methods

Study area description

Onyearugbulem river is found around the axis of Akure-Owo Expressway with geographical information system (GIS) coordinate of between latitude (7°12' N) and longitude (5° 15' E).

Collection of water samples

The water samples were taken with ethanol-sterilized 2.5-liter sampling bottles from 15 to 20 cm below the water surface in the early hours of the morning and taken to the Microbiology laboratory of the Federal University of Technology, Akure within one hour of collection for microbiological analysis [19].

Preparation of plant extracts

The *O. gratissimum* leaves were bought from a local market, Akure, Ondo State, Nigeria. The leaves were identified and authenticated by leaf and vegetable experts, called "Iya Alagbo or Iya elewe omo" in Yoruba [20]. The leaves were air-dried for six weeks and homogenized at the laboratory of Animal Production and Health Department of Federal University of Technology, Akure. The solvents used for the extraction were 100% ethanol and cold water. The *O.gratissimum* leaves were air-dried for three weeks and pulverized using an electric blender. The dried and milled plant materials were macerated with 3,500ml of distilled water and 2, 250ml of ethanol (90%) in a tightly sealed bucket for 72 hours at room temperature. The macerates were then sieved with muslin cloth and filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated in a vacuum using a rotary evaporator for solvent evaporation. The weight of the dried extract was measured and the percentage extract recovery was calculated

$$\text{Percentage extract recovered} = \frac{\text{dry weight of extract recovered after extraction}}{\text{Initial dry weight of plant}} \times 100\ \%$$

Enumeration of bacteria from contaminated water samples

Serial dilution protocol was adopted with minor adjustments via pour plate method as described by **Chouhan**, [21] on Eosine methylene blue (EMB) agar (Hi-media, Mumbai, India) Petri-plates were incubated for a duration of 24 hrs. at a temperature

of 37 ºC after rocking the plates clock-wisely, anti-clock-wisely, 25±2 ºC, and after the solidification of the media in the Petri plates. Plates were incubated in an inverted position.

Identification and characterization of bacterial isolates

Cultural, morphological, and biochemical characteristics of bacterial isolates obtained from the river water samples were carried out as described by **Ayo and Arotupin,** [22]. Colonial characteristics observed were colour, edge, shape, surface, elevation. Colonies were selected randomly and were characterized using biochemical tests such as Gram staining and other relevant confirmatory tests. Bacterial isolates were identified with reference to Bergey's manual of determinative Bacteriology [23]. The bacterial isolates identification was based on cultural characteristics descriptions on the EMB agar (Hi-media, Mumbai, India).

Reconstitution of the aqueous and ethanol extract of *Ocimum gratissimum*

Different concentrations of the extract (25mg, 50mg, and 100mg) were weighed and dissolved in 1ml of diluted DMSO (Dimethyl Sulfoxide) and a membrane filter was used to sieve out any contaminants in the extract [24].

Determination of the antibacterial activity of *Ocimum gratissimum* aqueous and ethanol extracts

Antibacterial activities of the plant extract were determined using the agar well diffusion method as described by **Adeyemi et al.** [25]. Ciprofloxacin (Oxoid, Basingstokes, UK) (0.15 g/ml) was used as a standard antibacterial agent for positive control. Different concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml, of the extracts were used for the bioassay using Mueller Hinton agar (MHA) (Hi-media, Mumbai, India). The surface of already solidified MHA plates was streaked with the evenly turbid bacterial cell suspension. A sterile cork borer was used to bore 4 holes on already solidified MHA (Oxoid, Basingstokes, UK) plates. The concentrations of the crude extract were filter-sterilized into respective holes using a sterile millipore membrane filter with pore sizes of 0.22 µm (Delson Pascal Laboratories, Nigeria) unto the MHA plates already seeded with the test organisms as conducted by **Esimone et al.** [26]. The antimicrobials present in the plant extract are allowed to disperse in the medium. The plates were incubated afterward at 37 ºC for 24 hours and the zones of inhibitions were recorded using a vernier caliper (500-197-20, Mitutoyo, New Delhi, India).

Determination of minimum inhibitory concentration (MIC) of the aqueous and ethanol extract of *Ocimum gratissimum*

The determination of the MIC and MBC of the plant extract was performed according to the procedure as described by **Dada and Faleye,** [27]. About 5 ml of freshly prepared Mueller Hinton Broth (MHB) was drawn out with sterile pipette into test tubes, then 0.1 ml of inoculum of the bacterial organisms (1×10^6 cell/ml) were inoculated into each test tube and mixed thoroughly. With the aid of a sterile pipette, one millimeter of the different concentrations of 25 mg/ml, 50 mg/ml, 100mg/ml of *Ocimum gratissimum* aqueous, and ethanol extracts was withdrawn into each test tube containing the broth culture of the various bacterial organisms enumerated in this study. The test tubes were then incubated at 37 °C for 24 hrs. The test tubes were examined for microbial growth by observing the turbidity with a spectrophotometer (QTECH Portable UV-VIS Spectrophotometer DU-8800D).

Determination of minimum bactericidal concentration (MBC) of the aqueous and ethanol extract of *Ocimum gratissimum*

To determine the MBC of bacterial isolates in the MIC assays which showed the lowest growth after incubation were streaked out on solidified nutrient agar plates using a sterile inoculating loop and incubated at 37 °C. The lowest concentration that showed the lowest growth on plates after 24 hours of incubation indicates bactericidal effect and was taken as MBC as described by **Ogata et al.** [28].

Structural elucidation of aqueous and ethanol extract of *Ocimum gratissimum*

The analysis of the sample was performed using GC-MS equipment on Varian 4000 GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Runtime was 40 minutes. MS Varian 3800 mass spectrometer, coupled to a Varian 4000 gas chromatograph was used according to the manufacturer's protocol. An Agilent column, HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. Experimental conditions of GC-MS system were as follows: carrier gas: nitrogen, injection temperature: 250 °C, split ratio 10:1, Film thickness: 0.25 µm Flow rate of mobile phase (carrier gas: N2) was set at 1.0 ml/min. In the gas chromatography part, programmed with an oven temperature of 40 °C raised to 280 °C at 5 °C/min

and the injection volume was 1 µl. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min as demonstrated by **Colombini et al.** [29].

Statistical data analysis

All experiments were performed in triplicates and data derived from the study were subjected to 2-way analysis of variance (ANOVA) and the level of significance was documented at $p \leq 0.05$. Separation of means was performed using means and standard error with the aid of SPSS (version '22).

Results

This study showed the percentage recovery of aqueous extract of *O. gratissimum* to be 13.9 % while that of ethanol extract to be 8.92 % as shown in **table (1)**.

Morphological characteristics and biochemistry of bacterial isolates from the water samples

The colony and cell features of the bacterial isolates were identified as, *Enterococcus faecalis (E. faecalis)* and *Escherichia coli (E. coli)* as shown in **table (2)**.

The biochemical characterization profile showed no disparity as both bacteria were catalase-positive. Both isolates were able to reduce nitrate and were oxidase-positive as shown in **table (3)**.

Antibacterial susceptibility profile of *Ocimum gratissimum* aqueous extract

Both test bacteria were susceptible to the *O. gratissimum* aqueous extract at wavering degrees producing zones of inhibition (ZOI). The 100 mg/ml concentration was the most effective. 100 mg/ml concentration had the highest ZOI on *E.coli* and *E. faecalis* at 30.67 ± 0.667 and 11.67 ± 1.202 mm and the least concentration (25 mg/ml) at 15.00 ± 1.555 mm (*E. coli*) and 4.33 ± 0.333 (*E. faecalis*) as shown in **table (4)**.

Antibacterial susceptibility profile of ethanol extract of *Ocimum gratissimum*

Both test bacteria were susceptible to the *O. gratissimum* ethanol extract at different degrees appreciable producing zones of inhibition (ZOI). The 100 mg/ml concentration was the most effective with the ZOI on *E. faecalis* and *E.coli* at 30.67 ± 0.667 and 26.67 ± 0.882 mm and the least concentration (25 mg/ml) at 8.33 ± 0.667 mm *E. faecalis* and 13.652 ± 0.667 *E. coli* as shown in **table (5)**.

Minimum inhibitory concentration and minimum bacteriocidal concentration of crude *Ocimum gratissimum* extracts on tested bacterial isolates

The aqueous extract of the *O. gratissimum* revealed that 50 mg/ml concentration was observed as the MIC for *E. coli* and *E. faecalis.* 25 mg/ml concentration was observed as the MIC of both *E. coli* and *E. faecalis* of the *O. gratissimum* ethanol extract. 100 mg/ml concentration was observed as the MIC and the MBC for the aqueous and ethanol extract of *O. gratissimum*

Chemical components of aqueous extract of *Ocimum gratissimum*

Table 6 shows the biochemical/bioactive compounds present in aqueous extract of *O. gratissimum* as revealed by gas chromatography and mass spectroscopy analysis. *Ocimum gratissimum* aqueous extract showed seventeen peaks which indicated the presence of seventeen phyto-constituents. The compounds identified were; Hexadecanoic acid, Phytol, Sabiene, β-Pinene, Myrcene, E-β-Ocimene, γ-Terpinene, Terpinolene, 1,8-Cineole, cis-Sabinene hydrate, Linalool, β-Cubebene, α-Bulnesene, Phenylpropanoids, β-Eudesmol, 1,10-di-epi-Cubenol, and Elemol**.** The result revealed that elemol had the highest retention time of 17.342 min while, Hexadecanoic acid had the least time of 1.234 minutes.

Structural elucidation of ethanol extract of *Ocimum gratissimum*

Table 7 shows the biochemical/bioactive compounds present in ethanol extract of *O. gratissimum* as revealed by gas chromatography and mass spectroscopy analysis. *Ocimum gratissimum* ethanol extracts displayed twenty-five peaks which indicated the presence of twenty-five phyto-constituents. The compounds identified were; Eugenol, Pentanedinitrile 2-methyl-, Benzenemethanamine, N-(1,1-dimethylethyl)-, Cyclohexane, 1,2,4-triethenyl-,Octanoic acid 2,7-dimethyloct-7-en-5-yn-4-yl ester, Caryophyllene, Phthalic acid, ethyl 3-methylbutyl ester, 4-Acetoxy-3-methoxystyrene, Phytol, Phthalic acid, 3-methylbut-3-enyl propyl ester, octadecanoic acid, Octadecanoic acid, methyl ester, 1-methyl-3-(1-methylethyl) benzene, Benzoic acid, 2-hydroxy-, pentyl ester, Hexadecanoic acid, Octadecanoic acid, Methyleugenol, Dodecane, 1,1-dimethoxy-, Hexadecanoic acid, methyl ester, Isopropyl palmitate, Octadecanoic acid, 2, 3-dihydroxypropyl ester, Z,Z-3,13-Octadecadien-1-ol acetate, Squalene

and Octadecyl vinyl ether. The result revealed that Octadecyl vinyl ether had the highest retention time of 33.93 minutes while, Eugenol acid had the least retention time of 4.87 minutes.

Table 1. Percentage recovery of *Ocimum gratissimum* aqueous and ethanol extracts.

Plant extracts	Initial dry weight of extract (g)	Weight after extraction (g)	Percentage recovery (%)
Aqueous *O. gratissimum*	421.5	58.6	13.9
Ethanol *O. gratissimum*	316.2	28.2	8.92

Table 2. Morphological characteristics of the bacterial isolates.

Colony characteristics			Cellular features		
Isolate no.	**Colour**	**Nature of colony**	**Gram's staining**	**Endospore staining**	**Probable organisms**
1.	Purple	Mucoid and entire	-ve, rod	-ve	*Enterococcus faecalis*
2.	Green metallic sheen	Shiny, mucoid, and entire	-ve, rod	-ve	*Escherichia coli*

Keys: +ve = positive; -ve = negative

Table 3. Biochemical characterization of bacterial isolates from the river water samples.

Biochemical tests	Isolate 1	Isolate 2
Catalase	+	+
Citrate	-	-
Indole	+	+
Nitrate reduction	+	+
Oxidase	-	-
Urease	+	+
Sugar fermentation	+	+

Keys: += positive; - = negative

Table 4. Antibacterial susceptibility of *Ocimum gratissimum* aqueous extract

Organisms	25 mg/ml	50 mg/ml	100 mg/ml
Enterococcus faecalis	4.33 ± 0.333	7.33 ± 0.667	11.67 ± 1.202
Escherichia coli	15.00 ± 1.555	23.67 ± 0.882	30.67 ± 0.667

Values are means ± standard error. Means carrying the same alphabet in the same column are not significantly different (p>0.5) .

Table 5. Antimicrobial sensitivity test for ethanol extract of *Ocimum gratissimim.*

Organisms	25 mg/ml	50 mg/ml	100 mg/ml
Enterococcus faecalis	8.33 ± 0.667	21.67 ± 1.202	36.00 ± 1.16
Escherichia coli	13.652 ± 0.667	21.67 ± 0.667	26.67 ± 0.882

Values are means ± standard error. Means carrying the same alphabet in the same column are not significantly different (p>0.5).

Table 6. Bioactive compounds in *Ocimum gratissimum* aqueous extract.

Peak #	RT (min)	Compound name	Molecular Formulae	M.W. (amu)	Abundance (%)
1	1.234	Hexadecanoic acid	$C_{16}H_{32}O_2$	290	5.76
2	2.342	Phytol	$C_{20}H_{40}O$	252	9.98
3	3.376	Sabiene	$C_{10}H_{16}O$	283	10.80
4	4.399	β-Pinene	$C_{10}H_{16}$	252	9.89
5	5.211	Myrcene	$C_{10}H_{16}$	204	7.78
6	6.432	E-β-Ocimene	$C_{10}H_{16}$	290	5.56
7	7.000	γ-Terpinene	$C_{10}H_{16}$	234	11.88
8	6.321	Terpinolene	$C_{10}H_{16}$	216	9.34
9	9.212	1,8-Cineole	$C_{10}H_{18}O$	234	10.34
10	10.233	cis-Sabinene hydrate	$C_{10}H_{18}O$	265	11.23
11	11.123	Linalool	$C_{10}H_{18}O$	212	10.65
12	12.222	β-Cubebene	$C_{15}H_{24}$	204	12.21
13	13.332	α-Bulnesene	$C_{15}H_{24}$	289	8.32
14	14.345	Phenylpropanoids		266	10.43
15	15.121	β-Eudesmol	$C_{15}H_{26}O$	204	11.34
16	16.211	1,10-di-epi-Cubenol	$C_{15}H_{26}O$	212	10.32
17	17.342	Elemol	$C_{15}H_{26}O$	289	9.54

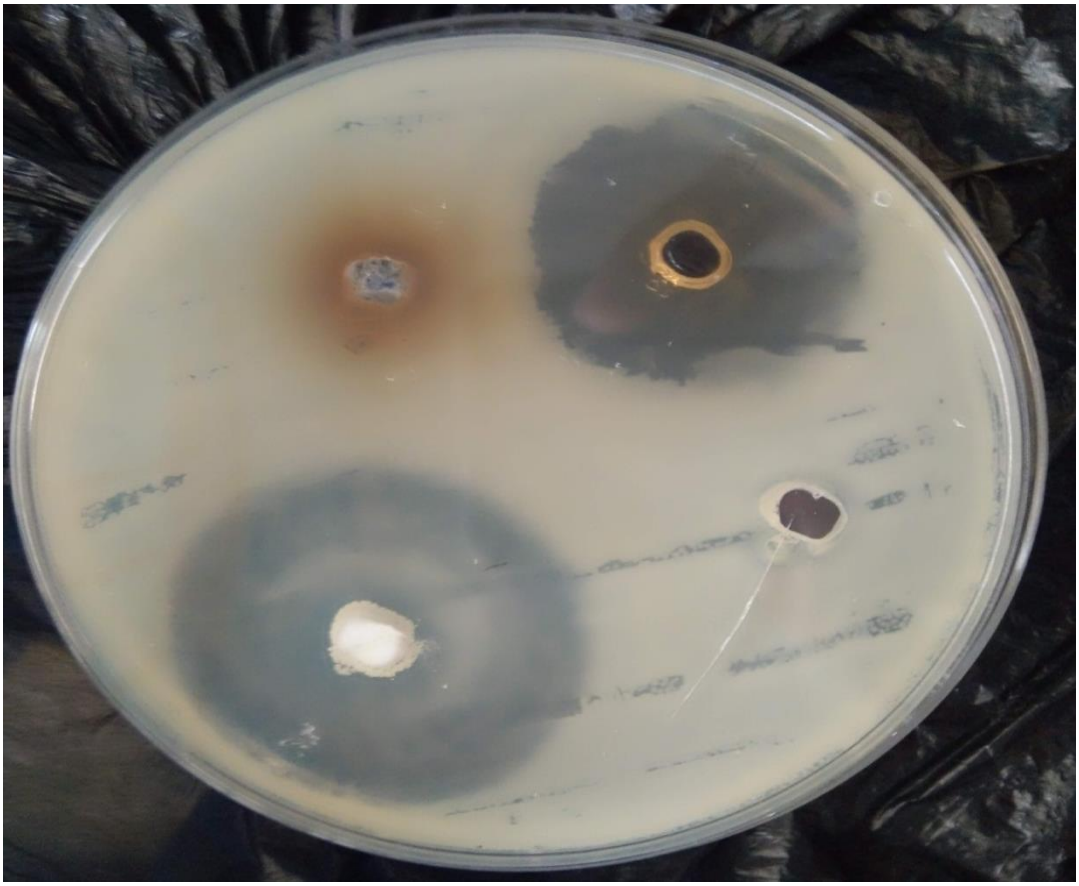
Key: RT- Retention Time; M.W- Molecular Weight; amu-atomic mass unit.

Table 7. Bioactive compounds in ethanol extract of *Ocimum gratissimum.*

Peak #	RT	Compounds Name	Molecular Formula	M.W. (amu)	Peak % area	% Composition
1	3.78	Unknown	C_8H_{12}	108	2.29	1.06
2	4.87	Eugenol	$C_{10}H_{12}O_2$	164	1.62	33.03
3	5.75	Pentanedinitrile, 2-methyl-	$C_6H_8N_2$	108	2.58	3.14
4	7.51	Benzenemethanamine, N-(1,1-dimethylethyl)-	$C_{11}H_{17}N$	163	3.44	1.07
5	9.50	Cyclohexane, 1,2,4-triethenyl-	$C_{12}H_{18}$	162	2.48	5.31
6	9.76	Octanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	$C_{18}H_{30}O_2$	278	6.12	1.05
7	10.26	Caryophyllene	$C_{15}H_{24}$	204	2.48	5.42
8	11.99	Phthalic acid, ethyl 3-methylbutyl ester	$C_{15}H_{20}O_4$	264	2.43	0.18
9	13.45	4-Acetoxy-3-methoxystyrene	$C_{11}H_{12}O_3$	192	3.63	0.09
10	13.93	Phytol	$C_{20}H_{40}O$	296	1.34	2.20
11	15.44	Phthalic acid, 3-methylbut-3-enyl propyl ester	$C_{16}H_{20}O_4$	270	3.63	1.09
12	17.87	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.57	2.11
13	19.14	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	2.29	2.15
14	21.45	1-methyl-3-(1-methylethyl) benzene	$C_{10}H_{14}$	134	2.48	0.25
15	23.78	Benzoic acid, 2-hydroxy-, pentyl ester	$C_{12}H_{16}O_3$	208	1.34	1.66
16	24.00	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.88	3.34
17	25.36	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.61	2.30
18	26.78	Methyleugenol	$C_{11}H_{14}O_2$	178	6.68	9.97
19	27.81	Dodecane, 1,1-dimethoxy-	$C_{14}H_{30}O_2$	230	3.82	1.47
20	28.41	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7.84	4.38
21	29.56	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	8.41	7.09
22	30.36	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	15.29	5.05
23	32.22	Z,Z-3,13-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	5.35	1.02
24	33.63	Squalene	$C_{30}H_{50}$	410	6.12	2.44
25	33.93	Octadecyl vinyl ether	$C_{20}H_{40}O$	296	2.29	0.83

Key: RT- Retention Time; M.W- Molecular Weight; amu-atomic mass unit.

Plate 1. Antibacterial activity of *Ocimum gratissimum* ethanol extract on *Escherichia coli*. Key: A=Negative control; B= Positive control; C=50 mg/ml, D= 100 mg/ml.



Positive Control = Ciprofloxacin

Discussion

Waterborne diseases as a result of the bacterial infections of surface water has caused a high mortality rate of people exposed to polluted water especially in rural and developing areas as reported by **Blaisi,** [30], hence the antimicrobial properties of aqueous and ethanol extracts of *O. gratissimum* (Linn) on bacteria isolated from surface water, Akure was evaluated in this study. The morphological and biochemical description of the bacterial isolates enumerated from the contaminated surface water of Onyearugbulem river includes; *E. faecalis* and *E. coli.* This is in accordance with **Muller et al.** [31] who gave a summary of the morphological characteristics of bacterial isolates associated with surface water. Most of the bacterial consortia enumerated from this study are pathogenic bacteria that are wholly responsible for water-borne diseases such as gastrointestinal disorders and diarrhea. The ingestion of pathogenic bacteria through water contamination may lead to water-borne outbreak epidemics as buttressed by **Craun et al.** [32]; **Pandey et al.** [33]. The reason for the pathogenicity of these water-borne related bacteria is that most of the bacteria cause gastrointestinal disturbances having symptoms such as diarrhea, fever, vomiting, and abdominal pain. Most of the above symptoms were caused by *E. coli*, which parallel to the findings of this study.

The percentage recovery of *O. gratissimum* extract shows the two different yields when subjected to the same extraction process with two different quantities of dry weight. The aqueous extract had a higher percentage recovery of 13.9 % than that of ethanol extract which had a percentage recovery of 8.92 %. This agrees with **Ibekwe et al.** [34] that aqueous extract of *Ocimum gratissimum* has a high polar state. This is due to the presence of pharmacologically-active constituents including carbohydrate and amino acids with their derivatives which is in agreement with **Azwanida,** [35]. The aqueous extract percentage yield result of this study also **Okoduwa et al.** [36] as they observed a high percentage yield of 33% for aqueous extract of *O. gratissimum* with water the least polar solvent used

in their extraction as n-hexane, chloroform, ethyl-acetate, and n-butanol were utilized in increasing order of polarity in their work.

The outcome of the gas chromatography and mass spectroscopy analysis conducted on *O. gratissimum* crude extracts to identify the bioactive compounds present revealed that Seventeen and twenty-five different compounds were identified in the aqueous and ethanol extracts of *O. gratissimum* respectively which are mainly categorized by the percentage abundance. Some of the identified compounds include eugenol, pentane-dinitrile, 2-methyl-, squalene, octadecyl vinyl ether, and methyleugenol. Eugenol had the highest percentage composition of 33.0% on the ethanol extract of *O. gratissimum*, while γ-Terpinene had the highest percentage composition of 11.88% in aqueous extract of *O. gratissimum*. This agrees with the study of **Rai,** [37] who stated that terpenoids constitute a group of compounds that occur in the family of Asteraceae. The reason for this disparity might be due to the difference in polarity of the extraction solvents employed in this study. The bioactive compounds identified in ethanol extract of *O. gratissimum* having high molecular weight were more of essential oils including; phytol (296 atomic mass unit), octadecanoic acid-methyl ester (298 atomic mass unit), and octadecanoic acid (284 atomic mass unit) which is in tandem with the works of **Matasyoh et al.** [38]; **Moghaddam et al.** [39] **and Mith et al.** [40]. The presence of eugenol as the highest percentage composition of bioactive compounds in ethanol extract of *O. gratissimum* is in agreement with **Igbinosa et al.** [41]; **[Onyebuchi and Kavaz,** [42] as they identified high phenolic compounds in ethanol extract of *O. gratissimum.*

All ethanol extract of *Ocimum gratissimum* showed more inhibitory activity against the bacterial isolates than the aqueous extract. This is in concord with the observation of Ibekwe and his co-researchers as they observe high inhibitory potentials of ethanol extracts of *O. gratissimum* compared to the aqueous extract in their study [35]. The ZOI of the plant extract diameters at a concentration of 100 mg/ml ranged between 4–30 mm for aqueous and ethanol extracts on *E. coli* and *E.faecalis* as observed by **Chinedu et al.** [43], as they observe high inhibitory potential of ethanol extract of *O. gratissimum* most especially against *Staphylococcus aureus* which is also evident in this study. Since *O. gratissimum* have been shown to exhibit strong antimicrobial tendencies when extracted with ethanol, the difference in solubility of ethanol and water used as extraction solvents in this study could be responsible in variation in antibacterial activity as supported by **Bamidele et al.** [44]. Some extracts showed ZOI higher than the positive control antibacterial agent; ciprofloxacin at 150 mg concentration against 100 mg concentration of the *O. gratissimum* leaf aqueous and ethanol extract most especially on *Escherichia coli.*

This study has shown that ethanol extract from the leaves of *O. gratissimum* possesses remarkable antibacterial activity against pathogenic bacterial consortia enumerated from the surface water analyzed in this study. *Ocimum gratissimum* contains phytoconstituents which have been found to inhibit the growth of the isolated bacteria; these phytochemicals may be responsible for the high medicinal values of *O. gratissimum.* The demonstration of antibacterial activity against the test bacteria is an indication that there is the possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents. The inhibition in the zone of inhibition of the extract on the isolates can be directly associated with the organism's susceptibility to the antibacterial components present in the extracts. This notion bears semblance to the observation of **Olusola-Makinde and Bayode,** [45] who both worked on the comparative antimicrobial study of *Vernonia amygdalina* and *Lawsonia inermis* against microorganisms from surface water environment.

The antimicrobial effect of aqueous extract was low and not as effective as ethanol extract, this may be attributed to the presence of soluble phenolic and poly-phenolic compounds that are readily extracted with a non-polar solvent such as ethanol when compared with a polar solvent (aqueous). **Okigbo et al.** [9]; **Amanze et al.** [46] reported that inactivity of plant extracts may be due to the age of the plant, extracting solvent, method of extraction, and time of harvesting of plant materials. Conversely, the ethanol extracts *O. gratissimum* showed a concentration-dependent gradient decrease in the level of inhibition against isolates. From the results, there is a variation in the degrees of antibacterial activities of the extracts on the isolates. The variation is presumed to be due to different active compounds present in these plants.

The MIC of *O. gratissimum* extracts was performed on *E. coli* and *E. faecalis.* The MIC values obtained on the test bacterial organisms

varied from one plant extract to another and it was observed that the MIC of ethanol extract for *E. coli* and *E. faecalis* 25 mg/ml. This result is in alignment with the observations of Hamma and his co-workers as they observe 25 mg/ml as the MIC values of *S. aureus* and *E. coli* [47]. The MIC values of aqueous extract of *O. gratissimum* on the tested bacterial isolates might be due to the low presence of phenolic compounds in the structurally-elucidated aqueous extract of *O. gratissimum* compared to the ethanol extract. This implies that *O. gratissimum* extracts have bacteriostatic effects on the tested bacterial isolates enumerated from contaminated surface waters, with ethanol extract having more inhibitory effect on most of the bacterial isolates than the aqueous extract in consonance with **Agatemor** [16]; **Olusola-Makinde and Bayode,** [45] who reported that Gram-negative bacteria (*E.coli*) are more resistant to than Gram-positive (*Staphylococcus aureus*) as demonstrated in this study. The MBC result of 100 mg/ml for all tested bacterial isolates enumerated in this study is analogous to the findings of **Hamma et al.** [47], as they observe 100 mg/ml as the MBC for *staphylococcus aureus* and *E. coli*. These findings indicate that the usage of *O. gratissimum* leaf ethanol extract in the treatment of water-borne infections and eugenol could also be explored for future contemporary studies due to its high percentage composition as a phenolic essential oil with potential for high ethno-botanical potentials.

Conclusion

This study revealed the bioactive compounds present in *O. gratissimum* leaves with Eugenol (33.03%) and γ-Terpinene (11.88%) having the highest percentage abundance in aqueous and ethanol extract respectively. The study also showed the antibacterial properties of *O. gratissimum* against bacteria associated with the surface water samples. These findings indicate that *O. gratissimum* leaf ethanol extract can be employed in the treatment of *E. coli* and *E. faecalis* infections associated with contaminated surface water. Eugenol could be used explored for contemporary antibacterial studies to curb contaminated surface water-related bacteria. The provision of portable water should be made available by the government to areas with little or no access to portable water supply to ameliorate problems associated with water supply.

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Authors' contributions

Author O. O. Olusola-Makinde designed the study concept and supervised the study. D. A. Lawrence conducted the literature search, methodology, analyze/interpreted the data. D. A. Lawrence wrote the first manuscript draft. O. O. Olusola-Makinde edited and reviewed the draft. Both authors approved the final manuscript.

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