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Original Research Article

Anti-vibrio potentials of acetone and aqueous leaf extracts of *Ocimum gratissimum* (Linn)

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Abstract

Purpose: *To evaluate the anti-vibrio potentials of acetone and aqueous leaf extracts of Ocimum gratissimum and determine its relevance in the treatment of vibrios infection.*
Methods: *The agar-well diffusion method was used for screening the extracts for their anti-vibrio activity. Broth micro-dilution assay was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. Time-kill assay was used to assess bactericidal and/or bacteriostatic activity.*
Results: *The acetone extract showed activity against 47.5 % (19/40) of the test bacteria, while the aqueous extract had activity against 30 % (12/40). MIC and MBC values range for the acetone extract were 0.625 – 5.0 mg/mL and 2.5 – 10 mg/mL respectively. The range of MIC exhibited by the antibiotic (gentamicin) against the vibrios is 0.002 mg/mL and >0.256 mg/mL. Significant reduction in the bacterial density was at 2 × MIC after a 4 h interaction period, while bacterial density after 6 and 8 h interactions with extract was highly bactericidal. Growth inhibition and efficacy of the crude acetone extract were observed to be both concentration- and time-dependent.*
Conclusion: *The bacteriostatic and bactericidal activities observed for Ocimum gratissimum leaf suggest that the plant is a potential source of bioactive components that may be effective in the treatment of vibrios infections.*

Keywords: *Ocimum gratissimum, Vibrios infection, Antibiotics, Multi-drug resistance, Minimum inhibitory concentration, Minimum bactericidal concentration, Time kill assay*

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INTRODUCTION

The increase of antimicrobial resistance among pathogenic bacteria has emerged as an important public health issues, this has showcase debate about the careful use of antimicrobial agents [1]. Most of these water bodies are used for drinking water, household, recreational purposes and fishing by the people living in the surrounding communities and they are at risk of acquiring *Vibrio* infections [2]. *Vibrio* species and other food-borne pathogens that have been exposed to antibiotics within or outside the effluents environment, can acquire antibiotics resistance transferable by mobile genetic elements and horizontal gene transfer [1]. This has resulted to increased resistance to different antibiotics groups. *Vibrio* specie is not

an exception when it comes to antibiotic resistant strains [1,3] several studies have reported the appearance of such strains. The development of antibiotic resistance outpaces the development of new drugs such that it has become a global challenge with detrimental long term effects [4].

The challenge is to develop effective approach that could help control antibiotic resistance in pathogens such as *Vibrio* species. Therefore the need to increase the body of knowledge on the antimicrobial activities of some traditional medicinal plants such as *Ocimum gratissimum* towards controlling the effects of antibiotic resistance bacteria becomes imperative. *Ocimum gratissimum* has been established to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria which have earlier been reported [5,6]. The medicinal properties of *Ocimum gratissimum* according to our study reveal that biologically active components of this plant have disease inhibiting ability potentials [7]. Pharmacologically active molecules may act individually, additively or in synergy to improve health [8].

Ocimum gratissimum has been reported to be active against bacteria and fungi species [9, 10]. There is paucity of information of the anti-vibrio potential of the aqueous and acetone extracts of *Ocimum gratissimum* leaves, especially against environmental strains of the bacteria such as those isolated from aquaculture environments. Preliminary data revealed the increasing trend of multiple antibiotic resistances in *Vibrio* species isolated from fish pond in Benin City environs. The exploration for new anti-vibrio compounds especially of plants origin becomes imperative. This study was designed to evaluate anti-vibrio potentials of aqueous and acetone extracts of *Ocimum gratissimum* leaves and justifies its relevance in the treatment of vibrios infections.

EXPERIMENTAL

Collection of plant material

Fresh leaves of *Ocimum gratissimum* (Linn) were collected in May and June, 2014 from a local farm in Benin City, Nigeria. The plant was authenticated by Dr Joseph Erhabor, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria, and a voucher specimen (UBHL 0281) was prepared and deposited in the herbarium of the Plant Biology and Biotechnology Department, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Preparation of extract

The plant leaves were allowed to air-dry at ambient temperature and pulverized using an electric blender (Pye Unicam, Cambridge, England) and stored in an air-tight container for further use. The pulverized leave powder (100 g) was steeped in the respective solvent (aqueous or acetone, 500 mL) and placed in an orbital shaker for 48 h. The resultant extract was centrifuged at 3,000 rpm for 5 min at 4 ^{o}C. The supernatant was then filtered through Whatman No.1 filter paper, while the residue was then used in the second extraction with 300 mL of the respective solvent. After the second extraction process, the aqueous extract was freeze-dried at -40 °C and dried for 48 h using a freeze dryer Savant Refrigerated Vapour Trap, (RVT 41404, CA, USA), whereas acetone extracts were concentrated under reduced pressure using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany) to remove the solvents. The concentrated extracts were allowed to dry to a constant weight under a stream of air in a fume cupboard at room temperature. The acetone extracts were reconstituted in dimethylsulphoxide (DMSO) at concentration of 5 % of the total volume made up with filtered sterile distilled water, while the aqueous extracts were reconstituted in filtered sterile distilled water.

Bacterial strains

Forty strains of *Vibrio* species were used in this study. The bacteria were isolated from fish pond (aquaculture environment) in Benin City, identified using Analytical Profile Index (API 20NE).The *Vibrio* isolates were found to belong to six species groups' which include:- *V. vulnificus, V. parahaemolyticus, V. fluvialis, V. mimicus, V. alglinolyticus* and *Vibrio* sp. The selections of these *Vibrio* strains were based on their phenotypic characterization to antibiogram profile to more than three groups of different antibiotics. *Vibrio* colonies were picked from 18 - 24 h old cultures grown on brain heart infusion agar and suspended in phosphate buffer solution (PBS) to give an optical density of approximately 0.5 at 600 nm.

Screening of crude extracts for anti-vibrio activity

The agar-well diffusion method was used in accordance with the method previously described by Irobi *et al* [11]. *Vibrio* strains inoculum were prepared as described above.

The prepared bacterial suspension (50 µL) was inoculated into sterile molten Mueller-Hinton agar medium at 50 ^{o}C in a MacCartney bottle, mixed gently and then poured into a sterile petri dish and allowed to solidify. A sterile 6 mm diameter cork borer was used to bore wells into the agar medium. The wells were inoculated with 50 µL of the respective extract solution at a concentration of 10 mg/mL. Gentamicin (0.002 mg/mL) was used as a positive control, and distilled water was used as the negative control while 5 % dimethylsulphoxide (DMSO) was also tested to determine its effect on each organism. All plates were incubated at 37 ^{o}C for 24 h. After incubation, zones of inhibition were measured and recorded.

Determination of minimum inhibitory concentration (MIC)

The MICs were determined for *Vibrio* strains that had shown susceptibility to the crude extracts using the broth microdilution method as described in EUCAST [12], with the aid 96-well microtiter plates. Two-fold serial dilutions using filtered sterile distilled water were carried out from 10 mg/mL stock plant extracts to make ten test concentrations ranging from 10 mg/mL to 0.0195 mg/mL for each solvent extract.

A 100 µL-volume of double strength Mueller-Hinton broth was introduced into the 96- well microtiter plates and 50 µL of the varying concentrations of the extracts were added in decreasing order with 50 µL of the test bacteria suspension. Control experiment were set up; the positive control wells contains 100 µL Mueller-Hinton broth, 50 µL of gentamicin and 50 µL of the test bacteria, and the negative control wells containing 100 µL Mueller-Hinton broth, 50 µL filtered sterile distilled water and 50 µL of the test bacteria. The plates were incubated at 37 ^{o}C for 18 - 24 h. Results were read visually by adding 40 µL of 0.2 mg/mL of ρ-iodonitrotetrazolium violet (INT) into each well. A colour change from colourless to purple, indicated actively growing bacteria based on the oxidation-reduction reaction in which electrons are transferred from NADH (a product of the oxidation of threonine to 2-amino-3-ketobutyrate) to INT which forms the red formazan which is purple in colour. The MIC was recorded as the lowest concentration of the extract that prevented the appearance of visible growth of the organism after 18 - 24 h of incubation.

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from the MIC broth micro-dilution assays by sub-culturing 10 µL from each well which did not show growth after 24 h of incubation and inoculating onto fresh Mueller-Hinton agar plates [13]. The plates were incubated for 48 h after which the numbers of colonies were counted. The MBC was defined as the lowest concentration that kill more than or equal to 99.9 % of the inoculum compared with initial viable counts [13].

Time-kill assay

The time kill assay was done following the procedure as described by Odenholt *et al* [15]. The turbidity of the 18 h old test *Vibrio* was first standardized to 10^{8}CFU/mL. Two different concentrations of the plant extract were made starting from the MIC and 2× MIC value for each test bacteria. A 0.5 mL of cell density from each bacteria suspension was added to 4.5 mL of different concentrations of the extracts solutions, and the time kill assay was determined at 0, 2, 4, 6 and 8 h. A 0.5 ml of each suspension was withdrawn at 2 h intervals and transferred to 4.5 mL of Mueller Hinton broth recovery medium containing 3 % Tween 80 to deactivate the effects of the antimicrobial agent on the test bacteria. The suspension was serially diluted and an aliquot of100 µL plated out on Mueller Hinton agar using pour plate technique, and incubating at 37 ^{o}C for 24 h. Emergent bacterial colonies were counted, CFU/mL calculated and compared with the count of the culture control without extract.

Statistical analysis

All incubations and determinations were performed two or more times and the mean taken. The data were analyzed using SPSS version 18.0 (SPSS Inc. PASW Statistics for Windows, Chicago: SPSS Inc.), and Excel 2007 version (Microsoft). One way ANOVA was used to compare the mean difference in inhibitory activities of extracts and antibiotics by Tukey's post hoc test. Differences were considered significant at $p < 0.05$ or $p < 0.01$.

RESULTS

Antibacterial activity of *Ocimum gratissimum* leaf extract

The results of the anti-vibrio activities of the acetone and aqueous crude extracts of *Ocimum gratissimum* leave are shown in Table 1.The acetone extract showed activity against 47.5 % (19/40) of the test bacteria, while the aqueous extract exhibited activity against 30 % (12/40).

Table 1: Sensitivity profile of antibiotic (gentamicin), crude acetone and aqueous leaf extracts of *Ocimum gratissimum* against *Vibrio* pathogens

Bacterial isolate	Inhibition zone diameter (mm)			
	Acetone extract (10 mg/mL)	***Aqueous extract (10 mg/mL)***	***Gentamicin (0.002 mg/mL)***	***P value***
Vibrio specie (ADW2)	15 ± 0.04	0 ± 0.00	22 ± 1.21	0.05
Vibrio specie (UM4)	10 ± 1.02	0 ± 0.00	11 ± 1.01	0.05
Vibrio specie (IKH12)	0 ± 0.00	0 ± 0.00	25 ± 1.11	ns
Vibrio specie (UM9)	18 ± 1.01	10 ± 1.05	24 ± 0.15	0.01
Vibrio specie (IKH10	15 ± 0.10	0 ± 0.00	23 ± 0.21	0.05
Vibrio specie (IKH15)	0 ± 0.00	0 ± 0.00	25 ± 0.17	ns
Vibrio mimicus (ADW14)	10 ± 0.15	5 ± 0.00	17 ± 1.55	0.05
Vibrio mimicus (IKH5)	0 ± 0.00	0 ± 0.00	13 ± 1.05	ns
Vibrio mimicus (UM10)	12 ± 1.25	8 ± 1.11	17 ± 0.14	0.05
Vibrio alglinolyticus (IKH20)	0 ± 0.00	0 ± 0.00	15 ± 1.08	ns
Vibrio alglinolyticus (ADW4)	18 ± 0.56	10 ± 0.67	27 ± 0.57	0.01
Vibrio alglinolyticus (UM5)	9 ± 0.77	0 ± 0.00	16 ± 0.02	0.05
Vibrio vulnificus (IKH25)	16 ± 0.08	10 ± 0.50	25 ± 0.28	0.01
Vibrio vulnificus (IKH30)	0 ± 0.00	0 ± 0.00	20 ± 0.32	ns
Vibrio vulnificus (IKH18)	12 ± 0.01	8 ± 0.15	15 ± 0.08	0.05
Vibrio vulnificus (UM15)	0 ± 0.00	0 ± 0.00	30 ± 0.09	ns
Vibrio vulnificus (UM9)	15 ± 0.19	6 ± 0.00	17 ± 1.26	0.05
Vibrio vulnificus (ADW10)	0 ± 0.00	0 ± 0.00	30 ± 0.20	ns
Vibrio vulnificus (ADW20)	11 ± 0.01	0 ± 0.00	25 ± 1.02	0.05
Vibrio vulnificus (IKH28)	0 ± 0.00	0 ± 0.00	21 ± 2.05	ns
Vibrio vulnificus (UM25)	0 ± 0.00	0 ± 0.00	29 ± 1.15	ns
Vibrio vulnificus (ADW15)	0 ± 0.00	0 ± 0.00	20 ± 0.50	ns
Vibrio parahaemolyticus (ADW3)	17 ± 1.25	8 ± 1.02	20 ± 0.21	0.05
Vibrio parahaemolyticus (ADW7)	0 ± 0.00	0 ± 0.00	24 ± 0.51	ns
Vibrio parahaemolyticus (UM8)	0 ± 0.00	0 ± 0.00	25 ± 0.41	ns
Vibrio parahaemolyticus (UM35)	0 ± 0.00	0 ± 0.00	26 ± 0.01	ns
Vibrio parahaemolyticus (UM40)	15 ± 0.18	8 ± 1.05	23 ± 1.24	0.05
Vibrio parahaemolyticus (IKH29)	0 ± 0.00	0 ± 0.00	24 ± 0.01	ns
Vibrio parahaemolyticus (IKH45)	0 ± 0.00	0 ± 0.00	22 ± 1.34	ns
Vibrio parahaemolyticus (IKH35)	14 ± 0.01	0 ± 0.00	22 ± 1.52	0.05
Vibrio fluvialis (UM45)	16 ± 0.21	7 ± 1.10	22 ± 0.10	0.05
Vibrio fluvialis (UM28)	13 ± 0.14	0 ± 0.00	27 ± 1.00	0.05
Vibrio fluvialis (IKH55)	0 ± 0.00	0 ± 0.00	18 ± 1.21	ns
Vibrio fluvialis (ADW22)	0 ± 0.00	0 ± 0.00	23 ± 0.02	ns
Vibrio fluvialis (IKH37)	0 ± 0.00	0 ± 0.00	28 ± 0.21	ns
Vibrio fluvialis (ADW38)	15 ± 1.05	9 ± 0.52	22 ± 0.10	0.05
Vibrio fluvialis (UM48)	0 ± 0.00	0 ± 0.00	15 ± 1.10	ns
Vibrio fluvialis (IKH16)	0 ± 0.00	0 ± 0.00	16 ± 0.26	ns
Vibrio fluvialis(ADW45)	16 ± 2.01	8 ± 0.72	20 ± 0.87	0.05
Vibrio fluvialis (IKH20)	0 ± 0.00	0 ± 0.00	23 ± 0.09	ns

Data are mean ± SD (n = 3). Differences were considered significant at $p < 0.05$ and $p<0.01$; ns- not significant; 5 % DMSO negative controls had no activity on all tested *Vibrio* species

All the isolates tested were screened for activity of the extract at a concentration of 10 mg/mL. The zones of inhibition ranged between 10 ± 0.15 mm and 18 ± 1.01 mm for acetone extracts and 5 ± 0.00 mm to 10 ± 1.05 mm for the aqueous extracts. The positive control (gentamicin) shows activity against all the isolates with inhibition zones ranging between 11 ± 1.01mm and 30 ± 0.20 mm. All the bacterial isolates used were resistant to 5 % (v/v) DMSO used as the negative control.

MICs and MBCs of the extracts

Table 2 shows the MIC and MBC results for both extracts against the susceptible *Vibrio* isolates. The acetone extract had MIC values range of 0.625–5.0 mg/mL, while the MBC values range of 2.5–10 mg/mL. The aqueous extract had MIC values between 5 and 10 mg/mL and MBC values 10 mg/mL for all the isolates. The range of MIC exhibited by the antibiotic (gentamicin) against the vibrios is 0.002 mg/mL and > 0.256 mg/mL.

Table 2: MIC and MBC of the crude leaf extracts of *Ocimum gratissimum* and standard antibiotic against *Vibrio* isolates

Bacterial isolate	Extract				Gentamicin (mg/mL)
	Acetone (mg/mL)		*Aqueous (mg/mL)*		
	MIC	MBC	MIC	MBC	MIC
Vibrio specie (ADW2)	1.25	5	-	-	0.032
Vibrio specie (UM4)	5	10	-	-	0.256
Vibrio specie (UM9)	1.25	5	10	10	0.016
Vibrio specie (IKH10)	1.25	5	-	-	0.016
Vibrio mimicus (ADW14)	5	10	10	10	0.064
Vibrio mimicus (UM10)	1.25	5	-	-	0.128
Vibrio alglinolyticus (ADW4)	0.625	2.5	5	10	0.002
Vibrio alglinolyticus (UM5)	5	10	-	-	0.128
Vibrio vulnificus (IKH25)	0.625	2.5	5	10	0.002
Vibrio vulnificus (IKH18)	2.5	5	10	10	0.128
Vibrio vulnificus (UM9)	1.25	5	10	10	0.128
Vibrio vulnificus (ADW20)	5	10	-	-	0.004
Vibrio parahaemolyticus (ADW3)	0.625	2.5	10	10	0.064
Vibrio parahaemolyticus (UM40)	1.25	5	10	10	0.016
Vibrio parahaemolyticus (IKH35)	1.25	5	-	-	0.032
Vibrio fluvialis (UM45)	0.625	2.5	10	10	0.004
Vibrio fluvialis (UM28)	1.25	5	-	-	0.008
Vibrio fluvialis (ADW38)	0.625	2.5	10	10	0.004
Vibrio fluvialis(ADW45)	0.625	2.5	10	10	0.016

Key: MIC - minimum inhibitory concentrations; MBC- minimum bactericidal concentrations; - MIC value not determined

Bactericidal activity

The time course of the extract at different concentrations was examined and result presented in Table 3.

Results are presented in terms of Log_{10}CFU/mL decrease in viable cell count and are based on the conventional bactericidal activity standard that is, a 3Log_{10}CFU/mL or greater reduction in the viable cell density. Average log reduction in viable cell count in time kill assay for 1× MIC at different time ranged between 1.173 Log_{10} and 3.324 Log_{10}CFU/mL at 2 h; 1.100 Log_{10} and 2.276 Log_{10}CFU/mL at 4 h; 0.572 Log_{10} and 2.058 Log_{10}CFU/mL at 6 h, and 0.122 Log_{10} to 1.447 Log_{10}CFU/mL at 8 h interactions. The 2 × MIC revealed the following:- 1.050 Log_{10} to 2.890 Log_{10}CFU/mL at 2 h; 0.410 Log_{10} to 1.871 Log_{10}CFU/mL at 4 h; -0.951 Log_{10} to 1.205 Log_{10}CFU/mL at 6 h and -0.727 Log_{10} to 0.614 Log_{10}CFU/mL at 8 h interactions. Significant reduction in the bacterial density was at 2 × MIC after a 4 h incubation period, while the bacterial population after 6 h and 8 h interactions with the extract was highly bactericidal. Growth inhibition and efficacy of the crude acetone extract were observed to be dose and time dependent.

DISCUSSION

The recognition of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics have led scientist to investigate the antimicrobial activity of several medicinal plants utilized as folk medicines. This study has revealed that both acetone and aqueous extracts of the *Ocimum gratissimum* leaves have antagonistic activities against vibrios isolated from fish ponds (aquaculture environment). In folk medicine practice, *Ocimum gratissimum* leaves are usually extracted in an aqueous medium for use. The antagonistic activity exhibited by the aqueous extract *in-vitro* validates the traditional use of the plant for the treatment of diarrhea, upper respiratory tract infections, headache, ophthalmic, skin diseases, pneumonia, cough fever and conjunctivitis [15].

Vibrio parahaemolyticus, V. alginolyticus and *V. vulnificus* are known to cause seafood-borne infections such as septicemia and wound infections, and *V. vulnificus* has been reported to be responsible for 95 % of seafood-related deaths [16]. The findings in this study concur with previous reports on the antibacterial activities of *Ocimum gratissimum* leaves [17]. Antibacterial activity of the ethanolic extracts against a range of pathogenic bacteria such as *Escherichia coli, Streptococcus viridians, Klebisiella pneumonia, Pseudomonas aeruginosa, Listeria monocytogenes* and *Proteus vulgaris* has been documented [18].

It has also been established that the eugenol isolated from *Ocimum gratissimum* possess

Table 3: Inhibition of crude acetone extracts of *Ocimum gratissimum* against vibrios strains

Bacterial isolate	MIC (mg/mL)	Log $_{10}$ Kill 1 × MIC					*P*-value	Log $_{10}$ Kill 2 × MIC					*P*-value
		0 h	*2 h*	*4 h*	*6 h*	*8 h*		*0 h*	*2 h*	*4 h*	*6 h*	*8 h*	
Vibrio specie (ADW2)	1.25	3.053	2.230	1.120	2.058	1.201	0.05	3.478	2.890	1.871	-1.521	-1.253	0.01
Vibrio specie (UM9)	1.25	3.217	2.050	1.121	1.090	1.022	0.05	3.185	2.018	1.810	1.421	-2.152	0.01
Vibrio specie (IKH10)	1.25	2.991	2.524	2.197	1.703	1.065	0.05	2.481	1.921	1.511	-1.241	-2.101	0.01
Vibrio mimicus (UM10)	1.25	3.501	2.852	2.210	1.503	1.221	0.05	3.235	2.015	1.511	-1.152	-2.312	0.01
Vibrio alglinolyticus (ADW4)	0.625	3.167	2.450	2.230	1.641	1.447	0.05	3.248	2.210	1.916	1.125	1.062	0.05
Vibrio vulnificus (IKH25)	0.625	2.375	2.023	1.185	1.091	0.233	0.01	3.427	2.345	1.543	-1.091	-2.638	0.01
Vibrio vulnificus (UM9)	1.25	4.519	3.324	2.276	1.199	0.122	0.01	2.375	1.050	0.982	-0.951	-2.387	0.01
Vibrio parahaemolyticus (ADW3)	0.625	2.248	2.015	1.894	1.271	1.092	0.05	2.461	1.592	0.410	-1.310	-2.201	0.01
Vibrio parahaemolyticus (UM40)	1.25	2.131	1.299	1.024	0.825	0.525	0.01	2.539	1.895	1.104	1.205	-2.155	0.01
Vibrio parahaemolyticus (IKH35)	1.25	2.083	1.392	1.100	0.572	0.491	0.01	2.729	2.052	1.260	0.863	0.255	0.05
Vibrio fluvialis (UM45)	0.625	2.270	1.832	1.501	1.108	1.074	0.05	2.288	1.185	1.071	0.852	-0.727	0.01
Vibrio fluvialis (UM28)	1.25	2.275	2.142	1.150	0.931	0.252	0.01	2.260	1.148	1.022	0.649	-1.564	0.01
Vibrio fluvialis (ADW38)	0.625	2.284	2.010	1.134	1.028	0.429	0.05	2.451	1.292	1.083	0.850	0.614	0.05
Vibrio fluvialis (ADW45)	0.625	2.633	1.173	1.100	0.873	0.530	0.05	2.303	1.358	1.059	0.782	-1.287	0.01

Values are means of triplicates. The mean difference is considered significant at $p<0.05$ and $p<0.01$

antimicrobial activities [19,20].

The ocimum oil extracted from *Ocimum gratissimum* plant is active against several species of bacteria and fungi [18-20]. These phytochemical compounds have been known to play different roles in the antimicrobial potential of medicinal plants. Previous reports have demonstrated the anti-diarrhoeal activity of tannin, flavonoid and saponin, these secondary metabolites were elucidated in our previous studies [7], these molecules may act individually, additively or in synergy to improve health. The phytochemicals present in *Ocimum gratissimum* leaves might be responsible for the anti-vibrios activities found in this study, more so as most *Vibrio* species are implicated in diarrhoea.

The result of the study revealed response of the bacteria to the tested extract varied among the strains which is dose and time course dependent. The differences in susceptibility may be as a result of the differences in cell wall composition and genetic content of their plasmids [21]. While the active components in the crude extract may be acting synergistically to produce good antimicrobial effects [22], the disparity between the activities of the extract and the standard antimicrobial drug may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotics [23].

Time-kill results for *Vibrio* species when tested against acetone extract show it to be concentration- and time-dependent. The time kill course show the bactericidal activity and the duration of a bacteriostatic effect of a fixed concentration of the antimicrobial agent, thereby providing a clear analysis of the relationship between the extent of microbial population mortality and the antimicrobial agent concentration [24]. The rate of kill activity of the acetone extract shown to be bacteriostatic at MIC values after 6 and 8 h interaction period. Bactericidal action or activity was shown at 2 × MIC values after 8 h exposure time for the test bacteria, since a reduction of the viable bacterial density of ≥ 99.9 % or ≥3Log_{10} in cfu/mL is used as a standard of measurement for bactericidal efficacy [25], thus suggesting *O. gratissimum* to be a potential source of active compounds of significant relevance in anti-vibrio chemotherapy.

CONCLUSION

The findings of this work indicate that the bacteriostatic and bactericidal activities of *Ocimum gratissimum* leaf extracts are significant, suggesting that the plant is a potential source of bioactive components that can be used in the treatment of vibrios infections. The acetone extract is more active and is bactericidal. Further studies are, however, ongoing to isolate some components of interest from the crude extract, in order to identify the active/functional groups that may be responsible for its bioactivity and hence the mechanism/mode of action.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHORS' CONTRIBUTION

The research idea, study concept and design were conceived by EOI and OGI. EOI and OGI were involved in drafting and revising the manuscript. All the authors read and approved the final manuscript.

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