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Ocimum gratissimum Extract's Effectiveness against *Vibrio cholerae* from Uli Streams

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Abstract

This study investigated the antimicrobial activity of *Ocimum gratissimum* extract against *Vibrio cholerae* strains isolated from stream samples. A total of 100 samples were collected and screened for *Vibrio* species using standard microbiological techniques. The phytochemical constituents of *Ocimum gratissimum* were quantitatively determined, and its antibacterial activity was evaluated using agar-well diffusion method. The study revealed the presence of three *Vibrio cholerae* strains: C6789 (VCC6), E7976 (VCE7), and R1979 (VCR1). VCR1 was the most predominant strain in the stream samples ($p < 0.05$). Phytochemical analysis of *Ocimum gratissimum* extract showed the presence of alkaloids, steroids, cardiac glycosides, phenolics, flavonoids, tannins, and saponins. The extract exhibited significant antimicrobial activity against the *Vibrio cholerae* strains ($p < 0.05$), with the highest inhibition observed against VCE7. Statistical analysis revealed a significant difference in the susceptibility of the strains to the extract ($p < 0.05$). The findings of this study demonstrate the potential of *Ocimum gratissimum* extract as an antimicrobial agent against *Vibrio cholerae* strains. The pronounced activity of the extract against VCE7 suggests its potential application in the treatment of infections caused by this strain. Further studies are needed to explore the mechanisms of action and the efficacy of *Ocimum gratissimum* extract *in vivo*.

Keywords: Strains, Antibacterial, Predominant, Phytochemical

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Introduction

Streams play a crucial role in shaping the landscape through erosion, wearing down rocks, and carrying sediment downstream. The morphology of streams varies depending on the landscape they flow through, with different shapes and characteristics. Streams support a diverse range of plants and animals, each adapted to thrive in these environments. However, streams face numerous threats, including pollution, dams, and urbanization, which can harm their health and the organisms that depend on them (Obu *et al*., 2024).

In Uli community, streams play vital roles, majorly anthropological roles. It serves many domestic purposes ranging from bathing, drinking, swimming, cleaning and other eccentric functions. Several reports have shown that most water from these streams are associated with cholera infection (Iheukwumere *et al*., 2025), and needs a natural and permanent remedy without any adverse effect to the ecosystem.

Despite the importance of streams, they are often vulnerable to contamination by pathogenic microorganisms, such as *Vibrio cholerae* (Madora and Momba, 2010). This bacterium can cause cholera, a diarrheal disease that can be life-threatening if left untreated. The emergence of multidrug-resistant *Vibrio cholerae* strains has further complicated the treatment of cholera (Bulus *et al*., 2015), highlighting the need for alternative antimicrobial agents. This study aims to investigate the antimicrobial activity of *Ocimum gratissimum* extract against *Vibrio cholerae* strains isolated from streams.

Materials and Methods

Sample collection, handling and transportation: The samples used for this study were drawn from the rivers. A total of 100 freshwater samples were collected from five different streams used in Uli community. Samples were taken from twenty different sites, each site in triplicates. The stream samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the river water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis.

Isolation of organisms: One milliliter (1.0 ml) water sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Thiosulphate Citrate Bile Sucrose agar (TCBS/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), Ekesiobi *et al*. (2025a), and Ekesiobi *et al*. (2025b) biochemical reactions as described in the study published by Iheukwumere *et al*. (2020), Ekesiobi *et al*. (2025c) and Ekesiobi *et al*. (2025d) and molecular characterization as described in the study published by Gabriela *et al*. (2014), Ekesiobi *et al*. (2025e) and Ekesiobi *et al*. (2025f). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates

Prevalence and Distribution of the Isolates in the Stream Samples

The number each bacterial isolate in each sampling area were enumerated, and these were calculated in percentage of the occurrences. The bacterial that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al*. (2021), Ekesiobi *et al*. (2025g) and Iheukwumere *et al*. (2025a).

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the study at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test as described in the study published by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) and Iheukwumere *et al*. (2025e).

Results

The isolates exhibited similar cultural characteristics on thiosulfate-citrate-bile-salt-sucrose agar, including elevation, edge, and surface morphology. They also showed similar morphological attributes, such as string test, Gram reaction, endospore, capsule, and motile nature (Table 1).

The isolates were characterized as hydrogen sulfide production, methyl red, urease, arabinose, and dulcitol negative. However, they were catalase, citrate, gelatin, oxidase, glucose, and galactose positive, with variations in their ability to utilize inositol, xylose, sorbitol, and lactose (Table 2).

The nucleic acid extracts had an absorbance ratio of 1.80-1.90 at 260/280 nm, confirming the presence of DNA (Table 3). Molecular analysis identified the isolates as *Vibrio cholerae* 01 biovar El Tor strains C6789 (VCC6), R1979 (VCR1), and E7976 (VCE7) (Table 4).

The plant extract contained alkaloids, phenolics, flavonoids, saponins, tannins, glycosides, and steroids, with alkaloids being the most abundant and steroids being the least abundant (Table 5).

The leaf extract of *Ocimum gratissimum* exhibited pronounced activity against *Vibrio* species, with the ethanolic extract showing greater activity than the aqueous extract ($p < 0.05$). However, the activity of the extract was significantly lower than that of ciprofloxacin ($p < 0.05$). The extract showed the most pronounced activity against VCP2 and the least activity against VCC6 (Table 6).

Table 1: Cultural and morphological characteristics of isolate

Parameter	L	M	N
Appearance in TCB	Yellow	yellow	Yellow
Edge	Smooth	smooth	Smooth
Elevation	Raise	raise	Raise
Surface	Smooth	smooth	Smooth
String test	+	+	+
Gramm test	-	-	-
Shape	rod/comma	rod/comma	rod/comma
Endospore	-	-	-
Capsule	-	-	-
Motility	+	+	+

Table 2: Biochemical characteristics of the isolate.

Parameter	L	M	N
Catalase	+	+	+
Citrate	+	+	+
Galatin	+	+	+
H^2S	-	-	-
Methyl red	-	-	-
Oxidase	+	+	+
Urease	-	-	-
Arabinose	-	-	-
Glucose	+	+	+
Galactose	+	+	+
Insitol	-	+/-	-
Dycitol	-	-	-
Xylose	+/-	-	+/-
Surbitol	-	+/-	-
Lactose	+/-	+/-	+/-

Table 3: Verification of the extraction nucleus

sample ID	conc(üg/ml)	260nm	270nm	260/280
L	121.20	3.0120	1.6119	1.86
M	125.70	3.1082	1.6810	1.85
N	132.82	3.2110	1.7643	1.82

Table 4: Molecular identity of the isolate

Parameter	L	M	N
Max score	5686	5686	5686
Total score	7595	7595	7595
Query cover (%)	100	100	100
E value	0.0	0.0	0.0
Identity(%)	100	100	100
Accession length	1070357	1070357	1071008
Accession number	CP047298	CP472300	CP472304
Description	*Vibro cholerae* 01 biovar EIT strain C6789(VCC6)	*Vibro cholerae* 01 biovar EIT strain E7976 (VCE7)	*Vibro cholerae* 01 biovar EIT strain R1979(VCR1)

Table 5: Verification of the extraction nuclues constituent of the *Ocimum gratissimum* leave extract.

Parameter	Value
Alkanoid	1. 99 ± 0.11
Phenolic	0.75 ± 0.07
Flavonoid	1.92 ± 0.12
Tanin	2.81 ± 0.14
Saponin	3.14 ± 0.17
Steroids	0.20 ± 0.00
Glycoside	2.41 ± 0.14

Table 6: Antibacterial activity
Diameter zone of inhibition (x±SD) mm

Inhibiting substance(100mg/ml)	VCC6	VCP2	VCE7
EEO	8.70 ± 0.07	9.70 ± 0.11	8.50±0.07
AEO	7.10 ± 0.07	7.30 ± 0.14	7.00±0.11
CPX	14.00 ± 0.17	17.30 ± 0.11	14.50±0.07

EEO - Ethanolic Extract of Ocimum gratissimum
AEO - Aqueous Extract of Ocimum gratissimum
CPX - Ciprofloxacin

Discussion

The role of water in living organisms cannot be overemphasized. Ensuring the availability of potable water for both drinking and domestic purposes is paramount for healthful living. This study investigates the susceptibility pattern of *Vibrio cholerae* isolated from streams at Uli community to extract of *Ocimum gratissimum*. The cultural, morphological, and biochemical characteristics of *Vibrio cholerae* in this study conform to the features reported by several researchers (Madora and Momba, 2010; Bulus *et al*., 2015; Obu *et al*., 2024) who isolated and characterized *Vibrio* species from different water sources. The ability of the bacterial isolates to utilize common sugars such as glucose, galactose and lactose, indicates their high enzyme-producing potentials. This ability had been described by other researchers (Madora and Momba, 2010; Bulus *et al*., 2015; Obu *et al*., 2024) who stated that the sugars serve as carbon and energy sources. Molecular characterization of the bacterial isolates revealed the presence of three strains of *Vibrio cholerae* namely; *Vibro cholerae* 01 biovar EIT strain C6789 (VCC6) and *Vibro cholerae* 01 biovar EIT strain E7976 (VCE7), and *Vibro cholerae* 01 biovar EIT strain R1979 (VCR1). The occurrence of *Vibrio cholerae* in strains had been reported by other researchers (Madora and Momba, 2010; Bulus *et al*., 2015; Obu *et al*., 2024) who stated that molecular technique provides a more sensitive and rapid technique for detecting bacterial pathogens.

The phytochemical compounds (alkanoids, flavonoids, glycosides, tannins, saponins, phenolics, and steroids) detected in the extract of *Ocimum gratissimum* indicate its bioactive enrichment. Similar observation was made by researchers (Kharisma *et al*., 2020; Charla *et al*., 2022; Chavan *et al*., 2025) who evaluated the phytochemical and antimicrobial activity of *Ocimum gratissimum* extract.

The ability of the medicinal extract from *O. gratissimum* to inhibit the growth of *Vibrio cholera*e could be attributed to the presence of the bioactive compounds, especially flavonoids and glycosides (Kharisma *et al*., 2020; Charla *et al*., 2022; Chavan *et al*., 2025). The highest zone of inhibition observed in Ciprofloxacin could be attributed to high potency, but the antibiotic is synthetic, which indicates that side effects and inconsistency in potency are possible. Similar conclusion was drawn by other researchers (Kharisma *et al*., 2020; Charla *et al*., 2022; Chavan *et al*., 2025).

Conclusion

This study confirms the presence of *Vibrio cholerae* strains C6789 (VCC6), E7976 (VCE7), and R1979 (VCR1) in stream samples, with VCR1 being the most prevalent. Notably, *Ocimum gratissimum* extract exhibited significant antimicrobial activity against these isolates, particularly against VCE7. Given its efficacy, *Ocimum gratissimum* extract holds potential as a natural antibacterial agent for controlling *Vibrio cholerae* infections, warranting further exploration of its applications.

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Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

References

Bulus, G. H., Ado, S. A., Yakubu, S. E. and Ella, E. E. (2015). Isolation and Characterization of Vibrio Cholerae from Water Sources in Zaria, Nigeria. *Annals of Experimental Biology* 3 (3):8 – 13

Charla R, Patil PP, Bhatkande AA, Khode NR, Balaganur V, Hegde HV, Roy S (2022) In vitro and in vivo inhibitory activities of selected traditional medicinal plants against toxin-induced cyto-and entero-toxicities in cholera. Toxins 14(10):649

Chavan, P., Naik, K., Ghosal, S. *et al.* Antibacterial efficacy and antibiotic sensitivity augmentation potential of selected traditional medicinal plant extracts against *Vibrio cholerae* O1 E1 Tor Ogawa. *Futur J Pharm Sci* **11**, 3 (2025). https://doi.org/10.1186/s43094-024-00750-x

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., & Ochibulu, S. C. (2025a). Hyping the Inhibitory Activity of Xylopia aethiopica against Vibrio cholerae using Azithromycin. IPS Journal of Basic and Clinical Medicine, 2(3), 93–98. https://doi.org/10.54117/ijbcm.v2i3.16

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., & Ochibulu, S. C. (2025b). Natural Product-Based Therapies: Exploring the Potential of Ocimum gratissimum and Vitamin C Combination against Vibrio cholerae Infections. IPS Interdisciplinary Journal of Biological Sciences, 4(3), 119–124. https://doi.org/10.54117/iijbs.v4i3.64.

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Dim, C. N., Okereke, F. O., & Ochibulu, S. C. (2025c). Soil Bacterial Dynamics: Assessing the Effects of Urine on Lipolytic and Cellulytic Bacteria. IPS Journal of Advanced and Applied Biochemistry, 1(2), 34–37. https://doi.org/10.54117/ijaab.v1i2.66

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., … Dim, C. N. (2025d). Public Health Implications of Shigella Contamination in Borehole Water Sources in Uli Community. IPS Journal of Public Health, 5(3), 265–269. https://doi.org/10.54117/ijph.v5i3.48.

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., Ochibulu, S. C., & Agbaugo, C. F. (2025e). Upshot of Urine on Beneficial Soil Bacteria. Journal of Pollution Monitoring, Evaluation Studies and Control, 4(2), 100–103. https://doi.org/10.54117/jpmesc.v4i2.18

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C.C., Ike, V. E., Ikejiaku, C. C., Okereke, F. O., & Ochibulu, S. C. (2025f). Cross-Sectional Study of Salmonella Species among Ready-To-Eat Fruit Salads. Journal of Pollution Monitoring, Evaluation Studies and Control, 4(2), 104–109. **https://doi.org/10.54117/jpmesc.v4i2.19**.

Ekesiobi, A. O., Iheukwumere, C.M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., & Ochibulu, S. C. (2025g). Combination Therapy: Investigating the Combined Effects of Zingiber officinale and Azithromycin against Vibrio cholerae. IPS Journal of Drug Discovery Research and Reviews, 3(2), 44–50. https://doi.org/10.54117/ijddrr.v3i2.34.

Gabriela, I. F., Cecilia, L. E., Teresa, I. C.and Maria, E. E. (2014).Detection and characterization of shiga toxin producing Escherichia coli, Salmonella species and Yersinia strains from human, animal and food samples in San Luis, Argentina. International Journal of Microbiology 2014:1–11.

Ihekwumere, C.M., Umedum, C.U. and Iheukwumere, I.H. (2020). Identities and prevalence of Aspergillus species on Phaseolus vulgaris(Bean) seeds sold in Ihiala, Anambra State, Nigeria. Greener Journal of Microbiology and Antimicrobials 5(1):16 –25.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., & Ihenatuoha, U. A. (2025). Cross-Sectional Study of Different Strains of Bacillus cereus among Pap Sold in Major Towns in Ihiala LGA, Anambra State. *IPS Journal of Public Health*, *5*(2), 199-204.

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. Journal of Natural Sciences Research 8: 21–33.

Iheukwumere, I. H., Opara , G. R., Iheukwumere, M. C., Okafor, . C. F., & Nwakoby, N. E. (2021). Prophylactic potential of Essential cream produced from Chromolaena odorata leaf extract against Cladosphialophora bantiana strain D12E. IPS Journal of Applied Microbiology and Biotechnology, 1(1), 1–11

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Okoli, U. O., Dim, C. N., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., Nwankwo, A. K., & Ochibulu, S. C. (2025a). Bacteriological Study of Urine Samples from Obstetric Patients in Onitsha Metropolis: Public Health Implications. IPS Journal of Basic and Clinical Medicine, 2(3), 99–107.**https://doi.org/10.54117/ijbcm.v2i3.17**

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Okoli, U. O., Ejike, C. E., Dim, C. N., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., Nwankwo, A. K. ., & Ochibulu, S. C. (2025b). Waterborne Pathogen Research: Examining Shigella species in Fish Ponds of Uli Community. IPS Interdisciplinary Journal of Biological Sciences, 4(3), 125–129. **https://doi.org/10.54117/iijbs.v4i3.65**

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Okoli, U. O., Ejike, C. E., Ilechukwu, C. C., … Ochibulu, S. C. (2025c). Public Health Risk of Vibrio cholerae Contamination in Streams of Uli Community. IPS Journal of Public Health, 5(3), 270–275. **https://doi.org/10.54117/ijph.v5i3.49**.

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Dim, C. N., & Ochibulu, S. C. (2025d). Dual Approach Therapy: Assessing Xylopia aethiopica and Ciprofloxacin Synergy against Salmonella enterica Serovar Typhi. IPS Intelligentsia Multidisciplinary Journal, 4(1), 27–31.

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Dim, C. N., Ochibulu, S. C., Unegbu, C. C., & Egbuna, C. (2025e). Food Safety Implications: Assessing the Potential of Desmodium velutinum Leaves Extracts to Control the Most Predominant Fungal Contamination in Ready-To-Eat Fried Chicken. IPS Journal of Nutrition and Food Science, 4(3), 494–500.

Kharisma A, Tjahjaningsih W, Sigit S (2020) Determination of minimum inhibitory and minimum bactericidal concentration of ketapang (*Terminatia catappa*) leaves extract against Vibrio harveyi. IOP Conf Ser Earth Environ Sci 441(1):012012

Madoroba, E. and Momba, M.N.B. (2010). Prevalence of *Vibrio cholerae* in rivers of Mpumalanga province, South Africa as revealed by polyphasic characterization. African Journal of Biotechnology (43), pp. 7295-7301 .

Obu, A.P., Mbata, C.A. and Monsi, T.P. (2024). "Prevalence of Vibrio Species Isolated from Water Bodies in Buguma Community in Rivers State, Nigeria". *International Journal of Pathogen Research* 13 (6):21-31.

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