

Evaluating the Combined Efficacy of *Zingiber officinale* Extract and Clarithromycin against Cholera Pathogen

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ABSTRACT

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Vibrio cholerae is a significant pathogen responsible for cholera outbreaks worldwide. The rise of antibiotic-resistant strains has necessitated the search for alternative antibacterial agents. This study aimed to characterize *V. cholerae* isolates and evaluate the antibacterial activity of *Zingiber officinale* rhizome extract against these isolates, alone and in combination with clarithromycin. *V. cholerae* isolates were characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. The phytochemical constituents of *Z. officinale* extract were analyzed, and its antibacterial activity was assessed using the disc diffusion method. Three *V. cholerae* O1 biovar El Tor strains (C6709/VCC6, P27459/VCP2, E7946/VCE7) were identified, exhibiting characteristic cultural, morphological, and biochemical features. The *Z. officinale* extract contained alkaloids, flavonoids, and tannins. The ethanolic extract (EEZ) showed higher inhibition zones against the *V. cholerae* isolates compared to the aqueous extract (AEZ). Combinations of *Z. officinale* extracts with clarithromycin (CLA) enhanced antibacterial activity, with EEZ+CLA showing the highest inhibition zones (20.87-26.33 mm). Statistical analysis revealed significant differences ($p < 0.05$) among the inhibition zones. The study suggests that combining *Z. officinale* extracts with clarithromycin enhances antibacterial activity against *V. cholerae* isolates. This study provides valuable data on the antibacterial activity of *Z. officinale* extract against *V. cholerae* isolates, highlighting its potential as a natural antibacterial agent against cholera.

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Keywords

Vibrio cholerae, *Zingiber officinale*, antibacterial activity, phytochemical constituents, clarithromycin, combination therapy

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INTRODUCTION

The emergence of antimicrobial resistance (AMR) represents one of the most critical challenges to global public health, severely compromising the treatment of infectious diseases such as cholera (World Health Organization, 2021; Okeke *et al.*, 2017; Dim *et al.*, 2025a). *Vibrio cholerae*, a Gram-negative waterborne pathogen, remains a leading cause of severe, dehydrating diarrhea in regions with inadequate sanitation, contributing to significant morbidity and mortality annually (Ali *et al.*, 2015). While antibiotic therapy is essential for managing severe cases and reducing transmission, the effectiveness of conventional agents is increasingly undermined by the proliferation of multidrug-resistant (MDR) strains, which have been reported across multiple cholera-endemic regions (Miwanda *et al.*, 2015; Dutta *et al.*, 2021; Amadi *et al.*, 2017; Dim *et al.*, 2025b).

In response to this therapeutic crisis, there is renewed scientific interest in integrating natural antimicrobials with conventional antibiotics to enhance efficacy and counteract resistance mechanisms. Phytomedicine offers a rich repository of bioactive compounds with the potential to act synergistically with existing drugs, thereby restoring or amplifying their activity (Abreu *et al.*, 2012). *Zingiber officinale* (ginger), a widely used medicinal plant with a long

history in traditional medicine systems, is rich in bioactive phytochemicals such as gingerols, shogaols, and flavonoids (Ali *et al.*, 2008). These compounds have demonstrated significant antimicrobial, anti-inflammatory, and antioxidant properties in various *in vitro* and pharmacological studies (Semwal *et al.*, 2015; Dim *et al.*, 2025c; Chude *et al.*, 2020). Ginger extracts have shown notable activity against a range of pathogenic bacteria, including Gram-negative species, suggesting their potential as a source of novel antimicrobial agents or potentiators (Kumar *et al.*, 2011).

Clarithromycin, a semi-synthetic macrolide antibiotic, is used clinically to treat various bacterial infections. Its mechanism of action involves binding to the bacterial 50S ribosomal subunit, thereby inhibiting protein synthesis (Zuckerman, 2004). However, like other antibiotics, its utility is threatened by rising resistance. Combining plant-derived extracts with antibiotics like clarithromycin may offer a synergistic advantage; phytochemicals can disrupt bacterial cell membranes, inhibit efflux pumps, or modulate bacterial virulence, thereby enhancing antibiotic penetration and efficacy while potentially lowering the required therapeutic dose (Cox *et al.*, 2021). Such combinations represent a promising strategy within the broader framework of antimicrobial stewardship. Therefore, this study aims to

evaluate the individual and combined antibacterial effects of *Zingiber officinale* extract and clarithromycin against clinical isolates of *Vibrio cholera*.

MATERIALS AND METHODS

Isolation and Characterization of Test Isolates

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. This was done using the method described in work published by Iheukwumere *et al.* (2025a), Iheukwumere *et al.* (2025b), Iheukwumere *et al.* (2025c), Egbe *et al.* (2025a).

Culture and Isolation of Enteric Bacteria

This was carried out using the modified method of Cheesbrough. The swab sticks were stricked on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated in inverted at $37\pm 2^{\circ}\text{C}$ for 24-48 h. (Egbe *et al.*, 2025b; Egbe *et al.*, 2025c; Iheukwumere *et al.*, 2025d; Iheukwumere *et al.*, 2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at $37\pm 2^{\circ}\text{C}$ 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.* (2018b), Iheukwumere *et al.* (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al.* (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al.* (2025), Ekechukwu *et al.* (2025a), Ekechukwu *et al.* (2025b), Ezedianafu *et al.* (2025a), and Ezedianafu *et al.* (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al.* (2020b), Idigo *et al.* (2025a), Idigo *et al.* (2025b), Idigo *et al.* (2025c), Idigo *et al.* (2025d), and Ezedianafu *et al.* (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al.*, 2017; Iheukwumere *et al.*, 2017a; Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2023a; Iheukwumere *et al.*, 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with

clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al.* (2017c), Iheukwumere *et al.* (2018c) Ike *et al.* (2025a), Iheukwumere *et al.* (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at $35\pm 2^{\circ}\text{C}$ for 24 h as described by Frank and Robert (2015), Iheukwumere *et al.* (2017d), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al.* (2025b) Ike *et al.* (2025c) Iheukwumere *et al.* (2022d), Idigo *et al.* (2025e), Obiefuna *et al.* (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al.* (2017), Obianom *et al.* (2024), Ekechukwu *et al.* (2025c), Obiefuna *et al.* (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al.* (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025h), Ike *et al.* (2025d), Idigo *et al.* (2025e), Ezedianafu *et al.* (2025d), Ezedianafu *et al.* (2025e) and Iheukwumere *et al.* (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the

sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37°C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al.* (2025d), Ike *et al.* (2025e), Idigo *et al.* (2025f), Idigo *et al.* (2025g) and Obiefuna *et al.* (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45°C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37°C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al.* (2017), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), and Idigo *et al.* (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. The presence pink/red colouration indicated positive urease test.

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al.* (2025h), Idigo *et al.* (2025i), Iheukwumere *et al.* (2025j) and Idigo *et al.* (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), Idigo *et al.* (2025k), Idigo *et al.* (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α -naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c), and Idigo *et al.* (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025l), Iheukwumere *et al.* (2025m). A smear of the isolate was made

on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H₂O₂) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c) Iheukwumere *et al.* (2025n), and Iheukwumere *et al.* (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al.*, 2025p; Iheukwumere *et al.*, 2025q; Chude *et al.*, 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al.*, 2025r; Iheukwumere *et al.*, 2025s; Ejike *et al.*, 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with $\geq 95\%$ similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al.*, 2017; Iheukwumere *et al.*, 2025t; Nwike *et al.*, 2017).

Preparation and extraction of the plant material

Preparations of ginger powder: The first step for extraction involved the preparation of dry powder from ginger rhizomes. For this, fresh gingers were purchased from the local market of Uli in Ihiala L.G.A of Anambra state, Nigeria. The Fresh ginger rhizomes were washed, peeled, sliced, shadow dried and dried in hot oven at 70° C. The dried ginger samples were ground into powder form using a sterile electric grinder and sieved to give a powdery form.

Ginger extracts preparation: This was done using the method published by (Iheukwumere *et al.*, 2025u). Firstly, 50 grams of ginger powder was weighed into a 1000 mL conical flask (Pyrex) and then 50 mL of 99 % ethanol was added. This was thoroughly shaken and then made up to 500 mL using absolute ethanol. The flasks were incubated at room temperature for 72 hours with intermittent manual shaking.

The crude extracts were filtered with Whatman No. 1 filter paper. The extracts were concentrated using a rotary evaporator at 78°C (Iheukwumere and Umedum, 2013).

Phytochemical analysis of the plant extracts

The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Iheukwumere and Umedum (2013).

Alkaloids: Five milliliters of the sample was mixed with 96% ethanol and 20% tetraoxosulphate (VI) acid (1:1). One milliliter of the filtrate from the mixture was added to 5 ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 5 minutes. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at an absorbance of 550 nm.

Glycosides: This was carried out using Buljet's reagent. One gram of the fine powder of the sample was soaked in 10 ml of 70% alcohol for 2 h and then filtered with Whatman No. 1 filter paper. The extract was then purified using lead acetate solution and disodium hydrogen tetraoxosulphate (VI) solution before the addition of freshly prepared Buljet's reagent. The absorbance was taken at 550 nm.

Flavonoids: Five milliliters of the extract was mixed with 5 ml of dilute hydrochloric acid and boiled for 30 minutes. The boiled extract was allowed to cool and then filtered with Whatman No. 1 filter paper. One milliliter of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% ammonia solution. The absorbance was taken at 420 nm.

Tannin: Ten milliliters was pipetted into 50 ml plastic containing 50 ml of distilled water. This was mixed for 1 h on a sterile mechanical shaker. The solution was filtered with Whatman No. 1 filter paper, and 5 ml of the filtrate was mixed with 2 ml of iron (III) chloride solution in 0.1 N hydrochloric acid. The absorbance was taken at 550 nm.

Steroids: The extract was eluted with normal ammonium hydroxide solution. Two milliliters of eluate was mixed with 2 ml of chloroform in a test tube. Three milliliters of ice cold acetic anhydride was added to the mixture and allowed to cool. The absorbance was taken at 420 nm.

Saponins: Five milliliters of the sample was dissolved in aqueous methanol. The 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm.

In Vitro Antibacterial Activity

Preparation of the inhibitory substance for in vitro antibacterial susceptibility Tests: In this study the concentration of 100 mg/ml of the extract was used to screen for the antibacterial activity. This was carried out using the modified method described in the study published by Iheukwumere *et al.* (2018). Here, 2.5 g of the extract was dissolved in 25.0 ml of peptone water. Similarly, equal concentration of the antibiotic was prepared, and then equal

volume of the extract and antibiotic were mixed, and this was used for the study

In vitro antibacterial susceptibility test: This was carried out using the method described in the study published by Iheukwumere *et al.* (2025v). Each labeled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labeled and then incubated at 37±2°C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

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 main effects and interactions 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.* (2017e), Manasseh *et al.* (2025), Idigo *et al.* (2025n), Idigo *et al.* (2025o), Idigo *et al.* (2025p), Idigo *et al.* (2025q), Idigo *et al.* (2025r), Idigo *et al.* (2025s), Idigo *et al.* (2025t), Ugwu *et al.* (2025a) and Ugwu *et al.* (2025b).

RESULTS

The *Vibrio cholerae* isolates (L, M, N) exhibited characteristic cultural and morphological features, including yellow appearance on TCBS, smooth edges, and rod/comma-shaped cells (Table 1). Biochemical analysis revealed that the isolates were positive for catalase, citrate, and oxidase tests, and fermented glucose and galactose (Table 2). Molecular analysis confirmed the isolates as *Vibrio cholerae* strains VCC6, VCP2, and VCE7, with 100% identity to reference strains (Table 4).

The *Zingiber officinale* rhizome extract was analyzed for phytochemical constituents, revealing the presence of alkaloids (6.20 g/100g), flavonoids (5.41 g/100g), and tannins (4.56 g/100g) (Table 5). The extract's antibacterial activity was assessed, showing moderate inhibition zones against the *V. cholerae* isolates (Table 6). The ethanolic extract (EEZ) exhibited higher inhibition zones (8.20-9.33 mm) compared to the aqueous extract (AEZ) (6.20-7.10 mm).

Clarithromycin (CLA) showed higher inhibition zones (16.80-19.80 mm) compared to ciprofloxacin (CPX) (14.00-17.30 mm). Combinations of *Zingiber officinale* extracts with clarithromycin enhanced antibacterial activity, with EEZ+CLA showing the highest inhibition zones (20.87-26.33 mm), followed by AEZ+CLA (18.67-22.70 mm). Statistical analysis revealed significant differences ($p < 0.05$) among the inhibition zones.

The p-values for the antibacterial activity were < 0.05 , indicating statistical significance. Specifically, EEZ+CLA and AEZ+CLA showed significantly higher activity than CPX and CLA alone ($p < 0.01$). The results suggest that combining *Zingiber officinale* extracts with clarithromycin enhances antibacterial activity against *Vibrio cholerae* isolates

Table 1: Cultural and morphological characteristics of the isolates.

Parameter	L	M	N
Appearance on TCBS	Yellow	Yellow	Yellow
Edge	Smooth	Smooth	Smooth
Elevation	Raised	Raised	Raised
Surface	Smooth	Smooth	Smooth
String Test	+	+	+
Gram reaction	-	-	-
Shape	Rods/Comma	Rods/Comma	Rods/Comma
Endospore	-	-	-
Capsule	-	-	-
Motility	+	+	+

Table 2: Biochemical characteristics of the isolates

Parameter	L	M	N
Catalase	+	+	+
Citrate	+	+	+
Gelatin	+	+	+
H ₂ S	-	-	-
Methylred	-	-	-
Oxidase	+	+	+
Urease	-	-	-
Arabinose	-	-	-
Glucose	+	+	+
Galactose	+	+	+
Inositol	-	+/-	-
Dulcitol	-	-	-
Xylose	+/-	-	+/-
Sorbitol	-	+/-	-
Ketose	+/-	-	+/-

Table 3: Verification of the extracted nucleic acids

Sample ID	Conc (Ng/ml)	26nm	280nm	260/280
L	121.20	3.0120	1.6194	1.86
M	125.70	3.1082	1.6801	1.85
N	132.80	3.2110	1.7643	1.82

Table 4: Molecular identities of the isolates

Parameter	L	M	N
Max Score	5686	5686	5686
Total Score	7295	7295	7295
Ovary Cover (%)	100	100	100
E-Value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession Length	1070351	1073537	1071008
Accession Number	Cp047298	Cp047300	Cp047304
Description	<i>Vibrio cholerae</i> 01biovar EITor strain C6709 (VCC6)	<i>Vibrio cholerae</i> 01biovar EITor strain P27459 (VCP2)	<i>Vibrio cholerae</i> 01biovar EITor strain E7946 (VCE2)

Table 5: Phytochemical constituents of *Zingiber officinale* rhizome extract

Parameter	Value (g/100g)
Alkaloids	6.20 ± 0.21
Phenolics	1.21± 0.01
Flavonoids	5.41± 0.14
Tannins	4.56± 0.17
Saponins	0.92 ± 0.01
Glycosides	1.02 ± 0.01
Steroids	0.02 ± 0.00

Table 6: Antibacterial activity

Inhibitory substance	Diameter Zone of inhibition [$\bar{x} \pm S\bar{D}$] mm		
	VCC6	VCP2	VCE7
EEZ	8.80 $\pm$ 0.14	9.33 $\pm$ 0.67	8.20 $\pm$ 0.17
AEZ	6.77 $\pm$ 0.21	7.10 $\pm$ 0.08	6.20 $\pm$ 0.03
CPX	14.00 $\pm$ 0.17	17.30 $\pm$ 0.82	14.56 $\pm$ 0.07
CLA	16.80 $\pm$ 0.22	19.80 $\pm$ 0.47	16.96 $\pm$ 0.13
EEZ + CLA	20.87 $\pm$ 0.11	26.33 $\pm$ 0.67	21.82 $\pm$ 0.13
AEZ + CLA	18.67 $\pm$ 0.33	22.70 $\pm$ 0.14	19.42 $\pm$ 0.41

EEZ- Ethanolic Extract of *Zingiber officinale* (Ginger)

AEZ – Aqueous Extract of *Zingiber officinale*

CPX – Ciprofloxacin CLA- Clarithromycin

DISCUSSION

The detection of *Vibrio cholerae* in stream water samples aligns with established research highlighting aquatic environments as critical reservoirs for this pathogen, particularly in areas with inadequate sanitation (Lutz *et al.*, 2013; Ekelozie *et al.*, 2018; Adabe *et al.*, 2022). The predominance of these isolates in the studied streams corroborates findings from similar ecological settings, reinforcing the concept that surface waters serve as a persistent transmission route (Huq *et al.*, 2012; Islam *et al.*, 2020). The specific isolation of strains VCC6, VCP2, and VCE7 underscores the ongoing environmental presence of toxigenic *V. cholerae* and reaffirms the necessity for continuous water quality surveillance to mitigate infection risks for exposed communities.

The observed prevalence of *V. cholerae*, particularly strain VCP2, can be attributed to a confluence of genetic, ecological, and anthropogenic factors. Genetic adaptability—including antigenic variation, genomic plasticity, and the horizontal acquisition of fitness genes—can enable specific lineages to thrive in particular environmental niches (Yap *et al.*, 2014). Furthermore, local conditions such as poor waste management, sewage contamination, and seasonal flooding likely enrich the aquatic environment with nutrients, creating favorable conditions for bacterial persistence and proliferation (Kumar *et al.*, 2013; Lutz *et al.*, 2013).

Phytochemical analysis of the *Zingiber officinale* (ZO) rhizome extract confirmed the presence of alkaloids, flavonoids, tannins, phenolics, and saponins, consistent with the well-documented bioactive profile of ginger (John-Dewole *et al.*, 2012; Arora & Sen, 2023). These compounds are recognized for their broad-spectrum antimicrobial properties, which likely mediate the extract's inhibitory effect against the *V. cholerae* isolates. The significant antibacterial activity observed aligns with the traditional use of ginger in managing gastrointestinal ailments and supports its potential as a source of antimicrobial agents.

A pivotal finding of this study was the enhanced antibacterial effect achieved when the ZO extract was combined with clarithromycin. This synergistic interaction suggests that ginger phytochemicals may potentiate the antibiotic's activity, possibly through mechanisms such as bacterial membrane disruption, efflux pump inhibition, or modulation of virulence factors. This approach is particularly relevant in an era of escalating antimicrobial resistance, which is frequently driven by plasmid-mediated horizontal gene transfer (Njimoh *et al.*, 2015; Musbal *et al.*, 2024). By employing a combination strategy, it may be possible to lower

the effective dose of conventional antibiotics, reduce the risk of resistance development, and improve therapeutic outcomes.

CONCLUSION

This study confirms the presence of *Vibrio cholerae* strains VCC6, VCP2, and VCE7 in local stream water, with VCP2 identified as the predominant isolate. The *Zingiber officinale* extract demonstrated significant intrinsic antibacterial activity against these pathogens. Importantly, its combination with clarithromycin resulted in a synergistic enhancement of efficacy, indicating a promising combined therapeutic strategy. These findings support further investigation into ginger-based adjunctive therapies as a means to augment conventional antibiotic regimens, potentially offering a more effective approach to managing cholera infections in the face of rising multidrug resistance.

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