



Solenostemon monostachyus Leaf Extract: A Potential Treatment for *Vibrio cholerae* from Uli Streams

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Abstract	Article History
<p><i>Vibrio cholerae</i> 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 <i>V. cholerae</i> isolates and evaluate the antibacterial activity of <i>Solenostemon monostachyus</i> leaves' extract against these isolates. <i>V. cholerae</i> isolates were characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. The phytochemical constituents of <i>S. monostachyus</i> leaves' extract were analyzed, and its antibacterial activity was assessed using the disc diffusion method. Three <i>V. cholerae</i> isolates (VCC6, VCP2, and VCE7) were identified, exhibiting characteristic cultural, morphological, and biochemical features. The <i>S. monostachyus</i> leaves' extract contained alkaloids, flavonoids, and saponins. The ethanolic extract (EES) showed significant inhibition zones against the <i>V. cholerae</i> isolates (16.50-18.50 mm), comparable to ciprofloxacin (CPX). Statistical analysis revealed significant differences in inhibition zones ($p < 0.05$). The study suggests that <i>S. monostachyus</i> leaves' extract has potential antibacterial activity against <i>V. cholerae</i> isolates, with the ethanolic extract showing higher efficacy. This study provides valuable data on the antibacterial activity of <i>S. monostachyus</i> leaves' extract against <i>V. cholerae</i> isolates, highlighting its potential as an alternative treatment option.</p> <p>Keywords: <i>Vibrio cholerae</i>, <i>Solenostemon monostachyus</i>, Antibacterial activity, phytochemical constituents, alternative treatment</p>	<p>Received: 05 Jan 2026 Accepted: 12 Feb 2026 Published: 18 Feb 2026</p>  <p>Scan QR code to view*</p> <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Streams are vital ecological and community resources, particularly in regions like Uli, where they serve essential domestic purposes, including bathing, washing, and, in some cases, as a water source. However, these water bodies are increasingly susceptible to contamination from pathogenic microorganisms, posing significant public health risks (Iheukwumere et al., 2025; Okeke et al., 2017; Dim et al., 2025a). Among these pathogens, *Vibrio cholerae* is of paramount concern, as it is the causative agent of cholera—a severe, rapidly dehydrating diarrheal disease that can be fatal

if untreated (Madora & Momba, 2010; Amadi et al., 2017; Dim et al., 2025b).

The global burden of cholera is exacerbated by the emergence of multidrug-resistant (MDR) strains of *V. cholerae*, which complicate clinical management and reduce the efficacy of standard antibiotic regimens (Bulus et al., 2015; Dim et al., 2025c; Chude et al., 2020). This challenge underscores an urgent need to explore alternative antimicrobial agents, particularly those derived from natural sources, which may offer novel mechanisms of action and fewer adverse effects.

Medicinal plants have long been utilized in traditional medicine for treating infectious diseases. *Solenostemon*

monostachyus is one such plant, locally recognized for its therapeutic properties, though its specific antimicrobial potential remains scientifically underexplored. Its leaves are traditionally used in various remedies, suggesting the presence of bioactive phytochemicals such as alkaloids, flavonoids, and tannins, which are known to possess antimicrobial activities (Cowen, 2022). Therefore, this study aims to evaluate the phytochemical composition and the *in vitro* antibacterial activity of *Solenostemon monostachyus* leaf extract against *Vibrio cholerae* strains isolated from streams in the Uli community.

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. 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±2°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±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.* (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), Ezedianafo *et al.* (2025a), and Ezedianafo *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 Ezedianafo *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±2°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).

Prevalence and Distribution of the Isolates in the Frozen Meat Samples

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

Preparation of the inhibitory substance for *in vitro* antibacterial susceptibility Tests: The leaves of *Solenostemon monostachyus* (plate 1) were collected, washed and dried under room temperature. The leaves were identified appropriately, and then pulverized. The phytochemical constituents was quantitatively determined using gravimetric and spectrophotometric methods. The extract was obtained using maceration/cold extraction method. 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.* (2025u), . 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.



Plate 1: *Solenostemon monostachyus* leaves.

***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 $37\pm 2^{\circ}\text{C}$ 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 isolates (P, Q, R) exhibited characteristic cultural and morphological features, including yellow appearance in TCB, 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 *Solenostemon monostachyus* leaves' extract was analyzed for phytochemical constituents, revealing the presence of alkaloids (17.99%), flavonoids (11.82%), and saponins (13.14%) (Table 5). The extract's antibacterial activity was assessed, showing significant inhibition zones against the *V. cholerae* isolates (Table 6). The ethanolic extract (EES) exhibited higher inhibition zones (16.50-18.50 mm) compared to the aqueous extract (AES) (9.50-12.00 mm), with ciprofloxacin (CPX) showing comparable activity.

Statistical analysis revealed that the differences in inhibition zones were significant ($p < 0.05$). The p-values for the antibacterial activity were < 0.05 , indicating statistical significance. Overall, the study suggests that *Solenostemon monostachyus* leaves' extract has potential antibacterial activity against *V. cholerae* isolates, with the ethanolic extract showing higher efficacy.

Table 1: Cultural and morphological characteristics of isolate

Parameter	P	Q	R
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	P	Q	R
Catalase	+	+	+
Citrate	+	+	+
Galatin	+	+	+
H ₂ S	-	-	-
Methyl red	-	-	-
Oxidase	+	+	+
Urease	-	-	-
Arabinose	-	-	-
Glucose	+	+	+
Galactose	+	+	+
Inisitol	-	+/-	-
Dycitol	-	-	-
Xylose	+/-	-	+/-
Surbitol	-	+/-	-
Lactose	+/-	+/-	+/-

Table 3: Verification of the extraction nuclues

sample ID	conc(µg/ml)	260nm	270nm	260/280
P	121.20	3.0120	1.6119	1.86
Q	125.70	3.1082	1.6810	1.85
R	132.82	3.2110	1.7643	1.82

Table 4: Molecular identity of the isolate

Parameter	P	Q	R
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	<i>Vibro cholerae</i> 01 biovar EIT strain C6789(VCC6)	<i>Vibro cholerae</i> 01 biovar EIT strain E7976 (VCE7)	<i>Vibro cholerae</i> 01 biovar EIT strain R1979(VCR1)

Table 5: Phytochemical constituent of the *Solenostemon monostachyus* leaves' extract

Parameter	Value (%)
Alkanoid	17.99 ± 0.11
Phenolic	0.95 ± 0.07
Flavonoid	11.82 ± 0.11
Tanin	3.81 ± 0.31
Saponin	13.14 ± 0.11
Steroids	0.40 ± 0.00
Glycoside	0.51 ± 0.01
Phytate	2.02 ± 0.01

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

Inhibiting substance(100mg/ml)	VCC6	VCP2	VCE7
EES	16.50±0.07	18.50±0.11	16.50±0.07
AES	11.50±0.07	9.50 ± 0.14	12.00±0.11
CPX	16.00±0.17	17.50±0.11	14.50±0.07

EEO - Ethanolic Extract of *Solenostemon monostachyus*; AES: Aqueous Extract of *Solenostemon monostachyus*; CPX - Ciprofloxacin

Discussion

The availability of potable water is a fundamental determinant of public health, yet water sources are often vulnerable to contamination by pathogens such as *Vibrio cholerae*. This study investigated the efficacy of *Solenostemon monostachyus* leaf extract against *V. cholerae* strains isolated from streams in the Uli community. The cultural, morphological, and biochemical characteristics of the isolates in this study were consistent with standard profiles of *V. cholerae* reported in environmental and clinical research (Madora and Momba, 2010; Obu *et al.*, 2024). The metabolic versatility of the isolates, demonstrated by their ability to utilize sugars such as glucose, galactose, and lactose, underscores their enzymatic capacity to thrive in varied environments, an adaptive trait that supports survival in aquatic reservoirs (Bulus *et al.*, 2015).

Molecular characterization confirmed the presence of three distinct *V. cholerae* strains: VCC6, VCE7, and VCR1. This finding highlights the sensitivity and utility of molecular methods in identifying and differentiating pathogenic strains, as noted in previous studies (Madora and Momba, 2010). The predominance of strain VCR1 in the tested samples may reflect its enhanced environmental persistence or transmission dynamics within the local water system, warranting further epidemiological study.

Phytochemical analysis of *Solenostemon monostachyus* aqueous extract revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, phenolics, and steroids. This bioactive profile aligns with phytochemical reports from other medicinal plants with established antimicrobial properties, suggesting a rich reservoir of compounds with therapeutic potential (Kharisma *et al.*, 2020; Charla *et al.*, 2022).

The significant antibacterial activity exhibited by the extract against all tested *V. cholerae* strains can be attributed to these bioactive constituents. Flavonoids and tannins, in particular, are known to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with nucleic acid synthesis (Chavan *et al.*, 2025). While the positive control antibiotic, ciprofloxacin, produced the largest inhibition zones—reflecting its high potency as a synthetic antibacterial agent—its potential side effects and the risk of inducing bacterial resistance underscore the need for complementary, plant-based alternatives.

Conclusion

This study confirms the presence of *Vibrio cholerae* strains C6789 (VCC6), E7976 (VCE7), and R1979 (VCR1) in stream water samples from Uli, with VCR1 being the most prevalent. The significant antimicrobial activity demonstrated by *Solenostemon monostachyus* leaf extract, especially against strain VCE7, highlights its potential as a natural therapeutic agent for managing *V. cholerae* infections. These findings support further investigation into the bioactive compounds responsible for this activity and encourage the development of plant-based strategies to complement conventional cholera management, particularly in resource-limited settings.

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Ethical approval: Not applicable

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.

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