International Journal of Contemporary Research In Multidisciplinary

*Research Article*

# Phytochemical Profiling and Antimicrobial Efficacy of *Couroupita Guianensis* Bark Extract Against *Aeromonas Hydrophila* and *Vibrio Harveyi* Isolated from Brackish Water Tilapia (*Oreochromis Spp.*)

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**DOI:** https://doi.org/10.5281/zenodo.21868923

## Abstract

Antibiotic-resistant bacterial pathogens are an escalating threat to brackish-water aquaculture, driving interest in plant-derived antimicrobials as sustainable alternatives to synthetic drugs. This study evaluated the phytochemical composition and antibacterial potential of methanolic Couroupita guianensis (cannonball tree) bark extract against Aeromonas hydrophila and Vibrio harveyi isolated from the skin and gills of Tilapia (Oreochromis mossambicus) collected from the Retteri river, Chennai, India. Qualitative screening confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides, amino acids, carbohydrates, phenolics and coumarins in the bark extract, while saponins, lignins and anthocyanins were absent. Quantitative assay showed a concentration-dependent increase in total flavonoid (77.3-186.1 µg quercetin equivalent) and total phenolic content (50.1-59.6 µg gallic acid equivalent). Both bacterial isolates were confirmed as Gram-negative rods by standard biochemical tests (triple sugar iron, oxidase, catalase, methyl red, urease). Disc diffusion assay demonstrated a dose-dependent, though modest, inhibitory effect of the extract (10-100 µg/mL) relative to the gentamicin positive control. Broth microdilution established a minimum inhibitory concentration (MIC) of 75 µg/mL for A. hydrophila and 100 µg/mL for V. harveyi. PCR amplification of the Tilapia Lake Virus (TiLV) segment from bacterial-isolate-associated gill and skin DNA yielded amplicons in the expected 400-500 bp range, consistent with reported TiLV detection protocols. These findings indicate that C. guianensis bark harbours a broad-spectrum phytochemical profile with measurable, dose-dependent antibacterial activity against two economically important brackish-water fish pathogens, supporting further investigation of the extract as a candidate eco-friendly phytotherapeutic for sustainable aquaculture disease management.

## Manuscript Information

- **ISSN No:** 2583-7397
- **Received:** 11-07-2026
- **Accepted:** 06-08-2026
- **Published:** 10-08-2026
- **IJCRM:**5(4); 2026: 633-649

- **Plagiarism Checked:** Yes
- **Peer Review Process:** Yes

## How to Cite this Article

D. Manoj, L. Srimathi, N. Tamilselvi. Phytochemical Profiling and Antimicrobial Efficacy of *Couroupita* Guianensis Bark Extract Against *Aeromonas Hydrophila* and Vibrio Harveyi Isolated from Brackish Water Tilapia (Oreochromis Spp.). Int J Contemp Res Multidiscip. 2026;5(4):633-649.

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**KEYWORDS:** Couroupita guianensis; Aeromonas hydrophila, Vibrio harveyi, phytochemical screening, minimum inhibitory concentration, antimicrobial resistance, aquaculture, Tilapia Lake Virus.

## 1. INTRODUCTION

Global aquaculture production is projected to rise from roughly 185 million tonnes in 2022 to over 205 million tonnes by 2032, with Asia continuing to dominate the sector [1]. This intensification, coupled with crowding and environmental stress, has increased the incidence of bacterial disease, with Aeromonas and Vibrio species responsible for substantial mortality and economic loss in finfish and shellfish culture [1,2]. Aeromonas hydrophila causes motile Aeromonas septicaemia in freshwater and brackish-water fish through haemolysin, aerolysin and enterotoxin-mediated virulence mechanisms [2], while Vibrio harveyi is a major luminescent-vibriosis pathogen of tilapia and shrimp [3]. Global antimicrobial consumption in aquaculture was projected to grow by roughly a third between 2017 and 2030, now accounting for close to 6% of total global antibiotic use [4], and this reliance on synthetic antibiotics has accelerated the emergence of multidrug-resistant strains, environmental antibiotic residues and resistance-gene dissemination between aquatic and terrestrial reservoirs [1,5]. The global burden of bacterial antimicrobial resistance is now recognised as one of the leading causes of death worldwide, intensifying the need for non-antibiotic disease-control strategies across food-animal production, including aquaculture [6].

Plant-derived antimicrobials (phytobiotics) are increasingly explored as biodegradable, low-residue alternatives to conventional antibiotics in fish and shrimp farming [2,7,8]. Medicinal-plant extracts rich in alkaloids, flavonoids, tannins, terpenoids and essential oils have demonstrated antibacterial activity against A. hydrophila and Vibrio spp. through mechanisms that include disruption of bacterial membrane integrity, inhibition of efflux and quorum-sensing systems, and interference with virulence-factor expression [2,7,9]. Couroupita guianensis Aubl. (Lecythidaceae), the cannonball tree, is a medicinal plant used traditionally against bacterial and fungal infections; recent phytochemical and pharmacological studies on its leaf and bark have confirmed antioxidant, antimicrobial and cytotoxic bioactivity attributable to its phenolic and flavonoid-rich profile [10,11]. However, the antibacterial potential of C. guianensis bark specifically against brackish-water fish pathogens, and its relationship to co-occurring viral pressure such as Tilapia Lake Virus (TiLV) - an orthomyxo-like virus responsible for mass tilapia die-offs worldwide - remains comparatively underexplored [12,13].

The present study therefore aimed to (i) qualitatively and quantitatively characterise the phytochemical composition of methanolic C. guianensis bark extract, (ii) evaluate its antibacterial efficacy against A. hydrophila and V. harveyi isolated from diseased brackish-water Tilapia, (iii) determine the minimum inhibitory concentration of the extract against both pathogens, and (iv) assess the presence of TiLV in the sampled fish population by PCR, to contextualise the bacterial findings within the broader disease pressure affecting the sampled population.

## 2. MATERIALS AND METHODS

### 2.1 Plant material and extraction

Bark of Couroupita guianensis was collected from Avadi, Chennai, Tamil Nadu, India, washed with deionised water, shade-dried at 28±2°C for 15 days, and pulverised in a sterile Wiley mill (60-mesh). Ten grams of powdered bark was macerated in 100 mL of HPLC-grade methanol (1:5 w/v) for 72 h at room temperature. The macerate was filtered sequentially through muslin cloth and 0.45 µm nylon syringe filters, then concentrated on a rotary evaporator (40°C, 150 rpm) to yield a semisolid extract (14.2% w/w).

**FIG. 2.1:** Couroupita guianensis (Cannonball Tree)


**FIG. 2.2:** Extraction of C. guianensis Bark

**2.2 Fish sampling and pathogen isolation**

Tilapia (Oreochromis mossambicus) exhibiting skin ulcers and haemorrhagic gills were collected from the Retteri River and transported in oxygenated brackish water at 25°C. Swabs from skin lesions and gills were enriched in 0.9% saline (37°C, 36 h), sub-cultured in nutrient broth, and spread onto Mueller-Hinton agar (37°C, 24 h). Isolates were identified by Gram staining and standard biochemical tests (triple sugar iron, oxidase, catalase, methyl red, urease).

**FIG. 2.3:** Oreochromis (Tilapia)

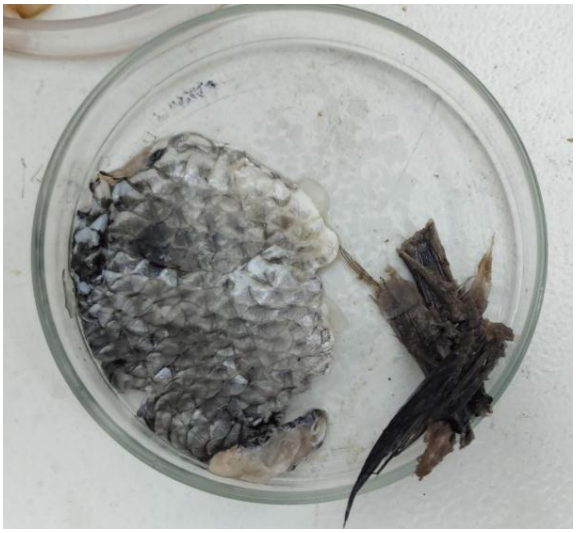

**FIG. 2.4:** Isolation of Fish Sample (Gills & Skin) Mixed with 9% Saline

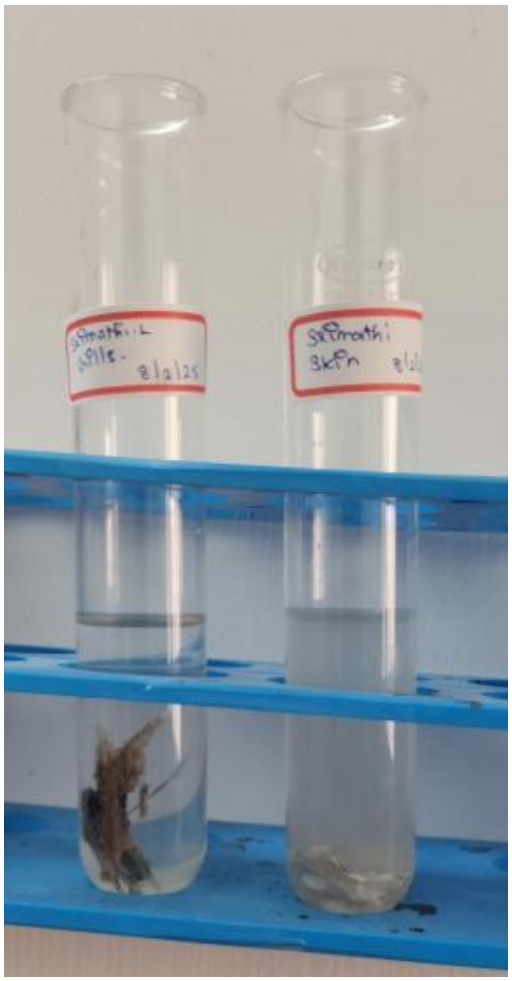

**FIG. 2.5:** Nutrient Broth Preparation


**FIG.2.6:** Sample Aliquot in Nutrient Broth

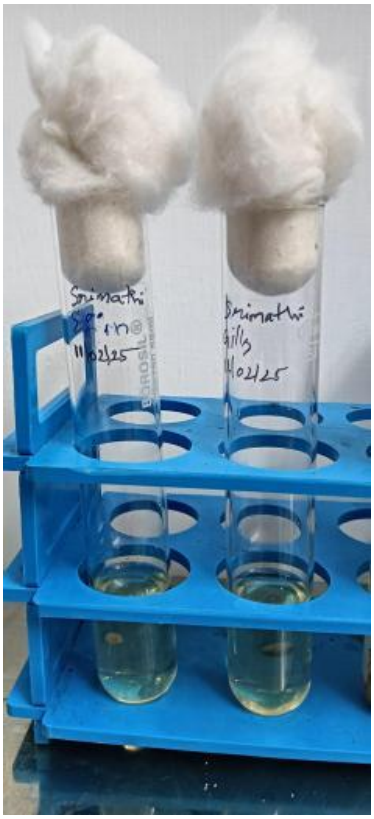

**FIG. 2.7:** Spread Plate

### 2.3 Phytochemical screening

Qualitative screening for alkaloids (Wagner's, picric acid tests), flavonoids (alkaline reagent test), tannins (gelatin test), saponins (foam test), terpenoids and steroids (Salkowski's test), glycosides (Fehling's test), amino acids (ninhydrin test), carbohydrates (Benedict's test), phenolics (ferric chloride test), coumarins (UV fluorescence) and anthocyanins (HCl test) followed standard protocols. Total flavonoid content was determined by the aluminium chloride colorimetric assay (absorbance at 510 nm; quercetin standard) and total phenolic content by the Folin-Ciocalteu method (absorbance at 765 nm; gallic acid standard), each performed in triplicate at 100 mg and 500 mg extract concentrations.

### 2.4 Antimicrobial susceptibility and MIC determination

Antibacterial activity was assessed by disc diffusion on Mueller-Hinton agar seeded with a 0.5 McFarland bacterial lawn. Discs were loaded with 10-100 µg/mL extract (serially diluted in 10% DMSO); gentamicin (10 µg) and 10% DMSO served as positive and negative controls, respectively. Zones of inhibition were measured after 24 h at 37°C. The minimum inhibitory concentration was determined by broth microdilution in Mueller-Hinton broth across a concentration gradient of 10-100 µg/mL, with bacterial growth quantified spectrophotometrically at OD 600 nm after 16 h incubation at 37°C.

### 2.5 Detection of Tilapia Lake Virus (TiLV)

Genomic DNA from gill- and skin-associated bacterial isolates was extracted using a commercial kit and quality-checked by 1% agarose gel electrophoresis. PCR targeting a TiLV gene segment used a 25 µL reaction (2X master mix, 10 pmol each primer, template DNA) under the following conditions: initial denaturation 95°C/5 min; 35 cycles of 95°C/30 s, 55-60°C/30 s, 72°C/1 min;
final extension 72°C/5 min. Amplicons were resolved on a 1.5% agarose gel with a 100 bp-1 kb ladder and visualised under UV transillumination.

## 3. RESULTS

### 3.1 Phytochemical screening of C. guianensis bark extract

The methanolic bark extract tested positive for alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides, amino acids, carbohydrates, phenolic compounds and coumarins, while saponins, lignins and anthocyanins were not detected (Table 1).

**Table 3.1:** Qualitative phytochemical screening of methanolic C. guianensis bark extract.

| Phytochemical | Test applied | Result |
|---|---|---|
| Alkaloids | Wagner's test | + |
| Flavonoids | Alkaline reagent test | + |
| Tannins | Gelatin test | + |
| Saponins | Foam test | - |
| Terpenoids | Salkowski's test | + |
| Steroids | Salkowski's test | + |
| Glycosides | Fehling's test | + |
| Amino acids | Ninhydrin test | + |
| Carbohydrates | Benedict's test | + |
| Phenolic compounds | Ferric chloride test | + |
| Coumarins | UV fluorescence test | + |
| Lignins | Labat test | - |
| Anthocyanins | HCl test | - |

**FIG. 3.1:** Phytochemical Analysis

Total flavonoid content increased from 77.3 µg quercetin equivalent (QE) at 100 mg extract to 186.1 µg QE at 500 mg, and total phenolic content increased from 50.1 to 59.6 µg gallic acid equivalent (GAE) over the same range (Table 2), indicating a concentration-dependent presence of these bioactive secondary metabolites.

**Table 3.2:** Total flavonoid and phenolic content of C. guianensis bark extract.

| Concentration | Total flavonoids (µg QE) | Total phenolics (µg GAE) |
|---|---|---|
| 100 mg | 77.30 | 50.11 |
| 500 mg | 186.14 | 59.59 |

### 3.2 Identification of bacterial isolates

Both isolates from skin and gill samples were Gram-negative rods forming smooth, circular, cream-coloured colonies. Biochemical profiling (Table 3) was consistent with Aeromonas hydrophila (acid slant/acid butt with gas on TSI) and Vibrio harveyi (acid slant/acid butt without gas), both oxidase- and catalase-positive and methyl red- and urease-negative.

**Table 3.3**: Biochemical characterisation of isolated fish pathogens

| Biochemical test | Aeromonas hydrophila | Vibrio harveyi |
|---|---|---|
| Gram staining | Gram-negative rods | Gram-negative rods |
| Catalase test | Positive | Positive |
| TSI test | Acid with gas | Acid without gas |
| Oxidase test | Positive | Positive |
| Methyl red test | Negative | Negative |
| Urease test | Negative | Negative |

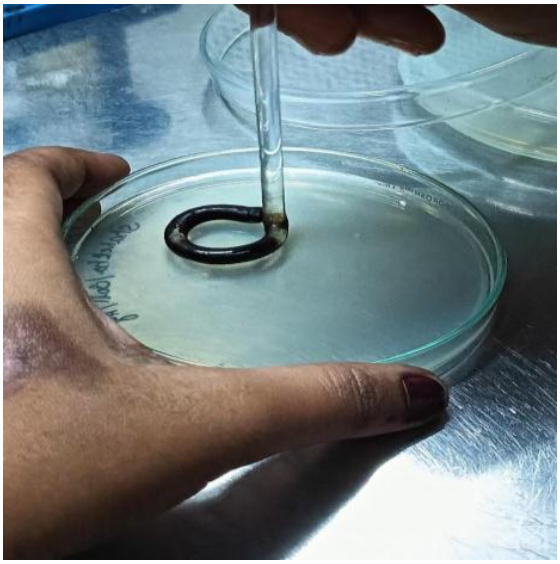

**FIG. 3.2:** MR Test

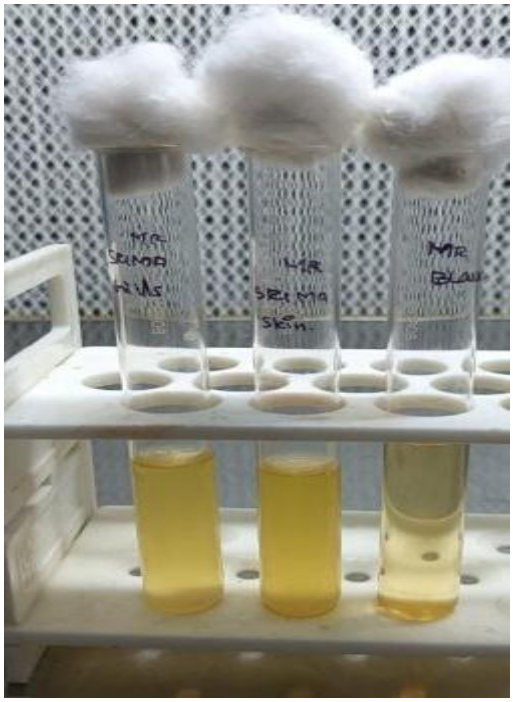

**FIG. 3.3:** Urease Test

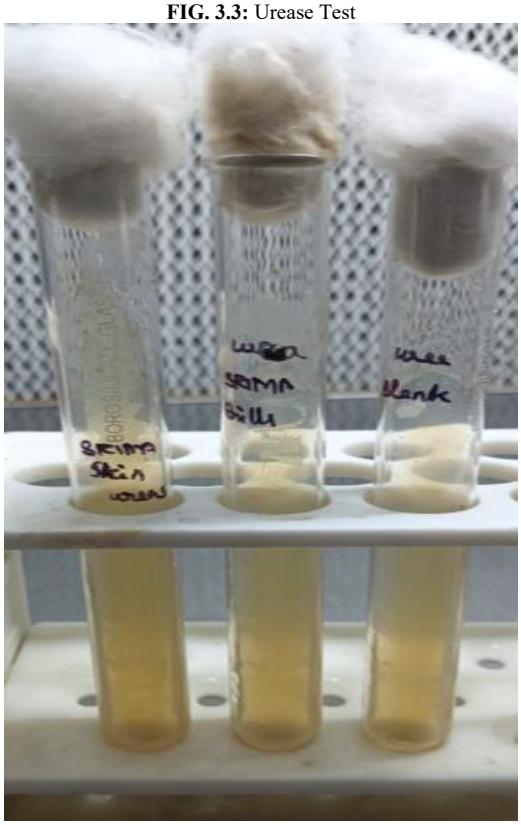

**FIG. 3.4:** TSI Test

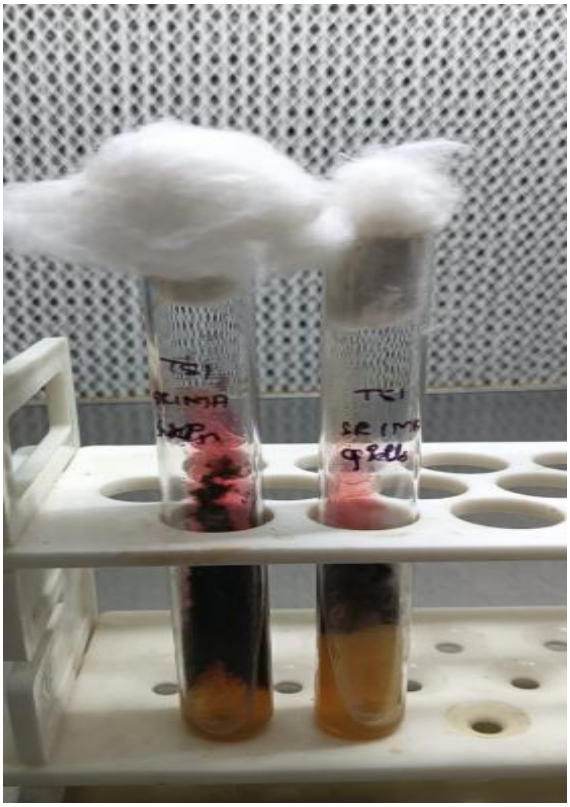

**FIG. 3.5:** Oxidase Test of Skin

**FIG. 3.6:** Oxidase Test of Gill

**FIG. 3.7:** Catalase Test

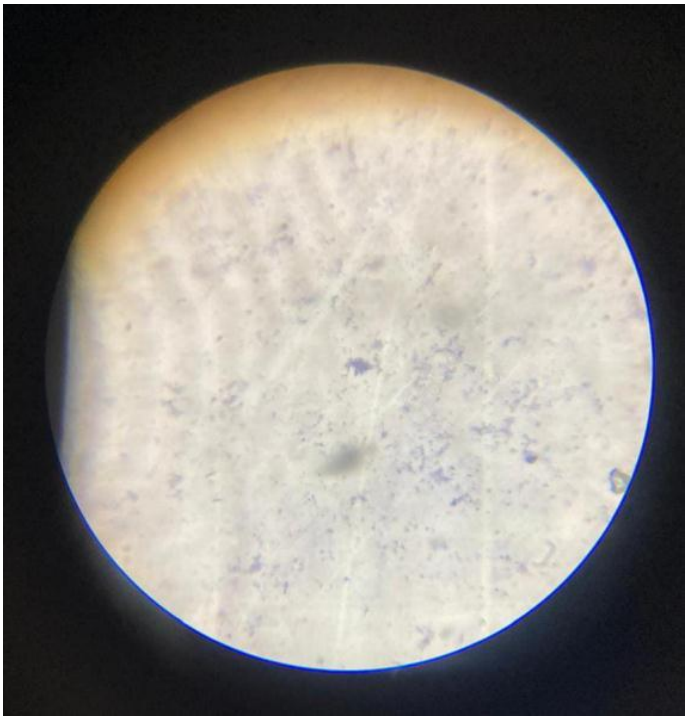

**FIG. 3.8:** Microscopic View of Skin and Gills

### 3.3 Antibacterial activity and minimum inhibitory concentration

Disc diffusion assay showed a dose-dependent increase in the zone of inhibition against both pathogens across the 10-100 µg/mL range, though inhibition remained markedly lower than the gentamicin (10 µg) positive control at all tested concentrations (Table 4). Gill isolates of A. hydrophila showed the largest zones among the extract-treated groups, followed by gill isolates of V. harveyi.

**Table 3.4:** Zone of inhibition (mm) of C. guianensis bark extract against A. hydrophila and V. harveyi.

| Extract concentration | A. hydrophila (gill) | A. hydrophila (skin) | V. harveyi (gill) | V. harveyi (skin) |
|---|---|---|---|---|
| Gentamicin 10 µg (+control) | 6.0 | 5.5 | 4.5 | 4.5 |
| 10 µg/mL | 1.2 | 0.6 | 0.4 | 1.0 |
| 25 µg/mL | 1.4 | 0.7 | 0.8 | 1.2 |
| 50 µg/mL | 1.3 | 1.2 | 1.2 | 1.2 |
| 75 µg/mL | 1.6 | 1.0 | 1.1 | 1.5 |
| 100 µg/mL | 1.5 | 1.2 | 1.8 | 1.7 |

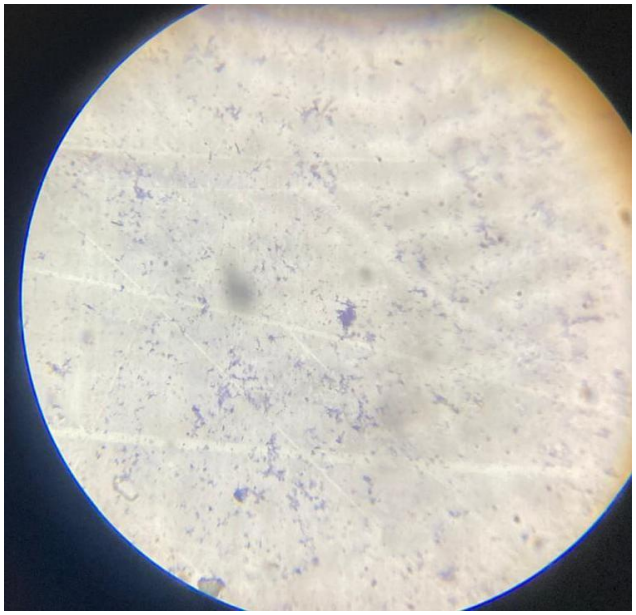

**FIG. 3.9:** Aeromonas hydrophila (G)

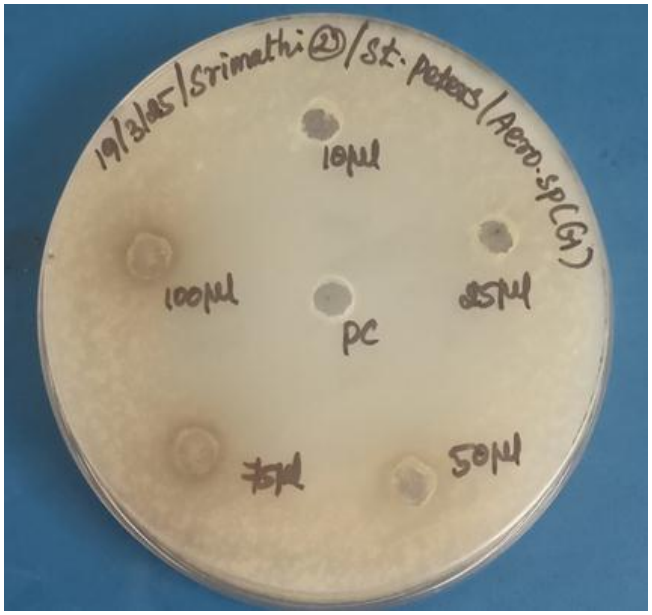


**FIG. 3.10:** Aeromonas hydrophila (S)

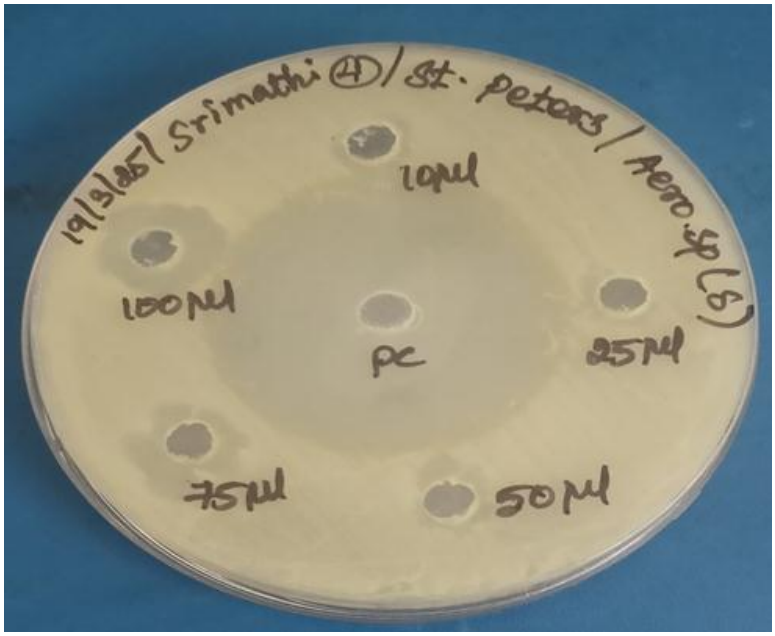


**FIG. 3.11:** Vibrio harveyi (G)

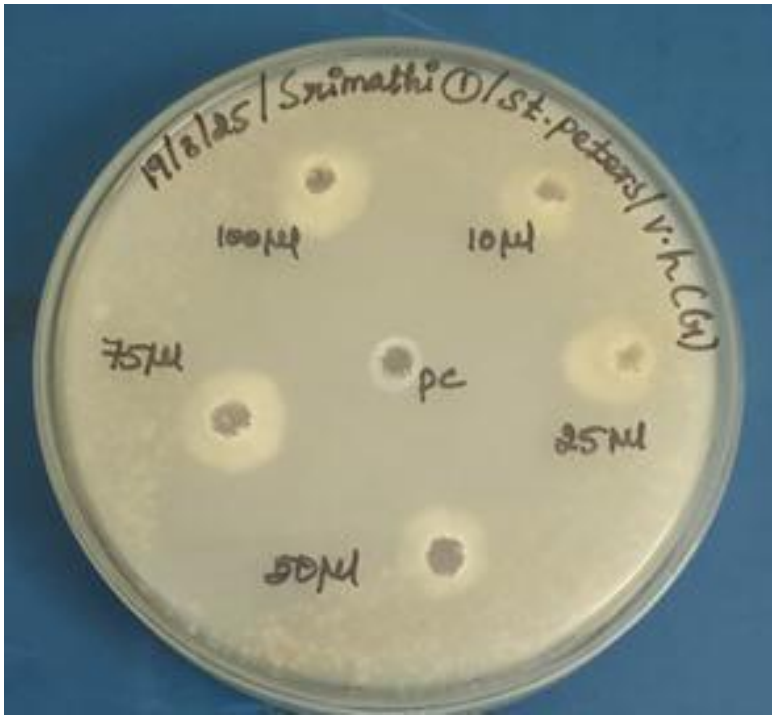


**FIG. 3.12:** Vibrio harveyi (S)

Broth microdilution established the MIC of C. guianensis bark extract at 75 µg/mL for A. hydrophila and 100 µg/mL for V. harveyi (Table 5), indicating relatively greater susceptibility of A. hydrophila to the extract.

**Table 3.5:** Minimum inhibitory concentration of C. guianensis bark extract.

| Pathogen | MIC (µg/mL) |
|---|---|
| Aeromonas hydrophila | 75 |
| Vibrio harveyi | 100 |


**FIG. 3.13**: MIC: A. hydrophila (G)

**FIG. 3.14:** MIC: V. harveyi (G)

**FIG.3.15:** MIC: A. hydrophila (S)

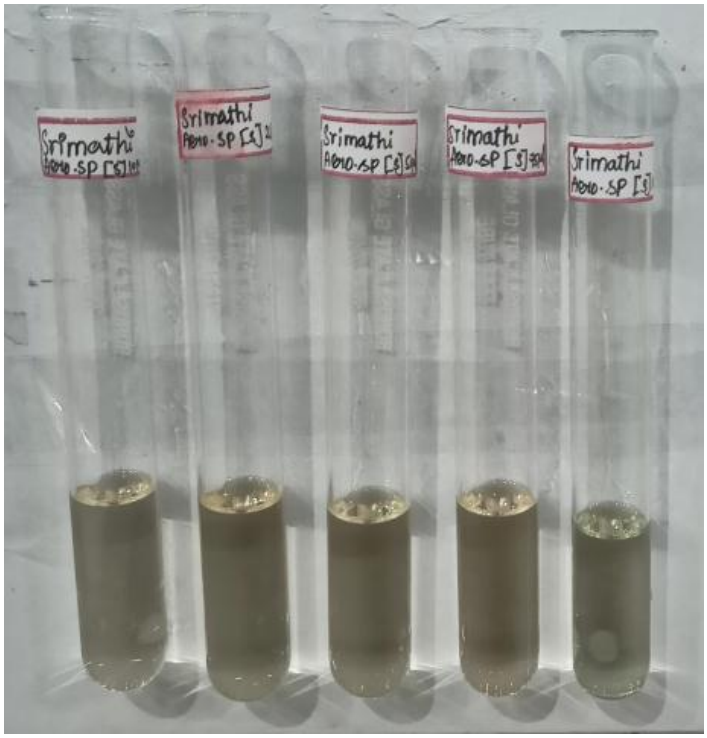


**FIG. 3.16:** MIC: V. harveyi (S)


**FIG. 3.17:** Bar Chart of MIC – A. hydrophila

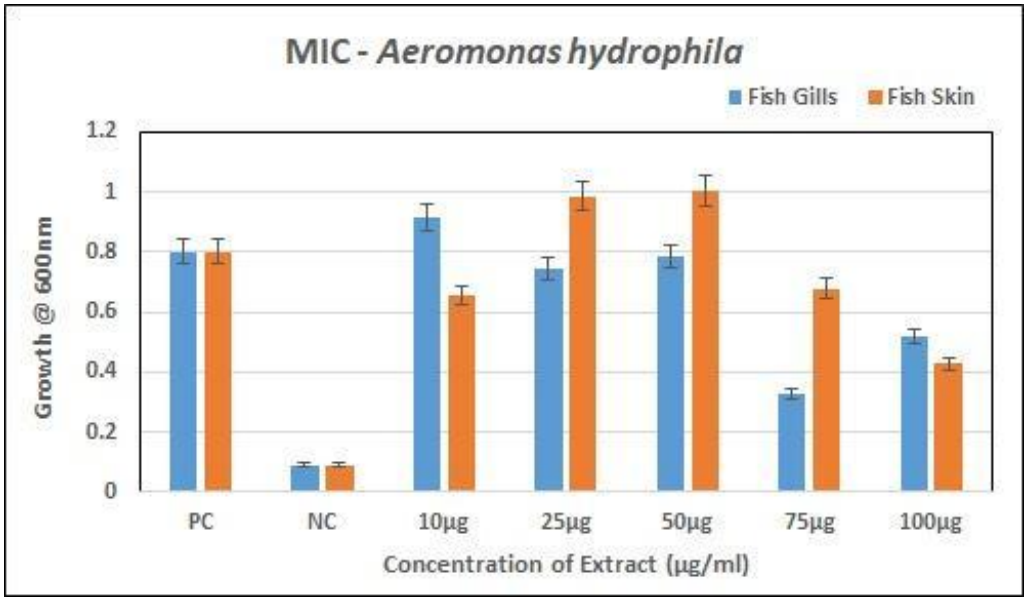


**FIG. 3.18:** Bar Chart of MIC – V. harveyi

### 3.4 TiLV gene detection

PCR amplification of bacterial-isolate-associated DNA from both gill and skin samples produced clear, distinct amplicons within the expected 400-500 bp range for the targeted TiLV gene segment, with comparable band intensity between the two tissue sources, consistent with concurrent viral pressure in the sampled Tilapia population alongside the bacterial pathogens characterised above.

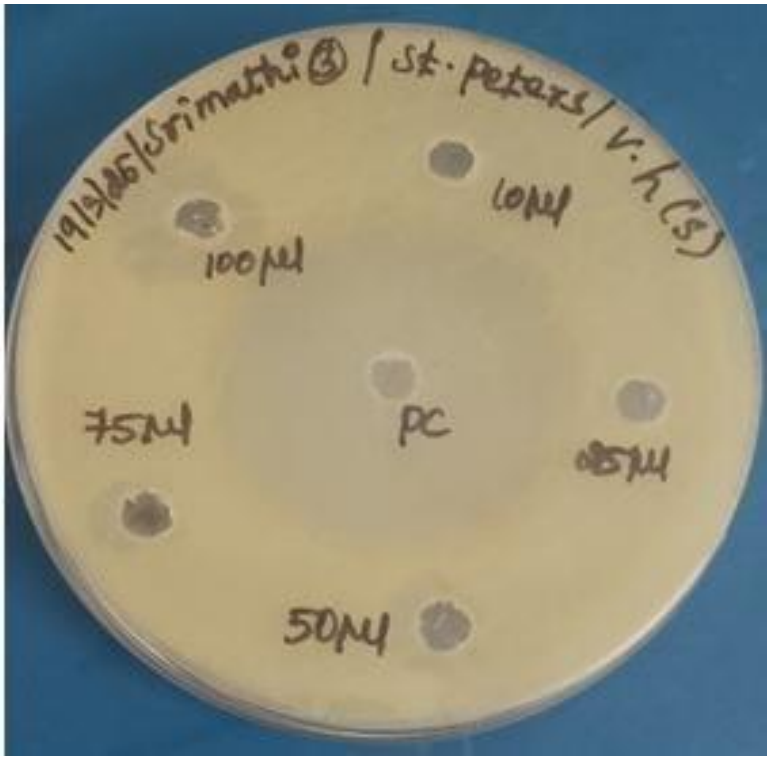


**FIG. 3.19:** TiLV Gene Band

## 4. DISCUSSION

The phytochemical profile of C. guianensis bark - rich in alkaloids, flavonoids, tannins, terpenoids and phenolics - is consistent with previous reports on the antioxidant and antimicrobial bioactivity of leaf and bark extracts of this species [10,11], and aligns with the broader literature identifying these compound classes as the principal drivers of antibacterial action in phytotherapeutic aquaculture agents,

through mechanisms including bacterial membrane disruption, enzyme inhibition and interference with quorum sensing [2,7,9]. The concentration-dependent rise in flavonoid and phenolic content observed here mirrors patterns reported for other medicinal-plant extracts used against Aeromonas and Vibrio pathogens in aquaculture settings [7,9].

The antibacterial activity recorded by disc diffusion, while dose-dependent, was modest relative to the gentamicin control, and the MIC values (75 µg/mL for A. hydrophila; 100 µg/mL for V. harveyi) indicate that inhibitory concentrations were reached only at the higher end of the tested range. This is broadly consistent with reviews noting that crude, unfractionated plant extracts typically show weaker in vitro activity than isolated bioactive fractions or essential-oil formulations, and that solvent choice, extraction method and bacterial strain variability strongly influence measured potency [2,7,8]. Comparable dose-dependent inhibition of A. hydrophila and Vibrio spp. by crude plant extracts has been reported using garlic, cinnamon and citrus-derived preparations, generally at extract concentrations in the low-to-mid mg/mL range rather than the µg/mL range achieved by isolated essential-oil constituents [7,8]. This suggests that fractionation, nanoencapsulation or combination with sub-inhibitory antibiotic doses may be needed to translate the present findings into a field-ready phytotherapeutic, an approach supported by studies combining plant bioactives with reduced antibiotic loads to restore efficacy against resistant aquaculture pathogens [2].

The concurrent detection of TiLV-consistent amplicons in the sampled fish population underscores the multifactorial disease pressure typical of intensified brackish-water aquaculture, where viral and bacterial co-infection can compound mortality and complicate disease management [12,13]. Given that global antimicrobial use in aquaculture continues to rise [1,4] against a backdrop of accelerating antimicrobial resistance [6], eco-friendly phytotherapeutic candidates such as C. guianensis bark extract merit continued investigation, including in vivo efficacy trials, toxicity and withdrawal-period assessment, and mechanistic studies (e.g., membrane integrity and biofilm assays) to substantiate the antibacterial mechanism suggested by the phytochemical profile reported here.

This study is limited by its in vitro scope, single extraction solvent, and reliance on PCR-based band-size confirmation rather than sequencing for TiLV identification; these constraints should be addressed in follow-up work before any practical aquaculture application is proposed.

## 5. CONCLUSION

Methanolic bark extract of Couroupita guianensis contains a broad spectrum of bioactive phytochemicals - alkaloids, flavonoids, tannins, terpenoids, steroids and phenolics - and exhibits dose-dependent, though moderate, antibacterial activity against Aeromonas hydrophila (MIC 75 µg/mL) and Vibrio harveyi (MIC 100 µg/mL) isolated from diseased brackish-water Tilapia, alongside evidence of concurrent TiLV pressure in the sampled population. These findings support C. guianensis bark as a candidate natural antibacterial agent for further development in sustainable aquaculture disease management, pending in vivo validation, toxicological assessment and mechanistic characterisation.

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