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Research Article

Antibacterial Activity of *Caulerpa racemosa* Methanolic Extract and Its Effects on Vibrio-Challenged *Litopenaeus vannamei*

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Abstract

Shrimp aquaculture faces increasing disease pressure from *Vibrio* spp., highlighting the need for sustainable alternatives to conventional antibiotics. This study evaluated the antibacterial activity and protective effects of a methanolic extract of *Caulerpa racemosa* collected from Sri Lanka. *In vitro* screening using the agar well diffusion method showed that only the undiluted crude extract (100%) exhibited significant antibacterial activity against a presumptive *Vibrio* sp. isolate, producing an inhibition zone of 15.67±0.67 mm, whereas diluted extracts and methanol solvent control showed no inhibition. During the *in vivo* trial, *Litopenaeus vannamei* post-larvae were fed a commercial diet surface-coated with the methanolic extract for seven days prior to bacterial challenge. The treatment group showed a numerically higher survival rate (90±5%) than the challenged control (73.33±7.64%), although the difference was not statistically significant. Growth performance parameters also did not differ significantly among treatments ($p > 0.05$). Following bacterial challenge, shrimp in the treatment group showed milder apparent clinical signs, including lethargy and reddish body coloration. Water quality parameters remained relatively stable among the groups throughout the experimental period. These findings provide preliminary evidence that *C. racemosa* methanolic extract possesses antibacterial activity against a presumptive *Vibrio* sp. isolate and may have potential as a natural dietary supplement for shrimp health management. Further studies should focus on standardized dietary dosage, identification of bioactive compounds, immune-response assessment, and longer-term feeding trials to evaluate its potential application in sustainable shrimp aquaculture.

Keywords: *Caulerpa racemosa*, Methanolic extract, *Litopenaeus vannamei*, *Vibrio* sp., Antimicrobial activity, Sustainable aquaculture

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1. Introduction

Shrimp aquaculture has emerged as one of the fastest-growing sectors in global food production, particularly in Southeast Asia and other tropical regions, driven by increasing demand, favorable climatic conditions, and the economic viability of high-value species such as *Litopenaeus vannamei* (Dashtiannasab *et al.*, 2016; Liu *et al.*, 2020). This species now dominates shrimp farming due to its adaptability to intensive culture systems, rapid growth, and high survival rates (Lin *et al.*, 2017; Sudaryono *et al.*, 2018a; Ding *et al.*, 2020; Janewanthanakul *et al.*, 2020; Ngo *et al.*, 2020; Shi *et al.*, 2020). The global shrimp market was valued at approximately USD 64.2 billion in 2025 and is expected to reach USD 88.1 billion by 2030, with a compound annual growth rate (CAGR) of approximately 6.5% (The Business Research Company, 2026).

The intensification of shrimp farming has been accompanied by an increasing incidence of disease outbreaks, including both bacterial and viral infections, which are often exacerbated by environmental stressors such as high stocking densities, fluctuating temperatures, and poor water quality (Manilal *et al.*, 2012; Huang *et al.*, 2013; Félix *et al.*, 2020; Pratiwi *et al.*, 2021). Among bacterial pathogens, *Vibrio* spp. including *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus,* and *V. campbellii* are main causing agents of vibriosis and other bacterial diseases in shrimp aquaculture such as acute hepatopancreatic necrosis disease (AHPND), luminous vibriosis, and white tail disease, which lead to substantial economic losses (Hettiarachchi *et al.*, 2005; Mine and Boopathy 2011; Dash *et al.*, 2017; Aguilera-Rivera *et al.*, 2019; Mohamad *et al.*, 2019; Sotomayor *et al.*, 2019; Klongklaew *et al.*, 2021; de Souza Valente and Wan 2021). At the same time, viral diseases, including White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Taura Syndrome Virus (TSV), Infectious Myonecrosis Virus (IMNV), and Yellow Head Virus (YHV), also continue to threaten shrimp production and can cause high mortality and production losses (Athula *et al.*, 2024).

Antibiotics have long been the primary strategy for controlling bacterial pathogens in aquaculture, but their extensive and often indiscriminate use has raised serious concerns, including the emergence of antimicrobial-resistant strains, residual drug accumulation in aquatic products, and contamination of surrounding environments (Klongklaew *et al.*, 2020; Pratiwi *et al.*, 2021). Sri Lanka has also experienced serious shrimp disease outbreaks. Monodon Baculovirus (MBV) disease was reported during 1989 – 1990 and caused mortality among hatchery post-larvae, while White Spot Syndrome Virus (WSSV) has affected shrimp production since 1996 and has caused substantial mortality in grow-out ponds. These recurring disease problems, together with the recent adoption of *L. vannamei* farming, underscore the urgent need for environmentally sustainable and biologically effective alternatives to conventional antibiotics, highlighting the importance of developing natural disease management strategies in shrimp aquaculture (Hettiarachchi *et al.*, 2005; Dashtiannasab *et al.*, 2016; Wahjuningrum *et al.*, 2020).

In response, recent research has focused on natural disease control strategies, including the use of functional feed additives such as probiotics, prebiotics, and marine macroalgae (Lim *et al.*, 2020; Shi *et al.*, 2020). Marine macroalgae are increasingly explored as natural feed additives due to their bioactive compounds, including sulfated polysaccharides and phenolics, which possess antimicrobial and functional properties (Yadav *et al.*, 2015; El Shafay *et al.*, 2016; Dashtiannasab *et al.*, 2016; Dugassa and Gaetan, 2018; Mulyadi *et al.*, 2020; Safavi *et al.*, 2019; AftabUddin *et al.*, 2021; Pratiwi *et al.*, 2021). Among these, *Caulerpa racemosa*, a green macroalgae widely distributed in tropical and subtropical marine environments, has shown significant pharmacological potential, including antibacterial activity against aquaculture pathogens (Pratiwi *et al.*, 2021). In Sri Lanka, *C. racemosa* commonly occurs in shallow coastal waters and coral reef areas, particularly along the southern and western coasts of Sri Lanka, where it grows on sandy or rocky substrates (Coppejans *et al.*, 2009).

Several studies have reported antibacterial activity of *Caulerpa* species against pathogens including *Vibrio parahaemolyticus*, *Pseudomonas* spp., and other Gram-negative and Gram-positive bacteria (El Shafay *et al.*, 2016; Mulyadi *et al.*, 2020; Pratiwi *et al.*, 2021). However, studies specifically evaluating methanolic extracts of Sri Lankan *C. racemosa* against *Vibrio* infections in *L. vannamei* post-larvae are lacking. Methanol was selected in the present study because it has been used to extract a range of bioactive constituents from marine macroalgae and was suitable for the preliminary antibacterial screening conducted here. The seven-day feeding period was selected as a preliminary short-term assessment before considering longer-term studies.

This study addresses this gap by investigating both the *in vitro* antibacterial activity and *in vivo* protective effects of methanolic extract of *C. racemosa* against a presumptive *Vibrio* sp. isolate. Specifically, it evaluates (i) the antibacterial activity of the extract against the presumptive *Vibrio* sp. isolate, and (ii) the effects of dietary supplementation on growth, survival, and clinical responses of *L. vannamei* post-larvae. Although several studies have evaluated seaweed extracts in shrimp aquaculture, limited information is available regarding the antibacterial and protective effects of methanolic extracts of Sri Lankan *C. racemosa* in *L. vannamei*. By exploring a locally available marine macroalgae as a functional feed additive, this research contributes to sustainable disease management strategies and reduces reliance on conventional antibiotics in shrimp aquaculture.

2. Materials and Methods

2.1. Ethical Approval

All experimental protocols involving *L. vannamei* post-larvae, including dietary supplementation, *Vibrio* challenge, monitoring of growth and survival, and assessment of clinical responses, were reviewed and approved by the Research Ethics Committee of Uva Wellassa University, Sri Lanka (Ref No. UWU/REC/2022/005). All procedures were conducted in accordance with institutional guidelines for the ethical care and use of aquatic animals.

2.2. Collection and Preparation of *Caulerpa racemosa*

Fresh *Caulerpa racemosa* (Figure 1) was collected from the Ahangama coastal region in southern Sri Lanka (5°57'59.1"N, 80°22'30.0"E; Figure 2). Samples were transported to the Aquaculture Laboratory, Uva Wellassa University, Sri Lanka in seawater-filled polyethylene bags. Upon arrival, the biomass was thoroughly rinsed under running freshwater to remove sand, epiphytes, animal debris, and calcareous deposits. The cleaned seaweed was chopped and subjected to overnight air-drying under an electric fan, followed by 48 hours of shade drying at ambient temperature. Once dried to a constant weight, the material was ground into a fine powder using a mechanical grinder and stored in airtight containers until extraction.

The fresh biomass decreased from 1900.10 g to 137.40 g after shade drying, corresponding to 92.77% water content and 7.23% dry matter.



Figure 1. *Caulerpa racemosa* collected from the Ahangama coastal region, Sri Lanka.



Figure 2. Sampling site for *Caulerpa racemosa*– Ahangama coastal region (5°57'59.1"N, 80°22'30.0"E) at low tide.

2.3. Extraction of Bioactive Compounds

The extraction method was adapted from Manilal *et al.* (2012), with slight modifications. Five grams of powdered *C. racemosa* were mixed with 50 mL of methanol (1:10 w/v ratio). Extraction was performed at 40 °C for 24 hours in a constant temperature shaking incubator (Model FS-50B, Faithful Instrument (Hebei) Co., Ltd., China) at 120 rpm. The mixtures were filtered through A1 Grade filter paper, and the filtrates were concentrated using a rotary evaporator. The concentrated crude extract was then re-dissolved in 1 mL of methanol to prepare a crude stock solution and stored in airtight vials at 4 °C protected from light. Methanol was selected as the extraction solvent based on its reported use for extracting diverse bioactive constituents from marine macroalgae and its suitability for preliminary antibacterial screening. Methanol was used only as a laboratory extraction solvent and was not intended as a component of the shrimp feed or as a therapeutic agent.

2.4. *In Vitro* Antibacterial Assay

Antibacterial activity of the methanolic extract was evaluated using the agar well diffusion method, following the methods described by Isebe (2016) and AftabUddin *et al.* (2021). The presumptive *Vibrio* sp. isolate was obtained from a diseased shrimp pond water sample in Chilaw, Sri Lanka by culturing on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. The isolate was identified based on colony morphology and culture characteristics, maintained as a laboratory culture, and used for the antibacterial assay. Mueller-Hinton agar plates were prepared and inoculated by evenly spreading the previously isolated presumptive *Vibrio* sp. suspension over the entire agar surface using a sterile cotton swab to obtain a uniform bacterial spread. The bacterial suspension was standardized to a

turbidity equivalent to a 0.5 McFarland standard (approximately 1×10^8 CFU mL^{-1}). Five wells, each 8 mm in diameter, were aseptically prepared in each agar plate and filled with 100 µL of the test solutions: undiluted crude extract (100%), a 10-fold dilution (1:10; 10%), and a 100-fold dilution (1:100; 1%). The corresponding solvent methanol used for extraction was included as the negative control, while ciprofloxacin (0.5 mg mL^{-1}) served as the positive control. The treatments were allowed to diffuse before incubation. After incubation at 37 °C for 24 hours, the diameters of inhibition zones were measured in millimeters using a sterile ruler. The complete set of five treatments was included on each replicate plate, and the assay was performed in triplicate. The mean inhibition-zone diameter of the three replicate plates was used for statistical analysis.

2.5. Experimental Design and Feeding Trial

A 7-day feeding trial was conducted using *L. vannamei* post-larvae aged 20 days (PL20), sourced from a commercial hatchery in Chilaw, Sri Lanka. A completely randomized design (CRD) with the three treatments in triplicate was employed:

- C– (Unchallenged Control): Fed basal diet, no *Vibrio* exposure
- C+ (Challenged Control): Fed basal diet, followed by *Vibrio* challenge
- T (Treatment): Fed diet supplemented with undiluted crude methanolic *C. racemosa* extract, followed by *Vibrio* challenge

Each of the nine fiberglass-reinforced plastic (FRP) tanks (250 L) was stocked with 10 shrimp (n = 90). Post larvae were fed six times daily with a commercial diet containing ≥34% crude protein. For the treatment diet, methanolic crude extract was dissolved in 100 mL of distilled water containing 2% gelatin as a binder. The resulting solution was evenly sprayed onto 1 kg of commercial feed and thoroughly mixed to ensure uniform coating. The coated feed was then dried in a hot-air oven at 40 °C and stored in airtight containers until use (Sarjito *et al.*, 2020). Owing to the limited yield, the crude extract was applied as a qualitative surface coating rather than a quantified dietary inclusion level.

The feeding trial duration was limited to seven days as the primary objective of the study was to evaluate the short-term protective effects of the extract against a *Vibrio* challenge rather than long-term growth performance.

2.6. Water Quality Monitoring

Water quality parameters were monitored daily to ensure uniform rearing conditions across all experimental tanks. Measurements were taken each morning before feeding and prior to water exchange. Water temperature was measured using a digital thermometer, salinity using a handheld refractometer (Extech RF20, Extech Instruments), dissolved oxygen using a portable DO meter (YSI Pro20 Dissolved Oxygen Instrument, YSI Inc., USA), and ammonia concentration using an API Ammonia Test Kit (API Fishcare, USA). During the experimental period, water quality conditions remained within acceptable ranges for shrimp culture, with salinity ranging from 26–28 ppt,

temperature from 30–31 °C, dissolved oxygen from 7.0–8.0 mg L^{-1}, and ammonia concentration from 1.0–1.5 mg L^{-1} (Sudaryono *et al.*, 2018b).

To maintain water quality, approximately 30% of tank water was exchanged daily in the morning. All values are presented as mean ± standard deviation (SD).

2.7. *Vibrio* Challenge and Clinical Observations

After the feeding period, shrimp from the C+ (challenged control) and T (treatment) groups (n = 30 per group) were exposed to a *Vibrio* sp. suspension (1×10^6 CFU mL^{-1}) by immersion for 1 h. Following exposure, shrimp were transferred to their respective tanks and monitored for 48 h.

Clinical observations were conducted to record behavioral, external, and internal signs of infection. Behavioral changes such as reduced feeding activity, lethargy, abnormal swimming, and weak response to disturbance were monitored through direct observation during the post-challenge period. External clinical signs including body discoloration, reddish body coloration, and reduced movement were visually assessed. Internal changes in hepatopancreatic appearance were examined through visual inspection of sampled individuals when mortality occurred. Mortality was recorded during the observation period to determine post-challenge survival rate.

2.8. Growth and Survival Assessment

Ten shrimp per tank were individually weighed at the beginning and at the end of the 7-day feeding trial. Growth performance and survival were calculated using the following equations (Klongklaew *et al.*, 2021):

$$Weight\ Gain = Final\ Weight - Initial\ Weight \quad (1)$$

$$Average\ Daily\ Growth = \frac{Final\ Weight - Initial\ Weight}{No\ of\ Days} \quad (2)$$

$$Specific\ Growth\ Rate = \frac{(ln\ Final\ Weight\ - ln\ Initial\ Weight\ )}{No\ of\ Days} \times 100 \quad (3)$$

$$Survival\ Rate\ (\%) = \frac{Number\ of\ Surviving\ Shrimp}{Initial\ Number} \times 100 \quad (4)$$

2.9. Statistical Analysis

Data from the *in vitro* antibacterial assay and *in vivo* experiment were expressed as mean ± standard deviation (SD). The *in vitro* antibacterial assay data, including inhibition zone diameters, and the *in vivo* experimental data were analyzed using SPSS Statistics ver. 26. (IBM, USA). One-way ANOVA was used to assess significant differences among

treatments, followed by Tukey's HSD post hoc test. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

This study evaluated the antibacterial activity and protective effects of methanolic extracts of *Caulerpa racemosa* in *Litopenaeus vannamei* challenged with *Vibrio* sp. The results highlight its potential application as a natural feed additive in shrimp aquaculture. The results provide preliminary evidence of antibacterial activity of the methanolic C. racemosa extract and its possible protective potential under bacterial challenge.

3.1. *In Vitro* Antibacterial Activity

The undiluted crude methanolic extract (100%) of *Caulerpa racemosa* exhibited antibacterial activity against the presumptive *Vibrio* sp. isolate, producing a clear inhibition zone measuring 15.67±0.67 mm. In contrast, the 10-fold and 100-fold diluted methanolic extracts showed no measurable inhibition, and the methanol-only negative control also showed no antibacterial activity. The positive control, ciprofloxacin (0.5 mg mL^{-1}), produced the largest inhibition zone (21.33 ± 0.33 mm), confirming the effectiveness of the assay. Among all tested treatments, only the undiluted methanolic extract demonstrated significant antibacterial activity against the presumptive *Vibrio* sp. isolate ($p < 0.05$), while diluted extracts showed no detectable inhibitory effect (Figure 3).

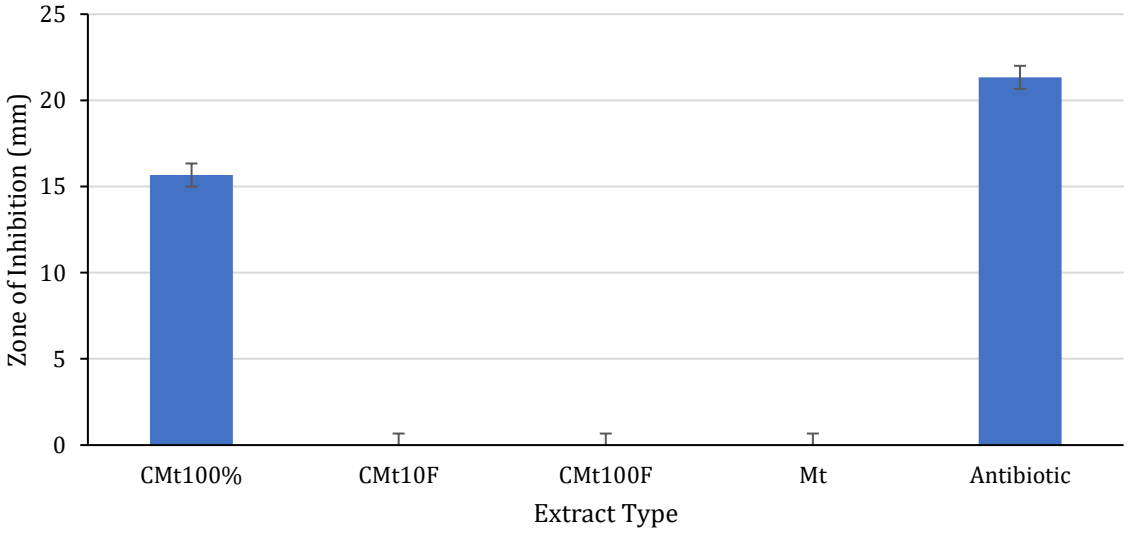


Figure 3. Antibacterial activity of *Caulerpa racemosa* methanolic extract against *Vibrio* sp. measured by agar well diffusion assay (mean ± SD). CMt100%: methanol 100% undiluted crude extract; CMt10F: methanol 10-fold dilution; CMt100F: methanol 100-fold dilution; Mt: methanol solvent control.

The *in vitro* assay demonstrated that only the undiluted methanolic extract (100%) of *Caulerpa racemosa* exhibited a measurable zone of inhibition against the presumptive *Vibrio* sp. isolate, whereas the 10-fold (10%) and 100-fold (1%) diluted extracts showed no detectable antibacterial activity. The inhibition observed with the undiluted extract

suggests that the concentration of extract was an important factor influencing the antibacterial response. Similar concentration-dependent antibacterial effects of seaweed extracts have been reported previously (Yadav *et al.*, 2015; El Shafay *et al.*, 2016; AftabUddin *et al.*, 2021; Pratiwi *et al.*, 2021). The absence of inhibition in the solvent-only control indicates that the observed antibacterial activity was associated with the *C. racemosa* extract rather than the solvent used for extraction. Methanol has been widely used for extracting diverse bioactive constituents from seaweeds, including phenolic and flavonoid compounds that have been associated with antimicrobial activity. Although methanol was effective for preliminary extraction and antibacterial screening, its toxicity limits its direct practical application in aquaculture (Yadav *et al.*, 2015; El Shafay *et al.*, 2016; AftabUddin *et al.*, 2021; Pratiwi *et al.*, 2021). Based on its measurable antibacterial activity against the presumptive *Vibrio* sp. isolate, the undiluted methanolic extract was selected for the subsequent feeding trial.

3.2. Growth Performance and Survival Rate

Growth performance parameters did not differ significantly among treatments ($p > 0.05$). Numerically, the treatment group (T) recorded the highest mean values for weight gain (0.99 ± 0.04 g), average daily growth (0.14 ± 0.01 g day^{-1}), and specific growth rate (1.51 ± 0.08%), compared with the unchallenged control (C−) and challenged control (C+). However, these numerical differences were not statistically significant ($p > 0.05$) (Table 1).

Table 1. Growth performance and survival rate of *Litopenaeus vannamei* under different treatments (mean ± SD).

Group	Weight Gain (g) ± SD	Average Daily Growth (g day^{-1}) ± SD	Specific Growth Rate (%) ± SD	Survival Rate (%)
C− (Unchallenged Control)	0.89 ± 0.17^{a}	0.13 ± 0.02^{a}	1.24 ± 0.29^{a}	100.00 ± 0.00^{a}
C+ (Challenged Control)	0.91 ± 0.05^{a}	0.13 ± 0.01^{a}	1.38 ± 0.24^{a}	73.33 ± 7.64^{b}
T (Treatment – Seaweed + Challenge)	0.99 ± 0.04^{a}	0.14 ± 0.01^{a}	1.51 ± 0.08^{a}	90.00 ± 5.00^{b}

Values are presented as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences ($p < 0.05$). C−, unchallenged control; C+, challenged control; T, seaweed extract supplemented and challenged group

Survival rates differed significantly among treatments ($p < 0.05$). The unchallenged control (C−) recorded the highest survival rate (100.00 ± 0.00%), while the challenged control (C+) and treatment group (T) recorded survival rates of 73.33 ± 7.64% and 90.00 ± 5.00%, respectively. The survival rate of the treatment group was numerically higher than that of the challenged control; however, the difference was not statistically significant (Table 1). Higher survival following dietary supplementation has also been reported in studies evaluating seaweed extracts against *Vibrio* challenges (Esquer-Miranda *et al.*, 2016; Rudi *et al.*, 2019).

The *in vivo* results showed numerical differences in growth performance among treatments; however, weight gain, average daily growth, and specific growth rate did not differ significantly among groups (p>0.05). The treatment group recorded the highest numerical values for these growth parameters, but these differences should not be interpreted as evidence of improved growth performance. Previous studies have reported effects of dietary seaweed supplementation on shrimp growth performance (Yadav *et al.*, 2015; Jasmanindar *et al.*, 2018; Mulyadi *et al.*, 2020; Pratiwi *et al.*, 2021). Differences among studies may be related to factors such as seaweed species, extraction method, dietary inclusion level, culture conditions, and experimental duration.

3.3. Clinical Signs of Infection

Compared with the challenged control (C+), shrimp in the treatment group (T) showed fewer apparent clinical signs during the post-challenge observation period. Clinical signs in the C+ group were first observed within 24 h post-challenge and included reduced activity, lethargy, and abnormal swimming behavior, and reddish body coloration. Internal examination of affected individuals revealed changes in hepatopancreatic appearance. These clinical signs were monitored throughout the experimental observation period.

In contrast, shrimp in the group T showed only mild behavioral changes and limited external symptoms such as body discoloration, and reduced movement, whereas shrimp in the unchallenged control (C−) remained apparently healthy throughout the observation period. These observations suggest a possible association between dietary supplementation and the apparent post-challenge response; however, the clinical observations were not quantitatively scored and specific immune parameters were not evaluated. These observations are consistent with previous reports describing protective effects of seaweed-derived bioactive compounds in shrimp aquaculture (Mulyadi *et al.*, 2020; Sarjito *et al.*, 2020; Pratiwi *et al.*, 2021). Although specific immune parameters were not evaluated in the present study, the lower apparent severity of clinical signs in the T group, together with its numerically higher survival rate than the C+ group, indicate an association between dietary supplementation and the observed post-challenge response.



Figure 4. Challenged control shrimp (C+) showing reddish body coloration and apparent external clinical signs at 24 h post-challenge.

Similar protective effects of seaweed-derived preparations have been reported in aquatic animals. Dashtiannasab et al. (2016) reported that an ethanol extract of the red seaweed *Laurencia snyderiae* improved resistance of *Litopenaeus vannamei* to *Vibrio harveyi* challenge. Mulyadi *et al.* (2020) reported increased resistance of *L. vannamei* to *Vibrio alginolyticus* following exposure to an ethanolic extract of the brown seaweed *Sargassum* sp. AftabUddin et al. (2021) evaluated methanol and ethanol-based extracts of the brown seaweeds *Padina tetrastromatica* and *Sargassum ilicifolium* in *Penaeus monodon* and reported improved growth, immune responses, and resistance to *Vibrio parahaemolyticus* following dietary supplementation. These findings indicate that bioactive preparations derived from different seaweeds may influence disease resistance and physiological responses in aquaculture species, although the effects may vary according to seaweed species, extraction method, preparation type, dose, and experimental species.

3.4. Water Quality Parameters

Throughout the experimental period, water quality parameters were maintained within acceptable ranges for *L. vannamei* culture and showed no statistically significant differences among the experimental groups ($p>0.05$). The stability of environmental conditions minimized potential variation in shrimp responses attributable to water quality (Table 2).

Table 2. Water quality parameters recorded during the experiment (mean ± SD).

Water Quality Parameter	Treatment		
	C-	C+	T
Temperature (°C)	30.53 ± 0.11[a]	30.52 ± 0.14[a]	30.47 ± 0.16[a]
Salinity (ppt)	26.86 ± 0.00[a]	26.86 ± 0.00[a]	26.86 ± 0.00[a]
DO (mg L^{-1})	7.41 ± 0.09[a]	7.47 ± 0.06[a]	7.51 ± 0.02[a]
Ammonia Concentration (mg L^{-1})	1.02 ± 0.08[a]	1.02 ± 0.11[a]	1.00 ± 0.12[a]

Values are presented as mean ± SD. C−, unchallenged control; C+, challenged control; T, extract-supplemented and challenged group; DO, dissolved oxygen. Different superscript letters within the same row indicate significant differences (p<0.05).

Environmental conditions remained relatively consistent among the experimental groups throughout the study, thereby minimizing potential variation in shrimp responses attributable to water quality. As noted by Hettiarachchi *et al.* (2005), Mohamad *et al.* (2019), Klongklaew *et al.* (2021), and Pratiwi *et al.* (2021), environmental stress can affect immune function, growth performance, and susceptibility to disease. Additionally, *Caulerpa* species may improve water quality, offering added value to water in integrated systems (Anh *et al.*, 2021; Margono *et al.*, 2021). Therefore, the relatively consistent water quality conditions observed among the experimental groups support the interpretation that differences in the measured responses were not associated with substantial variation in these environmental parameters.

Overall, these findings provide evidence of antibacterial activity of the methanolic *C. racemosa* extract and preliminary evidence of its potential as a dietary supplement in shrimp culture. Given the increasing antibiotic resistance and regulatory restrictions, seaweed-based supplements may represent a potential alternative. These findings are consistent with previous reports describing the potential of seaweed-derived bioactives to improve disease resistance in aquaculture species (Dashtiannasab *et al.*, 2016; Dugassa and Gaetan, 2018; Safavi *et al.*, 2019; AftabUddin *et al.*, 2021; Pratiwi *et al.*, 2021). However, further studies under controlled and larger-scale experimental conditions are required to confirm these effects and determine their practical application in shrimp culture.

The study had several limitations. The relatively short experimental period and limited number of experimental replicates may have restricted the ability to detect differences in growth performance, although the specific contribution of these factors cannot be established from the present results. Specific immune parameters were not assessed because of the small size of the shrimp post-larvae and the associated difficulty of collecting sufficient haemolymph for quantitative analysis. Therefore, specific antibody responses against the challenge organism and other immune parameters could not be evaluated. Future studies using larger shrimp and a species-level identified bacterial

challenge could investigate specific and nonspecific immune responses using appropriate immunological assays.

In addition, the exact dietary dose of the crude extract could not be quantified because the extract was applied as a surface coating to the feed during this preliminary evaluation. Future studies should therefore investigate standardized extract inclusion levels and dose-response relationships over longer experimental periods. The stability of the extract and its bioactive constituents during feed preparation and storage should also be evaluated.

Qualitative phytochemical screening of the methanolic *C. racemosa* crude extract detected flavonoids, terpenoids, carbohydrates, and cardiac glycosides. However, these findings are preliminary, as qualitative screening does not establish the identity or concentration of individual compounds or their respective contributions to the observed antibacterial activity. Further chemical characterization using appropriate chromatographic and spectrometric techniques is required to identify and quantify the specific bioactive constituents and to clarify their potential contribution to the observed antibacterial effects.

4. Conclusion

This study demonstrated that the methanolic extract of *Caulerpa racemosa* exhibited antibacterial activity against the presumptive *Vibrio* sp. isolate. Dietary supplementation with the extract was associated with lower apparent severity of clinical signs following the bacterial challenge, and the treatment group showed numerically higher survival than the challenged control. However, growth performance parameters did not differ significantly among treatments, and the survival difference between the treatment and challenged control groups was also not statistically significant. Therefore, the present findings provide preliminary evidence for the potential use of *C. racemosa* methanolic extract as a dietary supplement in *Litopenaeus vannamei*, but further studies are required to confirm its protective effects.

These findings highlight the practical value of utilizing locally available marine macroalgae as functional feed ingredients, supporting sustainable aquaculture and reducing reliance on conventional antimicrobial agents. Future studies should focus on longer-duration feeding trials, standardized extract inclusion levels and dose-response relationships, detailed characterization of bioactive constituents, and evaluation of appropriate immune parameters to clarify the mechanisms underlying the observed responses to further validate its application in commercial shrimp farming.

Author Contributions

Conceptualization, D. M. W; methodology, D. M. W., D. P. N. De S., B. V. A. S. M. B., J. A. A. and A. C.; formal analysis, D. M. W.; investigation, D. M. W.; data curation, D. M. W.; visualization, D. M. W.; validation, D. P. N. De S., B. V. A. S. M. B., J. A. A. and A. C.; resources, D. P. N. De S., B. V. A. S. M. B. and J. A. A.; writing – original draft preparation, D. M. W.; writing – review and editing, D. P. N. De S., B. V. A. S. M. B., J. A. A. and A. C.; supervision, D. P. N. De S., B. V. A. S. M. B., J. A. A. and A. C. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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