Volume 7, Issue 1: KUS: MariNEX 1504: xx-xx: 11012026: 2026

KHULNA UNIVERSITY
STUDIES

journal homepage: www.kus.ku.ac.bd

Research article

Assessment of Anti-*Vibrio alginolyticus* and -*Exiguobacterium qingdaonense* Activity of Seaweed Collected from the St. Martin's Island

Nurunnahar Nadira*, Sagar Roy, Anup Sardar, Alokesh Kumar Ghosh, Shikder Saiful Islam and Abul Farah Md. Hasanuzzaman

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh

ABSTRACT

Seaweeds are being increasingly assessed for their potential antimicrobial effects in controlling microbial infections in aquaculture units; however, despite the rapid growth of aquaculture in Bangladesh, studies on the antibacterial potential of indigenous seaweeds against aquaculture pathogens remain limited. In this study, four seaweeds (*Hypnea spinella*, *Padina australis*, *Chnoospora implexa*, *Sargassum carpophyllum*) collected from St. Martin's Island, Bangladesh, were studied to evaluate their antibacterial activity. All experiments were conducted in duplicate and followed a randomized experimental design to ensure reproducibility and statistical reliability. Initially, a comparative study of total viable bacterial count (TBC) between seawater and seawater extracts of seaweeds was done to explore seaweeds' antibacterial potentiality; the mean TBC in the seawater and seawater-extracts of seaweed samples was $1.24\pm0.01\times10^8$ and $1.105\pm0.02\times10^8$ CFU/mL, respectively, being likely associated with antibacterial potential of these seaweeds. Accordingly, the antibacterial activities of crude extracts prepared from each seaweed using water, methanol, ethanol, ethyl acetate, and hexane were evaluated against the gram-negative *Vibrio alginolyticus* and the gram-positive *Exiguobacterium qingdaonense* isolated from the Mud crab *Scylla olivacea*. The antibacterial activity of the extracts was evaluated at a concentration of 5 mg/disc compared to 6 commercial antibiotics; not a single extract exhibited an inhibitory zone against *V. alginolyticus* while it was sensitive to ciprofloxacin and tetracycline antibiotics; likely, the tested seaweed extracts might have insufficient active compounds at the tested dose or inherent resistance of *V. alginolyticus*, highlighting further dose optimization studies. Conversely, the extracts showed inhibitory zones against *E. qingdaonense*, with the exception of water extracts, and the strain was susceptible to all antibiotics. Since the ethanol extract of *P. australis* exhibited the largest zone of inhibition (11 mm), a dose-response (1-10 mg/disc) analysis was performed, which showed a strong positive linear ($R^2 = 0.961$) relationship. The IC_{50} of this extract, determined by the broth microdilution method, was 0.9220 mg/mL indicating weak to moderate antibacterial activity. The toxicity evaluation through *in vivo* brine shrimp assays demonstrated that the tested seaweed extracts had no significant toxicity observed at concentrations up to 1.0 mg/mL, suggesting the applicability of these extracts in crustacean aquaculture units. Nevertheless, further meticulous investigations are necessary to reveal the inhibitory effects of these seaweeds against a wider range of fish and shellfish pathogens.

ARTICLE INFO

Article timeline:
Date of Submission:
11 January, 2026
Date of Acceptance:
28 April, 2026
Article available online:
14 May, 2026

***Keywords*:**
Seaweeds
Bacteria
Toxicity
Antibacterial activity
IC_{50}

Introduction

Seaweeds, or marine macroalgae, are primitive non-flowering plants that lack true roots, stems, and leaves. Seaweeds are divided into three groups based on their color: Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae) (Lobban & Harrison, 1994). As key primary producers, seaweeds contribute significantly to the functioning of marine ecosystems. Extensive research has confirmed the nutritional potential of many seaweed species. Moreover, seaweeds have

---

*Corresponding author: <nadirakufmrt18@gmail.com>

DOI: https://doi.org/10.53808/KUS.2026.SI.7.01.MariNEX1504-ls

remarkable pharmacological effects, including antioxidant, anti-inflammatory, antibacterial, and even anticancer capabilities (El Gamal, 2010; Gupta & Abu-Ghannam, 2011a; Gupta & Abu-Ghannam, 2011b; Holdt & Kraan, 2011; Mohamed *et al*., 2012). Polysaccharides, different phytochemicals, carotenoids, minerals, peptides, and lipids are among the active ingredients (Gupta & Abu-Ghannam, 2011b; Holdt & Kraan, 2011; Balboa *et al*., 2013).

In recent years, seaweed-derived compounds have gained attention for their effectiveness in preventing and managing viral and bacterial infections in aquatic species (Cortés *et al*., 2014). Seaweed extracts have antibacterial action against aquatic pathogenic bacteria (Bansemir *et al*., 2006; Thanigaivel *et al*., 2014), and have been found effective against various food-borne pathogenic bacteria (Afrin *et al*., 2023; AftabUddin *et al*., 2021; Genovese *et al*., 2012). Defoirdt *et al*. (2006) reported that halogenated furanone extracted from the red alga *Delisea pulchra* exhibited antibacterial properties against shrimp pathogens, including *Vibrio campbellii*, *Vibrio harveyi*, and *Vibrio parahaemolyticus*. Another study reported antibacterial activity of *Padina australis* against *Aeromonas hydrophilla*, *V. harveyi*, and *V. parahaemolyticus* (Latifah *et al*., 2019). Several seaweed species, including *Caulerpa racemosa*, *Padina gymnospora*, *Sargassum wightii*, and *Ulva fasciata*, are effective against human pathogenic bacteria, including *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus mutans* (Vaithiyanathan *et al*. 2023).

However, various seaweeds such as *Laminaria* sp., *Acanthophora spicifera, Gracilaria edulis, Padina gymnospora, Ulva fasciata*, and *Enteromorpha flexuosa* have cytotoxic properties (Azis *et al*., 2023; Ganesan *et al*., 2020; Nursid *et al*., 2017). Jumaetri Sami *et al*. (2020), reported the LC_{50} value of methanol, ethyl acetate, and n-hexane extract of *Padina australis* against *Artemia salina L* were 785.03 ppm, 73.3 ppm, and 300 ppm, respectively.

St. Martin's Island is home to over 216 different kinds of seaweed (Aziz *et al*., 2023). Numerous seaweeds, including *Caulerpa* sp., *Hypnea* sp., *Padina* sp., *Sargassum* sp., etc., are found here (DoF, 2022; Ahmed & Taparhudee, 2005). Seaweeds have been shown to possess antioxidant and antibacterial properties by a large number of studies carried out worldwide over the last 20 years. However, despite the diversity of seaweeds along Bangladesh's coast, very few studies have evaluated their antimicrobial activity against pathogens relevant to aquaculture systems, and the effect of solvent extraction on their bioactivity remains largely unexplored (Uddin *et al*., 2020). Although pharmacological properties have been documented, there hasn't been a comprehensive investigation or reporting of seaweed extracts' toxicity (Ramu *et al*., 2020). Some studies on *Padina* sp., *Sargassum* sp., *Ulva* sp., etc have been carried out, and most of the seaweeds showed no toxicity (Klongklaew *et al*., 2020; Ramu *et al*., 2020; Ganesan *et al*., 2020; Banu & Umamageswari, 2011). Though research on *Hypnea spinella* (Díaz *et al*., 2011), and *Sargassum carpophyllum* (Tian *et al*., 2020) demonstrated their immunomodulatory qualities, antibacterial effects were not experimented.

The present study hypothesized that the seaweeds commonly available in the St. Martin's Island might have antibacterial properties against bacteria causing diseases in aquaculture production units; numerous pathogenic *Vibrio* species such as *V. anguillarum*, *V. alginolyticus*, and *V. harveyi*. (Genovese *et al*., 2012; Cavallo *et al*., 2013). Another bacterium, *Exiguobacterium* spp., was considered in this study; this bacterium has eight strains, including *E. qingdaonense, E. indicum*, and *E. arabatum*, and only a few cases, such as bacteremia and skin infections by *Exiguobacterium* spp., have been documented (Vishnivetskaya *et al*., 2009).

Given the lack of systematic evaluation of the antimicrobial activity of Bangladesh coastal seaweeds against aquaculture pathogens, a thorough assessment of their toxicity and antibacterial potential is crucial. To explore the potential of these seaweeds in aquatic animals' diseases and health management, the current study aimed to assess the antibacterial activity of these seaweeds against gram-negative *Vibrio alginolyticus* and gram-positive *Exiguobacterium qingdaonense* bacteria, and the toxicity on brine shrimp.

Materials and Methods

Sample collection and seaweed identification

Four seaweeds and seawater samples were collected from various points of St. Martin's Island, Bangladesh. Sampling was conducted in March 2023 during the dry season at four stations, and the GPS coordinates of the collection sites were recorded (Figure 1). Seaweeds were manually harvested from the intertidal zone and collected in 1 kg polythene bags for each species. The collected seaweeds were first washed with seawater, followed by tap water to remove sand and epiphytic organisms, and finally washed with distilled water to ensure that only fresh and healthy specimens were retained. The seaweed species were taxonomically identified by a scientific officer of Bangladesh National Herbarium, Dhaka, Bangladesh, where specimens are deposited. Sample details have been listed in Table 1. Finally, the samples were brought to the 'Fish and Shellfish Quality Control and Pathology Laboratory', Fisheries and Marine Resource Technology Discipline (FMRT) of Khulna University for further analysis.

Sample processing, crude extract preparation, and evaluation of yield (%)

After collection, some seaweed samples were kept in the bottles (washed with distilled water) containing seawater collected from the sampling sites, and then the bottles containing seawater and seaweeds were kept in ice boxes. After these bottles had been brought to the laboratory, the bottles containing seaweeds were frequently shaken to aid in the breakdown of seaweeds and dissolve the seaweeds' components in the seawater used as stock.

For seaweed extract preparation, seaweed samples were dried in the shade for a week at ambient temperature, and dried samples were made into powder using an electric grinder. Seaweed powder was weighed and packed in an airtight container and stored at room temperature before further use. Powdered seaweed samples were solubilized with 5 solvents, viz., hexane, acetone, ethanol, methanol, and water (From non-polar to polar). Crude extracts were prepared following AftabUddin et al. (2021) with some modifications; powdered seaweed samples (1g) were mixed with each solvent (1:10, w/v) using separate test tubes. Following hand mixing, samples were first sonicated for 15 minutes at 40°C to enhance solvent penetration. The tubes were then incubated for 18-20 hours at 35ºC and 135-140 rpm. A second sonication for 15 minutes at 40°C was performed to maximize the release of intracellular compounds. The mixtures were then centrifuged at 3000 rpm for 10 minutes, and the supernatants were collected in the tubes. Water extracts of seaweed were dried at 40°C, and other extracts were then dried at ambient temperature. Following drying, water extracts were cleaned with 70% ethanol and then dried once more at 40°C in an oven. Equation 1 was used to calculate the yield of each extract. In order to prevent contamination, crude extracts were lastly kept at 4°C in the dark, and water extract at -20°C. The following formula (Equation 1) was used to calculate the yield of each extract. Equation 1.

$$\text{Yield}\ (\%) = \frac{\text{Weight of extract \& tube} - \text{Empty weight of tube}}{\text{Weight of initial dried sample}} \times 100$$

(Anokwuru et al., 2011)

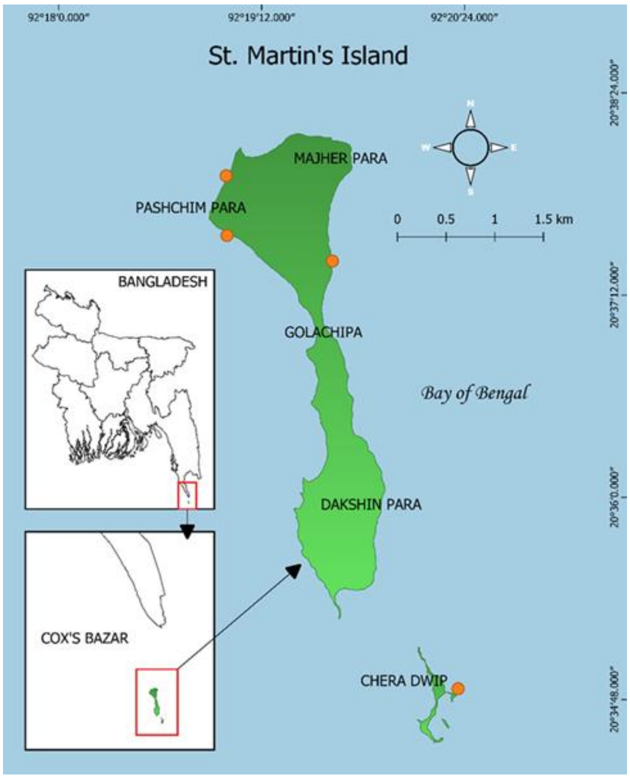

Figure 1: Location of the seaweed sampling area

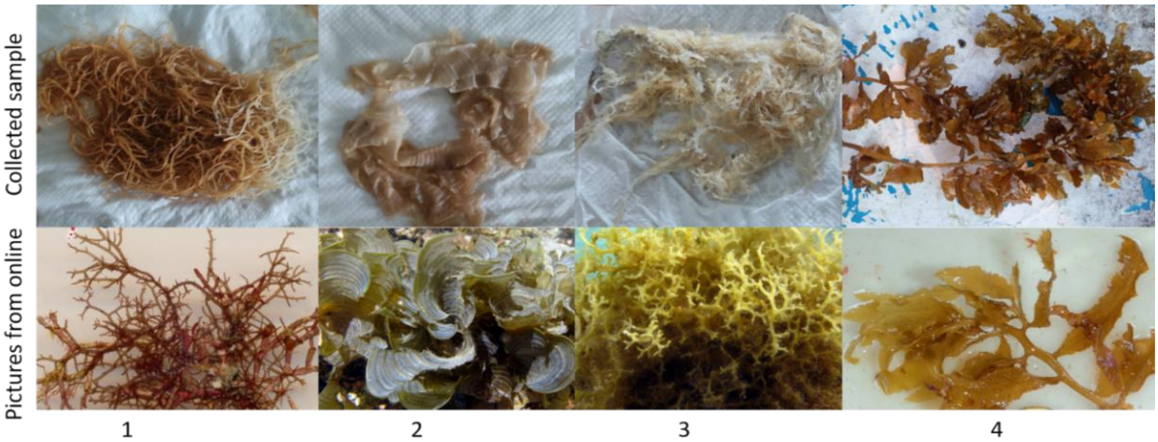

Figure 2: Pictures of samples. Label numbers are assigned in line with the label no. of seaweed species shown in Table 1.

Table 1: Details of seaweed samples

Group	Label no	Scientific name	Common name	Family	Accession no
Red algae	1	*Hypnea spinella* (C. Agardh) Kützing	No widely accepted common name	Cystocloniaceae	DACB 99114
Brown algae	2	*Padina australis* Hauck	Peacock's tail or Fan-leaf seaweed	Dictyotaceae	DACB 99112
	3	*Chnoospora implexa* J. Agardh	No widely accepted common name	Scytosiphonaceae	DACB 99113
	4	*Sargassum carpophyllum* J. Agardh	Gulfweed or Sea holly	Sargassaceae	DACB 94867

Comparison of Total Viable Bacteria Count (TBC) between seawater and seawater containing seaweeds

The seawater and seawater extract of seaweed samples were serially diluted using alkaline peptone water as a diluent in a ratio of 1:9 up to 10^{-9}. To make sure the dilutions were uniform, vortex mixing was done at each stage of the process. To determine total bacterial count (TBC), nutrient agar medium culture plates were used; each sample dilution was inoculated into the plate and evenly distributed using a glass rod. Each time, the glass rod was burned to prevent contamination. All dilutions were plated in duplicate (n = 2) to ensure accuracy and reproducibility of the results. The nutrient media plates with inoculum were incubated at 37±1°C for 24 hours. The plates with 30-300 colonies were selected as standard plates, and the number of colonies on the standard plates was counted. Finally, bacterial load was calculated using Equation 2.

Equation 2.

$$\text{Bacterial load (CFU/mL)} = \frac{\text{No. of colonies} \times \text{Total dilution factor}}{\text{Volume of culture plated in mL}}$$

(Ferdous *et al*., 2023)

Bacterial strain collection

To determine antibacterial activity, the crude extracts of seaweeds were evaluated using two different bacteria. One was gram-negative *Vibrio alginolyticus* NBRC 15630 (GenBank accession no PQ553460; Hasanuzzaman *et al*., 2025), which was collected from the 'Fish and Shellfish Quality Control and Pathology Laboratory', FMRT Discipline of Khulna University. According to Hasanuzzaman *et al.* (2025), this strain was isolated from Mud crab (*Scylla olivacea*); for the present investigation, this bacterial strain was sub-cultured. Another was gram-positive bacteria, which was isolated from the wild harvest Mud crabs. The isolated colony was pure cultured and molecularly identified as *Exiguobacterium qingdaonense* S82 according to the procedures outlined by Hasanuzzaman *et al.* (2025). In brief, genomic DNA from the representative bacterial isolate was extracted using a DNA isolation kit (Model: AS1010, Origin: Promega, USA) following the manufacturer's protocol. Universal 16S rRNA primers were used for amplification of PCR products. PCR amplification was performed in a 25 µl reaction mixture containing 12.5 µl of Master Mix (Promega, USA), 1 µl of T DNA (25–65 ng/µl), 1 µl of Primer F (10-20 pMol), 1 µl of Primer R (10–20 pMol), and 9.5 µl of Nuclease Free Water. The resulting PCR products were purified using the PCR CLEAN UP SYSTEM (Promega, USA) and WIZARD® SV GEL according to the manufacturer's guidelines. Purified amplicons were then sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA). To determine the identity of the sequences, BLAST analysis was carried out using the NCBI nucleotide database "16S rRNA sequences (Bacteria and Archaea)" with the search settings configured for "Highly similar sequences (MegaBLAST)."

Antibacterial sensitivity test (AST)

Dried crude extracts were dissolved in 250 mg/mL DMSO for impregnating the paper discs (6 mm diameter). Water extracts were dissolved in sterilized distilled water in same concentration. After dissolving, the extracts were properly mixed using a vortex machine. The antibiotic sensitivity of 2 bacterial isolates against 20 extracts (4 seaweeds and 5 solvents for each) and 6 commercial antibiotics (Ampicillin 10 µg, Chloramphenicol 30 µg, Ciprofloxacin 5 µg, Tetracycline 30 µg, Erythromycin 15 µg, Penicillin G 10 µg) was carried out by the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966). The representative isolates from the pure culture plates were inoculated in Mueller Hinton Broth (MHB; Himedia, India) and incubated for 24 h. The suspension's turbidity was adjusted to match a 0.5 McFarland standard, after which a sterile swab was immersed in the suspension and inoculated across the surface of the Mueller Hinton Agar (MHA) plate. A total of 20 seaweed extract discs (20 µl from stock), 6 commercial antibiotics discs, 1 blank disc (sterile paper only), and 1 solvent control disc (20 µl DMSO) were placed on the swabbed MHA plates. So, the final dose became 5 mg. Although 5 mg/disc is higher than the concentrations typically used for purified antibiotics, such doses are commonly employed for crude extracts, consistent with previous marine macroalgae studies (O'Keeffe *et al.*, 2019). Each of the plates contained 4 discs (3 test discs and 1 blank disc). Finally, plates were incubated upside down and incubated at 37°C overnight. All assays were performed in duplicate. After incubation, the zone of inhibition for each antibiotic was measured to assess the susceptibility (CLSI, 2013) of the representative bacterial isolates. The minimum detectable zone is considered the disc diameter, but the focus is on inhibition beyond the disc. If there was no zone at all, we marked the zone diameter as 'No inhibition'---even though the disc itself is around 6 mm. The result for commercial antibacterial agents was reported as sensitive, resistant, or intermediate compared with the zone diameter interpretative standard (CLSI 2020; Antimicrobial Disk Diffusion Zone Interpretation Guide).

Antibacterial activity of the most susceptible extract with different doses

A dose-response study was conducted; the most suitable extract with specific doses against the most susceptible strain was evaluated following the Kirby-Bauer disc diffusion method, and a dose-response effect was determined (Ericsson & Sherris, 1971; Murray *et al*, 2003). Dried crude extract was dissolved in 200 mg/mL DMSO for impregnating the paper discs. For 1-7 mg, 6 mm paper discs were used, and 12-14 mm for 8-10 mg doses. Larger discs were necessary for higher doses to accommodate the increased volume of solvent required for dissolving the extract. For this procedure, MHA media, MHB media, and bacterial suspension (0.5 McFarland standard) were prepared. Then, sterile blank paper discs were placed on the surface of the swabbed MHA plates. From the stock, 5-50 µl with 5 intervals were impregnated into discs. Thus, 1–10 mg were the final concentrations. For control, other

Half-maximal inhibitory concentration (IC_{50})

Ethanol extract of *P. australis* (2E) was the most effective extract against *E. qingdaonense,* which was used for evaluating IC_{50} using the broth microdilution method as recommended by CLSI with some modifications (CLSI, 2012; Klomjit *et al*., 2021; O'Keeffe *et al*., 2019).

Bacterial culture and OD (Optical Density) measurement

About 4-5 bacterial colonies were inoculated in 3 mL of nutrient broth media (1.5% NaCl) and then kept in an incubator for 18-20 hours at 135-140 rpm, 37ºC. After incubation, OD was taken through spectrophotometer at 620 nm. Then, it was diluted to 0.003 according to Equation 3. The resulting bacterial solution was added to 10 mL of broth media for further processing.

Equation 3. $\text{Bacterial solution} = \frac{0.003 \times 10}{\text{OD value}} \times 10$

Serial dilution process

From the stock seaweed extract (200 mg/mL), 50 µl was transferred to a sterile 96-well microtiter plate. Twofold serial dilutions were then prepared up to a 128-fold dilution using DMSO as the diluent. The dilutions (2.5-0.078125 mg/mL) were used as test samples.

Inoculation, incubation, and inhibition measurement

Firstly, each test sample (10 µl) was placed into wells of a 96-well plate that contained 190 µl of the diluted bacterial suspension, which served as the negative control. Secondly, extract control wells containing 190 µl of nutrient broth and 10 µl of each test sample were included to account for any absorbance contributed by the extract itself. There were also some wells containing 190 µl diluted bacterial solution and 10 µl DMSO to observe whether there was any inhibition from DMSO. Growth controls (no extracts but just 190 µl diluted bacterial solutions) were expected to show the growth of bacteria without the compound. The sterile controls (only 190 µl nutrient broth medium) were expected to show no bacterial growth after incubation. The microdilution plates were incubated in a shaker incubator at 37ºC and 135–140 rpm for 18–24 hours. Following incubation, turbidity in each well was recorded at 620 nm using a Microplate Reader. All assays were performed in duplicate. The percentage of relative inhibition of each test sample was calculated using Equation 4.

Equation 4.

$$\text{Inhibition (\%)} = 100 - \frac{(\text{Absorbance of negative controls}) - (\text{Absorbance of extract controls})}{\text{Average absorbance of bacterial solution and DMSO}} \times 100$$

Toxicity testing

Using brine shrimp (*Artemia* sp.), a microscopic crustacean, as the test organism, all seaweed extracts were evaluated for toxicity. For determining the *in vivo* toxicity of plant-derived complexes or isolated chemicals, the brine shrimp assay offers a practical and quick screening method. Because of its availability, ease of production, low cost, and capacity to adapt to harsh environments, it is a marine zooplanktonic organism that is considered the gold standard in toxicological assays (Danabas *et al*., 2020). The toxicity test was performed according to Borja *et al.* (2016).

Brine shrimp hatching

A beaker containing synthetic seawater (3.5% NaCl) was filled with 1g of brine shrimp eggs. In order for the container to hatch into nauplii, it was also maintained at 25–28°C with continuous light and aeration for 24 hours. The nauplii were drawn to a light source once they hatched in order to differentiate them from the unhatched eggs.

Preparation of the test Solution

The test extracts were produced in a stock solution (100 mg/mL) using the proper solvent (DMSO). For every concentration and extract, there were two duplicates. There were two doses used: 0.5 and 1 mg/mL.

Brine Shrimp Lethality Test (BSLT)

A micropipette was used to add 10 brine shrimp nauplii and 200 µl of saline water to each well. Then, each well received 1 µl or 2 µl of each extract (0.5 or 1 mg/mL, respectively). Two negative controls were used in this experiment. Control 1 solely included nauplii, while control 2 included nauplii with either 1 or 2 µl of DMSO. A 96-well plate was maintained for 24 hours at room temperature (25–28°C) with continuous light. Covering the plate prevented evaporation and contamination. Following the incubation period, the numbers of live and dead nauplii in each well were observed with a compound microscope and recorded. Finally, the percentage of death was computed for every concentration of the test solutions using the following formula (Equation 5):

Equation 5. $\text{Mortality (\%)} = \frac{\text{Dead nauplii}}{\text{Given no of nauplii}} \times 100$ (Ahmmed *et al*., 2025)

Biosafety compliance

Sample preparation, bacterial strain collection and culture, toxicity testing and handling of artemia samples were carried out in accordance with the code of practice (CoP) to care and use of animals for scientific purposes at Life Science School, Khulna University, Bangladesh under the ethical approval (Ref No: KUAEC-2023-04-08; Date: 30-04.2023) by Animal Ethics Committee of Khulna University, Bangladesh.

Statistical analysis

Using Microsoft Word Professional Plus 2016, Excel Professional Plus 2016, PowerPoint Professional Plus 2016, IBM SPSS Statistics 26, QGIS 3.44, and GraphPad Prism 5, the collected data were compiled, analyzed, and presented. According to CLSI, the zone diameter interpretative standard was used to evaluate and report the antibiotic susceptibility test results as either sensitive, resistant, or intermediate. To assess the significant difference between the extraction yield (%) based on solvents and extracts, one-way ANOVA was conducted. To show the dose-response effect of the most effective extract, a best-fit line was drawn. Inhibition (%) was mathematically analyzed to establish IC_{50}. The toxicity of seaweeds was interpreted on the basis of a literature review.

Results

Comparison of Total Viable Bacteria count (TBC) between seawater and seawater extract of seaweeds

In the seawater samples, the TBC ranged from 1.23×10^8 to 1.25×10^8 CFU/mL, with an average of $1.24\pm0.01\times10^8$ CFU/mL. The seawater containing decomposed seaweeds sample had TBC ranging from 1.09×10^8 to 1.12×10^8 CFU/mL, with an average of $1.105\pm0.02\times10^8$ CFU/mL (Fig. 3). An independent samples t-test was performed to compare the mean TBC values between the two groups, which demonstrated a significant difference ($p < 0.05$).

Extraction yields

The extraction yields (%) for each solvent and seaweed extract are shown in Fig. 4; the yield (%) of different seaweed species varied significantly ($P<0.05$), and *P. australis* exhibited the best yields (%) for all solvents. However, the yield of each solvent did not differ significantly ($P> 0.05$). But, apparently, water solvent yielded the highest amount of extracts (Fig. 5).

Molecular identification of bacterial isolates

Table 2 shows the putative identification of the sequences blasted against the nucleotide sequence of the bacteria from the NCBI database. The phylogeny analysis resulted in the tree (Fig. 6) showing taxonomic profile of the studied 2 isolates with other sequences homologous to their 16S rRNA gene sequences.

Antibiotic sensitivity test

V. alginolyticus and *E. qingdaonense* were the two bacterial strains employed in this study. Not a single extract exhibited an inhibitory zone against *V. alginolyticus,* while it was sensitive to ciprofloxacin and tetracycline (Table 3 and Table 4). In the case of the gram-positive bacterium (*E. qingdaonense*), except for water extracts, the remaining samples displayed an inhibitory zone against *E. qingdaonense*. The extracts of both *S. carpophyllum* and *P. australis* showed superior outcomes compared to the other two seaweeds. *E. qingdaonense* exhibited sensitivity to all of the antibiotics used in this study.

Antibacterial activity of the most susceptible extract with different doses (mg/disc)

P. australis ethanol extract was the most effective extract, showing high antibacterial activity (11 mm inhibition zone) against *E. qingdaonense* at 5 mg/disc. This extract underwent a concentration analysis, with the results demonstrating a dosage response effect, as indicated in Fig. 7 (a) and Table 5. As seen in Fig. 7 (b), DMSO did not exhibit any antibacterial properties. It is evident from the inhibitory zone that bacterial susceptibility rose with increasing dosage. Regression analysis with 95% confidence intervals was used to get the best-fit line. The zone of inhibition and extract concentrations had a strong, positive linear relationship, as seen in Fig. 8.

Determination of IC_{50}

P. australis ethanol extract (labeled as 2E) was utilized to assess IC_{50} since it showed the most potent antibacterial activity against *E. qingdaonense*. In a dose-response manner, the inhibitory impact began at 0.15625 mg/mL, and higher concentrations of the extract resulted in higher death rates (Table 6). Additionally, the extract's IC_{50} value was 0.9220 mg/mL, calculated using GraphPad Prism 5 by nonlinear regression analysis of the dose–response curve (Fig. 9). While sterile controls verified that the broth media was uncontaminated, the OD value of growth controls guaranteed that bacterial growth was good. The absorbance of wells with bacterial solution and DMSO demonstrated that DMSO did not affect the inhibition of bacterial growth.

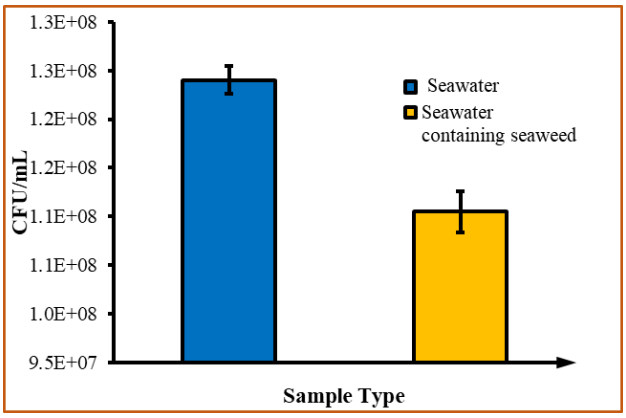

Figure 3: Comparison of TBC between seawater and seawater containing seaweeds

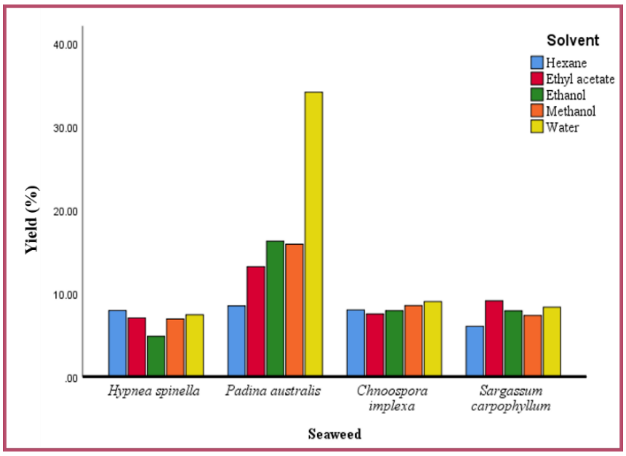

Figure 4: Extraction yields (%) for each extract

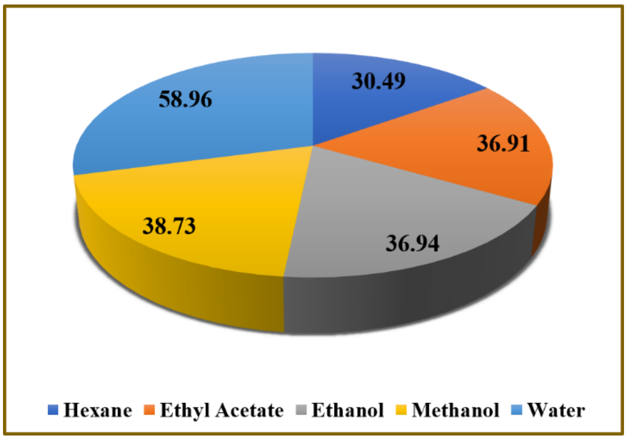

Figure 5: Total extraction yield (%) of solvents

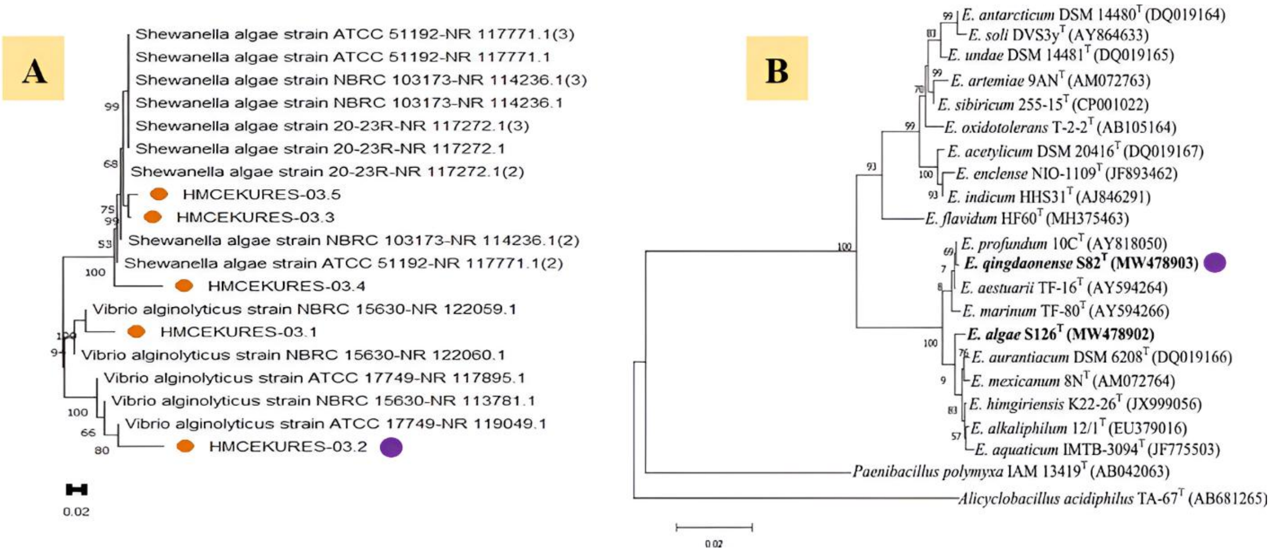

Figure 6: Phylogenetic tree. (A) *V. alginolyticus* HMCEKURES-03.2 strain collected (Hasanuzzaman et al., 2025); (B) *E. qingdaonense* isolated (Liu et al., 2021). Taxa labeled with colored (purple) circles indicate the bacterial strains in the present study.

Table 2: Identification of selected bacterial isolates by nucleotide BLAST search against the NCBI database

Isolate code	Length of sequences (bp)	Identified putative species	Most relevant strain name (Accession no.)	Max score	Query cover (%)	E value	Identity match (%)	GeneBank accession no.
HMCEKURES-03.2	1260	*Vibrio alginolyticus*	NBRC 15630 (NR 113781.1)	1803	88	0	95.96	PQ553460
Gram+ bacterium	1411	*Exiguobacterium qingdaonense*	S82 (NR 181609.1)	854	78	0	98.33	Not submitted

Table 3: Antibacterial activity of crude extracts against *E. qingdaonense* at 5mg/disc

Solvent	Inhibition zone (mm) for seaweed extracts			
	Hypnea spinella	*Padina australis*	*Chnoospora implexa*	*Sargassum carpophyllum*
Hexane	8	10	8	10
Ethyl Acetate	9	9	8	9
Ethanol	8	11	8	9
Methanol	8	9	8	10
Water	No inhibition	No inhibition	No inhibition	No inhibition

Table 4: Antibacterial activity of commercial antibiotics against both *E. qingdaonense* and *V. alginolyticus*

Antibacterial agent	Inhibition zone (mm) with susceptibility criteria	
	V. alginolyticus	*E. qingdaonense*
Ampicillin	No inhibition (R)	35 (S)
Chloramphenicol	13 (I)	23 (S)
Ciprofloxacin	41 (S)	25 (S)
Tetracycline	15 (S)	23 (S)
Erythromycin	No inhibition (R)	23 (S)
Penicillin G	No inhibition (R)	34 (S)

(S = Sensitive, I = Intermediate, R = Resistant)

Table 5: Concentration study on the antibacterial activity of ethanol extract of *P. australis* at 1-10mg/disc against *E. qingdaonense*

Extract	Inhibition zone (mm)									
	1 mg	2 mg	3 mg	4 mg	5 mg	6 mg	7 mg	8 mg	9 mg	10 mg
P. australis	0	0	7	9	11	12	14	17	19	20
DMSO	0	0	0	0	0	0	0	0	0	0

Toxicity testing

Four seaweeds in two different concentrations were introduced into the brine shrimp nauplii rearing units. Within 24 hours of exposure, brine shrimp mortality rates were calculated and documented (Table 7). The majority of the extracts exhibited absolutely no mortality, and with the exception of ethyl acetate and ethanol extracts of *Sargassum carpophyllum*, other extracts of seaweeds caused an average mortality of about 20% at both doses (0.5 and 1 mg/mL). Considering the LC_{50} value for a particular substance reported as very toxic for < 30 ppm, toxic for 30-1000 ppm, and not toxic for > 1000 ppm i.e. the extract's LC_{50} value is less than 1000 ppm is considered toxic (Meyer *et al.*, 1982; Jumaetri Sami *et al.*, 2020), all extracts at both doses had an average mortality rate of less than 50%. Thus, it might be safe to employ these seaweeds and/or seaweed extracts in aquaculture production units.

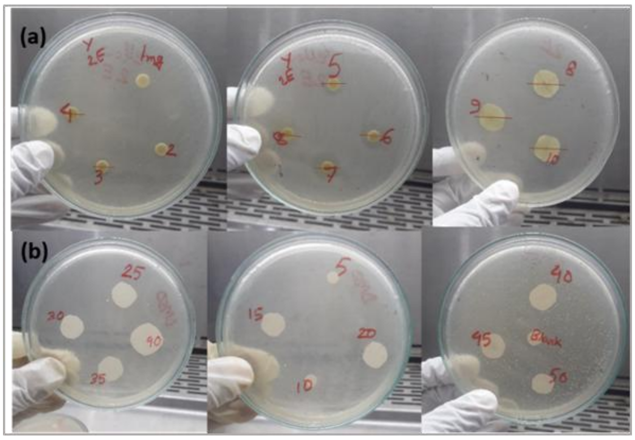

Figure 7: Dose response effect of crude ethanol extracts of *P. australis* at (a) 1-10 mg/disc against *E. qingdaonense* (b) Negative controls

Table 6: Determination of IC_{50} of ethanol extract of *P. australis* (2E)

Plate	Concentration (mg/mL)	Inhibition (%)	Inhibition (%) of replicate
2Ea	2.5	63	59
2Eb	1.25	58	56
2Ec	0.625	55	49
2Ed	0.3125	36	42
2Ee	0.15625	2	1
2Ef	0.078125	0	0

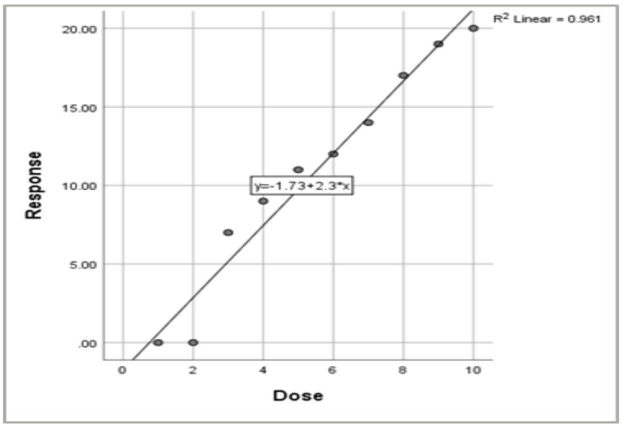

Figure 8: Best fit line for dose response effect

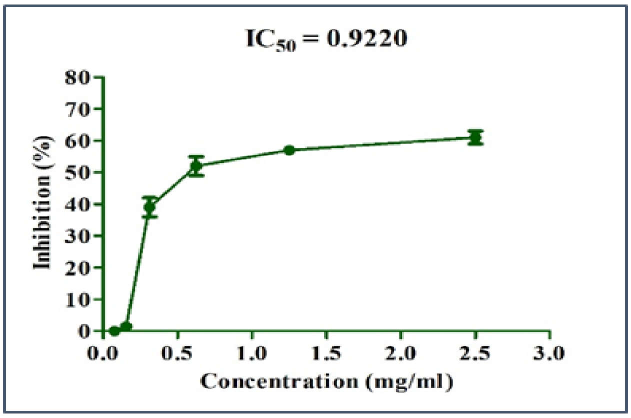

Figure 9: IC_{50} value

Discussion

Effective preventative and therapeutic methods are necessary to control infectious illnesses that impact aquatic animals farmed for food (Burridge *et al.*, 2010). Seaweed extracts have the potential to combat a variety of gram-positive and gram-negative bacteria pathogenic to fish as well as humans (Afrin *et al.*, 2023; Anusha & Bramhachari, 2023; Thanigaivel *et al.*, 2016). The antibacterial compounds found in seaweed extracts typically interact with the causing microorganisms' cells and cell membranes, interfering with their ability to absorb and transport vital intracellular chemicals (Mohamed *et al.*, 2012; Hierholtzer *et al.*, 2013).

In the present investigation, the significantly lower ($p<0.05$) total viable bacteria count (TBC) in the seawater with seaweeds sample compared to that of the only seawater sample might be attributed to the presence of various bioactive compounds in the seaweeds, being in accordance with Lee *et al.* (2010) and Kim *et al.* (2011) implicating seaweeds extracts' antibacterial activity in relation to reduced microbial counts in food products containing seaweed extracts. The total bacteria count in the presence of *Gracilaria textorii* was 7.5×10^6 cells/mL (Pang *et al*., 2006). The introduction of raw brown Irish edible seaweeds (*Himanthalia elongata*, *Laminaria saccharina*, and *Laminaria digitata*) into seawater resulted in the complete elimination of aerobic mesophiles and halophiles (Gupta *et al*., 2010).

While the polarity of the solvent and the degree of polarity of the different extract constituents seemed to have an impact on the seaweed extraction yield (Klomjit *et al.*, 2021), the present study recorded varied yields of seawater extracts using different solvents, and the solvent water yielded the largest quantity, and hexane resulted in the lowest quantity of seaweed extracts. Studies on natural product extraction report that extraction yield is influenced by both the solvent used and the composition of the material being extracted (e.g., species), and that solvent effects may not always be statistically significant when compared across a given material (Cardoso *et al.*, 2025).

In aquaculture, the disease vibriosis commonly occurs due to infection with *Vibrio* bacteria, including *V. harveyi*, *V. anguillarum*, *V. ordalii*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. salmonicida* (Cavallo *et al.*, 2013). Skin sores, respiratory disorders, muscular necrosis, eye damage or blindness, and tail rot disease are all caused by *V. alginolyticus* (Snoussi *et al.*, 2009). Additionally, these bacteria may grow in the cultivated fish's digestive systems, which could be extremely dangerous for human health (Normanno *et al.* 2006). The current investigation has globally demonstrated that the gram-negative pathogenic bacterium *V. alginolyticus* NBRC 15630 was not susceptible to any of the extracts, as well as to commercial antibiotics except Ciprofloxacin and Tetracycline (Table 4). Similarly, Thirunavukkarasu *et al.* (2013) discovered that diethyl ether extract of seaweeds, including *Ulva facita*, *Ulva lacta*, *Acanthophora spicifera*, had no antibacterial action against *V. alginolyticus*. On the contrary, the methanol extract of these seaweeds showed inhibitory zones. The solvent used in the extraction process could be the reason for the variation in antibacterial activity (Rosaline *et al*., 2012; Mishra *et al*., 2016); Tüney *et al.* (2006) also found antimicrobial activity of 11 seaweed species extracts prepared with ethanol, diethyl ether, methanol, and acetone against some human pathogenic bacteria, and Cox *et al.* (2010) proved that methanol was the best solvent for removing antimicrobials from Phaeophyceae, but acetone was effective for Chlorophyceae. Furthermore, the polysaccharide λ-carrageenan, which was extracted from red seaweeds, showed strong dose-dependent bactericidal activity against *Photobacterium damselae*, but no discernible effect on other gram-negative bacteria, such as *V. harveyi*, *V. anguillarum*, and *Tenacibaculum maritimum* (Campos-Sánchez *et al.*, 2024). This is because different seaweed species produce distinct bioactive compounds with diverse antibacterial activities. Moreover, antimicrobial action may be affected by other factors, such as the season and environment of algal collection, the different stages of macroalgae growth, experimental protocols, etc.

It is also important to note that *V. alginolyticus*, as a Gram-negative bacterium, possesses a distinctive cell envelope structure consisting of an inner membrane, a thin peptidoglycan layer, and an outer membrane enriched with lipopolysaccharides (LPS). This outer membrane acts as a selective permeability barrier that limits the penetration of many antimicrobial agents. The restricted diffusion through porin channels, together with active efflux systems, further reduces intracellular accumulation of antibiotics. In contrast, Gram-positive bacteria lack an outer membrane, which generally renders them more susceptible to externally applied antimicrobial compounds (Miller, 2016; Exner et al., 2017; Gupta & Datta, 2019). Hernández-Robles et al. (2016) reported that *V. alginolyticus* strains exhibited β-haemolytic and proteolytic activities, produced capsules, and in most cases synthesized siderophores. These virulence-associated traits enhance host colonization, iron acquisition, immune evasion, and biofilm formation, thereby promoting bacterial survival during infection. Although virulence factor–encoding genes are not direct antimicrobial resistance determinants, their presence may indirectly contribute to bacterial persistence and reduced susceptibility under antibiotic pressure.

Our study found that *E. qingdaonense* S82, a gram-positive bacterium, showed susceptibility to all extracts and selected commercial antibiotics, with the exception of aqueous extracts. Of them, the *Padina australis* ethanol extract displayed the largest inhibitory zone (11 mm) (Table 3), and it was selected for the study of dose-response effect. The maximal zone of inhibition was 20 mm at the maximum dosage of 10 mg/disc (Table 5). The zone of inhibition and extract concentrations showed a strong positive linear relationship, as shown in Fig. 8. Conversely, during the concentration trial, *Padina lanosa* demonstrated an 18.3 mm zone of inhibition against *Xanthomonas fragariae* at 10 mg/disc (O'Keeffe *et al.*, 2019). *Padina tetrastromatica*, *Sargassum muticum*, *Sargassum swartzii*, *Hypnea musciformis*, and other marine algae demonstrated inhibitory action against human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* (Afrin *et al.*, 2023; Hasan *et al.*, 2021; Sujatha *et al.*, 2019). Honey et al. (2024) investigated three well-recognized seaweed species (*Caulerpa racemosa*, *Padina tetrastromatica*, and *Hypnea musciformis*) collected from St. Martin's Island, Bangladesh, and reported limited antibacterial activity against five human pathogenic bacteria. These studies have supported our study; the extracts of the selected seaweeds in our study, under the genus *Padina*, *Sargassum*, and *Hypnea*, were found to have an inhibitory effect on *E. qingdaonense*.

According to the present study, the ethanol extract from *Padina australis* was tested for bacteriostatic characteristics by measuring its IC_{50} against *E. qingdaonense* (Hidayati *et al.*, 2022). Bioactive substances such as tannins, steroids, and flavonoid compounds are found in *Padina* sp. One particularly interesting source of phenolic chemicals is *P. australis* (Far *et al.*, 2023). In this study, the inhibitory action of *P. australis* started at about 0.15625 mg/mL in a dose-response manner, as shown in Table 6, and the antibacterial efficacy increases with extract concentration. This extract's estimated IC_{50} value was around 0.9220 mg/mL. IC_{50} values from various extracts have been reported to be varied. For example, *Dictyota humifusa* and *Ulva fasciata* had respective values of 4.75 and 4.82 mg/mL against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (Stirk *et al.*, 2007). When *Sargassum filipendula* was tested against human pathogenic bacteria such as *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*, the IC_{50} ranged from 89.8 to 144.0 µg/mL (Mofeed *et al.*, 2022). Methanol extract of *P. australis* demonstrated strong antibacterial properties against medically significant human pathogens, including *Pseudomonas aeruginosa* (MIC = 125 µg/mL), Methicillin-resistant *Staphylococcus aureus* (MIC = 250 µg/mL), *Klebsiella pneumoniae* (MIC = 125 µg/mL), and *Staphylococcus aureus* (MIC = 250 µg/mL) (Arguelles & Sapin, 2022).

Several physiologically active metabolites are found in seaweeds. They are particularly rich in functional metabolites such as proteins, lipids, peptides, polysaccharides, amino acids, polyphenols, vitamins, and minerals (Xu *et al.*, 2017). Many studies have already looked at the toxicity of different seaweeds to ensure the safety of their bioactive compounds for aquatic and human species. *In vivo* toxicological evolution was done on *Chaetomorpha aerea*, *Agardhiella sublata*, *Hypnea cornuta* at the concentration of 50–400 µg/mL, *Sargassum wightii* at 100-2000 mg/kg, *Padina gymnospora*, *Padina tetrastomatica* at 2000 mg/kg. The seaweeds did not appear to be toxic (Zammuto *et al.*, 2022; Ramu *et al.*, 2020; Ganesan *et al.*, 2020; Banu & Umamageswari, 2011). But *Laminaria* sp. displayed its LC_{50} at 114.05 ppm (Azis *et al.*, 2023). According to our experiment (Table 7), the majority of the extracts exhibited absolutely no mortality. As per the toxicity standards, a substance is considered non-toxic if 1000 parts per million of it cannot kill half of the population (Meyer *et al.*, 1982; Jumaetri Sami *et al.*, 2020). In the present investigation, no extract killed 50% of the *Artemia* at any dose. Therefore, the seaweeds under experimentation are not toxic.

Despite the promising results, certain limitations should be acknowledged. The study utilized crude seaweed extracts, therefore, the specific bioactive compounds responsible for the observed antibacterial activity were not identified. In addition, antibacterial assays were conducted against a limited number of bacterial strains, including a single strain of *V. alginolyticus* and *E. qingdaonense*, which may not fully represent strain-level variability in antimicrobial susceptibility. Furthermore, the experiments were performed under *in vitro* conditions, and the toxicity assessment was limited to the Artemia bioassay.

Table 7: Toxicity of each extract against brine shrimp (*Artemia*)

	Average Mortality (%)							
Dose	**0.5 mg/mL**				**1 mg/mL**			
Seaweed / Solvent	*Hypnea spinella*	*Padina australis*	*Chnoospora implexa*	*Sargassum carpophyllum*	*Hypnea spinella*	*Padina australis*	*Chnoospora implexa*	*Sargassum carpophyllum*
Hexane	13.39	0.00	8.12	0.00	0.00	0.00	8.01	0.00
Ethyl Acetate	0.00	6.51	7.69	36.04	0.00	16.52	0.00	21.59
Ethanol	6.97	0.00	6.80	7.69	0.00	0.00	0.00	38.69
Methanol	5.21	11.67	8.39	20.00	0.00	0.00	12.18	19.05
Water	0.00	9.72	8.50	17.14	0.00	0.00	0.00	0.00
Control 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Conclusions

This study reveals that seaweeds collected from St. Martin's Island, Bangladesh, exhibit promising antibacterial properties, particularly against the human pathogenic bacterium *Exiguobacterium qingdaonense* S82. Among the examined species, the ethanol extract of *Padina australis* showed the strongest inhibitory activity, with a clear dose–dependent relationship and a low IC_{50} value (0.9220 mg/mL). However, the observed inhibition zones were moderate, indicating preliminary antibacterial activity rather than strong pharmaceutical potency. Although none of the seaweed extracts were active against the aquaculture pathogen *Vibrio alginolyticus* NBRC 15630, their selective activity against *E. qingdaonense* suggests the presence of specific bioactive compounds. Therefore, direct aquaculture disease-control implications cannot be established from this study. The brine shrimp lethality assay indicated no detectable toxicity up to 1.0 mg/mL, supporting the preliminary safety profile of the tested extracts. Overall, the findings suggest that these seaweeds may serve as potential sources of bioactive compounds, but further validation is required before considering pharmaceutical or aquaculture applications. Future investigations should focus on the isolation and characterization of the active compounds, determination of minimum inhibitory concentration and minimum bactericidal concentration values, and *in vivo* validation through fish or shrimp challenge trials to evaluate efficacy and safety under practical aquaculture conditions.

Acknowledgement

The authors express their gratitude to the Bangladesh Ministry of Science and Technology for the National Science and Technology (NST) fellowship.

Conflict of interest

The authors declare no conflict of interest.

References

Afrin, F., Ahsan, T., Mondal, M. N., Rasul, M. G., Afrin, M., Silva, A. A., Yuan, C., & Shah, A. K. M. A. (2023). Evaluation of antioxidant and antibacterial activities of some selected seaweeds from Saint Martin's Island of Bangladesh. *Food Chemistry Advances*, *3*, 100393. https://doi.org/10.1016/j.focha.2023.100393

AftabUddin, S., Siddique, M. A. M., Habib, A., Akter, S., Hossen, S., Tanchangya, P., & Abdullah Al, M. (2021). Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus*. *Italian Journal of Animal Science*, *20*(1), 243-255. https://doi.org/10.1080/1828051X.2021.1878943

Ahmed, N., & Taparhudee, W. (2005). Seaweed cultivation in Bangladesh: Problems and potentials. *Kasetsart University Fisheries Research Bulletin*, *28*(1), 13-21.

Ahmmed, S. S., Sadia, H. T., Nasrin, F., Adhikary, U., Sarower, M. G., & Ghosh, A. K. (2025). Enhancing growth and immune responses in giant freshwater prawn (*Macrobrachium rosenbergii*) via oral administration of fennel

(*Foeniculum vulgare*) extract against *Vibrio parahaemolyticus*. *Comparative Immunology Reports, 9*, 200247. https://doi.org/10.1016/j.cirep.2025.200247

Anokwuru, C. P., Anyasor, G. N., Ajibaye, O., Fakoya, O., & Okebugwu, P. (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. *Nature and Science*, *9*(7), 53-61.

Anusha, K., & Bramhachari, P. V. (2023). Antimicrobial activities of marine macroalgal lipidic extracts against fish pathogenic *Vibrio* species of Kakinada coastal region. *Environment and Ecology, 41*(1), 73-80.

Arguelles, E. D. L. R., & Sapin, A. B. (2022). Bioactive properties and therapeutic potential of *Padina australis* Hauck (Dictyotaceae, Ochrophyta). *International Journal of Agricultural Technology*, *18*(1), 13-34.

Azis, M., Soekamto, N. H., & Firdaus, F. (2023). Identification of components and toxicity assessment of seaweed methanol extract *Laminaria* sp against larva *Artemia salina*. *AIP Conference Proceedings, 2673*(1), 040003. https://doi.org/10.1063/5.0127046

Aziz, A., Kabir, S., & Alfasane, M. A. (2023). Seaweed flora of the St. Martin's Reef, Bangladesh. *Bangladesh Journal of Plant Taxonomy*, *30*(1), 153-163. http://dx.doi.org/10.3329/bjpt.v30i1.67052

Balboa, E. M., Conde, E., Moure, A., Falqué, E., & Domínguez, H. (2013). *In Vitro* antioxidant properties of crude extracts and compounds from brown algae. *Food Chemistry, 138*(2-3), 1764-1785. https://doi.org/10.1016/j.foodchem.2012.11.026.

Bansemir, A., Blume, M., Schröder, S., & Lindequist, U. (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture, 252*(1), 79-84. https://doi.org/10.1016/j.aquaculture.2005.11.051

Banu, A. T., & Umamageswari, S. (2011). Toxicity study of seaweeds in rat. *Journal of Food Science and Technology, 5*(2), 23-31.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Borja, K. B., Buhion, V. L., & Canalita, E. E. (2016). Toxicity test on different brands of food seasonings using brine shrimp (*Artemia salina*) lethality test. *Biological and Chemical Research*, *3*, 227-33.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in Salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture*, *306*(1-4), 7-23. https://doi.org/10.1016/j.aquaculture.2010.05.020

Campos-Sánchez, J.C., Guardiola, F.A., & Esteban, M.Á. (2024). *In vitro* effects of a natural marine algae polysaccharide (λ-carrageenan) on seabream erythrocytes, tumour cell lines and marine bacterial pathogens. *Journal of Applied Phycology,* 36, 399–409. https://doi.org/10.1007/s10811-023-03133-6

Cardoso, C., Matos, J., & Afonso, C. (2025). Extraction of Marine Bioactive Compounds from Seaweed: Coupling Environmental Concerns and High Yields. *Marine Drugs*, *23*(9), 366. https://doi.org/10.3390/md23090366

Cavallo, R. A., Acquaviva, M. I., Stabili, L., Cecere, E., Petrocelli, A., & Narracci, M. (2013). Antibacterial activity of marine macroalgae against fish pathogenic *Vibrio* species. *Central European Journal of Biology*, *8*(7), 646-653. https://doi.org/10.2478/s11535-013-0181-6

Clinical and Laboratory Standard Institute (CLSI), (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard, 9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne. 87 pp.

Clinical and Laboratory Standards Institute (CLSI) (2013) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplements. M100 S21, 31:1. Clinical and Laboratory Standards Institute, Wayne.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

Cortés, Y., Hormazábal, E., Leal, H., Urzúa, A., Mutis, A., Parra, L., & Quiroz, A. (2014). Novel antimicrobial activity of a dichloromethane extract obtained from red seaweed *Ceramium rubrum* (Hudson) (Rhodophyta: Florideophyceae) against *Yersinia ruckeri* and *Saprolegnia parasitica*, agents that cause diseases in salmonids. *Electronic Journal of Biotechnology*, *17*(3), 126-131. https://doi.org/10.1016/j.ejbt.2014.04.005

Cox, S., Abu-Ghannam, N., & Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal, 17*, 205-220.

Danabas, D., Ates, M., Tastan, B. E., Cimen, I. C. C., Unal, I., Aksu, O., & Kutlu, B. (2020). Effects of Zn and ZnO nanoparticles on *Artemia salina* and *Daphnia magna* organisms: Toxicity, accumulation and elimination. *Science of the Total Environment*, *711*, 134869. https://doi.org/10.1016/j.scitotenv.2019.134869

Defoirdt, T., Crab, R., Wood, T. K., Sorgeloos, P., Verstraete, W., & Bossier, P. (2006). Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii*, and *Vibrio parahaemolyticus* isolates. *Applied and Environmental Microbiology*, *72*(9), 6419-6423. https://doi.org/10.1128/AEM.00753-06

Díaz, R. T. A., Chabrillón, M., Cabello-Pasini, A., Gómez-Pinchetti, J. L., & Figueroa, F. L. (2011). Characterization of polysaccharides from *Hypnea spinella* (Gigartinales) and *Halopithys incurva* (Ceramiales) and their effect on RAW 264.7 macrophage activity. *Journal of Applied Phycology*, *23*(3), 523-528. http://dx.doi.org/10.1007/s10811-010-9622-7

DoF 2022. National Fish Week 2022 Compendium (in Bangla). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh. 160p.

El Gamal, A. A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, *18*(1), 1-25. https://doi.org/10.1016/j.jsps.2009.12.001

Ericsson, H. M., & Sherris, J. C. (1971). Antibiotic sensitivity testing. Report of an international collaborative study. *Acta pathologica et microbiologica Scandinavica. Section B: Microbiology and immunology*, *217*, 1+.

Exner, M., Bhattacharya, S., Christiansen, B., Gebel, J., Goroncy-Bermes, P., Hartemann, P., Heeg, P., Ilschner, C., Kramer, A., Larson, E., Merkens, W., Mielke, M., Oltmanns, P., Ross, B., Rotter, M., Schmithausen, R. M., Sonntag, H. G., & Trautmann, M. (2017). Antibiotic resistance: What is so special about multidrug-resistant gram-negative bacteria?. *GMS Hygiene and Infection Control*, *12*, Doc05. https://doi.org/10.3205/dgkh000290

Far, Z. S., Naghdi, S., Almashkoor, H. S. A., Silakhori, D. A., Tahergorabi, R., & Lorenzo, J. M. (2023). Exploring the antioxidant and antibacterial capacities of *Padina australis* extracts, and their utilization in starch-based coatings for preserving rainbow trout (*Oncorhynchus mykiss*) fillets. *Algal Research*, *74*, 103234. https://doi.org/10.1016/j.algal.2023.103234

Ferdous, R., Sultana, N., Hossain, M. B., Sultana, R. A., & Hoque, S. (2023). Exploring the potential human pathogenic bacteria in selected ready-to-eat leafy greens sold in Dhaka City, Bangladesh: Estimation of bacterial load and incidence. *Food Science and Nutrition*, *12*(2), 1105–1118. https://doi.org/10.1002/fsn3.3825

Fouad, Z. (2011). Antimicrobial disk diffusion zone interpretation guide. http://dx.doi.org/10.13140/RG.2.2.13801.70240

Ganesan, A. R., Subramani, K., Balasubramanian, B., Liu, W. C., Arasu, M. V., Al-Dhabi, N. A., & Duraipandiyan, V. (2020). Evaluation of *in vivo* sub-chronic and heavy metal toxicity of under-exploited seaweeds for food application. *Journal of King Saud University-Science*, *32*(1), 1088-1095. https://doi.org/10.1016/j.jksus.2019.10.005

Genovese, G., Faggio, C., Gugliandolo, C., Torre, A., Spano, A., Morabito, M., & Maugeri, T. L. (2012). *In vitro* evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. *Marine Environmental Research*, *73*(117), 1-6. https://doi.org/10.1016/j.marenvres.2011.10.002

Gupta, S., & Abu-Ghannam, N. (2011b). Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innovative Food Science and Emerging Technologies*, *12*(4), 600-609. https://doi.org/10.1016/j.ifset.2011.07.004

Gupta, S., Rajauria, G., & Abu-Ghannam, N. (2010). Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds. *International Journal of Food Science and Technology*, *45*(3), 482-489. https://doi.org/10.1111/j.1365-2621.2009.02149.x

Gupta, V., & Datta, P. (2019). Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. *The Indian Journal of Medical Research*, *149*(2), 97–106. https://doi.org/10.4103/ijmr.IJMR_755_18

Gupta. S., & Abu-Ghannam, N. (2011a). Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science and Technology*, *22*(6), 315-326. https://doi.org/10.1016/j.tifs.2011.03.011

Hasan, M., Imran, M. A. S., Bhuiyan, F. R., Ahmed, S. R., Shanzana, P., Moli, M. A., Foysal, S. H., & Dabi, S. B. (2021). Phytochemical constituency profiling and antimicrobial activity screening of seaweeds extracts collected from the Bay of Bengal sea coasts. *Journal of Advanced Biotechnology and Experimental Therapeutics*, *4*(1), 25-34. https://doi.org/10.5455/jabet.2021.d103

Hasanuzzaman, A. F. M., Nadira, N., Sardar, A., and Islam, S. (2025). Antibiotic sensitivity of *Vibrio* spp. and *Shewanella algae* isolated from brood and egg of Mud crab hatchery. *Animal Research and One Health*, *4*(1), 55–66. https://doi.org/10.1002/aro2.70008

Hernández-Robles, M. F., Álvarez-Contreras, A. K., Juárez-García, P., Natividad-Bonifacio, I., Curiel-Quesada, E., Vázquez-Salinas, C., & Quiñones-Ramírez, E. I. (2016). Virulence factors and antimicrobial resistance in environmental strains of *Vibrio alginolyticus*. *International Microbiology*, *19*(4), 191-198.

Hidayati, J. R., Bahry, M. S., Karlina, I., & Yudiati, E. (2022). Antioxidant Activity and Bioactive Compounds of Tropical Brown Algae *Padina* sp. from Bintan Island, Indonesia. *Jurnal Kelautan Tropis*, *25*(3), 309-319. http://dx.doi.org/10.14710/jkt.v25i3.15562

Hierholtzer, A., Chatellard, L., Kierans, M., Akunna, J. C., & Collier, P. J. (2013). The impact and mode of action of phenolic compounds extracted from brown seaweed on mixed anaerobic microbial cultures. *Journal of Applied Microbiology*, *114*(4), 964-973. https://doi.org/10.1111/jam.12114

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology*, *23*, 543-597. http://dx.doi.org/10.1007/s10811-010-9632-5

Honey, O., Nihad, S. A. I., Rahman, M. A., Rahman, M. M., Islam, M., & Chowdhury, M. Z. R. (2024). Exploring the antioxidant and antimicrobial potential of three common seaweeds of Saint Martin's Island of Bangladesh. *Heliyon*, *10*(4), e26096. https://doi.org/10.1016/j.heliyon.2024.e26096

Jumaetri Sami, F., Hariani Soekamto, N., Firdaus, F., & Latip, J. (2020). Antioxidant activity, toxicity effect and phytochemical screening of some brown algae *Padina australis* extracts from Dutungan island of South Sulawesi Indonesia. *International Journal of Medical Science and Dental Research*, *3*(5), 16-21.

Kim, M. J., Kim, K. B. W. R., Lee, C. J., Kwak, J. H., Kim, D. H., SunWoo, C., Jung, S.A., Kang, J.Y., Kim, H.J., Choi, J.S., Choi, H.D., & Ahn, D. H. (2011). Effect of *Sargassum sagamianum* extract on shelf-life and improved quality of morning bread. *Korean Journal of Food Science and Technology*, *43*(6), 723-728.

Klomjit, A., Praiboon, J., Tiengrim, S., Chirapart, A., & ThamLikitkul, V. (2021). Phytochemical composition and antibacterial activity of brown seaweed, *Padina australis* against human pathogenic bacteria. *Journal of Fisheries and Environment*, *45*(1), 8-22.

Klongklaew, N., Praiboon, J., Tamtin, M., & Srisapoome, P. (2020). Antibacterial and antiviral activities of local Thai green macroalgae crude extracts in Pacific white shrimp (*Litopenaeus vannamei*). *Marine Drugs*, *18*(3), 140. https://doi.org/10.3390/md18030140

Latifah, L. A., Soekamto, N. H., & Tahir, A. (2019). Preliminary study: *Padina australis* Hauck's antibacterial activity and phytochemical test against pathogenic shrimp bacteria. *Journal of Physics: Conference Series*, *1341*(2), 022005. http://dx.doi.org/10.1088/1742-6596/1341/2/022005

Lee, C. J., Choi, J. S., Song, E. J., Lee, S. Y., Kim, K. B. W. R., Kim, S. J., Yoon, S.Y., Lee, S.J., Park, N.B., Jung, J.Y., Kwak, J.H., Kim, T.W., Park, N.H., & Ahn, D. H. (2010). Effect of *Myagropsis myagroides* extracts on shelf-life and quality of bread. *Korean Journal of Food Science and Technology*, *42*(1), 50-55.

Lobban, C. S., & Harrison, P. J. (1994). *Seaweed Ecology and Physiology*. Cambridge: Cambridge University Press.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, *45*(5), 31–34. https://doi.org/10.1055/s-2007-971236

Miller, S. I. (2016). Antibiotic resistance and regulation of the gram-negative bacterial outer membrane barrier by host innate immune molecules. *mBio*, *7*(5), e01541-16. https://doi.org/10.1128/mBio.01541-16

Mishra, J. K., Srinivas, T., Madhusudan, T., & Sawhney, S. (2016). Antibacterial activity of seaweed *Halimeda opuntia* from the coasts of South Andaman. *Global Journal of Bio-science and Biotechnology*, *5*(3), 345-348.

Mofeed, J., Deyab, M., Mohamed, A., Moustafa, M., Negm, S., & El-Bilawy, E. (2022). Antimicrobial activities of three seaweeds extract against some human viral and bacterial pathogens. *Biocell*, *46*(1), 247-261. https://doi.org/10.32604/biocell.2022.015966

Mohamed, S., Hashim, S. N., & Rahman, H. A. (2012). Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science and Technology*, *23*(2), 83-96. http://dx.doi.org/10.1016/j.tifs.2011.09.001

Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., and Yolken, R. H. (2003). Manual of clinical microbiology (8th edition). Washington DC: American society for microbiology.

Normanno, G., Parisi, A., Addante, N., Quaglia, N. C., Dambrosio, A., Montagna, C., & Chiocco, D. (2006). *Vibrio parahaemolyticus*, *Vibrio vulnificus* and microorganisms of fecal origin in mussels (*Mytilus galloprovincialis*) sold in the Puglia region (Italy). *International Journal of Food Microbiology*, *106*(2), 219-222. https://doi.org/10.1016/j.ijfoodmicro.2005.05.020

Nursid, M., Noviendri, D., Rahayu, L., & Novelita, V. (2017). Isolasi fukosantin dari rumput laut coklat *Padina australis* dan sitotoksisitasnya terhadap sel MCF7 dan sel vero. *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, *11*(1), 83-90. http://dx.doi.org/10.15578/jpbkp.v11i1.237

O'Keeffe, E., Hughes, H., McLoughlin, P., Tan, SP., & McCarthy, N. (2019). Antibacterial activity of seaweed extracts against plant pathogenic bacteria. *Journal of Bacteriology and Mycology*, *6*(3), 1105.

Pang, S. J., Xiao, T., & Bao, Y. (2006). Dynamic changes of total bacteria and *Vibrio* in an integrated seaweed–abalone culture system. *Aquaculture*, *252*(2-4), 289-297. https://doi.org/10.1016/j.aquaculture.2005.06.050

Ramu, S., Murali, A., Narasimhaiah, G., & Jayaraman, A. (2020). Toxicological evaluation of *Sargassum wightii* Greville derived fucoidan in wistar rats: Haematological, biochemical and histopathological evidences. *Toxicology Reports*, *7*, 874-882. https://doi.org/10.1016/j.toxrep.2020.07.009

Rosaline, X. D., Sakthivelkumar, S., Rajendran, K., & Janarthanan, S. (2012). Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S140-S146. https://doi.org/10.1016/S2221-1691(12)60145-2

Snoussi, M., Noumi, E., Hajlaoui, H., Usai, D., Sechi, L. A., Zanetti, S., & Bakhrouf, A. (2009). High potential of adhesion to abiotic and biotic materials in fish aquaculture facility by *Vibrio alginolyticus* strains. *Journal of Applied Microbiology*, *106*(5), 1591-1599. https://doi.org/10.1111/j.1365-2672.2008.04126.x

Stirk, W. A., Reinecke, D. L., & van Staden, J. (2007). Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. *Journal of Applied Phycology*, *19*(3), 271-276. http://dx.doi.org/10.1007/s10811-006-9134-7

Sujatha, R., Siva, D., & Nawas, P. M. A. (2019). Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *World Scientific News*, (115), 27-40.

Thanigaivel, S., Chandrasekaran, N., Mukherjee, A., & Thomas, J. (2016). Seaweeds as an alternative therapeutic source for aquatic disease management. *Aquaculture*, *464*, 529-536. https://doi.org/10.1016/j.aquaculture.2016.08.001

Thanigaivel, S., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N., & Thomas, J. (2014). Antioxidant and antibacterial activity of *Chaetomorpha antennina* against shrimp pathogen *Vibrio parahaemolyticus*. *Aquaculture*, *433*, 467-475. https://doi.org/10.1016/j.aquaculture.2014.07.003

Thirunavukkarasu, R., Pandiyan, P., Balaraman, D., Subaramaniyan, K., Edward Gnana Jothi, G., Manikkam, S., & Sadaiyappan, B. (2013). Isolation of bioactive compound from marine seaweeds against fish pathogenic bacteria *Vibrio alginolyticus* (VA09) and characterisation by FTIR. *Journal of Coastal Life Medicine*, *1*(1), 26-33.

Tian, H., Liu, H., Song, W., Zhu, L., Zhang, T., Li, R., & Yin, X. (2020). Structure, antioxidant and immunostimulatory activities of the polysaccharides from *Sargassum carpophyllum*. *Algal Research*, *49*, 101853. https://doi.org/10.1016/j.algal.2020.101853

TÜney, İ., Cadirci, B. H., Ünal, D., & Sukatar, A. (2006). Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*, *30*(3), 171-175.

Uddin, S. A., Akter, S., Hossen, S., & Rahman, M. A. (2020). Antioxidant, antibacterial and cytotoxic activity of *Caulerpa racemosa* (Forsskål) J. Agardh and *Ulva* (*Enteromorpha*) *intestinalis* L. *Bangladesh Journal of Scientific and Industrial Research*, *55*(4), 237–244. https://doi.org/10.3329/bjsir.v55i4.50959

Vaithiyanathan, S., Subramanian, A., & Tennyson, S. (2023). Antibacterial activity of Seaweed extracts against human pathogenic bacteria. *Research Journal of Pharmacy and Technology*, *16*(11), 5039-5044. https://doi.org/10.52711/0974-360X.2023.00816

Vishnivetskaya, T. A., Kathariou, S., & Tiedje, J. M. (2009). The *Exiguobacterium* genus: Biodiversity and biogeography. *Extremophiles*, *13*, 541-555. https://doi.org/10.1007/s00792-009-0243-5

Xu, S. Y., Huang, X., & Cheong, K. L. (2017). Recent advances in marine algae polysaccharides: Isolation, structure, and activities. *Marine Drugs*, *15*(12), 388. https://doi.org/10.3390/md15120388

Zammuto, V., Rizzo, M. G., Spanò, A., Genovese, G., Morabito, M., Spagnuolo, D., Capparucci, F., Gervasi, C., Smeriglio, A., Trombetta, D., Guglielmino, S., Nicolo, M., & Gugliandolo, C. (2022). *In vitro* evaluation of antibiofilm activity of crude extracts from macroalgae against pathogens relevant in aquaculture. *Aquaculture*, *549*, 737729. http://dx.doi.org/10.1016/j.aquaculture.2021.737729