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# Characterization of Antimicrobial Bioactive Compounds and Antibacterial Potential of *Ulva fasciata* Delile Isolated from West Coast of Kanyakumari, India

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Six seaweed species, *Bryopsis plumose* (Hudson)C.Agardh, *Caulerpa racemose* (Forskall) J.Agardh, *Ulva fasciata* Delile, *Sargassum longifolium* (Turner) C. Agardh, *Gracilaria corticate* (J.Agardh) J. Agardh and *Sargassum wightii* Greville ex J. Agardh were collected at Kadiyapattinum Coast of Kanyakumari District. They were shade dried, powdered and extracted with 100%

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methanol and screened the phytochemicals by standard analytical protocols. Among the seaweeds, *U. fasciata* showed the presence of alkaloids, glycosides, phenols, steroids, tannins and terpenoids also found in rich of total protein and carbohydrate. Based on the initial screening *U. fasciata* was selected for further antimicrobial study and serially extracted with methanol, chloroform, ethyl acetate, acetone and hexane and screened antibacterial and antifungal activity. Among the selected solvents, methanol extract of *U. fasciata* showed high activity against *Staphylococcus aureus*, *Bacillus* sp., *Klebsiella pneumonia* and *Aeromonas hydrophila*. The methanol extracts effectively controlled the bacterial pathogens at the zone of inhibition of 8.11, 11.33, 13.87 and 17.76 mm against *Bacillus sp., S. aureus*, *K. pneumonia* and *A. hydrophila* respectively. Antifungal screening result revealed that, very least activities, less than 3mm were observed against the fungal pathogens, *Candida albicans, Rhizopus oryzae* and *Aspergillus flavus.* Fourier-Transform Infrared Spectroscopy revealed the presence of alcohol, alkenes, aromatic, amine, phenyl, ether, methylene, primary amines, and aliphatic chloro compound in the methanolic extract of *U. fasciata*. In order to characterize the antimicrobial protein, crude extract was purified through sephadex G-75 gel filtration chromatography and characterized the antimicrobial protein found to be 13 kDa. Further the methanolic extract of *U. fasciata* at different concentrations (100,200 & 400 mg/Kg*)* was incorporated the diets were fed to the ornamental gold fish *Carassius auratus* for 30 days and challenged with virulent *A. hydrophila* and studied the survival, specific bacterial load reduction. The highest concentrations of *U. fasciata* diets fed fishes had significantly ($P<0.05$) improved survival and reduced Aeromonas count in the blood. This study concluded that, *U. fasciata* extract improve survival and also helped to reduce the Aeromonas load at *in vivo* level which was useful to develop antibacterial diets in the fresh water aquaculture industry.

*Keywords: Antimicrobial; Aeromonas hydrophila; Carassius auratus; Seaweeds; Ulva fasciata.*

## 1. INTRODUCTION

Seaweeds differ widely in terms of their biochemical compounds, consistency, quality, and color. Seaweeds are primarily categorized as red (Rhodophyceae), green (Chlorophyceae), or brown (Phaeophyceae) based accessory pigments that determine the color of the algae: phycoerythrin (red color), fucoxanthin (brown color) and green algae chlorophyll [1]. These are more diverse photosynthetic plant that may be of scientific interest recent years. Their ability to adhere to rocks, corals, or any other natural or artificial substrate makes them a highly adaptable product that has been used extensively in both food and medicine [2]. Because seaweeds are a remarkable source of bioactive compounds, recent research has shown an unexpected bloom.

Seaweeds are a good source of many different bioactive substances including carotenoids, phycocolloids, fatty acids, lectins, fibers, antioxidants, oils, sterols, unsaturated fatty acids, proteins, vitamins and amino acids. Tannins, carotenoids, and sterols are among the various compounds that have been identified and extracted from seaweeds and have demonstrated antioxidant properties [3]. Numerous biological characteristics of seaweeds, including their antibacterial, antifungal, antiviral, anti-inflammatory, cytotoxic, nematicidal, antifeedant, larvicidal, and anticoagulant qualities, have been demonstrated. These properties make seaweeds a potentially renewable resource in the marine environment [4].

According to Zbakh et al. [5], they are the most significant macro algae due to their wide range of biological activities, which includes antimicrobial activity. Numerous bioactive substances, including compounds with antibacterial properties, were extracted from macro algae [6]. They contain various compounds that have been integrated into medicine and pharmacotherapy, and some of the isolated compounds have the ability to both inhibit and kill bacteria [7]. The antibiotic cycloeudesmol, which contains sesquiterpene and is derived from *Chondria oppositiclada* [8], has been shown to be effective against both *Candida albicans* and *S. aureus.*

Ulva are a genus of edible algae that are found all over the world's ocean coasts [9]. Because of their unique chemical compounds, Ulva have a variety of interesting bioactivities [10]. According to Selvin and Lipton [11], *Ulva fasciata* has demonstrated antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria that are frequently found in human infections. According to Blunt et

al. [12], *U. fasciata* demonstrated antibacterial activity that may have been caused by the presence of terpenoids, tannins, phenolic compounds, phytochemicals, and steroids. The goal of this study was to characterize the phytochemicals and protein from *U. fasciata*, as well as investigate the antibacterial activity of the methanolic extract of *U. fasciata* against a range of bacterial pathogens and it's *in vivo* effects in *Carassius auratus* culture against *Aeromonas hydrophila* challenge.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Extraction of Seaweeds

Six seaweeds species including *Bryopsis plumosa*, *Caulerpa racemosa, Ulva fasciata, Sargassum longifolium*, *Gracilaria corticata* and *Sargassum wightii*) were collected from Kadiyapattinum (8.130885; 77.304380), South West coast of India. Following a distilled water wash and dried in the shade, they were ground into a fine powder. Ten grams of powder were extracted using the percolation method with 100 millilitres of methanol. The extracted material was concentrated and kept for future research at 4 °C.

### 2.2 Phytochemical and Biochemical Screening

The phytochemicals such as, alkaloids, flavonoids, glycosides, phenolic compounds, steroids, saponins, terpenoids and tannins were screened following the method described by Kamba and Hassan [13] from the methanolic extracts of six seaweeds. Bovine serum albumin was used as a standard and the results are expressed as % to dry weight of the sample. Total carbohydrate was estimated as suggested by Dubois et al. [14] using a UV-Visible spectrophotometer measured at 490 nm and glucose was used as the standard. Sulfophospho vanillin method was used for the estimation of lipid [15] and cholesterol was used as a standard and the results are expressed as % to dry weight of the sample.

### 2.3 Antimicrobial Screening

In order to perform the antimicrobial screening *U. fasciata* was extracted with ethanol, ethyl acetate, chloroform, methanol and acetone by percolation extraction method. The powder was immersed in a suitable solvent (1:1 ratio) for duration of 48 hours. Following filtration and washing to get rid of insoluble fractions, the filtrate was centrifuged for 10 minutes at 10,000 X g to provide clarification. Condensing the clarified extracts at 35 °C was done until the solvent residue disappeared. A known amount of *U. fasciata* organic extract condensate was impregnated in sterile paper discs with a 5-mm diameter from Himedia, India. Three replicates of the disc diffusion test were used to screen against the bacterial pathogens *Staphylococcus aureus, Bacillus sp., Enterobacillus sp., Klebsella pneumonia,* and *Aeromonas hydrophila* as well as the fungal pathogens *Candida albicans, Rhizopus sp* and *Aspergillus flavus* [16]. *U. fasciata* extracts were not impregnated in sterile disks used for the control experiments. Antibiotics such as Amikacin and Nystatin (30 µg) were used as the positive control. Antibacterial and antifungal activities were studied for inhibitory zone formation in the respective agar plates after 24 hrs incubation.

### 2.4 Functional Group Analysis by FT-IR

In order to study the functional groups, methanolic extract of *U. fasciata* was used for Fourier Transform Infrared Spectroscopy (FT-IR) analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disks. The powdered sample was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 $cm^{1}$.

### 2.5 Antimicrobial Protein Characterization

The antimicrobial protein from *U. fasciata* was purified by the combination of ammonium sulphate precipitation and gel filtration chromatography. 10 gm *U. fasciata* powder was weighed and ground with physiological saline (0.9% NaCl). It was centrifuged at 10,000 X g for 10 min and supernatant was retained. To this supernatant, ammonium sulphate was added at various saturations (10-80% saturation). Then the active fraction was loaded on sephadex G-75 gel filtration column and 15 fractions were collected. All fractions were subjected to total protein estimation and antibacterial activity against *A. hydrophila*. The highly active fraction was subjected to SDS-PAGE to determine the molecular weight.

### 2.6 Diet Preparation

45.1% protein, 7.2% lipid, 14.6% ash, 7.1% moisture, and 3.1% fiber made up the basic ration. For the experimental diets, the

concentration of the *U. fasciata* methanolic extract mixture and base ingredients was 100 (UFD1), 200 (UFD2), and 400 (UFD3) mg/Kg. The subjects were also given an identical control diet devoid of *U. fasciata* extract. After mixing the ingredients well and adding enough water, the mixture was cold-extruded, sliced into pellets, allowed to air-dry, and stored at room temperature.

### 2.7 Experimental Fish Culture and Feeding

For every treatment group, 30 healthy *Carassius auratus* fish, with a mean weight of 30±2 g (10 fish per tank), were stocked in triplicate tanks with a flow-through water system in a 50 l tank. During the study period, fish groups were fed the respective diets thrice daily at a rate of 10% of their body weight on an ad libitum basis. For duration of thirty days, a daily partial water exchange was carried out to eliminate waste feed and faecal materials.

### 2.8 Bacterial Challenge, Survival and Aeromonas Load Reduction

Following the feeding trial, 100 µl of virulent *A. hydrophila* suspension at a rate of $1\times 10^8$ cfu/fish was intraperitoneally injected into each tank's fish. For ten days, survival and pathological signs were recorded every three hours. For the Aeromonas count, 100 µl of blood was plated in Aeromonas agar (Hi media, India). Every sample was kept in triplicates, and they were incubated for 48 hours at 37º C.

### 2.9 Data Analysis

The data used in this study were expressed as mean ± SD. They were then analyzed using the ANOVA test, and post hoc multiple comparisons with the SNK test was carried out using the computer program SPSS at the 5% level of significance.

## 3. RESULTS

The methanolic extract of *U. fasciata* showed the presence of alkaloids, glycosides, phenols, steroids, tannins and terpenoids. However, the methanol extract of *S. longifolium* showed only two phytochemicals. The phytochemicals of methanol from other macro algae was tabulated in Table 1. The biochemical parameters including protein, carbohydrate and lipid of all seaweed extracts were given in the Fig. 1. The highest protein level observed of 24.5 mg/kg in *U. fasciata* and the level was decreased to 19.4, 18.4, 15.8, 11.3 and 8.3 mg/kg in *B. plumose, C. racemosa, S. longifolium, S. wightii* and *G. corticata* respectively. Likewise, the highest level carbohydrate (28.3 mg/kg) observed in *U. fasciata* and the lowest level observed of 8.3 mg/kg in *S. wightii*. The highest level lipid (13.4 mg/kg) observed in *G. corticata* and the lowest level observed of 7.1 mg/kg in *U. fasciata.*

The antimicrobial screening including antibacterial and antifungal activities results of *U. fasciata* were presented in Table 2. Among the different solvent extractions, methanol had effectively inhibited the pathogenic bacterial growth at *in vitro* level. The antibacterial activity observed of 11.33 mm against *S. aureus* and the activity was significantly ($P<0.05$) decreased to other extractions and the least activity observed of 1.05 mm in ethyl acetate extractions. Antibacterial activities observed of 8.26 and 8.11 mm of zone of inhibition in methanol and acetone extraction against *Bacillus* sp. Antibacterial

**Table 1. Phytochemical analysis of the methanolic extract of seaweed species isolated from Kadiyapattinum Coast of Kanyakumari District**

| Phytochemicals | Seaweed Species | | | | | |
|---|---|---|---|---|---|---|
| | ***Bryopsis plumosa*** | ***Caulerpa racemosa*** | ***Ulva fasciata*** | ***Sargassam longifolium*** | ***Sargassam wightii*** | ***Gracilaria corticata*** |
| Alkaloids | + | -- | + | -- | -- | + |
| Flavonoids | -- | + | -- | + | + | + |
| Glycosides | + | -- | + | + | -- | -- |
| Phenols | + | -- | + | -- | + | -- |
| Steroids | -- | -- | + | -- | + | -- |
| Saponins | -- | + | -- | -- | -- | -- |
| Tannins | + | + | + | -- | -- | -- |
| Terpenoids | -- | + | + | -- | -- | -- |

*+ Positive; -- Negative*

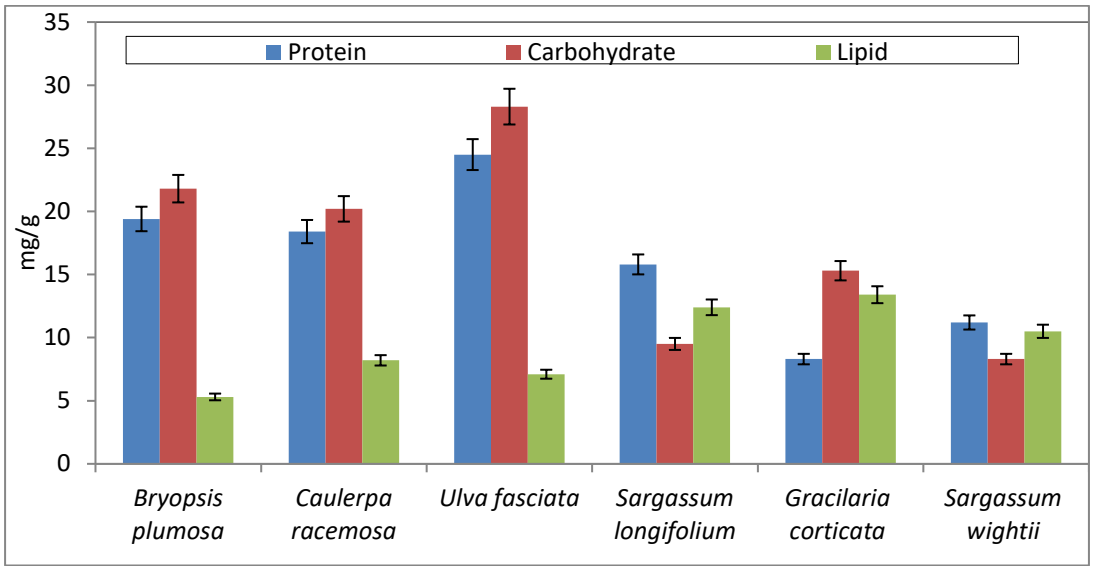


**Fig. 1. Biochemical composition (protein, carbohydrate and lipid) of the methanolic extracts of seaweeds species isolated from Kadiyapattinum Coast of Kanyakumari District (mean ± SD), (n=3)**

activities against *Enterobacillus sp,* the activity was observed of maximum 4.67 mm in acetone extraction and the least activity observed of 1. 88 mm of zone of inhibition was observed in hexane extraction. Methanol and chloroform extracts was effectively controlled *K. pneumonia* at the level of 13.87 and 12.78 mm of zone of inhibition respectively. The highest zone of imbibition (17.76 mm) observed against *A. hydrophila* by the methanolic extraction and this was significantly ($P<0.05$) decreased to other extractions. The antifungal activity results revealed that, there was a less activity (> 3 mm of zone of inhibition) by all extractions against the tested fungal pathogens, *C. albicans, Rhizopus* sp and *A. flavus* and the values were non significantly ($P>0.05$) differed.

The FT-IR spectrum of methanolic extract of *U. fasciata* showed the presence of alcohol, alkenes, aromatic, amine, phenyl, ether, methylene, primary amines, 1° and 2° amine and aliphatic chloro compound. The major band was observed at 3208.3 $cm^{-1}$ that mainly could be O-H stretching vibrations of alcohol group. The other detected functional group and their corresponding wave number are presented (Table 3; Fig. 2). The antimicrobial protein characterized through sephadex G-75 gel filtration chromatography followed by SDS PAGE results revealed that, the protein detected at molecular weight of the protein was found to be 13 kDa (Fig. 3). The elution profile of the dialysed samples numbers 7 and 8 had the protein content of 0.982 and 1.03 mg/ml respectively. The antibacterial activity performed against *A. hydrophila* were 14 and 133 mm of zone of inhibition in 7 and $8^{th}$ fractions respectively (Table 4).

The control fish group in the challenge experiment died totally in nine days, while the experimental group had the highest survival rate. Following the tenth day of the *A. hydrophila* challenge, the survival rates of 20, 60, and 75% in the fish groups fed the UFD1, UFD2, and UFD3 diets were observed (Fig. 4). The survival data were differed significantly each other's ($F$ = 25.87; $P \leq 0.001$(column); $F$ = 12.23; $P \leq 0.001$(Row)). It may due to the influence of the antibacterial compounds present in the methanolic extract *U. fasciata* that helped to arrest the multiplication of *A. hydrophila* at *in vivo* level that's reflected in the improved survival. Aeromonas load reduction result revealed that, UFD1 and 2 helped to reduce the load. Maximum load observed of $9.5 \times 10^5$ cfu/ ml after $6^{th}$ day of challenge in control group. The load observed of $2.8 \times 10^3$ cfu/ ml after $9^{th}$ day of challenge in UFD1 diet fed group. The load was reduced to $0.55 \times 10^2$ and $0.32 \times 10^2$ cfu/ ml in UFD2 and UFD3 diet fed groups respectively after $9^{th}$ days of challenge (Table 5). The *in vivo* influence of *U. fasciata* also helped to arrest the bacterial multiplication by cell wall disturbance and arresting the transcription binding the compounds to the specific receptor.

**Table 2. Antimicrobial activity of *Ulva fasciata* against various pathogenic bacteria and fungi. Each value is the mean ± SD of triplicate analysis; within each row means with different superscript letters are statistically significant (One way ANOVA test; *P*<0.05 and further *post hoc* multiple comparison with SNK test)**

| Extractions | Pathogenic Bacteria | | | | | Pathogenic Fungi | | |
|---|---|---|---|---|---|---|---|---|
| | SA | BA | *EB* | *KP* | AH | CA | RZ | AF |
| Methanol | 11.33[a] ± 0.67 | 8.11[a] ± 0.65 | 3.98[a] ± 0.12 | 13.87[a] ± 1.02 | 17.76[a] ± 1.75 | 2.11[NS] ± 0.01 | .32[NS] ± 0.04 | 1.58[NS] ± 0.04 |
| Chloroform | 3.23[b] ± 0.04 | 2.57[b] ± 0.15 | 2.06[b] ± 0.11 | 12.78[a] ± 0.88 | 3.87[b] ± 0.67 | 1.07[NS] ± 0.06 | 0.58[NS] ± 0.01 | 2.54[NS] ± 0.05 |
| Ethyl acetate | 1.05[c] ± 0.03 | 1.25[b] ± 0.04 | 1.97[a] ± 0.03 | 2.08[b] ± 0.045 | 2.05[c] ± 0.55 | 1.23[NS] ± 0.03 | 2.11[NS] ± 0.04 | 2.11[NS] ± 0.03 |
| Acetone | 7.87[d] ± 0.65 | 8.26[a] ± 0.76 | 4.67[a] ± 0.02 | 1.65[b] ± 0.05 | 3.86[b] ± 0.05 | 0.77[NS] ± 0.01 | 2.03[NS] ± 0.02 | 2.07[NS] ± 0.04 |
| Hexane | 2.97[b] ± 0.023 | 2.77[b] ± 0.32 | 1.88[a] ± 0.013 | 1.55[b] ± 0.07 | 3.11[b] ± 0.03 | 1.62[NS] ± 0.03 | 3.13[NS] ± 0.04 | 1.98[NS] ± 0.02 |

*SA: S.aureus; BA: Bacillus sp.; EB: Enterobacillus sp.; KP: K. pneumonia; AH: A. hydrophila; CA: C. albicans; RZ: Rhizopus sp.; AF: A. flavus. [NS]: Non Significant*

**Table 3. Fourier Transform Infrared spectroscopy spectra analysis of the methanolic extract of *U. fasciata***

| Wave number ($cm^{-1}$) | Components (peak) | Functional groups |
|---|---|---|
| 3208.3 | OH | Alcohol group |
| 2843.3 | C-H | Alkenes |
| 2700.6 | O-H | Alkenes |
| 660.5 | C-H | Aromatic |
| 1501.2 | NH | Amine |
| 1382.8 | OH | Phenyl |
| 1224.05 | C-O | Ether |
| 1162.75 | $CH_2$ | Methylene group |
| 1094.45 | C-O-C | Aromatic cyclic ether |
| 980.25 | C-N | Primary amines |
| 823.5 | N-H | 1° and 2° amines |
| 758.2 | C-Cl | Aliphatic chloro compound |
| 665.7 | OH | Alcohol |

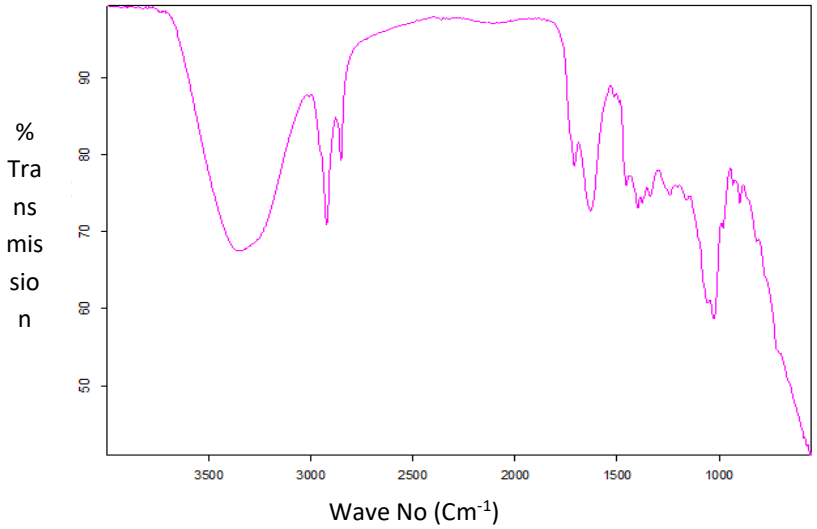


**Fig. 2. Fourier Transform Infrared spectroscopy spectra analysis of the methanolic extract of *U. fasciata*.**

**Table 4. Elution profile of antimicrobial protein from *U. fasciata.* About 1.0 ml dialyzed sample was loaded on sephadex G-75 chromatography column. Fraction 7 and 8 showed antibacterial activity against *A. hydrophila***

| Fraction No | Total protein content (mg/ml) | Zone of inhibition (mm) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0.029 | 0 |
| 5 | 0.053 | 0 |
| 6 | 0.072 | 0 |
| **7** | **0.982** | **14** |
| **8** | **1.03** | **13** |
| 9 | 0.8 | 0 |
| 10 | 0.73 | 0 |
| 11 | 0.62 | 0 |
| 12 | 0.292 | 0 |
| 13 | 0.02 | 0 |
| 14 | 0.04 | 0 |

| Fraction No | Total protein content (mg/ml) | Zone of inhibition (mm) |
|---|---|---|
| 15 | 0.06 | 0 |
| 16 | 0.982 | 0 |
| 17 | 0.872 | 0 |
| 18 | 0.042 | 0 |
| 19 | 0.002 | 0 |
| 20 | 0.04 | 0 |

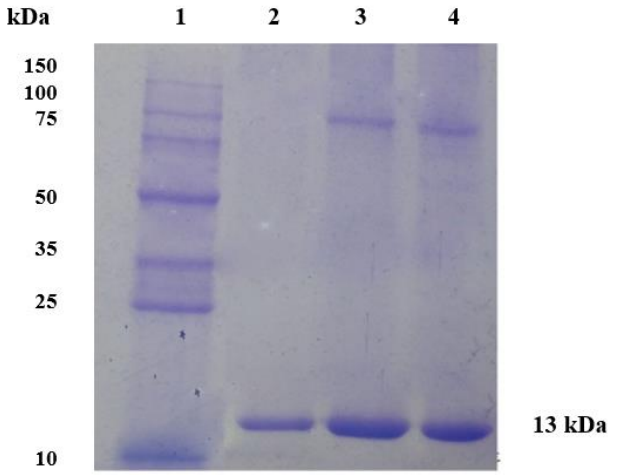


**Fig. 3. Antimicrobial activity of protein from *U. fasciata* extract (Wells1: Molecular marker; 2: sephadex G-75 gel filtration fraction; dialyzed ammonium sulphate fraction at 35% and dialyzed ammonium sulphate fraction at 80%)**

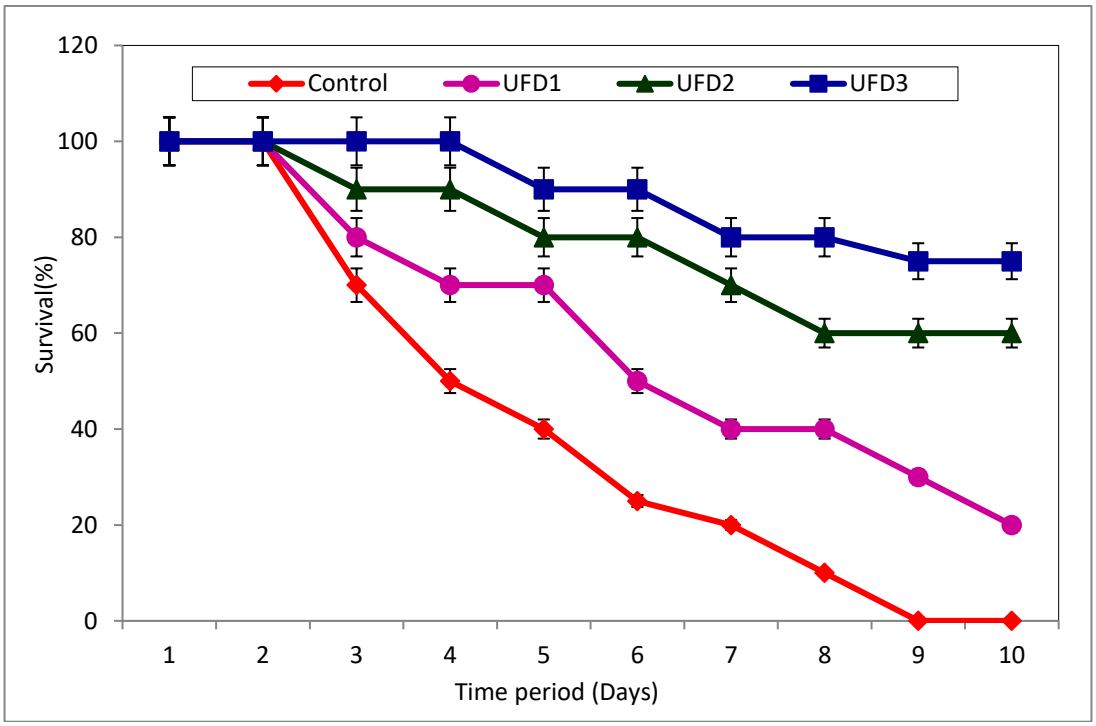


**Fig 4. Survival of *C. auratus* fed on different concentrations of *U. fasciata* extract supplemented diets after challenged with virulent *A. hydrophila*. The data were differed significantly each other's (*F* = 25.87; P ≤ 0.001(column); *F* = 12.23; P ≤ 0.001(Row)) -Two Way ANOVA**

**Table 5. Aeromonas count in blood samples of *C. auratus* fed on different concentrations of *U. fasciata* extract diets after challenged with virulent *A. hydrophila* in different days interval**

| Diets | Aeromonas count (Cfu/ ml) | | |
|---|---|---|---|
| | 3rd day | 6th day | 9th day |
| Control | $8.1 \times 10^5 \pm 0.33 \times 10^1$ | $9.5 \times 10^5 \pm 0.45 \times 10^1$ | - |
| UFD1 | $3.7 \times 10^4 \pm 0.15 \times 10^1$ | $1.8 \times 10^4 \pm 0.67 \times 10^1$ | $2.8 \times 10^3 \pm 0.22 \times 10^1$ |
| UFD2 | $3.4 \times 10^4 \pm 0.56 \times 10^1$ | $1.4 \times 10^3 \pm 0.21 \times 10^1$ | $0.55 \times 10^2 \pm 0.57 \times 10^1$ |
| UFD3 | $1.7 \times 10^4 \pm 0.12 \times 10^1$ | $1.2 \times 10^2 \pm 0.61 \times 10^1$ | $0.32 \times 10^2 \pm 0.11 \times 10^1$ |

## 4. DISCUSSION

The methanol extract of *U. fasciata* showed the presence alkaloids, glycosides, phenols, steroids, tannins and terpenoids. Seaweeds are observed to be used in various medicinal applications which are mainly due to the presence of bioactive components including the proteins, carbohydrates, lipids, crude fibers, carotenoids, vitamins, phenolics, amino acids, flavonoids etc [17]. The enriched bioactive compounds in *U. fasciata,* they have act as best antimicrobials and antioxidants. Saponins also have beneficial effects on the lowering of blood cholesterol, and in acting against cancer along with antibacterial and antiviral properties [18]. Glycosides are useful to treat cardiac arrhythmias and cardiac disorders [19]. Chandrasekaran et al. [20] identified the phytochemicals including terpenoids, tannins, cardiac glycosides and phenolic compounds from the *U. fasciata* organic solvents. The enrichment of bioactive compounds in the seaweed species due they are living in adverse environmental conditions such as varying pH, temperature, inconsistent tides and alternating salinity etc. The biochemical results revealed that, highest protein and carbohydrate levels observed in *U. fasciata* followed by *C. racemosa* and *B. plumosa.* It may due to the enriched level of polysaccharides and the polysaccharides are help to various bioactivities including antimicrobial, antioxidants and immunomodulation. Polysaccharides, which may serve structural and/or storage purposes, are typically the primary constituents of green, brown, and red seaweed. Alginic acid and alginates, carrageenans and agar, laminarans, fucoidans, ulvans, and derivatives are among the many polysaccharides that make up the cell walls of algae [21,22].

The antibacterial screening results revealed that, the methanolic extracts effectively suppressed the bacterial growth it may due to the compound polarity. The aloholic extracts of *U. reticulate, S. wightii* and *Halimeda macroloba* showed antibacterial activity against the biofilm producing bacterial strains viz., *Pseudomonas* sp., *Cytophaga* sp. *Flavobacterium* sp., and *Bascillus* sp. [23]. Methanol extracts of *Cladophora glomerata* exhibited bactericidal activity against *Vibrio anguillarum, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio fischeri, Bacillus cereus, Escherichia coli* and *Acinetobacter baumannii* [24]. Generally seaweeds are growing under the stress environments such as wave and tidal action, temperature variation, pressure, competition and predation. They have the defence mechanism naturally helped to synthesis of various bioactive secondary metabolites. That includes, pathogenic interruption, signalling among the seaweed species and associated microbes, maintain cellular health against stress and nutrient uptake etc. Theses metabolites are act as anticancer, antimicrobial, antifungal, anti-inflammatory and other therapeutic substances. Antimicrobials from seaweed limit or inhibit the growth and development of other competitive microorganisms in marine ecosystem. Metabolites from red, brown and green marine algae may be useful for inhibiting viruses, bacteria, viruses, fungi and other epibionts (e.g., antihelmintic activity). Algae extracts was exhibited anticoagulant [25], antioxidant [26], antiviral [27], anti-inflammatory [28], anticancer [29], activities.

FT-IR spectrum of methanol extract of *U. fasciata* showed the presence of various functional groups such as, alkenes, alcohol, aromatic, phenyl, amine, ether, methylene, primary amines, and aliphatic chloro compound. In our study, a major band was observed at 3208.3 $cm^{-1}$ that mainly could be O-H stretching vibrations of alcohol group. Previously, the FTIR spectra from *U. fasciata* showed various functional groups which agreed with the FTIR values reported for marine macro algae *Laminaria digitata* [30]. Similarly, Azizi et al. [31] observed various functional compounds like from marine algae *Sargassum muticum* using FTIR. Antimicrobial protein had the molecular weight found to be 13 kDa from the *U. fasciata* extracts. The antibacterial activity performed against the

freshwater fish pathogen had efficient results. Among the different fractions tested against the pathogen, two fractions had effectively inhibited *A. hydrophila* at 14 and 13 mm of zone of inhibition respectively. The two fractions had approximately 1 mg/ml of protein were observed. Hayes [32] stated that lectins and phycobiliproteins are two families of bioactive algal proteins which have been widely exploited for various industrial applications. Lectins are the important protein having antimicrobial activity extracted from macroalgal sources, while phycobiliproteins are typically isolated from microalgae [32]. Phycobiliproteins, especially phycoerythrin, can constitute a significant proportion of the overall protein content in algae, with levels of 1.2% reported for *P. palmata* [33]. Crude extracts of *Ulva sp.* often displayed antibacterial and/or antifungal activities [34].

The current study's survival results showed that the enrichment of diets with *U. fasciata* extract effectively inhibited the *in vivo* growth of *A. hydrophila,* as evidenced by the over 60% survival rate of *C. auratus.* According to Velmurugan et al. [35], shrimp *Fenneropenaeus indicus* treated with *Enteromorpha flexuosa* extract exhibited a 90%+ survival rate against the White Spot Syndrome Virus. When *C. auratus* was fed a diet supplemented with *Ixora coccinea* against an *A. hydrophila* challenge, the Aeromonas load was significantly reduced [36]. *S*. *wightii* contains bioactive substances that can enhance antibacterial and antioxidant properties, such as phenolics and flavonoids [37]. In the current investigation, the *in vivo* multiplication of *A. hydrophila* was effectively suppressed by the *U. fasciata* extracts. On the sixth day of the challenge, the *A. hydrophila* load from the control group ($9.5 \times 10^5$) was reduced to the lowest level of $1.2 \times 10^2$ cfu/ml by the highest concentration of the extract (400 mg/kg). Bioactive compounds derived from *Graciella tenuistipitata* have been employed medicinally to treat and manage diseases of shrimp, with documented success on a global scale [38]. According to Chotigeat et al. [39], oral administration of crude fucoidan (CF) derived from *Sargassum polycystum* can lessen the effects of WSSV infection in *P. monodon*. The present study clearly indicated that, the bioactive compounds of *U. fasciata* helped to improve the health of tested fish *C. auratus* and revealed that, this diet will help to treat the bacterial infections in ornamental aquaculture industry.

## 4. CONCLUSION

Based on the results of this investigation, it can be said that the methanolic extract of *U. fasciata* that contains active compounds effectively controls *A. hydrophila* both *in vitro* and *in vivo* while enhancing immunity against pathogenic disruption. The diets especially UFD2 (200 mg/kg) and UFD3 (400 mg/kg) were helped to improve the survival and reduced the specific bacterial load, *A. hydrophila* due to the high concentrations of active compounds of *U. fasciata* Our research also aided in the development of a novel immunostimulant and antibacterial medication to combat *A. hydrophila* in cultivable freshwater fish.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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