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SCREENING AND EVALUATION OF AQUEOUS EXTRACT OF *U. INTESTINALIS* FOR POTENTIAL BIOLOGICAL ACTIVITY AND AQUACULTURE APPLICATIONS

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ABSTRACT
Replacing synthetic feed supplements with natural compounds is recognized as a valuable approach to sustainable aquaculture. The antioxidant activity, contents, phytochemical assessments, and anti-inflammatory activities were tested in the aqueous extracts of *Ulva intestinalis* L. grown in the coastal areas of Kalijai, Chilika, Odisha. Aqueous extract of *U. intestinalis* was tested for total phenolic contents (TPC), total flavonoid contents (TFC), and total tannin contents (TTC). In vitro antioxidant activity was determined by total antioxidant activity, ferric reducing antioxidant power (FRAP) assay, 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, and 2, 2'-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS) assay. The bioactive compounds of seaweed were analyzed by liquid chromatography mass spectrometry (LC-MS). The values of TPC, TFC, and TTC were 1.10 ± 0.05 mg GAE g^{-1}, 8.27 ± 0.2 mg QE g^{-1}, and 1.79 ± 0.07 mg GAE g^{-1}, respectively. The total antioxidant activity, FRAP, DPPH, and ABTS were 76.87 ± 4.00 mg ascorbic acid g^{-1}, 84.05 ± 2.01 mg ascorbic acid g^{-1}, 36.21 ± 0.88 %, and 43.02 ± 5.10 % respectively. In LC-MS analysis, compounds like L-phenylalanine and Chlortetracycline were found. Overall, this study suggests its applicability as a sustainable functional feed additive and better health of fish in aquaculture.

HIGHLIGHTS
- An aqueous extract of *Ulva intestinalis* showed potential antioxidant activity.
- Antioxidant and anti-inflammatory activity of the aqueous extract was studied in vitro.
- Total phenols, flavonoids and tannins contributed to the bioactivity.
- LC–MS profiling identified several bioactive metabolites.
- Histopathology study of *Labeo rohita* showed no alterations in both gill and intestine

KEYWORDS: Seaweed, *U. intestinalis*, Secondary metabolites, Antioxidant activity, Anti-inflammatory activity, Aquafeed.

1. INTRODUCTION
Marine seaweeds, such as brown, red, and green algae, are primarily found growing on rocky substrates in intertidal and subtidal habitats worldwide. For decades, they have been used as folk remedies for various ailments, as edible food, and as animal feed (Alasalvar and Bolling 2015). In some seaweeds, bioactive compounds have been discovered to have biological activity and have become highly important products in the pharmaceutical, food, and other biotechnological industries (Sameeh et al. 2016). Most studies have demonstrated that seaweeds from different groups derive bioactive secondary metabolites, such as vitamins, enzymes, proteins, polyphenolic chemicals, sulphated polysaccharides, flavonoids, and terpenoids, which showed antioxidant, antimicrobial, antibacterial, antifungal, antidiabetic, and anti-inflammatory activities (Baghel et al. 2023; Carpena et al. 2023; Jayalakshmi et al. 2021).
Marine biodiversity represents an extensive and valuable source of exploitable natural resources, with global seaweed aquaculture production exceeding 30 million tonnes annually since 2015. Although marine algae have gained increasing scientific attention because of their diverse bioactive compounds and potential health benefits, they remain relatively underutilized (Senadheera et al. 2023). Extracts obtained from marine algae using aqueous and organic solvents have demonstrated considerable antimicrobial activity (Abou-Dobara et al. 2023). Among these extraction methods, aqueous extraction is particularly advantageous because of its non-toxic nature, environmental safety, cost-effectiveness, and suitability for aquaculture and nutraceutical applications (Wang et al. 2026). This method efficiently isolates polar bioactive compounds, including polysaccharides, proteins, phenolics, flavonoids, and sulfated metabolites, while eliminating the risk of hazardous solvent residues. Consequently, aqueous seaweed extracts are considered more biocompatible and environmentally friendly for pharmaceutical, feed, and nutraceutical applications (Ng et al. 2025).
Despite their promising potential, the application of marine-derived polyphenols is constrained by several challenges (Getachew et al. 2020). These include limited precision in qualitative and quantitative molecular-level

characterization, the presence of bioactive compounds in extremely low concentrations, and difficulties in standardizing analytical procedures such as sample preparation and extraction. The high and variable concentrations of dissolved salts in marine samples further complicate these challenges. Consequently, research in this field faces significant methodological obstacles

Finding natural feed ingredients that offer nutritional advantages as well as bioactive qualities and biological safety is crucial given the rising need for ecologically friendly aquaculture. *Ulva intestinalis* has been identified as a rich source of beneficial metabolites, but there hasn't been much focus on its integrated assessment as a functional aquafeed element for *Labeo rohita*; however, it was examined for the mucosal and immune parameters(Rouhani et al. 2022). In order to optimize feed and evaluate histopathology in *L. rohita*, this study thoroughly examined the nutritional constituents, phytochemical profile, antioxidant, and anti-inflammatory properties of *U. intestinalis* extract. The viability of using *U. intestinalis* as a sustainable and safe functional element for aquaculture is revealed by this comprehensive approach.

2. MATERIALS AND METHODOLOGY

2.1 Collection of Seaweed Material

The fresh biomass of native green seaweed, *U. intestinalis*, was collected in an autoclave bag during low tide along the coast of the Kalijai temple, Chilika, in the winter season of November 2025. The collected specimens were washed in tap water to remove extra sediments and then in distilled water to remove extra salts, identified based on morphological and taxonomic characteristics, thallus structure, branching pattern, colour, and cellular organization, using standard taxonomic keys and previously published literature. The identified specimen was confirmed as *Ulva intestinalis*. Then the DNA from the fresh weight of the Ulva sample was isolated and separated by agarose gel electrophoresis (0.8%), run in 1×TAE buffer at 50V for 30-45 minutes until the DNA fragments migrated well. Then the concentration of each DNA sample was measured using a Nanodrop (Biotech instruments, USA). The extracted DNA was amplified through PCR following the protocol of (Kumar et al. 2018). The amplicons obtained were resolved on a 1.5% agarose gel in 0.5x Tris-acetate-EDTA (TAE) buffer and used for sequencing. The sequencing data were submitted to the National Center for Biotechnology Information (NCBI). The samples were then washed, shaded in a well-ventilated room for 72 hours, and powdered using a mortar and pestle. The dried powder was stored in a plastic bag at 4°C for further use.

2.2 Aqueous Extract Preparation

The dried powder sample (12g) of *U. intestinalis* was dissolved in 150 mL of deionized water at room temperature for 24 h in the dark, then mixed with a magnetic stirrer for 1 h. The extraction process was repeated, and the mixture was filtered through a glass funnel lined with Whatman No. 1 filter paper. The filtrate was concentrated to dryness by using a rotary evaporator to reduce the volume of liquid into a concentrated residue. (Meenakshi et al. 2011).The nutritional compositions (carbohydrate, protein, and lipid) of the *U. intestinalis* were analyzed by following the standard protocol(Yemm and Willis 1954; Bradford 1976; Taha et al. 2012). Higher moisture content can promote microbial growth and may degrade the bioactive compounds. The moisture content was evaluated by the method of AOAC (1997).

$$\text{Moisture content at } 105^{\circ}\text{C}(\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,

$W_1 =$ Weight of empty desiccator (g)
$W_2 =$ Weight of desiccator with wet sample (g)
$W_3 =$ Weight of desiccator with dried sample (g)

2.3 Analysis of Total Phenolic Contents

The Folin-Ciocalteu reagent was used to quantify total phenolic content as gallic acid equivalents (GAE). 1.0 mg of the concentrated extract was diluted with 0.5 mL ethanol and mixed with 1.8 mL of deionized water and 0.2 mL of 0.1 M Folin-Ciocalteu reagent, and the mixture was thoroughly mixed. After 1 min of incubation, 1.0 mL of 20% sodium carbonate solution was added. Then, after 30 min of incubation at 35 °C, the optical density was measured at 750 nm, and the value was expressed as mg GAE g^{-1} (Velioglu et al. 1998).

2.4 Analysis of Total Flavonoid Contents

A total of 1 mg of concentrated seaweed extract was diluted with 300 µL of distilled water, followed by the addition of 150 µL of sodium nitrite (5%). The mixture was incubated for 5 minutes. Then, 2 mL of sodium hydroxide (4%) was added and made up to 5 mL with distilled water and left for 15 minutes at room temperature. The optical density was measured at 510 nm. The result was expressed as quercetin equivalent mg QE g^{-1} extract. (Janssen et al. 1998).

2.5 Analysis of Total Tannin Contents

total of 500 µg of concentrated extract was mixed with 200 mg of polyvinylpolypyrrolidone and 500 µL of distilled water and incubated at 4°C for 4 h. The mixture was then centrifuged at 5,000 rpm for 5 min, and 20 µL of the supernatant was collected after precipitation of tannin–polyvinylpolypyrrolidone complexes. The optical

density was measured at 725 nm and expressed as free phenolic content on a dry matter basis(Siddhuraju and Manian 2007). The total tannin content was calculated as follows:

$$\text{Tannins (mg GAE}^{-1}\text{ extract)} = \text{Total phenolic (mg GAE}^{-1}\text{ extract)} - \text{Free phenolic (mg GAE}^{-1}\text{ extract)}$$

2.6 Antioxidant Assessments

2.6.1 Total Antioxidant Activity (TAA)

Approximately 0.4 mg of the concentrated sample was mixed with 4.0 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate). Then the mixture was incubated at 95°C for 90 min. After that, the optical density of the sample was measured at 695 nm (Prieto et al. 1999). The result was expressed in mg g^{-1}.

2.6.2 DPPH Radical Scavenging Assay

Approximately 3.0 mg of the concentrated sample was added to 3.5 mL of 0.16 mM DPPH methanolic solution. Then the mixture was stirred for 1 min, left to settle for 30 min in the dark, and the optical density was measured at 517 nm (Yen and Chen 1995). The result was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{(A_1 - A_0)}{A_C}\right] \times 100$$

Where A_C is the absorbance of the control, A_1 is the absorbance of the concentrated sample, and A_0 is the absorbance of the sample only.

2.6.3 ABTS+Radical Scavenging Assay

Approximately 3.0 mg of the concentrated sample was added to the ABTS solution (5.0 mL, 7.0 mM), which was reacted with potassium persulfate (87.0 $\mu L, 141 mM$), kept in the dark overnight to obtain the ABTS+•, followed by dilution with 50% ethanol for absorbance at 734 nm (Yuan et al. 2005). The scavenging potency was expressed as the percentage of inhibition, which was calculated as follows:

$$\text{Scavenging Effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample

2.6.4 Ferric Reducing Antioxidant Power (FRAP) Assay

Approximately 1.0 mg of the concentrated sample was mixed with FRAP reagent (10 mM TPTZ solution + 0.3 M acetate buffer + 20 mM ferric chloride) in a 10:1:1 ratio. In the mixture, 1 mM $FeSO_4$ was added, then the absorbance was measured at 595 nm (Oyaizu 1986). The result was expressed as mg GAE g^{-1} on a dry weight basis.

2.7 Antiinflamatory Assays

2.7.1 Bovine Serum Albumin (BSA) Denaturation Assay

Approximately 0.08 mg of the concentrated sample was added to 0.45 mL of a 1% aqueous bovine serum solution (pH 6.3 using 1 N hydrochloric acid). The sample was then incubated at room temperature for 20 minutes, heated in a water bath at 55°C for 30 minutes, and, after cooling, the absorbance was measured at 660 nm (Das et al. 2019). Diclofenac sodium was used as the standard. The percentage of protein denaturation was determined using the following equation:

$$\text{\% Inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample

2.7.2 Egg Albumin Denaturation Assay

The samples (0.3 mL of egg albumin (fresh), 2.7 mL of phosphate-buffered saline (PBS) at pH 6.4, and 0.7 mL of the extract) were dissolved in 0.2% DMSO. The solution was incubated for 10 minutes at 37°C and then heated at 70°C for 20 minutes to induce denaturation of the egg albumin. After cooling the mixture, the optical density was measured at 660 nm (Ameena et al. 2023). The percentage of protein denaturation inhibition was calculated by the following equation:

$$\text{\% Inhibition} = \left(\frac{A_s}{A_c} - 1\right) \times 100$$

where, A_S represents the absorbance of the sample, while A_C represents the absorbance of the control.

2.8 LC-MS Analysis of the aqueous Extract

The concentrated aqueous extract of the seaweed sample was used for the analysis of Liquid Chromatography Mass Spectrometry (LC-MS). The obtained raw LC-MS data files were processed using the MZmine 2.5 software (Pluskal et al. 2010). First raw data (.d) files were converted to .mzml using MS Converter of Proteowizard. The "database search" module from MS-DIAL was used to search the MoNA LCMS Spectra Database (MassBank of North America).

2.9 Determination of Specific Growth Rate of Rohu

The *Labeo rohita* (Rohu) fingerlings were collected from the ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, and acclimatized in a water tank under a 12 h dark:12 h light photoperiod. Based on the water quality parameters, including ammonia concentration and pH, the tank water was renewed every 3 days. After 1

month of acclimatization, the fish were transferred to 25 × 30 cm tanks, containing four fingerlings. The nutritional compositions of seaweed extract are shown in Table 1. Feed ingredients were ground and thoroughly mixed with the addition of vegetable oil premix using a laboratory mixer and converted it in to small pellets with a handmade cone and dried to store in a zip-lock plastic bag for use (Serrano Jr and Aquino 2014). The survival rate was calculated by following the equation:

$$\text{Specific Growth Rate (SGR) (\% day}^{-1}) = \frac{\ln W_2 - \ln W_1}{t} \times 100$$

Where W_1 = Initial weight of fish, W_2 = Weight after feeding , t = Time period

2.10 Statistical Analysis

Every experiment was carried out in triplicate (n = 3), and the mean ± standard deviation (SD) was used to describe the results. The variability between repeat data for various biochemical, phytochemical, and antioxidant parameters was assessed using descriptive statistical analysis. The mean ± SD was used to display the results. Microsoft Excel was used for statistical analysis, and corresponding graphical representations were created.

3. RESULTS

3.1 Nutritional parameters of *U. intestinalis*

The nutritional composition of the *Ulva intestinalis* aqueous extract was determined and expressed as a percentage (%). The protein, carbohydrate, lipid, and moisture contents were 65.07 ± 1.25%, 29.77 ± 0.85%, 8.73 ± 0.42%, and 8.45 ± 0.35%, respectively (Figure 1).

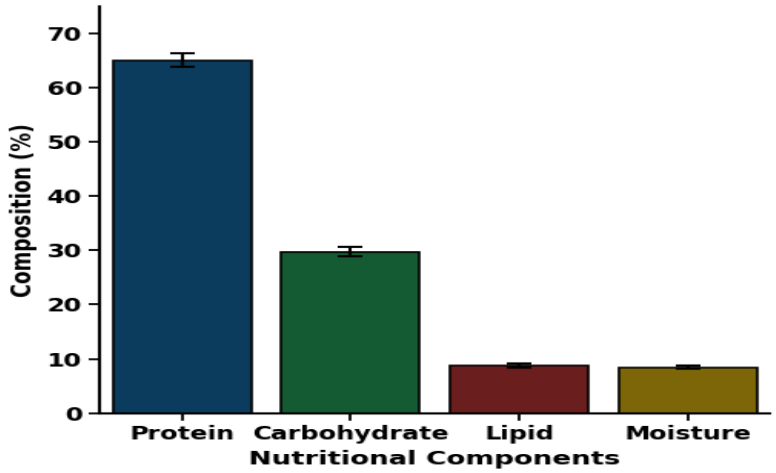


Figure 1. Nutritional constituents of *Ulva intestinalis* extract. Values are expressed as mean ± standard deviation (SD) of three replicates (n = 3)

3.2 Evaluation of TPC, TTC, and TFC Contents

The moisture content of the seaweed sample was stabilized to 10%, and then total phenolic contents (TPC) of the aqueous extract were evaluated using the Folin-Ciocalteu method, and it was 1.10. ±0.05 mg GAE g^{-1}. The total tannin content (TTC) was 1.79 ± 0.07mg GAE g^{-1}. The results are presented in **Figure 2**, and the total flavonoid content (TFC) was 8.27 ± 0.2mg QE g^{-1}.

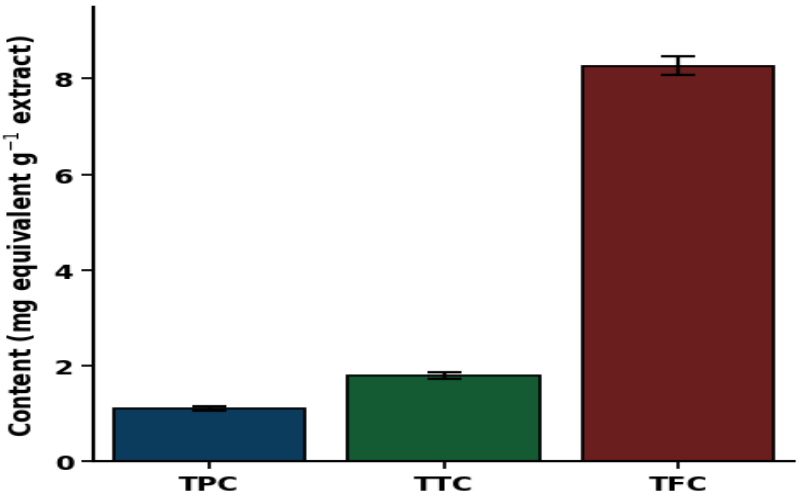


Figure 2. Phytochemical constituents of *Ulva intestinalis* extract. Values are expressed as mean ± standard deviation (SD) of three replicates (n = 3)

3.3 Analysis of TAA and FRAP Assay

The total antioxidant activity (TAA) of the aqueous extract was determined using the phosphomolybdenum method and expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE g^{-1}). In this assay, molybdenum (VI) is reduced to molybdenum (V), resulting in the formation of a green phosphate–Mo^{5+} complex. The aqueous extract of *U. intestinalis* exhibited a TAA value of 76.87 ± 4.00 mg AAE g^{-1} extract. The ferric reducing antioxidant power (FRAP) assay evaluates the ability of antioxidants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions through an electron-transfer reaction. The reducing capacity reflects the ability of antioxidant compounds to donate electrons, thereby reducing oxidized intermediates formed during lipid peroxidation and acting as both primary and secondary antioxidants. The FRAP value was 84.05 ± 2.01 mg AAE g^{-1} extract, which was higher than the TAA value, indicating a strong reducing and antioxidant potential of the extract. The results indicate that the aqueous extract of *U. intestinalis* possesses substantial antioxidant activity. The higher FRAP value suggests that the extract is particularly effective as an electron donor, enhancing its capacity to reduce oxidizing agents and potentially protect biological systems from oxidative damage. The values were depicted in Figure 3

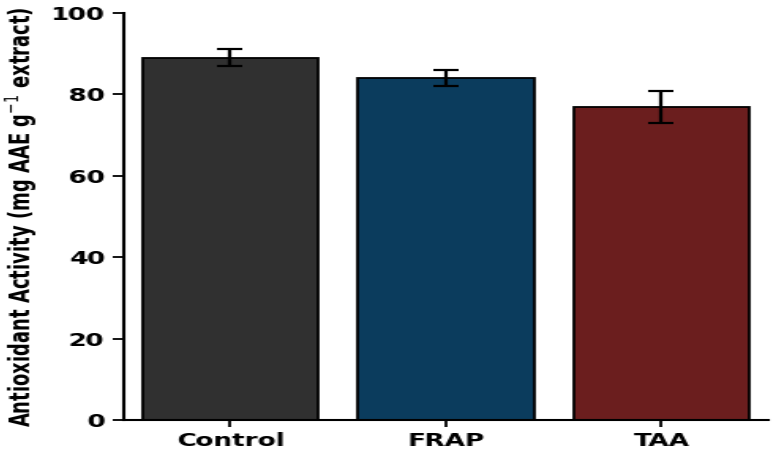


Figure 3. Activity of ferric reducing antioxidant power and total antioxidant activity compared with control (ascorbic acid) of *Ulva intestinalis* extract. Values are expressed as mean ± standard deviation (SD) of three replicates (n = 3)

3.4 Analysis of DPPH, ABTS+• Assay

The antioxidant potential of the extract was assessed using DPPH and $ABTS^{+\bullet}$ radical scavenging assays. Due to its hydrogen-donating capacity, the extract demonstrated a DPPH radical scavenging activity (RSA) of 36.21 ± 0.88%. In the $ABTS^{+\bullet}$ assay, the extract exhibited a higher RSA of 43.02 ± 5.10% (Figure 3), indicating moderate antioxidant activity against both radical species. Overall, the results suggest that the extract possesses moderate antioxidant activity, with a slightly greater effectiveness in scavenging $ABTS^{+\bullet}$ radicals compared to DPPH radicals.(Figure 4)

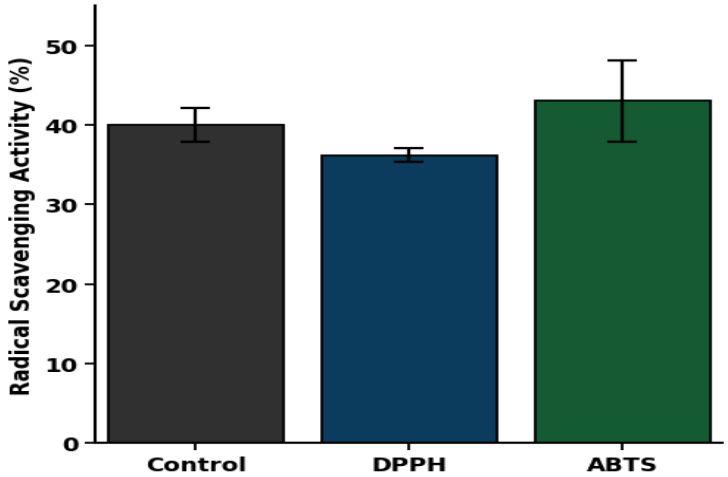


Figure 4. Radical scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) compared with control (ascorbic acid) of *Ulva intestinalis* extract. Values are expressed as mean ± standard deviation (SD) of three replicates (n = 3)

3.5 Analysis of Anti-Inflammatory Assay

The bovine serum albumin (BSA) denaturation assay is a very common and effective assay for the anti-inflammatory test that shows the percentage of protein denaturation. The percentage of inhibition was found to be 41.21 ± 4.01 %. Further, the egg albumin denaturation assay was conducted to estimate the percentage of protein denaturation, and the inhibition was 63.09 ± 2.01 % Figure 5.

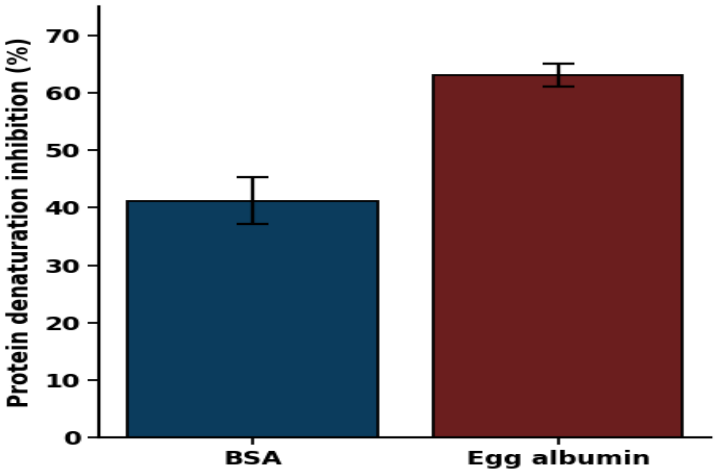


Figure 5. Bovine serum albumin and egg albumin assay of *Ulva intestinalis* extract. Values are expressed as mean ± standard deviation (SD) of three replicates (n = 3)

3.6 Liquid chromatography-mass spectrometry of Aqueous Extract

The liquid chromatography-mass spectrometry (LC-MS) analysis of the aqueous extract shows that the compounds that are elucidated in Table 1 have very important biological activities for pharmacological studies and in the biosynthesis of cellular proteins.

Based on retention duration, molecular formula, molecular weight, and precise m/z values, LC-HRMS analysis identified eight key bioactive chemicals in the *U. intestinalis* extract. Amino acids, peptide-related substances, alkaloid derivatives, and bioactive chemicals containing nitrogen were among the detected metabolites. Two significant amino acid components linked to protein synthesis were found to be L-phenylalanine and L-tyrosine. The extract's abundance of N-acetyl-L-glutamate was demonstrated by its greatest peak intensity (4.98×10^1). Other discovered compounds with antioxidant, antibacterial, anti-inflammatory, and immunomodulatory properties include (3-Arylcarbonyl)-alanine, Dodecylguanidine, 13-Hydroxylupanine, and chlortetracycline. The presence of many bioactive metabolites that might support the functional characteristics of *U. intestinalis* extract is highlighted by the LC–HRMS profile.

3.7 Optimization Data of Fish Feed Formulation vs Survival Rate

The experimental feeding trial was conducted using nine dietary treatments, including one control diet (D0) and eight experimental diets (D1-D8) supplemented with different concentrations of *U. intestinalis* extract. The control diet (D0) contained no supplementation, whereas the experimental diets contained 0.5%, 0.8%, 1.2%, 2.1%, 2.5%, 3.0%, 3.5%, and 4.5% *U. intestinalis* extract for D1, D2, D3, D4, D5, D6, D7, and D8, respectively (Table 2). The diets were formulated to be isonitrogenous and isolipidic, with the inclusion level of soybean meal adjusted to accommodate the varying concentrations of *U. intestinalis* extract while maintaining the overall nutritional balance of the feed.

Ingredient	**Control**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**
Rice and rice products	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Soybean meal	30.0	29.5	29.2	28.8	27.9	27.5	27.0	26.5	25.5
Maize and maize products	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Fish meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Fish oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Soy lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin–mineral premix	3								
Total	**100**								
Mineral premix					**Vitamin premix**				
Calcium (Ca)					Vitamin A				
Phosphorus (P)					Vitamin B				
Sodium (Na)					Vitamin C				
Magnesium (Mg)					Vitamin D_3				
Cobalt (Co)					Vitamin E				
Zinc (Zn)					Vitamin K_3				

Manganese (Mn)	
Iron (Fe)	
Copper (Cu)	
Selenium (Se)	

Note: Control = basal diet without *Ulva intestinalis* extract; D1–D8 represent experimental diets containing increasing levels of *Ulva intestinalis* extract.

The fingerlings, with an average body weight of 23 ± 4.00, were used in the experiment and were fed 5% of their total body weight of the respective experimental diets. The highest survival rate (95%) was recorded in the treatment containing 3.50% seaweed extract, as depicted in Figure 6. However, the survival rate declined as the concentration of seaweed extract in the feed increased beyond this level.

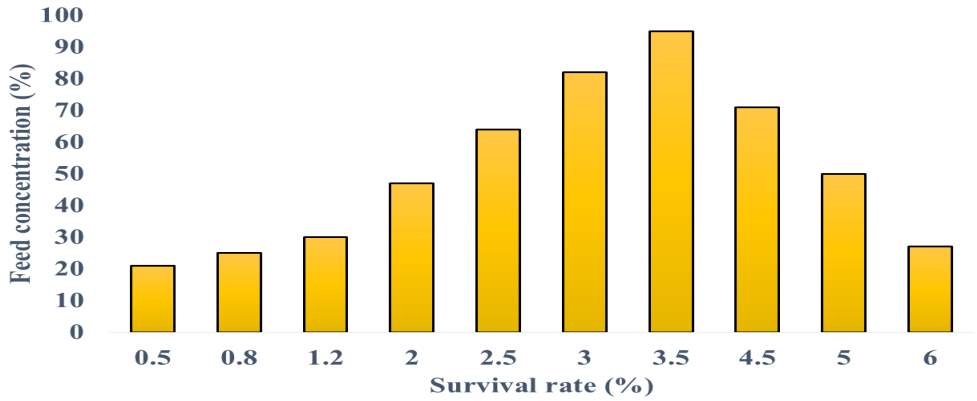


Figure 6. Different feed concentrations vs survival rate of *Labeo rohita* fingerlings

4. DISCUSSION

Seaweeds are major economic resources in the aquatic environment. Approximately 10,000 metabolites have been isolated from marine organisms that have a significant effect on the pharma industries (Carpena et al. 2026). In the present study, the presence of phytoconstituents such as phenolics, flavonoids, and tannins in green seaweeds indicates the possibility of their importance for their antioxidant activities (Gunathilake et al. 2022; Rahman et al. 2021; Jomova et al. 2025). Phenolic compounds are commonly found in terrestrial plants and have been reported to have numerous biological activities, including antioxidant and antimicrobial properties (Dash et al. 2023; Pradhan et al. 2025; Das et al. 2025; Jena et al. 2026). Many studies focused on the biological activities of phenolic compounds, which have antioxidant potential and free radical scavenging ability (Fernando et al. 2016; Rahman et al. 2021; Kruk et al. 2022; Sinha et al. 2024). In the present study, the phenolic content of *U. intestinalis* was evaluated using the Folin-Ciocalteu method and was expressed as GAE. The observed result was similar to the phenolic compound present in *U. intestinalis* (Farasat et al. 2014). Total tannin content was examined by treating with polyvinylpolypyrrolidone, and the result was nearly similar to the study of (Alghazeer et al. 2022). Total flavonoid content was comparatively higher than both the TPC and TTA, and the result was found to be similar to that of *U. intestinalis* (Farasat et al. 2014). Duan et al. 2006, suggested a significant correlation of antioxidant activity with phenolic content of the green seaweeds. Many green seaweed species contain phytochemicals such as phenols, flavonoids, and tannins, which could be responsible for the potential antioxidant activity by preventing many diseases through free radical scavenging activity (Ahmed et al. 2024; Golmakani et al. 2025), and in our study, the antioxidant activity of *U. intestinalis* could be due to these compounds. There are several methods for antioxidant activity tests, which differ in their experimental conditions and principle. Consequently, the antioxidant potential has varying contributions to the total antioxidant potential (Cao and Prior 1998). Antioxidant activities were examined using four different assays, i.e., total antioxidant activity, FRAP assay, DPPH radical scavenging activity, and ABTS assay. TAA was evaluated and found to be nearly similar to the result of (Farghl et al. 2021). The antioxidant potential is proportional to the combined ferric reducing power of the antioxidants in the sample (Benzie and Szeto 1999). The reducing power indicates that the antioxidant compounds behave like electron donors and have the ability to reduce the oxidized intermediates of lipid peroxidation. In our study, we found a good result of reducing the activity of the *U. intestinalis*. A stable free radical, DPPH, was used for another antioxidant activity. Decolorization of DPPH proves the presence of electron donors in aqueous extract. This could be attributed to the higher amount of total phenol compounds found in *U. intestinalis*. Our study was comparatively higher than the study of (Meenakshi et al. 2011). The radical scavenging activity of *Ulva intestinalis* indicates moderate antioxidant potential. Similar findings were reported in *Ulva* species, where ABTS scavenging activity ranged between 20–70% depending on extract type and concentration. The antioxidant activity may be associated with the presence of sulphated polysaccharides and phenolic compounds. Comparable results in related green seaweeds support the reliability of the present study. Therefore, *U. intestinalis* can be considered a promising natural antioxidant source (Peasura et al. 2015).

The anti-inflammatory activity of *Ulva intestinalis* aqueous extract was confirmed through BSA denaturation and egg albumin assay. The extract effectively inhibited protein denaturation, indicating its potential to stabilize proteins during inflammation. Similar activities have been reported in green seaweeds because of the presence of phenolics, flavonoids, and sulfated polysaccharides (Xu et al. 2023). These results suggest that *U. intestinalis* possesses promising natural anti-inflammatory properties and is similar to the previous investigation of (Sanniyasi et al. 2023). Liquid chromatography-mass spectrometry (LC-MS) analysis of the seaweed extract revealed a diverse array of bioactive compounds, including amino acids, alkaloids, and phenolic derivatives, which may contribute to its biological effects. The presence of L-phenylalanine and L-tyrosine underscores the nutritional and metabolic significance of seaweed extract, as these amino acids are essential for protein synthesis and cellular metabolism. Compounds such as N-acetyl-L-glutamate and N-acetylmuramoyl-alanine are associated with metabolic regulation and antimicrobial properties. Furthermore, the detection of (3-arylcarbonyl)-alanine and 13-hydroxylupanine suggests potential antioxidant and anti-inflammatory benefits. The identification of metabolites analogous to chlortetracycline indicates possible antimicrobial and immunomodulatory effects. Collectively, these findings suggest that the seaweed extract contains several pharmacologically significant metabolites that may account for its therapeutic and nutraceutical potential. Some of the compounds of our present study were similar to the methanolic extract of seaweed (Jhoomuck and Mund 2025).

4.1 Optimization of Feed Formulation and Survival Rate

The feed optimization study demonstrated that the survival rate of *Labeo rohita* fingerlings was significantly influenced by the concentration of seaweed extract incorporated into the feed formulation. However, a further increase in concentration resulted in a decline in survival, indicating that excessive supplementation may adversely affect fish health and feed acceptability (El-Tawil 2010). The enhanced survival observed at moderate supplementation levels may be attributed to the presence of bioactive compounds such as phenolics, flavonoids, amino acids, and antioxidant metabolites that may improve antioxidant defense mechanisms, immune response, and physiological stability in aquatic organisms. Similar findings have been reported in previous studies where seaweed-derived feed additives enhanced growth performance, stress tolerance, and survival of cultured fish species (Sumana et al. 2026). The decline in survival rate beyond the optimum concentration suggests that higher levels of seaweed extract may alter feed palatability, nutrient balance, or metabolic activity, thereby reducing feed utilization efficiency. Therefore, the present findings indicate that a 3.5% seaweed extract incorporation level may be considered optimal for improving the survival performance of rohu fingerlings.

Overall, the present study confirms that *Ulva intestinalis* possesses considerable antioxidant and anti-inflammatory potential, as evidenced by the diverse bioactive metabolites identified through phytochemical screening and LC–MS analysis. N-acetylmuramoyl stimulates the innate immune system; on the other hand, N-acetylmuramoyl-L-alanine, a metabolite produced from peptidoglycan, may serve as a natural immunostimulant (Guinand et al. 1984). Immune receptors' recognition of peptidoglycan fragments increases cytokine production, phagocytosis, and macrophage activity, strengthening the host defense system. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which shield tissues from oxidative stress generated by immune responses, are often activated in concert with these immune pathways (Jena et al. 2023). As a result, this molecule may lessen oxidative damage and increase disease resistance, enabling a higher allocation of metabolic resources toward development.

Furthermore, the production of thyroid hormones, catecholamines, and melanin, all essential for controlling metabolism, development, and stress adaptation, requires the multifunctional amino acid L-tyrosine. The combined presence of these bioactive metabolites suggests that *U. intestinalis* extract improved feed utilization and survival performance in rohu fingerlings. These findings support the synergistic action of the bioactive metabolites found by LC–MS, which may be responsible for the improved growth performance and survival of *L. rohita* fingerlings given diets enriched with *U. intestinalis* extract. While N-acetyl-L-glutamate promotes nitrogen metabolism and effective use of dietary amino acids, the availability of proteinogenic amino acids, such as L-phenylalanine and L-tyrosine, provides necessary substrates for protein synthesis and appropriate physiological development (Jiang et al. 2025). Additionally, N-acetylmuramoyl-L-alanine has immunostimulatory qualities that may boost innate immune responses, improving fish health and survival (Taziki et al. 2023). By scavenging reactive oxygen species and shielding biological components from oxidative damage, the antioxidant metabolites included in the extract are also expected to lessen oxidative stress (Hissen et al. 2023). The potential application of *U. intestinalis* as a sustainable and functional feed additive for aquaculture contributes to improved fish health and production efficiency.

5. CONCLUSION

The current study highlighted the potential of *Ulva intestinalis* as a functional component for aquafeed applications by showing that it has a positive nutritional profile and includes a variety of phytochemicals with noteworthy antioxidant and anti-inflammatory effects. Feed optimization in *Labeo rohita* determined an appropriate dietary inclusion level of *U. intestinalis* extract. According to these results, *U. intestinalis* shows promise as a sustainable and natural feed component for aquaculture. However, further research is necessary to assess its long-term impacts on immune response, disease resistance, and growth performance under commercial culture settings.

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Author Contributions

- **Sasmita Das:** Conceptualization, Investigation, Formal analysis, Visualization, Writing–original draft.
- **Lakshmi Singh:** Conceptualization, Supervision, Writing–review & editing.

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Competing Interests

- The authors have no conflict of interest.

Data Availability

- The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request, subject to institutional approval.

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