Exploring the Composition and Functional Attributes of Brown Seaweed for Promoting Nile Tilapia Growth under Cold Stress

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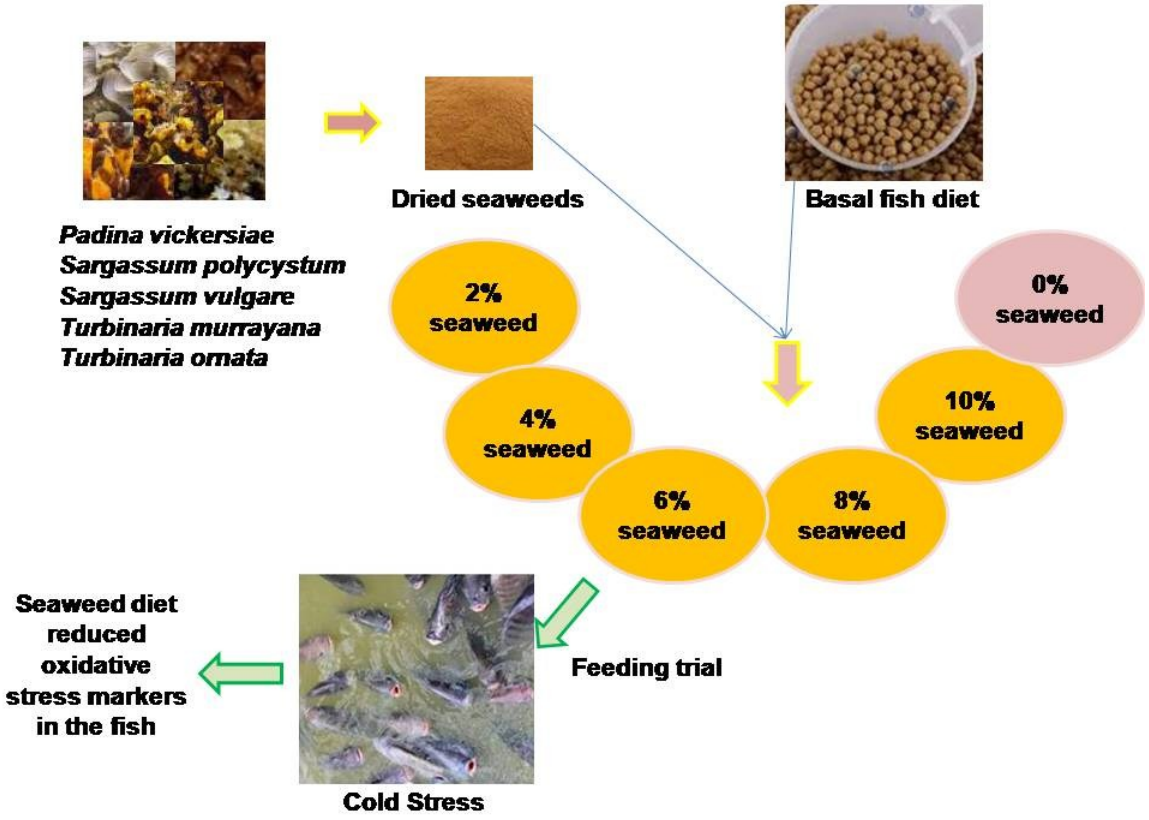
GRAPHICAL ABSTRACT



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The proximate composition and functional attributes were analyzed for brown seaweeds (*Padina vickersiae*, *Sargassum polycystum*, *Sargassum vulgare*, *Turbinaria murrayana*, and *Turbinaria ornata*)relative to the growth of Nile tilapia fish. The collected seaweeds were dried in an oven at 70 °C for 12 h. Seaweed contained a maximum of total protein, dietary fibre, and low concentration of lipids. The nutrient rich seaweeds were mixed with the fish diet at various concentrations (2% to 10%). The formulated feed was fed to tilapia, which improved growth performance, feed conversion ratio, and specific growth rate after 60 days of experiment. At 8% concentration of seaweed in the fish diet fish growth was improved significantly relative to the control diet ($p<0.05$). To analyse cold stress, *Oreochromis niloticus* were maintained at 15 °C for seven days. The administered seaweed diet significantly decreased catalase activity ($p<0.05$) and lipid peroxidation activity ($p<0.05$) in tilapia fish relative to the control (without seaweed mixture). The supplemented brown algal diet improved superoxide dismutase enzyme activity ($p<0.05$) and glutathione S-transferase activity than control fish ($p<0.05$). The present findings revealed that brown algae have the potential to be used as feed supplements to improve the growth and health benefits of Nile tilapia.

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Keywords: Seaweeds; Brown algae; Antioxidants; Stress mitigation; Functional feed; Nile tilapia; Growth performance; Oxidative stress; Cold stress

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INTRODUCTION

Seaweeds are nonflowering photosynthetic marine macroalgae that occur in oceans and seas. Seaweeds are broadly classified into three major groups, namely, green, brown, and red algae, and this classification is based on the colour of macroalgae. Seaweeds are rich in macro- and micronutrients and several bioactive secondary metabolites (García-Vaquero and Hayes 2016; Corino *et al.* 2019; Premarathna *et al.* 2022). In recent years, several natural sources have been identified as potent molecules with biological activity in fish. Seaweeds include numerous bioactive secondary metabolites, such as phenolic compounds, carotenoids, peptides, tocopherols, and several carboxylated polysaccharides

and sulfated polysaccharides, including ulvan, alginate, and fucoidan (Felipe *et al.* 2025). Seaweeds that contain sulfated polysaccharides have been identified as antioxidant molecules due to their hydrogen, which reacts with radicals, forming stable radicals and completely arresting radical chain reactions (Shao *et al.* 2013; Hardouin *et al.* 2016). In addition, seaweed sterols exhibit anti-inflammatory activity and cholesterol-lowering activities (Melchor-Martínez *et al.* 2025). Seaweed phytochemicals act as prebiotics to improve the health and growth profile of aquatic organisms, especially fish, *via* several molecular mechanisms (Wang *et al.* 2014; Véliz *et al.* 2023). Seaweeds contain various bioactive secondary metabolites. The chemical composition of marine macroalgae can be significantly influenced by various factors, including the harvesting season, drying methods, geographical conditions, seaweed types, and environmental conditions (Verma *et al.* 2017). Chemical composition of seaweeds can also be affected by climate change. *Ulva* was cultivated under controlled environmental conditions in order to understand the climatic change. Under culture conditions, increased temperature improved amino acids, and supplemented nitrate increased most of the amino acids and improved functional properties (Gao *et al*. 2028). In seaweeds, laminaran is a major storage of carbohydrate which has several biological functions, including, antimicrobial, antioxidant, wound healing and antioxidant activities. Laminaran production varied based on environmental temperature and CO_2-enrichment in the ocean (Chen *et al*. 2024). The fresh brown and red seaweed have 26.9 to 74.1% carbohydrate content, 76.0 to 96.0% moisture content, 6.0 to 45.0% ash content, 4.0 to 34.7% crude fibre content, 5.2 to 17.3% crude protein content, and 0.15 to 0.84% crude fat content (Ahmad *et al.* 2012). Previous studies revealed that seaweed has various health-promoting and growth-enhancing activities (Kidgell *et al.* 2019). Many macroalgal species have been used in industry, especially for the preparation of phycocolloids and pharmaceutically important substances. In addition, they are applied as fertilizers, herbal medicines, herbicides, fungicides, and human nutrition. Macroalgae are rich in vitamins, minerals, essential fatty acids, fibres, and proteins. They have been used in traditional medicine in Japanese, Chinese, and Korean diets (Dawczynski *et al.* 2007; Azizi *et al.* 2021). They can be consumed as soups, salads, condiments, and meals. Seaweeds are rich in carbohydrates, have low protein and fat contents, and add minimal calories to the diet. Brown seaweed varieties consist of laminaran (β-1,3-glucan), fucoidan, alginates, cellulose, and mannitol. The fibres of brown seaweed are rich in cellulose and insoluble alginates. These are magnesium, calcium, or sodium salts of alginic acid (Hughes *et al.* 2025). In contrast, red seaweed consists of various types of carbohydrates, including xylan, cellulose, Floridean starch (α-1,4-bindingglucan), and mannan. The water-soluble fibres of seaweed are composed of sulfur-containing galactans (*e.g.*, carrageenan and agar). Seaweeds contain minerals, such as K, Na, Mg, Ca, Zn, Fe, and Mn, that are essential for all life (Shen *et al.* 2025). Moreover, these essential minerals are rare in terrestrial plants and vary among seaweed types, geographical locations, and seasons. Macroalgae contained the maximum amount of calcium (7%), which was high in chalky seaweed (>25%). Brown seaweeds contain minerals, vitamins, proteins, and several bioactive compounds, including polysaccharides, fucoidan, and polyphenols. Because aquaculture production encourages the use of prebiotics and restricts the application of antibiotics, brown algae have drawn increased attention in recent years. Alginate is one of the key phytochemical compounds in brown algal cell walls and has anti-inflammatory, antibacterial, and antioxidant functions (Lu *et al.* 2022).

The oligosaccharides extracted from the seaweeds showed prebiotic activity and improved enzyme production. The administration of seaweed improves drug resistance,

improves growth and intestinal functions, and alleviates intestinal inflammation in animals (Samarasinghe *et al.* 2021). Supplementation with brown algae improved intestinal health, enhanced immune function, improved the intestinal morphology and height of long villi, induced microbial flora and improved beneficial microbial flora. The supplementation of brown algae with a fish diet improved feed conversion efficiency, growth, and immune function. The intensive culture of aquaculture species aims for sustainable development and high-quality protein (Sultana *et al.* 2023).Tilapia adapts to survive in warm water; however, they are less tolerant of cold water. A decrease in water temperature causes mass death in cultured tilapia, resulting in major economic losses. Therefore, minimizing this cold challenge in tilapia cultured in subtropical regions by improving their cold tolerance potential would help improve their growth and reduce mortality. The dietary interventions of tilapia with seaweed, especially brown algae, could improve digestibility and antioxidant activity and mitigate cold stress (Bezerra *et al*. 2025). In fish, temperature is an important physical factor involved in sex differentiation/determination during the sexual labile period. During this time, temperature affects sex-determining genes expression. The sexual labile period mainly occurs during very early phase of larval stage and the end during the juvenile stage. In early stages of development, fish are highly susceptible to external temperate, which influences sex differentiation and growth. It has been previously reported that low temperature induces feminization in cobalt cap silverside (*Hypoatherina tsurugae*) (Miyoshi *et al*. 2020). In tilapia, above 25 °C is ideal for spawning, and spawning is affected between 20 and 24 °C and it was less between 14 and 20 °C (Palmer *et al.* 2024).

The seaweed mixture in the fish diet improves fish growth, and adequate mixture can positively affect fish gut health. Notably, the optimum concentrations of bioactive compounds from the seaweeds, especially minerals and vitamins were associated with higher growth and these components improved assimilation of dietary nutrients in fish. Fish diet are prepared using more than one seaweed and are linked to the presence of optimum concentration of phytochemical compounds, reduced level of antinutritional factors and improved absorption of essential nutrients (Siddik *et al*. 2023). Moreover, most of the previously published research lacks the utilization of brown algae for fish feed formulation at optimum level and subsequent fish growth profile analysis and validation of the functional properties of the fish diet. In addition, analysis of brown algae on stress mitigation effect is not completely studied. To fill this research gap, the present study aimed to analyse the chemical composition of seaweed and incorporate seaweed powder to improve the growth, feed conversion efficiency of fish, and to mitigate cold stress. This research elucidates the identification of bioactive compounds, their composition, the growth profile of fish, and the mitigation of cold stress.

EXPERIMENTAL

Seaweed Collection

Seaweeds were collected from the Gulf of Mannar, Tuticorin, India, at a depth of 2 meters in July 2024. The collected seaweeds were washed with seawater to remove sand particles and associated epiphytes. The fresh seaweeds were identified as *Padina vickersiae*, *Sargassum polycystum*, *Sargassum vulgare*, *Turbinaria murrayana*, and *Turbinaria ornata* (Voucher numbers: SWD/148/2024, SWD/149/2024, SWD/150/2024, SWD/151/2024 and SWD/152/2024). They were dried in an oven at 70 °C for 12 h. The

samples were further ground mechanically, and the finely ground particles were <0.5 mm in size and stored at −4 °C.

Proximate Analysis of Seaweeds

The contents of ash, dry matter, moisture, lipids, dietary fibre, and protein were determined according to the AOAC method (AOAC 2005). The fresh seaweeds were utilized for the analysis of moisture content. Dried seaweed samples were used for the determination of total ash content. The dried samples were pre-weighed and placed in a muffle furnace for 6 h at 600 °C until a constant weight was obtained. To determine the dry matter content, the seaweed samples were continuously dried at 40 °C in an oven until a constant weight was obtained. Kjeldahl total nitrogen method was applied to assay the percentage total protein. The obtained total nitrogen content was further multiplied ×6.25 (ISO 1978). The total lipid content of the seaweed samples was determined *via* the chloroform:methanol (2:1, v/v) method with slight modifications. A total of 1 g of dried seaweed powder was mixed with 9 mL of the solvent mixture and vortexed for 60 s. The mixture was placed in an ultrasonic water bath for 30 min at 40 °C. Then, 3 mL of 2% sodium chloride solution was added. The mixture was shaken vigorously for 60 s and left undisturbed for 5 min. The chloroform layer contained lipid and was removed *via* a Pasteur pipette. Then it was subsequently allowed to evaporate at 31±1 °C. The solids remaining after solvent evaporated were measured, and the percentage lipid content was calculated (Folch *et al.* 1957).

Analysis of Trace Elements

The amount of seaweed minerals present was determined *via* an atomic absorption spectrometer (iCETM 3000 series, Thermo Scientific, Waltham, MA, USA). Approximately 0.2 g of dry ash was mixed with 20 mL of nitric acid and allowed to stand for 4 h. This mixture was further filtered with Whatman filter paper (No. 40), and the filtrate was diluted with Millipore water and minerals (Mg, Cu, Mn, Zn, Fe, Li, Ca, K, Ni, and Na) (Suárez *et al.* 2007).

Amino Acid Composition of Seaweeds

The amino acid content of the seaweed was determined using high-performance liquid chromatography (HPLC) (Agilent Technologies, Inc., Santa Clara, CA, USA). The seaweed powder (0.4 g) was hydrolysed with 4 mL of 6 N hydrochloric acid (HCl) and heated for 24 h at 110 °C. The hydrolysate obtained after acid treatment was cooled at room temperature (29±1 °C). It was transferred into a 100 mL Erlenmeyer flask containing 50 µmol/mL α-aminobutyric acid in 0.1 M HCl with Millipore water. The sample was filtered using 0.2 µm syringe filter and 20 µL filtrate was collected into a microcentrifuge tube. About 10 µL of filtered sample was injected into a HPLC (Waters 2475, Waters Co., Milford, MA, USA) system. The mobile phase was filtered through 0.45 µm cellulose membrane filters before it was used. The mobile phase used was: (A) AccQ Tag Eluent A (200 mL AccQ Tag to 2 L of ultrapure water) and (B) HPLC grade acetonitrile (60%). The standard amino acid mixture (10 µL) (18 amino acids) (2.5 µmol/mL in 0.1N HCl) was used as standard for HPLC analysis of seaweed protein hydrolysates. Elution was performed for 30 min with the flow rate set at 1 mL/min. A fluorescence detector(Agilent Technologies, Inc., Santa Clara, CA, USA) was used to detect amino acids at 340 nm (excitation wavelength) and 450 nm (emission wavelength). The amount of each amino acid was determined from the peak area of known quantity of amino acid standard mixture and peak area of individual amino acid in seaweed hydrolysate (Biancarosa *et al.* 2017).All

the measurements of amino acids were carried out in triplicate, and the results were expressed as mg/100 g dry seaweed.

Extraction of Seaweed Phytochemicals

A conventional reflux extraction method was followed with minor modifications. Briefly, 120 mL of ethanol and 60 mL of water were mixed and placed inside the extraction vessel. A total of 5 g of dried sample was used for analysis, and a paper cartridge was used for extraction. Solvent extraction was performed with solvent reflux for 6 h (AOAC 2005).

Analysis of Total Phenolic Content

The total phenolic content (TPC) of the seaweeds was determined as described previously (Singleton *et al.* 1999). The dried seaweed (50 g) was mixed with 500 mL methanol and extracted using a Soxhlet apparatus. It was dried and 0.1 g of dried extract was mixed with 10 mL methanol. Then, 0.2 mL of the solvent extract was mixed with 2 mL of double distilled water, 0.5 mL of Folin–Ciocalteu reagent, and 0.2 mL of a 6% sodium carbonate solution. The sample was mixed and incubated in the dark at room temperature for 30 min. The absorbance of the sample was measured at 730 nm using a UV–Vis spectrophotometer. Gallic acid (GA) was used as the positive control, and the results are expressed as mg of GA/100 g of sample.

Analysis of Antioxidant Activity

For the antioxidant assay, the seaweed extract (0.1 g) was mixed with 1.9 mL of 80% methanol and sonicated at room temperature for 30 min. The DPPH radical scavenging assay of the seaweed extract was performed according to the method described by Brand-Williams *et al*. (1995). A total of 0.05 mL of the sample was mixed with 1.95 mL of 60 µmol/L DPPH solution. The mixture was subsequently incubated in the dark for 30 min at ambient temperature. The absorbance of each sample was read at 515 nm against a reagent blank *via* a spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The ABTS radical scavenging assay was performed as suggested by Al-Momani *et al*. (2023), with slight modifications. Briefly, 0.05 mL of sample was mixed with 1.95 mL of ABTS reagent and incubated at room temperature for 5 min. The absorbance of the sample was measured at 734 nm *via* a spectrophotometer (AgilentTechnologies, Santa Clara, CA, USA). Triplicate experiments were performed, and the mean value was used for data analysis.

Formulation of Experimental Diets

The seaweeds were dried, and the finely powdered sample was used for the preparation of artificial feed. The chemical compositions of the experimental diets were determined and are presented in Table 1. The formulated crude protein content was 30%, and wheat gluten, soybean meals, corn powder and wheat bran were used as protein sources. The formulated experimental diets meet the requirements of tilapia juveniles. In the control group, seaweed powder was not incorporated into the basal diet. The experimental fish feed was prepared by maintaining the seaweed powder at 2% to 10% *P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, or the *T. ornata* mixture in the control diet. The equal ratios of all five seaweeds were selected at inclusion level. Macroalgae powder combined with fish feed contains equal amounts of all five algae (Ghosh *et al.* 2011). Fish meal is used as the major protein source in formulated fish diets, due to the presence of balanced amino acid, excellent digestibility, palatability, and absence of anti-nutritional factors and fat- and water-soluble vitamins (Galkanda-Arachchige *et al*. 2020;

Hardy 2010). An overview of the feed formulation and application is shown in Fig. 1. Control diet was prepared without any seaweed powder.

Table 1. Feed Ingredients and Chemical Content (% of Dry Matter) of a Fish Diet Containing a Mixture of 2 to 10% Seaweed

Ingredients	Composition (%)
Fishmeal	8.2 ± 0.02
Wheat flour	22 ± 0.27
Soybean meal	31 ± 2.2
Wheat gluten	12 ± 0.65
Vegetable oil	2 ± 0.04
Rice bran	15 ± 0.2
Fish oil	2 ± 0.01
Starch	3 ± 0.05
Lysine	1 ± 0.03
Sodium chloride	1 ± 0.09
Vitamin premix	1 ± 0.041
Mineral premix	1 ± 0.02
Seaweed mixture*	2 - 10

**P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, or *T. ornata*

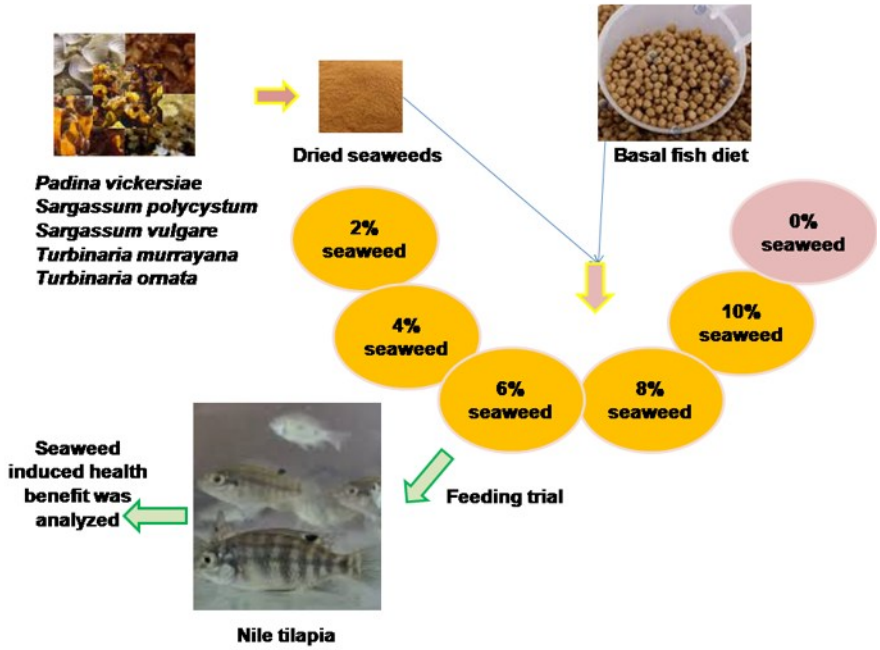

Fig. 1. Feed formulation and growth performance analysis of Nile tilapia fed various concentrations of five seaweeds (*P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, and *T. ornata*) at five different concentrations (2 to 10% seaweed) for 60 days in experimental trials

Nile Tilapia (*Oreochromis niloticus*) Culture Conditions

Oreochromis niloticus were introduced to six experimental tanks containing 75 L of filtered water with sufficient aeration, and the temperature was 28 ± 1 °C. In each tank, 25 randomly collected fish were introduced and further acclimatized in the culture tank for

15 days. During acclimation period, fish were fed with artificial pellet diet at 5% body weight two times daily. Then, the experimental or control fish were fed a commercial fish diet twice daily. After 15 days of acclimatization, the fish were fed either the experimental (commercial fish diet containing seaweed powder) or the control diet (without seaweed powder). The experimental animals fed the seaweed diet were considered the experimental groups, and the amount of fish feed was determined on the basis of preliminary experimental trials. The experimental period was two months, during which experimental and control animals were maintained at ambient temperature. The oxygen content was monitored regularly and maintained at >5 mg/L in all tanks. The unused and fecal pellets were siphoned regularly, and one-fourth of the tank water was exchanged daily. Following the two-month feeding trial, the tilapia were counted, and all the fish were weighed. (Hepher *et al.* 1983).

Fish Plasma

The starved fish were randomly collected from the experimental and control tanks and further anaesthetized *via* MS-222 solution at the optimum concentration (2 g/L) prepared in sodium bicarbonate buffer. A heparinized syringe was used for the collection of peripheral blood from the caudal vein. The collected blood (1 mL) was transferred to a microtube containing 20 µL of heparin solution prepared in physiological saline (3000 U/mL). After that, the blood was centrifuged at 5000 × *g* for 5 min at 4 °C, and the separated plasma sample was stored at −80 °C until use.

Analysis of Biochemical Parameters

Total protein, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels were determined *via* a clinical diagnostic kit according to the manufacturer's instructions (Habib *et al.* 2022).

Culture of Tilapia under Cold Stress and Determination of the Oxidative Stress Response

All experiments were performed in accordance with the ethics and animal care committee (Ethical approval number: JM2025-194). *O. niloticus* were maintained in culture tanks containing 75 L of water, and the temperature of the water was reduced to 15 °C from ambient temperature (31±1 °C).The fish were maintained in good condition, and a feeding trial was performed for one week. They were fed with experimental or control diets. After one week, fish were collected from both experimental and control tanks. Then, livers were dissected from the experimental and control fish and homogenized in a glass homogenizer with 1 mL of phosphate buffer (pH 7.4, 0.1 M). The crude sample was centrifuged at 5000 ×*g* for 5 min at 4 °C, and the clear supernatant was collected and stored at −80 °C until use. Oxidative stress markers (glutathione S-transferase (GST) activity, catalase (CAT) activity, lipid peroxidation (LPO), and superoxide dismutase (SOD) activity) were analysed. The results are expressed in milligrams of protein. The SOD activity was expressed as percentage inhibition (Marmelo *et al.* 2024).

Statistical Analysis

All experiments were performed in triplicate, and the mean value was used for analysis. The results are presented as the means ± standard deviations (SDs). Analysis of variance (ANOVA) was used to analyse the experimental results, and Tukey's post hoc test was used to compare the data. Statistical analyses were carried out *via* SPSS software

(version 24, IBM Corp., Armonk, NY, USA), and a p value <0.05 was assumed to indicate statistical significance.

RESULTS AND DISCUSSION

Proximate Analysis of Seaweeds

The approximate compositions of five selected seaweeds (*P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, and *T. ornata*) were analysed. The mean ash, dry matter, lipid, moisture, protein, and dietary fibre contents are shown in Table 2. In the study, the ash content of brown algae ranged between 5.24 ± 0.76% and 40.2 ± 0.21%. The dry matter content was high in *S. vulgare* (5.02 ± 0.14%), whereas the lipid content was high in *P. vickersiae* (4.11 ± 0.03%). The fresh seaweeds were utilized for the analysis of moisture content. The moisture content varied between 74.01 ± 0.52% and 83.4 ± 0.11%.The present finding was similar with the report of Pizarro-Oteíza *et al.*(2025) and has been reported as 87.5 ± 0.03% moisture in the fresh Brown Seaweed (*Lessonia spicata*). The total protein and dietary fibre contents were greatest in *S. polycystum* (17.4 ± 0.01%) and *P. vickersiae* (61.3 ± 0.52%). The protein content observed in this study was higher than that of previous report (Kasimala *et al.* 2017). In this study, ash content ranged from 5.24 ± 0.76 to 40.2 ± 0.21%. Earlier studies revealed that the ash content of seaweeds ranged between 19.07 ± 0.61% and 34.00 ± 0.11% (Sánchez-Machado *et al.* 2004), and the present result was consistent with earlier results. The continuous intake of brown seaweed has numerous health benefits and prevents several diseases, including fever prevention, fluid retention, and intelligence improvement. The proximate contents of *Gracilaria corticata*, *Enteromorpha clathrata*, and *Sargassum linearifolium* were analysed.

The seaweed was dried in an oven at 70 °C for only 12 h to reduce heat-labile nutrients and bioactive compounds. Drying is one of the important steps to preserve the seaweed and improve shelf life; dehydration can also negatively influence the colour, quality, aroma, and phytochemical content of the seaweed (Chan *et al*. 1997). In this study, an initially large volume of seaweeds was collected and shade-dried to reduce microbial growth. The quality of collected seaweed can be significantly reduced if useful phytochemical compounds in the seaweeds are lost as a result of the drying process. For example, amino acids, lipids, proteins, fatty acids and other bioactive metabolites in macroalgae can be affected significantly by drying (Sappati and Nayak 2018). The amount of phenolic compounds is often reduced when using excessive drying procedures, which may further result in reduced antioxidant potential (Cruces *et al*. 2016). These previous studies have shown the value of selecting suitable drying methods in order to preserve useful bioactive compounds within seaweeds that are used for the preparation of functional feed. Of the drying methods that have been described for seaweed, oven-drying and sun-drying are the common methods, and these methods are considered low-cost options (Kadam *et al*. 2015). The freeze-drying method has been considered one of the valuable methods for seaweed when compared to other methods (Wong and Cheung 2001). However, the freeze-drying method is a highly expensive method and is not common for the commercial processing of seaweeds. Despite its higher cost, freeze drying preserves the nutritional and bioactive properties of seaweed more effectively than other methods. As such, it may be more suitable for applications where quality is paramount, even if it limits broader commercial viability. The moisture content ranged between 85.3% and 90.9% and was high in *G. corticata*, whereas the carbohydrate content ranged between 23.5% and 29.0% and was greatest in *E. clathrata*. The protein content ranged from 6.9%

to 13.6%, and lower lipid contents have been reported (Kasimala *et al.* 2017). The ash content observed in this study was lower than that reported previously, and the protein content was lower (59to201 g kg^{-1}dw) than that reported in the present study (Olsson *et al.* 2020). The ash content ranged from 5 to 29.8% for brown algae, and the crude carbohydrate content ranges from 21 to 29.5% (Alghazeer *et al.* 2022). The dietary fiber comprises a mixture of polymers and carbohydrates present in seaweeds, including polysaccharides, and oligosaccharides such as hemicelluloses, cellulose, gums, pectin substances, and non-carbohydrate components (Lin *et al*. 2026). Brown seaweeds are excellent sources of nutrients and bioactive secondary metabolites, includingβ-carotene, fucoxanthin, vitamins, tannins, flavonoids, and phenols. The antioxidant power of brown seaweed is strongly correlated with phytochemicals (Ismail *et al.* 2023). The present investigation and earlier reports on proximate analysis indicated that brown seaweeds were enriched with high amounts of dietary fibre, ash, protein, and limited lipid content, indicating that they could be considered good prebiotic diets for fish.

Table 2. Proximate Content (%) of Seaweed

Seaweeds	Ash	Dry matter	Lipid	Moisture	Protein	Total Dietary Fibre
Padina vickersiae	5.24 ± 0.76	4.31 ± 0.14	4.11 ± 0.03	74.01 ± 0.52	10.3 ± 0.04	61.3 ± 0.52
Sargassum polycystum	39.8 ± 1.1	4.91 ± 0.02	3.42 ± 0.04	80.3 ± 0.42	17.4 ± 0.01	37.49 ± 0.53
Sargassum vulgare	40.2 ± 0.21	5.02 ± 0.14	3.38 ± 0.13	83.4 ± 0.11	16.2 ± 0.01	35.4 ± 0.48
Turbinaria murrayana	9.4 ± 0.31	3.28 ± 0.81	3.52 ± 0.08	81.32 ± 0.52	14.2 ± 0.02	59.21 ± 0.42
Turbinaria ornata	10.2 ± 0.62	4.52 ± 0.52	4.02 ± 0.12	82.10 ± 0.41	13.2 ± 0.03	48.3 ± 0.33

Amino Acid Content of Seaweed

Seaweeds are rich in free amino acids and have been used in the food industry (Ummat *et al.* 2021). The essential and nonessential amino acid contents of the seaweed samples were analysed, and the results are presented in Table 3. The present findings revealed that the arginine content was high (1.15 ± 0.002 mg/100 g) in *S. vulgare*, whereas the isoleucine content was high (1.04 ± 0.025 mg/100 g) in *T. ornata*. Essential amino acids, such as histidine, methionine, and tryptophan were very much less among the selected seaweeds (<0.010 mg/100 g seaweed). The leucine level was high (0.539 ± 0.005 mg/100 g) in *S. polycystum*, and the lysine content reached a maximum in *P. vickersiae* (1.2 ± 0.015 mg/100 g). In a previous study, Terriente-Palacios and Castellari (2022) detected tau (2-aminoethanosulfonic acid), an essential amino acid from the marine macroalgae *Gracilaria longissima*, *Palmaria palmata*, and *Porphyra* sp. Essential amino acids (lysine, histidine, tyrosine, isoleucine, threonine, lysine, tryptophan, leucine, phenylalanine, taurine, and valine) and nonessential amino acids were detected in brown seaweeds (*Sargassum ilicifolium*, *Sargassum wightii*, *Padina pavonica*, *Padina gymnospora*, and *Dictyota ciliolate*) collected from Southeast India (Perumal *et al.* 2023). The amount of amino acids detected in the current study was greater than that reported for seaweed from the Indian coast.

Table 3. Amino Acid Contents of the Selected Seaweeds (mg/100 g Dry Sample)

Amino Acids	Amino acid (mg/100 g)				
	Padina vickersiae	*Sargassum polycystum*	*Sargassum vulgare*	*Turbinaria murrayana*	*Turbinaria ornata*
Arginine	0.841 ± 0.001	0.524 ± 0.01	1.15 ± 0.002	0.853 ± 0.021	1.04 ± 0.003
Isoleucine	0.001 ± 0.0	1.04 ± 0.025	0.821 ± 0.002	0.24 ± 0.006	0.80 ± 0.005
Histidine	0.003 ± 0.0	0.002 ± 0.0	0.001 ± 0.0	0.003 ± 0.0	0.002 ± 0.0
Leucine	0.34 ± 0.04	0.539 ± 0.005	0.105 ± 0.002	0.25 ± 0.006	0.32 ± 0.001
Lysine	1.2 ± 0.015	0.905 ± 0.003	0.53 ± 0.002	1.0 ± 0.006	0.49 ± 0.055
Phenyl alanine	0.2 ± 0.005	0.10 ± 0.005	0.331 ± 0.006	0.10 ± 0.002	0.335 ± 0.005
Valine	0.1 ± 0.001	0.30 ± 0.003	0.005 ± 0.001	0.206 ± 0.003	0.10 ± 0.003
Threonine	1.9 ± 0.15	1.0 ± 0.009	0.50± 0.005	1.50 ± 0.002	0.705 ± 0.004
Methionine	0.001 ± 0.0	0.003 ± 0.0	0.001 ± 0.0	0.008 ± 0.001	0.001 ± 0.0
Glycine	1.7 ± 0.11	5.6 ± 0.002	0.007 ± 0.00	2.1 ± 0.005	1.54 ± 0.003
Tryptophan	0.003 ± 0.0	0.002 ± 0.0	0.001 ± 0.0	0.009 ± 0.001	0.007 ± 0.001
Alanine	0.2 ± 0.002	0.139 ± 0.022	0.342 ± 0.005	0.22 ± 0.005	0.28 ± 0.015
Aspartic acid	0.39± 0.005	0.525 ± 0.0055	0.058 ± 0.0005	0.243 ± 0.005	0.10 ± 0.004
Proline	0.002 ± 0.0	0.001 ± 0.0	0.003 ± 0.0	0.001 ± 0.0	0.001 ± 0.0
Serine	0.64 ± 0.001	1.05 ± 0.004	0.605 ± 0.003	0.406 ± 0.003	0.70 ± 0.002
Tyrosine	0.009 ± 0.001	0.001 ± 0.0	0.002 ± 0.0	0.001 ± 0.0	0.003 ± 0.0

Analysis of Phenolic Compounds and Antioxidant Activity

The phenolic content of the seaweed extract was determined, and the results are shown in Fig. 2A. In this study, the total phenolic contents were 6.4 ± 0.53, 9.2 ± 0.35, 10.2 ± 0.71, 6.5 ± 0.65, and 8.5 ± 0.22 mg GAE/100 g for seaweed, *P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, and *T. ornata*, respectively. The phenolic content of algae significantly influences their antioxidant activities, and compared with those of green and red algae, the antioxidant activity of algae is associated with increased amounts of phenolic compounds (Yuan *et al.* 2018; Keramane *et al.* 2023). Seaweeds are exposed to several adverse environmental factors, which induce the generation of various metabolites, including pigments, vitamins, enzymes, polysaccharides, tocopherols, phenolics, and phospholipids, to protect algae from external environmental stress. The seasonal variation, species, sample location, and environmental factors significantly influence the variation in seaweed phytochemicals.

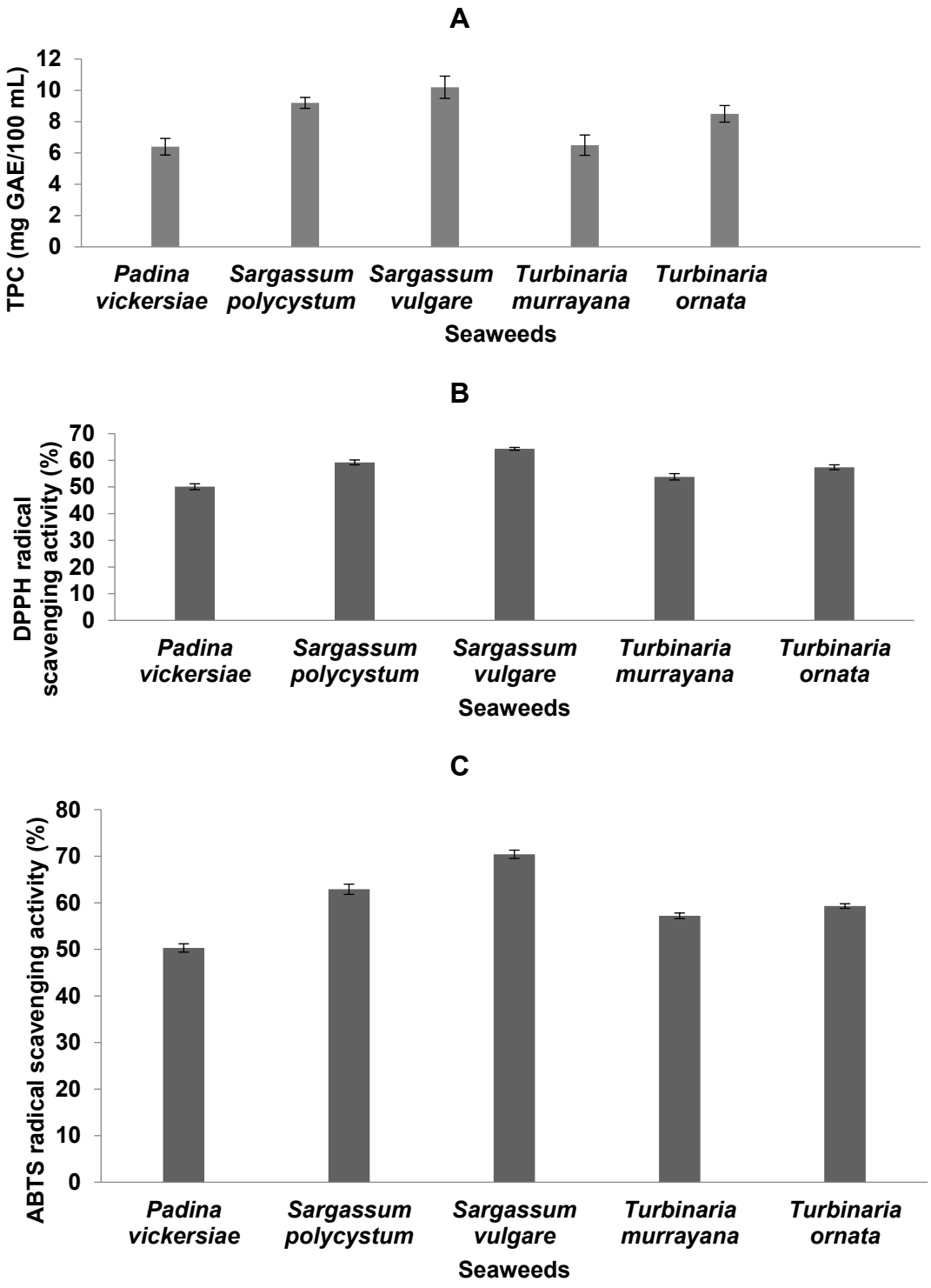

Fig. 2. Total phenolic content and antioxidant activity of brown seaweed extract: (A) Total phenolic content; (B) DPPH radical scavenging activity; (C) ABTS radical scavenging activity. Error bar represents standard deviation.

Previous investigations revealed that brown algae present increased levels of phenolic compounds, characterized by high biological activity and greater antioxidant activity than red and green algae do (Gupta and Abu- Ghannam 2011; Balboa *et al.* 2013; Montero *et al.* 2014). In the current study, ethanol was used for the extraction of phenolic compounds. It has been extensively used for the extraction of phenolic compounds from seaweed (Farvin and Jacobsen 2013). In this study, the increased phenolic content improved the antioxidant activities of seaweed. The tested seaweeds (*P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, and *T. ornata*) were rich sources of phenolic compounds and natural antioxidants. The DPPH radical scavenging activity varied from 50.1 ± 1.1 to 64.3 ± 0.52, whereas the *S. vulgare* extract exhibited the maximum ABTS radical scavenging activity (70.4 ± 0.9%) (Fig. 2B and C). The antioxidant activity observed in this study was correlated with the detected phenolic compounds, revealing that seaweed polyphenols are highly active components in these seaweed extracts. In addition to phenolic compounds, coextracted phytochemicals, such as tocopherols and pigments, in solvent extracts, peptides or proteins, and ethanolic extracts contribute to antioxidant activities (Farvin and Jacobsen 2013). The ethanol extract of *Sargassum* sp. increased the total phenolic content (25.33± 1.45 mg GAE/g) (Duan *et al.* 2023), which was greater than that reported in this study. The amount and number of polyphenols in the extract varied based on the extraction type and the solvents used for the extraction procedure. The seaweed *Sargassum filipendula* exhibited antioxidant activity and was detected *via* the ABTS, DPPH, and Ferric Reducing Antioxidant Power (FRAP) methods, and the antioxidant activity was directly related to the amount of phenolic compounds in the extract (Polo and Chow 2022). In brown algae, polyphenolic compounds are among the major compounds with antioxidant activity (Mahendran *et al.* 2024), and seaweed processing methods, including drying methods, also significantly affect the yield of polyphenolic compounds (Subbiah *et al.* 2023).

Growth Performance of Nile Tilapia

Compared with the control diet, the seaweed diet supplemented at various percentages (2 to 10%) improved growth performance (Fig. 3). The addition of seaweed significantly improved the growth of Nile tilapia during the culture period of 60 days. The present findings revealed that the final body weight, feed conversion ratio, and specific growth rate of Nile tilapia were affected by increasing concentrations of seaweed in the experimental fish diet. An increased SGR%/day (3.676 ± 0.02) and DGR (g/day) (0.336 ± 0.025) were obtained in the experimental diet containing 10% seaweed (Table 4). Moreover, 8% seaweed supplements also have significant effect on tilapia growth ($p<0.05$) and this percentage is considered as optimum for feed formulation.

The growth performance varied to an insignificant extent between 8% and 10% seaweed supplemented diet. The improvements in FCR were obtained when the percentage of brown algae in the fish diet increased from 6% to 8% and then to 10%, and the FCR values were found to be 1.49 ± 0.11, 1.41 ± 0.09, and 1.39 ± 0.06, respectively. These FCR values were significantly greater than those of the fish fed the control diet (1.54 ± 0.22). Similarly, the SGR values increased with increasing brown algae content in the experimental diet. A good SCR value was achieved with the experimental animals fed a 10% fish diet (3.676 ± 0.02). Similarly, the DGR (g/day) was high in the fish fed 8% seaweed (0.333 ± 0.12) and 10% seaweed (0.336 ± 0.25) (Fig. 2).

The present findings align with earlier studies that revealed the positive effects of applying macroalgae, especially seaweeds from the genus *Gracilaria,* to fish diets, which improved the growth performance of Persian sturgeon, *Acipenser persicus* (Adel *et al.* 2021), and Atlantic salmon (Kamunde *et al.* 2019), respectively. Previous work has shown that 5% seaweed in fish diet show positive impacts on fish growth performance; however, 10% seaweed in fish diet was found to neither decrease nor improve growth performance (Martínez-Antequera *et al*. 2021). Another study reported that up to 10% red seaweed (*Porphyra dioica*) in fish diet improved fish growth and 15% seaweed affected growth in rainbow trout (*Oncorhynchus mykiss*) (Soler-vila *et al.* 2009).

The improved growth performance observed in this study may be due to the presence of nutrients such as carbohydrates, polyunsaturated fatty acids, vitamins, and essential amino acids. In addition to these nutrients, the available phytochemicals in seaweed stimulate the digestion process and increase absorption due to the modification of the intestinal villi (Hashim and Saat 1992; Lordan *et al.* 2011). Furthermore, the improved growth observed in this study may also be due to the increased availability of polysaccharides and oligosaccharides in seaweed that perform prebiotic functions, thus improving digestion by stimulating the proliferation of beneficial bacteria, reducing the growth of pathogenic bacteria and improving nutrient assimilation by enhancing the activity of probiotic bacteria (O'Sullivan *et al.* 2010).

It has been previously reported that the diet supplemented with seaweed has been reported to improve the growth of Nile tilapia. These include *Gracilariopsis lemaneiformis* (Shahabuddin *et al.* 2024), *Hypnea* sp. (Sultana *et al.* 2023), *Sargassum dentifolium* (Abdelrhman *et al.* 2022), *Chaetomorpha aerea* (Sattanathan *et al.* 2024), and *Sargassum polycystum* (Nazarudin *et al.* 2025). The supplemented seaweeds with fish feed act as prebiotics, improve probiotic bacteria within the gastrointestinal tract, improve the function of the gut, and stimulate the production of secondary metabolites and digestive enzymes (Weththasinghe *et al.* 2025).

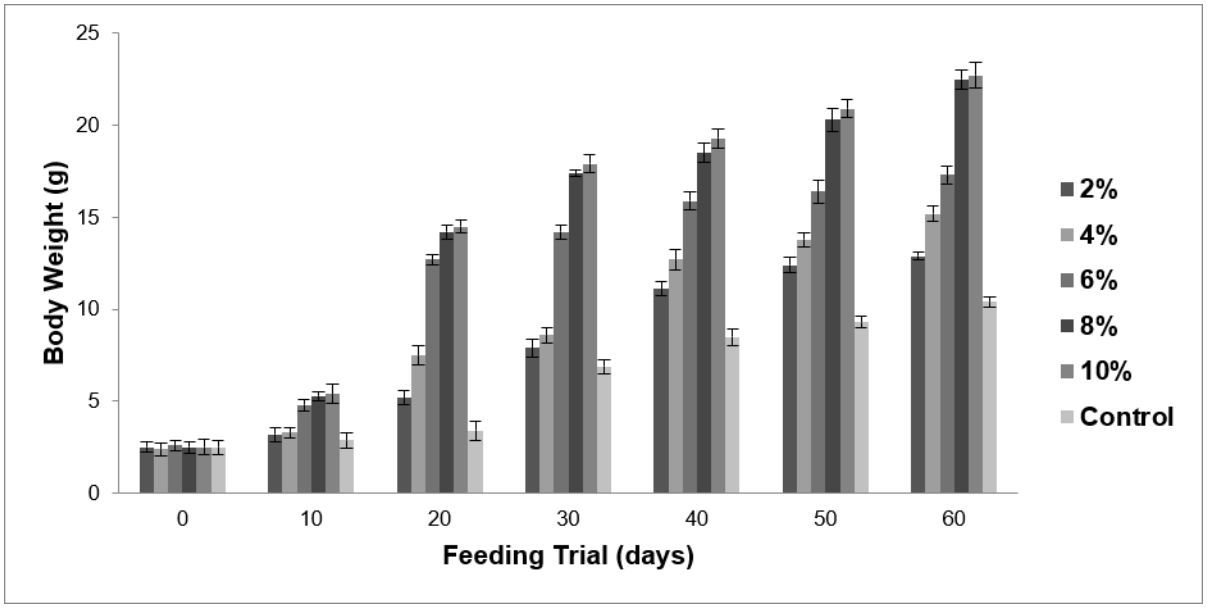

Fig. 3. Growth performance of Nile tilapia fed various dietary levels of seaweed (*P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, and *T. ornata*) for 60 days

Table 4. Weight Gain, Feed Conversion Ratio, and Specific Growth Rate of Nile Tilapia Fed Various Levels of Seaweed* for 60 Days

Treatments (Seaweed*)	BW (i) g	BW (f) g	WG g	FCR	SGR%/day	DGR (g/day)
2%	2.51 ± 0.29[a]	12.9 ± 0.23[a]	10.39 ± 0.21[a]	1.56 ± 0.35[a]	2.7282 ± 0.02[a]	0.1732 ± 0.09[a]
4%	2.4 ± 0.33[b]	15.2 ± 0.42[b]	12.8± 0.04[b]	1.53 ± 0.21[a]	3.076 ± 0.01[a]	0.2133 ± 0.05[a]
6%	2.6 ± 0.27[c]	17.3 ± 0.49[c]	14.7 ± 0.22[c]	1.49 ± 0.11[b]	3.158 ± 0.18[b]	0.245 ± 0.14[b]
8%	2.7 ± 0.33[d]	22.5 ± 0.53[d]	20 ± 0.18[d]	1.41 ± 0.09[b]	3.66 ± 0.04[c]	0.333 ± 0.12[c]
10%	2.6 ± 0.42[c]	22.7 ± 0.7[d]	20.2 ± 0.23[d]	1.39 ± 0.06[b]	3.676 ± 0.02[c]	0.336 ± 0.025[c]
Control	2.5 ± 0.27[a]	10.4 ± 0.29[e]	7.9 ± 0.22[e]	1.54 ± 0.22[a]	2.375 ± 0.15[a]	0.1317 ± 0.19[a]
**P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, or *T. ornata*)						

Effect of Seaweed on Fish Blood Parameters

The total protein content of the control and the fish fed 2% seaweed did not significantly differ. However, at higher concentrations, the total protein content in the plasma increased. The ALP and ALT levels decreased significantly with increasing seaweed concentration in the fish diet ($p<0.05$) (Table 5). Determination of AST and ALP in fish is very important for analyzing metabolic function and liver health. The supplemented seaweed protect fish by reducing or stabilizing these enzyme levels, which reduces toxicity and mitigates against stress, without affecting liver function and normal growth (Adnan and Pramaningtyas 2022). In this study, ALP and ALT levels decreased significantly, revealing that the supplementation of seaweed powder provides a hepatoprotective property, effectively reducing liver damage (Harikrishnan *et al*. 2011). Nile tilapia fed with seaweed lipid extract presented improved serum protein and lysozyme levels compared with control tilapia exposed to *Aeromonas hydrophila* (Ashour *et al.* 2020).

Table 5. Determination of Blood Parameters in Nile Tilapia Fed a Seaweed Diet and Cultured Under Cold Stress

Treatments (Seaweed*)	Total Protein (g/dL)	ALP U/L	ALT U/L
2%	3.01 ± 0.02	227 ± 1.5	19.4 ± 0.5
4%	3.2 ± 0.5	214 ± 0.8	18.2 ± 0.8
6%	3.7 ± 0.3	193 ± 3.8	17.5 ± 1.1
8%	4.1 ± 0.2	187 ± 2.2	16.5 ± 0.8
10%	3.9 ± 0.1	179 ± 1.3	15.9 ± 0.5
Control	3.01 ± 0.2	241 ± 4.1	20.3 ± 1.1
(**P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, or *T. ornata*)			

The hybrid tilapia (♂*Oreochromis niloticus* × ♀*O. mossambicus*) supplemented with increasing amounts of unsaturated fatty acids mitigated cold stress (13 °C for 15 days) and improved body nutrient composition, histopathological alterations, and liver enzymes after 98 days of the feeding trial. In control fish, cold stress affects fish growth and alanine transaminase and aspartate transaminase activities (Refaey *et al.* 2023).

Effect of Seaweed on Stress Mitigation

Catalase activity was lower in the fish fed the seaweed diet than in those fed the control diet. The supplemented seaweed decreased catalase activity and MDA level. In the control fish group, catalase activity was 4.6 ± 0.02 µmol/min/mg protein, and it was reduced in the experimental fish fed with fish diet containing 10% seaweed (2.8 ± 0.1 µmol/min/mg protein) ($p<0.05$). Similarly, MDA level was 0.49 ± 0.03 µmol/mg protein in the control fish (without seaweed diet), and it was reduced in the experimental fish fed with fish diet containing 10% seaweed (0.35 ± 0.01 µmol/mg protein) ($p<0.05$). Conversely, GST Activity and SOD activity was increased in the fish fed with seaweed powder at various concentrations. The GST activity was27.4 ± 1.8 µmol/min/mg protein in the control fish, and it was increased significantly in the fish fed with 10% seaweed (40.8 ± 1.1 µmol/min/mg protein) ($p<0.05$). Similarly, SOD activity was 77.4 ± 1.5% in the control fish, and it was increased in the fish fed with 10% seaweed (81.7 ± 1.5%) ($p<0.05$). These findings revealed the stress mitigation effect of seaweed (Table 6). The present finding was consistent with previous findings. The *Laminaria digitata* supplemented aquafeeds lower lipid peroxidation in juvenile gilthead seabream. Conversely, the expression of superoxide dismutase and glutathione S-transferase were increased which improved stress response (Pereira *et al*. 2025). It has been previously reported that juvenile fish (*Piaractus mesopotamicus*) fed red algae mitigate oxidative stress and trigger the biosynthesis of liver enzymes when exposed to relatively low temperatures (14 °C). At lower concentrations of seaweed, no mitigation effects have been reported in fish, and higher concentrations of seaweed have shown mitigating effects. In addition, lipid oxidative damage has been reported in both experimental and control diets of fish exposed to relatively low temperatures (Ale *et al.* 2021).

Table 6. Antioxidant Enzymes and Chemicals of Nile Tilapia Fed Seaweeds (2% to 10%) Cultured Under Cold Stress

Treatments (Seaweed*)	Catalase Activity (µmol/min/mg Protein)	GST Activity (µmol/min/mg Protein)	SOD Activity (% Inhibition)	MDA (µmol/mg Protein)
2%	4.5 ± 0.4^{a}	27.5 ± 0.9^{a}	77.5 ± 2.9^{a}	0.49 ± 0.01^{a}
4%	3.6 ± 0.2^{b}	29.2 ± 1.1^{b}	78.4 ± 1.1^{b}	0.48 ± 0.04^{b}
6%	3.4 ± 0.1^{b}	30.7 ± 1.2^{b}	79.2 ± 1.2^{c}	0.46 ± 0.04^{b}
8%	3.1 ± 0.2^{b}	40.5 ± 0.9^{c}	81.5 ± 1.1^{d}	0.41 ± 0.06^{b}
10%	2.8 ± 0.1^{c}	40.8 ± 1.1^{c}	81.7 ± 1.5^{d}	0.35 ± 0.01^{c}
Control	4.6 ± 0.02^{a}	27.4 ± 1.8^{a}	77.4 ± 1.5^{a}	0.49 ± 0.03^{a}
(**P. vickersiae*, *S. polycystum*, *S. vulgare*, *T. murrayana*, or *T. ornata*)				

Nile tilapia fed with seaweed extract presented mitigated ammonia stress and *Aeromonas hydrophila* infection. The experimental fish presented decreased antioxidant activity (CAT and SOD) and increased MDA levels under both stresses (Radwan *et al.* 2025). The present finding was similar to the study of Siddik *et al.* (2024). Nile tilapia fed with *Gracilaria* extract has been presented decreased levels of malondialdehyde (<0.05) and reduced nuclear and cellular abnormalities (Siddik *et al.* 2024). Brown seaweeds typically have higher antioxidant property than green and red algae due to their higher phenolic compound levels and prevent oxidative damage (Galindo *et al*. 2023).

CONCLUSIONS

1. Brown seaweeds are excellent sources of nutrients and phytochemical compounds. The present findings revealed that dietary brown algae, *Padina vickersiae*, *Sargassum polycystum*, *Sargassum vulgare*, *Turbinaria murrayana*, and *Turbinaria ornata* at 8% in the fish diet significantly improved the growth of Nile tilapia.
2. The supplemented seaweed mitigated cold stress by improving the antistress response, including a reduction in the production of catalase activity and lower lipid peroxidation significantly at 8% seaweed in the fish diet in comparison to 10% seaweed supplemented seaweed. The level of glutathione S-transferase and superoxide dismutase enzyme activity increased significantly at 8% seaweed in the diet.
3. The present findings revealed the importance of brown algae as an efficient feed supplement for tilapia to reduce cold-stress in tilapia culture in cold environments.

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Conflict of Interest

The authors do not have any conflict of interest on publishing this research article.

Use of Generative AI

The authors declare that no AI tools were used to prepare images, figures, graphs, or diagrams.

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