Nutritional and Functional Properties of Mixed Brown Seaweeds and the Growth Performance of Goldfish Fed a Pellet Diet Formulated with Seaweeds

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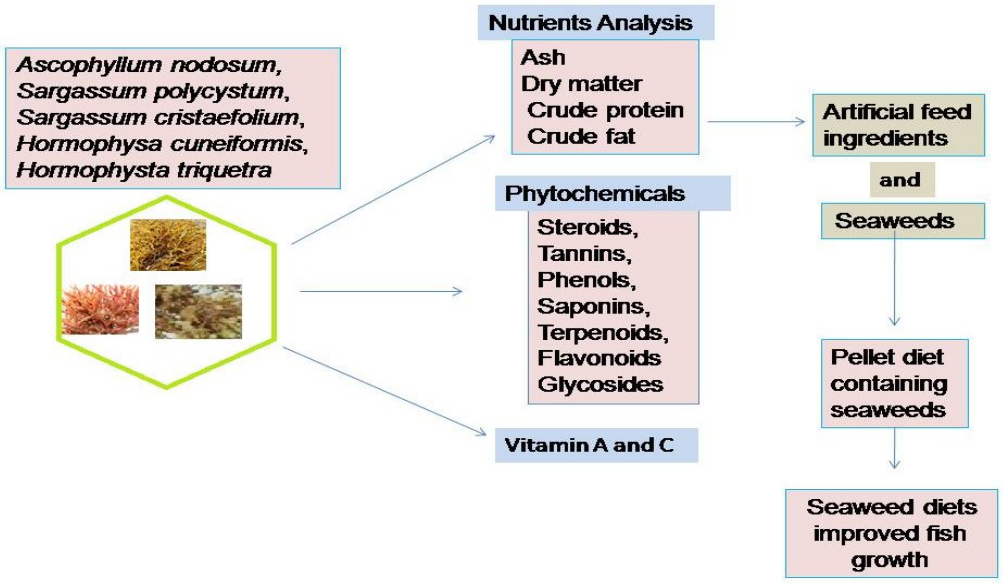
GRAPHICAL ABSTRACT



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The nutrient profile and pharmaceutical activities were analyzed for seaweeds (*Ascophyllum nodosum*, *Sargassum polycystum*, *Sargassum cristaefolium*, *Hormophysa cuneiformis*, and *Hormophysa triquetra*) in the fish diet. The total protein content was greatest (8.4±0.2%) in *S. polycystum*, and the highest lipid content was detected in *S. cristaefolium* (3.7±0.1%). The crude fiber content was greatest (13.8±0.7%) in *S. polycystum*, and the carbohydrate content was high (35.2±0.5%) in *S. polycystum*. The phenol (73.1±1.1 mg GAE/g DW) and flavonoid (85.2±1.1 mg CE/g DW) contents were high in *S. polycystum,* whereas the tannin content was high in *S. cristaefolium* (15.5±0.8 mg GAE/g DW). *Sargassum polycystum* presented the maximum DPPH activity (64.4±2.2%) and H_2O_2 activity (45.8±1.3%). Equal amounts of seaweed (*A. nodosum*, *S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, and *H. triquetra*) were mixed and used for feed formulation. The experimental diet was prepared by mixing 5% to 10% seaweed with other feed ingredients. The experimental diet was prepared with soybean oil, fish oil, and water. Compared with the control diet, the seaweed-supplemented diet improved fish growth ($p<0.05$). Overall, the results revealed the beneficial effects of seaweed on the feed of goldfish.

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Keywords: Nutrients; Antioxidant activity; Fish feed; Specific growth rate

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INTRODUCTION

Microalgae and macroalgae constitute more than 164,0000 species, and approximately 9,800 are seaweeds. However, only 0.17% of seaweeds are exploited for commercial use (Sultana *et al*. 2023). The interest in seaweed has increased exponentially, and several bioactive compounds have been identified and utilized for the preparation of functional foods and for nutraceutical applications (Li *et al*. 2021). These functional foods are utilized to reduce metabolic risk factors such as hypercholesterolemia, hyperglycemia, and hyperlipidemia (Collins *et al*. 2016). The fiber components present in seaweeds primarily consist of structural polysaccharides such as alginate and fucoidan in brown algae; carrageenan, agar, and porphyran in red algae; and ulvan in green algae. Seaweeds are rich in mineral content due to their natural habitat in the marine environment, which

allows sea weeds to absorb a diverse range of minerals. Studies conducted by various research groups have shown that the nutritional value of feeds is associated with the cultured species of seaweed, with the essential amino and fatty acid contents of seaweeds being important factors in their nutritional value. Consequently, essential amino acids and long-chain unsaturated fatty acids are the major dietary components of fish, supporting their growth rate and survival (Lu *et al*. 2022). This is very important because most aquatic organisms have a lower ability to produce these compounds from precursor molecules. Brown algae represent a significant proportion of the algae that are cultivated extensively in various countries because of their health benefits over green and red seaweeds. It is a valuable source of secondary metabolites and improves fish growth. They are rich in nutrients such as proteins, vitamins, and minerals, along with bioactive compounds such as fucoidan, polysaccharides, and polyphenols (Geng *et al*. 2017; Chen *et al*. 2023). With the increasing development of the global aquaculture sector and increasing restrictions on the application of commercial antibiotics in fish feed, interest in brown algae extracts has increased (Zhang *et al*. 2020).

In brown algae, alginate is a major component of the cell wall and has anti-inflammatory, antibacterial, and antioxidant properties. Moreover, its efficacy is affected by its high viscosity and large molecular weight. The oligosaccharides derived from alginates are considered alternatives to antibiotics, improve enzyme production, and show antibacterial activity (Huang *et al*. 2024). These oligosaccharides can improve disease resistance in fishes and are involved in their growth; in addition, these oligosaccharides act as anti-inflammatory agents. Several previous studies have shown that the addition of brown algae or their extracts to the diets of fish can improve their immunity, enhance the gut microbial flora, increase the absorption of nutrients in the gut, improve the food conversion ratio and digestibility, and positively impact the maintenance of bacterial diversity within fish intestines, thereby protecting intestinal health (Wan *et al*. 2021). In addition, the coculture of *Ulva* spp. with shrimp improved the functional properties of *Litopenaeus vannamei* (Gao *et al*. 2018; Gao *et al*. 2022). Seaweeds exhibit several bioactive compounds, including polyphenols, fucoidan, vitamins, and β-glucans, which are broadly considered immunostimulants and efficient antioxidant chemicals that improve the health benefits of farmed fish. Compared with single seaweed, a mixture of more than one seaweed species in a fish diet has been found to significantly improve overall health benefits because of a balanced bioactive profile, nutritional synergy, and minimized risks of antinutritional factors (Silva *et al*. 2015; Cárdenas *et al*. 2015; Abdelhamid *et al*. 2021; Seyedalhosseini *et al*. 2023). In this work, we hypothesized that adequate levels of these compounds would improve the overall growth of fish. Brown macroalgae are rich in alginate, fucoidan, laminaran and other polysaccharides (Chen *et al*. 2024). The supplementation of brown algae extracts in fish diets offers several advantages. It can improve digestive conditions, alter the rumen microbial flora, effectively reduce protein breakdown, and enhance the in vitro digestibility of feed. As aquaculture aims for sustainable practices and high-quality development, extracts rich in bioactive compounds have attracted significant interest (Pan *et al*. 2016).

However, the majority of existing research has focused on the preparation of fish diets with single or two seaweeds, and there is a lack of knowledge on the effects of multiple seaweeds on nutritional synergy in finfish. To fill this research gap, a mixture of brown algae was used for the preparation of the fish diet. The plants were fed goldfish for 30 days, and their growth performance was analyzed. The objective of this study was to assess the effects of the nutritional and pharmaceutical status of seaweed and the seaweed

mixture on fish growth. This research, therefore, fills this gap and highlights the potential use of seaweed mixtures in aquaculture.

EXPERIMENTAL

Seaweeds

Seaweeds (*Ascophyllum nodosum, Sargassum polycystum*, *Sargassum cristaefolium*, *Hormophysa cuneiformis*, and *Hormophysa triquetra*) were collected by hand at the Kanniyakumari coast, Arabian Sea, India (Latitude, 8° 4′ 41.13″ N. Longitude, 77° 33′ 19.35″ E), in the morning (temperatures – 29 °C and 31 ppt). The collected seaweeds were cleaned, and the sand, epiphytes, and other particles were removed. It was stored in plastic containers and transported to the laboratory, and morphological identification was carried out. The mixture was subsequently washed with double distilled water and lyophilized at -80 °C until a constant weight was reached. It was ground to a powder and sieved through a 200-micron sieve. The sample was labeled and stored in brown bottles at −20 °C for further experiments.

Analysis of the Proximate Composition of the Seaweed

The ash content, dry matter, crude protein, crude fat (by ether solvent extraction) and total dietary fiber contents were determined as described previously (AOAC 2006). The ash content of the seaweed was determined by placing the samples in a muffle furnace at 550 °C for 6 h (AOAC 942.05 for ash content**)**. The moisture content of each sample was analyzed by drying 2 g of algal powder at 105 °C until a constant weight was obtained (AOAC 935.29 for moisture). The protein content of the seaweed samples was estimated *via* AOAC (N × 6.25) (AOAC 990.03 for protein), and the fat content was determined after the seaweed samples were extracted with petroleum ether via Soxhlet extraction (AOAC 996.06 for fat). The amount of crude fiber was estimated after acid hydrolysis (0.26 N sulfuric acid) at 100 °C for 30 min, followed by alkaline treatment (0.31 N sodium hydroxide) for 30 min. An enzymatic-gravimetric method was used to determine crude fiber via the combination of amyloglucosidase and α-amylase AOAC 978.10 for crude fiber.

The amount of nitrogen-free extract (NFE) was calculated *via* the following formula.

$$\text{NFE (\%)} = 100 - (\text{crude protein} + \text{lipids} + \text{ash} + \text{crude fiber}) \quad (1)$$

Sample Preparation

The dried seaweed (*A. nodosum*, *S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, and *H. triquetra*) powder (10 g) was mixed with 100 mL of methanol at a 1:1 (w/v) ratio and vortexed for 10 min. The mixture was sonicated for 20 min with a sonicator at 28 ± 1 °C. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was dried using a rotary evaporator. The dried extract was stored at 0 ºC until further use.

Phytochemical Screening

To detect terpenoids, 0.1 mL of methanol extract was dissolved in 2 mL of chloroform and evaporated. Then, concentrated sulfuric acid (2 mL) was slowly added, and the mixture was incubated for 2 min. The development of a grayish color indicated the presence of terpenoids. To detect steroids from the seaweed extract, 0.1 mL of extract was mixed with chloroform (2 mL). To this mixture, 2 mL of sulfuric acid and 2 mL of glacial acetic acid were added and mixed. The development of a greenish color indicated the presence of steroids. To test the presence of phenols and tannins in the seaweeds, 0.1 mL of extract was mixed with 2 mL of 2% $FeCl_3$. The development of blue–green color indicates phenols, and the tannins are black. To determine the flavonoid content, 0.1 mL of sample was mixed with 2 mL of 1 N NaOH solution. The development of yellow color was observed, few drops of hydrochloric acid were added, and the solution became colorless. To detect saponins, 0.1 mL of extract was poured into a test tube, 5 mL of double distilled water was added, and the mixture was shaken vigorously. The development of foam indicated the presence of saponins.

Analysis of Total Phenols, Flavonoids, and Tannins

The total phenolic content (TPC) of the seaweed extract was estimated *via* the colorimetric method with the Folin–Ciocalteu reagent (Tambe and Bhambar 2014). The absorbance of the sample was read at 700 nm, and the result was expressed in milligrams of gallic acid equivalents per gram of seaweed (mg GAE/g DW). The flavonoid content of the extract was estimated by the colorimetric method of analysis with aluminum chloride (Yan *et al*. 1999). The absorbance of the sample was read at 415 m, and the result was expressed as milligrams of catechin equivalents (CE) per gram of dried seaweed (mg CE/g DW). The tannin content of each sample was estimated using Folin–Ciocalteu reagent, and the final color was read at 760 nm. The results are expressed as mg GAE/g DW (Tambe and Bhambar 2014).

Analysis of Vitamins A and C

Vitamins A and C are essential for fish health; hence, their contents were determined from seaweed extracts. The vitamin A content of the seaweed was tested as described previously by Prieto *et al*. (1999), with slight modifications. The result was expressed as α-tocopherol equivalents per gram of DW. The amount of vitamin C in the extract was determined as described by Pantelidis *et al*. (2007), with slight modifications. The results are expressed as milligrams of ascorbic acid/100 g DW of seaweed.

Antioxidant Activity

The antioxidant activity of the extract was analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and a H_2O_2 free radical scavenging assay. The DPPH activity of the seaweed extract was assayed as described previously by Yepez *et al*. (2002). The percentage of DPPH scavenging activity was calculated using Eq. 2.

$$\text{DPPH scavenger activity } (\%) = [(A_C - A_S)/A_C] \times 100 \quad (2)$$

The hydrogen peroxide activity of the extract was detected as described by Gülçin *et al*. (2004). Ascorbic acid was used as a positive control. The percentage of H_2O_2 free radical scavenging activity was calculated using Eq. 3.

$$\text{Free radical scavenging } (H_2O_2)(\%) = [(A_C - A_S)/A_C] \times 100 \quad (3)$$

Preparation of Experimental Diets Enriched with Seaweeds

Brown seaweed powder was used as one of the ingredients for the formulation of the fish diet. The chemical composition of the experimental diets prepared in this study is shown in Table 1. Fish meal was used as a major protein source. In the control diets, seaweeds were not included (T0). Equal amounts of all five seaweeds (*A. nodosum*, *S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, and *H. triquetra*) were mixed and used for further studies. The experimental diets were prepared by adding increasing concentrations of seaweed at 2% (T1), 4% (T2), 6% (T3), 8% (T4), and 10% (T5). The seaweeds were mixed with the ingredients, and stiff dough was prepared using soybean oil, fish oil, and water. The prepared dough was further extruded by a pellet feed maker using a 2 mm diameter die. The mixture was further dried at 60 °C for 12 h, packed in a container, and stored at 4 °C until further use. The isoenergetic content of the experimental and control diets was approximately 20±1.2 MJ/kg. The AOAC (AOAC 2005) method was used to analyze the chemical composition of the experimental diets. The proximate composition of the fish diet, including ash, protein, lipid, dry matter, moisture, and NFE, was analyzed, and the composition is described in Table 2.

Table 1. Ingredients Used for the Preparation of Artificial Pellet Diets Containing 2% to 10% Seaweed

Composition	T0	T1	T2	T3	T4	T5
	Ingredients (g/kg feed)					
Fish Meal	460	460	460	460	460	460
Corn Gluten Meal	121	121	121	121	121	121
Wheat Gluten Meal	121	121	121	121	121	121
Soybean Meal	50	40	30	20	10	0
A. nodosum	0	2	4	6	8	10
S. polycystum	0	2	4	6	8	10
S. cristaefolium	0	2	4	6	8	10
H. cuneiformis	0	2	4	6	8	10
H. triquetra	0	2	4	6	8	10
Fish Oil	20	20	20	20	20	20
Wheat Middling	135	135	135	135	135	135
Beef Gelatin	20	20	20	20	20	20
Canola Oil	20	20	20	20	20	20
Soy Lecithin	20	20	20	20	20	20
DL-Methionine	1	1	1	1	1	1
L-Lysine	2	2	2	2	2	2
Mineral Premix	10	10	10	10	10	10
Vitamin Premix	10	10	10	10	10	10
Vitamin C	5	5	5	5	5	5
Sodium Diformate	2.5	2.5	2.5	2.5	2.5	2.5
Butyric Acid	2.5	2.5	2.5	2.5	2.5	2.5

Table 2. Proximate Composition of an Artificial Pellet Diet Containing 2% to 10% Seaweed

Fish Diet	T0	T1	T2	T3	T4	T5
	Chemical Composition (% DW)					
Ash	8.3 ± 0.3	8.7 ± 0.1	8.5 ± 0.2	9.1 ± 0.1	9.2 ± 0.1	9.3 ± 0.3
Protein	40.5 ±1.1	41.3 ± 0.2	42.4 ± 1.2	44.5 ± 0.2	46.3 ± 0.1	47.2 ± 0.1
Lipid	14.2 ± 0.3	14 ± 0.1	13.5 ± 0.1	13.6 ± 0.3	13.3 ± 0.1	13.2 ± 0.2
Dry Matter	87.4 ± 1.1	88.2 ± 0.5	89.3 ± 1.1	87.2 ± 0.2	88.6 ± 0.3	89.2 ± 1.1
Moisture	21.5 ± 0.3	22.4 ± 0.1	21.3 ± 0.2	22.2 ± 0.3	21.9 ± 0.3	22.3 ± 0.4
Nitrogen Free Extract	15.5 ± 0.2	13.6 ± 0.3	14.5 ± 0.1	10.6 ± 0.2	11.3 ± 0.1	8 ± 0.2

T0 - Control; T1 to T5: Experimental Fish Diet

Fish and Culture Conditions

The goldfish were collected from the aquarium and transported to the laboratory. A total of 125 fish were acclimated to the confined environmental conditions for two weeks in 50 L fiber tanks and were fed a pellet diet. After two weeks of acclimation, 120 healthy fish were randomly distributed into six 20 L cylindrical polyethylene tanks. A total of 20 fish in each experimental or control tank were continuously fed the experimental or control diet for 30 days. The fish was fed two times daily, and the water quality parameters were measured (pH 7.2, 29.1 °C, 7.3 mg/L dissolved oxygen, 13:11 h light:dark photoperiod). Approximately 50% of the water was exchanged daily.

Analysis of Fish Growth

After one month of the experiment, the fish were collected and starved daily. The collected fish species was subsequently weighed, length was measured, and feed utilization parameters were subsequently calculated as described below:

$$\text{Weight gain (WG, \%)} = 100 \times (\text{final weight (g)} - \text{initial weight (g)})/\text{initial weight (g)} \quad (4)$$

$$\text{Specific growth rate (SGR, \%/day)} = 100 \times (\ln \text{final weight} - \ln \text{initial weight})/\text{days} \quad (5)$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)}/\text{weight gain (g)}. \text{Survival (\%)} = 100 \times (\text{final number of fish}/\text{initial number of fish}) \quad (6)$$

Statistical Tests

The experimental findings were tested using analysis of variance (ANOVA),and the differences among the treatments were tested at a significant level ($P \leq 0.05$) to compare the biological indices of the fish between the experimental and control groups. The data were examined using SPSS software.

RESULTS AND DISCUSSION

Proximate Composition of Seaweeds

The proximate compositions of the brown seaweeds *A. nodosum*, *S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, and *H. triquetra* are described in Table 3. The ash content of the seaweed ranged from 18.3±1.5% to 22.3±1.1% dry weight (DW), and the moisture

content was the highest (17.4±1.1%) in *S. cristaefolium*. The total protein content was highest (8.4±0.2%) in *S. polycystum*, whereas the maximum lipid content was detected in *S. cristaefolium* (3.7±0.1%). The crude fiber content was highest (13.8±0.7%) in *S. polycystum* and extremely low (8.4±0.3%) in *S. cristaefolium*. The amount of carbohydrates was greatest (35.2±0.5%) in *S. polycystum,* and the lowest amount (28.5±0.7%) was detected in *H. triquetra*. The total dietary fiber content was 1.7±0.1% in *H. triquetra,* and the maximum (2.5±0.1%) in *S. cristaefolium*. The amount of NFE was 58.4% (*A. nodosum*), 51.4% (*S. polycystum*), 61.4% (*S. cristaefolium*), 58.7% (*H. cuneiformis*), and 54.8% (*H. triquetra*). The physiological and morphological characteristics of seaweed and its chemical composition differ from those of terrestrial plants. The ash content of *Ascophyllum nodosum*is29.5 g/100 g DM, and the crude fat content is3 g/100 g DM (Nazarudin *et al*. 2021). In *S. polycystum*, the total protein, lipid, carbohydrate, and total dietary fiber contents are8.65 ± 1.06%, 3.42 ± 0.01%, 36.55 ± 1.09% and 2.75 ± 0.58%, respectively (Samarasinghe *et al*. 2021). The increased ash content in the seaweed reveals an appreciable amount of diverse minerals in the collected seaweeds compared with terrestrial plants, which generally have <10% ash content (Rupérez 2002). The ash contents of the seaweed samples detected in the present study were consistent with those reported previously (12 to 40% DW) for different seaweed samples reported elsewhere (Kumar *et al*. 2015). The protein content of the seaweed samples observed in this study was consistent with that reported previously (3 to 15% DW) for brown seaweed (Kumar *et al*. 2015). The variation in the protein content of seaweed may be due to seasonal changes, differences in the nutritional status of the marine ecosystem, and species differences. The lipid content of the seaweed was consistent with a previous report (Guo *et al*. 2018). The lipid content was high in *S. polycystum* and was consistent with that in other algal species. In seaweed, the presence of lipids is important because of the availability of monounsaturated and polyunsaturated fatty acids, which contribute to the antifungal, antibacterial, cytotoxic, and antiviral properties of seaweed. The amount of dietary fiber is high in the seaweed *S. polycystum*, which delays hunger in fishes and improves mineral and nutrient absorption.

Table 3. Proximate Composition of Seaweeds

Seaweeds	Composition (% Dry Weight)						
	Ash	Moisture	Protein	Lipid	CF	C	TDF
A. nodosum	19.4 ± 1.2	15.2 ± 0.9	7.9± 0.4	2.9 ± 0.1	11.4 ± 0.2	34.1 ± 1.3	2.1 ± 0.1
S. polycystum	22.3 ± 1.1	12.5 ± 0.2	8.4 ± 0.2	4.1 ± 0.2	13.8 ± 0.7	35.2 ± 0.5	1.8 ± 0.2
S. cristaefolium	18.3 ± 1.5	17.4 ± 1.1	8.2 ± 0.4	3.7 ± 0.1	8.4 ± 0.3	29.4 ± 1.1	2.5 ± 0.1
H. cuneiformis	19.8 ± 0.3	13.1 ± 0.8	7.9 ± 0.2	2.9 ± 0.3	10.7 ± 0.5	30.1 ± 0.9	2.2 ± 0.2
H. triquetra	21.8 ± 1.2	14.9 ± 0.4	7.6 ± 0.1	3.5 ± 0.4	12.3 ± 0.2	28.5 ± 0.7	1.7 ± 0.1

Mean ± SD; n=3, CF – Crude fiber, C - Carbohydrate; TDF – Total dietary fiber

Screening of Seaweed Phytochemicals

The methanolic extracts of the seaweeds (*A. nodosum, S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, and *H. triquetra*) contained steroids, tannins, phenols, saponins, terpenoids, flavonoids, and glycosides (Table 4). The qualitative analysis revealed the presence of all these screened compounds in *S. polycystum*, *S. cristaefolium*, *H. cuneiformis, A. nodosum*, and *H. triquetra*. Seaweeds are rich in secondary metabolites, including phenols, steroids, saponins, tannins, glycosides, and flavonoids, and these compounds have been detected in various seaweeds, including Sargassum species such as

S. boveanum, S. oligocystum, and *S. angustifolium* (Mehdinezhad *et al*. 2016). The available tannins in the methanolic extract of seaweeds exhibit antibacterial properties, bind to adhesion and contribute to enzyme inhibition, membrane disruption, and substrate deprivation (Guo *et al*. 2018). The saponins detected in the seaweeds exhibited anti-inflammatory, anticancer, and antimicrobial properties (Sidana *et al*. 2016). The phenolic compounds present in seaweed exhibit antioxidant activity (Sytar *et al*. 2018), and steroids exhibit antimicrobial and anti-inflammatory activities (Cuevas *et al*. 2015).

Table 4. Phytochemicals from the Seaweed

Seaweeds	*A. nodosum*	*S. polycystum*	*S. cristaefolium*	*H. cuneiformis*	*H. triquetra*
Phenols	+	+++	+	++	+
Steroids	++	+	-	+	+
Saponins	+	+	+	++	+
Tannins	+	++	+	+	+
Terpenoids	+	+	+	++	+
Flavonoids	+	+++	+	++	+++
Glycosides	+	+	+	+	+
+ Low amount		++ Medium amount		+++ High amount	

Phenol, Flavonoid and Tannins in Seaweeds

Polyphenols are important secondary metabolites that have various biological functions, including antioxidant and antibacterial activities. The amounts of phenols and flavonoids are high in seaweed, and the amount of polyphenols is generally greater in seaweed than in terrestrial plants. Among seaweeds, brown seaweeds produce significant amounts of flavonoids and phenols (Kalasariya *et al*. 2022). In the present study, phenol (73.1±1.1 mg GAE/g DW) and flavonoid (85.2±1.1 mg CE/g DW) contents were high in *S. polycystum*, whereas the tannin content was high in *S. cristaefolium* (15.5±0.8 mg GAE/g DW) (Table 5).

Table 5. Phytochemical Contents of Flavonoids, Phenol, and Tannin from the Seaweeds

Seaweed	Phenol (mg GAE/g DW)	Flavonoid (mg CE/g DW)	Tannin (mg GAE/g DW)
A. nodosum	64.2 ± 2.2	53.1 ± 3.4	13.8 ± 0.5
S. polycystum	73.1 ± 1.1	85.2 ± 1.1	15.3 ± 1.1
S. cristaefolium	54.5 ± 0.9	49.4 ± 2.3	15.5 ± 0.8
H. cuneiformis	49.4 ± 1.1	57.3 ± 0.9	10.3 ± 1.1
H. triquetra	36.9 ± 1.2	44.5 ± 1.1	6.1 ± 0.9

The phenolic content detected in this study was similar to that reported by Elkhateeb *et al*. (2021) in ethanol extracts from *S. subrepandum*. The total phenolic content varied among the selected seaweeds, and this variation might be related to the solvent used for extraction, method, and type of seaweed. In this study, the tannin content was highest (15.5±0.8 mg GAE/g DW) in *S. cristaefolium* and the lowest level was found in *H.*

triquetra (6.1±0.9 mg GAE/g DW). The amount of tannins detected in this study was greater than the amount of tannins detected in *C. sinuosa* (0.14 to 4.55 mg/g DW) (Shobier *et al*. 2023).

Vitamins A and C Contents in Seaweeds

Vitamins A and C are required for cellular functions in animal cells. The amount of vitamin A ranged from 2.9±0.2 to 7.4±0.3 mg α-tocopherol/g, which was higher than that of other macroalga, *T. decurren* (1.87 mg/g DW), and similar to *D. spiualis* (7.38 mg/g DW) (Ortiz *et al*. 2009). Most animals, including fish, cannot synthesize vitamin C due to the lack of L-gulonolactone oxidase enzyme. In the present study, the vitamin C level was found to be higher in *S. polycystum* (0.97±0.011 mg ascorbic acid/100 g) than in the other seaweeds tested (Table 6). The amount of vitamin C ranged from 0.36±0.03 to 0.97±0.011 mg ascorbic acid/100 g.

Table 6. Vitamins A and C Contents in Seaweeds

Seaweed	Vitamin A (mg α-tocopherol/g)	Vitamin C (mg ascorbic acid/100 g)
A. nodosum	5.8 ± 0.2	0.91 ± 0.02
S. polycystum	7.4 ± 0.3	0.97 ± 0.011
S. cristaefolium	4.3 ± 0.1	0.36 ± 0.03
H. cuneiformis	2.9 ± 0.2	0.42 ± 0.01
H. triquetra	3.5 ± 0.1	0.84 ± 0.02

Antioxidant Activity

The antioxidant activity of the seaweeds was determined by means of DPPH and H_2O_2 scavenging assays. Among the seaweeds, *S. polycystum* presented the maximum DPPH activity (64.4±2.2%) and H_2O_2 activity (45.8±1.3%) (Table 7).

Table 7. Antioxidant Activity of Seaweed Extracts

Seaweed	Antioxidant Activity	
	DPPH (%)	H_2O_2 (%)
A. nodosum	55.4 ± 1.9	35.4 ± 2.5
S. polycystum	64.4 ± 2.2	45.8 ± 1.3
S. cristaefolium	40.3 ± 1.1	17.2 ± 2.4
H. cuneiformis	53.9 ± 1.2	36.4 ± 1.1
H. triquetra	42.5 ± 2.3	37.3 ± 1.5

The antioxidant activity of seaweed has been detected previously. The phenolic and flavonoid contents were high in *S. polycystum*, and these phytochemicals improved its antioxidant activity. Phytochemical compounds such as pigments, phenolic compounds, vitamins, precursors, and polysaccharides are involved in antioxidant activity, and the presence of these antioxidant compounds, especially phenols and flavonoids, is positively correlated with antioxidant activity (Begum *et al*. 2021). The antioxidant molecules in seaweed can mitigate free radicals and exhibit free radical scavenging activity. These

molecules prevent the formation of hydroxyl radicals due to their chelation power and convert H_2O_2 to oxygen or water (Rajauria *et al*. 2013). Compared with green and red algae, the brown algae analyzed in this study exhibited significant activity (Vladkova *et al*. 2022). DPPH and H_2O_2 scavenging assays are frequently used to evaluate the antioxidant activity of seaweeds, and the antioxidant activity detected in this study was better than that of other brown and red algae (Ismail *et al*. 2022).

Growth Performance of Goldfish

Fish growth performance was analyzed, and the inclusion of seaweed up to 8% positively influenced fish growth. The feed utilization of goldfish fed 2% to 10% *A. nodosum, S. polycystum*, *S. cristaefolium*, *H. cuneiformis*, or the *H. triquetra* mixture after one month is illustrated in Table 8. Compared with the goldfish fed with the other seaweed mixtures, the goldfish fed with a mixture of 8% seaweed had greater final weights ($p<0.05$), specific growth rates ($p<0.05$), and weight gains ($p<0.05$) than did the goldfish fed with 10% seaweed powder. However, the FCR and SGR were greater than those of the control fish, which were not supplemented with seaweed. The dietary seaweed did not affect fish survival, and insignificant variation was observed in this study ($p>0.05$). The SGR was greater in the experimental fish group fed the 8% fish diet ($p<0.05$), and the FCR was good, which was associated with improved growth and good feed intake ($p<0.05$). The FCR value was affected, and growth performance was reduced at higher concentrations of seaweed (10%), suggesting that an increased amount of raw seaweed compromised fish growth. The increased percentages of seaweed powder in the formulated diet resulted from the presence of high levels of nonstarch polysaccharides, antinutritional factors, and sulfated polysaccharides. High levels of nonstarch polysaccharides, fiber, sulfated polysaccharides (*e.g.*, agar) and antinutritional factors can affect digestive enzyme activity, which affects fish growth (Sáez *et al*. 2013; Ragaza *et al*. 2015).

Table 8. Growth Performance of Goldfish Fed with Control Diet (T0), and Experimental Diet (T1 to T5)

Growth Parameters	Experiment					
	T0	T1	T2	T3	T4	T5
Initial Weight (g)	1.02	1.05	1..031	1.12	1.04	1.12
Final Weight (g)	1.41	1.73	1.82	2.53	2.92	2.53
Weight Gain (g)	0.39	0.68	0.829	1.41	1.88	1.41
Specific Growth Rate (SGR)	1.3	2.26	2.77	3.8	6.36	4.7
Feed Intake (g)	0.701	0.705	0.813	1.23	1.55	1.42
Feed Conversion Ratio (FCR)	1.79	1.03	0.98	0.87	0.82	1
Survival Percent (%)	98.3	98.5	99.2	99.43	98.3	99.4

The presence of increased levels of iron may affect fish growth (Nazarudin *et al*. 2020). The growth of *L. calcarifer* improved in the fish fed a diet containing brown seaweed (*S. polycystum*). In these fish, a 1.5% seaweed-supplemented diet improved the FCR, and a relatively high concentration of seaweed (3%) affected fish growth (Nazarudin *et al*. 2020). Shapawi and Zamry (2016) reported that the incorporation of seaweeds (*E. denticulatum*, *K. alvarezii*, and *S. polycystum*) improved the feed utilization of *L. calcarifer* juveniles. Similarly, Morshedi *et al*. (2018) reported that 9% *G. pulvinata* was optimal for

improving the growth and FCR of *L. calcarifer* juveniles. Notably, in the present study, a diet supplemented with up to 8% seaweed significantly improved goldfish growth. In addition, Zeynali *et al*. (2020) prepared a fish diet with *S. ilicifolium* meal, and up to 9% *S. ilicifolium* meal improved the FCR and growth of *L. calcarifer* juveniles. The variation in the FCR and growth of the fish varied among the experimental conditions, seaweed contents, experimental durations, fish species, and biochemical compositions of the fish diet. Owing to their synergistic effects, more than one seaweed species is recommended to improve FCR efficacy and fish growth. Seaweed metabolites can bind to enzymes, especially proteolytic enzymes, and inhibit enzyme activity. This can lead to reduced protein digestion and absorption, resulting in overall poor growth. In addition, the available antinutritional factors affect digestion and absorption in fish (Siddik *et al*. 2023).

CONCLUSIONS

1. The brown algae presented significant protein, lipid, crude fiber, and carbohydrate contents. The brown algae contained various phytochemical compounds (steroids, tannins, phenols, saponins, terpenoids, flavonoids, and glycosides).
2. The presence of polyphenols and flavonoids in seaweed improved its antioxidant activity. Brown algae contain vitamins A and C, which contribute to their antioxidant activity.
3. The combination of the five seaweed mixtures with the basal diet improved the overall growth performance of the goldfish.

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