Proximate Composition and Antioxidant Property, Anti-inflammatory and Anti-Diabetic Efficacies of Brown Seaweed Extracts

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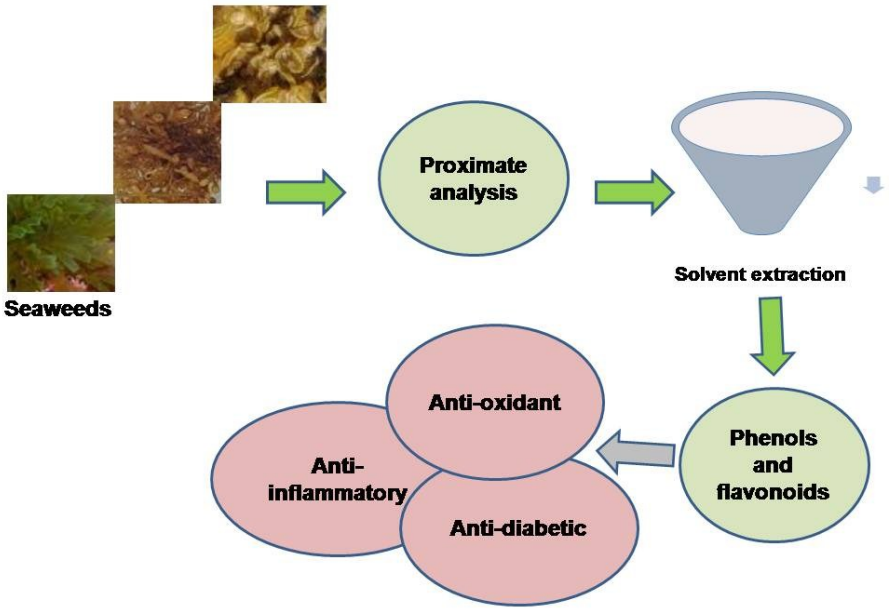
GRAPHICAL ABSTRACT



Proximate Composition and Antioxidant Property, Anti-inflammatory and Anti-Diabetic Efficacies of Brown Seaweed Extracts

Mansour K. Gatasheh *

The proximate compositions of seaweeds, namely *Sargassum boveanum*, *Padina gymnospora*, and *Dictyota dichotoma*, were analyzed. The dried powder was further extracted *via* ethanol, acetone, ethyl acetate, and chloroform, and the phenolic and flavonoid contents of the extracts were determined. The ethanolic extracts presented greater yields than the other solvents did, and the values ranged from 5.1±0.9 to 25.2±1.3, 2.2±0.4 to 35.3±1.1, and 3.3±0.5 to 20.6±1.2, for *S. boveanum*, *P. gymnospora*, and *D. dichotoma*, respectively. The highest total flavonoid and phenolic contents were found in the ethyl acetate extract of *P. gymnospora*, with values of 91.5±1.2 mg QE/g and 178.3±1.1 mg GAE/g, respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging assay, ferric reduction activity powder (FRAP) assay, and ABTS method were used. The ethyl acetate extract of *P. gymnospora* presented the maximum DPPH activity (65.3±1.1 TE/g), whereas the ethanol extract presented 27.6±0.5 AAE/g in the FRAP assay, and the ethyl acetate extract presented 37.3±0.8 TE/g activity. The ethyl acetate extract of *P. gymnospora* presented higher α-amylase inhibitory activity (0.28±0.04 mg/mL), whereas the ethanol extract of *S. boveanum* presented higher α-glucosidase inhibitory activity (0.31±0.02 mg/mL). The maximum human red blood cell protection activity (43.7±1.2%) and cyclooxygenase enzyme -2 inhibition(37.1±0.2%) activity were observed in the ethyl acetate fraction of *S. boveanum*.

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Keywords: Seaweeds; Biomass; Bioreserve; Antioxidant; Anti-diabetic; Anti-inflammatory

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INTRODUCTION

There are more than 15,000 seaweed species, and these have been widely used in several countries. They have been applied in Asian countries for medicine and food (Pérez-Lloréns *et al*. 2020). Seaweed has gained much more attention in recent years in several industries, including pharmaceuticals, because of their excellent industrially viable applications in food, textiles, and cosmetics (Janarthanan and Senthil Kumar 2018; Lomartire and Gonçalves 2022). They are used as laxatives, antimicrobial agents, and antiulcer agents owing to their potential as pharmaceutical compounds (Mendes *et al*. 2010). In addition, seaweeds are rich in several nutrients, including vitamins, proteins, and minerals (Mac Monagail *et al*. 2017). Secondary metabolites, which are extracted from seaweeds, are utilized by pharmaceutical industries and are considered a major nutrient reserve. Seaweeds present various colors and have bioactive potential. These include antifungal, antibacterial, anticancer, and antioxidant activities (Mutalipassi *et al*. 2021). In

seaweed, macromolecules and phytochemicals such as antioxidant enzymes, carotenoids, polyphenols, tocopherols, ascorbic acid and several other molecules exhibit antioxidant activity (Mutalipassi *et al*. 2021). The seaweed is also rich in minerals (iron, iodine, copper, and calcium), polysaccharides (alginate, agar–agar, and carrageenan), proteins, lipids, amino acids, and polyunsaturated fatty acids (Safafar *et al*. 2015; Schmid *et al*. 2018; Raja *et al*. 2022). Seaweeds are rich in vitamins, phlorotannins, flavonoids, bromphenols, and phenolic compounds. In addition, the availability of water-soluble and fat-soluble vitamins reduces the incidence of atherosclerosis, thrombosis, and cardiovascular diseases (Morais *et al*. 2020; Ferdous and Balia Yusof 2021).Seaweeds are rich in carotenoids and phenolic compounds that neutralize the free radicals in seaweeds, downregulate oxidative stress and prevent various degenerative diseases (Lobo *et al*. 2010).The development of these complications is associated with the progression of oxidative stress, which induces the development of free radicals and affects several metabolic pathways (Daoudi *et al*. 2022). Anti-diabetic drugs have been used to control blood sugar levels and maintain homeostasis, and these drugs have serious side effects.

Current epidemiological evidence reveals that diabetes affects more than 463 million people worldwide, and the estimated number of cases may be approximately 700 million by 2045. Diabetes is broadly classified into Type 1 and Type 2.Type 2 is the most common type of diabetes among individuals. *Diabetes mellitus* causes severe complications and affects the animal vascular system. Diabetes can induce microvascular complications (microangiopathy) and macrovascular complications (macroangiopathy) (Deshpande *et al*. 2008). Seaweeds are considered to maintain the balance of sugar metabolism in animals. The side effects of drugs vary based on the type of individual's response and the type of anti-diabetic drug. Common side effects include diarrhea, gastrointestinal disorders, edema, nausea, heart failure, severe hypoglycemia, weight gain, and vomiting (Ashaolu *et al*. 2024; Yuzbasioglu *et al*. 2022). Seaweeds have potential antidiabetic effects, and several studies have shown the positive impacts of seaweed on blood glucose control and the prevention of diabetes-related complications. Animals produce an enzyme called α-glucosidase, which regulates blood glucose levels and breaks down carbohydrates into sugars. α-Glucosidase inhibitors from the natural environment, including seaweed. Polyphenols from seaweed exhibit anti-inflammatory and antioxidant properties (Gómez-Guzmán *et al*. 2018; Michalak *et al*. 2022).

Brown seaweed is considered the major reserve of anti-inflammatory substances with various chemical compounds. These compounds are polysaccharides, polyphenols, halogenated compounds, carotenoids, and fatty acids (Fernando *et al*. 2016; Palanisamy *et al*. 2017). In brown algae, fucoidan is the major reserve of phytochemicals and contains approximately 20% to 60% fucose with varying α-glycosidic bonds. Fucoidan is considered the major phytochemical compound in brown algae (Jaswir and Monsur 2011). Brown algae contain phlorotannins, which are polyphenolic compounds that have anti-inflammatory effects. The available phlorotannins in seaweed inhibit the generation of reactive oxygen species in the cellular system and prevent cellular damage (Chouh *et al*. 2022). Most previous works on anti-diabetic and anti-inflammatory activity of brown seaweeds have been performed by extraction using different solvents rather than adequate phytochemical quantification and *in vitro* validation. In the present study, it was hypothesized that seaweed extracts alters physiological complications due to the presence of bioactive compounds. To evaluate the bioactive potential of brown algae, the present study was carried out to analyze the anti-inflammatory, antioxidant, and antidiabetic activities of seaweed.

EXPERIMENTAL

Seaweed Samples

Brown algae (*Sargassum boveanum*, *Padina gymnospora*, and *Dictyota dichotoma*) were collected from the Red Sea Coast of Jazan region, Saudi Arabia, and further washed with tap water to remove salt and epiphytes. The collected seaweeds were then lyophilized, blundered to make a fine powder and stored at −20 °C until use.

Proximate Composition of Seaweed

The moisture content of each sample was determined by heating it in a hot air oven (105 °C) for 12 h, and a constant weight was obtained. The ash content of the sample was determined *via* the gravimetric method by heating in a muffle furnace at 550 °C for 5 h (TAPPI 2007). The fat content of the seaweed was determined by AOAC 920.85 *via* petroleum ether solvent and Soxhlet extraction for 6 h (AOAC 2024). The total protein content of the seaweed extract was obtained using the Kjeldahl method, and the values were multiplied by 6.25 (Polat and Ozogul 2013). The carbohydrate content of each sample was calculated from the seaweed content. The lignin content of the seaweed was estimated following the method of Technical Association of the Pulp and Paper Industry(TAPPI) (TAPPI 2006). The contents of α-cellulose and hemicellulose were determined as described by Rowell (2005) and Wise *et al*. (1946).

Extraction

Seaweeds were subjected to solvent extraction, and the following solvent systems were used: ethanol, acetone, ethyl acetate, and chloroform. Five grams of seaweed powder was gently added to an amber flask with solvent (1:30 w/v), and the mixture was stirred at 125 rpm for 24 h at 50 °C. The extract was collected, 100 mL of fresh solvent was added, and this step was carried out three times. The collected extract was subsequently centrifuged, the residues were removed, and the final supernatant was dried and suspended in 5 mL of ethanol:water (75:25, v/v). To calculate the yield, 2 mL of seaweed extract was placed in a crucible and kept in an oven at 110 °C for 12 h. Then, it was placed in a desiccator, cooled, and weighed (Das *et al*. 2025). The extract yield was calculated following Eq. 1,

$$\text{Yield } (\%) = [(P_2 - P_1)/P_0] \times 100 \quad (1)$$

where P_0 denotes initial weight;P_1 is crucible weight; and P_2 denotes final weight after freeze-drying.

Determination of Total Phenolic Content

The total phenolic content (TPC) of the seaweed extracts were evaluated using a colorimetric test. Briefly, 0.025 mL of seaweed extract was mixed with 0.075 mL of double distilled water and 0.025 mL of 1 N Folin–Ciocalteu reagent and incubated in the dark for 10 min. Then, 0.1 mL of sodium carbonate (50 g/L) was added, and the mixture was incubated in the dark at ambient temperature. The absorbance of each sample was read at 765 nm, and the yield was expressed as gallic acid equivalents (GAE) per gram (Salar *et al*. 2012).

Determination of Flavonoid Contents

The amount of flavonoids in the extract was determined as suggested by Kim *et al*. (2006), with slight modifications. Briefly, 0.2 mL of crude extract was mixed with 1.0 mL of double distilled water and 0.05 mL of 5% $NaNO_2$ solution. The mixture was incubated for 5 min, and 0.12 mL of 10% $AlCl_3$ was added and incubated in the dark. Then, 0.4 mL of 1 M NaOH solution was added, and 0.25 mL of double distilled water was added to the blank and test samples. The absorbance values of the test, standard, and blank samples were read at 510 nm. Quercetin was prepared at various concentrations (0 to 1.0 mg/mL) and was used as a positive control.

Antioxidant Assay

DPPH Scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl)-scavenging assay is based on the antioxidant activity of seaweed extracts. The DPPH activity was assayed spectrophotometrically at 517 nm using the stable nitrogen radical DPPH. In this method, 0.025 mL of seaweed extract was mixed with a newly prepared 0.2 mL of DPPH solution (50 mg/mL) and incubated for 20 min in the dark at ambient temperature. Trolox (6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid) was used as a positive control. The final results were expressed as Trolox equivalents per gram of seaweed extract (TE/g) (Wu *et al*. 2003).

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) analysis was carried out by mixing 0.025 mL of the extract with 0.175 mL of FRAP reagent. The FRAP reagent was prepared by mixing sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), and Fe^{3+} at a 10:1:1 ratio. FRAP analyses were carried out by adding 0.025 mL of seaweed extract to 0.175 mL of FRAP reagent. The mixture was mixed and incubated for 5 min at 37 °C for the end point of the reaction, and the absorbance of the sample was read at 593 nm (Wu *et al*. 2003). The FRAP data are presented as ascorbic acid equivalents per gram of dry weight (AAE/g). Ascorbic acid was used as the positive control.

ABTS antioxidant assay

The 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method for the antioxidant assay was performed as described previously by Arnao *et al*. (2001). Briefly, 2 mM ABTS and 70 mM potassium persulfate were mixed and kept under darkness overnight at ambient temperature to generate radical cations from ABTS. Then, the mixture was further diluted with methanol (80%), and the absorbance of the mixture was tested. Absorbance values <0.700 were used for analysis at 734 nm. Then, 0.05 mL of sample was mixed with 1.95 mL of ABTS solution and incubated for 6 min. The absorbance of the sample was read at 734 nm at ambient temperature, and Trolox (6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid) was used as a positive control.

In vitro Anti-diabetic Activity of Seaweed Extract

a-Amylase inhibition assay

The *a*-amylase inhibition of the seaweed extract was tested as suggested by Daoudi *et al*. (2020), with slight modifications. Briefly, 0.2 mL of seaweed extract or acarbose (positive control) was mixed with 0.2 mL of phosphate buffer and 0.1 mL of enzyme (>25 IU). The tubes were preincubated for 10 min at 37 °C before the addition of 1% soluble

starch prepared in phosphate buffer. Furthermore, this reaction was performed for 20 min at 37 °C. Then, 0.5 mL3,5-dinitrosalicylic acid (DNS) reagent was added, and the mixture was incubated for 5 min at 100 °C in a water bath. Finally, the absorbance of the sample was read at 540 nm, and the percentage inhibition (% inhibition) was calculated *via* the following Eq. 2:

$$\text{Inhibition activity (\%)} = \frac{(\text{ODcontrol 540 nm} - \text{OD control blank 540 nm}) - (\text{OD sample 540 nm} - \text{OD sample blank 540 nm})}{\text{OD control 540 nm} - \text{OD control blank 540 nm}} \times 100 \quad (2)$$

a-Glucosidase inhibition assay

The *a*-glucosidase inhibitory effect of the seaweed extract was determined as suggested by Hbika *et al*. (2022). Briefly, the reaction mixture comprised 0.1 mL of 50 mM sucrose, 1.0 mL of phosphate buffer (50 mM, pH 7.5), and 0.1 mL of intestinal *a*-glucosidase enzyme (>10 IU). This mixture was mixed with 5 mL of double distilled water (negative control), seaweed extract, or acarbose (positive control). The mixture was incubated at 37 °C for 30 min in a water bath incubator. The amount of released glucose was measured, and the percentage inhibition was calculated.

Anti-inflammatory Activity

Human red blood cell membrane solubilization assay

The membrane solubilization assay using human red blood cell(HRBC) was performed as described earlier by Moualek *et al*. (2016). Briefly, heparinized blood was collected and centrifuged at 2000×g for 10 min at 4 °C. The collected HRBCs were subsequently suspended in isotonic phosphate-buffered saline (10 mM, pH 7.4). Salicylic acid was prepared at a 3.6 mM concentration and was used as the positive control, and DMSO (20%) was used as the negative control. The assay mixture consisted of HRBC (2%), mixed with 0.1 mL of extract and incubated for 10 min at 37 °C. The mixture was centrifuged at 2000 ×g for 5 min. The absorbance of the sample was read at 540 nm against a blank, and the percentage HRBC degradation was determined. The result was reported as a percentage (%) of inhibition. Diclofenac sodium was used as the standard anti-inflammatory drug.

Cyclooxygenase enzyme inhibition assay

A cyclooxygenaseenzyme (COX-2) inhibition assay was performed using the inhibitor screening assay kit based on the manufacturer's instructions. Briefly, the seaweed extract was suspended in DMSO at a concentration of 0.5 g/mL before being subjected to this assay. The percentage inhibition was calculated (Cayman Chemical, MI, USA).

Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD). The results were tested using one-way analysis of variance (ANOVA) and the $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Proximate Composition of Seaweed

The proximate composition of the seaweed samples was determined, and the results are presented in Table 1. The moisture content of the seaweed varies from 4.8±0.2 to 9.5±0.5, which is within the range as reported previously (Hossain *et al*. 2021). The ash content was greater in the *Padina gymnospora* extract (15.3±0.37%) than that in the other seaweeds ($p<0.05$). The ash content of the seaweed ranged from 8% to 40% (Bhuiyan *et al*. 2016), which was within the limit of seaweed extract. In general, seaweed contains minerals and high concentrations of metals and minerals that improve the ash composition of seaweed (Samarasinghe *et al*. 2021). The moisture content of seaweed observed in this study ranges from 4.8±0.2 to 9.5±0.5, which is similar to that of green, red, and brown algae (Patel *et al*. 2020). The ash content of seaweed was high in *P. gymnospora* (15.3±0.37%), and it was higher than that reported previously for other seaweed species (Cebrián-Lloret *et al*. 2024). The ash content of seaweed was greater than that of green and red algae and varies on the basis of heavy metal uptake and the physicochemical parameters of seawater (Manns *et al*. 2014; Olsson *et al.* 2020). Fat and protein contents were high in *S. boveanum* (15.3±0.9% and 17.4±2.2%), whereas carbohydrate levels were greater in *D. dichotoma* (59.5±2.2%) ($p<0.05$). The total protein content was 0.57 wt% in *P. gymnospora* (Yang *et al*. 2022) and 3.2 ± 0.12 wt% in *S. subrepandum* (Abou-El-Wafa *et al*. 2011). Moreover, Agustín *et al*. (2023) reported 49.05 ± 1.36 wt% protein content. The variation in protein levels in seaweed varies with geographical location, species, reproduction, growth, and other environmental conditions.

Table 1. Proximate Composition of Three Different Seaweeds

Proximate Composition (g/100 g dw)	*Sargassum boveanum*	*Padina gymnospora*	*Dictyota dichotoma.*
Ash	11.2±0.3	15.3±0.37	9.4±0.3
Fat	15.3±0.9	5.8±0.3	13.9±0.2
Carbohydrates	53.4±1.4	47.4±1.3	59.5±2.2
Moisture	4.8±0.2	9.5±0.5	8.7±0.55
Protein	17.4±2.2	13.5±0.9	14.2±0.99
Polysaccharides			
Soluble lignin	1.5±0.4	0.83±0.04	1.3±0.1
Insoluble lignin	5.41±0.2	8.5±0.2	11.3±0.2
α-Cellulose	14.2±0.41	11.5±0.5	15.9±0.3
Hemicellulose	4.5±0.33	6.4±0.2	5.2±0.1

Results are expressed in gram percentage dry weight. The dried seaweeds were analyzed and the results are expressed mean±standard deviation.

Effects of Organic Solvents on Phytochemical Extraction

The data on the extraction potential of organic solvents in terms of extraction yield (%) are presented in Table 2. The ethanolic extracts presented greater yields than the other solvents did (acetone, ethyl acetate, and chloroform), and the values ranged from 5.1±0.9 to 25.2±1.3%, 2.2±0.4 to 35.3±1.1%, and 3.3±0.5 to 20.6±1.2%, for *S. boveanum*, *P. gymnospora*, and *D. dichotoma*, respectively ($p<0.05$). The recovery efficiency was high

for ethanol because of its high polarity, which was associated with the nonselective extraction of several macroalgal phytochemical compounds, including polysaccharides and proteins. The present findings revealed that the recovery efficiency of plant components was dependent mainly on the chemical properties (polarity) of the organic solvent used. In addition, the yield of solvent extract has been found to be based on various factors, such as the extraction method, selected matrix, temperature, extraction time, and polarity of the solvent (Chan *et al*. 2014). Similarly, 50% ethanol has been considered to be the potent solvent for the extraction of phytochemicals from the *Clitoria ternatea* flowers (Jeyaraj *et al.* 2021).

Table 2. Effect of Organic Solvent on the Yield (%) of the Seaweed Extract.

Seaweed	Solvent	Extract Yield (%)
Sargassum boveanum	Ethanol	25.2±1.3
	Acetone	11.7±0.6
	Ethyl acetate	7.5±1.1
	Chloroform	5.1±0.9
Padina gymnospora	Ethanol	35.3±1.1
	Acetone	24.4±0.8
	Ethyl acetate	3.5±0.8
	Chloroform	2.2±0.4
Dictyota dichotoma.	Ethanol	20.6±1.2
	Acetone	14.3±0.9
	Ethyl acetate	9.5±1.1
	Chloroform	3.3±0.5

Seaweed powder was extracted with solvent at 1:30 (w/v) ratio for 24 h at 50 ºC.

Total Flavonoid and Phenolic Contents of the Seaweeds

The total flavonoid and phenolic contents of the seaweed extracts were tested on the basis of milligram quercetin equivalents (mg QEs), as described in Table 3. The highest total flavonoid content was found in the ethyl acetate fraction of *P. gymnospora*, with a value of 91.5±1.2 mg QE/g. This was followed by the ethyl acetate fraction of *S. boveanum*, with a value of 84.3±3.1 mg QE/g. These findings revealed that ethyl acetate is an effective solvent for seaweed flavonoid extraction and the extraction capacity varied significantly ($p<0.05$).

The acetone extract and chloroform extract presented the lowest values. For *S. boveanum*, *P. gymnospora*, and *D. dichotoma,* the mean amount of polyphenols was determined. The total flavonoid content of the ethyl acetate extract of *S. fusiforme*, with a value of 13.42 mg QUE/g, was investigated by Lee *et al*. (2022). The phenolic content of the methanol extract of *U. intestinalis* was 12.59 ± 2.27 mg GAE/g dry weight, and it was 88.70 ± 2.19 mg GAE/g in the methanol extract of *G. longissima* (Ullah *et al*. 2024). Moreover, methanol has been reported to be an efficient solvent for the recovery of phytochemicals from natural sources (Chan *et al*. 2014; Ullah *et al*. 2023).

These findings are in line with those of a previous report on the presence of bioactive compounds from brown seaweed (Tanna *et al*. 2019). The polyphenolic content of the seaweed extract was established after methanolic extraction in *G. bursapastoris* (Yildiz *et al*. 2011). The contents of phenols and flavonoids detected in the ethyl acetate

extract were greater than reported previously. In *P. tetrastromatica*, the methanolic extract contained 41.77 mg of QE/g of total flavonoids and 85.61 mg of GA/g of total phenolic content (Sobuj *et al*. 2021).

Table 3. Total Flavonoids and Polyphenols in the Solvent Extracts of Brown Seaweed

Seaweed	Solvent	Flavonoids (mg QE/g)	Polyphenols (mg GAE/g)
Sargassum boveanum	Ethanol	39.3±0.4	104.2±10.1
	Acetone	14.5±0.8	21.5±1.2
	Ethyl acetate	84.3±3.1	157.5±10.3
	Chloroform	14.3±0.3	35.2±1.5
Padina gymnospora	Ethanol	40.2±1.1	115.4±1.2
	Acetone	9.5±0.81	19.5±0.9
	Ethyl acetate	91.5±1.2	178.3±1.1
	Chloroform	50.2±0.2	15.3±0.9
Dictyota dichotoma	Ethanol	19.5±1.3	40.3±1.1
	Acetone	10.5±0.12	50.5±2.5
	Ethyl acetate	37.5±2.2	109.2±1.2
	Chloroform	18.5±0.9	16.4±0.8

The results are expressed as mean±standard deviation.

Antioxidant Activity of the Seaweed Extract

The antioxidant activity of the seaweed extract was determined *via* DPPH-radical scavenging, ABTS, and FRAPS assays, and the results are presented in Table 4. The antioxidant activity of plant phytochemicals is heterogeneous in nature; hence, analysis of antioxidant activity *via* different methods is suggested to explore the antioxidant mechanism of action.

The DPPH radical scavenging activity of *S. boveanum* ranged between 18.4±0.3 and 42.4±0.4 TE/g, whereas the ethyl acetate extract of *P. gymnospora* presented the maximum DPPH activity (65.3±1.1 TE/g). The ethanol extract of *P. gymnospora* had 27.6±0.5 AAE/g in the FRAP assay, whereas the ethyl acetate extract of *P. gymnospora* had 37.3±0.8 TE/g activity ($p<0.05$) (Table 4).

In this study, the increased amount of flavonoids and polyphenols presented maximum antioxidant activity due to the presence of high phytochemical content in the extract. The antioxidant activity of the solvent extracts of seaweed may be attributed to bioactive compounds such as phenols and tannins.

Dang *et al*. (2018) reported the ABTS radical scavenging activities of a solvent extract (0.06 mg/mL of *Sargassum* species such as *S. podocanthum*, *S. linearifolium*, and *S. vestitum*), and the activities were 13.30, 2.02, and 31.71 mg TE/g extract, respectively. Phytochemical compounds such as phenols and tannins significantly contribute to antioxidant activity, and these compounds may be present in solvent extracts (Phang *et al*. 2023). The antioxidant activity of seaweed confirmed that it can be used in the development of functional foods and antioxidant cosmetics.

Table 4. Antioxidant Activity of the Seaweed Extracted Using Four Different Solvents

Seaweed	Solvent	DPPH assay (TE/g)	FRAP assay (AAE/g)	ABTS assay (TE/g)
Sargassum boveanum	Ethanol	42.4±0.4	20.5±0.3	21.5±0.4
	Acetone	37.4±0.5	20.4±0.5	14.2±0.5
	Ethyl acetate	59.3±1.1	18.6±0.8	24.7±0.4
	Chloroform	18.4±0.3	10.4±0.3	3.9±0.2
Padina gymnospora	Ethanol	51.8±1.1	27.6±0.5	19.5±1.1
	Acetone	29.2±0.4	18.3±0.1	13.6±0.8
	Ethyl acetate	65.3±1.1	24.8±0.5	37.3±0.8
	Chloroform	30.5±0.8	18.4±0.2	12.5±0.2
Dictyota dichotoma.	Ethanol	38.5±0.4	29.5±0.3	21.8±0.4
	Acetone	15.4±0.3	20.3±0.2	18.5±0.3
	Ethyl acetate	40.5±1.1	19.5±0.4	20.5±0.4
	Chloroform	9.3±0.3	10.2±0.8	3.9±0.3

The antioxidant power was tested using three different methods and the results are expressed mean±standard deviation (SD).

Anti-Diabetic Activity of Seaweed Extract

The effects of seaweed extract on *a*-amylase and *a*-glucosidase inhibition are presented in Table 5. The results revealed that the seaweed extract inhibited *a*-amylase activity. Among the seaweeds, the ethyl acetate extract of *P. gymnospora* presented a low IC_{50} value (0.28±0.04 mg/mL). In addition, the present findings revealed that the seaweed extract significantly reduced *a*-glucosidase activity ($p<0.05$) (Table 5).

Table 5. Anti-diabetic Activities of the Seaweeds Extracted using Four different Solvents

Seaweed	Solvent	IC_{50} (mg/mL)	
		α-Amylase	α-Glucosidase
Sargassum boveanum	Ethanol	0.42±0.04	0.31±0.02
	Acetone	0.71±0.02	0.62±0.07
	Ethyl acetate	1.42±0.09	1.08±0.01
	Chloroform	0.39±0.02	0.42±0.02
Padina gymnospora	Ethanol	0.75±0.01	0.62±0.01
	Acetone	1.59±0.02	1.41±0.02
	Ethyl acetate	0.28±0.04	0.32±0.04
	Chloroform	0.58±0.01	0.64±0.05
Dictyota dichotoma.	Ethanol	0.49±0.02	0.34±0.02
	Acetone	1.28±0.03	1.03±0.03
	Ethyl acetate	0.95±0.05	0.37±0.02
	Chloroform	0.82±0.04	0.75±0.02

Results are presented as the mean IC_{50} values. Antidiabetic power was tested and the results are expressed mean±standard deviation (SD).

Among the solvent extracts, the ethanolic extract of *S. boveanum* presented significant activity compared with the other solvents. Natural inhibitors, either *a*-amylase or *a*-glucosidase, are considered effective hyperglycemia-controlling agents rather than inorganic drugs (Daoudi *et al*. 2020). The phenolic compounds detected in the seaweed extract exhibited enzyme inhibitor activity. Gallic acid is considered a good enzyme inhibitor that inhibits amylases (Lordan *et al*. 2013). Phytochemical compounds can stimulate insulin production by increasing peripheral glucose uptake in animals (Abdel-Moneim *et al*. 2018). The percentage inhibition of carbohydrate degrading enzymes indicated that seaweed can be used as effective antidiabetic agents (Shafay *et al*. 2021).

Anti-inflammatory Activity of Seaweed Extracts

The seaweed extracts were tested for their HRBC protection and COX-2 inhibition activities. The maximum HRBC protection activity was observed with the *S. boveanum* ethyl acetate extract (43.7±1.2%), followed by the *P. gymnospora* ethyl acetate extract (40.3±0.6%), and they varied significantly among the seaweeds ($p<0.05$). In the COX-2 inhibition assay, the highest percentage of inhibition was observed with the acetone extract of *S. boveanum* (37.1±0.2%) in comparison to the other solvent extracts ($p<0.05$) (Table 6). In addition, the other extracts moderately inhibited COX-2. COX enzymes are involved in the synthesis of prostaglandins and are involved in the mediation of various inflammatory reactions, and inhibiting the synthesis of these prostaglandins alleviates the development of fever and pain (Gautier and Choukem 2008; Bases *et al*. 2025). In this study, the anti-inflammatory response varied based on the available phytochemicals in the extract. An anti-inflammatory mechanism has been established. During the inflammatory process, lysosomal enzymes are released. The enzyme activity of lysosomes is considered the major cause and is associated with chronic or acute inflammation.

Table 6. Anti-inflammatory Activity of Seaweed Extract

Seaweed	Solvent	Anti-Inflammatory Effect (%)	
		HBRC Protection (%)	COX-2 Inhibition (%)
Sargassum boveanum	Ethanol	29.5±1.1	25.4±0.1
	Acetone	38.5±0.8	37.1±0.2
	Ethyl acetate	43.7±1.2	6.4±0.04
	Chloroform	9.4±0.1	13.5±0.03
Padina gymnospora	Ethanol	24.8±0.2	10.4±0.2
	Acetone	7.5±0.1	15.2±0.1
	Ethyl acetate	40.3±0.6	29.5±0.2
	Chloroform	22.1±0.2	20.4±0.1
Dictyota dichotoma.	Ethanol	20.5±0.2	22.6±0.2
	Acetone	9.2±0.1	3.1±0.04
	Ethyl acetate	39.4±1.5	1.5±0.02
	Chloroform	22.5±0.2	14.3±0.01

Human red blood cell membrane solubilization assay and cyclooxygenase enzyme inhibition assay were performed and the results are expressed percentage inhibition.

The functional properties of HRBC membranes are similar to those of lysosomal membranes, and this assay is used to indirectly determine the stability of lysosomal membranes (Wong and Cheung 2000). The *Laminaria* species exhibited anti-inflammatory activity and was evaluated *in vitro*. The extract of *Laminaria japonica* reduces the inflammatory response *via* nitrous oxide production and decreases reactive oxygen species generation (Lin *et al*. 2016).

CONCLUSIONS

1. Seaweeds are rich in various phytochemical compounds, including polyphenols, polysaccharides, sterols, and pigments. These contribute antioxidant, anti-inflammatory, and antidiabetic activities.
2. The availability of bioactive phytochemicals (flavonoids, terpenoids, and polyphenols)are significantly influenced by the type of solvent (methanol, ethanol, and acetone) used for extraction and the type of seaweed.
3. Seaweeds can be utilized as natural sources of antioxidant, anti-inflammatory, and antidiabetic compounds for the preparation of functional feed or as dietary supplements.

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Conflict of Interest

Authors do not have any conflict of interest in publication of this research article.

Use of Generative AI

Authors did not use any AI tools in the preparation of text, data analysis, and collation of references.

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