Antioxidant, Antiacetylcholinesterase, and Hypoglycemic Properties of Aqueous Extracts of Five Seaweeds

Arokya Glory Pushpa Thiraviam,[a] T. Renisheya Joy Jeba Malar,[b,]* Balamuralikrishnan Balasubramanian,[c] Nicholas Daniel Amalorpavanaden,[d] Bader O. Almutairi,[e] and Mikhlid H. Almutairi [e]

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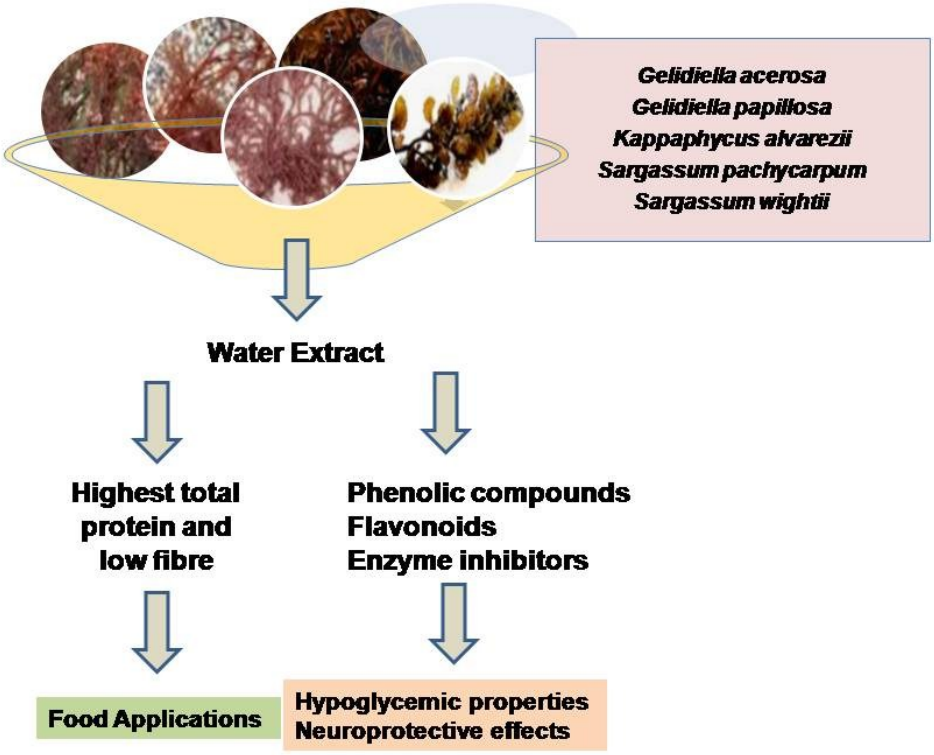
GRAPHICAL ABSTRACT



Antioxidant, Antiacetylcholinesterase, and Hypoglycemic Properties of Aqueous Extracts of Five Seaweeds

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The biochemical compositions and bioactive properties of red seaweed species *Gelidiella acerosa*, *Gelidiella papillosa*, *Kappaphycus alvarezii*, *Sargassum pachycarpum*, and *Sargassum wightii* were characterized. The crude protein (13.9 ± 0.28%) and dry matter (91.4 ± 2.1%) contents were highest in *K. alvarezii* and *G. acerosa*, respectively. The ash content was high in *S. pachycarpum* (28.9 ± 0.22%). The highest phenolic (3.27 ± 0.019 gallic acid equivalents (GAE)/g dry wt) and flavonoid (3.372 ± 0.002 catechin equivalents (CAE)/g dry wt) contents were detected in *S. wightii*. The antioxidant activity ranged from 58.5 ± 0.44 to 81.5 ± 1.1% in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The half maximal inhibitory concentration values (IC_{50} values) of the seaweed extracts ranged from 0.61 ± 0.021 mg/mL to 0.79 ± 0.027 mg/mL according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which were lower than those of the ferric reducing antioxidant power (FRAP) method (0.76 ± 0.027 to 1.39 ± 0.015 mg/mL). The seaweed extracts exhibited moderate acetylcholinesterase enzyme inhibition activity. The highest inhibition activity was observed for the *K. alvarezii* extract (49.3 ± 1.3%), while *S. wightii* presented the highest α-glucosidase (83.2 ± 1.8%) inhibition activity. The *G. papillosa* extract exhibited the highest *a*-amylase inhibition activity (57.4 ± 2.4%).

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Keywords: Seaweed; Antioxidant; Acetylcholinesterase inhibition; a-Glucosidase inhibitor; a-Amylase inhibitor; Hypoglycemic activity

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INTRODUCTION

Marine organisms are important sources of several bioactive substances used by the pharmaceutical and food industries. Bioactive compounds can be extracted from a range of marine organisms. More than 36000 bioactive compounds have been extracted from marine sources (Ebrahimi *et al*. 2023). The use of these antioxidant bioactive compounds can reduce chronic noncommunicable disease risk by decreasing the onset of inflammation and cellular oxidation (Cheung *et al*. 2016). In recent years, several phytochemical

compounds have been identified from seaweed and are considered major reservoirs of bioactive compounds (Lopez-Santamarina *et al*. 2020; Ebrahimi *et al.* 2024). Seaweeds are a set of halophytic, autotrophic, and complex groups of organisms that live in marine ecosystems and can be used as renewable resources (Anjum *et al*. 2014). Generally, seaweeds are classified into (i) Chlorophyta (green algae), (ii) Rhodophyta (red algae), and (iii) Phaeophyta (brown algae) (Kim *et al*. 2021). Both cultivated and wild seaweeds grow well in shallow coastal waters and can be collected for food consumption (Govindasamy *et al*. 2013). The use of seaweed as food is limited to within a few Asian countries, including China, Japan, and Korea (Lopez-Santamarina *et al*. 2020). The consumption of seaweed has several advantages for human health because of its essential fatty acids, protein, essential minerals, and vitamins (Manivannan *et al*. 2011). The proximate composition level and nutrient composition vary in response to several factors, including geographical area of origin, seaweed species, seawater temperature, and solar intensity (Lopez-Santamarina *et al*. 2020). In addition to food applications, seaweeds are also used in cosmetics and fertilizers. In addition, seaweed extracts are rich in antioxidant and antimicrobial compounds and are used in the pharmaceutical industry for the preparation of drugs (Chin *et al*. 2019). Extraction methods influence not only purity, yield, and efficacy, but also biofunctional activity and production cost. Selecting a suitable method is very important for obtaining secondary metabolites, improving their bioactive property, and maximizing their therapeutic properties. Traditional extraction methods, including chemical, physical, and enzyme-based methods are used to extract phytochemical compounds (Malar *et al.* 2020). The solvents such as ethanol, methanol, and n-hexanse are often applied for the extraction of the phytochemical compounds, and these chemicals are associated with safety hazards because of the presence of solvent residues. Seaweeds are rich in polysaccharides, water and lipid-soluble vitamins and minerals. Biochemicals such as terpenoids, alkaloids, carbohydrates, and glycosides can be best extracted using water (Sarikurkcu *et al.* 2020).

Seaweed from the genus *Kappaphyus* is one of the major commercially important red seaweeds and is cultivated in various countries, especially tropical countries, such as Indonesia, the Philippines, and Malaysia, as well as various Eastern African countries (Chin *et al*. 2019). This genus has attracted much more attention because it is relatively simple to cultivate in shallow waters, has a low production cost, and has a short production cycle (Adharini *et al*. 2020). It is consumed by people from coastal hamlets and is believed to have several health benefits. In India, the intertidal region of the southeast coast has a unique marine habitat with the availability of several seaweed species. The seaweed *Kappaphycus alvarezii* is widely distributed across Kanniyakumari to the Ramanthapuram coast (Prasad *et al*. 2013). Seeds from the genus *Gelidium*, especially *Gelidium sesquipedale* extracts, exhibit biological and nutritional effects, such as antimicrobial, antienzymatic, anti-inflammatory, antioxidant, and cytotoxic activities (Grozdanic *et al*. 2012; Pérez *et al*. 2016; Grina *et al*. 2020). In seaweed, phytochemicals, such as flavonoids, phenolics, functional low-molecular-weight carbohydrates, and mycosporine-like amino acids have been reported (Parailloux *et al*. 2020). The antiviral, antibacterial, anticancer, and anti-inflammatory properties of seaweed phytochemicals have been shown to play major roles in the prevention of osteoarthritis, cardiovascular disease, and diabetes (Jaswir and Mansur 2011). Seaweed contains various macroelements and trace minerals that are essential for human health and development. These essential micronutrients are required for several biological processes, including hormone metabolism, growth, reproduction, and antioxidant defense (Parailloux *et al*. 2020). The increased availability of vitamins is

associated with various health benefits, such as preventing cardiovascular disease, lowering blood pressure, and lowering cancer risk (Škrovánková 2011). Seaweeds contain several antioxidant substances that protect the host against oxidative stress caused by adverse environmental conditions. Macroalgae are rich in polyphenol compounds, and these compounds have potential antioxidant, antibacterial, anticancer, antidiabetic, antiallergic, anti-inflammatory, antiaging, and anti-HIV properties (Collins *et al*. 2016). The amounts of flavonoids and phenolic compounds vary widely, and are present even under extreme climatic conditions. These compounds are predominant in seaweed, revealing the significant role of these compounds in the antioxidant system. The nutrient composition of seaweed varies with topography, age, habitat, species type, and environmental conditions (Lopez-Santamarina *et al*. 2020). Seaweeds are excellent sources of bioactive compounds for treating Alzheimer's disease and lowering blood glucose levels. Therefore, it is necessary to study the antioxidant activity, antiacetylcholinesterase activity, and hypoglycemic properties of aqueous extracts of seaweed. Previous studies have focused mainly on screening phytochemicals and their antibacterial, antifungal, and anticancer activities. The present study aimed to analyze the biochemical content of seaweed, antioxidant activity, acetylcholinesterase enzyme inhibition activity, *a*-glucosidase activity, and *a*-amylase inhibition activity of five red seaweeds.

EXPERIMENTAL

Collection and Processing of Red Seaweeds

The red seaweeds *Gelidiella acerosa*, *Gelidiella papillosa*, *Kappaphycus alvarezii*, *Sargassum pachycarpum*, and *Sargassum wightii* were collected from the Kanniyakumari Coast, Tamilnadu, India. The collected seaweeds were cleaned and transported to the laboratory. The necrotic parts, epiphytes, dust, and mud were removed from the seaweeds. The collected seaweeds were identified using taxonomic keys based on their physical traits (Garreta *et al.* 2001; Shabaka 2018; Yang *et al*. 2021). The material was dried at 30 ± 1 °C for two weeks and ground into a fine powder. The mechanically powdered biomass was stored in an airtight container and used for further analysis.

Analysis of the Biochemical Content of Seaweed

Seaweed powder was used for the determination of dry matter, crude protein, ash, and crude fiber contents. The dried seaweed was kept in an oven for 24 h at 65 °C, and the dry matter content was determined. The amount of total protein in the dried seaweed was estimated *via* the Kjeldahl method, and a conversion factor (6.25) was used to calculate the total protein content (Lourenço *et al*. 2002). The seaweed was maintained for 2 h at 560 °C, and the ash content was determined. The crude fiber content of the seaweed was evaluated as described previously (Kasimala *et al*. 2017).

Crude Extract

Seaweed extract was prepared by adding 10 g of dried seaweed powder to 100 mL of double distilled water in an Erlenmeyer flask. The mixture was placed on a rotary shaker for one week at 110 rpm. After one week, the mixture was filtered through Whatman No. 1 filter papers, and the clear supernatant was used for analysis.

Phytochemical Analysis

Analysis of alkaloids

The alkaloid contents of *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* were assayed as described previously. Briefly, 1 mL of water extract was added to a bromocresol green (5 mL) solution. The mixture was mixed and shaken vigorously, after which 5 mL of chloroform was added. The absorbance of each sample was measured at 470 nm against a reagent blank (Shimadzu, Japan) (Lavanya *et al*. 2024).

Analysis of flavonoids

The flavonoid content of the seaweed extract was determined *via* the aluminum chloride method, and catechin was used as a standard. Then, 1 mL of extract was mixed with 4 mL of sterile double distilled water. The mixture was mixed and incubated for 5 min, and 0.25 mL of 10% aluminum chloride and 0.25 mL of 5% sodium nitrite were added. To the mixture, 2.5 mL of 1 M sodium hydroxide was added, and the final volume of the sample was increased to 10 mL with double-distilled water. The absorbance of each sample was read at 510 nm spectrophotometrically with a UV–Vis spectrophotometer. The results are expressed as mg catechin/g dried extracts (Shimadzu, Japan) (Lavanya *et al*. 2024).

Analysis of tannins

The amount of tannins in the seaweed extract was determined as described by Maliński *et al*. (2021). Briefly, 0.1 mL of aqueous extract was mixed with 2.5 mL of 40% vanillin (dissolved in methanol), and 1 mL of 1 N hydrochloric acid was added. The mixture was shaken well and left undisturbed for 15 min in the dark. The absorbance of each sample was read at 500 nm, and catechin (10 to 100 mg/L) was used as the standard (Shimadzu, Japan).

Analysis of phenolic compounds

The amount of phenolic compounds in the extract was assayed with Folin–Ciocalteu's reagent. Briefly, 0.5 mL of extract was mixed with 2 mL of 1 N Folin-Ciocalteu's reagent. The mixture was incubated for 5 min. Then, 5 mL of 2.5% sodium carbonate solution was added, and the mixture was incubated for 30 min. The absorbance of the sample was read at 750 nm *via* a spectrophotometer against the reagent blank. Catechol was used as the standard, and the amount of phenolic compounds was expressed as mg of catechol per g of dry seaweed weight (Shimadzu, Japan) (Nortjie *et al*. 2022).

Analysis of cardiac glycosides

The amount of cardiac glycoside in the extract was determined *via* Buljet's reagent. Briefly, 0.5 g of dried seaweed powder was soaked in alcohol (20 mL, 70%) for 2 h and filtered through Whatman No. 1 filter paper. Then, 0.1 M disodium hydrogen phosphate solution and lead acetate solution were added. Freshly prepared picric acid solution (9.5 mL of picric acid) and 0.5 mL of 10% sodium hydroxide were subsequently added. The mixture was incubated for 10 min, and the absorbance of the sample was read at 217 nm against a reagent blank (Shimadzu, Japan) (Maliński *et al*. 2021).

Analysis of steroids

One mL of seaweed extract was pipetted into a test tube. To 0.5 mL of sulfuric acid, 2 mL of 0.5% iron (III) chloride and 2 mL of 0.5% potassium hexacyanoferrate (III)

solution were added, and the mixture was incubated at 70 ± 1 °C for 20 min. Then, the absorbance was read at 780 nm against the reagent blank (Shimadzu, Japan) (Nortjie *et al*. 2022).

Antioxidant Activities

Free radical scavenging capacity using the DPPH assay

The antioxidant activity of the seaweed extract *via* the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Wada *et al*. (2015). Briefly, 0.5 mL of 8.66×10^{-5} M DPPH methanolic solution was added to 0.1 mL of standard/sample in a 96-well microtiter plate. The mixture was incubated in the dark for 30 min at room temperature (27 ± 1 °C). The absorbance of each sample was measured at 517 nm against a reagent blank. All the experiments were performed in triplicate, and the DPPH radical scavenging activity was calculated *via* the following equation,

$$\text{Scavenging effect (\%)} = \left[1 - (A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of the control (DPPH without sample), A_{sample} is the absorbance of the sample, and $A_{\text{sample blank}}$ is the absorbance of the sample without the DPPH reagent. Four milligram of ascorbic acid was dissolved in 5.0 mL of double distilled water. It was serially diluted and prepared at 50 to 200 µg/mL concentrations.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant power of the seaweed extracts was analyzed *via* a FRAP assay as described by Benzie and Strains (1996). Acetate buffer (300 mM, 3.6) was prepared, and 40 mMHCl, 10 mM TPTZ, and 20 mM $FeCl_3$ were mixed at a ratio of 1:10:1 (v/v/v). For the freshly prepared 2 mL of FRAP solution, 0.1 mL of seaweed extract was dispensed and incubated for 10 min. The absorbance of the reaction mixture was measured at 593 nm. Trolox was used as the standard, and the results are expressed as mg Trolox equivalents (TE)/100 g dry seaweed.

Acetylcholinesterase (AChE) Inhibition Activity

Acetylthiocholine iodide was used as the substrate for the AChE inhibition assay. The reaction mixture comprised 130 µL of sodium phosphate buffer (pH 8.0, 0.1 M) containing 100 µL of sample, and 10 µL of AChE (0.5 U/mL) was mixed and incubated at 28 ± 1 °C for 10 min. Then, 50 µL of DTNB (5,50-dithiobis (2-nitrobenzoic acid) (0.5 mM) and 10 µL of acetylthiocholine iodide (0.71 mM) were added. The development of the 5-thio-2-nitrobenzoate anion was measured at 412 nm, and kinetic changes were observed every 10 s (Ellman *et al*. 1961). The percentage (%) inhibition of AChE was determined via the following equation,

$$\%\ \text{Inhibition} = \left[(\Delta\text{Abs/min}_{\text{control}} - \Delta\text{Abs/min}_{\text{sample}})/\Delta\text{Abs/min}_{\text{control}}\right] \times 100$$

where Abs (control) is the absorbance of the assay without sample; and Abs (sample) is the absorbance of the assay in sample. The amount of sample that caused 50% inhibition of AChE activity was calculated *via* a nonlinear regression assay. Physostigmine was used as standard and was prepared at various concentrations ranging between 1.25 and 250 µg/mL.

a-Amylase Inhibition Assay

In vitro α-amylase inhibition analysis was performed as described previously by Daoudi *et al*. (2020). Briefly, 0.1 mL of the algal extract or acarbose (standard), 0.25 mL of buffer (sodium phosphate buffer, pH 7.2, 0.1 M), and 0.1 mL of amylase (100 IU/mL) were added. The mixture was mixed for 10 min, and 0.5 mL of starch (1% starch, dissolved by heating in sodium phosphate buffer, pH 7.2, 0.1 M) was added. The mixture was incubated for 30 min at 37 °C. The DNS reagent was added (0.1 mL), and the mixture was placed in a boiling water bath for 10 min. The absorbance of the sample was read at 540 nm against a reagent blank, and the percentage inhibition was determined (Daoudi *et al*. 2020). Acarbose was used as a positive control for the α-amylase inhibitory assay.

In vitro a-Glucosidase Inhibition Activity

The effects of the seaweed extracts on α-glucosidase activity were evaluated as described previously by Hbika *et al*. (2022), with slight modifications. Briefly, 1 mL of sucrose (50 mM) was mixed with 1 mL of α-glucosidase solution (10 IU). To the control, double distilled water was added, and acarbose was used as the positive control. The mixture was incubated for 10 min at 37 °C. The released glucose level was determined, and the percentage of enzyme inhibition was calculated.

RESULTS AND DISCUSSION

Chemical Composition of Seaweeds

The chemical composition of the seaweed revealed that the crude protein content was greatest in *K. alvarezii* (13.9 ± 0.28%), followed by *G. acerosa* (10.3 ± 0.41%), and *S. wightii* (8.3 ± 0.92%) (Table 1). The dry matter content was high in *G. acerosa* (91.4 ± 2.1%), whereas the ash content was high in *S. pachycarpum* (28.9 ± 0.22%). The crude fiber content was highest in *G. papillosa* (13.2 ± 0.42%) and extremely low in *G. acerosa* (4.8 ± 0.9%). Seaweeds have diverse chemical compositions of biochemical compounds, including lipids, polysaccharides, and proteins. The amount of these biochemical compounds varies with environmental factors, such as water temperature, season of harvest and nutrient availability in the water (Bikker *et al*. 2020; Ford *et al*. 2020). The amount of biochemical compounds determined in the current study was comparable with that reported in previous studies. The ash content of the seaweed was >20% and reached a maximum (28.9 ± 0.22%) in *S. pachycarpum*, which was similar to previous reports (Tayyab *et al*. 2016; Bikker *et al*. 2020). The seaweed contained >5% crude protein and less crude fiber. Thus, seaweeds can serve as rich sources of protein and minerals to meet livestock feed (Corino *et al*. 2019; Frazzini *et al*. 2022).

In feed formulations, the bioavailability of minerals is affected by the fiber content of seaweed, which reduces the bioavailability of minerals by forming insoluble complexes and colloidal structures. In the current study, a lower fiber content was detected in *G. acerosa* and *S. pachycarpum* than in the other seaweeds, revealing that these seaweed species are suitable for feed formulation. The crude protein and dry matter contents of the *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* powders were in line with previous reports (Manivannan *et al*. 2009; Syad *et al*. 2013; Suresh Kumar *et al*. 2015; Rasyid and Handayani 2019; Aziz *et al*. 2021; Kumar *et al*. 2021a,b).

Table 1. Chemical Compositions of the Five Selected Seaweeds

Seaweed	Crude Protein (%)	Dry Matter (%)	Ash (%)	Crude Fiber (%)
G. acerosa	10.3 ± 0.41	91.4 ± 2.1	27.3 ± 0.38	4.8 ± 0.9
G. papillosa	7.5 ± 0.19	88.2 ± 0.5	25.2 ± 0.51	13.2 ± 0.42
K. alvarezii	13.9 ± 0.28	87.1 ± 1.3	20.3 ± 1.1	10.1 ± 0.83
S. pachycarpum	6.5 ± 0.47	86.5 ± 2.2	28.9 ± 0.22	8.6 ± 0.38
S. wightii	8.3 ± 0.92	89.2 ± 0.5	21.9 ± 0.27	9.5 ± 0.42

Analysis of Phytochemical Components

Phytochemical analysis of *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, and steroids. In *G. acerosa*, 1.98 ± 0.04 CAE/g dry wt tannin and 1.18 ± 0.07 mg/g dry wt steroid were detected. The contents of phenolic compounds (3.27 ± 0.019 GAE/g dry wt.) and flavonoids (3.372 ± 0.002 CAE/g dry wt.) were greater in *S. wightii* than in the other seaweeds (Table 2). These phenolic compounds and tannins play significant roles as major bioactive compounds (Rengasamy *et al*. 2013), whereas tannins are reported to have antibacterial, antiviral, and antioxidant properties (Kytidou *et al*. 2020). A greater amount of glycosides (2.93 ± 0.029 mg/g dry wt) was detected in *K. alvarezii* than in the other seaweeds.

Glycosides have anti-inflammatory and antioxidant activities and find good application in the prevention of several human diseases (Kytidou *et al*. 2020). Flavonoids are major phytochemical compounds because of their antioxidant, antimicrobial, antiviral, and spasmolytic activities. These phytochemical compounds significantly contribute to the bioactivity of seaweed (Anitha *et al*. 2019). Similarly, the phytochemical compounds and biological activities of the seaweed extracts were reported previously. The major reported phytochemical compounds in seaweed are phenolics, flavonoids, polysaccharides, and carotenoids (Sheela and Uthayakumari 2013; Mahabaleshwara *et al*. 2016).

Table 2. Phytochemical Content of Aqueous Seaweed Extract

	Seaweed				
Phytochemicals	*G. acerosa*	*G. papillosa*	*K. alvarezii*	*S. pachycarpum*	*S. wightii*
Alkaloids (ATE/g dry wt)	0.591 ± 0.02^{a}	1.7 ± 0.06^{b}	0.172 ± 0.009^{b}	1.02 ± 0.05^{ab}	0.285 ± 0.05^{c}
Flavonoids (CAE/g dry wt)	0.497 ± 0.02^{a}	1.06 ± 0.03^{b}	2.05 ± 0.05^{c}	0.051 ± 0.001^{d}	3.372 ± 0.002^{d}
Tannins (CAE/g dry wt)	1.98 ± 0.04^{a}	1.08 ± 0.018^{b}	1.05 ± 0.021^{c}	1.02 ± 0.02^{b}	2.02 ± 0.03a
Phenolics (GAE/g dry wt)	0.192 ± 0.02^{a}	2.19 ± 0.03^{b}	1.05 ± 0.02^{ab}	3.03 ± 0.02^{c}	3.27 ± 0.019^{bc}
Glycosides (mg/g dry wt)	1.72 ± 0.04^{a}	1.03 ± 0.04^{b}	2.93 ± 0.029^{c}	1.06 ± 0.062^{b}	0.83 ± 0.054^{d}
Steroids (mg/g dry wt))	1.18 ± 0.07^{a}	0.514 ± 0.04^{b}	2.05 ± 0.04^{c}	0.051 ± 0.002^{d}	1.02 ± 0.04^{e}

The values are the means of three different analyses of the sample ± standard deviation (n=3)

Antioxidant Activity

Proinflammatory mediator inhibitors are potential candidates for anti-inflammatory therapeutics. In this study, the antioxidant properties of extracts of *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* were detected. Compared with the FRAP method, the present study demonstrated greater DPPH antioxidant activity. The samples analyzed in the current study differed in their antioxidant activity according to the DPPH and FARP assay methods, and the antioxidant content ranged from 58.5 ± 0.44 to 81.5 ± 1.1% DPPH activity (Table 3). The IC_{50} values of the seaweed extracts ranged from 0.61 ± 0.021 mg/mL to 0.79 ± 0.027 mg/mL according to the DPPH assay (Fig. 1). This result was in agreement with previous findings by Araújo *et al*. (2022) in *Kappaphycus alvarezii* strains (Rhodophyta) collected in Brazil. Moreover, the percentage of antioxidants varies based on the available phenolic compounds in the extract, which is determined by exposure to temperature and other factors that induce environmental stress (Sampath-Wiley *et al*. 2008; Almeida *et al*. 2021).

In addition to the major nutrient sources in seaweed, other available bioactive substances, such as pigments, polyphenols, vitamins, essential fatty acids, and amino acids, are considered feed supplements in aquaculture. These bioactive compounds possess antifungal, antiviral, antimicrobial, antioxidant, anti-stress, immunostimulant, and anti-inflammatory properties (Freitas *et al*. 2015). Table 4 shows the increase in FRAP activity (%) due to the scavenging potential of the seaweed extract. *S. wightii* extract exhibited maximum (81.5 ± 1.1%) activity at a concentration of 1 mg/mL among the seaweeds. These findings indicate that *S. wightii* is a potential source of antioxidants. The IC_{50} value was higher for the FRAP assay method than for the DPPH assay method. The IC_{50} values ranged between 0.76 ± 0.027 and 1.39 ± 0.015 mg/mL according to the FRAP method (Fig. 2). The IC_{50} value was 0.49 mg/mL in DPPH assay. Compared with synthetic antioxidants, natural antioxidants have several advantages. Synthetic antioxidant molecules are mutagenic and toxic to cellular systems. These natural antioxidants replaced synthetic antioxidants due to the presence of polysaccharides, pigments, fatty acids, phenolic compounds, and peptides in seaweeds. All these bioactive molecules improved oxidative stability of food products and associated with antioxidant activity (Tenorio-Rodriguez *et al.* 2017). The seaweed extract possesses antioxidant abilities, especially the maximum activity in the DPPH and FRAP assays.

Table 3. DPPH Antioxidant Activity of Seaweed Extracts at Various Concentrations

Sample Volume (mg/mL)	DPPH Activity (%)				
	G. acerosa	*G. papillosa*	*K. alvarezii*	*S. pachycarpum*	*S. wightii*
0.2	14.2 ± 0.12^{a}	20..8 ± 0.18^{a}	17.3 ± 0.19^{a}	8.5 ± 0.03^{a}	17.4 ± 0.29^{a}
0.4	20.2 ± 0.2^{b}	28.9 ± 1.5^{b}	24.3 ± 0.27^{b}	25.3 ± 1.2^{b}	29.5 ± 0.12^{b}
0.6	41.4 ± 0.14^{c}	37.5 ± 2.7^{c}	47.5 ± 0.18^{c}	39.5 ± 0.9^{c}	38.5 ± 0.33$_{c}$
0.8	52.5 ± 0.25^{d}	64.2 ± 3.1^{d}	57.4 ± 0.25^{d}	53.1 ± 0.63^{d}	58.1.6 ± 0.55^{d}
1	59.3 ± 1.9^{e}	78.3 ± 2.2^{e}	60.5 ± 0.62^{e}	58.5 ± 0.44^{d}	81.5 ± 1.1^{e}

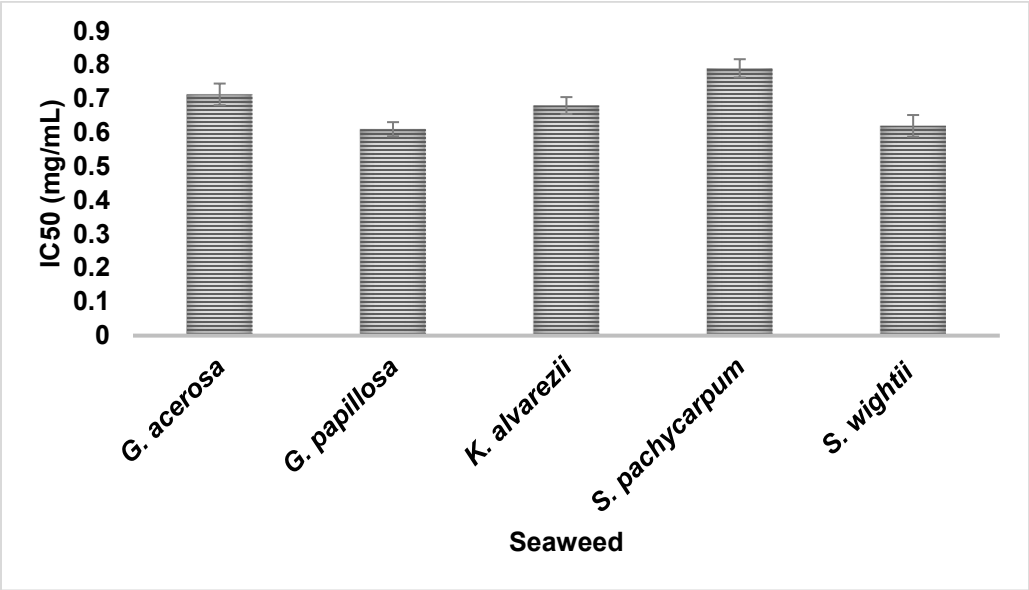

Fig. 1. The half maximal inhibitory concentration values (IC_{50} values) of the seaweed extracts according to the DPPH antioxidant assay

Table 4. FRAP Antioxidant Activity of Seaweed Extracts at Various Concentrations

Sample Volume (mg/mL)	FRAP Activity (%)				
	G. acerosa	*G. papillosa*	*K. alvarezii*	*S. pachycarpum*	*S. wightii*
0.2	15.5 ± 0.29	20.3 ± 0.02	18.3 ± 0.09	16.2 ± 0.11	19.8 ± 0.15
0.4	20.4 ± 0.11	23.7 ± 0.28	27.4 ± 0.11	23.2 ± 0.18	28.4 ± 0.27
0.6	29.2 ± 0.22	30.5 ± 1.1	40.4 ± 0.21	32.1 ± 0.08	37.5 ± 0.28
0.8	38.3 ± 0.11	49.2 ± 2.1	42.8 ± 0.33	39.7 ± 0.12	55.1 ± 0.24
1	53.2 ± 0.27	62.4 ± 1.9	54.2 ± 0.22	45.2 ± 0.16	67.5 ± 0.38

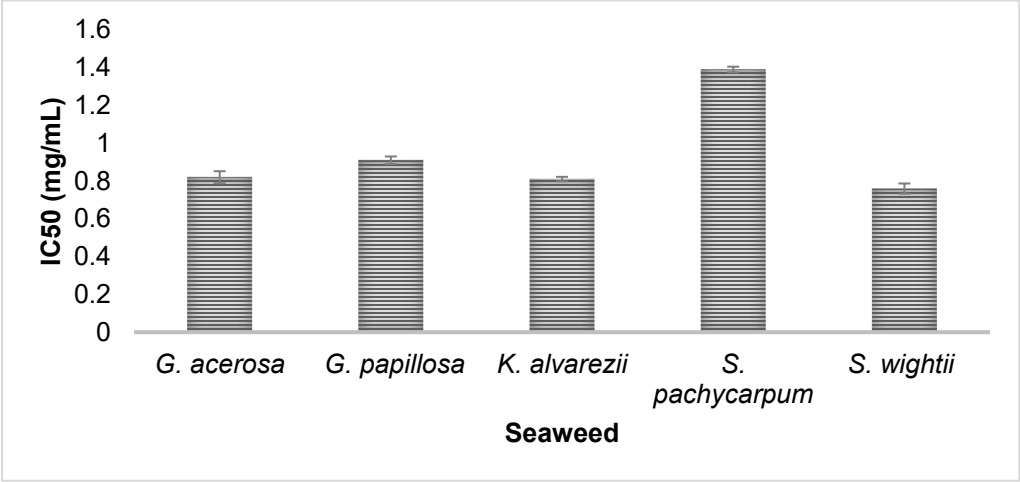

Fig. 2. The half maximal inhibitory concentration values (IC_{50} values) of seaweed extracts according to the FRAP antioxidant assay

AChE-Inhibitory Properties of Seaweed Extracts

The AChE enzyme inhibition activity of the seaweed extract was assayed, and the results are presented in Fig. 3. The seaweed extracts exhibited moderate AChE enzyme inhibition activity, and the maximum inhibition activity was observed in *K. alvarezii* (49.3 ± 1.3%). The other extracts had 36.5 ± 1.1% (*S. wightii*) and 33.9 ± 2.2% (*G. papillosa*) inhibitory activity against AChE. Many studies have reported the development of AChE enzyme inhibitors for the treatment of various diseases, including Alzheimer's disease. However, screening suitable AChE enzyme inhibitors is still in its infancy. The present study revealed the presence of AChE inhibitors in the extract; however, it was <50%. The results of the present study were similar to those of previous reports on *G. acerosa* (Devi *et al*. 2008), *Halimeda cuneata* (Rengasamy *et al*. 2015), *Ulva reticulata*, *Hypnea valentiae*, *Gracilaria edulis* (Suganthy *et al*. 2010), *Ecklonia maxima* (Osbeck) Papenfuss (Kannan *et al*. 2013), and *Gracilaria manilaensis* (Pang *et al*. 2022). The secondary metabolites, particularly monoterpenes, represent an important group of natural compounds that exhibit acetylcholinesterase inhibition activity. Algal extracts containing monoterpenes demonstrate the highest levels of acetylcholinesterase inhibition and act as reversible competitive inhibitors of the acetylcholinesterase enzyme (Machado *et al.* 2025). AChE inhibitors are used to treat neurodegenerative diseases. AChE contributes to various functions beyond the breakdown of ACh, including morphogenesis, inflammation, and oxidative stress. AChE is found in AChE-E or AChE-R forms and is found in neurodegenerative diseases. AChE is also involved in cell apoptosis, and the inhibition of this enzyme activity subsequently inhibits the development of apoptosis (Xie *et al*. 2011; Zhang and Greenberg 2012).

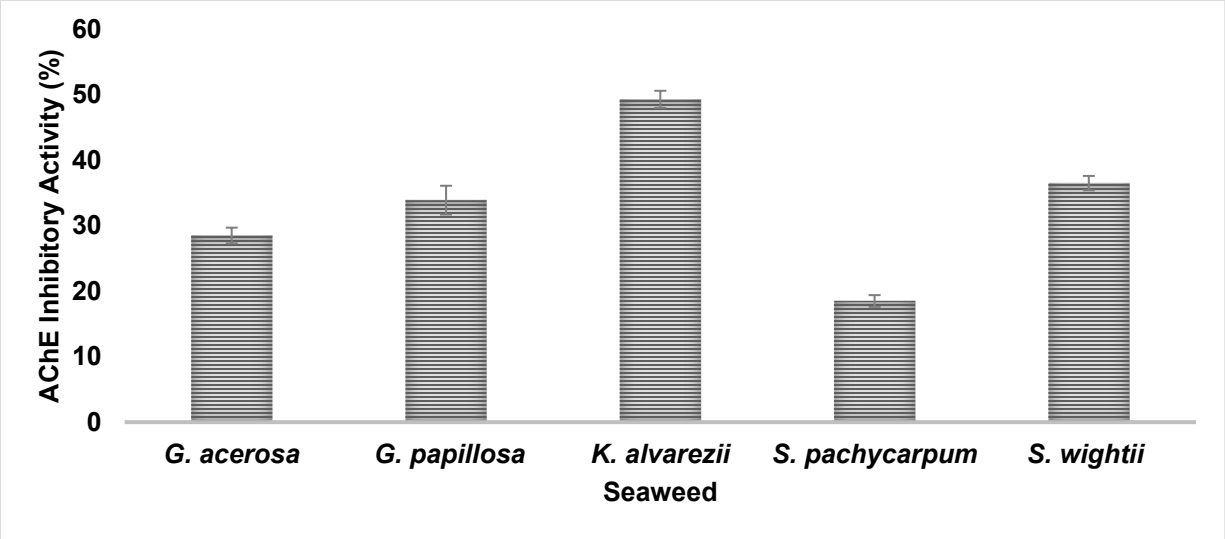

Fig. 3. Acetylcholinesterase inhibition activity of aqueous seaweed extract; AChE enzyme inhibition activity was assayed, and the percentage enzyme inhibition activity was calculated.

In vitro α-Amylase and α-Glucosidase Inhibition Activity

Figure 4 illustrates the effects of the *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* extracts on α-amylase and α-glucosidase inhibition activity *in vitro*. The results revealed that all five seaweed extracts inhibited α-amylase and α-glucosidase activity. Among the different aqueous extracts, *S. wightii* exhibited greater α-glucosidase (83.2 ± 1.8%) inhibition activity than the other extracts did (Fig. 4a). The other

extracts showed dissimilar α-glucosidase inhibition activities. Compared with the other extracts, the *G. papillosa* extract showed the highest α-amylase inhibition activity (57.4 ± 2.4%) (Fig. 4b).

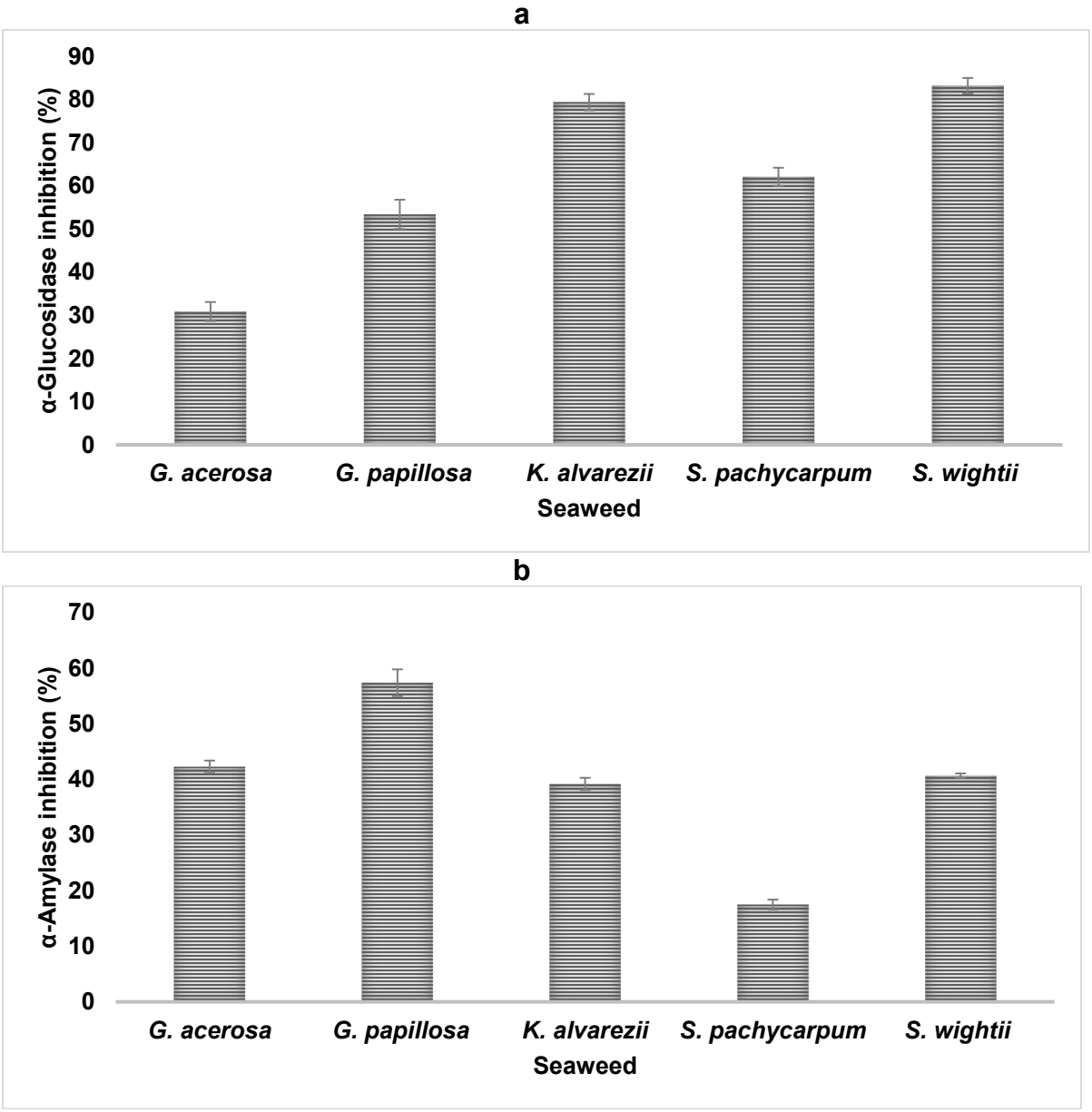

Fig. 4. Effect of seaweed extract on α-Glucosidase (a) and α-Amylase (b) inhibition

The results revealed that the enzyme inhibitory activity of the seaweed extract effectively reduced pancreatic glucosidase activity. The detected enzyme inhibitory functional properties of the seaweed extract may be attributed to the presence of phytochemical compounds in the extracts that can act as effective inhibitors involved in hydrolyzing disaccharide and polysaccharide chains. These molecules may be unique to α-glucosidase and α-amylase. The inhibitory effects of seaweed extracts obtained from *G. acerosa*, *G. papillosa*, *K. alvarezii*, *S. pachycarpum*, and *S. wightii* were lower than those of standards (acarbose). These results are in good agreement with those of a study performed by Chin *et al*. (2015), who reported that *H. macroloba* seaweed extract had

moderate activity even at relatively high concentrations (40 mg/mL). Hyperglycemia in the body can induce the generation of free radicals and tissue damage, and the presence of phenolic substances in the seaweed extract can reduce oxidative stress. The antioxidant molecules in the extract are strongly related to reducing hyperglycemic conditions and diabetes complications. The secondary metabolites of seaweed, flavonoids, alkaloids, and terpenoids are inhibitors of α-glucosidase (Xu 2010). These α-glucosidase inhibitors reduce blood glucose levels both *in vitro* and *in vivo* (Ghani 2015). Phytochemicals, such as flavonoids, alkaloids, glycosides, and steroids, have hypoglycemic effects (Al-Rajhi *et al.* 2023; Bakri *et al.* 2024). The seaweeds contained fatty acids, and these compounds inhibited the activity of carbohydrate-hydrolyzing enzymes, including α-glucosidase and α-amylase. These inhibitors bind to the active site of the enzyme substrate, resulting in the active site of α-glucosidase or α-amylase (Su *et al*. 2013).

CONCLUSIONS

1. The seaweeds *Gelidiella acerosa*, *Gelidiella papillosa*, *Kappaphycus alvarezii*, *Sargassum pachycarpum*, and *Sargassum wightii* were found to be major sources of protein, dry matter, and ash contents and had low fiber content. The decreased level of fiber content in the aqueous extract increased the availability of nutrients in feed applications.
2. The highest phenolic and flavonoid contents in the seaweed extract improved the antioxidant activity. The acetylcholinesterase enzyme inhibition activity of seaweed highlights the potential of these seaweeds in the treatment of Alzheimer's disease due to their neuroprotective effects.
3. The seaweeds inhibited a-glucosidase and a-amylase enzymes activity. These properties reduce the glucose content in the serum.

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