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Evaluation of the chemical, functional, antioxidant, and antidiabetic properties of *Kappaphycus alvarezii* (Red seaweed)

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Abstract
Kappaphycus alvarezii is one of the edible red seaweeds commercially cultivated in Sri Lanka and available in large quantities; however, people are unaware of their biochemical and bioactive importance. This study aimed to investigate the chemical and functional properties of *K. alvarezii* powder and the bioactivity of different solvent extracts of *K. alvarezii*. Compositional analysis on a dry-weight basis showed that it was rich in fiber (9.82%), ash (28.87%), and carbohydrates (58.41%), mainly carrageenan (37.70%), but low in fat (0.68%). It contained water-soluble red pigment phycobiliprotein (1.13 mg/g) and other health-promoting pigments such as chlorophylls and carotenoids. It showed good solubility (0.26 g/g), Swelling capacity (11.07 mL/g), water-holding capacity (8.87 g/g), and oil-holding capacity (3.04 g/g), with a significant correlation with increasing temperature. Extraction yield was significantly higher in the methanolic extract (22.16%). Water extract had significantly higher level of total phenolic (1.85 mg gallic acid equivalent/g extract) and flavonoid content (0.88 mg rutin equivalent/g extract), exhibited significantly higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC_{50}: 6.36 mg/mL), total antioxidant (6.52 mg ascorbic acid equivalent (AAE)/g extract, reducing capacity (2.86 mg AAE/g extract), and H_2O_2 scavenging activity (IC_{50}: 1.62 mg/mL), compared to ethanol and methanol extracts, whereas alpha - amylase inhibition activity was significantly high in ethanolic extract (IC_{50}: 12.13 mg/mL). This study suggested that *K. alvarezii* might be potentially used as a functional food or ingredient in the food and pharmaceutical industries to improve human health.

Keywords: Seaweed, Nutrition, Physicochemical, *K. alvarezii*, antioxidant, antidiabetic, bioactive

Introduction
As the world population grows, people are seeking alternative sources of food, medicine, and agriculture. Seaweed is economically significant as it is used in the food, medicine, and cosmetics sectors. Their natural abundance, diversity, and global availability make them a valuable source of biologically functional substances (Domínguez, 2013) [19]. Seaweeds have an excellent nutritional profile, being rich in macro- and micronutrients (Subbiah *et al*., 2022) [55]. Sudhakar *et al*. (2018) [56] indicated that macroalgae are high in carbohydrate, protein, and water and low in fat. The nutritional content and bioactive compounds of seaweeds vary with season, location, light intensity, water salinity, and temperature (Lafeuille *et al*., 2023) [37]. Bioactive molecules, such as polysaccharides, dietary fibre, pigments, proteins, minerals, vitamins, lipids, polyunsaturated fatty acids (PUFAs), steroids, phenolics, alkaloids, saponins, and terpene, have applications across various fields of study, such as biomedical and food industries (Ejaz *et al*., 2020) [20].
The edible red seaweed (*Kappaphycus alvarezii)*, commonly referred to as *Eucheuma cottonii,* is one of the algal species discovered to be high in nutrients and nutraceuticals (Chang *et al*., 2017) [14]. *K. alvarezii* is the fifth most widely cultivated macroalga with numerous industrial applications worldwide (Nunes *et al*., 2024) [43]. *K. alvarezii* is rich in carrageenan, as well as other phytochemicals such as phenolics, flavonoids, phytosterols, lectins, terpenoids, alkaloids, polysaccharides, proteins, and pigments has been found to display diverse bioactive properties, including anti-inflammatory, antibacterial, antifungal, antioxidant, anticancer, anticoagulant, antidiabetic, and antiviral effects (Jalal et

al., 2023 & Cadar *et al*., 2025) [29, 9]. It is commercially cultivated in Sri Lanka and available in large quantities; however, it remains underutilized, and people are largely unaware of its nutritional, physicochemical, and bioactive significance. Therefore, the present study aimed to evaluate the chemical, functional, and bioactive properties of *K. alvarezii*.

Materials and Methods

Materials

Fresh *Kappaphycus alvarezii* was collected from a cultivation site at Ponnalai, in the coastal area of Chulipuram (9.7841°N, 79.9397°E), located in the Jaffna District, Northern Province, Sri Lanka. The seaweed was harvested after 45 days of cultivation. All analytical grade solvents and chemicals were procured from Sigma-Aldrich.

Preparation of Seaweed Powder

The seaweeds were transported to the laboratory in a cooler box, thoroughly rinsed with tap water to remove epiphytes, sand, salt, and other debris, and then washed with distilled water to remove impurities. Then, the sample was dried in a dehydrator (Weston® Pro-1200, USA) at 40 °C until it reached constant weight. Dried seaweed samples were milled into powder using a laboratory milling machine (HR 2200, China). The ground sample was sieved to fine particles (< 475 µm) using a sieve (mesh size 40 ASTM). The powdered samples were vacuum-packed and maintained in a refrigerator until analysis.

Proximate composition of seaweed powder

Seaweed powder was analyzed using the AOAC (2006) [6] method: crude fibre (method 978.10), crude protein (method 978.04), crude fat (method 920.39), crude ash (method 923.03), and moisture (method 925.10). The carbohydrate content was determined by difference, subtracting the percentage of fat, ash, protein, and moisture from 100%. The total caloric content was determined using the Atwater system (Eq. 1).

$$\text{Energy} = (9 \text{ kcal. g}^{-1} \text{ x\% fat}) + (4 \text{ kcal g}^{-1} \text{ x\% protein}) + (4 \text{ kcal g}^{-1} \text{ x\% carbohydrate}) \quad (1)$$

Carrageenan content

A slightly modified version of Al-Alawi *et al*. (2011) [5] was used for the carrageenan extraction and alkali pre-treatment. In brief, 1g of powdered dried seaweed was steeped in 20 mL of 2% KOH at 80 °C for three hours. After filtering and repeatedly washing the extract with de-ionized water to remove any remaining KOH salt, 5 M HCl was added to bring the pH to 8 or 9. The suspension was heated to 90 °C for one hour to extract carrageenan. Ethanol precipitation was carried out by adding two volumes of 95% ethanol (1:2 v/v) to the concentrated extract. Carrageenan precipitate was dried and milled into powder. Carrageenan yield was calculated as the percentage of carrageenan obtained relative to the initial weight of the sample.

Pigment Analysis

Phycobiliprotein content

Phycobiliprotein content was determined using the method described by Castejón *et al*. (2021) [10] with slight modifications. The pigment was extracted using 0.1 M phosphate buffer (pH 6.8) with sonication for 30 minutes in an ultrasonic bath at room temperature, and then kept for 1 hour with continuous agitation.

Phycocyanin (R-PC), Phycoerythrin (R-PE), and Allophycocyanin (APC) concentrations were combined as total phycobiliprotein (PEP) and assessed using absorbance readings at 562 nm, 615 nm, and 652 nm from a UV-VIS spectrometer (Thermo Scientific 201, USA). Phycobiliprotein content was determined by applying the following equations (Eq. 2, 3, and 4):

$$\text{R-PC (mg/mL)} = (OD_{615} - 0.474\ OD_{652}) / 5.34 \quad (2)$$

$$\text{R-PE (mg/mL)} = (D_{562} - 2.41\ PC - 0.849 APC) / 9.62 \quad (3)$$

$$\text{APC (mg/mL)} = (OD_{562} - 0.208\ OD615) / 5.09 \quad (4)$$

The concentration of phycobiliprotein is represented as mg/mL, and the result is presented as mg/g dw.

Total chlorophyll and carotenoid Content

The technique outlined by Dere *et al*. (1998) [18], with minor adjustments, was used to assess the levels of carotenoid and chlorophyll. Supernatant absorbance was read at 470, 645, and 662 nm with a UV/VIS spectrophotometer (Thermo Scientific 201, USA). The following formulas (Eq. 5,6 and 7) were used to compute pigments, which were expressed as µg/g dw.

$$\text{Chlorophyl a} = 11.75\ (OD_{662}) - 2.350\ (OD_{645}) \quad (5)$$

$$\text{Chlorophyl b} = 18.61\ (OD_{645}) - 3.960\ (OD_{662}) \quad (6)$$

$$\text{Carotenoids} = 1000\ (OD_{470}) - 2.270\ (C_a) - 81.4\ (C_b) / 227 \quad (7)$$

Functional Properties

Solubility

The solubility of *K. alvarezii* powder was assessed using the methodology outlined by García-Vaquero *et al*. (2017) [24] with minor changes. After mixing 0.2 g of seaweed powder with distilled water (10 mL) and vortexing for 10 minutes, the mixture was centrifuged for 30 minutes at 6000 rpm. Supernatant containing soluble solids was carefully poured into a petri dish and dried in an oven at 105 °C for six hours. The solubility% was expressed as a ratio of soluble solids remaining in the supernatant after drying to the initial sample weight, multiplied by 100.

Swelling capacity

The technique outlined by Chan and Matanjun *et al*. (2017) [11], with some modifications, was used to assess the swelling capacity (SWC) of seaweed powder. Seaweed powder (0.5 g) and distilled water (20 mL) were combined in a measuring cylinder and vigorously stirred. After that, it was kept for 18 hours at room temperature (RT), 40 °C, 60 °C, and 80 °C. The swelling capacity was calculated as the volume occupied by the swollen sample per gram of dry sample.

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Water Holding and Oil Holding Capacity

The water-holding capacity (WHC) was calculated using the methods given by Chan and Matanjun (2017) [11], with minor adjustments. Distilled water (10 mL) and *K. alvarezii* powder (0.2 g) were combined in centrifuge tubes that had

been previously weighed. After mixing the dispersion and allowing it to stand at RT, 40 °C, 60 °C, and 80 °C for an hour. After centrifuging for 25 minutes at 3000 rpm, the supernatant was discarded, and the residue was weighed. WHC was calculated as the grams of water bound per gram of dry sample. Oil holding capacity (OHC) was measured using the same protocol; instead of water, sunflower oil was used to measure OHC, and measured as grams of oil bound per gram of dry sample. The density of sunflower oil is 0.92 g/mL.

Preparation of seaweed extract

The extraction procedure was modified slightly in accordance with Badmus *et al*. (2019) [7]. To prepare an extract, *K. alvarezii* fine powder was soaked in various solvents (70% methanol, 70% ethanol, and water) at a 1:10 (w/v) ratio. The mixture was stirred and then sonicated for 30 minutes in an ultrasonic bath (Soner 206H: 35kHz) at 25°C. Following that, samples were shaken at 120 rpm for 4 hours in a water bath at 25 °C. After centrifugation at 2500 rpm for 10 minutes, the supernatant was filtered using Whatman No. 1 filter paper (11 µm). Following that, the ethanol and methanol extracts were concentrated at 40 °C using a rotary evaporator (HS-2005V-N, South Korea). Concentrated ethanol and methanol extracts, as well as water extracts, were freeze-dried using a freeze dryer (Alpha 1-2 LD plus). The freeze-dried extract powder was kept in a tightly sealed container at -20 °C for future use. Extracts were redissolved in the appropriate solvents before analysis.

Yield of extract

The yield of extract (%) was measured as the percentage ratio of the weight of the dried extract to the initial weight of the dried sample,

Total phenolic and flavonoid content

Total phenolic content was determined by the Folin-Ciocalteu method, as described by Gunathilake and Ranaweera (2016) [25]. The total phenol content was presented as mg of gallic acid equivalents (GAE) per gram of dry extract. The total flavonoid content of *K. alvarazii* extracts was determined using aluminum chloride colorimetry as described by Chang *et al*. (2002) [13] and expressed as mg rutin equivalent (RE) per gram dry extract.

Antioxidant properties

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of seaweed extracts was measured using a modified method of Yen and Chen (1995) [62]. DPPH methanolic solution (2 mL, 0.1 mM) was mixed with seaweed extract (2 mL) at different concentrations (2.5 to 15 mg/mL) and vortexed for 15 seconds. The mixture was incubated in the dark at room temperature for half an hour. A UV/VIS spectrometer was then used to detect the optical density at 517 nm. Inhibition% was calculated using the following formula (Eq. 8).

$$\text{DPPH inhibition\%} = \frac{(\text{Absorbance of control - Absorbance of sample})}{\text{Absorbance of control}} \text{ x } 100 \quad (8)$$

Total antioxidant capacity

The total antioxidant capacity of seaweed extracts was measured using the Prieto *et al*. (1999) [46] technique, with some changes. 3 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to 0.3 mL of seaweed extract at different concentrations (2.5–15 mg/mL) and incubated in a water bath at 95 °C for 90 minutes. After cooling, absorbance was recorded at 695 nm. Total antioxidant capacity was reported as mg of ascorbic acid equivalent (AAE) per gram of dry extract, and absorbance was measured at 695 nm.

Reducing power

The reducing power of seaweed extracts was assessed using the method reported by Yen and Chen (1995) [62], with minor changes. phosphate buffer (0.2 M, pH 6.6, 1 mL) and 1% (w/v) potassium ferricyanide (1 mL) were mixed with 1 mL of extract (2.5–15 mg/mL). The mixture was then incubated at 50 °C for 20 minutes. After cooling, 10% trichloroacetic acid (1 mL) was added to the mixture and centrifuged at 3000 rpm for 10 minutes. Following centrifugation, $FeCl_3.6H_2O$ (0.1%, 0.3 mL) and distilled water (1.5 mL) were combined with 1.5 mL of supernatant in another test tube. At 700 nm, the optical density was measured. The findings are reported in mg of ascorbic acid equivalent per g extract.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of extracts was assessed using a slightly modified technique described by Ruch *et al*. (1989) [50]. Hydrogen peroxide solution (40 mM, 0.6 mL) and phosphate buffer (40 mM; pH 7.4, 2 mL) were combined with 0.4 mL of seaweed extracts at different levels of concentration (0.1–3 mg/mL) and vortexed. The absorbance was measured at 230 nm against a blank solution (phosphate buffer) following a 10-minute incubation period. Scavenging activity was determined using the following formula (Eq. 9):

$$H_2O_2 \text{ scavenging (\%)} = \frac{(\text{Absorbance of control - Absorbance of sample})}{\text{Absorbance of control}} \text{ x } 100 \quad (9)$$

Antidiabetic property

Alpha amylase inhibitory activity

Seaweed extract was tested for α-amylase enzyme inhibition using the Janarny and Gunathilake (2020) [30] protocols, with slight changes. 200 µL of Alpha amylase solution (0.275 mg/mL) with sodium phosphate buffer (0.02 M, pH 6.9 with 6.7 mM NaCl at 20 °C) was combined with 200 µL of the extract (2.5 to 15 mg/mL). The resulting mixture was incubated at 25 °C for 10 minutes. After that,100 µL of starch solution (1%) was added. Then the mixture was kept at 25 °C for 30 minutes. 400 µL of the DNSA color reagent was added to halt the reaction. The mixture was then incubated in a hot water bath for 5 minutes. After cooling, distilled water was added to dilute the reaction solution. At 540 nm, the absorbance was recorded. The following formula (Eq. 10) was used to determine the percentage of α-amylase inhibition.

$$\text{\% Inhibition} = \frac{(A - (B - b)}{A} \text{ x } 100 \quad (10)$$

Where,
A = (Control) was the absorbance in the presence of the buffer (no test sample) and enzyme
B = (Reaction) was the absorbance of the test sample and the enzyme
b = (Reaction blank), was the absorbance with the test sample without the enzyme

Statistical analysis
SPSS software (IBM SPSS Statistics 20, New York, USA) was used to analyze the experimental data. Samples were compared using one-way analysis of variance (ANOVA), and means were compared using Duncan's Multiple Range Test at the 95% confidence level.

Results and Discussion

Proximate composition

The water content of fresh *Kappaphycus alvarezii* was 89.79 ± 0.01% (wet basis), which indicates the low dry mass (10.21 ± 0.01%) was comparable to the studies stated by Adharini *et al*. (2020) [3], where red and green strains of *K. alvarezii* were 89.48 ± 0.15% and 90.66 ± 0.15%, respectively. The moisture content of dried *K. alvarezii* powder was 8.62 ± 0.05% (Table 1), and this value was comparable to the values of *K. alvarezii* (8.18 ± 0.41%; Charles and Alamsjah, 2019 [15], and 8.9%; Hardjani *et al*., 2017 [26]).

The ash content of *K. alvarezii* powder was 28.87 ± 0.14%, which was comparable to the *K. alvarezii* cultivated in the Mandapam area of Ramanathapuram district (28.9%; Abirami and Kowsalya, 2011) [1] and *K. alvarezii* from Gujarat, India (27.00 ± 1.62% dw; Kumar *et al*., 2014) [35] and was considerably higher than that of *K. alvarezii* from Langkawi (16.3%) and Sabah (17.1%), in Malaysia (Xiren, G.K. and Aminah, A, 2017) [61]. Kumar *et al*. (2014) [35] reported that *K. alvarezii's* ash content varied between 20.99 ± 0.08 and 33.81 ± 1.71% dw. This study also reveals that *K. alvarezii* contained high ash content, reflecting that it was rich in minerals.

K. alvarezii contained 3.42 ± 0.30% dw of protein, which was consistent with previously reported protein content of *K. alvarezii* (3.13%; Mustafa *et al*., 2018 [41] and 3.26%; Hardjani *et al*., 2017) [26]. Previous studies reported different amounts of protein content in *K. alvarezii* cultivated in various places; from Indonesia (1.79–1.94%; Adharini *et al*., 2020) [3], from Kalpitiya, Sri Lanka (5.63%) (Jayasinghe *et al*., 2018) [31], and from the Mandapam area of Ramanathapuram district (4.5%) (Abirami & Kowsalya, 2011) [1]. Seaweed harvesting age, species, environmental conditions, and geographical location all significantly impact the variation in seaweed protein content (Thiviya *et al*., 2022) [58]. Lectins, mycosporine-like amino acids, glycoproteins, peptides, and phycobiliproteins are bioactive substances found in seaweed protein (Thiviya *et al*., 2022) [58].

According to Hrstich-Manning and Aguirre (2024) [28], the lipid content of seaweeds typically ranges from 0.2% to 2.5%, making the least contribution to the nutritional composition. In this study, the fat content of *K. alvarezii* was 0.68%, which was comparable with other studies on fat content of *K. alvarezii* (0.64% dw; Kumar *et al*., 2014 [35], 0.67%; Mustafa *et al*., 2018 [41], and 0.66 to 0.72; Suresh Kumar *et al*., 2022) [57]. Rajaram *et al*. (2021) [48] reported that the fat content of K. alvarezii ranged from 0.57 ± 0.52% to 1.10 ± 0.12%. Despite having a low-fat content, seaweeds contain high polyunsaturated fatty acids (PUFAs) levels comparable to those of terrestrial plants (Chan and Matanjun 2017) [11]. Seaweeds are used as an ingredient in food products because of their low lipid content and superior fatty acid composition.

Seaweeds have more dietary fibre than most fruits, vegetables, and terrestrial foods. Consuming seaweeds increases fibre intake and reduces the risk of some chronic illnesses such as heart disease, diabetes, obesity, and colorectal and breast cancer (Waddell & Orfila, 2022) [60]. The crude fibre amount in seaweeds ranged between 2% and 27% (Choudhary *et al*., 2023) [16]. *K. alvarezii* investigated in this study (9.82 ± 0.17%) was comparable to the fibre content of *K. alvarezii* reported by Kumar *et al*. (2014) [35] (9.68 ± 0.08 to 18.57 ± 0.15% dw).

Carbohydrates are the major component of dried seaweeds. Ahmad *et al*. (2012) [4] reported that red seaweeds have the greatest carbohydrate content (57.79% to 74.11% dw). This study showed that *K. alvarezii* contained 58.41 ± 0.34% of carbohydrates, which was comparable to the red seaweed *Eucheuma denticulatum (*var yellow) (57.79% dw) (Ahmad *et al*., 2012) [4]. *K. alvarezii* gives 2141.73 ± 19.84 kcal/kg energy, which was lower than the value reported by Hardjani *et al*. (2017) [26] in *K. alvarezii* (2883.8 kcal.kg^{-1}). Gamero-Vega *et al*. (2020) [23] reported an average energy value of 2232 ± 73.95 kcal/kg, with a maximum of 3378 kcal/kg and a minimum of 1059 kcal/kg.

Table 1: Proximate composition of *Kappaphycus alvarezii*

Composition	% dw
Moisture	8.62 ± 0.05
Ash	28.87 ± 0.14
Crude Protein	3.42 ± 0.30
Crude Fat	0.68 ± 0.44
Crude Fibre	9.82 ± 0.17
Total Carbohydrate	58.41 ± 0.34
Energy (kcal.kg^{-1})	2141.73 ± 19.84

Values are expressed as mean ± standard deviation (n = 3).
dw- Dry weight

Carrageenan

Carrageenan is the most abundant sulfated polysaccharide in red seaweed and is typically extracted from *K. alvarezii* (*Eucheuma cottonii*) and *Eucheuma denticulatum* for commercial use (Shao & Duan, 2022) [53]. The carrageenan yield found in this study (37.70 ± 0.26%) aligns with Rajaram *et al*. (2021) [48], who mentioned that *K. alvarezii* in the monsoon and post-monsoon seasons (37.47 ± 1.66% and 37.90 ± 1.84%, respectively). Jayasinghe *et al*. (2016) [32] stated that the carrageenan yield from *K. alvarezii* from the south coast of Sri Lanka ranged from 29.70 to 37.30%. Carrageenan is in high demand because of its distinctive functional qualities, which can be utilized to gel, thicken, and stabilize the food systems (Saha and Bhattacharya, 2010) [51].

Pigments

Marine seaweeds, like other land plants, are regarded as the primary source of health-promoting pigments such as chlorophylls, carotenoids, and phycobiliproteins. Chlorophyll a, b, total chlorophyll, and carotenoids in *K. alvarezii* were 118.06 ± 1.08 µg/g dw, 32.55 ± 0.35 µg/g

dw, 150.62 ± 1.43 µg/g dw, and 12.69 ± 0.14 µg/g dw, respectively, and align with Hong *et al*. (2007) [27], who stated that the amounts of chlorophyll a and b in red seaweeds are in the range between 68 and 162 µg/g and 25 to 46 µg/g.

The main photosynthetic water-soluble pigment found in red seaweed is Phycobiliproteins (Castejón *et al*., 2021) [10], which make up about 50% of the seaweed's total protein content. *K. alvarezii* contained phycocyanin (R-PC) (0.32 ± 0.07 mg/g dw), phycoerythrin (R-PE) (0.26 ± 0.03 mg/g dw), Allophycocyanin (APC) (0.56 ± 0.07 mg/g dw) and Phycobiliprotein (PBP) (1.13 ± 0.04 mg/g dw), was higher than the value indicated by Pang *et al*. (2011) [45]; R - PC (0.22 ± 0.01 mg/g dw), R- PE (0.13 ± 0.01 mg/g dw), APC (0.33 ± 0.02 mg/g dw) and PBP (0.68 ± 0.04 mg/g dw).

Functional properties of seaweed powder

The solubility of *K. alvarezii* powder was increased significantly with temperature (Table 2). At ambient temperature, the solubility of *K. alvarezii* was comparable to *Ulva lactuca* (25.99 ± 2.50%), whereas higher than *C. linum* and *G. edulis* (13.04 ± 1.39% and 15.63 ± 3.89%, respectively), and at 80˚C was comparable to *U. fasciata* (35.83 ± 1.97%) (Bajad *et al*., 2024) [8]. The carrageenan compound present in *K. alvarezii* species is primarily responsible for the soluble nature of red seaweed (Nurshahida *et al*., 2020) [44].

The swelling capacity (SWC)significantly increased with increasing temperature, reaching a maximum of 22.83 mL/g at 60˚C, as conformational changes in polysaccharides and protein binding sites may increase the swelling ability of seaweed, and then decreased at 80˚C, as seaweed carrageenan gelatinizes and exhibits reduced swelling (Table 2). SWC of *K. alvarezii* at ambient temperature (11.07 ± 0.12 mL/g dw) was comparable to that of *H. elongate* (sea spaghetti) (10.97 mL/g dw) (Mohammed *et al*., 2021) [40] and higher than *G. edulis* and *G. corticata* at 25˚C (8.66 ± 0.53 mL/g and 7.70 ± 0.60 mL/g, respectively) and at 37˚C (7.90 ± 0.32 mL/g and 5.70 ± 0.65 mL/g, respectively) (Rosemary *et al*., 2019) [49].

The Water Holding Capacity (WHC) was significantly increased with increasing temperature due to the increased solubility of the proteins and fibres present in *K. alvarezii*, and was highest at 60˚C, then slightly decreased at 80˚C (Table 2). WHC of *K. alvarezii* at ambient temperature (8.87 ± 0.01 g/g dw was similar to *Porphyra* sp. (8.82 ± 0.40 g/g; Kumari *et al*., 2022) [36], *E. striatum* (8.45 g/g dw; Nurshahida *et al*., 2020) [44] and higher than the *Gracilaria* sp., *U. pinnatifida, Sargassum* sp. (7.67 ± 0.30, 7.99 ± 0.25 and 6.91 ± 0.15 g water/g sample, respectively; Kumari *et al*., 2022) [36]. Viscous foods, such as baked goods, sausages, dough, and custards, require high WHC levels (Kumari *et al*., 2022) [36].

The oil-holding capacity (OHC) of *K. alvarezii* increased with increasing temperature and was significantly high at 60°C (3.43 ± 0.09 g/g dw), then decreased (2.96 ± 0.09 g/g dw) at 80 °C (Table 2). OHC of *K. alvarezii* (3.04 ± 0.02 g/g dw) at room temperature was comparable to the OHC of *K. alvarezii and E. striatum* (3.07 g/g dw and 2.84 g/g dw, respectively; Nurshahida *et al*., 2020) [36] and higher than *U. pinnatifida* (1.98 ± 0.22 g oil/g; Kumari *et al*., 2022) [48]. SWC, WHC, and OHC values indicate that these properties may be utilized as texture and viscosity modifiers in the food sectors (Nurshahida *et al*., 2020) [44].

Table 2: Functional properties of *K. alvarezii* seaweed powder

Functional properties	Temperature			
	Room Temperature 25 °C	40 ˚C	60 ˚C	80 ˚C
Solubility (g/g dw)	0.26 ± 0.01^{a}	0.27 ± 0.01^{b}	0.30 ± 0.01^{c}	0.32 ± 0.01^{d}
Swelling Capacity (SWC) (mL/g dw)	11.07 ± 0.12^{a}	12.87 ± 0.23^{b}	22.83 ± 0.29^{d}	14.90 ± 0.17^{c}
Water Holding Capacity (WHC) (g/g dw)	8.87 ± 0.01^{a}	10.62 ± 0.05^{b}	16.87 ± 0.12^{d}	12.81 ± 0.08^{c}
Oil Holding Capacity (OHC) (g/g dw)	3.04 ± 0.02^{ab}	3.14 ± 0.04^{b}	3.43 ± 0.09^{c}	2.96 ± 0.09^{a}

Values are expressed as mean ± standard deviation (n = 3). dw- dry weight. Mean values with different superscript letters within the same row are significantly different at $p < 0.05$.

Extraction Yield

Extraction yield of *K. alvarezii* using methanol was significantly higher (22.16%) than ethanol (20.11%), and water (17.07%) (Table 3). The composition of the compounds found in seaweed, polarity of solvent, pH, extraction temperature, and duration affect the extraction yield (Sasadara & Wirawan, 2021; Ummat *et al*., 2020) [52, 59]. The extraction yield using methanol and ethanol in the current study was comparable to the extraction yield of *Gracilaria sp* using 75% aqueous methanol (27.39 ± 0.41%) and 75% aqueous ethanol (21.41 ± 0.65%) reported by Sasadara & Wirawan (2021) [52].

Total Phenolic Content (TPC)

TPC of different extracts of *K. alvarezii* ranged from 0.99 to 1.85 mg AAE/g dry extract. The highest TPC was observed in *K. alvarezii* water extract (1.85 ± 0.03 mg GAE/g dry extract), followed by 70% ethanolic extract (1.37 ± 0.05 mg GAE/g dry extract), and 70% methanolic extract (0.99 ± 0.03 mg GAE/g dry extract) (Table 3). A similar trend was observed in Naseri *et al*. (2019) [42] studies, where water extract of *K. alvarezii* exhibited higher phenolic content (2.95 ± 0.01 mg GAE/g dw) than ethanolic (0.14 ± 0.01 mg GAE/g dw) and methanolic (0.18 ± 0.02 mg GAE/g dw) extracts. Phenolic content in the methanolic extract of *P. capillacea* (red seaweed), reported by El-Din and El-Ahwany (2015) [21] as having a TPC of 0.93 mg GAE/g, was comparable to the present study's methanolic extract, which showed a TPC of 0.99 mg GAE/g.

Total Flavonoid Content (TFC)

Total flavonoid content of *K. alvarezii* ranged between 0.63 and 0.88 mg RE/g extract. It was significantly higher in water extract (0.88 ± 0.03 mg RE/g extract) compared to ethanolic and methanolic extracts (0.63 ± 0.04 and 0.40 ± 0.06 mg RE/g, respectively) (Table 3). The flavonoid content of *K. alvarezii* water extract in the present study was higher than that reported by Kanatt *et al*. (2015) [33] and Lalopua *et al*. (2011), with the values of 0.19 mg catechin equivalents (CAE)/g extract and 0.00187 mg quercetin equivalents (QE)/g extract, respectively.

Table 3: Extraction yield, total phenolic, and flavonoid content of different solvent extracts of *K. alvarezii*.

Extract	Extraction yield (% of dw)	Total Phenolic (mg GAE/g extract)	Total Flavonoids (mg RE/g extract
70% Ethanol	20.11 ±1.26[b]	1.37 ± 0.05[b]	0.63 ± 0.04 [b]
70% Methanol	22.16 ±0.49[c]	0.99 ± 0.03 [a]	0.40 ± 0.06 [a]
Water	17.07 ±0.08[a]	1.85 ± 0.03 [c]	0.88 ± 0.03 [c]

Values are expressed as mean ± standard deviation (n = 3). Mean values with different superscript letters within the same column are significantly different at $p < 0.05$. dw: dry weight. GAE: Gallic Acid Equivalent, RE: Rutin Equivalent.

Antioxidant properties

DPPH Scavenging Activity

Among the extracts, water extract exhibited significantly higher DPPH scavenging activity with the lowest IC_{50} of 6.36 mg/mL, followed by ethanol (IC_{50}: 8.82 mg/mL) and methanol (IC_{50}: 10.48 mg/mL) (Table 4), which was comparable to the previous studies reported by Kanatt *et al*. (2015) [33]; where highest DPPH scavenging activity was obtained in *K. alvarezii* water extract (IC_{50} of 5.03 mg/mL) compared to that of methanolic extract. Siriwardhana *et al*. (2003) [54] mentioned that *Hizikia fusiformis* (brown algae) water extracts exhibited the greatest DPPH scavenging activity compared to other extracts, and López *et al*. (2010) [39] stated that aqueous extract of *Stypocaulon scoparium* seaweed showed the maximum DPPH scavenging activity compared to ethanol and methanol.

Total antioxidant activity

Total antioxidant capacity of water extract was significantly high (6.52 ± 0.03 mg AAE/g extract), followed by extracts from ethanol (4.03 ± 0.06 mg AAE/g extract) and methanol (3.16 ± 0.06 mg AAE/g extract), and was double that of the methanol extract (Table 4). The total antioxidant capacity of aqueous extract in the current studies was higher than that of the aqueous extract of *Padina pavonia,* brown seaweed (5.1 ± 0.1 mg AAE/g extract) and *Corallina elongate*, red seaweed (4.0 ± 0.1 mg AAE/g extract), reported by El-Sheekh *et al*. (2020) [22].

Reducing Power

The water extract showed significantly higher ($p < 0.05$) reducing ability (2.86 ± 0.02 mg AAE/g extract) compared to ethanolic (1.81 ± 0.04 mg AAE/g extract) and methanolic (1.67 ± 0.02 mg AAE/g extract) extracts (Table 4) due to the presence of more hydrophilic compounds in water extracts. Kumar *et al*. (2008) [34] reported that, in addition to phenolics, carrageenan, a sulfated polysaccharide, is present in the cell wall of *K. alvarezii* and may increase its antioxidant potential. Similar findings were described by Kanatt *et al*. (2015) [33], who observed that *K. alvarezii* water extract had the highest reducing power, followed by ethanolic and methanolic extracts.

H_2O_2 Scavenging activity

In present study, water extract (IC_{50}: 1.62 ± 0.02 mg/mL) exhibited significantly higher H_2O_2 scavenging activity than ethanol and methanolic extracts (IC_{50}: 1.95 ± 0.06 and 2.06 ± 0.07 mg/mL, respectively) (Table 4), which may be due to the presence of water-soluble polar bioactive compounds such as sulfated polysaccharides, phenolic constituents and other hydrophilic antioxidants in water extract of *K.alvarezii* to decrease the the pro-oxidants substances like H_2O_2 (Quitério *et al*., 2022) [47]. A sililar trend was observed in Siriwardhana *et al*. (2003) [54], who found that *H. fusiformis* water extracts exhibited significantly higher H_2O_2 scavenging activity (over 74%) than other organic solvent extracts, while the scavenging activity of this study was higher than the activity reported by Chandrasekaran *et al*. (2022) [12], with an aqueous extract of *Gracilaria opuntia* exhibiting H_2O_2 scavenging activity (IC_{50}: 2.02 mg/mL).

Anti-diabetic: Alpha amylase inhibitory activity

According to Abo-Shady *et al*. (2023) [2], seaweeds are effective in treating diabetes. Ethanolic extract inhibition of alpha-amylase activity was significantly higher (IC_{50}: 12.13 ± 0.34 mg/mL) compared to extracts of water (IC_{50}: 14.65 ± 0.30 mg/mL) and methanol (IC_{50}: 16.40 ± 0.7 mg/mL) (Table 4). Similarly, Ethanolic extracts of *S.wightii* (32.55%), *G.corticata (*43.02%), and *K. alvarezii (*61.62%) exhibited excellent antidiabetic properties (Deepika, 2018) [17].

Table. 4: *In vitro* Antioxidant activities of different solvent extracts of *K. alvarezii*

Extract	DPPH Scavenging	H_2O_2 Scavenging	Total Antioxidant	Reducing Power	Alpha amylase inhibition
	IC_{50} (mg/mL)	IC_{50} (mg AAE /mL)	(mg AAE/g extract)	(mg AAE/g extract)	IC_{50} (mg/mL)
Methanol	10.48 ±0.22[c]	2.06 ±0.07[b]	3.16 ±0.06[a]	1.67 ±0.02[a]	16.40 ± 0.72 [c]
Ethanol	08.82 ±0.13[b]	1.95 ±0.06[b]	4.03±0.06[b]	1.81±0.04[b]	12.13 ± 0.34
Water	06.36 ±0.11[a]	1.62 ±0.02[a]	6.52 ±0.03[c]	2.86 ±0.02[c]	14.65 ± 0.30 [b]
Ascorbic acid (AA)	0.011 ±0.27	0.041± 0.002			

Values are expressed as mean ± standard deviation (n = 2). Mean values with different superscript letters within the same column are significantly different at $p < 0.05$. dw: dry weight, AA: Ascorbic acid, AAE: Ascorbic Acid Equivalent.

Conclusion

Red seaweed: *Kappaphycus alvarezii* was rich in carbohydrates, mainly sulfated polysaccharide- carrageenan, fibre, minerals, protein, and low in fat and caloric profile. *K. alvarezii* mainly contained water-soluble red pigment Phycobiliprotein (PBP), Phycocyanin (PC), Phycoerythrin (R-PE), and Allophycocyanin (APC), and also chlorophyll a, b, and carotenoids. *K. alvarezii* powder showed good solubility, swelling, water holding, and oil holding capacity. Methanol and ethanol at 70% gave higher yields than the water extract. All extracts contained phenolics and flavonoids and exhibited antioxidant properties (DPPH scavenging, reducing power, total antioxidant activity, and H_2O_2 scavenging) with significantly higher activity in water extract, whereas alpha-amylase inhibition showed significantly higher activity in ethanolic extract. Thus, it has the potential to be utilized as an essential natural source for

commercial food products as a nutritional, functional, and bioactive ingredient.

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Conflict of Interest
There is no conflict of interest between the authors.

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