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A comparative evaluation of nutritional composition and antioxidant properties of six Malaysian edible seaweeds

Ying Yee Chin [a], Kian Aun Chang [a], Wei Mei Ng [a], Zei Pei Eng [d], Lye Yee Chew [a,b,*], Yun Ping Neo [a,b], See Wan Yan [e], Ching Lee Wong [a], Kin Weng Kong [c], Amin Ismail [d]

[a] School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia
[b] Food Security & Nutrition Impact Lab, Taylor's University, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia
[c] Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[d] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[e] School of Hospitality Management, Macao Institute for Tourism Studies, Macau

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ABSTRACT

Agriculture in the 21st century faces challenges in adopting efficient and sustainable production methods to feed the growing population. In this context, seaweed offers greater advantages over terrestrial plants. This study investigated the nutritional composition and antioxidant properties of six edible seaweeds found in Malaysia. The seaweeds studied were brown (*Padina australis, Sargassum binderi, Sargassum polycystum*), green (*Caulerpa racemosa, Caulerpa sertularioides*), and red (*Garcilaria changgi*) seaweeds. The moisture, ash, protein, fat, and total dietary fibre contents of the seaweeds were analysed according to the Association of Official Analytical Chemists methods. Total available carbohydrate content was assessed using the Clegg-anthrone method. Mineral, amino acid, and fatty acid contents were determined through atomic absorption spectroscopy, high-performance liquid chromatography, and gas chromatography methods, respectively. Results revealed that the seaweeds were all high in total dietary fibre (53.96–76.97 g/100 g dried weight, dw) and ash (4.46–18.53 g/100 g dw) whereas their fat (0.05–4.62 g/100 g dw) content was generally low. The brown seaweeds were good sources of calcium. Red seaweed *G. changii* had the highest content of essential amino acids whereas brown seaweed *S. binderi* had the highest polyunsaturated fatty acid and lowest saturated fatty acid contents. Fucoxanthin could only be detected in brown seaweeds. Methanolic extracts of seaweeds showed good antioxidant activities measured using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA), ferric reducing antioxidant power (FRAP), and trolox equivalent antioxidant activity (TEAC) assays. Overall, this study contributed knowledge on underexploited Malaysian seaweeds and proposed them as an alternative source of nutrients for humans to meet food security challenges.

Introduction

Seaweed or macroalgae is one of the leading commodities of aquaculture fisheries with high economic value (Nor et al., 2020). Studies on seaweeds, particularly on their chemical composition, biological activity, and technological properties have been around for years due to the commercial interests in using seaweeds for various food and non-food applications, such as food additive, nutraceutical, pharmaceutical, cosmetic, biofertiliser, and biopackaging (Food & Agriculture Organization, 2018; McHugh, 2003). From the nutritional standpoint, seaweed is considered nutrient-dense as it can supply carbohydrate, protein, lipid, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and fibre (Natrah et al., 2007). Seaweed is also well recognised as nutraceutical owing to the presence of various functional components, for instance, dietary fibres reduce total cholesterol and low-density lipoprotein levels; omega-3 fatty acids exert anti-atherogenic properties; carotenoids and phenolic compounds serve as antioxidants (Plaza et al., 2008). Habitual consumption of seaweed can thus enhance the nutritional quality of daily diet and reduce the risk of non-communicable diseases.

The United Nations (2019) predicted an increase in global population to 9.7 billion by 2050 and an associated increase in demand for food

* Corresponding author at: School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia.
E-mail address: lyeyee.chew@taylors.edu.my (L.Y. Chew).

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and arable land. From the agricultural perspective, the greatest advantage of seaweed is that it is cultivated on non-arable land. This helps to reverse the desertification effect of fertile land (Karan et al., 2019). Moreover, the seaweed ecosystem can effectively recycle nutrients such as nitrogen and phosphorous in the contained environment (i.e., saline and/or wastewater), which helps to reduce eutrophication and reliance on energy-intensive chemical fertilisers (Karan et al., 2019; Neveux et al., 2018). The cultivation of seaweed contributes to achieving at least 7 out of 17 Sustainable Development Goals (SDGs) that include "clean water and sanitation, life below water, live on land, good health and well-being, clean energy, climate action, and sustainable cities and communities" (Phang, 2018). In Malaysia, the Department of Fisheries Malaysia intends to develop the seaweed aquaculture sector as a strategy to ensure food security threatened by population pressure (Nor et al., 2020).

Malaysia is reported to have 375 species of seaweed (Phang, 2006). Many of these species are still underexploited or not fully characterised. Over the past decade, the phycology research activity in Malaysia has constantly focused on exploring new or under-characterised seaweeds. Matanjun et al. (2009) evaluated the nutritional profile (proximate composition, fatty acid, amino acid, minerals, vitamin C, and α-tocopherol) of brown seaweed (*Sargassum polycystum*), green seaweed (*Caulerpa lentillifera*), and red seaweed (*Eucheuma cottonii*) from Bangi, Semporna, and Kota Kinabalu. Ahmad et al. (2012) determined the proximate composition and total phenolic content of 15 edible seaweeds from Semporna. Nazarudin et al. (2021) studied the chemical, nutritional, and physicochemical properties of *S. polycystum* from Port Dickson. Therefore, this study aimed to characterise the nutritional composition (carbohydrate including total available carbohydrate and dietary fibre; protein including essential and non-essential amino acids; lipid including saturated, monounsaturated and polyunsaturated fatty acids; ash including selected macro- and microminerals; and moisture) and antioxidant properties (fucoxanthin content and antioxidant activities) of six Malaysian edible seaweed species (brown seaweed, *Padina australis, Sargassum binderi*, and *Sargassum polycystum*; green seaweed, *Caulerpa racemosa* and *Caulerpa sertularioides*; and red seaweed, *Garcilaria changgi*). The findings from this study are important to provide scientific information on Malaysian seaweeds that have not been used to their full potential as an alternative food source. Further, the research data is also expected to promote the commercial farming activities of the seaweed species as a strategy to improve the economic prospects of seaweed aquaculture in Malaysia.

Materials and methods

Seaweed harvesting and preparation

P. australis, S. binderi, S. polycystum, C. racemosa, and *C. sertularioides* were harvested from a conveniently available pool of seaweeds from the shallow water of the intertidal zone of Teluk Kemang beach, Port Dickson (2.4823° N, 101.8482° E) in the middle of the year (between June and August). A previously harvested (between February and March) and dried-to-preserve *G. changii* was used as it was seasonally unavailable during the seaweed collection period. The seaweeds were cleaned with tap water to remove foreign matter and dried in a fume hood at room temperature of 26 °C and air velocity of 5 m/s for 24 h before they were ground into powder using a grinder (Philips, HR2860/55, Malaysia). The seaweed powder was stored in air-tight plastic bags at –40 °C before further use.

Determination of nutritional composition

The Association of Official Analytical Chemists (AOAC International, 2012) methods were used for the determination of seaweed nutritional composition. The moisture, ash, protein, and fat contents of seaweeds were determined according to AOAC 930.04, AOAC 930.05, AOAC 2001.11, and AOAC 930.09 methods, respectively with some modifications as described by Tee et al. (1996). Total available carbohydrate and total dietary fibre contents of the seaweeds were determined according to the Clegg-anthrone (Peris-Tortajada, 2015) and AOAC 985.29 methods, respectively.

Determination of mineral content

The contents of selected macro- (calcium, magnesium, potassium, sodium) and microminerals (copper, iron, zinc) of seaweeds were determined according to the method of Tee et al. (1996) using a flame atomic absorption spectrometer (PerkinElmer, AAnalyst 200 AA, Germany). The non-carbonaceous residue after dry ashing was dissolved in a diluted acid solution before the analyses. Calibration curves were established using analytical grade mineral standard solutions for quantification. The wavelength was adjusted accordingly during each mineral identification. The mineral content was expressed in unit mg per 100 g of dry weight seaweed.

Determination of amino acid composition

The amino acid composition of seaweeds was determined using the Pico-Tag method described by Chew et al. (2011) and Heinrikson and Meredith (1984). Briefly, 1 g of seaweed powder was hydrolysed with 10 mL of 6 N hydrochloric acid at 110 °C for 24 h in an oven (Memmert, VO200, USA). The hydrolysate was added with 10 ml of 2.5 mM L-α-aminobutyric acid (AABA) as an internal standard and made up to 50 mL with deionised water. Subsequently, 10 μL of the diluted hydrolysate was transferred into a microcentrifuge tube and dried under vacuum before 20 μL of coupling reagent (methanol:triethylamine: deionised water; ratio 2:1:2) was added and dried again under vacuum. This was followed by the addition of 20 μL derivatisation reagent (methanol:PITC:triethylamine:deionised water; ratio 7:1:1:1) and dried again under vacuum. Finally, the dried reaction mixture was dissolved in 100 μL of diluent (mobile phase A) before high-performance liquid chromatography (HPLC) analysis. The HPLC system used consisted of a DGU-20A5 degasser, LC-20AT pump system, and SPF-M20A diode array detector (Shimadzu Corporation, Japan) along with a C18 column (PerkinElmer, COL-Analytical; 5.0 μm, 150 mm x 4.6 mm, USA). Mobile phases used were buffer A (0.1 M ammonium acetate; pH 6.5) and buffer B (0.1 M ammonium acetate:acetonitrile:methanol; ratio 44:46:10; pH 6.5). The flow rate, operating temperature, and detection wavelength were set at 1 mL/min, 40 °C, and 254 nm, respectively. Relative response factors of amino acids in the mixed standard were calculated in relation to the internal standard, AABA, which was then used to quantify each amino acid in the seaweed. The content of amino acid was expressed in unit mg per 100 g of dry weight seaweed.

Determination of fatty acid composition

The fatty acid composition of seaweeds was determined according to the method of David et al. (2003) using a gas chromatography system equipped with a flame ionisation detector (PerkinElmer, Clarus 500, USA). Crude fat in 5 g of seaweed powder was extracted using 300 mL of hexane at room temperature for 24 h. The hexane in the extract was then removed using a rotatory evaporator (EYELA, N-1200BV-W, Japan). Extracted fat (100 mg) was transferred to a tube where 10 mL of hexane was added to redissolve the fat. Next, 100 μL of 2 N potassium hydroxide in methanol was added and the content was properly mixed. The mixture was centrifuged at 2700 x *g* for 10 min at room temperature. The clear supernatant, referred to as the fatty acid methyl ester (FAME), was filtered and transferred into a vial for fatty acid analysis. Approximately 10 μL of the FAME was injected into the gas chromatography system where separation was performed on a capillary column (SGE Analytical Science, BPX 70; length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm). The oven temperature was held at 50 °C for 1 min, increased

from 50 °C at 25 °C/min to 175 °C and then from 175 °C at 4 °C/min to 230 °C; and maintained at 230 °C for 20 min. The temperatures of the injection port and detector were 250 and 280 °C, respectively. Peaks of FAME were identified by matching their retention time with a standard of 37 components FAME mix (Supelco®, CRM47885, Sigma-Aldrich). The content of individual fatty acids in the seaweed was expressed in unit percent (%) of total fatty acid; calculated by comparing the peak area of the fatty acid in the seaweed with the peak area of the fatty acid in the mixed standard that has known content of the fatty acid.

Preparation of seaweed extract

The extraction was performed according to the method of Mise et al. (2011) with some modifications. Briefly, 3 g of seaweed powder was extracted with 90 mL of methanol for 5 h at room temperature and the extract was filtered. The solid residue was extracted again with 60 mL of methanol for another 3 h at room temperature, after which the extract was filtered. The extracts were then pooled together. Methanol in the extract was removed using a rotary evaporator. Seaweed extract at the concentration of 0.05 g/mL was prepared by redissolving the dried extract in methanol, kept in an amber bottle, and stored at –40 °C for determination of fucoxanthin content, DPPH radical scavenging activity, ferric-reducing antioxidant power, and trolox equivalent antioxidant capacity. Colourimetric measurements were performed using an ultraviolet-visible spectrophotometer (PerkinElmer, LAMBDA XLS, USA) in a quartz cuvette.

Determination of fucoxanthin content

The fucoxanthin content of seaweeds was determined using HPLC according to the method of Garcia-Plazaola and Esteban (2012). Seaweed extract was filtered through a 0.2 μm syringe filter. The two mobile phases used were solvent A (acetonitrile:methanol:tris hydrochloride; ratio 42:1:7; pH 8) and solvent B (methanol:ethyl acetate; ratio 17:8). The flow rate, injection volume, and temperature were set at 1.2 mL/min, 10 μL, and 40 °C, respectively. The applied gradient elution condition was: 0–12 min, linear gradient from 0% to 100% solvent B; 12–18 min, 100% solvent B isocratic; 18–19 min, linear gradient from 100% to 0% solvent B; 19–25 min, 0% solvent B isocratic. Fucoxanthin was identified by comparing the retention time and ultraviolet-visible absorption spectra characteristic of the eluted peak in extract with that of an authentic standard. Fucoxanthin standard (2–10 μg/mL) was prepared to construct a calibration curve. The result was expressed in unit mg fucoxanthin per g of dry weight seaweed.

Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH-RSA) of seaweeds was determined using the method of Brand-Williams et al. (1995) with some modifications. Seaweed extract (0.1 mL) was mixed with DPPH reagent (3.9 mL, 50 μM). The mixture was left to stand for 30 min in the dark at room temperature. The absorbance was then measured at 517 nm against methanol as a blank. The DPPH-RSA was calculated using the following equation: DPPH-RSA = [(Ao–Ac) / Ao)] x 100%, where Ao is the absorbance of methanol (blank) and Ac is the absorbance of seaweed extract mixed with DPPH reagent. The DPPH-RSA was expressed in percent inhibition as compared to control.

Determination of ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) of seaweeds was determined using the method of Benzie and Strain (1996) with some modifications. FRAP reagent composed of 400 mM acetate buffer (pH 3.6):10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TTPZ) in 40 mM hydrochloric acid:20 mM iron (III) chloride at a ratio of 10:1:1 was prepared. Seaweed extract (0.1 mL) was mixed with 6 mL of freshly prepared FRAP reagent. The mixture was incubated in a water bath (Memmert, WNB 22, Germany) at 37 °C for 30 min. The absorbance of the mixture was then measured at 593 nm against distilled water as a blank. Ferrous sulphate heptahydrate standard (0.1–1.0 mM) was used to construct a calibration curve for quantification. The FRAP was expressed in unit μmol Fe^{2+} per g of dry weight seaweed.

Determination of trolox equivalent antioxidant capacity

The trolox equivalent antioxidant capacity (TEAC) was determined using the method of Re et al. (1999) with some modifications. The 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagent at a concentration of 7 mM was prepared by dissolving ABTS salt in distilled water. ABTS radical cation ($ABTS^{+}$) was generated by reacting ABTS reagent with 2.45 mM potassium peroxodisulphate. The mixture was left in dark for 16 h at room temperature before use. A working solution was prepared by diluting 6 mL of $ABTS^{+}$ reagent with 360 mL of distilled water to obtain an absorbance of 0.70 at 734 nm. Seaweed extract (0.1 mL) was mixed with 10 mL of diluted $ABTS^{+}$ reagent and left for 6 min at room temperature. The absorbance of the reaction mixture was then measured at 734 nm against distilled water as blank. Trolox standard (0.2–1.0 mM) was used to prepare a standard calibration curve. The TEAC was expressed in unit μmol TE per g of dry weight seaweed.

Statistical analyses

The experimental data were obtained in triplicates ($n = 3$). Statistical analyses were performed using Minitab Statistical Software (Minitab Inc., Version 16, USA). The difference between mean of groups was analysed using One-way Analysis of Variance (ANOVA), followed by post-hoc multiple comparison test. The level of significance was set at $p < 0.05$.

Results and discussion

Nutritional composition

Table 1 shows the nutritional composition of the six seaweeds expressed on a dry weight (dw) basis. Results on a fresh weight (fw) basis (Supplementary 1) were also reported in the text for comparison between the seaweeds and vegetables that are commonly consumed by Malaysians. The moisture content of the seaweeds was found in the range of 84.52–91.56 g/100 g fw. The moisture content of *G. changii* was not determined due to methodological limitation. The seaweed was harvested at an earlier time compared to the other seaweeds, it was dried and stored directly to preserve its integrity and quality for analyses along with others. *Gracilaria changii* harvested from Santubong, Sarawak, Malaysia was reported to have a moisture content of 5.32% on a dw basis (Chan & Mantajun, 2017). According to Tee et al. (1997), vegetables commonly consumed by Malaysians had a moisture content in the range of 87.9–94.7 g/100 g fw. These vegetables include Chinese kale (*Brassica alboglabra*), fern shoots (*Diplazium esculentum*), broccoli (*Brassica oleracea*), spinach (*Spinacia oleracea*), Chinese mustard leaves (*Brassica juncea*), sweet potato shoots (*Ipomoea batatas*), common cabbage (*Brassica oleracea*), King's Salad (*Cosmos caudatus*), Chinese cabbage (*Brassica chinensis*) and lettuce (*Lactuca sativa*). In this study, brown seaweeds had the highest ash content, followed by green seaweeds and red seaweed. The ash content of brown seaweeds (1.06–2.56 g/100 g fw) was noted to be higher than those local vegetables often consumed, which ranged from 0.7 to 1.8 g/100 g fw (Tee et al., 1997). In contrast, the ash content of brown seaweeds (12.03–18.53 g/100 g dw) was found to be lower than those originating from Pramuka Island, Indonesia and Semporna Island, Malaysia, which were 22.26 g/100 g dw (*P. australis*) and 21.87 g/100 g dw (*S. polycystum*), respectively (Ahmad et al., 2012; Santoso et al., 2013). Ash is the inorganic residue remaining after food is

Table 1
Nutritional composition and mineral contents of six edible seaweeds.

	Brown Seaweed *P. australis*	*S. binderi*	*S. polycystum*	Green Seaweed *C. racemosa*	*C. sertularioides*	Red Seaweed *G. changii*	†††RNI per Day
Nutritional Component							
Moisture	84.52 ± 0.15^{a}	91.56 ± 0.20^{e}	86.17 ± 0.17^{b}	89.17 ± 0.21^{d}	88.44 ± 0.50^{c}	ND	NA
Ash	15.89 ± 0.02^{e}	12.03 ± 0.06^{d}	18.53 ± 0.08^{f}	4.46 ± 0.01^{a}	6.97 ± 0.54^{b}	8.83 ± 0.16^{c}	NA
Carbohydrate†	4.80 ± 1.11^{a}	8.50 ± 0.36^{b}	5.07 ± 0.67^{a}	19.08 ± 0.35^{d}	15.63 ± 1.07^{c}	38.03 ± 1.75^{e}	50–65% TEI
Fibre††	71.02 ± 2.74^{bc}	72.09 ± 2.98^{bc}	60.76 ± 1.50^{ab}	56.47 ± 1.28^{a}	53.96 ± 7.37^{a}	76.97 ± 9.14^{c}	25–30 g
Protein	3.06 ± 0.09^{a}	7.03 ± 0.04^{a}	7.64 ± 0.04^{a}	2.62 ± 0.70^{a}	19.39 ± 0.19^{b}	5.42 ± 0.17^{a}	10–20% TEI
Fat	1.82 ± 0.21^{c}	0.46 ± 0.28^{a}	0.63 ± 0.33^{b}	4.03 ± 0.04^{d}	4.62 ± 0.09^{e}	0.05 ± 0.03^{a}	25–30% TEI
Calorie (kcal)	190	210	178	236	290	328	*2240 kcal (M);*1840 kcal (F)
Macromineral							
Calcium	2488.58 ± 12.85^{e}	1902.87 ± 17.69^{d}	557.39 ± 8.66^{c}	80.68 ± 0.67^{a}	91.49 ± 0.54^{ab}	105.3 ± 0.27^{b}	1000 mg
Magnesium	126.57 ± 3.58^{b}	151.62 ± 1.22^{d}	251.51 ± 1.39^{e}	140.40 ± 4.75^{c}	260.01 ± 1.04^{f}	92.48 ± 0.96^{a}	400 mg (M);310 mg (F)
Potassium	86.63 ± 1.20^{a}	211.02 ± 8.59^{b}	2236.33 ± 37.53^{d}	24.49 ± 0.18^{a}	46.09 ± 0.25^{a}	424.64 ± 4.68^{c}	4.7 g
Sodium	65.64 ± 0.57^{a}	130.93 ± 0.82^{c}	275.13 ± 0.76^{e}	119.32 ± 1.65^{b}	120.69 ± 1.44^{b}	171.10 ± 0.87^{d}	1500 mg
Na:K ratio	0.76	0.62	0.12	4.87	2.62	0.40	NA
Micromineral							
Copper	0.84 ± 0.01^{a}	4.92 ± 0.04^{e}	4.31 ± 0.03^{d}	0.78 ± 0.02^{b}	0.57 ± 0.01^{a}	2.77 ± 0.03^{c}	900 μg
Iron	83.76 ± 0.01^{d}	45.61 ± 1.44^{b}	79.47 ± 2.32^{d}	8.28 ± 0.29^{a}	73.39 ± 1.87^{c}	41.35 ± 1.51^{b}	**14 mg (M);**29 mg (F)
Zinc	4.28 ± 0.02^{f}	3.61 ± 0.04^{d}	2.52 ± 0.02^{c}	0.81 ± 0.02^{a}	1.69 ± 0.01^{b}	4.01 ± 0.01^{e}	6.6 mg (M);4.7 mg (F)

Values are mean ± standard deviation ($n = 3$). Different superscript letters within the same row indicate a significant difference ($p < 0.05$). Nutritional composition is reported in unit g/100 g of dry weight seaweed except for moisture (g/100 g of fresh weight seaweed). Mineral content is reported in unit mg/100 g of dry weight seaweed.

† Total available carbohydrate.

†† Total dietary fibre.

††† RNI – Recommended nutrient intakes (for Malaysian adults aged 19–29 years).

* Energy requirement for moderately active individual.

** Based on 10% dietary iron bioavailability. TEI – Total energy intake. M – Male; F – Female (RNI is applicable for both gender if M and F are not specifically indicated). NA – not applicable. ND – not determined.

subjected to incineration, which also reflects the mineral content of the food.

Total available carbohydrate (TAC) content of the seaweeds was observed to be significantly different ($p < 0.05$) amongst the six species. Red seaweed *G. changii* had the highest TAC content compared to brown and green seaweeds. At the same time, *G. changii* also exhibited the highest total dietary fibre (TDF) content. Red seaweeds are rich in hydrocolloids such as agarose and carrageenan (Usov, 2011) while brown seaweeds are reported to contain alginate (Draget & Taylor, 2011). TAC refers to the carbohydrates that are digested and absorbed and are glucogenic in humans (McCleary et al., 2019). On the other hand, TDF represents carbohydrates that can be incorporated into the diet without a substantial calorie increase (McCleary et al., 2019). The TDF content of the seaweeds (6.12–10.99 g/100 g fw) was also found to be higher than those commonly consumed local vegetables (0.5–1.6 g/100 g fw) (Tee et al., 1997). Meanwhile, the TDF content of the six seaweeds (53.96–76.97 g/100 g dw) was remarkably higher than the edible tropical brown, green, and red seaweeds from the coastal areas of North Borneo, Malaysia that ranged between 25.05 and 39.67 g/100 g dw (Matanjun et al., 2009).

Protein content was also noted to be significantly different ($p < 0.05$) amongst the six seaweeds. The highest and lowest protein contents were determined in the green seaweeds, whereby *C. sertularioides* had the highest protein content whereas *C. racemosa* had the lowest protein content. The protein content of *C. sertularioides* (2.24 g/100 g fw) and brown seaweed *S. polycystum* (1.06 g/100 g fw) was comparable to local vegetables that ranged between 1.2 and 4.1 g/100 g fw (Tee et al., 1997). Besides, *C. sertularioides* also demonstrated significantly higher ($p < 0.05$) fat content compared to brown and red seaweeds. On fw basis, the fat content of the seaweeds was in the range of 0.05–0.53 g/100 g fw. These values were lower as compared to those of local vegetables, which ranged from 0.1 to 0.7 g/100 g fw (Tee et al., 1997). The protein (2.62–19.39 g/100 g dw) and fat (0.05–4.62 g/100 g dw) contents of the six seaweeds demonstrated some trends similar to the seaweeds from the Persian Gulf studied by Pirian et al. (2020). The researchers also reported significantly higher contents of protein (29.10–38.20 g/100 g dw) and fat (6.12–9.13 g/100 g dw) in the green Chlorophyta species (*B. corticolans, C. racemose*, and *C. sertularioides*) as compared to the brown Phaeophyta (protein, 14.64–21.22 g/100 g dw; fat, 1.27–2.02 g/100 g dw) and red Rhodophyta (protein, 17.82–32.05 g/100 g dw; fat, 2.12–4.02 g/100 g dw) species. Overall, findings revealed that the six seaweeds from Malaysia were low in fat and serve as good sources of TDF together with minerals, which make them favourable as vegetable alternatives.

Mineral content

Considerable amounts of calcium, magnesium, potassium, and sodium were found in the six seaweeds (Table 1, on a dw basis). Results on a fw basis (Supplementary 2) were also mentioned in the text to delineate a similarity or difference between the seaweeds and commonly consumed Malaysian vegetables such as sweet potato shoots, broccoli, common cabbage, lettuce, spinach, Chinese cabbage, fern shoots, Chinese mustard leaves, Chinese kale, and King's Salad. Brown seaweeds were noted to show higher calcium content than green and red seaweeds in this study. Brown seaweeds, especially *P. australis* and *S. binderi*, showed the highest calcium content at 2488.58 and 1902.87 mg/100 g dw, respectively. Interestingly, the calcium content of *S. polycystum* documented by another two groups of researchers was observed to be approximately two-fold (1079.75 mg/100 g dw; Port Dickson, Malaysia) (Nazarudin et al., 2021) and seven-fold (3792.06 mg/100 g dw; Kota Kinabalu, Malaysia) (Matanjun et al., 2009) higher than that determined in this study. The six seaweeds had calcium content that laid within the range of 8.74–385.23 mg/100 g fw, which was comparable to some

common local vegetables (11–270 mg/100 g fw) (Tee et al., 1997). Besides, the six seaweeds were significantly different ($p < 0.001$) in respect of their magnesium content. The highest magnesium content was found in green seaweed *C. sertularioides*, followed by brown seaweeds, whereas the lowest was in red seaweed *G. changii*.

While the sodium content of the six seaweeds (10.16–38.05 mg/100 g fw) was comparable to local vegetables with a range of 4–25 mg/100 g fw (Tee et al., 1997), the potassium content of the seaweeds (2.65–309.28 mg/100 g fw) was lower compared to commonly consumed vegetables in Malaysia (103–722 mg/100 g fw) (Tee et al., 1997). A low Na:K ratio is propitious to human health as potassium can neutralise the heart-damaging effect of sodium and lessens the effects of sodium on blood pressure and calcium loss, thereby reducing the risks for hypertension and osteoporosis (Debruyne et al., 2016; Weaver, 2013). Brown seaweed *S. polycystum* showed the lowest Na:K ratio, whereas the highest was found in green seaweed *C. racemose*. The Na:K ratio of 0.12 for *S. polycystum* in this study is comparable to that reported by Matanjun et al. (2009) at 0.16. Iron content was moderately high in the six seaweeds whereas copper and zinc contents were comparatively low. Iron content was highest in brown seaweed *P. australis* and lowest in green seaweed *C. racemosa*. Iron content of the seaweeds (0.90–12.97 mg/100 g fw) was comparable to local vegetables (0.6–5.2 mg/100 g fw) (Tee et al., 1997). In general, brown seaweeds appeared to be good sources of both macro- and microminerals for human, where the recommended nutrient intake (RNI) for Malaysian could be practically met by consuming the seaweeds as alternatives to commonly consumed vegetables.

Notwithstanding the considerable mineral contents found in the seaweed, they should be consumed in moderation. Seaweed consumption poses health risks to humans owing to the presence of heavy metals and other minerals such as iodine. The concentration of iodine in certain brown seaweed species was over 30,000 times that of seawater (Zava & Zava, 2011). This is inevitable because in seaweed, the cell wall polysaccharide and the proteins with anionic carboxyl, sulfate, and phosphate are excellent binding sites for metal retention (Yong et al., 2017). Heavy metals as well as algal toxins are present in seaweed at levels that vary according to the coastal environment and degree of contamination (Yong et al., 2017). Nonetheless, the toxicity caused by ingestion of heavy metals depends on various factors, which include chemical specification and chelation mechanism, dosage, exposure pathways, as well as sex and nutritional status of the individuals (Roleda et al., 2019). Often time, the levels of hazardous substances from aquatic and terrestrial plants are typically well below the threshold for acute and chronic toxicities in a usual balanced, moderate, and varied diet (World Health Organization, 2023). Hence, seaweed is recommended to be consumed in moderate amounts as part of best dietary practices to gain its nutritional benefits while minimising potential health hazards.

Amino acid composition

Essential amino acids (EAA) are amino acids that cannot be synthesised in animal cells or those that are insufficiently synthesised to meet the demand for maintenance, growth, development and health; and must be provided in the diet. They are different from non-essential amino acids (NEAA) that could be synthesised in adequate amounts by animal cells and may not be provided in the diet (Hou et al., 2015). As presented in Table 2, higher content of NEAA (63.29–89.94 mg/100 g dw) was found in the six seaweeds compared to EAA (10.06–36.71 mg/100 g dw) (Supplementary 3, results on a fw basis). NEAA cystine was the predominant amino acid found in all the seaweeds while the contents of EAA varied amongst the seaweeds. EAA isoleucine and phenylalanine; and NEAA cystine, serine, and tyrosine were present in substantial amounts in the six seaweeds. EAA threonine was not detected in the seaweeds except for red seaweed *G. changii*. *Gracilaria changii* was also found to contain all the EAA but histidine. Interestingly, these results contradicted the previous findings where all EAA were found to be present in brown seaweed *S. polycystum* according to Matanjun et al. (2009). Meanwhile, Barrow and Shahidi (2007) reported a greater amount of EAA in red seaweed *G. changgi* compared to NEAA. In this study, green (*C. racemosa*) and red (*G. changgi*) seaweeds were better sources of amino acids than brown seaweeds. Despite that, all six seaweeds were incomplete proteins because they are missing one or more essential amino acids.

Fatty acid content

Table 3 shows that all six seaweeds contained more saturated fatty acid (SFA) (43.87–93.76%) than monounsaturated fatty acid (MUFA) (8.57–23.41%) and polyunsaturated fatty acid (PUFA) (6.24–35.28%). Brown seaweeds exhibited a higher percentage of MUFA compared to

Table 2
Amino acid composition of six edible seaweeds.

Amino Acid	Brown Seaweed			Green Seaweed		Red Seaweed
	P. australis	*S. binderi*	*S. polycystum*	*C. racemosa*	*C. sertularioides*	*G. changii*
Essential amino acid						
Histidine	ND	ND	ND	3338.66 ± 994.94^{b}	1866.98 ± 47.91^{a}	ND
Isoleucine	341.11 ± 9.88^{a}	476.32 ± 4.29^{b}	461.35 ± 32.65^{b}	544.63 ± 4.40^{c}	352.76 ± 5.16^{a}	552.64 ± 3.05^{c}
Leucine	41.78 ± 20.48^{a}	171.91 ± 4.52^{bc}	241.59 ± 47.96^{c}	152.00 ± 18.50^{b}	ND	345.69 ± 13.58^{d}
Lysine	ND	ND	130.58 ± 2.85^{a}	ND	ND	235.72 ± 1.66^{b}
Methionine	ND	148.32 ± 1.89^{b}	112.28 ± 0.01^{a}	279.22 ± 6.50^{c}	142.50 ± 3.19^{b}	141.62 ± 0.06^{b}
Phenylalanine	58.51 ± 6.29^{a}	189.07 ± 8.75^{b}	152.29 ± 28.04^{b}	507.66 ± 15.66^{d}	167.73 ± 4.62^{b}	381.01 ± 6.03^{c}
Threonine	ND	ND	ND	ND	ND	571.58 ± 197.15
Valine	ND	194.87 ± 15.28^{a}	152.57 ± 25.81^{a}	357.33 ± 22.40^{b}	ND	382.53 ± 18.77^{b}
% EAA	10.06	20.55	23.33	36.71	30.06	30.75
Non-essential amino acid						
Alanine	ND	210.15 ± 6.25^{a}	ND	ND	275.72 ± 1.26^{b}	ND
Arginine†	ND	518.04 ± 8.85^{a}	373.04 ± 20.15^{a}	1896.20 ± 663.53^{b}	781.77 ± 18.12^{a}	780.10 ± 155.79^{a}
Asparagine/Aspartic acid	ND	ND	ND	ND	ND	ND
Cystine†	3717.22 ± 33.10^{d}	3245.57 ± 10.76^{ab}	3430.08 ± 124.08^{bc}	4276.54 ± 98.39^{e}	3669.20 ± 29.13^{d}	3429.04 ± 11.16^{c}
Glutamine/Glutamic acid†	ND	ND	ND	ND	ND	ND
Glycine†	ND	ND	ND	ND	ND	545.04 ± 9.19
Proline†	ND	ND	ND	ND	ND	488.67 ± 2.99
Serine†	167.43 ± 3.98^{a}	429.37 ± 6.76^{b}	214.79 ± 44.28^{a}	2114.13 ± 0.35^{e}	834.99 ± 4.93^{d}	488.47 ± 26.13^{c}
Tyrosine†	60.44 ± 5.40^{a}	160.32 ± 3.00^{b}	93.15 ± 7.72^{a}	643.57 ± 22.82^{d}	325.87 ± 7.02^{c}	149.54 ± 3.84^{b}
% NEAA	89.94	79.45	76.67	63.29	69.94	69.25

Values are mean ± standard deviation ($n = 3$). Different superscript letters within the same row indicate a significant difference ($p < 0.05$). Amino acid content is reported in unit mg/100 g of dry weight seaweed. ND – not detected.

† Conditionally essential amino acid (insufficient synthesis in humans due to pathophysiological disorders or severe catabolic anxiety).

Table 3
Fatty acid composition of six edible seaweeds.

Carbon No.	Fatty Acid Methyl Ester	Brown Seaweed *P. australis*	*S. binderi*	*S. polycystum*	Green Seaweed *C. racemosa*	*C. sertularioides*	Red Seaweed *G. changii*
Saturated Fatty Acid (SFA)							
C8:0	Caprylic	13.52 ± 0.16^{a}	ND	ND	ND	ND	26.47 ± 0.78^{b}
C10:0	Capric	ND	ND	ND	2.22 ± 0.08^{a}	8.46 ± 3.78^{a}	20.64 ± 0.04^{b}
C11:0	Undecanoic	ND	ND	ND	1.82 ± 0.07^{b}	0.71 ± 0.02^{a}	ND
C12:0	Lauric	ND	ND	ND	1.82 ± 0.08^{a}	3.54 ± 0.13^{b}	ND
C13:0	Tridecanoic	ND	ND	ND	0.54 ± 0.35^{a}	1.05 ± 0.29^{a}	ND
C14:0	Myristic	2.68 ± 0.14^{a}	4.99 ± 0.20^{d}	ND	3.26 ± 0.05^{b}	3.68 ± 0.01^{c}	2.85 ± 0.06^{a}
C15:0	Pentadecanoic	ND	ND	ND	ND	ND	32.57 ± 0.34
C16:0	Palmitic	29.94 ± 0.28^{b}	36.43 ± 0.31^{c}	47.18 ± 1.39^{d}	33.67 ± 0.04^{bc}	27.83 ± 1.15^{b}	11.23 ± 0.04^{a}
C17:0	Heptadecenoic	ND	ND	5.31 ± 0.17^{c}	3.74 ± 0.04^{b}	2.36 ± 0.25^{a}	ND
C18:0	Stearic	2.01 ± 0.01^{a}	2.45 ± 0.25^{a}	ND	2.26 ± 0.04^{a}	4.18 ± 0.21^{b}	ND
C20:0	Arachidic	3.15 ± 0.02	ND	ND	ND	ND	ND
C21:0	Henecosanoic	ND	ND	ND	ND	2.48 ± 0.15	ND
C22:0	Behenic	ND	ND	ND	ND	3.25 ± 0.27	ND
C23:0	Tricosanoic	4.18 ± 0.10^{b}	ND	6.61 ± 0.30^{c}	2.78 ± 0.14^{ab}	2.14 ± 0.07^{a}	ND
% SFA		55.48	43.87	59.10	52.11	59.68	93.76
Monounsaturated Fatty Acid (MUFA)							
C16:1	Palmitoleic	1.95 ± 0.02^{a}	6.99 ± 0.08^{c}	5.15 ± 0.24^{c}	3.87 ± 0.01^{b}	7.09 ± 0.58^{d}	ND
C17:1	Cis-10-Heptadecenoic	ND	ND	2.50 ± 0.04^{a}	6.63 ± 0.01^{b}	ND	ND
C18:1n9c	Oleic	21.46 ± 0.13^{e}	13.85 ± 0.11^{d}	9.66 ± 0.54^{c}	3.88 ± 0.14^{b}	1.48 ± 0.04^{ab}	ND
% MUFA		23.41	20.84	17.31	14.38	8.57	ND
Polyunsaturated Fatty Acid (PUFA)							
C18:2n6c	Linoleic	3.95 ± 0.01^{b}	6.04 ± 0.04^{c}	3.46 ± 0.12^{a}	10.24 ± 0.14^{d}	6.39 ± 0.22^{c}	ND
C18:2n6t	Linolelaidic	ND	ND	ND	ND	2.88 ± 0.09	ND
C18:3n6	γ-Linolenic	2.30 ± 0.01^{a}	3.97 ± 0.08^{b}	3.97 ± 0.18^{b}	15.76 ± 0.19^{d}	8.28 ± 0.24^{c}	ND
C18:3n3	α-Linolenic	4.41 ± 0.04^{c}	2.35 ± 0.01^{ab}	2.27 ± 1.72^{ab}	0.94 ± 0.01^{ab}	3.37 ± 0.22^{b}	ND
C20:3n3	Cis-11,14,17-Eicosatrienoic	ND	ND	ND	ND	6.09 ± 0.24	ND
C20:3n6	Cis-8,11,14-Eicosatrienoic	10.45 ± 0.04^{d}	22.92 ± 0.01^{f}	13.88 ± 0.16^{e}	3.65 ± 0.01^{b}	2.02 ± 0.06^{a}	6.24 ± 0.30^{c}
C20:4n6	Arachidonic	ND	ND	ND	ND	1.83 ± 0.03	ND
C20:5n3	Eicosapentaenoic	ND	ND	ND	1.31 ± 0.37^{a}	0.89 ± 0.11^{a}	ND
C22:2	Cis-13,16-Docosadienoic	ND	ND	ND	1.62 ± 0.28	ND	ND
% PUFA		21.11	35.28	23.58	33.52	31.75	6.24
ω−6/ω−3		3.79	14.01	9.39	13.90	2.07	6.24

Values are mean ± SD ($n = 3$). Different superscript letters within the same row indicate a significant difference ($p < 0.05$). Fatty acid content is reported in unit percent (%) of total fatty acid. ND – not detected.

green seaweeds, whereas no MUFA was detected in the red seaweed. Brown seaweed *S. binderi* showed the highest PUFA and lowest SFA contents. Unsaturated fatty acids are known as "good fats" due to their beneficial effects on blood lipid profile, blood pressure, inflammatory response, and endothelial function (Mišurcová et al., 2011). Furthermore, all six seaweeds, except for red seaweed *G. changii*, were found to contain both omega-3 (ω−3) and omega-6 (ω−6) fatty acids. According to Simopoulos (2002), an excessive amount of ω−6 would promote the pathogenesis of cardiovascular disease, cancer, inflammation, and autoimmune diseases while a low ratio of ω−6:ω−3 on the contrary would exert suppressive effects. Green seaweed *C. sertulariodes* in this study demonstrated an ω−6:ω−3 ratio of 2.07:1 (suggested ratio: 1–2:1), which is deemed ideal for optimal health benefits. Nonetheless, lower ω−6:ω−3 ratio of 0.68:1 and 1.34:1 were reported in *C. sertulariodes* from Kachchh Coast, India and Sinaloa Coast, Mexico, respectively (Dixit et al., 2018; Osuna-Ruiz et al., 2019).

Fucoxanthin content

Analysis using high-performance liquid chromatography (HPLC) was carried out to quantify fucoxanthin. Fucoxanthin was only detected in the brown seaweeds, whereby *P. australis* (2.09 mg/g dw) exhibited the highest fucoxanthin content, followed by *S. polycystum* (1.31 mg/g dw) and *S. binderi* (0.94 mg/g dw). Nagappan et al. (2017) reported a lower (0.31 mg/g dw) fucoxanthin content for *S. polycystum* collected from the same geographical origin, whereby extraction was also performed using methanol. Meanwhile, Yip et al. (2014) reported a higher (7.4 mg/g dw) fucoxanthin content for *S. binderi*, which could be attributed to the differences in seaweed origin (i.e., Sabah) and extraction solvent (i.e., methanol:chloroform:water, 4:2:1, v/v/v) used. In another study, the fucoxanthin content of *Sargassum* species from Malaysia as determined by Din et al. (2022) was in the range of 0.07–7.4 mg/g dw. Fucoxanthin had been documented to display anti-cancer (Jaswir, 2011), anti-diabetic (Oh et al., 2016), and anti-obesity (Maeda et al., 2007) properties that could be beneficial for human health.

Antioxidant activities

The antioxidant activities of the six seaweeds as determined by DPPH-RSA, FRAP, and TEAC assays are presented in Table 4. In general, all seaweed species showed considerable antioxidant activity, despite noticeably lower in *G. changii* and *S. binderi*. The seaweeds showed DPPH-RSA when compared to the control, which implied promising antioxidant activity. *Sargassum polycystum* revealed the highest (54.76%) DPPH-RSA whereas *G. changii* showed the lowest (2.39%) activity. Cruciferous vegetables, including Chine white cabbage, Chinese cabbage, green cabbage, mustard cabbage, and red cabbage in Malaysia were reported to demonstrate DPPH-RSA in the range of 79–97% (Lee et al., 2007). Popular leafy vegetables in Malaysia, including Chinese chive, Indian spinach, romaine lettuce, sweet potato leaves, and water spinach, were also reported to exhibit DPPH-RSA approximately between 5 and 85% (Bhat et al., 2013). Meanwhile, Mustafa et al. (2010) reported that 15 of 21 Malaysian tropical plants investigated showed DPPH-RSA between 70 and 90%.

The FRAP of the seaweeds was in the range of 3.28–28.87 μmol Fe^{2+}/g dw. The highest FRAP was found in *C. sertulariodes* while the lowest was in *G. changii*. The FRAP value of selected Malaysian salads, vegetables, and herbs was in the range of 1.62–63.61 mmol Fe^{2+}/g dw (Khalid & Babji, 2018). Differences in extraction procedure, concentrations of extract and reagent, and sample:reagent ratio, amongst others, are factors that may cause the disparity in antioxidant activities observed. Meanwhile, the TEAC of the seaweeds ranged between 0.04

Table 4
Antioxidant activities of six edible seaweeds.

Antioxidant Activities	Brown Seaweed			Green Seaweed		Red Seaweed
	P. australis	*S. binderi*	*S. polycystum*	*C. racemosa*	*C. sertularioides*	*G. changii*
DPPH-RSA†	36.06 ± 1.93^{d}	6.18 ± 0.01^{a}	54.76 ± 1.16^{e}	14.41 ± 3.88^{b}	24.15 ± 0.68^{c}	2.39 ± 0.59^{a}
FRAP	13.36 ± 0.32^{b}	6.32 ± 0.28^{a}	18.90 ± 0.21^{bc}	16.26 ± 0.23^{bc}	28.87 ± 3.43^{d}	3.28 ± 0.27^{a}
TEAC	12.21 ± 0.32^{e}	2.46 ± 0.21^{bc}	5.39 ± 0.59^{d}	1.57 ± 0.01^{b}	3.01 ± 0.44^{c}	0.04 ± 0.01^{a}

Values are mean ± standard deviation ($n = 3$). Different superscript letters within the same row indicate a significant difference ($p < 0.05$). DPPH – 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (%) for seaweed extract at the concentration of 0.05 g/mL; FRAP – ferric reducing antioxidant power (μmol Fe^{2+}/g of dry weight seaweed); TEAC – trolox equivalent antioxidant capacity (μmol TE/g of dry weight seaweed).
† Gallic acid (standard) at the same concentration provided 95.97% of DPPH-RSA.

and 12.21 μmol TE/g dw with significant differences ($p < 0.05$) observed amongst the seaweeds. *Padina australis* displayed the highest TEAC while *G. changii* had the lowest activity. According to Matanjun et al. (2008), the TEAC of *Padina* spp., *S. polycystum*, and *C. racemosa* from Sabah was 1.49, 1.86, and 2.01 mM TE/mg extract, respectively. The TEAC of *Padina* spp., *S. polycystum*, and *C. racemosa* in this study was 0.933, 0.238, and 0.095 mM TE/mg extract, respectively (Supplementary 4, results expressed in unit mM TE/mg extract). Although all the seaweeds were of Malaysian origin, TEAC could be influenced by multiple environmental factors such as air, sunlight, water temperature, nutrient availability, and salinity, amongst others (Fung et al., 2013).

In general, there was no specific trend that could be observed in terms of differences in antioxidant activities between the brown and green seaweeds. However, red seaweed *G. changii* was noted to exhibit the lowest activities in all three antioxidant assays. In terms of the disparity in antioxidant activities measured by different assays, it could be attributed to the differences in antioxidant reaction mechanisms between the assays (Santos-Sánchez et al., 2019). It must be highlighted that the concentration of seaweed extract used in all three antioxidant assays was 0.05 g extract/mL methanol, and the antioxidant activities determined were specific for this concentration of extract used in this study. It is difficult to compare results for antioxidant activity of similar species between different studies because the concentrations of extract and free radical solution used are not the same amongst researchers most of the time (Hwang & Lee, 2023). The methods adopted are often modified to suit the context and nature of different studies. Since there is limited literature data on the six underexploited seaweeds investigated, adopting an identical analytical method from the literature to ensure a fair comparison with previous studies had been a challenge and hence became the limitation of this study.

Conclusion

The seaweeds from Malaysia are valuable sources of nutrients and antioxidants that can be incorporated into the daily diet. Each seaweed species had different nutritional composition and antioxidant properties. The six seaweeds studied were generally rich in total dietary fibre and minerals in addition to being low in fat. The considerable amount of unsaturated fatty acids in brown seaweeds may serve as a source of good fat. Additionally, brown seaweeds can also be a good supply of calcium and potassium. Green and red seaweeds may help in obtaining the essential amino acids. All seaweeds exhibited antioxidant activities but fucoxanthin could only be detected in the brown seaweeds. Further study is warranted to identify bioactive compounds other than fucoxanthin that serve as antioxidants in these seaweeds. In addition, the contents of iodine, heavy metals, and algae toxins should be further explored and comprehensively documented.

CRediT authorship contribution statement

Ying Yee Chin: Formal analysis, Investigation, Methodology, Writing – original draft. **Kian Aun Chang:** Writing – review & editing, Visualization. **Wei Mei Ng:** Formal analysis, Investigation, Methodology. **Zei Pei Eng:** Formal analysis, Investigation, Methodology. **Lye Yee Chew:** Data curation, Project administration, Writing – review & editing. **Yun Ping Neo:** Writing – review & editing. **See Wan Yan:** Supervision, Writing – review & editing. **Ching Lee Wong:** Conceptualization, Resources. **Kin Weng Kong:** Writing – review & editing. **Amin Ismail:** Conceptualization, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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