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Research on the optimization of preparation and the application analysis of antioxidant activity of mycosporine-like amino acids derived from marine macroalgae

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Abstract

In recent years, mycosporine-like amino acids (MAAs) have demonstrated extensive application prospects across various fields such as food, pharmaceuticals, and cosmetics. In a previous study we conducted, the MAA extracts obtained from several macroalgae contained a large amount of pigments, which introduced numerous interfering factors to the study of the extract's activity. In this study, the extraction processes of MAAs of the six macroalgae, such as *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp., and *Undaria pinnatifida*, were optimized with the aim of reducing the pigment content in the MAA extracts. Additionally, their in vitro antioxidant activities, anti-browning effects, and mineral contents were also analyzed. Furthermore, the target MAA extracts were combined with several common food additives to explore their impact on the stability of the target MAA extracts. The results indicated that the removal of pigments along with multiple alcohol precipitation could remarkably diminish the pigment content within the MAA extracts and augment the extraction yield. Through this pretreatment method, the yields of the MAA extracts from *Bangia fuscopurpurea* and *Gelidium amansii* were elevated to 1.5 times and 1.1 times their original values, respectively. The MAA extracts from the six macroalgae predominantly consisted of palythine, palythenic acid, shinorine, and/or porphyra-334. Among them, the MAA extracts sourced from *Sargassum fusiforme* and *Bangia fuscopurpurea* exhibited stronger antioxidant capabilities and more pronounced anti-browning effects. These MAA extracts were also rich in both macro- and micro-elements essential for the human body, while being devoid of any harmful mineral elements. Carrageenan and agar proved effective in maintaining the stability of the MAA extracts from *Sargassum fusiforme* and *Bangia fuscopurpurea*. Overall, the MAA extracts from *Sargassum fusiforme* and *Bangia fuscopurpurea* demonstrated favorable potential for application in the food industry.

Keywords Mycosporine-like amino acids, Marine macroalgae, Antioxidant activity, Anti-browning effect, Stability

Marine macroalgae are renowned as "Biorefineries" owing to their high yield, rapid growth rate, and rich chemical composition. They represent an ideal option for large-scale cultivation and the sustainable production of high-value active substances. Marine macroalgae harbor a series of active substances possessing antioxidant [19], antiviral activities [20], and photo-protective [27]. Extracts derived from marine macroalgae

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have demonstrated extensive application prospects in the fields of medicine, cosmetics, and food [1, 32]. Since the 1990s, the antioxidant active substances present in marine macroalgae have garnered significant attention as an optimal source of natural antioxidants [47]. During the period from 2000 to 2023, CNKI was employed to conduct a search for studies related to the antioxidant activity of marine macroalgae. It was discovered that the principal antioxidant substances in marine macroalgae encompass polysaccharides [54], phenols [57], mycosporine-like amino acids (MAAs) [56], pigments and other substances [55], along with numerous unidentified components within the extracts of large seaweeds [15] (Fig. 1). Among them, MAAs are multi-functional active compounds that have come to the fore in recent years. They possess properties such as resistance to ultraviolet radiation and photoprotection [16, 53], exhibit diverse structures, and hold significant commercial potential [11].

To date, only Cyanobacteria, phytoplankton, and algae have been found capable of synthesizing MAAs [38]. Among them, marine macroalgae possess a more abundant and more easily accessible biomass compared to other primary producers of MAAs. Consequently, marine macroalgae are regarded by researchers as an ideal source of MAAs [30, 34, 58]. As far as we are aware, previous research on MAAs in marine macroalgae has mainly centered around their distribution [25, 31], induction by various environmental factors [12], chemical characterization and profiling [41, 45], as well as isolation [42]. However, relatively few studies have been dedicated to exploring their antioxidant activities [8, 18]. MAAs, which are extracted from seaweed, are safe and possess more intricate functions compared to existing chemical antioxidants. They hold great potential to serve as natural antioxidants. Additionally, it has been suggested that the antioxidant activities of MAAs might also contribute to their capacity to inhibit the activities of cancer cells [21, 59]. Hence, it is imperative to conduct an analysis of the antioxidant activities of MAAs derived from a broader range of marine macroalgae for the development of novel antioxidant and multifunctional drugs, among other applications.

In our previous studies, we have found that marine macroalgae such as *Bangia fuscopurpurea*, *Gelidium amansii*, *Palmaria palmata* (Rhodophyta), *Sargassum* sp., *Sargassum fusiforme* and *Undaria pinnatifida* (Phaeophyceae) contained MAAs [50, 51]. To the best of our current understanding, no prior studies have focused on the antioxidant activities of MAAs sourced from these six specific macroalgae species. In the present research endeavor, the extraction and preparation procedures of MAAs from the six macroalgae were initially optimized. Subsequently, a comprehensive evaluation was carried out to assess the total antioxidant capacity, the scavenging efficacy against superoxide anion radicals, as well as the anti-browning effect on fresh yam and apple slices exhibited by the MAA extracts from the six macroalgae. Furthermore, the mineral contents within the target MAA extracts were analyzed, and the influence of several common food additives on the stability of the target MAA extracts was investigated, with the aim of exploring the practical feasibility of applying the target MAA extracts in the food industry.

Materials and methods

Materials and reagents

Bangia fuscopurpurea, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp. and *Undaria pinnatifida* were purchased by Jiangsu Bilian Marine

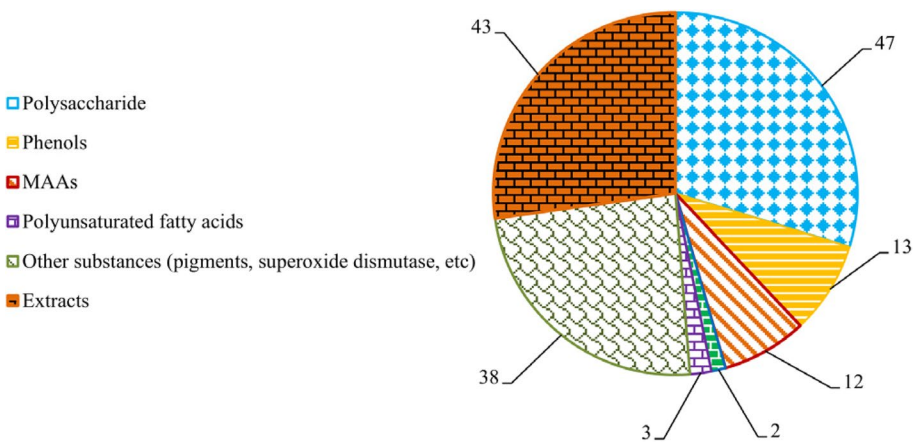

Fig. 1 Classification of antioxidant active substances of marine macroalgae (data are from China National Knowledge Infrastructure (CNKI) literatures from 2000 to 2023)

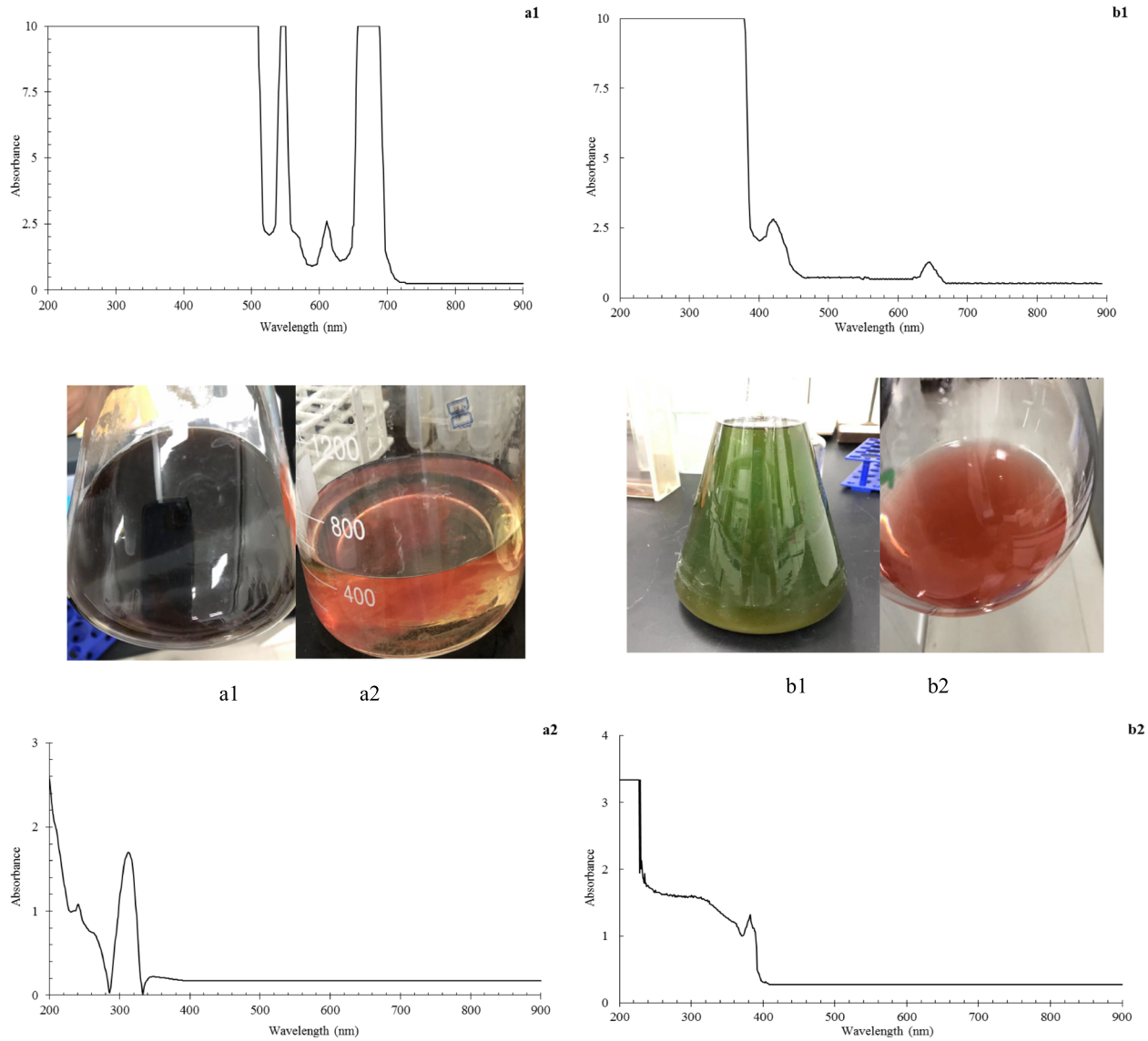

Fig. 2 Wavelengths scanning and appearance of the MAA extracts solution from *Bangia fuscopurpurea* (a1 and a2) and *Palmaria palmata* (b1 and b2) (a1 and b1 indicated the extracts solution that has not been treated to remove pigments and increase the number of alcohol precipitation before extraction; a2 and b2 indicated the extracts solution that was treated to remove pigment and increase the number of alcoholic precipitation before extraction)

Biotechnology Co., Ltd. It was washed, freeze-dried, crushed by an ultra-fine grinder, and then sieved through a 40-mesh sieve for backup.

Fresh yams and red Fuji apples were purchased from Lianyungang Farmers' market and produced locally. Antioxidant reagent kit was purchased from Nanjing Jiancheng Bioengineering Institute. The reagents used for high-performance liquid chromatography-mass spectrometry analysis, such as formic acid and acetonitrile, were chromatographic pure, while the other reagents were analytical pure.

Preparation of the MAA extracts from marine macroalgae

Referring to our previous research methods [50], the extraction MAAs from marine macroalgae was carried out. 10 g of marine macroalgae powder was added with 250 mL of anhydrous ethanol and extracted for 2 h in a constant temperature water bath shaker at 45 ℃. After being taken out and left at room temperature for 30 min, the supernatant was discarded and centrifuged at 8000 r·min^{-1} for 15 min. Anhydrous ethanol was added to wash the algae residue 2–3 times and then centrifuged. 25% methanol solution was added to the

algae residue mentioned above and shook for 2 h at 45 ℃. Subsequently, the supernatant was obtained through centrifugation at 8000 r·min^{-1} for 15 min. The above extraction was repeated 2–3 times and merged with the supernatants. The supernatants were rotated and evaporated at 40 ℃, and the concentrated volume did not exceed three-quarters of the original volume to ensure the removal of methanol. 4 times the volume of anhydrous ethanol was added to the concentrated supernatants and precipitated with ethanol at -20 ℃ for 6 h, and then centrifuged at 5 ℃ for 20 min to remove precipitation. The alcohol precipitation steps were repeated 2–3 times. Finally, ethanol was removed by rotary evaporation at 40 ℃, and MAAs were obtained through freeze-drying. The qualities of MAAs were 2.362 g, 1.103 g, 1.127 g, 1.136 g, 0.983 g and 1.182 g, respectively, from the dried powder of *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp. and *Undaria pinnatifida*. The yield of the MAA extracts was calculated according to the following formula:

$$\text{Yield} = 100\% \times \text{Quality of the MAA extracts} / \text{Quality of marine macroalgae powder}$$

Determination of antioxidant activity in vitro of the MAA extracts

Determination of total antioxidant capacity

10 mM Troloxs standard solution (the solution was prepared according to the reagent kit method) was accurately absorbed, and diluted with ultrapure water to 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM and 1.0 mM. Reaction reagents were added according to the steps in Table 1 and reacted at room temperature for 6 min. The absorbance of each well at 405 nm using microplate reader was determined to obtain Trolox standard curve $y = -1.1223x + 1.1283$ ($R^2 = 0.9957$). In the formula, x was the absorbance, and y was the Trolox concentration (mM). The antioxidant capacity of Trolox standard could be expressed as TEAC, and the formula for calculating the total antioxidant capacity was as follows:

$$\text{TEAC (mM)} = -1.1223 \times \text{absorbance} + 1.1283 \quad (1)$$

0.0100 g of the MAA extracts were accurately weighed and dissolved in ultrapure water to obtain 1% of the concentration. After filtering with 0.45 μM membrane, reaction reagents were added according to the steps in Table 1 and reacted at room temperature for 6 min. Absorbance of each well was obtained by microplate reader at 405 nm. Three parallel samples were set for the MAA extracts from each marine macroalgae. According to formula (1), the TEAC value could be calculated. The total antioxidant capacity of the MAA extracts was calculated using formula (2), where W (g) was the quality of the MAA extracts.

$$\text{Total antioxidant capacity of the MAA extracts (mmol/g)} = \frac{\text{TEAC}}{\text{W}} \quad (2)$$

The scavenging effect on superoxide anions

① Preparation of or the MAA extracts and V_C (vitamin C) solutions. 0.20 g, 0.30 g, 0.40 g, 0.50 g and 0.60 g of the MAA extracts from each marine macroalgae were dissolved in ultrapure water and diluted to 10 mL to prepare the solution to be tested with the concentrations of 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL and 60 mg/mL, respectively. 0.10 g of V_C was added to ultrapure water and diluted to a constant volume of 100 mL. And then 100 μL, 150 μL, 200 μL, 250 μL and 300 μL of V_C solution were transferred and diluted to 10 mL with ultrapure water to obtain 0.010 mg/mL, 0.015 mg/mL, 0.020 mg/mL, 0.025 mg/mL and 0.030 mg/mL of V_C solution to be assayed, respectively. ② The scavenging effect of superoxide anion radical. 4500 μL Tris–HCl buffer solution was added into the sample tube and then added 25 mmol/L of pyrogallol solution 400 μL (containing 1 mmol/L hydrochloric acid), then poured into a colorimetric dish and quickly mixed. The absorbance at 325 nm (recorded as A_0) was measured every 30 s and stopped at 300 s. 2 mL of or the MAA extracts solutions of different concentrations were transferred and added to the sample tube. The absorbance at

Table 1 Operation condition of total antioxidant capacity

	Blank holes	Standard holes	Assay holes
Ultrapure water (μL)	10		
Troloxs solution(μL)		10	
Sample (μL)		10	
Reagent IV application solution (μL)	20	20	20
ABTS operating fluid (μL)	170	170	170

325 nm (recorded as A_{sample}) was measured every 30 s until it stopped at 300 s. Ultrapure water was used instead of the sample solution to be tested, 1 mmol/L HCl was used as reference blank instead of pyrogallol solution for zero adjustment. Vc standard was used as positive control, and 3 parallel samples set for each experiment. The calculation formula for the scavenging effect of superoxide anion was:

The scavenging effect of superoxide anion (%) = 100% × [(A_0-A_{sample})/A_0], in the formula, A_0 was the absorbance of the superoxide anion without adding the sample solution to be tested. At this time, A_{sample} was equivalent to A_{sample} A_{blank}, and A_{blank} was the background absorbance of the sample solution to be tested; A_{sample} represents the absorbance of the sample solution to be tested. According to the correlation curve between the concentration and scavenging effect, the concentration EC_{50} of the sample solution could be obtained when the scavenging effect of superoxide anion reached 50% using the method reported in reference [43].

Anti browning experiment

Browning experiment of yam slices

Reference to the determination method in the literature [35] with slight adjustment, 0.050 g of MAAs (or isolated component of the MAA extracts from *Bangia fuscopurpurea* obtained by cation-exchange resin column) was added appropriate amount of ultrapure water for redissolution and diluted to a constant volume of 100 mL to obtain the concentration of 0.5 mg/mL. Fresh yams were sliced horizontally and divided into 8 groups (blank group, the MAA extracts from the six macroalgae, and isolated component of the MAA extracts from *Bangia fuscopurpurea*), with 3 pieces in each group. Each has a quality of 4.0 g ± 0.5 g and a thickness of 2.0 mm ± 0.5 mm. The yam slice was put in the corresponding solution mentioned above (ultrapure water, solution of the MAA extracts, and isolated component of the MAA extracts from *Bangia fuscopurpurea*), and shook for 30 min. And then, these yam slices were taken out and evaporated naturally to dry, sealed with a preservative film and avoided contact with yam slices. The experimental time was set at 3 days to observe the browning degree in 8 groups of yam tablets. The browning degree of yam slices was evaluated based on the browning index. Among them, 0 class was no brown, 1 class was light brown, 2 class was brown, and 3 class was dark brown. According to formula (3) mentioned in the literature [4], the browning index was calculated:

$$\text{The browning index (\%)} = 100\% \times [\sum(\text{Class} \times \text{number of browning yam slice})/(\text{Highest browning class} \times \text{total number of yam slices in this group})] \quad (3)$$

Browning experiment of apple slices

Fresh red Fuji apples were longitudinally sliced along the root, with a quality of 5.0 g ± 0.3 g and a thickness of 4.0 mm ± 0.6 mm. According to Method 1.4, the experiments were conducted and the browning index of apple slices calculated.

Determination of mineral content

0.20 g of the MAA extracts from macroalgae was poured into a platinum crucible. Subsequently, it carbonized on the electric furnace until there was no smoke, and then it was fully burned in the box-type resistance furnace until only the inorganic mineral elements were retained. When the furnace temperature drops to 200 °C, platinum crucible was removed and cooled in a dryer for 30 min. The platinum crucible was moistened with a small amount of ultrapure water, and then added 3 ~ 5 drops of sulfuric acid solution (1:1, *v*:*v*), 15 mL of nitric acid and 15 mL of hydrofluoric acid. This platinum crucible was placed on an electric hot plate for heating digestion, and the temperature was raised to promote acid volatilization until no smoke was emitted. After cooling, 2% nitric acid solution (volume concentration) for reconstitution was added, poured into a glass volumetric flask, set the volume to 30 mL, and then shaken well. The solution into the glass volumetric flask was poured and filtered by a 0.45 μm filter membrane. Finally, filtrates were detected using the machine (ICP-OES) to obtain mineral content.

Effect of food additives on the stability of the MAA extracts

0.50 g of the MAA extracts from *Bangia fuscopurpurea* or (*Sargassum fusiforme*) was dissolved in 3 mg/mL of Casson preservative aqueous solution and diluted to 500 mL to obtain 1 mg/mL of the solution of the MAA extracts. The solution was divided into 5 groups. And then citric acid, carrageenan, sodium glutamate, agar and sodium benzoate with a concentration of 3 mg/mL were added to the evenly divided the solution of the MAA extracts mentioned above, respectively, and mixed. Subsequently, the mixed solution was placed at 25 ℃ and 48 ℃ for 40 days. 3 mL of each group was sampled daily and measured the absorbance of the sample at 330 nm. During the experiment, three parallel samples were set.

HPLC-NMR analysis

Agilent Eclipse Plus C18 column (4.6 mm × 150 mm, 5 μm) was selected. Column temperature was 25 ℃, mobile phases A and B were 0.2% formic acid aqueous

solution and acetonitrile, respectively (elution gradient: 0 ~ 20 min, B%: 0 ~ 70%; flow rate of 1.0 mL/min), wavelength was set 330 nm, and injection volume was 50 μL.

According to the reference [15], NMR parameters were set. Namely, the spray pressure was 45 psi, the nitrogen flow rate was 10.0 L/min, the drying temperature was 350 ℃, the crushing voltage was 100 V, the capillary voltage was 4500 V; the full scan (Scan), and the mass charge ratio of the reference ion was 121.0509 and 922.0098, so as to conduct real-time correction for the determination results of the reference ion. The resolution (m/z) at 922.0098 was 11300 full scans, and the mass charge ratio (m/z) range was 120 ~ 1000.

Data processing

The experimental data was analyzed using the SPSS11.5 software package for independent sample testing, with $P<0.05$ indicating significant differences and $P<0.01$ indicating extremely significant differences.

Results

Preparation and composition of the MAA extracts from the six marine macroalgae

The solutions of the MAA extracts from *Bangia fuscopurpurea* and *Palmaria palmata* prepared using the extraction method from our previous research [50] exhibited black red or black green color, respectively. A substantial amount of pigment residue impeded the subsequent application the MAA extracts. Therefore, prior to the extraction of MAAs, this paper first used ethanol to extract and discard the pigments. In Fig. 2, it could be clearly seen that the pigment extracts from *Bangia fuscopurpurea* exhibited multiple obvious absorption peaks in the range of 450 nm to 700 nm,and the pigment extracts from *Palmaria palmata* showed absorption peaks in the range of 650 nm to 700 nm, and 390 nm to 440 nm. It was reported that the absorption peaks of chlorophyll were 420 nm ~ 460 nm, and 630 nm ~ 665 nm [49], and the absorption wavelengths of phycobiliprotein were 557 nm, 565 nm, 617 nm and 652 nm [33], β-carotene has an absorption peaks near 430 nm and 449 nm [52]. This indicated that the pigment extracts from *Bangia fuscopurpurea* contained chlorophyll and phycobiliprotein, while the pigment extracts from *Palmaria palmata* contained β-carotenoids and chlorophyll. The MAA extracts were obtained from macroalgae powder after pigment removal and increased the number of alcoholic precipitations to further remove non-target substances. In Fig. 3, the results showed that the color of the MAA extracts obtained was significantly lighter than that of the MAA extracts prepared using the method in previous research [50], and they have the transparent reddish color.

Based on the above experiments, HPLC–MS analysis of the MAA extracts from the six macroalgae was conducted (not listed in the figure). The results indicated that *Bangia fuscopurpurea*, *Sargassum fusiforme*, *Sargassum* sp. and *Undaria pinnatifida* mainly included the four kinds of MAAs, such as, palythine, palythenic acid, shinorine and porphyra-334; the MAA extracts from *Gelidium amansii* and *Palmaria palmata* were composed mainly of palythine, palythenic acid, and shinorine (or porphyra-334) (Table 2). In comparison with prior researches ([50], Zhu et al., 2023), the removal of pigment and increasing of alcohol precipitation times did not change the composition of the MAA extracts

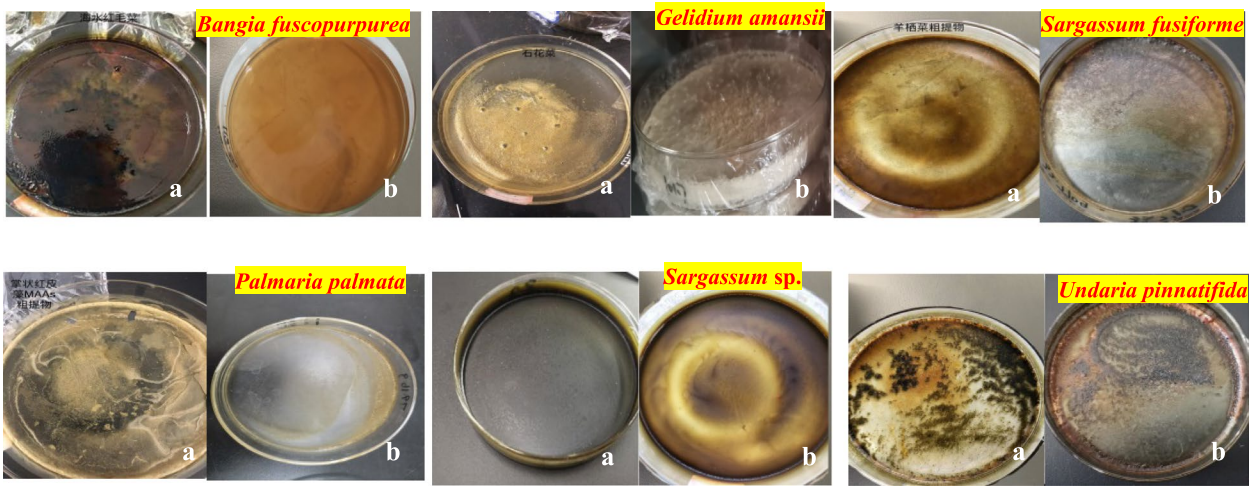

Fig. 3 The MAA extracts from the six marine macroalgae (**a**. No pre-pigment extraction was performed; **b**. Pre-pigmentation extraction and increase the number of alcoholic precipitations were performed)

Table 2 Yield, absorption wavelengths, information of MS and the kinds of the MAA extracts from the six marine macroalgae

MAAs	Yield (%)	Wavelength (nm)	$[M+H]^+$	Mass	Kind of MAA
Bangia fuscopurpurea	23.62	319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		333	333.1	332	Shinorine
		334	347.0	346	Porphyra-334
Gelidium amansii	11.27	268	205.0	204	Gadusol (precursor of MAA)
		328	222.7	226	unkown MAA
		319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		334	347.0	346	Porphyra-334
Sargassum fusiforme	11.36	260 ~ 280	205.0	204	Gadusol
		260 ~ 280	222.7	226	unkown MAA
		319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		333	333.1	332	Shinorine
		334	347.0	346	Porphyra-334
Palmaria palmata	11.03	268	205.0	204	Gadusol
		328	222.7	226	unkown MAA
		319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		333	333.1	332	Shinorine
Sargassum sp.	9.83	260 ~ 280	205.0	204	Gadusol
		260 ~ 280	222.7	226	unkown MAA
		319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		333	333.1	332	Shinorine
		334	347.0	346	Porphyra-334
Undaria pinnatifida	11.82	260 ~ 280	205.0	204	Gadusol
		260 ~ 280	222.7	226	unkown MAA
		319	245.1	244	Palythine
		337	329.0	328	Palythenic acid
		333	333.1	332	Shinorine
		334	347.0	346	Porphyra-334

from *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassumfusiforme*, *Sargassum* sp. and *Undaria pinnatifida*. Meanwhile, the removal of pigments and the increasing of alcohol precipitation times reduced impurities in the MAA extracts. The yields of the MAA extracts from *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp. and *Undaria pinnatifida* were 23.62%, 11.27%, 11.36%, 11.03%, 9.83% and 11.82% (Table 2), respectively. The yields of the MAA extracts from *Bangia fuscopurpurea* and *Gelidium amansii* respectively increased by 1.5 times and 1.1 times respectively compared with those in our previous study [50]. This indicated that such a combined operation approach was highly conducive to the preparation of the MAA extracts from *Bangia fuscopurpurea* and *Gelidium amansii*. However, the yields of the MAA extracts from *Sargassum fusiforme*, *Sargassum* sp. and *Undaria pinnatifida* decreased significantly ($P<0.05$), indicating that the removal of pigments and/or increasing the number of alcohol precipitation times caused the loss of MAAs, and the subsequent treatment methods need to be improved to ensure that the yield of the MAA extracts will not decrease significantly.

In vitro antioxidant activity of the MAA extracts from the six macroalgae

In Fig. 4, the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* showed highest the total antioxidant capacity. Moreover, their total antioxidant capacities were 1.8 to 5.1 times higher than those of the

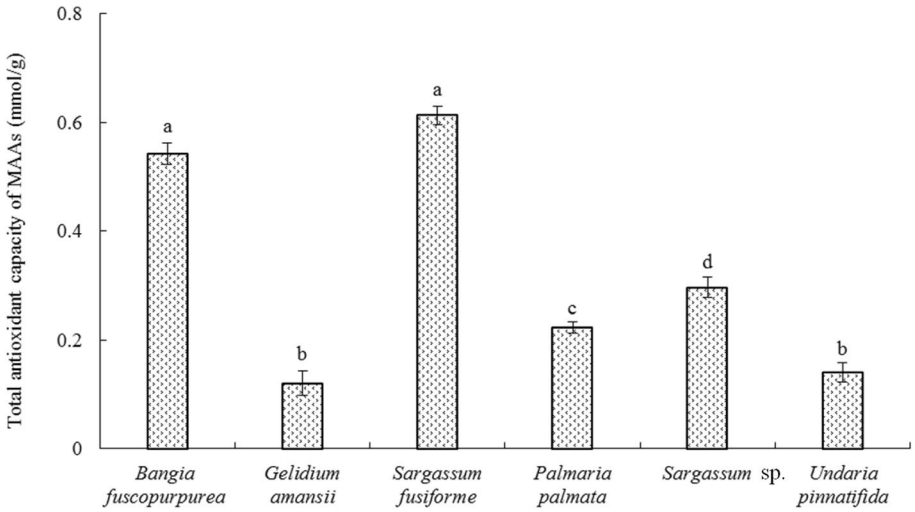

Fig. 4 Total antioxidant capacity of the MAA extracts from the six marine macroalgae (Different letters indicate significant differences)

MAA extracts procured from the other four macroalgae. Among them, the total antioxidant capacity of the MAA extracts from *Gelidium amansii* was weakest.

The scavenging effects of the MAA extracts from the six macroalgae on superoxide anions significantly increased with the increase of the extract's concentration ($P<0.05$) (Fig. 5). At 50 mg/mL, the scavenging effects of the MAA extracts from *Sargassum fusiforme*, *Bangia fuscopurpurea* and *Sargassum* sp. on superoxide anions were more than 93%, and the scavenging effects of the MAA extracts from other three macroalgae on superoxide anions were more than 64%. The order of

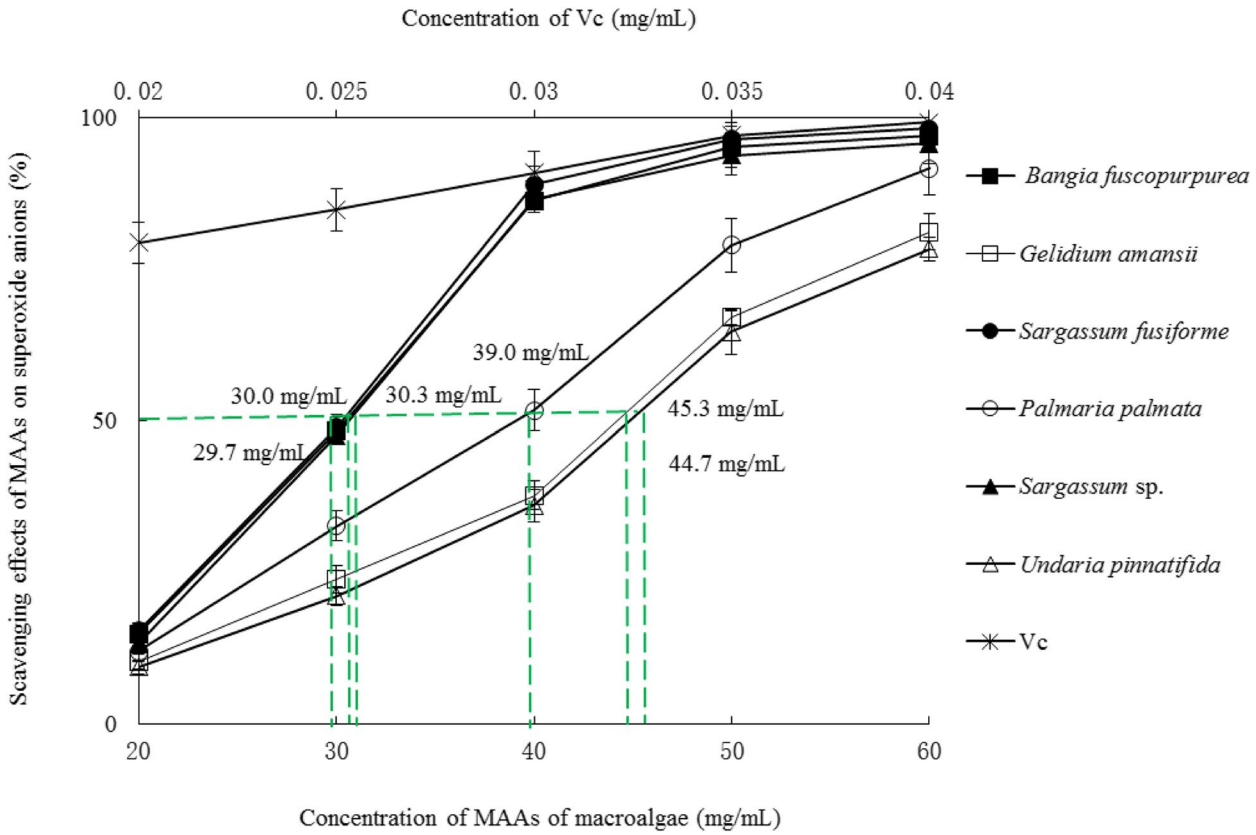

Fig. 5 Scavenging effects of Vc and the MAA extracts from the six marine macroalgae on superoxide anions

the scavenging ability of the MAA extracts from the six macroalgae on superoxide anions was as follows: *Sargassum fusiforme* and *Bangia fuscopurpurea* ≈ *Sargassum* sp. > *Palmaria palmata* > *Gelidium amansii* and *Undaria pinnatifida*. The scavenging ability of Vc on superoxide anions was significantly better ($P < 0.01$) than that of the MAA extracts from the six macroalgae, and it had a scavenging effect of more than 96% at a concentration of 0.035 mg/mL (Fig. 6). The EC_{50} values, representing the scavenging effect of the MAA extracts from *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp. and *Undaria pinnatifida* on superoxide anions, were determined using the linear regression algorithm based on the graph where the inhibition percentage was plotted against the MAA extracts. These EC_{50} values were 30.0 mg/mL, 44.7 mg/mL, 29.7 mg/mL, 39.0 mg/mL, 30.3 mg/mL and 45.3 mg/mL, respectively.

In subsequent experiments, the MAA extracts from *Bangia fuscopurpurea* with significant in vitro antioxidant activity were loaded on silica gel column chromatography (3.0 cm × 40 cm, 200–300 mesh silica gel), and methanol: ethanol: water (8:10:0.5, *v*: *v*: *v*) were as eluent. The first three tubes were combined and concentrated under reduced pressure to obtain isolated components from the MAA extracts. Also, this component, namely the isolated component of MAAs from *Bangia fuscopurpurea*, was also used for anti-browning experiments.

Anti-browning of the MAA extracts from the six macroalgae

The browning degree of yam slices and apple slices could directly reflect their oxidation during the storage period. From Fig. 6, it could be seen that these yam slices of the blank group showed the most obvious browning, and their browning index was higher than that of yam slices treated with the MAA extracts within 1–3 days. On the third day, the browning index of untreated yam slices was higher than that of yam slices soaked in the MAA extracts solution. Among them, the anti-browning effects of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* were the best ($P < 0.05$). It was clear that the isolated components of the MAA extracts from *Bangia fuscopurpurea* also showed good anti-browning effects on yam tablets, and anti-browning effects were significantly better ($P < 0.05$) than that of other macroalgae in the first two days of the experiments (Fig. 6).

On the 3th day, in the blank control groups, the browning of apple slices was highly conspicuous ($P < 0.05$). In contrast, the apple slices treated with the MAA extracts merely exhibited some faint browning, and the apple slices treated with the isolated component from the MAA extracts from *Bangia fuscopurpurea* showed the weakest browning (Fig. 7). These results clearly demonstrated that the MAA extracts from the six macroalgae exerted excellent anti-browning effects on the apple slices.

Analysis of mineral elements contents in the MAA extracts from the six macroalgae

According to the standards for food antioxidants, the presence of heavy metals was not permitted. To develop the MAA extracts for use as food antioxidants, it is essential to ascertain the mineral content in the MAA extracts. From Fig. 8, the MAA extracts from *Undaria pinnatifida* have the highest content of Ca, K content was highest in the MAA extracts from *Sargassum fusiforme*,

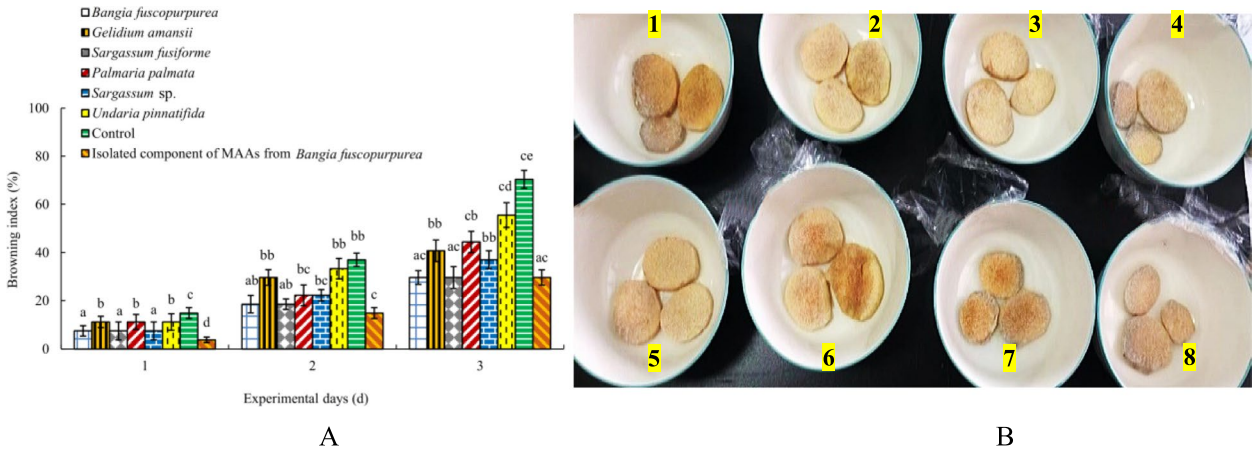

Fig. 6 Anti-browning effects of the MAA extracts from marine macroalgae on fresh yam slices. **A** Browning index of fresh yam slices; **B** Appearance of fresh yam slices. The numbers 1, 2, 3, 4, 5, 6, 7 and 8 represent the control, *Bangia fuscopurpurea*, isolated components of MAAs, *Palmaria palmata*, *Sargassum fusiforme*, *Gelidium amansii*, *Undaria pinnatifida* and *Sargassum* sp., respectively (Different letters indicate significant differences in the column chart)

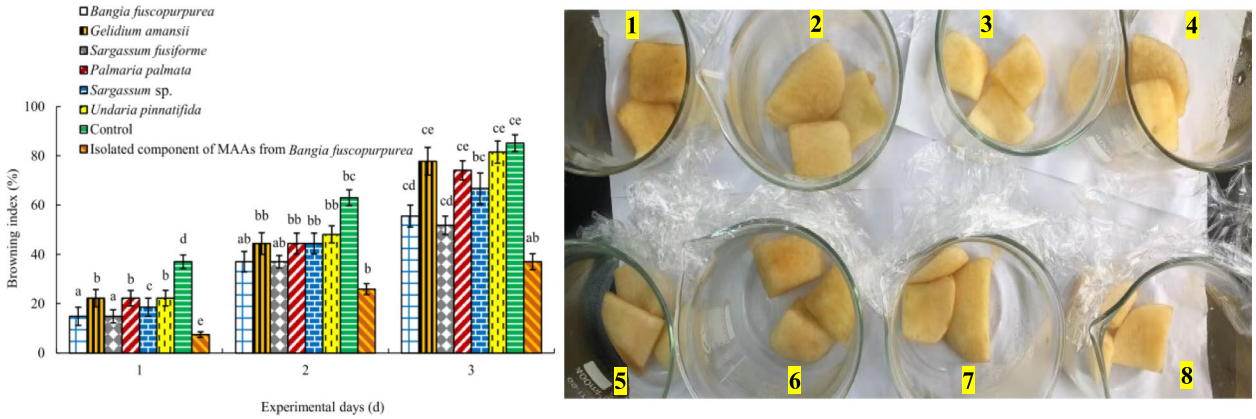

Fig. 7 Anti-browning effects of the MAA extracts from marine macroalgae on fresh apple slices. **A** Browning index of fresh apple slices; **B** Appearance of fresh apple slices. The numbers 1, 2, 3, 4, 5, 6, 7 and 8 represent the control, *Bangia fuscopurpurea*, isolated components of MAAs, *Palmaria palmata*, *Sargassum fusiforme*, *Gelidium amansii*, *Undaria pinnatifida* and *Sargassum* sp., respectively (Different letters indicate significant differences in the column chart)

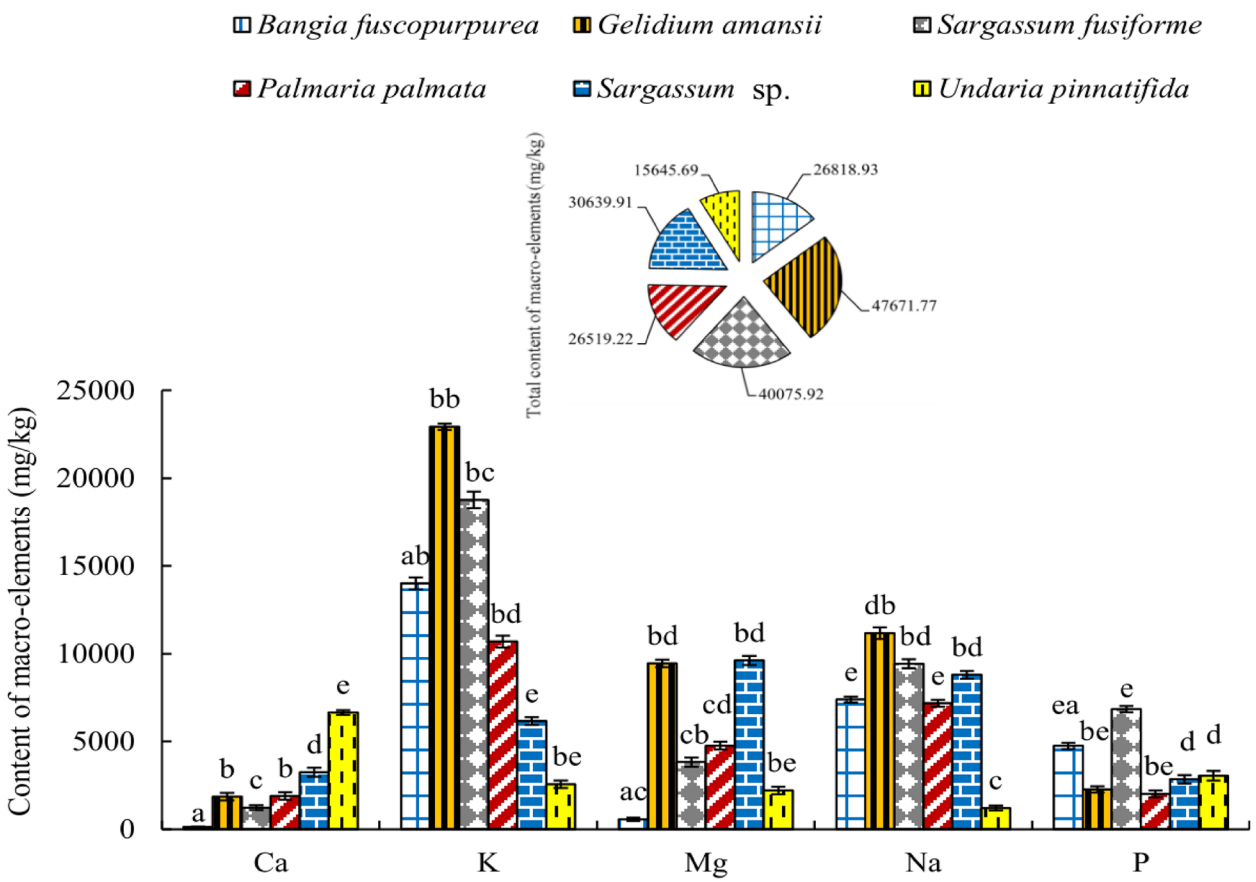

Fig. 8 The contents of macro-elements in the MAA extracts from the six marine macroalgae (The pie chart represents the total contents of macro-elements in the MAA extracts from each marine macroalgae. Different letters indicate significant differences in the column chart)

Mg has higher content in the MAA extracts from *Gelidium amansii* and *Sargassum* sp., the contents of Na and P were highest in the MAA extracts from *Gelidium amansii* and *Sargassum fusiforme*, respectively. These macro-elements were essential to the human body [48]. The principal elements present in the MAA extracts can effectively meet the human body's requirements for such major elements. The sequence of the total contents of the principal elements in the MAA extracts was as follows: *Gelidium amansii* > *Sargassum fusiforme* > *Sargassum* sp. > *Bangia fuscopurpurea* > *Palmaria palmata* > *Undaria pinnatifida*.

In Fig. 9, the trace elements in the MAA extracts from the six macroalgae were all essential trace elements for the human body [3, 40, 48]. The human body needed to consume 2 ~ 3 mg of Cu, 10 ~ 20 mg of Fe, 10 ~ 15 mg of Zn, and 2.5 ~ 5 mg of Mo daily. The MAA extracts from the six macroalgae were free from harmful heavy metal elements Pb, Cd, As and Hg to human health, and the content of Cu was much lower than 20 mg/kg specified in the Chinese Pharmacopoeia (2020 Edition), especially the content of Cu in the MAA extracts from five macroalgae (*Bangia fuscopurpurea*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp. and *Undaria pinnatifida*) was trace and beneficial to human health [3, 40]. The total contents of trace elements in the MAA extracts from the six macroalgae followed the order: *Undaria pinnatifida* > *Gelidium amansii* > *Sargassum* sp. > *Sargassum fusiforme* > *Palmaria palmata* > *Palmaria palmata*.

Effect of common food additives on the stability of the MAA extracts from the six macroalgae

The above-mentioned series of experiments validated that the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* had high yields, along with excellent antioxidant and anti-browning activities in vitro. To further explore the potential for applying these MAA extracts in food industry, an in-depth analysis was carried out on the impacts of five commonly-used food additives on the stability of the MAA extracts from these two macroalgae.

For the control groups, the MAA extracts from *Bangia fuscopurpurea* demonstrated remarkable stability. Throughout the entire experiment, whether at 25 °C or 48 °C, the absorbance did not experience a significant

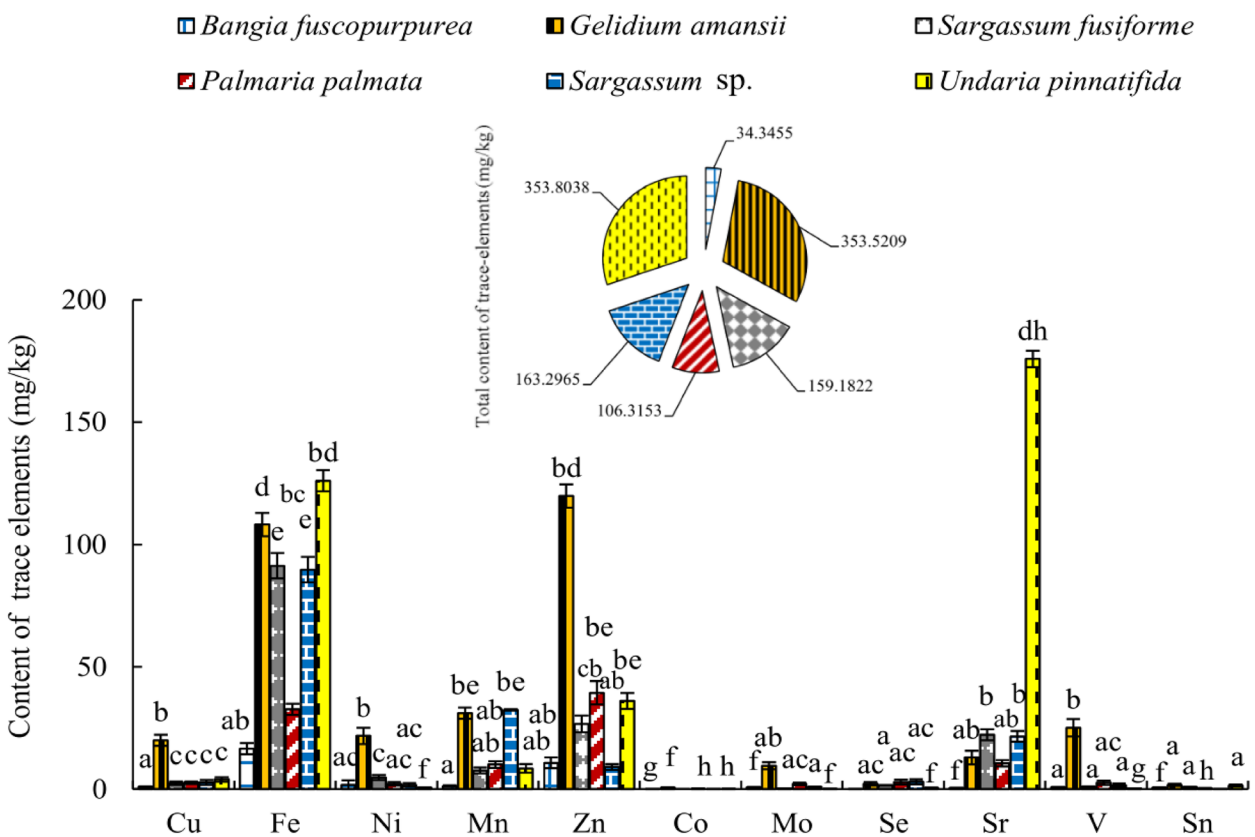

Fig. 9 The contents of trace-elements in the MAA extracts from the six marine macroalgae (The pie chart represents the total contents of trace-elements in the MAA extracts from each marine macroalgae. Different letters indicate significant differences in the column chart)

($P > 0.05$) decline. In contrast, the stability of the MAA extracts from *Sargassum fusiforme* was evidently influenced by temperature. Specifically, their stability was superior at 25 °C compared to 48 °C. From day 1 to day 28, the absorbance of the MAA extracts from *Sargassum fusiforme* at 25 °C remained relatively stable, showing no marked ($P > 0.05$) changes. However, at 48 °C, the absorbance started to decrease substantially ($P < 0.05$) from day 21 (Fig. 10). Figure 10 clearly illustrated the effects of five food additives on the stability of the MAA extracts. Among the five food additives, citric acid had a highly detrimental effect on the stability of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*. At both 25 °C and 48 °C, the absorbance of the MAA extracts from *Bangia fuscopurpurea* started to decline significantly ($P < 0.05$) from day 25. By the end of the experiment, compared with the control groups, the absorbance had decreased by more than 23%; the absorbance of the MAA extracts from *Sargassum fusiforme* started to decline significantly on the 20th and 11th days respectively. By the end of the experiment, compared with the control groups, the reduction was more than 40%. Sodium glutamate was found to have an adverse impact solely on the stability of the MAA extracts from *Bangia fuscopurpurea*. At both 25 °C and 48 °C, the absorbance of the experimental groups with the addition of sodium glutamate was significantly ($P < 0.05$) lower than that of the control groups. In contrast, the

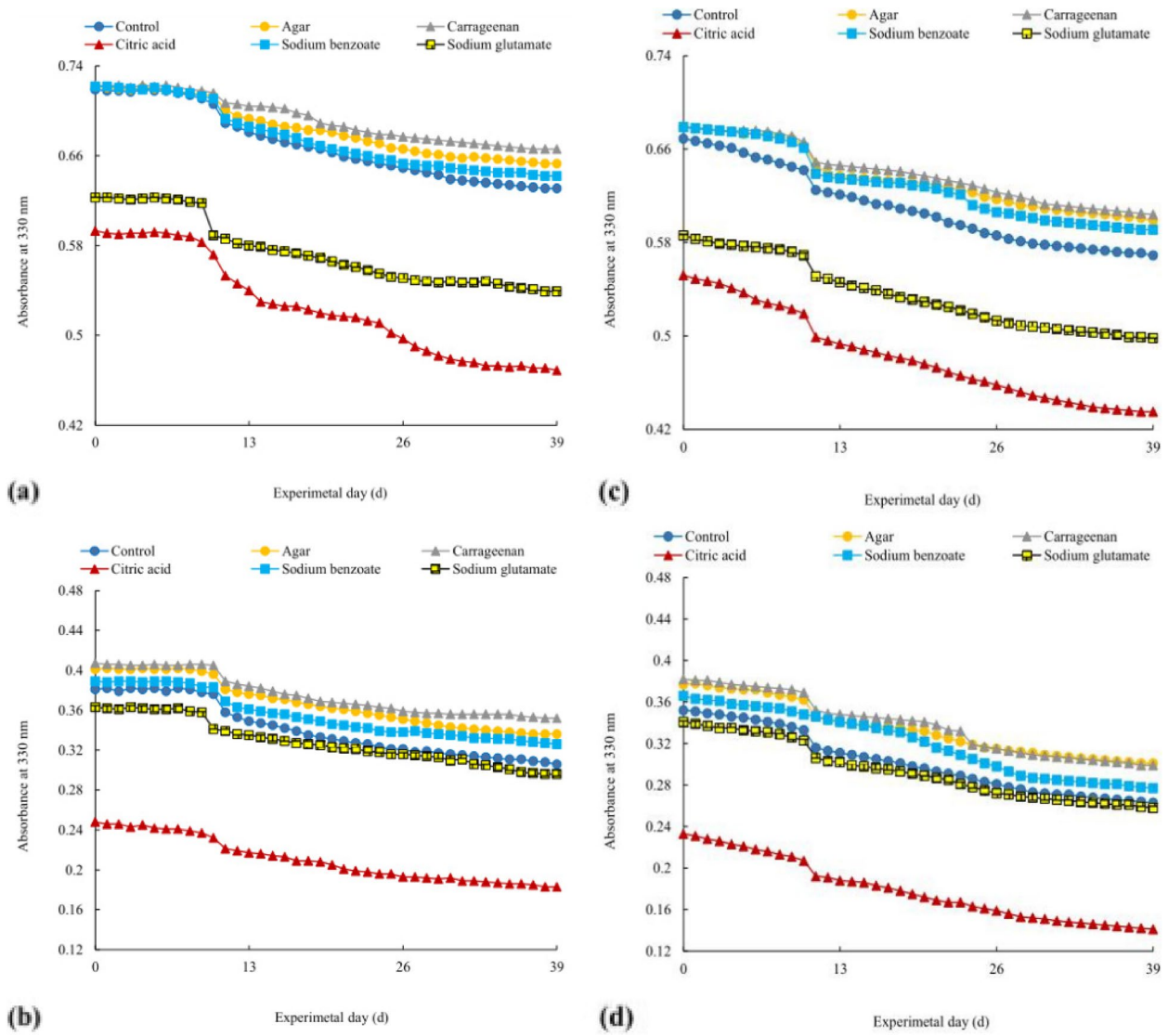

Fig. 10 Effects of five commonly used food additives on the stability of the MAA extracts from *Bangia fuscopurpurea* (**a**, **c**) and *Sargassum fusiforme* (**b**, **d**). "a" and "b" represented experiments were carried out at 25 ℃; "c" and "d" experiments were carried out at 48 ℃

other three food additives-agar, carrageenan, and sodium benzoate-not only exerted no negative effects on the stability of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*, but also enhanced their stability to some degree. Among these three food additives, sodium benzoate functions as an anti-bacterial and anti-corrosion agent under acidic conditions, particularly in strongly acidic environments [2]. However, all MAA extracts maintained stability under weakly acidic, neutral, and alkaline conditions [17]. As a result, the combination of sodium benzoate and the MAA extracts from two macroalgae (*Bangia fuscopurpurea* and *Sargassum fusiforme*) was not a suitable option.

Based on the above-mentioned analysis, it can be concluded that agar and carrageenan were appropriate for being mixed with the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*.

Discussion

With the robust development of the blue economy, the health industry that utilizes marine resources as raw materials is witnessing a remarkable upsurge. Marine macroalgae possess outstanding physiological activities and confer significant health benefits, thus emerging as an ideal source for food and natural products [7]. They have even been lauded as the "super plant" pivotal for sustainable human development. Marine macroalgae harbor a diverse array of physiologically active substances, among which the research on antioxidant active substances has garnered heightened attention [47]. Up to the present moment, the active substances in marine macroalgae exhibiting antioxidant activities predominantly comprise polysaccharides [60], phenols [61], pigments [22], and MAAs [53]. As far as we are aware, only a limited number of studies have been dedicated to exploring the antioxidant properties of MAAs in marine macroalgae. In China over the past two decades, the research focus on antioxidant active substances of marine macroalgae has mainly centered around polysaccharides [54], phenols [57], and pigments [55] (Fig. 1). Correspondingly, the research efforts devoted to investigating the antioxidant activity of MAAs have also been relatively scarce [39].

MAAs constitute a class of bio-active substances that are widely distributed in marine macroalgae. Previous investigations have indicated that over 570 species of marine macroalgae harbor MAAs [51]. Regrettably, the preparation and application of MAAs from only a small fraction of marine macroalgae have been delved into [39, 44, 50, 58]. MAAs possess notable advantages such as non-toxicity, excellent light and thermal stability [24, 56], as well as resistance to ultraviolet radiation [53], which render them optimal candidates for the development of novel antioxidants. However, despite the fact that porphyra-334 and shinorine have been verified to exhibit outstanding antioxidant activities [17, 24], there remains a lack of reports on MAAs antioxidants, both in the domestic and international arenas. Marine macroalgae typically contain a multiplicity of MAAs, which share similar structures, thereby posing difficulties in their isolation [51]. Over the past five years, research efforts focused on the isolation and purification of MAAs from marine macroalgae have played a pivotal role in facilitating their application [50, 58].

Bangia fuscopurpurea, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp., and *Undaria pinnatifida* were common marine macroalgae in China. This paper aims to explore whether their MAA extracts could be developed into novel antioxidants. In our preliminary study [50], the extraction procedures and compositional profiles of the MAA extracts from *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Sargassum* sp. and *Undaria pinnatifida* have been investigated. On this foundation, this paper focused on the optimal preparation of the MAA extracts from these five macroalgae species, namely *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Sargassum* sp., and *Undaria pinnatifida*, as well as *Palmaria palmata* (Figs. 2 and 3). By implementing the pre-removal of pigments and augmenting the ethanol precipitation times, the MAA extracts with a yield approximating that reported in previous research [50] and exhibiting lighter color characteristics were successfully obtained. Moreover, the compositional makeup of the MAA extracts (Table 2) was found to be consistent with the prior findings [50]. Notably, neither the pigment removal nor the increased ethanol precipitation times led to a significant depletion of the MAA extracts from the six macroalgae, signifying that this optimization process was conducive to the subsequent application of these extracts. It is particularly noteworthy that this article represents the first report of the presence of palythenic acid in *Palmaria palmata* (Table 2). Traditionally, this particular MAA has been predominantly detected in phytoplankton and microalgae [37], and to date, it has only been identified in the red macroalgae *Solieria chordalis* (Rhodophyta) [10], *Bangia fuscopurpurea*, *Gracilariopsis longissima* (formerly *Gracilaria confervoides*) (Rhodophyta) [50], as well as brown macroalgae *Sargassum fusiforme*, *Saccharina japonica* (formerly *Laminaria japonica*), *Sargassum* sp. and *Undaria pinnatifida* (Phaeophyceae) [56].

All MAA extracts derived from the six macroalgae exhibited a certain degree of total antioxidant capacity

as well as the capacity to scavenge superoxide anions (Figs. 4 and 5). Among them, the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* manifested the most prominent antioxidant capacities in vitro. Their EC_{50} values for scavenging superoxide anions were approximately 30.0 mg/mL, which surpassed those of the MAA extracts from *Gloiopeltis* sp. [56] and fucoidan of *Sargassum fusiforme* [36] in terms of superoxide anion scavenging. This phenomenon might be attributed to the relatively low contents of antioxidant-active MAAs within the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*. It is anticipated that, after subsequent purification procedures, the in vitro antioxidant capacity of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* could be substantially enhanced. A relevant report indicated that, when determined by the DPPH free-radical quenching assay, the antioxidant activities of two specific MAAs, namely porphyra-334 and shinorine, were comparatively low in relation to that of Vc (ascorbic acid) in vitro [23]. However, their capacity to quench free radicals through hydrogen atom transfer was remarkably significant. Given that *Bangia fuscopurpurea*, *Sargassum fusiforme*, *Sargassum* sp., and *Undaria pinnatifida* also contained porphyra-334 and shinorine (Table 2), the MAA extracts from these four marine macroalgae hold considerable potential for development as antioxidants.

During the processes of cutting or other mechanical operations, fresh fruits and vegetables are prone to tissue browning, which subsequently leads to consumer rejection. Consequently, the control of browning has emerged as one of the most crucial aspects in guaranteeing the quality of fresh-cut fruits and vegetables. Natural product extracts have demonstrated potential as effective anti-browning agents in the context of fresh-cut fruits and vegetables [5, 6, 46]. However, there is a dearth of research exploring the application of natural product extracts derived from marine macroalgae [5, 9]. In the current study, as illustrated in Figs. 6 and 7, it was evident that the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* exhibited favorable anti-browning effects on slices of both fresh-cut vegetables and fruits. These effects were congruent with their total antioxidant capacity and superoxide anion scavenging capacity, as depicted in Figs. 4 and 5. Notably, the isolated component from the MAA extracts from *Bangia fuscopurpurea* manifested even more pronounced anti-browning effects on yam and apple slices. In comparison to green tea extract [29], the anti-browning efficacy of this isolated component was superior, capable of inhibiting browning in over 52% of yam and apple slices after 48 h. A prior study reported that the MAA extracts from *Bangia fuscopurpurea* possess excellent moisture-adsorption and retention capabilities [26], suggesting that their anti-browning effect might be correlated with this property. In subsequent research endeavors, it is imperative to investigate the underlying mechanism of the anti-browning action of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*. At present, our focus has been primarily confined to the browning index of yam and apple slices. In future follow-up studies, a more comprehensive evaluation will be conducted, encompassing parameters such as weight loss, texture, color, and visual quality score.

Research findings have indicated that mineral elements play a crucial role in the physiological metabolism processes of the human body. Elements such as calcium (Ca) and iron (Fe), among others, are intimately associated with the body's immunity, disease resistance, as well as growth and development [14, 28]. The MAAs sourced from the six macroalgae encompassed nearly all the essential mineral elements required by the human body (Figs. 8 and 9). The concentrations of major elements like Ca and trace elements such as Fe were adequate to meet the body's mineral requirements. Notably, these MAA extracts contained no harmful heavy metals like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), which are strictly regulated with limited content thresholds in the Chinese Pharmacopoeia (2020 edition). Additionally, the copper (Cu) content in the MAA extracts from the six macroalgae was extremely low. In general, the MAA extracts from the six marine macroalgae exhibited outstanding safety characteristics and provided beneficial impacts on the human body.

Agar, carrageenan, citric acid, sodium benzoate and sodium glutamate are widely-used common food additives. When they are used in conjunction with the MAA extracts from marine macroalgae, it is likely to have an impact on the stability of these extracts. To date, no comparable studies have come to light. This paper was the first time to clarify the promoting effect of agar, carrageenan and sodium benzoate on the stability of the MAA extracts, and the negative effect of citric acid and sodium glutamate on the stability of the MAA extracts (Fig. 10). Sodium benzoate was used under acidic conditions, and MAAs exhibited relatively high stability within the pH range of 6 to 12 [24], so the MAA extracts were not suitable for mixed with sodium benzoate; Agar and carrageenan can be used together with the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme*.

These results showed the potential of the MAA extracts from *Bangia fuscopurpurea* and *Sargassum fusiforme* as the food antioxidants and mineral element supplements. To the best of our knowledge, this article represents the first report concerning the contents of both macro- and trace-elements, as well as the

impacts of food additives on the stability of the MAA extracts derived from marine macroalgae. This research endeavor has furnished a novel perspective for the practical application of MAAs sourced from marine macroalgae.

Conclusions

As a principal source of functional ingredients, the research and development of active extracts from marine macroalgae has emerged as one of the focal points in the health industry. One of the primary factors impeding the widespread utilization of active substances derived from marine macroalgae lies in the deficiency of key technologies for their extraction and preparation [13]. In the preparation process of MAAs, the pre-removal of pigments and an increase in the number of alcohol precipitation steps significantly augmented the yield of the MAA extracts from *Bangia fuscopurpurea* and *Gelidium amansii*. Moreover, these measures effectively enhanced the color quality of the MAA extracts from *Bangia fuscopurpurea*, *Gelidium amansii*, *Sargassum fusiforme*, *Palmaria palmata*, *Sargassum* sp., and *Undaria pinnatifida*. The research findings presented here provide a highly valuable reference for the isolation and purification of MAAs from marine macroalgae. The insights gleaned from this study can make a meaningful contribution to the extraction of high-value products from marine macroalgae.

The MAA extracts from the six marine macroalgae demonstrated specific antioxidant activities in vitro and displayed excellent anti-browning capabilities. Though their antioxidant activities in vitro were not outstanding so far, antioxidant activities of the MAA extracts are likely to be increased after further isolation and purification. The MAA extracts from the six macroalgae exhibited two indisputable advantages: they demonstrated remarkable anti-browning capabilities and contained beneficial macro- and trace-elements essential for the human body. Additionally, a third advantage emerged: two common food additives proved beneficial for enhancing the stability of the MAA extracts from *Sargassum fusiforme* and *Bangia fuscopurpurea*. These three advantages are of particular significance for the development of the MAA extracts, especially those intended to serve as food antioxidants or mineral element supplements. In future research, more in-depth investigations will be conducted to explore the wide-ranging applications of the MAA extracts from *Sargassum fusiforme* and *Bangia fuscopurpurea* in health foods.

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Authors' contributions
Ying-ying Sun conceived and designed the study. Yuxiang Li, Jingwen Wang, Siyu Wang, Xiujie Jiang and Sun Shi performed the experiments. Ying-ying Sun wrote the paper. Yuxiang Li, Jingwen Wang and Siyu Wang reviewed and edited the manuscript. All authors read and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Aabel LS, Hakme E, Wirenfeldt CB, Feyissa AH. Seaweed extract as an effective dough conditioner in bread manufacturing. LWT. 2024;197:115881.
2. Ahmed Firas A, Hussein RM. Evaluation of the antibacterial and antibiofilm activities of sodium benzoate against the soft rot disease of tomato in Iraq. Arch Phytopath Plant. 2022;55(5):636–51.
3. Aknaf A, Ahsan U, Baghour M, Rahhou A, Imperl J, Petrova P, Kolar M. Minerals and trace element compositions of some seaweeds from the Marchica lagoon, North-East Mediterranean coast of Morocco. Mar Pollut Bull. 2024;206:116795–116795.
4. Ali HM, El-Gizawy AM, El-Bassiouny REI, Saleh MA. The role of various amino acids in enzymatic browning process in potato tubers, and identifying the browning products. Food Chem. 2016;192:879–85.
5. Ana A, Tiago S, Sara CN, Geoffrey M, Marco FLL, Keshavan N, Susana FJS. An insight into the mechanisms underpinning the anti-browning effect of *Codium tomentosum* on fresh-cut apples. Food Res Int. 2022;161:111884–111884.
6. Androudis E, Gerasopoulos A, Koukounaras A, Siomos AS, Gerasopoulos D. Water extracts of cruciferous vegetable seeds Inhibit enzymic browning of fresh-cut mid ribs of Romaine Lettuce. Horticulturae. 2024;10:500.
7. Arias A, Feijoo G, Moreira MT. Macroalgae biorefineries as a sustainable resource in the extraction of value-added compounds. Algal Res. 2023;69:102954.
8. Athukorala Y, Trang S, Kwok C, Yuan YV. Antiproliferative and antioxidant activities and mycosporine-Like amino acid profiles of wild-harvested and cultivated edible Canadian marine red macroalgae. Molecules. 2016;21(1):1–17.
9. Augusto A, Simões T, Pedrosa R, Silva SF. Evaluation of seaweed extracts functionality as post-harvest treatment for minimally processed Fuji apples. Innov Food Sci Emerg. 2016;33:589–95.
10. Bedoux G, Hardouin K, Marty C, Taupin L, Vandanjon L, Bourgougnon N. Chemical characterization and photoprotective activity measurement of extracts from the red macroalga *Solieria chordalis*. Bot Mar. 2014;57(4):291–301.

11. Bhandari RR, Sharma P. KPhotosynthetic and biochemical characterization of pigments and UV-absorbing compounds in *Phormidium tenue* due to UV-B radiation. J Appl Phycol. 2011;23:283–92.
12. Briani B, Sissini MN, Lucena LA, Batista MB, Costa lO, Nunes JMC. The influence of environmental features in the content of mycosporine-like amino acids in red marine algae along the Brazilian coast. J Phycol. 2018;54(3):380–439.
13. Bruna M, Fernando P, Isabel SP, Catarina GA. Biorefinery as a tool to obtain multiple seaweed extracts for cosmetic applications. J Appl Phycol. 2023;35(6):3041–55.
14. Chen YH. Determination and analysis of trace elements in Chinese herbal medicine. Studies of Trace Elements and Health. 2018;35(2):39-40 (in Chinese with English abstract) http://wyjk.cbpt.cnki.net/WKB3/WebPublication/wkTextContent.aspx?colType=4&yt=2018&st=02.
15. Chen JM, Huang XD, Cai JX, Gu YQ. Study on Antioxiant active fraction from a seaweed, Ishige okamurai. J Minnan Normal University (Nat Sci). 2016;2:73–9. https://doi.org/10.16007/j.cnki.issn2095-7122.2016.02.012. (in Chinese with English abstract).
16. Chrapusta E, Kaminski A, Duchnik K, Bober B, Adamski M, Bialczyk J. Mycosporine-like amino acids: potential health and beauty ingredients. Mar Drugs. 2017;15:326–55.
17. Chuang LF, Chou HN, Sung PJ. Porphyra-334 isolated from the marine algae Bangia atropurpurea: conformational performance for energy conversion. Mar Drugs. 2014;12:4732–40.
18. De la Coba F, Aguilera J, Figueroa FL, et al. Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. J Appl Phycol. 2009;21(2):161–9.
19. Díaz AC, Espino ML, Arzoz NS, Velurtas SM, Ponce NMA, Stortz CA. Free radical scavenging activity of extracts from seaweeds *Macrocystis pyrifera* and *Undaria pinnatifida*: applications as functional food in the diet of prawn, *Artemesia longinaris*. Lat Am J Aquat Res. 2017;45(1):104–12.
20. Dinesh S, Menon T, Hanna LE, Suresh V, Sathuvan M, Manikannan M. In vitro anti-HIV-1 activity of fucoidan from *Sargassum swartzii*. Int J Biol Macromol. 2016;82:83–8.
21. Dunlap WC, Yamamoto Y. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol B: Biochem Molecular Biol. 1995;112(1):105–14.
22. Foo SC, Yusoff FM, Ismail M, Basri M, Yau SK, Khong NMH, Chan KW, Ebrahimi M. Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. J Biotech. 2017;241(1):175–83.
23. Gacesa R, Lawrence KP, Georgakopoulos ND, Yabe K, Dunlap WC, Barlow DJ, Wells G, Young AR, Long PF. The mycosporine-like amino acids porphyra-334 and shinorine are antioxidants and direct antagonists of Keap1-Nrf2 binding. Biochimie. 2018;154:35–44.
24. Geraldes V, Pinto E. Mycosporine-like amino acids (MAAs): Biology, chemistry and identification features. Pharmaceuticals. 2021;14(1):63.
25. Guihéneuf F, Gietl A, Stengel DB. Temporal and spatial variability of mycosporine-like amino acids and pigments in three edible red seaweeds from western ireland. J Appl Phycol. 2018;30:2573–86.
26. Han X, Sun YY, Cheng TJ, Deng XQ, Hu ZJ, Wang H. Study on the moisture-absorption and moisture-retention properties of mycosporine-like amino acids extracts from four species of marine red macroalgae. Modern Chemical Research. 2022;17:31–4 (in Chinese with English abstract).
27. Hermund DB, Torsteinsen H, Vega J, Figueroa FL, Jacobsen C. Screening for new cosmeceuticals from brown algae *Fucus vesiculosus* with antioxidant and photo-protecting properties. Mar Drugs. 2022;20:687.
28. Jia YN, Guo WJ, Zhou Q, Xu JC. Reference intervals validation of serum potassium, sodium, chloride, total calcium, and phosphorus for healthy population in Changchun. Chin J Lab Diagn. 2018;22(7):1115–1117 (in Chinese with English abstract) https://zszd.chinajournal.net.cn/WKA/WebPublication/wkTextContent.aspx?colType=4&yt=2018&st=07.
29. Inga K, Anna G. Green tea extract as an anti-browning agent for cloudy apple juice. J Sci Food Agri. 2017;97(5):1420–6.
30. Julia V, Geniane S, Bruna RM, Carolina H, José BB, Félix LF. Mycosporine-like amino acids from red macroalgae: UV-photoprotectors with potential cosmeceutical applications. Appl Sci. 2021;11(11):5112–5112.
31. Karsten U, Sawall T, Hanelt D, Bischof K, Figueroa FL, Flores-Moya A, Wiencke C. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. Bot Mar. 1998;41:443–53.
32. Kim YR, Park SY, Kim JY. Therapeutic potential of seaweed extracts: In vitro and in vivo studies on alleviating inflammation and enhancing intestinal barrier function. Food Biosci. 2024;59:103774.
33. Kursar TA, Alberte RS. Photosynthetic unit organization in a red alga: relationships between light-harvesting pigments and reaction centers. Plant Physiol. 1983;72(2):409–14.
34. Lalegerie F, Lajili S, Bedoux G, Taupin L, Stiger-Pouvreau V, Connan S. Photo-protective compounds in red macroalgae from Brittany: considerable diversity in mycosporine-like amino acids (MAAs). Mar Environ Res. 2019;147:37–14748.
35. Li M. Study on preservation effect of bioligical preservative on olecranon peach and establishment of storage model. Master's thesis, Guangzhou: South China University of Technology. 2019 (in Chinese) https://doi.org/10.27151/d.cnki.ghnlu.2019.001695.
36. Liu X, Wang GH, Zhao FJ, Zhou X, Kusaikin M, Liu CH. Structural characterization and antioxidant activities of fucoidan from *Sargassum fusiforme*. Sci Technol Food Industry. 2019;40(3):79–84. https://doi.org/10.13386/j.issn1002-0306.2019.03.013. (in Chinese with English abstract).
37. Llewellyn CA, Airs RL. Distribution and abundance of MAAs in 33 species of microalgae across 13 classes. Mar Drugs. 2010;8(4):1273–91.
38. Mason DS, Sehafer F, Shiek JM, Dunlap WC. Ultraviolet radiation absorbing mycosporine-like amino acids (MAAs) are acquired from their diet by medaka fish (*Oryzias latipes*) but not by SKH hairless mice. Comp Biochem Physiol. 1998;120:587–98.
39. Niu MY. The study on the isolation, purification, identification and antioxidant activity of mycosporine-like amino acids (MAAs) in *Eucheuma*. Master's thesis, Qingdao: Ocean University of China. 2014. (in Chinese with English abstract).
40. Barandiaran LN, Taylor VF, Karagas MR. Exposure to iodine, essential and non-essential trace element through seaweed consumption in humans. Sci Rep. 2024;1:13698–13698.
41. Orfanoudaki M, Hartmann A, Karsten U, Ganzera M. Chemical profiling of mycosporine-like amino acids in twenty-three red algal species. J Phycol. 2019;55:393–403.
42. Orfanoudaki M, Hartmann A, Miladinovic H, Ngoc HN, Karsten U, Ganzera M. Bostrychines A-F, six novel mycosporine-like amino-acids and a novel betaine from the red alga *Bostrychia scorpioides*. Mar Drugs. 2019;17(6):356–71.
43. Ozkan A, Erdogan A, Sokmen M, Tugrulay S, Unal O. Antitumoral and antioxidant effect of essential oils and in vitro antioxidant properties of essential oils and aqueous extracts from *Salvia pisidica*. Biologia. 2010;65(6):990–6.
44. Pedrosa VMD, Sanches AG, da Silva MB, et al. Production of mycosporine-like amino acid (MAA)-loaded emulsions as chemical barriers to control sunscald in fruits and vegetables. J Sci Food Agric. 2021;1:1–12.
45. Pandey A, Pandey S, Rajneesh JP, Ahmed H, Singh V, Singh SP, Sinha RP. Mycosporine-like amino acids (MAAs) profile of two marine red macroalgae, *Gelidium* sp. and *Ceramium* sp. Int J Appl Sci Biotechnol. 2017;5(1):12–21.
46. Qiao LP, Liu YH, Deng XL, Yu WY, Wang YY, Li XK, Lu LF, Liu X. A new strategy for browning regulation: Flos Sophorae Immaturus extract and thermal treatment modulates nitric oxide and reactive oxygen species network in fresh-cut potatoes. J Food Sci. 2023;88(11):4574–90.
47. Rastogi RP, Madamwar D, Pandey A. Algal Green Chemistry. In: Sonani RR, Rastogi RP, Madamwar D, editors. Natural antioxidants from algae: a therapeutic perspective. Elsevier; 2017. p. 91–120.
48. Rupérez P. Mineral conent of edible marine seaweeds. Food Chem. 2002;79:23–6.
49. Sims D, Gamon J. Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices based on liquid water and chlorophyll absorption features. Remote Sens Environ. 2003;84(4):526–37.
50. Sun YY, Han X, Hu ZJ, Cheng TJ, Tang Q, Wang H, et al. Extraction, isolation and characterization of mycosporine-like amino acids from four species of red macroalgae. Mar Drugs. 2021;19:615.
51. Sun YY, Zhang NS, Zhou J, Dong SS, Zhang X, Guo L, et al. Distribution, contents, and types of mycosporine-like amino acids (MAAs) in marine macroalgae and a database for MAAs based on these characteristics. Mar Drugs. 2020;18(1):43–66.
52. Tófoli RJ, Ferreira AL, Núñez EGF, Haminiuk CWI, Branco CCZ, Branco lG. Effects of solvent extraction on phenolic concentration and antioxidant

capacity of the Oedogonium sp. (Chlorophyta) using a simplex-centroid mixture design. Acta Sci-Technol. 2023;45:1807–8664.
53. Urrea-Victoria V, Furlan CM, Santos DYAC, Chow F. Antioxidant potential of two Brazilian seaweeds in response to temperature: *Pyropia spiralis* (red alga) and *Sargassum stenophyllum* (brown alga). J Exp Mar Biol Ecol. 2022;549:151706.
54. Wang Y, Lou YJ, Gong FF, Chen SM, Zhang YQ, Liu YD, et al. Purification, antioxidant activity and structure of *Enteromorpha prolifera* polysaccharides. Food Ferment Industr. 2020;46(12):66–72. https://doi.org/10.13995/j.cnki.11-1802/ts.023124. (in Chinese with English abstract).
55. Wang XN, Wang MJ, Zhang J, Xu YJ, Zhao YP, Liu F, et al. Study on antioxidant activity of pigment in 8 kinds of seaweed and three *Laminaria japonica*. Sci Technol Food Industry. 2018;39(3):65–70. https://doi.org/10.13386/j.issn1002-0306.2018.03.014. (in Chinese with English abstract).
56. Wu YY, Zhang W, Li LH, Wang JX, Hu X, Yang SL. Antioxidant capacity and category analysis of MAAs in *Gloiopeltis*. J Chinese Institute Food Sci Technol. 2018;18(5):264–72. https://doi.org/10.16429/j.1009-7848.2018.05.032. (in Chinese with English abstract).
57. Yang XQ, Lu HY, Li YP, Liu Y. Antioxidative Activities of phlorotannins with different molecular weight extracted from *Sargassum fusiforme*. Marine Sciences. 2013;37(4):47–51 (in Chinese with English abstract) http://qdhys.ijournal.cn/hykx/ch/reader/issue_list.aspx?year_id=2013&quarter_id=4.
58. Yamamoto R, Toriumi S, Kawagoe C, Saburi W, Kishimura H, Kumagai Y. Extraction and antioxidant capacity of mycosporine-like amino acids from red algae in Japan. Biosci Biotech Bioch. 2024;88(7):830–8.
59. Yvonne VY, Westcott ND, Hu C, Kitts DD. Mycosporine-like amino acid composition of the edible red alga, *Palmaria palmata* (dulse) harvested from the west and east coasts of Grand Manan Island, New Brunswick. Food Chem. 2009;112:321–8.
60. Zhao Y, Han C, Wu YYD, Sun QC, Ma M, Xie Z, Pei HY. Extraction, structural characterization, and antioxidant activity of polysaccharides from three microalgae. Sci Total Environ. 2024;931:172567–172567.
61. Zheng HL, Du HR, Ye EJ, Xu XY, Wang XR, Jiang X, Guo L. Optimized extraction of polyphenols with antioxidant and anti-biofilm activities and LC-MS/MS-based characterization of phlorotannins from *Sargassum muticum*. LWT. 2024;198:116069.

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