Extraction and antioxidant capacity of mycosporine-like amino acids from red algae in Japan

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ABSTRACT

Mycosporine-like amino acids (MAAs) are the natural UV absorbing compounds with antioxidant activity found in microalgae and macroalgae. We collected red algae *Asparagopsis taxiformis*, *Meristotheca japonica*, and *Polysiphonia senticulosa* from Nagasaki, where UV radiation is more intense than in Hokkaido, and investigated the effect of UV radiation on MAAs content. It was suggested that *A. taxiformis* and *M. japonica* contained shinorine and palythine, while UV absorbing compound in *P. senticulosa* could not be identified. The amounts of these MAAs were lower compared to those from Hokkaido. Despite an increase in UV radiation in both region from February to April, MAAs contents of red algae from Nagasaki slightly decreased, while that from Hokkaido significantly decreased. This difference was suggested the amount of inorganic nitrogen in the ocean. Antioxidant activity of MAAs increased under alkaline conditions. The extract containing MAAs from *P. senticulosa* showed the highest antioxidant activity among four red algae.

KEYWORDS: red alga, mycosporine-like amino acids, antioxidant capacity

Introduction

Mycosporine-like amino acids (MAAs) are unique UV protection compounds among marine organisms such as seaweed, cyanobacteria, phytoplankton, and marine animals (Jain *et al*. 2017; Karentz *et al*. 1991; Sinha, Singh and Häder 2007). Their UV absorption maxima range from 310 to 360 nm (Chrapusta *et al*. 2017; Geraldes and Pinto 2021). Red algae are main MAAs producers in the ocean. MAAs were detected in a total of 468 macroalgae strains between 1990 and 2019 (Sun *et al*. 2020), and 22 types of MAAs have been identified. Cyanobacteria contain 1-2 types of MAAs, commonly mycosporine-glycine (MG) and shinorine (Jain *et al*. 2017). Red algae often possess three or more types of MAAs, including shinorine, porphyra-334, MG, asterina-330, and palythine (Figure 1 and Figure S1). Some of them also contain trace amount of MAAs such as aplysiapalythine A and B, mycosporine-2-glycine, palythene, palythinol, and usujirene (Parailloux *et al*. 2020); while, many cyanobacteria contain these MAAs as major components (Kedar, Kashman and Oren 2002). Some MAAs are hydrolyzed to form simple structured palythine. Therefore, the accumulation of palythine suggests some indicator of stress in algae from environmental changes (Carreto and Carignan 2011).

MAA exhibits high water solubility, high stability, low toxicity, and antioxidant activity (Carreto and Carignan 2011; Torres *et al*. 2018). These diverse functions of MAA are useful as promising natural products in the cosmetic and biotechnology industries. Products containing MAAs from macroalga *Porphyra umbilicalis* are available. However, there are only a few sunscreen products commercially available as cosmetic ingredients (de la Coba *et al.* 2019; Sen and Mallick 2021; Singh *et al.* 2021). This limited usage is due to the small amount of MAAs contained in red algae (Briani *et al*. 2018; Jofre *et al*. 2020). Therefore, it is important to explore species with high MAAs content.

Antioxidant activity is one of the functions in MAAs (Browne *et al*. 2023; Figueroa 2021; Wada, Sakamoto and Matsugo 2015). Many cosmetics contain antioxidants because UV rays generate reactive oxygen species (ROS), contributing to the sings of skin aging (Kusumawati and Indrayanto 2013; Lupo 2001; Silva *et al*. 2019). The strength of antioxidant activity differs among MAAs. MG has particularly strong antioxidant activity (Dunlap and Yamamoto 1995; Suh *et al*. 2014). The IC_{50} value for antioxidant activity of MG in the ABTS radical scavenging assay was 3 μM, which was stronger compared to porphyra-334 and shinorine (de la Coba *et al*. 2009). Recent studies have suggested that the antioxidant activity of MAAs is influenced by pH condition. Palythine and porphyra-334 showed low activity at acidic conditions but showed stronger antioxidant activity under alkaline conditions (Torres *et al*. 2018; Nishida *et al*. 2020). Thus, some studies of antioxidant activity of MAAs have failed to compare properly due to not considering or lacking information on pH conditions. It has report that heated-MAAs change their structures, resulting in compounds with strong antioxidant active in various pH conditions (Nishida *et al*. 2022; Yoshiki *et al*. 2009). The accumulation of knowledge for antioxidant activity of MAAs is valuable for promoting the use of MAA from red algae.

In our previous studies, we investigated the amount of MAAs in red alga dulse (*Devaleraea inkyuleei*) (Nishida *et al*. 2020; Nishida *et al*. 2021; Yamamoto *et al*. 2023a; Yamamoto *et al*. 2023b). Red algae are also recognized as a valuable source of bioactive compounds (Holdt and Kraan 2011; Stengel, Connan and Popper 2011) such as proteins, polysaccharides, pigments, polyunsaturated fatty acids (Carpena *et al*. 2022; Gressler *et al.* 2010; Ruperez 2002). For instance, the dulse protein and phycoerythrin (PE) and their peptides showed angiotensin I-converting enzyme inhibitory activity (Fitzgerald *et al*. 2012; Furuta *et al*. 2016; Kitade *et al*. 2018; Miyabe *et al*. 2017). The chromophores from PE showed antioxidant capacity (Sato *et al*. 2019). Furthermore, dulse contains xylan as cell walls, and the hydrolysis product, β-(1→3)-xylosyl-β-(1→4)-xylobiose, exhibited prebiotic effects on intestinal bacteria (Fujii *et al*. 2021; Kobayashi *et al*. 2020; Yamamoto *et al*. 2019). Several studies have reported the relationship between MAAs content in red algae and environmental factors such as UV radiation levels and ocean nitrogen concentrations (Abraham *et al*. 2021; Hylander 2020; Singh *et al*. 2023). We have studied the relationship using red alga sample in Hokkaido (Yamamoto *et al*. 2023b). However, the studies on MAAs from red algae in other parts of Japan remains limited. Therefore, we have employed samples from Nagasaki Prefecture for the better understanding of the relationship.

In this study, we determined the extraction conditions to evaluate the MAAs content of red algae collected in Nagasaki Prefecture, Japan, and then investigated the monthly changes (February and April 2023). Finally, we evaluated the effect of pH on the antioxidant capacity of MAA containing fractions.

Materials and methods

Materials

Dulse (*Devaleraea inkyuleei*) was collected at a depth of one meter in Usujiri, Hakodate, Japan, in February 2023. Red algae (*Asparagopsis taxiformis, Meristotheca japonica*, and *Polysiphonia senticulosa*) were collected at a depth of five meters in Mushima, Nagasaki Prefecture, Japan in February and April 2023. Thalli were rinsed with tap water to remove sea salt and impurity. Samples were lyophilized and ground into a fine powder by using a Wonder Blender WB-1 (Osaka Chemical Co., Osaka, Japan).

Preparation of extraction containing MAAs

The extraction of MAAs from red algae was performed according to previous methods (Yamamoto *et al*. 2023a). The one gram of powdered sample was suspended in 40 volumes of 25% ethanol and extracted at 4 °C for 24 h. Then, the extracts were collected by centrifugation at 4 °C, 27,200 × g for 10 min. The supernatant was evaporated, dissolved in water, and again centrifuged. The supernatant was lyophilized. The resulting solid samples were designated as extract containing

MAAs and used for experimental purposes.

HPLC analysis of MAAs

After the extraction process, the extract containing MAAs sample was dissolved in water containing 0.1% formic acid (FA). The sample was filtrated via Millex-GV (pore size, 0.22 μm) (Merck Millipore, Billerica, MA, USA). The filtrated MAAs sample was isolated by reversed-phase HPLC (Shimadzu, Kyoto, Japan) with a Mightysil RP-18GP aqua column (5 μm, 10 × 250 mm) (Kanto Kagaku, Tokyo, Japan). The column oven was set at 40 °C, and the detection wavelength was set at 330 nm (SPD-20A, Shimadzu, Kyoto, Japan). The sample was eluted with an isocratic elution of pure water containing 0.1% FA for 7 min, and a linear gradient of acetonitrile (0-70%) containing 0.1% FA for 13 min, at a flow rate of 4.73 mL/min. MAAs content was expressed in terms of micro-molecules per gram of dry dulse powder (μmol g^{-1} DW (dry weight)). The maximum absorption wavelength (λ_{max}) of each MAA was identified using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) after the separation by HPLC. A standard curve for each MAA was constructed using HPLC based on the peak area and the extinction coefficient of each MAA employing Lambert–Beer law: MAAs were extracted from dulse and separated by HPLC. Then, each MAA was lyophilized, dissolved in water, and its absorbance was measured using a spectrophotometer. The concentration of each MAA was determined by using the molar extinction coefficient (Nishida *et al*. 2020). Subsequently, a standard curve for each MAA was obtained using the concentration and peak area of HPLC.

LC-MS analysis of MAAs

MAAs structure was determined by the liquid chromatography-mass spectrometry (LC-MS) (Shimadzu, Kyoto, Japan). The MAAs were dissolved in HPLC grade water containing 0.1% FA. The sample was subjected to sequential filtration via Millex-GV (pore size, 0.22 μm) (Merck Millipore, Billerica, MA, USA) and Millex-LG (pore size, 0.20 μm) (Merck Millipore). The filtrated MAA solution was analyzed using LCMS-2020 (Shimadzu, Kyoto, Japan) with a Mightysil PR-18GP aqua column (5 μm, 4.6 × 250 mm) (Kanto Kagaku, Tokyo, Japan) and eluted with an isocratic solvent of 0.1% FA at flow rate of 1.00 mL/min, with the column oven temperature at 40 °C and the detection of a wavelength at 330 nm. The mass spectral range was recorded from 200 to 400 *m/z* in positive mode.

Abiotic data in Nagasaki Prefecture

Monthly means of the daily maximum ultraviolet index (UVI) was obtained from the Japan Meteorological Agency (JMA: https://www.data.jma.go.jp/gmd/env/uvhp/info_uv.html, accessed on 5 December 2023). The erythemal UV intensity (mW/m^2) was calculated by multiplying the UVI by a factor of 25. Data on near surface chlorophyll concentration (mg/m^3) were obtained from NASA's

Ocean Color WEB (https://oceancolor.gsfc.nasa.gov, accessed on 5 December 2023).

Crude protein and phycoerythrin (PE) contents

The extraction of crude protein from red algae (*A. taxiformis, M. japonica*, and *P. senticulosa*) was performed according to our previous research (Yamamoto *et al*. 2023a; Yamamoto *et al*. 2023b). One gram of fine powder was dissolved in 20 mL water and extracted at 4 °C for 12 h. After centrifugation at 12,000 × *g* for 5 min, the supernatant was dialyzed against water at 4 °C and lyophilized. For each sample, PE, was prepared from the fine powders. A 10 mg amount of powder was dissolved in 1 mL distilled water and extracted at 4 °C for 12 h. After centrifugation at 12,000 × *g* for 5 min, the spectra of the supernatant were measured using a UV–visible spectrophotometer. The amount of PE was determined via the following equation (Beer 1985): PE (mg/mL) = $[(A_{564} - A_{592}) - (A_{455} - A_{592}) \times 0.2] \times 0.12$.

DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was performed according to the method of a previous study (Cheewinthamrongrod *et al*. 2016) with some modifications. Briefly, 0.5 mL of each concentration of the extract containing MAAs or control (distilled water) was mixed with 0.5 mL of 0.2 M phosphate buffer (pH 7.4), and then added 0.75 mL ethanol containing 0.5 mM DPPH. The mixtures were incubated for 20 min at room temperature under darkness. After the reaction, the solutions were centrifuged at 4 °C, 2,000 × g for 10 min, and the absorbance of each solution at 517 nm was measured using a UV–visible spectrophotometer.

The percentage of radical inhibition was calculated using the equation [1 − (Es − Esb) / (Ec − Ecb)] ×100), where Es is the absorbance of the sample with the DPPH reagent, Esb is the absorbance of sample with the phosphate buffer, Ec is the absorbance of distilled water with the DPPH reagent and Ecb is the absorbance of distilled water with the phosphate buffer. Ascorbic acid was used as a positive control. The IC_{50} was defined as the concentration of the sample required to consume 50% of DPPH free radicals.

ABTS radical scavenging assay

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay was carried out according to a conventional method. (Binsan *et al*. 2008) with some modifications. The ABTS radical cation was generated by mixing with equal volumes of 14.8 mM ABTS and 5.2 mM potassium persulphate and incubated at room temperature for 12 h under darkness before use. An ABTS reagent with an absorbance of 1.00 ± 0.02 at 734 nm was prepared by the dilution of ABTS working solution with 0.2 M phosphate buffer (pH 5.8, 6.6, 7.4, and 8.0). The ABTS radical scavenging assay was performed as follows: 50 µL of each concentration of the extract containing

MAAs sample or distilled water was mixed with 950 µL of the ABTS reagent or the phosphate buffer. Then, the mixture was incubated at room temperature for 12 h under darkness. After the incubation, the solution was centrifuged at 2,000× g for 5 min at 4 °C to remove insoluble substance. The supernatant was measured at 734 nm using a UV–visible spectrophotometer. The ABTS radical scavenging activity (%) was calculated using the equation [1 − (As − Asb) / (Ac − Acb)] ×100, where As is the absorbance of sample with the ABTS reagent, Asb is the absorbance of sample with the phosphate buffer, Ac is the absorbance of distilled water with the ABTS reagent, and Acb is the absorbance of distilled water with the phosphate buffer. Ascorbic acid was used as a positive control. The IC_{50} value was calculated from the percentage inhibition plotted against concentration.

Ferrous reducing power assay

Ferrous reducing power assay evaluates the ability to convert Fe^{3+} to Fe^{2+}. This assay was conducted following previous research. (Kuda and Yano 2009) with some modifications. First, 0.4 mL of each concentration of the extract containing MAAs or distilled water was mixed with 0.4 mL of 0.2 M phosphate buffer (pH 7.4) and 0.4 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, 0.4 mL of 10% trichloroacetic acid was added. Half of the samples was mixed with 960 µL of 0.017% ferric chloride and incubated for 10 min at room temperature under darkness. The absorbance was measured at 700 nm. The ferrous reducing power (OD) was calculated using the equation (As − Asb), where As is the absorbance of sample and Asb is the absorbance of control. Ascorbic acid was used as a positive control.

Statistical analysis

Data are expressed as the mean ± standard error. Each value represents the mean of triplicate independent analysis. Student's t test was applied for pairwise comparisons. Tukey–Kramer's multiple comparisons test and Student's t test were applied for multiple comparisons. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using Statcel 3 software (Version No. 3, OMS Publisher, Tokorozawa, Japan).

Results and Discussion

Preparation of extract containing MAAs and analysis of MAAs content by HPLC

Extracts containing MAAs from red algae were prepared by 25% ethanol extraction, and their MAAs contents were evaluated by HPLC (Table 1). Among the tested red algae, *M. japonica* yielded of the highest extract containing MAAs content (398 mg/g DW), while *P. senticulosa* yielded the least (167 mg/g DW). LC-MS analysis identified two types of 330 nm-absorbing compounds from *A. taxiformis* and *M. japonica*, and one type of the compound from *P. senticulosa* (Figure S2). The first peak in *A. taxiformis* and *M. japonica* was eluted at 3.00 min, showing a λ_{max} at 319 nm and a

prominent ion peak of protonated molecules ($[M+H]^+$) at *m/z* 245.1, suggesting the presence of palythine (Figure S3(a) and S4(a)). The second peak for these algae, eluted at 3.20 min, showed a λ_{max} at 331 nm and a ($[M+H]^+$) at *m/z* 333.1, suggesting the presence of shinorine (Figure S3(b) and S4(b)). A one peak in *P. senticulosa* was eluted at 3.50 min, showing a λ_{max} at 331 nm and a ($[M+H]^+$) at *m/z* 303.1. The total amount of fractions containing MAAs (total MAAs) is shown in Table 1. *D. inkyuleei* showed a high MAAs content (10.7 mg/g DW), while that from Nagasaki was around 1 mg/g DW.

The amount of MAAs in the extracts containing MAAs was low, suggesting that the main components of red algae, such as proteins, polysaccharides, and salts, were extracted (Zhang *et al*. 2010). The characteristics of the compound with absorption maximum at 331 nm from *P. senticulosa* (C-331) suggest the possibility of aplysiapalythine A, mycosporine-2-glycine, and palythinol (Figure S3(c) and S4(c)). The typical predominant MAAs in red algae are palythine, porphyra-334, and shinorine (Karsten *et al*. 1998; Karsten and West 2000). C-331 did not corresponded to those MAAs, suggesting the possibility of unique compound in terms of its major components. This study focused on the effect of UV radiation on MAAs contents. Therefore, the identification of C-331 will be the purpose of our next experiment.

Monthly changes in MAAs content and environmental factors

The monthly variation of MAAs in samples collected in February and April 2023 was investigated (Table 2 and Figure 2a). A higher amount of MAAs was obtained in *A. taxiformis* (4.91 μmol/g DW). The total MAAs content in *A. taxiformis* and *M. japonica* did not show a significant difference between February and April, while the contents were more abundant in the samples of February. *A. taxiformis* significantly decreased shinorine content from 49 to 41 mol%, while palythine content increased from 51 to 59 mol% with a statistically non-significant difference. In *M. japonica*, shinorine significantly decreased from February (65 mol%) to April (41 mol%), and palythine significantly increased in April (from 35 to 59 mol%). These results indicate an increase in palythine in both red algae in April and a decrease in total MAAs in February. The variations in nitrogen compounds in red algae, such as crude protein and PE contents, were investigated (Table 3). Crude protein and PE contents in *A. taxiformis* and *M. japonica* decreased from February to April. Crude protein in *A. taxiformis* significantly decreased from 26 to 18 mg/g DW. The content of crude protein in *P. senticulosa* was the lowest at 19 mg/g DW.

The environmental factors affecting MAAs content were investigated (Figure 2). UV radiation and chlorophyll levels increased from during this period. Namely, UV radiation rose monthly by approximately 96 mW/m^2 from February to April. Chlorophyll concentrations gradually increased from January to April (up to 5 mg/m^3) and rapidly decreased towards May (below 0.5 mg/m^3) (Figure 2b). These results suggest that the factors affecting MAA, crude protein and PE contents were related to the nutrient levels in the ocean.

UV radiation is known to be one of the factors affecting the increase in MAAs content in red algae (Karsten *et al*. 1998). The UV radiation levels in both Hokkaido and Nagasaki Prefecture increased monthly (Figure 2b and Yamamoto *et al*. 2023b). This study investigated the relationship between the MAAs content in red algae and UV radiation levels by comparing samples from Nagasaki Prefecture and Hokkaido. However, the correlation between the increase in UV radiation levels and MAAs content was not observed. Another factor affecting MAAs content is the availability of nutrients in the ocean. It has been showed that the nutrient levels in the ocean decrease with an increase in chlorophyll levels. Therefore, chlorophyll levels indicate a decrease in nutrient availability towards April.

For instance, *Palmaria palmata* promoted MAA accumulation by an increase in solar radiation exposure (Karsten and Wiencke 1999). *Porphyra columbina* increased MAA concentrations based on dry weight by the addition of ammonium to the medium and with different UV irradiation treatments (Peinado *et al*. 2004). These studies suggest a potential link between nitrogen availability and MAA production across diverse red macroalgal species. The paper evaluated the effect of UV radiation and nutrients, showing that limited usage of nitrogen compounds reduces MAAs content (Lalegerie *et al*. 2024). The increase in chlorophyll levels around February to March is more remarkable in Hokkaido compared to Nagasaki Prefecture, resulting in a rapid decrease in protein and MAAs contents in Hokkaido samples. In contrast, chlorophyll proliferation in Nagasaki Prefecture is weaker, resulting in a moderate decrease in those compounds. From these results, we suggested that due to the insufficient nitrogen compounds in the ocean, a correlation between MAAs content and UV radiation levels is not obtained.

Despite the increase in UV radiation, the molar concentration of palythine increased in red algae without an increase in the total MAAs content. This change was observed in samples from both Hokkaido and Nagasaki Prefecture. The accumulation of palythine in algae suggests an increase in stress from environmental changes (Carreto and Carignan 2011) due to the insufficient synthesis of MAAs caused by the shortage of nitrogen compounds in the ocean.

Antioxidant activity of MAAs

Antioxidant activities (DPPH radical scavenging activity, ABTS radical scavenging activity, and ferrous reducing power assay) of extract containing MAAs were measured at pH 7.4 (Figure 3, Table 4). Extract containing MAAs from *P*. *senticulosa* showed the highest antioxidant activity among tested algal species in three methods. On the other hand, the extract containing MAAs from *A*. *taxiformis* exhibited the lowest activity in both DPPH and ABTS radical scavenging activity, while the extract containing MAAs from *D*. *inkyuleei* exhibited the lowest activity in ferrous reducing power. Antioxidant activity of shinorine and C-331 containing fractions were assessed with different pH conditions (Figure 4). ABTS radical scavenging activity of shinorine and C-331 containing fractions

increased from acidic to alkaline conditions. The IC_{50} values of C-331 containing fractions at pH 8.0 was 5.9 μg/mL, and the putative IC_{50} values of shinorine containing fractions was 16.4 μg/mL.

In the case of ascorbic acid, the IC_{50} value at pH 7.4 was 1.68 μg/mL for DPPH radical scavenging assay, 1.98 μg/mL for ABTS radical scavenging assay and 0.43 μg/mL for ferrous reducing power assay. The antioxidant activity of extract containing MAAs was approximately 280 to 1,000 times weaker than that in ascorbic acid. The exact IC_{50} values of MAAs are unknown. Most studies reported that palythine exhibits relatively strong antioxidant activity, while shinorine shows weak activity (Wada, Sakamoto and Matsugo 2015; Torres *et al*. 2018). The MAAs in *A*. *taxiformis* and *M*. *japonica* were mixtures of shinorine and palythine. Despite containing a higher amount of MAAs in extract containing MAAs from *M*. *japonica*, the extract containing MAAs from *A*. *taxiformis* showed lower antioxidant activity. The major MAAs in February in dulse were palythine and porphyra-334, and MAAs content in extract containing MAAs was ten times higher than that from other red algae tested in this experiment (Table 1; Yamamoto *et al*. 2023b). This suggests the presence of antioxidant activity other than MAAs in the extract containing MAAs.

The fractions containing MAAs obtained by HPLC were compared with the fractions containing palythine or porphyra-334 previously obtained from dulse (Nishida *et al*. 2020). Combination with this study, the rank order of antioxidant activities as follows: palythine > C-331 and porphyra-334 > shinorine. Antioxidant activity is likely associated with the structure of MAAs. MAAs are composed of a cyclohexenone or cyclohexenimine rings (Chrapusta *et al*. 2017; Geraldes and Pinto 2021). These MAAs have a cyclohexenimine structure, and their differences are the amino acid attached to C1 of cyclohexenimine (Figure 1 and Figure S1). Shinorine, porphyra-334, and mycosporine-2-glycine are linked to serine, threonine, and glycine, respectively. Palythine is a compound formed by the decarboxylation and demethylation of the glycine group of mycosporine-2-glycine. All these MAAs form a resonance hybrid structure, leading to the delocalization of electrostatic charge between C1 to C3 of nitrogen atoms. The antioxidant activity of MAAs in response to pH conditions may be attributed to distinct tendencies for delocalization (Nishida *et al*. 2020). Furthermore, the diverse antioxidant activities of each MAA may be influenced by the structure of C1. The C1 group of palythine is composed only of NH, and is a shorter chain compared to the other three MAAs (Figure 1 and Figure S1). This structural feature may be responsible for the difference in antioxidant ability (Yang *et al*. 2018). The C4 or C6 group of cyclohexenimine contributed antioxidant activity of MAAs (Apak *et al*. 2016). Therefore, the structure bound to C1 may inhibit the antioxidant reaction carried out by C6. In fact, C1 of palythine has a simple structure and had the highest antioxidant activity. The activity was reduced with an increase in the size of C1 group. Until now, there has been no consideration of antioxidant activity due to differences in the structure of the C1 group, and this is a point that should be noted in the future.

Conclusion

In this study, we clarify the composition of MAAs in red algae from Nagasaki Prefecture. It was suggested that two species (*A. taxiformis* and *M. japonica*) contained shinorine and palythine, while one compound from *P. senticulosa* cannot be identified. It had been thought that macroalgae possess a variety of MAAs. However, these results showed that macroalgae from southern regions may have a limited type of MAAs. Moreover, our research suggested that the quantity of MAAs is influenced not only by the UV intensity but also by the nutrient content in the ocean. The tested MAAs exhibited strong antioxidant activity under alkaline conditions, and we determined the order of antioxidant activity among the four types of MAAs containing fractions. This research enhances the understanding of the antioxidant function of MAAs and provides valuable insights for the application of MAAs in various fields.

Supplementary material

Supplementary material is available at Bioscience, Biotechnology, and Biochemistry online.

Data availability

Data available upon request.

Author contributions

Y.K. and H.K. conceived and designed the research; R.Y. and C.K. performed the experiments; R.Y. and S.T. analyzed the data; Y.K., R.Y., W.S., and H.K. contributed to writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figure legends

Figure 1. Chemical structure of MAAs. (a) Shinorine, (b) Palythine, (c) Porphyra-334.

Figure 2. MAAs content, erythemal UV intensity, and chlorophyll concentration in Nagasaki Prefecture in February and April 2023. (a) MAAs content and erythemal UV intensity. The white and gray bars represent the MAAs content of *A*. *taxiformis* and *M*. *japonica* in February and April 2023. The red line shows the erythemal UV intensity from February to April 2023. Data were obtained from the Japan Meteorological Agency (https://www.data.jma.go.jp/gmd/env/uvhp/info_uv.html, accessed on December 5, 2023). (b) Chlorophyll concentration. Data were obtained from the Japan Meteorological Agency and NASA's Ocean Color WEB. All data were recorded in 2023. White arrow indicates Mushima, Nagasaki Prefecture.

Figure 3. Antioxidant activity of extract containing MAAs from red algae. (a) DPPH radical scavenging assay, (b) ABTS radical scavenging assay, (c) Ferrous reducing power assay. All assays were performed at pH 7.4. The data show the mean value ± SE, $n = 3$. Different letters above the bars in each assay indicate statistically significant differences in the mean values (Tukey–Kramer's multiple comparisons test, a, b, c, d, $p < 0.05$).

Figure 4. ABTS radical scavenging assay of MAA containing fractions. The assay was performed at pH 5.8, 6.6, 7.4 and 8.0. (a) Shinorine, (b) C-331. Data are presented as mean value ± SE, $n = 3$. Different letters for each antioxidant activity indicate significant differences in mean values (Tukey–Kramer's multiple comparisons test, a, b, c, d, $p < 0.05$). The concentration of 36 μM of shinorine and C-331 corresponds to 10.9 μg/mL and 12.0 μg/mL, respectively. The concentration of C-331 was estimated using the molar extinction coefficient of M2G, which has the weakest value (44,668 M^{-1} cm^{-1}) among the candidate MAAs, palythinol (43,500 M^{-1} cm^{-1}) and aplysiapalythine A (not determined) to reduce overestimation.

Graphical abstract caption

UV absorption compounds and antioxidant activity: MAAs from red algae

Table 1. Yield of extracts and MAAs from red algae

	Extract containing MAAs (mg/g DW)	Total MAAs (mg/g DW)
A. taxiformis	299 ± 16	1.4 ± 0.1
D. inkyuleei	382 ± 35	10.7 ± 0.4
M. japonica	398 ± 6	0.86 ± 0.05
P. senticulosa	167 ± 29	0.54*

Red algae were collected on February 2023. Data are expressed as mg/g DW. The data show mean values ± SE, $n = 3$. *, The concentration of total MAAs from *P. senticulosa* was estimated using the molar extinction coefficient of 44,668 M^{-1} cm^{-1} from mycosporine-2-glycine, which has the weakest value among the MAAs containing ($[M+H]^+$) at *m/z* 303.1, palythinol (43,500 M^{-1} cm^{-1}) and aplysiapalythine A (not determined) to reduce overestimation.

Table 2. Monthly variation of MAAs content and composition

	A. taxiformis		*M. japonica*	
(μmol/g DW)	February	April	February	April
Total MAAs content	4.91 ± 0.26	3.92 ± 0.59	2.86 ± 0.17	2.66 ± 0.38
Shinorine	2.42 ± 0.11^{a} (49.3 mol%)	1.61 ± 0.26^{b} (41.1 mol%)	1.86 ± 0.11^{A} (65.0 mol%)	1.09 ± 0.16^{B} (41.0 mol%)
Palythine	2.49 ± 0.15 (50.7 mol%)	2.31 ± 0.33 (58.9 mol%)	1.00 ± 0.06^{B} (35.0 mol%)	1.57 ± 0.22^{A} (59.0 mol%)

Red algae were collected on February and April 2023 from Mushima, Nagasaki, Japan. The MAAs content are expressed as μmol/g DW. The data show mean values ± SE, n = 3. The percentages in parentheses indicate the molar ratio of each MAA relative to the total MAAs. Different letters indicate significant differences in mean value (Student's t test, $^{a, b}$, $p < 0.05$ for *A. taxiformis*; $^{A, B}$, $p < 0.05$ for *M. japonica*).

Table 3. Monthly variation of crude protein and PE contents

	A. taxiformis		***M. japonica***		***P. senticulosa***
(mg/g DW)	February	April	February	April	February
Crude protein	25.5 ± 0.7[a]	18.1 ± 2.1[b]	36.8 ± 4.1	30.1 ± 1.9	18.7 ± 0.9
PE	0.20 ± 0.05	N.D.	13.6 ± 2.8	9.03 ± 1.83	N.D.

Red algae were collected on February and April 2023 from Mushima, Nagasaki Prefecture, Japan. The crude protein and PE contents are expressed as mg/g DW. The data show mean values ± SE, $n = 3$. Different letters indicate significant differences in mean value (Student's t test, [a, b], $p < 0.05$ for *A. taxiformis*). N.D., Not Detected.

Table 4. IC_{50} values for antioxidant activities in extract containing MAAs.

Red algae	DPPH radical scavenging activity (mg/mL)	ABTS radical scavenging activity (mg/mL)	Ferrous reducing power (mg/mL)
A. taxiformis	2.17	0.34	1.35
D. inkyuleei	1.80	0.41	1.00
M. japonica	1.81	0.21	0.68
P. senticulosa	1.60	0.12	0.64

Red algae were collected on February 2023. IC_{50} value for antioxidant activities are expressed as mg/mL. The data show mean values ± SE, $n = 3$.

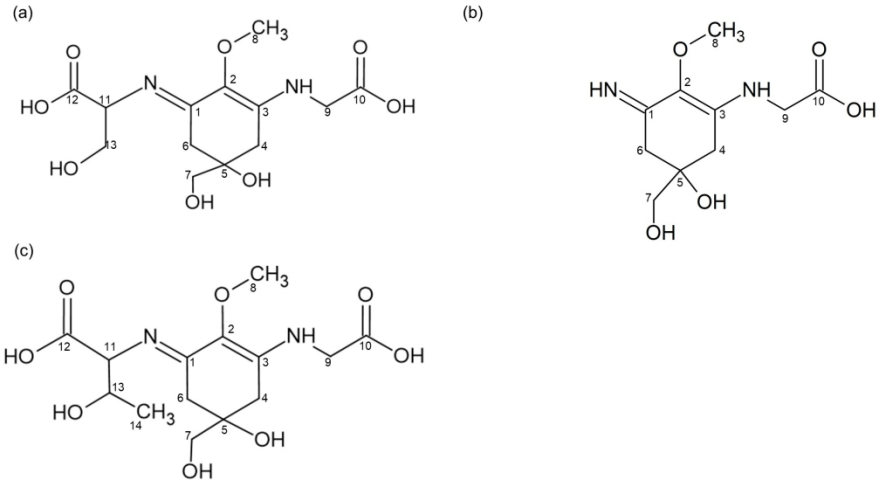

Fig1

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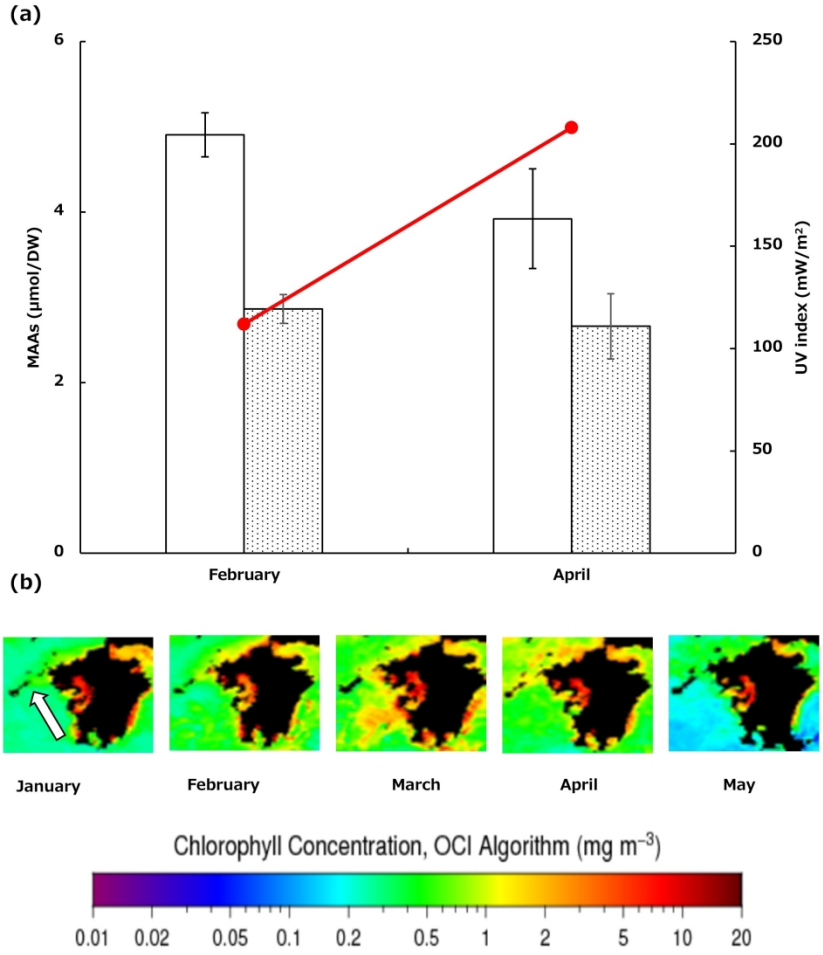

Fig2

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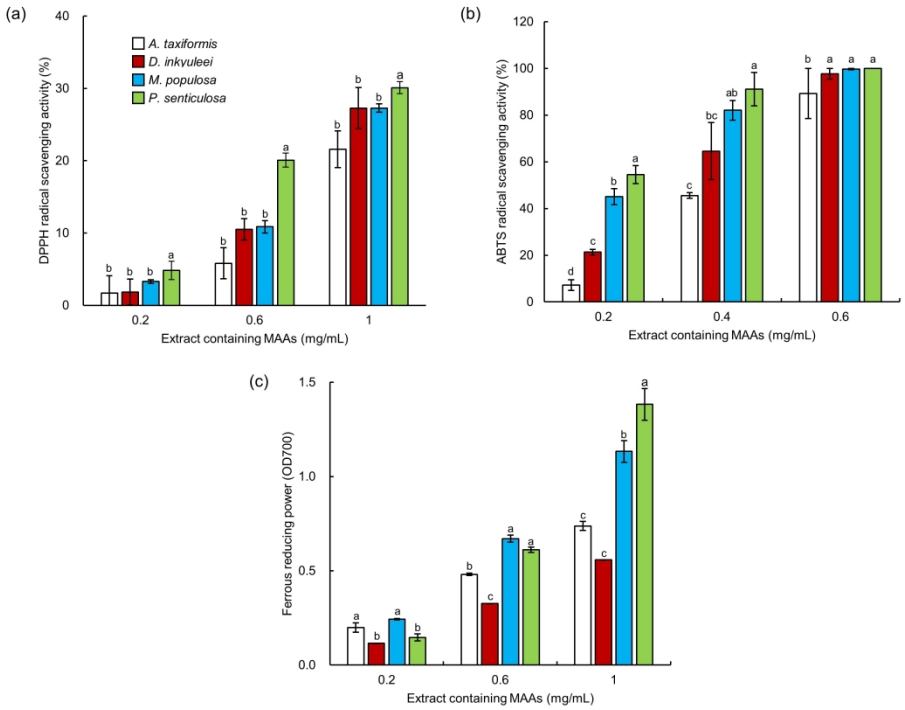

Fig3

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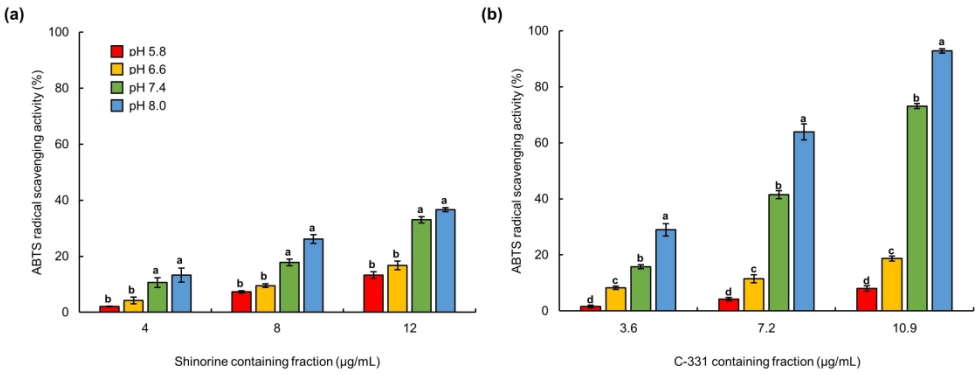

Fig4

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Supplementary Materials

Extraction and antioxidant capacity of mycosporine-like amino acids from four species of red algae in Japan

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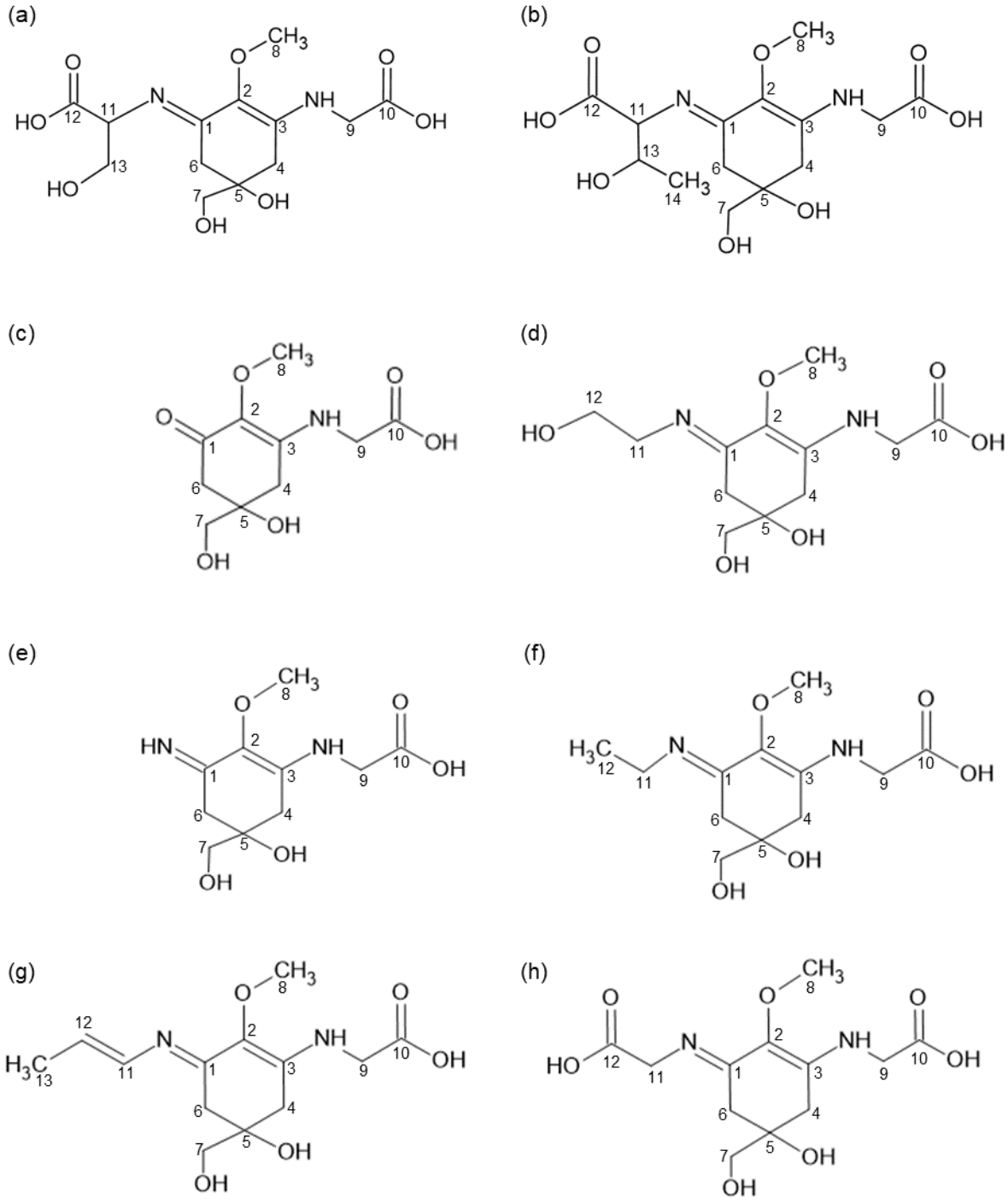


Figure S1. Chemical structure of MAAs. (a) Mycosporine-glycine, (b) Asterina-330, (c) Aplysiapalythine B, (d) Usujirene, (e) Palythene, (f) Mycosporine-2-glycine, (g) Palytinol, (h) Aplysiapalythine A.

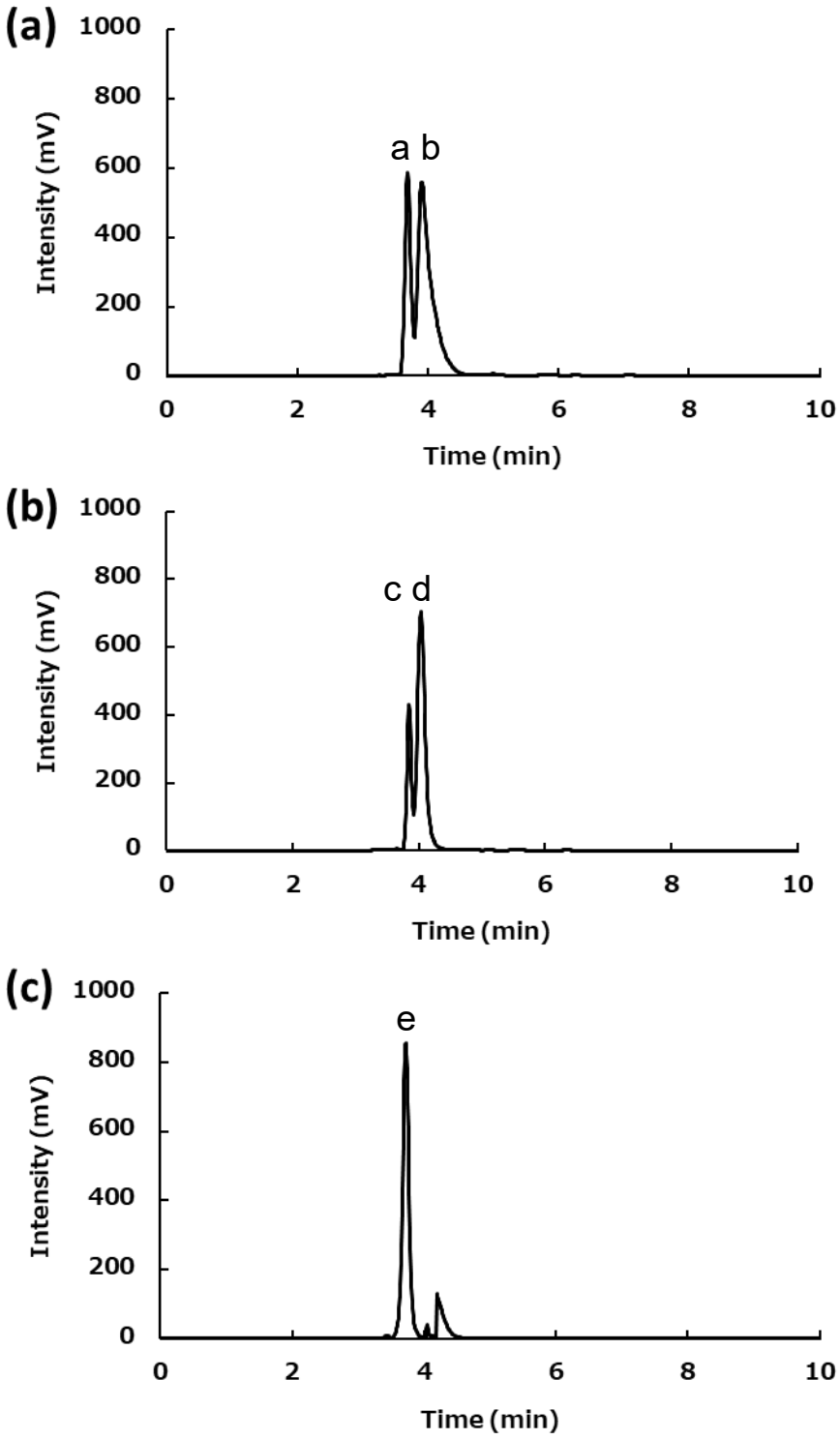


Figure S2. HPLC chromatogram. (a) *Asparagopsis taxiformis*, (b) *Meristotheca japonica*, (c) *Polysiphonia senticulosa*.

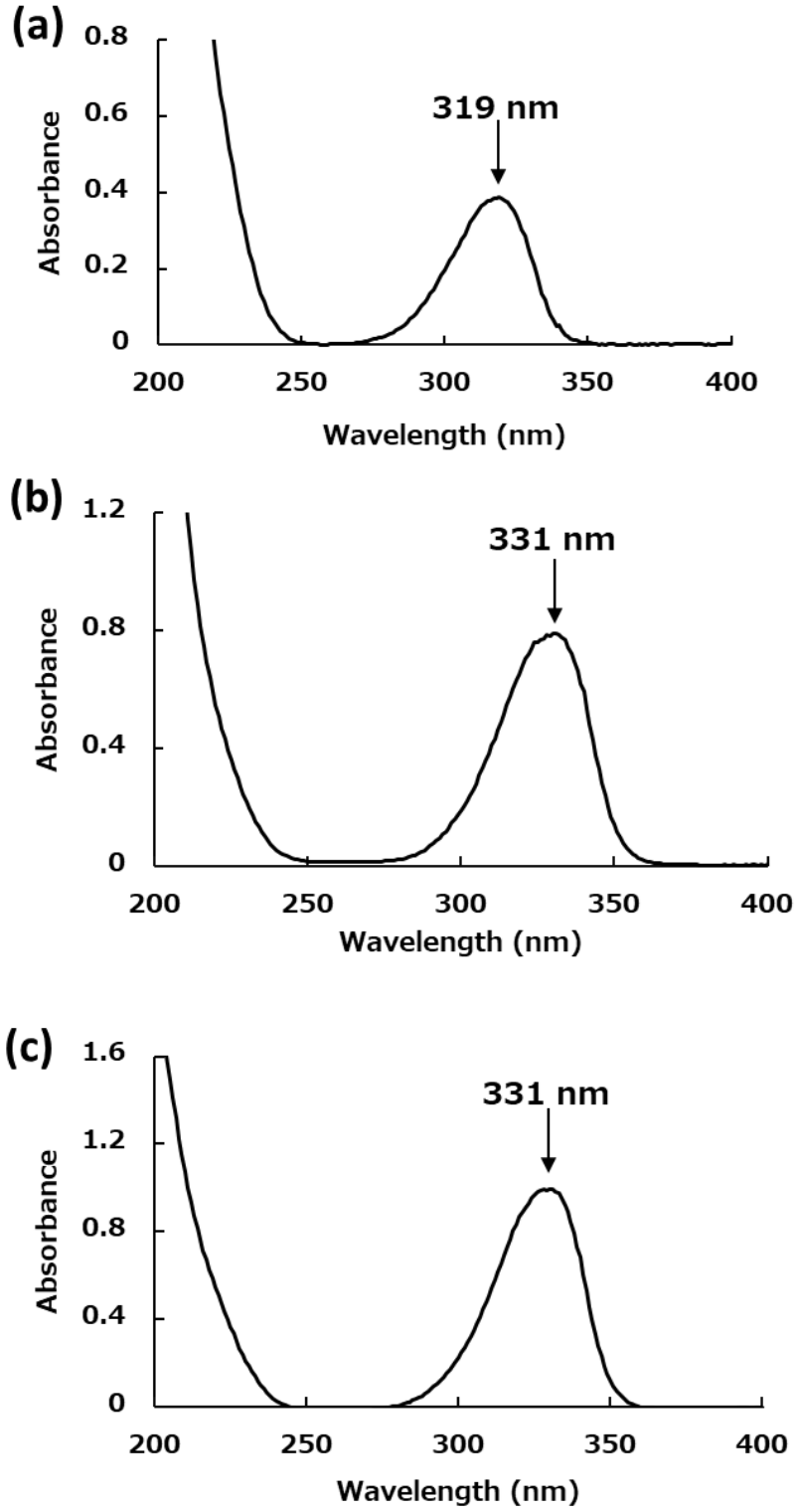


Figure S3. UV-Vis spectral absorbance of MAA. (a) Palythine (Figure S2 a and c), (b) Shinorine (Figure S2 b and d), (c) C-331 (Figure S2 e).

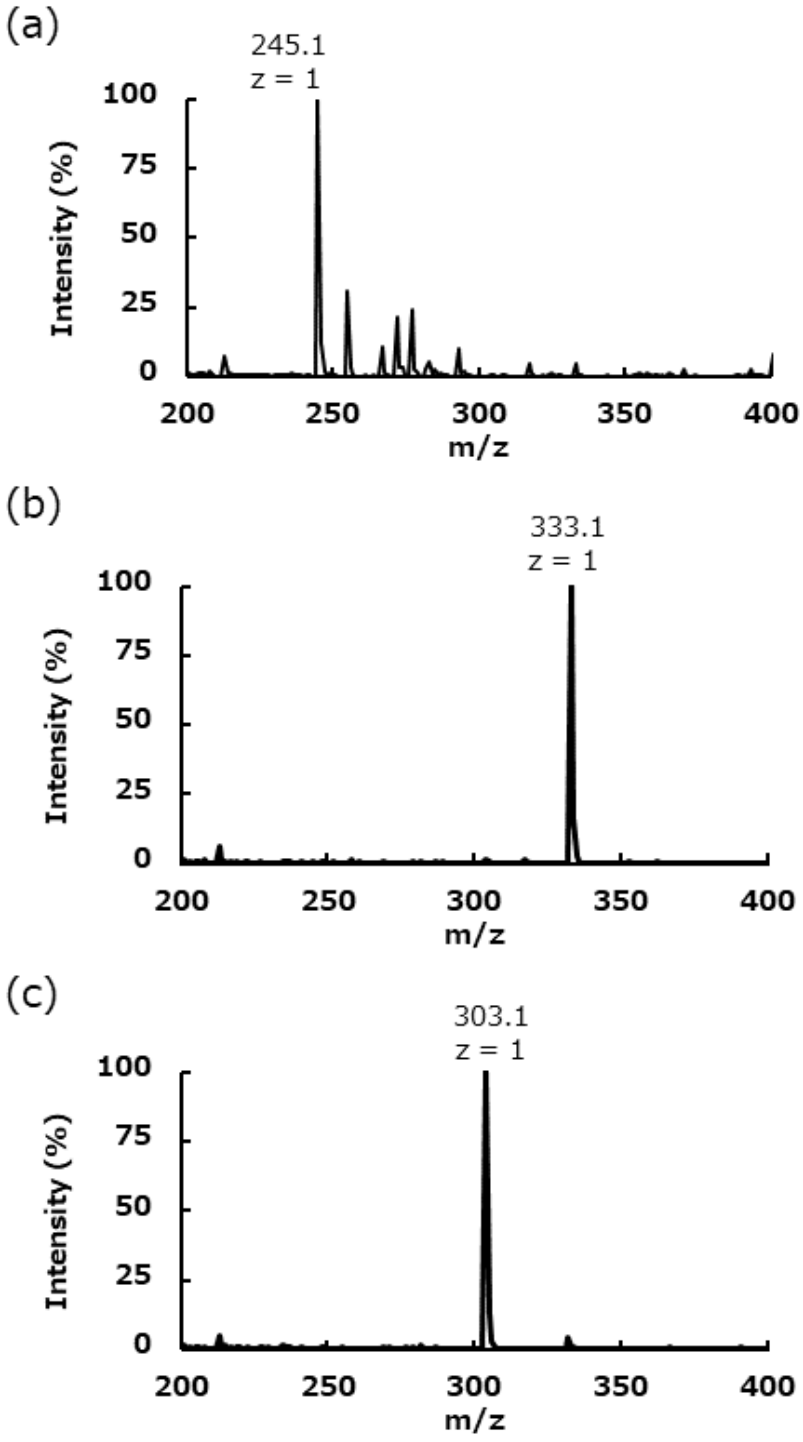
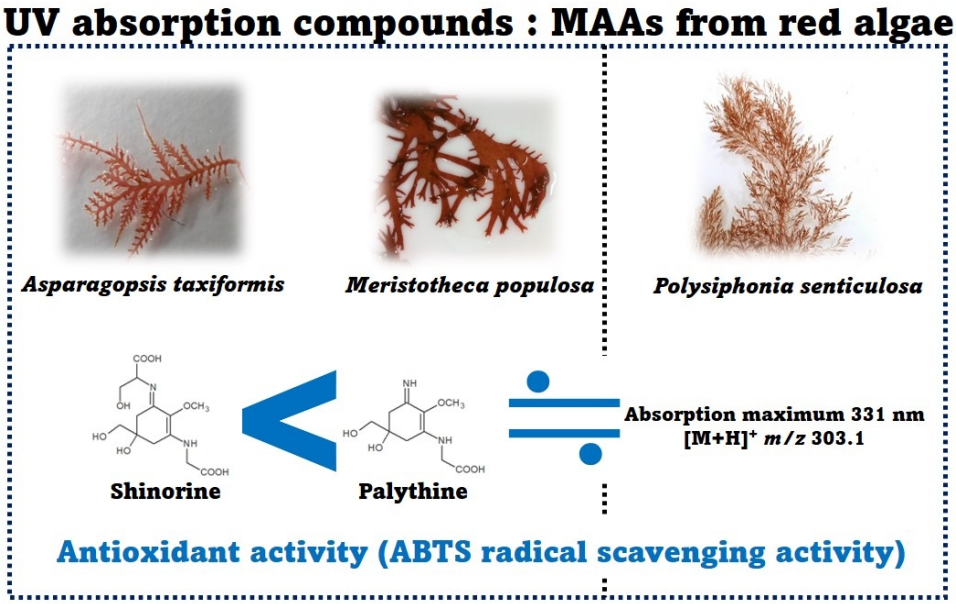


Figure S4. ESI-MS of MAA. Crude MAAs were separated and the mass of MAAs was detected by LCMS-2020 (Shimadzu, Kyoto, Japan). (a) Palythine (Figure S2 a and c), (b) Shinorine (Figure S2 b and d), (c) C-331 (Figure S2 e).



UV absorption compounds and antioxidant activity: MAAs from red algae

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