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Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan

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ABSTRACT

To clarify the useful properties of the macroalgal beach-casts for food, we determined mineral, water-soluble polysaccharide and total phenolic compound contents and antioxidant properties of aqueous solutions obtained from frond of eight brown and four green algae. These algae were washed ashore from the East Sea (the Sea of Japan) to the northeast beaches of the Noto Peninsula in autumn. There were fresh and large amounts of *Ecklonia stolonifera* (Es), *Ecklonia kurome* (Ek), *Sargassum ringgoldianum* subsp. *coreanum* (Sr) and *Sargassum macrocarpum* on the beaches. Potassium, magnesium and calcium ions were high in Sr-solution. High molecular weight water-soluble polysaccharide, mainly alginates, and relative viscosity were also high in Sr-solution. Both Es and Ek solutions showed high contents of total phenolic compounds and high antioxidant activities, including DPPH radical- and hydroxyl radical-scavenging and ferrous reducing power. On the other hand, the highest superoxide anion radical-scavenging activity was in the Sr solution. In the case of Sr, the same antioxidant properties were also found in the aqueous solution obtained from stem. These results suggest that some macroalgal beach-casts, especially Sr, can be utilised as new natural resources for functional foods, cosmetics, medical applications and fertilizer instead of being processed to landfill or incineration.

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1. Introduction

Since ancient times, in coastal regions of Far-East countries, peoples have discovered and collected edible algae from beachcasts (Ikehara & Hayashida, 2003). Ikemori and Tajima (2002) reported that one hundred or more species of algae can be collected from the coast of the Noto Peninsula facing the middle of the East Sea (the Sea of Japan). Though there are about 40 species edible algae in the coast, the most edible algae have a season for harvest to eat. As examples, Sargassum horneri and Ecklonia kurome are harvested from January to March. It is known that these algae have notable bioactive compounds, such as fucoidans and phlorotannins (algal polyphenols), regarded as antivirus, antibacterial and antioxidative compounds (Heo, Park, Lee, & Jeon, 2005; Hoshino et al., 1998; Kuda, Kunii, Goto, Suzuki, & Yano, 2007). On the other hand, a lot of inedible algae are left on the beaches, particularly in autumn. Recently, various kinds of macroalgae, including inedible seaweeds, are considered to be resources for bio-active compounds (Nahas et al., 2007) as well as for silage and fertilizer.

The antioxidants of edible plants interest many researchers. Oxidative modification of DNA, proteins, lipids and small cellular molecules by reactive oxygen species (ROS), such as superoxide anion radical and hydroxyl radical, plays a role in a wide range of common diseases and age-related degenerative conditions (Borek, 1993). These include cardiovascular disease, inflammatory conditions and neurodegenerative disease, including Alzheimer's disease, mutations and cancer (Byers & Guerrero, 1995; Kaur & Kapoor, 2001; Richardson, 1993). Since marine algae are rich in polysaccharides and minerals, a documented antioxidant activity would elevate their value as human food and pharmaceutical supplements.

In this study, to clarify the useful property of the macroalgal beach-casts for foods and healthy diets, we determined the contents of minerals, and water-soluble polysaccharides, as well as antioxidant properties of hot water extract solutions obtained from eight brown algae and four green algae. These algae were collected in autumn, which is out of season for most edible algae.

2. Materials and methods

2.1. Chemicals

(+)-Catechin (Cat), Folin-Ciocalteu's phenol reagent, the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, phenazine metho-sulphate (PMS), 3-(2-pyridyl)-5,6-di(p-sulfophenyl)1,2,4-triazine



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disodium salt (ferrozine), β -nicotinamide adenine dinucleotide (NADH) and nitroblue tetrazolium salt (NBT) were purchased from Sigma–Aldrich (St. Louis, MO). Phloroglucinol dihydrate (PG), potassium ferricyanide and trichloroacetic acid (TCA) were purchased from Wako Chemicals (Osaka, Japan). 1,10-phenanthroline was purchased from Nakalai Tesque (Kyoto, Japan). Other reagents were of analytical grade.

2.2. Seaweed materials and sample preparation

Marine algal species used for this study were collected from six beaches located on the northeast side of the Noto Peninsula (Wajima-city, Suzu-city and Noto-town; $37^{\circ}37-53'$ N; $137^{\circ}00-35'$ E) during October and November, 2007. The samplings were carried out after windy and rainy days to gather the fresh algal-casts. Then the samples were immediately transported to our laboratory and stored in a refrigerator (-30 °C) until experiments were begun. The scientific names and the Japanese names are summarised in Table 1.

Frond parts (10 g) of the wet sample were minced with 40 ml of distilled water for 60 s by a blender (16 Speed Blender, Osaka Chemical, Osaka, Japan). The water extract solution was collected after shaking (120 rpm) for 30 min at 80 °C, and centrifugation (2500g for 10 min).

2.3. Determination of minerals in the sample solutions

The sample solution (0.02 ml) was diluted with distilled water (9.98 ml) and filtered through a 0.45 μ m pore size filter. The dissolved and ionised mineral composition was determined by an ion analyzer (PIA-1000, Shimadzu, Kyoto, Japan) with each exclusive column and eluent for cations (Shim-pack IC-C3(S) and IC-MC3-1) and anions (Shim-pack IC-A3(S) and IC-MA3-1).

2.4. Relative viscosity and yield of water-soluble polysaccharides

Viscosities of the sample solutions were directly determined with an oscillation viscometer (Viscomate VM-1 G, Yamaichi Electronics, Osaka, Japan) under ice cooling. Relative viscosity was calculated as the quotient of the sample-viscosity divided by waterviscosity.

Table 1

Composition of ions in aqueous sample solutions (µmol/ml)

Proximate molecular size and quantity of water-soluble polysaccharides was determined by high performance liquid chromatography (HPLC) with a gel-filtration column (GFC) analysis. The column was Asahipack GS-520 (Asahi Chemical, Osaka, Japan) used at ambient temperature. The eluent was 50 mM NaCl, and the flow rate was 1 ml/min. Eluting compounds were detected by reflective index (RI). In the case of brown algae, crude fucoidan/laminaran and crude alginate, in the sample solutions were extracted and determined by alcohol precipitation methods described previously (Kuda, Taniguchi, Nishizawa, & Araki, 2002).

2.5. Determination of the amount of total phenolic compounds and UV absorbance

Total phenolic contents were determined as described previously (Kuda, Tsunekawa, Goto, & Araki, 2005; Oki et al., 2002) modified slightly. Briefly, 0.05 ml of a diluted sample solution, and 0.05 ml of 10% Folin–Ciocalteu solution were put into a 96 well microplate. After an interval of 3 min, 0.1 ml of a 10% sodium carbonate was added. The mixture was allowed to stand for 60 min at ambient temperature, and the absorbance was measured at 750 nm using a grating microplate-reader (SH-1000Lab, Corona Electric, Hitachinaka, Ibaraki, Japan). The phenolic content was expressed as phloroglucinol equivalent (PGEq)/ml.

The ultra-violet (UV) absorbance spectrum from 200 to 400 nm for the sample extract solution was measured by a spectrophotometer (U0080D, Hitachi High-Technologies, Tokyo) after dilution with distilled water.

2.6. Antioxidant activities

2.6.1. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Blois (1958) with slight modification (Kuda, Tsunekawa, Hishi, & Araki, 2005). Briefly, sample solution (0.025 ml), distilled water (0.075 ml) and ethanol (0.1 ml) were put into a 96-well microplate and 1 mM DPPH? in ethanol solution (0.025 ml) was added. After wrapping with vinylidene chloride copolymer (PVDC) film and incubation at 37 °C for 30 min, the absorbance was measured at 517 nm. Catechin (Cat) was used as positive control. From the concentration of sample and Cat for 50% radical-scavenging

Scientific name	Japanese name	Abbreviation in figures	Cation					Anion		
			NH	Na	K	Mg	Ca	H ₂ PO ₄	Cl	SO
Phaeophyceae										
Laminariaceae										
Ecklonia stolonifera	Tsuru-arame	Es	1.7	40.6	27.1	3.0	1.7	0.5	35.3	3.0
E. kurome	Kurome	Ek	2.0	44.5	27.6	3.6	2.0	0.4	40.5	3.6
Sargassaceae										
Sargassum ringgoldianum subsp. coreanum	Yanagi-moku	Sr	-	45.3	69.2	7.8	4.1	4.4	104.8	2.5
S. macrocarpum	Nokogiri-moku	Sm	0.8	27.7	39.8	5.6	2.3	3.9	63.1	1.4
S. silliquastrum	Yore-moku	Ss	0.7	33.3	33.1	4.7	1.9	4.0	60.6	1.9
S. confusum	Fushisuji-moku	Sc	0.8	26.1	32.5	4.3	1.5	1.1	51.3	1.2
S. patens	Yatsumata-moku	Spa	0.7	16.8	15.1	6.2	1.3	-	20.4	0.5
S. piluliferum	Mame-tawara	Spi	0.7	25.9	16.9	4.1	1.5	3.0	36.1	4.2
Chlorophyceae										
Ulvaceae										
Ulva pertusa	Ana-aosa	Up	0.7	16.7	20.4	16.2	1.7	_	9.4	3.1
Enteromorpha intetinalis	Usuba-aonori	Ei	6.4	12.4	17.6	8.8	1.3	3.1	9.5	2.9
Cladophoraceae										
Chaetomorpha moniligera	Tama-jyuzumo	Cm	0.6	10.0	13.6	1.3	0.9	-	14.3	0.2
Codiaceae										
Codium fragile	Miru	Cf	1.1	52.5	5.9	6.6	1.8	-	61.8	4.0

Values are mean of triplicate measuring.

(SC50), the radical-scavenging activity were expressed as CatEq/ml.

2.6.2. Superoxide anion radical-scavenging activity

Superoxide anion radical-scavenging activity was measured by a non-enzymatic method (Nishikimi, Rao, & Yagi, 1972) modified slightly (Kuda, Hishi, & Maekawa, 2006). Sample solution (0.025 ml) was treated with 0.1 ml of 25 mM phosphate buffer (pH 7.2), 2 mM NADH (0.025 ml) and 0.5 mM NBT (0.025 ml), and absorbance at 560 nm was measured as a blank value. After a 10 min incubation at ambient temperature with 0.025 ml of 0.03 mM PMS, the absorbance was again measured.

2.6.3. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging assay was carried out using Fenton's reaction method, described by de Avellar et al. (2004) and Li, Jiang, Zhang, Mu, and Liu (2008) with some modifications. Sample solution (0.025 ml), 0.1 ml of 25 mM phosphate buffer (pH 7.2), distilled water (0.075 ml), 6 mM 1,10-phenanthroline (0.025 ml) and 6 mM FeSO₄ (0.025 ml) were put into a 96-well microplate. Hydroxyl radical was generated by adding of 0.1% H_2O_2 (0.025 ml). The mixture was incubated at 37 °C for 60 min, and the absorbance was measured at 536 nm.

2.6.4. Ferrous reducing power

Total reducing power was determined as described by Zhu, Hackman, Ensunsa, Holt, and Keen (2002), but modified slightly (Kuda et al., 2007). Briefly, each 0.025 ml of the sample solution, 0.025 ml of 0.1 M phosphate buffer (pH 7.2) and 0.05 ml of 1% potassium ferricianide were put into a 96-well microplate. After incubation at 37 °C for 60 min, 0.025 ml of 10% TCA, 0.1 ml of distilled water and 0.025 ml of 0.1% FeCl₃ were added to the mixture. Increased absorbance, at 700 nm, of the reaction mixture indicated increased reducing power. Cat was used as a positive control. From the concentration for the increased absorbance of 0.25, 0.5 or 1.0, the reducing power was expressed as CatEq/ml.

3. Results and discussion

3.1. Macroalgal beach-casts used in this study

Although there were various seaweeds on the beaches, the mainly washed algae were eight brown algae and one green alga, *Ulva petrusa* (Up), summarised in Table 1. Particularly, there were fresh and large amounts of *Ecklonia stolonifera* (Es), *Ecklonia kurome* (Ek), *Sargassum ringgoldianum* subsp. *coreanum* (Sr) and *Sargassum macrocarpum* (Sm) on the beaches. These *Sargassum* species are drifting seaweeds that are very important for growth of small fishes. Yatsuya, Nishigaki, Douke, and Wada (2005) reported that the main drifting alga in September and October, Wakasa Bay, facing the middle of the East Sea, was Sr. In this study, Sr was also one of the main constituents in the beach-casts.

3.2. Mineral composition

Mineral composition of the sample solutions is also shown in Table 1. Sr solution showed high contents of potassium (69 μ mol/ml), magnesium (7.8 μ mol/ml) and calcium (4.1 μ mol/ml) ions. Sodium ion was the highest in *Codium fragile* (Cf). Ratio of potassium to sodium (K/Na) was the highest in Sr. The K/Na balance is regarded to be important for people who take diuretics, to control hypertension and suffer from excessive excretion of potassium (Cutler, 2006; Zillich, Garg, Basu, Bakris, & Carter, 2006). Minerals are also important as constituents of bones, teeth, soft tissues, hemoglobin, muscle, blood and nerve cells, and are vital for overall mental and physical well being (Miyake et al., 2005).

3.3. Relative viscosity and water-soluble polysaccharides

As shown in Fig. 1A, relative viscosity was the highest in Sr solution (10.2), followed by *Sargassum. patens* (Spa; 6.3) and *Ulva pertusa* (Up; 4.6) solutions. The viscosity in other sample solutions was about 2.0 or less. Alginates and ulvans are known as viscous polysaccharides in brown algae and green algae *Ulva* sp., respectively.



Fig. 1. Relative viscosity and GFC-HPLC chromatogram of aqueous solutions obtained from macroalgal beach-casts. See Table 1 for the sample abbreviations. A: Relative viscosity. Values are means and SD (*n* = 3). B: GFC-HPLC chromatogram of *S. ringgoldianum* subsp. *coreanum* (Sr). C: GFC-HPLC chromatogram of *E. stolonifera* (Ek). D: Regression of the relative viscosity and alginic acid in the sample solutions.



Fig. 2. Total phenolic content (A) and UV absorption spectrum (B) of the sample solutions. Values in A are means and SD (*n* = 3). Abbreviations correspond to the samples in Table 1.

These algal polysaccharides are beneficial dietary fibres for human and animal health (Brownlee et al., 2005; Kaeffer, Bénard, Lahaye, Blottière, & Cherbut, 1999).

In the brown algae samples, Sr and Spa solutions contained a quantity of high molecular weight (about 800 kDa) polysaccharides (Fig. 1B). According to the alcohol precipitation properties, these high molecular weight polysaccharides consisted of alginic acids and a trace of fucoidans. On the other hand, Es and Ek solutions contained some lower molecular weight polysaccharides (Fig. 1C). From saccharide composition, these polysaccharides were estimated to be laminaran. Laminaran is a beta-1,3 glucan and is easily fermented by intestinal microflora, like prebiotic-oligosac-charides (Kuda, Yano, Matsuda, & Nishizawa, 2005). As shown in Fig. 1D, the viscosity of sample solutions was correlated with high molecular weight polysaccharide (alginic acid) content ($r^2 = 0.958$).

3.4. Total phenolic content and UV-absorbance

3.4.1. Total phenolic content

The total phenolic contents in the sample solutions are shown in Fig. 2A. The phenolic concentrations in the sample solutions of Es and Ek solutions were higher than 40 µmol PGEq/ml. Among the six *Sargassum* samples, Sr showed the highest content, about 25 µmol PGEq/ml, and *S. piluliferum* (Spi) showed the lowest content (<2 µmol PGEq/ml). In the case of all four green algae Chlorophyceae samples, total phenolic content was very low (<0.6 µmol PGE/ml). It was reported that *Ecklonia* sp. have several bio-active compounds, particularly phlorotannins working as strong antioxidants (Kang, Chung, Jung, Son, & Choi, 2003; Kang et al., 2004; Kuda et al., 2007).

3.4.2. UV-absorbance

Most of the tested sample solutions showed maximal UVabsorbance at nearby 280 nm (Fig. 2B). The UV-absorbance at 280 nm correlated well with the concentration of phenolic compounds ($r^2 = 0.980$). Swanson and Druehl (2002) reported that short-term exposure of *Macrocystis integrifolia* Bory (a brown algae) blades to UVA and UVB radiation increased phlorotannins within their own tissues. The photoprotective effect of phenolic compounds or phlorotannins depends partially on the screen effect resulting, not only from their UV-absorbance, but also from their high antioxidant and radical-scavenging capacities, which may be involved in other cytoprotective roles (Connan, Dedlandes, & Gall, 2007).

3.5. Antioxidant properties

3.5.1. DPPH radical-scavenging activity

Both *Ecklonia*, Ek and Es, solutions showed high DPPH radicalscavenging activity from about 22–32 μmol CatEq/ml (Fig. 3A). Among the six *Sargassum* samples, Sr showed the highest activity, about 17 μ mol CatEq/ml and Spi solution showed the lowest activity (0.33 μ mol CatEq/ml). The radical-scavenging activity was very low in all four sample solutions obtained from Chlorophyceae (<0.1 μ mol CatEq/ml). These results were strongly correlated with phenolic compound content (r^2 = 0.958).

3.5.2. Superoxide anion radical-scavenging activity

The highest superoxide anion radical-scavenging activity was shown in Sr solution, about 120 µmol CatEq/ml (Fig. 3B), though the radical-scavenging activity of the two *Ecklonia* solutions was also high, about 70 µmol CatEq/ml. A Chlorophyta Up solution showed moderately high activity (40 µmol CatEq/ml), since the total phenolic content of this algal solution was very low. Consequently, the correlation between superoxide anion radical-scavenging activity and the phenolic compound content was not so high ($r^2 = 0.611$). Furthermore, the radical-scavenging capacities of Sr, Es and Ek solutions, expressed as CatEq/ml, were far higher than the DPPH radical-scavenging activities.

It is considered that the superoxide anion radical-scavenging activity of the algal samples was caused not only by phenolic compounds but also by other water-soluble compounds, such as peptides, fucoidan and Maillard reaction products (Kuda et al., 2005, 2006). Recently, the radical-scavenging activities of ulvans and ulvan derivatives were reported (Qi et al., 2005, 2006).

In most organisms, superoxide anion-radical is converted to hydrogen peroxide by superoxide dismutase. In the absence of transition metal ions, hydrogen peroxide is fairy stable. However, hydroxyl radicals can be formed by the reaction of superoxide with hydrogen peroxide in the presence of metal ions, usually ferrous or copper (Macdonald, Galley, & Webster, 2003). Hydroxyl radicals are much more reactive (toxic) than are superoxide anion radicals. Therefore, superoxide anion radical-scavenging activity of the sample solutions, particularly in the Sr solution shown in this study, suggests that the algal solution is beneficial for decreasing toxicity, not only of superoxide anion, but also of hydroxyl radicals and hydrogen peroxide.

3.5.3. Hydroxyl radical-scavenging activity

Although hydroxyl radical formation can occur in several ways, by far the most important mechanism *in vivo* is the Fenton's reaction, where a transition metal is involved as pro-oxidant in the catalysed decomposition of superoxide and hydrogen peroxide (Stohs & Baguchi, 1995). In this study, hydroxyl radical-scavenging activity was high in both *Ecklonia* solutions, about 60–90 µmol CatEq/ml (Fig. 3C), and was not detected in any of the four Chlorophyceae or two of the *Sargassum* (Sp and Spi) solutions. Regression of the radical-scavenging activity and total phenolic content fitted well with an exponential curve ($r^2 = 0.988$) rather than a line ($r^2 = 0.881$).



Fig. 3. Antioxidant properties, including DPPH radical-scavenging (A), superoxide anion radical-scavenging (B), hydroxyl radical-scavenging (C) and Fe-reducing (D) activities, of aqueous sample solutions. Values are means and SD (*n* = 3). Abbreviations correspond to the samples in Table 1.

3.5.4. Ferrous reducing power

Most non-enzymatic antioxidant activities, such as scavenging of free radicals and inhibition of peroxidation, are mediated by redox reactions (Zhu et al., 2002). Compounds with reducing power



Fig. 4. Shape of S. *ringgoldianum* subsp. *coreanum* body collected from the autumn beach in the Noto Peninsula.



Fig. 5. Relative viscosity and GFC-HPLC chromatogram of aqueous solutions obtained from different parts of *S. ringgoldianum* subsp. *coreanum*.



Fig. 6. Total phenolic content and UV absorption spectrum of aqueous solutions obtained from different parts of *S. ringgoldianum* subsp. *coreanum*.



Fig. 7. DPPH radical-scavenging (A), superoxide anion radical-scavenging (B) and Fe-reducing power (C) activities, of aqueous solutions obtained from different parts of *S. ringgoldianum* subsp. *coreanum*. Values are means and SD (*n* = 3).

indicate that they are electron donors, and can reduce the oxidised intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995). Fig. 3D shows ferrous-reducing power of the sample solutions. Two *Ecklonia* and Sr solutions showed strong activity, from about 13–28 µmol CatEq/ml. The activities of Spi and Chlorophyceae samples were low (<1 µmol CatEq/ml). The reducing power was well correlated with the phenolic compound content ($r^2 = 0.963$). This result is very similar to the result of DPPH radical-scavenging activity.

3.6. Properties of the solutions from different parts of S. ringgolidianum subsp. coreanum

As mentioned above, Sr solution from frond was rich in minerals, water-soluble polysaccharides and phenolic compounds. Sr body has specific parts of holdfast, stem, frond and upper (Fig. 4). Therefore we also determined the properties of the aqueous solutions from stem, frond and upper parts of Sr, as above.

Concentrations of minerals in the stem and upper part solutions were about 70% and 80%, respectively, of the concentrations in the frond solution (data not shown). High molecular weight polysac-charides and high viscosity in the upper part solution were found to be the same as in the frond solution (Fig. 5). In the case of the stem solution, mannitol was detected as in the frond solution.

Total phenolic compound content in the stem solution (33 μ mol PGEq/ml) equalled with the content in the frond solution (Fig. 6). On the other hand, the content in the upper part solution was low (16 μ mol PGEq/ml). UV absorbance of the solutions was also correlated with the phenolic compound content.

Though the stem solution showed high radical-scavenging activities (Fig. 7A and B) and reducing power (Fig. 7C), the antioxidant activities of the upper part solution was 50% or less of those of the frond and stem solutions.

Although there are no reports about food functions or toxicities of Sr, Ren, Noda, Amano, Nishino, and Nishizawa (1994) reported about antihyperlipidemic, antihypertensive and antiarteriosclerosis effects of *S. ringgoldianum*, called ooba-moku in Japan, on rats when comparing them with various marine algae. Perhaps these beneficial effects are related to the high contents of minerals, dietary fibres and antioxidants shown in this study. Therefore we think that Sr can be used as a functional food material.

4. Conclusion

In this study, we examined the minerals, water-soluble polysaccharides and antioxidant properties of hot water extract solutions obtained from 12 macroalgal beach-casts from the Noto Peninsula, Japan. Among the algae, *S. ringgolidinoum* subsp. *coreanum* (Sr) solution showed a high content of potassium and high viscosity. *E. stolonifera* and *E. kurome* solutions showed a high content of total phenolic compounds, strong DPPH radical-scavenging activities and reducing power. Moreover, Sr solution showed the highest superoxide anion radical-scavenging activity. Sr and the two *Ecklonia* were dominant washed-ashore seaweeds on the autumn beaches. From results in this study, it is considered that these macroalgal beach-casts can be utilised as natural resources for functional foods, cosmetics and medical applications, instead of being processed for incineration or landfill.

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