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# Antioxidant properties of dried product of 'haba-nori', an edible brown alga, *Petalonia binghamiae* (J. Agaradh) Vinogradova

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**Abstract**

Dried 'haba-nori' *Petalonia binghamiae*, a brown alga, is a traditional food in the fisheries towns in Japan. To determine the antioxidant properties of the dried *P. binghamiae*, assays for antioxidant activities, including ferrous-reducing power, ferrous ion chelating, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and scavenging of a superoxide anion radical-generated by non-enzymatic system were tested in this study. A water extract solution contained total phenols at about 75 μmol phloroglucinol equivalents/g dry sample and showed strong antioxidant activities in the reducing power, DPPH radical and superoxide anion radical scavenging assays. The antioxidant activities were detected in high-molecular (>100 kDa), 10–30 kDa, and low-molecular (<5 kDa) fractions and were correlated with, not only phenolic compounds, but also brown compounds. The radical- scavenging activities were increased by heat treatment at 121 °C for 1 h. These results suggest that *P. binghamiae* is both a useful seafood and a healthy food with antioxidant activity.


*Keywords:* *Petalonia binghamiae*; Antioxidant property; Radical scavenging activity; Reducing power

## 1. Introduction

A brown alga, *Petalonia binghamiae* (J. Agaradh) Vinogradova called 'haba-nori' in Japan, is widely distributed in the Pacific, for example along the coasts of Japan, China and West of the USA (Segawa, 1996). The shape of *P. binghamiae* is an aggregate of several leaves that are 15–50 mm in width and 100–250 mm in length. Although *P. binghamiae* grows well along many coasts of Japan and other countries, it is consumed as an edible alga and traditional food only in the fisheries town areas. Usually, the alga is eaten after drying and roasting lightly, like a dried product of 'nori' *Porphyra* spp., a red alga, that is one of the major algal products (Kitamura, Myouga, & Kamei, 2002).

Oxidative modification of DNA, proteins, lipid and small cellular molecules by reactive oxygen species (ROS) 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 diseases, such as Alzheimer's disease (Richardson, 1993), mutations and cancer (Byress & Guerrero, 1995). Though there are publications about the antioxidant activity of seaweeds (Yan, Nagata, & Fan, 1998), there are few reports about antioxidant activities in dried algal products (Kuda, Tsunekawa, Hishi, & Araki, 2005), and these studies are mainly confined to non-edible and/or fresh raw seaweeds. It is reported that drying and storage decrease the antioxidant compounds and activities (Araki, 1983).

There are some reports about fucoxanthin-related compounds (Mori et al., 2004), including retinoic acid (vitamin A) in *P. binghamiae*. These compounds have inhibitory activities against mammalian replicative

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DNA polymerases (Murakami et al., 2002). However, there are hardly any reports about any functional activity, such as antioxidant activity, in dried products of *P. binghamiae*.

The aim of the present work was to evaluate the profitable properties of *P. binghamiae* for human food. We investigated the antioxidant activities of water extract and ethanol extract by ferrous-reducing power assay, ferrous ion chelating assay, DPPH radical assay, and superoxide anion-scavenging assay. These antioxidant assays employ methodology widely used for plants and processed foods. Effects of heating on the antioxidant activities were also examined.

## 2. Materials and methods

### 2.1. Material

*P. binghamiae* was harvested in Wajima city (located in the temperate zone and facing the Sea of Japan), Ishikawa, Japan in April, 2004. The harvested material was spread in a mesh bottom frame about $25 \times 25$ cm and dried like 'nori'. The dried product was purchased from a retail shop in Wajima and used in this study.

### 2.2. Chemicals

(+)-Catechin (CA), Folin–Ciocalteu's phenol reagent, the stable radical DPPH, nitroblue tetrazolium salt (NBT), phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-di(*p*-sulfophenyl)-1,2,4-triazine, disodium salt (ferrozine) and ethylendiaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Phloroglucinol dihydrate (PG) and Potassium ferricyanide were purchased from Wako Chemicals Co., Ltd. (Osaka, Japan). Other reagents were of analytical grade.

### 2.3. Preparation of sample extract

The dried product sample (2 g) was weighed and 50 ml of distilled water or ethanol were added. The water extract (WE) and ethanol extract (EE) solutions were collected after shaking for 1 h at room temperature and centrifugation ($2220 \times 10$ min). The sample preparation was replicated three times. The WE and EE solutions were dark brown and dark green, respectively.

A portion (6 ml) of the WE solution was fractionated into six fractions (each 6 ml) of molecular weights, that were >100, 50–100, 30–50, 10–30, 5–10 kDa, and <5 kDa, using an ultrafiltration system, VIVASPIN 6 (Sartorius AG, Goettingen, Germany).

To determine the effect of heat treatment on the antioxidant activities, the WE and EE were extracted at 85 °C and 121 °C (WE85, WE121, EE85 and EE121, respectively).

### 2.4. Determination of the amount of total phenolic compounds, brown compounds, protein, saccharides and minerals

Total phenolic compound concentrations were determined as described previously (Kuda et al., 2005; Oki et al., 2002). Briefly, 0.4 ml of 10% Folin–Chiocalteu solution was added to 0.2 ml of a sample solution. After an interval of 3 min, 0.8 ml of a 10% sodium carbonate was added. The mixture was allowed to stand for 30 min at ambient temperature and the absorbance was then measured at 750 nm. The phenolic content was expressed as PG equivalent (Eq). To determine the relative content of brown compounds in *P. binghamiae*, 0.2 ml of WE was put into a microplate well and then absorbance was measured at 490 nm with reference to absorbance at 655 nm.

Protein content in the individual fractions was measured with a DC protein assay kit (Bio-Rad, CA). Total saccharide was determined by the phenol–sulfuric method (Dubois, Gilles, Hamilton, Rebens, & Smith, 1956). Salinity was measured by a salt meter (ES-421, Atago, Tokyo). Concentrations of sodium and potassium were determined by ion meters (C-122 and C-131, Horiba, Kyoto). Calcium and magnesium were measured with commercially available kits (for Ca and Mg Test-Wako series, Wako Pure Chemical, Osaka).

### 2.5. Reducing power

Total reducing power was determined as described by Zhu, Hackman, Ensunsa, Holt, and Keen (2002), but modified slightly. Briefly, each 0.2 ml of the sample solution was mixed with 0.2 ml of phosphate buffer (0.2 M, pH 7.2) and 0.2 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. Afterwards, 0.2 ml of 10% trichloroacetic acid was added to mixture. Finally, 0.125 ml of the mixture and 0.125 ml distilled water were put into a 96-well microplate and 0.02 ml of 0.1% $FeCl_3$ was added. Increased absorbance at 655 nm of the reaction mixture indicated increased reducing power. PG was used as positive control.

### 2.6. Ferrous ion chelating activity

The method of Decker and Welch (1990) was used. To a sample solution (0.1 ml), distilled water (0.1 ml) and 0.5 mM $FeCl_2$ (0.025 ml) were added. After measurement of absorbance at 550 nm, 2.5 mM ferrozine was added. After 20 min at room temperature, the absorbance was measured. EDTA was used as positive control.

### 2.7. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Blois (1958) with slight modification.

Thirty minutes after adding 1 mM DPPH/methanol solution (0.025 ml) to the sample solutions (0.2 ml), absorbance was measured at 550 nm. If the mixture was turbid, the absorbance was measured after centrifugation.

### 2.8. Superoxide anion radical-scavenging activity

The non-enzymatic generation of superoxide anion was measured by the method of Robak and Gryglewski (1988). Sample solution (0.1 ml) was treated with 0.1 ml of 0.1 M phosphate buffer (pH 7.2), 0.025 ml of 2 mM NADH and 0.025 ml of 0.5 mM NBT, and absorbance at 550 nm was measured as a blank value. After a 3 min incubation with 0.025 ml of 0.03 mM PMS, the absorbance was again measured.

## 3. Results and discussion

### 3.1. Chemical compounds in extract solutions

The total phenolic contents in WE and EE were 73.5 and 21.8 μmol PG Eq/g dry sample, respectively (Table 1). The other main compounds in WE were saccharides (polysaccharides). Protein and mineral contents in WE were not so high. Potassium content was four times higher than sodium content.

### 3.2. Antioxidant properties of the water and ethanol extract solutions

Ferrous-reducing power, DPPH radical scavenging activity and superoxide anion radical scavenging activity of WE were higher than that of EE (Table 2). The phenolic content, ferrous-reducing power and radical scavenging activity in WE was higher than that of common brown algae, such as *Fucus*, *Laminaria*, *Undaria*, (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto (2001)), besides of 'kajime' *Ecklonia cava* (Nagai & Yukimoto (2003)).

Table 1
Chemical compounds in extractions of dried 'haba-nori' *P. binghamiae*

| | WE | EE |
|---|---|---|
| Total phenolic compounds (μmol PG Eq/g dry sample) | 75.3 ± 7.5 | 21.8 ± 0.3 |
| Soluble saccharide (mg/g dry sample) | 78.1 ± 15.2 | – |
| Soluble protein (mg/g dry sample) | 5.15 ± 0.37 | – |
| Salinity (mg/g dry sample) | 53 ± 1 | – |
| Na | 3.3 ± 0.1 | – |
| K | 11.3 ± 0.3 | – |
| Mg | 1.68 ± 0.05 | – |
| Ca | 1.52 ± 0.19 | – |

WE, water extract solution; EE, ethanol extract solution.
PG, phloroglucinol; Eq, equivalent.
Values are means and SD ($n = 3$).

Table 2
Antioxidant properties of dried 'haba-nori' *P. binghamiae*

| | WE | EE |
|---|---|---|
| Ferrous-reducing power (μmol PG Eq/g dry sample) | 94.2 ± 2.9 | 18.9 ± 0.1 |
| Ferrous ion chelating activity (μmol EDTA Eq/g dry sample) | 2.32 ± 0.33 | <2 |
| DPPH radical-scavenging (mmol PG Eq/g dry sample) | 2.73 ± 0.49 | 0.53 ± 0.16 |
| Superoxide anion scavenging (μmol CA Eq/g dry sample) | 80.0 ± 1.9 | 5.9 ± 1.7 |

WE, water extract solution; EE, ethanol extract solution.
PG, phloroglucinol; Eq, equivalent; CA, (+) catechin.
Values are means and SD ($n = 3$).

DPPH has been used extensively as a radical to evaluate reducing substances (Cotelle et al. (1996)). In most cases, irrespective of the stage in the oxidative chain in which the antioxidant action is assessed, most non-enzymatic antioxidative activity, such as scavenging free radicals or inhibition of peroxidation, is mediated by redox reactions (Zhu et al. (2002)). In most organisms, superoxide anion radical is converted to hydrogen peroxide by superoxide dismutase. In the absence of transition metal ions, hydrogen peroxide is fairly 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)).

Ferrous ion chelating activity of both WE and EE was not so high comparing with 'kayamo-nori' *Scytosiphon lomentaria* (Kuda et al. (2005)). The metal binding capacities of dietary fibers are well known and the inhibitory effects on ferrous absorption of algal dietary fibers have been reported (Harmuth-Hoene & Schelenz (1980)). It is considered that the amount and/or ferrous binding capacities of water-soluble polysaccharides, such as alginate, fucoidan and laminaran, in *P. binghamiae* were lower than ones of *S. lomentaria*.

### 3.3. Antioxidant properties of individual P. binghamiae molecular weight fractions

As summarized in Table 1, WE of *P. binghamiae* was rich in phenolic compounds. With respect to the six molecular weight fractions isolated, the greatest amount of phenolic compounds was found in the >5 kDa, and >100 kDa fractions (Fig. 1(a)). The lowest amount of phenolic compounds was measured in the 50–100- and 30–50-kDa fractions.

Absorbance at 490 nm referenced by the absorbance at 655 nm was used as brown compounds (Fig. 1(b)). About 60%, 20% and 10% of the brown compounds were in the >100-, 10–30- and <5-kDa fraction, respectively.

As shown in Fig. 1(c), the highest ferrous-reducing power was observed in the >100 kDa, followed by <5, 10–30 and 5–10-kDa fractions. This result is thought

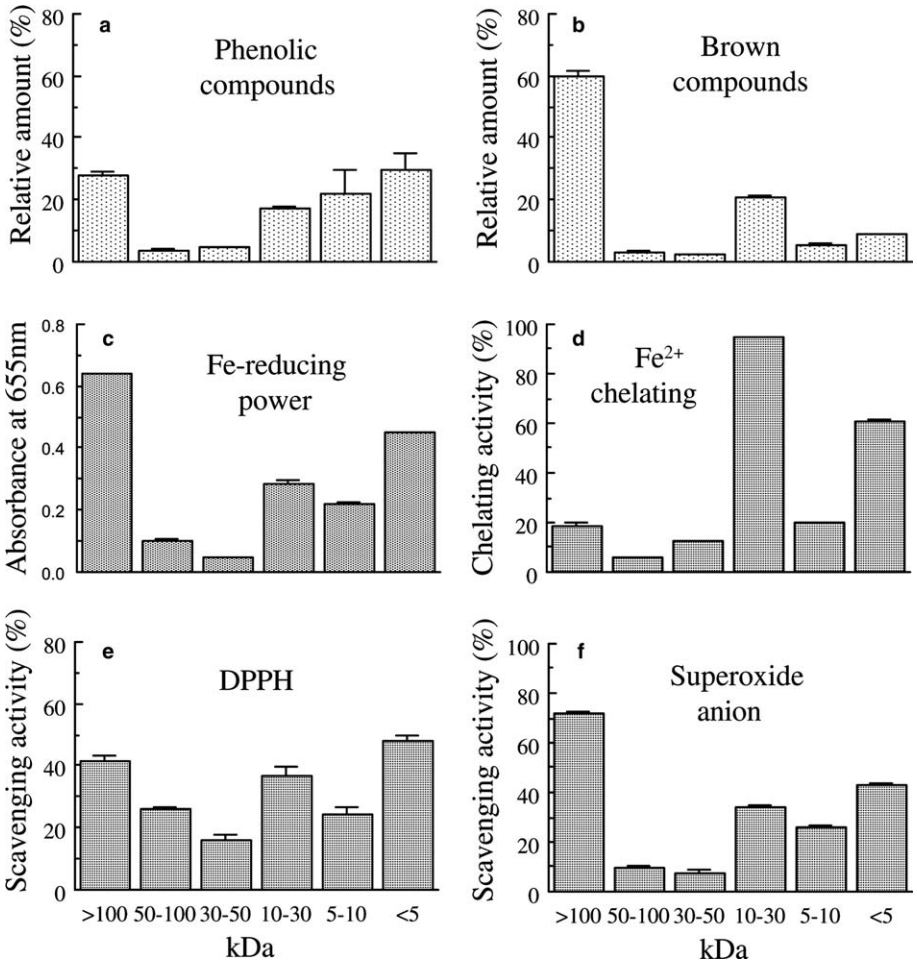


Fig. 1. Antioxidant properties of individual molecular weight fractions from dried *P. binghamiae*. (a) Amount of total phenolic compounds. (b) Relative content of brown compounds. (c) Ferrous-reducing power. (d) Ferrous ion chelating activity. (e) DPPH radical scavenging activity. (f) Scavenging activity in non-enzymatic assay. Sample solution volumes in the assays c–f were 0.1, 0.1, 0.05 and 0.05 ml, respectively. Values are means and SD ($n = 3$).

to correlate with the content of brown compounds rather than with the content of phenolic compounds, though many researchers have suggested that there may be a relationship between the amount of total phenolic compound and reducing power (Jiménez-Escrig et al., 2001; Zue et al., 2002).

The highest ferrous ion chelating activity was observed in the 10–30-kDa fraction, followed by <5-kDa fraction (Fig. 1(d)). Interestingly, the chelating activity of the 10–30-kDa fraction (95%) was higher than that of the WE (52%) at the same sample solution volume (0.1 ml) in this assay.

The highest DPPH scavenging activity was shown in the <5-kDa fraction, followed by <100- and 10–30-kDa fractions (Fig. 1(e)). This result is thought to correlate with the content of phenolic compounds rather than with the content of brown compounds. As with reducing power, it has been reported that the amount of DPPH-scavenging activity is dependent on the phenolic concentration of the algal samples (Jiménez-Escrig et al., 2001).

The highest superoxide anion radical scavenging activity was shown in the >100-kDa fraction followed by the 10–30- and <5-kDa fractions (Fig. 1(f)). This result is similar to that of the ferrous-reducing power (Fig. 1(c)).

### 3.4. Effect of extraction temperature on the antioxidant properties

To determine the effect of heating on the antioxidative activity of *P. binghamiae*, WE and EE were obtained from dried *P. binghamiae* at room temperature, 85 and 121 °C for 1 h.

The total phenolic compounds in WE extracted at 121 °C (WE121) was 65% higher than that of WE extracted at room temperature (WERT) (Fig. 2(a)). On the other hand, the content in WE heated at 85 °C (WE85) was decreased slightly. The phenolic content in EE was not so affected by the extraction temperature. The brown compound was increased about 2.5

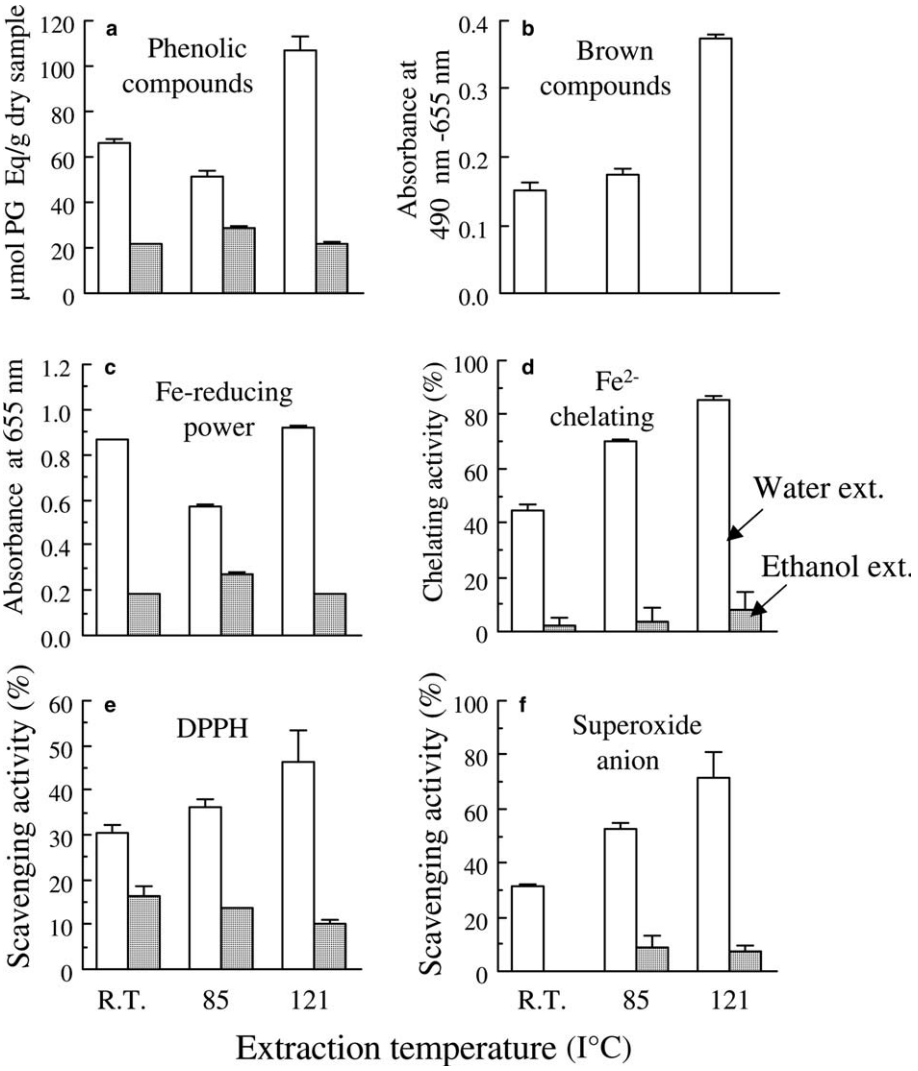


Fig. 2. Effect of heating on the antioxidant activities of water extract (open square) and ethanol extract (semi-closed square) solutions from dried *P. binghamiae*. (a) Amount of total phenolic compounds. (b) Relative content of brown compounds. (c) Ferrous-reducing power. (d) Ferrous ion chelating activity. (e) DPPH radical scavenging activity. (f) Scavenging activity in non-enzymatic assay. Sample solution volumes in the assays c–f were 25, 50, 1.25 and 12.5 μl, respectively. R.T., room temperature. Values are means and SD ($n = 3$).

times in WE121 (Fig. 2(b)). It is considered that the brown compound was generated by the Maillard reaction.

As shown in Fig. 2(c), the reducing power was not increased in WE121. Furthermore, the reducing power decreased by 35% in WE85. This result may be related to phenolic compound content. Jiménez-Escrig et al. (2001) reported that the phenolic content and reducing power in *Fucus* were decreased by drying at 50 °C for 48 h, and storage at room temperature.

The chelating activities were about 65% and 100% increased in WE85 and WE121, respectively (Fig. 2(d)). Perhaps, the increased activity was brought about by increasing solubility of polysaccharides. We had reported that the crude alginate and fucoidan from *Scytosiphon lomentaria*, heated at 121 °C for 15 min, have the ferrous ion binding activity (Kuda et al., 2005).

DPPH radical scavenging activities in WE were 20% and 50% increased by heating at 85 and 121 °C, respectively (Fig. 2(e)). On the other hand, the activity in EE was decreased slightly by the heat treatment. Superoxide anion radical-scavenging activity in WE was also clearly increased by the heating (Fig. 2(f)).

It is reported that drying and storage decrease antioxidant compounds, such as ascorbic acid and fucoxantins, and their activities (Araki, 1983). However, there are many reports about radical-scavenging activity of brown compounds (pigments) induced by Maillard reaction (Jing & Kitts, 2002; Morales & Babbel, 2002). It is considered that the brown compounds having radical scavenging activity are generated by the Maillard reaction in WE during the heating.

Although there are several reports about antioxidant activity of raw edible brown algae, such as 'kombu'

*Laminaria,* 'wakame' *Undaria* and 'kajime' *Ecklonia* (Jiménez-Escrig et al., 2001; Kang et al., 2004; Yan et al., 1998), we believe that the algal foods circulating as dried product should be examined after the drying process. Usually, these brown algae are dried and eaten after swelling with 20–40 and more volume of water. On the other hand, *P. binghamiae* is eaten after drying and only light roasting.

In summary, our observations demonstrate that WE and its >100- <5- and also 10–30-kDa fractions have reducing power and radical scavenging activity. Furthermore, the radical scavenging and ferrous-chelating activities are promoted by heat treatment such as retortring. In general, it is considered that the antioxidant activities depend on the phenolic and/or brown compounds. However, the antioxidant activities of sulfated polysaccharides, such as fucoidan have been reported (Kuda et al., 2005; Ruperez, Ahrazem, & Leal, 2002). From the results of the study and the processes, we consider that *P. binghamiae* is a useful healthy sea vegetable having antioxidant activities. It is necessary to begin experiments and purification of antioxidants in WE. We are especially interested in the 10–30 kDa fraction, of *P. binghamiae.* These studies are now in progress.

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