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Note

Fucoxanthin as the Major Antioxidant in *Hijikia fusiformis*, a Common Edible Seaweed

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The radical scavenging activity of Japanese edible seaweeds was screened by the DPPH (1-diphenyl-2-picrylhydrazyl) assay to evaluate the DPPH radical scavenging activity in organic extracts. The fresh brown alga *Hijikia fusiformis* showed the strongest DPPH radical scavenging activity, followed by *Undaria pinnatifida* and *Sargassum fulvellum*. The major active compound from *Hijikia fusiformis* in its acetone extract was identified as fucoxanthin by ^{13}C-NMR spectroscopy.

Key words: edible seaweed; antioxidant; free radical; fucoxanthin; *Hijikia fusiformis*

Edible seaweeds have high nutritional value as sources of minerals, vitamins, and noncaloric dietary fibers,[1] and as potential sources of antioxidants. Antioxidants neutralize potentially harmful reactive free radicals in body cells, and may reduce potential mutations and thereby help prevent cancer and heart disease.[2,3] Findings of antioxidative activity could elevate seaweed value as a food or additive, and expanding its market.[4] Although screening studies have shown that dozens of species of seaweed had antioxidative activity,[5–8] common edible seaweeds have not been systematically studied. We therefore studied the antioxidative activity in common edible seaweeds with the DPPH assay to assess the radical scavenging activity.

Fresh edible seaweeds were purchased from a retailer (see Table 1). Each seaweed sample was lyophilized and pulverized into powder. Ten millilitre of chloroform was added to 0.6 g of the sample in a tube. The mixture was sonicated twice for 10 min, and shaken at 60 rpm overnight. After filtration, the filtrate was centrifuged at 8000 rpm to remove any small pellets that might interfere with spectrophotometric measurements. The remaining residue was sequentially extracted with 10 ml of chloroform, ethyl acetate, acetone, and methanol. The organic solvent extracts, *i.e.*, the extracts of chloroform, ethyl acetate, acetone and methanol, were used in the DPPH assay, which was modified from that of Brand-Williams (1995).[9] The DPPH solution was prepared at a concentration of 3×10^{-5} mol/l in dimethylsulfoxide (DMSO). An organic solvent extract of 2 ml was mixed with 2 ml of the DPPH solution in a test tube, which was capped after nitrogen bubbling. After standing for 60 min, the absorbance at 517 nm was measured by a UV-vis spectrophotometer (UV-1200, Shimadzu Co., Japan), using DMSO as the blank. Meanwhile, the absorbance of the organic solvent extract after adding 2 ml of DMSO was also determined. All samples were taken in triplicate, and mean values of free radical scavenging activity were calculated. Trolox was used as a positive control.

Screening results of the DPPH assay (Table 1) show large differences in free radical scavenging activity among the different seaweed species. Green seaweed (Chlorophyta) and red seaweed (Rhodophyta) were less active than brown seaweed (Phaeophyta), the DPPH scavenging activities in all the extracts from green seaweed and red seaweed by chloroform, ethyl acetate, acetone and methanol being less than 20%. On contrary, the methanol extracts of some brown seaweed extracts exceeded 50%, the most active seaweed species being *Hijiki fusiformis* (65%), followed by *Undaria pinnatifida* (51.1% for its sporophyll), and *Sargassum fulvellum* (36.3%). Although the methanol extract usually showed the highest antioxidative activity in many seaweed species, it has been demonstrated that the active compound in brown seaweed such as *Sargassum* was phlorotannins,[10] whose structure is very complex and difficult to elucidate. *Hijikia fusiformis* also contained a large amount of phlorotannins, up to 1.5% (evaluated by the Folin-Denis method with phloroglucinol as the reference compound; personal data), so the acetone extract of *Hijikia fusiformis* was used for further chemical characterization.

During preliminary separation of the acetone extract of *Hijikia fusiformis*, it was found that it could be separated well as a colored band by silica gel TLC, eluting with hexane/ethyl acetate (1:1). The band with the most active DPPH scavenging activity was the most polar part (R_f=0), belonging to polyphenols, while some green bands (R_f=0.15, 0.18, 0.56 and 0.70, respectively) showing moderate activities were chlorophylls. Only one bright yellow band (R_f=0.25–0.28), the most abundant in carotenoids, had moderate activity (up to *ca.* 30% of the total activity in the acetone extract), the other yellow bands failing to show any activity. This bright yellow pigment was presumed to be fucoxanthin based on its typical UV-vis spectrum, with a maximum absorption peak at 450 nm.

Purification and identification of the active compound in the *Hijikia fusiformis* acetone fraction was then carried out. A 200-g amount of lyophilized *Hijiki*

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Table 1. Radical Scavenging Activities in the DPPH assay of Common Species of Edible Seaweed

Sample			Radical scavenging activity %[c] (mean value, n=3)			
Latin name	Japanese name	Classification	$CHCl_3$	EtOAc	Me_2CO	MeOH
Ulva pertusa[a]	Ana-aosa	Chlorophyta	9.0	6.5	0.6	16.8
Ulva fasciata[a]	Rinbo-aosa	Chlorophyta	11.2	14.0	3.9	19.5
Gloiopeltis tenax[a]	Mafunori	Rhodophyta	4.4	7.3	0.6	17.6
Grateloupia elliptica[a]	Tanba-nori	Rhodophyta	4.1	6.4	3.3	17.5
Gelidium amansii[b]	Makusa	Rhodophyta	3.8	1.3	9.4	12.8
Undaria pinnatifida (blade)[a]	Wakame	Phaeophyta	23.0	6.0	13.2	36.4
U. pinnatifida (sporophyll)[a]	Mekabu	Phaeophyta	23.5	5.4	8.3	51.1
U. pinnatifida (stipe)[a]	Wakame	Phaeophyta	10.4	—	4.2	36.9
Laminaria japonica[a]	Makonbu	Phaeophyta	29.2	7.5	5.2	37.0
Hijikia fusiformis[a]	Hijiki	Phaeophyta	44.7	14.9	42.2	65.0
Hijikia fusiformis[b]	Hijiki	Phaeophyta	6.1	6.5	3.5	27.4
Sargassum fulvellum[a]	Hondawara	Phaeophyta	10.1	10.1	32.2	36.3

[a] lyphilized sample.
[b] air-dried sample.
[c] The error range of DPPH assay was 5–10%. The reference compound, Trolox, had DPPH radical scavenging activity of 27% at the same concentration at that of DPPH.

fusiformis was milled and extracted twice with 1 l of acetone. After filtration, the extract was evaporated to a small volume and loaded into a silica gel column (400 mm, 50 mm i.d.), which was eluted by hexane/ethyl acetate (1:1), the fractions with the colored bands developed in the column being collected. The active fractions were identified by the DPPH assay. An active fraction, bright orange in color, was collected and concentrated *in vacuo*, and separated by flash chromatography in a silica gel column (250 mm, 22 mm i.d.), eluting with chloroform/acetone (10:1). The major pigmented band was collected and purified twice by the same flash chromatography. This carotenoid pigment, after purification, was identified as all-*trans*-fucoxanthin (Fig. 1), because its ^{13}C-NMR spectral data were identical with those of an authentic compound of all-*trans*-fucoxanthin according to Englert *et al.* (1990).[11] ^{13}C-NMR spectral data are shown in Table 2. It was found that distortionless enhancement by polarization transfer (DEPT) spectroscopy increased the ^{13}C-NMR signal and separated some mixed signals, especially around 35–45 ppm, 60–70 ppm, and 120–140 ppm.

Carotenoids, including fucoxanthin, are well known antioxidants due to their singlet oxygen-quenching activity,[12] but this study has indicated that fucoxanthin is also an effective radical scavenger. Fucoxanthin is the richest carotenoid in *Hijikia fusiformis*; therefore, it is

Table 2. ^{13}C-NMR Spectral Data of Fucoxanthin. Data were recorded at 75.48 MHz with a DRX-300 spectrometer (Bruker, Karlsruhe, Germany) at room temperature in *d*-DMSO.

Carbon No.	δ (ppm)	DEPT[a]	Carbon No.	δ	DEPT[a]
1	35.51	C	2′	45.96	CH_2
2	48.27	CH_2	3′	67.72	CH
3	62.02	CH	4′	45.58	CH_2
4	42.12	CH_2	5′	71.00	C
5	66.60	C	6′	117.33	C
6	65.70	C	7′	201.84	C
7	41.44	CH_2	8′	102.35	CH
8	198.13	C	9′	132.97	C
9	134.38	C	10′	128.26	CH
10	139.33	CH	11′	126.11	CH
11	124.96	CH	12′	136.78	CH
12	144.91	CH	13′	137.84	C
13	135.73	C	14′	132.35	CH
14	136.35	CH	15′	132.70	CH
15	129.06	CH	16′	28.90	CH_3
16	27.91	CH_3	17′	30.69	CH_3
17	24.72	CH_3	18′	32.11	CH_3
18	20.86	CH_3	19′	13.94	CH_3
19	11.79	CH_3	20′	12.87	CH_3
20	12.68	CH_3	21′	169.96	C
1′	34.97	C	22′	21.26	CH_3

[a] CH and CH_3 were distinguished by both the DEPT experiment and chemical shift.

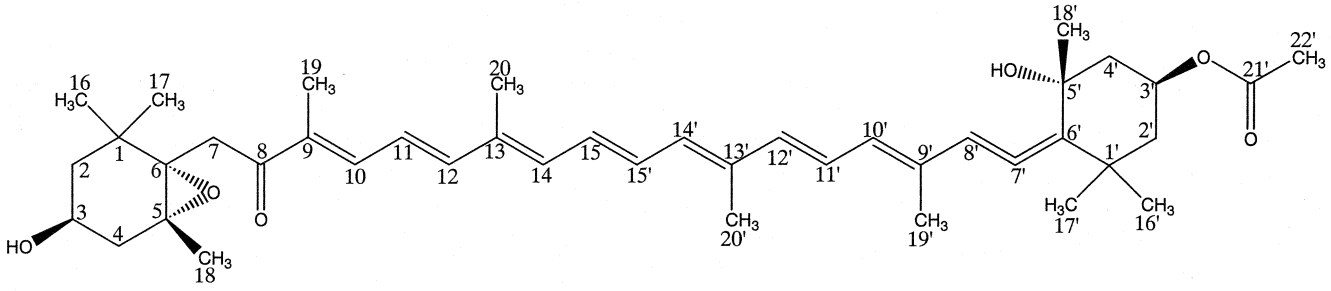

Fig. 1. Chemical Structure of All-*trans*-fucoxanthin.

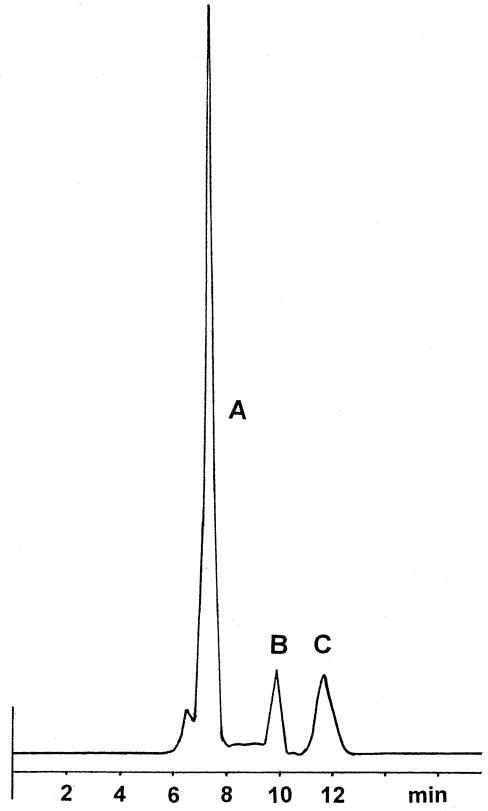

Fig. 2. HPLC Chromatogram of Purified Fucoxanthin.
Analytical HPLC of the purified fucoxanthin was conducted in a silica gel column (Spherisorb, gel size 5 μm, 4.6 × 250 mm) placed in a CO-965 (Jasco) thermostat at 40°C. A PU-980 (Jasco) pump and a UV-970 UV-Vis detector (Jasco) to detect at 450 nm was used. An isocratic solvent of chloroform/acetone (9:1) was applied at a flow rate of 1.0 ml/min with an injection volume of 10 μl.

regarded as a major antioxidant. Why only fucoxanthin had radical scavenging activity is not yet fully understood. From a structural point of view, fucoxanthin has an unusual double allenic carbon (C-7′, 201.84 ppm), and two hydroxyl groups. Nomura *et al.* (1997)[13] have also found that carotenoids such as β-carotene, β-cryptoxanthin, zeaxanthin and lutein did not show a DPPH-scavenging effect, except for fucoxanthin.

The purified pigment was subject to analytical HPLC, which showed that there were two minor components in the purified fucoxanthin, all-*trans*-fucoxanthin (A) along with two other peaks (B and C) appearing at 7.32 min, 9.88 min and 11.90 min, respectively (Fig. 2). Peaks B and C are believed to have been *cis*-isomers of fucoxanthin, as suggested by Haugan *et al.* (1992).[14] The ratio of all-*trans*-fucoxanthin to its two *cis*-isomers was calculated by peak area to be about 100:2:3.

Carotenoids, including fucoxanthin, have been demonstrated to have beneficial effects on health, such as anticarcinogenesis and antimutagenesis.[15,16] Fucoxanthin is one of the most abundant carotenoids in nature, especially in brown seaweed and diatoms, and its chemopreventive effects are well established.[17,18] It seems very worthwhile to explore the industrial utilization of fucoxanthin from seaweed resources.

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