

## Characterisation and Stability of Pigments Extracted from *Sargassum binderi* Obtained from Semporna, Sabah

(Pencirian dan Kestabilan Pigmen yang Diekstrak daripada *Sargassum binderi* dari Semporna, Sabah)

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### ABSTRACT

This study was carried out to identify the pigment extracted from Malaysian brown seaweed, *Sargassum binderi* and its stability in various conditions. Pigments were extracted using methanol:chloroform:water (4:2:1, v/v/v), which is part of fucoidan extraction process, where the pigments were waste. Carotenoid and chlorophyll were found in the extract using UV-vis spectrophotometer (420 and 672 nm, respectively). Fucoxanthin was identified as the carotenoid present using HPLC, while its functional groups and structure were determined using FTIR and <sup>1</sup>H NMR, respectively. The fucoxanthin-rich extract stability was tested on different pH (pH1-13), light exposure (dark and light) and storage temperature (4°C, 25°C and 50°C). The stability tests showed that it was most stable at pH5-7, stored in dark condition and at the storage temperature of 4°C and 25°C. The fucoxanthin-rich extract from *Sargassum binderi* has potential to be applied as bioingredient and functional food as it is stable in normal storage conditions.

**Keywords:** Carotenoid; chlorophyll; fucoxanthin; pigment; *Sargassum binderi*

### ABSTRAK

Kajian ini dijalankan untuk mengenal pasti pigmen yang diekstrak daripada rumput laut perang Malaysia, *Sargassum binderi* dan juga kestabilannya dalam pelbagai keadaan. Pigmen diekstrak menggunakan metanol:kloroform:air (4:2:1, v/v/v), yang merupakan sebahagian daripada proses pengekstrakan fukoidan, dengan pigmen merupakan bahan buangan/sisa. Kehadiran karotenoid dan klorofil telah ditentukan dengan menggunakan spektrofotometer UV-vis (masing-masing 420 nm dan 672 nm). Fukoxantin telah dikenal pasti sebagai karotenoid yang hadir dengan menggunakan kromatografi cecair prestasi tinggi (KCPT), manakala kumpulan berfungsi dan struktur fukoxantin masing-masing dikenal pasti dengan menggunakan spektroskopi transformasi Fourier inframerah (FTIR) dan spektroskopi resonans magnet nukleus proton (<sup>1</sup>H NMR). Kestabilan pigmen telah diuji pada beberapa parameter yang berbeza, iaitu pH (pH1-13), pendedahan cahaya (cerah dan gelap) dan suhu simpanan (4°C, 25°C dan 50°C). Ujian kestabilan menunjukkan bahawa pigmen adalah paling stabil pada pH5-7, disimpan dalam keadaan gelap dan pada suhu simpanan 4°C dan 25°C. Pigmen yang diekstrak daripada *Sargassum binderi* ini mempunyai potensi untuk diaplikasikan sebagai bioingredien dan makanan kerana ianya adalah stabil pada keadaan simpanan normal.

**Kata kunci:** Fukoxantin; karotenoid; klorofil; pigmen; *Sargassum binderi*

### INTRODUCTION

Seaweeds are excellent sources of bioactive compounds such as polyphenols, carotenoids and polysaccharides (Diplock et al. 1998; Lahaye, 1991; Shibata et al. 2002; Wijesinghe & Jeon 2011). These bioactive compounds can be applied in functional food, pharmaceuticals and cosmetic products as they bring health benefits to consumers (Pangestuti & Kim 2011; Sangha et al. 2013; Wijesinghe & Jeon 2011).

*Sargassum binderi* is a type of brown seaweed (Phaeophyceae) which is commonly found in Malaysia. According to Phang (1998), there are 21 species of *Sargassum* recorded in Malaysia. One of the bioactive compounds found in *Sargassum binderi* is fucoidan, a polysaccharide that contains L-fucose and sulfate, together with minor amounts of other sugars (Percival & McDowell

1967; Ponce et al. 2003). Fucoidan has been reported as anticoagulant agent (Chandia & Matsuhiro, 2008), antiviral agent (Li et al. 1995; Mandal et al. 2007) and showed antioxidant activity (Vo & Kim 2013; Zhao et al. 2005).

Before the fucoidan extraction, *Sargassum binderi* was treated with a solvent mixture of methanol:chloroform:water (4:2:1, v/v/v) at room temperature to remove coloured matter, fat and low molecular weight compounds (Lim et al. 2014). These compounds are considered as waste products of the fucoidan extraction. However, since the waste products removed from the *Sargassum binderi* contain coloured matter, it is therefore hypothesised that the coloured matter are the pigments of *Sargassum binderi*. The major pigment of brown seaweeds is fucoxanthin, which is one of the most abundant carotenoids in nature (10% estimated total production of carotenoids) (Pangestuti

& Kim 2011). It is an orange-coloured pigment, found in brown seaweeds along with chlorophyll, to give a brown or olive-green colour (Chandini et al. 2008; Hosokawa et al. 2009). Fucoxanthin has a unique structure, where it contains an unusual allenic bond and a 5,6-monoepoxide in its molecule. However, different brown seaweed strains produce different compositions and profile of fucoxanthin (Pangestuti & Kim 2011). Thus, it is suspected that the pigments from *Sargassum binderi* are rich in fucoxanthin. Previous studies reported that fucoxanthin has anticarcinogenic, antioxidation and antiobesity properties (Hosokawa et al. 1999; Maeda et al. 2005; Yan et al. 1999). Fucoxanthin is also a radical scavenger, whereby previous studies reported strong DPPH radical scavenging activity exhibited by fucoxanthin from various sources (Mise et al. 2011; Yan et al. 1999). It was also reported in a review paper by Pangestuti and Kim (2011) that there were no adverse effect of fucoxanthin in mice studies. Therefore, fucoxanthin is a pigment from brown seaweeds that has promising health enhancing properties and is suitable to be applied as bioingredient and functional food.

In order to use the pigments in the *Sargassum binderi* extract (waste) produced during fucoidan extraction, the types of pigments present in the seaweed extract must be determined first and its characteristics and stability should be studied. Hence, this research was aimed to determine the types of pigment present in the extract of *Sargassum binderi* from Semporna, Sabah and the stability of the pigment.

## MATERIALS AND METHODS

### MATERIALS

*Sargassum binderi* (originated from Semporna, Sabah, Malaysia – November 2010) was purchased from SIRIM Bhd (Shah Alam, Malaysia) in powder form. Fucoxanthin standard with purity of  $\geq 95\%$  was obtained from Sigma-Aldrich (St. Louis, USA). All other chemicals used in this research were analytical grade and purchased from Merck (Darmstadt, Germany) unless otherwise stated.

### PREPARATIONS OF SAMPLE

Pigments were extracted from dried *Sargassum binderi* powder (50 g) using 500 mL methanol:chloroform:water (4:2:1, v/v/v) at room temperature, for 24 h with mechanical stirring (Lim et al. 2014). The extract was collected in a Schott bottle and the residue was extracted with 500 mL methanol:chloroform:water (4:2:1, v/v/v) again for 3 times. The extracts were then combined and filtered before being centrifuged at 2500 rpm for 10 min (Kubota Table Top Centrifuge 2420) at room temperature to remove any solid residues (Dere et al. 1998). The seaweed extract was then evaporated to dryness at 30 to 35°C in a rotary evaporator until the final product was in the dried form (Heidolph, Laborota 4000 Efficient, Schwabach, Germany).

## CHARACTERISATION OF FUCOXANTHIN

*Uv-vis Spectrophotometric Analysis* The *Sargassum binderi* extract obtained was dissolved in methanol:water (1:9, v/v) and the pigment profile was determined using a double-beam UV-Visible spectrophotometer (Shimadzu UV-2450, Kyoto, Japan) at the wavelength range of 350-750 nm at room temperature. The wavelength range of 350-750 nm was chosen as most carotenoids absorb lights in the region between 400 and 500 nm and chlorophylls absorb light at 500 to 700 nm at room temperature (Dere et al. 1998). The yield of fucoxanthin in the crude extract was determined using a fucoxanthin standard curve (dissolved in methanol:water (1:9, v/v)) with concentrations of 1, 2, 4, 6, 8 and 10 µg/ml, at the wavelength of  $\lambda = 420$  nm.

*Purification of Fucoxanthin* The purification of fucoxanthin method was adopted from Noviendri et al. (2011) with some modification on the extraction solvent. The fucoxanthin-rich extract obtained was dissolved in adequate amount of methanol. The reconstituted fucoxanthin-rich extract was partitioned in a separation funnel between n-hexane and 90% (v/v) aqueous methanol for three times. The hexane phase was discarded. The aqueous phase was then partitioned with diethyl ether in a separation funnel to extract the fucoxanthin into the diethyl ether. The diethyl ether phase was evaporated to dryness using a rotary evaporator. The residue was re-dissolved in minimum amount of benzene (Noviendri et al. 2011). The benzene containing residue was loaded into an open column (300 mm, i.d. 24 mm) containing silica (Silica 60G, 0.040 - 0.063 mm, Merck). Elution was initially performed with n-hexane (100%) to remove chlorophyll and carotenoids other than fucoxanthin. Elution was continued with n-hexane:acetone (3:2, v/v) to recover fucoxanthin. Finally, residual fucoxanthin was eluted with absolute acetone. The hexane:acetone (3:2, v/v) and acetone fractions were pooled and evaporated to dryness using rotary evaporator (Noviendri et al. 2011).

*High Performance Liquid Chromatography (HPLC) Analysis* The purified fucoxanthin was dissolved in adequate amount of methanol. The presence of fucoxanthin in the *Sargassum binderi* was identified by comparing the retention time of fucoxanthin standard and purified fucoxanthin from *Sargassum binderi* using HPLC. The parameters of HPLC were obtained from Noviendri et al. (2011) with some modifications on the column brand and detector used. The HPLC Shimadzu system (Shimadzu LC-20AT) with auto-injector (Shimadzu Prominence HPLC Autosamplers SIL 20A HT) was equipped with a C18 column (Hypersil Gold, 5.0 µm particle size, 250 mm × 4.6 mm i.d.). The fucoxanthin was eluted using methanol:acetonitrile (7:3, v/v) mobile phase, with a flow rate of 1.0 mL/min, at 28°C. The eluent was detected using a UV-Vis detector (Shimadzu Prominence HPLC UV-Vis Detectors) at 450 nm (Noviendri et al. 2011).

**Fourier Transforms Infrared (FTIR) Spectroscopy Analysis** The functional groups present in the purified fucoxanthin were determined by Fourier transform infrared spectroscopy (FTIR) with Perkin Elmer Spectrum 400 FT-IR/FT-NIR & Spotlight 400 Imaging System at room temperature by comparison with fucoxanthin standard. The samples were ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets. A total of 4 scans were used on the samples in KBr pellets form, with a scanning range of 4000 - 650  $\text{cm}^{-1}$  and a resolution of 4  $\text{cm}^{-1}$  (Rodríguez-Jasso et al. 2011; Souza et al. 2012).

**Proton Nuclear Magnetic Resonance ( $^1\text{H}$  NMR) Analysis** Structure of fucoxanthin was determined using proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$  NMR). The purified fucoxanthin was dissolved in deuterated methanol (MeOD) and analysed using Fourier transform nuclear magnetic resonance 600 MHz equipped with Cryoprobe (Bruker/AVANCE III 600 MHz) under room temperature.

#### STABILITY STUDIES OF FUcoxANTHIN CRUDE EXTRACT

**Effect of pH** Fucoxanthin-rich extract in methanol was mixed with buffer solutions of different pH. All solutions were sealed in air-tight universal bottles and kept at 4°C for 4 weeks. The solvents used for buffer solutions preparation were shown in Table 1. The pH values were measured with a pH-meter.

**Effect of Light Exposure** Light exposure effect on fucoxanthin-rich extract stability was tested by comparing the absorbance of the extract (in methanol) in the dark and exposed to light conditions. Fucoxanthin-rich extracts in methanol form were kept in air-tight universal bottles with (dark condition) and without (exposed to light condition) sealing of aluminium foil. All bottles containing fucoxanthin-rich extracts were placed in a fume cupboard with a 20 W fluorescent lamp with the bottles at perpendicularly 1 meter below the light source, at room temperature.

**Effect of Storage Temperature** The effect of temperature on fucoxanthin-rich extract stability was tested by keeping

the fucoxanthin-rich extract (in methanol) inside air-tight universal bottles covered with aluminium foil and kept at 4°C (refrigerator), 25°C (laboratory bench) and 50°C (incubation oven).

The absorbance of fucoxanthin-rich extracts for all three pH, light and temperature effects were measured at 420 nm every week for four weeks. The absorbance represents the fucoxanthin concentration in the fucoxanthin-rich extract. The absorbance is directly proportional to the fucoxanthin concentration, meaning that the higher the absorbance the higher the fucoxanthin concentration in the fucoxanthin-rich extract.

#### STATISTICAL ANALYSIS

Stability tests were done in triplicates ( $n=3$ ) and data were obtained as the mean and standard deviation ( $SD$ ) and analysed using one-way ANOVA (two-tailed) followed by Duncan's multiple range test (DMRT) using SAS version 6.12 for Windows. Difference in mean values were considered significant when  $p<0.05$ .

#### RESULTS AND DISCUSSION

##### CHARACTERISATION OF FUcoxANTHIN

**Pigment Profile and Yield of Fucoxanthin** Two absorption peaks were shown at 420 nm and 672 nm in the UV-Vis spectrum (Figure 1). Different pigments absorb light at different wavelengths. Most carotenoids absorb light in the region between 400-500 nm (Mercadante 2008a) and chlorophylls absorb light at the wavelength around 660 nm (Marquez & Sinnecker 2008). It was also determined that the yield of fucoxanthin from *Sargassum binderi* was  $7.4 \pm 0.4$  mg/g sample. Based on the previous study by Mise et al. (2011), the yield of fucoxanthin from *Cladosiphon okamuranus* ranges from 39.6 to 270.0  $\mu\text{g/g}$ , depending on extraction solvent and particle size. Another study by Jaswir et al. (2012) reported that the fucoxanthin content in *Sargassum binderi* and *Sargassum duplicatum* are  $0.73 \pm 0.39$  and  $1.01 \pm 0.10$  mg/g, respectively. This shows that Malaysian *Sargassum binderi* has high fucoxanthin yield, thus suitable to be extracted for food applications.

TABLE 1. Weak acid/base and their conjugate base/acid in buffer solutions preparation

pH	Weak acid/base	Conjugate base/acid
1	0.2 M HCl	0.2 M KCl
3	0.2 M citric acid	0.2 M $\text{Na}_2\text{HPO}_4$
5	0.2 M citric acid	0.2 M $\text{Na}_2\text{HPO}_4$
7	0.2 M citric acid	0.2 M $\text{Na}_2\text{HPO}_4$
9	0.1 M NaOH	0.1 M boric acid
11	0.1 M NaOH	0.05 M $\text{Na}_2\text{HPO}_4$
13	0.2 M NaOH	0.2 M KCl

From the UV-Vis spectrum, this pigment extract consisted of higher proportion of carotenoid compared with chlorophyll. Since carotenoids have beneficial function and values in food and pharmaceutical industries, hence further analysis in this research was focused on the carotenoid. According to Chandini et al. (2008) and Hosokawa et al. (2009), fucoxanthin is the major carotenoid found in brown seaweeds. In order to determine whether the carotenoid present in pigment extract was fucoxanthin, fucoxanthin standard (Sigma-Aldrich) was dissolved in methanol:water (1:9, v/v) and analysed using UV-Vis spectrophotometer. The UV-Vis spectrum showed a maximum absorption peak at 423 nm (Figure 1). According to Papagiannakis et al. (2005), the absorbance of fucoxanthin ranges from 420-470 nm. Since the maximum absorption wavelengths of both the standard and sample were similar, this indicates that the carotenoid in the *Sargassum binderi* extract was fucoxanthin. The crude extract was purified using column chromatography and analysed using HPLC, FTIR and NMR to serve as further confirmation steps on the presence of fucoxanthin in this *Sargassum binderi* crude extract.

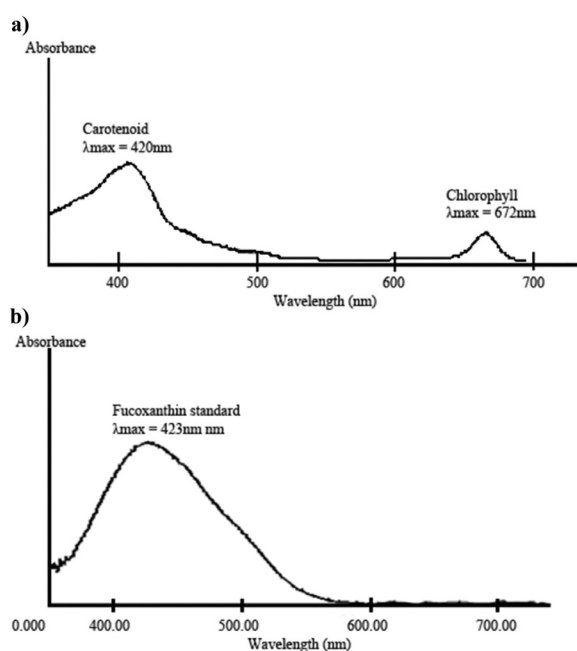


FIGURE 1. Absorption peaks of (a) fucoxanthin-rich extract from *Sargassum binderi* and (b) fucoxanthin standard (methanol:water (1:9, v/v)) (350-750 nm)

**High Performance Liquid Chromatography (HPLC) Analysis** The presence of fucoxanthin in fucoxanthin-rich extract from *Sargassum binderi* was identified using reversed-phase HPLC (RP-HPLC). From the chromatogram shown in Figure 2, the retention time for fucoxanthin from the purified fucoxanthin was 3.425 min and the retention time for fucoxanthin standard was 3.414 min. The similar retention time confirmed that both compounds were the same, i.e. fucoxanthin. The chromatogram also showed

that there were two minor components in the purified fucoxanthin. These two minor components are believed to be cis-isomers of fucoxanthin as suggested by previous studies (Yan et al. 1999; Zailanie & Purnomo 2011).

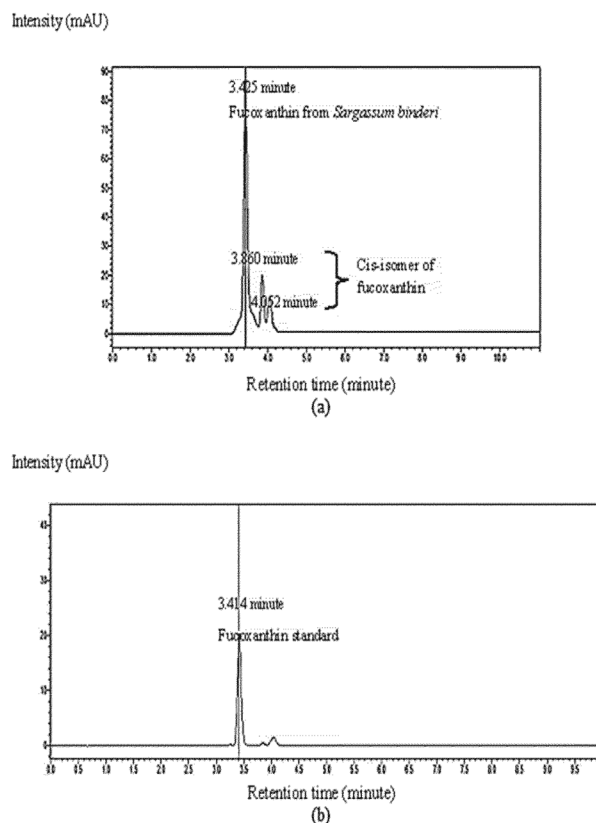


FIGURE 2. HPLC chromatogram of (a) purified fucoxanthin from *Sargassum binderi* and (b) fucoxanthin standard (methanol)

#### Fourier-transform Infrared (FTIR) Spectroscopy Analysis

The FTIR spectrum of purified fucoxanthin is shown in Figure 3 and it was found that the purified fucoxanthin showed similar absorption bands with fucoxanthin standard. The functional group that is descriptive of fucoxanthin is the presence of allenic bond ( $\text{C}=\text{C}=\text{C}$ ), where it is found in the spectrum at  $1929.21\text{ cm}^{-1}$  and  $1929.03\text{ cm}^{-1}$  for fucoxanthin standard and purified fucoxanthin from *Sargassum binderi* respectively (Pangestuti & Kim 2011). However, it must be noted that the presence of the allenic bond peak in the fucoxanthin from *Sargassum binderi* spectrum is very low. It is believed that the sample contains high amount of moisture which interfere with the infrared absorbance. The presence of moisture is attributed to the intense peak at  $3381.85\text{ cm}^{-1}$ , which is assigned to hydrogen bonded O-H symmetrical and asymmetrical stretching vibrations ( $\text{H}_2\text{O}$  contains the O-H bonds). This peak is significantly more intense compared to that found in the fucoxanthin standard spectrum. Besides that, the lump of peak from  $1528.19\text{ cm}^{-1}$  to  $1647.72\text{ cm}^{-1}$  in the fucoxanthin from *Sargassum binderi* spectrum is due to the bending vibrations of HOH,

which also indicate the presence of moisture in the sample (Silverstein & Webster 1998). This lump of peak is not observed in the fucoxanthin standard spectrum, where it has three sharp peaks at  $1645.82\text{ cm}^{-1}$ ,  $1606.88\text{ cm}^{-1}$  and  $1577.75\text{ cm}^{-1}$  respectively.

The absorption frequencies of purified fucoxanthin at  $3381.85\text{ cm}^{-1}$  and  $3011.90\text{ cm}^{-1}$  showed the presence of  $\text{-OH}$  bonds. Absorption at  $2956.34\text{ cm}^{-1}$  and  $2853.88\text{ cm}^{-1}$  showed the presence of alkanes with  $\text{C-H}$  bonds. Absorption at  $1732.54\text{ cm}^{-1}$  showed the presence of ketones with  $\text{-C=O}$  bonds. Absorption at  $1456.82\text{ cm}^{-1}$ ,  $1433.27\text{ cm}^{-1}$ ,  $1377.53\text{ cm}^{-1}$  and  $1363.65\text{ cm}^{-1}$  showed the presence of scissoring and bending alkanes with  $\text{-C-H}$  bonds. The presence of esters with  $\text{-C-O}$  bonds were presented by the absorption at  $1034.58\text{ cm}^{-1}$ . All these bonds are typically found in fucoxanthin (Zailanie & Purnomo 2011). Therefore, the presence of all these functional groups in the spectrum validates the UV-VIS and HPLC results that the purified fucoxanthin is indeed fucoxanthin.

#### Proton Nuclear Magnetic Resonance ( $^1\text{H}$ NMR) Analysis

The spectrum of chemical shift for purified fucoxanthin was shown in Figure 4 and the high-resolution peaks were identified with the functional groups in the molecule. The chemical shifts obtained from  $^1\text{H}$  NMR was also compared with the previous research of Zailanie and Purnomo (2011) using purified fucoxanthin from *Sargassum filipendula* (Table 2) and the predicted structure of the fucoxanthin molecule was shown in the inset of Figure 4. The comparison of chemical shifts present in the spectrum

shows that both are quite similar, thus allowing us to come out with the predicted structure of fucoxanthin (Zailanie & Purnomo 2011). However, there a few protons (4, 7, 19, 2' and 19' in Table 2) with chemical shifts that differ slightly from that of Zailanie and Purnomo (2011). This might be due to the different species of seaweed and also the different solvent used for NMR analysis (MeOD for current study, and  $\text{CDCl}_3$  for Zailanie and Purnomo (2011)).

#### STABILITY TEST ON FUCOXANTHIN-RICH EXTRACT

**Effect of pH** The stability of fucoxanthin is measured through its absorbance, where at higher the absorbance, more fucoxanthin are present. A lower absorbance signifies that the fucoxanthin had undergone chemical reactions, which renders it unstable and degrades or destroyed. Figure 5 shows that fucoxanthin-rich extract achieved the highest stability at pH6.1, which was its original pH (without treatment). The decrease of absorbance at pH1, 3, 9, 11 and 13 showed that the stability decreases in the high acidic and high alkaline condition. The absorbance of fucoxanthin was the least in pH1 suggested that fucoxanthin was relatively unstable in high acidic condition. The presence of acid causes pigment degradation by promoting trans-cis isomerization (Gliemmo et al. 2009; Ye & Eitenmiller 2006). Trans-cis isomerization occurred through the formation of carotenoid carbocation ( $\text{CarH}^+$ ) intermediate (Konovalov & Kispert 1999). Compared with sample kept in high acidic condition (pH1), samples kept in alkaline buffer (pH11 and 13) showed greater stability. According to Ye and Eitenmiller (2006), carotenoids are

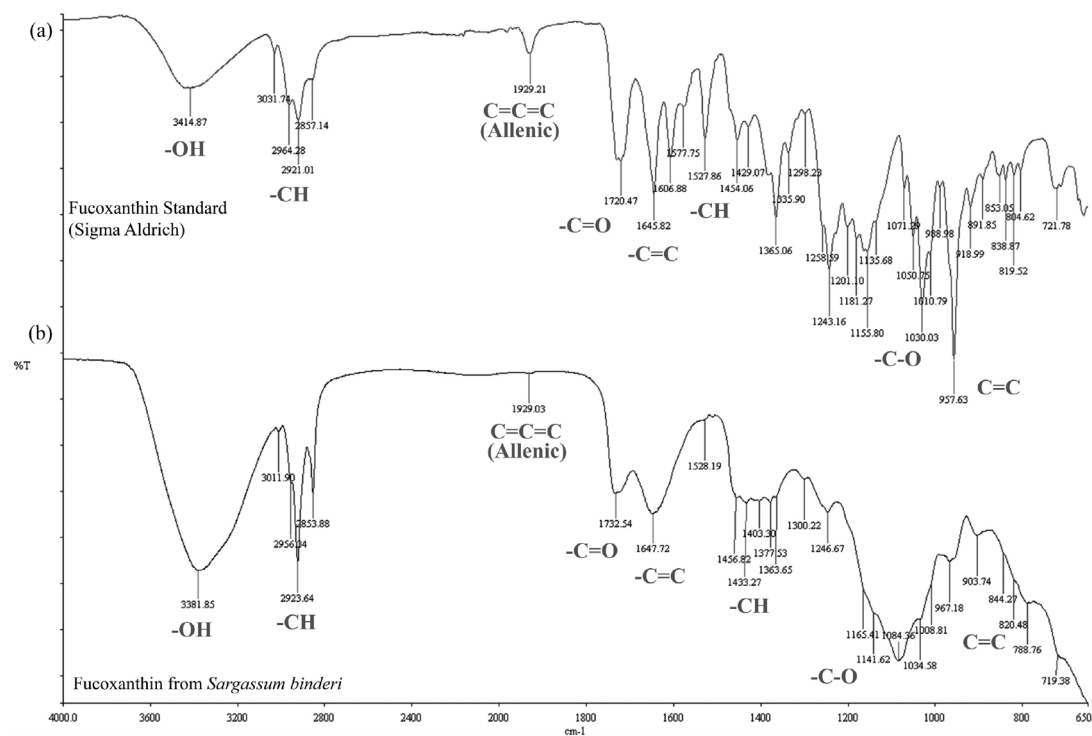


FIGURE 3. FTIR spectrum of (a) fucoxanthin standard (Sigma-Aldrich) and (b) purified fucoxanthin from *Sargassum binderi*

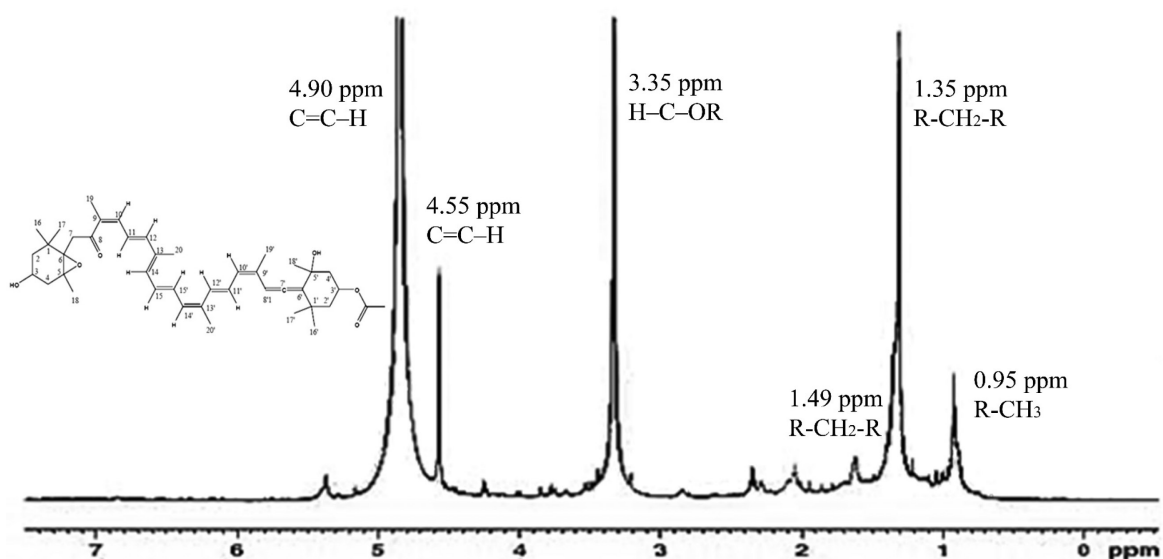


FIGURE 4.  $^1\text{H}$  NMR spectrum of purified fucoxanthin from *Sargassum binderi* and the predicted structure of fucoxanthin (inset)

TABLE 2. Comparison of chemical shift (ppm) between fucoxanthin from *Sargassum binderi* extract<sup>^</sup> and fucoxanthin from *Sargassum filipendula*<sup>#</sup>

Proton (Figure 4)	$^1\text{H}$ NMR fucoxanthin from <i>S. filipendula</i> extract (ppm) <sup>#</sup>	$^1\text{H}$ NMR fucoxanthin from <i>Sargassum binderi</i> extract (ppm) <sup>^</sup>
2	1.36	1.35
	1.49	1.49
3	3.80	3.80
	3.80	3.80
4*	1.77	1.65
	2.29	2.25
7*	2.59	-
	3.64	3.35
16	1.37	1.35
17	0.95	0.95
18	1.34	1.34
19*	1.80	1.85
20	1.98	1.95
2'*	1.41	1.35
	2.00	2.05
3'	5.37	5.40
4'	1.53	1.50
	2.29	2.30
16'	1.37	1.35
17'	1.06	1.05
18'	1.34	1.34
19'*	1.80	1.85
20'	1.98	1.95

<sup>^</sup> Current study – MeOD, 600 MHz

<sup>#</sup> Zailanie & Purnomo 2011 - CDCl<sub>3</sub>, 500 MHz

\* Proton with slight differ in chemical shift

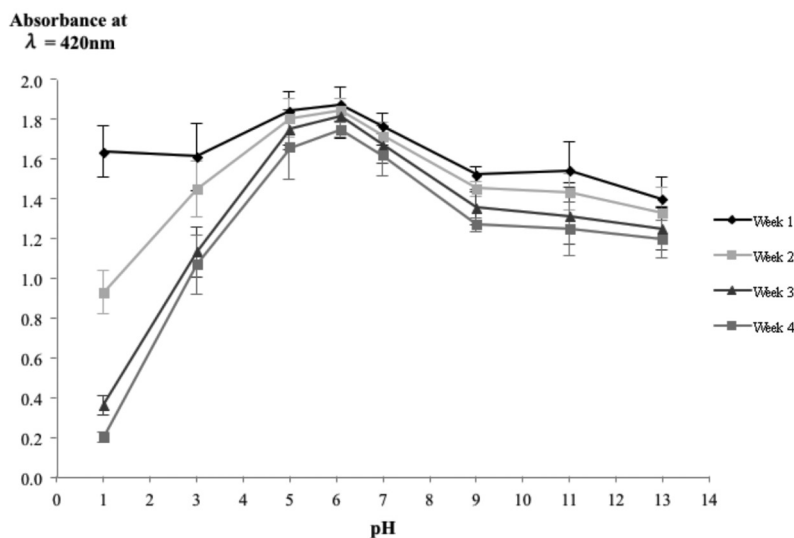


FIGURE 5. Comparison of fucoxanthin content in fucoxanthin-rich extract from *Sargassum binderi* ( $\lambda = 420\text{ nm}$ ) in regards to pH and time ( $n=3$ )

relatively stable at alkaline condition. A study conducted by Chen et al. (1996) reported that the rate of degradation of carotenoids were significantly slower at alkaline pH than at acidic pH. The absorbance of fucoxanthin-rich extract at pH5 and 7 did not differ significantly ( $p>0.05$ ) with pH6.1 throughout the experiment showing that the fucoxanthin-rich extract was suitable to be applied in food products at pH range of 5–7.

#### EFFECT OF LIGHT EXPOSURE

Light is another factor which has the great influence on the stability of carotenoids (Chen et al. 1996; Hii et al. 2010; Mercadante, 2008a; Rodriguez-Amaya, 2001; Ye & Eitenmiller 2006). Light effect on the fucoxanthin stability affects its use in food industries as light exposure may occur during processing and storage. The absorbance of fucoxanthin-rich extract kept in dark and exposed to light throughout the experiment was shown in Figure 6. The samples which were exposed to light showed more reduction in the absorbance value compared with the samples kept in the dark. This indicates that the fucoxanthin-rich extract was sensitive to light and its stability decreased when exposed to light.

Carotenoids undergo photodegradation and isomerization when exposed to light (Chen et al. 1996; Mercadante 2008b; Vásquez-Caicedo et al. 2007). Light can excite and lead to the formation of singlet oxygen. The singlet oxygen reacts with carotenoids to produce excited state carotenoids. The excited state carotenoids may follow a chemical degradation pathway that might involve a direct attack on the double bonds of the carotenoids by singlet oxygen, forming biradicals that can eventually lead to carbonyl chain cleavage products (Garavelli et al. 1998). Light can also promote trans-cis isomerisation reactions that caused pigment degradation.

**Effect of Storage Temperature** Different storage temperatures have different effects on carotenoid stability. There was no significant difference ( $p>0.05$ ) in terms of absorbance between fucoxanthin-rich extract kept at 4°C and 25°C throughout the experiment period (Figure 7). This indicates that fucoxanthin was stable when stored at low (4°C) and room (25°C) temperature. On the other hand, the absorbance of fucoxanthin-rich extract kept in 50°C was significantly ( $p<0.05$ ) lower than the other two samples stored in 4°C and 25°C from the third week onwards. The absorbance differences of the fucoxanthin-rich extract stored at 50°C to that of 4°C and 25°C are 19.2% and 17.0% respectively (results not shown). This indicates that the fucoxanthin was unstable at higher temperature and hence not suitable to be kept in temperature higher than room temperature. High temperature may cause the breakage of double bonds in the carotenoid molecule and caused pigment degradation (Boon et al. 2010; Mercadante, 2008b). Therefore, fucoxanthin-rich extract is more suitable to be applied into food products that are kept at room temperature or lower.

#### CONCLUSION

Fucoxanthin was found to be present in *Sargassum binderi* extract, which was detected using UV-Vis spectrophotometer at  $\lambda_{\text{max}} = 420\text{ nm}$  and identified using HPLC, FTIR and NMR. The yield of fucoxanthin from *Sargassum binderi* was  $7.4 \pm 0.4\text{ mg/g}$  sample. The fucoxanthin-rich extract from *Sargassum binderi* was found to be most stable in pH5 – 7, dark condition and it is suitable to be kept at room (25°C) or lower (4°C) temperature. Therefore, the fucoxanthin-rich extract from *Sargassum binderi* has potential to be applied in food products as bioingredient and functional food.

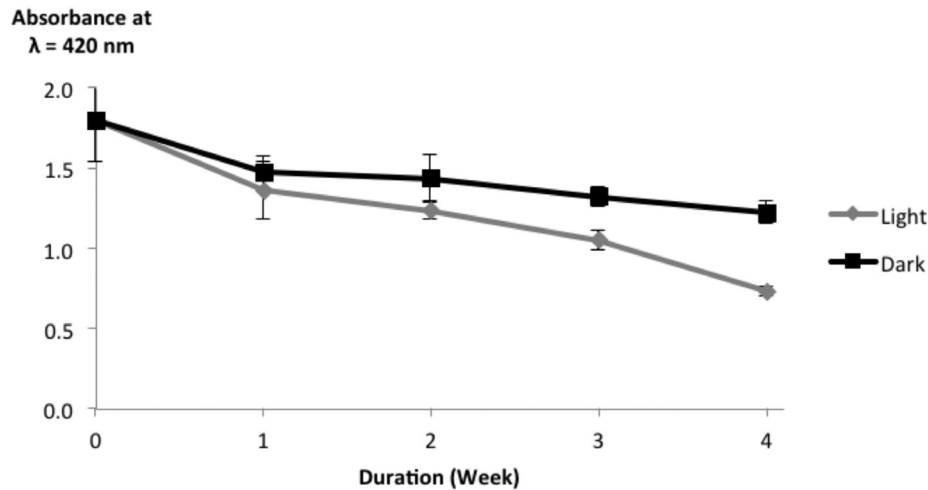


FIGURE 6. Comparison of fucoxanthin content in fucoxanthin-rich extract from *Sargassum binderi* ( $\lambda = 420$  nm) in regards to light exposure and time ( $n=3$ )

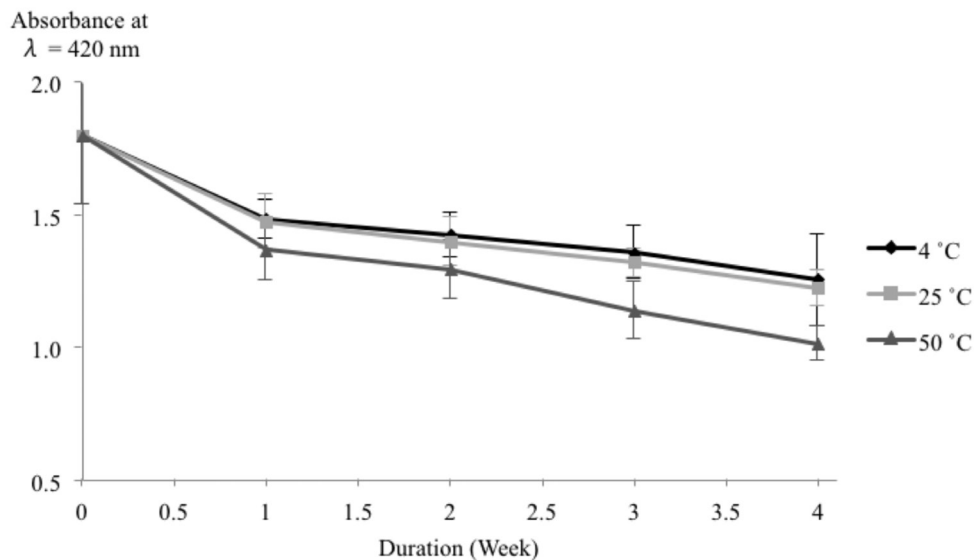


FIGURE 7. Comparison of fucoxanthin content in fucoxanthin-rich extract *Sargassum binderi* ( $\lambda = 420$  nm) in regards to temperature and time ( $n=3$ )

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#### REFERENCES

- Boon, C.S., McClements, D.J., Weiss, J. & Decker, E.A. 2010. Factors Influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition* 50: 515-532.
- Chandia, N.P. & Matsuhira, B. 2008. Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *International Journal of Biological Macromolecules* 42: 235-240.
- Chandini, S.K., Ganesan, P., Suresh, P.V. & Bhaskar, N. 2008. Seaweeds as a source of nutritionally beneficial compounds - A review. *Journal of Food Science and Technology* 45(1): 1-13.
- Chen, H.E., Peng, H.Y. & Chen, B.H. 1996. Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chemistry* 57(4): 497-503.
- Dere, S., Günes, T. & Sivaci, R. 1998. Spectrophotometric determination of chlorophyll - A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany* 22: 13-17.



- Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C. & Roberfroid, M. 1998. Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* 80: 77-112.
- Gliemmo, M.F., Latorre, M.E., Gerschenson, L.N. & Campos, C.A. 2009. Color stability of pumpkin (*Cucurbita moschata*, Duchesne ex Poiret) puree during storage at room temperature: Effect of pH, potassium sorbate, ascorbic acid and packaging material. *LWT - Food Science and Technology* 42: 196-201.
- Garavelli, M., Bernardi, F., Olivucci, M. & Robb, M.A. 1998. DFT study of the reactions between singlet-oxygen and a carotenoid model. *Journal of the American Chemical Society* 120: 10210-10222.
- Hii, S.W., Choong, P.Y., Woo, K.K. & Wong, C.L. 2010. Stability studies of fucoxanthin from *Sargassum binderi*. *Australian Journal of Basic and Applied Sciences* 4(10): 4580-4584.
- Hosokawa, M., Wanezaki, S., Miyauchi, K., Kurihara, H., Kohno, H. & Kawabata, J. 1999. Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. *Food Science and Technology Research* 5: 243-246.
- Hosokawa, M., Okada, T., Mikami, N., Konishi, I. & Miyashita, K. 2009. Biofunctions of marine carotenoids. *Food Science and Biotechnology* 18: 1-11.
- Jaswir, I., Noviendri, D., Salleh, H.M. & Miyashita, K. 2012. Fucoxanthin extractions of brown seaweeds and analysis of their lipid fraction in methanol. *Food Science and Technology Research* 18(2): 251-257.
- Konovalov, V.V. & Kispert, L.D. 1999. AMI, INDO/S and optical studies of carbocations of carotenoid molecules. Acid induced isomerization. *Journal of the Chemical Society, Perkin Transactions* 20: 901-909.
- Lahaye, M. 1991. Marine algae as sources of fibres: Determination of soluble and insoluble dietary fibre contents in some 'sea vegetables'. *Journal of the Science of Food and Agriculture* 54: 587-594.
- Li, F., Tian, T.C. & Shi, Y.C. 1995. Study on anti-virus effect of fucoidan *in vitro*. *Journal of Norman Bethune University of Medical Science* 21: 255-257.
- Lim, S.J., Wan Aida, W.M., Maskat, M.Y., Mamot, S., Ropien, J. & Mazita Mohd., D. 2014. Isolation and antioxidant capacity of fucoidan from selected Malaysian seaweeds. *Food Hydrocolloids* (in press). DOI: 10.1016/j.foodhyd.2014.03.007.
- Maeda, H., Hosokawa, M., Sashima, T., Funayama, K. & Miyashita, K. 2005. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications* 332: 392-397.
- Mandal, P., Mateu, C.G., Chattopadhyay, K., Pujol, C.A., Damonte, E.B. & Ray, B. 2007. Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica*. *Antiviral Chemistry & Chemotherapy* 18: 153-162.
- Marquez, U.M.L. & Sinnecker, P. 2008. Analysis of chlorophylls. In *Food Colorants: Chemical and Functional Properties*, edited by Socaciu, C. New York: CRC Press. pp. 429-446.
- Mercadante, A.Z. 2008a. Analysis of Carotenoids. In *Food Colorants: Chemical and Functional Properties*, edited by Socaciu, C. New York: CRC Press. pp. 447-478.
- Mercadante, A.Z. 2008b. Carotenoids in foods: Sources and stability during processing and storage. In *Food Colorants: Chemical and Functional Properties*, edited by Socaciu, C. New York: CRC Press. pp. 213-240.
- Mise, T., Ueda, M. & Yasumoto, T. 2011. Production of fucoxanthin-rich powder from *Cladosiphonokamuranus*. *Advance Journal of Food Science and Technology* 3(1): 73-76.
- Noviendri, D., Jaswir, I., Hamzah, M.S., Muhammad Taher, Kazuo, M. & Nazaruddin, R. 2011. Fucoxanthin extraction and fatty acid analysis of *Sargassum binderi* and *S. Duplicatum*. *Journal of Medicinal Plants Research* 5(11): 2405-2412.
- Pangestuti, R. & Kim, S.K. 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods* 3: 255-266.
- Papagiannakis, E., Van Stokkum, I.H.M., Fey, H., Büchel, C. & Van Grondelle, R. 2005. Spectroscopic characterization of the excitation energy transfer in the fucoxanthin-chlorophyll protein of diatoms. *Photosynthesis Research* 86(1): 241-250.
- Percival, E. & McDowell, R.H. 1967. *Chemistry and Enzymology of Marine Algal Polysaccharides*. New York: Academic Press.
- Phang, S.M. 1998. The seaweed resources of Malaysia. In *Seaweed Resources of the World*, edited by, Critchley, A.T. & Ohno, M. Japan: JICA. pp.79-91.
- Ponce, N.M.A., Pujol, C.A., Damonte, E.B., Flores, M.L. & Stortz, C.A. 2003. Fucoidans from the brown seaweed *Adenocystis utricularis*: Extraction methods, antiviral activity and structural studies. *Carbohydrate Research* 338: 153-165.
- Rodriguez-Amaya, D.B. 2001. *A Guide to Carotenoid Analysis in Foods*. USA: ILSI Press.
- Rodriguez-Jasso, R.M., Mussatto, S.I., Pastrana, L., Aguilar, C.N. & Teixeira, J.A. 2011. Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers* 86: 1137-1144.
- Sangha, J.S., Fan, D., Banskota, A.H., Stefanova, R., Khan, W., Hafting, J., Craigie, J., Critchley, A.T. & Prithviraj, B. 2013. Bioactive components of the edible strain of red alga, *Chondrus crispus*, enhance oxidative stress tolerance in *Caenorhabditis elegans*. *Journal of Functional Foods* 5: 1180-1190.
- Shibata, T., Yamaguchi, K., Nagayama, K., Kawagushi, S. & Nakamura, T. 2002. Inhibitory activity of brown algal phlorotannins against glycosidases from the viscera of the turban shell *Turbo cornutus*. *European Journal of Phycology* 37: 493-500.
- Silverstein, R.M. & Webster, F.X. 1998. *Spectrometric Identification of Organic Compounds*. 6th ed. New York: John Wiley & Sons, Inc. pp. 71-111.
- Souza, B.W.S., Cerqueira, M.A., Bourbon, A.I., Pinheiro, A.C., Martins, J.T., Teixeira, J.A., Coimbra, M.A. & Vicente, A.A. 2012. Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*. *Food Hydrocolloids* 27: 287-292.
- Vásquez-Caicedo, A.L., Schilling, S., Carle, R. & Neidhart, S. 2007. Impact of packaging and storage conditions on colour and  $\beta$ -carotene retention of pasteurised mango puree. *European Food Research and Technology* 224: 581-590.
- Wijesinghe, W. A. J. P. & Jeon, Y.J. 2011. Enzyme-assisted extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: A review. *Fitoterapia* 83(1): 6-12.
- Yan, X., Chuda, Y., Suzuki, M. & Nagata, T. 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Bioscience, Biotechnology and Biochemistry* 63(3): 605-607.

- Ye, L. & Eitenmiller, R.R. 2006. Fat-soluble vitamins. In *Handbook of Food Science, Technology, and Engineering*, edited by Hui, Y.H. USA: CRC Press. pp. 212-241.
- Zailanie, K. & Purnomo, H. 2011. Studi kandungan dan identifikasi fukosantin dari tiga jenis rumput laut cokelat (*Sargassum cinereum*, *Sargassum echinocarpum* dan *Sargassum filipendula*) dari Padike, Talongo, Sumenep, Madura. (Study of fucoxanthin content and identification from three types of brown seaweeds (*Sargassum cinereum*, *Sargassum echinocarpum* and *Sargassum filipendula*) from Padike, Talongo, Sumenep, Madura. *Berkala Penelitian Hayati Edisi Khusus* 7(A): 143–147 (In Indonesian).
- Zhao, X., Xue, C.H., Cai, Y.P., Wang, D.F. & Fang, Y. 2005. The study of antioxidant activities of fucoidan from *Laminaria japonica*. *High Technology Letters* 11: 91-94.

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