

Identification of fucoxanthin from brown algae (*Sargassum filipendula*) from Padike village, Talango district, Sumenep regency, Madura islands, using nuclear magnetic resonance (NMR)

¹*Zailanie, K. and ²Purnomo, H.

¹Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, East Jawa, Indonesia.

²Department Animal Food Technology, Faculty of Animal Husbandry, Brawijaya University, Malang, East Jawa, Indonesia

Article history

Received: 3 September 2015

Received in revised form:

7 March 2016

Accepted: 17 March 2016

Abstract

Thallus color on brown algae (*S. filipendula*) is influenced by the different types of pigments and pigment content therein dominant. Therefore, brown algae (*S. filipendula*) that have been identified using column chromatography (CC), then analyzed by using High Performance Liquid Chromatography (HPLC), Fourier Transform Infra-Red (FTIR), and Nuclear Magnetic Resonance (NMR) to determine the molecular structure of the pigment content and the dominant fucoxanthin from the stems, leaves and whole parts of plant from brown algae (*S. filipendula*). One to six, 7-62, 63-78, 79-135 types of pigment have been separated from *S. filipendula* respectively, in a single work step by Column Chromatography (CC) using a gradient elution of hexane:ethyl acetate (5:5 v/v) to obtain the separation of pigments. Identification of pigments made by order of polarity, R_f values, and structure of each pigment molecule compared to the literature data that uses samples of brown algae (*S. filipendula*) and mobile phase were relatively the same. Type of brown algae was then explored for the stems, leaves, and whole part of plant, the leaves that have been proven to have highest fucoxanthin content (1.08 ± 0.080). FTIR identification of fucoxanthin crude extract showed the functional groups of OH, CH, C=O, C=C, CH₂, C=O, COC and C=C trans substituted and isolated fucoxanthin had functional groups of OH, CH, allenic bond, C=O, C=C, CH₂, COC and C=C trans substituted. HPLC analysis results showed the chromatogram peaks increase linearly and with spiking can be proved that the samples are identified as fucoxanthin. While NMR analysis showed the presence of O₂ at 5.6 epoxide groups shown in the C5 and C6 atoms and the compounds were allene and acetate.

Keywords

Fucoxanthin

Brown algae (*S. filipendula*)

HPLC, FTIR

¹H-NMR

¹³C-NMR

© All Rights Reserved

Introduction

Brown algae are very popular, especially in Japan, China, Korea, and as one of the main components in the daily diet (Sachindra *et al.* 2010). Brown algae (*S. filipendula*) is one type of brown algae that grows scattered in Indonesian waters, especially in waters that are coral reefs, and is not widely used by the community (Handayani *et al.* 2004). *S. filipendula* contain polysaccharides, minerals and vitamins (Ruperez and Calixto, 2001). Besides the brown algae contain carotenoid pigments as a source of antioxidants and it is also anticancer as reported by Mori *et al.* (2004). Sachindra *et al.* (2007) found fucoxanthin isolated from wakame (brown algae) which have antioxidant activity. Furthermore, Sachindra *et al.* (2010) suggested efforts to increase the utilization of brown algae for foods, because the brown algae have been shown to contain antioxidants that are useful for human health. Christiana *et al.* (2008) have also identified antioxidants in the brown algae

Udotea fucoxanthin, *Amphiroa rigida* and *Turbinaria conoides* and they reported that fucoxanthin has an absorption properties at a wave length of 446 and 468 nm. Basically, brown algae contain chlorophyll and carotenoid pigments (β-carotene and fucoxanthin) (Goodwin, 1974; Atmadja *et al.* 1996).

However, research on the various pigments and fucoxanthin content in brown algae that grows in the waters of the Madura islands has not been done. Therefore, it becomes very important to do exploratory research on fucoxanthin from brown algae. The aims of this study were to determine the content of fucoxanthin of the stems, leaves and whole parts of brown algae *S. filipendula* and characterization of fucoxanthin from the leaves of brown algae *S. filipendula* by using Nuclear Magnetic Resonance (NMR).

The results showed the discovery of a suitable solvent for extraction and purification to obtain high purity of fucoxanthin. Physicochemical characteristics of fucoxanthin were expected to be a

*Corresponding author.

Email: kartindocor@gmail.com

reference basis for the isolation and purification of fucoxanthin with high amount and purity, and such methods can be suggested for other types of brown algae.

Materials and Methods

The materials used in this study were fresh brown algae *S. filipendula* obtained from the Padike Village, Talango District, Sumenep Regency, Madura Islands, East Java. Coordinates of sampling sites in Padike Village for the brown algae measured by the Global Positioning System (GPS) and decision-transect method. The chemicals used were DMSO (Dimethylsulfoxide), acetone, methanol, n-hexane, ethyl acetate, diethyl ether, Isoproponil Acid (IPA), and toluene, and all of them were Pro Analysis grade (PA, Merck).

Samples preparation and extraction of pigment

Pigment extraction procedure using Seely *et al.* (1992) was modified. The samples (*S. filipendula*) used in this study were taken from the Padike Village waters. Brown algae that was used for the first experiment was part of leaves, while the second experiment used the stems, leaves and whole part of plant.

The first stage, namely the brown algae washed, purged from impurities, and dried with a tissue, brown algae was diced with thickness of ± 1 cm, then weighed as much as 25 g to facilitate the extraction (sample was easily mixed with solvent). Thus the pigment extract can be obtained optimally. Samples were crushed with a mortar and CaCO_3 was added as neutralizing reagent.

The next stage, the brown algae was extracted with DMSO (Dimethylsulfoxide) (1:10, w/v) for 20 min and then filtered with filter paper (Whatman 40) and labeled as X_1 (DMSO extract volume). X_1 fraction partitioned (separation) in a separating funnel with ethyl acetate (1/2 extract volume of X_1) and 0.5 M ammonium sulfate $[\text{NH}_4 (\text{SO}_4)_2]$ (a volume of X_1 extract). While the upper phase partitioning results was further partitioned again with the same solvent as above.

If lower phase is still colored, therefore twice partition was needed with 10 ml diethyl ether and added saturated salt of ammonium sulfate and distilled water to enhance the separation. The results of partitioning phase were collected and evaporated with a vacuum rotary evaporator at 30°C and 100 rpm.

The result of the evaporation was added with anhydrous Na_2SO_4 to absorb water, and then dried

with argon gas and dry pigment extract was obtained (X_1). The rest of the DMSO extract or X_1 residue then re-extracted with acetone (1:10, w/v) at 100 rpm for 10 minutes and the crude extract was then filtered with filter paper (Whatman 40). The rest of the extract or residue was again extracted with acetone and added with distilled water (until all residue submerged) (1:10, w/v), and it was extracted at a speed of 100 rpm for 10 minutes. Extraction results was filtered with filter paper (Whatman 40) and labeled as X_2 . Volume of X_2 extract was then partitioned with hexane (1/3 volume of X_2) and distilled water (1/4 volume of X_2). Upper phase was partitioned twice with 75% methanol (1/9 volume of X_2). Upper phase again partitioned with 80% methanol (1/9 volume of X_2) and phase on the results of the partition labeled as X_{2A} fraction (hexane phase) and lower phase fraction was labeled as X_{2B} (phase of acetone-methanol-distilled water). X_{2A} fractions were dried with argon gas and dry pigment extracts obtained was X_{2A} . While, X_{2B} fraction (acetone-methanol-distilled water) was partitioned with diethyl ether (2/5 volume of X_{2B}) and added with saturated salt (anhydrous Na_2SO_4) and distilled water to enhance the separation. Upper phase, plus X_{2B} fraction, then dried with argon gas and dry pigment extracts obtained was labeled as X_{2B} . Bottle containing dry pigment extracts were wrapped in aluminum foil and stored in a freezer.

Dry extract of fucoxanthin that has been obtained from the stems, leaves and whole parts of plant *S. filipendula* were analyzed and the type and part of the plant that contain the highest fucoxanthin were determined.

Fucoxanthin analysis by using chromatography columns

Fucoxanthin isolation of the leaves from *S. filipendula* that grow predominantly in Padike Village was used in column chromatography with silica gel as stationary phase and mobile phase was hexane: ethyl acetate (6:4, v/v) ± 100 ml. Silica gel was weighed as much as 20 g and suspended with mobile phase of hexane: ethyl acetate (6: 4, v/v) ± 50 ml and stirred with a magnetic stirrer for 1 hour, so that when the silica gel was filled into the column does not crack. While it was mounted to the column and to begin with a little stationary and mobile phase using wet cotton. The next phase, mobile phase through the column filled nearly full slowly through the column wall. Slurry of silica gel column chromatography incorporated into fully formed until the column, and a column of silica gel in achieving as high as $\frac{3}{4}$ columns. The next phase, sea sand included in the objectives that were not on silica gel and as

a filter when the sample is introduced. Dry samples were dissolved in 0.5 ml of mobile phase hexane: ethyl acetate (6: 4, v/v) further, put in the column slowly so was not to damage the condition of silica gels. Column faucet was opened and the color bands were formed and accommodated in a test tube of each color corresponding with a mobile phase being added little by little so was not to dry silica gel.

Fucoxanthin analysis by using HPLC

Quantitative analysis of brown algae fucoxanthin using HPLC was also carried out on isolation results using the specific column for fucoxanthin and as a comparison using standard of fucoxanthin.

Fucoxanthin analysis by using FTIR

FTIR performed on crude extracts of brown algae that grows predominantly in Padike village, also on fucoxanthin as result of isolation and purification (stems, leaves and whole parts of plant). FTIR of crude extract of algae was compared to the isolated fucoxanthin from chromatography column.

Determination of molecular structure of fucoxanthin by using ¹H-NMR and ¹³C-NMR

Molecular structure of isolated fucoxanthin from Sargassum leaves was determined with methods of ¹H-NMR and ¹³C-NMR. While test to determining the location of the proton in the molecule by using ¹H-NMR (JEOL 2005).

Data analysis

Fucoxanthin of brown algae *S. filipendula* was further tested on parts of the stems, leaves, and whole parts of plant (intact). Experiment design used was Completely Randomized Design (CRD) with 3 replications of experiments and 3 replications of analysis, and the collected data were analyzed using SPSS.

Results and Discussion

Fucoxanthin analysis result by using HPLC

Analysis of the pigment composition of brown algae was also done using HPLC, and the chromatogram of crude extract of brown algae, fucoxanthin standards and co-chromatogram of fucoxanthin standard and fucoxanthin crude extract of *S. filipendula* detected at a wavelength of 450 nm as shown in Figure 1.

Fucoxanthin analysis result by using FTIR

Results of column chromatography showed that the condition column was 60 cm long and 3.5 cm

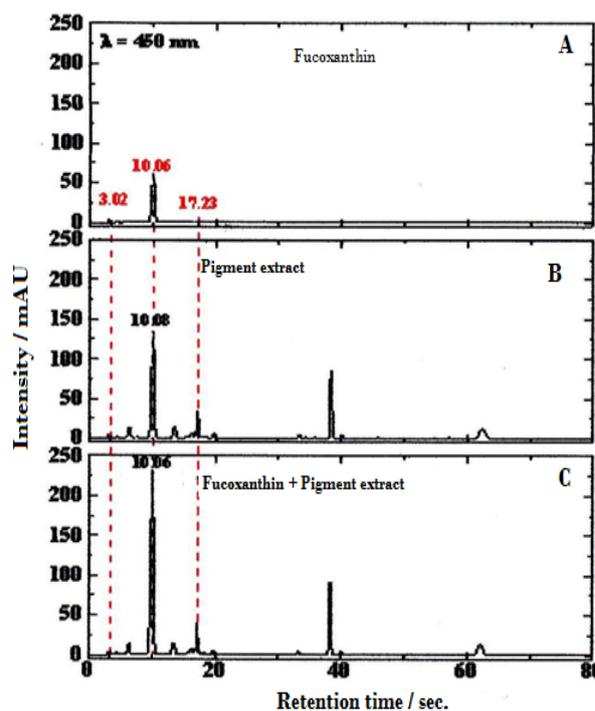


Figure 1. HPLC chromatograms of the crude extract pigment (A) fucoxanthin standard, (B) crude pigment extract of *S. filipendula*, (C) co-chromatogram of fucoxanthin standard and crude pigment extract of *S. filipendula*.

diameter, with a mobile phase of silica gel (20 g) with the stationary phase using hexane: ethyl acetate (6:4). (A) = V_{max} (cm^{-1}) of fucoxanthin extract (*S. filipendula*) based on the FTIR test, and it was conducted in LIPI (Indonesia Institute of Science), Serpong, Jakarta. Hydroxyl group (OH) was characterized by a strong broad absorption in the area of 3423.41 cm^{-1} for leaves, 3434.98 cm^{-1} for the stems and on the area 3426.30 cm^{-1} for the whole parts of plant. OH bond in the carboxylic acid obtained in the wave number of 2958.60 ; 2925.81 ; and 2854.45 cm^{-1} for the leaves, 2921.96 ; 2856.58 ; and 1733.89 cm^{-1} for the stems and 2958.60 ; 2925.81 ; and 2854.45 cm^{-1} for seaweed intact as shown in Table 1.

Identification Results of fucoxanthin from S. filipendula Leaves by using NMR

Bernhard *et al.* (1976) in his research on group's fucoxanthin from algae, also reported that these compounds were characterized by the presence of 5.6 epoxide groups, in the form of trans ce and groups at the C5 and C6 atoms, so it has the 5R configuration. While the sample under study was also found fucoxanthin 5.6 epoxide groups, which contained C5 and C6 atoms, so it can be concluded that the samples of fucoxanthin in this study was also in the form of trans with 5R configuration.

Fucoxanthin compounds were extracted and

Table 1. Functional groups of fucoxanthin samples from *S. filipendula* identified using Fourier Transform Infra-Red (FTIR) Spectrometer

No.	Type of fibration	Wave Numbers (cm ⁻¹)
		V _{max} (cm ⁻¹) (A)
1	OH stretching (4000 – 3200 cm ⁻¹)	3462
2	CH stretching, hydrogen bonded carboxylic acid / CH asymmetric stretching CH ₃ (3300 – 2500 cm ⁻¹)	2923, 2853
3	Allene	1930
4	C=O, asetat C=O, stretching alifatik ester (1750 – 1725 cm ⁻¹)	1742 1732
5	C=C conjugated	1650
6	C=O, conjugation	1654
7	CH ₂	1527, 1459
8	C-O, acetate	1242
9	C=C, Trans substituted	1201, 1175-1157, 1071, 1053, 1032, 958

identified in this study showed a shift in the structure of the chemical similarity to those reported by Yan *et al.* (1999) and Heo *et al.* (2008) was between 35-45, 60-70, and 120-140 ppm, respectively. In this study shifts the C71 atoms, amounting to 202.4 ppm indicating the presence of compounds allenic (C=C or C quarter), while Yan *et al.* (1999) reported that only shift 201.84 ppm. The big difference was apparently due to a shift in the location of the different sampling due to geological factors.

Strengthening the notion that compound 1 is fucoxanthin also performed with ¹³C-NMR measurements. ¹³C-NMR measurement results also showed 11 methyl group were at δ 25.0; 28.1; 21.2; 11.8; 12.8; 29.2; 32.1; 31.3; 14.0 and 12.9, respectively. Acetyl group signals appear at δ 23.19, and 170.4. Signals on δ 202.9; 198.41; 66.32; 67.32; 67.23 and 72.20, respectively showed the presence of a quaternary C atom, carbonyl and hydroxyl. NMR analysis was conducted to determine the frame work of hydrogen molecules of carbon atoms that form the structure of a compound. 0:00 ppm chemical shift is a shift TMS as an internal standard to calibrate the chemical shift of each proton were analyzed. Some research's show that most identification fucoxanthin (C₄₈H₅₆O₆) used spectrophotometric methods (UV, IR and NMR) and Liasen and Jensen (1989), in their study reported on brown algae that contain carotene had a group of Phaeophyceae Dicyclic xanthophylls, 5.6 epoxide, Allene, Acetate, and keto. The results of the analyzed samples of fucoxanthin found the presence of oxygen (O₂ group) at 5.6 epoxide, and it was shown in C5 and 6C atoms to the presence of group O.

Allene and acetate compounds and keto as shown in Figure 2.

¹H-NMR spectrums data

Table 2 shows the presence of 11 methyl singlet

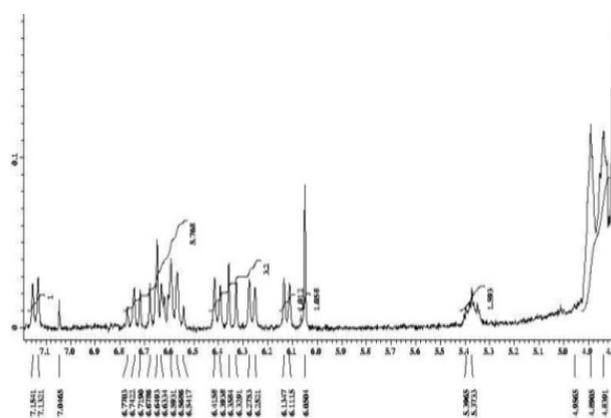


Figure 2. ¹H-NMR spectrums of fucoxanthin

form (s) that was at 1.37 (16-CH₃, s), 0.95 (17-CH₃, s), 1.34 (18-CH₃, s), 1.86 (19-CH₃, s) and 2.0 (20-CH₃, s). Another methyl-methyl at C-16'-C-20' which appears at 1.07; 0.92; 0.99; 1.34; 1.98 and 2.03, respectively. The presence of an acetyl group (CH₃-CO-), which appears at 2.05 (s), 1 Methyl in proton (=CH) in the form of a singlet at 6.07 (s, H-8'), and 10 protons (H) of a double bond (H-C=), namely the H7.14 (H-10, d, J=12.8), 6.58 (H-11, m), 6.66 (H-12, t, J=12.8), 6.40 (H-14, d, J=12.8), and 6.67 (H-15, m). Besides, the presence of considerable value downfield chemical shearing of a hydroxyl methyl (H-C-OH) was at 3.65 (m, H-3) and 5.39 (tt, J = 8, 11.5 Hz, H-3'). Confirmation fucoxanthin molecular structure and chemical shift values prediction was done by measuring the ¹H-NMR spectrums with solvent CDCl₃ (500MHz). Comparison of the results of ¹H-NMR spectra and chemical shift values (δ) for fucoxanthin. Phase IV study, Daley and Daley (2005) suggested that the chemical shift of 0.00 ppm is the chemical shift of tetramethylsilane (TMS) as an internal standard compound which serves to calibrate the chemical shift of each proton were analyzed.

Singlet peaks of the up field chemical shift range was δ 1.37; 0.95; 1.34; 1.86; 2.0; 1.07; 0.92; 0.99;

Table 2. Fucoxanthin spectrums data of ¹H-NMR

No	¹ H-NMR (δ, m, Hz) Fucoxanthin	¹ H-NMR (δ, m, Hz) Compound 1
1		
2	1,36 (1H, dd, J = 8,7; 14,2) 1,49 (1H, dd, J = 14,2)	1,33 m 1,44 m
3	3,80 (1H, m)	3,85 (1H, m)
4	1,77 (1H, dd, J = 8,7; 14,2) 2,29 (1H, dd, J = 2,9; 17,8)	1,67 (1H, dd, J = 9,5; 14,5) 2,21 (1H, m)
5		
6		
7	2,59, 3,64 (2H, d, J = 20,4)	(d 11) 3,65 (m)
8		
9		
10	7,14 (1H, d, J = 12,8)	7,36 (1H, d, J = 11,5)
11	6,58 (1H, m)	6,76 (1H, t, J = 11,5)
12	6,66 (1H, t, J = 12,8)	6,74 (1H, d, J = 11,5)
13		
14	6,40 (1H, d, J = 11,6)	6,38 (1H, d, J = 11,5)
15	6,67 (1H, m)	6,86 (1H, t, 11,5)
16	1,37 (3H, s)	1,37 (3H, s)
17	0,95 (3H, s)	0,95 (3H, s)
18	1,34 (3H, s)	1,34 (3H, s)
19	1,80 (3H, s)	1,86 (3H, s)
20	1,98 (3H, s)	2,0 (3H, s)
1'		
2'	1,41 (1H, dd, J = 10,4; 14,9) 2,00 (1H, dd, J = 2,9; 14,9)	1,37 (1H, m) 2,17 (1H, m)
3'	5,37 (1H, m, J = 8,8; 12,0)	5,39 (1H, m, J = 8,0; 11,6)
4'	1,53 (1H, dd, J = 10,4; 14,9) 2,29 (1H, dd, J = 2,9; 17,8)	1,45 (1H, m) 2,20 (1H, m)
5'		
6'		
7'		
8'	6,04 (1H, s)	6,07 (1H, s)
9'		
10'	6,12 (1H, d, J = 11,6)	6,18 (1H, d, J = 11,5)
11'	6,71 (1H, t, J = 12,0)	6,69 (1H, t, J = 11,5)
12'	6,34 (1H, d, J = 11,6)	6,50 (1H, d, J = 11,5)
13'		
14'	6,26 (1H, d, J = 11,6)	6,34 (1H, d, J = 11,5)
15'	6,71 (1H, t, J = 12,0; 14,2)	6,82 (1H, dd, J = 11,5; 15,3)
16'	1,37 (3H, s)	1,07 (3H, s)
17'	1,06 (3H, s)	0,92 (3H, s)
18'	1,34 (3H, s)	0,99 (3H, s)
19'	1,80 (3H, s)	1,34 (3H, s)
20'	1,98 (3H, s)	1,98 (3H, s)
3'-OAc, -CH ₃	2,03 (3H, s)	2,03 (3H, s)
3'-OAc, - C=O		
No	¹ H-NMR (δ, m, Hz) Fucoxanthin	¹ H-NMR (δ, m, Hz) Compound 1
1		
2	1,36 (1H, dd, J = 8,7; 14,2) 1,49 (1H, dd, J = 14,2)	1,33 m 1,44 m
3	3,80 (1H, m)	3,85 (1H, m)
4	1,77 (1H, dd, J = 8,7; 14,2) 2,29 (1H, dd, J = 2,9; 17,8)	1,67 (1H, dd, J = 9,5; 14,5) 2,21 (1H, m)
5		
6		
7	2,59, 3,64 (2H, d, J = 20,4)	(d 11) 3,65 (m)
8		
9		
10	7,14 (1H, d, J = 12,8)	7,36 (1H, d, J = 11,5)
11	6,58 (1H, m)	6,76 (1H, t, J = 11,5)
12	6,66 (1H, t, J = 12,8)	6,74 (1H, d, J = 11,5)
13		
14	6,40 (1H, d, J = 11,6)	6,38 (1H, d, J = 11,5)
15	6,67 (1H, m)	6,86 (1H, t, 11,5)
16	1,37 (3H, s)	1,37 (3H, s)
17	0,95 (3H, s)	0,95 (3H, s)
18	1,34 (3H, s)	1,34 (3H, s)
19	1,80 (3H, s)	1,86 (3H, s)
20	1,98 (3H, s)	2,0 (3H, s)
1'		
2'	1,41 (1H, dd, J = 10,4; 14,9) 2,00 (1H, dd, J = 2,9; 14,9)	1,37 (1H, m) 2,17 (1H, m)
3'	5,37 (1H, m, J = 8,8; 12,0)	5,39 (1H, m, J = 8,0; 11,6)
4'	1,53 (1H, dd, J = 10,4; 14,9) 2,29 (1H, dd, J = 2,9; 17,8)	1,45 (1H, m) 2,20 (1H, m)
5'		
6'		
7'		
8'	6,04 (1H, s)	6,07 (1H, s)
9'		
10'	6,12 (1H, d, J = 11,6)	6,18 (1H, d, J = 11,5)
11'	6,71 (1H, t, J = 12,0)	6,69 (1H, t, J = 11,5)
12'	6,34 (1H, d, J = 11,6)	6,50 (1H, d, J = 11,5)
13'		
14'	6,26 (1H, d, J = 11,6)	6,34 (1H, d, J = 11,5)
15'	6,71 (1H, t, J = 12,0; 14,2)	6,82 (1H, dd, J = 11,5; 15,3)
16'	1,37 (3H, s)	1,07 (3H, s)
17'	1,06 (3H, s)	0,92 (3H, s)
18'	1,34 (3H, s)	0,99 (3H, s)
19'	1,80 (3H, s)	1,34 (3H, s)
20'	1,98 (3H, s)	1,98 (3H, s)
3'-OAc, - CH ₃	2,03 (3H, s)	2,03 (3H, s)
3'-OAc, - C=O		

1.34; 1, 98; 2.03 a methyl groups on the H atom positions 16, 17, 18, 19, 20, 16', 17', 18', 19' and 20', respectively. Chemical shift of the alkyl group has a wide range depending on the group and other atoms are bound or directly. C atoms at positions 16, 17, 16' and 17' are methylene groups (-CH₂-) a cyclic

C6 alkane chain where electron resonance of protons (H) and electrons of an atom environment shield protons of the influence of the external magnetic field. This causes the signal to the methylene group raises up field region. The greater the shielding effect of the position of the proton resonance signal

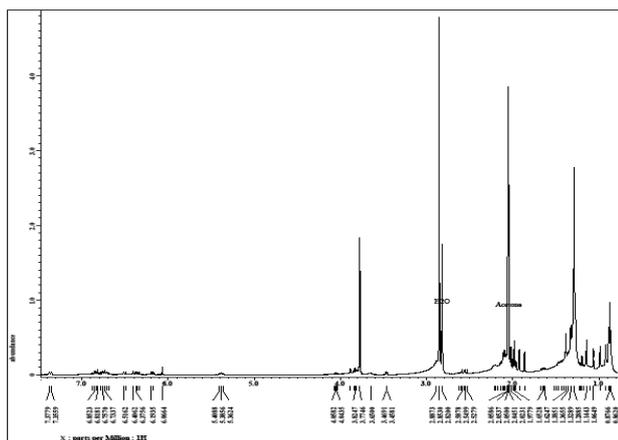


Figure 2. $^1\text{H-NMR}$ spectrums of fucoxanthin

results will increasingly lead to a smaller chemical shift concentration (Daley and Daley, 2005). Based on these results and compared with the value of the chemicals of fucoxanthin, and then the compound is fucoxanthin. Based on the measured data $^1\text{H-NMR}$ (Table 2) shows that in this sample there is fucoxanthin chemical compound with the following characteristics. The value of friction the chemistry of compounds of a sample of leaves *S. filipendula* that were detected by the method of $^1\text{H-NM}$. While $^{13}\text{C-NMR}$ spectrums can be seen in Figure 3.

$^{13}\text{C-NMR}$ spectra data

$^{13}\text{C-NMR}$ measurement data can be seen in Figure 3. It showed that the samples contained fucoxanthin compound with the following characteristics:

1. 10 methyl group (CH_3) is at 25.0; 28.1; 21.2; 11.8; 12.8; 29.2; 32.1; 31.3; 14.0 and 12, 9.
2. The presence of an acetyl group is shown at 23.19 (CH_3 -) and 170.4 ($-\text{C}=\text{O}$).
3. Signal at 202.90; 198.41; 66.32; 67.23 and 72.20 respectively indicate quaternary atom C ($=\text{C}=\text{}$), carbonyl ($-\text{C}=\text{O}$), 3 x-C-OH (5,6 and 5'). Reinforces roommates that this compound was a fucoxanthin.

Conclusion

The content of fucoxanthin was determined based on a calculation using De Garmo Effectiveness Index. The content of fucoxanthin on brown algae (*S. filipendula*) of 78.9 ± 4.71 (%) is indicated by the color orange in accordance with the standards. FTIR identification on fucoxanthin obtained crude functional groups OH, CH, C=O, C=C, CH_2 , C=O, COC and C=C trans substituted and isolated fucoxanthin had functional groups of OH, CH, allenic bond, C=O, C=C, CH_2 , COC and C=C trans substituted. In the HPLC analysis results showed the chromatogram peaks increase linearly and with spiking can gave evident that the samples was

identified as fucoxanthin. NMR showed that O_2 at 5.6 epoxide groups and it was shown at the C5 and C6 atoms and the compounds were allene and also acetate.

References

- Atmadja, W. S., Kadi, A., Sulistijo and Rachmaniar. 1996. The introduction of types of Indonesia seaweed. Puslitbang Oseanologi LIPI. Jakarta: 204-210.
- Bernhard, K., Moss, G. P., Toth, G. and Weedon, B.C.L. 1976. Absolute configuration of fucoxanthin. Tetrahedron Letters 2: 115-118.
- Christiana, R., Susanto, A. B. and Limantara, L. 2008. The analysis pigment acetone extract *Seaweed Udotea* sp., *Amphiora rigidadan*, dan *Turbinaria conoides*. Proceedings of Science and Technology of Natural Pigments. Semarang:194-210.
- Richard, D. F. and Dalley, J. S. 2005. Organic chemistry, p. 45-49. Aley Press, New York.
- De Garmo, E. P., Sullivan, W. G. and Canada, J. R. 1979. Engineering economy. 7th Ed. p. 1-15. McGraw-Hill Science. New York.
- Goodwin, T. W. 1974. Carotenoid and bill proteins in algal physiology and biochemistry. p. 57-68. University of California Press, Berkeley.
- Hall, D. O. and Rao, K. K. 1999. Photosynthesis. 6th Edition, p.106-121. Cambridge University Press, United Kingdom.
- Handayani, T., Sutarno and Setyawan, A. D. 2004. Nutrition composition analysis seaweed *Sargassum crassifolium*. Jurnal Agardh. Biofarmasi 2(2): 45:52.
- Heo, S. J., Kang, K. S. M., Kang, H. S., Kim, J. P., Kim, S. H., Lee, K. W., Chom, M. G. and Jeon, Y. J. 2008. Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H_2O_2 induced cell damaged. European Food Research and Technology 228: 145-151.
- Jeffrey, S. W., Mantoura, R. F. C. and Wright, S. W. 1997. Phytoplankton pigments in oceanography. Guidelines to modern methods. p.550-557 Unesco Publishing, Paris.
- Lenny, S. and Zuhra, C. F. 2005. Isolation and test bioactivity top chemical ingredients pudding red (*Graptophyllum pictum* L. Griff) brine shrimp with test methods. Journal of Communication Research 17(5): 56-59.
- Liasen, S. and Jensen, S. L. 1989. Studies on algae carotenoid. Pure and Applied Chemistry 61: 369-372.
- Mori, K., Ooi, T., Hiraoka, M., Oka, N., Hamada, H., Tamura, M. and Kusumi, T. 2004. Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. Marine Drugs 2: 63-72.
- Rupérez, P. and Calixto, F. S. 2001. Dietary fibre and physicochemical properties of edible Spanish seaweeds. European Food Research and Technology 212: 349-354.
- Sachindra, N. M., Sato, E., Maeda, E., Hosokawa, M., Niwano, Y., Kohno, M. and Miyashita, K. 2007.

Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry* 55: 8515-8522.

- Sachindra, N. M., Airanthi, M. K. W. A., Hosokawa, M. and Miyashita, K. 2010. Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweed. *Journal of Food Science and Technology* 47(1): 94-99.
- Seely, G. R., Duncan, M. J. and Vindaver, W. E. 1992. Preparative and analytical extraction of pigment from brown algae with dimethyl sulfoxide. *Journal of Marine Biology* 12: 184-199.
- Thorne, S. W., Newcomb, E. H. and Osmond, B. 1977. Identification of chlorophyll B in extracts of prokaryotic algae by fluorescence spectroscopy 74(2): 575-578.
- Yan, X., Chuda, Y., Suzuki, M. and Nagata, T. 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Bioscience, Biotechnology, and Biochemistry* 63(3): 605-607.
- (*Graptophyllum pictum* L. Griff) brine shrimp with test methods. *Journal of Communication Research* 17(5): 56-59.
- Liasen, S. and Jensen, S. L. 1989. Studies on algae carotenoid. *Pure and Applied Chemistry* 61: 369-372.
- Mori, K., Ooi, T., Hiraoka, M., Oka, N., Hamada, H., Tamura, M. and Kusumi, T. 2004. Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. *Marine Drugs* 2: 63-72.
- Rupérez, P. and Calixto, F. S. 2001. Dietary fibre and physicochemical properties of edible Spanish seaweeds. *European Food Research and Technology* 212: 349-354.
- Sachindra, N. M., Sato, E., Maeda, E., Hosokawa, M., Niwano, Y., Kohno, M. and Miyashita, K. 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry* 55: 8515-8522.
- Sachindra, N. M., Airanthi, M. K. W. A., Hosokawa, M. and Miyashita, K. 2010. Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweed. *Journal of Food Science and Technology* 47(1): 94-99.
- Seely, G. R., Duncan, M. J. and Vindaver, W. E. 1992. Preparative and analytical extraction of pigment from brown algae with dimethyl sulfoxide. *Journal of Marine Biology* 12: 184-199.
- Thorne, S. W., Newcomb, E. H. and Osmond, B. 1977. Identification of chlorophyll B in extracts of prokaryotic algae by fluorescence spectroscopy 74(2): 575-578.
- Yan, X., Chuda, Y., Suzuki, M. and Nagata, T. 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Bioscience, Biotechnology, and Biochemistry* 63(3): 605-607.
- Atmadja, W. S., Kadi, A., Sulistijo and Rachmaniar. 1996. The introduction of types of Indonesia seaweed. *Puslitbang Oseanologi LIPI. Jakarta*: 204-210.
- Bernhard, K., Moss, G. P., Toth, G. and Weedon, B.C.L. 1976. Absolute configuration of fucoxanthin. *Tetrahedron Letters* 2: 115-118.
- Christiana, R., Susanto, A. B. and Limantara, L. 2008. The analysis pigment acetone extract *Seaweed Udotea* sp., *Amphiora rigidadan*, dan *Turbinaria conoides*. *Proceedings of Science and Technology of Natural Pigments. Semarang*: 194-210.
- Richard, D. F. and Dalley, J. S. 2005. *Organic chemistry*, p. 45-49. Aley Press, New York.
- De Garmo, E. P., Sullivan, W. G. and Canada, J. R. 1979. *Engineering economy*. 7th Ed. p. 1-15. McGraw-Hill Science. New York.
- Goodwin, T. W. 1974. Carotenoid and bill proteins in algal physiology and biochemistry. p. 57-68. University of California Press, Berkeley.
- Hall, D. O. and Rao, K. K. 1999. *Photosynthesis*. 6th Edition, p.106-121. Cambridge University Press, United Kingdom.
- Handayani, T., Sutarno and Setyawan, A. D. 2004. Nutrition composition analysis seaweed *Sargassum crassifolium*. *Jurnal Agardh. Biofarmasi* 2(2): 45:52.
- Heo, S. J., Kang, K. S. M., Kang, H. S., Kim, J. P., Kim, S. H., Lee, K. W., Chom, M. G. and Jeon, Y. J. 2008. Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H₂O₂ induced cell damaged. *European Food Research and Technology* 228: 145-151.
- Jeffrey, S. W., Mantoura, R. F. C. and Wright, S. W. 1997. *Phytoplankton pigments in oceanography. Guidelines to modern methods*. p.550-557 Unesco Publishing, Paris.
- Lenny, S. and Zuhra, C. F. 2005. Isolation and test bioactivity top chemical ingredients pudding red