Quantitative fucoxanthin extract of tropical *Padina* sp. and *Sargassum* sp. (Ocrophyta) and its’ radical scavenging activity

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**Abstract.** Fucoxanthin is a potential compound in medicinal and nutritional ingredients due to its antioxidant activity. This study was carried out to determine the fucoxanthin contents and antioxidant activity of crude fucoxanthin extract from *Padina* sp. and *Sargassum* sp. The crude fucoxanthin was partitioned using some solvents ie. methanol-water and followed by using n-hexane. The total fucoxanthin contents were analysed using High Performance Liquid Chromatography (HPLC) instrument, quantitatively and qualitatively. The total of fucoxanthin contents in *Padina* sp. was relatively high (2.44 mg/g) and followed by *Sargassum* sp (1.95 mg/g) wet weight, respectively. The antioxidant activity was done spectrophotometrically using diphenylpicrylhidrazyl (DPPH) method. The crude fucoxanthin extract from *Padina* sp. was showing a moderate antioxidant activity (209.93 ppm), while *Sargassum* sp was 639.22 ppm. *Padina* sp. from tropical waters is rich in fucoxanthin. Concerning the unstable fucoxanthin compound, next methods to stabilize the fucoxanthin extract need to explore.

1. **Introduction**

*Padina* sp. and *Sargassum* sp. are classified in brown seaweed that can be easily found in Indonesia. Brown seaweeds are rich in natural antioxidants including phenolic [1], fluorotanin and fucoxanthin, carotenoids [2] and isoprenoid [3]. Carotenoid is brown seaweed’s pigment in yellow-red-ish color [4]. Carotenoid could be classified into carotene (i.e: α-carotene and β-carotene) and xantophill (i.e: fucoxanthin, zeaxanthin and lutein). The carotenoid content in seaweed is a natural compound and has a great potency for commercial purposes.

Fucoxanthin (xantophill group) which presents ini brown seaweed, has a brown-yellow-ish pigment. Fucoxanthin is applicated as anti-inflammatory, antiobesity, antidiabetic, anticancer, and antihypertensive agents [5]. Fucoxanthin also has a high antioxidant activity [6] by reducing free radicals [7]. Fucoxanthin has a unique structure due to its an unusual allenic bond and some oxygenic functional groups such as epoxy, hydroxyl, carbonyl and carboxyl moites in its molecule [8]. This allenic bond was responsible for the high antioxidant activity. Antioxidants have an important role for health by reducing the risk of chronic diseases such as cancer and heart disease [9]. Antioxidants are substances that can inhibit oxidative damage to the target molecule. Study from [10] revealed that fucoxanthin extracted from *Sargassum polycystum* showed a high antioxidant activity (99±8.49).
Another study from [7] showed high antioxidant activity from fucoxanthin extracted from *Padina australis*.

The 1,1-diphenyl-2-picrylhydrazil (DPPH) assay method is a simple, rapid and easy method and also sensitive in low concentration sample. The DPPH method has been widely used to test the ability of compounds act as radical scavengers or hydrogen donors. DPPH compounds are synthetic free radicals that are commonly used to evaluate antioxidant activity [11]. The reduction of DPPH radical results in a change from purple colour into yellow colour which indicates antioxidant activity. High Performance Liquid Chromatography (HPLC) method is a quantitative methods to identify fucoxanthin pigments. HPLC has been widely used to obtain the quantity of fucoxanthin contents [12]. The principle of HPLC methods is the separation of analyte components based on their polarity. However, research on the antioxidant activity of crude fucoxanthin extract from *Padina* sp. and *Sargassum* sp. has grown in Panjang Island, Central Java, Indonesia has not been done. Therefore it is very important to explore crude fucoxanthin extract from local *Padina* sp. and *Sargassum* sp. and determine their antioxidant activity. This study has been undertaken to evaluate crude fucoxanthin extract from *Padina* sp. and *Sargassum* sp. and their antioxidant activity by DPPH assay method.

### 2. Materials and methods

#### 2.1. Sample collection and preparation

The materials used in this study were fresh brown seaweeds *Padina* sp. and *Sargassum* sp. obtained from Panjang Island, Central Java, Indonesia – in June 2019. Cordinates of sampling sites in Panjang Island for *Padina* sp. was (06.57812° S 110.62971° E) and *Sargassum* sp. was (06.57827° S 110.62997° E). The location of *Padina* sp. and *Sargassum* sp. sampling is presented in figure 1. The island is surrounded by sandy beaches, a coral reef base and grown by vegetation such as mangroves, seagrasses, seaweeds, etc.

The samples were collected in a cool box, washed them with fresh water, then drained. The samples were cut into small pieces and put in the refrigerator for 4 days for drying. Dry samples were weighed and stored in a container to protect from the direct light exposure.

![Figure 1. The sampling locations of Padina sp. and Sargassum sp. in Panjang Island, Central Java, Indonesia.](image)

#### 2.2. Sample extraction of crude pigment

Pigment extraction procedure was referred to [13] with some modification. Pigments were extracted from dried *Padina* sp. and *Sargassum* sp. (9 g) using 90 mL analytical grade acetone (Merck) (1:10)
and refrigerated for 2 x 24 h. The extracts were filtered using Whatman no 42 and put in a vial bottle and wrapped in aluminium foil. The seaweed extracts were concentrated using rotary evaporator at a temperature of 30 to 35°C [14]. The extracts were stored in vial bottles for further testing.

2.3. Partition of crude fucoxanthin extract
The partition of crude fucoxanthin method were carried out based on [14] with some modification. As much as 27 mL of methanol and 3 mL of water were put into the Erlenmeyer. Each of Padina sp. extract and Sargassum sp. extract were partitioned using a mixture of methanol and water followed by n-hexane (10 mL) in a separation funnel. The extract were separated and produced two parts different colors, i.e. yellowish-green (n-hexane fraction) and reddish-brown (fraction of methanol-water). Finally, the methanol-water fractions were concentrated and evaporated to dryness at the similar methods above.

2.4. Characterization of crude fucoxanthin extracts
Two samples of crude fucoxanthin extracts from Padina sp. and Sargassum sp. were analyzed quantitatively and qualitatively using High Performance Liquid Chromatography (HPLC). Pigment analysis was performed using RP-HPLC LC-20A equipped with a diode array detector (Shimadzu) with a gradient elution program for ammonium acetate solution (1M), a mixture of MeOH and acetone at a flow rate of 1 mL/ min at 30°C in 70 mins according to the method of [15] with some modification. Each sample of Padina sp. extract and Sargassum sp. was injected into HPLC (20 µL) and detected using visible light at λ 450 nm.

2.5. Antioxidant Activity Analysis of Crude Fucoxanthin Extract
The antioxidant activity was determined using Inhibit Concentration 50 (IC₅₀) value. The antioxidant activity of the crude fucoxanthin extracts from Padina sp. and Sargassum sp. were conducted by the free radical method using DPPH according the method described by [16]. The control solution was made by mixing 1 mL DPPH solution with 3 mL methanol. Crude fucoxanthin extract from Padina sp. and Sargassum sp. were diluted in a concentration series (0-1000) using methanol. 1 mL of extract samples at each concentration were added 3 mL of DPPH solution, then placed in vial bottles wrapped with aluminium foil. The extract samples were incubated for 30 minutes at a room temperature and absorbance was measured using Shimadzu UV-1280 spectrophotometer at a wavelength of 517 nm. The IC₅₀ values were determined using probit linear regression analysis with MS Excel. The percentage inhibiton data was matched with the probit analysis table. Graph was made so that a linear regression equation in the form of \( y = ax+b \) was obtained to calculate the IC₅₀ value. Percentage value of inhibition (% inhibition) against DPPH free radicals was calculated using the formula :

\[
\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100
\]

Where : Ac is absorbance of control and As is absorbance of sample.

3. Results and discussion
3.1. Yield and characterization of crude fucoxanthin extract from Padina sp. and Sargassum sp.
The yield of crude pigment extract from Padina sp. and Sargassum sp. were 3.67 and 0.89 %, respectively. Then, the yield of crude fucoxanthin extract from Padina sp. and Sargassum sp. were 3.00 and 0.55 % respectively. The yield of the crude pigment extract from Padina sp. was higher than Sargassum sp. as well as the yield of crude fucoxanthin extract. Furthermore, the total of fucoxanthin content of Padina sp. was also higher than Sargassum sp. The presence of fucoxanthin and the total of fucoxanthin contents in crude fucoxanthin extract from Padina sp. and Sargassum sp. were identified using reversed-phase HPLC. The total of fucoxanthin contents in crude fucoxanthin extract from Padina sp. and Sargassum sp. were 2.44 mg/g and 1.95 mg/g wet weight extract, respectively.

The total of fucoxanthin content from each sample obtained was in accordance with the results by the study of [17] using the fucoxanthin standard curve line equation between the standard fucoxanthin concentration (g/ml) (X) and the peak area of the fucoxanthin in the area (detected at λ 450 nm). The
fucoxanthin standard curve line equation was $y = (2 \times 10^{11} \times X) – 35997$ dan $R^2 = 0.999$. Fucoxanthin produced by Padina sp, was higher than Sargassum sp. These results were consistent with the result of study by Limantara and Heriyanto [17] and [18]. Padina sp. is a unique brown seaweed that contains striates on its thallus.

3.2. *High performance liquid chromatography (HPLC) analysis*

The chromatogram of crude fucoxanthin extract from Padina sp. is shown in figure 2. The results show 5 dominant pigments in the chromatogram of Padina sp. and Sargassum sp. Those peaks are fucoxanthin and its isomers as well as other xanthophyll carotenoids. Peak number 4 is the highest peak in Padina sp. extract and peak number 2 was the highest in Sargassum sp. extract. The absorption peaks in crude fucoxanthin extracts from Padina sp. and Sargassum sp. were shown at 446, 450, 426, 442 and 442 nm (figure 2). The retention times for fucoxanthin in crude fucoxanthin extract from Padina sp. were 4.99, 6.16, 8.20, 9.32 and 16.20 min. While, the retention times for fucoxanthin in the crude fucoxanthin extract from Sargassum sp. were 6.17, 9.44, 10.99, 15.26, and 16.29 min. Based on the retention time and the absorption peaks of Padina sp. fucoxanthin, peak number 1, 2, 3, 4, and 5 are dinoxanthin, fucoxanthin, trans fucoxanthin isomer, violaxanthin, and a mixture of trans and cis fucoxanthin isomers, respectively.

The absorption peaks of fucoxanthin from Sargassum sp. peak number 1, 2, 3, 4, and 5 are dinoxanthin, fucoxanthin isomer, trans fucoxanthin isomer, fucoxanthin, and a mixture of trans and cis fucoxanthin isomers, respectively. Padina sp. and Sargassum sp. have similarity in dominant pigments. This was because both macroalgae are brown seaweed (Ocrophyta). The results of the pigment analysis were according to [19], [20], and Limantara and Heriyanto [17]. The absorbance of fucoxanthin ranges from 420-470 nm depending on the species. Moreover, most carotenoids absorb light in the region between 400-500 nm [21].

![Figure 2a. HPLC chromatogram of crude fucoxanthin extract from Padina sp.](image)

![Figure 2b. HPLC chromatogram of crude fucoxanthin extract from Sargassum sp.](image)

3.3. *Antioxidant activity analysis of crude fucoxanthin extract*

Antioxidant activity tests were carried out using 1,1-diphenyl-2-picrylhydrazil (DPPH). This method is based on the reduction of DPPH by antioxidant compounds carrying out a hydrogen atom transfer mechanism hidrogen [22]. This process caused discoloration of DPPH to pale yellow. This was in accordance with the results obtained in this study, crude fucoxanthin extract dissolved with methanol which originally orange color turned into purple after being given DPPH solution. Then, after being incubated at room temperature the color of samples appeared to be yellow. This study was used a some correction factor because in the crude fucoxanthin extracts from Padina sp. and Sargassum sp. was contained compounds whose peak absorption was also in lambda which was similar to DPPH.
The inhibitory activity was determined by % inhibition and Inhibition Concentration (IC\textsubscript{50}). The DPPH radical scavenging activity of crude fucoxanthin extract from \textit{Padina} sp. and \textit{Sargassum} sp. can be seen in table 1 and table 2. IC\textsubscript{50} value of crude fucoxanthin extract from \textit{Padina} sp. and \textit{Sargassum} sp. were 209.93 ppm and 639.22 ppm respectively. IC\textsubscript{50} value is the concentration of compound that can reduce 50% activity of free radical (DPPH). IC\textsubscript{50} values were determined by probit linear regression analysis based on [23]. This research determined probit linear analysis with Ms. Excel software. The probit analysis graphs of crude fucoxanthin extract from \textit{Padina} sp. and \textit{Sargassum} sp. is depicted in figure 3. The equations for the linear regression were y = 2.1586x + 0.0756 with R\textsuperscript{2} = 0.9857 (\textit{Padina} sp.) and y = 1.7205x + 0.1728 (\textit{Sargassum} sp.) with R\textsuperscript{2} = 0.9704.

The equation for the linear regression was used to find the IC\textsubscript{50} value by stating a value of y of 5 which was then obtained for the value of x. IC\textsubscript{50} value was obtained after antilog calculation from x value. This means the crude fucoxanthin extracts from \textit{Padina} sp. and \textit{Sargassum} sp. were able to inhibit 50% of DPPH free radicals at concentration of 209.923 ppm and 639.22 ppm. The crude fucoxanthin extract from \textit{Padina} sp. had stronger ability to scavenge DPPH radical in compared to crude fucoxanthin extract from \textit{Sargassum} sp. [24] reported that the IC\textsubscript{50} value is classified into three categories. The classification is: less than 50 ppm (IC\textsubscript{50} < 50 ppm) is very strong, strong (IC\textsubscript{50} 50-100 ppm), moderate (IC\textsubscript{50} 100-250 ppm), weak (IC\textsubscript{50} 250-500 ppm) and inactive (IC\textsubscript{50} > 500 ppm).

Based on these data, the antioxidant activity of the crude fucoxanthin from \textit{Padina} sp. is classified as moderate category and more potentially as an antioxidant compound. The higher fucoxanthin content, the higher antioxidant activity. These results were consistent with the statement of [10] that antioxidant activity contains fucoxanthin composition because fucoxanthin has a role in scavenge DPPH free radicals. [7] reported fucoxanthin extract derived from \textit{Padina australis} obtained stronger antioxidant activity with inhibition values of DPPH free radicals of 53% at a dose of 50 ppm compared to fucoxanthin extracts from \textit{Sargassum ilicifolium} taken from Binanguneun Beach, Banten.

\begin{figure}[h]
\centering
\includegraphics[width=0.48\textwidth]{figure3a.png}
\caption{Probit analysis graph of crude fucoxanthin extract from \textit{Padina} sp.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.48\textwidth]{figure3b.png}
\caption{Probit analysis graph of crude fucoxanthin extract from \textit{Sargassum} sp.}
\end{figure}
Table 1. Concentration log (ppm), probit and IC$_{50}$ of crude fucoxanthin extract from *Padina* sp.

| Concentration (ppm) | Log (ppm) | % Inhibition | Probit | IC$_{50}$ (ppm) |
|---------------------|-----------|--------------|--------|-----------------|
| 0                   | 0         | 0            | -      | -               |
| 100                 | 2         | 36           | 4.64   |                 |
| 200                 | 2.30      | 48           | 4.95   |                 |
| 300                 | 2.48      | 76           | 5.71   | 209.93          |
| 400                 | 2.60      | 78           | 5.77   |                 |
| 500                 | 2.70      | 68           | 5.47   |                 |

Table 2. Concentration log (ppm), probit and IC$_{50}$ of crude fucoxanthin extract from *Sargassum* sp.

| Concentration (ppm) | Log (ppm) | % Inhibition | Probit  | IC$_{50}$ (ppm) |
|---------------------|-----------|--------------|---------|-----------------|
| 0                   | 0         | 0            | -       | -               |
| 100                 | 2         | 23           | 4.26    |                 |
| 200                 | 2.30      | 21           | 4.19    |                 |
| 300                 | 2.48      | 28           | 4.42    |                 |
| 400                 | 2.60      | 30           | 4.48    | 639.22          |
| 500                 | 2.70      | 36           | 4.64    |                 |
| 600                 | 2.78      | 40           | 4.75    |                 |
| 700                 | 2.85      | 54           | 5.10    |                 |

The result of crude fucoxanthin extract in this present study was not in accordance to study by [10] which has a very strong antioxidant activity (IC$_{50}$ 99±8.49 ppm) in fucoxanthin extract from *Sargassum polycystum*. In addition, fucoxanthin should have a high antioxidant activity because of the allenic bond in its structure [8]. This was suspected that our fucoxanthin undergo a process of degradation that lead to decrease the antioxidant activity. In order to protect from degradation process, fucoxanthin must be avoided from several factors such as light and temperature during the storage. Samples can be degraded and lost of antioxidant activity caused by several condition such as humidity, oxygen, light, ant temperature during the storage process [25]. However, based on their study, [14] stated that the presence of the allenic bond peak in the fucoxanthin from *Sargassum Binderi* spectrum (FTIR analysis) was very low because sample was contained high amount of moisture which interefere with the infrared absorbance.

Fucoxanthin is unstable at high temperatures which can lead for breaking of conjugated double bonds resulting in decreased antioxidant activity. In addition, because fucoxanthin has an unstable structure, this makes fucoxanthin easily influence by heat, air and light exposure. Formation of some cis isomers by isomerization would occur due to storage conditions, media and type of carotenoid [5]. According to [26], this condition can reduce antioxidant activity in fucoxanthin. This means that high fucoxanthin content does not necessarily have high antioxidant activity due to these storage factors. In fact, this crude fucoxanthin extract was still mixed with other pigments. The content of other pigments which has similar polarity to fucoxanthin was also contributed as an antioxidant agent so this need to be purified. This was adjusted to the statement of [17] that the brown color of brown seaweed was influenced by a mixture of pigments consisting of carotenoid and chlorophyll group pigments. Based on this facts, the antioxidant activity of the crude fucoxanthin extracts obtained was not strong.
Therefore, crude fucoxanthin extract is more suitable to be applied into antioxidant agent products and surely keep in a good storage condition.

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