The effect of different type of solvents on the antioxidant activity of fucoxanthin extract from brown seaweed
*Sargassum duplicatum*

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Abstract. Fucoxanthin is the main part of carotenoid in brown seaweed. It have been reported as an antioxidant by donating an electron to free radicals so the oxidative reaction could be prevented. Fucoxanthin could be an alternative to substitute the use of synthetic antioxidant. The purpose of this study was to determine antioxidant activity of fucoxanthin extract from *Sargassum duplicatum* with different solvent and determine the solvent that can produce the highest antioxidant activity of fucoxanthin extract. This research was a descriptive exploratory. The extraction method in this study was maceration, while the solvent used are ethanol, methanol, and ethyl acetate. The result showed that methanol solvent was the best solvent to produce fucoxanthin with relatively strong antioxidant activity (IC₅₀ 78.52±21.23 ppm). And followed by ethanol solvent with strong antioxidant activity (93.77±21.19 ppm) and ethyl acetate solvent with moderate antioxidant activity (112.30±15.79 ppm). Beside that, methanol solvent has the highest fucoxanthin content (145.86 µg/g) than ethanol and ethyl acetate.

1. Introduction
The development of various degenerative disease in lately is very fast. Degenerative diseases caused by antioxidant in human body is unable to reduce the increase of free radicals [1]. Free radicals in the human body can be prevented by producing antioxidants that come from outside the body [2]. But recently, people often used the synthetic antioxidant. Synthetic antioxidants have high effectiveness but can be harmful to health. Therefore natural antioxidants can be selected as a source of antioxidants that are safe to be developed.

Indonesian seas with a coastline of about 81,000 km have the potential of seaweed is very high. Brown seaweed produce some secondary metabolites such as phenolic compounds, flavonoids, tannins, terpenoids, saponins, alkaloids and sterols [3]. Beside those compounds, the potential of seaweed that need to be explored bioactive compounds one of which is a pigment. Pigments of seaweed in addition to potentially as a dye, also has benefits in the field of health [4].

*Sargassum* is one of brown seaweed found in Talango Island, Madura. *Sargassum* is known to have bioactivity beneficial to human. Carotenoids are pigments groupson seaweed that potential to be developed, it generally grouped into two, carotene and xanthophyll. Fucoxanthin is part of xanthophyll that potential to be explored it’sbiological activity. *Sargassum duplicatum* is one of brown seaweed which exploration has not been maximized.
According to Nursid and Novendri [5] states that fucoxanthin from Padinaaustralis seaweed has antioxidant activity. Therefore, fucoxanthin could be an alternative to substitute the use of synthetic antioxidant. The purpose of this study was to determine antioxidant activity of fucoxanthin extract from Sargassum duplicatum with different solvent and determine the solvent that can produce the highest antioxidant activity of fucoxanthin extract.

2. Materials and methods

2.1. Tools and materials

The materials used in this study was S. duplicatum obtained from Talango, Madura. The ingredients used in the extraction process are ethanol, methanol, ethyl acetate were purchased from Chemical Multi Sentosa Indonesia, Surabaya, silica gel, n-hexane, and acetone were purchased from BRATACO, Surabaya.

The ingredients for phytochemical analysis were Meyer’s reagent, Dragendorf’s reagent, Wagner’s reagent, Magnesium powder, hydrochloric acid (HCl), sulfuric acid (H2SO4), sodium hydroxide (NaOH), acetic acid anhydride, iron (III) chloride (FeCl3) 5 % iron (III) chloride (FeCl3) 1%, and distilled water. The antioxidant activity of fucoxanthin extracts were determined using the DPPH free radical scavenging assay.

The equipment used for this study were 210 gr analytical balance (Ohauss pioneer), knife, grinder, beakers, erlenmeyer flask, glass beaker, vacuum pump, funnel glass, rotary evaporator, TLC plates, column chromatography, stative, clamps, cotton, test tube, flask, and UV-Vis spectrophotometer.

2.2. Preparation of the extract of S. duplicatum

The extraction process was done by maceration. A total of 300 grams of dried seaweed S. duplicatum put in Erlenmeyer flask, then the solvent was added to each Erlenmeyer flask, such as ethanol, methanol, and ethyl acetate (1:3). After that, each sample was macerated for 3x24h at room temperature. Then liquid extract was evaporated with a rotary evaporator to remove the solvent contained in the extract to obtain a thick extract of S. duplicatum [6].

2.3. Fractination of S. duplicatum crude extract

The fractionation process that refers to Noviendri et al. [7] using silica gel in a column chromatography. Preparation was done by soaking the silica gel in a column chromatography with n-hexane for 24h which put into the column with a height of 15 cm. The crude extract put into the chromatography column.

Fractionation process was done by adding n-hexane to separate the green to brownish orange color. The addition of a solution of n-hexane and acetone (6:4 v/v) recently performed to obtain the active fraction of the target with an orange indicator. Fractionation process continued on the active fraction obtained solution and then dried. Then fucoxanthin yield was counted and stored for use phytochemical analysis and antioxidant activity test.

2.4. Fucoxanthin analysis by TLC

Fucoxanthin analysis refers to research [8, 9]. The mobile phase used are n-hexane: acetone (6:4 v/v). The Plates was preheated in oven then underlined 2 cm and 1 cm above the line. Then the mobile phase was added to the chamber and closed. Fucoxanthin extract dissolved into ethanol and then spotted to a TLC plate. After the TLC plate was added to the chamber and the solvent reached the upper limit of the plate. Fucoxanthin analysis be identified by determining the value of Retention factor (Rf).

2.5. Fucoxanthin content analysis

25 mg of fucoxanthin pigment extract on each solvent dissolved into ethanol pro analysis then added the volume up to 10 mL in the flask. Then absorbance spectrum of fucoxanthin pigment was measured using a UV-Vis spectrophotometer at a wavelength of 300-800 nm in cuvette width is 1 cm.
Preparation of standard curve fukosantin refers to Noviendri et al. [5] by dissolving 1 mg fucoxanthin as standard with 1 mL of methanol pro analysis. Dilution series used is 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. Standard curve was constructed by connecting a standard solution concentration with absorption results obtained.

2.6. Antioxidant activity test
25 mg of extract fucosanthin dissolved in 25 mL of ethanol pro analysis dilution to obtain a solution with a concentration of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. The 3 mL of each concentration of the sample solution (extract) inserted in a test tube, add 1 mL of DPPH 100 µg/mL were homogenized [10]. Each solution then incubated with temperature 30°C for 30 minutes and the absorbance was measured using a spectrophotometer with a wavelength of 517 nm. Absorbance of the reference solution is measured to perform the calculation of inhibition. A total of 1 mL of DPPH 100 µg / mL was added to the test tube and then added 3 mL of ethanol were homogenized. Incubation solution in 37°C for 30 minutes and then measured the absorbance at a wavelength of optimum [11].

2.7. Phytochemical analysis
a. Alkaloids
Samples were extracted with chloroform and amonia inside the test tube and then shaken and filtered. The filtrate is added a few drops of 2N sulfuric acid. After that, the sample was treated using the reagent alkaloids such as Meyer, Dragendorff, and Wagner. Characterized alkaloid compounds are yellowish-white sediment at reagent Meyer, reddish-brown sediment on Dragendorff reagents and reagent Wagner [12].

b. Flavonoids
The extract is dissolved with ethanol and then filtered using a cotton swab. Then the sample is added and shaken Mg powder. Followed by the addition of each of concentrated HCl, concentrated H₂SO₄ and NaOH few drops. The presence of flavonoids characterized by the emergence of red, yellow, or orange [12].

c. Triterpenoids and steroids
The extract was weighed then diluted with ethanol. Then the sample is added 2 mL of chloroform, 10 drops of acetic acid anhydride and 3 drops of concentrated sulfuric acid until obtain two layers of liquid. The triterpenoids was characterized by producing brown rings on the two layers of liquid, steroids marked by a bluish-green color changes [12].

d. Saponins
The sample is dissolved into hot water of 20 mL. Samples were shaken and then added 1 drop of HCl 2 N. The samples were then shuffled back. Saponins is shown by foam that is consistent [12].

e. Phenolics and tannins qualitative test
The extract was added 10 drops of 1% FeCl₃. The presence of phenols and tannins is shown in green, red, purple, blue, or black color [12].

3. Result and discussion
The highest yields of fucosanthin is produced with methanol. It caused methanol has the highest polarity index. The results of the yield can be seen in Figure 1. This is consistent with research [13] that methanol is able to extract fucosanthin from Padina australis more optimal than ethanol, DMSO, acetonitrile and acetone. According to Xia et al. [14] fucosanthin extraction efficiency is very dependent on the type of solvent. The yield fucosanthin from methanol solvent is higher because methanol is known as a solvent capable of extracting fucosanthin of fresh seaweed [13].
Based on fucoxanthin analysis using thin layer chromatography (TLC), Retention factor (Rf) value of fucoxanthin from ethanol, methanol and ethyl acetate was 0.25 with an orange-brown color. According to Zaelani and Hartati [15] Rf value of fucoxanthin pigment is 0.25 (orange) and [16], Rf value with range from 0.26 to 0.28 (orange). Rf value used for estimate the type of extract contained in the extract [17].

![Figure 1. Yield of fucoxanthin](image)

| Variable Test | Solvent Treatment | Ethanol | Methanol | Ethyl Acetate |
|---------------|------------------|---------|----------|---------------|
| Fucoxanthin (µg / g) | 142.37 ± 0.106 | 145.86 ± 0.109 | 123.84 ± 0.015 |
| IC₅₀ (ppm ± SD) | 93.77 ± 21.19 | 78.52 ± 21.23 | 112.30 ± 15.79 |

Based on table 1, the methanol has the highest fucoxanthin content of 145.86 µg/g. This is consistent with Mise et al. [18] that methanol is able to produce higher fucoxanthin as much as 185.5 µg/g than other solvents. The high fucoxanthin was produced from methanol showed that S. duplicatum fucoxanthin is polar and attracted by methanol.

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**Table 1. Data on fucoxanthin content and IC₅₀ of fucoxanthin**

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Based on table 1, the highest antioxidant activity generated by fucoxanthin which extracted using methanol (IC₅₀ 78.52 ± 21.23 ppm). This is consistent with Fung et al. [19] that fucoxanthin from Undaria pinnatifida were extracted using methanol has antioxidant activity of 32.09 ± 3.03 ppm. According Nursid et al. [20] methanol is a polar solvent that is able to optimize the extraction process. Fucoxanthin are also classified for their polar hydroxyl (OH) groups, therefore it contributes to antioxidant activity. According Arifulloh [21] polar solvents such as methanol and ethanol can attract xanthin compounds and other polar compounds.

Based on the test results of phytochemical, fucoxanthin S. duplicatum extracted with ethanol and methanol showed positive results against triterpenoids compound. The active component from fucoxanthin is terpenoids. This is in accordance with Mikami and Hosokawa [22] that fucoxanthin is a pigment that belonging to the carotenoids, while the carotenoids are classified into terpenoids compound. Triterpenoids compound has not identified in the ethyl acetate solvent because triterpenoids have polar groups, while the ethyl acetate solvent is semi-polar solvent [23]. Triterpenoids are natural compounds that formed by biosynthesis and it’s widely distributed in the world of plants and animals [24].

Besides terpenoids, fucoxanthin from S. duplicatum showed positive results against phenolics and tannins compound which is evidenced by a change in the color green. Phenolics compound are
secondary metabolites of plants which has potential as an antioxidant. It caused by a hydroxyl group contained in the phenol compound. Hydroxyl groups can donate a hydrogen atom so the oxidation process can be inhibited [25].

The results on the phenolics of fucoxanthin S. duplicatum can be concluded to have a positive correlation between phenolic compounds and antioxidant activity. Antioxidant activity in fucoxanthin S. duplicatum is caused by the presence of phenolics in fucoxanthin S. duplicatum. According to Molyneux [11] if a material contains the phenolics compound, there is antioxidant activity available in materials. Tannins are also found in fucoxanthin S. duplicatum. According to Siregar et al. [26], tannins have a phenolics compound which has a hydroxyl group in it. Based on the results of the phytochemical analysis, triterpenoids, phenolics and tannins compound can affect to fucoxanthin S. duplicatum have strong antioxidant activity in ethanol and methanol.

Phytochemical analysis of each type of seaweed is different. This is influenced by several things including polar solvent is used, the method of drying seaweed and the species of seaweed [27, 23]. Alkaloids, flavonoids, steroids and tannins in fucoxanthin S. duplicatum showed negative results.

4. Conclusion
Based on the results of this study concluded that the different types of solvent affect on the antioxidant activity of fucoxanthin S. duplicatum. Methanol is the best solvent to produce fucoxanthin with the relatively strong antioxidant activity (IC50 78.52 ± 21.23 ppm). Followed by ethanol with strong antioxidant activity (93.77 ± 21.19 ppm) and ethyl acetate solvent with antioxidant activity was (112.30 ± 15.79 ppm).

5. References
[1] Sangkala S A, Minarni R J, and Made I T 2014 J Akademia Klm 3(4), 198-205 [in Indonesian].
[2] Ingrid M, and Henry S 2014 Extraction of Antioxidants and Active Compounds from Kiwi Fruit (Actinidia deliciosa) (Bandung: Institute for Research and Community Service, Parahyangan Catholic University).
[3] Baleta F, Bolanos N, Ruma J M, Baleta O C, and Cairel J D 2017 J Med. Plants Stud. 5(1), 382-387.
[4] Sanger G, Kaseger B E, Rarung L K, and Damongilala L 2018 JPHPI 21(2), 208-217 [in Indonesian].
[5] Nursid M, and Novendri D 2017 JP K 12(2), 117-124 [in Indonesian].
[6] Gazali M, Nurjannah and Neviati P Z 2018 JPHPI. 21(1), 167-178 [in Indonesian].
[7] Noviendri D, Jaswir I, Salleh M T, Mayashita K, and Ramli N 2011 J. Med. Plant Res. 5(11), 2405-2412.
[8] Jaswir I, Novendri D, and Salleh H M 2013 J. Liq. 36(10), 1340-1354.
[9] Noviendri D 2014 Isolation and Microencapsulation of Fucoxanthin for Drug Delivery System of Human Lung Cancer (H1299) Cell Line Dissertation (Malaysia Kuala Lumpur: International Islamic University Malaysia).
[10] Putranti R I 2013 Maspari 2, 82-88 [in Indonesian].
[11] Molyneux P 2004 J. Sci. Technol. 26(2), 211-215.
[12] Harborne J B 1998 Phytochemical Methods, Guide to Modern Ways of Analyzing Plants 3rd Edition Phytochemical Methods (Netherlands: Springer) p 302.
[13] Limantara L, and Heriyanto 2011 IJMS. 16(2), 86-94 [in Indonesian].
[14] Xia S, Wang K, Wan L, Li A, Hu Q, and Zhang C 2013 Mar. Drugs 11, 266-268.
[15] Zaelani K, and Hartati K 2014 Study Identification of Crude Fukosant and Fucoanthine Result of Isolation of Brown Algae (Padinaaustralis) by Spectroscopic Testing (Malang: Faculty of Science and Technology UIN Malang) pp 140-144.
[16] Kartikaningsih H, Eka D F, and Ardian E N 2017 AIP Conference Proceedings p 10.
[17] Arifah R U, Sri S, Endang S, and Ali R 2019 Marina Oceanography Bulletin 8(1), 25-32.
[18] Mise T, Ueda M, and Yasumoto T 2011 Int. J. Food Sci. 3(1), 73-76.
[19] Fung A, Nazimah H, and Jun L 2013 Food Chem. 136, 1055-1062.
[20] Nursid M, Dedi N, Lestari R, and Virza N 2016 JPK. 11(1), 83-90 [in Indonesian].
[21] Arifulloh 2013 Extraction of Lycopene from Tomato Fruit (Lycopersium esculatun Mill.) with Various Composition of Solvents Essay (Jember: University of Jember).
[22] Mikami K, and Hosokawa M 2013 J. Mol. Sci. 14, 13763-13781.
[23] Nasution A I S 2018 Characteristics of Fraction, Active Biopigments, Fucosantin, Grass, Sea, Brown as Antioxidant and UV Protector Essay Departement of Technology Results of Aquatic (Bogor: Faculty of Science and Science of Marine Technology Bogor Agricultural Institute).
[24] Hidayat T, Nurjanah, Mala N, and Effionora A 2018 J. Chem. Res. 4(2), 49-62.
[25] Diachanty S, Nurjanah, and Asadatun A 2017 JPHPI. 20(2), 305-318 [in Indonesian].
[26] Siregar A F, Agus S, and Delianis P 2012 J. Mar. Res. 1(2), 152-160.
[27] Lantah P L, Lita A D Y M, and Albert R R 2017 J Media Technology Results of Fisheries 5(3), 167-172.