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by Dhena Ria Barleany

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Fucoxanthin Extraction by Ultrasonic-Assisted from Brown Seaweed (Padina Sp) Origin Pulau Merak Banten

Denni Kartika Sari^{1a}, Dhena Ria Barleany^{1b}, Indar Kustiningsih^{1c}, Diansih^{1d},
 Eka Aprillia^{1e}

¹Chemical Engineering, University of Sultan Ageng Tirtayasa, Indonesia

^adenni.kartikasari@untirta.ac.id, ^bria.barleany@untirta.ac.id, ^cIndar.kustiningsih@untirta.ac.id,
^ddiansih1215@gmail.com, ^eeka.aprillia@gmail.com,

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Abstract. Seaweed is an underutilized resource with great potential in the food industry. Such as cosmetics, agrochemicals, biomass ingredients, bioenergy modifies. Indonesia is a country where two-thirds of its territory is marine. Fucoxanthin is a group of carotenoids found in abundance in marine macroalgae and has various health benefits. Traditional extraction (CE) of such potential biomolecules entails multiple steps that are both laborious and time-consuming. This study used ultrasound-assisted extraction (UAE) to extract high percentages of Fucoxanthin and phenolic compounds from the macroalga Padina sp. This research aims are to identification fucoxanthin compound in Padina Sp, determine the total phenolic content and composition of the pigment of Padina sp. This study also evaluates the effect of the extraction method concerning the time of exposure and solvent concentration on total phenolic content. Extract Padina Sp was further purified and characterized using FTIR. The pigment identification process is carried out by the thin layer chromatography method (TLC) and weigh mol using LC-MS. The results of the analyses present the highest content of TPC in Padina (755.633 mg GAE/mg) on 70 0C at 80% solvent concentration. Based on RSM methodology at 70 0C and 90 % solvent concentration. The active fraction of Fucoxanthin was identified at a Rf 0.96. The results of the RSM (Response Surface Methodology) showed the effect of concentration and temperature on the extraction of Fucoxanthin. The molecular weight of Fucoxanthin was found to be 659.43 m/z

Introduction

Indonesia is known as an island chain nation, with natural resources in the sea. Seaweeds, one of them, seaweed are classified into three groups based on their pigmentation green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Phaeophyta). Seaweeds are a vastly underutilized resource with enormous potential to isolate natural ingredients with potential for use in food and medicine. Padina, Sargassum, Turbinaria, and Dictyota are common brown seaweeds found throughout Indonesia's seas. The studies carried out previously have an antioxidant activity that exceeds red and green algae [1]. Brown seaweed morphological structure influenced pigment composition and Fucoxanthin concentration. Most brown algae contain fucoxanthin, an orange pigment-containing chlorophyll a, c, and -carotene. It has a one-of-a-kind structure that includes an phenolic bond and functional carbonyl, hydroxyl, and carboxyl moieties with various properties [2]. Fucoxanthin is one of the most abundant carotenoids, especially in brown seaweeds. Fucoxanthin is a pigment that belongs to the xanthophyll family. Recent research suggests that it has antioxidant, antimicrobial, antiobesity, and anticancer properties [3]. Padina sp is a brown seaweed found on the Merak island which is not utilized and becomes a waste for the community. Although there are potential fucoxanthin applications in the pharmaceutical industries and food, commercialization is hampered by inadequate extraction methods [4]. Solvent extraction, precipitation, freeze-thawing, and grinding are traditional pigment extraction methods [5]. The successful application and commercialization of ultrasonic extraction are mainly due to simple, quick, low-cost technology, reducing solvent consumption and time, resulting high yields [6].

Several types of polar solvents were used to extract fucoxanthin from microalgae. Ethanol proved to be the most effective solvent for maximizing fucoxanthin extraction [7]. Fucoxanthin and other oxygenated carotenoids can be degraded when ethanol is heated to high temperatures. Traditional organic solvent extraction methods are time-consuming and necessitate a large organic solvent [8]. The study on the content of fucoxanthin *Padina* sp from the Indonesian Merak island had never been carried out. Experiments were conducted using ultrasonic-assisted extraction with a variation of time and temperature. Fucoxanthin was characterized using UV-Vis, FT-IR, and LC-MS analytical techniques and determining its total phenolic content.

Materials and Methods

Materials. Sonicator Device (APSlap), Rotary Evaporator (B-One), Blender (Sanken), Erlenmeyer 250 ml (Pyrex), 25 ml measuring cup (Pyrex), Funnel (Pyrex), *Padina* sp. brown seaweed, Ethanol 96%, Aquades, Folin-Ciocalteu reagent, gallic acid.

Collection of samples. *Padina australis* (Hauck) (Dictyotaceae) that had grown naturally in Pulau Merak, Banten, were collected. Merak Island Cilegon Banten can be seen in Fig. 1. which is located at the position of 5°941 South Latitude (LS) and 105° 997 East Longitude (BT). *Padina australis* were collected and cleaned of epiphytes, epibionts, and debris, then rinsed with aquadest.

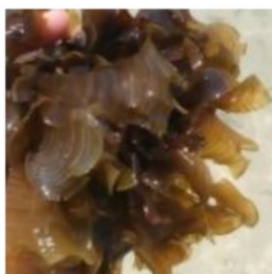


Figure 1. *Padina* sp.

Ultrasonic assisted extraction (UAE) of fucoxanthin. The initial stage is to determine the optimum time for the extraction process with a solvent concentration of 80% based on previous research, followed by optimizing the solvent concentration at 60,70,80,90% and extraction temperature at 50 °C, 60 °C, 70 °C. UAE extraction was performed using ethanol at various concentrations and temperatures over the specified periods (Table 1). The filtrate was centrifuged at 6000 rpm for 10 minutes and concentrated at 40 °C using rotary vacuum evaporation [9].

Total Phenolic compound. To determine the total phenolic content (TPC) of phenolic extracts, the Folin-Ciocalteu reagent was used. In 15 ml clean centrifuge tubes, 1 ml extract were reacted with 0.5 ml of 10% (v/v) Folin-Ciocalteu reagent and 2.0 ml of 7 percent (w/v) sodium bicarbonate solution. Each reaction mixture's absorbance was measured spectrophotometrically at 765 nm after 60 minutes of incubation at 40°C (Pharmacia UV 1601, Shimadzu, Kyoto, Japan). The positive control was gallic acid, and the results were expressed as milligram gallic acid per gram of dry weight biomass (mg GAE. g-1 DW).

FTIR. Fucoxanthin was examined using Fourier-Transform Infrared Spectroscopy (FTIR, IRAffinity-1S Shimadzu, Japan) with spectral ranges of 400–4000 cm⁻¹. A high-performance liquid chromatography system was used to test the purified fucoxanthin (2545, Waters, USA) with a UV-Vis detector (2489, Waters, USA) [9].

TLC. The purity of fucoxanthin and fucoxanthin was determined using TLC. TLC plates (10 x 20 cm) coated with silica gel 60 were used (Merck, USA) for neutral lipids: hexane/diethyl

ether/acetic acid; and polar lipids: hexane/diethyl ether/acetic acid. Co-chromatography with authentic standards revealed bands corresponding to fucoxanthin and lipids were visualized using copper sulfate [10].

LC-MS. Padina sp Hydrolyzate extract dissolved in 5 mL distilled water are injected into an LC column with a size of 2.1 mm x 50 m and a flow rate of 0.2 mL/minute. The temperature column used was 50 °C, and the experiment's total duration was 35 minutes. The separated fucoxanthin was then subjected to MS analysis to determine the chemical compounds' components [11].

Results and Discussion

Total phenolic content. The extraction yield depends on the solvent's polarity, pH, temperature, time of extraction, and sample composition. In this study, the initial stage to vary the extraction time with the fixed solvent concentration based on previous research. The most critical parameters are the extraction time and temperature, the solvent used, and the sample's composition. Antioxidant activities were also affected by the extraction method and conditions (temperature and time). The primary antioxidant component was phenolics, and their total concentration was directly proportional to their antioxidant activity [12]. Based on Table 1, extraction time affected the total phenol content. A relationship between extraction time and TPC was established, increasing exposure time, indicating the highest TPC presence at 55 minutes.

12
Table 1. Effect of Extraction Time on Total Phenolic Content

Temperature [°C]	Concentration [%]	mass [gr]	Absorbance [nm]	Concentration [ppm]	TPC [mg GAE/mg]
50	70%	0.840	0.906	67.113	479.377
	75%	0.930	0.833	61.624	397.575
	80%	0.889	1.126	83.617	564.341
	85%	0.860	1.099	81.624	569.470
	90%	0.840	0.925	68.541	489.581
60	70%	0.895	0.897	66.398	445.130
	75%	0.896	1.020	75.647	506.562
	80%	0.995	1.460	108.767	655.881
	85%	0.926	1.246	92.639	600.253
	90%	0.900	1.052	78.053	520.351
70	70%	0.958	0.177	12.301	77.064
	75%	0.985	0.211	14.820	90.262
	80%	0.954	1.611	120.083	755.633
	85%	0.959	1.579	117.714	736.712
	90%	0.998	1.592	98.429	713.947

At high temperatures, fucoxanthin becomes unstable, resulting in the deformation of conjugated double bonds and reduced antioxidant activity. Furthermore, because fucoxanthin's structure is unstable, it is easily influenced by heat, air, and light exposure [13]. TPC of ethanol extracts from the Padina sp is presented in Table 2. The results show that at temperature 70 °C and solvent concentration 70 % exhibited the highest TPC (755.633 mg GAE/mg). Polyphenol content had a strong correlation to DPPH's antioxidant activity. Phenolic compounds act as an antioxidant by their ability to act as a reducing agent, a buffering agent, and an anti-radical. Phenolic compounds reportedly serve as the primary antioxidants in seaweeds [1]. Phenolic molecules protect metal-catalytic centre's in radical initiator processes [14].

Higher temperatures can be caused by particles' movement to the solvent faster because temperature affected the mass transfer coefficient value. The increase in temperature also causes the cell permeability to get weaker, making it easier for ethanol as a solvent to extract the active substance in the material so that the results obtained are higher than at the low temperature. The longer extraction time caused a more significant heating effect and prolonged exposure to the solvent. The concentration of the active ingredient will increase the total number of broken cells. This condition will continue until an equilibrium condition is reached between the concentration of compounds in the Seaweed *Padina* sp. with the concentration of the compound in the solvent [15].

Table 2. Total phenolic content of *padina* sp seaweed

Concentration [%]	Time [minute]	mass [gr]	Absorbance [nm]	TPC [mg GAE/mg]
80	35	1.45	1.316	405.268
	45	1.005	1.199	532.196
	55	0.995	1.459	655.881
	65	0.985	1.207	545.979
	75	0.995	1.072	479.964

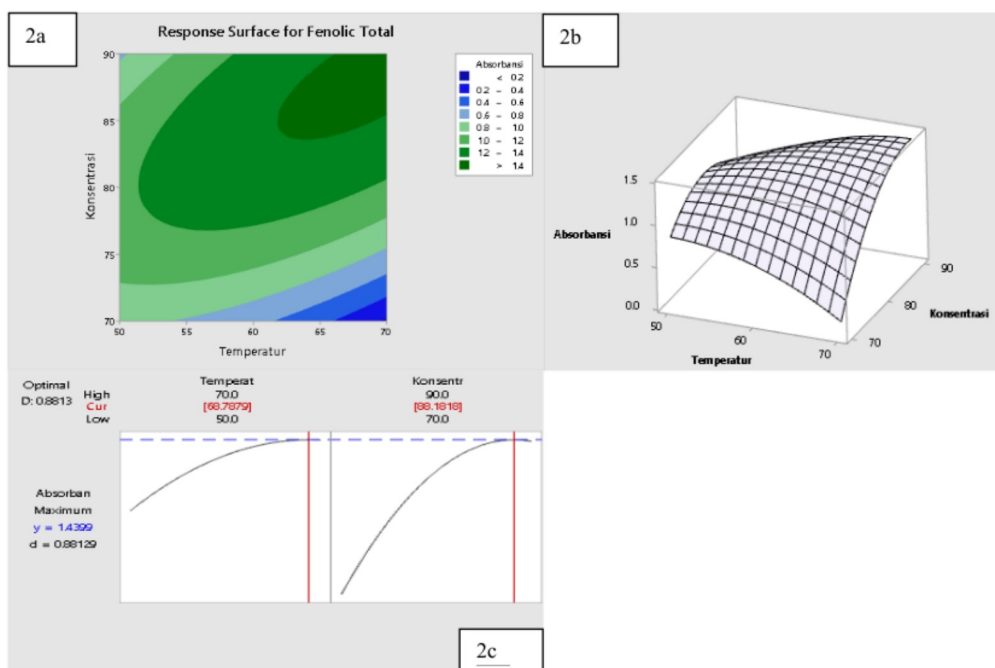


Figure 2. Response Surface for Total Phenolic content (a. plot contour b. Surface Plot c. Optimum Plot)

RSM is used to assess the impact of each factor and the interaction among elements and make the optimization activities more efficient. Fig.2a. Shows of various color variations, where each variation shows the range of the resulting response. The maximum conditions for the above plot are dark green with a compressive strength value above 14 MPa. Fig. 2b. Optimization area will be obtained by determination of the optimization are based on the average sample value. Figure 2c showed the proportions for each independent variable which will produce the most significant response variable. Where temperature variation 68.7897% and Concentration 88.1818%.

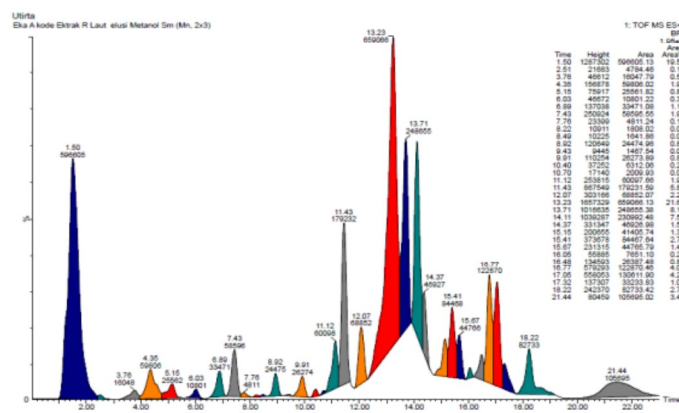
Pigment Identification by Thin Layer Chromatography Method. Since fucoxanthin is the primary carotene in seaweed, it was used as reference material. On a single plate, the pure and crude fucoxanthin were found to be present in equal quantities, along with a distinct chromatographic profile. The TLC experiment was done using the same solvent composition as the column.

The pop-up spot and Rf data confirm the presence of the findings of fucoxanthin in the crude extract. The existence of fucoxanthin in the natural extract could be ascertained using TLC and HPLC [16]. Pigment compounds in *Padina* sp., among others, are chlorophyll a with a Rf value of 0.96-0.98 with a dark green pigment. Chlorophyll c with an Rf value of 0.91-0.93 with a light green pigment and a Rf value of 0.94-0.96 with an orange pigment color [17]. From Fig. 2. The fucoxanthin can be calculated with a Rf of 0.96 from converting the compound distance (8 cm) and solvent movement (distance). The TLC analysis represented in Fig. 3



Figure 3. TLC based detection of compounds of *Padina* sp seaweed

Liquid Chromatography-Mass Spectrometry (LC-MS). The LC-MS method was used to analyze fucoxanthin from brown algae. LC-MS with mass-to-charge ratio (m/z). Fucoxanthin has been quantified primarily using LC-MS with electrospray or atmospheric pressure chemical ionization (APCI) or atmospheric pressure chemical ionization (ESI) sources in Fig. 4. along with other detection techniques such as UV in addition [16]. Fucoxanthin was found with a molecular weight of 659.43 m/z at a retention time of 16.15 minutes can be seen table 3. The abundance of fucoxanthin compounds in the extract of *Padina* sp. amounting to 5.8%. From the previous study by [18] using the maceration method for 24 hours. The total content of fucoxanthin was much over This approach, which uses ultrasounds to produce micro-bubbles inside the solvent, is thought to be cost-effective and efficient. The disintegration of the macroalgae cell wall occurs as the bubbles develop and collapse, allowing the solvent to penetrate. [16].



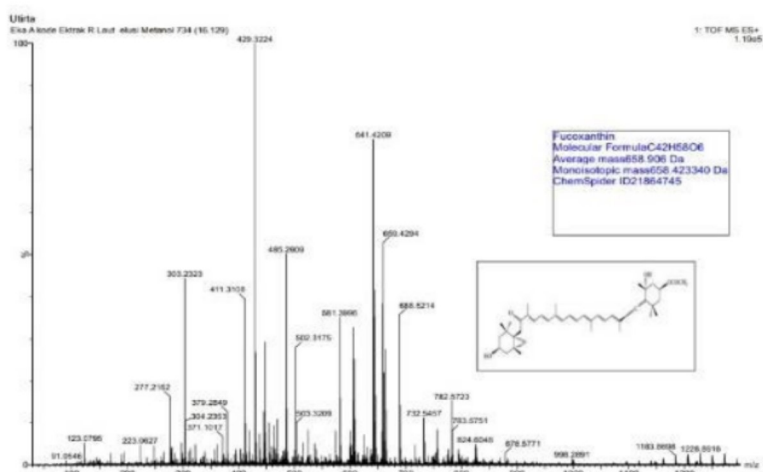


Figure 4. LC-MS profiles of Fucoxanthin

Table 3. Chemical compounds found in *Padina* sp From LC-MS

Retensi time [minute]	Molecular mass [m/z]	Molecular formula	compound	Abundance of compounds
4,57	205,01	C ₁₁ H ₁₂ N ₂ O ₂	DL-Tryptophan	6%
4,92	295,13	C ₁₄ H ₁₈ N ₂ O ₅	Aspartame	6%
5,19	246,17	C ₁₂ H ₂₃ NO ₄	Isovalerycarnitine	5,27%
6,03	265,07	C ₁₃ H ₁₂ O ₆	Methyl (7,8-dihydroxy-4-methyl-2-oxo-2H-chromen-3-yl)	0,7%
6,03	279,05	C ₁₃ H ₁₀ O ₇	Exifone	0,35%
7,45	197,12	C ₁₁ H ₁₆ O ₃	Zingerol	1,92%
8,94	427,28	C ₂₇ H ₃₈ O ₄	Garcionic Acid	0,8%
12,05	351,25	C ₂₁ H ₃₄ O ₄	10-Gingerol	2,26%
16,15	659,43	C ₄₂ H ₅₈ O ₆	Fucoxanthin	5,8%

FTIR. TLC-based preliminary identification suggested that the purified compound could be fucoxanthin. Additionally, spectroscopic identification was performed compared to an authentic fucoxanthin standard to confirm the purified compound's identity. The functional groups present in the solvent fractions of *Padina* sp. were identified using the FTIR spectrum. FT-IR data analysis was used to compare the structure between the crude extract sample and the active fraction of fucoxanthin.

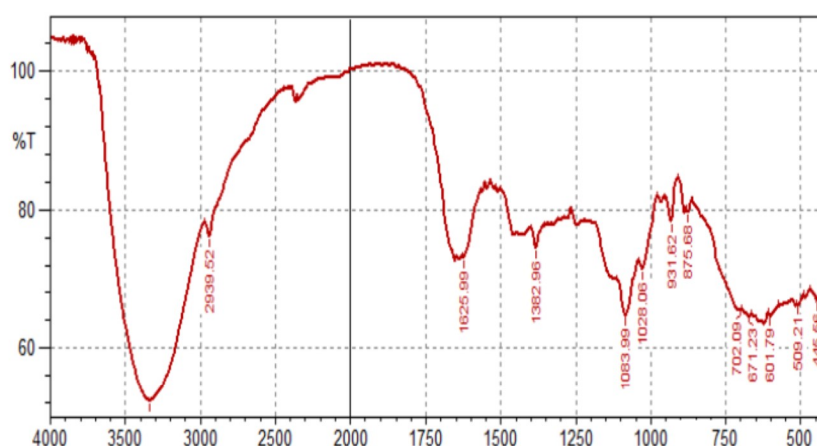


Figure 5. FTIR Result extract of Padina Sp

From Fig. 5. the sample Padina sp shows the presence of the hydroxyl functional group OH (alcohol) at wave number 3350.35 cm^{-1} , alkane CH at wave number 2939.52 cm^{-1} , strong absorption at wave number 1625.99 cm^{-1} C = O alkene, wave number 1382.96 cm^{-1} indicates the presence of methyl COH, and at the number 1083.99 cm^{-1} indicates an aromatic alkene. The presence of alcohol compounds, ketones, alkanes, esters, and weak allenic bonds differentiates fucoxanthin. [18]. Hydroxyl functional groups, alkyl chains, alkyl amine chains, nitrogen, and oxygen are antibacterial functional groups[19].

Table 4. Selected Functional Group Absorption[20]

Functional group	Frequency [cm^{-1}]
-OH	3600-3200
-NH and Nh ₂ (H bounded)	3300-3000
-CH ₃	2962(\pm 10),2872(\pm 10)
-CH ₂ -	2962(\pm 10),2853(\pm 10)
=C-H	3082-3000
-C=N	2260-2200
-C=C	2250-2040
-C=O	
Acids	1770-1750
Acid salts	1610-1550
Esters	1745-1725
Aldehydes	1735-1715
Ketones	1720-1710
Amides	1700-1600
-C=N	1670-1618
-C=C-trans	1678-1665
-C=C-cis	1662-1648
-N-H	1590-1500
-N-D	1490-1400
-C-N	1280-1030

Conclusion

Microalgae demonstrate tremendous potential as sustainable feedstocks for a wide variety of bioproducts. The UAE was discovered to be an efficient method due to the high extraction rate over

a short period and at a low temperature. The ethanol solvent concentration of 80% at 70 °C obtained the highest total phenolic yield, namely the 755.633 mg GAE / g sample. The active fraction of fucoxanthin was identified with a Rf value of 0.96. The fucoxanthin was placed at a Rf value interval of 0.94-0.96 with an orange pigment color. Infrared spectrophotometry (FTIR) shows the presence of functional groups of fucoxanthin compounds. Identification by LC-MS / MS found bioactive compounds of fucoxanthin.

16

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