Fucoxanthin Pigment Extraction from Brown Algae Species from Sumenep Waters Using High-performance liquid chromatography

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Sumenep waters, Madura are one of abundant marine brown algae. Brown algae is particularly rich in carotenoids, especially fucoxanthin. The aim of the research is to determine the content of fucoxanthin of 5 species brown algae from Sumenep waters, Madura (*Sargassum duplicatum, Sargassum polycystum, Sargassum filipendula, Padina australis* dan *Turbinaria conoides*). The pigment extract was separated by reversed-phase high performance liquid chromatography with Shim-pack ODS C-18 5µm column using an elution gradient of methanol, acetone and ammonium acetate 1 M solution. Identification fucoxanthin use thin layer chromatography using silica gel F-254 and elution hexane: ethyl acetate. The results show that fucoxanthin separation using high performance liquid chromatography indicate several peaks which not detected by thin layer chromatography. The main pigments of brown algae are chlorophyll c, fucoxanthin, β carotene and chlorophyll a. The highest content of fucoxanthin is gained by *Padina australis* (0.27 ± 0.0046) mg/g, and the lowest by *Sargassum polycystum* (0.158 ± 0.0091) mg/g. Fucoxanthin contents of *Turbinaria conoides, Sargassum filipendula, Sargassum duplicatum* are 0.21 ± 0.0108 mg/g, 0.19 ± 0.0173 mg/g, dan 0.165 ± 0.0092 mg/g.

Key words: brown algae species, extraction fucoxanthin pigment

Introduction

There are six genus of brown algae (*Dictyota, Padina, Hormophysa, Sargassum, Turbinaria,* and *Hydroclathrus*) which can germinate in Indonesia oceans, especially in the tropical clean water, coral reef, 0.5-10 m depth, waves and current smoothly, water temperature 27,25°C – 29,30°C and 32 – 33,5 ppt salinity (Atmadja, 2007). Sumenep waters, Madura is one of water which has coral reef environment that kinds of sea weed can blossom and growth rapidly. Almost all of that seaweed grow naturally, not creature of culturing.

Seaweed is the kind of photosynthetic plant which did not have root, leaf, stem differentiation. Its look like real plant, but it is thallus. *Sargassum* is builded from thallus which it resembles with leaf and stem. It also has bladder. *Padina* arranged from slices of thallus, resemble with leaf fans. *Turbinaria* is arranged from thallus which spin branch resemble with horn (Aslan, 1998). In means that brown algae (*Sargassum, Padina,* and *Turbinaria*) have different forms of thallus. Brown algae have photosynthetic pigment,

fucoxanthin, and a kind of pigment which arrange brown color. Most of the pigment are in the leaf and the a little in root, stem and branch (Rahayu and Limantara (2005)

Pigment identification can be done by chromatography and spectrophotometri. Pigment absorption is compared with refference (Gross, 1991). Brown algae pigment identification can be used in other applications (Christiana, *et al.,* 2008). The research about pigment composition from brown alga from Sumenep water is a litlle known.

Research Method

1. Pigment Extraction

Pigment extraction phase is done according to Seely *et al.,* (1972). Five brown algae species (*Sargassum duplicatum, Sargassum polycystum, Sargassum filipendula, Turbinaria conoides,* and *Padina australis*) from Sumenep waters, Madura are rinsed, cleaned, sliced 1 cm, weighed 10 g, blended, added $CaCO_3$. Pigment extraction used DMSO (*Dimetyl sulfoxide*) (1:10, w/v), 20 minutes, filtered with filter paper and marked as X_1 (DMSO extract volume). X_1 is separated with ethyl acetate (1/2 X_1 (v)) dan 0.5 M ($NH_4(SO_4)_2$) (X_1 extract volume). The upper phase is separated again with the same solution. The lower phase is twice separated with 10 ml diethyl ether and added with salt saturated. The upper phase is collected, dryed rotary vacum evaporator, 30°C 100 rpm.The yield is added Na_2SO_4 anhidrate, dryed with argon gas and collected as dryed pigment extract (X_1).

Extract DMSO (X_1) residue is extracted with acetone (1:10, w/v), 10 minutes, and 100 rpm, filtered with filter paper. Extract residue is extracted again with acetone, added with aquadest until all of the residue are dipped (1:10, w/v), 10 minutes, 100 rpm, filtered with filter paper and collected as X_2. X_2 is separated with heksana (1/3 $X_{2\ volumes}$) dan aquadest (1/4 $X_{2\ volumes}$). The upper phase is twice separated with methanol 75% (1/9 $X_{2\ volumes}$). The upper phase is separated again with methanol 80% (1/9 X_2 volume) and collected as X_{2A} (heksana phase) and the lower phase is collected as X2B (aseton-metanol-aquadest phase). X_{2A} is dried with argon gas and collected as dried pigment extract X_{2A}.

X_{2B} (aseton-metanol-aquadest) is separated with diethyl ether (2/5 X_{2B} volume), added salt saturated and water in order to clear the separation. The upper phase X_{2B} is added Na_2SO_4 anhydrate, dryed with argon and collected as dried pigment $X_{2B.}$

2. Fucoxanthine analysed

X1 dried pigment extract is dissolved with DMSO: aquadest (4:1 v/v). X2B dried pigment is dissolved with acetone: methanol: aquadest (3:1:1, v/v/v). All of them are

measured with UV-Vis spectrophotometri with 3 repetitions. Calculation of fucoxanthin pigment is done according Seely *et al.,* (1972).

$X_1 = (A_{480} – 0.722 (A_{631} + A_{582} – 0.297 A_{665}) – 0.049 A_{665}) / 130$
$X_{2B} = (A_{470} – 1{,}239 (A_{631} + A_{581} – 0.300 A_{664}) – 0.0275 A_{664}) / 141$

3. High-performance liquid chromatography

The pigment extract was separated by reversed-phase high performance liquid chromatography with Shim-pack ODS C-18 5µm column using an elution gradient of methanol, acetone and ammonium acetate 1 M solution (80:10:10, v/v/v), eflluent flow 1.0 ml/minutes (Hegazi *et al.,* 1998). Dried pigment extract of 5 brown algae species from Sumenep waters are dissolved with 5 ml elution gradient, filtered with 0.2 µm filter membrane. 20 µl of this solution is injected in high-performance liquid chromatography and analyzed at 450 nm.

4. Thin Layer Chromatography

Fucoxanthin identification is done by tin layer chromatography according to Yan *et al.* (1999) using gel silica F254 as reversed phase and elution hexane: ethyl acetate (1: 1, v/v) ± 10 ml. Dried extract fucoxanthine dissolve with 1 ml elution solution. 6 µl of this solution is dropped in plate TLC, dipped in the elution solution, wrapped, waited until the solution reach the upper TLC, acoounted the Rf value.

Result and Discussion

1. TLC (Thin Layer Chromatography)

Brown algae pigment composition using TLC is based on color of splatter, and Rf value of all the brown algae extract. From color and sum of splatter, *Padina australis, Turbinaria conoides, Sargassum filipendula, Sargassum duplicatum,* and *Sargassum polycystum* are same. They have 8 spllaters, yellow, grey, green-blue, yellow-orange, dark orange, orange, light orange and green (Table 1). Grey pigment is assumed as pheophitin (Jeffrey *et al.,* 1997). Green-blue pigment is presumed as chlorophyl a, green pigment as chlorophyl c and yeloow, yellow-orange, dark orange, orange are assumed as carotenoids (Gross (1991).

Rf value is used to support the color spllater. Yellow pigment has Rf value 0.94. Soviani *et al.,* (2004) and Pratikno *et al.,* (2004) used eluent acetone: methanol: isopropyl

alcohol (5%:4%; 1% in toluene) showed yellow pigment in Rf 0.91-0.94. Grey pigment has Rf 0.81, the same range Rf (0.74-0.82) with Soviani *et al.,* (2004), Heriyanto and Limantara (2006), Pratikno *et al.,* (2004) research. Green-blue pigment has Rf 0.59. It is assumed as chlorophyll a with Rf range 0.57-0.64 (Heriyanto and Limantara, 2006). Dark orange pigment has Rf value 0.23. It is presumed as fucoxanthine. According to **Y**an *et al.,* (1999), Rf value fucoxanthine pigment with eluent hexane: ethyl acetate (1:1, v/v) is in the range 0.25-0.28. Yellow-orange, orange, light orange pigment has Rf value 0.31, 0.2, 0.14. It identified as xantophyl. According to Heriyanto and Limantara (2006), xantophyl has Rf value 0.10 -0.30 with hexane: acetone (95:5 v/v) eluent. Green pigment has Rf value 0.07. It is presumed as chlorophyll c. According to Jeffrey *et al.,* (1997), chlorophyl c is green color.

Table 1. Spllater color and Rf value of five species brown algae

Brown algae	Color spllater							
	1	2	3	4	5	6	7	8
Padina australis	yellow	grey	green-blue	yellow-orange	dark orange	orange	light orange	greeen
Turbinaria conoides	yellow	grey	green	yellow	dark orange	orange	light orange	greeen
Sargassum filipendula	yellow	grey	green	yellow	dark orange	orange	light orange	greeen
Sargassum duplicatum	yellow	grey	green	yellow	dark orange	orange	light orange	greeen
Sargassum polycustum	yellow	grey	green	yellow	dark orange	orange	light orange	greeen
Rf value	0.94	0.81	0.59	0.31	0.23	0.2	0.14	0.07

2. High-performance liquid chromatography

Brown algae pigment (*Padina australis, Turbinaria conoides, Sargassum filipendula, Sargassum duplicatum,* and *Sargassum polycystum*) analysed using HPLC showed the retention time (tR) for 5 peak chromatogram (Table 2). According to Christiana, *et al.,* (2008), the first peak chromatogram is the pigment which has more polarity than the next peak. The research showed that chlorophyll c and fucoxanthine have the retention time more lowly than other pigment, so they identified as the first and the second peak chromatogram. Carotenoid pigment has high retention time, non polar, so it is showed in the last peak. Maximum absorbance from five peaks chromatogram support the brown algae pigment identification (Table 3).

Table 2. Pigment time retention

Pigment	Retention time (tR)					
	Padina australis	*Sargassum duplicatum*	*Sargassum filipendula*	*Sargassum polycystum*	*Turbinaria conoides*	**(**Hegazi, *et al.*, 1998)
Chlorophyl c	6.432	6.41	6.389	6.421	6.44	2.86
Fucoxanthine	10.22	10.09	10.112	10.13	10.13	5.85
Chlorophyl a	38.5	38.3	38.389	38.432	38.5	38.01
Pheofitine	56.928	56.64	56.8	56.98	56.9173	62.55
β-carotene	62.144	61.984	62.09	62.25	62.19	57.14

Table 3. Maximum absorbance pigment

Pigment	Maximum absorbance (nm)					
	Padina australis	*Sargassum duplicatum*	*Sargassum filipendula*	*Sargassum polycystum*	*Turbinaria conoides*	**(**Hegazi, *et al.*, 1998)
Chlorophyl c	444, 583, 634	442, 583, 634	442, 583, 634	445, 583, 634	445, 583, 634	444, 584, 632
Fucoxanthine	450, 465	450, 466	450, 465	450, 465	450, 465	452
Chlorophyl a	414, 431, 537, 585, 618, 664	412, 431, 537, 585, 618, 664	414, 432, 537, 585, 618, 664	412, 434, 537, 585, 618, 664	410, 431, 537, 585, 618, 664	412, 432, 532, 580, 616, 664
Pheofitine	409, 476, 505, 537, 559, 608, 665	409, 476, 505, 537, 559, 608, 665	409, 476, 505, 537, 559, 608, 665	409, 476, 505, 537, 559, 608, 665	409, 476, 505, 537, 559, 608, 665	412, 448, 472, 508, 536, 560, 608, 668
β-carotene	426, 451, 476	426, 451, 479	426, 451, 476	426, 451, 479	426, 451, 479	428, 452, 476

3. Fucoxanthine Content

From TLC, fucoxanthine is identified by color (dark orange), Rf value 0.21 - 0.23. Form Selly *et al.*, (1972) equation, fucoxanthine content brown algae (*Padina australis*, *Turbinaria conoides*, *Sargassum duplicatum*, *Sargassum polycystum*, and *Sargassum filipendula*) are 0.2674, 0.2134, 0.1957, 0.1649 and 0.1578 mg/g. It is showed that *Padina australis* has fucoxanthine content higher than *Sargassum* spesies. It is supposed that *Padina australis* build from thallus which looks like slices of fans. Fucoxanthine content higher in leaf than stem, branch or root (Rahayu and Limantara, 2005). On the other hand, *Sargassum* has thallus like long rope. The rope can reach 3-5 m. Besides morphology, fucoxanthine content of brown algae is influenced by sea water depth. *Sargassum* is tent to in the sea level surface. On the contrary, *Padina* tent to grow in the sea bottom. According to Nurdiana *et al.* (2008), fucoxanthine content in the brown algae increases suitable with the depdt of sea level, although this bottom sea has to be reached by solar sun.

Conclusion

From the research, it can be conclused that five species brown algae (*Padina australis*, *Turbinaria conoides*, *Sargassum filipendula*, *Sargassum duplicatum*, and *Sargassum polycystum*) from Sumenep waters, Madura have fucoxanthine, chlorophyll a, chlorophyll c and β-carotene pigment. Fucoxantine content from these brown algae are *Padina australis* 0,2674 mg/g, *Turbinaria conoides* 0,2134 mg/g, *Sargassum filipendula* 0,1957 mg/g, *Sargassum duplicatum* 0,1649 mg/g, and *Sargassum polycystum* 0,1578 mg/g. It is suggested that researcher in pigment extraction has to give attention in the length and diameters coloumn and the kind of extraction solution. The differentiation of that can make the yield differences.

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