

Analysis of Pigment Composition of Brown Seaweeds Collected from Panjang Island, Central Java, Indonesia

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Composition of pigments from four species of brown seaweeds (Phaeophyceae) collected from Panjang Island, Central Java, Indonesia, was investigated with spectroscopic method and reverse-phase high-performance liquid chromatography (RP-HPLC). Identification of pigments was based on their spectral and chromatographic properties and also confirmed by electrospray ionization-mass spectrometry (ESI-MS/MS) analysis. The experimental results showed that concentrations of chlorophyll *a* (Chl *a*) and total carotenoids (Cars) from brown seaweeds, estimated by spectroscopic method, varied depending on species from 1.73 mg · g⁻¹ to 8.84 mg · g⁻¹ and from 0.55 mg · g⁻¹ to 4.06 mg · g⁻¹ dry weight (dw), respectively. In addition, the order of concentrations of Chl *a* and total Cars in four species of seaweed was as follows: *Dictyota dentata* > *Padina australis* > *Sargassum crassifolium* > *Turbinaria conoides*. This order was in agreement with the concentrations of dominant pigments calculated by HPLC method, i.e., fucoxanthin (Fuco) (0.43 mg · g⁻¹ to 4.11 mg · g⁻¹ dw), Chl *a* (1.70 mg · g⁻¹ to 7.89 mg · g⁻¹ dw), β -carotene (0.16 mg · g⁻¹ to 0.78 mg · g⁻¹ dw). These results suggest that *D. dentata* is likely potential source material to explore the industrial utilization, especially functional food and biomedical ingredients, of Fuco and Chl *a*.

Key words: brown seaweed, chlorophyll *a*, ESI-MS/MS, fucoxanthin, Panjang Island, pigment composition

INTRODUCTION

Indonesia is well known as an archipelago country having abundant marine natural resources. One of them is seaweeds which are classified based on their pigmentation into brown, red and green seaweeds (Dawczynski et al. 2007).

Brown seaweeds (Phaeophyceae) have not been optimally explored, although they have been recognized to have several beneficial effects on human health. In addition to sodium alginate, fucoxanthin (Fuco), the major marine carotenoid (Car), is a commercial importance in brown seaweeds. Fuco has demonstrated anti-inflammatory (Shiratori et al. 2005), anticancer (Kotake-Nara et al. 2001), and anti-obesity (Miyashita 2009) activities.

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In spite of increasing research progress on beneficial effects and identification of Fuco in brown seaweeds, quantification and occurrence of Fuco, a useful marine pigment as similar to chlorophyll (Chl), β -carotene and other Cars need to be investigated. Previously, Limantara & Heriyanto (2010) have investigated the pigment composition and Fuco content from five species of brown seaweeds from Madura Island by reverse-phase high-performance liquid chromatography (RP-HPLC). Also Le Lann et al. (2012) investigated the measurement of Fuco content by HPLC in Sargassaceae species from the South Pacific Ocean. Brotsudarmo et al. (2017) used electrospray ionization-mass spectrometry (ESI-MS) to identify the dominant pigments from *Kappaphycus alvarezii* (Doty ex P. C. Silva) and *Padina australis* (Hauck). Hegazi et al. (1998), Dhargalkar (2004), and Bischof et al. (2006) reported that specific photosynthetic pigments and their concentrations in brown seaweeds varied depend on various environmental factors, such as nutrient, temperature, season, light intensity, and growing depth. Moreover, species and morphological structure of brown seaweeds influenced the pigments composition and Fuco concentration (Terasaki et al. 2009, Heriyanto et al. 2010, Le Lann et al. 2012).

Sargassum, *Padina*, *Turbinaria* and *Dictyota* are well known as genera of brown seaweeds spreading in all Indonesia waters (Atmadja et al. 1996). Information of composition and quantification of the pigments in brown seaweeds might be useful for selecting the sources of pigments for further applications, because of the reason described above. In this study, we determine the pigment composition and concentrations by spectroscopic and HPLC methods in brown seaweeds collected from Panjang Island.

MATERIALS AND METHODS

Chemicals

Acetone, methanol (MeOH), calcium carbonate (CaCO_3), sodium ascorbate, diethyl ether (DE), ammonium acetate, water, and formic acid were purchased from Merck (Darmstadt, Germany) and used without further purification. Standard pigments – Fuco, Chl *a*, and β -carotene obtained from NATChrom (Malang, Indonesia).

Collection of sample

Abundant brown seaweeds: *Dictyota dentata* (J. V. Lamouroux) (Dictyotaceae), *Padina australis* (Hauck) (Dictyotaceae), *Sargassum crassifolium* (J. Agardh) (Sargassaceae), and *Turbinaria conoides* (J. Agardh) Kützinger (Sargassaceae) (Figure 1) that grown naturally were collected by hand in Panjang Island, Central Java,

Indonesia (6°34'31.9"S, 110°37'51.8"E) on 6 August 2015. The collected site is a small area near seashore including sand-mud and scattered rocks up to around 1.0-1.5 m depth. Brown seaweeds were collected randomly several specimens per one species. Upon collection, these were cleaned from epiphytes, epibionts and debris and rinsed with seawater. Samples were then put into plastic bags and placed in cooling boxes with enough ice during transport to the laboratory.

Water content

For precise estimation of dry weight (dw) of the samples, water content of the samples was measured by using a Moisture Tester OC630U (Shimadzu, Kyoto, Japan) as described previously (Mulyadi et al. 2017). Approximately 0.1 g of ground sample was used for the analysis.

Extraction of pigments

Thalli were initially frozen by immersion in liquid nitrogen (N_2) and then finely ground into small particles with a mortar and pestle. The pigment extraction was carried out by vortexing approximately 0.1 g of the sample in 1 mL of acetone and MeOH mixture (7:3, v/v) in the presence of CaCO_3 and sodium ascorbate and followed by centrifugation to separate the crude pigment extract and residue. The residue was continuously extracted with the same solvent mixture until completely pigment extraction. The pigment extracts were stored at -30°C after drying with a stream of N_2 gas. The extraction of pigments was performed at room temperature under a dim light.

Concentrations of Chl *a* and total Cars

Absorption spectra of crude pigment extracts in DE were recorded by a UV-1700 spectrophotometer (Shimadzu) in the range of 300-900 nm. Concentrations of Chl *a* and total Cars were calculated according to the equation of Lichtenthaler (1987).

Separation, identification, and quantification of pigments

Analysis of pigments was performed by RP-HPLC using LC-20A equipped with a SPD-20MA diode array detector (Shimadzu). The separation of pigment was carried out on a Shim-pack VP-ODS C18 column (250 × 4.6 mm ID, Shimadzu) with a gradient elution program of ammonium acetate solution (1 M), MeOH and acetone mixture at the flow rate of 1 mL/min at 30°C in 70 min according to the modified method of Hegazi et al. (1998). Chromatographic and spectroscopic properties of the separated pigments were used for the identification of pigments. Further identification of dominant pigments was carried out by electrospray ionization-mass spectrometry (ESI-MS) (Shimadzu) according to the method of Brotsudarmo et al. (2017). The standard curve of pigments obtained from the linear equations was used for

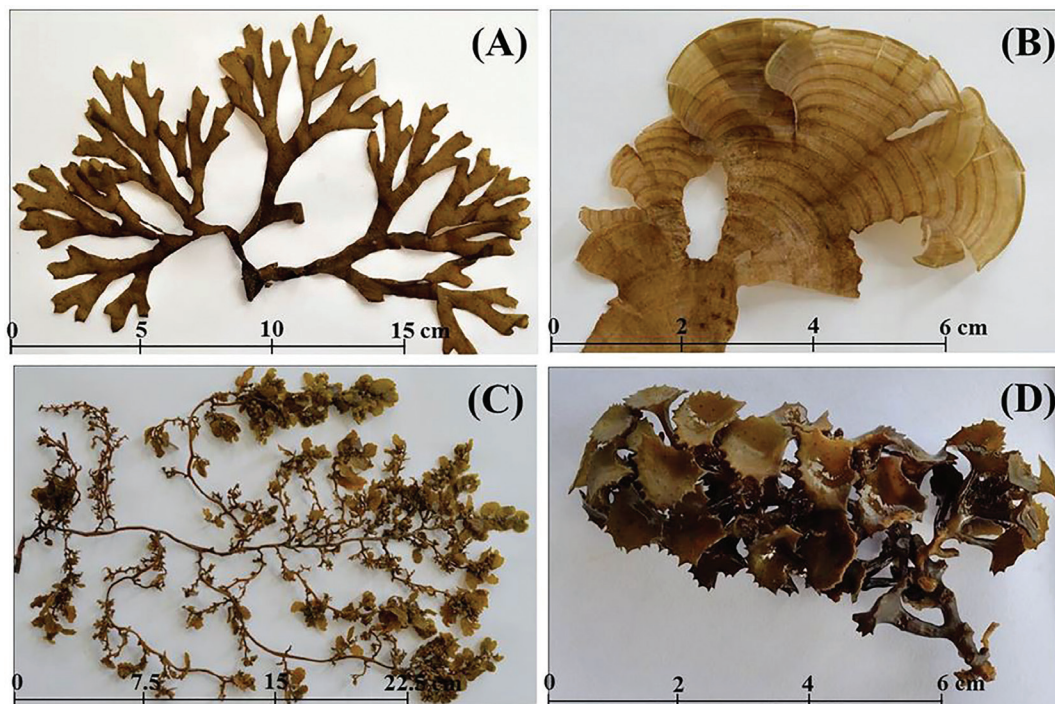


Figure 1. Four species of brown seaweeds (Phaeophyceae) collected in from Panjang Island (Central Java, Indonesia) and used in this study. (A) *Dictyota dentata*; (B) *Padina australis*; (C) *Sargassum crassifolium*; and (D) *Turbinaria conoides*.

calculating concentrations of Chl *a* ($R^2 = 0.9987$), Fuco ($R^2 = 0.9989$) and β -carotene ($R^2 = 0.9954$) in $\text{mg} \cdot \text{g}^{-1}$ dry weight (dw) (Brotosudarmo et al. 2017). Relative concentrations of the identified pigments were calculated from the area and percentage of area (% area) at their maximum absorption wavelengths (λ_{max}) and at 430 nm.

Data analysis

The concentration of pigments was averaged from three replications and then the standard error (SE) was calculated with the 95% confidence level.

RESULTS AND DISCUSSION

Absorption spectra of pigment extracts from brown seaweeds showed typical absorptions of Chl *a* at 430 nm and 660 nm and also Cars at around 400-500 nm which is partially overlapped with the Soret band of Chl *a* (data not shown). The pattern of absorption spectrum as well as its λ_{max} was quite the same for each pigment extract among 4 species examined. These spectroscopic properties indicate that the four brown seaweeds may have a similar pigment composition. On the other hand, absorbance values were different among them. *D. dentata* had a higher absorbance in spectrum than those of any other brown seaweeds used, indicating that this alga likely contains high amount of pigments.

High-performance liquid chromatography (HPLC) chromatograms of pigment extracts from 4 brown seaweeds are shown in Figure 2. Good separations of brown seaweed pigments, which have broad polarities, were achieved within 60 min by RP-HPLC. At least 11 distinct pigments out of approximately 30 pigments were clearly separated. In *D. dentata* and *P. australis*, 26 pigments were detected, while *S. crassifolium* and *T. conoides* contain 21 and 23 pigments, respectively. From the HPLC analyses, the four brown seaweeds seem to have the same pigment composition. Furthermore, the differences in pigment composition were observed mainly in minor pigments, such as degradation products of Chls.

Pigments were identified based on the combined characteristics of chromatography, UV-Vis spectrophotometry and mass spectrometry (Table 1), and also co-chromatographic results with the standard pigments (data not shown) including references (Limantara & Heriyanto 2010, Hegazi et al. 1998). Fuco, Chl *a*, and β -carotene were detected as the main pigments, while other minor pigments, such as antheraxanthin, zeaxanthin, violaxanthin, Chl *c*₁ and degradation products including *cis*-Fuco, pheophytin *a*, epimer Chl *a* (Chl *a'*), and chlorophyllide *a* were also observed. While other degradation products of Cars namely *cis*-isomer forms, Chl *a* species and Chl *c*₂ were also found in detectable quantity.

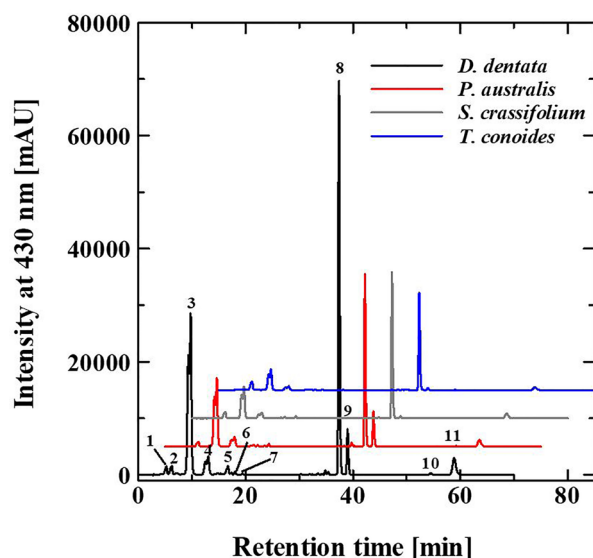


Figure 2. HPLC chromatograms of pigments extracted from 4 species of brown seaweeds (*Dictyota dentata*, *Padina australis*, *Sargassum crassifolium* and *Turbinaria conoides*). The sample, each 1 g dw, was used for the pigment extraction and the separated pigments were detected at 430 nm. HPLC and other experimental conditions are described in the text and the peak numbers in elution profile correspond to those in Table 1.

Peaks 1, 8 and 9 showed the same absorption spectra with the λ_{\max} at 431 nm, 618 nm, and 665 nm, but those pigments have the different retention times (t_R) especially for peak 1 compared with other two peaks probably due to the difference of their polarity (Limantara & Heriyanto, 2010).

Peak 8 which had the highest peak area was identified as Chl *a* according to the λ_{\max} and its molecular ions. From MS analysis of peak 8 (Figure 3A), protonated molecular ion $[M + H]^+$ was detected at m/z 893.5 and a fragment ion at m/z 871.6 corresponding to the loss of Mg as the central metal of Chl *a*. In nature, Chl *a* exists as two epimers, Chl *a* and Chl *a'*. These two epimers cannot be distinguished by their absorption spectra, but by different chromatographic behavior (Kobayashi et al. 2006). Therefore, peak 9 was identified as Chl *a'*. Shioi (2006) revealed that Chl *a'* is easily formed in air and at room temperature in MeOH solution. Formation of Chl *a'* may be taken place during the pigment extraction by acetone and MeOH mixture.

Peak 1 was identified as chlorophyllide *a*, which resulted from enzymatic hydrolysis of Chl *a* into chlorophyllide *a* and phytol by chlorophyllase. Therefore, chlorophyllide *a* is a more polar than Chl *a* due to the loss of phytol. This enzymatic reaction occurs when the brown seaweed was disrupted in a solvent by mortar leading to the contact between chlorophyllase and Chl *a*. Pheophytin *a* was identified at peak 10 based on its absorption spectrum (λ_{\max} at 409 nm, 505 nm, 536 nm, 610 nm, and 665 nm) and t_R which are in agreement with those of the standard. Pheophytin *a* is more hydrophobic than Chl *a* because of the absence of central metal Mg. Other Chl species of peak 2 was identified as Chl *c*₁ that has an intense absorption at 455 nm and belongs to protoporphyrins. Chl *c*₁ is known to be non-esterified Chl lacking phytol or other aliphatic long-chain alcohol. Therefore, it was separated at the beginning of elution in the polar region closely to chlorophyllide *a*.

Table 1. Chromatographic, spectrophotometric and mass properties of the pigments separated from brown seaweeds.

| Peak No | t_R [min] | λ_{\max} [nm] in the HPLC eluent | Molecular ion species [m/z] | Fragment ions [m/z] | Identification |
|---------|-------------|--|-----------------------------|--|--|
| 1 | 5.2 | 432,617,666 | – | – | Chlorophyllide <i>a</i> |
| 2 | 6.0–6.2 | 445,584,633 | – | – | Chlorophyll <i>c</i> ₁ |
| 3 | 9.2–9.7 | –,450,– | 659.6 [M + H] ⁺ | 641.6 [M + H – H ₂ O] ⁺ ; 581.5 [M + H – OH – C ₂ H ₃ O ₂] ⁺ | Fucoxanthin |
| 4 | 12.4–13.0 | 416,440,469 | – | – | Violaxanthin |
| 5 | 16.7 | 333,–,443,– | 659.6 [M + H] ⁺ | 641.5 [M + H – H ₂ O] ⁺ ; 581.5 [M + H – OH – C ₂ H ₃ O ₂] ⁺ | 13- & 13'- <i>cis</i> isomers of Fucoxanthin |
| 6 | 17.3 | –,445,472 | – | – | Antheraxanthin |
| 7 | 19.3–19.4 | –,450,476 | 569.6 [M + H] ⁺ | 537.6 [M + H – 2(OH)] ⁺ | Zeaxanthin |
| 8 | 37.2–37.4 | 431,618,665 | 893.5 [M + H] ⁺ | 871.6 [M + H – Mg + H ₂] ⁺ | Chlorophyll <i>a</i> |
| 9 | 38.8–39.0 | 431,618,665 | – | – | Chlorophyll <i>a'</i> |
| 10 | 54.2–54.5 | 409,505,536, 610,665 | – | – | Pheophytin <i>a</i> |
| 11 | 58.5–58.9 | –,451,477 | 536.7 [M] ⁺ | – | β -carotene |

Peak No., see Fig. 2.

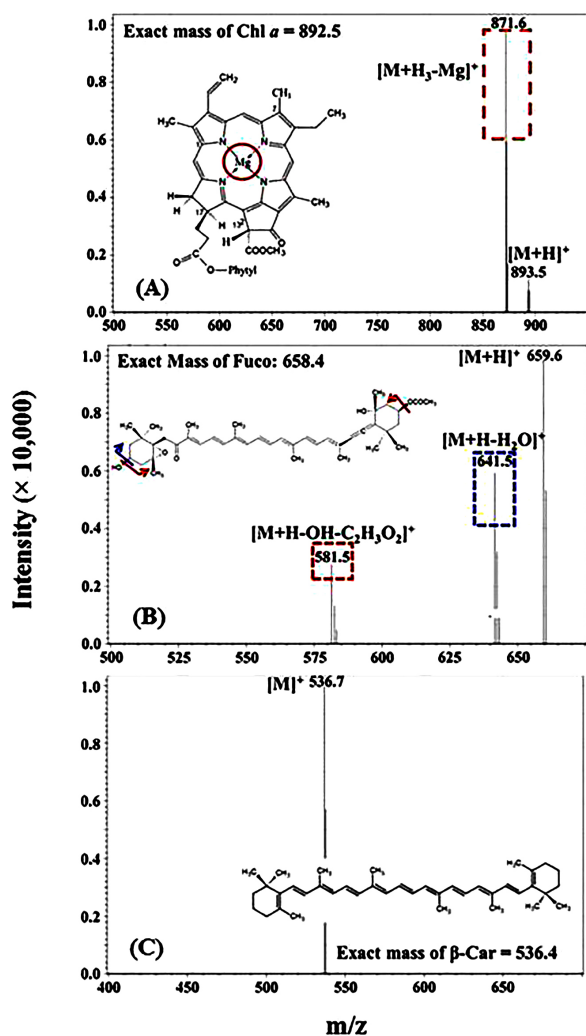


Figure 3. Positive ion ESI mass spectra of identified Chl *a* (A); Fuco (B), and β -carotene (C) showing their molecular structure, molecular ion species and fragment ions.

Fuco and its 13-*cis* isomer were identified at peaks 3 and 5, respectively. As shown in Fig. 3B, the mass spectra of these Fuco isomers showed the $[M + H]^+$ at m/z 659.6 and the fragment ions detected at m/z 641.6 and m/z 581.5 correlating to the losses of the hydroxyl group $[M + H - OH]^+$ and hydroxyl-ester groups $[M + H - OH - C_2H_3O_2]^+$ from the cyclic end groups. $[M + H]^+$ and the fragmentation pattern of Fuco have also been described by Maoka et al. (2002) and de Quirós et al. (2010). The distinct differences in these Fuco isomers are observed from their spectral properties. Absorption spectra of 13- and 13'-*cis* isomers of Fuco showed a reduction of intensity and a blue shift around 7 nm in vibrational fine structure and also appearance of an intense *cis*-peak located at 333 nm. Previously, Heriyanto & Limantara (2010) identified these isomers of Fuco as 13- and 13'-*cis* isomers which are the dominant degradation products of all *trans*-isomer of Fuco by thermal treatment. Peak 7 was identified as zeaxanthin. $[M + H]^+$ of zeaxanthin was detected at m/z 569.6 with a fragment ion at m/z 537.6, indicating the absence of two hydroxyl groups $[M + H - 2(OH)]^+$ which resembles the molecular structure of β -carotene (Figure not shown; cf. Table 1). MS analysis of peak 11 showed molecular ion $[M]^+$ at m/z 536.7 and identified as β -carotene in accordance with the results of de Quirós et al. (2010) that the $[M + H]^+$ of β -carotene was at m/z 537 (Fig. 3C). The presence of zeaxanthin is usually associated with the xanthophyll cycle (Couso et al. 2012). This cycle is the reversible deepoxidation of violaxanthin to zeaxanthin via the intermediate antheraxanthin to protect plants from photooxidative damage (see below). Thus, this cycle comprises above three species of Cars. According to the chromatographic and spectral properties, peaks 4 and 6 were identified as violaxanthin and antheraxanthin, respectively. The addition of one epoxide group in the cyclic end group caused a 5 nm blue shift of λ_{max} .

Brown seaweeds have a typical characteristic in the presence of Chl *c* as an accessory pigment in their photosynthetic membranes instead of Chl *b* which is usually found in

Table 2. Concentrations of pigments measured by spectrophotometry and HPLC methods in 4 species of brown seaweeds.

| Seaweed | Concentration (mg · g ⁻¹ ± SE) | Spectrophotometry | | HPLC | | |
|------------------------|---|----------------------|------------------|----------------------|-------------|-------------------|
| | | Chlorophyll <i>a</i> | Total Carotenoid | Chlorophyll <i>a</i> | Fucoxanthin | β -carotene |
| <i>D. dentata</i> | dw | 8.84 ± 0.83 | 4.06 ± 0.47 | 7.89 ± 0.59 | 4.11 ± 0.55 | 0.78 ± 0.09 |
| | fw | 0.82 ± 0.08 | 0.38 ± 0.04 | 0.66 ± 0.05 | 0.29 ± 0.05 | 0.08 ± 0.01 |
| <i>P. australis</i> | dw | 3.56 ± 0.45 | 1.68 ± 0.22 | 3.34 ± 0.35 | 1.64 ± 0.19 | 0.35 ± 0.03 |
| | fw | 0.61 ± 0.29 | 0.29 ± 0.14 | 0.53 ± 0.07 | 0.22 ± 0.04 | 0.08 ± 0.01 |
| <i>S. crassifolium</i> | dw | 2.70 ± 0.15 | 1.01 ± 0.04 | 2.70 ± 0.10 | 0.75 ± 0.09 | 0.31 ± 0.06 |
| | fw | 0.46 ± 0.03 | 0.17 ± 0.01 | 0.37 ± 0.02 | 0.09 ± 0.02 | 0.07 ± 0.01 |
| <i>T. conoides</i> | dw | 1.73 ± 0.11 | 0.55 ± 0.05 | 1.70 ± 0.37 | 0.43 ± 0.11 | 0.16 ± 0.01 |
| | fw | 0.56 ± 0.08 | 0.18 ± 0.02 | 0.54 ± 0.13 | 0.13 ± 0.04 | 0.07 ± 0.00 |

The values are average of three replications and SE.
dw: dry weight; fw: fresh weight.

higher plants (Boczar & Prezelin 1989). Moreover, Fuco is another distinctive pigment occurring in brown seaweeds and contains more than 50 % of the total Cars (Haugan & Liaaen-Jensen 1994). The occurrence of these pigments as well as Chl *a* in Fuco-Chl *a/c* protein complexes are responsible for harvesting light energy and transfer the harnessed energy to reaction centers in brown seaweeds (Kosumi et al. 2012). In the stressful high light condition, tightly bound Cars in the light harvesting antenna and reaction center provide the photoprotective function by dissipation of the accepted energy. A reversible switch of photosynthetic light harvesting complexes between a light harvesting state under low light and a dissipative state under high light is regulated by xanthophyll cycle, i.e., high light induces the de-epoxidation of violaxanthin, whereas the epoxidation of zeaxanthin occurs in the dark-adapted stage (Jahns et al. 2009).

As shown in Table 2, *D. dentata* had the highest contents of Chl *a* ($7.89 \pm 0.59 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$), Fuco ($4.11 \pm 0.55 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$) and β -carotene ($0.78 \pm 0.09 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$) compared to other studied brown seaweeds. The concentrations of pigments determined by spectrometric method were $8.84 \pm 0.83 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$ for Chl *a* and $4.06 \pm 0.47 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$ for total Cars. The concentrations of these pigments in four species of seaweeds follow this order: *D. dentata* > *P. australis* > *S. crassifolium* > *T. conoides*. This order was applicable to that of HPLC determination. There was a small variance in this pattern, although *P. australis* and *S. crassifolium* had close in β -carotene concentrations. Pigments concentrations of *D. dentata* were more than two times higher than other brown seaweeds, i.e., 2.5-5.1 times for Chl *a*, and 2.4-7.4 times for total Cars in spectrometric methods, 2.4-4.6 times for Chl *a*, 2.5-9.6 times for Fuco, and 2.2-4.9 times for β -carotene in HPLC method (Table 2). These results indicate that *D. dentata* is a possible candidate in brown seaweeds as a potential source of pigments. In fact, *D. dentata* composed of soft and thin branching thalli and therefore, its pigments are easily extractable (see Fig. 1A). Moreover, *D. dentata* appears to have higher surface area and biomass ratio compared to other three species. *P. australis* belongs to the same family, but it has wide fan type hard thalli due to accumulation of CaCO_3 in its tissue (Fig. 1B). On the other hand, thalli of *S. crassifolium* and *T. conoides* belong to Sargassaceae consisted of a small and hard independent leaf-like structure with many braches (Fig. 1C and D). Stiger et al. (2004) compared both morphological features between these two genera. Thus, *D. dentate* is suitable and potential source for the pigment production.

It is known that specific photosynthetic pigments and their concentrations in brown seaweeds varied depend on various environmental factors and morphological structure. However, comparative study in closely related species might give useful information concerning

differences among habitats. Concentrations of Chl *a* obtained from this study were in the range of $0.46 \text{ mg} \cdot \text{g}^{-1}$ to $0.82 \text{ mg} \cdot \text{g}^{-1}$ fresh weight (fw) basis (Table 2). Recently, Sudhakar et al. (2013) reported pigment concentrations of *Padina* sp., *Turbinaria* sp., and three species of *Sargassum* collected from Mandapam, Gulf of Mannar coast, Rameswaram, India. Chl *a* concentrations (0.19 - $1.13 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$) extracted with 90% acetone, 90% ethanol, and 100% acetone were lower compared to the results of the present study. They also measured total Cars (0.02 - $0.41 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$) and these values were 10 times lower than those of *D. dentata*. In addition, Fuco concentrations of these seaweeds were in the range of 0.09 - $0.38 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$. Terasaki et al. (2009).

$\text{g}^{-1} \text{ dw}$. Terasaki et al. (2009) determined concentrations of Fuco ($0.1 \pm 0.1 \text{ mg} \cdot \text{g}^{-1}$ to $3.7 \pm 1.6 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$) from six brown seaweeds in the Sargassaceae family and eight brown seaweeds from other families from Hakodate (Hokkaido, Japan). Edible Japanese brown seaweed, *S. horneri* had the highest Fuco concentration and species from Sargassaceae were generally rich in Fuco compared with species from other groups. Similarly, *S. horneri*, collected from the same locality Hakodate and extracted by MeOH, showed the slightly lower Fuco value of $1.09 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$ (Airanthi et al. 2011). *S. horneri* and *Saccharina japonica* collected from southern part of Korea and extracted with the different solvents and extraction methods had low amounts of Fuco ranging 0.08 - $0.77 \text{ mg} \cdot \text{g}^{-1} \text{ dw}$ (Sivagnanam et al. 2015). Le Lann et al. (2012) also determined the Fuco content of several species of Sargassaceae from South Pacific Islands and observed that *Sargassum* species always produced more Fuco than *Turbinaria* species.

Relative concentrations of Chl *a* derivatives, i.e., Chl *a'*, chlorophyllide *a* and pheophytin *a*, were much higher in *D. dentata* compared to those concentrations in *P. australis*, *S. crassifolium* and *T. conoides* (data not shown). The lowest relative concentration of chlorophyllide *a* in *T. conoides* may be correlated with the low amount of chlorophyllase which catalyzes the hydrolysis of Chl *a* and transesterification of phytol. It is known that all photosynthetic organisms contain pheophytin *a* in their reaction centers (Blankenship et al. 1995). The relative concentration of pheophytin *a* in *D. dentata* was 2.5-11.3 times higher than other brown seaweeds. Although, the occurrence of acid causes demetallation of Mg from Chl *a*, we neutralized seaweed tissues by the addition of CaCO_3 in the extraction. *D. dentata* had the highest relative concentration of Chl *a'* and it was 1.99-24.6 times higher than other brown seaweeds. It is known that the rate of formation of the Chl *a'* increases in the base condition (Porra et al. 1997). However, we have no information on this high concentration of Chl *a'*. Carotenoid occurrence in brown seaweeds depends

on the species and environmental factors. The depth of habitat of brown seaweeds correlates with the received light intensity and quality. *P. australis* and *S. crassifolium* were naturally grown at approximately 1 m depth, while *D. dentata* and *T. conoides* at 1.5 m depth. *P. australis* and *S. crassifolium* had a higher relative concentration of zeaxanthin and also a low ratio of relative concentrations between violaxanthin and zeaxanthin (7.0-9.8). In contrast to this ratio, *D. dentata* and *T. conoides* were 28.4 and 23.3, respectively. Accumulation of zeaxanthin or de-epoxidation of violaxanthin occurred in *P. australis* and *S. crassifolium* at high light intensity, suggesting the photoprotection function of zeaxanthin. On the other hand, *D. dentata* and *T. conoides* grown under low light accumulate violaxanthin or epoxidation of zeaxanthin in a light-harvesting state. Chl c_1 that plays a role in the light harvesting antenna of brown seaweeds was also found in *D. dentata* and *T. conoides* in higher amounts than in *P. australis* and *S. crassifolium*.

CONCLUSIONS

Pigments from brown seaweeds were clearly separated by RP-HPLC. At least 11 pigments were identified based on the chromatographic and spectral properties and confirmed by electron spray ionization-mass spectrometry (ESI-MS/MS) analysis. Four brown seaweeds had the same pigment composition and Fuco, Chl a and β -carotene were the main pigments. *D. dentata* had high contents of pigments and could be used as a potential source material for the preparation of these pigments.

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