CHEMICAL COMPOSITIONS OF TWO BROWN SEAWEEDS SPECIES FROM KARIMUN JAWA, INDONESIA

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ABSTRACT

Seaweeds are potentials to be developed as an alternative source in food and pharmaceuticals. In this study, chemical compositions of two brown seaweeds species from Karimun Java Indonesia *Padina australis* and *Turbinaria conoides* were investigated. Proximate results showed that both seaweeds contain a high amount of carbohydrates and ashes. Mineral contents of *P. australis* and *T. conoides* follow the orders of Na>Mg>Fe>K>Ca>Zn>Cu and Na>Mg>K>Fe>Zn>Ca>Cu, respectively. Almost all essential amino acids (including histidine, isoleucine, leucine, phenylalanine, threonine, valine and lysine) were present in both seaweeds. Fatty acid profiles showed that both seaweed contains polyunsaturated fatty acids (PUFA) with *T. conoides* contain a higher amount of EPA (8.58 ± 0.22 g per 100 g of total fatty acids) and DHA (6.05 ± 0.21 g per 100 g of total fatty acids). The findings of this study have provided evidence that brown seaweeds were nutritious and potential to be utilized for producing functional ingredients in food. Further, *P. australis* and *T. conoides* can be used as a candidate in food industries to increase shelf-life of food items for human consumption, and used to deter deleterious free radical-induced life-threatening diseases.

Keywords: Chemical compositions, antioxidant, brown seaweed, Indonesia.

INTRODUCTION

The exploding human population is of serious concern today (Kastenbaum, 2015). Hence, scientists never stop to look for future food resources. The future food resources should be nutritious, easy to cultivate, do not compete with agriculture, and are productive. Seaweeds have the potential to deliver new biomass flows for human use, since they can grow faster than terrestrial plants and are easy to cultivate. Moreover, seaweed grows in seawater where 70% of the world's surface is covered by ocean and vast coastal waters. In addition, these organisms are rich sources of nutrients including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals and vitamins, antioxidants, enzymes and bioactive peptides (Wijesekara *et al.*, 2010).

The tropical, extended and nutritious coastal area in Indonesia is a suitable and potential environment for seaweeds to grow. The earliest report of seaweed in Indonesia is obtained based on the Siboga Expedition (1899-1900). Presently, more than 1000 species of Indonesia seaweeds have been reported (Wibowo, 2013). In contrary to seaweed diversity, these organisms are still being recognized as under-exploited marine resources in Indonesia. Presently, the principal uses of seaweed in Indonesia are as a source of phycocolloids or exported in raw materials,

only a few seaweeds species in Indonesia have been authorized for human consumption as food or traditional medicine by local people. The consumption level of seaweeds is still low because there is not enough information and no database about their nutritional and non-nutritional compounds. Moreover, nutritional properties and antioxidant activity of many other seaweed species of Indonesia, particularly brown seaweeds, are not completely known. Therefore, it will be significant work to investigate nutritional value as well as antioxidant activities of seaweed from Indonesia.

Particular species with potential to be developed for food and widely abundant in Indonesia is *Padina australis* and *Turbinaria conoides*. These species belong to the brown algae (*Phaeophyceae*). In the present study, we investigated proximate composition, mineral, amino acids, fatty acids, natural pigment contents of *P. australis* and *T. conoides*. We also evaluated the effectiveness of different solvent systems [ethanol (EtOH), EtOH : acetone (DMK) (7:3, v/v), and EtOH : H_2O (7:3, v/v)] to simultaneously identify the best extraction solvent to finally produce an antioxidant-rich extract from the seaweeda. Extracts using different solvent system were compared based on their antioxidant activities.

MATERIALS AND METHODS

Materials

Brown algae *P. australis* and *T. conoides* were collected from the coast of Karimun Java. The collected samples were dried under the shade, powdered using a grinder and kept at -20°C until usage. EtOH, DMK was purchased from Merck with 2,2-Diphenyl-1-picrylhydrazyl (DPPH), *β*-carotene, ascorbic acid, linoleic acid, 3,5-Di-tert-4-butylhydroxytoluene (BHT) purchased from Sigma Chemical Co. The other chemicals and reagents used were of analytical grade commercially available.

Proximate Composition

The proximate compositions (moisture, fat, protein and ash) of brown seaweeds were evaluated according to the Association of Official Analytical Chemist (AOAC, 1997) method. The moisture content was determined by drying the samples, at 105°C, to a constant weight. Crude lipid was determined by using soxhlet system; crude protein content was calculated using the Kjeldahl method; crude carbohydrates estimation was evaluated by following phenol-sulfuric acid method, and crude ash content was calculated using the muffle-furnace technique.

Mineral Determination

The seaweed samples *w*ere immediately acidified with HNO_3 to a pH of < 2 and stored in a precleaned (rinsed with 10% HNO_3 followed by rinsing with MilliQ water) high-density polyethylene vial. Filtration was done just before measurement to eliminate tiny materials present in the sample, which are undesirable for PerkinElmer Elan 3300 (PerkinElmer, Waltham, MA, USA) measurement.

Identification and Quantification of Fatty Acid by GC Mass Spectroscopy

Lipids for fatty acid analysis were extracted with a mixture of chloroform and methanol (Choi *et al.*, 2014). Fatty acid composition was determined after methylation with 14% BF_3 in methanol (Sigma-Aldrich, St. Louis, MO, USA) using a gas chromatograph (HP-6890N; Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector and a SP™-2560 capillary column (100 m × 0.25 mm, film thickness 0.20 μm; Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas. Fatty acids were identified by comparison with known standards.

Amino Acid Analysis

The dry sample (50 mg) was placed into a vial and 4 mL 6 N HCl containing 0.1% thioglyocolic acids was added. The hydrolysis was conducted at 110°C in vacuum for 24 hours. Amino acids which derivatized with phenylisothiocyanate were identified and quantified using an automatic amino acid analyzer (Biochrom 20, Pharamacia Biotech, UK).

Extraction and Extraction Yield

Seaweeds were ground to powder and extracted with different solvent [EtOH, EtOH : DMK (7:3, v/v), and EtOH : H_2O (7:3, v/v)]. The

extraction was performed for 3 days in the dark at room temperature and repeated three times. After the extraction, the solvent was filtered out and vacuum evaporated to obtain the concentrated seaweed extract. The yield of each extract was calculated and kept at -20°C prior to further analysis.

DPPH Radical Scavenging Assay

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored by conducting the protocol of Yepez *et al.* (2002). The seaweed extracts were prepared in different concentrations, ranging from 0.1 mg/mL^{-1} to 1.0 mg/mL^{-1} for each sample and analyzed in triplicate. 160 µL of an ethanolic solution or sample of the tested sample were added to 40 µL of 50 µM DPPH solution in 96 well plates. DPPH solution was added and incubated in the dark for 30 min. Absorbance values were read at 517 nm using microplate reader Infinite® 200 PRO (Tecan Austria GmbH). The BHT were used as reference compounds under the same experimental conditions. Radical scavenging activity was calculated compared with the absorbance of the untreated control group.

RESULTS

Proximate Composition

The proximate composition of both brown seaweed species is summarized in Figure 1. According to the result, both *P. australi*s and *T. conoides* powder contained a high percentage of carbohydrate (65.58%, 59.84%) and ash (14.09%, 28.56%) respectively. *P. australi*s powder had 5.5% moisture, 0.06% lipid, 3.9% protein and 2.14% fiber. Powdered *T. conoides* had 9.56% moisture, 0.61% lipid, 4.59% protein and 5.56% fiber (all contents on the dry weight basis).

Mineral Contents

Atomic absorption spectrophotometry determination of seaweed samples showed the various mineral components such as macrominerals (sodium [Na], calcium [Ca], magnesium [Mg], potassium [K]), and trace elements (iron [Fe], zinc [Zn], copper [Cu]). The mineral components in the *P. australis* follow the orders: Ca>Mg>Na>Fe>K>Cu>Zn. Meanwhile, mineral compositions of *T. conoides* follow the orders: Ca>Na>Mg>K>Fe >Zn> Cu. Table 1 showedsummarizes the mineral contents of seaweed samples.

Table 1. Mineral contents of brown seaweeds .

Minerals (%)	*P. australis*	*T. conoides*
Calsium	9.81 ± 0.07	4.70 ± 0.03
Potassium	0.01 ± 0.00	0.05 ± 0.00
Magnesium	1.60 ± 0.00	0.68 ± 0.01
Natrium	1.15 ± 0.00	1.69 ± 0.03
Copper	0.006 ± 0.00	0.003 ± 0.00
Iron	0.02 ± 0.00	0.03 ± 0.00
Zinc	0.005 ± 0.00	0.01 ± 0.00

* All values given are means of three determinations (means ± SD: standard deviation)

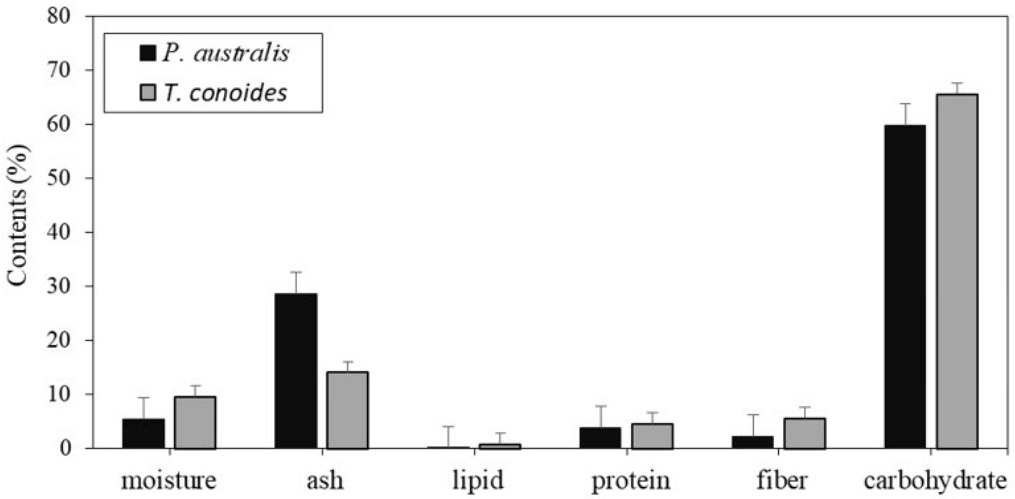

Figure 1. Proximate contents of *P. australis a*nd *T. conoides*. All values given are means of three determinations.

Amino Acids Contents

The amino acid composition of brown seaweeds (mg/100 g of total protein) is presented in Table 2. Both seaweeds contained eight essential amino acids (EAAs), *i.e.* histidine, isoleucine, leucine, phenylalanine, threonine, valine and lysine, and seven nonessential amino acids (NEAAs): aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine and arginine.

Table 2. Amino acid compositions of brown seaweeds *P. australis* and *T. conoides* (g per 100 g protein).*

Amino acids	*P. australis*	*T. conoides*
Histidine	0.15 ± 0.01	0.14 ± 0.01
Valine	0.23 ± 0.01	0.29 ± 0.01
Proline	0.16 ± 0.01	0.20 ± 0.03
Isoleucine	0.18 ± 0.01	0.23 ± 0.01
Lysine	0.16 ± 0.00	0.21 ± 0.00
Threonine	0.18 ± 0.02	0.21 ± 0.00
Leucine	0.27 ± 0.02	0.35 ± 0.01
Phenylalanine	0.18 ± 0.01	0.23 ± 0.00
Aspartic acid	0.43 ± 0.03	0.48 ± 0.00
Serine	0.14 ± 0.02	0.16 ± 0.00
Glutamic acid	0.43 ± 0.03	0.53 ± 0.01
Glycine	0.21 ± 0.02	0.26 ± 0.00
Alanine	0.21 ± 0.03	0.28 ± 0.00
Tyrosine	0.09 ± 0.02	0.12 ± 0.00
Arginine	0.21 ± 0.01	0.24 ± 0.01
Total EAA	1.51 ± 0.01	1.86 ± 0.01
Total	3.23 ± 0.18	3.93 ± 0.04

Fatty Acids Contents

Thirty fatty acids were identified (Table 3). Total monounsaturated and polyunsaturated fatty acid (MUFA and PUFA) were found in much greater quantity in *T. conoides* samples, whereas those of saturated fatty acid (SFA) were higher in *P. australis* samples. The predominant SFA was palmitic acid ranged from 16.29 ± 0.45 to 30.24 ± 0.65 g per 100 g. The highest amount belonged to *P. autralis*. The most abundant MUFA is oleic acid ranged from 20.18 ± 0.05 (*T. conoides*) to 20.83 ± 0.76 g per 100 g (*P. australis*). Important PUFA such as EPA and DHA were present in both seaweed samples.

Table 3. Fatty acid profile of brown seaweeds (g per 100 g of total fatty acids).*

Fatty acids	*P. australis*	*T. conoides*
Butyric acid $C_{4:0}$	1.84 ± 0.11	0.03 ± 0.04
Caproic acid $C_{6:0}$	1.08 ± 0.17	0.17 ± 0.15
Myristic acid $C_{14:0}$	7.43 ± 0.08	4.81 ± 0.34
Cis-10-Pentadecanoic acid $C_{15:1}$	nd	0.13 ± 0.01
Palmitic acid $C_{16:0}$	30.24 ± 0.65	16.29 ± 0.45
Margaric acid $C_{17:0}$	1.73 ± 0.17	0.41 ± 0.03
Margaroleic acid $C_{17:1}$	nd	0.37 ± 0.02
Stearic acid $C_{18:0}$	4.97 ± 0.10	2.36 ± 0.02
Behenic acid $C_{22:0}$	1.14 ± 0.53	1.13 ± 0.03
Erucic acid $C_{22:1n9}$	nd	0.28 ± 0.40
Σ SFA	48.43 ± 0.26	25.98 ± 0.15
Myristoleic acid $C_{14:1}$	nd	0.13 ± 0.09
Pentadecanoic acid $C_{15:0}$	1.01 ± 0.35	0.31 ± 0.16
Palmitoleic acid $C_{16:1}$	8.21 ± 0.27	13.48 ± 0.47
Arachidinic acid $C_{20:0}$	1.08 ± 0.09	0.25 ± 0.21
Eicosenoic acid $C_{20:1}$	nd	5.09 ± 0.12
Heneicosanoic acid $C_{21:0}$	nd	0.27 ± 0.21
Elaidic acid $C_{18:1n9t}$	nd	0.24 ± 0.01
Oleic acid $C_{18:1n9c}$	20.83 ± 0.76	20.18 ± 0.05
Linolelaidic acid $C_{18:2n6t}$	nd	0.14 ± 0.02
Tricosanoic acid $C_{23:0}$	nd	0.11 ± 0.01
Σ MUFA	31.13 ± 0.37	40.2 ± 0.14
Linoleic acid $C_{18:2n6c}$	7.70 ± 0.33	1.72 ± 0.05
ɣ-linolenic acid $C_{18:3n6,9,12c}$	1.46 ± 0.23	9.29 ± 0.09
Linolenic acid $C_{18:3n9,12,15c}$	2.01 ± 0.50	0.99 ± 0.02
cis-11,14-Eicosadienoic acid $C_{20:2}$	1.16 ± 0.34	0.30 ± 0.03
cis-8,11,14-Eicosatrienoic acid $C_{20:3n6}$	nd	5.47 ± 0.09
cis-11,14,17-Eicosatrienoicacid acid $C_{20:3n3}$	nd	0.05 ± 0.07
Arachidonic acid $C_{20:4n6}$	0.61 ± 0.08	1.29 ± 0.02
cis-13,16-Docosadienoic acid $C_{22:6n3}$	nd	0.04 ± 0.05
cis-5,8,11,14,17-Eicosapenteenoic acid (EPA) $C_{20:5n3}$	3.71 ± 0.51	8.58 ± 0.22
cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA) $C_{22:6n3}$	3.79 ± 0.48	6.05 ± 0.21
Σ PUFA	20.44 ± 0.35	33.78 ± 0.09
Σ ω6	9.77 ± 0.28	17.77 ± 0.06
Σ ω3	7.5 ± 0.5	14.72 ± 0.14
Ratio ω6/ω3	1.3	1.2

* All values given are means of three determinations (means ± SD: standard deviation); nd means not detected.

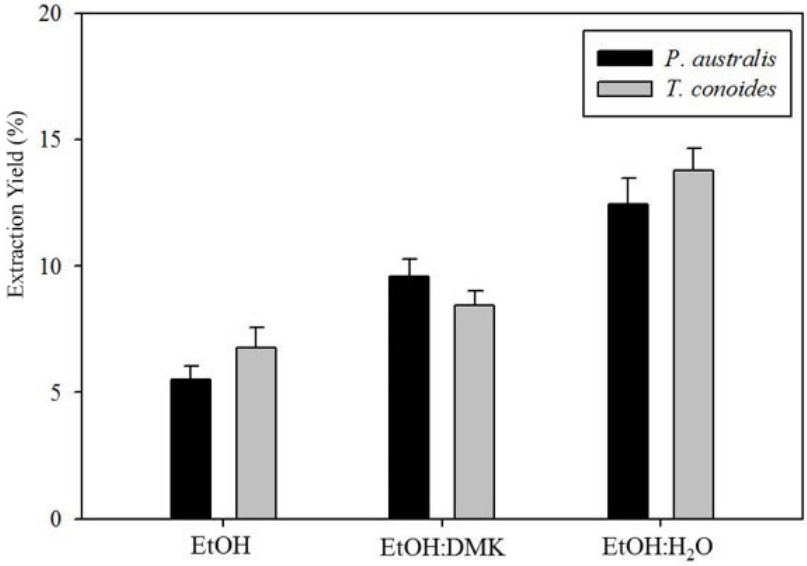

Figure 2. Extraction yield of brown seaweeds.

The Extraction Yield

In the present study, brown seaweeds were extracted with various solvents. The type of extraction solvents used in this study was EtOH, acetone (DMK) and H_2O. The extraction yields of *P. australis* and *T. conoides* are shown in Figure 2. The quantities of seaweed extracts ranged from 5.5-12.44% and 6.76-13.77%, respectively. The highest quantity of both seaweed extraction was obtained by using EtOH : H_2O (7:3, v/v), and the lowest from EtOH extraction.

Antioxidant Activity

Among different extracts, EtOH:DMK extracts from *T. conoides* appeared to possess the highest DPPH radical scavenging activity with an inhibitory concentration (IC_{50}) of 1.86 mg/mL (Table 4). The order of DPPH radical scavenging activity of *T. conoides* extract was: EtOH:DMK > EtOH:H_2O > EtOH.

Table 4. The IC_{50} value of brown seaweeds extracts on DPPH radical scavenging activity .

Seaweed extract	*P. australis*	*T. conoides*
EtOH	4.83	3.95
EtOH:DMK (7:3, v/v)	2.76	1.86
EtOH:H_2O (7:3, v/v)	3.63	2.92

DISCUSSION

According to the proximate contents of seaweed samples, the analysis data showed that the total lipid of both brown seaweed was in low amounts. This finding was similar to the previous work of brown seaweeds with < 4% lipid content (Herbreteau *et al.*, 1997; Rupérez and Saura-Calixto, 2001). Carbohydrates and ashes were the most abundant chemical components in both brown seaweed species (Figure 1). Carbohydrate and ash contents in *T. conoides* were 65.9% and 14.1% of dry weight, respectively. *P. australis* had 59.8% of carbohydrate and 28.6% of ash. Carbohydrate is one of the important constituents in metabolism since it supplies energy for respiration and other metabolic processes. The typical carbohydrates in brown seaweeds are alginates, fucoidan, cellulose, and laminaran (Dawczynski *et al.*, 2007). The total carbohydrates of both brown seaweeds are found to be in considerable high amounts. Its occurrence is a function of the intensity of sunlight (El-Tawil and Khalil, 1983). Brown seaweeds are rich in carbohydrates. The carbohydrate content found in the present study was found to be higher than an earlier study by Santoso *et al.,* 2006 (9.6%); as they examined the proximate contents based on the wet weight basis (Santoso *et al.*, 2006). The levels of carbohydrates from brown seaweeds detected in these studies were higher than most terrestrial plants and within the ranges previously reported for other brown seaweeds species (Goecke *et al.*, 2012; Matanjun *et al.*, 2009).

Ash contents of both seaweeds were quite high, the highest level being in *P. australis* (Figure 1). Many studies of nutritional and chemical

compositions of seaweeds have been reported. On average, ash content is higher in marine macroalgae (8-40%) than in terrestrial plants (Heiba *et al.*, 1997; USDA, 2001). Moreover, a later report suggested that ash content in brown seaweeds was significantly higher compared to that determined in red and green seaweeds (Rohani-Ghadikolaei *et al.*, 2012). The protein content found in the present study was relatively high (8.9% dry weight). Fleurence (1999) suggested that variations in the protein content of seaweeds can be attributed to species differences, seasonal periods and environmental growth conditions. Interestingly, the protein contents of seaweeds in this study conformed with the value of other brown seaweeds species from Chile reported in the previous study (Goecke *et al.*, 2012).

It has been known that brown seaweeds had a high mineral content, as their cell walls contain alginate that formed an insoluble salt, comprising calcium with minor amounts of magnesium, sodium, and potassium (Meillisa *et al.*, 2015). Generally, the mineral content in seaweeds was higher compared to that found in terrestrial vegetables, such as lettuces and spinach (Rohani-Ghadikolaei *et al.*, 2012). The marine environments in which seaweeds grow, allow them to absorb a wide diversity and high amounts of minerals; therefore, trace elements and minerals are more abundant in seaweeds compared to terrestrial food sources (Matanjun *et al.*, 2009). Hence, brown seaweeds were a potential to be consumed as a food supplement to help fulfill the recommended daily intake of essential minerals and trace elements.

The amino acid has an important role in molding protein structure and as intermediaries in human metabolism (Saravana *et al.*, 2016). This investigation showed that the brown seaweed samples contained all the essential amino acids (in a different amount, excluding methionine and tryptophan). According to the data, the total amount of EAAs in *T. conoides* was slightly higher than in the *P. australis* (Table 2). Both seaweeds contain a high amount of two acidic amino acids; aspartic acids and glutamic acids. In the human's body, aspartic acid is essential for energy production and plays a key role in metabolism. Glutamic acid supports the immune and digestive systems as well as energy productions (Ruth and Field, 2013). It is known that the main role of glutamic acid is as a principal excitatory neurotransmitter in the central nervous system. From the nutritional stand view, the amino acid content of *P. australis* and *T. conoides* should be compared with human amino acid requirements and three factors should be considered: amino acid balance, essential relative amino acid content compared with the egg protein reference and the ratio of essential amino acids. Brown seaweeds amino acids are of high quality in this respect because, as shown in this study, the essential amino acids represented around 30% of total amino acids.

The fatty acid composition of seaweeds is fundamentally different from meat and vegetables; seaweeds have a low lipid content compared with vegetables such as soy, thus make seaweeds a low source of nutritional energy. Nevertheless, it is worth mentioning that seaweeds polyunsaturated fatty acid (PUFA) composition is superior to those of terrestrial vegetables in regards to the human diet. The most abundant PUFA were eicosapentaenoic acid (EPA, $C_{20:5n3}$) and cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA, $C_{22:6n3}$). Compared to *P. australis*, *T. conoides* contain a higher amount of EPA (8.58 ± 0,22 g/100 g of total fatty acids) and DHA (6.05 ± 0,21 g/100 g of total fatty acids). The *P. australis* and *T. conoides* also contained the short chain PUFA linoleic acid ($C_{18:2n6c}$), linolenic acid ($C_{18:3n9,12,15c}$), and the eicosanoid precursors arachidonic acid ($C_{20:4n6}$). In the human body, EPA and DHA are important for maintenance of normal blood flows; and alleviate certain diseases (Venugopal, 2008). It must not be forgotten that at the current moment the elevated intake of seed oils plays a central role in the unbalance of the ω6/ω3 ratio and the development of cardiovascular disease (CVD) and coronary heart disease (CHD). According to FAO (Burlingame *et al.,* 2009), the ratio of ω6/ω3 should be lower than 10 in the diet. The ω6/ω3 ratio observed in *P. australis* and *T. conoides* was lower than 10, making these edible species for dietary food. Thus, fatty acid profiles of brown seaweeds particularly *T. conoides* demonstrated that the brown seaweed species is a potent low-fat food with a significant amount of PUFA.

Solvent extraction is the most common method used in sample preparations from plants and microorganisms, both terrestrials and marine.

The extraction yield depends on extraction solvents, time and temperature of extraction as well as on the chemical nature of the sample. In the present study; brown seaweeds were extracted with the various solvent. In both seaweed species, extraction with EtOH : H_2O (7:3, v/v) yielded the highest extraction yield followed by EtOH : DMK (7:3, v/v) and EtOH. These results indicate that different solvents give different impact on the yield. The extraction yields of EtOH : H_2O (7:3, v/v) than other extraction solvents which indicated that most of the soluble components in both seaweeds were high in polarity; moreover, the extracts were very viscous and difficult to filtrate through the filter paper due to the high content of polysaccharides in the extracts. Brown seaweeds are known to contain high levels of polysaccharides such as alginates.

The DPPH assay was used to evaluate the antioxidant activity of brown seaweeds extracts. DPPH is characterized as a stable free radical that is able to accept an electron or hydrogen and become a stable diamagnetic molecule. Thus, it has been widely used to measure the antioxidative capacity of natural antioxidants (Ahn *et al.*, 2007). As shown in Table 4, free radical scavenging activity was found to be higher in EtOH:DMK extract from *T conoides* when compared to other solvents from *P. australis*. The results in this study revealed that the radical scavenging activity of brown seaweed extract was affected by the extraction solvent which may be correlated with the bioactive substances content. A possible explanation of the free radical scavenging activity is the neutralization of free radical by the antioxidant components of crude extract/fractions, either by transfer of hydrogen or of an electron. Mortensen and Skibsted (1998) reported that the free radical scavenging mechanism of carotenoids was brought by the hydrogen atoms donation to free radicals (Mortensen and Skibsted, 1998).

CONCLUSION

The result of this study indicated the nutritional value and potential use of *P. australis* and *T. conoides* as a candidate to be used in food industries to increase shelf-life of food items for human consumption, and nutraceuticals to deter deleterious free radical-induced life-threatening diseases.

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