Preliminary Evaluation of the Biochemical and Antioxidant Properties of Seaweed Species Predominantly Distributed in Peninsular Malaysia

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ABSTRACT

Seaweeds are rich sources of nutritional and biochemical components. In this study, five marine macroalgal species were collected from the coast of Peninsular Malaysia: *Halimeda macroloba, Ulva intestinalis, Codium* sp., *Hydropuntia edulis* and *Sargassum ilicifolium*. The seaweeds were explored biochemically (lipids, total carotenoids, chlorophyll *a* and *b*), their metabolites were identified using GC-MS analysis, and their antioxidant activity was determined using DPPH free radical scavenging. The highest total lipids (4.10 and 3.42 %) was found in *H. macroloba* and *S. ilicifolium*, the highest total carotenoids (162.00 and 159.18 $\mu g \cdot g^{-1}$) in *U. intestinalis* and *Codium* sp., and the highest chlorophyll *a* content (313.09±2.53 $\mu g \cdot g^{-1}$) in *U. intestinalis. Codium* sp. also contained the highest chlorophyll *b* (305.29±7.09 $\mu g \cdot g^{-1}$) content. Of the metabolites identified from the seaweeds, hexadecanoic acid, stigmast-5-en-3-ol, neophytadiene, and 2-Pentadecanone,6,10,14-trimethyl- were the most abundant. In the assay for antioxidant activity, *U. intestinalis* extract displayed significantly ($p<0.05$) higher DPPH inhibition (65.02 %) than the other species at the highest concentration (1,000 $\mu g \cdot mL^{-1}$) tested; however, the difference was small. At the lowest tested concentration (200 $\mu g \cdot mL^{-1}$), DPPH inhibition by *U. intestinalis* (58.42 %) extract was also the highest, and differed significantly from three of the other species. These findings highlight the potential of these seaweed species for cultivation as a sustainable source of functional food for human consumption.

Keywords: Antioxidant, Carotenoid, Lipid, Metabolite, Seaweed

INTRODUCTION

Seaweeds, a group of marine macroalgae, are found ubiquitously in all maritime areas. Global attention to seaweeds has recently increased, as utilization of products from these marine resources supports a multi-billion-dollar industry, led by Asian markets (Smit, 2004). The uses of seaweeds are numerous, and include alternative sources for high-value food products. In this regard, seaweeds are seen as tremendous resources of vitamins, minerals, proteins, lipids, polysaccharides and unique bioactive metabolites, including polyphenols and carotenoids (Matanjun *et al*., 2009). Several studies have confirmed the benefits of seaweeds for human well-being, primarily in their uses as food, in industry, and in pharmaceutical applications (Holdt and Kraan, 2011; Ismail, 2017).

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Chen *et al*. (2016) reported a very significant amount (26 %) of polyunsaturated fatty acids (PUFAs) in the brown seaweed *Sargassum fusiforme*, with a balanced proportion of omega-6 (n-6) and omega-3 (n-3) PUFAs. Consumption of a balance of these fatty acids (FA) is associated with reduction in glucose intolerance, obesity, inflammation and other metabolic dysfunctions and diseases. FAs derived from the lipids found in seaweeds have been identified to play crucial roles in metabolism, as the basic unit of all biomembranes, as well as being gene regulators (Rustan and Drevon, 2005). The n-3 and n-6 FAs are known to be essential, as humans and animals are not capable of synthesizing them. However, they are readily available when seaweeds are consumed (Schmid *et al*., 2018). In addition to PUFAs, carotenoids found in edible seaweeds serve as antioxidants (Young and Lowe, 2018). Due to the vital metabolic roles that they play, the global demand for carotenoids is increasing; its global market value was forecast to reach around 1.5 billion USD in the year of 2021 (Poojary *et al*., 2016). In addition to these valuable nutritional properties previously identified, seaweeds exhibit biological activities owing to their metabolites with potential nutritive and therapeutic uses (Ismail, 2017). Effectively, seaweed has been suggested to be able to diminish the potential peril of chronic diseases in humans due to the fact that it contains an abundance of natural antioxidants (Rajauria *et al*., 2013).

In addition, studies have suggested that seaweeds possess numerous beneficial anticancer, anti-obesity, antioxidant, and anti-angiogenic properties, as well as providing neuroprotection for the human body due to the photosynthetic pigments they possess, including chlorophylls (*a, b, c*), carotenoids (carotene and xanthophylls) and phycobilins (phycocyanin and phycoerythrin) (Pangestuti and Kim, 2011; Chen *et al*., 2017).

Numerous beneficial seaweeds are available worldwide, yet few of them have been properly studied for potential consumption as functional human foods, since most of the work has focused on the commercially important and edible species. Seaweeds are highly productive freshwater and marine organisms that are well suited to growing on non-arable lands and can utilize waste materials as nutrient sources. Therefore, the present study evaluates the biochemical composition (lipids, total carotenoids, chlorophyll *a* and chlorophyll *b*) and identifies the metabolites of five species of seaweed, and investigates their antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Such knowledge will enable the exploitation of these species for commercial cultivation.

MATERIALS AND METHODS

Sample collection and preparation

Seaweed samples were obtained from three locations in Malaysia where they were most rich in density: (i) Teluk Kemang in Negeri Sembilan, west coast of Malaysia, (ii) Tanjung-Adang and (iii) Merambong in Johor, south coast of Malaysia (Nazarudin *et al*., 2020). The samples were collected during low tide between September 2015 and September 2016. The seaweed samples were cleaned to remove foreign materials such as sand, pebbles, shells, and debris using a soft-bristle brush and then thoroughly washed with sea water. The cleaned samples were then placed in transparent polyethylene bags and transported to the Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Malaysia. Upon arrival at the laboratory, the samples were further rinsed with distilled water and placed in a -80 °C freezer (Thermo Scientific, USA). All frozen samples were lyophilized and ground to a powder using a blender before being passed through a 200-micron sieve. Powdered samples were stored in sealed bags in a -80 °C freezer until further use.

Lipid extraction

The fine-powdered samples of seaweed were subjected to lipid extraction following Nazarudin *et al*. (2020) with slight modification. All samples (0.5 g each) were homogenized (Power Sonic 505 Ultrasonic bath, Korea) with 20 mL chloroform: methanol (2:1, v/v) for 5 min. Then, the homogenate was filtered using Whatman No.1 filter paper before mixing with 10 mL of 9% sodium chloride solution. The mixture was centrifuged for

8 min (Eppendorf 5810R, Germany) at 2,000 rpm. The lower (organic) layer containing chloroform was collected while the upper (aqueous) layer was discarded. The residual biomass left after filtration was subjected to extraction three more times following the same steps. The multiple-extracted chloroform was concentrated using a rotary evaporator (EYELA N-1000, with EYELA Oil Bath OSB-2000, Japan). Then, the samples were dried using nitrogen gas and maintained at 40 °C in a heat block until constant weight was reached for gravimetric quantification of total lipid content for each seaweed.

Total carotenoid and chlorophyll content determination

Total carotenoids and chlorophylls were determined following Lichtenthaler and Buschmann, (2001) with slight modification. The dried seaweed lipid extract was diluted with methanol. Meanwhile, the determination of total carotenoids, chlorophyll *a* and chlorophyll *b* were carried out by measuring the absorbance at 470, 665.2 and 652.4 nm, respectively, using a UV-spectrophotometer (Shimadzu UV 1601, Japan) and then calculated using the Lichtenthaler equations as follows (with values expressed as $\mu g \cdot g^{-1}$ dry weight [$\mu g \cdot g^{-1}$ DW]):

$$C_{(x+c)}\ (\mu g \cdot mL^{-1}) = (1000\, A_{470} - 1.63\, C_a - 104.96\, C_b)/\ 221$$

$$C_a\ (\mu g \cdot mL^{-1}) = 16.72\, A_{665.2} - 9.16\, A_{652.4}$$

$$C_b\ (\mu g \cdot mL^{-1}) = 34.09\, A_{652.4} - 15.28\, A_{665.2}$$

Where $C_{(X+C)}$ = total carotenoids, C_a = chlorophyll *a*, C_b = chlorophyll *b*, A_{470} = absorbance at 470 nm, $A_{665.2}$ = absorbance at 665.2 nm and $A_{652.4}$ = absorbance at 652.4 nm.

Gas chromatography-mass spectrometry analysis

All seaweed extracts were subjected to gas chromatography-mass spectrometry (GC-MS) analysis using the GC-MS QP2010 Ultra (Shimadzu Co., Japan) system to characterize and quantify their bioactive components. Initially, a 0.5 µL sample was separated on a DB-1 (0.25 µm film × 0.25 mm ID × 30 m length) column. Splitless injection was selected, with a purge time of 1 min, 1 $mL \cdot min^{-1}$ flow rate, helium as the carrier gas, and using EI (electron impact) mode with 70 eV of ionization energy. The column temperature was set at 50 °C (maintained for 3 min), then increased by 10 °C per min to 250 °C, and held at that temperature for 30 min. The metabolites were detected later by assessing the spectral patterns acquired with those of mass spectral archives (NIST3208 and WILEY libraries). Quantification of metabolites was done by measuring corresponding peak area and presented as a percentage of total components identified.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of methanolic extracts from seaweeds was analyzed based on a method of Blois (1958), with minor modification. DPPH solution was prepared by dissolving 1.95 mg DPPH powder into 50 mL of methanol. Then, stock solution of methanolic extract was prepared by dissolving 5 mg of methalonic extract of seaweed into 1 mL of methanol. Subsequently, serial dilutions were made to prepare concentrations of 200, 400, 600, 800 and 1,000 $\mu g \cdot mL^{-1}$ with working volume of 500 µL. Next, ascorbic acid was prepared as a positive control. For negative control, 500 µL of methanol was added into 500 µL of DPPH solution. Then, 500 µL of DPPH solution was added into each sample and left in a dark room for 30 min. An amount of 100 µL from each sample was transferred into 96-well plates and absorbance was measured at wavelength of 517 nm by ELISA reader. The capability of scavenging the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0\text{-}A_1/A_0) \times 100]$$

Where A_0 = absorbance of control and A_1 = absorbance of sample.

Statistical analysis

Values in the results are presented as mean±SD from three replications. The data for total lipids, carotenoids, and chlorophylls were analyzed

using one-way analysis of variance (ANOVA). The mean values were compared using Tukey's Test to determine significant differences. The differences were considered significant at 95 % confidence level. The mean values of DPPH radical scavenging activity for seaweed samples were compared to that of ascorbic acid (control) using Dunnett's test.

RESULTS AND DISCUSSION

Total lipid content of seaweeds

The total lipid content (presented as % DW) varied among the seaweed samples evaluated, as shown in Table 1. The total lipid content in *Halimeda macroloba* (4.10±1.011 %) was the highest ($p<0.05$), followed by *Sargassum ilicifolium* (3.42±0.014 %), whereas lipids in *Hydropuntia edulis* (1.12±0.10 %) were significantly lower than the others. Total lipids in *Ulva intestinalis* (1.84± 0.013 %) and *Codium* sp. (1.72±0.002 %) were comparable and not significantly different.

Algae grow rapidly through photosynthesis, which enables them to capture and recycle carbon dioxide from their environment to develop biomass (Gosch *et al*., 2012). Seaweeds, therefore, generally differ from terrestrial plants in their morphological and physiological characteristics, as well as their chemical composition, as is evident in their different appearance. In the present exploration, the content of lipids, total carotenoids and chlorophyll *a* and *b* in five different unused but common seaweeds found along the coast of Peninsular Malaysia were measured, with further investigation of the composition of the lipids (e.g., unsaturated fatty acids, glycerolipids, sterol lipids, etc.) using GC-MS and by measuring antioxidant activity (free radical scavenging via DPPH assay) in their lipophilic extracts.

According to Montgomery and Gerking (1980) and Kumari *et al*. (2010) the typical perception about most seaweed species is that they contain low total lipid content (<5 % DW) and are thus not suitable to accumulate oil-based products. Consistent with this perception, (Özçimen and İnan, 2015) opined that algae contain amounts of lipids, proteins and carbohydrates relative to the capabilities of their photosynthetic pigments. The highest total lipid (4.10 %) measured in this study was in a green seaweed species (*Halimeda macroloba*) sampled from Merambong shoal in Johor, and was within the perceived range (<5 %). However, this contrasted with results reported in another study by Manivannan *et al*. (2009), where total lipid content of the same species sampled in India was recorded as only 0.26 % DW. In the present study, similarly low lipid content was observed in *Ulva intestinalis* and *Codium* sp., both green seaweed species (sampled from Teluk Kemang and Port Dickson), and in *H. edulis*, a brown species (from Tanjung Adang).

The brown seaweed *Sargassum ilicifolium* (sampled at Port Dickson), on the other hand, contained relatively higher lipid content (3.42 %) than *Hydropuntia edulis*, another species of Phaeophyta. From other studies, the content of total lipids in *Ulva armoricana* was reported to be 2.6 % in France by Kendel *et al*. (2015), 1.2 % in Pakistan by Ambreen *et al*. (2012), and 3.1 % in

Table 1. Total lipid content (% dry weight, mean±SD [n = 3]) of the studied seaweed species.

Seaweed species	Sampling location	Percentage yield (% DW)
Halimeda macroloba	Merambong, Johor	4.10 ± 1.011^{a}
Ulva intestinalis	TanjungAdang, Johor	1.84 ± 0.013^{c}
Codium sp.	Teluk Kemang, Port Dickson	1.72 ± 0.002^{c}
Hydropuntia edulis	Tanjung Adang, Johor	1.12 ± 0.10^{d}
Sargassum ilicifolium	Teluk Kemang, Port Dickson	3.42 ± 0.014^{b}

Note: Mean values with different superscript letters are significantly different ($p<0.05$).

California by Khotimchenko *et al*. (2002). The differences in lipid content of seaweeds at different locations could be attributed to differences in season of sample collection, location of sampling, and growing conditions of the species such as salinity, light intensity and nutrient availability (Kendel *et al*., 2015).

A report by Gosch *et al*. (2012) previously suggested that one of the most important steps for assessing seaweed species as feedstock for oil-based products is determining individual differences, even within the same species. This is because genotypic and environmental factors both clearly play roles that affect the quality and quantity of lipids in seaweed. The most critical and significant environmental factors affecting total lipid (and fatty acid) accumulation in seaweeds have been noted to include light (Hotimchenko, 2002), temperature (Holdt and Kraan, 2011) and availability of nutrients (Rodolfi *et al*., 2009).

Nutritionally, seaweed lipid extracts naturally contain a variety of high value polyunsaturated, polysaturated, monosaturated and monounsaturated fatty acids (FA) that act as catalysts in the antibacterial, antifungal, antiviral and cytotoxic activities in the species (Goecke *et al*., 2010). Their lipid content also adds potential benefits to their use as feed material capable of supplying energy for metabolic processes during embryonic and pre-feeding stages of larval fish (Evans *et al*., 2002); as such, they are high energy resources for marine fish nutrition.

Total carotenoid and chlorophyll content

The values for total carotenoid content (in $\mu g \cdot g^{-1}$ DW) determined in the selected seaweed samples studied are displayed in Table 2. The total concentration of carotenoids from both *Ulva intestinalis* (162.00±0.84) and *Codium* sp. (159.18± 0.47) were equivalent and significantly higher than the other species ($p<0.05$). These were followed by *Halimeda macroloba* (117.36±1.30), which had higher total carotenoid content than *Sargassum ilicifolium* (45.28±1.77). The extract from *Hydropuntia edulis* (11.51±0.93) contained the least carotenoid content among the seaweed species studied.

Results also reveal differences among seaweed samples in their content of chlorophyll *a* and *b* ($\mu g \cdot g^{-1}$ DW), as shown in Table 2. Chlorophyll *a* content in *Ulva intestinalis* was significantly higher than the others (313.09±2.53), and was roughly twice the quantity measured in the other seaweeds studied except *Hydropuntia edulis*, which had the lowest amount (37.19±1.65). Chlorophyll *b*, on the other hand, was particularly abundant (between 237.96 and 305.29 $\mu g \cdot g^{-1}$) in the majority of the species studied, while *Sargassum ilicifolium* and Hydropuntia edulis had relatively lower quantities (111.29 and 21.94 $\mu g \cdot g^{-1}$, respectively).

The high concentrations of carotenoids (ranging from 117.36 to 162.00 $\mu g \cdot g^{-1}$ DW) measured from the green macroalgae (*Halimeda macroloba* and *Codium* sp.) and red macroalga

Table 2. Total carotenoids and chlorophyll *a* and *b* ($\mu g \cdot g^{-1}$ dry weight, mean±SD [n = 3]) in extracts of the studied seaweed species.

Seaweed species	Total carotenoids ($\mu g \cdot g^{-1}$)	Chlorophyll *a* ($\mu g \cdot g^{-1}$)	Chlorophyll *b* ($\mu g \cdot g^{-1}$)
Halimeda macroloba	117.36±1.30^{b}	154.42±1.73^{b}	237.96±8.25^{b}
Ulva intestinalis	162.00±0.84^{a}	313.09±2.53^{a}	292.52±8.84^{a}
Codium sp.	159.18±0.47^{a}	142.3 ±1.09^{b}	305.29±7.09^{a}
Hydropuntia edulis	11.51±0.93^{d}	37.19±1.65^{c}	21.94±3.05^{d}
Sargassum ilicifolium	45.28±1.77^{c}	141.98±1.18^{b}	111.29±2.28^{c}

Note: Mean values in a column with different superscript letters are significantly different ($p<0.05$).

(*Ulva intestinalis*) in the present study are concurrent with previous reports suggesting that carotenoids are more abundant in green (Chlorophyta) and red (Rhodophyta) seaweeds, as they have higher photosynthetic activity than brown seaweeds (Phaeophyta) (Yokoya *et al*., 2007). Results of this study, however, differed from those published by Kumar *et al*. (2009), where high carotenoid content was measured in brown seaweeds and low content was recorded in green seaweeds. Other researchers (Collins *et al*., 2016), however, explained that such opposing results (i.e., Kumar *et al*., 2009) may have been influenced by a high content of siphonaxanthin, a compound that is dominant in green seaweeds.

The concentrations of chlorophyll *a* among the five seaweed species included here (varying between 141.98 and 313.09 $\mu g \cdot g^{-1}$ DW) are consistent with those reported from previous work by Gordillo *et al*. (2006), where the highest content of chlorophyll *a* was demonstrated in the green seaweed *Ulva intestinalis*, while the lowest was demonstrated in a brown seaweed, *Hydropuntia edulis*. Kumar *et al*. (2009) reported high chlorophyll *b* content in green seaweed and the least in red seaweed. Similarly, in this study, chlorophyll *b* was recorded to be richest in a green algae, *Codium* sp., and lowest in red algae, *Hydropuntia edulis*.

It is widely accepted that the concentration of specific photosynthetic pigments in seaweeds varies according to the species as well as the prevailing environmental conditions (Heriyanto *et al*., 2017). In a study of marine algae-derived pigments, Pangestuti and Kim (2011) elucidated various beneficial biological activities that may be considered when selecting seaweed species for the cosmetic, food and pharmacological industries.

GC-MS analysis and metabolite identification

The compounds identified in the five seaweeds, along with their retention time (RT), peak area (%) and molecular formulas are presented in Tables 3-7. The GC–MS analysis of lipid extracts

Table 3. Chemical components in *Halimeda macroloba* extract.

Retention time (min)	Compounds	Concentration (%)
	Ketones	
48.88	2-Pentadecanone, 6,10,14-trimethyl-	1.92
	Alcohol	
57.90	Phytol	0.89
	Phenol	
35.77	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	0.53
	Fatty acid	
53.28	Hexadecanoic acid	26.16
	Fatty acid methyl ester	
51.80	Hexadecanoic acid, methyl ester	0.70
	Sterol	
83.83	Stigmast-5-en-3-ol, (3*β*)	0.73
84.52	Cholesterol	2.41
89.83	Stigmast-5-en-3-ol, (3*β*)	43.14
91.44	Cholest-5-ene, 3-methoxy-, (3*β*)	0.63
	Hydrocarbons	
43.66	Dodecane, 2,6,10-trimethyl-	0.62
48.70	Neophytadiene	6.78
57.57	2-methyltetracosane	0.33

Table 4. Chemical components in *Ulva intestinalis* extract.

Retention time (min)	Compounds	Concentration (%)
	Ketones	
48.87	2-Pentadecanone, 6,10,14-trimethyl-	7.84
51.20	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	20.06
	Alcohol	
57.12	Heptadecanol	21.95
	Phenol	
35.77	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	0.53
	Fatty acid	
53.11	Pentadecanoic acid	18.41
	Fatty acid methyl ester	
61.83	Hexadecanoic acid, 2-hydroxyethyl ester	1.84
	Sterol	
84.53	Cholesterol	6.34
	Hydrocarbons	
48.69	Neophytadiene	2.60
57.57	2-Methyltetracosane	1.77
60.66	Hexadecane, 2,6,10,14-tetramethyl-	1.91
	Other	
62.43	14-*β*-H-Pregna	1.72
63.64	2-Hexyldodecyl propionate	4.06

Table 5. Chemical components in *Codium* sp. extract.

Retention time (min)	Compounds	Concentration (%)
	Ketones	
48.885	2-Pentadecanone, 6,10,14-trimethyl-	2.47
51.196	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.67
	Fatty acid	
45.912	Tetradecanoic acid	0.59
53.188	Hexadecanoic acid	9.65
	Fatty acid ester	
69.56	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.93
	Sterol	
89.322	Stigmasterol	4.57
	Hydrocarbons	
85.984	Triacontane	5.85

of *Halimeda macroloba*, *Ulva intestinalis*, *Codium* sp., *Hydropuntia edulis* and *Sargassum ilicifolium* revealed the following main chemical constituents: stigmast-5-en-3-ol (43.14 %), hexadecanoic acid (26.16 %) and neophytadiene (6.78 %) in *H. macroloba*; 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (20.06 %), heptadecanol (21.95 %) and pentadecanoic acid (18.41 %) in *U. intestinals*; hexadecanoic acid (9.65 %) and stigmasterol (4.57 %) in *Codium* sp.; pentadecanoic acid (22.16 %), cholesterol (6.98 %) and 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (5.58 %) in *H. edulis*; and hexadecanoic acid (42.48 %) in *S. ilicifolium*.

Generally, *Hydropuntia edulis* was noted to contain the highest number of metabolites. Of the two seaweed extracts in which the highest yield of total lipids were isolated (Table 1), stigmas-5-en-3-ol (43.14 %), hexadecanoic acid (26.16 %) and neophytadiene (6.78 %) were the most abundant metabolites in *Halimeda macroloba* (Table 3),

Table 6. Chemical components in *Hydropuntia edulis* extract.

Retention time (min)	Compounds	Concentration (%)
	Ketones	
48.88	2-Pentadecanone, 6,10,14-trimethyl	3.49
51.20	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	5.58
	Alcohol	
57.13	Heptadecanol	5.40
69.07	*n*-Heptadecanol-1	0.89
	Fatty acid	
53.16	Pentadecanoic acid	22.16
59.55	9-Octadecenoic acid (*Z*)	2.25
	Fatty acid ester	
61.84	Octadecanoic acid, 2-hydroxyethyl ester	0.50
69.55	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.58
	Sterol	
84.55	Cholesterol	6.98
87.79	Cholest-4-en-3-one	0.97
90.01	Cholest-3,5-diene-3β-ol acetate	0.88
91.11	3α,7β-Dihydroxy-5β, 6β -epoxycholestane	5.07
92.61	1,2-Epoxycholestan-3-one	2.30
	Hydrocarbons	
54.35	Hentriacontane	0.85
57.57	2-Methylhexacosane	0.38
60.67	Hexadecane, 2,6,10,14-tetramethyl-	0.60
74.36	Tetratetracontane	0.93
81.42	Triacontane	2.04
83.62	Eicosane	2.69
	Other	
60.05	Hexadecanamide	0.71
66.15	Tetradecanamide	0.78

whereas *Sargassum ilicifolium* extract contained hexadecanoic acid (42.48 %) and 2-pentadecanone, 6,10,14-trimethyl- (5.53 %) as the major metabolites (Table 7). The saturated fatty acid hexadecanoic acid is therefore noteworthy as a major constituent of the two lipid-rich seaweed extracts.

Use of GC-MS analysis in the present study revealed the presence of several different major bioactive metabolites in lipid extracts of the selected seaweeds, such as hexadecanoic acid (Patra *et al*., 2015; Kamariah *et al*., 2017), neophytadiene (El Gamal, 2010; Anjali *et al*., 2019), stigmast-5-en-3-ol, (3*β*) (Belkacemi *et al*., 2020), heptadecanol, and pentadecanoic acid (Nazarudin *et al*., 2020), which are known to display different pharmaceutical activities. Various other metabolites were also identified in the samples.

One of the forms of fatty acid found in plants, stigmast-5-en-3-ol, (3*β*) (43.14 %) was shown to be abundant in *Halimeda macroloba*. Stigmast-5-en-3-ol, (3*β*) or *β*-sterol along with *β*-sitosterol, and campesterol are reported to be typical phytosterols. Mujeeb *et al*. (2014) reported that phytosterol acts chemically as a compound through elevated antioxidant activity as well as moderating radical scavenging. Relatively high abundance of neophytadiene was also noted in the extracts. Neophytadiene is a member of a group of compounds known as sesquiterpenoids, the natural defense compounds and pheromones (Mujeeb *et al*., 2014). It has been demonstrated to show powerful antioxidant and anti-inflammatory properties (Venkataraman *et al*., 2012). Meanwhile, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione found in *Ulva intestinalis* (20.06 %), *Codium* sp. (0.67 %), *Hydropuntia edulis* (5.58 %) and *S. ilicifolium* (3.45 %) is an antioxidant naturally found in the ethanolic extract of *C. speciosus* leaves (Conforti *et al*., 2009).

Heptadecanol (21.95 %) was among the metabolites detected with the highest concentration in some of the species explored. This metabolite has been suggested to show antioxidant activity (Saishri *et al*., 2016). Hexadecanoic acid was also found in high concentrations in *Codium* sp. and

Table 7. Chemical components in *Sargassum ilicifolium* extract.

Retention time (min)	Compounds	Concentration (%)
	Ketones	
46.388	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4*H*)-one	3.89
48.867	2-Pentadecanone, 6,10,14-trimethyl-	5.53
51.179	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	3.45
	Alcohol	
57.12	1-Octadecanol	3.30
	Fatty acid	
38.092	Dodecanoic acid	1.42
45.916	Tetradecanoic acid	3.64
53.17	Hexadecanoic acid	42.48
	Sterol	
89.696	Stigmasta-5,24(28)-dien-3-ol, (3*β*)-	1.43
	Hydrocarbons	
48.694	Neophytadiene	3.10
	Other	
63.638	2-Hexyldodecyl propionate	1.15
76.371	9-Octadecenamide, (*Z*)	1.50

Sargassum ilicifolium extracts, while pentadecanoic acid was identified in higher concentration in *Hydropuntia edulis*. Results of evaluations by Schmid *et al*. (2014) suggested that apart from differences between species, variation in fatty acid composition and concentration among seaweeds is mostly due to various abiotic factors including light, salinity, and nutrients. Hexadecanoic acid and pentadecanoic acid are fatty acids, and both have been reported to possess antioxidant properties (Mujeeb *et al*., 2014). Consequently, it is noteworthy that the availability of different categories of fatty acids can be correlated with antioxidant activity of the extracts in the species explored.

DPPH assay in seaweeds

Comparison of the free radical scavenging activity of seaweed extracts to that for ascorbic acid showed similar patterns, although the DPPH inhibition values were lower in the seaweeds than the control, as shown in Figure 1. At the lowest concentration of seaweed extracts tested (200 µg·mL^{-1}), DPPH inhibition was comparable among *Sargassum ilicifolium* (53.80 %), *Halimeda macroloba* (54.72 %), *Hydropuntia edulis* (57.30 %) and *Ulva intestinalis* (58.42 %). The DPPH inhibition for *Codium* sp. (47.28 %) was significantly lower than the other extracts. At the same concentration (200 µg·mL^{-1}), the control (ascorbic acid) exhibited 77.69 % free radical inhibition. Progressive increases in the radical scavenging activity were recorded when the concentration of seaweed extracts was increased in a dose-response manner, consistent with the trend observed in the control. Thus, the highest scavenging activity for all seaweed extracts was attained at a concentration of 1,000 µg·mL^{-1}. The extract that showed the highest inhibition was *U. intestinalis* (65.02 %), and although this value was significantly different from all the other seaweed extracts, the size of the difference was small; the lowest inhibition at this concentration was measured in *S. ilicifolium* (62.13 %). Radical inhibition activity measured in the control at the highest concentration was 86.67 % (Figure 1).

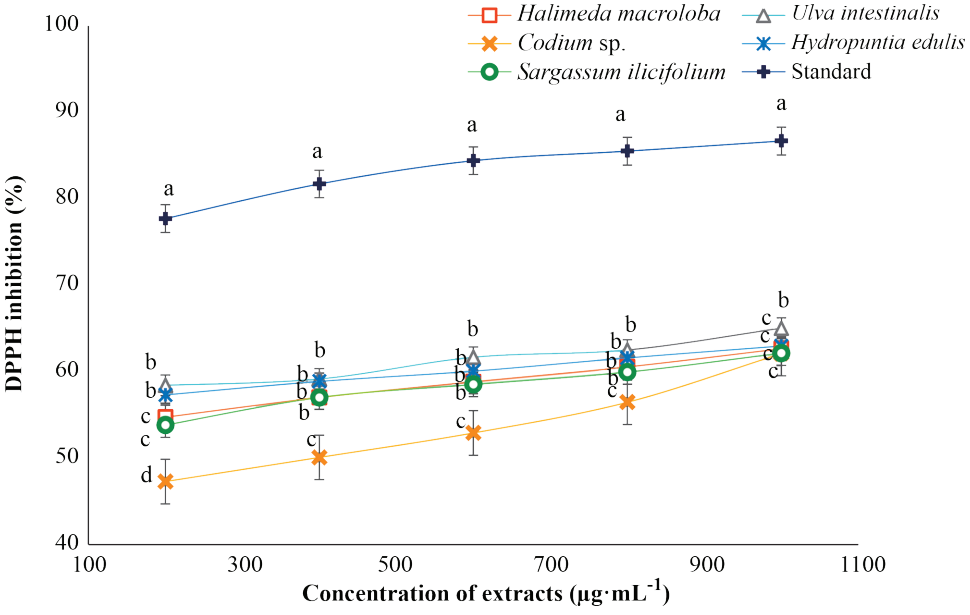

Figure 1. DPPH inhibition assay summary (mean±SE, n = 3) showing free radical scavenging activity for extracts of selected seaweed species compared to control (ascorbic acid) measured at five concentrations (200-1,000 µg·mL^{-1}).

Note: Error bars represent SE; Different lowercase letters at the same concentration denote significant differences among means ($p<0.05$, Dunnett's test).

Results show that extracts from the five seaweed species have significantly lower ($p<0.05$) scavenging activity compared to the standard (ascorbic acid). Among the seaweed species, *Ulva intestinalis* extract produced the highest DPPH reduction, ranging between 58.42 % and 65.02 % from the lowest to highest doses tested. Consequently, *U. intestinalis* was shown to exhibit significant antioxidant activity, as over 50 % DPPH inhibition was achieved even at the lowest concentration of 200 $\mu g \cdot mL^{-1}$. This is consistent with results of another study on the same *Ulva* species reported by Indu *et al*. (2013); the authors attributed the high antioxidant activity to seasonal factors and the prevailing weather during sample collection. The observation was also supported by a study by Khairy and El-Sheikh (2015), where an *Ulva* species was demonstrated to show maximum antioxidant activity during the summer, a season with conditions comparable to those experienced in Malaysia during sampling for the present study.

The five tested seaweed samples all demonstrated antioxidant activity, since the DPHH inhibition values increased in a dose-response manner. The seaweeds possess significant levels of natural bioactive compounds such as pigments and their derivatives (Leelavathi and Prasad, 2014) known to be beneficial in preventing oxidative damage. Cox *et al*. (2010) reported that fucoxanthin, a carotenoid pigment of the xanthophyll group and which is found abundantly in brown seaweed species, is the reason for their greater antioxidant activity compared to green and red seaweed species. A similar conclusion was proposed by Airanthi *et al*. (2011). It is worth noting, however, that other carotenoids in red and green seaweed species such as β-carotene, β-cryptoxanthin, zeaxanthin and lutein do not appear to have as much DPPH scavenging effect as found in the brown seaweeds (Nomura *et al*., 1997). Meanwhile, Plaza *et al*. (2010) suggested that antioxidant activity can never be attributed to only one compound, since seaweed extracts usually comprise a complex matrix of compounds which can act synergistically.

Based on the total lipid content assessment of the five seaweeds, *Halimeda macroloba* and *Sargassum ilicifolium* were the most favorable species, although *S. ilicifolium* was noted to be lower in total carotenoids and chlorophyll *b*. *Ulva intestinalis* and *H. macroloba* were notable as the most favorable species for their content of total carotenoids and chlorophyll *a* and *b*. Nonetheless, the seaweed species demonstrating the highest antioxidant activity (via DPPH assay) were *S. ilifolium* and *U. intestinalis*, in which the highest levels of hexadecanoic acid were also detected. Consequently, results from this study clearly support the conclusions of previous researchers, namely that morphological characteristics of seaweed species and the controlling environmental factors, along with sampling location and time (Heriyanto *et al*., 2017) are the key factors responsible for their content of pigments and bioactive components.

For example, the green seaweed *Halimeda macroloba* was sampled at different locations at approximately the same time in this study, and although samples exhibited similar content of pigments (total carotenoids and chlorophylls), the content of total lipids was significantly ($p<0.05$) different. Furthermore, even though *Ulva intestinalis* is also a green seaweed and was sampled at a location near *H. macroloba*, total lipid content of *U. intestinalis* was significantly lower. A similar observation can be made for total lipid content of two brown seaweed species (*Hydropuntia edulis* and *Sargassum ilicifolium*) sampled at different locations. In addition to the observation of differences in lipid content among similar seaweed species sampled at different locations, it is noteworthy that the antioxidant potential (measured via DPPH assay) of *S. ilicifolium* (a brown seaweed) and *U. intestinalis* (a green seaweed) sampled at different locations was higher than the other species sampled.

CONCLUSION

This study successfully demonstrates that the five examined seaweed species possess essential photosynthetic pigments (carotenoids, chlorophyll *a* and *b*) responsible for high antioxidant activity, as well as other bioactive compounds such as hexadecenoic acid and biochemical compounds such as lipids. The amount of these valuable compounds within the seaweed extracts can differ significantly,

not only due to variation in species or higher taxa (red, green or brown seaweeds), but also depends to a large extent on season of sample collection, location of sampling and the growing conditions such as degree of salinity, light intensity and nutrient availability. More studies are therefore required in order to correlate the specific factors responsible for the biological activities observed in these species. Nonetheless, the identification of a saturated fatty acid like hexadecanoic acid in high concentration cannot be ignored, as it is a vital metabolite playing a key role in the antioxidant activity of seaweeds. In general, these findings highlight the potential of these seaweed species for cultivation as a sustainable source of functional food for human consumption.

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