

# Northumbria Research Link

Citation: Balasubramaniam, V., June Chelyn, L., Vimala, S., Mohd Fairulnizal, M.N., Brownlee, Iain and Amin, I. (2020) Carotenoid composition and antioxidant potential of *Eucheuma denticulatum*, *Sargassum polycystum* and *Caulerpa lentillifera*. *Heliyon*, 6 (8). e04654. ISSN 2405-8440

Published by: Elsevier

URL: <https://doi.org/10.1016/j.heliyon.2020.e04654>  
<<https://doi.org/10.1016/j.heliyon.2020.e04654>>

This version was downloaded from Northumbria Research Link:  
<http://nrl.northumbria.ac.uk/id/eprint/44370/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)



## Research article

Carotenoid composition and antioxidant potential of *Euचेuma denticulatum*, *Sargassum polycystum* and *Caulerpa lentillifera*V. Balasubramaniam<sup>a,\*</sup>, L. June Chelyn<sup>b</sup>, S. Vimala<sup>c</sup>, M.N. Mohd Fairulnizal<sup>a</sup>, I.A. Brownlee<sup>d</sup>, I. Amin<sup>e</sup><sup>a</sup> Nutrition, Metabolism & Cardiovascular Research Centre, Institute for Medical Research, Ministry of Health Malaysia, Level 3, Block C7, No.1, Jalan Setia Murni U13/52, Setia Alam, 40170, Shah Alam, Selangor, Malaysia<sup>b</sup> Herbal Medicine Research Centre, Institute for Medical Research, Ministry of Health Malaysia, Level 3, Block C7, No.1, Jalan Setia Murni U13/52, Setia Alam, 40170, Shah Alam, Selangor, Malaysia<sup>c</sup> Forest Research Institute Malaysia, 52109, Kepong, Selangor Darul Ehsan, Malaysia<sup>d</sup> Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne NE1 8ST, UK<sup>e</sup> Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

## ARTICLE INFO

## Keywords:

Food science  
Food technology  
Food analysis  
Nutrition  
*Caulerpa lentillifera*  
*Sargassum polycystum*  
Orbitrap  
LC-MS/MS  
Carotenoid  
*Euचेuma denticulatum*  
Antioxidant

## ABSTRACT

Three species of Malaysian edible seaweed (*Euचेuma denticulatum*, *Sargassum polycystum* and *Caulerpa lentillifera*) were analyzed for their carotenoid composition using a combination of high-performance thin layer chromatography (HPTLC) and ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS), while the antioxidant capacities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays. The HPTLC analysis exhibited a distinct carotenoid pattern among the three seaweed groups. The UHPLC-ESI-MS/MS analysis showed fucoxanthin as the major carotenoid present in *S. polycystum* while lutein and zeaxanthin in *E. denticulatum*. For *C. lentillifera*,  $\beta$ -carotene and canthaxanthin were the major carotenoids. Some of the carotenoids, such as rubixanthin, dinoxanthin, diatoxanthin and antheraxanthin, were also tentatively detected in *E. denticulatum* and *S. polycystum*. For antioxidant activity, *S. polycystum* (20 %) and *E. denticulatum* (1128  $\mu\text{mol TE/g}$ ) showed the highest activity in the DPPH and ORAC assays, respectively. The findings suggest the three edible varieties of seaweeds may provide a good dietary source with a potential to reduce antioxidative stress.

## 1. Introduction

Seaweed remains a popular food ingredient in many parts of Asia and is an important source of high-value hydrocolloids, such as agar, alginates and carrageenan [1, 2]. In addition, seaweeds are increasingly recognized for their health promoting benefits, leading to a growing interest and number of utilization in the nutraceutical, pharmaceutical and cosmetic industries [3]. Previous studies have further shown that seaweed-derived extracts and compounds possess many interesting bio-activities, such as anti-inflammatory, anti-diabetic, anti-obesity and anti-cancer properties [4, 5, 6]. The observed activities could partly be contributed by the range of antioxidant compounds found in seaweeds [7, 8, 9, 10].

Seaweeds are exposed to many environmental factors that favor the generation of free radicals and strong oxidizing agents [11, 12, 13]. As a

result of these harsh conditions, seaweeds are highly resistant to oxidative damage, possibly contributed by the antioxidant compounds within their cells [14].

Carotenoids are one of the essential antioxidant compounds in seaweeds [15]. Carotenoids, which have a characteristic linear C40 chain, contain up to 11 conjugated bonds (allenic bonds) that may participate in antioxidant activities via the transfer of the excess energy of singlet oxygen ( $\text{O}^\bullet$ ) in the long central allenic chain [16]. Moreover, the allenic bonds and other functional groups at the terminal rings of the structure, as seen in astaxanthin and fucoxanthin, may also react with free radicals and thus further contribute to its antioxidant potential [9]. Although profiling of carotenoids has been used for the taxonomic classification of seaweed, data on their concentrations in different phyla remain scarce.

Traditionally, the separation and characterization of carotenes (nonpolar carotenoids) and xanthophylls (polar carotenoids) have been

\* Corresponding author.

E-mail addresses: [vimala.rmt@moh.gov.my](mailto:vimala.rmt@moh.gov.my), [balavimala@hotmail.com](mailto:balavimala@hotmail.com) (V. Balasubramaniam).<https://doi.org/10.1016/j.heliyon.2020.e04654>

Received 13 March 2019; Received in revised form 30 April 2020; Accepted 4 August 2020

2405-8440/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

achieved using thin-layer chromatography (TLC) and high-performance liquid chromatography coupled to a UV or photodiode array detector (HPLC-UV/DAD) [15]. However, the complexity of the extract matrices, as well as close structural similarities between the carotenoids, necessitates new methods that are more precise and sensitive to distinguish closely related isomers and accurately quantify them [15, 17]. The development of high-resolution and accurate mass spectrometry (MS) has allowed complex mixtures of carotenoids to be distinguished according to their accurate masses and fragmentation patterns [18]. Various MS ionization methods have also been investigated in the analysis of carotenoids, such as atmospheric pressure chemical ionization (APCI) and the electrospray ionization (ESI) method [19, 20]. Moreover, substantial improvements have been made in the development of ultra-high-performance liquid chromatography (UHPLC), which allows better sensitivity and a shorter run time for carotenoid analysis [18, 21].

To date, information regarding the bioactive components present in the Malaysian local seaweeds remain insufficient. The current work is a continuation of our previous research [4, 5]. These studies reported that the ethanol extracts of *E. denticulatum* manifest anti-diabetic, anti-obesity and anti-inflammatory properties. The health benefits shown in the ethanol extracts may be due to the presence of polyphenols or other antioxidant components, such as carotenoids. The evaluation of polyphenols content has been reported previously on the selected seaweeds [22, 23]. Therefore, in the present study, we investigate the carotenoid composition of *E. denticulatum* (red seaweed), *S. polycystum* (brown seaweed) and *C. lentillifera* (green seaweed) and their antioxidant capacity. Brown and green seaweed were included for comparison with the red seaweed for this study. A rapid, HPTLC system was used for the quick visualization and identification of carotenoids in the different seaweeds, while UHPLC coupled to an ESI-MS detector (UHPLC-ESI-MS) was used to further validate and quantify the identified carotenoids. The antioxidant activities were assessed by the 2-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay and oxygen radical absorbance capacity (ORAC) assay.

## 2. Materials and methods

### 2.1. Chemicals

HPLC-grade ethanol (EtOH) and dichloromethane were purchased from Merck (Merck Co., Darmstadt, Germany). The LC-MS-grade solvents acetonitrile (ACN), methanol (MeOH) and formic acid (FA) were purchased from Fisher Chemical (New Jersey, USA). Chemical standards of lutein,  $\beta$ -carotene, zeaxanthin, and lycopene were purchased from Sigma (St Louis, MO, USA); those of  $\beta$ -cryptoxanthin, all-trans-canthaxanthin, all-trans-fucoanthin, all-trans-astaxanthin, violaxanthin and neoxanthin were obtained from ChromaDex (Santa Ana, CA). All other reagents and solvents were of high analytical grade unless otherwise stated. Water was obtained from arium® pro Ultrapure Water Systems (Sartorius, Goettingen, Germany). All solvents were degassed before use.

### 2.2. Seaweed collection and processing

The seaweeds (*E. denticulatum*, *S. polycystum* and *C. lentillifera*) were collected from the coast of Sabah with the assistance of the Aquaculture Division, Department of Fisheries, Malaysia. Samples were processed immediately upon arrival at the Institute for Medical Research, Malaysia, as previously discussed [4, 5]. Briefly, the collected seaweed samples were cleaned thoroughly to remove epiphytes, salt and sand using tap water and finally rinsed with deionized water three times before being cut into small pieces. The samples were placed in glass jars and sealed with parafilm. Then, the seaweeds were frozen at  $-80^{\circ}\text{C}$  prior to freeze drying at  $-85^{\circ}\text{C}$  (Beta 2–8 LDplus freeze dryer, Martin Christ, GMBH, Germany) and ground to a fine powder (40 mesh size) using a Waring blender. Samples were stored in airtight containers at  $-80^{\circ}\text{C}$  until further analysis.

### 2.3. Sample preparation

Freeze-dried seaweed powder was mixed in a ratio of 1:20 w/v with the extraction solvent (100 % ethanol; EMSURE® ACS) by stirring at room temperature for 24 h. The crude extracts were obtained by filtration using a Whatman filter paper (No. 1), and the filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) under reduced pressure at  $45^{\circ}\text{C}$ . The concentrated extracts were subsequently aliquoted into a 5 ml Bijou bottle. Next, the extracts were dried overnight ( $45^{\circ}\text{C}$ ) by vacuum centrifugation using MiVac (Genevac, UK) to yield a dark-green extract for *E. denticulatum* and *C. lentillifera*, while for *S. polycystum*, a dark-brown extract was obtained. The extracts were maintained at  $-20^{\circ}\text{C}$  until further use. Each so-obtained dried pellet (seaweed extract) was reconstituted with relevant solvents according to analysis. For the LC-MS analysis, the seaweed extract was diluted with a 50:50 DCM/MeOH solution and then filtered through a  $0.22\ \mu\text{m}$  nylon filter into a LC vial to remove any impurities before loading to the UHPLC. A concentration of 1 mg/ml was injected in UHPLC.

### 2.4. Preparation of stock standard solutions

Stock standard solutions (10 mg/ml) were prepared individually by dissolving the analytes in a 50:50 DCM/MeOH solution. The solutions were stored in the dark at  $-20^{\circ}\text{C}$ . The standard stock solution was used for spiking, as different spiking levels are required for validating the method. Only primary grade standards ( $\sim 99.5\%$  purity) were used for the LC-MS/MS analysis.

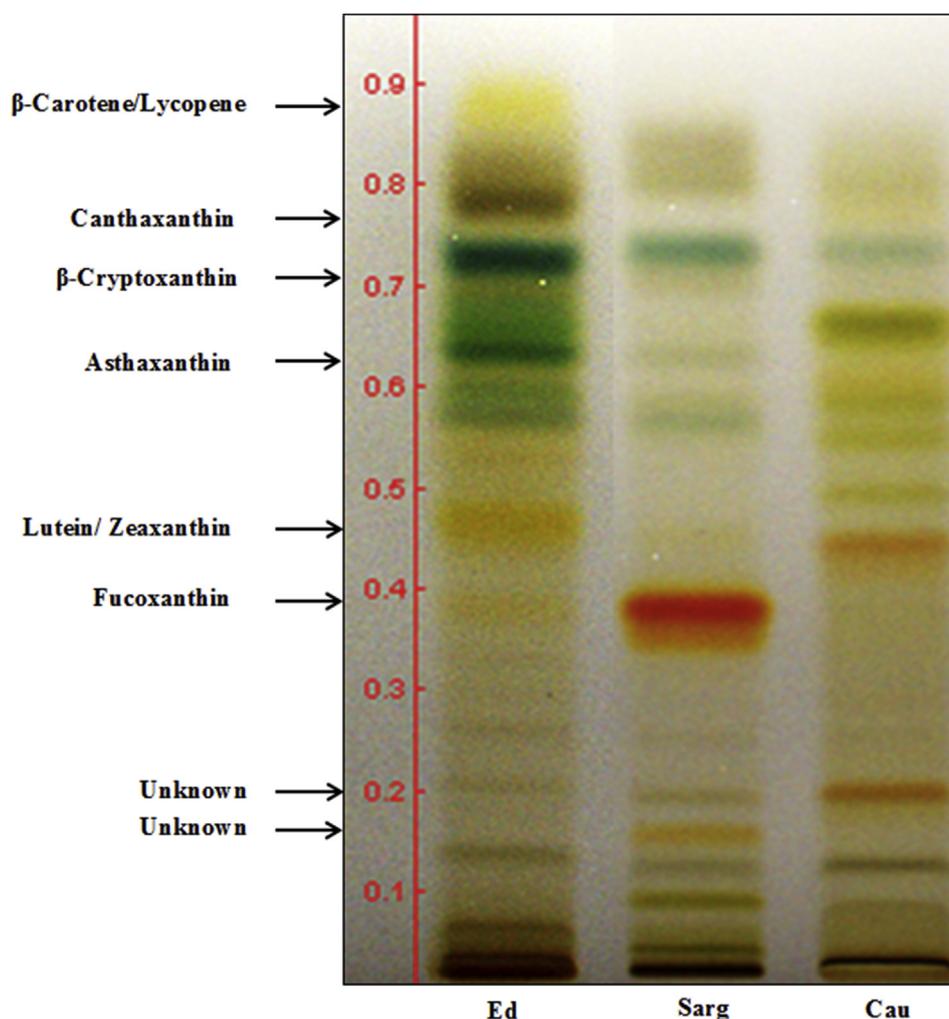
### 2.5. Identification of carotenoids

#### 2.5.1. Carotenoid analysis by HPTLC

HPTLC analysis was performed using a HPTLC plate precoated with silica gel 60 F<sub>254</sub> (Merck, Darmstadt, Germany). Briefly, ethanolic extracts of each seaweed (*E. denticulatum*, *C. lentillifera* and *S. polycystum*) (10 mg) and standards (1 mg) were dissolved in a 50:50 DCM/MeOH solution by sonication to make 10 mg/ml and 1 mg/ml stock solutions, respectively. The crude extract solution (10  $\mu\text{l}$ ) and standard solutions (2  $\mu\text{l}$ ) were applied to the HPTLC plate as 8 mm-wide bands using a Camag LINOMAT V applicator (Muttenez, Switzerland). A HPTLC developing chamber was presaturated with the mobile phase consisting of toluene: acetone (70:30, v/v) for 20 min. Then, the HPTLC plate was inserted into the chamber and developed until the developing distance reached 70 mm. Finally, the plate was removed from the chamber, air-dried and visualized under white light without any further derivatization.

#### 2.5.2. Carotenoid analysis by UHPLC-ESI-MS/MS

Carotenoids were identified according to a modified method described previously [24] using a UHPLC-ESI/HRMS/MS<sup>n</sup> system that consisted of a Dionex Ultimate 3000 UHPLC system (Thermo Scientific, CA, USA) and a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, CA, USA). A MS-compatible C18 reversed-phase column (50 mm  $\times$  2.1 mm i.d., 1.7  $\mu\text{m}$  particle size), Acquity UPLC BEH C18 (Waters, MA, USA), was used in this study. The autosampler was set at  $4^{\circ}\text{C}$ , and sample volumes of 3  $\mu\text{l}$  were loaded onto the column. The column was run at a temperature of  $35^{\circ}\text{C}$ . The gradient program was composed of two mobile phases, water with 0.1 % formic acid (A) and ACN with 0.1 % formic acid (B). The flow rate was 0.3 ml/min, and the gradient program was as follows: 0–2 min, 5 % B; 2–20 min, from 5 % B to 99 % B, 20–25 min, 99 % B; 25–30 min 5 % B. The MS analysis was carried out in positive and negative ion electrospray ionization (ESI) modes. The mass spectrometer was used with a resolving power setting of 140,000 FWHM and a scan range of  $m/z$  100–1000, the capillary temperature was set at  $320^{\circ}\text{C}$ , and the sheath gas and auxiliary gas flow rates were 35 and 12 arbitrary units, respectively. The spray voltage was at 3.7 kV. The S-tube lens was set at 55 V for both the positive and the negative ionization modes. The MS/MS spectra were acquired



**Figure 1.** HPTLC analysis of carotenoids in ethanolic extracts of red seaweed, *E. denticulatum* (Ed), brown seaweed, *S. polycystum* (Sarg) and green seaweed, *C. lentillifera* (Cau) developed on a silica gel HPTLC plate with toluene: acetone (70:30,v/v) as the mobile phase (Full picture is provided in supplementary 1).

using a collision energy of 35 V. Data were generated using X-calibur 2.1.0 (Thermo Scientific, CA, USA). A column guard was used to avert damages and blockage in the column due to the complex nature of seaweed. In addition, washing steps were taken between long sample runs to prevent the column from becoming dirty.

### 2.5.3. Peak identification

Compound identification was based on accurate mass determination at a resolution of 140,000 and additional higher-energy collision dissociation (HCD) fragments with a mass deviation below 5 ppm. Most peaks were identified using standards by comparing the chromatographic retention times. A tentative identification of compound based on a comparison with data in the literature was applied when no standards were available.

### 2.5.4. UHPLC-MS/MS method validation

A quantitation method using Thermo Scientific Q-Exactive Xcalibur 2.2 software, which combines chromatography with high-resolution mass spectrometry, was developed for the determination of astaxanthin,  $\beta$ -cryptoxanthin, canthaxanthin, lutein, fucoxanthin and zeaxanthin. The validation procedure was performed through determination of the linearity, limit of detection (LOD), limit of quantification (LOQ) and recovery. To ascertain the validity of the retention times, standards were run frequently. Quantitative calibration was carried out using a five-point calibration.

## 2.6. Antioxidant capacity

### 2.6.1. 2-Diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay

The scavenging capacity of the DPPH radical was estimated according to the method of Blois [25] with a modification using a microplate system. A sample (50  $\mu$ l of 1.0 mg/ml) was added to 50  $\mu$ l of DPPH (1 mM in ethanolic solution) and 150  $\mu$ l of ethanol (absolute) in a 96-well microtiter plate in triplicate. The plate was shaken at 500 rpm for 15s and followed by incubation at room temperature for 30 min. Finally, the absorbance was measured spectrophotometrically at 520 nm.

### 2.6.2. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed as described previously [26] with some modifications. AAPH (2,2'-azobis (2-amidino-propane) dihydrochloride) (0.65 g) was dissolved in 10 ml of 75 mM phosphate buffer (pH 7.4) to a final concentration of 240 mM (made fresh daily). A fluorescein stock solution (1 mM) was made in 75 mM phosphate buffer (pH 7.4) and stored at 5  $^{\circ}$ C, wrapped in foil. Immediately prior to use, the stock solution was diluted 1:100,000 with 75 mM phosphate buffer (pH 7.4) (made fresh daily). To all experimental wells, 150  $\mu$ l of working sodium fluorescein solution was added. The blank wells were added with 25  $\mu$ l of Trolox 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) dilution. The sample wells were added with 25  $\mu$ l of samples. Following that, the plate was equilibrated by incubating for 10 min at 37  $^{\circ}$ C. A BMG Omega Fluostar<sup>TM</sup> fluorescent spectrophotometer with an

**Table 1.** Measured theoretical and accurate masses of the targeted carotenoids in *E. denticulatum*, *S. polycystum* and *C. lentillifera* by Orbitrap-MS (identity was confirmed by standard compound).

Peak	Retention time (min)	Selected ion	$m/z^a$ experimental	$m/z^a$ observed	Error (ppm)	Compounds
1	17.99	$[M + H - H_2O]^+$	641.42005	641.41809	-3.05	Fucoxanthin
2	20.11	$[M + H - H_2O]^+$	551.42474	551.4237	-3.21	Lutein
3	19.10	$[M + H]^+$	597.39384	597.39185	-2.52	Astaxanthin
4	21.17	$[M + H]^+$	565.40401	565.40167	-3.05	Canthaxanthin
5	20.14	$[M]^{+•}$	568.42803	568.42560	-3.31	Zeaxanthin
6	22.50	$[M]^{+•}$	552.43311	552.43036	-3.99	$\beta$ -Cryptoxanthin

<sup>a</sup>  $m/z$  (relative abundance).

injector was used with an excitation filter of 485 nm and emission filter of 528 nm. Reactions were initiated by the addition of 25  $\mu$ l of AAPH solution (240 mM) using the microplate reader's injector for a final reaction volume of 200  $\mu$ l. The plate was shaken at maximum intensity for 50 s. The fluorescence was then monitored kinetically with data taken every minute. The fluorescence of each well was measured every 60 s. ORAC values were calculated using MARS Data Analysis Reduction Software.

### 2.7. Statistical analysis

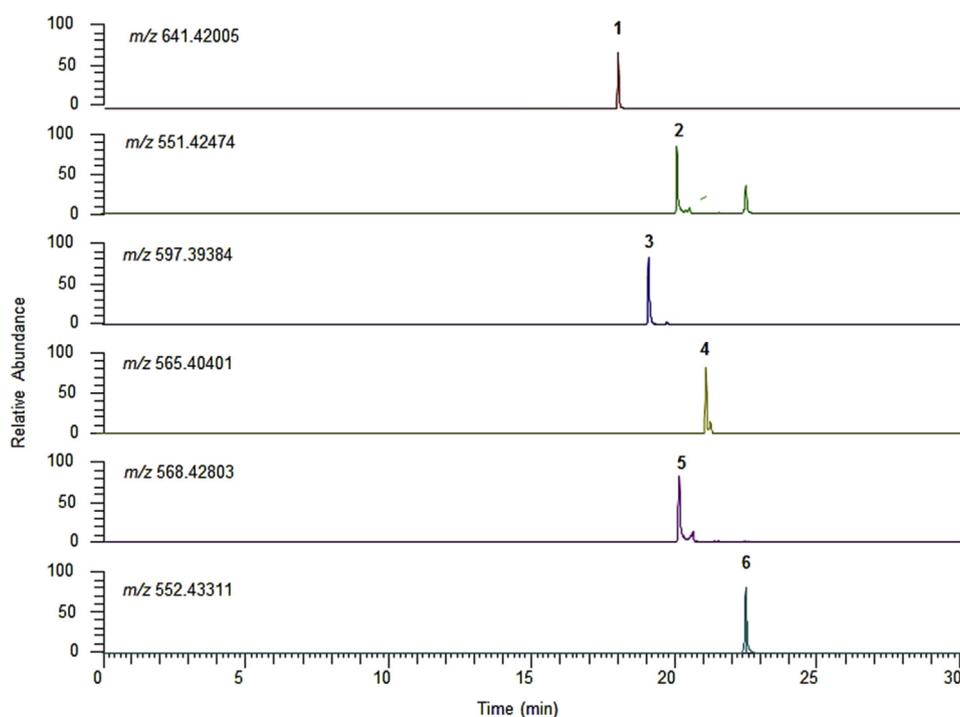
Data were analyzed using GraphPad Prism version 5.0 for Windows (San Diego, California, USA). Seaweed extraction and analysis were performed in triplicate for each sample. Quantitative results were expressed as mean  $\pm$  SD. A comparison of means was performed by one-way ANOVA, and a significant difference was accepted at a probability of  $p < 0.05$  determined by Bonferroni's test.

## 3. Results and discussion

### 3.1. Carotenoid composition in *E. denticulatum*, *S. polycystum* and *C. lentillifera* by HPTLC analysis

Carotenoids are usually categorized into two groups: xanthophylls, which contain oxygen in their molecular structure, and carotenes, which

are hydrocarbons. Both groups absorb light at different wavelengths during photosynthesis, and hence, xanthophylls appear more yellow, while carotenes are orange. In this study, HPTLC was used to analyze the color profiles and distribution pattern of carotenes and xanthophylls, as well as to tentatively identify the carotenoids present in the selected seaweed. According to Giri et al. [27], HPTLC-based solute separation can be useful in the development of validation and standardization processes, as well as isolation of pure compounds and facilitate the determination of classes of compounds in plants. Hence, seaweed fingerprinting is helpful in identifying a seaweed. A comparison of the carotenoid contents in the three species of seaweed by HPTLC is shown in Figure 1. The HPTLC patterns of the three seaweeds suggest the presence of distinct phytochemicals, where the major components detected were carotenoids along with chlorophylls and pheophytins. The HPTLC results clearly demonstrate that the seaweeds manifest a class-specific composition of xanthophylls. *E. denticulatum* contains mainly zeaxanthin or/and lutein ( $R_f = 0.44$ ) and  $\beta$ -carotene ( $R_f = 0.88$ ), present as light-yellow bands. Another tentatively detected xanthophyll,  $\beta$ -cryptoxanthin, is an intermediate in the biosynthesis pathways of lutein or zeaxanthin. A similar finding was reported by Marquardt and Hanelt [28]. Additionally, fucoxanthin, which is commonly found in brown seaweed, was detected in this red seaweed as a light-yellow band, indicating a smaller concentration compared to that of fucoxanthin in *S. polycystum* (brown seaweed), which appeared as a bright orange band.

**Figure 2.** Chromatograms for selected carotenoids by gradient reversed-phase UHPLC full scan Orbitrap-MS. Peak 1, fucoxanthin; Peak 2, lutein; Peak 3, astaxanthin; Peak 4, canthaxanthin; Peak 5, zeaxanthin and Peak 6,  $\beta$ -cryptoxanthin. Further details of the peaks are described in Table 1.

**Table 2.** Carotenoid contents in *E. denticulatum* (red seaweed), *S. polycystum* (brown seaweed) and *C. lentillifera* (green seaweed).

	Carotenoid concentration (mg/100 g of DW extract)						
	Lutein	Zeaxanthin	Fucoxanthin	$\beta$ -Cryptoxanthin	Canthaxanthin	Astaxanthin	$\beta$ -Carotene
<i>E. denticulatum</i>	87.7 $\pm$ 0.1 <sup>a</sup>	21.3 $\pm$ 0.1 <sup>a</sup>	4.0 $\pm$ 0.0 <sup>a</sup>	3.6 $\pm$ 0.0 <sup>a</sup>	<0.001	3.0 $\pm$ 0.0 <sup>a</sup>	4.7 $\pm$ 0.1 <sup>a</sup>
<i>S. polycystum</i>	11.7 $\pm$ 0.0 <sup>b</sup>	13.9 $\pm$ 0.0 <sup>b</sup>	2740.0 $\pm$ 0.5 <sup>b</sup>	6.1 $\pm$ 0.0 <sup>ab</sup>	21.7 $\pm$ 0.0 <sup>a</sup>	25.5 $\pm$ 0.0 <sup>b</sup>	<0.04
<i>C. lentillifera</i>	<0.02	3.6 $\pm$ 0.0 <sup>c</sup>	<0.001	1.3 $\pm$ 0.0 <sup>ac</sup>	14.6 $\pm$ 0.0 <sup>a</sup>	3.0 $\pm$ 0.1 <sup>a</sup>	19.5 $\pm$ 0.0 <sup>b</sup>

\*All carotenoids except  $\beta$ -carotene were quantified using UHPLC-ESI-MS on the basis of their retention times compared with authentic standards;  $\beta$ -carotene was detected and quantified by UHPLC. DW, dry weight. Values represent mean  $\pm$  SD of triplicate analysis (n = 3) with six replicates. Different letters (a-c) denote a significant difference between mean values within the same column.

According to previous findings [29, 30], red seaweed (Rhodophyta) does not present a unique carotenoid profile; nonetheless, the ubiquitous carotenoids  $\beta$ -carotene and/or  $\alpha$ -carotene and their dihydroxylated derivatives, zeaxanthin and lutein, were detected, which agrees with our finding.

Fucoxanthin at  $R_f = 0.39$  was detected as the major xanthophyll in *S. polycystum*, which was consistent with scores of previous studies reporting the abundant presence of fucoxanthin in brown seaweeds [28, 29]. Fucoxanthin was also noted as an intermediate of the lutein and zeaxanthin biosynthesis pathways [28].

The HPTLC analysis of *C. lentillifera* (green seaweed) showed it to be similar to higher plants in that zeaxanthin or/and lutein ( $R_f = 0.44$ ) were the dominant xanthophyll(s) present [31]. Other xanthophyll, such as canthaxanthin, was found in trace amounts in *C. lentillifera*, and this finding is in agreement with the results of Goodwin [32]. In contrast,  $\beta$ -carotene was not detected in the HPTLC analysis of *C. lentillifera*, as predicted based on previous findings [15]. The green pigments present in the analysis of *C. lentillifera* were probably chlorophylls and pheophytins involved in photosynthesis, which are generally found in green seaweeds as well as green terrestrial plants [33]. Hence, this finding suggests that different species of seaweed contain different kinds of carotenoids based on the phylum, which has been affirmed by previous studies; however, certain carotenoids were absent in contradiction to other findings [15, 33], which can be explained in terms of the different methods and species used.

### 3.2. Identification and quantitation of carotenoids

MS (ESI) was applied to confirm the identities of carotenoids from all three seaweed ethanolic extracts that were tentatively identified by HPTLC fingerprinting. These carotenoids were eluted under gradient conditions, and their identities were confirmed by their retention times, the accurate mass ( $m/z$  value) of their generated ions and the MS/MS patterns of their respective reference standards. The corresponding <sup>13</sup>C isotopic ion was also monitored as additional information for determining a compound's identity. Studies have demonstrated that isotope abundances provide increased confidence of identification [34, 35]. Nevertheless, the instability of carotenoids due to factors such as heat, light and oxygen may create challenges in the identification and quantitation of these compounds. Therefore, abovementioned factors should be taken account during sample preparation and analytical steps to ensure accurate analytical results.

Individual carotenoids were identified by ESI in positive ionization mode. In addition, the presence of generated molecular ions, particularly protonated  $[M + H]^+$ , dehydrated  $[M + H - H_2O]^+$  and the radical molecular ion  $[M]^{\bullet+}$ , was studied (Table 1). From the MS analysis (Figure 2), it was observed that lutein (Peak 1) forms predominantly the dehydrated molecule (i.e.,  $[M + H - H_2O]^+$ ), and this finding agrees with previously reported data [36], while for fucoxanthin (Peak 2), the dehydrated molecule  $m/z$  641  $[M + H - H_2O]^+$  was selected to facilitate the comparison with the standard, although it is noteworthy that the protonated ion ( $m/z$  659) was detected abundantly in the sample. For astaxanthin and canthaxanthin (Peaks 3 and 4), the protonated molecule  $[M + H]^+$

was noted as the most abundant species, while zeaxanthin (Peak 5) and  $\beta$ -cryptoxanthin (Peak 6) were characterized by the molecular ion  $M^{\bullet+}$  due to loss of one electron and formation of a stable radical system. Notably, in this study, the MS system applied with an ESI positive mode ionized xanthophylls (astaxanthin,  $\beta$ -cryptoxanthin, canthaxanthin, fucoxanthin and lutein) well but did not ionize hydrocarbon carotenes (such as lycopene and  $\beta$ -carotene). Hence, UHPLC- diode array detector was utilized to detect and quantify the  $\beta$ -carotene present in all three seaweed species tested.

The carotenoid concentrations of the three species of seaweeds are shown in Table 2. The detectable carotenoids for *E. denticulatum* (red) consisted of lutein, zeaxanthin,  $\beta$ -cryptoxanthin, and  $\beta$ -carotene with a few unsubstantiated carotenoids. Lutein was present as the major xanthophyll for this class of seaweed with a concentration of 87.7 mg/100 g DW. This result agrees with the findings of Marquardt and Hanelt [28], who describe lutein, in general, as the predominant carotenoid in most red seaweeds.  $\beta$ -Cryptoxanthin (3.6 mg/100 g) was the least abundant detected carotenoid in *E. denticulatum* at  $m/z$  552. This adduct species was selected for quantitating  $\beta$ -cryptoxanthin due to its better peak resolution and higher abundance compared to those of the peak at  $m/z$  535.

Researchers have noted that red seaweeds generally lack a xanthophyll biosynthesis pathway after zeaxanthin, which results in an accumulation of zeaxanthin and lutein as major carotenoids [15] and agrees with the present finding. Consequently, this explains the minor presence of other carotenoids in this species. The  $\beta$ -carotene content of *E. denticulatum* (4.7 mg/100 g DW) was comparable to the previous findings of 5.4 mg/100 g DW of  $\beta$ -carotene detected in the red seaweed of the *Gracilaria* spp [37]. and 5.7 mg/100 g in *Kappaphycus alvarezzi* [38]. Interestingly, the tentative identification of other xanthophylls, such as rubixanthin, antheraxanthin, dinoxanthin, diatoxanthin and diadinoxanthin, in *E. denticulatum* (data not shown) adds a new perspective to the carotenoid profile of the red seaweeds of this species. In the present study, however, an exact quantification analysis of the abovementioned carotenoids based on pure compounds could not be carried out due to the absence or costliness of the pure standard. Further research is needed for the exact determination of these carotenoids. Authentication of this finding may contribute new information to the *E. denticulatum* carotenoid profile.

Fucoxanthin (2.76 g/100 g DW) is the predominant carotenoid found in the brown seaweed *S. polycystum*, as well as in most other brown seaweeds [15]. Consequently, the finding of a high fucoxanthin content in *S. polycystum* adds to the value and increases the potential commercial prospects of this seaweed, which currently remain underexploited. Moreover, this species is abundantly available along coastal Malaysia, and no firm commercial application has been established in Malaysia or elsewhere. In general, the fucoxanthin found in brown seaweed is in the form of trans-fucoxanthin. Abidov et al. [39] reported that a 2.4 mg of fucoxanthin intake per day can significantly increase the energy expenditure of obese female volunteers and promoted weight loss, decreased body fat, plasma triacylglycerol as well as liver lipid contents. Judging from the content of fucoxanthin in *S. polycystum* seaweed, the concentration will be sufficiently high to show the physiological activity of these

**Table 3.** Carotenoids detected in the studied seaweeds.

Pigment	Seaweed		
	<i>E. denticulatum</i>	<i>S. polycystum</i>	<i>C. lentillifera</i>
Antheraxanthin	+	+	-
Astaxanthin*	+	+	+
β-Carotene*	+	-	+
β-Cryptoxanthin*	+	+	+
Canthaxanthin*	-	+	+
Dinoxanthin	+	+	-
Diatoxanthin	+	+	-
Diadinoxanthin	+	+	-
Fucoxanthin*	+	+	-
Lutein*	+	+	-
Lycopene*	-	-	-
Neoxanthin*	-	-	-
Rubixanthin	+	-	-
Violaxanthin*	-	-	-
Zeaxanthin*	+	+	+

‘+’ denotes presence; ‘-’ denotes not detected; asterisk denotes compound confirmed with standard.

substances when they are incorporated to general foods. The LC-MS/MS result correlates well with the HPTLC findings, which exhibited an intense, thick orange band ( $R_f = 0.39$ ), denoting a high concentration. This finding proves the presence of fucoxanthin in *S. polycystum* seaweed, which suggests that it is responsible directly, partially or/and synergistically with other phenolic compounds for the anti-radical activity observed in the present study. Apart from fucoxanthin, appreciable amounts of astaxanthin, canthaxanthin, lutein and zeaxanthin were detected (at 25.5, 21.7, 11.7 and 13.9 mg/100 g DW, respectively). β-Cryptoxanthin was present in the lowest amount at 6.1 mg/100 g DW, while β-carotene was not detected in this species of seaweed. The carotenoid composition of *S. polycystum* obtained in the present study contradicts the finding of Motshakeri et al. [40] regarding the same seaweed. This previous study reported the presence of β-carotene (75 μg/100 g DW) but showed an absence of lutein, canthaxanthin and astaxanthin. In both studies, the carotenoids zeaxanthin, fucoxanthin and diatoxanthin were detected. Quantitation of carotenoids showed variation within the same species due to differences in sample preparation and possibly in the method and instruments used. However, this study [40] did not provide a clear explanation of the method used for quantitation. Hence, we could not make a comparison. Other compounds, such as diadinoxanthin, diatoxanthin and antheraxanthin, were tentatively identified in *S. polycystum* on the basis of their molecular mass ( $m/z$  583, 567, and 585, respectively), as described in the literature [41, 42]. Since there were no reference compounds for these xanthophylls, the data could not be confirmed by authentic materials. Diatoxanthin is a xanthophyll commonly found in seaweed that contributes to the “diadinoxanthin cycle”. The presence of diadinoxanthin in seaweed is deemed a possible precursor for fucoxanthin synthesis [43]. Neoxanthin

was not detected in *S. polycystum*, and this result is supported by Mikami and Hosokawa [15] due to the lack of a gene encoding neoxanthin synthase (NXS) in brown seaweed.

The highest carotenoid contents in *C. lentillifera* (green) were those of β-carotene and canthaxanthin (14.6 mg/100 g and 19.5 mg/100 g DW, respectively), while lutein and fucoxanthin were not detected, which is in accordance with the HPTLC finding. The other xanthophylls detected in this seaweed, zeaxanthin, β-cryptoxanthin and astaxanthin, had contents ranging between 1.3 and 3.0 mg/100 g, which agrees with the findings of Young et al. [44]. Zeaxanthin, found in *C. lentillifera*, is a common carotenoid present in other green seaweeds and higher plants. However, in contrast to terrestrial plants, green seaweed possesses extra xanthophylls, such as canthaxanthin and astaxanthin, as detected in *C. lentillifera*. Neoxanthin and violaxanthin were not detected in the tested green seaweed (*C. lentillifera*), as expected. The detection was based on comparing the mass and retention time with the available standards (cis-neoxanthin and all-trans-violaxanthin). Contrarily, other studies have reported the presence of violaxanthin and/or neoxanthin as prominent carotenoids in green seaweed [29, 45]. Nevertheless, it is worth noting that an unknown peak was detected at  $m/z$  601 at a different retention time ( $RT = 19.14$  min) from those of the violaxanthin ( $RT = 18.93$  min) and neoxanthin ( $RT = 17.42$  min) standard peaks. The detected compound was more nonpolar and eluted later than the violaxanthin peak from the referral standard. Further structural analysis is needed to verify this compound.

Table 3 shows the tentatively detected carotenoids in each species of seaweed, with some of the compounds having been confirmed with standards. Twelve carotenoid compounds were detected in the studied seaweeds, some for the first time. *E. denticulatum* displayed a wide variety of carotenoid contents compared to the other two species. *C. lentillifera* had the fewest detected pigments.

From this analysis, it is evident that high-resolution mass spectrometry enables the determination of accurate masses with <5 ppm mass accuracy and detection of targeted compounds. An additional route to compound identification is provided by the fragmentation of ions produced in a higher-energy collision dissociation (HCD) cell. Because of the limitations of the ESI source, it was not suitable for detecting the carotenes, and hence, UHPLC was used to quantitate β-carotene in this study.

### 3.3. Validation of parameters for carotenoid quantitation

The linearity of the developed methods was evaluated by preparing five-point calibration curves in solvents for the various carotenoids analyzed. The determination coefficients ( $R^2$ ) obtained for these compounds all exceeded 0.97, which suggests good linearity. The relative recoveries of astaxanthin, β-carotene, canthaxanthin, fucoxanthin, lutein, and zeaxanthin ranged from 85 % to 104 % (Table 4) at the level tested. However, the relative recovery of β-cryptoxanthin was the lowest at 74 %. This low recovery may be attributed to the β-cryptoxanthin instability, which was noted to be higher than those of other carotenoids, and to the lower solubility of this carotenoid in the injection solvent. Except for β-cryptoxanthin, satisfactory recoveries were obtained for the tested analytes. Nevertheless, according to Rivera et al. [46], the selection of the

**Table 4.** Performance criteria for Orbitrap-MS.

Seaweed	LOD (ng/μl)	LOQ (ng/μl)	$R^2$	Linear range (ng/μl)	Recovery (%)
Lutein	0.0170	0.0567	0.9966	0.0–1.0	99
Zeaxanthin	0.0017	0.0057	0.9731	0.0–1.0	99
Fucoxanthin	0.0014	0.0047	0.9995	0.0–2.0	104
β-Cryptoxanthin	0.0067	0.0150	0.9993	0.0–0.5	74
Canthaxanthin	0.0011	0.0044	0.9870	0.0–1.0	93
Astaxanthin	0.0023	0.0058	0.9975	0.0–1.0	88
*β-Carotene	0.0418	0.1393	0.9981	0.0–1.0	85

\*Performance based on UHPLC.

**Table 5.** Antioxidant capacity of seaweed ethanolic extracts as determined by DPPH and ORAC.

Sample	DPPH (%)	ORAC ( $\mu\text{mol TE}/100\text{ g}$ )
<i>E. denticulatum</i>	7.99 $\pm$ 0.5 <sup>a</sup>	112,762 $\pm$ 8,575 <sup>a</sup>
<i>S. polycystum</i>	20.36 $\pm$ 0.7 <sup>b</sup>	42,060 $\pm$ 5,153 <sup>b</sup>
<i>C. lentillifera</i>	5.74 $\pm$ 0.9 <sup>a</sup>	68,372 $\pm$ 1,596 <sup>c</sup>

DPPH expressed as a percentage of radical scavenging activity, and ORAC expressed in  $\mu\text{mol trolox equivalent per } 100\text{ g}$ . Data are expressed as mean  $\pm$  standard error mean (SEM) (n = 3). Different letters (a-c) denote a significant difference between mean values within the same column.

injection solvent was a compromise between the satisfactory solubility of carotenoids, the compatibility with the mobile phase, and the absence of peak distortions. Retention time repeatability was checked with 6 successive runs of the targeted analytes. The limit of detection (LOD) and lower limit of quantitation (LOQ) were determined based on the signal-to-noise ratio (S/N) of the analytes. The lowest LOD and LOQ values were 0.0011 and 0.0044 ng/ $\mu\text{l}$ , respectively, obtained for canthaxanthin, compared to 0.0418 and 0.1393 ng/ $\mu\text{l}$ , respectively, for  $\beta$ -carotene, for the UHPLC-DAD performance. The results obtained in this work attest to the performance of the high-resolution Orbitrap-MS with respect to ESI.

### 3.4. Antioxidant capacity of *E. denticulatum*, *S. polycystum* and *C. lentillifera*

The inhibition of radical scavenging activity by the ethanolic extracts of *E. denticulatum*, *S. polycystum* and *C. lentillifera* (200  $\mu\text{g}/\text{ml}$  respectively) is reported in Table 5. The results indicate that the ethanolic extract of *S. polycystum* (200  $\mu\text{g}/\text{ml}$ ) had the highest radical scavenging capacity (20.36  $\pm$  0.7 %) among the three seaweeds. The scavenging capacities of *E. denticulatum* and *C. lentillifera* were considerably lower at 7.99  $\pm$  0.5 % and 5.74  $\pm$  0.9 %, respectively. This finding supports the previous reports on brown seaweed having better DPPH scavenging activity than red seaweeds [47, 48]. Furthermore, fucoxanthin, a non-phenolic carotenoid that is commonly found in brown seaweed, also exerts good DPPH radical scavenging activity [49].

Conversely, the antioxidant capacity determined by ORAC assay (Table 5) showed that the *E. denticulatum* (112,762  $\pm$  8,575  $\mu\text{mol TE}/100\text{ g}$ ) extract had the highest antioxidant ability against the peroxy radicals with significant differences between species, while *S. polycystum* (42,060  $\pm$  5,153  $\mu\text{mol TE}/100\text{ g}$ ) had the lowest, which explains the various other mechanisms of antioxidant. ORAC assays directly measure the scavenging capacity against the peroxy radical, which is the exiting and emission of 2, 2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH). The ORAC values of *E. denticulatum* in the present study were comparable to those reported in the literature, including those of a 70% acetone extract of *Ascophyllum nodosum* (141,700  $\mu\text{mol TE}/100\text{ g}$  extract) and *Laminaria hyperborea* (97,500  $\mu\text{mol TE}/100\text{ g}$  extract) [50].

## 4. Conclusions

The HPTLC spectrum displayed a distinct carotenoid pattern for each class of seaweed, which reflects the potential for different effects on health. By using the LC-MS/MS technique, the molecular masses of carotenoids can be determined, and their structure can be explored based on the fragmentation pattern from collision-induced dissociation. Twelve types of carotenoids were detected in the seaweed ethanolic extracts based on the calculated mass ( $m/z$ ). The seaweeds also showed potential antioxidant properties and a peroxy radical scavenging effect, which is at least partly contributed by the carotenoid presence in the extracts. Previous research suggested several potential health benefits of the selected seaweeds, and the carotenoids may play a pivotal role in the health-promoting activities. In general, these seaweeds appear to be a

promising, sustainable ingredient in future functional food product development.

## Declarations

### Author contribution statement

Vimala Balasubramaniam, Lee June Chelyn: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vimala Subramaniam: Performed the experiments.

Mohd Fairulnizal Mohd Noh, Iain Brownlee, Amin Ismail: Contributed reagents, materials, analysis tools or data.

### Funding statement

This work was supported by Ministry of Health Malaysia (NMRR-12-500-12439; 12-011).

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e04654>.

## Acknowledgements

The authors thank the Director General of Health Malaysia and the Director of Institute for Medical Research (IMR), Malaysia, for giving the permission to publish this article. We also thank the staff of the Nutrition Unit, Institute for Medical Research for their continuous support. Special thanks to Ang Jo-Lyn, Azerul and Rathi for their invaluable assistance and advice for this project. We sincerely thank the Department of Fisheries Sabah, Malaysia for assisting us during the collection of seaweed samples.

## References

- [1] N. Rhein-Knudsen, M. Ale, A. Meyer, Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies, *Mar. Drugs* 13 (6) (2015) 3340–3359.
- [2] Y.X. Chin, Y. Mi, W.X. Cao, P.E. Lim, C.H. Xue, Q.J. Tang, A pilot study on anti-obesity mechanisms of *Kappaphycus alvarezii*: the role of native K-carrageenan and the leftover sans-carrageenan fraction, *Nutrients* 11 (2019) 1133.
- [3] E.M. Brown, P.J. Allsopp, P.J. Magee, C.I. Gill, S. Nitecki, C.R. Strain, et al., Seaweed and human health, *Nutr. Rev.* 72 (3) (2014) 205–216.
- [4] V. Balasubramaniam, J.C. Lee, M.F.M. Noh, S. Ahmad, I.A. Brownlee, A. Ismail, Alpha-amylase, antioxidant, and anti-inflammatory activities of *Euचेuma denticulatum* (N.L. Burman) F.S. Collins and Hervey, *J. Appl. Phycol.* 28 (3) (2016) 1965–1974.
- [5] V. Balasubramaniam, S. Mustar, N. Mustafa Khalid, A. Abd Rashed, M.F. Mohd Noh, M.D. Wilcox, et al., Inhibitory activities of three Malaysian edible seaweeds on lipase and  $\alpha$ -amylase, *J. Appl. Phycol.* 25 (5) (2013) 1405–1412.
- [6] K.K.A. Sanjeeva, W.W. Lee, Y.J. Jeon, Nutrients and bioactive potentials of edible green and red seaweed in Korea, *Fish Aquat Sci* 21 (1) (2018) 1–11.
- [7] Y. Sharifuddin, Y.X. Chin, P.E. Lim, S.M. Phang, Potential bioactive compounds from seaweed for diabetes management, *Mar. Drugs* 13 (8) (2015) 5447–5491.
- [8] R. Pangestuti, S.K. Kim, Biological activities and health benefit effects of natural pigments derived from marine algae, *J. Funct. Foods* 3 (4) (2011) 255–266.
- [9] H. Maeda, T. Tsukui, T. Sashima, M. Hosokawa, K. Miyashita, Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient, *Asia Pac. J. Clin. Nutr.* 17 (SUPPL. 1) (2008) 196–199.
- [10] A. Gomez-Zavaglia, M.A. Prieto Lage, C. Jimenez-Lopez, J.C. Mejuto, J. Simal-Gandara, The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value, *Antioxidants* 8 (9) (2019) 406.
- [11] J. Kubanek, P.R. Jensen, P.A. Keifer, M.C. Sullards, D.O. Collins, W. Fenical, Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi, *Proc. Natl. Acad. Sci. U. S. A.* 100 (12) (2003) 6916–6921.

- [12] D.B. De Alencar, S.R. Da Silva, K.M.S. Pires-Cavalcante, R.L. De Lima, F.N. Pereira, M.B. De Sousa, et al., Antioxidant potential and cytotoxic activity of two red seaweed species, *Amansia multifida* and *Meristiella echinocarpa*, from the coast of northeastern Brazil, *An. Acad. Bras. Cienc.* 86 (1) (2014) 251–263.
- [13] B.W.S. Souza, M.a. Cerqueira, J.T. Martins, M. a C. Quintas, A.C.S. Ferreira, J.a. Teixeira, et al., Antioxidant potential of two red seaweeds from the Brazilian coasts, *J. Agric. Food Chem.* 59 (10) (2011) 5589–5594.
- [14] J. Peng, J.P. Yuan, C.F. Wu, Wang JH. Fucoxanthin, A marine carotenoid present in Brown seaweeds and diatoms: metabolism and bioactivities relevant to human health, *Mar. Drugs* 9 (10) (2011) 1806–1828.
- [15] K. Mikami, M. Hosokawa, Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in Brown seaweeds, *Int. J. Mol. Sci.* 14 (7) (2013) 13763–13781.
- [16] K. Miyashita, S. Nishikawa, F. Beppu, T. Tsukui, M. Abe, M. Hosokawa, The allenic carotenoid fucoxanthin, A novel marine nutraceutical from Brown seaweeds, *J. Sci. Food Agric.* 91 (7) (2011) 1166–1174.
- [17] C.W. Huck, M. Popp, H. Scherz, G.K. Bonn, Development and evaluation of A new method for the determination of the carotenoid content in selected vegetables by hplc and HPLC-MS-MS, *J. Chromatogr. Sci.* 38 (10) (2000) 441–449.
- [18] S. Bijtbeier, E. D'Hondt, B. Noten, N. Hermans, S. Apers, S. Voorspoels, Ultra high performance liquid chromatography versus high performance liquid chromatography: stationary phase selectivity for generic carotenoid screening, *J. Chromatogr. A* 1332 (2014) 46–56.
- [19] R.M. Pop, Y. Weesepeol, C. Socaciu, A. Pinte, J.P. Vincken, H. Gruppen, Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties, *Food Chem.* 147 (2014) 1–9.
- [20] B. Sivathanu, S. Palaniswamy, Purification and characterization of carotenoids from green algae *Chlorococcum humicola* by HPLC-NMR and LC-MS-APCI, *Biomed Prev Nutr* 2 (4) (2012) 276–282.
- [21] Y. Weesepeol, H. Gruppen, W. de Bruijn, J.-P. Vincken, Analysis of palmitoyl apo-astaxanthinols, apo-astaxanthinones, and their epoxides by UHPLC-PDA-ESI-MS, *J. Agric. Food Chem.* 62 (42) (2014) 10254–10263.
- [22] F. Ahmad, M.R. Sulaiman, W. Saimon, C.F. Yee, P. Matanjun, Proximate compositions and total phenolic contents of selected edible seaweed from semporna, Sabah, Malaysia, *Borneo Sci* 31 (2012) 74–83.
- [23] S. Mohd Rosni, A. Faisal, A. Azwan, F.Y. Chye, P. Matanjun, Crude proteins, total soluble proteins, total phenolic contents and SDS-PAGE profile of fifteen varieties of seaweed from semporna, Sabah, Malaysia, *Int Food Res J* 22 (4) (2015) 1483–1493.
- [24] S.M. Rivera, R. Canela-Garayoa, Analytical tools for the analysis of carotenoids in diverse materials, *J. Chromatogr. A* 1224 (2012) 1–10.
- [25] M.S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature* 181 (1958) 1199–1200.
- [26] D. Huang, B. Ou, M. Hampsch-Woodill, J.A. Flanagan, R.L. Prior, High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format, *J. Agric. Food Chem.* 50 (16) (2002) 4437–4444.
- [27] L. Giri, H.C. Andola, V. Kant Purohit, M.S.M. Rawat, R.S. Rawal, I.D. Bhatt, Chromatographic and spectral fingerprinting standardization of traditional medicines: an overview as modern tools, *Res. J. Phytochem.* 4 (4) (2010) 234–241.
- [28] J. Marquardt, D. Hanelt, Other marine red algae from polar and temperate habitats, *Eur. J. Phycol.* 39 (3) (2004) 285–292.
- [29] N. Schubert, E. García-Mendoza, I. Pacheco-Ruiz, Carotenoid composition of marine red algae, *J. Phycol.* 42 (2006) 1208–1216.
- [30] M. Andersson, H. Schubert, M. Pedersen, P. Snoeijs, Different patterns of carotenoid composition and photosynthesis acclimation in two tropical red algae, *Mar Biol* 149 (3) (2006) 653–665.
- [31] M. Kakinuma, N. Shibahara, H. Ikeda, M. Maegawa, H. Amano, Thermal stress responses of a sterile mutant of *Ulva pertusa* (chlorophyta), *Fish Sci* 67 (2) (2001) 287–294.
- [32] T.W. Goodwin (Ed.), *The Biochemistry of Carotenoids*. Plants, Chapman and Hall, New York, 1980.
- [33] M.M. Hegazi, A. Pérez-Ruzafa, L. Almela, M.E. Candela, Separation and identification of chlorophylls and carotenoids from *Caulerpa prolifera*, *Jania rubens* and *Padina pavonica* by reversed-phase high-performance liquid chromatography, *J. Chromatogr. A* 829 (1–2) (1998) 153–159.
- [34] G. Stoev, Y. Xuan, M. Peycheva, M. Scigelova, Quantitative assessment of the contribution of high resolution mass spectrometric analysis to the reliability of compound confirmation, *Talanta* 98 (2012) 19–27.
- [35] S.M. Lehner, N.K.N. Neumann, M. Sulyok, M. Lemmens, R. Krska, R. Schuhmacher, Evaluation of LC-high-resolution FT-orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food, *Food Addit. Contam.* 28 (10) (2011) 1457–1468.
- [36] Z. Hao, B. Parker, M. Knapp, L. Yu, Simultaneous quantification of alpha-tocopherol and four major carotenoids in botanical materials by normal phase liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry, *J. Chromatogr. A* 1094 (1–2) (2005) 83–90.
- [37] M.H. Norziah, C.Y. Ching, Nutritional composition of edible seaweed *Gracilaria changgi*, *Food Chem.* 68 (1) (2000) 69–76.
- [38] M. Fayaz, K.K. Namitha, K.N.C. Murthy, M.M. Swamy, R. Sarada, S. Khanam, et al., Chemical composition, iron bioavailability, and antioxidant activity of *Kappaphycus alvarezii* (doty), *J. Agric. Food Chem.* 53 (3) (2005) 792–797.
- [39] M. Abidov, Z. Ramazanov, R. Seifulla, S. Grachev, The effects of xanthigen<sup>tm</sup> in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat, *Diabetes Obes. Metabol.* 12 (1) (2010) 72–81.
- [40] M. Motshakeri, M. Ebrahimi, Y.M. Goh, P. Matanjun, S. Mohamed, *Sargassum polycystum* reduces hyperglycaemia, dyslipidaemia and oxidative stress via increasing insulin sensitivity in A rat model of type 2 diabetes, *J. Sci. Food Agric.* 93 (7) (2013) 1772–1778.
- [41] T. Bjørnland, Chlorophyll and carotenoids of five isolates of the red alga *Antithamion plumula*, *Biochem. Systemat. Ecol.* 11 (2) (1983) 73–76.
- [42] N.-E. Rmiki, C. Brunet, J. Cabioch, Y. Lemoine, Xanthophyll-cycle and photosynthetic adaptation to environment in macro- and microalgae, in: S.C. Lindstrom, D.J. Chapman (Eds.), *Fifteenth International Seaweed Symposium: Proceedings of the Fifteenth International Seaweed Symposium Held in Valdivia, Chile, in January 1995*, Springer Netherlands, Dordrecht, 1996, pp. 407–413.
- [43] E. Jin, J.E.W. Polle, H.K. Lee, S.M. Hyun, M. Chang, Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application, *J. Microbiol. Biotechnol.* 13 (2) (2003) 165–174.
- [44] A.J. Young, D. Phillip, J. Savill, Carotenoids in higher plant photosynthesis. *Handbook of Photosynthesis*, Marcel Dekker Inc., New York, 1997, pp. 575–596.
- [45] S. Takaichi, Distributions, biosyntheses and functions of carotenoids in algae, *Agro Food Ind. Hi-Tech* 24 (1) (2013) 55–58.
- [46] S.M. Rivera, F. Vilaro, C. Zhu, C. Bai, G. Farre, P. Christou, C.-G. Ramon, Fast quantitative method for the analysis of carotenoids in transgenic maize, *J. Agric. Food Chem.* 61 (22) (2013) 5279–5285.
- [47] T. Wang, R. Jónsdóttir, G. Ólafsdóttir, Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds, *Food Chem.* 116 (1) (2009) 240–248.
- [48] A. Jiménez-Escrig, I. Jiménez-Jiménez, R. Pulido, F. Saura-Calixto, Antioxidant activity of fresh and processed edible seaweeds, *J. Sci. Food Agric.* 81 (5) (2001) 530–534.
- [49] X. Yan, Y. Chuda, M. Suzuki, T. Nagata, Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed, *Biosci Biotech Biochem* 63 (1999) 605–607.
- [50] Z. Wei, W. Wang, J. Chen, D. Yang, R. Yan, Q. Cai, A prospective, randomized, controlled study of omega-3 fish oil fat emulsion-based parenteral nutrition for patients following surgical resection of gastric tumors, *Nutr. J.* 13 (1) (2014) 1–6.