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Biochemical composition of some Egyptian seaweeds with potent nutritive and antioxidant properties

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Abstract

The present study investigated the biochemical composition of three seaweeds; *Ulva fasciata* (Chlorophyta), *Sargassum linifolium* (Phaeophyta) and *Corallina officinalis* (Rhodophyta). Total chlorophyll content was maximum in *U. fasciata* (34.06mg/g dry wt.) while carotenoid content was the highest in *C. officinalis* (3.8 mg/g dry wt.). The uppermost level of carbohydrates was (27.98% of dry wt.) in *C. officinalis* and proteins were maximum (14.89%) in *S. linifolium*. Aspartic, glutamic, alanine, leucine and proline were common amino acids in the three tested species. The polyunsaturated ω6 and ω3 essential fatty acids were recorded in *S. linifolium* (3.28%) and in *U. fasciata* (3.18%). The results showed that *U. fasciata* contained the highest amounts of lipids (2.96%), phenols (11.95mgGA/g dry wt.), flavonoid (7.04 mgCA/g dry wt.) and ascorbic acid (4.11mg/100g), respectively. β-Carotene was maximum (3940.12 IU/100 g) in *C. officinalis*. DPPH antioxidant activities were the highest in *U. fasciata* (81.3%) followed by *S. linifolium* (79.8%) then *C. officinalis* (72.6%). Among the 12 analyzed minerals, most of them were high in *S. linifolium* in which ion quotient ratio was the smallest (0.343). Since these algal species are common in the Egyptian coastal waters, their biochemical composition and antioxidant activities made them promising candidates for nutritional, pharmaceutical and medicinal applications.

Keywords: seaweeds; biochemical composition; elemental analysis; antioxidant activity.

Practical Application: Seeking for new supplies for food, feed, drugs and treatments Macroalgae (seaweeds) proved to be safe, economic and eco-friendly natural sources for multi-purpose applications.

1 Introduction

Seaweeds are macroalgae which represented a virtual component of the marine ecosystems. According to their nutrient value and chemical composition, they are classified as red (Rhodophyta), brown (Phaeophyta), and green seaweeds (Chlorophyta) (Dawczynski et al., 2007). In orient countries, mainly Japan, China and Korea about (5%) of green algae, (66.5%) of brown algae and (33%) of red algae were nutritionally consumed in daily diets (Gade et al., 2013; Valentina et al., 2015). Most studies were carried out on naturally collected seaweeds from many parts of the world; their nutritious capacity were varied depending on species, habitats, environmental conditions and maturity of the seaweeds (Ganesan & Kannan, 1994; Fleurence, 1999; Cardozo et al., 2007; Saroja, 2016). Nowadays, seaweeds consumption is increasing due to their natural composition. They were recorded to have many beneficial nutritive bioactive compounds such as vitamins (ascorbic and β carotene), polyphenols, pigments, minerals, fibers and polysaccharides (Lahaye, 1991). They were also low in fat and in calorific value with high levels of essential fatty acids and essential amino acids in addition to about 80-90% water. In many studies, these bioactive compounds confirmed antioxidant, antimicrobial, antitumor and antiviral activities (Mabeau & Fleurence, 1993; Ortiz et al., 2006; Seenivasan et al., 2012).

Antioxidant compounds act as free radical scavengers to protect living organisms from the systemic production of reactive oxygen species (ROS), lipid peroxidation, protein damage and DNA breaking (Kokilam & Vasuki, 2014). Many seaweed species verified natural antioxidant capacity that can protect the human body from free radicals and retard the progress of many chronic diseases such as hypertension, heart diseases, diabetes and cancer (Ruberto et al., 2001; Shanab, 2007; Kokabi et al., 2013; Kolanjinathan et al., 2014; Collins et al., 2016).

Phenolic and flavonoid compounds were broadly recognized in seaweeds confirming their potent role in chelating metal ions, preventing radical formation and improving the internal antioxidant system under stress environmental conditions. These activities protect the body from progressive diseases caused by the adverse effects of reactive oxygen species (ROS) (Chakraborty et al., 2013). Similar attitude was reported for carotenoid pigments of seaweeds, especially β carotene, for which activity against cancer diseases was postulated (Plaza et al., 2010). In addition to their valuable contents of carbohydrates (about 50%), proteins (10:47%) and lipids (1:3%), seaweeds were considered a good source for minerals and trace elements (8-40%) which are necessary for the metabolic reactions of the human health. (Nelson et al., 2002; Shanmugam & Palpandi, 2008; El-Said &

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El-Sikaily, 2013; Valentina et al., 2015). For this, investigations on seaweeds as a new natural food source in animal and human diets are current need of research. The aim of this study is to explore the biochemical and nutrient content of three different seaweeds *Ulva fasciata, Sargassum linifolium* and *Corallina officinalis* which are commonly found in the coastal waters of Egypt. The antioxidant activity of these seaweed extracts were also studied to confirm their nutritional, pharmaceutical and medicinal possible applications.

2 Materials and methods

2.1 Samples collection

Three species of seaweeds: green *Ulva fasciata;* brown *Sargassum linifolium* and red *Corallina officinalis* were collected during autumn and winter seasons 2015. The seaweeds were collected from Abu Qir Bay submerged rocks (30°05'-30°22'E and 31°16'-31°21'N) located about 36 km east of Alexandria Mediterranean coast, Egypt. In laboratory, the samples were washed with tap water to remove epiphytes, sand and potential contaminants; then were shade-air dried until constant weight. The samples were powdered and stored in plastic bags in the refrigerator for further analysis. The seaweed samples were identified following (Aleem, 1993) and (Guiry & Guiry, 2011). They belonged to three divisions: *U. fasciata* (Delile) of Chlorophyta, *S. linifolium* (C. Agardh) of Phaeophyta and *C. officinalis* (Linnaeus) of Rhodophyta.

2.2 Extraction and estimation of chlorophyll

Five hundred mg seaweed fresh material was extracted twice with 10 ml of 80% acetone (Arnon, 1949). The samples were centrifuged at 3000 rpm for 15 minutes. Absorbance of the extract was read at 663 and 645nm for Chlorophyll a and Chlorophyll b, respectively using UV-spectrophotometer. The results were finally expressed as mg/g dry weight of seaweeds.

$$Chlorophyll\ 'a' = \frac{\left[12.7\left(A_{663}\right) - 2.69\left(A_{645}\right)\right] * vol.of\ extraction}{Weight\ of\ the\ sample}$$

$$Chlorophyll\ 'b' = \frac{\left[22.9\left(A_{645}\right) - 4.68\left(A_{663}\right)\right] * vol.\ of\ extraction}{Weight\ of\ the\ sample}$$

$$Total\ Chlorophyll = \frac{\left[20.2\left(A_{645}\right) - 8.02\left(A_{663}\right)\right] * vol.\ of\ extraction}{Weight\ of\ the\ sample}$$

Where (A_{663}) is the absorbance at 663nm and (A_{645}) is the absorbance at 645nm.

2.3 Extraction and estimation of carotenoids

Carotenoids content of seaweeds was determined spectrophotometrically at 480nm according to (Kirk & Allen, 1965) using the same extract used for chlorophyll estimation. The content was expressed as mg/g dry weight.

$$Carotenoids = \frac{4 * \left(A_{480}\right) * vol.of\ extraction}{Weight\ of\ the\ sample}$$

Where (A_{480}) is the optical density at 480nm and 4 is the correction factor.

2.4 Extraction and estimation of protein

The dry seaweeds material was extracted into Tris HCL buffer (0.1 M pH7.5) overnight at 4 °C with stirring. After centrifugation, the total protein in the supernatant was estimated spectrophotometrically at 750 nm (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard. Proteins were expressed as percentage of algal dry weight. The Amino acids profile of the extract was determined using SYKAM AAA amino acids analyzer.

2.5 Extraction and estimation of carbohydrate

The total carbohydrates were assayed by the phenol-sulphuric acid method (Dubois et al., 1956) after extraction with 2.5N HCL for 3 h at 100 °C. The content was calculated in percentage by referring to glucose standard curve.

2.6 Extraction and estimation of lipids

Total lipids of seaweeds were extracted by the chloroform-methanol method (Bligh & Dyer, 1959) and expressed in percentage. After methylation according to (Francavilla et al., 2013); the fatty acids content of lipids was analyzed by GC-MS spectrophotometry (Claurs 580, 560S Perkin Elemer) under oven initial temperature 140 °C for 2 min, ramp10 °C/min to 200 °C, hold 2 min, ramp 5 °C/min to 250 °C, hold 9 min, Injection = 250 °C, Volume = 1 µl, Split = 20:1, Carrier gas = He, Solvent delay = 3 min, Transfer temp = 250 °C, Source temp = 200 °C, Scan = 50 to 500Da and Column = 30m × 250 µm.

2.7 Total phenolic content

The amount of total phenols in seaweeds extracts was determined with Folin– Ciocalteu reagent according to (Singleton & Rossi, 1965) method. The developed color was read at 750 nm with Gallic acid stock solution (10 mg/10 ml) as standard. The results were expressed as milligram Gallic acid equivalent (mg GA/g dry weight of seaweed).

2.8 Total flavonoids content

Flavonoids were assayed using aluminum chloride colorimetric technique (Zhishen et al., 1999). The absorbance of the reaction mixture was measured at 415 nm. A calibration curve using (+) Catechin solution (20-100 µg) in methanol was prepared. The results were expressed as milligram (+) Catechin equivalents (mg CE /g dry weight of seaweed).

2.9 Beta carotene (Vitamin A) content

The β-carotene in the sample was extracted according to (Tee et al., 1996) method with slight modifications adopted by (Ismail & Fun, 2003). Briefly, the seaweed sample (10 g) was added to 40 ml of absolute ethanol and 10ml of 100% (w/v) potassium hydroxide then homogenized for 3min using a blender. The mixture was saponified using a refluxing apparatus for 30 min then cooled to room temperature. Further extraction steps were made using *n*-hexane solution. β carotene was determined by a reverse-phase HPLC technique (Hewlett Packard HPLC Series

1100, USA). The resulted peak of β-carotene was identified based on their retention time and spiking test with that of standard trans-β-carotene.

2.10 Ascorbic acid (Vitamin C) content

Vitamin C was extracted and estimated according to the modified method of (Abushita et al., 1997). The seaweed sample (10g) was homogenized with an extracting solution containing meta-phosphoric acid (0.3M) and acetic acid (1.4M). The ratio of the sample to extraction solution was 1:1. The mixture was agitated at 100 rpm for 15min under dark conditions then filtered. Like previously mentioned, vitamin C content was determined in the extract by reverse-phase HPLC technique and identified by comparing the retention time with that of L-ascorbic acid standard.

2.11 Antioxidant assays

DPPH radical scavenging capacity

The scavenging capacity of seaweeds extracts for DPPH (1,1-diphenyl-2-picrylhydrazyl) radical were examined according to (Yen & Chen, 1995). Briefly, 2ml of test sample was added to 2 ml of 0.16 mM DPPH methanol solution. The mixture was vortexed then left for 30min in the dark. The absorbance was read at 517nm and percentage of DPPH radical scavenging activity was calculated using the following equation: DPPH scavenging activity (%) = [(Ac-As) /Ac] ×100. Where, Ac is the absorbance of the control (100µL of methanol solvent with 100 µL of the DPPH solution), and As is the absorbance of the sample.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity of seaweed extracts was determined according to (Prieto et al., 1999). Sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) were mixed together in 250 ml with distilled water and known as (TAC) reagent. 3 ml of TAC reagent was added to 0.3 ml of seaweed methanolic extract and incubated at 95 °C for 90min in caped tubes. Absorbance was read at 695nm against blank. Total antioxidant capacity was determined as a percentage with ascorbic acid as a standard. Total Antioxidant capacity (TAC) % = [(Ac-As) /Ac] ×100. Where, Ac is the absorbance of the control and As is the absorbance of the sample or standard.

Estimation of Total Reducing Capacity (TRC)

The reducing power of the extracts were determined by (Oyaizu, 1986) method. Extract sample (1ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide was added. The mixture was incubated at 50 °C for 20 min. Trichloroacetic (2.5 ml, 10%) acid was added and centrifuged at 3000 rpm for 10min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was read at 700 nm after 10min of incubation. Increased absorbance of the reaction mixture indicated increased reducing power.

2.12 Estimation of minerals content

Dry seaweed (0.5 g) was digested in 6ml of HNO_3 (65% v/v) by using advanced microwave digestion system (ETHOS 1).Total contents of different elements were determined in the digested solution by using an Inductively Coupled Plasma Spectrometer (Perkin Elemer Emission Specrrophotometer- 6000 Series, Thermo Scientific) following (Allen et al., 1997).

2.13 Statistical analysis

All the experiments were run in triplicates and the results were expressed as means ± standard deviation. Analysis of variance (one-way ANOVA) was used to identify the statistically significant difference between the studied seaweeds parameters. Relationships between the different bioactive compounds and the antioxidant activities were tested using simple linear correlation coefficient (r). All statistical analyses were performed using SPSS statistics software.

3 Results and discussion

In the present study, pigments composition was considerably different between the tested species (Figure 1). Chlorophyll a recorded (23.82 ± 0.14), (11.93 ± 0.8) and (20.29 ± 0.16) mg/g dry wt.; chlorophyll b was (10.24 ± 0.87), (6.06 ± 0.11) and (0.69 ± 0.89) mg/g dry wt. for *U. fasciata, S. linifolium* and *C. officinalis*, respectively. Total chlorophyll was thus maximum in *U. fasciata* followed by *C. officinalis* then *S. linifolium.* Meanwhile, carotenoids presented (1.97 ± 0.08), (2.70 ± 0.03) and (3.84 ± 0.13) mg/g dry wt. for the three species in the same order. Like green plants, many studies reported that green algae contained higher concentrations of chlorophyll a than the red and the brown algae. Alternatively, carotenoids content was fluctuated among algal groups with the highest in the red *Corallin*a and the lowest in the green *Ulva* species. These findings were in accordance with (Chandraprabha et al., 2012; Valentina et al., 2015; Ismail et al., 2016). According to (Plaza et al., 2010) pigments help in cell communications and human health maintenance, have probable antimicrobial activities and promising applications in food and pharmaceutical fields. Also, algal carotenoids have an antioxidant activity against human diseases related to oxidative stress and cancer cells proliferation (Astorg, 1997; Collins et al., 2016).

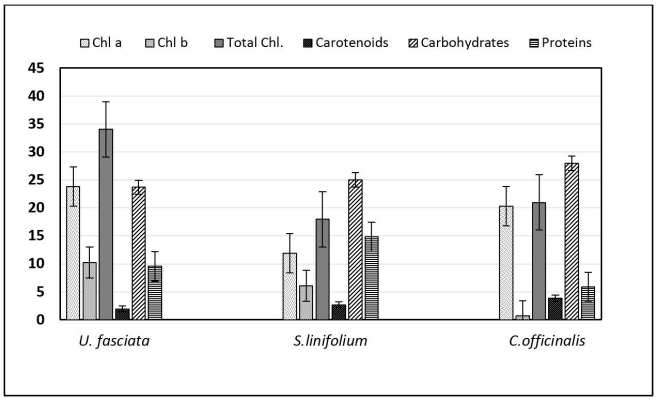

Figure 1. Biochemical composition of the studied seaweeds.

Protein, carbohydrate and lipid are the most vital biochemical constituents of algae. In the present study, protein content of *S. linifolium* was higher (14.89%) than that of *U. fasciata* (9.56%) and *C. officinalis* (5.91%) of algal dry wt. (Figure 1). Similar results were observed by (Ganesan & Kannan, 1994; Anitha et al., 2008) who reported that Phaeophycean species generally contained significant protein content. However, variations in protein content may be proportional to the temperature values, seasonal period or to its consumption by the seaweeds in growth and reproduction. Also might be related to the differences between species, the geographical locations and the surrounding environmental conditions of seaweeds (Ganesan & Kannan, 1994; Fleurence, 1999). The protein amino acids profile was shown in (Table 1). The amount of each amino acid varied in the different studied species. *S. linifolium* contained the highest amount of total amino acids. However, the total percentage of essential amino acids recorded (40.79%) for *U. fasciata* followed by *C. officinalis* (37.92%) and *S. linifolium* (37.20%) of the total amino acids content. These results were in conformity with (Dawczynski et al., 2007) who reported that essential amino acids in seaweeds comprised over 30% of the total amino acids content. Proline recorded the highest values of all amino acids in *S. linifolium* (16.6%) and *C. officinalis* (13.81%). In higher plants, proline was stored as a nontoxic defensive osmolyte under saline conditions and in response to environmental stresses (Khatkar & Kuhad, 2000). Aspartic acid, glutamic acid, alanine and leucine were common and ranged (12-13%), (10-14%), (7-11%) and (6-8%) of total amino acids percentage in the three tested species, respectively. The ratio values of essential to nonessential amino acids were 0.69, 0.59 and 0.61 implying good proteins quality (Table 1). In most seaweeds aspartic and glutamic acids constitute together a large part of the amino acids fraction and were responsible in addition to alanine and glycine for their special flavor and taste (Peng et al., 2013). These findings were in accordance with (Fleurence, 1999) who concluded that seaweeds, especially Chlorophyceae & Rhodophyceae, could be a complementary source of food proteins for human and animal nutrition and their amino acids content was of nutritional interest.

Carbohydrates are considered the most important biochemical constituent in algae since they represent the main energy source for the metabolic routes. Generally, carbohydrates were more abundant than proteins in all tested seaweeds (Figure 1). *C. officinalis* recorded the highest carbohydrates value (27.98%) followed by *S. linifolium* (25.03%) and finally *U. fasciata* (23.7%) of algal dry weight. These findings were in conformity with those of (Chandraprabha et al., 2012; Kokilam & Vasuki, 2014). The high content of carbohydrate in red algae might be due to higher phycocolloid content in their cell walls. Seaweeds contain large amounts of polysaccharides as cell wall structural components that were already captured by the hydrocolloid industry. Many algal soluble polysaccharides were related with hypocholesterolemia and hypoglycemic activities, whereas water insoluble polysaccharides were related with digestive tract transit time reduction (Lahaye, 1991; Kolanjinathan et al., 2014).

Total lipids were found to be generally low. *U. fasciata* was the maximum (2.96%) and *C. officinalis* was the minimum (1.37%) while *S. linifolium* presented (2.16%) of algal dry weight (Figure 1). Seaweeds were not normally a worthy source of lipids since, in many studies, the total lipids content was recorded to be less than 4%. However, their polyunsaturated fatty acids content can be adequately compared to that of higher plants. The present results were supported by the findings of (Ortiz et al., 2006; Shanmugam & Palpandi, 2008; Francavilla et al., 2013; Saroja,

Table 1. Amino acids composition of the three studied seaweeds.

Amino acids (% dry wt) / Nonessential amino acids	*U. Fasciata*	*S. linifolium*	*C. officinalis*
Aspartic	0.0282 ± 0.02	0.0662 ± 0.03	0.0243 ± 0.01
Serine	0.0136 ± 0.001	0.0287 ± 0.01	0.0122 ± 0.003
Glutamic	0.0278 ± 0.03	0.0736 ± 0.02	0.0195 ± 0.005
Proline	0.0111 ± 0.001	0.0889 ± 0.04	0.0259 ± 0.02
Glycine	0.0174 ± 0.002	0.0283 ± 0.03	0.0112 ± 0.003
Alanine	0.0247 ± 0.01	0.0379 ± 0.01	0.0153 ± 0.005
Tyrosine	0.0051 ± 0.003	0.0069 ± 0.002	0.0046 ± 0.004
Argnine	0.0039 ± 0.001	0.005 ± 0.002	0.0033 ± 0.003
Total nonessential amino acids	0.1318	0.3355	0.1163
Essential amino acids			
Threonine	0.0131 ± 0.005	0.0269 ± 0.01	0.0099 ± 0.002
Valine	0.0138 ± 0.003	0.0245 ± 0.03	0.0094 ± 0.004
Methionine	0.0016 ± 0.001	0.0054 ± 0.006	0.0011± 0.004
Isoleucine	0.0099 ± 0.006	0.0193 ± 0.003	0.0082 ± 0.003
Leucine	0.0177 ± 0.003	0.0352 ± 0.02	0.0139 ± 0.005
Phenylalanin	0.016 ±0.005	0.0255 ± 0.01	0.0105 ± 0.002
Histidine	0.0069 ± 0.001	0.0360 ± 0.01	0.0077 ± 0.003
Lysine	0.0118 ± 0.003	0.0259 ± 0.01	0.0105 ± 0.001
Total essential amino acids	0.0908	0.1987	0.0711
Total amino acids	0.2226	0.5342	0.1875

Values are means of three replicates ± standard deviations SD.

2016). Considering the fatty acids composition, 17 components were identified with a varied quantities among the studied seaweeds (Table 2). *U. fasciata* contained the major percentage of saturated fatty acids (SAFA) followed by *C. officinalis*. Palmitic acid (C16:0) prevailed constituting 31.01%, 22.89% and 15% of total fatty acids content in *U. fasciata*, *S. linifolium* and *C. officinalis*, respectively. Pentadecanoic acid (C15:0) recorded 13.24%, 22.44% and 27.63%; while Myristic acid (C14:0) was of 11.01, 11.90 and 17.37% for the same three species, respectively. Stearic acid (C18:0) was higher in *Ulva* and docosanic acid (C23:0) was higher in *Corallina* if compared to *Sargassum* (Table 2). Four monounsaturated fatty acids (MUFA) were recorded in the three tested species namely tetradecanoic acid (C14:1), 14, Pentadecenoic acid (C15:1), 9 Hexadecenoic acid (ω7) (C16:1) and Oleic acid (ω9) (C18:1). These acids constituted (25.66%) in *S. linifolium* followed by *C. officinallis* (18.37%) and then *U. fasciata* (14.72%) of the total fatty acids content. These results were in conformity with many other studies (Dawczynski et al., 2007; Shanmugam & Palpandi, 2008; Francavilla et al., 2013).

Polyunsaturated essential fatty acids (PUFA) were also detected in this study. Arachidonic acid ω6 (C20:4) was present in the three seaweeds; linoleic acid ω6 (C18:2) occurred in *U. fasciata* (1.72%) and *S. linifolium* (0.82%) while 11, 14 Eicosadienoic acid ω6 (C20:2) appeared only in *S. linifolium* (1.18%). The ω3 group of essential fatty acids was represented by α Linolenic acid (ω3) (C18:3) and Eicosapentaenoic acid (EPA) ω3 (C20:5) in all studied seaweeds (Table 2). Usually, arachidonic acid ω6, (EPA) acid ω3 and decosahexadecanoeic acid ω3 (DHA), which are the main dietary basis for fish, constituted the major sources of ω3 and ω6 long-chain PUFAs in seaweeds. PUFAs play important roles in algal physiology processes and thus may be very sensitive to environmental changes especially temperature, genetic differences between species, season of collection and drying conditions (Nelson et al., 2002). Additionally, the ratio of ω6/ω3 was 1.67, 2.56 and 4.42 for the green, brown and red algae, respectively. According to WHO this ratio should not be higher than 10 in diets (Sánchez-Machado et al., 2004) which endorse the studied seaweeds for nutritive purposes after further investigations.

The examined seaweeds displayed a considerable content of total phenols and flavonoids that were significantly different at $P \leq 0.05$ (Table 3). The maximum value was recorded for *U. fasciata* (11.95) followed by *S. linifolium* (10.35) and *C. officinalis* (4.89) mg GA/g dry wt. The same pattern was observed for flavonoid content with (7.04), (4.53), (3.48) mg CA/g dry wt. for the three seaweeds, respectively. According to (Chakraborty et al., 2013) seaweeds phenolic compounds can chelate metal ions and prevent radical formation therefore improving the antioxidant intrinsic coordination. In this way, phenols convey hydrogen atoms to peroxyl in the lipid peroxidation cycle forming the aryloxyls. Aryloxyls are unable to act as chain carriers for free radicals and thus delaying the peroxidation process. Depending on their molecular structure, flavonoids evidenced a broad spectrum of chemical and biological activities including antioxidants, inhibitors of lipids peroxidation and as therapeutic agents for many diseases (Sarojini et al., 2012). Flavonoid exhibited anti-inflammatory, antihepatotoxic and antiulcer effects besides protecting against cardiovascular mortality as well (Plaza et al., 2010; Kokilam & Vasuki, 2014). As shown in (Table 3), ascorbic acid (vitamin C) content showed notable significant value in

Table 2. Fatty acids composition of the three studied seaweeds (% of total of fatty acid).

Fatty acids	*U. fasciata*	*S. linifolium*	*C. officinalis*
Caprylic acid (C8:0)	0.204 ± 0.02	----------	----------
Lauric acid (C12:0)	2.652 ± 0.31	1.917 ± 0.38	4.076 ± 0.54
Tetradecanoic acid (C13:0)	6.079 ± 0.35	4.818 ± 0.05	8.504 ± 0.09
Tetradecanoic acid (C14:1)	1.436 ± 0.18	2.770 ± 0.28	3.945± 0.08
Myristic acid (C14:0)	11.013 ± 0.62	11.895 ± 0.4	17.374 ± 0.32
14,Pentadecenoic acid (C15:1)	7.686 ± 0.28	10.128 ± 0.04	12.862 ± 0.13
Pentadecanoic acid (C15:0)	13.235 ± 0.59	22.441± 0.37	27.631 ± 0.35
9 Hexadecenoic acid (ω7) (C16:1)	1.323 ± 0.08	3.266 ± 0.34	----------
Palmitic acid (C16:0)	31.013 ± 0.47	21.887 ± 0.50	15.007 ± 0.25
α Linolenic acid (ω3) (C18:3)	1.049 ± 0.09	0.630 ± 0.08	0.043 ± 0.01
Linoleic acid (ω6) (C18:2)	1.715 ± 0.12	0.823 ± 0.16	----------
Oleic acid (ω9) (C18:1)	4.270 ± 0.54	9.496 ± 0.47	1.562 ± 0.12
Stearic acid (C18:0)	17.034 ± 0.48	7.056 ± 0.12	4.843 ± 0.54
Eicosapentaenoic acid (EPA) ω3 (C20:5)	0.142 ± 0.02	0.291 ± 0.04	0.013 ± 0.01
Arachidonic acid (φ6) (C20:4)	0.272 ± 0.08	0.354 ± 0.03	0.221 ± 0.03
11,14 Eicosadienoic acid (ω6) (C20:2)	----------	1.184 ± 0.06	----------
Docosanic acid (C23:0)	0.879 ± 0.05	1.047 ± 0.12	3.924 ± 0.12
Saturated fatty acids (SAFA)	82.108	71.06	81.358
Monounsaturated fatty acids (MUFA)	14.72	25.66	18.37
Polyunsaturated fatty acids PUFA (ω6)	1.987	2.360	0.221
Polyunsaturated fatty acids PUFA (ω3)	1.19	0.92	0.05
Ratio ω6/ω3	1.67	2.56	4.42

Values are means of three replicates ± standard deviations SD.

U. fasciata (4.11 mg/g dry wt.) though markedly decreased in the other two algae. Oppositely, the highest content of β-carotene was detected in *C. officinalis* (3940 IU/100 g) followed by *S. linifolium* (2616.5 IU/100 g) and then *U. fasciata* (1912.9 IU/100 g). The content of β-carotene was also significantly different between species at $P \leq 0.05$. These findings were in agreement with (Zubia et al., 2007; Ismail et al., 2016). Natural antioxidants such as vitamins, polyphenols and β-carotene in higher plants were used to capture reactive oxygen species (ROS) which cause lipid peroxidation. Therefore, these compounds became used in food industry to protect the human body from free radicals and hinder many chronic diseases expansion. Many experimental studies strongly recommended natural sources of β-carotene to prevent the free radical-damage leading to aging progression and to inhibit cancer initiations (Astorg, 1997; Kolanjinathan et al., 2014; Collins et al., 2016).

A rapid, simple and inexpensive method to measure antioxidant capacity of algae as foods involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH was widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to quantify antioxidants in complex biological systems. The other two methods were used to asses and compare the antioxidant activity of different seaweed components as in case of total antioxidant capacity and reducing capacity (electron donors). Results in (Table 3) showed that DPPH antioxidant scavenging activity was significantly pronounced for all studied seaweeds. *U. fasciata* recorded (81%) followed by *S. linifolium* (79.8%) and *C. officinalis* (72.6%); these values were not only close to that recorded for ascorbic acid standard (75.5% ± 0.04) but even better in case of the green and brown algae. The other two assays, total antioxidant capacity and reducing capacity exhibited the same attitude with different scavenging percentages (Table 3) which was close to the estimated value of ascorbic acid standard (15.6% ± 0.03 and 74.7% ± 0.01) for the two assays, respectively. The phenolic and flavonoid contents in the present study were significantly correlated to the DPPH antioxidant activity at 0.01 level. Oppositely, β-carotene content was negatively correlated while ascorbic acid content was insignificantly correlated with the DPPH activity for all the tested seaweed extracts (Table 4). The same pattern of antioxidant mechanism was exhibited by the TAC assay although less dependent on the phenolic and flavonoid compounds at 0.05 level. While the TRC antioxidant activity was highly positively correlated with the flavonoid and ascorbic acid contents at 0.01 level. However, the results in (Table 4) declared positively significant correlations between the three antioxidant assays at 0.05 level. The same dependency of antioxidant activity was reported by (Chakraborty et al., 2013) with some edible brown, green and red seaweeds of rich phenolic content.

A synergistic effect between pigments, phenolic compounds and essential oils present in *Enteromorpha compressa* ethyl acetate extract was suggested by (Shanab et al., 2011); to which attributed the highest antioxidant activity by DPPH method. On the contrary, lower pigment contents, phenolic compounds and undetermined compounds in the crude extract caused reduced antioxidant activity due to antagonism effects. This supports the results of the present study and also recommended the tested seaweed species, especially *Ulva fasciata*, for food, pharmaceutical and agricultural applications.

Calcium (Ca), Sodium (Na), Potassium (K) and Magnesium (Mg) were among the nutritive minerals which are present in considerable amounts in marine algae. Seaweeds were famed as good sources of minerals and vitamins (Lahaye, 1991).

Table 3. Total phenol, flavonoid, β-carotene and ascorbic acid contents. The DPPH radical scavenging activity, Total antioxidant capacity (TAC) and Total reducing capacity (TRC) for the three studied seaweeds.

Biochemical content/ Antioxidant assay	*U. fasciata*	*S. linifolium*	*C. officinalis*	F value
Total phenol content (mg GE/g dwt.)	11.95 ± 0.784	10.35 ± 0.922	4.89 ± 0.98	65.296*
Flavonoid content (mg CE/g dwt.)	7.04 ± 0.635	4.53 ± 0.501	3.48 ± 0.822	23.70*
β-Carotene content (IU/100 g)	1912.93 ± 99.97	2616.47 ± 100.06	3940.12 ± 99.89	317.99*
Ascorbic acid content (mg/100g)	4.11 ± 0.96	0.25 ± 0.02	0.677 ± 0.08	43.42*
DPPH (%)	81.26 ± 0.845	79.78 ± 0.09	72.63 ± 1.17	92.22*
TAC (%)	13.70 ± 0.99	12.19 ± 0.95	11.37 ± 0.89	4.70*
TRC (%)	64.72 ± 0.98	53.11 ± 0.91	50.29 ± 1.07	195.75*

Values are means of three replicates ± standard deviations SD. F value * means that difference is significance at $p \leq 0.05$ level.

Table 4. Simple linear correlation coefficient (*r*) matrix between the potent antioxidant substances and different assays.

	Total phenol content	Total flavonoid content	β-carotene content	Ascorbic acid content	DPPH	TAC	TRC
Total phenol content	-----						
Total flavonoid content	0.822**	-----					
β-carotene content	–0.937**	–0.823**	-----				
Ascorbic acid content	0.555	0.899**	–0.655	-----			
DPPH	0.990**	0.807**	–0.949**	0.553	-----		
TAC	0.713*	0.795*	–0.715*	0.590	0.669*	-----	
TRC	0.771*	0.970**	–0.849**	0.941**	0.763*	0.786*	-----

*Correlation is significance at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

The results showed that all estimated elements were significantly different between species at $P \leq 0.05$ (Table 5). Mineral contents proved to be varied according to algal species, waving contact, seasonal fluctuation, environmental and physiological factors, routine of mineralization and type of processing (Mabeau & Fleurence, 1993). Na and K were higher in *S. linifolium* followed by *U. fasciata* while sharply decreased in *C. officinials*. The Na/K ratio in the studied algae recorded 0.8, 0.4 and 7.4 for *Ulva*, *Sargassum* and *Corallina* species, respectively, which was in the range of the former reports (Ruperez, 2002; El-Said & El-Sikaily, 2013). The preferential accumulation of potassium over sodium (except for *C. officinials*) could be due to the difference in the geochemical reactivity between the two similar elements and /or to the algal alginates and sulphate-containing polysaccharides with high negatively charged densities (Valentina et al., 2015). Consumption of foods with a high Na level may cause hypertension, therefore Na, K, and Cl are key factors for conservation of body fluid balance (Insel et al., 2007).

These conclusions advised the intake of seaweed to balance Na/K ratio of diets. Calcium is an important element for human health and known to be accumulated in seaweeds at much higher levels than in terrestrial foodstuffs. However, in this study Ca content was moderate being most abundant in the red alga *C. officinalis* (54.4 µg/g). Also, Mg is vital for chlorophyll structure and in other metabolic processes in algae. Mg was copious in *U. fasciata* (198.9 µg/g) then in *S. linifolium* (168.5 µg/g) and median in *C. officinalis* (77.5 µg/g) (Table 5). In seaweeds, Ca and Mg were the predominant elements for nutrients uptake binding sites and enhance accumulation of Si and Cl elements during stress conditions (Munda & Hudnik, 1991). Generally, mineral elements are important as constituents of bones, teeth, soft tissues, hemoglobin, muscle, blood, and nerve cells, and are vital for overall mental and physical wellbeing (Kuda & Ikemori, 2009). By decreasing sodium absorption and increasing potassium absorption in the gastrointestinal tract; potassium, calcium and magnesium are implicated in lowering blood pressure and lessening the risk of strokes (Vaskonen, 2003; Smith et al., 2010). The ion quotient ratio can be calculated for all living organisms. It affords better dietary and healthy characteristics than the simple Ca/Mg or Na/K ratios with concentrations given in moles (Kiss et al., 2004):

$$Ion\ quotient = \left[Ca^{+2}\right] + \left[Na^{+}\right] / \left[Mg^{+2}\right] + \left[K^{+}\right]$$

This molar ratio was calculated to be 0.414, 0.343 and 1.097 for *U. fasciata*, *S. linifolium* and *C. officinalis*, res≤ectively. The ion quotient generally vary between 2.5 and 4.0 in human body (El-Said & El-Sikaily, 2013). These results imply that using seaweed species in foods can decrease this range in human body and reduce related diseases such as hypertension, preeclampsia, and heart disease.

Iron was present in seaweeds at higher levels than in many familiar sources (e.g. meats and spinach) due to their metabolic capacity of directly absorbing elements from the seawater (Smith et al., 2010). Ferrous is a vital constituent in hemoglobin and interfere in many other metabolic processes of plants and animals. The results of this study showed that the tested species contain considerable amounts of Fe. The maximum amount (11.45 µg/g) was present in *S. linifolium* followed by the red and the green species (Table 5). These values were below the levels recorded by (Gebhart & Thomas, 2002) for the Dietary Recommended Intake (DRI) of iron; suggesting the possibility of using seaweeds as iron nutritive sources without risk of overdoses toxicity. Similar findings were recorded for copper with a highest content in *S. linifolium* (1.74 µg/g) and a lowest in *C. officinalis* (0.798 µg/g). These levels lied within the ranges in previous reports for the dietary intake of copper from seaweed sources (Mabeau & Fleurence, 1993; Ruperez, 2002).

Mn, Zn, Pb, Cd, Co and Cr were also estimated in this study recording trace fluctuating amounts among seaweeds (Table 5). Therefore would not contribute significantly to the

Table 5. Mineral composition of the three studied seaweeds.

Minerals (µg/g dry wt)	*U. Fasciata*	*S. linifolium*	*C. officinalis*	F value
Ca	6.698 ± 0.7	28.35 ± 0.535	54.38 ± 1.1	1683.03*
Na	146.4 ± 9.99	203.9 ± 1.005	35.96 ± 0.995	643.4*
K	170.6 ± 0.95	509.3 ± 0.798	4.82 ± 0.22	350067.06*
Mg	198.9 ± 2.11	168.5 ± 10.01	77.5 ± 0.99	343.39*
Fe	2.54 ± 0.999	11.45 ± 1.00	3.74 ± 0.999	70.27*
Mn	0.382 ± 0.04	0.561 ± 0.056	0.465 ± 0.045	9.44*
Zn	0.517 ± 0.072	0.606 ± 0.019	0.478 ± 0.035	5.65*
Cu	1.46 ± 0.092	1.74 ± 0.099	0.798 ± 0.002	113.23*
Pb	0.758 ± 0.099	0.653 ± 0.099	0.393 ± 0.098	10.81*
Cd	0.297 ± 0.008	0.319 ± 0.009	0.3 ± 0.007	6.57*
Co	ND	ND	ND	
Cr	ND	ND	ND	
Total content	528.552	925.379	178.834	
Na/K ratio	0.858	0.400	7.460581	
Ion quotient ratio (in moles)	0.414	0.343	1.097425	

Values are means of three replicates ± standard deviations SD. F value * means that difference is significance at $P \leq 0.05$ level. ND = not detected.

Dietary Recommended Intake (DRI) if seaweed were consumed in single gram quantities. However, zinc was implicated in enzyme function, protein stability and in the regulation of gene expression while manganese was involved in the formation of bone and the metabolism of lipids, amino acids and carbohydrates (Smith et al., 2010). Co and Cr were not detected in the tested seaweeds. Generally, the content of heavy metals in seaweeds reflects its concentration in the medium and the capacity of the alga to chelate them up.

4 Conclusion

Seaweeds *U. fasciata, S. linifolium* and *C. officinalis* collected from Alexandria beach, Egypt, were considered as low calories foods with high levels of carbohydrates, proteins, fatty acids, vitamins and minerals implying a promising role in food, feed and industrial applications. Due to their investigated content, these seaweeds offer new potential sources of bioactive compounds such as phenols, flavonoids and β carotene. These compounds exhibited a high antioxidant activities with immense pharmaceutical, biomedical and nutraceutical prospected applications. Intensive future studies should be performed to use and develop these naturally economical resources.

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