

ORIGINAL ARTICLE

## Evaluating the chemical composition and antioxidant activity of three Egyptian seaweeds: *Dictyota dichotoma*, *Turbinaria decurrens*, and *Laurencia obtusa*

Avaliação da composição química e atividade antioxidante em três algas marinhas do Egito: *Dictyota dichotoma*, *Turbinaria decurrens* e *Laurencia obtusa*

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### Abstract

Seaweeds have a growing number of successful applications in the food industry, medicine and in the cosmetic industry, which increases the importance of evaluating their chemical composition. In the present study, three common Egyptian seaweeds (*Dictyota dichotoma*, *Turbinaria decurrens* and *Laurencia obtusa*) were collected from the Red Sea coast, Suez, Egypt. The chemical profile of the three seaweeds was studied beside the antioxidant activity of their extracts. The results indicated that the amount of carbohydrate was greater than the protein and lipid contents in the three seaweeds, with a natural richness in minerals and antioxidants besides considerable amounts of monounsaturated and polyunsaturated fatty acids, including Omega-3 and Omega-6 fatty acids. All essential amino acids for human were found in the three seaweeds, with significant amounts of aspartic and glutamic acids. Furthermore, the results of the antioxidant activity assays were consistent with the antioxidant contents (phenols, flavonoids, alkaloids, vitamin C, carotenoids) of each seaweed. *D. dichotoma* was the most valuable seaweed of the three species studied, due to its relatively high protein content of  $7.28 \pm 0.25\%$ , moderate carbohydrate content of  $25.35 \pm 0.32\%$ , and highest pigment and antioxidant contents. In conclusion, these three seaweeds, especially *Dictyota dichotoma*, have an interesting chemical composition with a prospective nutritional and pharmaceutical value.

**Keywords:** Marine macroalgae; Chemical constituents; Amino acid profile; Fatty acid profile; Antioxidant activity.



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## Resumo

Algas marinhas têm um número crescente de aplicações bem-sucedidas nas indústrias de alimentos, de produtos farmacêuticos e de cosméticos, o que aumenta a importância de se avaliar sua composição química. No presente estudo, três algas marinhas comuns no Egito (*Dictyota dichotoma*, *Turbinaria decurrens* e *Laurencia obtusa*) foram coletadas do Mar Vermelho, Suez, Egito. O perfil químico de cada alga marinha foi estudado como também a atividade antioxidante dos seus extratos. Os resultados indicaram que as quantidades de carboidratos foram maiores que os conteúdos de proteína e lipídeo nas três algas marinhas, com uma riqueza natural em minerais e antioxidantes, além de quantias razoáveis de ácidos graxos monoinsaturados e poli-insaturados, incluindo os ácidos graxos Omega-3 e Omega-6. Todos os aminoácidos essenciais para humanos foram encontrados nas três algas marinhas, com quantias significantes dos ácidos aspártico e glutâmico. Ademais, os resultados obtidos nos ensaios de atividade antioxidante foram de acordo com os conteúdos antioxidantes (fenóis, flavonoides, alcaloides, vitamina C e carotenoides) de cada alga marinha. Das três espécies de algas marinhas estudadas, *D. dichotoma* apresentou o maior valor devido ao seu conteúdo relativamente alto de proteína, de  $7,28 \pm 0,25\%$ , ao conteúdo moderado de carboidrato, de  $25,35 \pm 0,32\%$ , e conteúdos mais altos de pigmentos e antioxidantes. Em conclusão, as três algas marinhas, e especialmente *Dictyota dichotoma*, têm composições químicas interessantes, cujos valores apresentam perspectivas nutricionais e farmacêuticas.

**Palavras-chave:** Macroalgas marinhas; Constituintes químicos; Perfil de aminoácidos; Perfil de ácidos graxos; Atividade antioxidante.

## 1 Introduction

Marine macroalgae, known as seaweeds, are aquatic organisms very similar to plants, but they live in coastal regions. This group includes at least 8000 species (Lüning et al., 1990), which are divided into three categories; green, brown and red. Their classification is based mainly on the colour of the thallus, pigment composition, ultrastructure and biochemical features (Dawczynski et al., 2007; Guiry, 2017). Several articles reported on the chemical composition of different seaweed species collected from different parts of the world, to evaluate their commercial and nutritional value (Renaud & Luong-Van, 2006; Banerjee et al., 2009; Gressler et al., 2011; Ahmad et al., 2012; Rohani-Ghadikolaei et al., 2011; Khairy & El-Shafay, 2013). The seaweeds investigated showed variable chemical compositions which depended on the species, collection season, geographic habitat and other physicochemical parameters (Marinho-Soriano et al., 2006; Marsham et al., 2007; Dawczynski et al., 2007). Seaweeds are being used in the food and cosmetic industries, in agriculture (Kelman et al., 2012; Guiry, 2017) and as a promising source for biofuel production (Gosch et al., 2012).

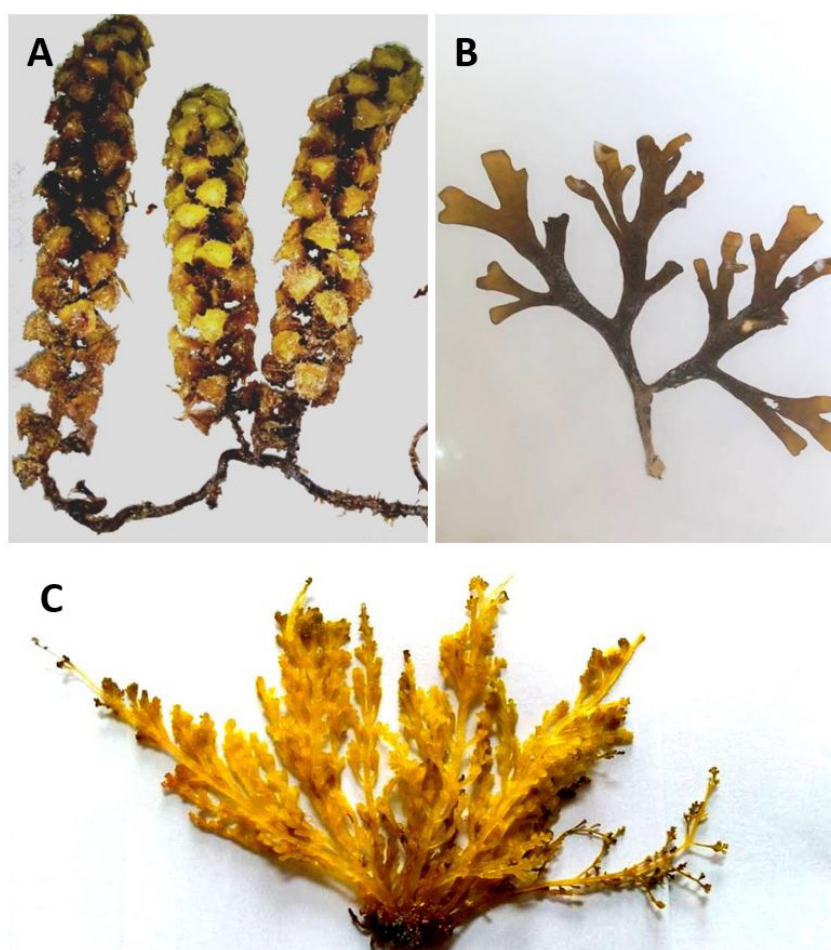
In general, seaweeds contain 90% water; and therefore the chemical composition is usually evaluated based on its dry weight. This includes the contents of carbohydrate, protein, lipids, minerals, vitamins and other metabolites with health benefits (Zvyagintseva et al., 2005; Artan et al., 2008; Choi et al., 2009). Carbohydrates are considered the major fraction of the dry weight of seaweeds and can reach up to 50%. Most of the seaweed carbohydrates are categorized as dietary fibres since they are not digested by the human gastrointestinal tract (Rupérez & Saura-Calixto, 2001). The protein content can vary between 3% to 15% (Fleurence, 1999) and may reach up to 47% in some cases, with large amounts of essential amino acids (García-Casal et al., 2007). The lipid contents are generally low (Kumari et al., 2010) and range from 1% to 3% of the dry weight. Also, seaweeds usually have large amounts of trace elements and essential minerals important for human needs, including large amounts of potassium and calcium (Ruperez, 2002).

Furthermore, marine macroalgae have become a really promising alternative source of bioactive compounds, since they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological behaviours such as antibacterial, antiviral and antifungal properties (Marinho-Soriano et al., 2006; Yaich et al., 2013). However, there is no fixed value even for the same genus or species;

and only the range or ratio of a certain chemical component in a given taxon can be known. Antioxidants are probably among the most interesting metabolites extracted from seaweeds, and they are valuable in the treatment of various serious diseases (Kohen & Nyska, 2002). The algae produce antioxidants in large amounts to protect the functional macromolecules inside their cells from oxidation via reactive oxygen species (ROS), which causes structural damage inside the algal cells. Thus seaweeds are considered to be rich in natural antioxidants (Lim et al., 2002; Kuda et al., 2005; Yuan et al., 2005; Duan et al., 2006) such as ascorbic acid, phenols, flavonoids (Wu et al., 2010) and carotenoids (Plaza et al., 2008), which have been found in brown, red and green algae (Cox et al., 2012).

Certain seaweed species, not included in this study, may contain toxic metabolites, which may prevent their use as food or animal feedstuffs. For example, *Caulerpa taxifolia* contains sesquiterpenic toxins such as caulerpenyne (Guerriero et al., 1992). However, such toxic compounds may be successfully used in other applications such as drug formulations for certain diseases, for instance due to their anticancer activity (Barbier et al., 2001), or even used to synthesize metallic nanoparticles (Aboelfetoh et al., 2017). Therefore, more attention should be paid to the choice of suitable seaweed species for use as food.

In the present study, the chemical compositions of three Egyptian seaweeds; *Dictyota dichotoma*, *Turbinaria decurrens* and *Laurencia obtusa* (Figure 1) were estimated, and their use proposed as food additives and as promising sources of antioxidants with a view to the nutrition and health of both humans and animals.



**Figure 1.** Optical images of the three seaweeds studies; *Turbinaria decurrens* (A), *Dictyota dichotoma* (B) and *Laurencia obtusa* (C).

## 2 Materials and methods

### 2.1 Collection of the seaweed samples

Sufficient amounts of the three marine macroalgae were collected from the Red Sea coast of Suez, Suez Bay, Egypt (29° 55' N, 32° 28' E) during June 2015. The samples were washed thoroughly with distilled water directly after collection to remove epiphytes and excess salts, placed in sterilized polyethylene bags and transferred to the laboratory in an icebox for the experimental work. In the laboratory, the samples were washed with sterile distilled water, air dried, oven dried at 45 °C to 50 °C, cut into small pieces, and then ground to a fine powder using a tissue grinder. The samples were identified according to Aleem (1978) and Guiry & Guiry (2011) as belonging to two algal divisions (See Figure 1): Phaeophyta (*Dictyota dichotoma*, *Turbinaria decurrens*) and Rhodophyta (*Laurencia obtusa*).

### 2.2 Analysis of the chemical composition

#### 2.2.1 Extraction and estimation of the carbohydrate content

The total carbohydrate content was quantitatively determined by the phenol-sulphuric acid method as described by Kochert (1978), after extraction by 1 N NaOH in a boiling water bath for 2 hours as described by Payne & Stewart (1988). The content was calculated in percentage using a standard glucose curve.

#### 2.2.2 Extraction and estimation of the protein and amino acid contents

The protein content was extracted from about 0.1 g powder. The extraction was carried out by adding 1 N NaOH to the seaweed powder and leaving the mixture in a boiling water bath for 2 hours as described by Payne & Stewart (1988). After centrifugation, the total soluble protein content was estimated using the Bradford method (Bradford, 1976) and expressed as a percentage of the algal dry weight. Furthermore, the amino acid profile was determined according to the methods described by Khairy & El-Shafay (2013). The algal samples (3 g) were prepared for hydrolysis according to Blackburn (1978) and the amino acids determined using an amino acid analyser (AAA).

#### 2.2.3 Extraction and estimation of the lipid and fatty acid contents

The total lipid content was extracted using chloroform-methanol according to the modified Folch method (Folch et al., 1957), and expressed as a percentage. After methylation according to Francavilla et al. (2013), the fatty acid contents of the lipids were analysed using a GC-MS spectrophotometer (Clarus 580, 560 S Perkin Elmer). The injection volume was 0.1 µl at 220 °C, the carrier gas was helium and the flow rate 0.2 mL/min.

#### 2.2.4 Extraction and estimation of the pigments

The total carotene and chlorophyll (Chlorophyll a, Chlorophyll b) contents were extracted according to Khuantrairong & Traichaiyaporn (2012), as a modified method of Yoshii et al. (2004) and of Dere et al. (1998). The total carotene content and chlorophylls a and b were determined spectrophotometrically using a UV-visible spectrophotometer at 470, 645 and 662 nm, respectively, according to the equations 1-3 proposed by Wellburn & Lichtenthaler (1984):

$$\text{Chlorophyll a} = 11.75A_{662} - 2.35A_{645} \quad (1)$$

$$\text{Chlorophyll b} = 18.61A_{645} - 3.960A_{662} \quad (2)$$

$$\text{Carotene} = (1000A_{470} - 2.270C_a - 81.4 C_b) / 227, \quad (C_a = \text{chlorophyll a}, C_b = \text{chlorophyll b}) \quad (3)$$

Where ( $A_{662}$ ) is the absorbance at 662 nm, ( $A_{645}$ ) is the absorbance at 645nm, ( $A_{470}$ ) is the absorbance at 470 nm, ( $C_a$ ) is chlorophyll a and ( $C_b$ ) is chlorophyll b. The results were expressed as ( $\mu\text{g g}^{-1}$ ) of seaweed.

### 2.2.5 Mineral analysis

About 10 ml of a diacid mixture (2:5 of nitric and perchloric acids) were added to 0.2 g powder and left for a few hours. The mixture was then placed on a hot plate and the contents digested by increasing the temperature. The digestion was continued until the contents became colourless. The digested material was filtered through Whatman N<sup>o</sup>. 40 paper and the filtrate diluted to a suitable volume and fed into an inductively coupled plasma spectrometer (ICP) (Perkin Elmer emission spectrophotometer-6000 series), according to Allen et al. (1997).

## 2.3 Secondary metabolites analysis

### 2.3.1 Estimation of the total phenolic compound content

The total phenolic compound content was determined using the Folin–Ciocalteu phenol reagent according to the method described by Kumar & Balamuruga (2015). The total phenol content of the samples was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE  $\text{g}^{-1}$  dry weight).

### 2.3.2 Total flavonoid content

The total flavonoid content was determined according to the method of Chang et al. (2002), using quercetin as the standard. The flavonoid content was expressed as mg quercetin equivalents per gram of extract (mg QE  $\text{g}^{-1}$  extract).

### 2.3.3 Estimation of the total alkaloid content

The total alkaloid content was measured quantitatively according to the method described by Harborne (2008) and expressed as milligram per gram of dry weight (mg  $\text{g}^{-1}$  dry weight).

### 2.3.4 Ascorbic acid (Vitamin C) content

The Ascorbic acid content was estimated according to Oser (1979) and calculated as milligrams per gram of dry weight (mg  $\text{g}^{-1}$  dry weight) using an ascorbic acid calibration curve.

## 2.4 Antioxidant activity assays

### 2.4.1 Determination of DPPH free radical scavenging activity

The radical scavenging activity of the methanolic seaweed extract against DPPH radicals was determined using the method of Blois (1958) and Yamasaki et al. (1994) and calculated using the following equation 4:

$$\text{DPPH scavenged}(\%) = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (4)$$

Where ( $A_{\text{control}}$ ) is the absorbance of the control reaction and ( $A_{\text{test}}$ ) is the absorbance in the presence of the extracts.

## 2.4.2 Total Antioxidant Capacity (TAC)

The total antioxidant activity of the seaweed extracts was determined using the phosphomolybdate assay method (PMA) (Ahmad et al., 2012) and expressed in mg ascorbic acid equivalents (AAE).

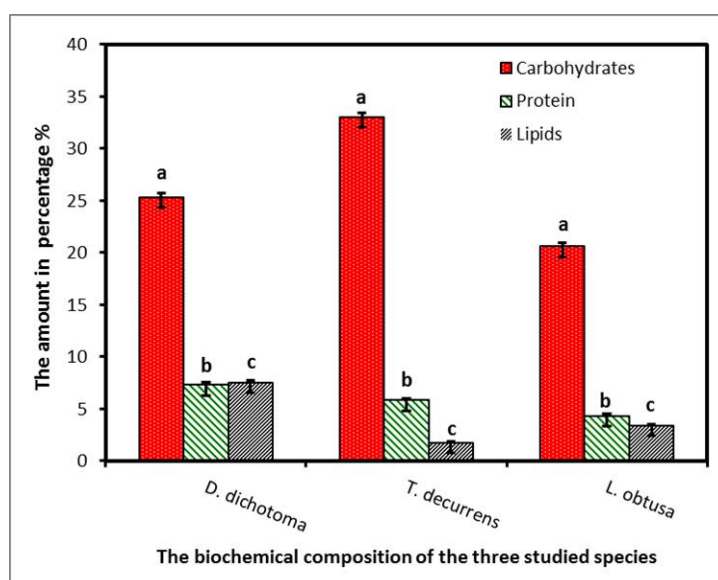
## 2.5 Statistical analysis

All the experiments were carried out in triplicate and the results expressed as the means  $\pm$  standard deviation (SD). Statistically significant differences between the seaweed parameters studied were identified by the analysis of variance (one-way ANOVA) using the SPSS software for windows. When the F values showed significance, the individual means were compared using the Duncan multiple range test (DMRT) method for data with significant differences. Significant differences were considered when  $p < 0.05$ .

## 3 Results and discussion

### 3.1 Chemical composition of the seaweeds studied

Protein, carbohydrate and lipids are the major vital chemical constituents of seaweeds. Figure 2 shows the carbohydrate, protein and lipid contents of the three seaweeds, showing highly significant differences ( $p \leq 0.05$ ), the fractions of each component varying between the three species depending on the taxon (Yaich et al., 2011). The results indicated that the carbohydrate contents were greater than the protein and lipid contents.



**Figure 2.** Diagram illustrating the carbohydrate, protein and lipid contents in the three seaweeds studied. Error bars show the standard deviations for the replicates. Different letters indicate statistically significant differences at  $p \leq 0.05$ .

The average carbohydrate contents of the seaweeds studied ranged from 20.6% to 33%. The maximum value was recorded for *T. decurrens* and the minimum for *L. obtusa*. This large amount of carbohydrate is important for the metabolism of the organism, since it supplies the energy needed for respiration and other metabolic processes and usually includes considerable amounts of polysaccharides, for example alginate and fucoidan in the brown seaweeds and carrageenan in the red seaweeds. These polysaccharides are edible and show promising biomedical applications including antiviral, antibacterial, anticoagulant and anticancer properties, the use as drug coatings in drug delivery systems and antioxidant activities (Venkatesan et al., 2017).

In general, the protein content of seaweeds is relatively low, between 3% to 15% of the dry weight (Fleurence, 1999), similar to the results obtained in the present study (Figure 2). According to the literature, the red seaweeds usually contain a larger protein fraction than both green and brown seaweeds (Cian et al., 2015; Pangestuti & Kim, 2015), but the results obtained here showed the largest protein fraction in the brown seaweed (*D. dichotoma*), and the lowest amount in the red seaweed (*L. obtusa*), results consistent with those of Parthiban et al. (2013). The reason for these differences could be that the protein content of seaweeds varies between species and even within the same species due to different habitats, time of the year and levels of maturity (Stirk et al., 2007; Gressler et al., 2010).

The amino acid profile analysis was carried out to determine the nutritional value of the three seaweed protein contents, and Table 1 shows the amino acid contents of the seaweeds studied. These amino acids may occur in the combined form or in the free state (Munda & Gubenšek, 1976; Dave & Parekh, 1978). Ten essential and six non-essential amino acids were detected in the protein hydrolysates of the seaweeds, including all the essential amino acids, and they were especially rich in aspartic and glutamic acids, also showing significant amounts of proline and alanine (Table 1), similar to results from previous studies (Fleurence et al., 2012; Cian et al., 2013). The amino acid profiles of the seaweeds studied were comparable with the FAO reference pattern (Food and Agriculture Organization of the United Nations, 1981) and the amino acid profiles of other food proteins (Orr & Watt, 1968) indicating the high nutritional value of seaweed protein, which can provide the total amount of essential amino acids required as food.

**Table 1.** Amino acid profile, concentrations ( $\mu\text{g g}^{-1}$  dwt) in the three seaweeds investigated.

Amino acids		Seaweeds		
		<i>D. dichotoma</i>	<i>L. obtusa</i>	<i>T. decurrens</i>
No.	Common name	Conc.	Conc.	Conc.
<b>Essential Amino Acids</b>				
1	Threonine	72	8.7	68.5
2	Valine	63.9	7.4	56.5
3	Methionine	29	2.3	10.7
4	Isoleucine	44.0	5.4	46.3
5	Leucine	105	10.6	89.5
6	Tryptophan	24.6	2.7	40.5
7	Phenylalanine	41.0	4.9	59.8
8	Histidine	48.5	8.0	41.3
9	Lysine	21.4	6.0	7.4
10	Arginine	58.5	6.2	72.3
<b>Total Essential Amino Acids (EAA)</b>		<b>507.9</b>	<b>62.2</b>	<b>492.8</b>
<b>The percentage of Total EAA (%)</b>		<b>40.7</b>	<b>40.7</b>	<b>44.2</b>
<b>Non-Essential Amino Acids</b>				
11	Aspartic acid	162.5	20.1	155
12	Serine	79.3	8.6	58.8
13	Glutamic acid	181.2	20.1	141.1
14	Proline	58.3	6.3	48.2
15	Glycine	89.5	10.1	78.8
16	Alanine	115.1	13.3	89.0
<b>Total Non-Essential Amino Acids (Non-EAA)</b>		<b>685.9</b>	<b>78.60</b>	<b>570.5</b>
<b>The percentage of Total Non-EAA (%)</b>		<b>54.9</b>	<b>51.4</b>	<b>51.1</b>
17	Ammonia	55.6	12.13	52.6

Conc. stands for concentration; dwt stands for dry weight.

The average total lipid content of the three seaweeds studied varied from 1.7% to 7.5% (Figure 2). The maximum lipid content was recorded in *D. dichotoma* followed by the red algae *L. obtusa*, while the minimum lipid content was found in *T. decurrens*. The total lipid content of seaweeds is generally lower than 5% of the dry weight in most species (Kumari et al., 2010; Gosch et al., 2012). However, some species are different, such as the members of the order Dictyotales, which can have total lipid contents of up to 20% of

the dry weight (Gosch et al., 2012), which explains the relatively high total lipid content ( $7.5\% \pm 0.24\%$ ) recorded in *D. dichotoma*. Furthermore, the results obtained for *L. Obtusa* were consistent with the total lipid fraction found in the two *Laurencia* species previously studied, which varied between 1.1% and 6.4% of the dry weight (Gressler et al., 2010).

The results obtained in the fatty acid analyses indicated that saturated fatty acids (SFAs) were predominant in the three seaweeds studied (Table 2). *L.obtusa* recorded the highest percent followed by *T. decurrens* and *D. dichotoma*. These results are in agreement with previous studies (Khotimchenko, 1995; Vaskovsky et al., 1996), where SFAs were found to be the dominate fraction amongst the total fatty acids in seaweeds. Palmitic acid (C16:0) was the largest fraction amongst the SFAs, showing 50.86%, 42.89% and 26.51% for *T. decurrens*, *L.obtusa* and *D. dichotoma*, respectively, followed by myristic acid (C14:0) and stearic acid (C18:0). Palmitic acid was also the major SFA fraction in *Gellidium micropterum* (Venkatesalu et al., 2004) and in *Porphyra* spp. (Sanchez-Machado et al., 2004). In addition, some brown seaweeds were found to be rich in myristic acid (Gosch et al., 2012).

**Table 2.** Fatty acid profile, concentrations ( $\mu\text{g } 100 \text{ g}^{-1} \text{ dwt}$ ) and percentages in the three seaweeds investigated.

Fatty Acids		Seaweeds					
		<i>D. dichotoma</i>		<i>L. obtusa</i>		<i>T. decurrens</i>	
Common name	Lipid Number	Conc.	%	Conc.	%	Conc.	%
<b>SFA</b>							
<i>Caproic acid</i>	C6:0	21.7	1.8	32.2	0.8	N.D.	N.D.
<i>Caprylic acid</i>	C8:0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Capric acid</i>	C10:0	N.D.	N.D.	N.D.	N.D.	29.4	0.2
<i>Undecylic acid</i>	C11:0	18.3	1.5	296.8	7.5	503.9	4.3
<i>Lauric acid</i>	C12:0	N.D.	N.D.	107.5	2.7	61.0	0.5
<i>Tridecylic acid</i>	C13:0	7.6	0.6	109.1	2.8	540.3	4.6
<i>Myristic acid</i>	C14:0	85.3	7.1	356.6	9.0	563.8	4.8
<i>Pentadecylic acid</i>	C15:0	9.4	0.8	94.6	2.4	355.7	3.0
<i>Palmitic acid</i>	C16:0	318.5	26.5	1691.4	42.9	6006.3	50.9
<i>Margaric acid</i>	C17:0	9.3	0.8	N.D.	N.D.	60.3	0.5
<i>Stearic acid</i>	C18:0	52.9	4.4	245.3	6.2	527.2	4.5
<i>Arachidic acid</i>	C20:0	24.2	2.0	45.9	1.2	N.D.	N.D.
<b><math>\Sigma</math> SFA</b>		<b>546.9</b>	<b>45.5</b>	<b>2979.5</b>	<b>75.6</b>	<b>8647.9</b>	<b>73.2</b>
<b>MUSFA</b>							
<i>Myristoleic acid</i>	C14:1 n-5	19.4	1.6	108.4	2.8	486.4	4.1
<i>Pentadecenoic acid</i>	C15:1	N.D.	N.D.	51.4	1.3	113.3	1.0
<i>Palmitoleic acid</i>	C16:1 n-7	63.8	5.3	69.4	1.8	413.5	3.5
<i>Heptadecenoic acid</i>	C17:1	N.D.	N.D.	N.D.	N.D.	51.3	0.4
<i>Oleic acid</i>	C18:1 n-9	274.5	22.9	407.5	10.3	1522.2	12.9
<i>Eicosenoic acid</i>	C20:1 n-9	60.7	5.1	N.D.	N.D.	115.8	1.0
<b><math>\Sigma</math> MUSFA</b>		<b>418.4</b>	<b>34.8</b>	<b>636.7</b>	<b>16.1</b>	<b>2702.4</b>	<b>22.9</b>
<b>PUSFA</b>							
<i>Linoleic acid</i>	C18:2 n-6	32.7	2.7	33.7	0.9	227.5	1.9
<i><math>\alpha</math>-Linolenic acid</i>	C18:3 n-3	149.7	12.5	N.D.	N.D.	70.3	0.6
<i>Eicosatrienoic acid</i>	C20:3 n-3	53.5	4.5	N.D.	N.D.	159.9	1.4
<i>Eicosapentaenoic acid</i>	C20:5 n-3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Docosadienoic acid</i>	C22:2 n-6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Docosahexaenoic acid</i>	C22:6 n-3	N.D.	N.D.	294.1	7.5	N.D.	N.D.
<b><math>\Sigma</math> PUSFA</b>		<b>235.8</b>	<b>19.6</b>	<b>327.8</b>	<b>8.3</b>	<b>457.6</b>	<b>3.9</b>
<b>Total % &amp; Total conc. of FA (<math>\mu\text{g.g}^{-1}.\text{dwt}</math>)</b>		<b>1201</b>	<b>100</b>	<b>3944</b>	<b>100</b>	<b>11808</b>	<b>100</b>
<i>SFA / MUSFA</i>		1.31		4.7		3.2	
<i>SFA / PUSFA</i>		2.3		9.1		18.9	
<i>SFA / TUSFA</i>		0.8		3.1		2.7	
$\Sigma$ U-3		16.9		7.5		2.0	
$\Sigma$ U-6		2.7		0.9		1.9	
$\Sigma$ U-6/ $\Sigma$ U-3		0.2		0.1		1.0	

SFA: Saturated fatty acids; MUSFA: Mono-unsaturated fatty acids; PUSFA: Poly-unsaturated fatty acids, and TUSFA: Total unsaturated fatty acids; U-6: omega 6 fatty acids; U-3: omega 3 fatty acids; N.D.: Not Detectable; dwt: dry weight.



The monounsaturated fatty acids (MUSFA) were in second place in the fatty acid content of the three seaweeds, and similar results were obtained by Khairy & El-Shafay (2013). The brown seaweeds recorded the highest percentage, while the red seaweeds recorded the lowest one. Oleic acid, an omega-9 fatty acid, presented the highest percentage amongst the MUSFA in all the seaweeds studied, in agreement with the results of Dawczynski et al. (2007), who claimed that oleic acid represented the highest fraction of the MUSFA in *Porphyra* spp. It is important to mention that the fatty acid contents of the three seaweeds may indicate their suitability for biofuel production, since the three species show very high percentages of SFAs as compared to the MUSFA and PUSFA (Hu et al., 2008; Knothe, 2008), with a relatively high content of MUSFA which would enhance the quality of the biofuel (Knothe, 2008).

Concerning the polyunsaturated fatty acids (PUSFA), the brown algae *D. dichotoma* attained the highest percent followed by the red algae *L. obtusa*, and *T. decurrens* recorded the lowest percent. The Omega-3 fatty acids ( $\alpha$ -Linolenic acid and Eicosatrienoic acid) were only found in the brown *D. dichotoma* and *T. decurrens*. Meanwhile decosahexaenoic acid (DHA), an omega-6 fatty acid, was only found in the red algae *L. obtusa*. These results are consistent with those of Khairy & El-Shafay (2013) who reported that DHA was the predominant component of PUFA in the red algae *Jania rubens*. Furthermore, linoleic acid, an omega-6 fatty acid, was present in all the seaweeds studied. Recently, the importance of the  $\omega$ -6/ $\omega$ -3 ratio has been widely discussed in scientific reports. The original value of 1 of the  $\omega$ -6/ $\omega$ -3 ratio involved the balance of intake of both PUSFA  $\omega$ -6 and  $\omega$ -3 fatty acids (Francavilla et al., 2013). The  $\omega$ 6/ $\omega$ 3 ratios found here were 0.161, 0.114 and 0.987 for *D. dichotoma*, *T. decurrens* and *L. obtusa* respectively. According to WHO, this ratio should not be higher than 10 in the diets (Sánchez-Machado et al., 2004), which endorses the need for further investigations concerning the use of the seaweeds studied for nutritive purposes. The PUFAs were reported to play key roles in cellular and tissue metabolism, including the regulation of cell membrane fluidity, electron and oxygen transport and thermal adaptation, and could reduce the risk of coronary heart disease (Funk, 2001; Mozaffarian et al., 2005).

### 3.2 Pigment contents

Table 3 shows the estimated contents for the photosynthetic pigments chlorophyll “a” and chlorophyll “b”, and the total carotenoid contents. *D. dichotoma* showed the highest chlorophyll “a”, chlorophyll “b” and carotenoid contents, followed by the other two species. *T. decurrens* and *L. obtusa* which showed quite similar results; although *T. decurrens* showed slightly, but still significantly lower contents than the red seaweed. Similarly, Utami et al. (2017) found that the pigment concentrations in *Dictyota* were higher than those in *Turbinaria*. In general, the variation in pigment content is associated with the species type, light intensity, water depth and temperature (Sampath-Wiley et al., 2008; Schmid et al., 2017).

Photosynthetic pigments could be a promising component for applications in the food industry and pharmaceutical fields, since the pigments could help in cell communication, human health maintenance, and also probably have antimicrobial activities (Plaza et al., 2010). Furthermore, carotenoids have major functions as antioxidants, that could play a role in preventing human diseases linked to oxidative stress (Mitra et al., 2006).

**Table 3.** Chlorophyll a, chlorophyll b and the total carotene contents of the three seaweed species *Dictyota dichotoma*, *Turbinaria decurrens* and *Laurencia obtusa*.

Seaweeds	Chlorophyll a ( $\mu\text{g g}^{-1}$ dwt)	Chlorophyll b ( $\mu\text{g g}^{-1}$ dwt)	Total Carotene content ( $\mu\text{g g}^{-1}$ dwt)
<i>D. dichotoma</i>	15.2 $\pm$ 0.161 <sup>a</sup>	2.5 $\pm$ 0.017 <sup>a</sup>	1146.6 $\pm$ 26.510 <sup>a</sup>
<i>T. decurrens</i>	1.7 $\pm$ 0.005 <sup>b</sup>	0.1 $\pm$ 0.012 <sup>b</sup>	117.0 $\pm$ 3.339 <sup>b</sup>
<i>L. obtusa</i>	0.6 $\pm$ 0.011 <sup>c</sup>	0.1 $\pm$ 0.014 <sup>b</sup>	45.5 $\pm$ 1.324 <sup>c</sup>

Each value is the mean of three readings  $\pm$  standard deviation; different letters represent significant differences amongst the pigments at  $p \leq 0.05$  (Duncan); dwt stands for dry weight.

### 3.3 Mineral analysis

Table 4 presents the results obtained in the analysis of minerals. *T. decurrens* showed the highest contents of potassium, sodium and iron, while *L. obtusa* recoded the highest nitrogen and zinc contents. The nitrogen, phosphorus, magnesium and copper contents showed slight variations between the three species. In general, seaweeds are rich in minerals (Cian et al., 2015), especially in sodium and iodine, due to their high polysaccharide content (Lahaye, 1991). The heavy metal content in seaweeds reflects its concentration in the medium, and the capacity of the seaweed to chelate them.

**Table 4.** Average results for the mineral contents of the three seaweed species *Dictyota dichotoma*, *Turbinaria decurrens*, and *Laurencia obtusa*.

Seaweeds	Nitrogen (%)	Phosphorus (%)	K (mg.g <sup>-1</sup> .dwt)	Mg (mg.g <sup>-1</sup> .dwt)	Zn (mg.g <sup>-1</sup> .dwt)	Na (mg.g <sup>-1</sup> .dwt)	Fe (mg.g <sup>-1</sup> .dwt)	Cu (mg.g <sup>-1</sup> .dwt)
<i>D. dichotoma</i>	1.45 ± 0.04 <sup>b</sup>	0.459 ± 0.001 <sup>c</sup>	68.2 ± 0.25 <sup>c</sup>	26.48 ± 0.77 <sup>c</sup>	0.001 ± 0.005 <sup>c</sup>	93.13 ± 1.1 <sup>b</sup>	0.162 ± 0.002 <sup>b</sup>	0.271 ± 0.02 <sup>c</sup>
<i>T. decurrens</i>	1.43 ± 0.05 <sup>b</sup>	0.309 ± 0.001 <sup>b</sup>	125.87 ± 0.97 <sup>b</sup>	22.01 ± 0.13 <sup>b</sup>	0.006 ± 0.0006 <sup>b</sup>	130.4 ± 1.5 <sup>a</sup>	0.62 ± 0.05 <sup>a</sup>	0.102 ± 0.006 <sup>b</sup>
<i>L. obtusa</i>	1.84 ± 0.02 <sup>a</sup>	0.25 ± 0.003 <sup>a</sup>	24.60 ± 1.25 <sup>a</sup>	23.94 ± 0.91 <sup>a</sup>	0.048 ± 0.002 <sup>a</sup>	100.9 ± 1.15 <sup>b</sup>	0.162 ± 0.002 <sup>b</sup>	0.24 ± 0.008 <sup>a</sup>
<b>F-value</b>	112.1*	7186.9*	8945.2*	31.4*	848.1*	4.1*	250.8*	141.3*

Each value is the mean of three readings ± standard deviation; different letters represent significant differences for that mineral at  $p \leq 0.05$  (Duncan). \*Highly significant at  $p \leq 0.05$  using the one-way analysis of variance (ANOVA); dwt stands for dry weight.

The Na/K ratio ranged from 1.1 to 4.1 in the species studied, representing desirable values for considering these species as food to provide a balanced Na/K ratio (Insel et al., 2007). Potassium, sodium and chlorides are responsible for maintaining the body fluid balance by decreasing sodium absorption and increasing potassium absorption in the gastrointestinal tract, whereas potassium together with calcium and magnesium are implicated in lowering the blood pressure and lessening the risk of strokes (Vaskonen, 2003; Smith et al., 2010).

The high iron levels present in seaweeds could compete with other sources, such as meat and spinach, by comparing with the data reported by Tee et al. (1988). The iron content of edible seaweeds could take part as a vital constituent in hemoglobin biosynthesis, and also interfere in many other human and metabolic processes.

Furthermore, the high zinc concentration present in the red seaweed, *L. obtusa*, is important for enzyme function, protein stability and in the regulation of gene expression (Smith et al., 2010).

### 3.4 Antioxidant contents

Natural antioxidants are not limited to terrestrial sources and reports have shown that seaweeds are also rich in natural antioxidant compounds (Lim et al. 2002; Duan et al., 2006; Kuda et al., 2007) including phenolic compounds, flavonoids, alkaloids and vitamin C. These antioxidants aid in the treatment of various serious diseases such as cancer and the cardiovascular and aging diseases, by scavenging ROS and free radicals (Kohen & Nyska, 2002).

Phenolic compounds are commonly found in plants as well as in seaweeds and have been reported to have a wide range of biological activities including antioxidant properties (Duan et al., 2006; Kuda et al., 2007; Wang et al., 2009). The total phenolic compound content (TPC) of the seaweeds studied ranged from  $16.87 \pm 3.2$  to  $474.46 \pm 29.3$  mg GAE g<sup>-1</sup> (Table 5), and that of the *D. dichotoma* extract was significantly higher. This higher TPC of the *D. dichotoma* extract resulted in its greater antioxidant capacity.

**Table 5.** Total phenolic compound content, total flavonoid content, DPPH inhibition, total antioxidant activity, ascorbic acid content and total alkaloid content of the three seaweed species *Dictyota dichotoma*, *Turbinaria decurrens* and *Laurencia obtusa*.

Seaweeds	<i>T. phenolic compounds</i> (mg GAE g <sup>-1</sup> )	<i>T. flavonoids</i> (mg QE g <sup>-1</sup> )	<i>Ascorbic acid content</i> (mg g <sup>-1</sup> )	<i>T. alkaloids</i> (mg g <sup>-1</sup> )	<i>DPPH inhibition</i> (%)	<i>T. antioxidant activity</i> (mg ascorbic acid g <sup>-1</sup> )
<i>D. dichotoma</i>	474.5 ± 29.3 <sup>a</sup>	38.2 ± 2.4 <sup>a</sup>	358.1 ± 23 <sup>a</sup>	15.6 ± 0.45 <sup>a</sup>	43 ± 0.56 <sup>a</sup>	555.9 ± 34.5 <sup>a</sup>
<i>T. decurrens</i>	16.9 ± 3.2 <sup>b</sup>	2.1 ± 0.81 <sup>b</sup>	135.1 ± 10.9 <sup>b</sup>	2.1 ± 0.002 <sup>c</sup>	4.9 ± 0.74 <sup>c</sup>	14.1 ± 2.6 <sup>c</sup>
<i>L. obtusa</i>	54.1 ± 16.5 <sup>b</sup>	4.5 ± 1.6 <sup>b</sup>	333.9 ± 56.6 <sup>a</sup>	4.2 ± 0.3 <sup>b</sup>	8.2 ± 1.16 <sup>b</sup>	168.9 ± 11.7 <sup>b</sup>
<i>F- value</i>	505.6*	390.0*	34.9*	314.5*	1795.6*	524.9*

Each value is the mean of three readings ± standard deviation; different letters represent significant differences for that compound at  $p \leq 0.05$  (Duncan). \* Highly significant at  $p \leq 0.05$  using the one-way analysis of variance (ANOVA).

Table 5 shows the total flavonoid and total alkaloid contents of the seaweed extracts. The *D. dichotoma* extract showed higher total flavonoid and total alkaloid contents. Flavonoids have been reported to be antioxidants, scavengers of a wide range of ROS and inhibitors of lipid peroxidation, and also to be potential therapeutic agents against a wide variety of diseases (Ross & Kasum, 2002; Williams et al., 2004).

The vitamin C (ascorbic acid) contents presented in table 5 show similar results for both *D. dichotoma* and *L. obtusa*, while *T. decurrens* showed a much smaller amount. Vitamin C is also one of the important antioxidants that are helpful in the growth and repairing of different body tissues, including cartilage, bones, tendons, ligaments and teeth.

### 3.5 Antioxidant activity assays

Two simple methods were used to evaluate the antioxidant capacity of the algal extracts including the DPPH free radical scavenging activity and the total antioxidant activity of seaweeds and the results are presented in Table 5. Higher values were observed in the *D. dichotoma* extract, followed by those of *L. obtusa* and *T. decurrens*. These results are consistent with those of Parthiban et al. (2013) who found that the acetone extract from *D. dichotoma* showed strong DPPH radical scavenging activity.

The antioxidant activity of the three seaweeds arose from their chemical compositions including their pigment contents (such as carotenoids), vitamin and vitamin precursor contents (such as ascorbic acid), total phenolic compound contents (such as polyphenolics, hydroquinones and total flavonoids), total alkaloid content and those of other antioxidative substances, which directly or indirectly contribute to the scavenging of both ROS and free radicals (Shahidi, 2008). Several reports have shown a close relationship between the total phenolic compound content and high antioxidant activity, and many researchers have demonstrated that phenolic compounds are one of the most effective antioxidants in marine algae (Luo et al., 2010, Zakaria et al., 2011).

In agreement with these facts, *D. dichotoma* showed the highest contents of total phenolic compounds, total flavonoids, ascorbic acid, total alkaloids, pigments, (including carotenoids) and total lipids, followed by *L. obtusa*, which could be the reason for the higher DPPH scavenging and antioxidant activities of its extract. The brown seaweed, *T. decurrens*, showed the lowest contents of all the antioxidant compounds, and hence its extract showed the lowest antioxidant activity amongst the three seaweeds. The results indicated that the brown alga *D. dichotoma* can be used as a source of natural antioxidant compounds since its extracts exhibited significant antioxidant activity.

## 4 Conclusion

In conclusion, the present study provided evidence that the seaweeds under investigation (*Dictyota dichotoma*, *Turbinaria decurrens* and *Laurencia obtusa*) showed promising potential for use as food, animal

feed and in medical applications due to their chemical contents. Carbohydrate represented the largest fraction of the dry weight of the seaweeds, followed by proteins and then lipids, except for *D. dichotoma*, where the lipid content was very close to the protein content. All the essential amino acids were present in the protein fractions, which were especially rich in aspartic and glutamic acids. The saturated fatty acids predominated in all the three seaweeds with an abundance of palmitic acid, followed by the monounsaturated fatty acids with an abundance of oleic acid, and finally the polyunsaturated fatty acids with an abundance of the omega 3 and omega 6 fatty acids. Also, they are a remarkable natural source of minerals and of some antioxidant compounds (phenols, flavonoids, alkaloids, carotenes, Vitamin C) that could be used as functional ingredients and provide dietary alternatives.

*D. dichotoma* was found to be the most nutritionally rich species, with appreciable protein contents (with large amounts of essential amino acids), a moderate carbohydrate content (with a useful polysaccharide content), large amount of polyunsaturated fatty acids (especially omega-3), with a suitable  $\omega 6/\omega 3$  ratio, and also relatively high levels of minerals (with the required Na/K balance) and high antioxidant activity, which makes this species very valuable for human health as a low-calorie food.

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