

Biochemical Constituents and Antioxidant Capacity of Some Seaweeds from Red and Mediterranean Coasts of Egypt

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PHYTOCHEMICAL screening was performed on six algal genera belonging to three marine macroalgal divisions (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coastal region of Red and Mediterranean coasts, Egypt during spring (2012). The antioxidant potential of the candidate seaweeds was evaluated by measuring the ferric reducing power (FRAP), total antioxidant capacity (TAC), total phenol and flavonoids, ascorbic acid as well as glutathione contents were determined. Significant individual differences of the biochemical parameters were recorded in all tested marine algae. The macroalgal phytochemical analysis showed quantitative variations in the total soluble and insoluble carbohydrates and proteins as well as the glycerol contents. The highest accumulation of carbohydrate was estimated in the members of Chlorophyta followed by Phaeophyta and Rhodophyta divisions. The protein content was higher in the species of Rhodophyta, moderate in Chlorophyta and the lowest was estimated in the members of Phaeophyta. In addition, the maximum glycerol content was recorded in *Dictyota*. Green algae exhibited greater levels of chlorophylls *a* and *b*. However, among all the studied three groups, the highest carotenoids were estimated in *Padina* followed by *Dictyota*; both belonging to Phaeophyta while the lowest carotenoid content was recorded in *Gracilaria* (Rhodophyta). The highest carbohydrate content was recorded in *Enteromorpha* where the lowest phenol was estimated. Results also revealed that, there was no relation between antioxidant activity and total phenols or flavonoid content. All the studied species are considered to be a rich source of antioxidants (ASA and GSH). Accordingly, these seaweed species have a high anti-oxidative potential and can provide dietary alternatives.

Keywords: Antioxidant, Biochemical composition, Ferric reducing power; Seaweeds.

Abbreviations: ASA Ascorbic acid, FRAP Ferric reducing ability power, GSH Reduced Glutathione, H₂O₂ Hydrogen peroxide, ROS reactive oxygen species and TAC Total antioxidant capacity.

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Increasing demand for natural antioxidants having less or no side effects is one of the main issues for marine biologists. Edible seaweeds have historically been consumed by coastal populations as a source of pharmacological and food additives with high antioxidant capacity (Rimber and Indy 2007; Gupta and Abu-Ghannam, 2011; Peng *et al.*, 2013 and Rameshkumar *et al.*, 2013). In fact, seaweeds live in complex habitats under extreme conditions (salinity, temperature, nutrients, UV etc.), therefore, the adaptation process produce a great variety of biologically active metabolites, which cannot be found in other organisms (Carlucci, *et al.*, 1999). Seaweeds had been used for many years directly for human consumption, animal feed and as manure because of their high calcium and protein content (Kumari *et al.*, 2010). It is also an ingredient for cosmetics industries and is used as fertilizer (Rupérez, 2002). Interestingly, seaweeds are used in several industries as the gelling, thickening, emulsifying, binding, stabilizing, clarifying and agars (Chopin, 2007). Moreover, macroalgal polysaccharides are used in the food, cosmetics, paints, textile, paper, rubber and building industries. In addition, they are used in medicine and in pharmacology for their antimicrobial, antiviral, antitumor and anticoagulant properties (Fleurence, 1999 and Sabina *et al.*, 2005). Recent studies show the antioxidant abilities of many types of seaweeds (Lim *et al.*, 2002; Park *et al.*, 2005; Duan *et al.*, 2006 and Kuda and Ikemori, 2009). Marine algae, like other photosynthesizing plants, are exposed to different environmental conditions that lead to the generation of reactive oxygen species (Asada, 1999). The injurious effects occurs when the capacity of antioxidant defense system are lower than the generation of reactive oxygen species (ROS). However, the stability of their structural components suggests the presence of protective defense systems (Fujimoto 1990; Matsukawa *et al.*, 1997 and Lim *et al.*, 2002) to alleviate the injurious effects of ROS. Ascorbic acid (ASA) and reduced glutathione (GSH) are responsible for scavenging such ROS by the Hallwell-Asada pathway (Horemans *et al.*, 2000). A major function of ascorbic acid is to scavenge H₂O₂ through the ascorbate / glutathione pathway (Zhang and Kirkham, 1996). Ascorbate works in co-operation with GSH providing synergetic protection of the membranes.

Natural pigments also have received particular attention as they exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. Therefore, various natural pigments isolated from marine algae have attracted much attention in the fields of food, cosmetic and pharmacology (Pangestuti and Kim, 2011). Estimation of total antioxidant capacity is therefore a valuable tool in evaluation of seaweeds nutritional value (Stajner *et al.*, 2011). In addition to the well known antioxidant system, the light was thrown on the essential role of flavonoids, and phenolic compounds as effective antioxidants (Michalak, 2006). The antioxidant activity of these compounds are mainly attributed to scavenging activity against superoxide and hydroxyl radicals, chelating ability, quenching singlet and triplet oxygen and reducing power (Ruberto, *et al.*, 2001 and Athukorala *et al.*, 2006). In this respect, algae can be considered as an important

Naguib (1963) respectively. Soluble sugars and those obtained after polysaccharides hydrolysis were estimated using the anthrone reagent as described by Blakeney and Mutton (1980). Moreover, glycerol content was estimated according to Lambart and Neish (1950). Soluble proteins were extracted according to the method described by Guy *et al.* (1992). Meanwhile, the water insoluble residue remaining after extraction of soluble proteins was extracted with 1 N NaOH. Soluble proteins and those resulting after insoluble residue hydrolysis were measured using BIO-RAD protein assay dye reagent according to the method adopted by Bradford (1976).

Antioxidants as glutathione and ascorbic acid were extracted and estimated following the methods described by Smith (1985) and Mukherjee and Choudhuri (1983), respectively. The amount of total phenol was determined with the Folin-Ciocalteu reagent using the method of Malik and Singh (1980) and the absorbance was measured at 765 nm using spectrophotometer (Spectronic 601, Milton Roy Company). Total flavonoid content can be determined by Aluminum chloride method (Harborn, 1998) and the absorbance was measured at 415 nm. Ferric reducing power (FRAP) was estimated according to Dorman *et al.* (2003). Moreover, total antioxidant capacity was determined according to the method of Oyaizu (1986).

Statistic Analysis

Data were subjected to an analysis of variance, and the means were compared using the least significant difference (LSD) test at the 0.05 level according to the method of Snedecor and Cochran (1982).

Results and Discussion

Totally six seaweeds were tested for their nutritive properties viz. carbohydrates, proteins, glycerol, flavonoids and total phenols. Measurable differences in nutritional composition were apparent among the candidate macroalgal species. Carbohydrates and proteins are the most important biochemical components in algal biomass. Data presented in (Table 2) showed that, the concentration of carbohydrate was higher in the specie of Chlorophyta followed by Phaeophyta and Rhodophyta respectively. Similarly, Haroon (2000) and Anantharaman *et al.* (2013) recorded highest carbohydrates content in green seaweeds. In Chlorophyta, the highest carbohydrate content ($p > 0.05$) was observed in *Enteromorpha* (192.2 mg g⁻¹FW). In Phaeophyta, the highest carbohydrate content ($p > 0.05$) was observed in *Padina* (93.6 mg g⁻¹FW). Meanwhile, there was no any significant difference in total carbohydrate content ($p < 0.05$) between *Sargassum* and *Dictyota*. Moreover, the red seaweed *Gracilaria*, recorded the lowest carbohydrate ($p > 0.05$) concentration (20.4 mg g⁻¹FW). Such variations may be attributed to species difference and to differences in their metabolic preferences (Pádua *et al.*, 2004).

TABLE 2. Metabolic constituents of some Egyptian seaweeds.

Seaweeds	Total soluble sugars mg g ⁻¹ FW	Total Insoluble Sugars mg g ⁻¹ FW	Total Sugars mg g ⁻¹ FW	Sucrose mg g ⁻¹ FW	Total Soluble Proteins mg g ⁻¹ FW	Total Insoluble Proteins mg g ⁻¹ FW	Total Proteins mg g ⁻¹ FW	Glycerol µmole/g F.W
Phaeophyta								
<i>S. dentifolium</i>	1.54 ^e ±0.01	49.79 ^f ±0.07	51.31 ^f ±0.09	0.87 ^d ±0.2	4.46 ^c ±0.02	4.36 ^c ±0.003	8.82 ^d ±0.02	144.04 ^b ±1.2
<i>P. pavonia</i>	0.87 ^b ±0.03	92.76 ^e ±0.16	93.59 ^e ±0.16	2.82 ^{bc} ±0.1	1.97 ^h ±0.01	1.86 ^c ±0.017	3.83 ^b ±0.02	148.47 ^b ±2.2
<i>D. dichotoma</i>	1.45 ^{e,f} ±0.03	49.72 ^f ±0.94	51.21 ^f ±0.96	0.52 ^d ±0.1	2.97 ^{ie} ±0.08	1.20 ^f ±0.096	4.18 ^b ±0.07	238.51 ^a ±2.9
Rhodophyta								
<i>G. latifolium</i>	5.63 ^a ±0.2	20.85 ^b ±1.4	26.63 ^b ±1.2	2.49 ^{bc} ±0.3	5.55 ^b ±0.18	4.66 ^c ±0.02	10.21 ^b ±0.16	136.35 ^c ±3
<i>G. dura</i>	3.75 ^b ±0.03	16.72 ^b ±0.67	20.4 ^b ±0.67	3.41 ^b ±0.07	6.42 ^a ±0.01	5.27 ^b ±0.23	11.69 ^a ±0.23	129.49 ^d ±0.9
Chlorophyta								
<i>E.intestinales</i>	1.78 ^d ±0.01	190.5 ^c ±6.08	192.2 ^c ±6.08	3.48 ^b ±0.1	3.40 ^c ±0.15	6.13 ^a ±0.11	9.53 ^c ±0.21	52.23 ^f ±0.4
LSD (>0.05)	species	0.234	17.219	17.161	1.132	0.216	0.33	5.962
D (0.05)	Division	0.128	9.431	9.399	0.62	0.118	0.181	3.265

All the values are mean ± SE. Column wise values with same superscripts of this type indicate no significant difference (P > 0.05).

The potential production of glycerol by seaweeds is of obvious economic benefit (Ben-Amotz and Avron, 1980; Chen and Chi, 1981). Most seaweeds produce glycerol exclusively by degradation of starch. This is the case in our study; the glycerol content was in inverse to the insoluble carbohydrate content. The maximum (p>0.05) glycerol content was recorded in the members of phaeophyta where the lowest one was measured in Chlorophyta specie (Table 2). It is interesting to note that, there was no significant difference in glycerol content (p<0.05) between *S. dentifolium* and *P. pavonia*. In seaweeds, carbohydrate metabolism may be shifted toward glycerol production (Avron, 1992). Similarly, Bentalel *al.*, (1990) on his study on *Dunaliella*, suggested a dynamic inter-conversion between the two major carbon pools (glycerol and starch) to meet the osmotic requirements.

Like carbohydrates and glycerol, there was a significant differences ($p > 0.05$) of protein contents among all studied seaweeds. In the present study, protein content showed remarkable variation with highest values in Rhodophyta followed by Chlorophyta and Phaeophyta. Protein content varied from 3.8 to 11.6 mg g⁻¹FW; maximum protein ($p > 0.05$) was recorded in *Gracilaria* (11.6 mg g⁻¹FW) followed by *Geledium* (10.2 mg g⁻¹FW and *Enteromorpha* (9.5 mg g⁻¹FW). The minimum protein ($p > 0.05$) concentration was estimated in *Padina* (3.8 mg g⁻¹FW) followed by *Dictyota* (4.1 mg g⁻¹FW) from Phaeophyta (Table 2). Nevertheless, higher protein contents were recorded in red and green algae; the protein content of brown algae is generally low. The findings of the present study coincided well with Dere *et al.*, (2003) and Rameshkumar *et al.* (2013) who reported a similar trend of higher protein content in species of Rhodophyta, moderate in Chlorophyta and the lowest in Phaeophyta. According to the study of Marinho-Soriano *et al.* (2006), the high carbohydrate content was concomitant with decreasing the nitrogen and proteins content. This is the case in our study; the higher protein content in red algae which recorded the lowest carbohydrates. Protein content varied among different genera and also in different species of the same genus (Dhargalkar *et al.*, 1980). On the contrary, Burtin (2003) estimated high protein content in green seaweeds. However, Wong and Cheung (2001) demonstrated that protein content of the red seaweeds was significantly higher than those of green seaweeds.

The environment in which seaweeds grow is harsh and can lead to the formation of strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. Such fact implies that seaweed cells have evolved complex protective system (Matsukawa *et al.*, 1997) to tolerate salinity. Attention of researchers was focused on the ability of algae to accumulate antioxidant compounds. Heo *et al.* (2005) identified the potential antioxidant compounds as some pigments (e.g. chlorophyll, carotenoid) and polyphenols (e.g. phenolic acid, flavonoid). Data presented in Table 3 indicated that, amongst all the species of the three algal groups, the order of chlorophyll *b* content is as follows: Chlorophyta > Phaeophyta > Rhodophyta. The results of the current study corroborated with the findings of Francisco *et al.* (2006) and Kumar *et al.* (2009) stating highest chlorophyll *a* and *b* contents in green algae and lowest in red algae. Among all the three groups, highest carotenoids ($p > 0.05$) were reported in *Padina* (6.62 ug g⁻¹ FW) followed by *Dictyota* (5.56 ug g⁻¹ FW), both belonging to the Phaeophyta while the lowest carotenoid content ($p > 0.05$) was observed in *Gracilaria* (1.3 ug g⁻¹ FW) belonging to the Rhodophyta. Similar results were reported by Fritsch (1971) and Dere *et al.* (2003). Indeed, chlorophyll *a* exhibited antioxidant activity (Endo *et al.*, 1985), act as potent synergist of vitamin E (Mendiola *et al.*, 2005). Carotenoids are known to quench the excited molecules and singlet oxygen (Bondarev, 1997). In this study, the higher antioxidant activity of *Dictyota* (Table 3) might be attributed to the high content of the lipid soluble total chlorophylls especially Chl *a* (Stajner *et al.*, 1999) and / or to the high content of total carotenoids (Rivero *et al.*, 2003).

TABLE 3. Chlorophyll a and b, carotenoid contents of some Egyptian seaweeds.

Seaweeds	Chlorophyll a	Chlorophyll b	Carotenoids	Ch _a /Ch _b	Total Pigments
	µg/g FW				
Phaeophyta					
<i>S. dentifolium</i>	7.50 ^{bc} ±0.5	2.64 ^{bc} ±0.06	4.14 ^c ±0.3	2.87 ^b ±0.3	14.28 ^c ±1
<i>P. pavonia</i>	10.07 ^c ±0.6	3.55 ^{de} ±0.1	6.62 ^a ±0.5	2.85 ^b ±0.05	20.24 ^b ±0.8
<i>D. dichotoma</i>	10.17 ^{de} ±0.2	2.89 ^{cd} ±0.01	5.56 ^b ±0.07	3.52 ^a ±0.1	18.62 ^b ±0.1
Rhodophyta					
<i>G. latifolium</i>	2.94 ^d ±0.3	1.94 ^b ±0.01	1.4 ^{bc} ±0.08	1.52 ^c ±0.09	6.28 ^d ±0.2
<i>G. dura</i>	0.1±2.36	2.07 ^{ab} ±0.1	1.32 ^a ±0.03	1.16 ^{cd} ±0.2	5.75 ^d ±0.2
Chlorophyta					
<i>E. intestinales</i>	10.65 ^{de} ±1.1	9.44 ^a ±0.5	3.02 ^d ±0.05	1.13 ^{cd} ±0.01	23.12 ^a ±1.3
LSD	0.560	0.673	0.622	0.413	2.069
(>0.05)					
Species					
Division	00.23	0.369	0.341	0.226	1.134

All the values are mean ± SE. Column wise values with same superscripts of this type indicate no significant difference ($P > 0.05$).

Phenols are the aromatic metabolites which trigger various biochemical processes of the organisms. Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical intermediates, which prevent the oxidation of various ingredients, particularly fatty acids and oils (Yuan *et al.*, 2005). Data presented in Table 4 revealed that, the total phenol was in inverse to carbohydrate content in most species under investigation. The highest carbohydrate content ($p > 0.05$) was recorded in *Enteromorpha* (19c mg g⁻¹FW) where the lowest phenol (0.34 mg g⁻¹FW) was estimated. The carbohydrate metabolism might be channeled for the production of total phenols (Tongnetti and Johnson, 1999), thus explaining the reason why the production of secondary metabolites was up-regulated with decreasing carbohydrates. Moreover, data in Table 4 indicated that, there was no significant difference in total phenols content ($p < 0.05$) between green algae members. Likewise, the highest flavonoid contents were showed in the investigated macro-algal species of Phaeophyta followed by Chlorophyta and Rhodophyta respectively. In the members of the Phaeophyta the highest flavonoid contents ($p > 0.05$) were 5.4 mg g⁻¹ DW, 3.4 mg g⁻¹ D.W. and 3.2 mg g⁻¹ DW in *Padina*, *Dictyota* and *Sargassum* respectively. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelating of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS (Chanda and Dave, 2009). Previous study gave similar results and explained that, there was no correlation between antioxidant activity and total phenol or flavonoid content (Yu *et al.* 2003; Dasgupta, 2007; Alam *et al.*, 2012).

Data presented in Table 4 also indicated variations in TAC which may be attributed to species differences. Accordingly, all the studied species are considered to be a rich source of antioxidants. The antioxidant activity of the plant extract has been found to be associated with the reducing power property. Among the seven species studied, *Sargassum* had the highest concentration of ascorbic acid whereas *padina* had the lowest. *Sargassum* also had the richest source of glutathione. Furthermore, the highest reducing power ($p > 0.05$) was observed in *Dictyota* (g⁻¹ DW) and the lowest reducing power was estimated in the green algae *Enteromorpha* (g⁻¹ DW). Interestingly, *Dictyota* also exhibited the highest antioxidant activity suggesting the relationship between antioxidant activities and reducing power (Table 4). Seenivasan (2013) reported that brown seaweeds exhibited good antioxidant activity when compared to red and green seaweeds. Indeed, all species under studies showed high reducing power and the studied seaweed extracts were able to reduce Fe⁺³ to Fe⁺² as a function of reducing power.

In conclusion, the present study evident that, the seaweeds under investigation (*Sargassum dentifolium*, *Padina pavonia*, *Dictyota dichotoma*, *Gelidium latifolium*, *Gracilaria dura* and *Enteromorpha intestinales*) are interesting natural source of some antioxidant compounds that could be used as functional ingredients and can provide dietary alternatives. Moreover, further research is underway for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicines to replace synthetic antioxidant.

TABLE 4. Antioxidant capacity, ferric reducing power, ascorbic acid, glutathione, total phenols and flavonoid contents of some Egyptian seaweeds.

Seaweeds	Ascorbic Acid μMole/g F.W	Glutathione mMole/g F.W	Total Phenols mg/g F.W	Total Flavonoids mg/g D.W	Reducing Power μg/g D.W	Total Antioxidant Capacity mg/g D.W
Phaeophyta						
<i>S. dentifolium</i>	0.70 ^a ±0	13.17 ^a ±0.6	0.82 ^b ±0.06	3.21 ^{de} ±0.09	0.20 ^c ±0.01	189.08 ^{bc} ±1.3
<i>P. pavonia</i>	0.27 ^d ±12.17	11.13 ^b ±1	0.77 ^b ±0.04	5.43 ^b ±0.05	0.46 ^b ±0.02	165.51 ^{bc} ±3.6
<i>D. dichotoma</i>	0.47 ^b ±15.68	8.47 ^{cd} ±0.2	0.61 ^{bc} ±0.09	3.42 ^d ±0.1	1.04 ^a ±0.05	1471.26 ^a ±123.5
Rhodophyta						
<i>G. latifolium</i>	0.31 ^{cd} ±1.42	8.22 ^{de} ±0.5	1.21 ^a ±0.02	2.37 ^f ±0.1	0.31 ^{cd} ±0.02	249.42 ^b ±9.9
<i>G. dura</i>	0.33 ^c ±13.58	8.42 ^{cd} ±0.3	1.08 ^a ±0.07	1.79 ^e ±0.1	0.22 ^{de} ±0.01	194.82 ^{bc} ±11.4
Chlorophyta						
<i>E.intestinales</i>	0.45 ^b ±37.9	7.73 ^c ±0.2	0.39 ^{cd} ±0.03	3.83 ^c ±0.2	0.19 ^e ±0.01	134.86 ^{bc} ±8.3
LSD (0.05<)						
Species	0.045	1.479	2.258	0.359	0.093	118.67
Division	0.025	0.81	1.236	0.196	0.051	64.99

All the values are mean ± SE. Column wise values with same superscripts of this type indicate no significant difference ($P > 0.05$).

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المكونات البيوكيميائية وقدرة مضادات الأكسدة لبعض أنواع الطحالب البحرية من سواحل البحر الأحمر والأبيض المتوسط لمصر.

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تهدف هذه الدراسة الى تقييم التركيب الكيميائي والقدرة الكلية لمضادات الأكسدة لستة أنواع من الطحالب البحرية من المنطقة الساحلية في مصر خلال ربيع 2012 ولقد أسفرت النتائج عن وجود اختلافات كمية في تراكم المواد الكربوهيدراتية والبروتينية وكذلك بعض مضادات الأكسدة مثل حمض الاسكوربيك, الجلوتاثيون, الفينولات وكذلك الفلافينويدات.

حيث أظهرت النتائج زيادة معنوية واضحة في تراكم المواد الكربوهيدراتية والبروتينية في الطحالب الخضراء يليها الطحالب البنية ثم الحمراء. بالإضافة الي ذلك تم تسجيل أقصى محتوى للجلسرين في *Dictyota* بينما كان أدنى مستوي له في *Enteromorpha* ومما هو جدير بالذكر أن الطحالب الخضراء أظهرت أعلى مستويات من الكلوروفيل (أ) و (ب) بين المجموعات الثلاث، وسجلت أعلى قيمة للكاروتينيدات في *Padina* تليها *Dictyota*، وكلاهما ينتميان إلى الطحالب البنية بينما سجلت أدنى قيمة في *Gracilaria* (الطحالب الحمراء). ولقد لوحظ وجود علاقة عكسية بين تراكم الفينولات و المواد الكربوهيدراتية في المجموعات الثلاثة. وكشفت النتائج أيضا أنه لم تثبت العلاقة بين القدرة الكلية لمضادات الأكسدة من ناحية و الفينولات و محتوى الفلافونويد من ناحية أخرى. وتبين من النتائج أن جميع الأنواع قيد الدراسة كانت مصدرا غنيا لمضادات الأكسدة مثل حمض الاسكوربيك و الجلوتاثيون (ASA و GSH).

وخلاصة القول، إن هذه الأنواع من الأعشاب البحرية أعطت نتائج واعدة خاصة كمضادات للأكسدة ويمكن أن تستعمل كبداية غذائية.