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Variation of photosynthetic pigments and biochemical screening in some seaweeds from Eastern Harbor, Alexandria, Egypt

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ABSTRACT

The current study examined the biochemical composition of four seaweeds (*Ulva fasciata, Ulva compressa, Corallina officinalis, and Corallina elongate)* which were collected from Eastern Harbor located at Alexandria Mediterranean coast, Egypt. Total chlorophyll content was the maximum in *Ulva compressa* (2.7 mg g^{-1} FW) and the minimum value was observed in *Corallina elongate* (0.90 mg g^{-1} FW). In comparison, the maximum carotenoids were registered in *Corallina officinalis* (1.04 mg g^{-1} FW) followed by *Corallina elongate* (0.86 mg g^{-1} FW). The lowest ratio was recorded in *Ulva fasciata* (0.45 mg g^{-1} FW). The results showed that the green seaweed (*Ulva compressa)* contained the highest amounts of phenols (12.7 mgGA/g dry wt.), flavonoid (9.42 mgCA/g dry wt.) and has the maximum percentage of DPPH radical scavenging capacity, Total antioxidant capacity (TAC) assay, and Total Reducing Capacity (TRC) (80.45, 12.5 and 71.6 respectively). On the other hand, the red seaweed (*Corallina elongate)* contained the lowest amounts of phenols (5.9 mgGA/g dry wt.), flavonoid (8.29 mgCA/g dry wt.), and has the maximum percentage of DPPH, TAC, and TRC (70.3, 8.4, and 57.8 respectively). Due to seaweed's biochemical composition, these findings recommend being used as an antioxidant agent for food supplements, cosmetics, medicinal applications, and pharmaceutical industries.

INTRODUCTION

In the last decade, the aquatic ecosystem has gained researchers' interest because many species contain or obtain compounds with strong biological activity. Marine

species are the source materials of pharmacological and biological processes for structurally unique natural products (**Faulkner, 2001**). Macroalgae (seaweed) occupy an important role as a source of medicinal compounds for marine species (**Manilal *et al*., 2010**). Approximately 19,000 various species of macroalgae exist (**Dawes, 2016; Guiry and Guiry, 2019**).

Seaweeds are heterogeneous communities of photosynthetic species rather than land plants inhabiting coastal waters (**Lauritano *et al*., 2016; Abdelhamid *et al*., 2018 and Lezcano *et al*., 2018**). Often characterized based on their photosynthetic pigments, but also by variations in many ultra-structural and biochemical characteristics, including the form of storage material, the composition of the cell wall, presence/absence of flagella, mitosis ultrastructure, contacts between neighboring cells, and the fine chloroplast structure (**Rindi *et al*., 2012; Alves *et al*., 2013 and Balboa *et al*., 2013**). Algae are photosynthetic organisms; they are, however, obscured by photosynthetic pigments that give them a distinctive color used to describe main divisions (**Menetrez, 2012)**. Algae are usually split into two main groups, macroalgae and microalgae, depending on their morphology and scale. Macroalgae, generally referred to as seaweed, are typically present both in intertidal and subtidal environments in coastal areas and consist of numerous cells that organize into structures resembling higher plant roots, stems, and leaves; some species have gas-filled structures to provide buoyancy (**Chen *et al*., 2009**). Typically, they are classified into three divisions: green (Chlorophyta), red (Rhodophyta), and brown (Phaeophyceae) (**Dawes, 2016**).

Macroalgae are often subject to detrimental environmental conditions and the negative effects on them in vivo are not evident, suggesting their capacity to develop different metabolites (enzymes, pigments, polysaccharides, antioxidants, phenolics, tocopherols, phospholipids) that shield them from external influences (**Cox *et al*., 2012; Liu *et al*., 2012; Herrero *et al*., 2013; Chakraborty *et al*., 2015 and Dixit *et al*., 2018**). In seaweeds, phenolic and flavonoid compounds have been commonly identified, supporting their strong role in chelating metal ions, avoiding radical formation, and strengthening the internal antioxidant system in environmental conditions of stress. These activities defend the body from progressive diseases caused by the detrimental effects of reactive oxygen species (ROS) (**Chakraborty *et al*., 2013**).

In affecting public health, free radicals play an important role by causing such illnesses (e.g., heart diseases, cancer, hypertension, diabetes, and atherosclerosis). Antioxidants have proven their value in stopping multiple diseases containing free radicals over the last decade (**Lee *et al*., 2007)**. Due to their wide range of biological activities, such as antioxidant activities, marine macroalgae are the most interesting algae community (**Devi *et al*., 2011**). The natural antioxidant potential has been confirmed by many seaweed species that can preserve the human body from free radicals and delay the

progress of many chronic diseases such as hypertension, heart disease, diabetes, and cancer (**Ruberto *et al*., 2001; Shanab, 2007; Kokabi *et al*., 2013; Kolanjinathan *et al*., 2014 and Collins *et al*., 2016**). The study focused on the biochemical properties of species that are among the most abundant in our study area, which could become a natural supply for the nutritional, pharmaceutical, and medical sectors.

MATERIALS AND METHODS

1- Sampling Location and Algal Collection

Four species of seaweeds "green and red" (*Ulva fasciata, Ulva compressa, Corallina officinalis, and Corallina elongate)* were collected manually in June 2019. The seaweeds were collected from Eastern Harbor (latitude: 31°12'02.5"N and longitude: 29°53'33.4"E) located at Alexandria Mediterranean coast, Egypt, **(Fig.1)**.

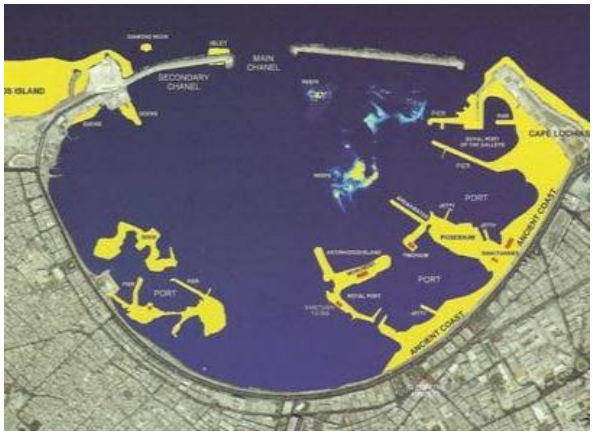
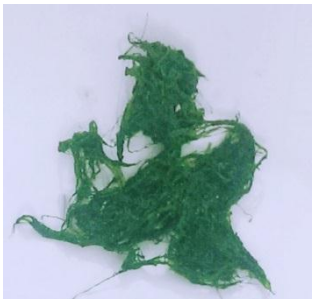


Figure (1): Samples collection area (Eastern harbor), Alexandria, Egypt

The chosen samples were collected and subsequently washed with seawater at the sampling site to extract the foreign particles, sand particles, and epiphytes. The samples were kept in an icebox after washing and immediately shipped to the laboratory, rinsed in deionized water to prevent metal loss during treatment. Seaweeds belonged to two divisions: *Ulva fasciata* (Delile) and *Ulva compressa* of Chlorophyta, and *Corallina officinalis* (Linnaeus) and *Corallina elongate* of Rhodophyta (**Guiry and Guiry, 2013**). of Rhodophyta **Figure 2**. Spread out at room temperature (25ºC) for drying, the dried samples were then homogenized with pestle and mortar and stored at 4ºC for further analysis.

Chlorophyta

Ulva fasciata



Ulva compressa

Rhodophyta

Corallina elongate

Corallina officianalis

Figure (2): Images of collected seaweed species

2- Pigment Analysis

Estimation of Chlorophyll contents

Estimation of Chlorophyll contents according to **Arnon (1949**). For Chlorophyll a and Chlorophyll b, the absorption of the extract was read at 663 and 645nm respectively, using UV spectrophotometers.

Extraction and estimation of carotenoids

The carotenoid content of seaweed was measured spectrophotometrically at 480 nm using the same extract used for chlorophyll estimation according to **Kirk and Allen (1965**).

3- Phytochemical screening of the algal extract

The method used in this experiment was the inclusion of some reagents giving a positive reaction. This analysis determined the concentration of total phenolic compounds, flavonoids, and antioxidant activity.

Preparation of seaweed extracts

One gram of dried sample was extracted with 50 ml of 80% methanol twice at room temperature followed by centrifugation for 10 minutes. The supernatant has been pooled and filtered into fresh tubes using (Whattman No. 1) filter paper and stored at 4ºC for further analysis.

Total phenolic content

The number of total phenols in seaweed extracts was determined by the Folin-Ciocalteu reagent method according to **Singleton and Rossi (1965**).

Total Flavonoids content

The colorimetric technique of aluminum chloride was used to analyze flavonoids by **Zhishen *et al*. (1999**).

4- Determination of Antioxidant Activity

The antioxidant activity in extracts and fractions of selected seaweeds was determined by the following assays:

DPPH radical scavenging capacity

The scavenging potential of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical seaweed extracts has been evaluated according to **Yen and Chen** (**1995**).

Total antioxidant capacity (TAC) assay

The total antioxidant potential of the extracts of seaweed was estimated according to the method of **Prieto *et al*. (1999**).

Total Reducing Capacity (TRC)

The extract's reducing power was calculated by **Oyaizu (1986**) method.

5- Statistical analysis

All the experiments were run in triplicates and the findings were expressed as means ± standard deviation. All statistics were carried out using statistics 8.1software. To describe the statistically significant variation between the studied seaweed parameters, variance analysis (one-way ANOVA).

RESULTS

1- Estimation of pigment content

The pigment composition of seaweeds is illustrated in **Figure 3.** The highest chlorophyll-*a* was remarked in *Ulva compressa* (1.6 mg g^{-1} FW), followed by *Ulva fasciata* (1.4 mg g^{-1} FW), then *Corallina officinalis* (0.80 mg g^{-1} FW), and *Corallina elongate* (0.60 mg g^{-1} FW). Chlorophyll *b* was observed to be the richest *Ulva compressa* (1.06 mg g^{-1} FW), while the lowest chlorophyll *b* was noted in *Corallina elongate* (0.33 mg g^{-1} FW). Consequently, the greater total chlorophyll value was reported in *Ulva compressa* (2.7 mg g^{-1} FW) and the minimum value was observed in *Corallina elongate* (0.90 mg g^{-1} FW).

In comparison, the maximum carotenoids were registered in *Corallina officinalis* (1.04 mg g^{-1} FW) followed by *Corallina elongate* (0.86 mg g^{-1} FW). The lowest ratio was recorded in *Ulva fasciata*(0.45 mg g^{-1} FW).

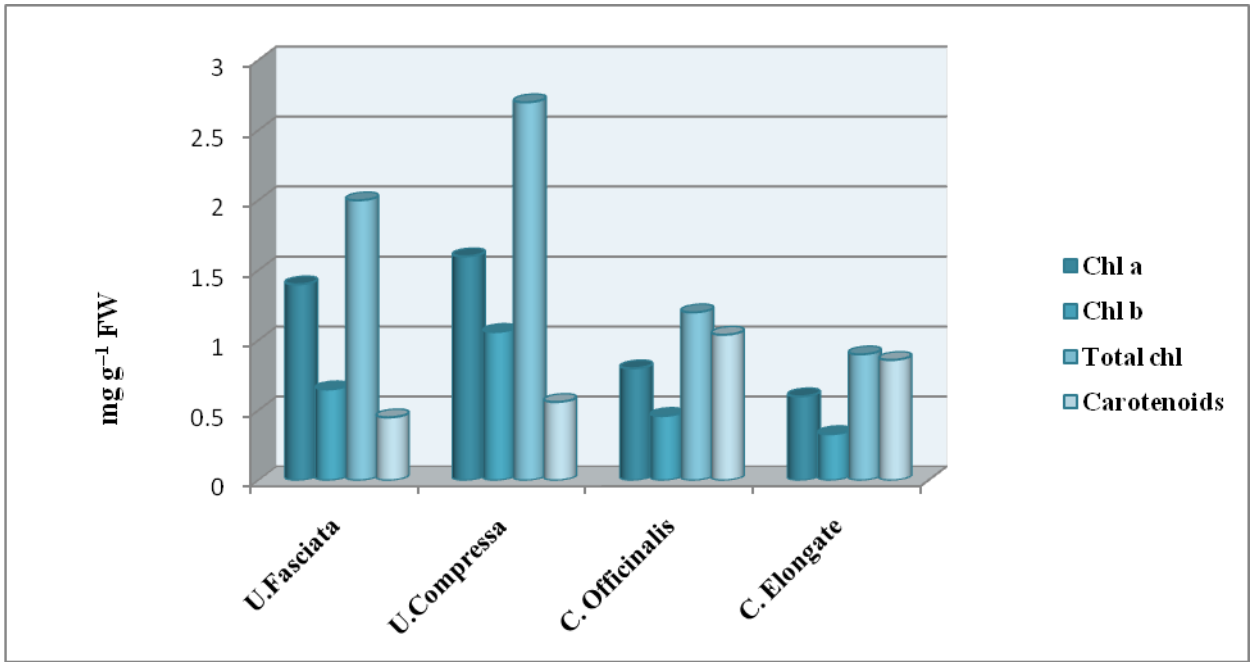


Figure (3): The pigment content of seaweeds

2- **Determination of total phenolic and flavonoids content**

Results of total phenolic and flavonoids content were presented in Table 1. Phenolic and flavonoids compounds play an important role in the cell defense against biotic and abiotic stresses in macroalgae. The highest phenolic content is recorded in the green seaweed species (12.7 and 10.5 mg/g DW in *Ulva compressa* and *Ulva fasciata* respectively) followed by red seaweeds (6.0 and 5.9 mg/g DW in *Corallina officinalis* and *Corallina elongate* respectively). Furthermore, flavonoids compounds registered the highest content in the green seaweed species (9.42 and 8.29 mg/g DW in *Ulva compressa* and *Ulva fasciata* respectively) followed by red seaweeds (3.82and 3.17 mg/g DW in *Corallina officinalis* and *Corallina elongate*, respectively).

Table 1: Determination of total phenolic and flavonoids content

Phenolic and flavonoids content (mg GE/g d.wt.)	***Ulva fasciata***	***Ulva compressa***	***Corallina officinalis***	***Corallina elongate***	**LSD**
Total phenolic content	10.5 ± 0.46^{b}	12.7 ± 0.10^{a}	6.0 ± 0.02^{c}	5.9 ± 0.13^{c}	0.79
Total Flavonoids content	8.29 ± 0.16^{b}	9.42 ± 0.18^{a}	3.82 ± 0.09^{c}	3.17 ± 0.04^{d}	0.42

3- Antioxidant activity

Three approaches have been used to determine the antioxidant activity of various seaweeds (DPPH, TAC, and TRC), results showed in Table 2. *Ulva compressa* has the maximum percentage of DPPH, TAC, and TRC (80.45, 12.5, and 71.6 respectively). While, the low percentage of DPPH, TAC, and TRC recorded in *Corallina elongate* (70.3, 8.4, and 57.8 respectively*).*

Table 2: Antioxidant activity

Species	DPPH (%)	TAC (%)	TRC (%)
Ulva fasciata	80.69±0.59^{b}	11.8±0.38^{a}	66.2±3.87^{a}
Ulva compressa	82.45±0.32^{a}	12.5±0.28^{a}	71.6±0.55^{a}
Corallina officinalis	71.67±0.35^{c}	9.7±0.09^{b}	59.3±0.09^{b}
Corallina elongate	70.3±0.32^{d}	8.4±0.15^{c}	57.8±0.02^{b}
LSD	1.33	0.8	6.37

DISCUSSION

The photo-protective plant pigment concentration and distribution vary with the season in micro-and macro-algae and the form of tissue in macro-algae species (**Paerl, 1984; Rowan, 1989).** Photosynthetic pigments are essential components of organic plant food processing, and photosynthetic activity is consistent with cellular viability (**Bezerra *et al*., 2008**). Three primary photosynthetic pigments (chlorophylls, carotenoids, and phycobilins), are found in seaweeds. These pigments protect the high strength of light and also help to capture light and transfer energy to the reaction center (**Khan and Khorshid Abbas, 2015**).

Chlorophylls (Chls) are greenish, non-polar pigments containing rings of porphyrin or hydro-porphyrin centrally bound to a magnesium atom present in all autotrophic algae, as they allow light to be converted into biological energy (**Senge *et al*., 2014).** Carotenoids are also non-polar pigments and by inactivating reactive oxygen species (ROS) produced during light exposure, play a key role in photoprotection. They belong structurally to the terpenoid pigment class and have strongly conjugated polyene chains that give them various colors, such as purple, red, orange, or yellow (**Poojary *et al*., 2016).** The pigment contents differed significantly concerning the algal taxa, stations, and depth observed by **Dere *et al*. (2003**).

The findings suggest that green algae contain higher levels of Chl a, Chl b, and total Chl. Several researchers have found that green algae contain higher levels of

chlorophyll than red algae. Alternatively, between the algal types, the carotenoid content fluctuated, with the highest in the red Corallina and the lowest in the green seaweeds. These observations were in line with the results of **Chandraprabha *et al*. (2012); Valentina *et al*. (2015) and Ismail *et al*. (2016**). The total pigment of *Ulva* spp. ranged between 2.52–5.22 mg/g DW stated by **Moustafa and Saeed (2014).** Also, a change in the concentration of pigments is a reaction to environmental changes that cause an organism to respond to a specific ecosystem (**Khan and Khorshid Abbas, 2015**). Pigments aid in cell connectivity and human health maintenance, have possible antimicrobial activities, and have promising applications in the food and pharmaceutical sectors, according to **Plaza *et al*. (2010**). Furthermore, algal carotenoids also provide a protective function against oxidative stress and cancer cell proliferation-related human diseases (**Astorg, 1997; Collins *et al*., 2016**).

The study of phenolics is affected by their composition; the extraction method used the particle size of the sample, storage conditions, and period, as well as the assay used in extracts such as waxes, fats, pigments for their determination and presence of intervening substances (**Shahidi and Naczk, 2003**). Due to several controlling factors, such as algal type, geographical origin or the region of production, seasonal, physiological, and environmental variations, various algal products have given various total phenolic contents (**Marinho-Soriano *et al*., 2006**). Seaweed phenolic compounds can chelate metal ions and prevent radical formation, thus strengthening the intrinsic antioxidant coordination, according to **Chakraborty *et al*. (2013**). In this way, phenols in the lipid peroxidation cycle that forms the aryloxyls convey hydrogen atoms to peroxyl. Aryloxyls are unable to function and thereby delay the peroxidation process as chain carriers for free radicals.

Flavonoids have demonstrated a wide variety of chemical and biological functions, including antioxidants, lipid peroxidation inhibitors, and medicinal agents for many diseases (**Sarojini *et al*., 2012**). In addition to defending against cardiovascular mortality, Flavonoid has shown anti-inflammatory, anti-hepatotoxic, and anti-ulcer effects (**Kokilam and Vasuki, 2014**). Flavonoids have strong anti-allergic, antiviral, and free radical abilities to scavenge (**Kähkönen *et al*., 1999**). Different works have been recorded by others **Ganesan *et al*. (2011); Mhadhebi *et al*. (2014) and Güner *et al*. (2015**) observed a higher amount of phenolic and flavonoids contents in *Ulva Compressa*.

There has been a strong association between total phenolic and flavonoid content with high antioxidant activity in some studies of **Senthil and Kamaraj (2011) and Farasat *et al*. (2014**). Our findings also suggest that the higher Antioxidant activity was found in extracts with greater Contents of phenolics and flavonoids, which are in agreement with **Duan *et al*. (2006) and Boonchum *et al*. (2011**).

Extensively, the DPPH test has been used as a free radical to measure reducing substances and it is a valuable reagent for the investigation of compounds' free radical

scavenging operation (**Duan *et al*., 2006**). The highest activity of free radical scavenging was detected, in various species of algae belonging to distinct species of phyla (**Bianco *et al*., 2015**). Antioxidants in the sample convert ferric (III) to ferrous (II) in a redox-related colorimetric reaction in the reduction strength assay (**Li *et al*., 2006**). The reduction capacity means that the antioxidant compounds are electron donors and that the oxidized intermediate of the lipid peroxidation phase is minimized so that they can serve as primary and secondary antioxidants (**Yen and Chen, 1995**). As a function of decreasing strength, concentration dependence of antioxidant activity was investigated as this provided a general view of reductones present in the sample. With rising concentrations in all the samples, decreasing strength improved.

CONCLUSION

The present study revealed that seaweeds are regarded as a source of bioactive compounds with antioxidant effects which immense pharmaceutical, biomedical, and nutraceutical prospected applications. Intense future studies should be conducted to use and develop these naturally economical resources.

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