Comparative assessment of antioxidant activity and biochemical composition of four seaweeds, Rocky Bay of Abu Qir in Alexandria, Egypt

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Abstract

The current study estimated the antioxidant activity and biochemical composition of four seaweeds; *Toania atomaria* (Phaeophyta), *Padina pavonia* (Phaeophyta), *Jania rubens* (Rhodophyta) and *Corallina elongate* (Rhodophyta). The highest total antioxidant activity was observed with ethanol extract of *T. atomaria* (44.6 ± 1.6 mg ascorbic acid equivalents/g crude extract). Among the four seaweeds, ethanol extract of *T. atomaria* has recorded the highest value in ferric reducing antioxidant power (FRAP) assay (32.3 ± 0.0 mg ascorbic acid equivalents/g crude extract). The uppermost level of carbohydrates was (20.4% ± 0.4 and 19.5% ± 0.7 dry wt.) in *P. pavonia* and *T. atomaria*. Proteins were maximum (10.5% ± 0.6 and 8.0% ± 0.0 dry wt.) in *P. pavonia* and *T. atomaria*. The results detected that *T. atomaria* had the largest amounts of lipids (5.92% ± 0.09 dry wt.). The polyunsaturated ω6 essential fatty acids were recorded in *T.atomaria*, *P. pavonia,* and *J. rubens* (11.39%, 8.09%, and 6.28%), and ω3 essential fatty acids were recorded in *T. atomaria* and *P. pavonia* (0.65% and 0.97%). The seaweeds are interesting candidates for medicinal, pharmaceutical, and nutritional applications owing to their antioxidant activities, biochemical composition; they are not widespread in the coastal waters of Egypt and has few studies, especially *T. atomaria*.

Keywords: marine macroalgae, carbohydrate, lipid, protein, ω3and ω6 essential fatty acids, total antioxidant capacity, ferric-reducing antioxidant power.

Practical Application: The search for new natural resources has proven to be healthy, economical, and environmentally friendly, like seaweeds applied as food, antioxidants, and biofuel producers.

1 Introduction

In living organisms, reactive oxygen species (ROS) are produced during the metabolic processes and caused oxidative stress impact (Santos-Sánchez et al., 2019). Various chronic diseases, such as atherosclerosis, cancer, and aging may be due to the oxidative damage of the reactive oxygen species on biomolecules (nucleic acids, proteins, and lipids) (Pirian et al., 2017). Antioxidants may mitigate the negative effects of oxidative stress (Larson, 1995; Adwas et al., 2019). Butylated hydroxyl anisole (BHA) and Butylated hydroxyl toluene (BHT), which are synthetic antioxidants, were showed to be poisonous and carcinogenic in animal models, so need to be replaced with natural antioxidants (Safer & Al-Nughamish, 1999; J Mbah et al., 2019). Furthermore, novel resources of safe and cheap antioxidants of the natural source should be identified.

Seaweeds are marine, photosynthetic algae that are abundant in every ocean. There are three main phyla of seaweed: Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) (Shannon & Abu-Ghannam, 2019). Strong antioxidant properties have shown with seaweed extracts (Ibrahim et al., 2016; Mohy El-Din & El-Ahwany, 2016; Ismail et al., 2019; El-Shenody et al., 2019; Al-Araby et al., 2020). Barba (2017) and Lopez-Santamarina et al. (2020) reported that marine macroalgae contain an excellent source of bioactive components like dietary fibers, carotenoids, essential fatty acids, protein, minerals, and vitamins. In many Asian countries such as China, Japan, and Korea, seaweeds have been used as a food resource (Wijesekara et al. 2012).

The most remarkable biochemical compounds of algae are carbohydrates, proteins, and lipids. Khairy & El-Shafay, (2013) mentioned that lipids are commonly found in many algal resistance stages. Studies on the fatty acids of seaweeds and microalgae were few (Lie Ken Jie, 1989; Khairy & El-Shafay, 2013). The biomass of seaweeds can store high contents of oil, which can be consumed for biodiesel production (John & Anisha, 2012; Peng et al., 2020). For several coastal areas of the world, a few populations of *Padina* have usually been used as a food supply, generally recognized as gelatin-like sweetmeat (Robledo & Freile Pelegrín, 1997).

In the present study, *T. atomaria* is an annual photophilic species of the infralittoral zone, found in calm areas, with a greater occurrence in the early summer and very few individuals from late summer to late winter (Sala & Boudouresque, 1997). Coralline algae (Rhodophyta) play a vital role in fostering dominion of other benthic organisms, becoming the source of food source for herbivores, involving in the stabilization of reef networks, and in the production of carbonate. One of the most popular

coralline species, *Jania rubens*, that may survive as epiphytic or epilithic tufts in shallow environments (Porzio et al., 2018).

The purpose of this study is to search the biochemical content and fatty acid profile of four different seaweeds *T. atomaria*, *P. pavonia*, *J. rubens*, and *C. elongate*, which are commonly found in Rocky Bay of Abu Qir in Alexandria, Egypt. The pharmaceutical, medicinal, and nutritional applications of the studied seaweeds were confirmed by estimating the antioxidant activities of these seaweed extracts.

2 Materials and methods

2.1 Seaweeds collection

Jania rubens, *Coralina elongate*, *Padina pavonia*, and *Toania atomaria* were collected freshly from Rocky Bay of Abu Qir in Alexandria, Egypt (Figure 1). Collected samples were washed carefully with marine water then tap water, transferred in an iced condition to the Phycology laboratory. The collected samples were air-dried in the shade at room temperature then in the oven at 38 ± 2 °C; the dried seaweeds were ground to a fine powder and stored for further studies in tightly closed containers. For taxonomical identification, a portion of the collected samples were stored in 3-4% formalin in seawater or distilled water. The seaweeds were identified according to (Aleem 1993; Jha et al. 2009; Kanaan & Belous 2016), then using the Algae Base website to confirm their identification and the habitat details (Guiry & Guiry, 2019).

2.2 Preparation of the algal extracts (Conventional method)

The extraction was performed with several solvents (methanol, ethanol, hexane, diethyl ether, aqueous cold, aqueous hot) by steeping the material in the corresponding solvents (1:30 w/v) within a conical flask then closed with cotton wool. Next, retained on a rotatory shaker at 120 rpm at 20-30 °C for 2 days. The extracts were filtered, and the filtrate put in the oven at 45 °C to get rid of the solvent. The crude extracts were suspended in the corresponding solvents to obtain a final concentration of 5 mg/mL then kept at -20 °C in an airtight container to control the microbial contamination.

For the hot water extraction, soaking 1 g of seaweed powder in 30 mL distilled water for 2 hours at 60 °C then repeated the previous steps for extract preparation as mentioned above.

The extraction yield percentage of each extract was calculated according to (Maisuthisakul & Pongsawatmanit, 2004): Extraction yield%= (W1/W2)*100.

Where W1 is the dried crude extract weight, and W2 is the sample weight before extraction (1 gm).

2.3 Antioxidant activities estimation

Total Antioxidant Capacity (TAC) assay

The total antioxidant capacity (TAC) was determined, as reported by (Sun et al., 2011). In brief, 0.3 mL of the crude extracts was added to 3 mL of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid). Absorbance was measured at 695 nm against a blank). Total antioxidant capacity was expressed (mg ascorbic acid equivalents/g crude extract).

Ferric- reducing antioxidant power (FRAP) assay:

Ferric- reducing antioxidant power (FRAP) was estimated by the method reported by (Zubia et al., 2007). Aliquots (0.5 mL) of the extracts were added to 1.25 mL of sodium phosphate buffer (0.2 M, pH 6.6), 1.25 mL of (1%) potassium ferricyanide ($K_3Fe(CN)_6$). Afterward, the mix was kept at 50 °C for 20 min in a water bath. The mixture was cooled and mixed with 1.25 mL of (10%) trichloroacetic acid, and then 1.25 mL of the mixture was added to 1.25 mL distilled water, 0.25 mL of (0.1%) ferric chloride solution ($FeCl_3.6H_2O$). Absorbance was

Figure 1. Map of Abu Qir showing collection site (Abu Qir bay located in Abu Qir, Alexandria).

measured against blank. Higher absorbance marked increased reducing power. Ferric- reducing antioxidant power (FRAP) was reported as mg ascorbic acid equivalents/g crude extract.

2.4 Chemical composition of Seaweeds

Estimation of total soluble carbohydrates and total soluble protein

The sample (0.1 g powder) was extracted with NaOH (1 N) in a water bath at 100 °C for 2 hours, cooled the extract, and centrifugation done as represented by (Payne & Stewart, 1988).

- A- Total content of soluble carbohydrates

Total soluble carbohydrate was quantitatively estimated by the method of Phenol-Sulphoric acid described by (Kochert, 1973). The absorbance was determined at 490 nm against a blank. The carbohydrate concentration was stated after the creation of a calibration curve using glucose as a standard. Total carbohydrate content was given as a percentage of the algal dry weight %DW.

- B-Total content of soluble protein

Total soluble protein was assessed using the method of Bradford (1976). The absorbance was measured at 595 nm against a blank. Bovine Serum Albumin was used as a standard to evaluate the calibration curve. Total protein content was given as a percentage of the algal dry weight %DW.

Estimation of lipids

Total lipid content

Lipid was determined as reported by the modified Folch method (Folch et al., 1957) with some modifications.

Composition of fatty acids

- A- Preparation of methyl ester of fatty acids

The transmethylation of lipids and extraction of fatty acid methyl esters (FAMEs) were prepared from aliquots comprising total lipids, as mentioned by (Radwan, 1978), stored at 4 °C in the dark before GC–MS analysis.

- B- Gas liquid chromatography of methyl esters of fatty acids.

GC-MS spectrophotometry (Perkin Elmer model: Clarus 580/560 S) was used for the analysis of the fatty acid methyl esters (FAMEs). Acquisition parameters were oven initial temperature 60 °C for 2 min which ramped from 10 °C/min to 280 °C, hold 6 min, Injector temperature = 250 °C, Source temp = 200 °C, Transfer temp = 250 °C, Column (Rxi-5Sil MS column 30 m, 0.25 mm ID, 0.25 df), Scan = 50 to 500Da, Split ratio = 20:1, Injectionvolume = 1.0 µL of fatty acid methyl esters, Carrier gas = Helium, Solvent delay = 5.00 min.

2.5 Statistical analysis

All the assays were performed in triplicate, and the means ± standard deviation (SD) were recorded. The seaweed extraction yield and antioxidant activities were compared by custom tables using the SPSS 23.0 software, significant values at $p < 0.05$. The statistically significant difference between the chemical composition of seaweed was identified by the analysis of variance (One-way ANOVA) followed by Duncan's multiple range test for data with significant differences, at $p < 0.05$, using the SPSS 23.0 software.

3 Results and discussion

The extraction efficiency is strongly influenced by the extraction method, extraction time, the solvent used, temperature, and the phytochemicals composition (Turkmen et al., 2006; van Ngo et al., 2017). According to the findings of this study, the solvent is identified as one of the most important parameters under the same extraction conditions. The current study used distilled water (cold and hot) and organic solvents (methanol, ethanol, diethyl ether, and hexane) to extract bioactive compounds from collected seaweeds.

3.1 Taxonomic description of collected seaweeds

The collected species were identified as presented in the literatures, checked for synonyms and latest accepted names, referred to its systematic groups, and described. The collected species were *Padina pavonia* (Linnaeus) Thivy or *Padina pavonia* (Linnaeus) J.V.Lamouroux and *Taonia atomaria* (Woodward) J. Agardh from Phaeophyceae, *Jania rubens* (Linnaeus) J.V.Lamouroux, *Corallina elongate* Ellis et Solander from Rhodophyceae (Figure 2A, B, C and D).
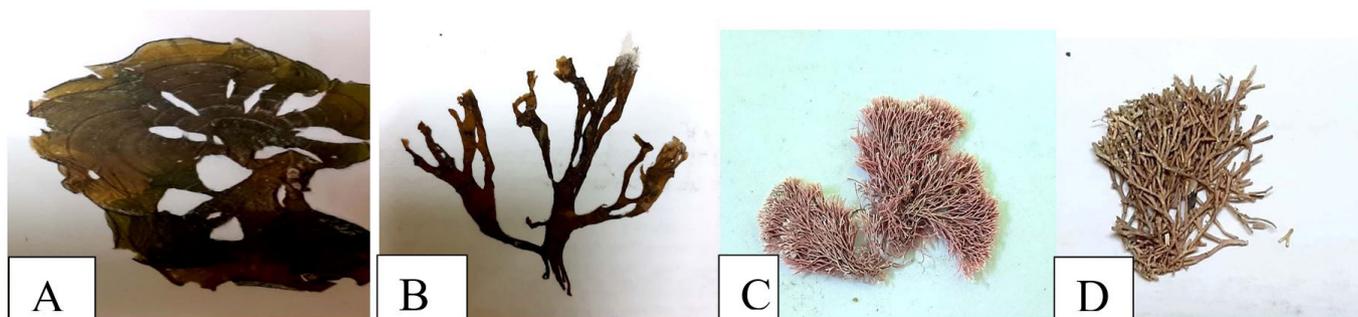
Figure 2. Photos of the studied seaweeds (A) *Padina pavonia* (B) *Taonia atomaria* (C) *Jania rubens* (D) *Corallina elongate*.

3.2 Extraction yield

Different solvents with various polarities were used for extraction. Also, the obtained crude extracts were used to evaluate the antioxidant activities using different methods due to the presence of different bioactive components with antioxidant potential. Moreover, the polarity index of extraction solvents was: methanol (5.1), ethanol (5.2), water (9.0), diethyl ether (2.8), and hexane (0.1). Table 1 indicates that the extraction yield increased with increasing solvent polarity. The trend indicated relatively greater yields of polar solvents vis a vis non-polar solvents in red and brown algae, while the brown alga remains the highest yielding. This result is consistent with (Truong et al., 2019), who observed that the extraction efficiency favors the highly polar solvents in *Severinia buxifolia*. This may be due to the plant material has great levels of polar compounds which are soluble in solvents with a high polarity such as water, methanol, and ethanol. In order to better understand the solvents effect on extraction yield, further analysis was performed to measure the antioxidant potential of bioactive compounds in the extract. These large variations in extraction yield detected among different seaweed species may be due to the fact that the polarities of different components present in the seaweeds and also species–species differences (Agregán et al., 2017, 2018).

Extraction yield of four seaweeds collected extracted with different solvents (aqueous cold, aqueous hot, methanol, ethanol, diethyl ether, and hexane) are recorded in (Table 1). With regard to the effect of different extraction solvents on the yield of the studied seaweeds, *T.atomaria* (16.5% ± 0.2) recorded the highest yield value with methanol extract; however, *J. rubens* (0.2% ±0.0) showed the lowest yield value in hexane extract. The yield of methanolic extract in the current study was larger as compared to the earlier study by (Chakraborty et al., 2015), who recorded a 5.32 g/100 g dry sample of total methanol extract in *J. rubens* (red seaweed). In the present study, the methanol extract yield was higher than the values previously reported, including *Padina pavonica* (6.5%), *Colpomenia sinuosa* (4.7%), *Cystoseira myrica* (4.3%) and *Ulva lactuca* (3.3%) (Kokabi et al., 2013). Furthermore, the yield of the extracts varies, ranging from 1.5 to 4.1% in other types of seaweeds, such as red seaweeds (Ganesan et al., 2008).

3.3 Evaluation of antioxidant activities

Antioxidants turn reactive oxygen species to non-toxic compounds, inhibit the adverse effects of reactive oxygen species and reduce some disorders, such as infections and ischemia,

diabetes infections, cardiovascular and cancer diseases (Rady et al., 1994; Al-Dabbas, 2017). Hence, in this study, the evaluation of antioxidant activities of the collected seaweeds extracted with different solvents carried out by two methods:

1. Total Antioxidant Capacity (TAC) assay.

2. Ferric reducing antioxidant power (FRAP) assay.

Total Antioxidant Capacity (TAC) assay

The attention of researchers was focused on the ability of algae to accumulate antioxidant compounds, as the environment in which seaweeds grow is harsh and can cause the formation of strong oxidizing agents; however, seaweeds rarely know any severe photodynamic damage during metabolism. Such a fact means that the cells of seaweed have developed a complex protective system to bear salinity (Zubia et al., 2007). The quantitative total antioxidant capacity (TAC) of the algal extract was assessed by a phosphomolybdenum assay. The TAC of the extracts or standards was measured depend on the reduction of molybdenum (Abifarin et al., 2019). Biologically, antioxidants perform their health-useful roles by transferring an electron or hydrogen (H) atom to reactive species, so inhibit them (Apak et al., 2016). Thus, measurements of the antioxidant potential may generally be through hydrogen atom transfer (HAT) and single electron transfer dependant assays (SET). SET assays determine the potential of an antioxidant to reduce an oxidant, which alters colour when reduced. SET assays such as Total Antioxidant Capacity (TAC) and Ferric reducing antioxidant power (FRAP) assays were able to measure the reduced capacity. Pérez-Jiménez & Saura-Calixto, (2006) stated that the type and polarity of solvent might affect the hydrogen atom transfer and the single electron transfer, which are major aspects in the estimation of antioxidant capacity. However, the total antioxidant activity of 245– 376 mg ascorbic acid/g crude extract has been detected in extracts of a higher plant (Kumaran & Karunakaran, 2007). Published studies on the total antioxidant activity of seaweed extracts are not available. As well, it has been mentioned that solvents used for extraction have a significant effect on the chemical species (Yuan et al., 2005).

The total antioxidant activity of the four seaweeds extracted with different solvents was recorded in Table 2. The present results showed that methanol, ethanol, diethyl ether, hexane, aqueous cold, and hot aqueous extracts of four seaweeds (Rhodophyta and Phaeophyta) possessed antioxidant activities. The methanol

Table 1. Yield value of four seaweeds collected from Rocky Bay of Abu Qir in Alexandria, Egypt extracted with different solvents (as % w/w of seaweed on dry weight basis).

Seaweeds	Solvents					
	Methanol	Ethanol	Aqueous hot	Aqueous cold	Diethylether	Hexane
	Yield (%)					
P. pavonia	7.9 ± 0.8[Aa]	15.6 ± 0.2[Ba]	5.5 ± 0.3[Ca]	6.9 ± 0.7[Aa]	1.1 ± 0.0[Da]	0.3 ± 0.0[Da]
J. rubens	2.7 ± 0.0[Ab]	2.0 ± 0.1[Bb]	1.4 ± 0.1[Cb]	5.0 ± 0.2[Db]	0.5 ± 0.0[Eb]	0.2 ± 0.0[Fa]
T.atomaria	16.5 ± 0.2[Ac]	10.5 ± 0.1[Bc]	12.3 ± 0.2[Cc]	13.5 ± 0.1[Dc]	4.8 ± 0.1[Ec]	4.3 ± 0.0[Fb]
C.elongate	2.1 ± 0.2[Ab]	1.8 ± 0.2[Ab]	4.6 ± 0.1[Bd]	4.4 ± 0.2[Bb]	0.8 ± 0.2[Cb]	1.6 ± 0.2[Ac]

Values are the mean of three replicates ± SD; Values with the same capital letter in the same row showed insignificant differences (at P< 0.05); Values with the small letter in the same column showed insignificant differences (at P< 0.05).

extract showed the highest antioxidant activities for all tested seaweeds except *T. atomaria*. *T. atomaria* observed the highest antioxidant activity in ethanol extract (44.6 ± 1.6 mg ascorbic acid equivalents/g crude extract), and it recorded the highest value between different solvents and seaweeds this action may be due to Lipids (soluble Lipids, PUFAs, and steroids) (El Baz et al., 2014), sterol fraction (Ibrahim et al., 2016), taondiol and epitaondiol metabolites (Nahas et al., 2007) which had high antioxidant activity. Hexane extract showed the lowest antioxidant activities for all tested seaweeds except *P. pavonia*, which had the lowest antioxidant activity with diethyl ether extract (0.5 ± 0.1 mg ascorbic acid equivalents/g crude extract). *J. rubens* showed the lowest antioxidant activity in hexane extract with 0.14 ± 0.0 mg ascorbic acid equivalents/g crude extract. Data presented in Table 2 indicated variations in TAC, which may be attributed to species differences. Accordingly, all the studied species are considered a rich source of antioxidants. Indu & Seenivasan (2013) reported that brown seaweeds possessed good antioxidant activity when compared to red and green seaweeds, and this explains that brown algae *T. atomaria* and *P. pavonia* exhibited the highest TAC. In *J. rubens,* methanol and ethanol extract give nearly equal results in TAC. In general, methanol was the most effective solvent for the extraction of antioxidant compounds from seaweeds, which may be because methanol having a larger dielectric constant than ethanol. We found that methanol extracts of *J. rubens*, *P. pavonia,* and *C. elongate* more reactive than the other extracts. Similar results were previously obtained by (Mohy El-Din & El-Ahwany, 2016), who stated that methanol extract of *Corallina mediterranea* was found to have the highest total antioxidant capacity. Other reports informed that methanol, etahanol, acetone and water extract of *Padina pavonica* (Ismail et al., 2019), sterol fraction of *Toania atomaria* (Ibrahim et al., 2016), methanol, ethanol, acetone and chloroform extract of *Jania rubens* (Mohy El-Din & El-Ahwany, 2016) and chloroform extract of *Jania rubens* (Saeed et al., 2020) had a significant antioxidant activity in Total Antioxidant Capacity assay. It is still unobvious what type of solvent is the most efficient and favorable for seaweeds extraction. Although different solvents have been used in screening algae for antioxidants (Yi et al., 2001; Manch et al., 2014). The antioxidant activity might be attributable to the presence of PUFAs and lipophilic antioxidants such as phenolic and terpenoid compounds, total carotenoids, and α-tocopherol (El Baz et al., 2014).

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) in the crude extracts of the four collected algae were recorded in Table 3. In FRAP, The ability of the antioxidant compounds in the extract to reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction that includes single electron transfer determined the antioxidant activity (Li et al., 2006; Sumayya & Murugan, 2019). With regard to different solvents, ethanol extract of *T. atomaria* recorded the highest ferric reducing antioxidant power (FRAP) (32.3 ± 0.0 mg ascorbic acid equivalents/g crude extract), and these values were the highest between solvents and seaweeds. Hexane and diethyl ether extracts showed the lowest ferric, reducing antioxidant power (FRAP) for all tested seaweeds. Duh (1998) stated that reducing properties are generally related to the presence of reductions. Reductions also prevent peroxide formation as they react with certain precursors of peroxide. The results indicate that the remarkable antioxidant activity of the tested seaweeds extracts was due to their reducing power. The components from these seaweeds may work as reductions by donating electrons and reacting with free-radicals to convert them to more stable products and ending the free-radical chain reaction.

Table 2. Total antioxidant activity (mg ascorbic acid equivalents/g crude extract) of four seaweeds extracted with different solvents (concentration of extracts used = 5 mg/mL).

Seaweeds	Solvents					
	Methanol	Ethanol	Aqueous hot	Aqueous cold	Diethyl ether	Hexane
	TAC (mg ascorbic acid equivalents/g crude extract)					
P. pavonia	30.8 ± 2.1[Aa]	19.8 ± 0.4[Ba]	15.3 ± 0.4[Ca]	5.1 ± 0.1[Da]	0.5 ± 0.1[Ea]	2.6 ± 0.2[Ea]
J. rubens	31.2 ± 1.1[Aa]	30.4 ± 1.2[Ab]	13.7 ± 0.3[Bb]	16.5 ± 0.1[Cb]	7.6 ± 0.1[Db]	0.14 ± 0.0[Eb]
T. atomaria	37.6 ± 2.0[Ab]	44.6 ± 1.6[Bc]	14.6 ± 0.2[Ca]	19.9 ± 0.2.7[Db]	7.6 ± 0.0[Eb]	5.7 ± 0.2[Ec]
C. elongate	25.1 ± 0.3[Ac]	17.3 ± 1.0[Ba]	17.3 ± 0.1[Bc]	4.0 ± 0.1[Ca]	2.6 ± 0.0[Dc]	0.38 ± 0.1[Eb]

Values are the mean of three replicates ±SD; Values with the same capital letter in the same row showed insignificant differences (at P< 0.05); Values with the small letter in the same column showed insignificant differences (at P< 0.05). TAC= total antioxidant capacity.

Table 3. Ferric reducing antioxidant power (FRAP) assay (mg ascorbic acid equivalents/g crude extract) of four seaweeds extracted with different solvents (concentration of extracts used = 5 mg/mL).

Seaweeds	Methanol	Ethanol	Aqueous hot	Aqueous cold	Diethylether	Hexane
	FRAP(mg ascorbic acid equivalents/g extract)					
P. pavonia	13.6 ± 0.2[Aa]	11.2 ± 0.3[Ba]	6.7 ± 0.2[Ca]	4.3 ± 0.1[Da]	0.4 ± 0.1[Ea]	0.8 ± 0.2[Ea]
J. rubens	22.7 ± 1.0[Ab]	22.4 ± 1.7[Ab]	15.4 ± 0.4[Bb]	18.5 ± 1.4[Cb]	0.7 ± 0.0[Db]	0.35 ± 0.0[Db]
T. atomaria	22.6 ± 0.8[Ab]	32.3 ± 0.0[Bc]	21.7 ± 0.0[Ac]	21.8 ± 0.1[Ac]	2.5 ± 0.2[Cc]	4.1 ± 0.2[Dc]
C. elongate	4.1 ± 0.1[Ac]	3.3 ± 0.2[Bd]	3.5 ± 0.0[Bd]	0.1 ± 0.0[Cd]	0.1 ± 0.0[Cd]	0.25 ± 0.0[Cb]

Values are the mean of three replicates ±SD; Values with the same capital letter in the same row showed insignificant differences (at P< 0.05); Values with the small letter in the same column showed insignificant differences (at P< 0.05).

The present data are in accordance with (Kelman et al., 2012; Ismail et al., 2017), who reported that the antioxidant activity of brown algae was higher than red groups. Ismail et al. (2017) found that FRAP values of 31.098 and 25.858 mg ascorbic acid equivalents/g extract for *Sargassum wightii* and *Jania rubens*, respectively showed the greatest reducing power activity and this findings appeared in line with our results, showed that methanol extract of *J. rubens* recorded higher reducing power than *P. pavonia*.

3.4 Estimation of primary metabolites (Carbohydrate, protein, and lipid)

Algae have been one of the most versatile sources of bioactive compounds, and investigation on their chemical composition has significantly extended in the past three decades (Cardozo et al., 2006; O'Sullivan et al., 2010). In general, the nutritional values of seaweeds are defined according to biochemical composition like carbohydrates, proteins, lipids, and ash content (McDermid & Stuercke, 2003). Carbohydrates, protein, and lipids are the most important biochemical components in algal biomass. Significant differences ($p \leq 0.05$) were recorded in carbohydrate, protein, and lipid contents (Table 4). Yoshii et al. (2004) stated that the ratios of each constituent change between the species based on the taxon. The results showed that the carbohydrate contents were more numerous than the protein and lipid contents.

The total soluble carbohydrate contents of the studied seaweeds ranged from 6.4% to 20.4%. *P. pavonia* (20.4% ± 0.4) and *T. atomaria* (19.5% ± 0.7) was recorded the highest value in carbohydrate contents, while the lowest value showed with *C. elongate* (6.7% ± 0.5) and *J. rubens* (6.4% ± 0.4). Similar values were found in the brown alga *Sargassum horneri* (19.93% DW) (Hossain et al., 2003). These variations may be due to the species difference, growth stage of each species, habitat, metabolic preferences, and photosynthetic activity (Pádua et al., 2004). The brown algae were rich in carbohydrate content than red algae, and these findings are in agreement with (El-Shenody et al., 2019). Carbohydrate usually contains excellent amounts of polysaccharides, for example, carrageenan in the red seaweeds, alginate, and fucoidan in the brown seaweeds, so this high content of carbohydrate is essential for the metabolism of the organism. Venkatesan et al. (2017) and Barbosa et al., (2019) demonstrated that promising biomedical applications, including antibacterial, antiviral, anticoagulant, anti-inflammatory, and anticancer properties, the use as drug coatings in drug delivery systems and antioxidant activities were showed by polysaccharides. The fermentation of macroalgal carbohydrates can be consumed for the production of bioethanol (John & Anisha, 2012; Sudhakar et al., 2018; Offei et al., 2019).

Similar to the results obtained in the present study (Table 4). Fleurence et al. (2012) showed that the seaweeds protein content is comparatively low, among 3% to 15% on the dry weight basis. The results explained here recorded the highest content of protein in the brown seaweeds (*P. pavonia* and *T. atomaria*), and the lowest amount in the red seaweeds (*C. elongate* and *J. rubens*). Similar protein contents for these macroalgae classes were reported in other studies (Polat & Ozogul, 2013; El-Shenody et al. 2019). Stirk et al. (2007) and Gressler et al. (2010) reported that the cause of these variations could be that the seaweeds protein content changes between species and within the same species because of levels of maturity, time of the year and different habitats.

Lipids supply much more power in oxidation processes than other biological components as they have abundant -C=O- bonds. For living organisms, they comprise a suitable storage material, mainly due to their large reduction levels. The differences in lipid contents were assigned to either environmental parameters or species types, or both of them (Chandini et al. 2008). Brown seaweeds usually had the highest total lipid content and then red seaweeds (Table 4) and this evaluation is in accordance with the findings of Gosch et al. (2012) and El-Shenody et al. (2019).

Total lipids were showed to be in general low. On the dry weight basis (%), the lipid contents of the four species were between 0.33% and 5.92%, depending on the algal species (Table 4). The highest lipid content was recorded in *T. atomaria* (5.92% ± 0.09), and the lowest in *C. elongate* 0.33% ± 0.06. Herbreteau et al. (1997) stated that total lipid contents were always below 4% in most species. This generalized evaluation is in agreement with the results of, Gosch et al. (2012). The relatively high total lipid content (5.92% ± 0.09) observed in *T. atomaria* are explained by Gosch et al. (2012) and El-Shenody et al. (2019) who found that some species are distinct, as the species of the order Dictyotales, which can record total lipid contents more than 20% of the dry weight. While the result showed for the red algae *J. rubens* (1.42% ±0.0) was higher than the result reported by Polat & Ozogul (2013), who mentioned the total lipid content (%DW) for *J. rubens* in different seasons, which varied between 0.19% and 0.85%. The result obtained for *P. pavonia* (1.42% ± 0.0) was consistent with the total lipid content detected in the *P. pavonia* earlier investigated, which ranged from 2.87% to 3.87% of the dry weight in different seasons (Polat & Ozogul, 2013).

3.5 Fatty acids profile of collected seaweeds

Variations in fatty acid contents are due to both environmental and genetic diversity (Nelson et al., 2002). This diversity may be owing to their having been collected at different sites and/or to the drying effects (Chan et al., 1997). Considering the fatty acids composition, 25 components were showed with varying amounts between the four collected species (Table 5 and Figure 3). The percentage of saturated fatty acids (SAFA) of the collected seaweeds could be arranged in the following sequence

Table 4. Total soluble carbohydrate, total soluble protein, and lipid content in the four studied seaweeds.

Primary metabolites (%DW)			
Seaweeds	Carbohydrate	Protein	Lipid
P. pavonia	20.4 ± 0.4[a]	10.5 ± 0.6[a]	1.28 ± 0.01[c]
J. rubens	6.4 ± 0.4[c]	5.1 ± 0.3[d]	1.42 ± 0.0[b]
T. atomaria	19.5 ± 0.7[b]	8.8 ± 0.3[b]	5.92 ± 0.09[a]
C. elongate	6.7 ± 0.5[c]	8.0 ± 0.0[c]	0.33 ± 0.06[d]
F-value	851.54	119.08	6699.82
p-value	0.0001	0.0001	0.0001

Data are expressed as the mean ± standard deviation (SD) of three replicates. Different letters represent the statistical comparisons between groups by using one-way ANOVA and post hoc Duncan's test (p<0.05). DW = Dry Weight.

J. rubens>P. pavonia >C. elongate>T. atomaria. Red algae showed high percentages of SAFA than the brown algae, and these results agree with previous studies (Khairy & El-Shafay, 2013; Polat & Ozogul, 2013; Ismail, 2017; El-Shenody et al., 2019). Nine saturated fatty acids (SAFA) were detected in the tested seaweeds. Palmitic acid methyl ester (C17:0) was the major saturated fatty acids (SAFA) found in all species with different percentages, and the highest percentages were showed in *J. rubens* (68.3%). Palmitic acid methyl ester (C17:0) was the largest constituent between the SAFAs in the present study, and this in agreement with most studies (Gressler et al., 2010; Fatma et al., 2015).

Eight monounsaturated fatty acids (MUSFA) were investigated in the four studied species; the highest percentage of MUSFA was (50.09%) in *C. elongate*. The percentages of MUFA in red algae *C. elongate* were greater than *P. pavonia* and *T. atomaria* and this in agreement with those of (Khairy & El-Shafay, 2013). Palmitoleic acid methyl ester (ω7) (C17:1) and Oleic acid methyl ester (ω9) (C19:1) were the most dominant monounsaturated fatty acids (MUSFA) and this in agreement with (Ismail et al.,

2016; El-Shenody et al., 2019). Oleic acid, an ω9 fatty acid, showed the largest percentage between the MUSFA in *T. atomaria* and *P. pavonia*, in accordance with the findings of El Baz et al. (2014) and Fatma et al. (2015) observed that oleic acid presented the greatest fraction of the MUSFA in *T. atomaria* and *P. pavonia*. It is important to the point that the fatty acid contents of the four seaweeds may showed their suitability for production of biofuel, since the four studied seaweeds record very large percentages of SAFAs when compared to the MUSFA and PUSFA (Gosch et al., 2012; Piligaev et al., 2019), with a relatively high content of MUSFA which would improve the biofuel quality (Knothe, 2008; Zhang et al., 2020).

Seven Polyunsaturated essential fatty acids (PUSFA) were appeared in (Table 5 and Figure 3), Eicosapentaenoic acid (EPA) acid (omega-3), which is the major dietary base for aquaculture, comprised the main sources of omega-3 and omega-6 long-chain PUFAs in seaweeds. The omega (ω3) fatty acids are stated as α-Linolenic acid (ALA) methyl ester (C19:3), Stearidonic acid methyl ester (C19:4) and Eicosapentaenoic

Table 5. Fatty acids composition of the four studied seaweeds (% of the total of fatty acid).

IUPAC name	Common name	Number of carbon atoms	*P. pavonia*	*J. rubens*	*T. atomaria*	*C. elongate*
			Area%			
Tridecanoic acid, methyl ester	Methyl tridecanoate	C14:0	ND	ND	0.68	ND
Methyl tetradecanoate	Myristic acid ME	C15:0	5.47	6.6	6.3	12.09
pentadecanoic acid ME	-	C16:0	0.56	1.92	ND	ND
4,7,10,13-Hexadecatetraenoic acid methyl ester	-	C17:4	ND	ND	ND	ND
9-Hexadecenoic acid, methyl ester, (Z)-	Palmitoleic acid ME	C17:1(ω7)	8.07	8.12	4.25	ND
7-Hexadecenoic acid, methyl ester, (Z)-	-	C17:1	0.28	ND	0.27	ND
Hexadecanoic acid, methyl ester	Palmitic acid ME	C17:0	43.9	68.3	36.12	37.8
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	-	C18:0	0.27	ND	ND	ND
cis-10-Heptadecenoic acid, methyl ester	-	C18:1	ND	ND	ND	50.09
Heptadecanoic acid, methyl ester	Margaric acid ME	C18:0	0.26	ND	ND	ND
all-cis-9,12,15-octadecatrienoic acid	(α-Linolenic acid) (ALA) ME	C19:3 (ω3)	0.44	ND	2.34	ND
Methyl stearidonate	Stearidonic acid ME	C19:4 (ω3)	6.87	ND	9.42	ND
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid ME	C19:2(ω6)	4.29	ND	4.57	ND
9-Octadecenoic acid (Z)-, methyl ester	Oleic acid ME	C19:1 (ω9)	18.7	6.59	22.71	ND
15-Octadecenoic acid, methyl ester	-	C19:1	1.39	ND	ND	ND
trans-13-Octadecenoic acid, methyl ester	-	C19:1	ND	ND	ND	ND
14-Octadecenoic acid, methyl ester	-	C19:1	0.25	ND	ND	ND
Methyl stearate	Stearic acid ME	C19:0	2.08	2.13	0.81	ND
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)	Arachidonic acid ME	C21:4 (ω6)	3.79	6.28	6.8	ND
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)	Eicosapentaenoic acid (EPA) ME	C21:5 (ω3)	0.99	ND	5.72	ND
8,11,14-Eicosatrienoic acid, methyl ester	Dihomo- gamma- linolenic acid ME	C21:3	0.71	ND	ND	ND
8,11,14,17-Eicosatetraenoic acid, methyl ester,(all-Z)-	-	C21:4	0.53	ND	ND	ND
cis-11-Eicosenoic acid, methyl ester	gondoic acid ME	C21:1	0.58	ND	ND	ND
Eicosanoic acid ME	Arachidic acid ME	C21:0	0.48	ND	ND	ND
Tetracosanoic acid, methyl ester	Lignoceric acid ME	C25:0	0.32	ND	ND	ND
Saturated fatty acids (SAFA)			53.35	79	43.92	49.9
Monounsaturated fatty acids (MUFA)			29.27	14.7	27.23	50.09
Polyunsaturated fatty acids PUFA (ω6)			8.09	6.28	11.37	ND
Polyunsaturated fatty acids PUFA (ω3)			8.3	ND	17.48	ND
Ratio ω6/ω3			0.97	-	0.65	-

ND not detected, ME= methyl ester, IUPAC=International Union of Pure and Applied Chemistry.
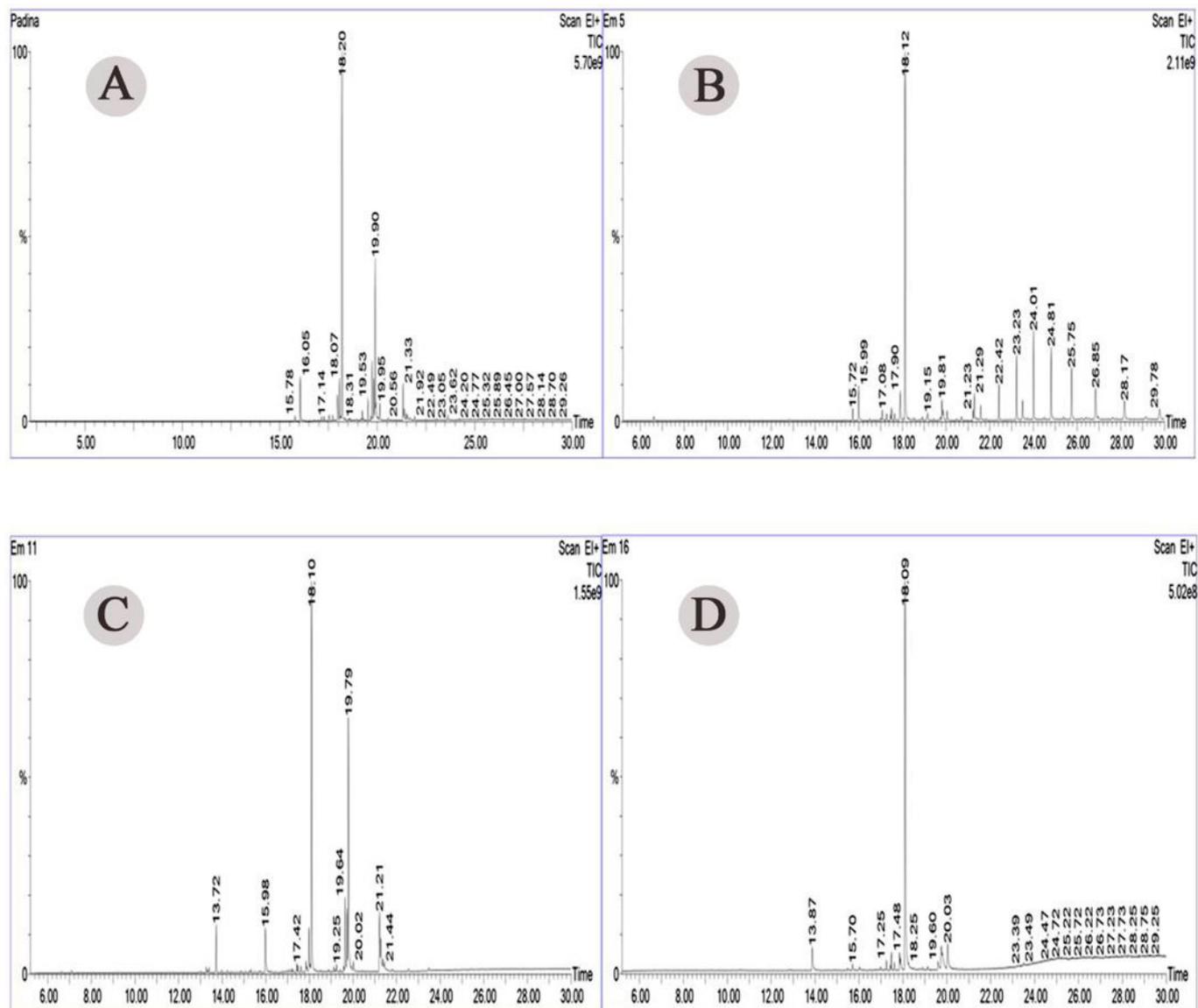
Figure 3. A chromatogram of the fatty acid methyl esters (FAMEs) of a dried seaweed sample. (A) *P. pavonia*, (B) *J. rubens*, (C) *T. atomaria* and (D) *C. elongate*.

acid methyl ester (EPA) (C21:5) in *T. atomaria* and *P. pavonia* only, while *T. atomaria* was recorded the highest percentages in the ω3 fatty acids (2.34%, 9.42%, and 5.72% respectively). Moreover, Linoleic acid methyl ester (ω6) (C19:2) was detected in *T. atomaria* and *P. pavonia* only with percentages (4.57% and 4.29%), respectively. These data are in agreement with those of Fatma et al. (2015), who reported that important long-chain polyunsaturated fatty acids (PUFAs) were found at the highest level in *T. atomaria* and *P. pavonia*.

The ω6 and ω3 are the two groups of essential fatty acids. EFAs can't be synthesized within the body, so they must be supplied in the food. Furthermore, ω6 fatty acids also play an important role in normal growth and development, in addition to brain function. *P. pavonia* and *T. atomaria* included ω6 and ω3 fatty acids, which is one of the essential fatty acids. Linoleic acid (ω6, C18:2) and α-linolenic acid (ω3, C18:3) are PUFA, which cannot be synthesized by vertebrates, including humans and

these fatty acids are present in this study with *T. atomaria* and *P. pavonia* and this in agreement with (Fatma et al., 2015). In the body, both can be changed into other PUFAs as eicosapentaenoic acid (EPA, C20:5, ω3), docosahexaenoic acid (DHA, C22:6, ω3) and arachidonic acid (C20:4, ω6) (Van Ginneken et al., 2011). Depend on fatty acid compositions, seaweeds have a shortage of DHA, but they can produce arachidonic acid and eicosapentaenoic acid at very large levels (Sánchez-Machado et al., 2004). In the literature, DHA was not noticed in seaweeds or presents in very small amounts in various phaeophytes (Li et al., 2002).

Differential use of the studied seaweeds to decrease the risk of multiple sclerosis, inflammation, diabetes mellitus, cancer, and coronary heart disease may be based on the variations in the contents of ω3 fatty acids. Simopoulos (2002) mentioned that the ω3 fatty acids have beneficial health effects, which included benefits concerning inflammatory bowel disease, cancer, psoriasis, and rheumatoid arthritis. In scientific reports, the

significance of the ω6/ω3 ratio has been widely argued recently. The equilibrium of assimilation of both PUSFA ω6 and ω3 fatty acids achieved when the ω6/ω3 ratio was equal to 1 (Francavilla et al., 2013).The total percentage of fatty acids of ω6 and ω3 of the collected seaweeds could be arranged in the following sequence *P. pavonia* >*T. atomaria* (Table 5 and Figure 3). However, the ratio of ω6/ω3 of *T. atomaria* (0.65%) and *P. pavonia* (0.97%) is the lowest among the studied seaweeds. This demonstrates that *T. atomaria* and *P. pavonia* are more desirable in decreasing the risk of many chronic diseases. The ω6 and ω3 fatty acids of *P. pavonia* in the current study were higher than the values previously reported, including for *Padina tetrastromatica* (2.2% and1.02%, respectively) (Ismail et al., 2016). In addition, the ratio of ω6/ω3 was 0.65 and 0.97 for the *T. atomaria* and *P. pavonia*, respectively. According to WHO this ratio should not be greater than 10 in diets (Sánchez-Machado et al. 2004), which recommend the studied seaweeds for nutritive purposes after further investigations.

4 Conclusion

The seaweeds *T. atomaria*, *P. pavonia*, *J. rubens*, and *C. elongate* collected from Rocky Bay of Abu Qir in Alexandria, Egypt, are rich with high levels of proteins, carbohydrates, lipids, and fatty acids; therefore they consider as foods with low calories. Furthermore, they may have a promising role in industrial applications, feed, and food. These seaweeds extracted using the traditional extraction methods and exhibited high antioxidant activities with massive biomedical, pharmaceutical, and nutraceutical applications. The *in vitro* antioxidant activities of different solvents of these seaweeds showed dose dependency. In general, methanol and ethanol extracts of the seaweeds were more efficient than diethyl ether, hexane, aqueous cold, and hot aqueous extracts. *T. atomaria* was the most nutritionally promising species, with intermediate carbohydrate content, suitable protein contents, and high lipid content with a great amount of polyunsaturated fatty acids (especially omega-3and omega-6), with a suitable ω6/ω3 ratio and high antioxidant activity. *T. atomaria* is a good candidate for oil-based products due to their total lipid content > 5% DW. These natural economic resources should be utilized and improved; therefore, many future studies must be carried out.

Acknowledgments

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