JFBE 1(2): 17-22,2025

Journal of Nutraceutical Foods and Bioactive Extracts

https://sanad.iau.ir/journal/nutfood

Original research

Evaluation of Physicochemical and Functional Properties of *Enteromorpha* and *Chaetomorpha* Macroalgae

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ABSTRACT

The use of lipid-based active compounds of algae in foods is growing. Therefore, the objective of this study was to evaluate the biochemical properties of the macroalgae *Enteromorpha* and *Chaetomorpha* species. In the present study, *Enteromorpha* and *Chaetomorpha* were extracted, and fat content, total protein, total phenolic content using the Folin-Ciucalteu colorimetric method, antioxidant capacity by ABTS radical scavenging assay, and fatty acid profiles using gas chromatograph (GC) with a flame ionization detector (FID) were measured. The results showed that the total fat contents of *Chaetomorpha* and *Enteromorpha* were 0.3 and 0.4, respectively. The protein content of *Chaetomorpha* was 12.54 mg/mL. The phenolic content increased as the concentration of the algae extract increased, indicating a positive relationship between the concentration and phenolic content of the extracts. Also, the highest antioxidant capacity was observed for *Chaetomorpha* (the highest percent inhibition of ABTS free radical and lowest IC50 values), and the lowest antioxidant capacity was found for *Enteromorpha* (the lowest percent inhibition of ABTS free radical and highest IC50 values) ($p < 0.05$). Among the identified fatty acids, the highest amount was observed for palmitic acid (*Enteromorpha* < *Chaetomorpha*), followed by oleic (*Enteromorpha* < *Chaetomorpha*) and linoleic (*Enteromorpha* < *Chaetomorpha*) acids. Therefore, macroalgae can be introduced as species with bioactivity because of their large amounts of beneficial bioactive compounds such as phenols, high antioxidant capacity and the presence of fatty acids/omega-3 and omega-6.

Keywords; *Enteromorpha*; Bioactive compounds; Antioxidant properties, *Chaetomorpha*; Macroalgae
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Received 03 April 2025; Accepted 04 July 2025

1. Introduction

Technological and economic aspects of algae production suggest their various applications. Algae are considered the most primitive energy sources and the oldest inhabitants of the oceans and freshwaters. They are considered a source of valuable biochemical compounds and a rich source of nutrients such as pigments, essential amino acids, vitamins, minerals, and unsaturated fatty acids (Bahrani et al., 2013; Vijayaram et al., 2024). Seaweeds are environmentally and commercially important. They are widely used as food for direct human consumption and are considered a food supplement in the 21st century, because they contain protein, lipids, polysaccharides, minerals, vitamins, and enzymes (Ahmadi et al., 2021). It is also used as an ingredient in the food and cosmetic industries, as a fertilizer, and as an additive in animal feed. Red and brown seaweeds are also used to produce hydrocolloids (Ahmadi et al., 2021). Macroalgae are plant-like organisms that generally live in coastal areas attached to rocks or on other hard surfaces (Assadi et al., 2013; Auwal et al., 2018; Ahmadi et al., 2021). *Enteromorpha* green algae have great potential for commercial use because they are a rich source of chemical compounds, nutrients, and food components that are

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essential for other organisms (Bao et al., 2011). *Chaetomorpha* are dark green, upright, brittle, filamentous, elongated and hard algae. *Chaetomorpha* extract has antimicrobial, anti-inflammatory, and antimalarial effects. It is also effective against hypertension, tumors and diabetes (Biris-Dorhoi et al., 2020). Microalgae refer to phytoplankton that are the primary food of all animals in aquatic ecosystems, providing all higher organisms in the food chain with the energy they need. Most phytoplankton are unicellular and are considered primary producers in aquatic habitats. Microalgae are a rich source of protein, carbohydrates and especially essential fatty acids. They also produce the main pigment in fish and vertebrates. They are important for fish nutrition, including phytophagous fish and zooplankton, and are also used in various industries, including the food and pharmaceutical industries, green manure (biofertilizer), and biodiesel (green fuel) (Bonilla-Ahumada et al., 2018).

Algae have a relatively high omega-3 content. Omega-3 polyunsaturated fatty acids are essential nutrients (e.g. docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), the main sources of which are seafood and some products such as walnuts, canola, and flaxseed. Fish oil has a high risk of chemical contamination. Commercial production of omega-3 polyunsaturated fatty acids for both food and feed is very promising, because sufficient and suitable raw materials are available for development in tropical regions (Cai et al., 2021; Cagalj et al., 2021). Brown and red algae contain more EPA and DHA than green algae (Castejon et al., 2021). Omega-3 fatty acids play a significant role in keeping the heart healthy. They can be used to fortify low-fat foods or produce functional foods. However, the incorporation of health-promoting omega-3 PUFAs into supplements, medicines and functional foods is limited. Since they are hydrophobic and practically insoluble in water, it is very difficult to fortify aqueous food and beverage products. These compounds are highly susceptible to oxidative degradation because of their unsaturated bonds, and when oxidation occurs, off-odor and off-flavor (rancidity) are developed and their health benefits and consumer acceptance are reduced. In addition, some of the lipid oxidation products are toxic, which may cause chronic health problems if consumed regularly over long periods (Chellappan et al., 2020). The objective of this study was to identify secondary metabolites and fatty acid profiles and determine some biological, phytochemical, and nutritional properties of *Enteromorpha* and *Chaetomorpha* green algae. Identification of the amounts of nutrients and essential elements, such as fatty acid profile in seaweed composition can greatly help evaluate the potential of different species for future uses in the food industry.

2. Material and Methods

2.1. Preparation of *Enteromorpha* and *Chaetomorpha* algae

Dried *Enteromorpha* (100 g) and *Chaetomorpha* (100 g) were obtained from Algae Resource Development Technology Company, Fars (López-López et al., 2009).

2.2. Extraction

Extract of powdered algae samples was prepared using ethanol/water (70 : 30 v/v) in an ultrasound bath at 50 °C for 30 min at 40 kHz with a solids to solvent ratio of 1: 20 (w/v) (114).

2.3. Measurement of fat content

40 g of the sample was weighed using a digital balance and placed in a Soxhlet apparatus at 70 °C. Then, 70 cc of n-hexane solvent was added. The oil content was calculated using Equation (1) (Wijesinghe and Jeon., 2011):

Fat content (%) = Extracted oil weight /sample weight × 100 (1)

2.4. Measurement of protein content

To measure the total protein content of macroalgae, the dried sample was powdered, and the protein content was determined using the Kjeldahl method. Finally, the total protein content of the sample was expressed as the dry weight percentage (%DM) (Wong and Cheung, 2002).

2.5. Measurement of total phenolic content

The total phenolic content was measured using the Folin-Ciucalteu colorimetric method and UV-Visible spectrophotometer at a wavelength of 765 nm. Gallic acid was used as a standard solution, and the phenolic content was calculated based on the gallic acid equivalent using the equation of the standard curve ($y = 0.0049x + 0.0117$; $R^2 = 0.991$).

2.6. Measurement of antioxidant capacity

In this study, the antioxidant capacity of algae was measured using the ABTS method. It is based on the relatively stable blue-green ABTS radical scavenging, which is converted into a colorless product. The color change indicates the amount of ABTS radical inhibited by antioxidants, which is measured using an ELISA reader. This method can be used to measure water- and fat-soluble antioxidants and/or impurities and food extracts. The percent inhibition of free radicals was calculated using Equation (3) (Yakubu et al., 2022; Tamaskani Zahedi et al., 2016):

Percent inhibition = (Control absorbance / (Sample absorbance - Control absorbance)) X100 (3)

2.7. Fatty acid/omega-3 and omega-6 profile

To methyl esterify fatty acids, 1 g of the obtained oil was refluxed in a flask with 20 mL of 1% w methanolic potassium hydroxide for 25 min. Then, 12 mL of fluorobromine was added using a condenser and simmered for 10 min. After turning off the heat, sodium chloride solution was added to the aqueous phase. The upper hexane phase containing the methyl-esterified fatty acids was removed from which 1 µL was immediately injected into the GC/FID. The gas chromatography device was equipped with a flame ionization detector (FID) and a fused silica capillary column of the bonded phase type (30 m × 0.22 mm ID × 0.25 µm film thickness). Helium at a pressure of 25 bar with 99.99% purity was used as the carrier gas. The detector and injector temperatures were 255 °C and 270 °C, respectively. The temperature of the device was initially 125 °C for 30 s and then 150 °C at a rate of 250 °C/min for 2 min. The flow rates of nitrogen, hydrogen and air in the FID detector were 25, 30 and 300 mL/min, respectively. The inlet temperature was 130-135 °C, the outlet temperature was 80-85 °C, the nozzle diameter was 6-7 mm, the dry air flow was 700 L\h and the pump speed was 15%. After the sample was injected into the gas chromatography device, the curve was drawn and the retention time of each fatty acid was compared with the curve of the standard fatty acid and its retention time. So, the type and amount of omega-3 and omega-6 fatty acids in the test sample were determined (Wijesinghe and Jeon, 2011).

2.8. Methods and data analysis

The statistical population included algae extracts and microcapsules from which the samples were randomly selected. All tests were performed in triplicate. To compare the means of two samples and more than two samples, the Duncan test and independent t-test were used, respectively, at a significance level of 0.05. To analyze data, SPSS software (version 21) was used.

3. Results and Discussion

3.1. Protein and fat content

The protein content of *Chaetomorpha* was 12.54 mg/mL. The total fat content of *Chaetomorpha* and *Enteromorpha* was 0.3 and 0.4, respectively.

3.2. Total phenolic content

The results of the measurement of phenolic content showed a significant ($p < 0.05$) difference between *Chaetomorpha* and *Enteromorpha* extracts. *Chaetomorpha* had a higher phenolic content. The TPC of *Chaetomorpha* and *Enteromorpha* was 8.63 and 6.48 mg GAE, respectively. The total phenolic content of *Enteromorpha* and *Chaetomorpha* is shown in Table 1.

Table 1 – Total phenolic content (mg gallic acid/L) of *Enteromorpha* and *Chaetomorpha*

Algae	Total phenolic content (TPC)
Enteromorpha	4.48 ± 0.15^{b}
Chaetomorpha	8.63 ± 0.00^{a}

Different small letters represent significant difference ($p < 0.05$)

The relationship between the concentration of *Enteromorpha* and *Chaetomorpha* extracts and gallic acid equivalent (mg/L) is shown in Figures 2 and 3.

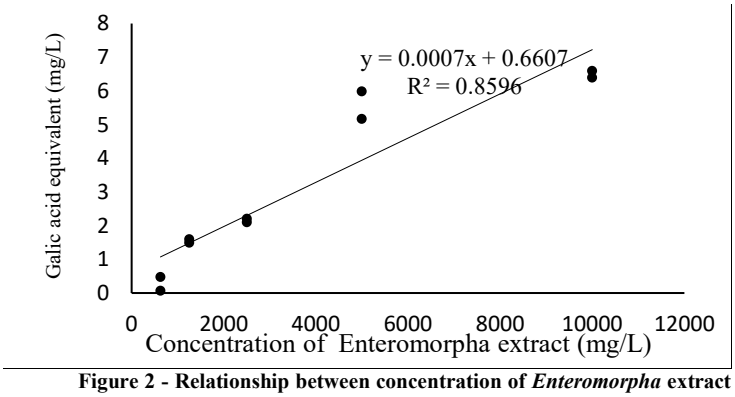

Figure 2 - Relationship between concentration of *Enteromorpha* extract and gallic acid equivalent (mg/L)

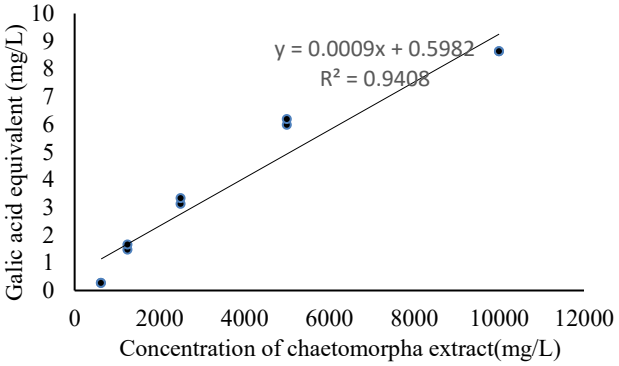

Figure 3 - Relationship between concentration of Chaetomorpha extract and gallic acid equivalent (mg/L)

In recent years, there has been growing interest in the use of algae extracts or active compounds of algae in the food industry [44]. The results of this study showed that *Chaetomorpha* and *Enteromorpha* had the highest and lowest phenolic content, respectively ($p< 0.05$). Also, it was found that *Chaetomorpha* and *Enteromorpha* extracts contained antioxidants, including phenols, and therefore had good antioxidant activity. Phenolic compounds are the most abundant secondary metabolites in seaweeds. However, the amounts of these compounds depend on the quality and intensity of light, salinity, concentration of nutrients and season of the year (Lang et al., 2011). It has been reported that phenolic compounds and flavonoids are associated with the antioxidant function of biological systems and absorb singlet oxygen and free radicals. Polyphenols can trap free radicals such as peroxy radicals, which are one of the key intermediate chain reactants, and thus terminate the oxidative degradation reactions (Lang et al., 2011).

3.3. Antioxidant capacity

The highest and lowest antioxidant capacities were found for *Chaetomorpha* (the highest percent inhibition of ABTS free radical and the lowest IC50 values) and *Enteromorpha* (the lowest percent inhibition of ABTS free radical and the highest IC50 values), respectively ($p < 0.05$). The antioxidant capacity of *Enteromorpha* and *Chaetomorpha* is shown in Table 3. In the present study, the antioxidant capacity of *Chaetomorpha* and *Enteromorpha* extracts was 69.75 and 48.09%, respectively, which supported the results of the measurement of phenols. The antioxidant capacity of algae extracts can be attributed to the fact that algae species are exposed to a wide range of solar radiation, especially UV-B and UV-A. Therefore, these marine organisms require internal antioxidants to protect cell membrane against damage to the phospholipid membrane and subsequent leakage of intracellular materials, and UV-induced lipid oxidation (Lang et al., 2011).

Table 2 – Inhibition of ABTS free radical (%) and inhibitory concentration of 50% of free radical (IC_{50}, mg/mL) of Enteromorpha and Chaetomorpha extracts

Algae	ABTS free radical inhibition (%)	IC_{50}
Enteromorpha	48.09 ± 9.35^{b}	10.751 ± 0.550^{a}
Chaetomorpha	69.75 ± 0.47^{a}	7.336 ± 0.002^{b}

Different small letters represent significant differences ($p < 0.05$)

Figures 4 and 5 show the relationship between the concentration of *Enteromorpha* and *Chaetomorpha* extracts and the percent inhibition of ABTS free radical.

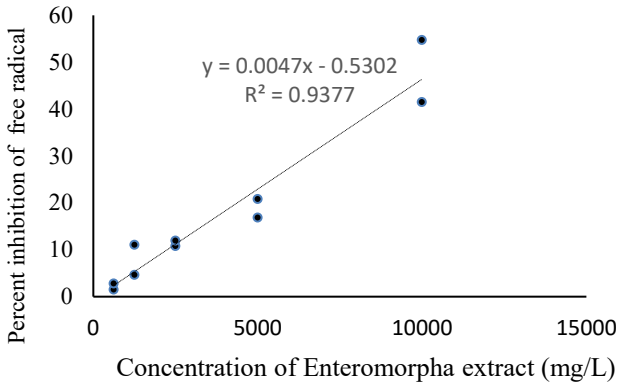

Figure 4 – Relationship between concentration of *Enteromorpha* extract and percent inhibition of free radical

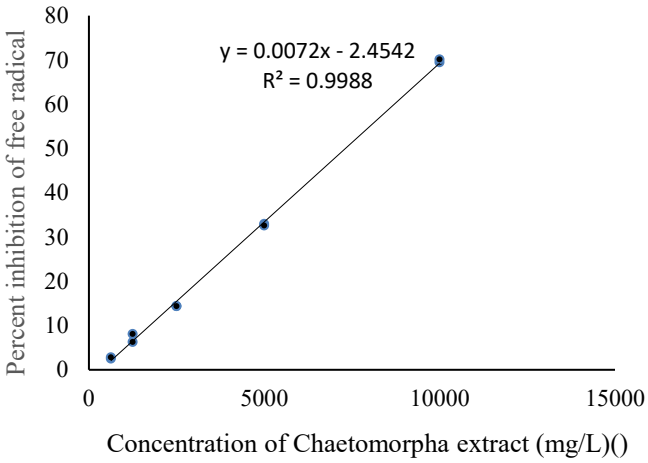

Figure 5 – Relationship between concentration of *Chaetomorpha* extract and percent inhibition of free radical

The antioxidant potential of the encapsulated *Spirogyra* extract was also demonstrated for fish oil stabilization by estimating the peroxide values of fish oil (Ponnanikajamideen et al., 2014). In another study, lower peroxide and anisidine values in food samples fortified with nanoliposomes containing omega-3 (PUFA) prepared using the method of Mozaffari compared to nonencapsulated PUFAs were demonstrated (Ponnanikajamideen et al., 2014). *Padina pavonica*, *Taonia atomaria*, *Jania rubens* and *Corallina elongata* algae with the highest phenolic content (176.7 ± 6.9 mg GAE/g crude extract) were identified (Setha et al., 2013). The total phenolic content of *Padina* brown algae was 4.43 mgGAE/g and its antioxidant activity, determined using the DPPH method, was 564.99 µg/mL (Shaghuli et al., 2017).

The presence of phenolic compounds and the antioxidant activity in other seaweeds, such as *Sargassum* (Liu et al., 2008), *Padina* (Sinha and Amed Asimi., 2007; Liu et al., 2008; Shaghuli et al., 2017; Savaghebi et al., 2021; Mu et al., 2022), *Dictyota dichotoma* (Savaghebi et al., 2021), *Taonia atomaria*, *Jania rubens* and *Corallina elongata* (Setha et al., 2013) have also been reported.

3.4. Fatty acid profile

Among the identified fatty acids, the highest amount was found for palmitic acid (*Enteromorpha* < *Chaetomorpha*), followed by oleic (*Enteromorpha* < *Chaetomorpha*) and linoleic (*Enteromorpha* < *Chaetomorpha*) acids (Table 3). These fatty acids are more susceptible to epoxide formation caused by reaction with atmospheric oxygen. A very high tendency to oxidation can occur in the absence of an antioxidant. Therefore, the addition of natural or synthetic antioxidants is necessary to retard the oxidation process.

Table 3 – Some of the fatty acid of Enteromorpha and Chaetomorpha extracts (mg/mL)

Fatty acid	Enteromorpha	Chaetomorpha
C6:0	1.98	1.130
C8:0	1.21	0.527
C10:0	0.921	0.9
C11:0	1.86	2.11
C12:0	4.27	–
C13:0	–	1.42
C14:1	2.55	–
C16:0	15.36	3.43
C18:1c	13.36	3.65
C18:2c	14.89	5.64

Ganesana et al. (2014) reported that *Enteromorpha* algae had relatively high amounts of n-3 fatty acids. In addition, they had more unsaturated fatty acids than saturated fatty acids. The monounsaturated fatty acids (MUFA) content of *Enteromorpha* ranged from 6.56 to 7.94% and the saturated fatty acids content ranged from 37.93 to 47.60%. In a study conducted by Kalasariya et al. (2021), octadecanoic, n-hexadecanoic and oleic acid acids showed the highest amounts (Kalasariya et al., 2021). In general, seaweeds can be a good source of nutrients. They are balanced sources of omega-3 and omega-6 fatty acids (López-lópez et al., 2009). For example, the EPA and DHA content of *Sargassum* was 0.03 and 0.11 mg/g, respectively (Kuznetcova et al., 2020). In *Padina* algae, saturated fatty acids included palmitic, myristic and stearic acids, while unsaturated fatty acids included oleic, linoleic, linolenic and elaidic acids (Oliver et al., 2020). Palmitic acid was the predominant fatty acid in ulva samples from the coast of Bushehr (Prieto et al., 2021). Our findings are in agreement with the results obtained by Ahmadi et al. (2021), who showed that the particle size of nanoliposomes carrying white tea polyphenols was 100 nm and that of empty liposomes was about 60 nm. Rashidinejad et al. (2014) also showed that liposomes containing catechins and epigallocatechin gallate had larger particle sizes than empty liposomes.

4. Conclusions

Today, the algae are increasingly used in industrial, agricultural, pharmaceutical and food industries and modern technology is used for the production and exploitation of algae in industrially developed countries. These valuable plants also have found applications in the medical, pharmaceutical and food industries. Given the presence of large resources of algae in Iran, they can be used optimally in various fields. Therefore, investigation of the biochemical value of these algae, which unfortunately have not been used economically so far, will not only help evaluate their nutritional composition but will also make the sources of protein, fat, carbohydrates, vitamins and minerals available for commercial purposes. Bioactive compounds of microalgal origin can be prepared directly from primary metabolites such as proteins, fatty acids and vitamins or they can be synthesized by secondary metabolites. Such compounds have several bioactivities that may be used for the reduction of incidence and prevention of diseases. Among the biochemical components, lipids have attracted the most attention for extraction and commercialization. The results showed that the total fat content of

Chaetomorpha and *Enteromorpha* was 0.3% and 0.4%, respectively. In terms of phenolic content, there was a significant ($p < 0.05$) difference between *Chaetomorpha* and *Enteromorpha* extracts. *Chaetomorpha* had a higher phenolic content. Also, the highest and lowest antioxidant capacities were observed for *Chaetomorpha* and *Enteromorpha* ($p < 0.05$). Among the identified fatty acids, the highest amount was found for palmitic acid (*Enteromorpha* < *Chaetomorpha*), followed by oleic (*Enteromorpha* < *Chaetomorpha*) and linoleic (*Enteromorpha* < *Chaetomorpha*) acids. Therefore, macroalgae can be introduced as species with bioactivities due to their high amounts of beneficial bioactive compounds such as phenols, high antioxidant capacity, and the presence of fatty acids/omega-3 and omega-6.

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