

# ANTIOXIDANT PROPERTIES OF SOME MACROALGAE HARVESTED FROM THE ISKENDERUN BAY TURKEY

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## ABSTRACT

Pharmacological and biological activities of seaweeds are significant in terms of human health, functional food and cosmetic applications. One of the most striking properties of seaweeds is their antioxidant activity that protects cells from oxidative damage caused by free radicals that cause many diseases such as cancer, type 2 diabetes, obesity, metabolic disorder, hypertension and cardiac diseases. In this context, evaluation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferrous ion chelating activity of methanolic extracts of seaweeds, *Cystoseira elegans* (Brown algae), *Cystoseira amentacea* (Brown algae), *Padina crassa* (Brown algae), *Jania rubens* (Red algae) and *Corallina elongata* (Red algae) harvested from Iskenderun Bay, Turkey, were carried out in this study. The total phenolic and total flavonoid contents of the extracts were also determined. According to the obtained results, *Cystoseria elegans* revealed the best free radical scavenging capacity with the highest flavonoid content and quite high total phenolic content among five seaweed extracts. This study is the first report to examine the antioxidant potentials of *Cystoseira elegans* and *Padina crassa* species. As a result of this study, it has been shown that all algae species examined may be potentially rich sources of natural antioxidants.

## KEYWORDS:

Seaweed, 1, 1-diphenyl-2-picrylhydrazyl scavenging activity, ferrous ion chelating activity, phenolic compounds, flavonoids

## INTRODUCTION

Seaweeds, also known as macroalgae, are photosynthetic eukaryotic organisms which are abundant in almost every aquatic environment. They are divided into three main groups: brown algae (Phaeophyceae), green algae (Chlorophyta) and red algae (Rhodophyta) [1]. The pharmacological and biological activities of seaweeds have been extensively investigated for human health, functional food and cosmetic applications, as they are one of the most commonly researched and used marine resources [2].

Many seaweeds are used as a functional food due to their high content of vitamins, minerals, proteins, carbohydrates, fibers, amino acids and lipids [1]. Furthermore, due to the antimicrobial [3], anti-allergic [4], anticoagulant [5], cytotoxic [2], antiviral [6], and antioxidant activities [2] of other bioactive compounds found in marine algae, they are also used in the exploration of less side-effect drugs intended for use in the treatment of diseases such as cancer, type 2 diabetes, obesity, metabolic disorder, hypertension and cardiac diseases [1].

One of the most striking properties of seaweeds is their antioxidant activity that protects cells from oxidative damage caused by free radicals. Free radicals formed as reactive oxygen species (ROS) and reactive nitrogen species (RNS) during normal metabolic pathways of organisms can damage the DNA, lipids and proteins within the cell structure. These radicals are scavenged by antioxidant defense mechanisms usually found in the human body, thereby maintaining the oxidation-antioxidation balance. However, some oxidative substances taken into the body from the outside cause this balance to deteriorate and oxidative damage occurs in cells. Oxidative damage may cause some health problems such as heart disease, stroke, arteriosclerosis, diabetes, cancer and inflammation [7, 8]. In order to achieve this balance again, exogenous antioxidants from natural sources need to be taken through foods or food supplements [9].

Biological activity studies on many foods and medicinal plants that have been a natural source of antioxidants for many years are still ongoing. Additionally, due to the numerous phenolic compounds they produce, seaweeds have begun to attract attention as an antioxidant source. Phenolic compounds, which are classified into two groups as flavonoids and non-flavonoid polyphenols, have some important properties such as antidiabetic, antimicrobial, antiallergic, antiinflammatory, antiviral, hepatoprotective, neuroprotective and anticancer besides their antioxidant activities [10]. These wide-ranging bioactivities of the phenolic compounds synthesized by seaweeds make them candidates for the development of products or ingredients used in food, health and cosmetics industry [11].

In addition, establishing the antioxidant activity of seaweeds would increase their value in the human diet as food and pharmaceutical supplements [12].

While there are many methods for determining the antioxidant activity of natural or synthetic substances, the DPPH method is the most commonly used method since it is a simple and inexpensive application of in vitro assays. Another method used to determine the antioxidant activity of a substance is the determination of the activity of chelation of iron ions.

Iron, which plays a key role in almost all cellular and physiological events by participating in the structure of many proteins and metabolic pathways, is an essential element for living organisms. However, the absence or more of the optimal amount of iron in the human body adversely affects the cells, leading to the occurrence of various pathological conditions. Iron, which participates in the structure of proteins such as ferritin when taken in a certain amount, gains the ability to create free radicals if taken in excessive amounts. As a result, oxidative stress occurs in tissues, causing the onset of cancer, neurodegeneration and other diseases. Therefore, evidence suggests that natural iron chelators from dietary and non-dietary sources can reduce oxidative stress by regulating oxidative damage induced by iron [13, 14]. Although iron chelation activities have been identified in some of the seaweeds consumed as food in many countries, there are still shortcomings in this regard [15].

Previous studies have shown that *Cystoseira elegans* [16], *Cystoseira amentacea* [17], *Jania rubens* [18], and *Corallina elongata* [18] macroalgae species have antioxidant effects. However, there are no studies in the literature that determined the iron chelation effect of *Cystoseira elegans*, *Cystoseira amentacea*, *Padina crassa* and *Corallina elongata* species, which are used in this study. In addition, antioxidant activity studies of *Padina crassa* have not yet been conducted. To that end, this study aims to evaluate the antioxidant activities, total phenolic and flavonoid contents of five methanolic seaweed extracts harvested from Iskenderun Bay, Turkey.

## MATERIALS AND METHODS

**Sample collection.** Antioxidant activity analysis were performed on *Cystoserira elegans*, *Cystoseira amentacea*, *Padina crassa*, *Jania rubens*, and

*Corallina elongata* species (Table 1). Sampling studies, were made by 0-20 m depth from the free dives in Iskenderun Gulf coast of Iskenderun, Hatay, Turkey, in June 2018. The materials were collected underwater in gathered mesh bags. Some of the materials collected were kept in jars in a 4-6% neutralized formaldehyde solution prepared with sea water, to be determined and defined later in the laboratory. The collected materials were separated and washed with distilled water in order to be free of epiphytes, rocks, sand and mud that may be present in them. The cleaned materials were dried in the laboratory in a shaded area without exposure to the sun for further analysis. The identification studies of the materials were carried out with the Olympus brand Ckx41sf model stereo inverted light microscope.

**Preparation of algal extracts.** The dried algal samples were extracted by maceration in 1:4 (w/v) biomass/solvent ratio with methanol for 2 weeks at room temperature in a dark environment. The obtained methanolic extract was filtered through filter paper. After filtration, the solvent was evaporated at 50 °C under reduced pressure in a rotary evaporator (Heidolph, Germany), and deposited at +4 °C before further usage.

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.** The free radical scavenging potential of the extracts was evaluated by DPPH assay comparing the EC50 value of synthetic chemical BHT with minor modifications according to the method mentioned in [19]. 0.5 mL of extracts of different concentrations was mixed with a freshly prepared methanolic solution of DPPH radical (0.1 mM). Following the incubation at room temperature in the dark for 30 min, absorbance was read by a spectrophotometer (Shimadzu UV-1800, Japan) at 517 nm against a blank (extract only). The same method was applied as a control group, with a solution without the extract. Butylated hydroxytoluene (BHT) was used as a reference standard. The percentage of DPPH radical scavenging effect was calculated using the equation below:

DPPH scavenging activity (% inhibition) =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ , where  $A_{\text{control}}$  is the absorbance of the control and  $A_{\text{sample}}$  is the absorbance of the reaction mixture or standard. An extract concentration curve versus percentage inhibition was developed to determine the extract

**TABLE 1**  
**Seaweed species which were used in this study**

Seaweeds	Divisions
<i>Cystoseira elegans</i> Sauvageau 1912	Phaeophyta (Brown algae)
<i>Cystoseira amentacea</i> (C.Agardh) Bory 1832	
<i>Padina crassa</i> Yamada 1931	
<i>Jania rubens</i> (Linnaeus) J.V.Lamouroux 1816	Rhodophyta (Red algae)
<i>Corallina elongata</i> J. Ellis & Solander 1786	

concentration required to cause the starting DPPH concentration to decrease by 50%. This value determined by the analysis of linear regression is referred to as EC<sub>50</sub>. The lower EC<sub>50</sub> value suggests greater antioxidant activity. All measurements were performed in triplicate.

**Ferrous ion chelating activity.** The ferrous ion chelating capacity of the extracts was determined with minor modifications according to the method mentioned in [20]. 0.5 mL of the extracts at different concentrations was mixed with 1.35 mL of methanol. To the extract solution, 50 µL of 2 mM FeCl<sub>2</sub> was added and stayed for 5 min. Thereafter, 100 µL of 5 mM ferrozine solution were added to this mixture and incubated for 10 min. After incubation, absorbance was read at 562 nm by spectrophotometer (Shimadzu UV-1800, Japan) against a blank (extract and FeCl<sub>2</sub> only). The extract was replaced with methanol in the control group. Percentage of the ability of the sample to chelate ferrous ion was calculated using equation below:

Ferrous ion chelating ability (%) =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ , where  $A_{\text{control}}$  is the absorbance of the control and  $A_{\text{sample}}$  is the absorbance of the reaction mixture. The EC<sub>50</sub> value was determined by linear regression curve, which is the concentration of the extracts that chelate 50% of the ferrous ion. All measurements were performed in triplicate.

**Determination of total phenolic contents (TPC).** Total phenolic content of each algal extract was quantified according to the method of Folin-Ciocalteu [21] using gallic acid as standard. 0.1 mL (1 mg / mL) of extracts was mixed with 0.2 mL of diluted Folin-Ciocalteu reagent (1:1 with water). After incubation at room temperature for 3 min, 1 mL 2% sodium carbonate was added to the reaction mixture. The absorbance was read by spectrophotometer (Shimadzu UV-1800, Japan) at 760 nm after 1 h of incubation at room temperature in the dark. The total phenolic content values are expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g). All measurements were performed in triplicate.

**Determination of total flavonoid contents (TFC).** The total flavonoid content of the extracts was calculated using quercetin as standard with the AlCl<sub>3</sub> method [22]. 1 mL of extracts (1 mg / mL) was mixed with 2% methanolic solution of AlCl<sub>3</sub>.6H<sub>2</sub>O in the same amount. After incubation at room temperature for 10 min, absorbance was read at 367 nm by spectrophotometer (Shimadzu UV-1800, Japan). The total flavonoid content values are expressed as quercetin equivalent (QE) in milligrams per gram of dried extract (mg QE/g). All measurements were performed in triplicate.

**Statistical analysis.** All analysis were run in

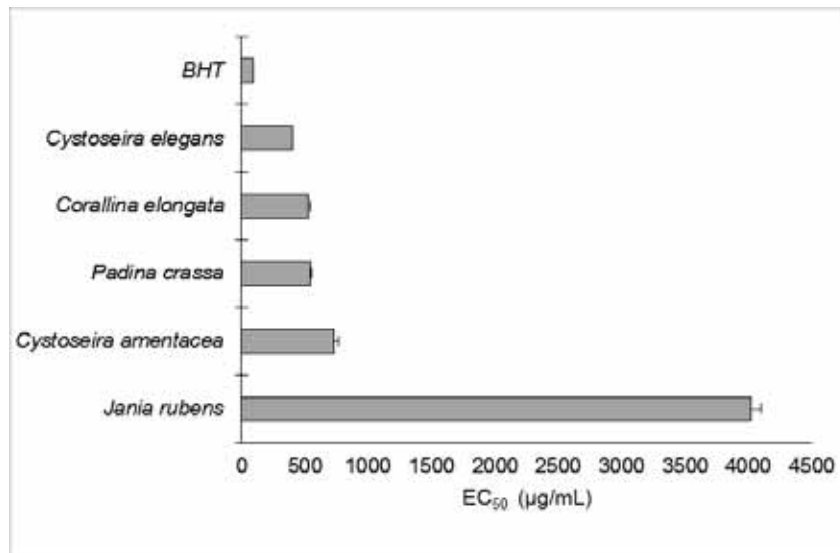
triplicate, and results are presented as mean values ± standard deviation (SD). The data obtained were analyzed using one-way analysis of variance (ANOVA) test to determine significant differences between groups by using the SPSS software (Version 16.0; SPSS; Chicago, IL, USA). Differences were considered significant at the 95% confidence level ( $p < 0.05$ ).

## RESULTS

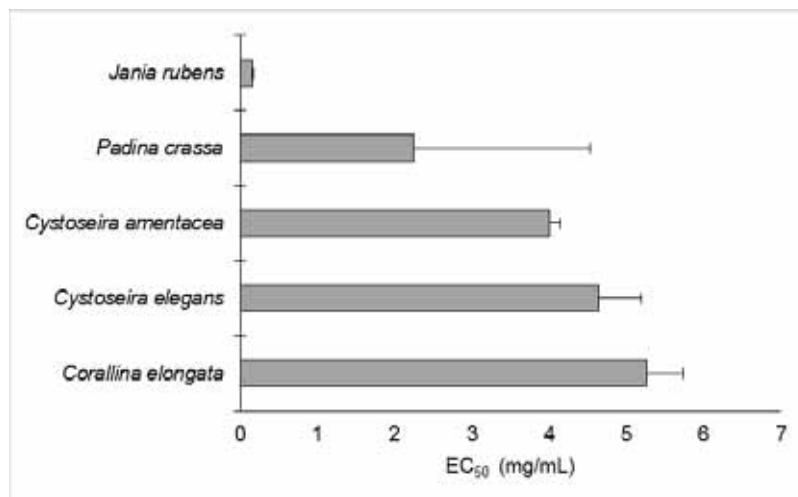
**DPPH radical scavenging activity.** In the present study, the DPPH radical scavenging activity method evaluated the antioxidant ability of the methanol extracts by comparing the EC<sub>50</sub> values (Figure 1). The free radical scavenging effect of the extracts according to the EC<sub>50</sub> values is indicated as follows: *Cystoseira elegans* ( $402.25 \pm 1.47^a \mu\text{g mL}^{-1}$ ) > *Corallina elongata* ( $528.97 \pm 13.20^a \mu\text{g mL}^{-1}$ ) > *Padina crassa* ( $545.85 \pm 9.58^a \mu\text{g mL}^{-1}$ ) > *Cystoseira amentacea* ( $731.27 \pm 33.02^a \mu\text{g mL}^{-1}$ ) > *J. rubens* ( $4025.56 \pm 76.90^b \mu\text{g mL}^{-1}$ ). The results showed that all extracts had weak antioxidant activity compared to BHT ( $96.47 \pm 0.54 \mu\text{g mL}^{-1}$  EC<sub>50</sub>) which was used as a positive control. Brown algae *C. elegans* extract was the most effective species against DPPH free radical. The red algae *J. rubens* demonstrated the lowest scavenging effect among the extracts examined ( $P < 0.05$ ).

**Ferrous ion chelating activity.** The ferrous ion chelating activities of the extracts according to the EC<sub>50</sub> values is indicated as follows: *J. rubens* ( $0.16 \pm 0.01^a \text{ mg mL}^{-1}$ ) > *P. crassa* ( $2.24 \pm 2.294^{ab} \text{ mg mL}^{-1}$ ) > *C. amentacea* ( $4.00 \pm 0.15^{ab} \text{ mg mL}^{-1}$ ) > *C. elegans* ( $4.64 \pm 0.56^{ab} \text{ mg mL}^{-1}$ ) > *C. elongata* ( $5.26 \pm 0.47^b \text{ mg mL}^{-1}$ ) (Figure 2). EC<sub>50</sub> values of *C. elegans*, *C. amentacea*, and *P. crassa* species were found to be statistically insignificant compared to themselves and other species ( $P > 0.05$ ). Red algae *J. rubens* methanolic extract had the highest chelating activity compared to other species. The difference between EC<sub>50</sub> values of *J. rubens* and *C. elongata* species was found to be statistically significant ( $P < 0.05$ ).

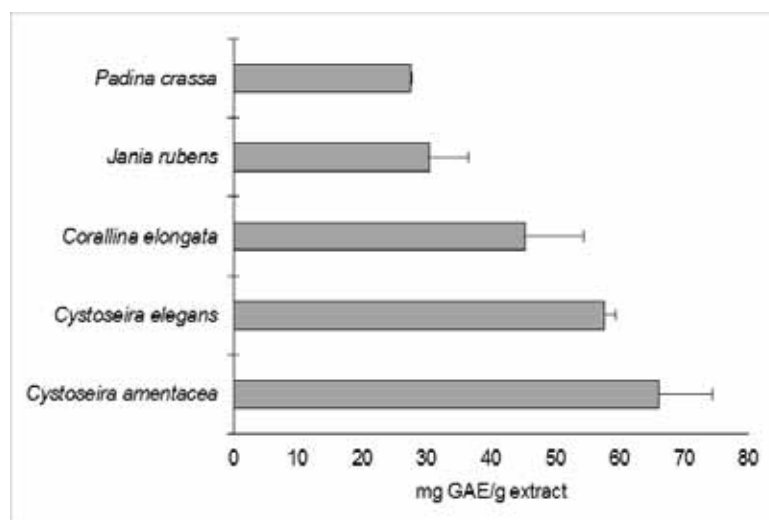
**Total phenolic contents (TPC).** The total phenolic content of the algae extracts found out as follows: *C. amentacea* ( $66.02 \pm 8.43^{ab} \text{ mg GAE g}^{-1}$  extract) > *C. elegans* ( $57.58 \pm 1.77^a \text{ mg GAE g}^{-1}$  extract) > *C. elongata* ( $45.32 \pm 9.03^{ab} \text{ mg GAE g}^{-1}$  extract) > *J. rubens* ( $30.43 \pm 6.02^{ab} \text{ mg GAE g}^{-1}$  extract) > *P. crassa* ( $27.58 \pm 0.12^b \text{ mg GAE g}^{-1}$  extract) (Figure 3). The phenolic content values of *C. amentacea*, *J. rubens*, and *C. elongata* species were found to be statistically insignificant compared to themselves and other species ( $P > 0.05$ ). *C. amentacea* and *C. elegans* extracts were found to have the highest



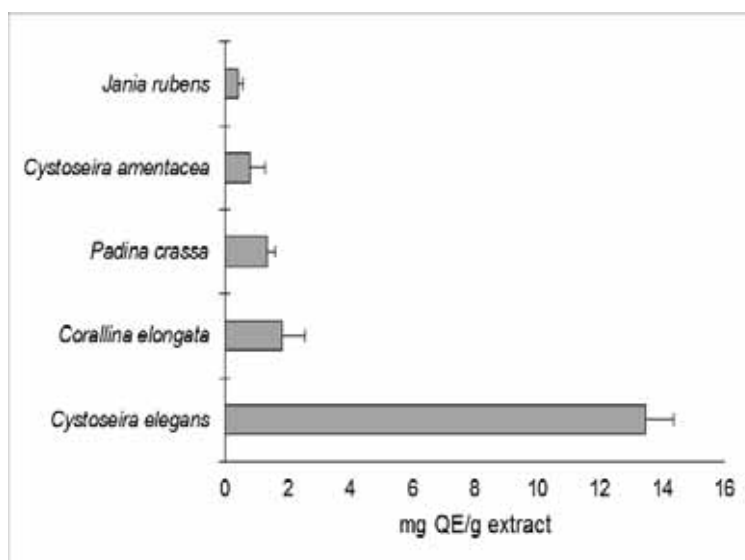
**FIGURE 1**  
DPPH radical scavenging activities (EC<sub>50</sub> µg/mL) of the extracts and BHT



**FIGURE 2**  
Ferrous ion chelating activities (EC<sub>50</sub> mg/mL) of the extracts



**FIGURE 3**  
Total phenolic contents (mg GAE/g extract) of the extracts



**FIGURE 4**  
Total flavonoid contents (mg QE/g extract) of the extracts

TPC values compared to other species. The difference between TPC values of *C. elegans* and *P. crassa* extracts was found to be statistically significant ( $P < 0.05$ ).

**Total flavonoid contents (TFC).** The total flavonoid content of the methanol extracts found out as follows: *C. elegans* ( $13.47 \pm 0.88^a$  mg QE g<sup>-1</sup> extract) > *C. elongata* ( $1.82 \pm 0.75^b$  mg QE g<sup>-1</sup> extract) > *P. crassa* ( $1.36 \pm 0.25^b$  mg QE g<sup>-1</sup> extract) > *C. amentacea* ( $0.82 \pm 0.48^b$  mg QE g<sup>-1</sup> extract) > *J. rubens* ( $0.44 \pm 0.13^b$  mg QE g<sup>-1</sup> extract) (Figure 4). Differences between TFC values of *C. amentacea*, *P. crassa*, *J. rubens* and *C. elongata* species were found to be statistically insignificant ( $P > 0.05$ ). It was determined that *C. elegans* methanolic extract had the highest flavonoid content. This value was found to be significantly higher than the TFC values of the other four species ( $P < 0.05$ ).

## DISCUSSION

Oxidative stress induced by the overproduction of reactive oxygen species and reactive nitrogen species, which are the products of normal cell metabolism, is a harmful process that is a significant mediator of damage to cell structures and, thus, of various diseases and aging. Among the disorders caused by oxidative stress are diabetes mellitus, cancer, hypertension, cardiovascular disease, atherosclerosis, ischemic heart diseases, neurodegenerative diseases, and liver diseases [23, 24].

Enzymatic and nonenzymatic antioxidants react with free radicals in single-electron reactions and inhibit oxidative damage [25]. The use of natural antioxidants among non-enzymatic antioxidants is preferred over synthetic antioxidants due to possible toxicity and food safety. Phenolic compounds from

various natural sources (plants, seaweeds, etc.) have been reported as natural antioxidants [10, 11]. Therefore, in this article, total phenolic and flavonoid contents and antioxidant activities of the seaweed extracts harvested from Iskenderun Bay were evaluated.

There are no studies on antioxidant activities or phenolic and flavonoid contents of methanolic extracts of *Cystoseira elegans* and *Padina crassa* species in the literature. In our study, according to the DPPH assay and total flavonoid content, *Cystoseira elegans* methanolic extract demonstrated the strongest radical scavenging activity and had the highest flavonoid content among other species. This is the first report on the antioxidant activity of this species and it is noteworthy that it has the highest scavenging effect among the species studied. Stanojković et al. and Kosanić et al. determined the DPPH radical scavenging activity of acetone extract of *Cystoseira amentacea* with EC<sub>50</sub> value of  $150.2 \pm 2.9$  and  $409.81 \pm 1.36$  µg/mL, respectively [2,17]. Since the EC<sub>50</sub> value of the *C. amentacea* methanol extract used in this study was  $731.27 \pm 33.02$  µg/mL, it appears to have lower antioxidant activity than the results in other studies. However, because of acetone instead of methanol, it is thought that the different components in the algae have shown different activities by switching to solvent. Saeed et al., Pinteus et al. and Alghazeer et al. studied the DPPH scavenging activity of the methanolic extracts of the *Jania rubens* collected from different countries and they obtained different EC<sub>50</sub> values as  $8.43 \pm 0.39$  mg/mL,  $>1000$  µg/mL, and  $130.5 \pm 31.53$  µg/mL, respectively [26-28]. In addition to these data, the EC<sub>50</sub> value of *J. rubens* methanol extract used in our study was determined as  $4025.56 \pm 76.90$  µg/mL. The antioxidant activities of the extracts vary according to the region in which they are located. Moreover, Khairy et al. claim that antioxidant activity and phe-

nolic compounds can vary even according to the seasons [29]. Pinteus et al. and Oucif et al. determined the EC<sub>50</sub> values of the *Corallina elongata* methanolic extract as >1000 µg/mL and 1780 µg/mL for DPPH scavenging activity, respectively [27, 30]. In our study, free radical scavenging activity of the *C. elongata* extract with a EC<sub>50</sub> value of 528.97 ± 13.20 µg/mL was found higher than these studies.

According to the EC<sub>50</sub> shown in Table 1 and Figure 2, the ferrous ion chelating activity of the methanolic extracts of five seaweeds exhibited the following order: *Jania rubens* (0.16 mg/mL) > *Padina crassa* (2.24 mg/mL) > *Cystoseria amentacea* (4.00 mg/mL) > *Cystoseria elegans* (4.64 mg/mL) > *Corallina elongata* (5.26 mg/mL). Earlier studies showed high ferrous ion chelating activities in ethanol (EC<sub>50</sub> value 0.78 mg/mL) and methanol extracts (28.37% at a concentration of 0.6 mg/mL) of *J. rubens* [31, 32]. However, the chelation effect of the extract used in our research (EC<sub>50</sub> value 0.16 mg / mL) was noted to be greater than the reports in these studies. This is the first report on chelating effect of other species studied. It is suspected that the use of these species in foods can be useful in balancing the oxidative damage caused by iron due to its natural chelator properties.

Kosanic et al. (2015) evaluated the total phenolic content of the acetone extracts of three different *Cystoseria* species *C. amentacea*, *C. barbata* and *C. compressa*. They determined the highest phenolic content in *C. amentacea* extract with a value of 81.28 ± 1.065 µg pyrocatechol equivalent/mg of extract [17]. There are no studies on the total phenolic content of the *C. elegans* species in the literature. In our study, the amount of phenolic substances of this species was found to be quite higher than other species studied and shows that this species has a high antioxidant potential. Zouaoui and Ghalem (2017) compared total phenolic contents of different *Corallina elongata* extracts (ethanol, diethyl ether and chloroform) and they found that diethyl ether extract has the highest phenolic content with a value of 206.22 ± 0.13 mg GAE/100 g dry algae powder [33]. Pinteus et al. (2017) studied the methanol extract of *C. elongata* and determined 10.39 ± 0.010 mg GAE/g extract total phenolic content in this extract and this value represents one-third of the value we found in our study [27]. The total amount of phenolic compound present in the *C. elongata* methanol extract was reported by Oucif et al. (2017) as 4.58 + 0.14 mg GAE / g dry weight [30]. This value is also quite low according to the amount of phenolic substances found in our study. The reason why the amount of phenolic matter contained in the same species varies so much can be attributed to external factors such as the type of solvent used, environmental conditions, and so on. There are several studies on the total phenolic content of different *Jania rubens* extracts and fractions by using Folin–Ciocalteu method and different results were found in all of them [32,34,35]. Some of

the results from these studies are higher than our results, while others are lower. It is thought that the reason for this diversity in the results is that the species changes the content of phenolic compound due to being found in different environmental conditions.

The highest flavonoid content of the extract was found in *Cystoseira elegans* among other studied species and there is no previous study on this species in the literature. In many studies, positive correlation between flavonoid content and antioxidant activity of seaweed extracts has been shown [11]. The fact that *C. elegans* extract, which has the highest flavonoid content, was also found as the extract with the highest free radical scavenging effect in this study supports this information. There is only one study in the literature that determines the amount of total flavonoids contained in the *Cystoseira amentacea* extract. Kosanic et al. (2015) found 64.58 ± 1.009 µg rutin equivalents / mg of extracts in acetone extract of *C. amentacea* [17]. The amount of total flavonoids contained in *Jania rubens* methanolic extract detected in our study is similar to the results obtained by El-Din and El-Ahwany [35]. There is no other study in the literature examining the amount of flavonoids contained in the extracts of *Corallina elongata* or *Padina crassa*.

## CONCLUSIONS

Antioxidant potentials of *Cystoseira elegans* and *Padina crassa* species, whose antioxidant activities were not determined before, was investigated. Furthermore, iron chelating effects of *C. elegans*, *C. amentacea*, *P. crassa* and *C. elongata* species were shown for the first time in this study. The brown algae *Cystoseria elegans* methanolic extract harvested from Iskenderun Bay revealed the best free radical scavenging capacity with the highest flavonoid content and quite high total phenolic content among five seaweed extracts. Seaweeds are currently undergoing detailed investigations of natural sources of antioxidant compounds that could be used as functional foods. All of the algal species studied may potentially be rich sources of natural antioxidants. These results tend to be useful in contributing to further studies to recognize and classify particular compounds responsible for the relatively high degree of antioxidant activity in these marine algae.

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