Acta Aquatica Turcica

Home Page: https://dergipark.org.tr/actaquatr
E-ISSN: 2651-5474
21(2): 158-166, 2025
DOI: 10.22392/actaquatr.1600679

Research Article
Araştırma Makalesi

Evaluation of Biochemical Content and Antioxidant Activity of *Pterocladiella capillacea* Algae

Pterocladiella Capillacea Alginin Biyokimyasal İçeriğinin ve Antioksidan Aktivitesinin Değerlendirilmesi

Yiğit Suyun[1], Selenay Ertan[1], Soner Atik[1], Alican Bahadır Semerci[1,2,*], Ayşe Gül Tekbaba[1], Tuğba Ongun Sevindik[1]

[1]Sakarya University, Faculty of Science, Department of Biology, 54050, Sakarya-TÜRKİYE
[2]Necmettin Erbakan University, Ereğli Vocational School of Health Services, 42310, Konya-TÜRKİYE

*Corresponding Author: alicanbahadirsemerci@gmail.com

Received: 13.12.2024 **Accepted:** 28.02.2025 **Published:** 01.06.2025

How to Cite: Suyun, Y., Ertan, S., Atik, S., Semerci, A. B., Tekbaba, A. G. & Ongun Sevindik, T. (2025). Evaluation of biochemical content and antioxidant activity of Pterocladiella capillacea algae. *Acta Aquatica Turcica, 21*(2), 158-166. https://doi.org/10.22392/actaquatr.1600679

Abstract: Red macroalgae are the basis of many commercially important food, pharmaceutical and other important industries. Research on these species has generally focused on improving seaweed cultivation, developing new methods to extract useful compounds or identifying new applications. In our study, the biochemical contents (total protein, carbohydrate and total phenolic substance) of *Pterocladiella capillacea* algae collected from the Black Sea were determined. In addition, antioxidant activity of *P. capillacea* species extracted by Soxhlet and ultrasonic-assisted maceration methods was investigated using 2 different methods (DPPH scavenging and iron reduction). The carbohydrate value of *P. capillacea* dry biomass was determined as 42% and protein value as 17%. In our study, where the effect of the extraction method on antioxidant activity and total phenolic substance was evaluated, it was determined that the Soxhlet method was more effective in total phenolic substance and iron reduction tests, while the ultrasonic-assisted maceration method was more effective in DPPH scavenging activity. In conclusion, the high carbohydrate content of *P. capillacea* species collected from the Black Sea (Türkiye) coasts and its potential use as a source of bioactive compounds causing antioxidant activity were highlighted.

Keywords
- Rhodophyta
- Extraction
- Antioxidant activity
- Total carbohydrate

Özet: Kırmızı makroalgler, ticari açıdan önemli birçok gıda, ilaç ve diğer önemli endüstrinin temelini oluşturur. Bu türler üzerine yapılan araştırmalar genellikle deniz yosunu yetiştiriciliğini iyileştirmeye, yararlı bileşikleri çıkarmak için yeni yöntemler geliştirmeye veya yeni uygulamalar belirlemeye odaklanmıştır. Çalışmamızda Karadeniz'den toplanan *Pterocladiella capillacea* alginin biyokimyasal içerikleri (toplam protein, karbonhidrat ve toplam fenolik madde) belirlendi. Ayrıca soxhlet ve ultrasonik destekli maserasyon yöntemleriyle ekstrakte edilen *P. capillacea türünün* 2 farklı metodla (DPPH süpürme ve demir indirgeme) kullanılarak antioksidan aktivitesi araştırılmıştır. *P. capillacea* kuru biyokütlesinin karbonhidrat değeri %42, protein değeri %17 olarak tespit edilmiştir. Ekstraksiyon metodunun antioksidan aktivite ve toplam fenolik madde üzerindeki etkisinin değerlendirildiği çalışmamızda toplam fenolik madde ve demir indirgeme testinde soxhlet metodunun, DPPH süpürme aktivitesinde ultrasonik destekli maserasyon metodunun daha etkili olduğu belirlenmiştir. Sonuç olarak, Karadeniz (Türkiye) kıyılarından toplanan *P. capillacea* türünün yüksek karbonhidrat içeriğine sahip olduğu ve antioksidan aktiviteye neden olan biyoaktif bileşik kaynağı olarak potansiyel kullanımını vurgulanmıştır.

Anahtar kelimeler
- Rhodophyta
- Ekstraksiyon
- Antioksidan aktivite
- Toplam karbonhidrat

1.INTRODUCTION

Marine macroalgae resources are gaining attention in the health and food cost industries due to their low cost and easy production. Among them, red algae (Rhodophyta): the largest group containing valuable bioactive compounds are used in cosmetics, food industry, pharmaceuticals, fertilizers and various supplements in food formula. Red algae are multicellular organisms that mostly live in the sea. It contains about 6000 species. (Aziz et al., 2021). Its cellular components, especially the cell wall polysaccharide composition, are quite different from other algal groups (Yoon et al., 2010). Red algae have been shown to contain a number of significant bioactive substances, including polysaccharides (alginate, agar, and carrageenan), lipids and polyphenols, steroids, glycosides, flavonoids, tannins, saponins, alkaloids, triterpenoids, anthraquinones, and cardiac glycosides. All of these bioactive compounds are now widely used as dietary and food supplements, emulsifiers, stabilizers and thickeners in the textile, food, cosmetics and pharmaceutical industries. Bioactive compounds obtained from marine macroalgae have attracted the attention of many researchers due to their various emerging biological activities and new beneficial properties. As a result, these substances can be used as a foundation for the development of new pharmaceutical substances intended for use as therapeutic and preventive agents, as well as dietary supplements, nutraceuticals, and functional food products (Torres et al., 2019; Khotimchenko et al., 2020). Macroalgae are especially preferred as a source of antioxidants.

Nowadays, the efforts of researchers to find natural alternatives to chemical compounds have increased considerably. It has been seen in studies in the literature that various techniques should be tried to reveal the best bioactive compounds. Extraction is of great importance in the recovery of phytochemicals from plant matrix and biomass. Many methods such as maceration, supercritical fluid extraction, filtration, microwave-assisted extraction, soxhlet method and ultrasonically assisted extraction are used for the recovery of bioactive molecules. Compared with traditional extraction techniques, ultrasonic assisted extraction is an efficient method as it can reduce the working time and solvent usage (Pollini et al., 2020; Tavakoli et al., 2021). Furthermore, this technique allows for low-temperature extraction, which minimizes heat loss from high temperatures and guarantees the preservation of bioactive compounds. Studies in the literature have shown that ultrasonically assisted extraction to extract bioactive compounds from different plant materials significantly reduces the extraction time and improves the overall targeted compound extraction yield compared to conventional methods (Lee and Lin 2007; Gam et al., 2020; Rashad et al., 2023).

In this study, it was aimed to determine the biochemical contents (chlorophyll a, chlorophyll b, carotenoids, total proteins, total carbohydrates) of dry biomass of red algae *Pterocladiella capillacea* collected from the Black Sea. In addition, the effects of ethanolic (70%) extracts prepared with different extraction methods (soxhlet and ultrasonic assisted extraction) on total phenolic substance and antioxidant activity were compared.

2.MATERIAL AND METHODS

2.1.Sample collection

Algae samples were collected (approximate wet weight 1 kg) from the coast of Kandıra district of Kocaeli province on September 29, 2024. Algae were brought to the laboratory, sorted and cleaned, and identified using identification books (Braune and Guiry, 2011; Bunker et al., 2017; Bothwell, 2023). The identified *Pterocladiella capillacea* (S.G.Gmelin) Santelices & Hommersand 1997 were dried in a drying oven (7 days at 50^{o}C).

2.2.Biochemical contents

2.2.1.Chlorophyll-a, chlorophyll-b, and total carotenoids contents measurement of dried biomass

The determination of chlorophyll-a, chlorophyll-b and total carotenoids of dried samples was performed by spectrophotometric methods according to Tavakoli et al. (2021). For pigment analysis, 20 mg of dried biomass was extracted with 3 mL of 95% glacial acetone. The absorbances of the samples were measured at 470, 648 and 664 nm spectrophotometrically. The amounts of chlorophyll-a, chlorophyll-b and total carotenoids (mg/g dried extract) were calculated according to the following equations.

$$Chla = 13.36 \times A664 - 5.19 \times A648 \quad (1)$$

$$Chlb = 27.43 \times A648 - 8.12 \times A664 \quad (2)$$

Carotenoids total = [(1000 × A470–1.63 × Chla - 104.96 × Chlb)/221] (3)

Chla: Chlorophyll-a; Chlb: Chlorophyll-b

2.2.2.Total protein

Total protein (TP) was included in the Bradford (1976) administration. Using 2 milliliters of 0.031 M citrate-phosphate buffer (pH: 5.5) solution, 0.01 grams of dried algal biomass were homogenized. The homogenized samples were centrifuged for 20 minutes at 14,000 rpm and +4°C, and the supernatants were then separated. 0.031 M Citrate-Phosphate buffer (pH: 5.5) and 0.01% Coomassie Brilliant Blue G-250 were added to the supernatants to create the combination. Using the standard chart (bovine serum albumin), the levels of protein were determined in milligrams per gram.

2.2.3.Total Carbohydrate

The total carbohydrate (TC) content was determined using the phenol-sulfuric acid method (Kochert, 1978). 1 mL of 5% phenol, 1 mL of an algal sample at a concentration of 0.5 mg/mL, and 3 mL of concentrated sulfuric acid were mixed together. After five minutes of incubation at 90 °C in a water bath, the mixture's absorbance at 490 nm was measured. The produced d-glucose standard graph was used to determine the samples' carbohydrate content.

2.2.4.Extract production

Soxhlet methods: 10 g of algal biomass was placed in a Soxhlet cartridge and extracted with 100 mL of ethanol (70%) for 8 hours in a Soxhlet device. Ultrasound assisted maceration (UAM): It was carried out using an ultrasonic bath (Wisd WiseClean) providing 100 W maximum power (W) at a fixed frequency of 30 kHz (f). 100mL of ethanol was added to 10g of algae biomass and mixed for 2 hours. Then, the mixtures placed in the ultrasound bath were extracted for 60 minutes by applying a power of 100 W (Tavakoli et al 2021). The extract obtained at the end of the extraction process was filtered through filter paper. Then, the solvents of the samples were removed under vacuum in a rotary evaporator at 55°C. A stock (at a concentration of 10 mg/mL) was prepared from the extracts and stored at +4°C until its use for the experiment (Semerci et al., 2020).

2.2.5.The total phenolic contents (TPC)

The TPC of the extracts was determined, as mentioned in the previous work (Semerci et al., 2020). First, 200 µL of 50% Folin-Ciocalteu reagent was combined with 100 µL of extract (0.5 mg/mL) and left for three minutes. The absorbance at 760 nm was then measured after 1 mL of a 3% sodium carbonate solution was added and left in the dark for 60 minutes. A calibration curve was employed for the gallic acid standard, and TPC extract was expressed as milligram per gram (mg GA g^{-1}).

2.3.Antioxidant activity

2.3.1. 2,2-difenil-1-pikrilhidrazil (DPPH) radical scavenging

The modified Blois method was used to determine the DPPH radical scavenging activity (Blois, 1958). 1 mL of the algae extract prepared at different concentrations (0.1-1 mg/mL) was taken and 1 mL of 0.04% DPPH was added. The quickly mixed mixtures were incubated in the dark at 25°C for 30 minutes. At the end of the period, the samples were read on a spectrophotometer at 517 nm. The DPPH% radical scavenging activity was determined using the Equation (4):

$$DPPH\%\ radical\ scavenging = \left(\frac{control\ absorbance - extract\ absorbance}{control\ absorbance}\right) x\ 100 \quad (4)$$

2.3.2. Reducing power

According to Oyaizu (1986), the extract's reducing power was measured. 1 mL of extract was mixed with 2.5 mL of phosphate buffer and 1% potassium ferricyanide at a certain concentration (0.1-1 mg/mL). For 25 minutes, this mixture was incubated at 50°C. 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 2500 g for 15 minutes. 0.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 500 µL of 1% (w/v) $FeCl_3$. Distilled water served as a blank when the absorbance value was measured at 700 nm.

2.4. Statistical analysis

Results of experiments performed in 3 replicates are presented as mean values ±95% confidence limits. Analysis of variance was performed using ANOVA procedures. Significant differences between means in antioxidant activity were determined at $P<0.05$ level by Tukey's pairwise comparison test.

3.RESULTS AND DISCUSSION

3.1. Biochemical contents

The use of different marine macroalgae (seaweed) as sources of bioactive compounds has the potential to industrialize a renewable natural

resource that has so far been underutilized. Macroalgal biomasses have been shown to produce a wide range of nutrients and bioactive secondary metabolites (Patarra et al., 2011; Biris-Dorhoi et al., 2020). In our study, the chlorophyll content and carotenoid values of the red alga *P. capillacea* are given in Figure 1. The chlorophyll a value in dry biomass was determined as 2.8 mg/g, chlorophyll b as 0.6 mg/g and carotenoid value as 0.86 mg/g ($p<0.05$). In a study conducted in Egypt, *P. capillacea* species was reported to have approximately chlorophyll a in 0.5 mg/g, chlorophyll b 0.17, and carotenoids 0.1 mg/g (El-Din and El-Ahwany 2016). Differences between studies are possible because the physical conditions of the algae collection site affect the pigment content of the algae. In particular, conductivity, turbidity, dissolved oxygen, sulfate and geographical location were found to be important in explaining the differences in pigment content(Hodgson et al., 2004; . Voerman et al.,2022).

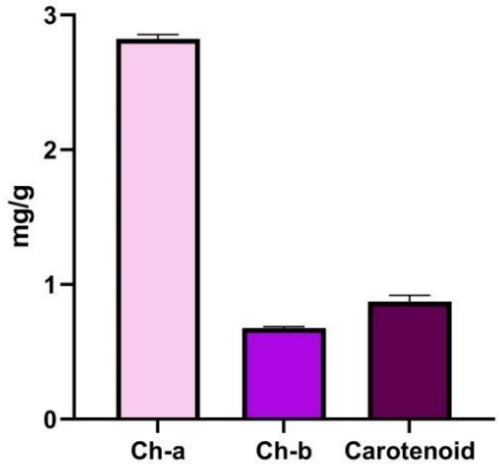

Figure 1. Chlorophyll-*a*, chlorophyll-*b*, and total carotenoid content [mg g^{-1}] (±standard deviation) in *P. capillacea*.

Combined with their textural properties, the use of algae as functional foods seems worth investigating. Regardless of the nutritional value of food products, their acceptability depends on the consumer's cognitive experiences and organoleptic properties. Some algae can bring bitterness from protein-derived peptides, saltiness from high mineral content (e.g. Na and K), and sweetness from soluble sugars (e.g. mannitol). Algae are known as representatives of the fifth taste, umami. In addition, algae have very characteristic aromas and smells transmitted by volatile compounds. Determination of the biochemical contents of new algae groups is important in revealing organoleptic properties (Mouritsen et al., 2012; Francezon et al., 2021). Macroalgae have a protein content that can range from 7% to 31% of dry weight and a lipid content that can range from 2% to 13% of dry weight (Fleurence et al., 2018; Kazir et al., 2019). Significant amounts of carbohydrates can also be found in macroalgae (32–60% dry weight). In our study, the carbohydrate value of *P. capillacea* dry biomass was determined as 42% and protein value as 17% ($P<0.05$) (Table 1). The protein and carbohydrate content were found to be within the range of the values given in the literature. In a study investigating the biochemical content of *P. capillacea* species collected from the Gulf of Alexandria (Egypt), the total protein value of the dry weight was reported as 18.47% and the total carbohydrate value as 51.36% (Ashour et al., 2020). The protein value of *P. capillacea* species collected from the coast of Portugal was found as 20.19% and 19.76% carbohydrate (Paiva et al., 2017). Since it is known that light, temperature and inorganic contents of the collection region affect the biochemical content of algae, it is possible that there are differences in the biochemical contents of algae collected from different regions (Voerman et al.,2022).

Table 1. Biochemical content of *P. capillacea* algae (DW: dry weight).

Biochemical contens	***P. capillacea***
Total protein content (% DW)	17±0.8
Total carbohydrate content (% DW)	42±0.4
Soxhlet TPC (mg GA/g)	22±1.2
UAM TPC (mg GA/g)	19±0.7

The high carbohydrate content of the strain we used in our study (such as polysaccharides beta-glucans, sulfated polysaccharides, cellulose, and others) suggested that these strains could aid bowel movements as dietary fiber for the digestive system. Additionally, these species might be helpful in controlling blood serum cholesterol and glucose levels by the use of nutraceuticals or dietary additives.

Ethanol and water mixtures are widely used in the isolation of antioxidant compounds due to their many advantageous properties such as low toxicity and suitability for food use (Dai et al., 2010; Do et al., 2014; Semerci et al., 2020). In

the studies conducted in the literature, it has been found that aqueous alcohol mixtures are effective in the recovery of other compounds, including phenolic compounds, from various macroalgae and microalgae (Monteiro et al., 2020; Andriopoulos et al., 2022). Polar solvents are generally the preferred solvent when the target compounds are polar antioxidants such as polyphenols and tannins (Semerci et al., 2024). In our study, based on this information, *P. capillacea* species was extracted using the Soxhlet and UAM methods by diluting the aqueous ethanolic solvent. It was determined that the total phenolic compound was higher in the extract prepared by the Soxhlet method (22 mgGA/g). In a previous study on *P. capillacea*, the TPC value of the ethanolic extract obtained by the maceration method was reported as 15.23 mgGA/g (De Alencar et al., 2016). In another study, the total phenolic content of *P. capillacea* was evaluated with a different method and the total phenolic compound content was found to be approximately 1100 µg g^{-1} FW. It was also found that the phenolic content decreased due to increased Cd toxicity (Schmidt et al., 2016). In a study investigating the phenolic content of *P. capillacea* species in Egypt in two different seasons, it was reported that the total phenolic content varied between 17.79 - 16.85 mg/g (Ashour et al.,2020). The method used, the place where the algae are collected and the nutrient medium produced appear to be effective on the phenol content.

3.2. Antioxidant activity

Antioxidants prevent or slow down the oxidation of these molecules by providing an electron-rich environment to the compounds that are likely to undergo oxidation. The complex properties of herbal antioxidants, which have few negative health impacts, include solubility, structure, production, mechanism of action, and kinetics (Neupane and Lamichhane 2020). Therefore, at least two test systems have been proposed to determine the in-vitro antioxidant activities of crude plant extracts. In the current investigation, two different methods have been used to assess the antioxidant activity of extracts. While the DPPH approach focuses on the radical scavenging characterisation of pure compounds, the reducing power method focuses on reducing antioxidant characterization (Gupta, 2015). In our study, the %DPPH scavenging activity of ethanolic extracts obtained using soxhlet and ultrasonically assisted maceration methods is given in Figure 2.

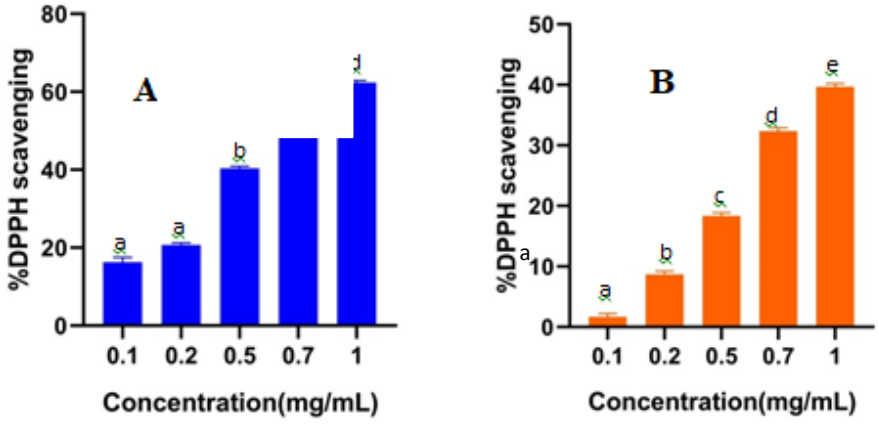

Figure 2. %DPPH scavenging activity of extracts prepared by ultrasonic-assisted maceration (A) and soxhlet methods (B). Bars with the same lower case letter (a–e) are not significantly (P>0.05) different.

At a concentration of 1 mg/mL, the extract produced by the ultrasonically assisted maceration approach was shown to scavenge the DPPH radical by 60%. At the same concentration, it was found that the extract made using the Soxhlet technique scavenged DPPH by 40%. In our study, it was observed that the extracts obtained with the ultrasonic-assisted maceration method had higher DPPH scavenging activity than the extracts obtained with the Soxhlet method. Another study reported that the DPPH scavenging rate of *P. capillacea* ethanolic

extract at a concentration of 1 mg/mL was 30% (De Alencar et al., 2016). It has been seen in the literature that different extraction methods are effective in revealing antioxidant activity (Ashour et al., 2020).

The reducing capacity of *P. capillacea* macroalgae was determined by measuring the amount of reducing agent in the sample. Reducing agents are substances that show antioxidant activity by donating a hydrogen atom and breaking free radical chains. In our study, the iron reducing capacities of extracts adjusted in the concentration range of 0.1-1 mg/mL were determined. The reducing agents present in the solution promote the reduction of the Fe 3+ /ferrocyanide complex to the ferrous form (Fe 2+), which can be measured in the absorbance at 700 nm. The greater the absorbance of the mixture at 700 nm, the greater the antioxidant activity of iron reduction.(Saadatmand et al., 2011). The results showed a dose-dependent increase in reducing power in both extraction methods ($P<0.05$) (Figure 3). The iron reducing power of the extract prepared by ultrasonic assisted maceration method increased in the concentration range of 0.1-1 mg/mL depending on the dose. There was a significant increase in the iron reducing power of the extract obtained by Soxhlet extraction between 0.1-0.7 mg/mL depending on the dose. But the iron reducing power reached saturation in the concentration range of 0.7-1 mg/mL. Unlike the DPPH scavenging test, it was observed that the extracts made with the Soxhlet method showed higher reducing power than the extracts made with UAM.

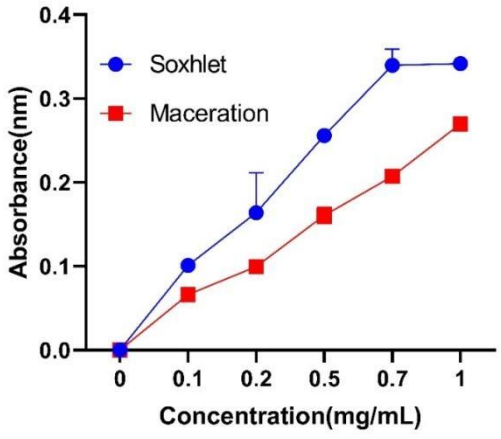

Figure 3. Iron reduction analysis results of the extract.

It was revealed that Soxhlet technique was more effective in revealing iron reducing power and phenolic content. The use of total phenolic compounds as reducing agents has been documented in the literature. It is also known that chemicals that easily enter into redox reactions can produce high levels of activity in the Folin-Ciocalteu method (Singleton et al., 1965; Dorman and Hiltunen 2004). The connection between phenolic compounds and iron reduction is supported by these data. Despite the strong iron binding properties of polyphenols, it is a matter of debate whether the iron chelation ability of polyphenol groups containing catechol or gallol plays an important role in their antioxidant activity (Perron and Brumaghim, 2009). However, researchers have found that catechins, which are important representatives of polyphenols, have antioxidant properties at all concentrations and have attributed the antioxidant behavior to iron chelation (Sugihara et al., 2001). According to Wang et al. (2009), phenolic compounds have low metal chelation abilities. Other compounds such as carbohydrate/protein (agar, carrageenan and alginate), carotenoids and lipids found in algae are also known to have metal chelation abilities and there is evidence that they have inhibitory effects on the absorption of iron ions (De Alencar et al., 2016; Hentati et al., 2022; Premarathna et al., 2024). In our study, it is thought that the iron reducing power is significantly high due to the carotenoid group and other secondary and primary compounds in red algae in addition to phenolic compounds.

4. CONCLUSION

Our study determined that *P. capillacea*, a red algae (Rhodophyta) with rich biochemical content, has a carbohydrate value of 42% and a protein value of 17% in dry weight. Pigment values in dry biomass were determined as 2.8 mg/g for chlorophyll a, 0.6 mg/g for chlorophyll b and 0.86 mg/g for carotenoids. Due to the high carbohydrate content of *P. capillacea* species, this macroalgae can be evaluated as a potential candidate in food insecurity, micronutrient deficiencies and sustainable food scarcity problems. Ultrasonic maceration method was first tried in the extraction of *Pterocladiella capillacea* species and the effect of this method on antioxidant activity was evaluated comparatively with the Soxhlet method. In the

results, it was determined that the Soxhlet method was more effective in total phenolic substance and iron reduction tests. It was determined that the ultrasonically assisted maceration method was more effective in revealing the DPPH scavenging activity of *P. capillacea* species.

ACKNOWLEDGEMENT

The authors thank Nisanur Kutlu and Baran Karaduman for their help during the laboratory part of the study.

FUNDING

No financial support was received for the present study.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: YS, ABS; Literature: AGT, YS, SE, TOS; Methodology: AGT, YS, SE, SA, TOS; Performing the experiment: ABS, TOS; Data analysis: YS, SA, AGT; Manuscript writing: YS, ABS, TOS Supervision: ABS, TOS. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Research data is not shared.

REFERENCES

Andriopoulos, V., Gkioni, M. D., Koutra, E., Mastropetros, S. G., Lamari, F. N., Hatziantoniou, S., Kornaros, M. (2022). "Total phenolic content, biomass composition, and antioxidant activity of selected marine microalgal species with potential as aquaculture feed", *Antioxidants*, 11(7), 1320

Ashour, M., El-Shafei, A. A., Khairy, H. M., Abd-Elkader, D. Y., Mattar, M. A., Alataway, A., & Hassan, S. M. (2020). Effect of Pterocladia capillacea seaweed extracts on growth parameters and biochemical constituents of Jew's Mallow. *Agronomy*, 10(3), 420.

Ashour, M., El-Shafei, A. A., Khairy, H. M., Abd-Elkader, D. Y., Mattar, M. A., Alataway, A., & Hassan, S. M. (2020). Effect of Pterocladia capillacea seaweed extracts on growth parameters and biochemical constituents of Jew's Mallow. Agronomy, 10(3), 420.

Aziz, E., Batool, R., Khan, M. U., Rauf, A., Akhtar, W., Heydari, M., ... & Shariati, M. A. (2021). An overview on red algae bioactive compounds and their pharmaceutical applications. *Journal of Complementary and Integrative Medicine*, 17(4), 20190203.

Biris-Dorhoi, E. S., Michiu, D., Pop, C. R., Rotar, A. M., Tofana, M., Pop, O. L., ... & Farcas, A. C. (2020). Macroalgae—A sustainable source of chemical compounds with biological activities. *Nutrients*, 12(10), 3085.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*. 4617, 1199-1200. https://doi.org/10.1038/1811199a0

Bothwell, J. (2023). Seaweeds of the World: A Guide to Every Order (Vol. 4). *Princeton University Press*.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.

Braune, W., & Guiry, M. D. (2011). Seaweeds: A colour guide to common benthic green, brown and red algae of the world's oceans. Germany: *ARG Gantner Verlag* KG; ISBN 978-3-906166-90-2.

Bunker, F., Brodie, J. A., Maggs, C. A., & Bunker, A. R. (2017). Seaweeds of Britain and Ireland (Vol. 9). *Princeton University Press*.

Dai, J., Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313-7352.

De Alencar, D. B., de Carvalho, F. C. T., Rebouças, R. H., Dos Santos, D. R., dos Santos Pires-Cavalcante, K. M., de Lima, R. L., ... & Saker-Sampaio, S. (2016). Bioactive extracts of red seaweeds Pterocladiella capillacea and Osmundaria obtusiloba (Floridophyceae: Rhodophyta) with

antioxidant and bacterial agglutination potential. *Asian Pacific Journal of Tropical Medicine*, 9(4), 372-379.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., Ju, Y. H. (2014). "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*", *Journal of Food and Drug Analysis*,

Dorman, H. J. D., & Hiltunen, R. (2004). Fe (III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis* L.) extract and subfractions. *Food Chemistry*, 88(2), 193-199.

El-Din, S. M. M., & El-Ahwany, A. M. (2016). Bioactivity and phytochemical constituents of marine red seaweeds (Jania rubens, Corallina mediterranea and Pterocladia capillacea). *Journal of Taibah University for Science*, 10(4), 471-484.

Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In Proteins in food processing (pp. 245-262). *Woodhead Publishing*.

Francezon, N., Tremblay, A., Mouget, J. L., Pasetto, P., & Beaulieu, L. (2021). Algae as a source of natural flavors in innovative foods. *Journal of Agricultural and Food Chemistry*, 69(40), 11753-11772.

Gam, D. H., Kim, S. Y., & Kim, J. W. (2020). Optimization of ultrasound-assisted extraction conditions for phenolic compounds, antioxidant activity, and epigallocatechin gallate in lipid-extracted microalgae. *Molecules*, 25(3), 454.

Gupta, D. (2015). Methods for determination of antioxidant capacity: A review. International *Journal of Pharmaceutical Sciences and Research*, 6(2), 546.

Hentati, F., Tounsi, L., Pierre, G., Barkallah, M., Ursu, A. V., Ben Hlima, H., ... & Abdelkafi, S. (2022). Structural characterization and rheological and antioxidant properties of novel polysaccharide from calcareous red seaweed. *Marine drugs*, 20(9), 546.

Hodgson, D. A., Vyverman, W., Verleyen, E., Sabbe, K., Leavitt, P. R., Taton, A., ... & Keely, B. J. (2004). Environmental factors influencing the pigment composition of in situ benthic microbial communities in east Antarctic lakes. *Aquatic microbial ecology*, 37(3), 247-263.

Kazir, M., Abuhassira, Y., Robin, A., Nahor, O., Luo, J., Israel, A., ... & Livney, Y. D. (2019). Extraction of proteins from two marine macroalgae, Ulva sp. and Gracilaria sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocolloids*, 87, 194-203.

Khotimchenko, M., Tiasto, V., Kalitnik, A., Begun, M., Khotimchenko, R., Leonteva, E., ... & Khotimchenko, Y. (2020). Antitumor potential of carrageenans from marine red algae. *Carbohydrate Polymers*, 246, 116568.

Kochert, G. (1978). Carbohydrate determination by the phenol-sulfuric acid method. Handbook of phycological methods, *Physiological and Biochemical Methods*., 95.

Lee, M. H., & Lin, C. C. (2007). Comparison of techniques for extraction of isoflavones from the root of Radix Puerariae: Ultrasonic and pressurized solvent extractions. *Food Chemistry*, 105(1), 223-228.

Monteiro, M., Santos, R. A., Iglesias, P., Couto, A., Serra, C. R., Gouvinhas, I., Barros, A., Oliva Teles,A., Enes, P., Díaz-Rosales, P. (2020). Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro-and microalgae extracts, *Journal of Applied Phycology*, 32, 349-362

Mouritsen, O. G.; Williams, L.; Bjerregaard, R.; Duelund, L. (2012). Seaweeds for umami flavour in the New Nordic Cuisine. *Flavour* 1 (1), 4,

Neupane, P., & Lamichhane, J. (2020). Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. *Vegetos*, 33, 360-366. https://doi.org/10.1007/s42535-020-00116-7

Oyaizu, M. (1986). Studies on products of browning reactions prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Paiva, L., Lima, E., Neto, A. I., Marcone, M., & Baptista, J. (2017). Nutritional and functional bioactivity value of selected Azorean macroalgae: Ulva compressa, Ulva rigida, Gelidium microdon, and Pterocladiella capillacea. *Journal of Food Science*, 82(7), 1757-1764.

Patarra, R. F., Paiva, L., Neto, A. I., Lima, E., & Baptista, J. (2011). Nutritional value of selected macroalgae. *Journal of Applied Phycology*, 23, 205-208.

Perron, N. R., & Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell biochemistry and biophysics*, 53, 75-100.

Pollini, L., Tringaniello, C., Ianni, F., Blasi, F., Manes, J., & Cossignani, L. (2020). Impact of ultrasound extraction parameters on the antioxidant properties of Moringa oleifera leaves. *Antioxidants*, 9(4), 277.

Premarathna, A. D., Ahmed, T. A., Kulshreshtha, G., Humayun, S., Darko, C. N. S., Rjabovs, V., ... & Hincke, M. T. (2024). Polysaccharides from red seaweeds: Effect of extraction methods on physicochemical characteristics and antioxidant activities. Food Hydrocolloids, 147, 109307.

Rashad, S., El-Chaghaby, G., Lima, E. C., & Simoes dos Reis, G. (2023). Optimizing the ultrasonic-assisted extraction of antioxidants from Ulva lactuca algal biomass using factorial design. *Biomass Conversion and Biorefinery*, 13(7), 5681-5690.

Saadatmand, S., Khavarinejad, R., Nejadsattari, T., & Soltani, S. (2011). Antioxidant and antibacterial activities of Cladophora glomerata (L.) Kütz. in Caspian Sea coast, *Iran. African Journal of Biotechnology*, 10(39), 7684-7689.

Schmidt, É. C., Felix, M. R. D. L., Kreusch, M. G., Pereira, D. T., Costa, G. B., Simioni, C., ... & Bouzon, Z. L. (2016). Profiles of carotenoids and amino acids and total phenolic compounds of the red alga Pterocladiella capillacea exposed to cadmium and different salinities. *Journal of Applied Phycology*, 28, 1955-1963.

Semerci, A. B., İnceçayir, D., Konca, T., Tunca, H., Tunç, K. (2020). Phenolic constituents,antioxidant and antimicrobial activities of methanolic extracts of some female cones of gymnosperm plant. *Indian Journal of Biochemistry and Biophysics*, 57(3), 298-303.4

Semerci, A. B., Tunç, K., Sağiroğlu, M. (2024). Investigation of antioxidant and antibacterial activities, phenolic contents of Allium staticiforme bulb fractions in different polarities. *Indian Journal of Traditional Knowledge*, 23(2), 182-188.

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158..

Sugihara, N., Ohnishi, M., Imamura, M., Furuno, K. (2001). Differences in antioxidative efficiency of catechins in various metal-induced lipid peroxidation in cultured hepatocytes. *Journal of health science*, 47(2), 99-106.

Tavakoli, S., Hong, H., Wang, K., Yang, Q., Gahruie, H.H., Zhuang, S., Li, Y., Liang, Y., Tan, Y., Luo, Y. (2021). Ultrasonic-assisted food-grade solvent extraction of high-value added compounds from microalgae Spirulina platensis and evaluation of their antioxidant and antibacterial properties. *Algal Research*. 60, 102493. https://doi.org/10.1016/j.algal.2021.102493

Torres, M. D., Flórez-Fernández, N., Domínguez, H. (2019). Integral utilization of red seaweed for bioactive production. *Marine drugs*, 17(6), 314.

Voerman, S. E., Ruseckas, A., Turnbull, G. A., Samuel, I. D., Burdett, H. L. (2022). Red algae acclimate to low light by modifying phycobilisome composition to maintain efficient light harvesting. *BMC biology*, 20(1), 291.

Wang, T., Jónsdóttir, R., Ólafsdóttir, G. (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food chemistry*, 116(1), 240-248.

Yoon, H. S., Zuccarello, G. C., & Bhattacharya, D. (2010). Evolutionary history and taxonomy of red algae. In Red algae in the genomic age (pp. 25-42). *Dordrecht: Springer Netherlands.*