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RESEARCH ARTICLE

Seasonal Changes in Proximate and Bioactive Compounds of Brown and Red Seaweeds from İskenderun Bay, the North-Eastern Mediterranean Sea

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Abstract: Proximate and bioactive compounds (total phenolic, flavonoid, chlorophyll-*a* and total carotenoid contents) of three brown seaweeds (*Dictyota dichotoma, Padina pavonica, Stypopodium schimperi*) and a red seaweed (*Jania rubens*) from the north-eastern Mediterranean Sea (İskenderun Bay) were investigated seasonally at three sampling sites. Seasonal variations were found for all of the parameters studied. The highest ash content was in *J. rubens* (77.7%) in the spring. The results showed that *J. rubens* is a rich source with respect to mineral content. *D. dichotoma* had the highest crude protein content, whereas *S. schimperi* contained the most lipids. Phenolics ranged between 34.6 - 107.0 mg GAE/g dw. The highest total phenolics were found in *S. schimperi* in the summer, and the lowest in *P. pavonica* in the spring. The flavonoid contents (9.05-10.6 mg QE/g dw) were higher in brown seaweeds than that in the red seaweed. Moreover, chlorophyll-*a* and carotenoids levels were highest in *D. dichotoma* (4.53 and 2.83 mg/g, respectively) during the autumn. The results revealed that the biochemical composition of the examined seaweeds showed significant changes depending on the species, location and seasons.

Anahtar kelimeler:

Makroalg
Temel besin maddesi kompozisyonu
Biyoaktif bileşikler
Fenolik
Flavonoid

İskenderun Körfezi'ndeki (Kuzeydoğu Akdeniz) Kahverengi ve Kırmızı Makroalglerin Temel Besin Maddesi ve Biyoaktif Bileşiklerindeki Mevsimsel Değişimler

Öz: Kuzeydoğu Akdeniz'de (İskenderun Körfezi) dağılım gösteren üç kahverengi (*Dictyota dichotoma, Padina pavonica, Stypopodium schimperi*) ve bir kırmızı makroalgin (*Jania rubens*) temel besin maddesi ve biyoaktif bileşikleri (toplam fenolik, flavonoid, klorofil-*a* ve toplam karotenoid içerikleri) üç örnekleme istasyonunda mevsimsel olarak incelenmiştir. İncelenen tüm parametrelerin mevsimsel değişimler gösterdiği belirlenmiştir. En yüksek kül içeriği ilkbaharda *J. rubens* türünde (%77.7) bulunmuştur. Sonuçlar, bu türün zengin bir mineral kaynağı olduğunu göstermiştir. *D. dichotoma* en yüksek ham protein içeriğine, *S. schimperi* ise en fazla lipit içeriğine sahip tür olmuştur. Makroalglerde fenolik madde içeriği 34.6 ile 107.0 mg GAE/g kuru ağ. arasında değişmiştir. Toplam fenolik madde miktarı yazın *S. schimperi* türünde en yüksek düzeye ulaşırken, en düşük değer ilkbaharda *P. pavonica* türünde bulunmuştur. Flavonoid içerikleri (9.05-10.6 mg QE/g kuru ağ.) kahverengi deniz yosunlarında kırmızı deniz yosunundan daha yüksek bulunmuştur. Klorofil-*a* ve karotenoid içeriği ise *D. dichotoma* türünde sonbahar mevsiminde en yüksek düzeylerde (sırasıyla 4.53 ve 2.83 mg/g) bulunmuştur. Sonuçlar, incelenen makroalg türlerinin biyokimyasal kompozisyonunun türe, lokaliteye ve mevsimlere bağlı olarak belirgin değişimler gösterebileceğini ortaya koymuştur.

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Introduction

Seaweeds are divided into three groups depending on pigmentation, namely red, green and brown algae. As primary producers, they provide food and shelter for many organisms living in the coastal waters. They are utilized as food for humans, feed for animals and as a source of various chemicals (Nedumaran and Arulbalachandran, 2015). They have been traditionally consumed by humans and used to extract ingredients. Seaweeds contain protein, lipids, dietary fibre, carotenoids, minerals and vitamins (Kumar *et al.,* 2008; Peñalver *et al.,* 2020). Many species red and brown seaweeds are utilized to produce alginate, agar and carrageenan, which are used as thickeners in foods, cosmetics and medicine. Moreover, seaweeds are particularly rich in biologically active compounds, i.e., functional foods such as phenolic and flavonoids, which have antioxidant activities (Machu *et al.,* 2015; Yılmaz et al., 2021). The antioxidant activity of seaweeds has been studied and strong relations between phenolic compounds and antioxidant activities were found. Wang *et al.* (2009) showed that the antioxidant activity of red seaweed was closely correlated with its extracted phenolics. Antioxidants are known to have a protective effect since they can defend the human body against damage by free radicals (Kalasariya *et al.,* 2021). Free radicals are associated with human diseases, including cardiovascular disease, cancer, diabetes, hypertension, ischemia, ageing, and Alzheimer's disease (Chauhan and Chauhan, 2006; Matanjun *et al.,* 2008).

Although Turkey is surrounded by the Black Sea, the Mediterranean Sea, the Sea of Marmara and the Aegean Sea with >8000 km of coastline, the bioactive compounds of seaweeds have still not been adequately studied. The previous studies in Turkish coastal waters are generally concentrated on the taxonomy and distribution of seaweeds (Aysel *et al*., 2006a; Aysel *et al.,* 2006b; Taşkın, 2014). Data on the chemical composition of seaweeds from the coasts of Turkey have increased in recent years but are still limited (Polat and Özoğul, 2008; Polat *et al.,* 2012; Turan *et al.,* 2015; Güner and Yavasoglu, 2018; Caf *et al*., 2019; Saygılı *et al.,* 2022). Moreover, there are only few studies on seasonal variations in the biochemical contents of seaweeds on our coasts (Polat and Özoğul, 2013; İrkin and Erduğan, 2016; İrkin and Erduğan, 2017; Yeşilova *et al.,* 2017). This study is aimed to investigate the proximate composition, total phenolic, flavonoid and pigment contents of seaweeds seasonally collected from İskenderun Bay on the north-eastern Mediterranean coast of Turkey.

Material and Methods

Collection of samples

Samples of three brown seaweeds (*Padina pavonica* (Linnaeus) Thivy, *Stypopodium schimperi* (Kützing) Verlaque & Boudouresque and *Dictyota dichotoma* (Hudson) J.V.Lamouroux), and one red seaweed (*Jania rubens*) (Linnaeus) J.V. Lamouroux were collected from three localities along the coast of İskenderun Bay (Fig. 1). The samples were collected seasonally over one year (between 2013-2014); in the spring, summer, autumn, and winter. Samples were taken from depths of up to 3 m.

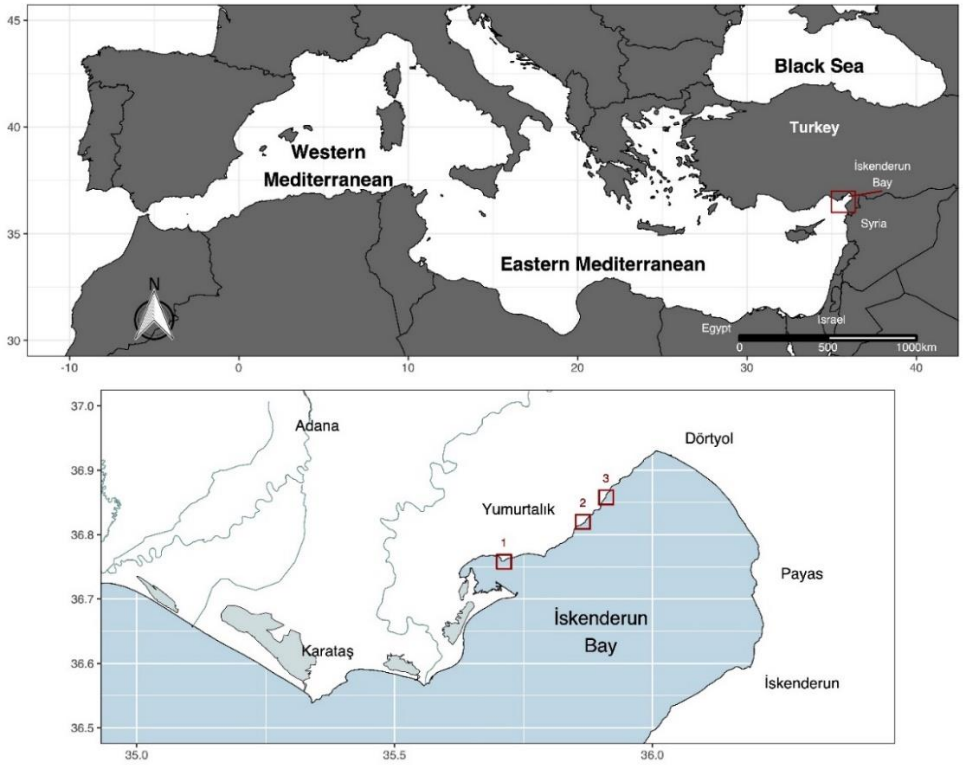

Figure 1. The study area and location of seaweed sampling sites in İskenderun Bay

Some species were only found in some seasons and locations. *P. pavonica* was not found in the winter, *S. schimperi* in the autumn and the winter, and *D. dichotoma* in the spring. Fresh samples of four selected seaweed species were washed with local seawater and immediately transported to the laboratory in an icebox with ambient seawater. Samples were gently washed with distilled water to remove epiphytes, salts, epifauna and sand. Samples were stored at -80 °C until further analysis. The temperature and salinity were measured with a YSI model SCT probe during the sampling periods, and the values for each sampling site are shown in Table 1.

Table 1. Seasonal variations of temperature and salinity during seaweed sampling

	Sampling sites	Spring	Summer	Autumn	Winter
Temperature (°C)	1	27.9	29.2	24.1	15.3
	2		31.6	25.4	17.6
	3	27.4	30.9	24.7	16.7
Salinity (‰)	1	35.5	37.2	37.4	37.2
	2		35.5	36.4	38
	3	34.8	37.1	36.5	37.3

Proximate composition analysis

Moisture content was determined using the AOAC method 950.46 (1990), and crude ash content was analyzed using the AOAC method 938.08 (1998a). Lipid analysis was carried out according to the method of Bligh and Dyer (1959). Samples were homogenized with a 1:2 mixture of chloroform and methanol and left in a dark place overnight after adding $CaCl_2$. The chloroform layer was removed and vaporized in an evaporator and finally in an oven at 60◦C for one hour. Total crude protein was determined using the AOAC method 955.04 (1998b). Seaweed samples were placed in Kjeldahl tubes, two Kjeldahl tablets and 20 mL H_2SO_4 were added to the incinerator, and the sample inside the tubes was burned at 420 °C. After digestion, 75 mL of water was added to the sample tube. Distillation was performed for 6 min with 40% NaOH by placing 25 mL of 40% boric acid (H_3BO_3) solution. Then, the distillate was titrated with 0.1 M HCl, and the amount of protein was found by recording the HCl consumed. The percentage of protein was determined with a conversion factor of 6.25.

Total phenolic content (TPC)

TPC of seaweed extracts was determined using the Folin-Ciocalteu method (Gámez-Meza *et al.*, 1999). The gallic acid was used as the standard, and the total phenol content was expressed as milligrams of GA equivalents per gram of extract (mg GAE/g dw).

Total flavonoid content (TFC)

The TFC of seaweeds were determined using the method described by Chang *et al.* (2002). The amount of TFC was determined from a standard calibration curve and expressed as mg QE/g dw (QE, quercetin equivalents) per gram of seaweed extract.

Chlorophyll-*a*

Chlorophyll-*a* was estimated according to the method described by Arnon (1949) and Thirumaran *et al.* (2009) with minor modifications. Ground seaweed (500 mg) was extracted with 10 ml of 80 % acetone (Merck, 99 %) and centrifuged at 3000 rpm for 15 min. The absorbance was measured at 645 and 663 nm. The content of chlorophyll-*a* was calculated based on the equation given by Thirumaran *et al.* (2009).

Total carotenoid

The total carotenoid level of seaweeds was determined spectrophotometrically using the same extract for chlorophyll-*a* estimation. The total carotenoid content was calculated from absorbance values at 480 nm using the equation of Kirk and Allen (1965).

Statistical analysis

The general linear models were used to compare biochemical compounds (ash, moisture, crude protein and lipid) among seasons and sampling sites. The models were separately fitted for each compound and species and then checked against the violations of normality and homogeneity using the Levene and Shapiro-Wilk tests on model residuals. Normality was met with all models. When heterogeneity was observed, a heteroscedasticity-corrected coefficient covariance matrix was calculated using the White Adjustment argument in the "car" package (Fox and Weisberg, 2011). After linear models were fitted, multiple comparisons were made using general linear hypothesis tests with Tukey's adjustment using the "multcomp" package (Hothorn *et al.*, 2008). In case of a violation of the homogeneity of the variance, the heteroskedasticity-consistent covariance matrix was estimated using the "sandwich" package (Zeileis, 2004). All statistical analyses used the R 3.4.4 statistical language (R Core Team, 2018).

Results and Discussion

The results of the four seaweed species' moisture, crude ash, lipid, total crude protein, total phenolic, flavonoid and pigment contents are shown in Tables 2-5. Ash content in seaweeds was generally higher than those in most land plants (Rupérez, 2002; Radha, 2018). The highest ash content was in *J. rubens* (77.7 %) collected from Site (St) 2 in the spring, whereas the lowest ash content was found in *D. dichotoma* (3.12 %) collected from St. 2 in the winter (Tables 2, 5). The high ash level of *J. rubens* shows that this species is rich in minerals. A similar high ash value was found in *Corallina officinalis* (77.8 %) by Marsham *et al.* (2007). The ash contents of all seaweed species showed significant differences between seasons and sampling sites ($p<0.01$, $p<0.05$), respectively. Marsham *et al.* (2007) reported that high ash indicates of a reasonable amount of mineral content. The maximum moisture was found in *D. dichotoma* (80.74 %) collected from St.1 in the winter and minimum content in *J. rubens* (3.15 %) collected from St.2 in the spring. The moisture content differed significantly ($p<0.01$, $p<0.05$) between seasons and sampling sites in all seaweed species. The moisture and ash composition of seaweeds differs depending on the species, geographical location and seasons.

Table 2. The proximate composition, total phenolics, flavonoids and pigments of *D. dichotoma*

D. dichotoma	**Sampling sites**	**Summer**	**Autumn**	**Winter**
Ash (%)	1	$3.32 \pm 0.29^{Aa**}$	n.a	3.12 ± 0.89^{Aa}
	2	n.a.	5.07 ± 0.10^{Ab}	3.12 ± 0.19^{Aa}
	3	4.98 ± 0.16^{Ba}	5.98 ± 0.78^{Bb}	5.49 ± 0.89^{Ba}
Moisture (%)	1	$75.67 \pm 1.09^{bc*}$	n.a	80.74 ± 3.67^{c}
	2	n.a.	72.87 ± 1.68^{ab}	79.87 ± 1.10^{c}
	3	69.42 ± 0.35^{ab}	70.25 ± 1.11^{ab}	67.81 ± 4.16^{a}
Lipid (%)	1	3.74 ± 0.01^{b}	n.a	0.90 ± 0.40^{a}
	2	n.a.	3.19 ± 0.32^{b}	1.02 ± 0.32^{a}
	3	5.13 ± 0.17^{c}	3.72 ± 0.08^{b}	2.72 ± 0.31^{ab}
Crude protein (%)	1	4.42 ± 0.29	n.a	5.16 ± 0.38
	2	n.a.	5.56 ± 1.67	4.66 ± 0.89
	3	6.15 ± 0.47	6.15 ± 3.33	4.92 ± 1.27
Phenolic content (mg GAE/g dw)	1	65.85 ± 3.95	n.a	47.06 ± 2.82
	2	n.a.	50.58 ± 3.03	46.48 ± 3.11
	3	52.70 ± 3.16	51.20 ± 3.07	46.28 ± 2.77
Flavonoid (mg QE/g dw)	1	9.23 ± 0.55	n.a	9.47 ± 0.61
	2	n.a.	9.16 ± 0.55	9.44 ± 0.56
	3	9.16 ± 0.54	9.05 ± 0.54	9.63 ± 0.57
Chlorophyll-*a* (mg/g)	1	1.43	n.a	3.51
	2	n.a.	4.50	3.69
	3	2.98	4.53	4.09
Total Carotenoid (mg/g)	1	1.05	n.a	2.04
	2	n.a.	2.46	2.23
	3	2.24	2.83	2.53

The values are expressed as means ± standard deviation, n = 3, n.a.: sample was not found, *Different superscript letters in the same variable indicate significant differences, **Lower case letters indicate significant differences between seasons, and caps indicate the differences between sampling sites when only two main effects are significant at 0.05 significance level.

The lipid contents of the seaweeds varied between 0.25 - 6.35%. The highest lipid content was found in *S. schimperi* collected from St.3 in the summer, while the lowest content (0.25%) was in *J. rubens* collected from St.3 in the autumn (Tables 4, 5). The differences in the lipid contents of all seaweed species were statistically significant ($p<0.05$) among seasons. However, the difference between sampling sites was only significant in *D. dichotoma* and *S. schimperi*. Similarly, Polat and Özoğul (2013) found the highest lipid in *S. schimperi* in

the summer, ranging between 2.03% - 2.16%. In another study, Polat and Özoğul (2009) reported the lowest total lipid content for *J. rubens*, as 0.12%. Parthiban *et al.* (2013) reported that the lipid contents of some seaweeds collected from Tuticorin varied from 3.15 to 5.30%. The results of this study were consistent with those of Nelson *et al.*, (2002) and Kostetsky *et al.* (2004) and showed that the lipid concentration of seaweeds may vary according to species, seasons and sampling sites.

Table 3. The proximate compositon, total phenolics, flavonoids and pigments of *P. pavonica*

P. pavonica	**Sampling sites**	**Spring**	**Summer**	**Autumn**
Ash (%)	1	$22.68 \pm 1^{bd*}$	25.14 ± 0.2^{cd}	n.a.
	2	18.17 ± 0.23^{a}	24.11 ± 0.60^{cd}	22.20 ± 1.99^{bc}
	3	19.97 ± 0.71^{ab}	32.00 ± 2.42^{e}	25.75 ± 0.78^{d}
Moisture (%)	1	32.74 ± 0.82^{a}	56.41 ± 0.31^{e}	n.a.
	2	53.67 ± 0.28^{cd}	55.24 ± 1.33^{de}	58.30 ± 2.77^{e}
	3	53.63 ± 0.28^{cd}	43.14 ± 4.04^{b}	52.10 ± 1.25^{c}
Lipid (%)	1	0.34 ± 0.05^{a}	2.16 ± 0.06^{d}	n.a.
	2	0.83 ± 0.15^{abc}	1.80 ± 0.47^{abcd}	1.20 ± 0.00^{b}
	3	1.36 ± 0.14^{bc}	1.76 ± 0.20^{bcd}	1.27 ± 0.01^{c}
Crude protein (%)	1	3.35 ± 0.15^{a}	4.03 ± 0.11^{b}	n.a.
	2	3.46 ± 0.14^{a}	5.30 ± 0.21^{b}	5.31 ± 0.60^{b}
	3	3.39 ± 0.58^{a}	4.53 ± 0.07^{b}	5.69 ± 1.28^{b}
Phenolic content (mg GAE/g dw)	1	34.61 ± 2.07	51.86 ± 2.01	n.a.
	2	n.a.	53.21 ± 3.19	49.44 ± 2.96
	3	42.50 ± 2.55	51.49 ± 3.02	61.41 ± 3.68
Flavonoid (mg QE/g dw)	1	10.07 ± 0.78	10.10 ± 0.31	n.a..
	2	n.a.	10.49 ± 0.62	10.00 ± 0.97
	3	10.47 ± 0.62	10.09 ± 0.60	10.00 ± 0.54
Chlorophyll-*a* (mg/g)	1	1.40	1.45	n.a.
	2	0.75	0.91	1.34
	3	1.11	1.12	1.62
Total Carotenoid (mg/g)	1	0.92	0.91	n.a.
	2	0.47	0.34	0.76
	3	0.79	0.94	0.96

The values are expressed as means ± standard deviation, n = 3, n.a.: sample was not found. *Different superscript letters in the same variable indicate significant differences, **Lower case letters indicate significant differences between seasons, and caps indicate the differences between sampling sites when only two main effects are significant at 0.05 significance level.

Crude protein ranged from 2.94 to 6.15%. The maximum crude protein was found in the brown seaweed *D. dichotoma* collected from St.3, during the summer and autumn (6.15%), followed by *S. schimperi* (6.05%) from St.1 in the spring. The minimum crude protein (2.94%) was in the *S. schimperi* collected from St.3 in the spring. The differences in crude protein were significant ($p<0.05$) among seasons in all species except *D. dichotoma*. However, the difference was not significant between the sampling sites in all species except *S. schimperi*. Similar results were reported by Tabarsa *et al.* (2012), who found relatively high crude protein in the brown seaweed *D. dichotoma* from the coastal area of Kuvehei (Iran). Polat and Özoğul (2013) found lower crude protein values ranging from 2.37 % to 2.68 % in *S. schimperi* than those reported in the present study. Higher crude protein levels (9.47 and 14.7%) were also reported by Parthiban *et al.* (2013) for some seaweed species collected from the Tuticorin and Mandapam coasts (India). The protein contents of the other species, *J. rubens* and *P. pavonica*, in the present study were higher than those previously found by Polat and Özoğul (2013). Factors such as the

physiological status of seaweed species and life cycle may have contributed to these results. In addition to species-specific differences, water quality, the season and the geographic area could influence the lipid and crude protein content of seaweeds (Fleurence, 1999; Haroon, 2000; Ratana-arporn and Chirapart, 2006).

Table 4. The proximate composition, total phenolics, flavonoids and pigments of *S. schimperi*

S. schimperi	**Sampling sites**	**Spring**	**Summer**
Ash (%)	1	$5.42 \pm 0.53^{bc*}$	3.96 ± 0.06^{a}
	2	n.a.	6.09 ± 0.24^{c}
	3	5.71 ± 0.25^{bc}	5.18 ± 0.10^{b}
Moisture (%)	1	$63.72 \pm 3.09^{Ca**}$	74.95 ± 0.15^{Cb}
	2	n.a.	66.26 ± 0.92^{Bb}
	3	61.21 ± 0.77^{Aa}	69.49 ± 0.20^{Ab}
Lipid (%)	1	3.28 ± 0.20^{Aa}	$4.73 \pm 0,14^{Ab}$
	2	n.a.	3.04 ± 1.82^{Bb}
	3	5.18 ± 0.08^{Ca}	6.35 ± 0.19^{Cb}
Crude protein (%)	1	6.05 ± 0.23^{b}	3.60 ± 0.06^{a}
	2	n.a.	3.96 ± 0.20^{a}
	3	2.94 ± 0.22^{a}	3.44 ± 0.04^{a}
Phenolic content (mg GAE/g dw)	1	92.85 ± 5.57	102.84 ± 5.23
	2	n.a.	106.05 ± 6.36
	3	52.90 ± 3.17	100.61 ± 6.03
Flavonoid (mg QE/g dw)	1	10.41 ± 0.62	10.62 ± 0.51
	2	n.a.	10.50 ± 0.72
	3	10.55 ± 0.63	10.61 ± 0.66
Chlorophyll-*a* (mg/g)	1	3.68	2.49
	2	n.a.	1.59
	3	3.04	1.18
Total Carotenoid (mg/g)	1	2.61	2.73
	2	n.a.	2.11
	3	2.33	1.95

The values are expressed as means ± standard deviation, n = 3, n.a.: sample was not found, *Different superscript letters in the same variable indicate significant differences, **Lower case letters indicate significant differences between seasons, and caps indicate the differences between sampling sites when only two main effects are significant at 0.05 significance level.

TPC of the seaweed species varied from 34.6 to 106.05 mg GAE/g dw. *S. schimperi* collected from St.2 in the summer showed the highest TPC, followed by *J. rubens* collected from St.1 in the autumn. The lowest TPC was found in *P. pavonica* collected from St.1 in the spring (Table 3). Kumar *et al.* (2011) reported phenolic contents in three green algae species *Caulerpa veravelensis*, *Caulerpa racemosa* and *Caulerpa scalpelliformis* as 32.57, 61.69 and 36.00 mg/g dw, respectively. Connan *et al.* (2007) reported that total phenolic contents in seaweeds may vary according to the difference between day and night temperatures and light intensity during the day. In the present study, the concentration of total phenolics in *S. schimperi* for the summer period (100.61-106.05 mg/g dw) was higher than all seaweed species sampled and those recorded in previous studies. *S. schimperi* seems to be a good antioxidant source for human health.

Table 5. The proximate compositon, total phenolics, flavonoids and pigments of *J. rubens.*

J. rubens	Sampling sites	Spring	Summer	Autumn	Winter
Ash (%)	1	n.a.	n.a.	$59.24 \pm 0.64^{A**}$	n.a.
	2	77.73 ± 0.20^{d}	72.62 ± 1.48^{c}	58.56 ± 0.43^{bA}	51.26 ± 2.85^{a}
	3	n.a.	n.a.	70.35 ± 4.22^{B}	n.a.
Moisture (%)	1	n.a.	n.a.	26.84 ± 0.92^{B}	n.a.
	2	3.15 ± 0.43^{a}	8.68 ± 1.56^{b}	27.38 ± 0.25^{cB}	3.69 ± 1.24^{a}
	3	n.a.	n.a.	6.31 ± 4.22^{A}	n.a.
Lipid (%)	1	n.a.	n.a.	0.39 ± 0.08^{ab}	n.a.
	2	$0.42 \pm 0.39^{ab*}$	0.78 ± 0.10^{b}	0.61 ± 0.25^{ab}	0.26 ± 0.01^{a}
	3	n.a.	n.a.	0.25 ± 0.00^{ab}	n.a.
Crude protein (%)	1	n.a.	n.a.	3.70 ± 0.04^{a}	n.a.
	2	4.64 ± 0.14^{b}	4.69 ± 0.45^{ab}	3.74 ± 0.06^{a}	3.36 ± 0.21^{a}
	3	n.a.	n.a.	3.77 ± 0.20^{a}	n.a.
Phenolic content (mg GAE/g dw)	1	n.a.	n.a.	70.35 ± 4.22	n.a.
	2	41.14 ± 2.46	52.65 ± 3.17	51.49 ± 3.08	40.67 ± 2.44
	3	n.a.	n.a.	68.03 ± 5.03	n.a.
Flavonoid (mg QE/g dw)	1	n.a.	n.a.	1.72 ± 0.10	n.a.
	2	1.80 ± 0.10	1.80 ± 0.10	1.75 ± 0.10	1.39 ± 0.08
	3	n.a.	n.a.	1.74 ± 0.07	n.a.
Chlorophyll-*a* (mg/g)	1	n.a.	n.a.	0.66	n.a.
	2	0.47	0.46	0.55	2.24
	3	n.a.	n.a.	0.37	n.a.
Total Carotenoid (mg/g)	1	n.a.	n.a.	0.19	n.a.
	2	0.17	0.15	0.16	1.06
	3	n.a.	n.a.	0.14	n.a.

The values are expressed as means ± standard deviation, n = 3, n.a.: sample was not found, *Different superscript letters in the same variable indicate significant differences, **Lower case letters indicate significant differences between seasons, and caps indicate the differences between sampling sites when only two main effects are significant at 0.05 significance level.

The flavonoid contents of seaweed species ranged from 1.39 to 10.62 mg QE/g dw. The maximum flavonoid content was found in *S. schimperi* collected from St.1 in the summer, followed by *P. pavonica*, collected from St.2 in the summer (Table 3, 4). The minimum flavonoid content was recorded in *J. rubens* collected from St.2 in the winter (Table 5). Sahayaraj *et al.* (2014) reported a similar flavonoid content for *P. pavonica* (11.53 mg/g dw). Similar to results obtained for *S. schimperi* and *P. pavonica* in the present study, Marinho *et al.* (2019) reported that the total flavonoid content of brown seaweed, *Saccharina latissima* reached a maximum level in the summer. However, the highest flavonoid value (4.83 mg RE g^{-1} dm) reported by Marinho *et al.* (2019) for *S. latissima* was lower than those found for seaweed species except *J. rubens* in this study. The primary photosynthetic pigment, chlorophyll-*a* content, varied between 0.37 and 4.53 mg/g, with the lowest in *J. rubens* and the highest in *D. dichotoma* (Tables 2, 5). Both seaweeds were collected from St.3 in the autumn. This result shows that the contents of chlorophyll-*a* may show species-specific differences. Palanivelu *et al.* (2012) reported comparatively lower chlorophyll-*a* content in *J. rubens* (0.07 mg/g ww). Chakraborty and Bhattacharya (2012) found chlorophyll-*a* content of *D. dichotoma* as 1.38 mg/g from the Gulf of Kutch (India). These results are consistent with the results of the present study. The total carotenoid contents of seaweed species ranged from 0.14 to 2.83 mg/g. The highest carotenoid level was found in *D. dichotoma* collected from St.3 in autumn, while the lowest value was recorded for *J. rubens* collected from the same site in the autumn (Tables 2, 5). Similar to the results of the present study, Sukalyan and Santra (2008) found the highest carotene level in *Dictyota ceylanica* Kützing. Moreover, Etemadian *et al.* (2017) stated that there was more carotene in brown algae, as was found in this study. The carotenoid levels of seaweed species found in the

present study were relatively higher than those reported by Chinnadurai *et al*. (2013) for six seaweed species (0.26 - 0.63 mg/g). However, the carotenoid contents of the seaweed species in the present study were lower than those of eight different seaweeds whose carotenoid levels were found between 18.85-29.02 mg/g in Sunderban (India) (Sukalyan and Santra, 2008). Godinez-Ortega *et al.* (2008) expressed that chlorophyll-*a* and carotenoid contents of seaweeds could vary according to light intensity. Similarly, Necchi and Zucchi (2001) highlighted that environmental conditions such as temperature, light intensity and period of light might affect the pigment content.

The biochemical analyses showed marked variations among species. Although comparatively low crude protein, lipid, flavonoid and pigment levels were observed, the highest ash levels were found in *J. rubens*, a heavily calcified alga. Renaud and Luong-Van (2006) stated that calcified seaweeds were rich in ash but low in nutrients. This suggests that red seaweed *J. rubens* may be a good source of minerals (Dixit and Reddy, 2017). On the other hand, brown seaweed *S. schimperi* contained the highest amount of TPC and TFC, which may have potential important roles in promoting human health due to their antioxidant, anti-aging and anti-carcinogenic properties. Moreover, seaweed carotenoids are strong antioxidants that prevent cardiovascular and neurodegenerative diseases and cancer (Boominathan and Mahesh, 2015). In this study, *D. dichotoma* showed the highest chlorophyll-*a* and carotene content, while red seaweed *J. rubens* had the lowest pigment levels.

Conclusion

Apart from their direct consumption, the substances extracted from seaweeds can be used in many applications, such as antioxidant and antibacterial agents in the food industry and for human health. The biochemical composition of the sampled seaweeds has the potential as antioxidant and mineral sources for functional uses, but differences were observed depending on the species, geographical location and season. Among the investigated species, *S. schimperi* and *J. rubens* seem to be the best source of phenolic and minerals, respectively. More studies are needed to evaluate the nutritional value and functional properties of these seaweeds as food supplements and for other industrial uses.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Sevim Polat and İbrahim Gür planned and designed the research. İbrahim Gür performed the sample collection and chemical analysis. All authors contributed to the writing of the final manuscript.

Ethics Approval

This article does not contain any studies with human or animal subjects.

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