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Original Scientific Paper

Biochemical Analysis of Some Brown Seaweeds from the Aegean Sea

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ABSTRACT: The purpose of this project was to investigate the total protein, sugar and phenolic content in phosphate extracts of marine macroalgae species from the Aegean Sea. *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier, *Petalonia fascia* (O.F. Müller) Kuntze, *Scytosiphon lomentaria* (Lyngbye) Link., *Dilophus spiralis* (Montagne) G. Hamel, and *Padina pavonia* J.V. Lamouroux were collected from Izmir Bay during April, as well as *Cystoseira* sp. collected on the Urla, Bodrum, Fethiye and Kas coastlines during the spring and summer months. The phenolic content of five brown algae extracts varied from 2.5 mg to 21.8 mg GAE/10 g. *Dilophus spiralis* extracts demonstrated the lowest concentrations based on total protein assay, while *Scytosiphon lomentaria* extracts showed the highest sugar contents. The extract of *Cystoseira* sp. collected from Fethiye and Kas, gave the highest concentrations in May.

Key words: Brown algae, protein assay, total phenolic content, total sugar

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INTRODUCTION

Marine organisms, especially algae are rich sources of natural bioactive products (Demirel *et al*. 2009). Since ancient times, macroalgae have been used as animal and human food, fodder and fertilizer in Asian countries (Yilmaz-Koz *et al*. 2009), associated with their high contents of amino acids, polysaccharides, minerals and vitamins (Durmaz *et al*. 2008; Kuda & Ikemori 2009). Brown algae are economically valuable seaweeds as a source of raw material for the extraction of polysaccharides (e.g. laminarin, alginate and fucoidan), mannitol and phlorotannins (phenol) (Davis *et al*. 2003; Zubia *et al*. 2008). Species of Phaeophyta are abundant in temperate areas and tropical regions (Zubia *et al*. 2007). Polysaccharides are the major structural components of cell walls and consist of acid units (b-D-mannuronic acid and a-L-guluronic acid). Brown algae are harvested for polysaccharides using industrial applications (water holding, gelling, emulsifying and stabilizing properties) (Davis *et al*. 2003). Mannitol (sugar alcohol) displays hydrating and antioxidant properties for cosmetic and pharmaceutical applications. Bioactive metabolites of brown algae are phenolic compounds, such as phlorotannins (oligomers of phloroglucinol) (Nagayama *et al*. 2003). Phlorotannins play ecological roles, such as antifouling substances, antioxidant, anti-plasmin inhibitor, chemical defenses against pathogens, epiphytes and grazers, and are photoprotection against solar radiation (UV radiation) (Kuda & Ikemori 2009; Davis *et al*. 2003; Zubia *et al*. 2008; Nagayama *et al*. 2003). Seaweed has a wide range of biological activities involved as antimicrobial, antivirus, and antioxidant, antitumor and anti-inflammatory (Patra *et al*. 2009; Kuda & Ikemori 2009; Zubia *et al*. 2008; Demirel *et al*. 2009; Tuney *et al*. 2007).

The objectives of this study were: 1) to analyze total protein, sugar and phenolic contents of *Colpomenia sinuosa*, *Petalonia fascia*, *Scytosiphon lomentaria*, *Dilophus spiralis*, *Padina pavonia,* which were collected from Izmir Bay in the springtime; 2) to analyze total protein, sugar

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and phenolic contents of *Cystoseria* sp., collected from the Urla, Bodrum, Fethiye and Kas coastlines during the spring and summer months (April to August 2009).

MATERIAL AND METHODS

Algae material. Brown macroalgae were collected from Izmir Bay in April in Turkey. Field specimens of brown seaweed, *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier (no EGE 40777), *Petalonia fascia* (O.F. Müller) Kuntze (no EGE 40773), *Scytosiphon lomentaria* (Lyngbye) Link (no EGE 40776), *Dilophus spiralis* (Montagne) G. Hamel (no EGE 40780), *Padina pavonia* J.V. Lamouroux (no EGE 40781) were deposited at Ege University, Herbarium Department, Izmir, Turkey. Macroalgae were collected from several reefs (depths of 1-2 m) along the Urla/Izmir coast (Turkey) and identified by Dr. Atakan Sukatar. In addition, *Cystoseria* sp. was collected from Urla (38.3705°N, 26.8367°E), Bodrum (36.9965°N, 27.2570°E), Fethiye (36.5405°N, 29.1259 °E) and Kas (36.1960°N, 29.6457°E) during the spring and summer months (April to August), 2009.

Freshly-harvested macroalgae samples were cleaned from their epiphytes by washing extensively in sterile sea water and voucher specimens were also stored in the Hydrobiology Laboratory in Ege University, Faculty of Science, Department of Biology, Izmir, Turkey.

Preparation of algae extracts. Freeze-dried samples were ground in liquid nitrogen with a mortar and pestle. Each algal powder sample (0.01 and 0.02 g) was extracted with 10 mL of phosphate buffer (pH 7.00) for 24 h using a shaking incubator and then a sonic bath for 30 min. The extracts were centrifuged at 6500 rpm for 5 min at +4 °C and the supernatant was transferred to new test tubes. The supernatant was collected for analysis of total proteins, total phenolic content and total sugars. Buffered seaweed extracts were kept at +4 °C until use.

Protein Assay. Brown seaweed total proteins were estimated by the method of Bradford (Bradford, 1976). Bovine serum albumin standards ranging in concentration from 0.05-0.5 mg/ml were run simultaneously with the test samples.

Total phenolic content. Total phenolic compound concentrations were determined as described previously (Kuda *et al*. 2005). Briefly, 0.4 ml of 10% Folin–Ciocalteu solution was added to 0.2 ml of sample solution. After 5 min, 0.8 ml of a 10% sodium carbonate solution was added. The mixture was allowed to stand for 1 h at ambient temperature and the absorbance was then assessed at 750 nm. The phenolic content was expressed as gallic acid equivalent (GAE) R^2=0.998.

Total sugar content. Total sugar content was estimated based on the phenol-sulphuric acid reaction of sugars (Dubois *et al*. 1956). D-Glucose standards ranging in concentration from 0 to 100 µg/ml, were also run.

Statistical analysis. The results were analyzed by one-way ANOVA using SPSS 12.00. All data (n=3) were expressed as mean ± standard deviation. A significant difference was considered at the level of $p<0.05$ (Choi *et al*. 2006).

RESULTS

Total protein, phenol and sugar contents analysed in the five freeze-dried brown algae are summarized in Table 1. *P. pavonia* extract showed the highest total phenol content (21.81±0.50 mg GAE/10 g). The highest percent of total sugar was measured in the brown alga *S. lomentaria* (86.88±4.72%), followed by *P. fascia* (75.34±13.36%). *D. spiralis* (2mg/mL) had the lowest protein, phenol and sugar contents among the brown seaweeds (0.09±0.04, 3.99±0.14, 27.49±7.36, respectively).

Table1. Total protein, total phenolic content and sugar of brown algae

Brown Algae	Concentration (mg/mL)	Total Protein (mg/mL)	Total Phenol (mg/10g)	Total Sugar %
Scytosiphon lomentaria	1	3.49± 0.22	8.11±.098	48.96±5.02
	2	5.10±0.14	12.53±0.24	86.88±4.72
Dilophus spiralis	1	n/a	2.46±0.23	16.88±7.38
	2	0.08±0.04	3.98±0.14	27.48±7.36
Colpomenia sinuosa	1	3.39±0.21	5.87±0.06	25.84±3.52
	2	4.79±0.27	10.85±0.22	50.43±4.77
Petalonia fascia	1	0.68±0.20	3.73±0.31	39.89±7.29
	2	0.87±0.09	5.45±0.24	75.33±13.36
Padina pavonia	1	4.19±0.23	12.81±0.29	55.07±4.48
	2	6.68±0.13	21.80±0.50	70.82±4.88

Table 2. Total protein, total phenolic and sugar content of *Cystoseira* sp. collected from Fethiye, Kas, Bodrum, Urla on April to August.

	Total Protein (mg/mL)	(mg/mL)	Fethiye	Kas	Bodrum	Urla
April		1	3.21±0.38	0.99±0.05	6.62±0.19	5.55±0.22
		2	4.35±0.21	1.21±0.03	7.44±0.39	7.27±0.39
May		1	2.12±0.026	6.75±0.10	-	-
		2	3.64±0.30	7.66±0.75	-	-
June		1	3.53±0.14	1.08± 0.05	-	-
		2	5.49±0.10	3.61±0.16	-	-
July		1	1.82±0.31	1.15±0.21	-	-
		2	3.76±0.20	2.00±0.17	-	-
August		1	0.16±0.14	0.16±0.08	-	0.98±0.03
		2	0.61±0.01	1.25±0.24	-	2.03±0.16
	Total Phenol (mg GAE/10 g)	**(mg/mL)**	**Fethiye**	**Kas**	**Bodrum**	**Urla**
April		1	10.1702±0.76	4.6292±0.58	35.3806±0.22	19.5045±0.11
		2	16.8468±0.15	7.7722±0.23	65.7340±0.77	35.8700±0.59
May		1	29.8737±0.54	39.0298±0.44	-	-
		2	43.6704±0.19	69.5405±0.34	-	-
June		1	11.5465±0.07	5.6927±0.71	-	-
		2	21.8058±0.16	13.6014±0.21	-	-
July		1	6.6234±0.20	7.3229±0.18	-	-
		2	14.8222±0.07	12.1095±0.05	-	-
August		1	2.9610±0.18	4.6747±0.08	-	6.3903±2.79
		2	4.2596±0.18	7.8120±0.26	-	9.3600±0.22
	Total Sugar (%)	**(mg/mL)**	**Fethiye**	**Kas**	**Bodrum**	**Urla**
April		1	30.81±2.00	26.28±2.57	22.94±0.29	31.41±3.76
		2	78.40±4.02	46.29±4.74	26.69±2.59	87.78±2.41
May		1	53.36±4.36	35.53±5.60	-	-
		2	10.30±3.27	53.84±2.34	-	-
June		1	16.72±2.04	34.32±3.43	-	-
		2	42.43±7.38	58.85±2.63	-	-
July		1	19.78±3.51	33.77±2.06	-	-
		2	30.49±1.94	52.90±3.58	-	-
August		1	22.66±9.86	23.27±2.69	-	13.63±1.66
		2	36.11±2.70	46.70±5.74	-	29.79±7.05

All the samples collected from Fethiye and Kas were collected monthly (spring to summer) from the Aegean Sea. As shown in Table 2, *Cystoseira* sp. had protein contents varying across the five months from 0.16±0.08 (Kas, August) to 7.67±0.75 mg/mL (Kas, May). In May, *Cystoseira* sp. extract also showed the highest total phenol content in Kas. Sugar contents determined in the present study on the brown macroalgae varied from 10.30±3.27% to 13.63±1.66%and the highest level of total sugars was detected in *Cystoseira* sp..

DISCUSSION

Colpomenia sinuosa, *Petalonia fascia*, *Scytosiphon lomentaria*, *Dilophus spiralis*, *Padina pavonia* and *Cystoseira* sp. were analyzed for protein, sugar and phenolic contents of the coast of Turkey. Factors like habitat, maturity and time of the year are known to affect the bioactive compounds of macroalgae (Durmaz *et al*. 2008; Stirk *et al*. 2007). Generally, macroalgae are valuable food sources for polysaccharides, proteins, vitamins and minerals (Sanchez-Machado *et al*. 2004). In addition, seaweeds have been regarded as new sources of dietary fibre and food ingredients (Gamal-Eldeen *et al*. 2009).

Burtin *et al.* 2003 reported that the protein concentration of brown macroalgae was low at 5-11% of dry weight. However, Marsham *et al.* 2007 found high levels of protein concentrations in *Laminaria digitata* (15.9% dw) and *Fucus serratus* (17.4% dw). Phenols form a significant part of plant organic matter, and their

quantity changes in response to environmental factors. Phaophyceae species contain various phlorotannins, including fucolls, florethols, fucoflorethols, fuhalols, isofuhalols, as well as halogenated and sulphited phlorotannins (Chkhikvishvili & Ramazanov 2000). Phenolic compounds (phlorotannins-polyphenols) play a significant role in the cell defence against biotic and abiotic stresses, in macroalgae. Chkhikvishvili & Ramazanov 2000 showed that the highest content of phenolic substances was determined in *Cystoseira compressia* and *Sargassum furcatum* in the Canary Islands (Spain). In general, it is considered that biological activities depend on phenolic and brown compounds. However, brown algae cell wall is storage of sulphated polysaccharides such as fucoidan and alginate (Zubia *et al*. 2008; Queiroz *et al*. 2008).

CONCLUSION

Turkey is surrounded by sea on three sides, so brown macroalgae existing along the Turkish coasts should be investigated to establish their chemical contents and their potential uses the as animal food sources. Animal feeding trials would be required to examine nutritional value of brown algae and some potential is showen in this paper.

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REZIME

Biohemijska analiza mrkih algi iz Egejskog mora

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Cilj rada bio je da se ispita ukupni sadržaj proteina, šećera i fenolnih jedinjenja u algama *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier, *Petalonia fascia* (O.F. Müller) Kuntze, *Scytosiphon lomentaria* (Lyngbye) Link., *Dilophus spiralis* (Montagne) G. Hamel i *Padina pavonia* J.V. Lamouroux, sakupljenih u Izmirskom zalivu tokom proleća. *Cystoseira* sp. Sakupljana je sa različitih lokaliteta tokom dužeg perioda (April – August 2009). Sadržaj fenola mrkih algi varira od 2.46 mg GAE/10 g do 21.81 mg GAE/10 g. *Dilophus spiralis* ima najmanji sadržaj proteina, dok *Scytosiphon lomentaria* sadrži visok procenat šećera. Sadržaj ispitivanih jedinjenja varira tokom sezone i u ekstraktima *Cystoseira* sp., sakupljenih na lokalitetima Fethiye i Kas, ima najviše vrednosti u maju mesecu.

Ključne reči: mrke alge, protein, sadržaj fenola, šećeri