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COMPONENT COMPOSITION AND ANTIOXIDANT POTENTIAL OF *CYSTOSEIRA BARBATA* FROM THE BLACK SEA

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Abstract: *The purpose of this study was to analyze some chemical elements and to determine the content of total phenols and pigments in the brown seaweed Cystoseira barbata from Rusalka Cape near the Black Sea. In addition, the antioxidant potential of this algae was also evaluated by four methods based on different mechanisms. The obtained results were compared with other scientific studies related to inorganic and organized compositions of Cystoseira barbata from different marine regions. It was found that the seaweed from Rusalka Cape contained high values of manganese, chromium and boron and a high concentration of carotenoids. Therefore, this seaweed was evaluated as a source of microelements, carotenoids and antioxidants.*

Key words: *Cystoseira barbata, mineral composition, total phenols, pigments, antioxidants.*

1. Introduction

The Bulgarian algoflora is composed of 165 species of algae, divided into three genera [26]. Among them the most numerous are red seaweed (Rhodophyta), followed by green (Chlorophyta) and brown (Phaeophyta) [11]. *Cystoseira barbata* (Stackh.) C. Agardh is one of the largest algae in the Black Sea basin, that belongs to the Phaeophyta (class Phaeophyceae, order Fucales, family Cystoseiraceae). It reaches a maximum length of 170 cm. The study of the association of these seaweeds is used for biomonitoring, to calculate indices that help determine the ecological status and the level of eutrophication of a habitat.

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Cystoseira barbata is an indicator of water purity [19]. Aqueous areas with dominant species of *Cystoseira* sp. are indicators of high water quality [10], [24].

Seaweeds, as well as plants, contain various inorganic and organic substances that can have a beneficial effect on human health. All seaweed contain large amounts of macro elements such as calcium, magnesium, sodium, phosphorus and potassium, as well as micro-elements such as zinc, iodine and manganese [21], [29]. Seaweed is one of the most important sources of calcium and phosphorus, as it contains these elements in higher amounts in comparison to some fruits such as apples, oranges, carrots and potatoes [7].

Brown seaweed *Cystoseira barbata* is known to possess functional nutritional properties and contain compounds with antimicrobial, antihypertensive and antioxidant properties [2], [4], [9], [32]. It is an edible brown alga, traditionally used as a functional food and as a source for the extraction of alginates [34]. *Cystoseira barbata* has been scientifically proven to contain compounds that have biological activity, such as laminarin, which has antibacterial, antioxidant and healing properties [32], fucoxanthin, which is used as an enhancer of colorants and oxidative stability of topical products, and polyphenolic protein polysaccharides [31].

2. Objectives

The purpose of this study is to quantify some chemical elements, to determine the content of total phenols and pigments and to evaluate the antioxidant potential of the brown seaweed *Cystoseira barbata* (Stackh.) C. Agardh from Cape Rusalka near the Black Sea and to compare the data with other similar scientific studies.

3. Material and Methods

3.1. Collection of Seaweed Samples

The sampling of the brown seaweed *Cystoseira barbata* was performed by the method of squares [28]. The samples were taken from the Black Sea in the summer of 2012 near the Rusalka cape, which is located 80 km from the city of Varna in the Republic of Bulgaria. The fresh brown algae was washed and cleaned of accompanying mechanical impurities and cut into 1 cm long strips and then frozen in a refrigerator at a temperature of -18°C. After thawing, 50 cm^3 of 0.5 N HCl was added to 10 g of seaweed. The resulting acid mixture was kept overnight at room temperature and stirred periodically. Then the filtration was performed through a nylon cloth and the algae were washed several times with distilled water to neutralize 0.5N HCl. Finally, the algae was dried for 3 days at 40°C.

3.2. Quantitative Analysis of Micro- And Macroelements

The mineral composition of brown seaweed was determined by a validated method developed at the Institute of Food Canning and Quality - Plovdiv, Bulgaria according to BNS 1179-84 and EN 14082:2003 using microwave mineralization of a sample (1 g) with 3 cm^3 of 0.2% HNO_3 and 2–3 cm^3 H_2O_2. The mineralized sample was filtered and analyzed by ICP-OES Spectroflame Modula - FTMOA81A (Spectro Analytica Instruments).

3.3. Pigment Analysis

The seaweed was extracted with 100% acetone (1:5 w/v) in triplicate in an ultrasonic bath SIEL UST 5.7-150 at 40 °C for 15 min. The combined acetone extracts were measured at three wavelengths 662, 645 and 470 nm against a blank. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids were calculated according to Lichtenthaler and Wellburn [18]. The results were presented as µg/g dry weight (dw).

3.4. Extraction of Total Phenols

Cystoseira barbata was extracted with two solvents with different polarity (acetone and 95% ethanol) in a solid to liquid ratio of 1:5 (w/v). The extraction was performed in triplicate into an ultrasonic bath (VWR, Malaysia) with a frequency of 45 kHz and a 30 W power at 45°C [27]. The obtained extracts were used for the determination of the total phenolic content and of antioxidant activity.

3.5. The Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocâlteu reagent. Seaweed extracts (0.2 cm^3) were mixed with 1 cm^3 Folin–Ciocâlteu and then 0.8 cm^3 7.5% Na_2CO_3 was added. After 20 min the absorption was measured at 765 nm against a blank. The results were expressed in mg equivalent of gallic acid (GAE) per g dw [14, 15].

3.6. Antioxidant Activity

3.6.1. DPPH (1,1-diphenyl-2-picrylhydrazyl) Method

Each extract (0.15 cm^3) was mixed with 2.85 cm^3 freshly prepared 0.1 mM methanol solutions of DPPH. The sample was incubated for 15 min at 37°C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank prepared with methanol [1].

3 6.2. ABTS+ Radical Scavenging Ability

The seaweed extracts (0.15 cm^3) were mixed with the ABTS+ solution (2.85 cm^3). After 15 min at 37°C in darkness, the absorbance was measured at 734 nm against ethanol [14].

3.6.3. Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP reagent (3.0 cm^3) consisting of 10 parts 0.3M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in distilled water was mixed with 0.1 cm^3 extract. After 10 min at 37°C in darkness, the absorbance of the sample was measured at 593 nm [14, 15].

3.6.4. Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

Sample (0.1 cm^3) was mixed with 1 cm^3 $CuCl_2 \times 2H_2O$, 1 cm^3 methanol solution of Neocuproine, 1 cm^3 0.1 M ammonium acetate buffer and 1 cm^3 distilled H_2O. After 20 min at 50°C in darkness, the samples were cooled and the absorbance was measured at 450 nm [14].

All the results were expressed as mM Trolox equivalents (mM TE) on dry weight (dw) and dry weight (dw) [14].

The data were presented as mean values ±standard deviation (SD). Statistical analysis was performed using MS Excel 2010.

3. Results and Discussion

3.1. Mineral Composition

The mineral composition of *Cystoseira barbata* was summarized in Table 1. The elemental composition of seaweed varies between species depending on cultivation conditions, growth phase and environmental factors [20], [36].

The presented results (Table 1) showed that calcium and magnesium was 1.9 and 1.5 times higher as compared to the content of these elements in the brown alga *Cystoseira barbata* collected from the city of Varna in Bulgaria [26]. The iron and manganese content of *Cystoseira barbata* from Cape Rusalka was 1.2 and 11.8 times higher than that of the algae *Cystoseira barbata* from the northeastern province of Sinop in Turkey near the Black Sea [5]. The content of the zinc microelement in *Cystoseira barbata* from Cape Rusalka was 3.4 and 3.7 times higher than the content of the same element in seaweed collected from the Sevastopol region (Cape Pavlovsky, southwestern Crimea, Black Sea) [16]. The concentration of nickel of the algae (*Cystoseira barbata*) from Cape Pavlovsky (southwestern Crimea, Black Sea) [16] was 2.3 and 2.2 times lower than the current results. However, the content of arsenic in the samples from Cape Rusalka was 1.6 and 1.3 times higher than the determined amount of the same element in *Cystoseira barbata* from the protected marine areas of the Crimea peninsula near the Black Sea [17].

Table 1

Mineral composition of Cystoseira barbata

Elements	Content [mg/kg dw[a]]
Calcium (Ca)	41600±500[b]
Magnesium (Mg)	6600±1000
Iron (Fe)	620±13
Manganese (Mn)	252±16
Zinc (Zn)	130±4
Nickel (Ni)	6±0.5
Arsenic (As)	48±1
Copper (Cu)	27±1
Chromium Cr	7±0.5
Boron (B)	1600±40

Notes: [a]dw – dry weight; [b] SD – standard deviation

According to the literature, the copper content of some species of algae from contaminated regions was from 200 to 300 μg/g [6]. The copper content in the alga from Cape Rusalka was 4 times higher as compared to the same chemical element in samples from the eastern Aegean Sea near Turkey [6]. Copper content in *Cystoseira barbata* from the present study showed a greater presence of anthropogenic sources of pollution from this metal near Cape Rusalka in the Black Sea as compared to the Aegean Sea (Turkey). The determined concentration of the chromium element in this study was 2 times higher than the chromium content in *Cystoseira barbata* from the Adriatic Sea in the area of the Makarska Riviera near the town of Mimice in Croatia [22]. The content of boron in the seaweed from Cape Rusalka was from 7 to 19 times higher as compared to the same species of algae from different regions of the Makarska Riviera in Croatia [23].

3.2. Phenolic Content and Antioxidant Activity

Phenolic compounds are secondary metabolites that are indirectly involved in physiological processes. The biosynthesis of phenolic compounds are carried out by the pathway of shikimic acid and acetate-malonate [13]. In most of the cases phenolic compounds bring about the antioxidant potential. Phenolic content and antioxidant activity in *Cystoseira barbata* were presented (Table 2). The content of total phenols in the ethanol extract was higher as compared to the acetone extract of *Cystoseira barbata* from Cape Rusalka. This fact is due to the type of solvent that selectively affects the phenolic compounds [8]. A significantly higher content of total phenols in the acetone and methanol extracts was found in *Cystoseira barbata* from the Black Sea coast of Romania [35] as compared to the brown alga from Cape Rusalka (the Black Sea, Bulgaria).

Table 2

Total phenolic content and antioxidant activity of Cystoseira barbata

Solvents	Total phenols [mg GAE/g dw]	DPPH	ABTS	FRAP	CUPRAC
		[mM TE/g dw]			
Acetone	0.26±0.05	1.49±0,43	1.84±0.35	1,06±0.02	7.96±0.12
95 % Ethanol	0.37±0.05	0.86±0.13	1.05±0.05	3.09±0.12	10.89±0.15

It was found that in our study the content of total phenols was 8.8 times (in ethanol extract) and 12.6 times (in acetone extract) less than the aqueous extract of *Cystoseira barbata* from Chanakkale in Turkey [3]. This variation in the amount of total phenols identified was due to differences in extraction methods, aquatic environmental parameters and reproductive development of *Cystoseira* seaweeds [2], [33]. To the best of our knowledge the antioxidant potential of *Cystoseira barbata* was not evaluated in detail. In the current study, four methods based on different mechanisms were used (Table 2). The ethanol extracts demonstrated higher metal reduction potential (FRAP and CUPRAC assays) than radical scavenging ability (DPPH and ABTS assays). The highest antioxidant activity was evaluated by CUPRAC assay -7.96 and 10.89 mM TE/g dw for acetone and ethanol extracts, respectively. The similar reports for ethanol as the proper solvent for extracting the active substances were reported earlier [2], [8].

3.3. Pigments Content

Carotenoids are color tetraterpenes with great structural diversity and an important role in human nutrition, providing provitamin A [3]. In addition, carotenoids are pharmacologically active substances that have antimicrobial, antioxidant and anticancer effects [3]. The results from pigment content in *Cystoseira barbata* were shown in (Figure 1). The carotenoid content was in the highest values - 515.8 µg/g dw. Our resulting values were higher than the content of carotenoids in *Cystoseira barbata* [4] from Turkey. In addition, the same species of brown algae from Iskenderun Bay in the northeastern Mediterranean coast of Turkey [25] contained 2.86 times less carotenoid content than *Cystoseira barbata* from Cape Rusalka.

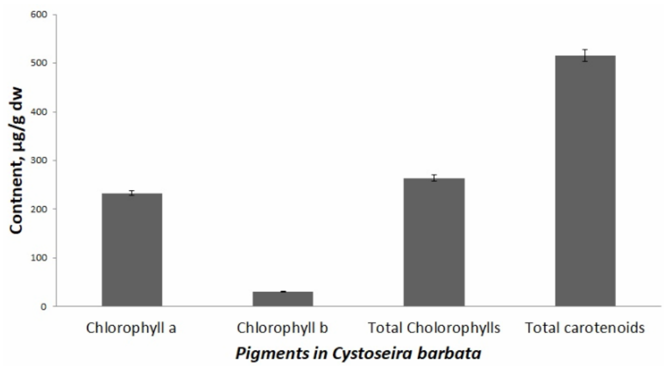

Fig. 1. *Pigments in Cystoseira barbata*

Fucoxanthin, the main pigment in brown algae, and chlorophyll-a together form protein complexes in thylakoids, which are involved in energy collection and transmission [30]. The content of chlorophyll in *Cystoseira barbata* (263.8 µg/g dw) from our study was 1.5 times less than the chlorophyll in the same seaweed from Sinop Bay [12] and 4.5 times lower content than in the methanol extract *of Cystoseira barbata* from Iskenderun Bay in Turkey [25]. From the conducted experiments and comparing the results with the data of other authors, it can be said that not only the location, but also the type of extracting agent used affect the content of the natural pigment chlorophyll in brown algae.

4. Conclusion

The obtained results demonstrated the potential use of the brown seaweed *Cystoseira barbata* from the Rusalka Cape as a valuable food source of micro and macro elements. As compared to other marine regions, *Cystoseira barbata* from the current study contained high values of manganese, chromium and boron. The presence of natural pigments such as chlorophylls and carotenoids reveals the potential use as a food colorant. In addition *Cystoseira barbata* were evaluated as a source of polyphenols with antioxidant potential. Therefore, *Cystoseira barbata* can be used not only as indicator for water quality, but also as a source of antioxidants, carotenoids, macro- and microelemets.

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