DPPH ANTIRADICAL ACTIVITY AND TOTAL PHENOLIC CONTENT OF METHANOL AND ETHANOL EXTRACTS FROM MACROALGAE (*ULVA RIGIDA*) AND MICROALGAE (*CHLORELLA*)

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ABSTRACT

INTRODUCTION: Algae are widely popular as dietary supplement. Furthermore, they can be a great source of antioxidants (pigments, alkaloids, carotenoids, phenolic acids, sulfated polysaccharides and long-chain polyunsaturated fatty acids etc.) and can be used instead of synthetic ones. The different nutrient compositions of algae depend on class, species, habitats, maturity, and environmental conditions.

AIM: The present study aims to investigate the differences in the antioxidant activity (AOA) and total phenolic content (TPC) of macroalgae *Ulva rigida* from the Black Sea and microalgae *Chlorella*. In addition, the obtained results will show their potential as natural sources of antioxidants.

MATERIALS AND METHODS: The marine macroalgae *Ulva rigida* and the microalgae *Chlorella* were used to perform different solvent extracts, which were analyzed for antiradical activity and total phenol content.

RESULTS AND DISCUSSION: All analyzed extracts (methanol and ethanol) showed positive results of the DPPH test and TPC. Both methanol extracts of microalgae *Chlorella* and macroalgae *Ulva rigida* had higher scavenging effect on used radicals for antioxidant activity compared to both ethanol extracts of the same plant material. The results show high potential as natural source of antioxidants of both algae species due perhaps to the phenolic content and other compounds having antioxidant activity.

CONCLUSION: Both *Ulva rigida* and *Chlorella* can be used as a source of antioxidants and phenolic acids, which can be added to new functional foods and supplements, as well as be the basis of pharmaceutical and cosmetic products.

Keywords: *Ulva rigida, Chlorella, DPPH, Folin-Ciocalteu, total phenolic content*

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INTRODUCTION

Green algae are the most diverse group of algae, with more than 7000 species growing in a variety of habitats. They are found in both sandy and rocky coasts. One of the common seaweed species is *Ulva rigida*. It cause "green tides" in the sea. It is a commercially important, renewable marine resource containing significant quantities of proteins, lipids,

minerals, and vitamins. These nutrient contents vary with geographical location, season, salinity of water, temperature and environmental conditions (1).

Chlorella vulgaris is a single-celled eukaryotic green microalgae normally found in freshwater basins. In its chloroplast, it contains the highest amount of chlorophyll of any other known plant (2). Chlorella's chemical nutrient composition includes a wide range of potent antioxidants, in particular beta-carotene, vitamin E, vitamin C, polyphenolic compounds and also significant quantities of the carotenoid lutein (3). Microalgae have been touted as a suite of biologically active dietary supplements with almost panacea-like properties. This kind of microalgae has the capabilities to produce secondary metabolites, including polysaccharides. The algae extracts can be considered as a source of dietary fiber, (4), which has identical functionality as prebiotics since they are indigestible in the human digestive system. Therefore, they are widely distributed and readily available in the market as a dietary supplement in tablets and powder forms (3,4).

Free radicals, in particular reactive oxygen species (ROS), damage DNA and therefore cause cells to reproduce improperly, which can lead to certain diseases, including coronary heart disease, cancer, premature aging, inflammatory diseases, Parkinson's disease, periodontal disease, and cataracts. In living systems, various metabolic processes and environmental stresses generate different reactive species (5, 6). Protection against free radicals in living systems is regulated by an important biochemical equilibrium between free radicals and antioxidants. If the balance is disturbed and cannot be resumed, living cells are damaged or die. Because of that the dietary daily intake of natural antioxidants is important and provides protection against free radicals. Many studies show that foods containing nutrients with antioxidant potential, may be of major importance in disease prevention and improving the quality of life (7). The antioxidant action can occur in various reactions and mechanisms prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity, and radical scavenging. The substances, which scavenge free radicals, play an important role in prevention of free radicalinduced diseases by donating hydrogen radicals, the primary radicals are reduced to nonradical chemical compounds and then converted to oxidized antioxidant radicals. This action helps in protecting the body from degenerative diseases. Antioxidants are generally defined as any substance that effectively prevents or delays the adverse effects caused by free radicals, even when the amount of the antioxidant substance is less than the oxidized substance (6, 8-10).

The antioxidant potential of algae and their phytochemical composition defines them as natural sources of free radical scavengers and antioxidant agents. Many studies report that the phytochemical screening of algae shows the presence of phenolic compounds, glycosides, flavonoids, alkaloids, steroids, terpenoids, sugars, fats, etc. Due to those facts, both *Ulva rigida* and *Chlorella* can be used to develop new functional foods and supplements, as well as be the basis of pharmaceutical and cosmetic products.

AIM

The present study aims to investigate the differences in the antioxidant activity (AOA) and total phenolic content (TPC) of two different extracts (methanol & ethanol) of macroalgae *Ulva rigida* from the Black Sea and microalgae *Chlorella*. Furthermore, the obtained results will show their potential as natural sources of antioxidants.

MATERIALS AND METHODS

The marine macroalgae *Ulva rigida* and the microalgae *Chlorella* were used to perform different solvent extracts, which were analyzed for antiradical activity and total phenol content.

Macroalgae Collection

The green macroalgae were collected during the summer season of 2018 year from the northern Black Sea region, where they are wildly growing in the wild. Their class and genus were determined by colleagues-biologists at the Institute of Oceanology at the Bulgarian Academy of Sciences. The microalgae were cultivated in culture, which purchased from the University's pharmacy in the same year.

Sample Preparation

Once harvested, seaweeds were thoroughly washed three times with fresh water to remove sands, salts and epiphytes. They were air-dried at room temperature for two days, and then further, dried in a drier at low temperature (35°C-40°C) to constant

weight. Dried algae were milled into powder to provide homogeneous samples before extraction.

Extraction

The extraction procedure include two different solvents-methanol and ethanol. Dried seaweed sample powder (1 g) was extracted with 100 mL pure methanol in an ultra sonic bath for 30 minutes after which the extraction continue in a shaker with constant stirring for two hours and again 30 minutes in an ultra sonic bath. The same procedure was used for ethanol extract preparation. All extraction procedures were prepared in triplicate in dark conditions.

Both sample extracts (methanol and ethanol) were analyzed spectrophotometrically for DPPH radical scavenging activity and TPC, by double beam UV-via a spectrophotometer.

Antioxidant Activity

The DPPH radical scavenging potential (1, 1-diphenyl -2-picrylhydrazyl, Sigma Aldrich) of different solvent extracts of *Ulva rigida* and *Chlorella* was determined by the described method of Wei Fu et al (11) with some modifications. The free radical scavenging activity of extracts was measured *in vitro* by mixing 7 μM methanol solution of DPPH and various concentrations of methanol or ethanol extract (200–600 μg/mL). This mixture was allowed to stay at room temperature (30 min in dark conditions) to obtain the reaction. Then the absorption of solutions was measured at λ=517 nm. The positive controls were referred against standard methanol solution of vitamin C (ascorbic acid).

Total phenolic content

TPC of the two solvent extracts was determined using the colorimetric method with the Folin-Ciocalteu's reagent described by Ludmila Machu et al. (12) with some modifications. Briefly, 500 μL of each sample extract was mixed with 2.5 mL of 10% Folin–Ciocalteu's reagent and 2.0 mL of 7.5-% sodium bicarbonate solution. After incubation at room temperature for 60 min., the reaction mixture absorbance was measured at 765 nm, against deionized water as a blank. A Gallic acid standard solution was used for a calibration curve construction. The TPC results were expressed in Gallic acid's equivalent.

Statistical analysis

All results were expressed as the average of triplicates ± standard deviation (mean ± SD). Standard curve for analysis of total phenolic content was obtained using five different concentrations of standard solutions of Gallic acid. The coefficient of correlation of standard curve was 0.9987. To assess the statistically significant differences of the results analysisq the t-test (nonparametric tests) procedure of a Graph Pad Prism 6 program at a significance level of 5% was used. The results were considered statistically significant at $p<0.005$.

RESULTS AND DISCUSSION

Antioxidant activity

The radical scavenging activity of two different solvent extracts was determined by DPPH radical scavenger assay. The data of three different concentrations for methanol and ethanol solutions are presented in Table 1.

Table 1. DPPH Inhibition of Ulva rigida and Chlorella methanol and ethanol extracts

Concentration, μg.mL^{-1}	Inhibition of DPPH, %			
	Ulva rigida		*Chlorella*	
	CH_3OH extract	C_2H_5OH extract	CH_3OH extract	C_2H_5OH extract
200	5.39 ± 0.42	2.08 ± 0.31	23.5 ± 1.24	10.57 ± 0.77
400	7.41 ± 0.51	6.04 ± 0.42	36.3 ± 1.88	20.76 ± 1.14
600	13.64 ±0.77	7.17 ± 0.38	40.8 ± 2.09	30.06 ± 1.64

According to the obtained data, both tested solvent extracts possessed the ability of scavenging DPPH at various degrees. It depended on the concentration of the extract. The results, presented in the table for both algae species, show strong dependence on the type of extractor. The methanol extracts in the three concentration levels indicated higher AOA, in both cases. The microalgae methanol extract (600 μg.mL^{-1}) demonstrated three times higher radical in-

hibition 40.8-%, compared with that of macroalgae 13.64-% ($p<0.005$) (Table 1). These and the results of the other two concentrations of both type algae are shown in Table 1.

The IC50 value is the most used and accurate assessment of the AOA of various extracts-this is the concentration of extract needed to inhibit 50% of the radicals. The lower IC_{50} value the stronger antiradical activity.

The IC_{50} values of the DPPH radical scavenging activity, of the two analyzed algae, are, presented in table 2. The data presented in the table show the highest antioxidant potential of the methanol extract of microalgae *Chlorella* (0.8 $mg.mL^{-1}$). The other three extracts have very close values, with the AOA being three times lower.

Table 2. Radical scavenging activity of Ulva rigida and Chlorella

Solvent	DPPH-radical scavenging activity - IC_{50}, $mg.mL^{-1}$	
	Ulva rigida	*Chlorella*
C_2H_5OH	2.65 ± 0.43	2.59 ± 0.44
CH_3OH	2.40 ± 0.50	0.78 ± 0.12

Mezghani et al. analyzed AOA of the same macroalgae species (2). The AOA of microalgae Chlorella has been explored widely by different scientific groups. The investigation results of the groups of Mezghani and Jayshree show comparable values with our results of IC_{50} of DPPH inhibition for methanol extracts of Ulva rigida and Chlorella (2,13).

Total phenolic content

The present study observed at the TPC of both extracts (methanolic and ethanolic) of marine algae *Ulva rigida* and microalgae *Chlorella* using the Folin-Ciocalteu method. The observed results arre present in Table 3 as microgram equivalents of Gallic acid per gram dry matter ($\mu gEqGA.g^{-1}$ d. m.).

Table 3. Total Phenolic Contents of Ulva rigida and Chlorella

Solvent	Total phenolic contents, μgEqGA/g d. m.	
	Ulva rigida	*Chlorella*
C_2H_5OH	2.29 ± 0.94	26.65 ± 2.07
CH_3OH	32.80 ± 2.16	74.23 ± 3.64

The phenolic content varied strongly-the lowest content was recorded from 2.3 $\mu gEqGA.g^{-1}$ d. m. (in ethanolic extract of *Ulva rigida*) and the highest from 74.2 $\mu gEqGA.g^{-1}$ d. m. (in methanolic extract of *Chlorela*). According to the data in Table 3 the microalgae is a better source of phenolic compounds, compared with analyzed green macroalgae species. The results show that methanol extracts of both analyzed objects have higher TPC values compared to ethanol extracts (5).

Ludmila Machu et al. (12) present results for TPC of green freshwater algae *(Chlorella pyrenoidosa)* and other seaweeds. They analyzed TPC in different solvent extracts, including methanol. The presented data for TPC of *Chlorella* were from the same order as ours for this species.

CONCLUSION

The obtained results confirmed that all prepared extracts showed AOA and contained phenolic components. The highest antioxidant activity was established for the methanol extract of *Chlorella*, with IC_{50} values of DPPH radical scavenging activity-0.8 $mg.mL^{-1}$.

The observed data of different extracts from *Ulva rigida* and *Chlorella*, showed higher phenolic content in the methanol extract, therefore methanol is a better extractor of phenolic acids than ethanol. According to the obtained results the microalgae *Chlorella*, was a better source of phenolic compounds than the analyzed macroalgae. Therefore, the analyses showed a good basis for subsequent HPLC separation and determination of individual phenolic acids and flavonoids to which antioxidant activity could be attributed.

Both *Ulva rigida* and *Chlorella* can be used as a source of antioxidants and phenolic acids which can be added to new functional foods and supplements, as well as be the basis of pharmaceutical and cosmetic products.

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