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

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 pheno- lic 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 macroalgae 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

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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.

| Concentration, μg.mL⁻¹ | *Ulva rigida* | *Chlorella* |
|------------------------|---------------|-------------|
|                        | Inhibition of DPPH, % |               |
|                        | CH₃OH extract | C₂H₅OH extract | CH₃OH extract | C₂H₅OH 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-
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