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Free Radical Scavenging Activity and Biochemical characteristics of *Ulva rigida* (Ulvophyceae) and *Arthrospira platensis* (Cyanophyceae)[#]

İlknur Ak[1,a,*], Gülen Türker[2,b]

[1]*Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, 17100, Çanakkale, Turkey.*
[2]*Department of Food Technology, Çanakkale School of Applied Sciences, Çanakkale Onsekiz Mart University, 17100 Çanakkale, Turkey*
[*]*Corresponding author*

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ABSTRACT

In this study, the antioxidant activities and biochemical characteristics of *Ulva rigida* (Ulvophyceae) and *Arthrospira platensis* (Cyanophyceae) were determined. The extracts from two seaweed species were evaluated for their free radical scavenging activity, using the 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) method, their total phenolic, flavonoid, and condensed tannin contents, through Folin–Ciocalteu, Quettier-Deleu, and Price methods, respectively. The extracts of two seaweeds showed a low free radical scavenging capacity in comparison with commercial antioxidant BHT (butylated hydroxytoluene), and vitamin C. The extract of *U. rigida* demonstrated greater antioxidant potential with a low IC_{50} (3.76±0.02 mg/g Ext.). The total phenolic contents were ranged from 2.21±0.08 (*U. rigida*) to 8.59±0.62 (*A. platensis*) mg GAE/g of extract. The highest flavonoid content was found in *A. platensis* as 22.70±0.65 mg rutin/g of extract. The contents of condensed tannin were measured 3.01±0.11 mg CE/g of extract for *A. platensis* and 3.76±0.06 mg CE/g of extract for *U. rigida*. According to results obtained, *U. rigida* and *A. maxima* possess antioxidant activity and could be used in for future applications in medicine, functional foods, and agriculture.

[a] *ilknurak@comu.edu.tr* *https://orcid.org/0000-0002-0233-0025* | [b] *gulenturker@hotmail.com* *https://orcid.org/0000-0002-7554-1544*

Introduction

Algae are known to possess an expansive range of physiological and biochemical properties and that can be harnessed for commercial applications such as food, pharmaceutical, cosmeceutical, biochemical, and bioenergy production (Kumar et al., 2019). They also contain bioactive substances like polysaccharides, proteins, lipids, and polyphenols, with antioxidant, antibacterial, antiviral and antifungal properties and this gives them great potential as a supplement in functional food or for the extraction of compounds (Yuan et al., 2005; Kumar et al. 2008; Ak and Türker, 2018).

Antioxidants are substances that at low concentrations delay the oxidative stress, DNA mutations, malignant transformations, as well as other parameters of cell damage (Sindhi et al., 2013; Pisoschi and Pop, 2015). They neutralize free radicals and protect the body from free radicals by maintaining redox balance (Pisoschi and Pop, 2015). Phenolic compounds or polyphenols are considered as one of the most important classes of natural antioxidants (Duan et al., 2006). Moreover, they can be divided into several classes, such as phenolic acids, flavonoids, isoflavonoids, stilbenes, lignans, and phenolic polymers (condensed tannins and hydrolysable tannins) (Manach et al., 2004). Polyphenols can behave as reactive oxygen species (ROS) and they can inhibit lipid oxidation with chelating metal ions (Rodrigo and Bosco, 2006).

Interest in new sources of natural antioxidants has increased in recent years in order to reduce the use of synthetic forms (Cox et al., 2010). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), generally are used. However, it is suspected that they are responsible for several side effects, such as carcinogenesis and liver damage (Alenisan et al., 2017; Shalaby, 2015). Consequently, alternatives to

artificial antioxidants are increasingly demanded (Vasconcelos et al., 2018). Antioxidants from plant origin also can retard or alleviate the extent of oxidative deterioration and increase the shelf life of foods (Cox et al., 2010; Choe, 2017). Commercially available natural antioxidants are mostly derived from terrestrial plants (Goiris et al., 2012). However, recent studies showed that micro and macro algae are an alternative source of natural antioxidants (Wang et al., 2007; Cox et al., 2010; Goiris et al., 2012; Jensen et al., 2015; Ak and Türker, 2018; Kumar et al., 2019). Algae and their and their secondary metabolites like various phenolic and pigment components and polysaccharides offer the opportunity in this respect (Matanjun et al., 2008; Jerez-Martel et al., 2017; Ismail, 2017). A strategic extraction of these metabolites from algae would increase the potential for new discoveries targeting high value-added products (Trigui et al. 2013).

Ulva sp., a green macroalga is common in the coast of Mediterranean. It is a good candidate for culture due to its nutritional value and high protein content (Gordillo et al., 2001; Peña-Rodríguez et al., 2011; Ak et al., 2015). Because of their protein levels, *Ulva* species are used as vegetables for human nutrition, especially in Asian countries (Fleurence, 1999). Moreover, *Ulva* species also contains minerals, fats, and phytochemicals (Kazir et al., 2019). *Arthrospira* sp., is a free-floating filamentous cyanobacterium that has received considerable attention for its high protein content and high concentrations of phenolic acids, essential fatty acids, vitamins (such as tocopherols and B_1, B_2, B_6, B_{12}, E and D), minerals and other constituents which have antioxidants activity (Vonshak, 1997; Wang et al., 2007; Jensen et al., 2015).

The main objective of this study was to determine the antioxidant activity and biochemical characteristics of extracts obtained from *Arthrospira platensis* (Cyanophyceae) and *Ulva rigida* (Ulvophyceae) with regard to their potential uses. We also used Pearson's correlation to estimate and compare the contribution of polyphenolic compounds to the measured antioxidant activities.

Materials and Methods

Chemicals

All chemicals were purchased from Fluka Chemie (Switzerland), Merck (Germany), Sigma-Aldrich (USA), and SPA (Milan, Italy). All reagents used were of analytical grade.

Algae Samples

Ulva rigida collected quantitatively by hand from intertidal zones at three locations in the Dardanelles (Çanakkale) Strait (40° 6' 43¨ N, 26° 24' 15¨ E). The Dardanos and Kepez, areas were chosen as sampling areas where *Ulva* species were distributed intensively. The macro alga material was identified according to Guiry and Guiry (2019). The samples were cleaned from their epiphytes, dried at 30°C and milled into powder before extraction.

Arthrospira platensis was supplied from the Algae Culture Collection in the Department of Aquaculture, Ege University (İzmir, Turkey). The cultures were grown in 50 L polyethylene bags with Zarrouk (1966) medium. Cultures were continuously aerated with a blower at 29 ±2°C and illuminated with fluorescent lamps 100 μmol photons m^{-2} s^{-1} photon flux density. Biomass samples were harvested and dried at 30°C before extractions were carried out.

DPPH Free Radical Scavenging Activity Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined according to the Brand-Williams et al. (1995). Each sample was diluted in methanol prior to the analysis (1 mg/ml). An aliquot (0.1 mL) of the solution was added to 3.9 mL of DPPH solution ($6x10^{-5}$ M in methanol), thoroughly mixed, and the absorbance of the sample at 515 nm was recorded after the time necessary for the reaction to reach a plateau. The absorbance of DPPH solution in methanol, without any antioxidant (control), was also measured. The percentage of DPPH radical scavenging (%) activity was calculated by the following equation:

$$DPPH = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where A sample is the absorbance of the sample after the time necessary to reach the plateau (30 min) and A control is the absorbance of DPPH. Butylated hydroxytoluene (BHT), α tocopherol, and Vitamin C were used as positive controls. Extract concentrations providing IC_{50} inhibition values were calculated from graph plotting using nonlinear regression.

Determination of Total Phenolic Contents

The total phenolic content of algae was measured using Folin-Ciocalteu reagent according to Djeridane et al. (2006). The gallic acid was used as a standard and a standard curve was drawn. Different concentrations of gallic acid were prepared in 80% of methanol, and their absorbance was measured at 765 nm. The seaweed samples were dissolved with the Folin–Ciocalteu. The solutions were incubated at room temperature for 1 min and 20% sodium carbonate solution was added. The mixture was shaken vigorously and then it was incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using UV–Vis spectrophotometer (Thermo Aquamate). The results are expressed in mg of gallic acid per g (GEA) of extract.

Determination of Total Flavonoid Contents

The total flavonoid content was determined according to Quettier- Deleu et al. (2000). Briefly, 1 ml of methanolic extract solution was added to 1 ml of 2% methanolic $AlCl_3$, $6H_2O$. Following thorough mixing of the solution, the absorbance against blank was determined at 430 nm after 10 min later. Rutin was used to prepare the standard curve and results were expressed as mg rutin/ g extract.

Determination of Condensed Tannin Contents

Condensed tannin content was evaluated according to Price et al., (1978). 20 ml of 1% HCl in methanol was added to an aliquot of 0.5 g of the seaweed extracts. Then, the samples placed in a water bath at 30°C with constant shaking for 20 min. and then they were centrifuged. Aliquots of the supernatants were placed in two separate assay tubes, one for the sample determination and the other

for blank determination. Samples and blanks were incubated for exactly 20 min after adding 5 ml of the vanillin reagent to the samples and 4% HCl in methanol to the blanks. The absorbance against blank was read at 500nm using a UV–Vis spectrophotometer (Thermo Aquamate). The catechin was used to prepare the standard curve and results were expressed as mg catechin equivalents per gram of extract (µg CE/g).

Statistical Analysis

The significance of difference was calculated by Student's *t*-test or one-way analysis of variance (ANOVA). Before Student's *t*-test or ANOVA, all data were checked for homogeneity of variance and normal distribution. Pearson's correlation analysis was used to assess correlations between means. A significant difference was considered at the level of $P<0.05$.

Results and Discussion

DPPH reagent has been used for investigating the free radical scavenging activities of compounds (Cox et al., 2010). The scavenging activity of *U. rigida* and *A. platensis* on DPPH free radical are summarized in Table 1. *U. rigida* showed the highest antioxidant potential with a low IC_{50} (3.76±0.02 mg/g Ext.) and significantly lower than three commercial antioxidants tested, BHT, α-tocopherol and Vitamin C, respectively. The antioxidant activities of two algae were significantly different ($P<0.05$), and Inhibition % values ranged from 36.46±0.09 to 41.40±0.05 mg/g of extract. Thus, both *U. rigida* and *A. platensis* showed medium potential DPPH radical scavenging activity.

The phenolic compounds belonging to the flavonoid, phenolic acids, and tannin groups are recognized as the dominant contributors to the antioxidant capacity (Duan et al., 2006; Rodrigo and Bosco, 2006). Many studies showed that those phenolic compounds are one of the most effective antioxidants in algae (Duan et al., 2006; Cox et al., 2010; Goiris et al., 2012; Jensen et al., 2015). The total phenolic contents of extracts of two algae are presented in Table 2. The total phenolic content of samples ranged from 2.21±0.08 to 8.59±0.62 mg/g GAE of extract. Blue-green alga *A. platensis* exhibited highest total phenolic content, as compared to a green alga, *U. rigida*. Liu et al. (2011) and Gargouri et al. (2016) reported that blue-green algae extracts had a phenolic content of 2.49 to 19.47 mg/g GAE of extract. The total phenolic contents of *A. platensis* of this study are among these values. Flavonoids, the largest group of phenolic compounds and they show antioxidant mechanisms by metal chelation and by inhibiting lipoxygenase (Goiris et al., 2012). They are important in plant defense mechanisms against the stressed environmental conditions such as excessive light or ultraviolet (UV) radiation and pollutants (Mierziak et al., 2014). The total flavonoid content of two algae changed from 12.61±0.07 to 22.70±0.65 mg/g of extract (Table 2). The total flavonoid content of *A. platensis* was significantly higher than *U. rigida* ($P<0.05$). Klejdus et al. (2009) stated that phenolic compounds were more abundant in microalgae compared to cyanobacteria species. However, several studies showed that cyanobacteria species could produce a great variety of secondary bioactive metabolites (Plaza et al., 2009; Shalaby 2015). Tannins are secondary plant metabolites and are widespread among terrestrial and marine plants (Mueller-Harvey, 2006; Zubek et al., 2012). The total condensed tannin content of algae extracts of *U. rigida* and *A. platensis* can be seen in Table 2. Condensed tannins of the studied algae changed from 3.01±0.11 to 3.76±0.06 mg CE/g of extract. *U. rigida* contained significantly higher total condensed tannin contents ($P<0.05$). According to Cox et al. (2010) and Gargouri et al. (2016) condensed tannins found in blue-green and green algae an in lower amounts in brown and red algae. Our results show a similarity with these studies.

Table 1 The DPPH radical scavenging activities of *U. rigida* and *A. platensis*. Different lowercase letters show the significant differences between the groups according to the ANOVA results ($P<0.05$).

Species	IC_{50} inhibition values (mg/g Ext.)	Inhibition %
U. rigida	$3.76±0.02^{b}$	$41.40±0.05^{b}$
A. platensis	$3.89±0.07^{a}$	$36.46±0.09^{c}$
Butylated hydroxytoluene	$1.33±0.01^{d}$	$99.00±0.11^{a}$
α-tocopherol	$1.48±0.02^{c}$	$96.00±0.15^{a}$
Vitamin C	$1.35±0.02^{d}$	$98.00±0.10^{a}$

Table 2 The total phenolic, flavonoid, and condensed tannin of *U. rigida* and *A. platensis*. Different lowercase letters show the significant differences between the groups according to the Student's *t*-test results ($P<0.05$).

Species	Total Phenolic (mg/g GAE Ext.)	Total Flavonoid (mg/g Ext.)	Condensed Tannin (mg CE/g Ext.)
U. rigida	$2.21±0.08^{b}$	$12.61±0.07^{b}$	$3.76±0.06^{a}$
A. platensis	$8.59±0.62^{a}$	$22.70±0.65^{a}$	$3.01±0.11^{b}$

Table 3 Pearson's correlation coefficients between the variables.

Variables	IC50 inhibition values	Total Phenolics	Total Flavonoids
Total Phenolics	0.887*		
Total Flavonoids	0.883*	0.999*	
Condensed Tannins	-0.848*	-0.981*	-0.987*

*Correlation is significant at the 0.05 level.

Pearson's correlation coefficient analysis was performed to determine the relationship between IC_{50} inhibition values and total phenolic, flavonoid and condensed tannin contents (Table 3). Positive significant correlations ($P<0.05$) were found between IC_{50} inhibition value and total phenolic content ($r= 0.887$). In agreement with previous studies (Zubia et al., 2007; Goiris et al., 2012; Ak and Türker, 2018), there was a strong correlation between antioxidant activity and phenolic content of two algae. According to Pearson's correlation coefficient analysis, a strong positive relationship was found between IC_{50} inhibition value and total flavonoid content ($r= 0.883$). There was a negative correlation between IC_{50} inhibition value and condensed tannin content ($r=-0.848$). Our results show similarities with Ak and Türker (2018). According to Piluzza and Bullitta (2011), the antioxidant activity of photosynthetic organisms not only dependent on the concentration but also depends on the structure and the interaction between the antioxidants. This can explain why *U. rigida* and *A. platensis* which had different phenolic, flavonoid, and condensed tannin content, exhibited a similar antioxidant activity.

Conclusions

Algae are good sources of polyphenols such as phenols, flavonoids, carotenoids, and tannins. In the present study, the antioxidant activity of *U. rigida* and *A. platensis* was evaluated. The results clearly showed that tested algae have high antioxidant activity with low IC_{50} inhibition values. We conclude that the main contributors to antioxidant activity these two algae according to the significant correlation between IC_{50} inhibition values and total phenolic and flavonoid contents. The results of the present study are promising as algal polyphenolic compounds are effective antioxidants and they could have potential in food applications. These results also show that polyphenolic compounds present in these algae extracts would be capable of functioning as free radical scavengers. Our findings appear useful in leading to further study in the identification and characterization of specific compounds responsible for antioxidant activities in these algae species.

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