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Antioxidant Activity and Total Phenolic Content in Two Alga Species from the Persian Gulf in Bushehr Province, Iran

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Abstract: ***The antioxidant properties of methanol extracts in two brown and red algae were studied in the present research. The maximum amounts of phenolic compounds (2.42±0.35 mg gallic acid/g extract) as well as the best antioxidant activities according to such tests as Di Phenyl Picryl Hydrazyl (DPPH) and Ferric Reducing Ability of Plasma (FRAP) were measured in red alga Gracilaria corticata. Brown alga Sargassum tenerrimum, had lower phenolic compounds (2.13±0.19 mg gallic acid/g extract) and antioxidant activities with the same tests. According to the results, both algae had no antioxidant activity with ABTS (µmol / g extract) test (in 50mg/ml concentration). There were no significant differences (p>0.05) between the studied algae in phenolic compounds and antioxidant activities according to DPPH and FRAP tests. However, there were significant differences between the algae in phenolic compounds and antioxidant activities according to DPPH and FRAP tests (sig<0.05).***

Keywords: antioxidant activity; FRAP; Persian Gulf; DPPH; total phenolic content; *Gracilaria corticata*, *Sargassum tenerrimum*

1. Introduction

Algae, in addition to their very important ecological role in nature, have been used in human diet for centuries as a healthy food or for medicinal purposes due to being rich in minerals and vitamins [1]. Marine algae are one of the largest producers in marine environment [2]. They have a wide variety of chemical metabolites in their potential environment. These active metabolites are known as biogenic compounds [2, 3].

Algae are a rich source of useful bioactive compounds. Many biological compounds have been identified and derived from multicellular algae with various applications such as antibiotic, antifungal, antiviral, and antitumor effects. Many of the primary or secondary metabolites in these plants can be active agents of interest to the pharmaceutical industry [4].

Plant-derived secondary metabolites such as phenol and total flavonoid have great potentials to clean up free radicals in all parts of a plant such as leaves, fruits, seeds, roots, and bark [5]. Antioxidant activities in some red, brown, and green algae were studied. It was shown that the antioxidant properties of extracts in marine algae are different and proportional to their content of antioxidant compounds [6].

The diversity and chemical nature of antioxidants make it rather impossible to separate and measure them in different algal samples just with one experiment. Therefore, it is necessary to design a set of experiments to measure antioxidant activities. Commercialization potential of antioxidant compounds from marine algae as a food supplement is an attempt to develop antioxidant products [7].

The algae (in the Persian Gulf in southern Iran) are one of the valuable biological potentials of the country. Yet, much attention is not paid to them and there are not principled codified plans to exploit such marine reserves [8].

2. Materials and methods

Sampling operations of brown and red algae (*S. tenerrimum* and *G. corticata*) were conducted in May 2013 at the tidal and rocky shores in Bushehr Province (at two stations of Bushehr University (N28° 54' and E50 °49') and Bushehr Nuclear power plant (N28°50' and E50°52')) as the habitat of such algae.

To determine the highest tides at the stations, the website of Iran Hydrography Organization showing the tidal positions of the coasts was used. The collected algae were washed with seawater and completely removed from sand and epiphytic organism. Then, they were put into plastic bags labeled with the station name, sampling date , stored in boxes of ice , and were transferred to the laboratory.

Some of the algae were placed in 4% formalin for maintenance and identification. In the laboratory, algae were washed thoroughly and carefully with tap and immersed in distilled water (to get out salts) . The water was replaced every few hours. The experiment was repeated up to three stages and the algae were then spread on a clean cloth in the shade and were dried within three days. After drying, the samples were completely ground to powder with an electric grinder [9].

The ground algae were extracted with methanol by maceration method for 24 hours and filtered through Whatman No. 1 filter paper. The extracts were then collected and concentrated using a rotary evaporator (Heidolph Laborota, model 4000; Germany) and remained frozen prior to the study [9].

2.1 Antioxidant activity of the extracts

2.1.1. Measurement of total phenolic content

Total phenolic contents were measured in samples with little change in algal extracts by the Folin–Ciocalto method [10]. According to this method, in tube to 0.1 ml of extract (at a concentration of 1 mg/ ml) or ethanolic solution of standard gallic acid (concentration 25-300 µg) 0.5 ml reagent Folin–Ciocalto (diluted with distilled water with a ratio of 1 to 10), and 0.4 ml sodium bicarbonate 5.7% were added and mixed.

After 30 minutes of storage at laboratory ambient temperature, its light absorption by spectrophotometer (Pharmacia LKB. Nova Space II, England) was read at a wavelength of 765 nm. Total phenolic content in extract samples, using the standard curve, was expressed in terms of mg gallic acid per gram of the extract.

2.1.2. Evaluation of Antioxidant activity by DPPH Free Radical

Total antioxidant activity of extract samples was evaluated by Von Gadow and colleagues [11]. According to this method, 2.4 mg of DPPH powder was dissolved in 100 ml of pure ethanol. In the tube, 1 ml of DPPH ethanol solution was added and mixed with 0.025 ml sample or Trolex standard solution.

In addition, DPPH solution was used as control. After 10 minutes of placing in darkness at ambient temperature, the optical absorbance of the samples was read. For the standard curve, Trolex solution with a concentration of 100-1000 µmol was used. Radical scavenging activity (RSA) of extracts was calculated by the following formula:

$A_{Control}$ = control absorption at time zero (t = 0)

A_{Sample} = absorbance of sample at a rate of 6 min (t = 6 min)

$$\text{RSA \%} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100$$

Antioxidant activity of extract samples, using the standard curve, was expressed in terms of µ mol Trolex / g dry extract (µ mol / g). Percentage of radical scavenging activity (RSA) was calculated for each sample and the antioxidant activity of extract samples was obtained using a standard curve in terms of µ mol Trolex /g of extract (µ mol / g).

2.1.3. Measurement of antioxidant properties by FRAP

In this test, Benzie and Strain method was used with slight modifications [12]. The FRAP solution was prepared daily by mixing 10 ml acetate buffer 300 mmol (pH = 3.6), 1 ml of 2, 4, 6- tri -2 - pyrydyl – S-triazine (TPTZ) 10 mM (dissolved in 40 mmol hydrochloric acid) and 1 ml of 20 mM ferrous chloride.

In the test tube, 1 ml of FRAP solution was added and mixed to 0.02 ml of extract (at a concentration of 1 mg / ml) or aqueous standard solution of ferrous sulfate (concentration 0./185-0./37 µ mol). The above mixture was placed at room temperature for 5 minutes and then the absorbance of the samples was read. The reducing activity of the extract samples was calculated, using the standard curve,in terms of µ mol iron / gr extract.

2.1.4. Evaluation of antioxidant activity by azinobis ethylbenzthiazoline sulphonic acid ($ABTS^+$) radical or Trolex-equivalent antioxidant capacity

The antioxidant activity of plant extracts was evaluated through the method developed by Re *et al*. [13]. In order to generate $ABTS^+$, 7 mM ABTS and 2.45 mM potassium persulfate was kept in distilled water for 12-16 hours at room temperature in the dark.

The pure $ABTS^+$ solution was diluted with ethanol to the extent that its absorption reached 0.7±0.02 wavelength of 734 nm. An amount of 2 ml of ethanol solution of $ABTS^+$ was added and mixed with 2 ml of extract (at a concentration of 1 mg / ml) or standard Trolex ethanol solution. Also, the $ABTS^+$ solution was used as the control sample. To draw the standard curve, soluble Trolex with concentrations of 100 – 1000 mol was used. The antioxidant activities of the extract samples were expressed, using the standard curve, in terms of micro molar Trolex / g dry extract (µmol / g).

Statistical Analysis

Experimental results were expressed as means ± SD. Moreover, all measurements were replicated three times. The data were analyzed by analysis of variance ($P<0.05$) and the means were separated by Duncan's multiple range test [14].

3. Results

Antioxidant and total phenolic content of algae

3.1. Total phenolic content

Amounts of total phenolic content in the extract samples, using the standard curve, were expressed in terms of mg gallic acid per gram of extract. The highest amount of phenol (2.42 ± 0.35 mg of gallic acid per gram of extract) belonged to the red alga *G. corticata* , while the least amount of phenol (2.13 ± 0.19 mg of gallic acid per gram of extract) belonging to the alga *Sargassum tenerrimum*.

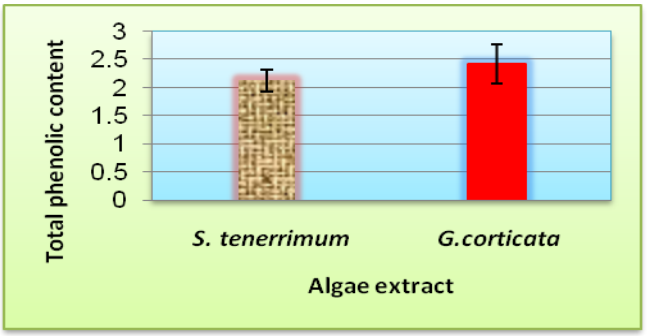

Figure 1: Total phenolic contents in the alga samples (mean ± SD)

3.1.1. Evaluation of antioxidant activity by DPPH free radical

According to Figures 2 and 3, the highest antioxidant activity (6000 ± 4108.19) and the percentage of inhibition (12.56 ± 8.61) with DPPH free radical test (µmol / g) belonged to the alga *G. corticata* , while the lowest amounts (1877.73 ± 782.00 and 3.92 ± 1.64) belonging to the alga *S. tenerrimum*.

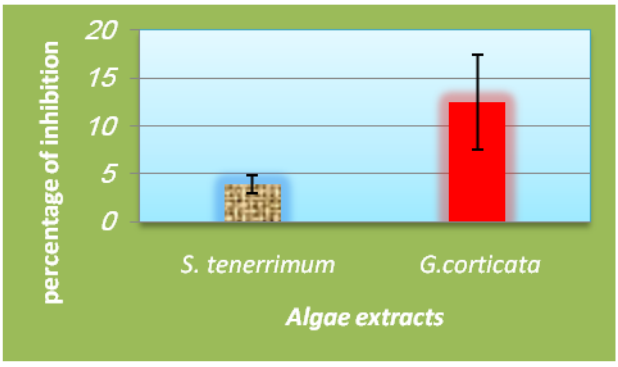

Figure 2: Percentages of inhibition in the samples with DPPH free radical test (mean ± SE)

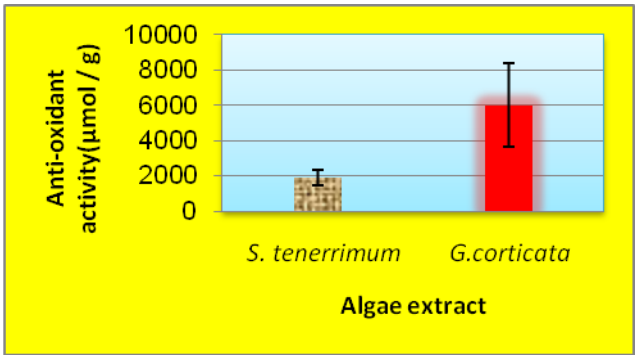

Figure 3: Amounts of antioxidant activity in the samples with DPPH free radical test (mean ± SE)

3.1.2. Measurement of antioxidant properties by FRAP

According to Figure 2, the maximum antioxidant activity with this test (4422.4 ± 588/8µmol trolex/g) belonged to the red alga *G. corticata*, and the minimum activity (4076.54-± 585.4 µmol trolex/g) belonged to the brown alga *Sargassum tenerrimum*.

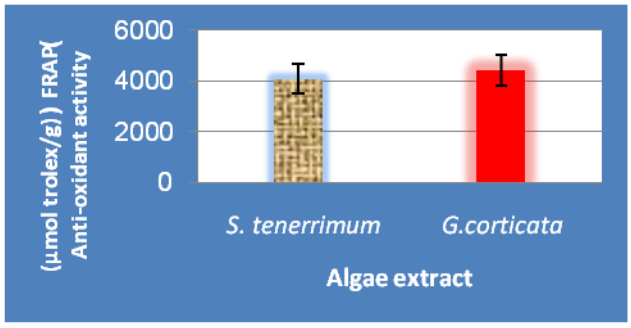

Figure 4: Antioxidant activity in extract samples through FRAP test (µmol/g)

3.1.3. Evaluation of antioxidant activity by Trolex-equivalent antioxidant capacity

No antioxidant activity and percentage of inhibition for the methanol extracts of the algae were obtained by Trolex-equivalent antioxidant capacity test (at a concentration of 50 mg/ml per solvent).

4. Results and Discussion

The amount of extract used to check the amount of total phenolic content was 50 mg / ml. Also, the total phenolic content of the studied alga extracts ranged between 2.13-2.42 mg gallic acid/ gr of extracts. There was no significant difference between algae phenolic contents (sig> 0.05).

In this study, the antioxidant activity of the brown alga *S. tenerrimum* was low. This low antioxidant activity can be attributed to the low, and perhaps the lack of, antioxidant compounds with high antioxidant property such as phenol and flavonoid in the non-polar extracts of the algae [15].In fact, antioxidant compounds such as total phenolic content follow several external factors (e.g., vegetarian pressure, sun exposure, depth, salinity, material, etc.), and internal intrinsic factors (e.g., species, age, and reproductive stages) [16].

The red alga *G. corticata* grows at the highest levels in tidal areas undergoing environmental stresses such as solar radiation, high temperatures, and is exposed to drought [17]. Exposure to high environmental stress may be among the reasons that it has shown lowest antioxidant property. In this research, the brown alga *S. tenerrimum* showed the highest antioxidant property in the deeper tidal areas exposed to fewer stresses.

The percentage of inhibition and antioxidant activity in these algae showed no significant difference using DPPH test (sig> 0.05).DPPH is a purple compound that has changed into a radical due to the presence of phenyl groups in its structure the source of free radicals. Taking an electron from an antioxidant compound, its color changes from purple to yellow. The DPPH free radicals have an absorption at 517 nm, following Beer-Lambert law. Decrease in their absorption has a linear relationship with the antioxidant.

The more the antioxidant increases, the more DPPH is consumed and its purple color tends to yellow. Trolex-equivalent antioxidant test and DPPH are both synthetic free radicals with similar applications. However, Trolex-equivalent antioxidant potential can be used for measuring the antioxidant activities of other polar and nonpolar compounds [18].

Phenolic compounds (in marine algae) are correlated with antioxidant activity and also dependent on the solvent and algal species used for extraction [19]. Basically, increase in total phenolic content may increase antioxidant properties [20]. Phenolic compounds act as electron donors and may neutralize the unwanted reactions created by free radicals in the body [21].

We can readily extract phenolic compounds with water and use them to have a high antioxidant effect [19]. Considering that the extracted compounds are mostly nonpolar compounds due to the solvent, amounts of phenolic compounds is certainly low in these extracts. The antioxidant activity of the reduced iron is measured based on ferric (III) ion reducing ability to ferrous (III) ion. The results are reported in terms of µ mol of ferrous ion /g extract [22, 23].

The antioxidant activity test of iron reduction is measured that directly measures antioxidants or reductants in the sample, and has a linear relationship with their antioxidant concentration [24].There is no significant relationship between the antioxidant activity of the algae by FRAP test considering their antioxidant property (sig> 0.05). However, there was a significant relationship between the antioxidant property with FRAP test and the total phenolic contents of the algae(sig <0.05).

There is a weak positive relationship between phenolic content and antioxidant activity with ferric antioxidant reducing ability test (R^2 = 0.43). There was a very weak positive relationship between phenolic content and antioxidant activity with DPPH test (R^2 = 0.012). The amount of phenolic compounds is likely to be very low in these compounds; therefore, no particular antioxidant activity was recorded with ABTS test. It is proposed that extract chemicals depend on the type of solvents with different pHs, polarity, extraction time and temperature, as well as chemical compositions of the samples [25].

Generally, the highest amount of total phenolic content and the best antioxidant activity with the tests belonged to the red alga *G. corticata*, and the lowest antioxidant activity and phenolic content with the same tests were recorded for the brown alga *S. tenerrimum*.

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