World Journal of Fish and Marine Sciences 7 (4): 237-242, 2015
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2015.7.4.94245

The Comparison of Antioxidant Power of Two Marine Algae Species with the Skin of Oak Fruit (*Querqus brantii*)

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Abstract: Antioxidants reduce damage caused by free radicals. The purpose of this study was to evaluate and comparison the antioxidant power of two red algae species of Persian Gulf (*Laurencia snyderia* and *Acanthophora nayadiformis*) with the skin of Oak fruit (*Querqus brantii*) using ABTS and FRAP tests. The highest percentage of inhibition (89.33±0.06%) and antioxidant activity using Azino Bisethyl Thiazoline Sulphonictest (ABTS) (297.304±200 mmol Trulks/g) and the lowest percentage of inhibition (4.09±2.76 %) and antioxidant activity (1.368±0.9216mmol Trulks/g) were related to the aqueous extract (distilled water) skin of Oak fruit and the red alga *L. snyderi,* respectively. Using Ferric Reduction Antioxidant Power test (FRAP) the most (94.498±7.7559 mmol Fe/g) and the lowest (4.010±2.43 mmol Fe/g) antioxidant activity were related to ethanol extract of the skin of Oak fruit and the red algae *A. nayadiformis*, respectively. Between the antioxidant Properties of aqueous extract and chloroformic extract of the skin of Oak fruit with the algae extract using ABTS test, as well as between the antioxidant Properties of hydro-alcoholic extract (ethanol 70 %) of skin of Oak fruit with the algae extract using FRAP test have been shown significant difference.

Key words: Red algae · Persian Gulf · Antioxidant activity · Skin of Oak fruit (*Querqus brantii*) · *ABTS* · *FRAP*

INTRODUCTION

Free radicals are highly unstable and reactive molecules that can damage cells, DNA and ultimately become mutagenesis [1]. Antioxidants reduce the damage caused by free reactive radicals in cells [2]. Considering that the immune system may be strong enough to deal with persistent or severe oxidative stress and antioxidants, so do not delay the process of oxidation, polymerization cycle from the beginning by free radicals and other oxidation reactions [3]. Thus a certain amount of foreign antioxidants proven to maintain a balance between the levels of antioxidants in the human body is needed. In many countries, in order to delay or prevent the oxidative degradation products, synthetic antioxidants such as butyllathydroxyAnyzel (BHA), butyllathydroxytoluene (BHT) and brings 3-butyl hydroquinone (TBHQ), widely used as an additive in their food. Food diet may be the most important sources of antioxidant tocopherol, glutathione, ascorbic acid and salts ascorbate, carotenoids and phenolic compounds mentioned [4]. Algae are rich source of beneficial bioactive compounds. Since many biological compounds with a wide range of applications such as the effects of antibiotics, anti-fungal, anti-viral and anti-cancer multicellular algae have been identified and derivatives [5]. Algae in the Persian Gulf are one of the valuable biological capacities and were not paid much attention to them and there is not planning principles for the operation of the marine resources. Secondary metabolites derived from plants such as phenols and flavonoids have a strong potential to neutralize free radicals (free Radical Scavenging) are in all parts of the plant, such as leaves, fruits, seeds, roots and bark [6]. In various studies, antioxidant activity in some red algae, brown and green have been studied [7]. Medicinal plants contain compounds called polyphenols (Antrokinon, Flavonoids, aromatic acids and tannins) which are called natural

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antioxidants. Since the use of some synthetic antioxidants because of their toxicity is limited. Therefore, medical and food and pharmaceutical industries tend to use natural antioxidants, especially its medicinal plants. Diversity and chemical nature of antioxidants due to a series of tests designed to measure the activity of antioxidants [8]. FRAP test is based on electron transfer and antioxidant compounds, such as thiols and proteins are not applicable [9]. The purpose of this study was to compare the antioxidant power of two algal species and skin of Oak fruit (*Querqus brantii*) with using the ABTS and FRAP tests.

MATERIALS AND METHODS

Samples of red algae *(L. snyderia* and *A. nayadiformis)* were investigated in June 2013 on the shores of the Persian Gulf in Bushehr province. After washing algae, we put them into plastic bags containing ice, then transported to the laboratory. A number of algae were placed in formalin4% to store and identify in the laboratory Algae were washed thoroughly and carefully again with tap water and then were immersed in distilled water (to remove the salts). This procedure was repeated three times, after which the algae were placed on a clean cloth over the three days were dried in the shade. Skin of Oak fruit (*Quercus brantii*) was prepared in the fall of 2012 from the forests around the city of Yasuj. Skin of fruit Oak samples was derided with the electrical grinding into powder (10).

Extracts of Algae and Skin of Oak Fruit: Extraction by maceration method using different solvents such as ethanol 70% (the skin of oak fruit), chloroform (red algae and the skin of oak fruit) and distilled water (the skin of oak fruit) were done for 48 hours at the laboratory and in the dark environment. Extracts taken into vials were kept in the fridge until use [10].

Evaluation of Antioxidant Activity of Algae

Antioxidant Activity Using ABTS Test or Equivalent Antioxidant Potential Trulks: Antioxidant activity of plant extracts were evaluated by Re and colleagues [11]. To produce $ABTS^+$, 7 mmol ABTS and 2.45 mmol potassium persulfate in distilled water for 12-16 hours at ambient temperature that was kept in the dark and natural environment. $ABTS^+$ solution was diluted with ethanol so that absorption at a wavelength of 734 nm, was 0.7± 0.02. Then 2ml of ethanol solution $ABTS^+$ to 0.02 ml of ethanol extract (at a concentration of 1 mg /ml) or standard Trulks ethanol solution, were added and mixed. The $ABTS^+$ solution was used as a control sample. After 6 minutes the samples at room temperature, the spectrophotometer (Pharmacia LKB. Nova SpaceII, England) at a wavelength of 734 nm wavelength is calibrated by ethanol and then the samples were read. For drawing the standard curve were used the Trulks solution with the concentration of 100-1000 μmol. Radical Scavenging Activity of the extract (RSA) was based on the following formula and antioxidant activity of the extract samples was expressed using a standard curve in µmol/g dry weight of the extract.

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

A Control =Control the absorption rate at of zero (time)
A sample= the absorption of the sample at the time of 6 minutes

Measuring Antioxidant Properties Using FRAP Test: FRAP way to measure the ability to restore the sample produced by Benzie. FRAP working solution was produced by mixing10 ml acetate buffer 300 mmol (pH =6.3), 1 mL of 2, 4, 6 tri-2 Pyrydyl-S triazines (TPTZ) 10 mmol (40 mmol dissolved in hydrochloric acid) and 1 ml iron chloride20mmol. In test tube, 1 ml of the FRAP working solution to 0/02 ml of the extract (at a concentration of 1 mg/ml) or water standard solution of ferrous sulfate (concentration of 0.185-0.37 µmol), was added and mixed. The above mixture was placed 5 minutes at room temperature and then absorbance of the sample was gathered. of the extract samples using the standard curve in µmol /g, were measured.

Statistical Analysis: The comparison T-test was used for the antioxidant algae extract. Shapiro-Wilk normality test were analyzed the data. Data analysis to evaluate the results of tests comparing different extracts analyzed using one-way ANOVA was used in the software package version 18 of SPSS. Differences between the groups at 95 percent ($p<0.05$) was considered significant. All measurements are repeated three times for each sample of skin of oak fruit and algae and the values were reported as mean ± standard deviation [12].

RESULTS

Evaluation of Antioxidant Activity Using ABTS Test: According to Figure 1, the highest (89.33%±0.06) and lowest (4.09%±2.76) percentage of inhibition were related to the aqueous extract of the skin of oak fruit and chloroformic extraction of red algae *L. snyderia* respectively.

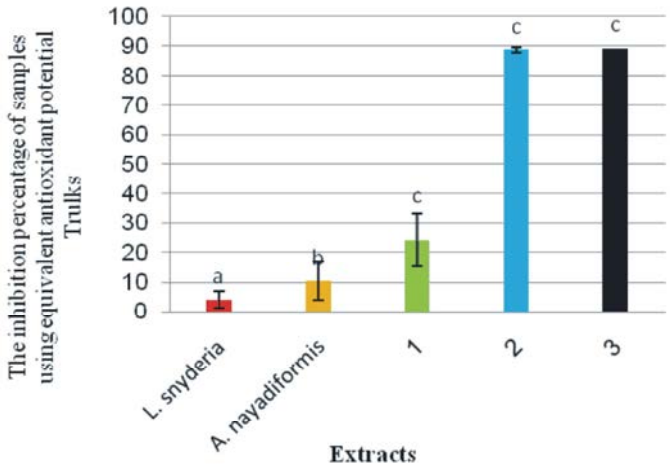

Fig. 1: Inhibition percentage of samples using Equivalent Antioxidant Potential Trulks test in µmol/g dry weight of the extract (1: chloroformic extract of the skin of Oak fruit, 2: hydro-alcoholic extract (ethanol 70 %) of skin of Oak fruit, 3: aqueous extract (distilled water) skin of Oak fruit)

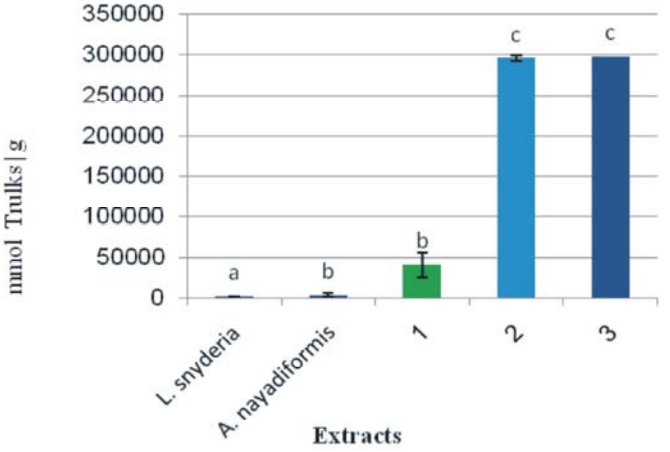

Fig. 2: Antioxidant activity of samples using Equivalent Antioxidant Potential Trulks test in µmol/g dry weight of the extract (1: chloroformic extract of the skin of Oak fruit, 2: hydro-alcoholic extract (ethanol 70 %) of skin of Oak fruit, 3: aqueous extract (distilled water) skin of Oak fruit)

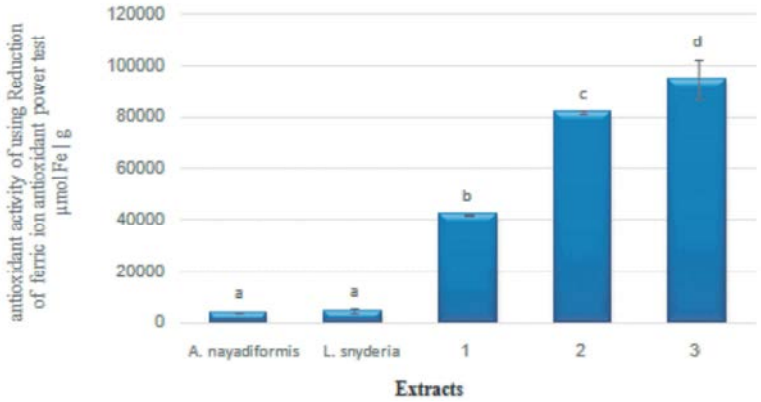

Fig. 3: Antioxidant activity of samples using Ferric Reduction Antioxidant Power test in µmol/g (1: chloroformic extract of the skin of Oak fruit, 2: aqueous extract (distilled water) skin of Oak fruit, 3: hydro-alcoholic extract (ethanol 70 %) of skin of Oak fruit)

According to Figure 2, using ABTS test, the most (297304±200 Trulks µmol/g of extract or 297.304 ±0.2mmol/g) and the lowest (1368.8±921.6 Trulks µmol/g of extract or 1.368 ±0.927 mmol/g) antioxidant activity were found of the aqueous extract of skin of oak fruit and chloroformic extract of red algae *L. snyderia* respectively.

Evaluation of Antioxidant Activity Using FRAP Test: According to Figure 3, using FRAP test, the most (94.498 ± 7.755 mmol Fe/g of extract) and the lowest (4.010±24.3 mmol Fe/g of extract) antioxidant activity were related to the methanol extract of skin of Oak fruit and red algae chloroformic extract *(A. nayadiformis)* respectively.

DISCUSSION AND CONCLUSION

In many recent studies researchers concern is to find natural antioxidants. The effects of antioxidant phenolic compounds and flavonoids of plant material partly attributed them that they exist in all different parts [13]. Therefore, identification and isolation of new compounds with antioxidant properties aquatic and terrestrial plants is considered [14]. The impact of synthetic and natural antioxidants can be determined from primary and secondary lipid peroxidation products in the food and biological systems. Nowadays there are other methods of assessing the antioxidant activity of synthetic and natural. Some of these methods include the oxygen radical absorption capacity, scavenging free radicals, Berry color of beta-carotene, the antioxidant capacity of Trulks, regenerative power produced and total antioxidant capacity. Basically, phenol compounds with antioxidant property increases more and more. High molecular weight phenolic compounds (tannins) great ability to neutralize free radicals and the ability to more aromatic rings depends on the number and nature of the hydroxyl groups are repulsive [15]. In this study, the largest and best antioxidant and Radical Scavenging were related to aqueous and hydro-alcoholic extracts of skin of Oak fruit. Also chloroformic extract of red algae had the weakest antioxidant activity. Rumbayua *et al*. [16] reported low polarity solvents such as hexane grade, acetone, butanol and chloroform extraction of these compounds are less capable than polar solvents. Generally, solvents, ethanol and methanol mixed with water (40-80%) more than pure ability to extract phenolic compounds in plant tissues [17]. The use of water as a solvent extraction, a polar environment created in which some phenolic compounds with a low degree of polarization of low amount are extracted. Add water to organic solvents formed a relatively polar environment is associated and therefore extraction amounts and a greater variety of phenolic compounds in these conditions will ensure. In addition, the water extract contains large amounts of impurities such as organic acids, proteins and soluble sugars which can interfere with the detection and quantification of phenolic compounds [18]. Algal species may have different components to the activities of antioxidant enzymes or improve or reduce the risk of enzymes used. But the relationship between chemical compounds and antioxidant activity of the extract of the algae need to be investigated further. Trulks equivalent antioxidant potential can be measured in polar and nonpolar compounds with antioxidant activity. Trulks equivalent antioxidant test (ABTS) is free radical synthesis [19]. In this study, using Azinobis ethyl thiazolinesulphonic (ABTS), a significant difference ($P<0.05$) have been shown between the percent inhibition of chloroformic extract of skin of oak fruit and extract of algal samples. Also using ABTS test, there were the significant difference between the percent inhibition of aqueous and hydro-alcoholic extracts of the skin of oak fruit and other extracts. Using ABTS test, there were the significant difference between the antioxidant properties of aqueous and hydro-alcoholic extracts from the skin of Oak fruit with other extracts of samples. In this research using ABTS test, between antioxidant activities of chloroformic extract of red algae with each other and between antioxidant activity of aqueous and hydro-alcoholic extracts of the skin of Oak fruit, are also parallel to each other. Compounds in chloroformic extracts which derived from algae possibly nonpolar compounds and similar. Compounds in aqueous and hydro-alcoholic extracts derived from the skin of Oak fruit include polar compounds (tannin). Such behavior can be attributed mainly to the presence of these compounds. Between phenolic content and antioxidant activity using ABTS is a positive relationship. Namely to increase the amount of phenol, the antioxidant activity of the test also showed a high level. tannins are including phenolic compounds with high molecular weight in the fruit and leaves of oak. Crust of oak tannin special TanykKuyrsy acid (Quercitanic acid) is water soluble. Using FRAP test, have been shown significant difference between the antioxidant properties of algae extract with other extracts of samples. Also using FRAP test, were observed the significant difference between the antioxidant properties of hydro-alcoholic extract from the skin of Oak fruit with other extracts of samples. Up to 70% ethanol in common

solvents for extraction of plant material and non-polar to polar compounds, has the highest performance. Studies show that high level phenolic compounds because of high antioxidant, including some extract is polar. As evidence that a positive correlation between phenolic compounds and antioxidant power plants is there. On the other hand seems to be the phenolic compounds widely found plants have high antioxidant power than can be extracted from plant extracts [20]. In this study, the polarities of the extracted compounds have more antioxidant activity of the extract have shown better. Ferric Reduction Antioxidant Power test (FRAP) of the antioxidant has the greatest similarity between chloroform extract of algae studied. With this test, any similarity between the antioxidant activity of ethanol extracts (hydro-alcoholic), water (aqueous) and chloroformic from were obtained the skin of oak fruit. Because of differences in the activity of antioxidant plant extracts can be caused by using different measured methods, extraction of different standards to express the results, the soil type and climate. The test of the antioxidant activity of iron reduction is method that directly antioxidants or reductionings measured in samples and linear relationship with the concentration of antioxidant. Although the genus *Laurencia* is rich in secondary metabolites, but their antioxidant activity is not comparable with Oak fruit skin extract. In summary, the largest and most antioxidant activity and radical scavenging using ABTS and FRAP tests were related to aqueous and hydro-alcoholic extracts derived from the skin of Oak fruit. Also the chloroformic extract of red algae has been shown the weakest antioxidant activity by these tests.

ACKNOWLEDGEMENT

Gratitude of all employees and Professor of microbiology and biochemistry laboratories of Yasuj University.

REFERENCES

1. Bergamini, C.M., S. Gambetti, A. Dondi and C. Cervellati, 2004. Oxygen, reactive oxygen species and tissue damage. Curr. Pharm. Des., 10(14): 1611-1626.
2. Anchana, C., T. Aphiwat and R. Nuansri, 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem., 92: 491-497.
3. Bahramikia, S. and R. Yazdanparast, 2008. Antioxidant and free radical scavening activities of different fractions of *Anethum graveolens* leaves using in vitro models. Pharmacol. online; 2: 233-219. [In Persian]
4. Pokorny, J., 2007. Are natural antioxidants better and safer than synthetic antioxidant?. European Journal of Lipid Science and Technology, 109: 629-642.
5. Barsanti, L. and P. Gualtieri, 2006. Algae anatomy, biochemistry and biotechnology, New York: Taylor and Francis Group, 2006.
6. Mathew, S. and T.E. Abraham, 2006. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem. Toxicol., 44: 198-206.
7. Zubia, M., D. Robledo and Y. Freile-Pelegrin, 2007. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J. Appl. Phycol., 19: 449-458.
8. Bocanegra, A., S. Bastida, J. Benedí, S. Ródenas and F.J. Sánchez-Muniz, 2009. Characteristics and nutritional and cardiovascular-health properties of seaweeds. J. Med. Food, 12: 236-258.
9. Huang, D., B. Ou and R.L. Prior, 2005. The Chemistry behind antioxidant capacity assays. J. Agricul. Food Chem., 53: 1841-56.
10. Salehi, P., A. Sonboli, F. Eftekhar, S. Nejad Ebrahimi and M. Yousefzadi, 2005. Essential oil composition, antibacterial and antioxidant activity of the oil and various extracts of *Ziziphora clinopodioides* subs. rigida (BOISS.) RECH. F. from Iran. Bio. Pharm. Bul., 28: 1892-6.
11. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice- Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology, 26: 1231-1237.
12. Jimenez, E., F. Dorta, C. Medina, A. Ramirez, I. Ramirez and H. Peña-Corté, 2011. Anti-Phytopathogenic Activities of Macro-Algae Extracts. Mar. drug., 9: 739-756.
13. Horincar, V., G. Parfene and G. Bahrim, 2011. Evaluation of bioactive compounds in extracts obtained from three Romanian marine algae species. Romani Biotecnol. Letterr, 6: 71-78.
14. Nshida, T., R. Matsukawa, K. Masaki, Z. Dubinsky and I. Karube, 1996. A method for screening potential antioxidant activity Biotechnology, 51: 149-155.
15. Lagouri, V. and D. Boskou, 1996. Nutrient antioxidants in oregano. Int. J. Food Sci. Nutr., 47: 493-497.

16. Rumbaoa, R.G.O., D.F. Cornago and I.M. Geronimo, 2009. Phenolic content and antioxidant capacity of Philippine potato (*Solanum tuberosum*) tubers. Journal of Food Composition and Analysis, 22: 546-550.
17. Suzuki, M., T. Watanabe, A. Miura, E. Harashima, Y. Nakagawa and K. Tsuji, 2002. An extraction solvent optimum for analyzing polyphenol contents by Folin-Denis assay. Nippon Shokuhin Kagaku Kaishi, 49: 507-511.
18. Chirinos, R., H. Rogez, D. Campos, R. Pedreschi and Y. Larondelle, 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruiz and Pavon). Separation and Purification Technology, 55: 217-225.
19. Amoudi, O.A.H., H.H. Mutawie, A.V. Patel and G. Blunden, 2009. Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia. Saudi Jour of Biolog. Sci., 16: 23-29.
20. Halliwell, B. and O.I. Aruoma, 1991. DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. FEBS Lett., 281(2): 9-19.