



Списание за наука

„Ново знание“

ISSN 2367-4598 (Online)

Академично издателство „Талант“

Висше училище по агробизнес и развитие на
регионите - Пловдив

New Knowledge

Journal of Science

ISSN 2367-4598 (Online)

Academic Publishing House „Talent“

University of Agribusiness and Rural Development -
Bulgaria

<http://science.uard.bg>

ANTIOXIDANT ACTIVITY OF FIVE SEAWEED EXTRACTS

Ilknur Ak¹, Gulen Turker²

¹Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology,
Department of Aquaculture, Çanakkale, Turkey

²Çanakkale Onsekiz Mart University, Çanakkale School of Applied Sciences, Department of
Food Technology, Çanakkale, Turkey

Abstract: In this study, aqueous extracts of five seaweeds collected from Çanakkale, Turkey were studied for free radical scavenging activity and phenolic compounds. The extracts of two brown algae (*Cystoseira barbata*, *Scytosiphon lomentaria*), two green algae (*Ulva rigida*, *Enteromorpha intestinalis*) and one red algae (*Gigartina acicularis*) were prepared with boiling. The extract of *S. lomentaria* demonstrated greater antioxidant potential with a low IC₅₀ (2,67 mg/g Ext.). It was also determined that the free radical scavenging activity of other seaweed extracts were close to this value. The total phenolics, flavonoids and carotenoids of the aqueous extracts ranged from 0,10 (*G. acicularis*) to 0,66 (*C. barbata*) mg/g Ext., 1,15 (*S. lomentaria*) to 1,30 (*E. intestinalis*) mg/g Ext. and 414,2 (*U. rigida*) to 2194,5 µg/g Ext. (*C. barbata*) mg/g, respectively. It was determined that seaweeds collected from Çanakkale exhibited high free radical scavenging capacity. We also found that, the aqueous extracts of brown algae *C. barbata* had high amounts of phenolic compound. According to our results, aqueous seaweed extracts could be use as organic fertilizers to increase antioxidant levels of agriculture products.

Keywords: seaweed, extraction, scavenging activity, phenolic compounds, organic fertilizer.

INTRODUCTION

Seaweeds contain large quantities of macronutrients and micronutrients as well as other bioactive compounds some of which are pharmacologically active such as phenolics, terpenes and carotenoids, which have antioxidant, antimicrobial and anticancer activities (Hoppe 1982; Blunden, 1991; Whapham et al.1993). Antioxidant activity of seaweed or their extracts have been studied during the last decade (Ahn et al., 2004; Cornish and Gabary,

2010, Kelman et al., 2012; Matanjun et al., 2008). These studies suggest that seaweed cells have protective antioxidative mechanisms as well as antioxidative compounds (Dykens et al., 1992; Matsukawa et al., 1997; Sukenik et al., 1993). Also, they have been used as food, feed, fertilizer, a soil conditioning agent, animal feed supplement and a human nutritional supplement (Ak, 2015; Fan et al., 2011; Mabeau and Fleurence, 1993; Blunden, 1991).

The extract of seaweeds in agriculture as an organic fertilizer are used because of their beneficial effects on crop production, nutrient uptake, stress resistance, and the quality of products after harvest (Akila et al., 2010; Fan et al., 2011; Fan et al., 2013; Sivasankari et al., 2006). The application of eco-friendly seaweed liquid fertilizers is recommended to the farmers for attaining better growth, biochemical constituents and antioxidant enhancement (Akila et al., 2010). The present study aimed to investigate the antioxidant properties of five seaweed species; *Cystoseira barbata*, *Scytosiphon lomentaria*, *Ulva rigida*, *Enteromorpha intestinalis* and *Gigartina acicularis*, from Çanakkale, Turkey for improving the nutritional quality of fruits and vegetables will be beneficial for human health.

MATERIALS AND METHODS

Seaweeds

Brown algae *Cystoseira barbata* and *Scytosiphon lomentaria*, Green algae *Ulva rigida* and *Enteromorpha intestinalis*, and Red alga *Gigartina acicularis* were manually collected at infralittoral zone from Çanakkale (40°6'43"N, 26°24'15"E) and identified according to Bourrelly (1972) and Guiry and Guiry (2018). The harvested fresh seaweed samples were cleaned from their epiphytes, and stored at 4°C until they were extracted.

Preparation of Seaweeds Extracts

100 g of seaweed was cut into small pieces and boiled separately with 100 ml of distilled water for an hour and filtered. The filtrate was taken as 100% concentration of the seaweed extract (Sivasankari et al., 2006).

Chemical reagents

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

DPPH free radical-scavenging activity assay

The effect of the oxidized seaweed extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated as described by Brand - Williams et al. (1995). Each sample was diluted in methanol prior to the analysis (1 mg/ml). The DPPH solution was added to the diluted sample, thoroughly mixed, then left for 30 min for the reaction to occur. After that, the absorbance of the sample as measured a 515 nm using a UV-Vis spectrophotometer (Thermo Aquamate). The absorbance of DPPH solution in methanol, without any antioxidant (control), was also measured. The percentage of DPPH radical scavenging activity was calculated by using the following equation:

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

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.

Extract concentrations providing IC50 inhibition values (defined as the concentration of the compounds that was able to inhibit 50% of the total DPPH radicals) were calculated from graph plotting using nonlinear regression and expressed in microgram material equivalents per gram for sample extracts. Butylated hydroxytoluene (BHT) was used as a positive control. A lower value of IC50 indicates a higher antioxidant activity and vice versa.

Analysis of total phenolic content

The amount of total phenolics in the seaweed extracts was measured using the Folin-Ciocalteu reagent method of Djeridane et al. (2006). The solution of each seaweed extracts (0.2 ml, 500 mg/ml) was taken in a test tube. 0,5 ml distilled water and 0,5 ml Folin-

Ciocalteu reagent was added and the tubes were shaken thoroughly. After 1 min 0,8 ml of sodium carbonate solution (7,5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Thermo Aquamate). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram extract.

Analysis of total flavonoids content

The total flavonoid content was determined according to Quettier- Deleu et al. (2000) using as a standard. The total flavonoid content was expressed as milligram per gram extract.

Analysis of total carotenoids content

The carotenoid content present in the seaweed extracts was determined spectrophotometrically (UV-Vis spectrophotometer, Thermo Aquamate) and the absorbance was measured at 480 nm. The carotenoid content was calculated using the following equation:

$$A = \alpha \cdot c \cdot l$$

where A is the absorbance at 480 nm, α is the specific absorbance coefficient of the solvent, c is the concentration of the carotenoids in $\mu\text{g/g}$ Ext., and l is the path length of the cuvette (1 cm) (Lichtenthaler and Buschmann, 2001).

RESULTS AND DISCUSSION

The DPPH free radical scavenging capacities of seaweed extracts are presented in Table 1. In the current study, the antioxidant activities of five seaweed extracts were evaluated. The results indicated that all the tested seaweeds in this investigation possess antioxidant activity. *S. lomentaria* demonstrated greater antioxidant potential with a low IC50 (2,67 mg/g Ext.) and DPPH percentage inhibition in comparison with those of the other species. The sequence of antioxidant activity of the seaweed extracts was as follows: *S. lomentaria* > *C. barbata* > *U. rigida* > *G. acicularis* > *E. intestinalis*. In the present study, the brown seaweeds *S. lomentaria* and *C. barbata* had greater antioxidant activities compared to the green and red seaweeds. In brown seaweeds, fucoxanthin is the dominant carotenoid (Dembitsky and Maoka 2007). The scavenging activity by fucoxanthin was 13,5 times higher than that of α -tocopherol (Airanthi et al., 2010). Radical scavenging activity of brown seaweed extracts would be correlated with fucoxanthin content as well as the amount of phenolics.

Total phenolic contents and flavonoid contents of seaweed extracts are presented in Table 2. The content of phenolic compounds varied from 3,29 (*C. barbata*) to 0,51 (*G. acicularis*) mg GAE /g Ext. The sequence of total phenolic content of seaweed extracts was as follows: *C. barbata* > *E. intestinalis* > *U. rigida* > *S. lomentaria* > *G. acicularis*. In general, the higher total phenolic content resulted in higher antioxidant capacity. The extracts of brown seaweed *C. barbata* and green seaweed *E. intestinalis* showed significantly higher phenolic content than the red seaweed extract. Jiménez-Escrig et al. (2001) and Matanjun et al., (2008) also reported similar findings that brown seaweeds contained higher phenolic content than the red seaweeds.

The seaweed extracts were found to be rich in total flavonoids (Table 2). The highest total flavonoid content was detected in extract of *E. intestinalis* (13,01 mg/g Ext.), while the lowest was found in extract of *S. lomentaria* (11,50 mg/g Ext.). All these results indicate flavonoids extracted from all seaweeds could be an important source of antioxidant molecules. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Meenakshi et al., 2009).

Table 1. The DPPH radical scavenging activity of seaweed extracts

Species	IC50 inhibition values (mg/g Ext.)	% Inhibition
<i>C. barbata</i>	3,14	43
<i>S. lomentaria</i>	2,67	43,5
<i>U. rigida</i>	3,76	42
<i>E. intestinalis</i>	4,04	42,7
<i>G. acicularis</i>	3,86	42,28
Butylated hydroxytoluene	1,33	99
α -tocopherol	1,48	96
Vitamin C	1,35	98

Total carotenoid content of seaweed extracts was shown Table 2. The highest total carotenoid content was found in *C. barbata* extracts (2194,5 μ g/g Ext.) and lowest in *U. rigida* extracts (414,2 μ g/g Ext.). The sequence of total carotenoid content of seaweed extracts was as follows: *C. barbata* > *S. lomentaria* > *G. acicularis* > *E. intestinalis* > *U. rigida*.

Table 2. The total phenolics, flavonoids and carotenoids of seaweed extracts

Species	Total Phenolics Content (mg GAE/g Ext.)	Total Flavonoids (mg/g Ext.)	Total Carotenoids (μ g/g Ext.)
<i>C. barbata</i>	3,29	11,71	2194,5
<i>S. lomentaria</i>	1,95	11,50	794,2
<i>U. rigida</i>	2,21	12,61	414,2
<i>E. intestinalis</i>	2,56	13,01	497,8
<i>G. acicularis</i>	0,51	12,36	585,2

The correlation between IC50 inhibition values and the total phenolics, total flavonoids and total carotenoids are as shown in Figure 1. There was a high correlation only between IC50 inhibition values and the total flavonoids ($R^2 = 0,90$). Yu et al., (2002) reported that there are no correction between total phenolics and radical scavenging capacity. Our results show similarities with this study. The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical (Nijveldt et al., 2001).

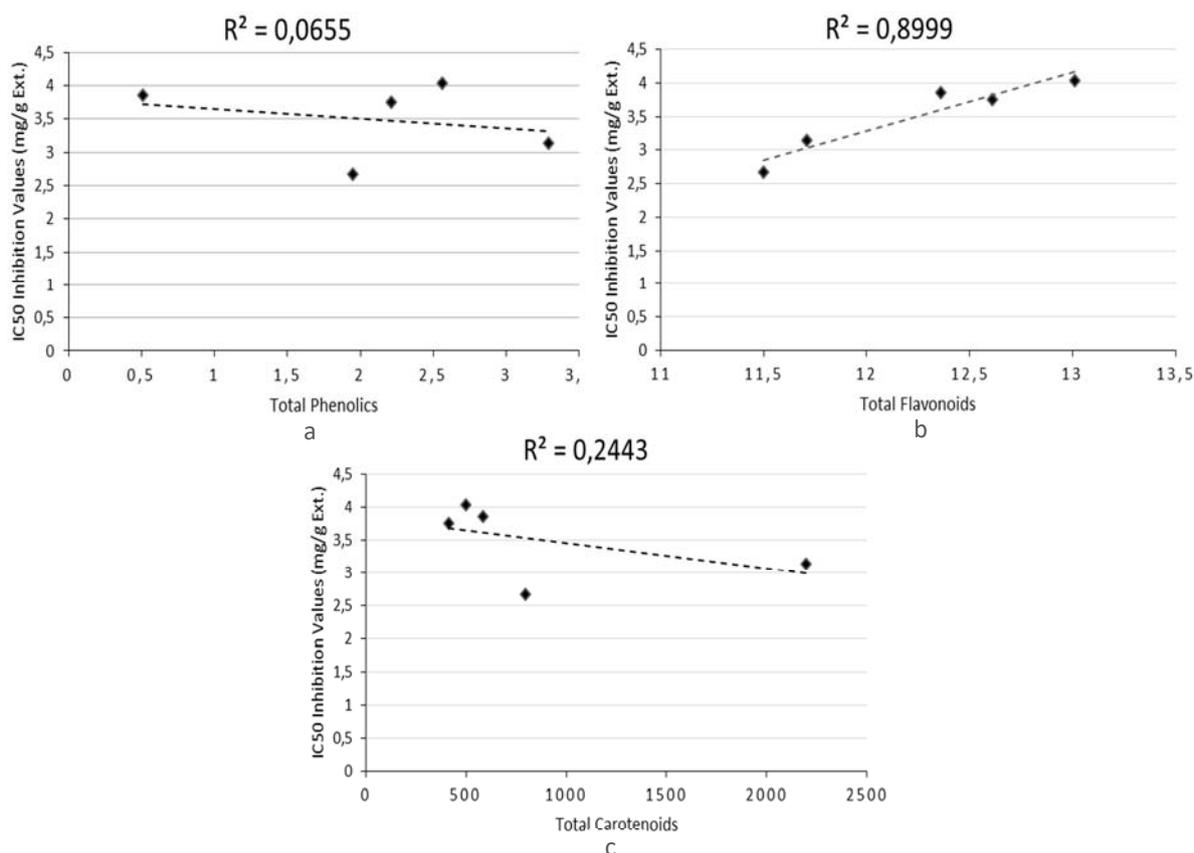


Figure 1. Correlation between IC₅₀ inhibition values ((mg/g Ext.) and (a) Total Phenolics (mg GAE/g Ext.), (b) Total Flavonoids (mg/g Ext.) and (c) Total Carotenoids ((µg/g Ext.).

CONCLUSION

In this, the antioxidant activities of the extracts of *C. barbata*, *S. lomentaria*, *U. rigida*, *E. intestinalis* and *G. acicularis* were evaluated. The results clearly indicated that all the tested seaweeds in this investigation possess antioxidant activity. *S. lomentaria* demonstrated greater antioxidant potential with a low IC₅₀. *C. barbata* exhibited high phenolics and total carotenoid contents. *E. intestinalis* showed high total flavonoid compounds. Positive and significant correlations between DPPH radical scavenging and flavonoid compounds displayed that, flavonoids are the main contributors of antioxidant activity in these seaweed extracts.

The results suggest that seaweeds possess antioxidant potential which could be considered for future applications in agriculture products. Seaweed extracts are used as organic fertilizer for culturing agricultural crops. But, no there is no study found on antioxidant activity of seaweed extracts used as fertilizers. Future researches should be focus on effects of antioxidant activities of seaweed extracts on antioxidant enhancement of crops.

REFERENCES

1. Ahn, C. B., Jeon, Y. J., Kang, D. S., Shin, T. S., Jung, B. M., 2004. Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphon lomentaria* by electron spin resonance spectrometry, *Food Research International*, 37(3): 253-258.
2. Ak, İ., 2015. Sucul ortamın ekonomik Bitkileri; Makroalgler. *Dünya Gıda Dergisi*. 12: 88-97.

3. Akila, N., Jeyadoss, T., 2010. The potential of seaweed liquid fertilizer on the growth and antioxidant enhancement of *Helianthus annuus* L. *Oriental Journal of Chemistry*. 26(4): 1353–1360.
4. Airanthi, M.K. W., Hosokawa, M., Miyashita, K., 2010. Comparative Antioxidant Activity of Edible Japanese Brown Seaweeds. *Journal of Food Science*. 76(1): 104 – 111.
5. Blunden, G., 1991. Agricultural uses of seaweeds and seaweed extracts. *Seaweed resources in Europe uses and potential* ed. Guiry, M.D. and Blunden, G. New York: Wiley.
6. Bourrelly, P., 1972. Les algues d'eau douce. Initiation à la systématique. T 1: Les algues vertes. Éditions N. Boubée et Cie. Paris. 572 p.
7. Brand-Williams W., Cuvelier, M. E., Berset C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol*. 28:25-30.
8. Cornish, M.L.; Garbary, D.J., 2010. Antioxidants from macroalgae: Potential applications in human health and nutrition. *Algae*, 25, 155–171.
9. Dembitsky, VM, Maoka T. 2007. Allenic and cumulenenic lipids. *Prog Lipid Res* 46:328–75.
10. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocher, Vidal N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. 97:654–660.
11. Dykens, J. A., Shick, J. M., Benoit, C., Buettner, G. R., & Winston, G. W., 1992. Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. *Journal of Experimental Biology*, 168: 219–241.
12. Fan, D., Hodges, D. M., Zhang, J., Kirby, C. W., Ji, X., Locke, S. J., Critchley, A. T., Prithiviraj, B., 2011. Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress, *Food Chemistry*, 124(1):195-202.
13. Fan, D., Hodges, D. M., Critchley, A. T., Prithiviraj, B., 2013. A Commercial Extract of Brown Macroalga (*Ascophyllum nodosum*) Affects Yield and the Nutritional Quality of Spinach In Vitro. *Communications in Soil Science and Plant Analysis*. 44(12): 1873 – 1884.
14. Guiry, M.D., Guiry, G.M., 2018. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 9 February 2018.
15. Hoppe, H. 1982. Marine algae: their products and constituents. In *Marine Algae in Pharmaceutical Science*. Vol. 2. (H. Hopper and T. Lovring, eds.) pp. 3-48, Walter de Gruyter, Berlin.
16. Jiménez-Escrig, A., Jimé'nez-Jime'nez, I., Pulido, R., Saura-Calixto, F., 2001. Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture*. 8: 530-534.
17. Lichtenthaler, H.K., Buschmann, C., 2001. *Current Protocols in Food Analytical Chemistry*. John Wiley and Sons, Inc., New York F4.3.1- F.4.3.8
18. Mabeau, S., Fleurence, J., 1993. Seaweed in food products: biochemical and nutritional aspects. *Trends in Food Science and Technology* 4 (4):103-107. Total Flavonoid and in vitro Antioxidant Activity of Two Seaweeds of Rameshwaram Coast. *Global Journal of Pharmacology*. 3 (2): 59-62.
19. Matanjun, P, Suhaila, M., Mohamed, M. N., Kharidah, M., Hwee, M. C., 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology*. 367(20):1573-5176.

20. Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masak, K., Masuda, Y., Takeuchi, T., Chihara, M., Yamamoto, Y., Niki, E., Karube, I., 1997. A comparison of screening methods for antioxidant activity in seaweeds. *Journal of Applied Phycology*, 9:29–35.
21. Meenakshi, S., Gnanambigai, D. M., Tamilmozhi, S., Arumugam, M., Balasubramanian, T., 2009.
22. Nijveldt, R.J., van Nood, E., van Hoorn, D.E.C., Boelens, P.G., Van Norren, K., van Leeuwen, P.A.M. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.* 74: 418-425.
23. Kelman, D., Posner E. K., McDermid, K. J., Tabandera, N. K., Wright, P. R., Wright, A.D., 2012. Antioxidant Activity of Hawaiian Marine Algae. *Marine Drugs*, 10(2):403-416.
24. Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, J., Luyck, M., Cazin, M., Cazin, J.C., Bailleul, F., Trotin, F., 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol.* 72:35-40.
25. Sivasankari, S., Venkatesalu, V., Anantharaj, M., Chandrasekaran, M., 2006. Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresource Technology* 97: 1745–1751.
26. Sukenik, A., Zmora, O., Carmeli, Y., 1993. Biochemical quality of marine unicellular algae with special emphasis lipid composition: II. *Nannochloropsis* sp. *Aquaculture*, 117, 313–326.
27. Whapham, C. A., Blunden, G., Jenkins, T., Hankins. S. D., 1993. Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *Journal of Applied Phycology* 5:231–234.
28. Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M., 2002. Free radical scavenging properties of wheat extracts. *J. Agr. Food Chem.* 50:1619-1624.