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Antioxidant Activities of *Eucheuma* sp. (Rhodophyceae) and *Laminaria* sp. (Phaeophyceae)#

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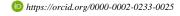
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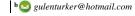
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ABSTRACT

In this study, antioxidant activities of two macroalgae, Eucheuma sp. J.Agardh and Laminaria sp., J.V.Lamouroux used as food supplements were investigated. The antioxidant activity, free radical scavenging activity, the total content of phenolics, flavonoids and condensed tannins in extracts were determined. The IC₅₀ values of the extracts of Eucheuma sp. and Laminaria sp. on DPPH scavenging activity was 2.26±0.03 mg/g and 2.63±0.03 mg/g of extract, respectively. The maximum total phenolic (5.31±0.04 mg GAE/g Ext.) were determined. According to our results, all extracts exhibited low radical scavenging activity when compared to the standards used, i.e. α-tocopherol and butylated hydroxytoluene (BHT) with the inhibition percentage in the range of 48.45±0.06% (Eucheuma sp.) - 58.49±0.03% (Laminaria sp.). The highest flavonoid content was measured as 18.78±0.10 mg rutin/g of extract in Laminaria sp. The condensed tannins were not detected in Eucheuma sp. However, this value was measured as 6.24±0.60 mg CE/g of extract for Laminaria sp. According to our results, Eucheuma sp. and Laminaria sp., which are commercially sold in medicinal herb stores, are good sources of antioxidant components because of their phenolic and flavonoid contents







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Introduction

Macroalgae or seaweeds are photosynthetic organisms and they are renewable resources in the marine environment (Chandini et al., 2008). They are also rich for bioactive compounds (such as protein, essential fatty acids, carbohydrates, vitamins, and minerals) and they can be used for direct human consumption or processed into food additives, pharmaceuticals, feeds, fertilizers, biofuels and cosmetics etc. (Pomponi, 1999; Ak et al., 2015; Pereira, 2018; Kumar et al., 2019). The cultivation of these photosynthetic organisms has accelerated for the last 50 years due to the high demand in the food and pharmaceutical industries. According to FAO statistics (FAO, 2018), 96% of seaweed is produced via aquaculture while only 4 % of seaweed is harvested. About seven seaweed genera are intensively cultivated such as Eucheuma sp., Kappaphycus sp. Doty, Gracilaria sp. Greville, Laminaria sp., Undaria sp. Suringar, Pyropia sp. J.Agardh and Sargassum sp. C.Agardh (Buschmann et al., 2017). Among this genus, Laminaria sp. accounts for over 33 % of global cultivated seaweed production, followed by Eucheuma sp. at 17 % of total production (FAO, 2018).

Laminaria is a brown algae genus, which lives along rocky coastal seashores (Margulis and Chapman, 2009). And, Laminaria sp. are common species of kelp that inhabits temperate and cold waters in the northern hemisphere and temperate waters in the southern hemisphere (Kim and Bhatnagar, 2011). This brown seaweed is an excellent source of dietary fibers (over 50%, mainly alginic acid, fucoidan, and laminarin), vitamins (A, C, D, B1, B2, B3, B6, B12, E, K), minerals (K, Na, Ca, Mg, I, S, Si, etc.) and proteins and has been used in agriculture, food, feed, pharmaceutical and cosmetic industries (Kim et al., 2010). Also, East Asian cultures have traditionally exploited Laminaria for food and medicinal

values (Kim and Bhatnagar, 2011). Laminaria species, especially L. japonica Areschoug and L. saccharina (L.) J.V.Lamouroux has been traditionally used in southeast countries as medicine to treat goiter, scrofula, urinary disease, dropsy, cancer, stomach ailments, and hemorrhoids (Chengkui and Junfu, 1984; Seok et al., 2015). In our country, people prefer this brown seaweed as a vitamin and mineral source. The red seaweed Eucheuma sp. is an economically important tropical seaweed, which is highly demanded its cell wall polysaccharides (Kumar et al., 2008). This macro alga naturally found the Philippines, Indonesia and the island coasts of East Africa (Ask and Azanza, 2002). Also, this red seaweed is cultivated for its hydrocolloids (Santos, 1989). In addition, it contains high amounts of dietary fibers, minerals, vitamins, antioxidants, polyphenols, phytochemicals, proteins, and polyunsaturated fatty acids (Matanjun et al., 2009; Balasubramaniam et al., 2016; Wardani et al., 2017). Eucheuma species traditionally used in Chinese herbal medicine to treat goiter, cough, bronchitis, tonsillitis, asthma with excessive phlegm, stomach ailments, hemorrhoids and anal fistulas (Chengkui and Junfu, 1984). This red seaweed is used as a cough suppressant by Turkish people.

Reactive oxygen species (ROS) or other free radicals are generated continuously via normal physiological processes (Raja et al., 2016; Sevindik, 2019). Under external stimulations (e.g., ionizing radiation, certain pollutants, organic solvents, pesticides etc.), the levels of reactive species can increase dramatically, and they can cause irreversible oxidative damage to all cellular components such as proteins, lipids, amino acids and DNA (Karawita et al., 2005; Tierney et al. 2013; Vasconcelos et al., 2018). The oxidative damages cause aging and many other diseases such as diabetes, arthritis, atherosclerosis, and cancers (O'Sullivan et al. 2011; Tierney et al. 2013; Zhang et al. 2017; Sevindik, 2018). ROS reacts with lipids, proteins, sugars, and vitamins and changes their functionalities (Choe and Min, 2006). In addition, ROS can aggravate the oxidative deterioration and decrease the shelf life of foods (Cox et al., 2010; Choe, 2017). Antioxidants delay the oxidative stress and neutralize free radicals (Sindhi et al., 2013; Pisoschi and Pop, 2015; Sevindik et al., 2018). Seaweeds have polyphenolic compounds such as phenols, flavonoids and condensed tannins which can behave as can behave metal chelators, enzyme modulators and can improve the internal antioxidant system under stress environmental conditions (Duan et al., 2006; Matanjun et al., 2009; Cox et al., 2010; Choe, 2017; Jerez-Martel et al., 2017; Ismail, 2017; Ak and Türker, 2018). Recently, researchers have focused on to find new and effective antioxidant anti-inflammatory and anticancer compounds in seaweeds. Among them, antioxidant activity is intensively studied due to the current growing demand from the pharmaceutical industry where they are interested in antiaging and anti-carcinogenic compounds, which possess health benefits (Raja et al., 2016).

In Turkey, interest in seaweed has been increasing in recent years. These photosynthetic organisms are consumed as a source of vitamins and minerals as well as medicinal herbs. There are no studies to determine the antioxidant properties of these products sold in herbal stores. The aim of the present study is to evaluate the antioxidant activity and biochemical characteristics of the

dried thallus of *Eucheuma* sp. and *Laminaria* sp., which are recently used by Turkish people as food supplements and medicinal herbs. We also used correlation to estimate and compare the contribution of polyphenolic compounds to the measured antioxidant activities.

Materials and Methods

Chemicals

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

Algal Samples

In the present study, algal products from *Eucheuma* sp. and *Laminaria* sp. were analyzed. Dried *Eucheuma* sp. and capsuled *Laminaria* sp. were purchased in medicinal herb stores. Seaweeds were homogenized (IKA, Germany) to a particle size of 1 mm and stored in glass bottles in a dry, dark environment.

DPPH Free Radical Scavenging Activity Assay

The free-radical-scavenging activity of the extracts was determined by the DPPH assay as described by Brand-Williams et al. (1995). A 0.06 mM solution of DPPH in methanol was prepared and an aliquot of 100 L of the antioxidant/seaweed extract solution was added to 3.9 ml of the DPPH solution. The ability of the samples to scavenge DPPH radical was evaluated by measuring the decrease in absorbance (Abs) at 515nm using 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 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. Extract concentrations providing IC_{50} 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.

Determination of Total Phenolic Contents

The amount of total phenolics was determined with the Folin–Ciocalteu reagent using the method of Djeridane et al. (2006). A standard curve was plotted using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% of methanol, and their absorbances were measured at 765 nm. Samples were dissolved with the Folin–Ciocalteu in distilled water. The solutions were mixed. And, then they incubated at room temperature for 1 min. After 1 min, 20% sodium carbonate (Na₂CO₃) solution was added. The final mixture was shaken 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) and the results are expressed in mg of gallic acid per g (GEA) of the dry weight of seaweed.

Determination of Total Flavonoid Contents

The flavonoid content was estimated according to Quettier- Deleu et al. (2000). 1 mL of methanolic extract solution was added to 1 mL of 2% methanolic AlCl $_3$, 6H $_2$ O. The absorbance was measured 10 min later at 430 nm. The results were expressed in mg rutin/100 g dry matter by comparison with standard rutin treated in the same conditions.

Determination of Condensed Tannin Contents

Condensed tannin content was evaluated according to Price et al., (1978). An aliquot of 0.5 g of the seaweed extracts was placed in centrifuge tubes and 20 mL of 1% HCl in methanol was added to each sample. Then, the tubes were placed in a water bath at 30°C with constant shaking for 20 min. After incubation, the samples 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. Afterward, the absorbance was measured at 500 nm using a UV–Vis spectrophotometer (Thermo Aquamate). The results were expressed as microgram catechin equivalents per gram of seaweed (μ g CE/g).

Results and Discussion

The results of the scavenging activity of two edible seaweeds on DPPH free radical are given in Table 1. *Laminaria* sp. displayed the highest antioxidant potential with a low the half-maximal inhibitory concentration (IC₅₀). But, this value was significantly higher than commercial antioxidants tested (Table 1). Statistical significant differences were determined among the comparing groups (P<0.05). The highest Inhibition % value was calculated in *Laminaria* sp. as 58.49±0.03% (P<0.05). Our findings showed that both *Eucheuma* sp. and *Laminaria* sp. have high potential DPPH radical scavenging activity.

The polyphenolic compounds such as phenolics, flavonoids, and tannins show antioxidant properties (Duan et al., 2006; Mohammed et al., 2018). Among these compounds, phenolics are dominant in algae and they are

one of the most potent antioxidant sources (Cox et al., 2010; Machu et al., 2015). The total phenolic content of two edible seaweed was analyzed using the Folin-Ciocalteu reagent. The obtained results together with statistical evaluation are presented in Table 2. Specifically, the highest total phenolic content was determined in the brown seaweed *Laminaria* sp. (5.31±0.04 mg GAE/g Ext). Also, quite high values were found in the red seaweed Eucheuma sp. (4.16±0.03 mg GAE/g Ext). According to Student t-test results, statistically significant differences were found between two groups (P<0.05). The high scavenging activity of Laminaria sp. may be due to hydroxyl groups existing in the phenolic compounds. We couldn't properly compare our results with other studies because researchers used different methods of extractions for example; methanol-chloroform extract of L. japonica contained 0.3 mg GAE /g (Zhang et al., 2007), ethanolextract of Laminaria digitata (Hudson) J.V.Lamouroux 37.00 mg GAE/g (Heffernan et al., 2015), methanol extract of Eucheuma/Kappaphycus 1.5 mg GAE/g (Ganesan et al., 2008) and 160% methanol extract of Eucheuma cottonii Weber Bosse 3.96 mg GAE /g (Damongilala et al., 2013). Also, the characteristics of the samples could affect the phenolic content because all mentioned studies were done with fresh samples contrary to the processed seaweeds analyzed in this work.

Flavonoids play an important role in defense systems of seaweeds are the largest group of polyphenolic compounds and they show antioxidant properties by metal chelation (Goiris et al., 2012). Also, they have been associated with reducing the risk of major chronic diseases (Manach et al., 2004). Therefore, the total flavonoid content of two seaweed extracts was estimated by aluminum chloride method (Quettier- Deleu et al., 2000). The total flavonoid contents of Eucheuma sp. and Laminaria sp. were 16.85±0.11 and 18.78±0.10 mg rutin/g of extract, respectively. The flavonoid contents of Laminaria sp. was significantly higher than Eucheuma sp. (P<0.05). This variation in flavonoid content may be due to the algae classes and environmental conditions. According to Farasat et al. (2014), the same species were from the same collection season, had a different level of flavonoid contents. Our results showed that Laminaria sp. could be a good candidate for flavonoid sources.

Table 1 The DPPH radical scavenging activities of *Eucheuma* sp. and *Laminaria* sp. 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 %
Eucheuma sp.	2.63 ± 0.03^{a}	48.45±0.06°
Laminaria sp.	2.26±0.03 ^b	58.49 ± 0.03^{b}
Butylated hydroxytoluene	1.33±0.01 ^d	99.00 ± 0.11^{a}
α-tocopherol	$1.48\pm0.02^{\circ}$	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 *Eucheuma* sp. and *Laminaria* sp. Different lowercase letters show the significant differences between the groups according to the Student's *t*-test results (P<0.05).

Species	Total Phenolic	Total Flavonoid	Condensed Tannin
	(mg GAE /g Ext.)	(mg rutin/g Ext.)	(mg CE/g Ext.)
Eucheuma sp.	4.16±0.03 ^b	16.85±0.11 ^b	0.07±0.01 ^b
Laminaria sp.	5.31±0.04 ^a	18.78 ± 0.10^{a}	6.24 ± 0.60^{a}

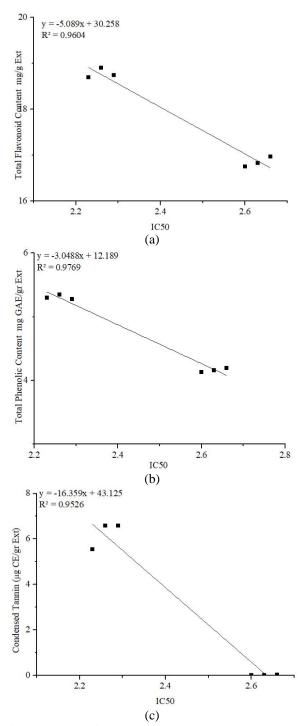


Figure 1 Correlation graphs for IC₅₀ values and (a) total flavonoid contents, (b) total phenolic contents, (c) condensed tannin contents in two edible seaweeds.

Tannins are naturally occurring polyphenolic compounds and they are secondary plant metabolites (Mueller-Harvey, 2006; Zubek et al., 2012). The total condensed tannin content of *Eucheuma* sp. and *Laminaria* sp. can be seen in Table 2. Condensed tannins of the studied seaweeds ranged from 0.07 ± 0.01 to 6.24 ± 0.60 mg CE/g of extract. The content of condensed tannins from *Laminaria* sp. was significantly higher than *Eucheuma* sp. (P<0.05). Many studies have shown that phlorotannins are the only phenolic group detected in brown algae (Jormalainen and Honkanen, 2004; Koivikko et al., 2007). Our results show a similarity with these studies.

Because of some similarities in results, statistical correlations between IC_{50} and the amount of phenolic, flavonoid, condensed tannin compounds have been investigated. According to our findings, the phenolic, flavonoid, and condensed tannin contents were significantly correlated to IC_{50} inhibition values. As can be seen in Figure 1, all evaluations show significant relationships. The same correlation of antioxidant activity was reported Chakraborty et al., (2013) and Ismail (2017) with some edible brown, green, and red seaweeds of rich polyphenolic content.

Conclusion

Seaweeds are not only a good source of carbohydrates, proteins, vitamins, oils, polyunsaturated fatty acid, and minerals, but also contain a large concentration of antioxidants compounds such as polyphenols which deserve attention because of the many health benefits they provide (Wells et al., 2017). In this study, we found that seaweed products also have considerable amounts of polyphenolic compounds, which are comparable to polyphenolic compounds in unprocessed seaweeds. The results clearly showed that two edible seaweeds have antioxidant activity with low IC50 inhibition values. We conclude that the main contributors of antioxidant activity these two seaweeds according to a significant negative correlation between IC₅₀ inhibition values and total phenolic, flavonoid, and condensed tannin contents. Therefore, polyphenols, in these two edible seaweeds, appear to not be the major contributors to the high scavenging activities. However, this needs to be determined other active components such as lowmolecular-weight polysaccharides, proteins, peptides, amino acids and essential fatty acids in extracts may have synergistic effects on the scavenging activities, which can give contradictory results. According to our results, Eucheuma sp. and Laminaria sp., which are commercially sold in medicinal herb stores, are good sources of antioxidant components because of their phenolic and flavonoid contents.

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