Antioxidant activity of macroalgae from the Azores

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Free radical scavenging activity (FRSA) was studied to determine the antioxidant activity of 8 common macroalgae species found in the Azores. Species under study were *Porphyra* sp., *Osmundea pinnatifida*, *Pterocladiella capillacea*, *Sphaerococcus coronopifolius* and *Gelidium microdon*, from Rhodophyta; *Ulva compressa* and *Chaetomorpha pachynema*, from Chlorophyta and *Fucus spiralis* from Phaeophyta.The antioxidant activity was evaluated in methanolic extract by a decolourisation solution test of 2,2-diphenyl-1-picrylhydrazyl (DPPH), used as a stable radical. The methanolic extracts were obtained from dried biomass by sequential extractions, attaining a final concentration of 2 $mg.mL^{-1}$. The FRSA values ranged from 19.54% for *S. coronopifolius* to 60.05% for *F. spiralis* with standard deviation (SD) varying between 1.41% and 6.80%. Results indicated that the studied seaweeds are a very promising source of biological active compounds with antioxidant properties. The seaweeds were collected in the Azorean islands where seawater pollution levels are low. Consequently, these seaweeds represent a valuable and good source of antioxidant material with superior beneficial effects on human health.

Key words: DPPH, functional food, macroalgae screening, radical scavenging activity

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INTRODUCTION

In living organisms, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to biomolecules, such as lipids, nucleic acids, proteins, enzymes and other cellular constituents (Halliwell 1991). The most important reaction is lipid peroxidation of unsaturated fatty acids. This oxidative degradation of lipids, proceeds by a free radical chain reaction mechanism, has been known to cause many pathological effects (Spencer et al. 1994). A lipid radical (L•) from a lipid (LH) is formed in the initiation step that then reacts with oxygen to form a lipid peroxyl radical (LOO•) which reacts with an additional lipid molecule to give a lipid hydroperoxide (LOOH) in the propagation step. Fatty acid hydroperoxides are known to be one of the active oxygen species (Ohkawa et al. 1979). The degradation and/or modification of these molecules have been related with various chronic diseases implicated in the processes of aging, as well as in a wide range of degenerative diseases, such as: coronary heart disease, atherosclerosis, cancer, cataracts, diabetes, liver injury, Alzheimer´s and Parkinson´s diseases, muscular dystrophy and some others neurological disorders (Duan et al. 2006). It is well known that antioxi-

dants can neutralise potentially harmful reactive free radicals in body cells before they cause lipid damage. In addition, lipid peroxidation is one of the major causes of deterioration during storage and food processing. To compensate these deleterious effects, synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used, which in excess may contribute to some negative health side effects, according to the published literature (Santoso et al. 2004). Therefore, there is a growing interest on the discovery of natural antioxidants, mainly for two reasons: (1) epidemical and clinical evidence suggests that consumption of fruits and vegetables reduce the risk of developing chronic disease, e.g. cancer and coronary heart disorders, and (2) phytochemicals are generally safer than synthetic chemicals (Dastmalchi et al. 2007). For these reasons, many products with antioxidant properties are widely used, particularly from natural sources, in order to minimise oxidative damage to living cells and to prevent oxidative deterioration (Rekka & Kourounakis 1991; Conforti et al. 2005; Rackova et al. 2007). Antioxidant activity of seaweeds is being intensively investigated due to the currently growing demand from the food and pharmaceutical industries where there is interest in anti-aging and anticarcinogenic natural bioactive compounds (Matsukawa et al., 1997; Siriwardhana et al. 2003; Dias et al. 2005; Senevirathne et al. 2006; Kuda et al. 2005; Ganesan et al., 2008). Furthermore, seaweeds have also been used as a source of novel functional foods with potential nutritional benefits (Santoso et al. 2004). More recently, much attention has been paid to the anti-tumour and anticholesterolemic activities of seaweed constituents (Park et al. 2005; Villaño et al. 2007; Duh 1998). Like plants, seaweeds contain various types of inorganic (high levels of minerals) and organic compounds (vitamins, proteins, essential amino acids, indigestible carbohydrates and dietary fibre) (Jiménez-Escrig et al. 2001; Yoshie et al. 2002; Patarra et al. 2011).

The objective of our study was to determine the antioxidant activity of the most common macroalgae in the Azores islands taking into account the low pollution levels of seawater in the region (Neto et al. 2009) and, consequently, the great potential of its use for human consumption and/or for the extraction of novel compounds with added value for the food and pharmaceutical industries. In addition, this study also comparatively evaluated the FRSA of the referred macrophytes with synthetic antioxidant BHT.

MATERIAL AND METHODS

SEAWEEDS SAMPLES AND CHEMICALS

All seaweed samples used in this study were collected during January/February 2007, April 2008 and June 2009, from the littoral zone of São Miguel Island, in the Azores Archipelago. The seaweeds were washed with water and alcohol, air-dried and stored in an air-tight container in a freezer (-20 ºC) until further analysis. Seaweeds analysed belonged to Phaeophyta (*Fucus spiralis* Linnaeus), Chlorophyta (*Ulva compressa* Linnaeus and *Chaetomorpha pachynema* (Montagne) Kützing), and Rhodophyta (*Porphyra* sp. C. Agardh, *Osmundea pinnatifida* (Hudson) Stackhouse, *Pterocladiella capillacea* (S.G. Gmelin) Santelices & Hommersand, *Sphaerococcus coronopifolius* Stackhouse and *Gelidium microdon* Kützing). BHT, DPPH and the methanol HPLC (high performance liquid chromatography) grade solvent were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals used were of analytical grade. Deionized water used for the samples preparation was obtained with an in-house Milli-Q water purification system (Millipore, Bedford, MA, USA).

SEAWEEDS SAMPLE PREPARATION

The dried seaweed samples were cut into small pieces and ground into a fine powder using a dry grinder. The ground samples were sieved to obtain a uniform particle size and stored at -20 ºC until further analysis. Each ground sample was weighed and transferred into a beaker. Methanol was added in the ratio of 1:10 and stirred for 2 hr with the aid of a magnetic stirrer. The extraction mixture was left to sediment for about 60 min before separating the extract from the residue by filtration through a cellulose acetate filter of 0.45 µm porosity. The residue was re-extracted twice with methanol in the same conditions and extracts were combined. The residual solvent was removed under reduced pressure at 45 ºC using a

rotary evaporator (BÜCHI Labortechnik AG in Flawil, Switzerland). Extracts were prepared in duplicates and used to determine the FRSA.

FRSA ASSAY

The FRSA of the seaweeds species were determined in a methanolic solution of DPPH, used as a stable radical, according to the method described by Molyneux (2004) and Rainha et al. (2011a). This methodology measures the hydrogen atom or electron donor capacity of an extract to the stable radical DPPH formed in solution. In other words, it measures the capacity of the extract to scavenge free radicals in solution.

An aliquot of 2.0 mL of the methanolic solution of seaweed species or BHT at 2.0 mg.mL-1 concentration was added to a test tube, with 1 mL of DPPH methanolic solution (4.5 mg.100 mL^{-1} in methanol, w/v). Methanol was used as a blank to zero the absorbance (Abs), BHT was used as reference sample and a mixture without seaweed extract or BHT was used as the control (c). The Abs was measured at 517 nm over a period of 30 min and after each 5 min of reaction time using a Shimadzu 160-A UV/VIS spectrophotometer. All determinations were performed in triplicate and averaged. The FRSA of the samples (s) were calculated as a percentage of DPPH decolouration using the following equation (Rainha et al. 2011a):

$$FRSA\ (\%) = (1 - Abss/Absc) \times 100$$

Results are expressed as mean values ± SD of three different Abs measurements of two extracts per seaweed species.

RESULTS

The antioxidant activity of the Azorean macroalgae compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Figure 1 shows the FRSA of the selected seaweed species that ranged from 19.54±2.40% to 60.05±4.29% after 30 minutes reaction. The SD of FRSA lower than 6.80% reveals good repeatability. The FRSA results of all seaweed samples show slightly increased values with increasing reaction time, particularly from 10 to 30 min (Table 1). Among the methanolic seaweed extracts, the greatest anti-free radical activity after 30 minutes reaction was observed for F. spiralis (60.05±4.29%) followed by *G. microdon* (45.86±5.05%), *C. pachynema* (43.52±4.79%), *U. compressa* (39.12±4.30%), *O. pinnatifida* (33.97±3.74%), *P. capillacea* (27.44±5.90%), *Porphyra sp.* (22.73±4.88%) and *S. coronopifolius* (19.54±2.40%). The differences in FRSA values for different species reflect their different chemical compositions, probably due to differences in the type and amount of phenolic compound contents. However, the differences for the same species from different origins also reflect the influence of geographic origin, climate, season, variety (Dawes et al. 1998; Jiménez-Escrig & Cambrondón 1999) and seawater quality.

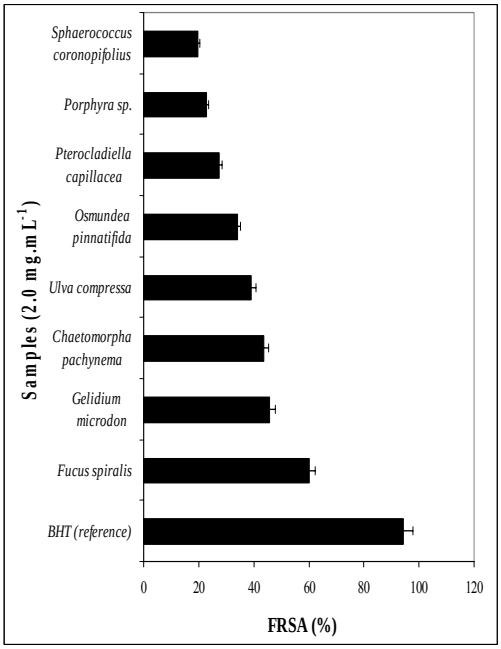

Fig. 1. Comparison of free radical scavenging activity (FRSA) of methanolic extract from selected Azorean macroalgae with standard butylated hydroxytoluene (BHT), at 2.0 $mg.mL^{-1}$ concentration, after 30 min reaction.

Table 1. Determination of free radical scavenging activity of selected Azorean macroalgae (reaction time from 5 min to 30 min)[a]

Samples	Free radical scavenging activity (FRSA) %[a]					
	5 min	10 min	15 min	20 min	25 min	30 min
Sphaerococus coronopifolius	7.05±3.41	10.72±5.20	13.50±1.84	15.82±2.16	17.69±1.54	19.54±2.40
Porphyra sp.	7.70±3.73	11.59±5.62	14.33±1.96	16.74±1.46	18.94±2.32	22.73±4.88
Pterocladiella capillacea	17.34±1.51	19.19±2.35	21.33±3.27	22.80±4.90	25.56±5.49	27.44±5.90
Osmundea pinnatifida	20.50±3.14	25.86±5.56	27.97±6.01	29.57±6.35	31.50±3.47	33.97±3.74
Ulva compressa	16.15±1.41	23.04±4.95	29.15±6.26	32.86±3.62	35.98±3.96	39.12±4.30
Chaetomorpha pachynema	28.03±6.02	33.97±3.74	36.73±4.04	39.67±4.36	41.59±4.58	43.52±4.79
Gelidium microdon	30.44±6.54	34.90±3.84	38.68±4.26	41.40±4.55	44.31±4.88	45.86±5.05
Fucus spiralis	38.03±4.18	46.18±5.75	50.34±6.37	54.46±6.80	58.24±4.16	60.05±4.29
BHT	59.67±4.26	72.45±5.97	78.98±5.27	85.45±2.66	91.38±3.07	94.22±1.88

[a]Values are expressed as mean ± SD (n = 6). Three determinations from two different batches of seaweeds were performed; BHT, butylated hydroxytoluene.

DISCUSSION

Generally, there is a preference for antioxidants from natural rather than from synthetic sources. The DPPH methodology allows one to determine the FRSA of different seaweeds with good repeatability. The results showed different values for different species, and *F. spiralis* and *G. microdon* presented superior antioxidant activities, revealing them to be valuable species that could be explored from biotechnology and commercial perspectives, taking into account the low seawater pollution levels in the Azores. The next step of this study will be the polyphenolic determination in order to establish the relationship with the antioxidant activity and also a fully antioxidant characterisation using other methodologies. These include: the β-carotene/linoleic acid assay (Dapkevicius et al. 1998), the reducing power effect assay (Oyaizu 1986), the superoxide anion scavenging activity assay (Liu et al. 1997), the ferrous ion-chelating assay (Dinis et al. 1994) and the bovine serum albumin (BSA) oxidative damage assay (Makris & Rossiter 2001), that were recently published with some experimental modifications by Rainha et al. (2011b).

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