Food Chemistry 218 (2017) 591–599

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cytoprotective effect of seaweeds with high antioxidant activity from the Peniche coast (Portugal)

Susete Pinteus [1], Joana Silva [1], Celso Alves, André Horta, Nádia Fino, Ana Inês Rodrigues, Susana Mendes, Rui Pedrosa *

[a] MARE – Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, 2520-641 Peniche, Portugal

ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 25 August 2016
Accepted 9 September 2016
Available online 10 September 2016

Keywords:
Marine natural products
Total phenolic content
Radical scavenging activity
Oxidative stress
Algae extracts
Tumor cell line

ABSTRACT

Screening of antioxidant potential of dichloromethane and methanolic extracts of twenty-seven seaweeds from the Peniche coast was performed by: total phenolic contents (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and oxygen radical absorbance capacity (ORAC). Seaweeds revealing the highest antioxidant activity were screened for cytoprotective potential in MCF-7 cells, including the mitochondrial membrane potential analysis and the caspase-9 activity. High correlation was found between TPC of seaweed extracts and their scavenging capacity on DPPH and peroxyl radicals. The highest antioxidant activity was displayed by the methanolic fraction of brown seaweeds belonging to Fucales, however *Ulva compressa* presented the highest cytoprotective effect by blunting the apoptosis process. These results suggest that high antioxidant activity may not be directly related with high cytoprotective potential. Thus, seaweeds reveal to be a promising source of compounds with potential against oxidative stress.

1. Introduction

Although essential to life, the molecular oxygen is the main producer of reactive oxygen species (ROS) like superoxide anion ($O^{\cdot -}$), hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2). These molecules react with biological membranes, DNA, proteins and other cellular constituents promoting their degradation and consequently promoting the aging process and the occurrence of several pathologies such as atherosclerosis, cancer, malaria, neurodegenerative diseases and arthritis (Heo, Park, Lee, & Jeon, 2005; Murray et al., 2013). Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation or rancidity and formation of undesirable secondary lipid peroxidation products which ultimately causes a decrease in nutritional value of foods affecting their safety, appearance and edibility.

Antioxidants have wide ranging applications as sacrificial reducing agents. They elicit their benefits by preventing, delaying, or neutralizing the effects of oxidative change and suppression and/or scavenging of free radicals. Therefore, antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation, reducing potential mutations as well as act as stabilizers in order to increase the shelf-life of food products (Devi, Suganthy, Kesika, & Pandian, 2008; Murray et al., 2013).

In food, cosmetics and pharmaceutical industries, many synthetic commercial antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) have been used to retard the oxidation and peroxidation processes. However, some physical properties of these synthetic antioxidants such as their high volatility and instability at elevated temperature, strict legislation on the use of synthetic food additives, and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants. Moreover, several studies have revealed that the use of synthetic antioxidants produce carcinogenic effects and liver damage in animals (Saad et al., 2007).

Consequently, the use of natural antioxidants in food, cosmetic, and therapeutic industry are promising alternative for synthetic antioxidants with respect to low cost, highly compatible with dietary intake and no harmful effects inside the human body (Lobo, Patil, Phatak, & Chandra, 2010). Moreover, natural products are generally safer than synthetic chemicals due to the absence of chemical contamination and therefore better accepted by the society and by food industries than synthetic chemicals (Heo et al., 2005; Patra, Rath, Jena, Rathod, & Thatoi, 2008). The principal sources of natural antioxidant compounds chiefly include those

* Corresponding author.
E-mail address: rpedrosa@ipleiria.pt (R. Pedrosa).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2016.09.067
0308-8146/

of herbs, spices, and medicinal plants. However, recent works in marine organisms reveals that seaweeds are also rich sources of antioxidant compounds as well as other bioactive compounds. In order to survive in harsh environments, seaweeds produce a variety of bioactive compounds such as polysaccharides, antioxidants, carotenoids, dietary fibre, protein, essential fatty acids, vitamins, and minerals making them interesting candidates to be used as food supplement, source of vitamins, food additives and for the isolation of bioactive compounds with therapeutic applications (Wijesekara, Pangestuti, & Kim, 2011; Yan, Nagata, & Fan, 1998). Therefore, the aim of this study was to evaluate the antioxidant activity of twenty-seven seaweeds from the Peniche coast (Portugal) by the quantification of total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and by the Oxygen Radical Absorbent Capacity (ORAC). Seaweeds that showed the highest antioxidant potential were evaluated for their ability to revert the oxidative stress condition promoted by H_2O_2 in an *in vitro* cell line model of human breast adenocarcinoma (MCF-7). The apoptosis process was also followed by the evaluation of the mitochondrial potential and caspase-9 activity.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent; gallic acid; DPPH; butylated hydroxytoluene (BHT); dimethyl sulphoxide (DMSO); sodium carbonate (Na_2CO_3); AAPH (2,2V-azobis (2-amidinopropane)); fluorescein (FL); disodium phosphate (Na_2HPO_4); monosodium phosphate (NaH_2PO_4); trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-car boxylic acid); RPMI-1640; fetal bovine serum (FBS); antibiotic/antimycotic; human insulin; MEM – non-essential amino acids; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT); hydrogen peroxide (H_2O_2); trypsin-EDTA and sodium pyruvate were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Methanol, *n*-hexane and dichloromethane of analytical grade were purchased from Fisher Scientific (Leicestershire, United Kingdom).

2.2. Seaweeds collection and identification

Seaweeds were collected along the Peniche coast (Portugal) and Berlenga Nature Reserve and immediately transported to the laboratory. They were cleaned and washed with sea water and then in fresh water to remove epiphytes, detritus and encrusting material. Seaweeds were identified as *Asparagopsis armata*, *Ceramium ciliatum*, *Plocamium cartilagineum*, *Corallina elongata*, *Porphyra linearis, Gelidium pulchellum*, *Jania rubens*, *Nitophyllum punctatum* and *Sphaerococcus coronopifolius* (Rhodophyta Division); *Fucus spiralis*, *Halopteris filicina*, Saccorhiza *polyschides*, *Bifurcaria bifurcata*, *Padina pavonica, Colpomenia peregrina*, *Dictyota dichotoma, Sargasssum muticum, Cystoseira tamariscifolia*, *Cladostephus spongiosus*, *Cystoseira usneoides*, *Taonia atomaria*, *Sargassum vulgare* and *Stypocaulon scoparium* (Heterokontophyta Division); *Codium adhaerens*, *Codium tomentosum*, *Codium vermilara* and *Ulva compressa* (Chlorophyta Division). Finally seaweeds were frozen at −80 °C, (Thermo Scientific, Electron Corporation, Waltham, Massachusetts, USA) lyophilized and hermetically stored at −20 °C until further use.

2.3. Preparation of seaweeds extracts

Lyophilized seaweeds were ground with a mixer grinder to make a powder. Each alga sample was sequential extracted in 1:4 (w/v) biomass/solvent ratio with methanol and dichloromethane at constant stirring for 12 h. Liquid-liquid extraction was also performed for the methanolic fraction, using *n*-hexane. The *n*-hexane was used to clean and enrich the methanol fraction by removing some nonpolar compounds. The solvents were evaporated in a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany) at 40 °C and the extracts were then solubilized in DMSO and stored at −20 °C until further use.

2.4. Quantification of total phenolic content (TPC)

The TPC of seaweed extracts were determined using Folin-Ciocalteu method adapted to microscale (Singleton & Rossi, 1965). Briefly, 2 μL of sample were added to 158 μL of distilled water, 10 μL of Folin-Ciocalteu reagent and 30 μL of 20% sodium carbonate. After one hour of reaction in the dark, the absorbance was measured at 755 nm (Synergy H1 Multi-Mode Microplate Reader, BioTek® Instruments, Winooski, VT, USA) and used to calculate the phenolic content using gallic acid as the standard.

2.5. DPPH radical scavenging activity

The DPPH free radical scavenging method was performed according to Brand-Williams, Cuvelier, and Berset (1995) adapted to microscale with slight modifications. DPPH radical was dissolved in methanol (0.1 mM). Various concentrations of 2 μL of sample solution were added to 198 μL of the DPPH radical solution. The mixture was vortexed for 1 min and allowed to stand at room temperature in the dark for 30 min, at which time the decrease in absorbance at 517 nm was measured (Synergy H1 Multi-Mode Microplate Reader, BioTek® Instruments). The radical solution was freshly prepared each day. The synthetic antioxidant BHT was used as a standard. The ability to scavenge the DPPH radical was calculated using the follow equation:

$$\text{Scavenging effect}\,(\%) = \left[1 - \left(\frac{A_{sample} - A_{sampleblank}}{A_{control}}\right)\right] \times 100$$

where the $A_{control}$ is the absorbance of the control (DPPH solution with DMSO), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and the $A_{sample\ blank}$ is the absorbance of the sample in methanol (sample without DPPH solution).

2.6. Oxygen radical absorbent capacity (ORAC)

The ORAC assay was performed as described by Dávalos, Gómez-Cordovés, and Bartolomé (2004) as follows: The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. Sample (20 μL) and fluorescein (120 μL; 70 nM, final concentration) were placed in the well of the microplate. The mixture was pre-incubated for 15 min at 37 °C. AAPH solution (60 μL; 12 mM, final concentration) was added rapidly using a multichannel pipet. The microplate was immediately placed in the reader and the fluorescence recorded every minute for 240 min. The microplate was automatically shaken prior each reading. A blank using phosphate buffer instead of the fluorescein and eight calibration solutions using Trolox (1–8 μM, final concentration) as antioxidant were also carried out in each assay. All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample.

Antioxidant curves (fluorescence versus time) were first normalized to the curve of the blank corresponding to the same assay by multiplying original data by the factor $\text{fluorescence}_{blank,t=0}/\text{fluorescence}_{sample,t=0}$. From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as:

$$AUC = 1 + \sum_{i=1}^{i-80} f_i/f_0$$