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In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland

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ABSTRACT

The antioxidant potential of methanolic extracts of brown seaweeds was assessed by Total Phenol Content, Ferric Reducing Antioxidant Power (FRAP), β-carotene bleaching and the DPPH· scavenging assays. *Ascophyllum nodosum*, *Pelvetia canaliculata*, and *Fucus serratus* contained the highest phenol concentrations while *Fucus vesiculosus* and *F. serratus* exhibited the highest FRAP activities. *F. vesiculosus* and *A. nodosum* were the most effective extracts at scavenging DPPH radicals and preventing β-carotene bleaching. The antioxidant activity of the seaweed extracts was also evaluated in Caco-2 cells. All extracts significantly ($P < 0.05$) increased glutathione (GSH) content of cells after 24 h. Caco-2 cells were also pretreated with seaweed extract for 24 h followed by exposure to hydrogen peroxide (H_2O_2). Antioxidant enzyme activity (catalase (CAT) and superoxide dismutase (SOD)) was assessed and DNA damage was measured using the comet assay. *P. canaliculata* was the most effective at preventing H_2O_2-mediated SOD depletion in Caco-2 cells while *F. serratus* exhibited the best DNA protective effects.

1. Introduction

Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), superoxide anion (O_2^{-}) and nitric oxide (NO) are deleterious to various physiologically important molecules including proteins, lipids and DNA (Wijeratne, Cuppett, Schlegel, 2005). ROS, generated in living organisms during metabolism, are very unstable and highly reactive, and they tend to initiate chain reactions which result in irreversible chemical changes in proteins or lipids. These deleterious reactions can result in cellular dysfunction and cytotoxicity. A number of cellular defence systems have evolved to counteract the accumulation of ROS. These include enzymatic scavengers such as catalase, glutathione peroxide, and superoxide dismutase (SOD). Antioxidant enzymes are the primary defence system directly involved in the detoxification of ROS (Aruoma, 1998). Physiological and dietary antioxidants constitute non-enzymatic antioxidant defence systems, which are the secondary defence system and include glutathione, carotenoids, polyphenols and other non-nutrient compounds (Aruoma, 1998).

Oxidative stress is implicated in a wide variety of diseases, including the development of various human chronic diseases such as cardiovascular disease, certain cancers, and a number of neurodegenerative diseases (Aruoma, 1998). Therefore, over the last two decades, a wide range of phytochemicals from terrestrial plant materials have been evaluated for their ability to protect tissues against oxygen-induced damage and hence lower the risk of human chronic diseases (Halvorsen et al., 2006).

Oxidative stress not only affects the body but can also have detrimental effects on foods due to the production of rancid flavours and odours while also reducing the shelf-life, nutritional quality, and safety of food products (Zainol, Abd-Hamid, Yusof, Muse, 2003). In order to lower the risk of oxidative deterioration, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been added to many food products. Due to safety issues and consumer demand, there has been considerable interest in replacing synthetic antioxidants with natural plant-based alternatives (Devi, Suganthy, Kesika, Pandian, 2008). Natural antioxidants derived from marine sources may have

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potential for inclusion in fish and meat products as alternatives to synthetic antioxidants such as BHA and BHT.

Many types of seaweed contain a wide range of bioactive compounds with potential antioxidant activity, for example phlorotannins a range of polyphenolic compounds exclusively found in brown seaweed, can account for between 1% and 10% of the dry weight of these seaweeds (Ragan Glombitza, 1986). Polyphenols derived from seaweeds may be more potent than analogous polyphenols derived from terrestrial plant sources due to presence of up to eight interconnected phenol rings (Hemat, 2007). In addition, compounds identified in seaweeds including alkaloids, terpenes, ascorbic acid, tocopherols and carotenoids have demonstrated antioxidant activity in a variety of *in vitro* studies (Heo et al., 2009; Hu, Lin, Lu, Chou, Yang, 2008).

In contrast to terrestrial plant materials, less research has been conducted on the antioxidant potential of marine seaweeds. In recent years a number of studies reported that seaweed extracts demonstrate strong antioxidant properties (Gamal-Eldeen, Ahmed, Abo-Zeid, 2009). A rich diversity of seaweed exists around the coast of Ireland with over 500 different species documented. Wild stocks of seaweed in Ireland yield approximately 36,000 tonnes annually while world output stands at close to 8 million tonnes (McHugh, 2003; Werner, Clarke, Kraan, 2004). The extent of the Irish seaweed resource has not been fully determined. Furthermore, reports on the potential antioxidant properties of seaweeds extracts from the coast of Ireland are limited.

In this study, five species of seaweed harvested from the west coast of Ireland in spring were examined for their antioxidant potential. These included *Ascophyllum nodosum*; a mid shore species found on sheltered coastlines, *Laminaria hyperborea*; a lower shore species, *Pelvetia canaliculata*; an upper shore species, and two *Fucus* species; *Fucus vesiculosus*; a mid shore and *Fucus serratus*; a lower shore species.

The objectives of the present study were to firstly, measure the antioxidant capacity of the five different seaweed extracts using a range of chemical reaction based assays. Secondly to investigate the cytotoxicity and antioxidant potential of the extracts using a human colon carcinoma cell line, Caco-2 cells, as a model system. In the present study, the five extracts were investigated for their potential cytoprotective and genoprotective effects against H_2O_2-induced stress in Caco-2 cells.

2. Materials and methods

2.1. Materials

Human colon adenocarcinoma Caco-2 cells were purchased from European Collection of Animal Cell cultures (Salisbury, UK). Foetal bovine serum was purchased from Invitrogen (Paisley, Scotland). Cell culture plastics were purchased from Cruinn Diagnostics (Greiner Bio-One, Frickenhausen, Germany). Seaweed extracts were prepared from *A. nodosum*, *L. hyperborea*, *P. canaliculata*, *F. vesiculosus* and *F. serratus* in the National Seaweed Centre at the National University of Ireland, Galway (NUI Galway). Calbiochem Catalase Colorimetric Activity Kit and Calbiochem SOD Assay Kit II were purchased from Merck Chemicals Ltd. (Nottingham, UK). All other cell culture chemicals and reagents were purchased from Sigma Chemical Co. (Dublin, Ireland).

2.2. Preparation of seaweeds extracts

Seaweeds were harvested from the seashore in Galway in spring and washed to remove any undesired material. The seaweeds were chopped and either frozen or dried for storage. The seaweed extracts were prepared according to the method of Connan, Goulard, Stiger, and Deslandes (2004) with modifications. Seaweed samples (5 g) were ground in a mortar with liquid nitrogen, and mixed with 75 ml of 60% methanol/40% distilled water. This mixture was homogenised at 24,000 rpm for 1 min and was then placed in the dark at 40 °C for 3 h, after which the mixture was centrifuged at 9000 rpm for 15 min. The mixture was then filtered with cotton wool to extract the supernatant which was subsequently subjected to solvent evaporation to remove methanol. Extracts were freeze-dried and stored in desiccators. The final weights of recovered extracts were 10.0, 12.3, 11.0, 14.6 and 13.0 mg for *A. nodosum*, *L. hyperborea*, *P. canaliculata*, *F. vesiculosus* and *F. serratus*, respectively. Extracts were reconstituted in distilled water to a concentration of 10 mg/ml.

2.3. Determination of Total Phenol Content (TPC)

The TPC of the extracts was quantified according to the method of Singleton and Rossi (1965). Gallic acid was used as a standard and TPC was expressed in terms of mg gallic acid equivalents/g dry weight of sample (GAEq/gdw).

2.4. Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain (1999). Ascorbic acid was used as a standard and FRAP values were expressed as μM ascorbic acid equivalents/g dry sample (AAEq/gdw).

2.5. DPPH· (2,2-diphenyl-2-picrylhydrazyl hydrate) scavenging assay

Serial dilutions of the seaweeds extracts (10 mg/ml) at concentrations ranging from 0.2 to 5 mg/ml in methanol were prepared. Stock solutions of trolox (2 mM, 0.53 mg/ml) and ascorbic acid (10 mM, 1.8 mg/ml) were diluted with methanol to give concentrations ranging from 0.01 to 0.27 mg/ml and 0.04 to 0.9 mg/ml, respectively. Hundred microlitres of each dilution was taken and added to cuvettes. 3.9 ml DPPH/methanol working solution were added to each cuvette and the absorbances were read immediately at 515 nm. After the initial measurement all samples are placed in the dark and the absorbance was subsequently measured at 30 min intervals. The sample results were compared to that of trolox standards expressed as % DPPH radical scavenging (Brand-Williams, Cuvelier, Berset, 1995).

2.6. β-Carotene Bleaching Assay (BCBA)

The BCBA was performed based on the method by Duan, Zhang, Li, and Wang (2006) with some modifications. Briefly, oxygenated water was mixed with β-carotene, Tween-40 and linoleic acid. The mixture (200 μl) was incubated with or without 50 μl (10 mg/ml) of seaweed extract or trolox standards (0–2 mM, 0–0.53 mg/ml) for 180 min at 50 °C and the absorbance was read at 450 nm at time 0 and every 30 min thereafter. The results were expressed as % β-carotene bleaching inhibition.

2.7. Cell culture

Human colon adenocarcinoma Caco-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with FBS (10% v/v) and non-essential amino acids (1% v/v). Cells were incubated in an atmosphere of CO_2–air (5:95, v/v) at 37 °C and were maintained in the absence of antibiotics. Caco-2 cells were plated at a density of 1×10^5 cells/ml, depending on the experimental procedure. After 24 h the growth media was replaced with fresh media containing reduced FBS (2.5% v/v) which were supplemented with 100 μg/ml seaweed extracts for 24 h in 6-well plates