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Characterization and *in vitro* evaluation of seaweed species as potential functional ingredients to ameliorate metabolic syndrome

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ABSTRACT

This study aimed at identifying seaweed species with optimal characteristics to develop multifunctional foods for metabolic syndrome (MetS) management. Mass spectrometry chemical characterization and bioactive profile evaluation of methanolic extracts from seven commonly consumed seaweeds were compared. Monomeric sugars namely mannitol, fucitol, xylitol and their sulphated analogs as well as lipids (phosphatidic acid, octadecenoic acid, and prostaglandin 2α) were detected in seaweeds. *Himanthalia elongata* showed the highest phenolic content (24.04 μmol GAE/g), and antioxidant activity. This species was the only one showing angiotensin converting enzyme-I inhibitory activity (IC_{50} = 65 μg/mL). *U. pinnatifida* and *H. elongata* extracts were notably more effective reducing pro-inflammatory molecules in lipopolysaccharide-induced RAW 264.7 macrophages. Finally, *Ulva* spp., *Palmaria palmata*, *U. pinnatifida* and *H. elongata* significantly inhibited triglyceride accumulation in mature 3T3-L1 adipocytes (43–52% inhibition). Among seaweeds, *H. elongata* showed the highest potential to be used as ingredient in the development of new functional foods for MetS management.

1. Introduction

The modern lifestyle is leading to changes in the eating patterns of global population towards diets rich in refined sugars, salt, saturated fats and energy-dense foods (Hyseni et al., 2017). These modern diets contribute to the development of chronic diseases responsible for 70% of all deaths worldwide (WHO, 2017). Metabolic syndrome (MetS) is becoming one of the most important health concerns in the last years, due to its rising prevalence (Grundy, 2016). This syndrome encompasses a constellation of several risk factors including hyperglycemia, raised blood pressure, dyslipidemia and visceral obesity that predispose to the development of cardiovascular disease and type 2 diabetes mellitus (Alberti et al., 2009). According to the National Cholesterol Education Program Adult treatment Panel III (NCEP ATP III, 2002) definition, MetS is present if three or more of the following five criteria are met: waist circumference over 102 cm (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl. Indeed, visceral obesity and insulin resistance are key etiological factors of MetS development. Obesity causes an increased concentration of free fatty acids (FFA) that may cause insulin resistance, oxidative stress and inflammation (Dandona, Aljada, Chaudhuri, Mohanty, & Garg, 2005). The search for efficient and economic strategies for prevention of MetS is crucial to ameliorate its impact on global health and economy. Healthy diets may play a key role in reducing the incidence of MetS (Grosso et al., 2017). In this context, many studies have provided evidence on the role of foods rich in bioactive compounds to counteract the different components of MetS.

Due to the current consumers trend to embrace organically grown, natural and healthy foods from clean environments, edible macroalgae

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(seaweed) are receiving an increasing acceptance. Edible seaweeds have been consumed as food and traditional medicines since ancient times in Asian countries such as Japan, Korea and China (Levine, 2016). Seaweed consumption in western societies is lower than in Asia, but they have been traditional consumed in countries such as England, Ireland, Scotland, Scandinavia, and Canada for human and domestic animal feeding (Delaney et al., 2016). The nutritional and beneficial health effects derived from seaweed consumption has stimulated their inclusion as functional ingredients in a broad range of foodstuffs (sushi, pasta, biscuits, bread and beverages, among others), and can be also consumed fresh or dried as whole foods (Rioux, Beaulieu, & Turgeon, 2018). Moreover, seaweeds are commonly used in the production of gelling agents such as alginate or agar by the food industry in western countries (Smit, 2004). The inclusion of seaweed extracts enriched in different bioactive compounds as powdered ingredients could represent a promising tool for the design of multifunctional foods or nutraceuticals.

Among the eight most important seaweeds used for human consumption are species from *Porphyra* spp. (Nori), *Laminaria japonica* (Kombu), *Undaria pinnatifida* (Wakame), *Ulva* spp. (Sea Lettuce), *Palmaria palmata* (Dulse) and *Chondrus crispus* (Irish moss) (Hassan, 2009). They are attracting growing interest nowadays as excellent sources of essential fatty acids with a nutritionally ideal n-6/n-3 fatty acid ratio, polysaccharides, with relatively high protein content, including all the essential amino acids, minerals, vitamins and trace elements. In addition, several *in vivo* studies support the positive effect of seaweed products when consumed directly or as dietary supplements on health by reducing body weight (Okada, Mizuno, Sibayama, Hosokawa, & Miyashita, 2011), lipid oxidation (Grasa-López et al., 2016), oxidative stress and insulin resistance (Tong, Ko, Kim, Ham, & Kang, 2015). Seaweed extracts have also shown antimicrobial (Cox, Abu-Ghannam, & Gupta, 2010), anti-inflammatory (Han, Ali, Woo, Jung, & Choi, 2015), anti-diabetic (Kang et al., 2013) and prebiotic effects (Charoensiddhi et al., 2017). All these effects have been attributed to the presence of bioactive compounds such as polysaccharides, bioactive lipids, trace elements and phenolic compounds (Gupta & Abu-Ghannam, 2011).

All these characteristics suggest a promising potential to develop novel seaweed products with commercial interest for functional food applications to enhance human health (Murray, Dordevic, Ryan, & Bonham, 2017). For this purpose, consideration must be given to the selection of seaweed species with the best phytochemical and bioactivity profile. Different algal species contain varying combinations and concentrations of bioactive compounds and bioactive properties (Murray et al., 2017). To date there is no research that has examined and compared the potential of different seaweeds with the aim to develop multifunctional food products for amelioration of MetS progression. Therefore, the aim of the present study was to chemically characterize seven seaweed species and to evaluate their potential for *in vitro* antihypertensive, anti-inflammatory, antioxidant and triacylglyceride-lowering activities.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS) from *Escherichia coli* O5:B55, Folin-Ciocalteau phenol reagent, Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein, 2,2′-azobis (2-methyl-propionamidine dihydrochloride) (AAPH), captopril and 3,4,5-dimethylthiazol-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin/streptomycin (5000 U/mL), and phosphate buffer saline (PBS) were purchased from Lonza (Cultek S.L.U, Madrid, Spain). Fetal bovine serum (FBS) of South American origin was obtained from Fisher Scientific (Madrid, Spain). Cell culture flasks and plates were obtained from Sarstedt (Nümbrecht, Germany).

2.2. Sample material

Seaweed species *Porphyra* spp. (Nori), *Undaria pinnatifida* (Wakame), *Ulva* spp. (Sea lettuce), *Himanthalia elongata* (Sea thong), *Chondrus cripus* (Irish moss), *Laminaria ochroleuca* (Kombu), and *Palmaria palmata* (Dulse) were collected and provided by a local producer (Portomuiños, Galicia, Spain), which commerzialize seaweed-derived products and certified the authenticity of the seaweed species analyzed in the present work. Fresh raw materials were lyophilized and powdered using a grinding mill and kept at −80 °C until further analysis.

2.3. Preparation of methanolic extracts

Seaweed extraction was carried out using 1 g of powdered sample and homogenized in 2 × 10 mL of 50% aqueous methanol by magnetic stirring for 1 h. The two extracts were centrifuged at 1635*g* for 10 min at 4 °C. Supernatants were combined and filtered using Whatman Grade 1 filter paper. Filtrates were evaporated under reduced pressure (SpeedVac Concentrator, Thermo Fisher Scientific, Madrid, Spain) to remove solvent and held at −80 °C until analysis. For all cell assays, samples were reconstituted in free-serum cell culture media to the appropriate concentration for testing.

2.4. Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

LC–MS/MS analysis of the seaweed extracts were performed on a Q-Tof Premier mass spectrometer (Waters Corporation, Milford, MA, USA) coupled to an Alliance 2695 HPLC system (Waters Corporation, Milford, MA, USA). Separation of compounds was achieved on an Atlantis T3 C18 column (Waters Corporation, Milford, USA, 100 mm × 2.1 mm; 3 μm particle size) using 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Column temperature was maintained at 40 °C. A stepwise gradient from 10% to 90% solvent B was applied at a flow rate of 0.3 mL/min for 18 min. Electrospray mass spectra data recorded on a negative ionisation mode for a mass range *m/z* 100 to *m/z* 1000. Capillary voltage and cone voltage were set at 3 kV and 30 V, respectively. Collision induced fragmentation (CID) of the analytes was achieved using 12–20 eV energy with argon as the collision gas.

2.5. Total phenolic content

Folin-Ciocalteu method (Slinkard & Singleton, 1977) was used with slight modifications. A volume of 140 μL of the sample extract was mixed with 280 μL of Folin-Ciocalteu reagent previously diluted (1:10, v/v) and 980 μL of 42.86 mM sodium carbonate. The mixture was shaken and allowed to stand for 100 min in darkness, following centrifugation at 15,000*g* for 3 min. The absorbance was measured at 760 nm with a microplate reader (Fluostar Omega, BMG Ortenberg, Germany). Results were expressed as mg gallic acid equivalents (GAE)/g of sample.

2.6. Antioxidant activity of seaweed and seaweed extracts

Antioxidant activity was measured in extracts using different assays: DPPH radical scavenging activity (DPPH), Oxygen Radical Absorbance Capacity (ORAC), FRAP (Ferric Reducing Ability of Plasma) and Trolox Equivalent Antioxidant Capacity (TEAC). In addition, total antioxidant activity was measured in samples without any extraction procedure (direct method) by the DPPH and TEAC methods.

DPPH was evaluated according to the procedure described by Brand-Williams, Cuvelier, and Berset (1995) with modifications. A total of 0.1 mL of extract was added to 3.9 mL of 63.41 μM DPPH methanolic