Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae

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**Abstract**

Phenolic-rich extracts from four edible marine macroalgae commonly found in UK waters were tested for their potential biological effects towards cultured colon cancer cells and for their ability to inhibit digestive enzymes to achieve potential anti-diabetic effects. Extracts from *Palmaria, Ascophyllum* and *Alaria*, but not *Ulva*, gave reasonable recoveries of phenolics and inhibited the proliferation of colon cancer cells in a dose-responsive manner. *Alaria* extracts were more effective than *Palmaria* or *Ascophyllum* extracts, but *Palmaria* and *Ascophyllum* would provide greater amounts of phenolics per gram intake.

Extracts from *Palmaria, Ascophyllum* and *Alaria* all inhibited α-amylase activity to some extent, but *Ascophyllum* extracts were very effective with an IC₅₀ of ~0.1 µg/ml GAE. The *Ascophyllum* extracts also inhibited α-glucosidase, the other key enzyme involved in starch digestion and blood glucose regulation, at low levels (e.g. IC₅₀ ~20 µg/ml GAE).

After fractionation on Sephadex LH-20, the inhibitory activity from *Ascophyllum* was concentrated in the fraction which, from mass spectrometric evidence, was enriched in phlorotannins. These components have the capacity to inhibit α-amylase and α-glucosidase activities at µM levels, which are easily achievable in the gut. This may explain the anti-diabetic properties associated with algal extracts and algal phenolics in various in vivo studies.

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**1. Introduction**

Edible marine macroalgae or seaweeds have formed an important part of the diet of many Far Eastern countries for centuries (Chan, Ho, & Phang, 2006) and although their use as foods in Western countries is well documented, they are less used. These marine macroalgae have been classified based on pigmentation into brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) types. Seaweeds are greatly used in Eastern cuisine (Yuan, Carrington, & Walsh, 2005) and demand for ingredients, such as “Kombu” from *Laminaria* species and “Nori” used in sushi from *Porphyra* species, has been largely met from cultivation of the seaweeds. Apart from food uses, including their main industrial use as thickeners and gelling agents, seaweeds are widely used as ingredients in cosmetics and as fertilisers (McHugh, 2003). Seaweeds are ecologically important in the food chain as primary producers and some have demonstrated potential as chelators of heavy metals (Chan et al., 2006).

Edible seaweeds contain a range of components which have potential health benefits (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007; Smit, 2004). They are good sources of dietary fibre, especially soluble fibre such as alginates, which can influence satiety and glucose uptake from foods (Brownlee et al., 2005). These soluble polysaccharides may also act as prebiotics, stimulating growth of “beneficial” bacteria in the colon (Wang, Han, Hu, Li, & Yu, 2006). As well as being sources of polysaturated fatty acids, minerals and certain vitamins (MacArtain et al., 2007), edible seaweeds can contain appreciable amounts of polyphenols (Rodriguez-Bernaldo de Quiros, Lage-Yustay, & Lopez-Hernandez, 2010), which are effective antioxidants and may have particular biological activities. For example, polyphenol-rich extracts and isolated phlorotannin components have been shown to inhibit proliferation of cancer cells (Kwon et al., 2007; Yuan et al., 2005) and to influence anti-inflammatory responses (Kim et al., 2009).

Polyphenols from edible seaweeds have also been suggested to influence responses relevant to diabetes through modulation of glucose-induced oxidative stress (Lee, Han, Heo, Hwang, & Jeon, 2010), as well as through inhibition of starch-digestive enzymes (Lee, Li, Karadeniz, Kim, & Kim, 2008). Indeed, polyphenol-rich extracts from *Ascophyllum* inhibited glucosidase and showed promising anti-diabetic effects in mouse models (Zhang et al., 2007) and...
polyphenols from Ecklonia have shown positive effects on genetically diabetic mice (Iwai, 2008).

In this study, the polyphenol content of four edible seaweeds was measured and their potential anti-proliferative activity and ability to inhibit starch digestive enzymes (McDougall & Stewart, 2007) was assessed.

2. Materials and methods

2.1. Material and extraction of polyphenols

Dry Ascophyllum nodosum was obtained from the Hebridean Seaweed Company (Plot 1.3, Arnish Point Industrial Estate, Isle of Lewis, Scotland) over three years (2007, 2008 and 2010). Dry Ulva lactuca (sea lettuce), Palmaria palmata (dulse) and Alaria esculenta (often referred to as wakame) were obtained from CyberColloids (Strand Farm, Cork, Ireland) in autumn 2007. All samples were supplied air-dried, but were frozen on arrival and freeze-dried at the Scottish Crop Research Institute (SCRI) to <2% residual moisture, as assessed by drying samples to a constant weight at 105 °C. These were ground to powder using a Cyclone Sample mill with 0.1 mm² sieves (UDY Corporation, Fort Collins, Colorado). The first extraction was as previously recorded by Yuan et al. (2005). Seaweed samples (25 g) were extracted overnight in 250 ml methanol (100%) on a rotary shaker (180 rpm: Model R100/TW, Rotatest Shaker, Luckham) covered with aluminium foil to protect against light. The extract was subsequently extracted with 200 ml of chloroform and ultra pure water (UPW) in a ratio 1:1 using a separating funnel. All extractions were carried out in duplicate.

Prior to further purification on solid phase extraction (SPE) columns into bound and unbound fractions, the water extracts were concentrated on a rotary evaporator (Buchi Rotavap, Switzerland) at 45 °C then acidified with acetic acid prior to SPE. Centrifugation was carried out if necessary to remove insoluble material before evaporation.

The second extraction was performed using 50% acetonitrile/50% UPW containing 0.2% formic acid and used the same proportions of powder and extractant. This simple method has proved effective for extraction of polyphenols from berry samples (McDougall, Kulkarni, & Stewart, 2009). Finally, the third extraction procedure was essentially similar to extraction 2 and only differed in the solid phase extraction procedure.

SPE columns (Giga C18E tubes, Phenomenex Ltd., Macclesfield, UK) were mounted on a suction flask attached to a pump and primed using acetonitrile, washed with UPW then equilibrated with 0.2% (v/v) formic acid. The different seaweed samples were added to separate columns and the run-through collected as the unbound fractions. The column was washed with 0.2% (v/v) formic acid (extraction 2) or UPW (extraction 3) before elution of the bound material with acetonitrile. In some cases, the column was re-equilibrated and the SPE procedure repeated on the unbound material to ensure that all phenolic material was collected. All data provided are based on assays utilising extracts obtained by the third extraction procedure.

2.2. Total phenol content

Samples were assayed for phenol content using a modified Folin–Ciocalteu method (Singleton & Rossi, 1965; Deighton, Brennan, Finn, & Davies, 2000) and the phenol content calculated using a standard curve of gallic acid. Aliquots of each seaweed samples at fixed phenol contents of gallic acid equivalents (GAE) were dried in a Speed Vac (Thermo Fisher, Basingstoke, UK), then stored frozen.

2.3. TEAC assay (Troxol equivalent antioxidant capacity)

This assay was adapted from Deighton et al. (2000). The working ABTS reagent [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, diammonium salt] at 7 mM was prepared in a ratio of 1:1 with potassium persulphate (33.1 mg/100 ml distilled water). The working reagent was maintained at 30 °C and diluted with ethanol to give an absorbance of ~0.75 at A734. Trolox standard (1 mM 6-hydroxy-2,5,7,8-tetramethylychroman-2-carboxylic acid) was prepared in methanol at 25 mg/100 ml. Controls containing ABTS (1 ml) and Trolox (10 µl and 1 ml ABTS) were run at the start of run and at the end of run. Samples (10 µl containing 1 µg GAE) were mixed by the addition of 1 ml ABTS (working solution) at 30 s intervals. Then absorbance was read at A734 exactly after 6 min with water as blank or reference on the CECIL CE 7200 spectrophotometer. The TEAC values were calculated as before (Deighton et al., 2000). Significant difference was assessed using Student’s paired T-test in the Excel programme.

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

Samples (containing 20 µg GAE by Folin assay) were analysed on a LCQ-DECA system, comprising a Surveyor autosampler, pump and photo diode array detector (PDAD) and a ThermoFinnigan mass spectrometer iontrap. The PDAD scanned three discrete channels at 280, 365 and 520 nm. Samples were eluted on a gradient of 5–100% acetonitrile over 30 min on a C18 column (Synergi Hydro C18 with polar end capping, 2 mm × 150 mm, Phenomenex Ltd.) at 200 µl/min. The LCQ-DECA LC–MS was fitted with an electrospray ionisation interface and the samples were analysed in positive and negative mode. There were 2 scan events; full scan analysis, followed by data dependent MS/MS of the most intense ions. The data dependent MS/MS used collision energies (source voltage) of 45% in wideband activation mode. The MS detector was tuned against cyanidin-3-O-glucoside (positive mode) and against ellagic acid (negative mode).

2.5. Fractionation on Sephadex LH-20

The procedure was adapted from the Tannins Handbook (see www.users.muohio.edu/hagermae/tannin.pdf) and involves sorption to Sephadex LH-20 in ethanol and selective debinding with aqueous acetone. This is an established method for the separation of tannins from non-tannin phenolics. Firstly, A. nodosum powder was extracted as above, but with 50% ethanol rather than acetonitrile, and filtered through a glass microfibre filter (Whatman GF/A). Sephadex LH-20 (GE Healthcare Ltd., London) was swollen in 50% aqueous acetone then poured into a column and equilibrated with three volumes of 50% ethanol. The extract (50 ml) was applied to column and run through with 50% ethanol (and increased to 80% after a column volume) and the combined eluate collected as the unbound LH-20 fraction. The column was then washed with two column volumes of 80% ethanol. Elution with 50% acetone then 80% acetone recovered the bound fraction. The total phenol content of fractions was assayed and evaporated to dryness in suitable aliquots.

2.6. Cell culture and measurements of cell viability

Human colon cancer (Caco-2) cells were grown as a monolayer in Dulbecco’s Modified Eagle Medium (DMEM), containing D-glucose and L-glutamine (BioWhittaker product No. BE12-604F) and supplemented with 10% fetal calf serum (Gibco Product No. 10500-065), 1% penicillin/streptomycin (Sigma Product No. P-0781), 1% non-essential amino acids (ICN Product No. 1681049) and 25 µg/ml gentamycin (Gibco product No. 15750)
as described previously (Ross, McDougall & Stewart, 2007). The cells were grown in 50 ml flasks at 37 °C in a constant humidified atmosphere of 5% CO₂/95% air. After trypsin detachment, Caco-2 cells were counted and sub-cultured at 50,000 cells/ml.

The cell suspension (100 μl/well) was added to wells on a TC Microwell plate (Nunc Product No. 167008). The plates were pre-incubated overnight. Using a template, each experimental treatment (extract type and concentration) was replicated four times in randomly assigned positions on each of the four 96-well plates. All extracts were filter sterilised in phosphate buffered saline (PBS) prior to addition to the plates and all additions were in 10 μl volumes. Phenol contents were routinely checked to assess phenol losses due to filter sterilisation. There were three controls; blanks which contained only PBS, controls with cells and PBS only; and treatment controls for each sample and concentration of extract that contained no cells, only PBS and extract. The plates were incubated for 72 h at 37 °C. Cell viability was assayed using the Dijindo CCK-8 kit following the manufacturer’s instructions (NBS Biologicals, Cambridge, UK).

2.7. α-Amylase assay

This assay measures the production of reducing termini from starch and is based on the p-hydroxybenzoic acid hydrazide (PAHBH) method described by McDougall, Shipiro, Dobson, Smith, Blake & Stewart, (2005). Artificial saliva (AS) solution (pH 6.8) was prepared as before. A 5% (w/v) PAHBAH stock solution in 0.5 M HCl was diluted 1:4 with 0.5 M NaOH to give the working reagent. 1% Soluble potato starch (SIGMA product S-2360) was suspended at 1% in AS and gelatinised in a water bath at >80 °C for 15 min and used as the stock substrate preparation. The α-amylase enzyme stock was prepared from 19 mg of α-amylase (Sigma Chem. Co., Ltd., product A-3176) in 50 ml of AS.

The control reaction tube contained 100 μl of α-amylase enzyme suspension and 900 μl of AS and reaction was started by addition of 500 μl of stock starch solution. The sample reaction tubes contained different seaweed extracts with AS adjusted accordingly to a total volume of 1 ml. The reactions were staggered to start 1 min apart. At 0, 5 and 10 min, 50 μl of the reaction tubes, were added to 1 ml PAHBH solution in 2.0 ml eppendorf tubes in triplicate. After heating in a heating block at 100 °C for 10 min, followed by subsequent cooling for another 20 min, the absorbance was read at 410 nm. The PAHBH solution was taken as reference or blank. Varying amounts of seaweed extracts were added to determine the IC₅₀ value (50% inhibition) and each assay was repeated at least twice. Acrabose (SIGMA product A8980) was used as a positive control and gave an IC₅₀ value of ~0.8 μg/ml (1.24 μM) under these conditions, which is similar to previously published values of 1.6 μM (e.g. Desseaux, Koulikolo, Moreau, Santimone, & Marchis-Mouren, 2002).

2.8. Lipase assay

This procedure has been previously reported (McDougall et al., 2009). The assay buffer was 100 mM Tris–HCl, pH 8.2. The substrate was 0.08% (w/v) 4-nitrophenyl laurate in 5 mM sodium acetate pH 5 containing 1% Triton X-100 and was heated in boiling water for 1 min to aid dissolution. The enzyme was the soluble supernatant (16,000 rpm for 2.5 min) from 10 mg/ml solution of porcine pancreatic lipase type II (Sigma product L3126) in UPW. Each extract required a blank without enzyme to take into account the possible colour of the extracts. The assays were incubated at 37 °C for 2 h and the absorbance read at 410 nm. Further assays were performed using various amounts of the different seaweed extracts. Each assay was repeated at least twice.

2.9. α-Glucosidase assay

The procedure was the same as used previously (McDougall et al., 2005). The source of α-glucosidase enzyme was 10 mg/ml of rat intestinal acetone powder (SIGMA product product I1360) in ultra pure water. The assay buffer was 100 mM HEPES [4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid] at pH 6.8 and the substrate was 2 mM p-nitrophenyl α-D-glucopyranoside. The assay constituents were added in order of buffer, enzyme, different seaweed extracts and finally substrate in triplicate. After incubation at 37 °C for 2 h, the samples were centrifuged at 16,000 rpm for 2.5 min and the absorbance measured at 410 nm. The reaction volume was adjusted for different volume of samples of seaweed extracts to generate an IC₅₀ value and each assay was repeated at least twice.

3. Results

Extracts from the most common edible seaweeds contained appreciable polyphenol content (Table 1), however the yield of polyphenols was influenced by the extraction method. Using the simple aqueous acetoniitre extraction (extraction 2), rather the method of Yuan et al. (2005), markedly increased yield for all seaweed samples. However, the small changes involved in extraction 3 only further improved the yield of the Palmarias and Ascophyllum polyphenols. Ascophyllum yielded substantially more polyphenols than the others. The Ulva samples were consistently low in polyphenol content and were not analysed any further. Differences in yield and the extractability of phenolics from seaweeds are well documented (e.g. Rodriguez-Bernaldo de Quiros et al., 2010) and Ulva has been found to have low polyphenol content compared to species of red and brown algae (Garcia-Casal, Ramirez, Leets, Pereira, & Quiroga, 2009).

In addition, because the solid phase extraction steps involved in extractions 2 and 3 focused on recovering polyphenols, any lipid-like material and sugars, minerals and other metabolites were removed and cannot subsequently interfere with phenol quantification (using the Folin method; George, Brat, Alter, & Amiot, 2005) or inhibition assays. Samples from extraction 3 were also preferred for assessment of biological activities, as they have the least chance of carry-over of acid from the SPE step into the dried samples.

The antioxidant capacities, expressed as TEAC values /μg phenol content were in the same range as similar extracts obtained from raspberries (variety Glen Ample; Deighton et al., 2000).

Polyphenol-rich extracts from Alaria, Ascophyllum and Palmaria inhibited the proliferation of Caco-2 colon cancer cells grown in culture (Fig. 1). Extracts from Alaria were more effective than Ascophyllum and Palmaria. The IC₅₀ values (concentration of extracts required for 50% inhibition) for Ascophyllum and Palmaria were similar at approximately 33 μg/ml and 38 μg/ml, respectively. These values are in the same range as previously quoted in our laboratory for phenolic-rich extracts from raspberries (Ross, McDougall, & Stewart, 2007). The Alaria extracts were more effective with an IC₅₀ value estimated at ~7 μg/ml.

All extracts caused some inhibition of α-amylase activity but Ascophyllum extracts abolished activity at 50 μg/ml (Fig. 2a). Indeed, the Ascophyllum extracts caused complete inhibition at 2–5 μg/ml (Fig. 2b) and the IC₅₀ value was estimated at ~0.1 μg/ml (Fig. 2c).

The Ascophyllum extracts were also tested for inhibition of α-glucosidase, the other main enzyme involved in starch digestion (McDougall & Stewart, 2007). The extracts caused good inhibition with an IC₅₀ value of ~19 μg/ml, which is lower than quoted in some previous studies (77 μg/ml; Zhang et al., 2007). It is
noteworthy that the phenolic yield (by extraction 3) and the IC\textsubscript{50} values for amylase and glucosidase inhibition were similar for Ascophyllum supplied in the three different years studied. Extracts were tested for their ability to inhibit pancreatic lipase, but none of them showed any inhibition at 100 l g GAE/ml (results not shown).

The Ascophyllum extracts were fractionated on Sephadex LH-20 into unbound and bound fractions by a procedure known to enrich tannin-like components in plants (Porter, 1990). The ability to inhibit \( \alpha \)-amylase (and \( \alpha \)-glucosidase) was retained in the bound “tannin-like” fraction (Figs. 3 and 4). When applied to LC–MS, the bound sample eluted in a unresolved set of peaks (results not shown) with negative mode \( m/z \) spectra (Fig. 5) that could be assigned to a series of phlorotannin structures (Ragan & Glombitza, 1986; Lee et al., 2008).

For example, there is a series of \( m/z \) signals that could result from C–C linked addition of phloroglucinol (i.e. +124 amu) to a core C–O–C linked dimer of phloroglucinol (see insert Fig. 5; \( M – H = 247 \)) to give \( m/z \) signals at 371, 495, 619, etc.). Indeed,
the phlorotannin tetramer, 7-phoroeckol, has this general structure (Lee et al., 2008) and has a mass of 496 \(M = H = 495\). A series composed of C–C linked addition of phloroglucinol units to a fucodiphenone core (see insert Fig. 5, \(m/z\) 497) could provide the higher signals at \(m/z\) 621, 745, 869, 993, 1117, 1241, 1365, 1489, 1613, 1738, 1861 and 1985. Another “series” could arise from addition of phloroglucinol units (+124 amu) to a core based on the signals at \(m/z\) 353, 477, 601, 725, 849, etc. This series is 18 amu lower than the major series, which suggests a loss of water. The structure of these “series” remains a matter of speculation and requires further analysis, however, it is interesting to note that phlorofucofuroeckol (Lee et al., 2008) has mass of 602 \(M = H = 601\).

4. Discussion

All of the polyphenolic-rich extracts from the seaweeds inhibited proliferation of cultured colon cancer cells, but the Alaria extracts were the most effective (Fig. 1). Anti-proliferative activity has been noted previously for extracts from a range of seaweed species (Yuan et al., 2005), but also with purified or semi-purified phenolics from Fucus (Parys et al., 2010), Laminaria (Yang, Zeng, Dong, Liu, & Li, 2010) and Ecklonia (Athukorala, Kim, & Jeon, 2006). Due to differences in the means of quantification and the types of cancer cells used, it is difficult to compare the effectiveness of the extracts in this study against previous work. Extracts from Alaria have been shown to protect macrophages against oxidative damage caused by hydrogen peroxide (Choi et al., 2009). Although the Alaria extracts had slightly, but not significantly, higher antioxidative potential (Table 1), it seems unlikely that this slight difference was responsible for the marked difference in anti-proliferative effectiveness between the different seaweed extracts and have significant differences in phenolic composition may be involved. Further work to identify the active ingredients responsible for this difference in effectiveness is required.

In addition, any comparison of the physiological relevance of the anti-proliferative effects of these extracts must be assessed with regard to the amount of phenolic material available from the different seaweeds. For example, Ascophyllum extracts were approximately 3- to 4-fold less effective than Alaria in preventing colon cancer cell proliferation in vitro, but Ascophyllum yielded over 40 times more phenols (Table 1). Initial studies also confirmed that phenolics were extracted from these edible seaweeds under simulated gastrointestinal digestion (results not shown) and may be available in the colon.

Extracts from Palmaria, Alaria and Ascophyllum inhibited \(\alpha\)-amylase activity in vitro, whereas Ascophyllum extracts were very potent with an IC\(_{50}\) value of \(\sim 0.1 \mu g\) GAE/ml. This is approximately 500-fold more potent than reported for similar extracts from raspberries (McDougall et al., 2005) and lower than the IC\(_{50}\) value for acarbose, determined under the same assay conditions at \(\sim 0.8 \mu g/mL\). Assuming (with backing from the MS data) that the active ingredients have an average molecular mass of between 500 and 1000 amu, this amounts to 0.1–0.2 \(\mu M\). The inhibitory components were retained on Sephadex LH-20 using conditions employed to purify plant tannins and are most probably phlorotannins (Ragan & Glombitza, 1986), which are known to bind to this matrix (Jung, Oh, & Choi, 2010; Lee et al., 2010). The initial LC–MS results strongly suggested the presence of MS signals assignable to phlorotannin structures, but further work is required to define the active components.

Inhibition of \(\alpha\)-amylase has been noted for purified phlorotannins from another brown alga, Ecklonia cava (Lee et al., 2008) and the IC\(_{50}\) value for the most effective component, dieckol, was calculated to be around 90 \(\mu g/mL\). However, phlorotannins from Ascophyllum have a different composition than Ecklonia (Koivikki, Loponen, Pihlaja, & Jormalainen, 2007; Ragan & Glombitza, 1986) and it should be noted that the crude phlorotannin-rich fraction from Ecklonia was considerably more effective in inhibiting \(\alpha\)-amylase than the most inhibitory single component (Lee et al., 2008), suggesting synergetic interactions between components. Apostolidis and Lee (2010) recently confirmed inhibition of \(\alpha\)-amylase and \(\alpha\)-glucosidase by extracts of A. nodosum and compared their effectiveness with acarbose, a known inhibitor of \(\alpha\)-amylase and \(\alpha\)-glucosidase. However, their extracts were more effective against \(\alpha\)-glucosidase than \(\alpha\)-amylase compared to the extracts used in this study. In addition, the IC\(_{50}\) value quoted for amylase inhibition by Ascophyllum at 1.34 \(\mu g\) phenols/ml was \(\sim 10\)-fold higher than reported here. However, these differences may be explained by differences in extraction procedures as the extracts used in this study were enriched in phenolics using SPE, whereas they prepared extracts in water, which may contain non-phenolic components. These may have influenced glucosidase activity more than amylase, yet could still have reacted with the Folins reagent. There is obvious opportunity for synergy in reducing starch breakdown and elevation of blood glucose levels through inhibition of both amylase and \(\alpha\)-glucosidase. Indeed, phenolic-rich extracts from Ascophyllum have already been shown to have positive anti-diabetic effects in mouse models (Zhang et al., 2007) and phlorotannins from Ecklonia have shown hypoglycaemic
effects in genetically diabetic mice (Iwai, 2008). In addition, recent, but limited, information suggests potential anti-diabetic effects of seaweed phenolic components in humans (Lemarche, Paradis, & Couture, 2010). However, it should also be noted that extracts from Ulva, with presumably low phenolic content, also have anti-diabetic effects in animal models (Celikler et al., 2009).

It has been suggested that seaweeds may accumulate phlorotannins to deter herbivory by molluscs, as well as other predators, and phlorotannins have been shown to potently inhibit digestive glycosidases from the guts of marine snails (Shibata, Yamaguchi, Nagayama, Kawaguchi & Nakamura, 2002b). Indeed, phlorotannins have been reported to inhibit hyaluronidase (Shibata, Fujimoto,
Nagayama, Yamaguchi & Nakamura, 2002a) and to inhibit the protease, β-secretase, involved in amyloid precursor protein processing (Jung et al., 2010).

In summary, this paper outlines potential biological activities of polyphenols from edible seaweeds and, in particular, suggests that phlorotannin components of A. nodosum have potential anti-diabetic effects through the inhibition of both α-amylase and α-glucosidase.

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