Macroalgae of Izmir Gulf: *Cystoseira barbata*, *Cystoseira compressa* and *Cystoseira crinita* species have high α-glucosidase and Moderate Pancreatic Lipase Inhibition Activities

Fatma Gül Çelenk* and Atakan Sukatar

*Department of Medical Genetics, Faculty of Medicine, Ege University, Izmir, 35040, Turkey.

bDepartment of Biology, Faculty of Science, Ege University, Izmir, 35040, Turkey.

Abstract

Hyperglycemia and hyperlipidemia have been symptoms of many serious diseases such as diabetes and atherosclerosis overall the world. Thus, drug researchers have focused on new, natural and healthy drug alternatives. Marine macroalgae is a great source of hypoglycemic, hypolipidemic or hypocholesterolemic agents. In this study, we investigated that hypoglycemic, hypolipidemic and cytotoxic potentials of 22 marine macroalgae from the Gulf of Izmir. According to our results, the cold methanol extract of *Polysiphonia denudata* exhibited the highest antioxidant activity (93.6%) compared to BHA (95.3%). Three *Cystoseira* species, *Cystoseria crinita* (91.9%), *Cystoseria barbata* (90.7%), *Cystoseria compressa* (89.8%) showed higher α-glucosidase inhibition rates than oral antidiabetic acarbose (79.5%). It has also been observed that same species are potent inhibitors of pancreatic lipase. Cytotoxicity test revealed that these extracts did not cause viability inhibition on MCF-7. The results of maltose-glucose assay indirectly displayed that *Cystoseira* cold methanolic extracts inhibited maltose consumption better than acarbose on HT29. The results of this screening study show that these *Cystoseira* species may provide non-toxic bioactive agents to control non-communicable diseases (NCDs) such as cardiovascular disease and diabetes mellitus.

Keywords: Inhibitors; Alpha-glucosidase; Pancreatic lipase; *Cystoseira*; HT29.

Introduction

International Diabetes Federation (IDF) has published in IDF Diabetes Atlas – 7th editions that there are 415 million adults aged 20-79 with diabetes worldwide and a further 318 million adults are estimated to have impaired glucose tolerance. World Health Organization (WHO) has noticed that among the non-communicable diseases, cardiovascular diseases (CVDs) are the number one cause of death.

Diabetes mellitus (DM) is classified Type 1 DM, Type 2 DM and gestational DM. Type 1 DM is certain insulin deficiency and is treated with insulin preparations. Type 2 DM is associated with insulin resistance in which cells fail to respond to insulin and main goal of its treatment is to keep stable blood glucose level via glucosidase inhibitors such as acarbose, miglitol and voglitol (1) usually leading to absolute insulin deficiency. The powerful synthetic α-glucosidase and α-amylase inhibitors such as acarbose, voglitol and miglitol cause sharp decrease in blood glucose level (2). Therefore, the main approach to alleviate diabetes is to inhibit the enzymes...
that hydrolase carbohydrates, and by this way, create a retardation to glucose absorption (3, 4). Because the higher glucose concentrations in blood cause higher ROS productions in endothelial cells via mitochondrial electron transport chain and advanced glycation end products (AGES) (5). Then ROS activates NFκB and this leads to inflammatory response (including cytokines, acute phase reactants and cell adhesion molecules) and vasomotor imbalance. ROS resulting hyperglycemia could lead to lipid peroxidation, which yields advanced lipoxidation end products (ALEs) (6). As a result a cardiovascular disease might occur, such as atherosclerosis (7). CVD is more complex in both diagnosis and treatment. The preliminary treatment of CVD is focused on diet and lifestyle interventions (8). Then, lipase inhibitors, such as orlistat, are used to decrease gastrointestinal absorption of lipids. By this way, elevated lipid levels in blood is decreased (9). On the other hand, it is commonly known the adverse effects of these kinds of synthetic agents for long-term use, such as flatulence, abdominal discomfort, kidney disease (2, 10 and 11) Natural antioxidants can attenuate these damaging effects of ROS related diabetic cardiovascular complications (12–14)

It is evident that macroalgae provide a great source for definable crude natural products which have less adverse effects and toxicity than their synthetic equivalents (15–18). Fucoidans, phycocolloides and phenolic compounds produced by brown macroalgae exhibit α-glucosidase inhibition potentials (19–22). Some of the macroalgal polysaccharides and fibers such as alginates, carrageenan, fumaran, laminaran, porfiran and ulvan, cause hyperlipidemia and hypoglycemia (21, 23–26) Researches have suggested different mechanism of action for macroalgal substances: 1) Enzyme inhibition studies for glucosidases and/or lipase are used to determine inhibitor potential of individual crude extract or identified compound (19, 25 and 27–31), 2) Responses of cells to high glucose concentration or glucose uptake assays, or lipid accumulation in adipocytes demonstrate the protective effects (22, 29 and 32), 3) oral or intraperitoneal administrations of macroalgal substances to experimental animals give information about vital effects on insulin, plasma glucose and fatty acid levels in blood (20, 23 and 33–36). These parameters help investigate the effects of macroalgal substances on hyperglycemia, postprandial hyperglycemia, hyperinsulinemia or hyperlipidemia.

**Experimental**

**Collection of Macroalgal Material**

Marine macroalgal species were collected from coastlines of Izmir Gulf. All macroalgal specimens belong to Chlorophyta, Rhodophyta and Phaeophyceae were placed in the labelled plastic bags (approximately 0.5-1 kg wet weight) and immediately transported to the laboratory in cooled containers. To remove sand and epiphytes, algal thalli were gently rinsed in seawater. Taxonomic identification was done in terms of their morphology. Specimens had been stored at -20 °C until experiments begun. The voucher specimens are preserved in the Herbarium of Ege University.

**Preparation of Macroalgal Extracts**

Wet tissues from each species were lyophilized. One gram of powder was extracted with 10 mL of methanol (Labscan, TH) by shaking in an orbital shaker at 300 rpm overnight at +4 °C. Extracts were then centrifuged at 4000 rpm for 10 min and supernatants were collected. Collected supernatants were placed in orbital shaker and extraction was initiated again. This procedure had been repeated for 10 days. After 10 days of extraction/centrifugation cycle, the collected solvent was removed by rotary evaporation and powders of the crude extracts were dissolved in methanol for Thin Layer Chromatography (1 mg mL⁻¹) and DMSO (dimethyl sulfoxide, Sigma-Aldrich, USA; 10 mg crude extract per mL) for further experiments. All sample stored at -20 °C.

**Thin Layer Chromatography**

Extraction efficiency was roughly evaluated by thin layer chromatography. Crude macroalgal methanol extracts were dissolved in methanol (1 mg mL⁻¹). Individual samples were loaded four times on silica coated aluminum TLC (Merck, DE) plate as a dash. The solvent system was chloroform: ethyl
acetate: methanol (5: 5: 1, v/v; Sigma-Aldrich, USA, Riedel- del Hansen, DE; Labscan, TH, respectively). As the solvent slowly migrated up the plate, the components of the extract migrated up at different rates. Individual extracts were separated into different coloured dashes. The TLC profiles of plates were visualized after dipping into hydrochloric acid/methanol (1: 9, v/v; Sigma-Aldrich, USA; Merck, DE) and heating until getting dark. All the coloured or invisible dashes were observed as dark dashes on plates.

**Antioxidant Assay with β-carotene/linoleic acid system**

The β-carotene/linoleic acid activities of samples were evaluated by β-caroten bleaching system with the minor modifications for 96-well plate (37)gallic acid (GA. Eight microliter of linoleic acid (Sigma-Aldrich, USA) were pipetted into eppendorf containing 800 µl of β-carotene (Sigma-Aldrich, USA; 1 mg mL⁻¹) in chloroform (Sigma-Aldrich, USA; 1 mg mL⁻¹) and added 80 µL of Tween 40 (Sigma-Aldrich, USA). The chloroform was blown away by nitrogen flow. This mixture was pipetted slowly in 20 mL of distilled water with vigorous agitation to form an emulsion. Twenty microliter of blank (ethanol), positive control (BHA: Buthyl-4-hydroxyanisole, 30 mg mL⁻¹) or samples (5 mg mL⁻¹) were loaded 96-well plate and added 200 µL of the emulsion and the absorbance was measured immediately at 450 nm. The plate was placed in a dark room for 90 min. The absorbance was measured again. All measurements were carried out in triplicate. The antioxidant activity of extracts was evaluated using the following Equation:

\[ \text{AA} (%) = \left[1 - \frac{(A_0 - A_s)}{(A_0' - A_s')}\right] \times 100 \]

Where the \( A_0 \) and \( A_0' \) are the absorbance values measured at zero time of the sample and the control, respectively, and \( A_s \) and \( A_s' \) are the absorbance values measured at 90 min of the sample and the control.

**Cell Culture and Microscopy**

All studied cell lines were kindly provided by Ege University Medical School Department of Medical Oncology. Breast cancer cell line MCF-7 was cultured in RPMI medium (Lonza, CH) with 10% fetal bovine serum (Biological Industries, USA) and penicillin-streptomycin (100 U mL⁻¹; Sigma-Aldrich, USA) supplement. Cultures were maintained in CO₂ incubator with standard incubation conditions. Cells were treated with different concentrations of macroalgal methanol extracts reconstituted in dimethyl sulphoxide (DMSO; Sigma-Aldrich, USA) and diluted with proper media for viability experiments. For each experimental procedure, appropriate concentration of vehicle (DMSO) was used as a carrier control. Human colon adenocarcinoma cell line HT29 was cultured in McCoy’s 5A Medium (Biochrom, UK) with 10% fetal bovine serum (Biological Industries, USA), penicillin-streptomycin (100 U mL⁻¹; Sigma-Aldrich, USA) and 1% L-glutamine (Gibco, USA) supplement. Cultures were maintained in CO₂ incubator with standard incubation conditions in 96-well plates.

**Enzyme Inhibition Tests**

**α-glucosidase enzyme inhibition test**

The enzyme inhibition activity of extracts for α-glucosidase was assessed using the PNG (4-nitrophenil-α-D-glucopyranoside; Sigma-Aldrich, USA) as substrate and *Saccharomyces cerevisiae* α-glucosidase as enzyme (Sigma-Aldrich, USA) with 96-well plates. Fifty microliter of PBS (100 mM; pH 7.5) were loaded into wells and 2 µL of samples (1 mg mL⁻¹), acarbose (250 mg mL⁻¹; as positive control) or PBS (for enzyme reaction) were added. Fifteen microliter of α-glucosidase was added, except for blank. The plate was pre- incubated at 37 °C for 10 min. Fifteen microliter of PNG (3 mM) was added into wells and the plate was incubated at 37 °C for 30 min. The enzymatic hydrolysis of substrate was monitored by the amount of ρ-nitrophenol released in the reaction mixture at 410 nm where the enzymes were replaced buffer. The inhibition percentage of α-glucosidase was assessed by following Equation:

\[ \text{Inhibition} (%) = \left[1 - \frac{(A_e - A_s)}\right] \times 100 \]

Where the \( A_e \) is the absorbance value of enzyme reaction and \( A_s \) is the absorbance value of extract added reaction.
Pancreatic lipase enzyme inhibition test

Pancreatic lipase enzyme inhibition test was designed as fluorometric assay for 96-well plates (black flat bottom). Fifty microliter phosphate buffer with CaCl$_2$ (Ca-PBS; 0.1 mM CaCl$_2$, pH 7.5), 50 µL of pancreatic lipase (Sigma-Aldrich, USA; 2 mg mL$^{-1}$) were loaded into wells. Twenty microliter of sample (5 µg mL$^{-1}$), orlistate (Sigma-Aldrich, USA; positive control, 15 mg mL$^{-1}$) or Ca-PBS (for enzyme reaction) were added and then the plate was pre-incubated at 37 °C for 10 min. After the pre-incubation, 100 µL of 4-methylumbelliferyl oleate (4-MU; Sigma-Aldrich, USA; 0.1 mM) was pipetted into wells and the plate was incubated 37 °C for 30 min. The amount of 4-MU released by the lipase measured using a fluorescence spectrometer at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. The inhibition percentage of pancreatic lipase was assessed by following Equation:

\[
\text{Inhibition} \% = 100 - \left( \frac{A_s \times 100}{A_c} \right)
\]

Where $A_s$ is the absorbance value of extract added reaction, $A_c$ is the absorbance value of enzyme reaction.

Inhibition of Cell Viability

Growth inhibitory effects of extracts were investigated via mitochondrial dehydrogenase activity by WST-8 colorimetric assay kit (Sigma-Aldrich, USA). Briefly, MCF7 cells were seeded in 96-well plate $10 \times 10^3$ cells per well and incubated overnight for both cell attachment and growth. Macroalgal extracts were added to wells in different final concentrations (0, 1, 5, 10, 25, 50 and 100 µg mL$^{-1}$) with six repeats. Maximum DMSO concentration was 1%. After 72 h of incubation period, 10 μL of WST-8 reagent was added to wells and plates were incubated at 37 °C for 6 h incubation period. 100 µL of experimental media were transferred clean Eppendorf tubes. Maltose- Glucose Assay Kit (Abcam, UK) was used to determine and calculate the decrease of maltose concentrations.

Statistical Analysis

Standard deviations of enzyme inhibition tests were calculated using Microsoft Office Excel and IC$_{50}$ values were determined by CalcuSyn 2.0 software.

Results

Samples of 22 macroalgae belonging to Chlorophyta, Rhodophyta and Phaeophyceae collected from Izmir Gulf were listed Table 1. Their collection dates, collection places and coordinates were published in our previous study (39).

Inhibition activities of macroalgal extracts for β- carotene/linoleic acid bleaching were presented in Table 1. Polysiphonia denudata (Dillwyn) Greville ex Harvey (EGE41707), a member of Phaeophyceae, exhibited the maltose disaccharide was used as sole carbon source in experimental media to observe α-glucosidase activity and effects of extracts on it. Maltose concentrations were adjusted to glucose concentration of McCoy’s 5A medium. Acarbose was positive control for inhibition. The decrease of maltose level in experimental media showed us maltose consumption, indirectly. Stable maltose levels were considered as indicator for inhibition activity of acarbose or extracts.
Table 1. Antioxidant activity and enzyme inhibition effects of macroalgae.

| Family           | Voucher ID | Species                                         | Antioxidant Activity (%) | α-glucosidase inhibition (%) | Pancreatic lipase inhibition (%) |
|------------------|------------|-------------------------------------------------|--------------------------|------------------------------|----------------------------------|
| Ulvaceae         | EGE41706   | Ulva linza Linnaeus                             | 91.4                     | 5.8 ± 0.82                   | 69.3 ± 3.0                       |
|                  | EGE41705   | Ulva rigida C. Agardh                           | 15.2                     | 54.6 ± 0.10                  | 70.5 ± 4.74                      |
|                  | EGE41711   | Codium fragile (Suringar) Harioit                | 44.3                     | 24.5 ± 0.05                  | 14.4 ± 0.7                       |
| Cladophoraceae   | EGE41703   | Chaetomorpha aerea (Dillwyn) Kützing             | 68.5                     | 12.5 ± 1.11                  | 71.1 ± 2.3                       |
|                  | EGE41700   | Cladophora sp.                                   | 41.2                     | 13.3 ± 0.10                  | 40.6 ± 9.2                       |
|                  | EGE41704   | Osmundea pinnatifida (Hudson) Stackhouse         | 55.5                     | 2.2 ± 1.42                   | 45.3 ± 4.0                       |
|                  | EGE41726   | Laurencia obtusa (Hudson) J. V. Lamouroux       | 50.5                     | 10.7 ± 0.06                  | 57.2 ± 2.9                       |
| Rhodomelaceae    | EGE41725   | Palissa perforata (Bory de Saint-Vincent) K. W. Nam | 35.5                     | 0 ± 0.09                     | 55.9 ± 1.8                       |
|                  | EGE41707   | Polysiphonia denudata (Dillwyn) Greville ex Harvey | 93.6                     | 10.1 ± 0.27                  | 0 ± 2.0                          |
|                  | EGE41712   | Acanthophora nayadiformis (Delile) Papenfuss    | 37.7                     | 31.5 ± 0.79                  | 20.2 ± 2.5                       |
| Gracilariaceae   | EGE41701   | M. Steentoft, L. M. Irvine and W. F. Farnham    | 22.3                     | 1.1 ± 1.94                   | 42.1 ± 4.8                       |
| Ceramiaceae      | EGE41716   | Ceramium virgatum Roth                          | 54.6                     | 6.2 ± 0.58                   | 44.4 ± 6.1                       |
|                  | EGE41717   | Gelidium spinosum (S. G. Gmelin) P. C. Silva    | 6.8                      | 11.0 ± 1.32                  | 0 ± 1.0                          |
| Gelidiaceae      | EGE41702   | Gelidium pulchellum (Turner) Kützing             | 59.8                     | 7.7 ± 1.66                   | 24.7 ± 12.0                      |
| Scytosyphonaceae | EGE41709   | Petalonia fascia (O. F. Müller) Kuntze          | 52.6                     | 13.9 ± 0.10                  | 46.2 ± 2.38                      |
|                  | EGE41833   | Colpomenia simusso (Mertens ex Roth) Derbès and Solier | 9.0                      | 8.6 ± 0.00                   | 5.9 ± 1.0                        |
|                  | EGE41708   | Dictyota spiralis Montagne                      | 20.0                     | 0 ± 0.36                     | 23.8 ± 8.8                       |
|                  | EGE41715   | Dictyota dichotoma (Hudson) J. V. Lamouroux     | 56.3                     | 3.7 ± 0.19                   | 58.4 ± 22.5                      |
| Stypocaulaceae   | EGE41718   | Halopteris filicina (Grateloup) Kützing          | 27.6                     | 16.2 ± 0.70                  | 36.3 ± 11.0                      |
|                  | EGE41719   | Cystoseria barbata (Stackhouse) C. Agardh       | 58.7                     | 90.7 ± 0.01                  | 73.8 ± 2.17                      |
| Sargassaceae     | EGE41721   | Cystoseria compressa (Esper) Gerloff and Nizamudd | 53.5                     | 89.8 ± 0.02                  | 79.5 ± 2.9                       |
|                  | EGE41722   | Cystoseria crinita Duby                         | 45.3                     | 91.9 ± 0.00                  | 61.5 ± 6.0                       |
| BHA              | -          | -                                               | 95.3                     | -                            | -                                |
| Acarbose         | -          | -                                               | -                        | 79.5 ± 0.07                  | -                                |
| Orlistat         | -          | -                                               | -                        | -                            | 95.2                             |
Figure 1. TLC profiles of macroalgae. Some of the macroalgal extracts were excluded from study (3, 4, 12, 17, 20, 22 and 25). (a) The members of Chlorophyta: (1) Cladophora sp., (2) Ulva linza, (5) Ulva rigida, (6) Chaetomorpha aerea, (7) Codium fragile. (b) The members of Rhodophyta: (8) Gracillaria gracilis, (9) Polysiphonia demudata, (10) Osmundea pinnatifida, (11) Gelidium pulchellum, (13) Acanthophora amygdalina, (14) Ceramium virgatum, (15) Gelidium spinosum, (16) Laurencia obtusa, (18) Palisada perforata. (c) The members of Phaeophyceae: (19) Petalonia fascia, (21) Colpomenia sinuosa, (23) Halopteris filicina, (24) Dictyota dichotoma, (26) Cystoseira compressa, (27) Cystoseira crinita, (28) Cystoseira barbata, (29) Dictyota spiralis.

highest inhibition percentage (93.6%). The highest average antioxidant activity was calculated for Chlorophyta (52.1%). Ulva linza Linnaeus (EGE41706), with the 91.4%, showed the highest antioxidant activity. Other species belong to Chlorophyta exhibited lower activities, and this situation cause decrease in the average activity of Chlorophyta. Rhodophyta (46.3%) and Phaeophyceae (40.4%) follow it. Except for Polysiphonia demudata (P. demudata) and Ulva linza (U. linza), it is observed that all macroalgal species have moderate antioxidant activities when compared to positive control BHA (95.3%).

The α-glucosidase inhibitory activities of the methanol extracts were shown in Table 1 as
percentage inhibitions. The inhibition rates were ranged between 0.0–91.9%. The members of Rhodophyta exhibited lower inhibition average (8.9%) than those of Chlorophyta (32.6%). However, the members of Phaeophyceae displayed stronger inhibitor effects (39.4%). Especially, three Cystoseira species, *Cystoseria crinita* Duby (EGE41722; 91.9%), *Cystoseria barbata* (Stackhouse) C. Agardh (EGE41720; 90.7%), *Cystoseria compressa* (Esper) Gerloff and Nizamudd (EGE41721; 89.8%) showed quite higher α-glucosidase inhibition rates than positive control acarbose (79.5%).

The pancreatic lipase inhibition percentages of the methanol extracts are seen in Table 1. Orlistat was used as positive control (95.2%). However, *Cystoseira compressa* (*C. compressa*) displayed lower pancreatic lipase inhibitory activity than orlistat (79.5%); it showed the highest pancreatic lipase inhibitory activity among macroalgal specimens. The zero inhibition rates were exhibited by both *P. denudata* and *Gelidium spinosum* (S.G. Gmelin) P.C. Silva (EGE41717). Interestingly, the members of Phaeophyceae, which showed the higher α-glucosidase inhibitory activities also, showed the higher pancreatic lipase inhibition rates: *C. compressa* (79.5%), *Cystoseira barbata* (*C. barbata*; 73.8%), *Cystoseira crinita* (*C. crinite*; 61.5%). In addition to them, three members of Chlorophyta, *Chaetomorpha aerea* (Dillwyn) Kützing (EGE41703), *Ulva rigida* C. Agardh (EGE41705) and *U. linza* also had the higher inhibitory rates for pancreatic lipase; 71.1, 70.5 and 69.3%, respectively.

Three members of Phaeophyceae, which exhibited good α-glucosidase and pancreatic lipase enzyme inhibition activity, were preferred to determine their cytotoxic activity. These macroalgal species did not exhibit a viability inhibition on MCF-7 at the administered doses (Figure 2).

Post- incubation maltose concentrations of experimental media were measured using maltose- glucose assay kit. The decrease of maltose level was considered as an indirect sign for inhibition of α-glucosidase enzyme of HT29 cells. Although, the inhibition percentages was variable, the cold methanol extracts of *Cystoseira* species, *C. crinita*, *C. barbata* and *C. compressa*, were effective on maltose consumption. The results showed that these *Cystoseira* species exhibited strong inhibition on α-glucosidase enzyme of HT29 cells (94.8%, 88.2% and 76.1%, respectively) compared to positive control acarbose (53.2%, Figure 3).

![Figure 2](image_url)

**Figure 2.** *Cystoseira* species do not inhibit the cell viability at the administered doses. EGE41721: *C. compressa*, EGE41722: *C. crinita*, EGE41720: *C. barbata*.

**Discussion**

Diabetes mellitus and cardiovascular diseases affect about millions of adults overall the world. The factors, such as mortality rates, drug resistance and adverse effects of medicines, prompt exploration for new agents.
In this study, as a second part of our screening study, we have evaluated the antioxidant hypoglycemic and hypolipidemic activities of marine macroalgae collected from Izmir Gulf. The first results of this screening study showed that collected macroalgae from this region displayed antioxidant and anticancer activities (39). The results revealed that *P. denudata* had exhibited strong 2, 2-diphenyl-1-picryl-hydrazyl inhibition activity (DPPH; 86.7%) and high total phenolic content (TPC; 245.9 mg GAD). Current study provides a crosscheck to show strong antioxidant capacity of *P. denudata* in β-carotene/linoleic acid system. It has also been reported that *P. denudata* displayed moderate cell viability inhibition on MCF7 cells (39) and *Artemis salina* (40). There has been no data about hypoglycemic or hypolipidemic activity of *P. denudata* in literature, until today. Our findings are the first records that adduce the hypoglycemic and hypolipidemic potential of *P. denudata*. However, the solvent types for extraction and preferred organisms for viability test are variable; the researchers have noticed high antioxidant activity and moderate cytotoxicity of *P. denudata* (39, 41 and 42).

*Cystoseira* genus consists of 293 species (and infraspecific), of which 46 have been flagged as currently accepted taxonomically and *Cystoseira* genus was represented by three species in our study (42). It had been observed that they have variable antioxidant activity in DPPH system and total phenolic content. Our recent results also substantiated their variable antioxidant activities via β-carotene/linoleic acid system. It was reported that the hydroquinons isolated from *C. crinita* have high antioxidant activity and its meroterpenoids exhibit weak cytotoxicity on HM02, HEPG2 and MCF7 cells (43). According to our results based on MCF-7 cytotoxicity of *C. compressa*, the former findings proved that its methanol extract showed very low cell viability inhibition on HEP3B cells (44). The crude methanol extract of *C. barbata* exhibited lower antioxidant activities; however, it has been indicated that its fucan and fucoidan displayed higher antioxidant activities (45, 46). In addition, it has been reported that the fucoidans isolated from *C. crinita* and *C. compressa* showed similar anti-radical, anti-inflammatory and gastroprotective effects and their aqueous extracts displayed anti-proliferative effects on cell lines and anti-inflammatory effects on male adult Wistar rats (47, 48). Researchers suggested that the techniques used for determining DPPH scavenging activity, reducing activity, and β-carotene-linoleic acid system assays present simple, fast, and reliable evidence to assessment of the total antioxidant activity of the extracts (49). Their finding of a significant correlation...
between the DPPH radicals scavenging activities and the reducing activities were explained by the fact that both assays rely on electron/hydrogen donation. On the other hand, the researchers reported that the emulsified lipid used in the β-carotene-linoleic assay introduced additional variables that affect the oxidation process in comparison to the other methods. In terms of our former findings, there was significant correlation between DPPH scavenging activity and TPC, and Phaeophyceae members displayed higher antioxidant activity. Nevertheless, our last results obtained by β-carotene-linoleic assay showed that Chlorophyta features the highest antioxidant activity potential than Phaeophyceae and Rhodophyta (52.1, 46.3 and 40.4%, respectively).

The extracts of Cystoseira members have generally distinguished their enzyme inhibiton capabilities (50–54). We observed that both α-glucosidase and pancreas lipase enzyme inhibition assays showed that their extracts might include strong inhibitor molecules. It has shown that C. crinita is a source of sulphated polysaccharides which inhibit the key enzymes of diabetes and hypertension (51). These researchers have reported that the administration of C. crinita sulphated polysaccharides protects pancreas β-cells from death and damage and attenuates α-amylase activity in serum, pancreas and small intestine in diabetic rats. They suggested that the decrease in α-amylase level and increase in insulin level lead to a decrease in glucose rate.

Our screening study revealed that three members of Chlorophyta have good inhibitory rates for pancreatic lipase compared to orlistat. Cold methanol extract of Chaetomorpha aerea (C. aerea) may probably include inhibitor compounds for pancreatic lipase. However, it did not inhibit α-glucosidase enzyme. Similarly, recent results in literature, the chloroform extract of C. aerea did not show effective inhibition against α-glucosidase, but it was reported that when used alpha-amylase, instead of this enzyme, the extracts exhibited good inhibitor activity (55). There is no record for hypolipidemic activity of this species. Ulva rigida (U. rigida) exhibited hypoglycemic activity on a certain level, but its hypolipidemic activity was slightly higher than this. In-vivo study conducted with streptozotocin-induced diabetic rats revealed that ethanol extract of U. rigida showed both antidiabetic and antihyperlipidemic effects and improves carbohydrate metabolism (56).

This screening research stated the antioxidant, hypoglycemic and hypolipidemic activities of marine macroalgae of İzmir Gulf in-vitro. Based on the results, with the certain levels of tested biological activities, these macroalgal species appear as good producers of bioactive molecules. Hence, further isolation and identification studies and in-vivo studies based on these bioactive molecules are going to be our next step to lead to evaluate for medical potential.

References

(1) Association AD. 2. Classification and Diagnosis of Diabetes. Diabetes Care (2017) 40: S11 LP-S24.
(2) Saito N, Sakai H, Suzuki S, Sekihara H and Yajima Y. Effect of an alpha-glucosidase inhibitor (voglibose), in combination with sulphonylureas, on glycaemic control in type 2 diabetes patients. J. Int. Med. Res. (1998) 26: 219–32.
(3) Casirola DM and Ferraris RP. α-Glucosidase inhibitors prevent diet-induced increases in intestinal sugar transport in diabetic mice. Metabolism (2006) 55: 832–41.
(4) Charpentier G, Dardari D and Riveline JP. How should postprandial glycemia be treated? Diabetes Metab. (2006) 32: 2521–27.
(5) Giacco F and Brownlee M. Oxidative stress and diabetic complications. Circ. Res. (2010) 107: 1058–70.
(6) Wright EJ, Scism Bacon JL and Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int. J. Clin. Pract. (2006) 60: 308–14.
(7) Haffner SM. Pre-diabetes, insulin resistance, inflammation and CVD risk. Diabetes Res. Clin. Pract. (2003) 61: S9–S18.
(8) Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corra U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FDR, Lochen ML, Lollgen H, Marques Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I and Verschuren WMM. 2016 European Guidelines on Cardiovascular Disease Prevention in Clinical Practice. The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. (Constituted by Representatives of 10 Societies and by Invited
(18) Syad AN, Rajamohamed BS, Shunmugaiah KP and Kasi PD. Neuroprotective effect of the marine macroalga Gelidiella acerosa: identification of active compounds through bioactivity-guided fractionation. Pharm. Biol. (2016) 54: 2073–81.

(19) Heo SJ, Hwang JY, Choi J, Han JS, Kim HJ and Jeon YJ. Diphloretohydroxycarmalol isolated from Ishige okamurae, a brown alga, a potent alpha-glucosidase and alpha-amyrase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. Eur. J. Pharmocol. (2009) 615: 252–6.

(20) Lee SH, Park MH, Heo SJ, Kang SM, Ko SC, Han JS and Jeon YJ. Dieckol isolated from Ecklonia cava inhibits α-glucosidase and α-amylase in vitro and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice. Food Chem. Toxicol. (2010) 48: 2633–7.

(21) Nwosu F, Morris J, Lund VA, Stewart D, Ross HA and McDougall GJ. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. Food Chem. (2011) 126: 1006–12.

(22) Lee SH, Kang SM, Ko SC, Lee DH and Jeon YJ. Octaphlorethol A, a novel phenolic compound isolated from a brown alga, Ishige foliacea, increases glucose transporter 4-mediated glucose uptake in skeletal muscle cells. Biochem. Biophys. Res. Commun. (2012) 420: 576–81.

(23) Min KH, Kim HJ, Jeon YJ and Han JS. Ishige okamurae ameliorates hyperglycemia and insulin resistance in C57BL/KsJ-db/db mice. Diabetes Res. Clin. Pract. (2011) 93: 70–76.

(24) Gómez Ordóñez E, Jiménez Escrig A and Rupérez P. Effect of the red seaweed Mastocarpus stellatus intake on lipid metabolism and antioxidant status in healthy Wistar rats. Food Chem. (2012) 135: 806–11.

(25) Wilcox MD, Brownlee IA, Richardson JC, Dettmar PW and Pearson JP. The modulation of pancreatic lipase activity by alginates. Food Chem. (2014) 146: 479–84.

(26) Shakambari G, Ashokkumar B and Varalakshmi P. Phlorotannins from Brown Algae: inhibition of advanced glycation end products formation in high glucose induced Caenorhabditis elegans. Indian J. Exp. Biol. (2015) 53: 371–9.

(27) Kim KY, Nam KA, Kurihara H and Kim SM. Potent alpha-glucosidase inhibitors purified from the red alga Grateloupia elliptica. Phytochemistry (2008) 69: 2820–5.

(28) Lee SH, Park MH, Heo SJ, Kang SM, Ko SC, Han JS and Jeon YJ. Dieckol isolated from Ecklonia cava inhibits alpha-glucosidase and alpha-amyrase in vitro and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice. Food Chem. Toxicol. (2010) 48: 2633–7.

(29) Park MK, Jung U and Roh C. Fucoidan from marine brown algae inhibits lipid accumulation. Mar. Drugs (2011) 9: 1359–67.

(30) Lordan S, Smyth TJ, Soler Vila A, Stanton C and Experts). Developed With the Special Contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). G. Ital. Cardiol. (Rome) (2017) 18: 547–612.

(9) Tucci SA, Boyland EJ and Halford JC. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. Diabetes. Metab. Syndr. Obes. (2010) 3: 125–43.
Ross RP. The alpha-amylase and alpha-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chem.* (2013) 141: 2170–6.

(31) Zhang LX, Zhang N, Li J and Wang Z. New α-Glucosidase Inhibitory Polysaccharides Isolated from Marine Green Algae Enteromorpha Linza. *Adv. Mater. Res.* (2013) 634–638: 1010–5.

(32) Seo MJ, Lee OH, Choi HS and Lee BY. Extract from Edible Red Seaweed (Gelidium amansii) Inhibits Lipid Accumulation and ROS Production during Differentiation in 3T3-L1 Cells. *Prev. Nutr. Food Sci.* (2012) 17: 129–35.

(33) Kim MJ and Kim HK. Insulinotrophic and hypolipidemic effects of Ecklonia cava in streptozotocin-induced diabetic mice. *Asian Pac. J. Trop. Med.* (2012) 5: 374–9.

(34) Lee SH, Min KH, Han JS, Lee DH, Park DB, Jung WK, Park PJ, Jeon BT, Kim SK and Jeon Y.J. Effects of brown alga, Ecklonia cava on glucose and lipid metabolism in C57BL/KsJ-db/db mice, a model of type 2 diabetes mellitus. *Food Chem. Toxicol.* (2012) 50: 575–82.

(35) Zha XQ, Xiao JJ, Zhang HN, Wang JH, Pan LH, Yang XF and Luo JP. Rodsaccharides in Laminaria japonica (LP) Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis. *Food Chem.* (2012) 134: 244–52.

(36) Lee C, Park GH, Ahn EM, Kim BA, Park CI and Jang JH. Protective effect of Codium fragile against UVB-induced pro-inflammatory and oxidative damages in HaCaT cells and BALB/c mice. *Fitogeterapia* (2013) 86: 54–63.

(37) Zhang WN, Duan XJ, Huang HL, Zhang Y and Wang BG. Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from Symplyochladia latisula (Rhodomelaceae). *J. Appl. Phycol.* (2007) 19: 97–108.

(38) Chiba S. Molecular mechanism in alpha-glucosidase and glucoamylase. *Biosci. Biotechnol. Biochem.* (1997) 61: 1233–9.

(39) Çelenk FG, Özkaya AB and Sukatar A. Macroalgae of Izmir Gulf: Dictyotaaceae exhibit high in-vitro anti-cancer activity independent from their antioxidant capabilities. *Cytotechnology* (2016) 68: 2667–76.

(40) De Rosa S, Kamenarska Z, Bankova V, Stefanov K, Dimitrova Konaklieva S, Najdenski H, Tzevtkova I and Popov S. Chemical composition and biological activities of the Black Sea algae Polysiphonia demudata (Dillw.) Kutz. and Polysiphonia demudata f. fragilis (Sperk) Woronich. *Z. Naturforsch. C.* (2001) 56: 1008–14.

(41) Kamenarska Z, Serkedjiyeva J, Najdenski H, Stefanov K, Tvetkova I, Dimitrova Konaklieva S and Popov S. Antibacterial, antiviral and cytotoxic activities of some red and brown seaweeds from the Black Sea. *Bot. Mar.* (2009) 52: 80–6.

(42) Guiry MD and Guiry GM. AlgBase. *Nat. Univ. Ireland* World-wide Electron Publisher, Galway (2017). Available from: URL: http://www.algaebase.org.

(43) Fisch KM, Bohm V, Wright AD and Konig GM. Antioxidative meroterpenoids from the brown alga Cystoseira crinita. *J. Nat. Prod.* (2003) 66: 968–75.

(44) Guner A, Koksal C, Erel SB, Kayalar H, Nalbantsoy A, Sukatar A and Karabay Yavusoglu NU. Antimicrobial and antioxidant activities with acute toxicity, cytotoxicity and mutagenicity of Cystoseira compressa (Esper) Gerloff and Nizamuddin from the coast of Urla (Izmir, Turkey). *Cytotechnology* (2015) 67: 135–43.

(45) Sellimi S, Kadri N, Barragan Montero V, Laouer H, Haji M and Nasri M. Fucans from a Tunisian brown seaweed Cystoseira barbata: Structural characteristics and antioxidant activity. *Int. J. Biol. Macromol.* (2014) 66: 281–8.

(46) Kosanic M, RANKOVIC B and STANOJKOVIC T. Biological potential of marine macroalgae of the genus Cystoseira. *Acta Biol. Hung.* (2015) 66: 374–84.

(47) Hadj Ammar H, Lajili S, Ben Said R, Le Cerf D, Bouraoui A and Majdoub H. Physico-chemical characterization and pharmacological evaluation of sulfated polysaccharides from three species of Mediterranean brown algae of the genus Cystoseira. *Darú* (2015) 23: 1.

(48) Mhadhebi L, Mhadhebi A, Robert J and Bouraoui A. Antioxidant, anti-inflammatory and antiproliferative effects of aqueous extracts of three mediterranean brown seaweeds of the genus Cystoseira. *Int. J. Pharn. Res.* (2014) 13: 207–20.

(49) Zubia M, Fabre MS, Kerjuean V, Lann K Le, Stiger Pouvreau V, Fauchon M and Deslandes E. Antioxidant, anti-inflammatory and antiproliferative activities of some Phaeophyta from Brittany coasts. *Food Chem.* (2009) 116: 693–701.

(50) Hetta M, Hassan S, Abdel Tawab S, Bastawy M and Mahmoud B. Hypolipidaemic effect of Acanthophora spineifera (Red Alga) and Cystoseira trinode (brown alga) on albino rats. *Iran. J. Sci. Technol. Trans. A Sci.* (2009) 33: 287–97.

(51) Ben Gar A, Ben Abdallah Kolsi R, Jardak N, Chaaen B, El Feki A, Fki L, Belghith H and Belghith K. Inhibitory activities of Cystoseira crinita sulfated polysaccharide on key enzymes related to diabetes and hypertension: in vitro and animal study. *Arch. Physiol. Biochem.* (2017) 123: 31–42.

(52) Nagy MA and Amin KA. Biochemical and histopathological analysis of Cystoseira myrica aqueous extract on alloxan induced diabetic rats.

---

**Hipogliceamic and Hypolipidemic Activities of Cystoseira Species**
(53) Hongayo MC. The effect of brown alga cystoseira moniliformis (Kützing) hauck extract on the blood glucose level of alloxan-induced hyperglycemic albino mice (mus musculus linne, 1758). J. Pharm. Clin. Sci. (2011) 3: 1–12.
(54) Gulf P, Yegdaneh IA, Ghannadi A, Plubrukarn A, Zandi K and Sartavi K. Acetylcholinesterase inhibitory activity of some seaweeds from. Res. Pharm. Sci. (2012) 7: 775.
(55) Unnikrishnan PS, Suthindhiran K and Jayasri MA. Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. Pharmacogn. Mag. (2015) 11: S511–S15.
(56) Tas S, Celikler S, Ziyanok-Ayvalik S, Sarandol E and Dirican M. Ulva rigida improves carbohydrate metabolism, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. Cell Biochem. Funct. (2011) 29: 108–13.

This article is available online at http://www.ijpr.ir