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Investigation of α-Glucosidase Inhibitory Activity of Ethanolic Extracts from 19 Species of Marine Macroalgae in Korea

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Abstract – In the present work, we have collected 19 species of macroalgae (9 Phaeophta and 10 Rhodophyta) from all around of Korea: *Dictyopteris divaricata*, *D. prolifera*, *Myelophycus cavus*, *Papenfussiella kuromo*, *Petalonia zosterifolia*, *Petrospongium rugosum*, *Rugulopteryx okamurae*, *Sargassum fulvellum*, *S. muticum*, *Callophyllis japonica*, *Gloiopeltis tenax*, *Gracilaria longissima*, *Gracilaria vermiculophylla, Grateloupia asiatica*, *Grateloupia lanceolata*, *Grateloupia sparsa*, *Grateloupia turuturu*, *Grateloupia* sp, and *Polyopes affinis*. The macroalgal species were extracted by 70% ethanol (EtOH) for 24 h and evaluated its inhibitory effects on α-glucosidase. Among ethanol extracts, *Myelophycus cavus* showed the most effectively inhibitory activity (IC_{50}, 2.17 μg/ml) against α-glucosidase, followed by *Sargassum fulvellum* (IC_{50}, 8.13 μg/ml), *Dictyopteris prolifera* (IC_{50}, 16.66 μg/ml), *Rugulopteryx okamurae* (IC_{50}, 50.63 μg/ml), and *Petrospongium rugosum* (IC_{50}, 101.62 μg/ml). Furthermore, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay showed no cytotoxicity on mouse pre-adipocytes cell line (3T3-L1). These results suggest that some edible macroalgae merit further evaluation for clinical usefulness as anti-diabetic functional foods.
Keywords – Macroalgae, Ethanol extracts, α-Glucosidase, Inhibitory activity

Introduction

Korean marine macroalgae have a rich diversity because of the diverse habitats and the mixing of both warm and cold currents. Approximately, 870 macroalgal species have been reported in Korea: 128 Chlorophyta, 176 Phaeophyta, and 566 Rhodophyta (Lee and Kang, 2001). This magnificent diversity and abundance of macroalgal populations reflect the dramatic sweep of diverse environments and habitats. Although they are identified according to morphological characters as cell wall, cell structure, growth patterns, branching pattern, holdfasts, types of sporangia, carpogonial branches and cystocarps, the macroalgal identification is very hard because of their morphological simplicity. Some of Phaeophyta can form a marine forest, drawing in abundant marine resources. Some of Rhodophyta are edible and are used to extract agar and carrageenan. Macroalgae have been identified as an under-exploited plant resource and a source of functional food (Heo *et al*., 2009). They have also been identified as rich sources of diverse bioactive compounds with great pharmaceutical and biomedical potential. Phaeophyta has fucoxanthin pigment and alginate in its cell walls and Rhodophyta has phycoerythrin pigment and agar or carrageenan in its cell walls. In particular, the Phaeophyta has a variety of biological compounds, including pigments, fucoidans, phycocolloids, and phlorotannis (Halliwell and Gutteridge, 1999). Several studies have been focused on the isolation of compounds and have been pointed out that those compounds have a variety of biological activities such as antioxidant, anticoagulant, antihypertension, antibacterial, and antituomor activities (Kotake-Nara *et al.*, 2005; Mayer and Hamann, 2005; Heo *et al.*, 2008).

It is well known that α-glucosidases (EC 3.2.1.20) are exo-acting carbohydrases, which catalyze release of α-D-glucopyranose from the non-reducing ends of various carbohydrate substrates (Frandsen and Svensson, 1998). These enzymes play an important role in the biochemical

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processes of glycoproteins and glycolipids (Bertozzi and Kiessling, 2001). Therefore, it has been proposed that α-glucosidase inhibitors might be useful in development of treatments for carbohydrate-mediated diseases, such as diabetes, certain forms of hyperlipoproteinemia and obesity. There has been increased interest in the past few years in identifying α-glucosidase inhibitors that can be used as an important tool to understand the biochemical processes and as prospective therapeutic agents (Markad *et al.*, 2006; Liu *et al.*, 2007).

α-Glucosidase inhibitors are oral antihyperglycemic agents that act by competitive inhibition of α-glucosidase, delaying intestinal carbohydrate absorption and lessening postprandial increases in glucose levels (Casirola and Ferraris, 2006). Some α-glucosidase inhibitors, such as acarbose and voglibose, are used clinically in combination with either diet or other antidiabetic agents to control blood glucose levels of patients (Van de Laar *et al*., 2005). However, they often cause severe gastrointestinal side effects, such as flatulence and diarrhea. Many synthetic compounds have been used in the treatment of diabetes. However, they have in general been associated with marked toxic or undesirable side effects (Lee and Lee, 2001). Therefore, macroalgae have become good candidates for the source of natural anti-diabetic materials (Kim *et al.*, 2008; Heo *et al.*, 2009).

The objective of our research was to identify macroalgal species and investigate the α-glucosidase inhibitory effects of ethanol extracts from 19 species of marine macroalgae in Korea.

Experimental

Plant materials – Nineteen macroalgal species were collected from three coast lines of Korea from January to June 2011. Twelve macroalgae were collected from five different localities (Jeju, Cheongsan-Do, Namhae, Jin-Do, and Geoje) along South Coast. Six macroalgae were collected from three different localities (Gampo, Kyeongju, Ulsan) along East Coast. One sample was collected from Gyeokpo beach of West Cost. All macroalgae sorted in the field and were transported to the laboratory for their identification and ethanol extraction.

Reagents – *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG), α-glucosidase (0.1 Unit), Acarbose, and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent were purchased from Sigma Chemical Co. Mouse fibroblast (3T3-L1) cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, penicillin/streptomy-cin/amphotericin (10,000 U/mL, 10,000 μg/mL, and 2,500 μg/mL, respectively), and fetal bovine serum (FBS) were obtained from Gibco BRL, Life Technologies (USA). All other chemicals and solvents were of analytical grade commercially available.

Identification – Macroalgae were preserved in a 5% formaldehyde-seawater solution for identification. Each macroalga was identified according to its morphological and anatomical features. Microscopic observations were made from materials stained with aniline blue (1% w/v, acidified with 5% 1 N HCl). Photographs were taken with an Olympus microscope (BX51TRF, Olympus, Tokyo, Japan) with Olympus DP71 camera. Voucher specimens of each species were deposited in the herbarium of Chosun University (CUK), Gwangju, Korea. All of macroalgae were washed over the three times with tap water to remove salts, epiphytes, and sands attached to the surface of the samples, and then dried in the dry machine. Dried samples were individually cut into small pieces, and then ground into powder.

Ethanol extraction – For ethanol extractions, 50 g of each macroalgal defatted barley powder was weighted and put into a 500 mL bottle. 200 mL of 70% ethanol (v/v) was added to each bottle, respectively. After 24 h of extractions at 25 °C, the supernatant and the sediment were separated by vacuum filtration. The residue was re-extracted as the first extraction, and then the obtained extraction solutions were combined and concentrated to extracts were weighed and the yields were calculated. The ethanol extracts were kept in dark at −80 °C until further analyses.

α-Glucosidase inhibitory activity – The inhibitory activity of α-glucosidase was determined according to the modified method described by Lee *et al.* (2001) of using a readily available enzyme. Briefly, α-glucosidase (0.1 U/mL, Wako) was dissolved in 100 mM sodium phosphate buffer (pH 6.8) and used as an enzyme solution. 1 mM *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) in the same buffer (pH 6.8) was used as a substrate solution. The 95 μL of enzyme solution, 10 μL of extracts, and 10 μL of ethanol and PBS solution (1 : 1) mixed in same 96 well plate to each concentration (250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL) and measured absorbance at 405 nm after incubation for 5 min. After substrate solution (95 μL) was added, sample solution incubated for another 10 min at 37 °C incubator. Enzymatic activity was quantified by measuring absorbance at 405 nm using an enzyme-linked immunosorbent assay (ELISA) micro plate reader (D.I Biotech Ltd.). The IC_{50} value was defined as the concentration of α-glucosidase inhibitor

that inhibited 50% of α-glucosidase activity. Acarbose was used as a positive control and all assays were conducted in triplicate.

Cell culture and cell viability assay – 3T3-L1 cells were grown in DMEM medium containing 10% of fetal bovine serum (BS), 2 mM glutamine, and 100 μg/mL penicillin-streptomycin at 5% CO_2 and 37 °C humidified atmosphere. The cytotoxicity was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay as described by Hansen *et al*. (1989). The cells were gown in 96-well plates at a density of 5×10^3 cells/well. After 24 h, cells were washed with fresh medium and were treated with different concentrations of crud extracts. After 48 h incubation, cells were rewashed and 100 μL of MTT (1 mg/mL) was added and incubated for 4 h. Finally, DMSO (150 μL) was added to solubilize the formazan salt formed and amount of formazan salt was determined by measuring the OD at 540 nm using an GENios® microplate reader (Tecan Austria GmbH, Austria). Relative cell viability was determined by the amount of MTT converted into formazan salt. Viability of cells was quantified as a percentage compared to of control (OD of treated cells – OD of blank/OD of control – OD of blank × 100%) and dose response curves were developed. The data were expressed as mean from at least three independent experiments and $P < 0.05$ was considered significant.

Results and Discussion

Observations of macroalgal species for identification – Nineteen macroalgal species were found for extracts with α-glucosidase inhibitory activity. Of them, one taxon, *Grateloupia* sp., was not identified to species level because part of plant was collected. We observed their morphological features and identified them: 9 Phaeophyta (*Dictyopteris divaricata*, *D. prolifera*, *Myelophycus cavus*, *Papenfussiella kuromo*, *Petalonia zosterifolia*, *Petros-*

Fig. 1. Specimens of 19 macroalgal species used in ethanol extracts: A, *Dictyopteris divaricata*; B, *Dictyopteris prolifera*; C, *Myelophycus cavus* D, *Papenfussiella kuromo*; E, *Petalonia zosterifolia*; F, *Petrospongium rugosum*; G, *Rugulopteryx okamurae*; H, *Sargassum fulvellum*; I, *Sargassum muticum*; J, *Callophyllis japonica*; K, *Gloiopeltis tenax*; L, *Gracilaria longissima*; M, *Gracilaria vermiculophylla*; N, *Grateloupia asiatica*; O, *Grateloupia lanceolata*; P, *Grateloupia sparsa*; Q, *Grateloupia turuturu*; R, *Grateloupia* sp.; S, *Polyopes affinis*.

Table 1. Information about 19 macroalgal species used in α-glucosidase inhibitory activity.

Species	Morphological features	Collecting information	Vaucher speciman	Distribution in the world
Dictyopteris divaricata (Okamura) Okamura 1932	Thalli dichotomous, with percurrent midrib, membranecous, 12.5 cm high	Kyeokpo, West Coast, Korea, 4.vi. 2011, Coll. T.O.Cho	CUK 7754	Asia, Australia, New Zealand
D. prolifera (Okamura) Okamura 1930	Thalli epilithic, complanate, subdichotomous with midrib, greenish to dark brown, 21 cm high	Jeju, Korea, 28.vi.2011, Coll. T.O.Cho	CUK 7755	Atlantic islands, Asia
Myelophycus cavus J.Tanaka & Chihara 1984	Thalli erect, , unbranched, cylindrical, hollow, twisted, up to 10 cm high	Cheongsan, South Coast, Korea, 28.v.2011, Coll. T.O.Cho	CUK 7756	Korea, Japan
Papenfussiella kuromo (Yendo) Inagaki 1958	Thalli erect, , cylindrical, slimy, tommentose, greenish brown	Gampo, East Coast, Korea, 21.v.2011, Coll. T.O.Cho	CUK 7332	Atlantic islands, Asia
Petalonia zosterifolia (Reinke) Kuntze 1898	Thalli erect, flattened, unbranched, twisted, up to 15.4 cm high	Kyeongju, East Coast, Korea, 21.v. 2011, Coll. T.O.Cho	CUK 7341	Ireland, Europe, North America, Western Atlantic, Asia
Petrospongium rugosum (Okamura) Setchell & Gardner 1924	Thalli gelatinous, dark brown, circular,	Namhae, South Coast, Korea, 6.vi.2011, Coll. T.O.Cho	CUK 7757	Asia, North America, Central America, Australia and New Zealand
Rugulopteryx okamurae (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim 2009	Thalli epilithic, dichotomous, 15 cm high	Jeju, Korea, 28.vi.2011, Coll. T.O.Cho	CUK 7758	Australia, New Zealand, Asia, Central Africa
Sargassum fulvellum (Turner) C.Agardh 1820	Stem twisted. Leaves, elliptical to lanceolate in basal part. Vesicles elliptical to pyriform	Ulsan, East Coast, Korea, 21.v.2011, Coll. T.O.Cho	CUK 7337	Asia
S. muticum (Yendo) Fensholt 1955	Stem solitary, terete. Leaves arranged spirally. ovoid to to serrulate. Vesicles stipitate, spherical to pyriform	Jindo, South Coast, Korea, 4.vi.2011, Coll. T.O.Cho	CUK 7759	Ireland, Europe, Asia, America
Callophyllis japonica Okamura in De Toni & Okamura 1895	Thalli alternate, dissected, red to dark purplish-red, cartilaginous, 10 cm high	Jeju, Korea, 28.vi.2011, Coll. T.O.Cho	CUK 7760	Asia
Gloiopeltis tenax (Turner) Decaisne 1842	Thalli erect, irregularly dichotomous, brownish-purple, , 16 cm high	Cheongsan, South Coast, Korea, 28.v.2011, Coll. T.O.Cho	CUK 7761	Asia
Gracilaria longissima (S.G.Gmelin)M.steentoft, L.M.Irvine & W.F.Farnham 1995	Thalli erect, cylindrical, , radially branched, in coarse sand or on rocks, 8 cm high	Cheongsan, South Coast, Korea, 28.v.2011, Coll. T.O.Cho	CUK 7762	Ireland, Europe, Atlantic islands, America, Africa, Pacific islands, Australia, New Zealand
Gracilaria vermiculophylla (Ohmi) Papenfuss 1967	Thalli saxicolous, erect, cylindrical to complanate, cartilaginous, 21 cm high	Jeju, Korea, 28.vi.2011, Coll. T.O.Cho	CUK 7763	Asia, Europe, North America, Western Atlantic
Grateloupia asiatica S.Kawaguchi & H.W.Wang 2001	Thalli erect, flattened, , with numerous proliferous branchlets along the margins, 24 cm long	Namhae, South Coast, Korea, 6.vi.2011, Coll. T.O.Cho	CUK 7764	Europe, Asia
Grateloupia lanceolata (Okamurae)Kawaguchi 1997	Thalli erect, , greenish-brown, gelatinous, compressed, lanceolate, 18.3 cm high	Gampo, East Coast, Korea, 21.v. 2011, Coll. T.O.Cho	CUK 7335	Asia, Europe, North America
Grateloupia sparsa (Okamura) Chiang 1970	Thalli erect, , reddish-brown, gelatinous, compressed, lanceolate, 6 cm high	Ulsan, East Coast, Korea, 21.v.2011, Coll. T.O.Cho	CUK 7339	Asia
Grateloupia turuturu Yamada 1941	Thalli erect, simple, gelatinous, foliose, undulate, pinkish 12 cm high	Geoje, South Coast, Korea, 21.i.2011, Coll. T.O.Cho	CUK 7765	Europe, Atlantic islands Africa, Asia, Australia, New Zealand
Grateloupia sp.	Thalli erect, gelatinous, foliose, undulate, pinkish, 15.1 cm high	Ulsan, East Coast, Korea, 21.v.2011, Coll. T.O.Cho	CUK 7338	
Polyopes affinis (Harvey) Kawaguchi & Wang	Thalli, dichotomous near base, cylindrical on lower axes, flattened in the upper part, 8 cm high	Cheongsan, South Coast, Korea, 28.v.2011, Coll. T.O.Cho	CUK 7766	Asia, Pacific islands, Africa

pongium rugosum, *Rugulopteryx okaumrae*, *Sargassum fulvellum*, and *S. muticum*) and 10 Rhodophyta (*Callophyllis japonica*, *Gloiopeltis tenax*, *Gracilaria longissima*, *Gracilaria vermiculophylla*, *Grateloupia asiatica*, *Grateloupia lanceolata*, *Grateloupia sparsa*, *Grateloupia turuturu, Grateloupia.* sp., and *Polyopes affinis*) (Table 1). The others have narrow or filamentous shape, whereas *Petrospongium rugosum*, *Grateloupia lanceolata*, *Grateloupia sparsa*, *Grateloupia turuturu*, and *Grateloupia* sp. have blade shape. *Myelophycus cavus* (Phylum Phaeophyta, Class Phaeophyceae, Order Dictyosiphonales, Family Punctariaceae) just reported in Korea and Japan (Cho *et al.*, 2003). This species occurred in the upper intertidal on the west and south coasts, and is regarded as edible Phaeophyta. The several species of *Grateloupia* (Phylum Rhodophyta, Class Rhodophyceae, Order Halymeniales, Family Halymeniaceae) compressed to foliose, linear to lanceolate, rarely unbranched, and usually branched proliferously to one or more. It is distributed throughout in the warm temperate (to tropical) waters throughout the world (Fig. 1).

Ethanol extraction – In this study anti-Glucosidase potential was investigated using several macroalgae, which are edible and abundant along the coasts of western sea, eastern sea, and southern sea in Korea. In the extracts with 70% ethanol solvents, *Gracilaria longgisima* resulted in the highest yield (18.2%), followed by *Sargassum fulvellum* (16.3%), *Papenfussiella kuromo* (15.1%), *Petrospongium rugosum* (14.4%), *Sargassum muticum* (13.9%), *Gracilaria vermiculophylla* (10.8%), *Grateloupia asiatica* (8.6%), *Callophyllis japonica* (6.5%), *Grateloupia sparsa* (6.1%), *Myelophycus cavus* (4.7%), *Rugulopteryx okamurae* (4.2%), *Polyopes affinis* (4.0%), *Grateloupia lanceolata* (3.8%), *Gloiopeltis tenax* (3.6%), *Grateloupia* sp. (3.5%), *Dictyopteris prolifera* (3.4%), *Dictyopteris divaricata* (3.3%), *Petalonia zosterifolia* (1.1%), and *Grateloupia turuturu* resulted in the lowest yield (1.0%).

α-Glucosidase inhibitory activity of ethanol extracts – Determination of α-glucosidase inhibitory activities of macroalgal ethanol extracts which were screened using *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) as a substrate, showed that all of the tested extracts (250 μg/mL, each) exist potential α-glucosidase inhibition activity and this was compared with acarbose used as positive control present in Table 2. The α-glucosidase inhibition activity of Phaeophyta ethanol extracts was more powerful than one of Rhodophyta extracts. In Phaeophyta extracts, *Myelophycus cavus* (99.3%), *Dictyopteris prolifera* (99.2%), *Sargassum fulvellum* (99.1%), *Rugulopteryx okamurae*

Table 2. α-Glucosidase inhibitory activities of 70% ethanol extracts from 19 macroalgal species. Inhibition effect was determined using *p*NPG as a substrate and acarbose was used as positive control. Each value is expressed as mean ± S.D. in 250 μg/ml concentration.

	Macroalgae (70% ethanol extracts)	α-glucosidase inhibitory activity (%)
Positive control	Acarbose	70.3 ± 2.2
Phaeophyta	*Dictyopteris divaricata*	29.6 ± 1.27
	D. prolifera	99.2 ± 0.10
	Myelophycus cavus	99.3 ± 0.08
	Papenfussiella kuromo	9.7 ± 2.89
	Petalonia zosterilolia	31.3 ± 0.64
	Petrospongium rugosum	88.2 ± 0.41
	Rugulopteryx okamurae	90.9 ± 1.02
	Sargassum fulvellum	99.1 ± 0.04
	S. muticum	17.4 ± 3.03
Rhodophyta	*Callophyllis japonica*	5.3 ± 1.51
	Gloiopeltis tenax	1.2 ± 6.78
	Gracilaria longissima	2.6 ± 3.59
	Gracilaria vermiculophylla	1.2 ± 2.02
	Grateloupia asiatica	5.8 ± 0.71
	Grateloupia lanceolata	1.2 ± 1.41
	Grateloupia sparsa	1.2 ± 4.74
	Grateloupia turuturu	7.9 ± 0.47
	Grateloupia sp.	10.5 ± 2.92
	Polyopes affinis	1.2 ± 2.01

Table 3. IC_{50} values of α-glucosidase inhibitory effect from 5 macroalgal species.

Inhibitor	IC_{50}[1)] value of a-glucosidase inhibitory activity (μg/ml)
Acarbose	173.11 ± 0.0
Myelophycus cavus	2.17 ± 0.012
Sargassum fulvellum	8.13 ± 1.55
Dictyopteris prolifera	16.66 ± 1.20
Rugulopteryx okamurae	50.63 ± 1.98
Petrospongium rugosum	101.62 ± 6.72

IC_{50} is the concentration of extracts required for 50% inhibition. Each value is expressed as mean ± S.D. in quadruple experiments.

(90.9%), and *Petrospongium rugosum* (88.2%) were more powerful than the positive control (Acarbose, 70.3%). The order of inhibition ability could be arranged as extracts which the IC_{50} values at 2.17 μg/mL (*Myelophycus cavus*), 8.13 μg/mL (*Sargassum fulvellum*), 16.66 μg/mL (*Dictyopteris prolifera*), 50.63 μg/mL (*Rugulopteryx okamurae*), 101.62 μg/mL (*Petrospongium rugosum*), respectively (Table 3). Therefore, these five macroalgal extracts showed strong inhibitory effects on α-glucosidase activity.

Targeting postprandial hyperglycemia may be difficult with conventional diabetes therapy and in this regard, the availability of α-glucosidase inhibitors is helpful (Van de Laar *et al.*, 2005; Scheen, 2003). α-Glucosidase inhibitors, such as acarbose, have shown to be nontoxic and well tolerated, and with mild antihyperglycemic activity, either used as monotherapy or adjuncts to any other oral diabetic agents (Charpentier, 2002). Presence of α-glucosidase inhibitor in diets can inhibit the activity of α-glucosidase and reduce absorption of dietary carbohydrates. Recent reports revealed that high postprandial plasma glucose level is more harmful than fasting blood glucose. It can not only cause serious complications but also increase the mortality, so it is important to control postprandial blood glucose level, so as to reduce complications and mortality. Now α-glucosidase inhibitors were selected as first line drugs for reducing postprandial blood glucose. So the study of those bioactive constituents represents a promising approach to the discovery of new diabetes drugs.

Among natural resources, marine macroalgae, which are abundant and widely consumed as food in many Asian countries, especially in Korea and Japan, can be a very useful source for therapeutic purpose. Many of them were reported to possess many biological activities, for example, anti-cancer, anti-oxidation and anti-allergy. Especially, Phaeophyta and Rhodophyta have gained great interest due to their potential ability to produce various bioactive derivatives (Ryu *et al.*, 2009; Porto *et al.*, 2009; Gomes *et al.*, 2008).

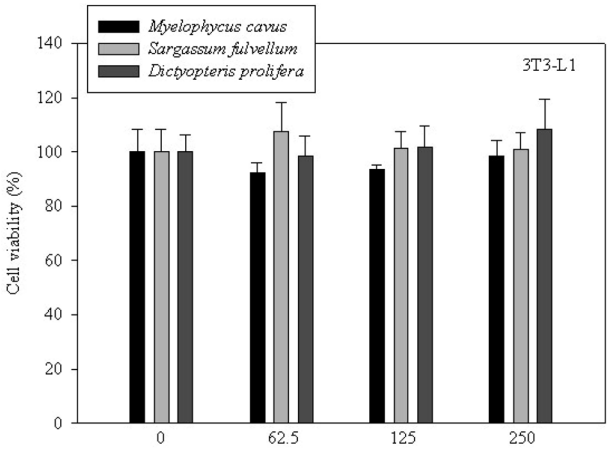

Fig. 2. Cytocompatible effects of extracts (*Myelophycus cavus*, *Sargassum fulvellum*, and *Dictyopteris prolifera*) on 3T3-L1 cells. Different concentrations (62.5, 125, and 250 μg/mL) of extracts were applied to the cell for 24 h and cell viability was assessed by MTT assay. Results are means ± standard error of three independent experiments.

Cytotoxic effect of three extracts in 3T3-L1 cells – Among ethanol extracts, *Myelophycus cavus*, *Sargassum fulvellum*, and *Dictyopteris prolifera* showed the most effectively inhibitory activity against α-glucosidase. So, MTT assay was conducted with extracts of *Myelophycus cavus*, *Sargassum fulvellum*, and *Dictyopteris prolifera*. To evaluate whether these macroalgal extracts have toxic effect to the cells, 3T3-L1 cells were treated with each extracts for 48 h, and cell viability was measured by MTT assay. The results demonstrate that macroalgal extracts were not exerting any cytotoxic effect at various concentrations (0, 62.5, 125, 250 μg/ml) in 3T3-L1 cells (Fig. 2). Therefore, based on the above results, those concentrations of three macroalgal extracts were used for all *in vitro* anti-diabetic activity experiments.

Conclusions

Recently many researchers are interested in finding any natural α-glucosidase inhibitor having safety and effectiveness, which can be substituted for current commercial inhibitors such as acarbose and voglibose. In this study, we have 19 species of macroalgae (9 Phaeophta and 10 Rhodophyta) from all around of Korea and were extracted by 70% ethanol. Among ethanol extracts, *Myelophycus cavus* showed the most effectively inhibitory activity against α-glucosidase, followed by

Sargassum fulvellum, *Dictyopteris prolifera*, *Rugulopteryx okamurae*, and *Petrospongium rugosum*. Furthermore, MTT assay showed no cytotoxicity on mouse pre-adipocytes cell line (3T3-L1). This study suggests that α-glucosidase inhibition activity extracts could be utilized to develop physiologically functional foods and pharmaceutical industry. Further studies on the isolation of these compounds are in progress.

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