Evaluation of α-glucosidase Inhibitory Activity of Jeju Seaweeds Using High Throughput Screening (HTS) Technique

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Abstract As a rapid and quick bioactive compound evaluation technique, we utilized an automatic system of high throughput screening (HTS) to investigate α-glucosidase inhibitory efficacy of seaweeds, collected from Jeju Island in Korea. In this study, different extracts with methanol at 20℃ and 70℃ from 23 species of brown seaweeds and 22 species of red seaweeds and 9 species of green seaweeds were subjected to HTS. Of the brown seaweeds tested, *Myelophycus simplex* (20B3), *Ishige sinicola* (20B5, 70B5), *Colpomenia sinuosa*, (20B14, 70B14), *Hizikia fusiforme* (20B21), *Ishige okamurai* (70B22) and *Ecklonia cava* (70B23) showed significantly high α-glucosidase inhibitory activity with 96.52%, 98.34%, 98.37%, 80.49%, 96.16%, 76.32%, 98.32% and 98.12%. *Schizymenia dubyi* (20R15), *Gelidium amansii* (20R16) and *Polysiphonia japonica* (70R22) amomng the red seaweeds showed remarkable α-glucosidase inhibitory activity more than 95%. On the other hand, the green seaweeds showed poor α-glucosidase inhibitory activities (less the10%) at 1 mg/ml.

Key words : High Throughput Screening (HTS), α-Glucosidase, Seaweeds, Inhibitory activity

Introduction

Diabetes mellitus is one of the most serious and chronic disease that is developing increasingly with the increasing obesity and ageing in the general population [1]. Persistent hyperglycemia in diabetic patients despite appropriate therapeutic measures leads to several complications, such as retinopathy, neuropathy, and cardiovascular diseases [2,3]. Diabetes mellitus is primarily classified into insulin-dependent (type I diabetes) and non-insulin-dependent (type II diabetes) [4]. The prevalence of type II diabetes is increasing globally [5]. Postprandial hyperglycemia plays an important role in the development of type II diabetes and complications associated with the disease, including macro-vascular and micro-vascular diseases [6].

One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate-hydrolyzing enzymes including α-glucosidase and α-amylase, in the digestive organs [7,8]. The synthetic α -glucosidase inhibitor, such as miglitol, acarbose and voglibose are known to reduce postprandial hyperglycemia primarily by delaying glucose absorption and interfering with the carbohydrate-digesting enzymes [9,10]. However, the continuous use of those synthetic agents should be limited because those agents may induce side effects such as diarrhea, vomiting, abdominal cramp and flatulence [11]. Therefore, a number of studies have been conducted in the search for natural α-glucosidase inhibitors that induce no deleterious side effects [12,13].

Marine bioresources are known to be attractive as they sometimes yield new compounds showing several kinds of different bioactivities which are not possible in land plants. In particular, seaweeds are known to pro-

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vide an abundance of bioactive materials with valuable pharmaceutical and biomedical potential. According to the results of the previous studies, some bioactive substances are contained in seaweeds, and various constituents have been demonstrated to possess antioxidant, anticoagulant, antibacterial and anticancer effects [14-17].

High throughput screening (HTS) is an instrument for quick screening and a large number of materials are automatically tested, for activity as activators and inhibitors of a particular biological target [18]. Assay systems and robotics that were capable of screening thousands of materials per day in the latter half of the 1990s had to evolve into ultraHTS (uHTS) methods capable of 100,000 assays per day or more [19]. Today, most pharmaceutical companies use HTS to find lead compounds from millions of materials or compounds on the primary screening.

This study has adopted HTS technique to investigate the α-glucosidase inhibitory activity of the methanolic extract from 54 seaweed species.

Materials and Methods

Regents

α-glucosidase and p-Nitrophenyl- α-D-glucopyranoside were all purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Preparation of seaweed extracts

The 54 seaweed species were collected along the coast of Jeju Island in South Korea (Table 1). The sample was washed 3 times with tap water in order to remove salt, epiphytes, and sand attached to the surface of the samples. Finally, the sample was carefully rinsed using fresh water, and stored at −20°C. The frozen samples were then lyophilized and homogenized with a grinder prior to the extraction. For preparation of the extracts from dried seaweeds, one gram of the seaweed powders were mixed with 100 ml of 95% methanol and placed in shaking incubator for 24 h at 20°C and 70°C. The mixtures were centrifuged at 3,500 rpm for 20 min at 4°C and filtered with Whatman filter paper to remove the residue, there after evaporated under vaccum at 40°C to remove all methanol and then dissolved in DMSO and used for the experiments.

α-glucosidase inhibitory activity by HTS system

The α-glucosidase inhibitory activity assay was done by the chromogenic method described by Watanabe *et al.* [20] and using a polara 2.0 program in HTS system, which is fully controlled system (Fig. 1). Briefly, yeat α-glucosidase (0.7 mU/ml) was dissolved in 0.1 M phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3 and used as an enzyme solution. A 5 mM ρ-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as a substrate solution. The 50 μl of enzyme solution and 50 μl of sample dissolved in DMSO at the 4 mg/ml concentration were added to each well of 96-well plate using liquid handling system. Then, the plate was transferred to the minitrack using Robotic arm. Next, 100 μl of substrate solution was added to each well. Next, the 96-well plate was transferred to the Carousel using Robotic arm and incubated for 10 min at room temperature. Finally, reacted with Victor 3 (detector) and measured absorbance at 405 nm.

Results and Discussion

Although many α-glucosidase inhibitors have been developed and used, their side effects to

α-glucosidase inhibitors are serious problems to be overcome in the treatment of diabetes mellitus. There is a need, therefore, to develop safer and better therapeutic agents from natural bioresources. Recently, there has been increasing interest in the α-glucosidase inhibitory therapeutic potential of natural resources, suggested that many plants have α-glucosidase inhibitory activities. Thus, several natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine [13]. α-glucosidase is one of the glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion [21]. The inhibition of α -glucosidase, in the human digestive tract, is considered to be effective to control diabete mellitus by diminishing the absorption of glucose decomposed from starch by this enzyme [22].

The purpose of the present study was to investigate the α-glucosidase inhibition effects of the methanolic extract of 54 seaweed species. α-glucosidase inhibitory effects of green seaweed extracts are shown in Fig. 2.

Table 1. Jeju seaweeds used in this study

Scientific name	Korean name	Lot number	
		20℃ MeOH ext.	70℃ MeOH ext.
Green seaweeds			
Enteromopha linza	잎파래	20G1	70G1
Enteromorpha intestinalis	창자파래	20G2	70G2
Monostroma nitidum	참홑파래	20G3	70G3
Codium fragile	청각	20G4	70G4
Codium contractum	몽우리청각	20G5	70G5
Ulva conglobata	모란갈파래	20G6	70G6
Ulva pertusa	구멍갈파래	20G7	70G7
Enteromorpha compressa	납작파래	20G8	70G8
Scytosiphon lomentaria	잘록이고리매	20G9	70G9
Brown seaweeds			
Sargassum fulvellum	모자반	20B1	70B1
Hydroclathrus clathratus	그물바구니	20B2	70B2
Myelophycus simplex	바위수염	20B3	70B3
Leathesia difformis	바위두룩	20B4	70B4
Ishige sinicola	넓패	20B5	70B5
Dictyota dichotoma	참그물바탕말	20B6	70B6
Desmarestia tabacoides	담배산말	20B7	70B7
Sargassum coreanum	큰잎모자반	20B8	70B8
Sargassum siliquastrum	꽈배기모자반	20B9	70B9
Myagropsis myagroides	외톨개모자반	20B10	70B10
Padina arborescens	부챗살	20B11	70B11
Pachydictyon sp.	참가죽그물바탕말	20B12	70B12
Sargassum thunbergii	지충이	20B13	70B13
Colpomenia sinuosa	불레기말	20B14	70B14
Petrospongium rugosum	바위주름	20B15	70B15
Endarachne binghamiae	미역쇠	20B16	70B16
Undaria pinnatifida	말미역	20B17	70B17
Sargassum horneri	괭생이모자반	20B18	70B18
Sagassum piluliferum	구슬모자반	20B19	70B19
Laminaria ochotensis	다시마	20B20	70B20
Hizikia fusiforme	톳	20B21	70B21
Ishige okamurai	패	20B22	70B22
Ecklonia cava	감태	20B23	70B23
Red seaweeds			
Gracilaria verrucosa	꼬시래기	20R1	70R1
Grateloupia elliptica	참도박	20R2	70R2
Grateloupia lanceolate	가는개도박	20R3	70R3
Sinkoraena lancifolia	털지누아리	20R4	70R4
Grateloupia filicina	빈참지누아리	20R5	70R5
Capopeltis affinis	참까막살	20R6	70R6
Laurencia okamurae	쌍발이서실	20R7	70R7
Chondria cassicaulis	개서실	20R8	70R8
Ahnfeltiopsisflabelliformis	부채살	20R9	70R9
Lomentaria catenata	마디잘록이	20R10	70R10
Pterocladiella capillacea	큰개우무	20R11	70R11
Prionitis cornea	붉은까막살	20R12	70R12
Gloiopeltis furcata	불둥풀가사리	20R13	70R13
Chondrophycus undulatus	혹서실	20R14	70R14
Schizymenia dubyi	갈래잎	20R15	70R15
Gelidium amansii	굵은참우뭇가사리	20R16	70R16
Scinaia okamurae	매끈껍질	20R17	70R17
Lithophyllum okamurai	혹돌잎	20R18	70R18
Chondrus crispus	주름진두발	20R19	70R19
Martensia denticulata	비단망사	20R20	70R20
Acrosorium flabellatum	부채분홍잎	20R21	70R21
Polysiphonia japonica	왜떨기나무붉은실	20R22	70R22

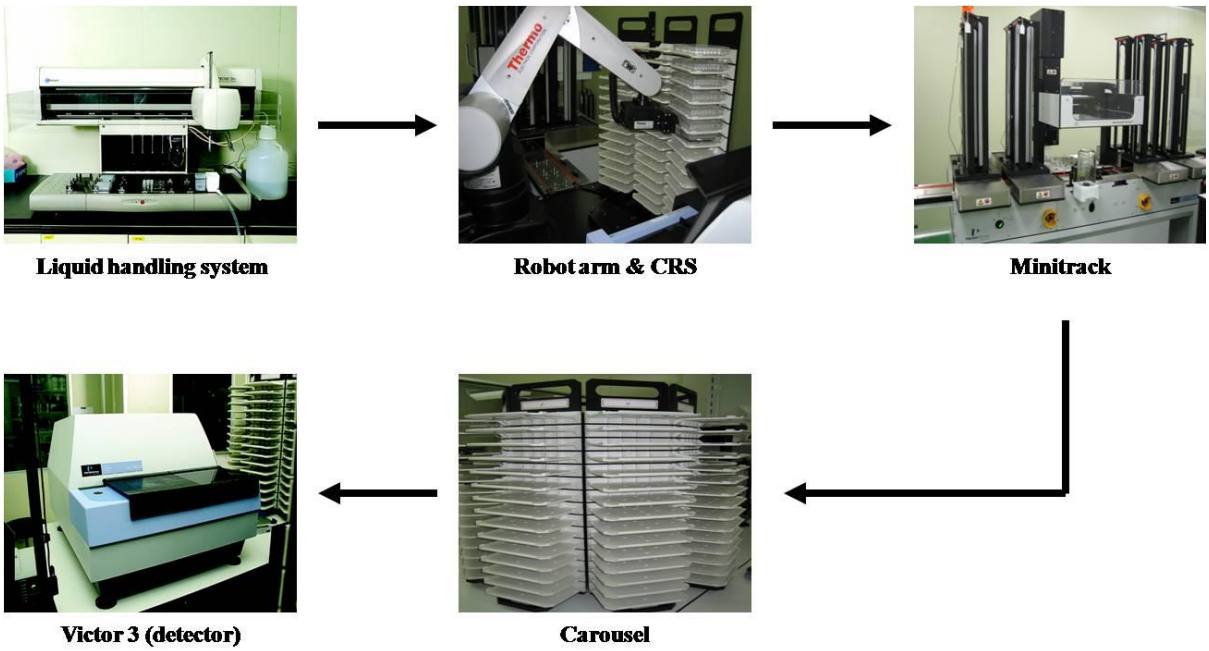

Fig. 1. High throughput screening system for assessing α-glucosidase inhibitory activity.

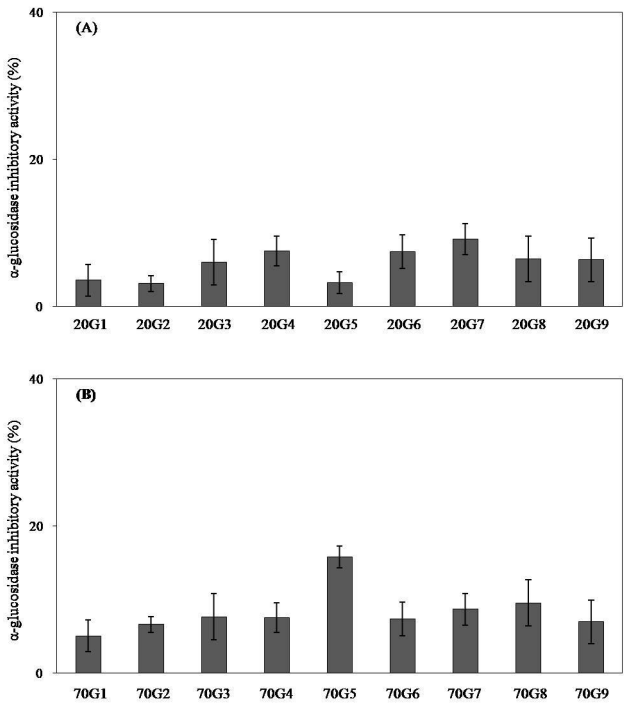

Fig. 2. α-glucosidase inhibitory activity of methanolic extract (1 mg/ml) from Jeju green seaweeds. (A) 20°C, (B) 70°C.

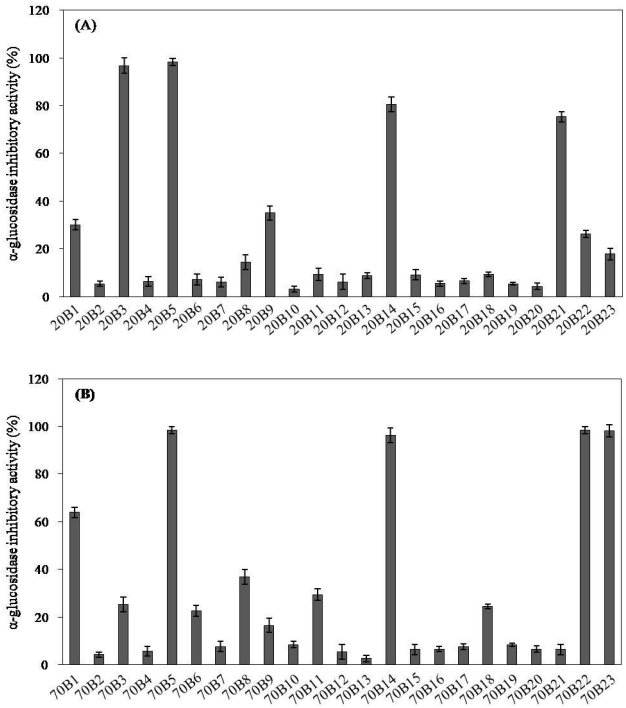

Fig. 3. α-glucosidase inhibitory activity of methanolic extract (1 mg/ml) from Jeju brown seaweeds. (A) 20°C, (B) 70°C.

All the green seaweed extracts showed poor α -glucosidase inhibitory activities (less 15%).

α-glucosidase inhibitory effects of brown seaweed extracts are shown in Fig. 3. Among the tested brown seaweed extracts at 20°C, *Ishige sinicola* (20B5), *Myelophycus simplex* (20B3), *Colpomenia sinuosa* (20B14) and *Hizikia fusiforme* (20B21) showed highly potent α-glucosidase inhibitory effects with 98.34%, 96.82%, 80.49% and 75.32%, respectively (Fig. 3(A)). As shown in Fig. 3(B), *Ishige sinicola*

(70B5), *Colpomenia sinuosa* (70B14), *Ishige okamurai* (70B22) and *Ecklonia cava* (70B23) inhibited the α-glucosidase inhibition effects by more than 95%. Of those extracts, *Ishige sinicola* (70B5), *Ishige okamurai* (70B22), *Ecklonia cava* (70B23) and *Colpomenia sinuosa* (70B14) exhibited α-glucosidase inhibitions of 98.37%, 98.32%, 98.12% and 96.16%, respectively.

α-glucosidase inhibitory effects of red seaweed extracts are shown in Fig. 4. Among the tested red seaweed extracts at 20°C, *Schizymenia dubyi* (20R15) and *Gelidium amansii* (20R16) showed highly potent α-glucosidase inhibitory effects with 97.39% and 96.47%, respectively (Fig. 4(A)). As shown in Fig. 4(B), *Polysiphonia japonica* (70R22) inhibited the α-glucosidase inhibition effects by more than 95%. However, other red seaweed extracts showed poor α-glucosidase inhibitory activities (less 10%).

Oxidative stress induced by increase of hyperglycemia cause diabetes-associated pathological damage [23,24]. Thus, to reduce the risk of diabetes-associated pathological damage including diabetes, attenuation of oxidative stress mediated by hyperglycemia is important. Reactive oxygen species (ROS) are considered to be important mediators of several biologic responses such as cell proliferation, and extracellular matrix deposition. Recent observations indicate that hyperglycemia triggers the generation ROS, and oxidative stress [25]. In addition, ROS-induced highly reactive oxidative damage was associated with diabetes [26,27].

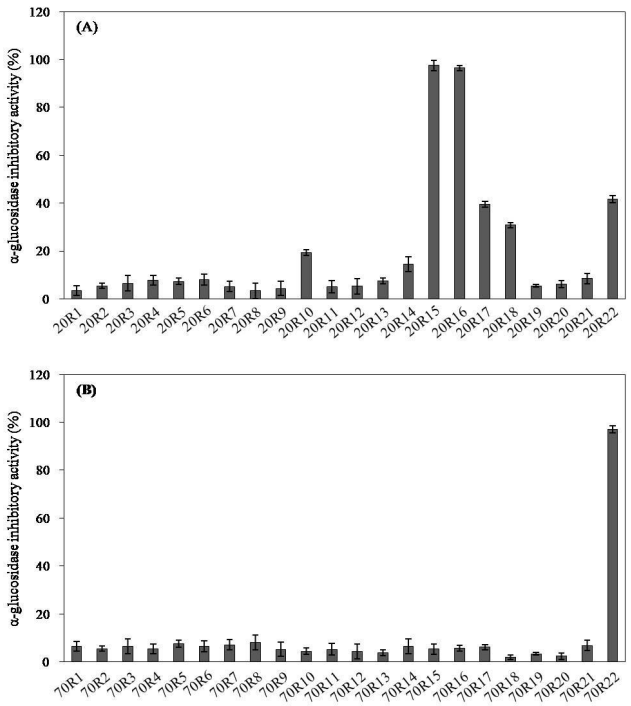

Fig. 4. α-glucosidase inhibitory activity of methanolic extract (1 mg/ml) from Jeju red seaweeds. (A) 20°C, (B) 70°C.

Seaweeds have been well-known as an important source to produce natural bioactive secondary metabolites including polyphenolic compounds with unique linkage [28]. The polyphenolic compounds of the seaweeds are referred to as phlorotannins and those are readily soluble in polar solvents like methanol [29]. The previous reports on seaweeds have revealed that it contains a variety of phlorotannin derivates which are commonly known to have strong antioxidant effect [30-32]. Also, phlorotannins inhibits the α-glucosidase and protective effect against high glucose-induced oxidative stress in human umbilical vein endothelial cells [1,4]. This suggests that the α-glucosidase inhibitory activities of the methanol extracts of seaweeds can be attributed to the phlorotannins.

In conclusion, methanolic extracts from 54 seaweed species collected from Jeju Island were evaluated for their α-glucosidase inhibitory activities by HTS technique. Most seaweed species tested in this study showed potential α-glucosidase inhibitory activities. Of the seaweed species *Ishige sinicola* (20B5 and 70B5), *Myelophycus simplex* (20B3), *Colpomenia sinuosa* (70B14), *Ishige okamurai* (70B22), *Ecklonia cava* (70B23), *Schizymenia dubyi* (20R15), *Gelidium amansii* (20R16) and *Polysiphonia japonica* (70R22) showed excellent α-glucosidase inhibition effects by more than 95%. However, further studies are essential to purify α-glucosidase inhibition compounds to elucidate relationships between structure and activity which might help with future drug design. Therefore, seaweeds present in Jeju Island are possible candidates for future α-glucosidase inhibition drug discovery.

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References

1. Heo, S. J., Hwang, J.Y., Choi, J.I., Han, J. S., Kim, H. J. and Jeon, Y. J. 2009. Diphlorethohydroxycarmalol isolated from *Ishige okamurae,* a brown algae, a potent α -glucosidase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Eur J Pharmacol* **615**, 252-256.
2. He, Z. H. and King, G. L. 2004. Microvascular complications of diabetes. *Endocrin. Metab. Clin.* **33**, 215-238.
3. Krentz, A. J., Clough, G. and Byren, C, D. 2007. Interaction between microvascular and macrovascular disease in diabetes: pathophysiology and therapeutic implications. *Diabetes Obes. Metab.* **9**, 781-791.
4. Lee, S. H., Park, M. H., Heo, S. J., Kang, S. M., Ko, S. C., Han, J. S. and Jeon, Y. J. 2010. Dieckol isolated from *Ecklonia cava* inhibits α-amylase in vitro and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice. *Food Chem. Toxicol.* In Press.
5. Zimmet, P., Alberti, K. and Shaw, J. 2001. Global and societal implications of the diabetes epidemic. *Nature* **414**, 782-787.
6. Baron, A. D. 1998. Postprandial hyperglycemia and α -glucosidase inhibitors. *Diabetes Res. Clin. Pract.* 40, S51-S52.
7. Clissold, S. P. and Edward, C. 1990. A preliminary review of its pharmacodynamic and pharmacokinetics properties, and therapeutic potential. *Drugs* **35**, 214-243.
8. Bhandari, M. R., Jong-Anurakkun, N., Hong, G. and Kawabata, J. 2008. α-glucosidase and α-amylase inhibitory activities on Nepalese medicinal herb Pakhanbhed (*Bergenia ciliate*, Haw.). Food Chem. 106, 247-252.
9. Lee, H. S. and Kim, M. K. 2001. Rat intestinal α -glucosidase and lens aldose reductase inhibitory activities of grain extracts. *Food Sci. Biotechnol.* **10**, 172-177.
10. Mooradian, A. D. and Thurman, J. E. 1999. Drug therapy of postprandial hyperglycemia. *Drugs* **57**, 19-29.
11. Hanefeld, M. 1998. The role of acarbose in the treatment of non-insulin-dependent diabetes mellitus. *J. Diabetes Complicat.* **12**, 228-237.
12. Kim, K. Y., Nam, K. A., Kurihara, H. and Kim, S. M. 2008. Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry* **69**, 2820-2825.
13. Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Miyata, Y., Tanaka, K. and Matsumoto, K. 2007. Alpha-glucosidase inhibitory profile of catechins and theaflavins. *J. Agric. Food Chem.* **55**, 99-105.
14. Heo, S. J., Park, E. J., Lee, K. W. and Jeon, Y. J. 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresour. Technol.* **96**, 1613-1623.
15. Kashiwagi, M., Mynderse, J. S., Moore, R. E. and Norton, T. R. 1980. Antineoplastic evaluation of pacific basin marine algae. *J. Pharm. Sci.* **69**, 735-738.
16. Gonzalez, A. G., Darias, V. and Estevez, E. 1982. Chemo-therapeutic activity of polyhalogenated terpenes from Spanish algae. *Planta Med.* **44**, 44-46.
17. Kosovel, V., Avanzini, A., Scarcia, V. and Furlani, A. 1988. Algae as possible sources of antitumoral agents. Preliminary evaluation of the *in vitro* cytostatic activity of crude extracts. *Pharmacol. Res. Commun.* **20**, 27-31.
18. Silverman, L., Campbell, R. and Broach, J. R. 1998. New assay technologies for high-throughput screening. *Curr . Opin. Chem. Biol.* **2**, 397-403.
19. Hertzberg, R. P. and Pope, A. J. 2000. High-throughput screening: new technology for the 21st century. *Curr . Opin. Chem. Biol.* **4**, 445-451.
20. Watanabe, J., Kawabata, J., Kurihara H. and Niki, R. 1997. Isolation and identification of alpha-glucosidase inhibitors from Tochucha (Eucommia ulmoides). *Biosci. Biotechnol. Biochem.* **61**, 177-178.
21. Lebovitz, H. E. 1997. Alpha-glucosidase inhibitors. *Endocrinol. Metab. Clin.* **26**, 539-551.
22. Hara, Y. and Honda, M. 1990. The inhibition of α -amylase by tea polyphenols. *Agric. Biol. Chem.* **54**, 1939-1945.
23. Uemura, S. Matsushita, H., Li, W., Glassford, A. J., Asagami, T., Lee, K. H., Harrison, D. G. and Tsao, P. S. 2001. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ. Res.* **88**, 1291-1298.
24. Fraga, C. G., Leibovita, R. M. and Roeder, R. G. 1998. Lipid peroxidation measured as thiobarbituric-reactive substances in tissue slices: Characterization and comparison with homogenates and microsomes. *Free radic. Biol. Med.* **4**, 155-161.
25. Lee, Y. J., Kang, D. G., Kim, J. S. and Lee, H. S. 2008. *Lycopus lucidus* inhibits high glucose-induced vascular inflammation in human umbilical vein endothelial cells. *Vasc. Pharmacol.* **48**, 38-46.
26. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. and Freeman, B. A. 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87**, 1620-1624.
27. Mandrup-Poulsen, T., Helqvist, S. Wogensen, L. D., Molvig, J., Pociot, F., Johannesen, J. and Nerup, J. 1990. Cytokine and free radicals as effector molecules in the destruction of pancreatic β cells. *Curr. Top. Microbiol. Immunol.* **164**, 169-193.
28. Torres, M. A., Barros, M. P., Campos, S. C., Pinto, E., Rajamani, S., Sayre, R. T. and Colepicolo, P. 2008. Biochemical biomarkers in algae and marine pollution: a review. *Ecotox. Environ. Safe* **71**, 1-15.
29. Van Alstyne, K. L., Whitman, S. L. and Ehlig, J. M. 2001. Differences in herbivore preferences, phlorotannin production, and nutritional quality between juvenile and adult tissues from marine brown algae. *Mar. Biol.* **139**, 201-210.
30. Ahn, G. N., Kim, K. N., Cha, S. H., Song, C. B., Lee, J. H., Heo, M. S., Yeo, I. K., Lee, N. H., Jee, Y. H., Kim, J. S., Heu, M. S. and Jeon, Y. J. 2007. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated

DNA damage. *Eur*. *Food Res*. *Technol*. **226**, 71-79.
31. Kim, J. A., Lee, J. M., Shin, D. B. and Lee, N. H. 2004. The antioxidant activity and tyrosinase inhibitory activity of phlorotannins in *Ecklonia cava*. *Food Sci*. *Biotechnol*. **13**, 476-480.
32. Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H. and Hama, Y. 2008. Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J*. *Appl*. *Phycol*. 20, 705-711.