Food Sci. Biotechnol. Vol. 17, No. 5, pp. 983 ~ 989 (2008)

Food Science and Biotechnology

Antioxidant Activity and Inhibition of MMP-9 by Isorhamnetin and Quercetin 3-*O*-β-D-Glucopyranosides Isolated from *Salicornia herbacea* in HT1080 Cells

Chang-Suk Kong[1], You Ah Kim, Moon-Moo Kim[2], Jin-Sook Park[3], Se-Kwon Kim[3], Burm-Jong Lee[4], Taek Jeong Nam[5], and Youngwan Seo*

Division of Marine Environment and Bioscience, Korea Maritime University, Busan 606-791, Korea
[1]*Research Institute of Marine Science and Technology, Korea Maritime University, Busan 606-791, Korea*
[2]*Department of Chemistry, Dong-Eui University, Busan 614-010, Korea*
[3]*Department of Chemistry, Pukyong National University, Busan 608-737, Korea*
[4]*Department of Chemistry, Inje University, Gimhae, Gyeongnam 621-749, Korea*
[5]*Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea*

Abstract Two flavonoids, isorhamnetin 3-*O*-β-D-glucopyranoside (**1**) and quercetin 3-*O*-β-D-glucopyranoside (**2**), from slander glasswort (*Salicornia herbacea,* Korean name *hamcho*) were isolated. Antioxidative and matrix metalloproteinase-9 (MMP-9) inhibitory effects of these compounds were investigated in HT 1080 cell lines. These compounds suppressed the electron spin resonance (ESR) signal intensity on generation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in a free-cellular system. Their scavenging effects on generation of intercellular reactive oxygen species (ROS) also exhibited similar trends with DPPH radical in the free cellular system. Also, a control group combined only with Fe(II)-H_2O_2 resulted in DNA apoptosis by oxidative stress, whereas treatments with these compounds suppressed radical-mediated DNA damage. Intracellular glutathione (GSH) levels were slightly increased in the presence of compound **1** and **2**. Moreover, these compounds led to the reduction of the expression levels of MMP-9 without cytotoxic influence. These results suggest that these compounds have a potential as a valuable natural antioxidant and MMP inhibitor related to oxidative stress. Therefore, these compounds not only can be developed as a candidate for a therapeutic potential but also a source for use as ingredients of health foods or functional foods to prevent metastasis involving MMP-9, closely related to ROS.

Keywords: *Salicornia herbacea*, isorhamnetin and quercetin 3-*O*-β-D-glucopyranosides, reactive oxygen species (ROS), DNA damage, matrix metalloproteinase-9 (MMP-9)

Introduction

Oxidative stress is in general used to describe the level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species (ROS). ROS are very unstable and react easily with other groups or substances in a body leading to cell damage or injury. ROS include hydrogen peroxide (H_2O_2), hydroxyl radical, and superoxide anion, generated from various cells during normal metabolism. Although some ROS have important functions as secondary biological messengers (1), enhanced production of ROS to overwhelm cellular antioxidant defense causes oxidative damage of DNA, lipids, and proteins (2). Since the damage or injury by occurrence of oxidative stress due to excessive production of active oxygen or imbalance in the body's redox potential is closely related to chronic inflammatory diseases (3), the search for dietary antioxidants has great potential. Recently, with the growing interest in the protective biochemical function of naturally occurring antioxidants in biological systems and their mechanism, the search for the new antioxidants is still actively in progress.

*Corresponding author: Tel: +82-51-410-4328; Fax: +82-51-404-3538
E-mail: ywseo@hhu.ac.kr
Received November 30, 2007; Revised February 13, 2008;
Accepted February 16, 2008

Interestingly, several studies have shown that ROS can positively activate several different matrix metalloproteinases (MMPs) so that both seem to contribute vitally to an inflammatory network and this activation can be blocked by antioxidants (4). Thus, ROS are closely related with MMPs which are principal enzymes in extracellular matrix degradation and play a substantial role in pathological process including inflammation, arthritis, cardiovascular diseases, pulmonary diseases, and cancer (5,6). Of the MMPs, MMP-9 (gelatinase B, 92 kDa) is a member of the MMP family that is most involved in tumor initiation and growth, angiogenesis, and metastasis in several cancers (7,8). Elevated MMP-9 levels in fibrosarcoma cells resulted in markedly enhanced metastasis potential (9). Therefore, much attention has focused on development of MMP inhibitors, as a new class of cancer therapeutic targets is of current interest.

Recent studies have focused on finding the biologically active compounds or functional food from natural resources. Many bioactive compounds have been isolated from terrestrial plants (10). It was reported that flavonoids and other phenolic compounds from terrestrial plants exhibited antioxidative effect and inhibited metastasis activities by reducing MMP production (11). Slander glasswort (*Salicornia herbacea,* Korean name *hamcho*) is one of the salt marsh plants that can grow in salt marshes and on muddy seashores along the western coast of Korea (12). It has

been not only used as a seasoned vegetable but also used as a folk medicine for disorders such as constipation, obesity, diabetes, and cancer (13).

A number of investigators have examined antioxidant activity of *S. herbacea* (12,14,15). In addition to antioxidant activity, this salt marsh plant has been reported to have a number of other important activities such as anti-inflammatory, immunomodulatory, antihyperglycemic, and antihyperlipidemic activities (16,17). Two flavonoids, isorhamnetin 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-glucopyranoside, and tungtungmadic acid have also been isolated from *S. herbacea* with their 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effects (18,19). We have also isolated isorhamnetin 3-*O*-β-D-glucopyranoside (**1**) and quercetin 3-*O*-β-D-glucopyranoside (**2**) from *S. herbacea*. Although these compounds have been reported to have potent scavenging effect on DPPH radical in a free cellular assay system, there are no reports about their inhibitory effects on generation of ROS in the cell system in detail.

In the present study, inhibitory effects of compound **1** and **2** on MMP-9 activity closely related with ROS as well as their scavenging effect on ROS in HT1080 cells were in vestigated.

Materials and Methods

Plant material and isolation of compounds Whole plant of slander glasswort (*Salicornia herbacea*) was collected in Daebudo(Island), Gyeonggi, Korea in July, 2004. The plant was identified by Sung Gi Moon by its morphological character. A voucher specimen was deposited in the laboratory of Sung Gi Moon (voucher No. 04D-3, Department of Biology, Kyungsung University). The collected sample was briefly dried under shade and kept at –25°C until use. The components of slander glasswort were isolated as following. The air-dried material of slander glasswort was chopped into small pieces and extracted with CH_2Cl_2 (3 L ×2) for 24 hr at room temperature. After removal of the solvent, the residue was extracted with MeOH (3 L×2) for 24 hr at room temperature. The combined crude extracts (340 g) were suspended between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aq. MeOH and *n*-hexane and the aqueous layer was fractionated with *n*-BuOH and H_2O, respectively, to afford the *n*-hexane (10.7 g), 85% aqueous MeOH (39.6 g), *n*-BuOH (53.7 g), and water (50.8 g) fractions after evaporation. A portion of the *n*-BuOH fraction (53.7 g) was separated into 6 subfractions by C_{18} (YMC-GEL ODS-A, 12 nm, S-75 μm) reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH and H_2O (50, 60, 70, 80, 90% aqueous MeOH, and 100% MeOH). The 60% aqueous MeOH fraction was separated into 10 subfractions by silica gel (silica gel 60, 0/063-0.20 mm, Merck, Damstadt, Germany) column chromatography eluting with gradient mixtures of $CHCl_3$ and MeOH. Finally, a bioactive fraction 5 was purified using a LH-20 Sephadex (bead size 25-100 μm, Sigma-Aldrich, St. Louis, MO, USA) column eluting with methanol to afford pure flavonoid glycosides, isorhamnetin 3-*O*-β-D-glucopyranoside (19 mg), and quercetin 3-*O*-β-D-glucopyranoside (3.1 mg). The chemical structures of these flavonoid compounds were illustrated in Fig. 1.

Determination of DPPH radical scavenging activity by electron spin resonance spectroscopy DPPH radical was generated and spin adducts were recorded using a JES-FA electron spin resonance (ESR) spectrometer (Jeol, Tokyo, Japan) at 25°C. Instrument setting was as follows; magnetic field 336±5 mT, sweep time 30 sec, sweep width 10 mT, modulation width 0.1 mT, and modulation frequency 100 kHz. DPPH radical was measured using the method described by Nanjo *et al.* (20). DPPH was purchased from Sigma-Aldrich. Sixty μL of compound sample in ethanol (or ethanol itself as a control) was added to 60 μL DPPH (60 μM) and vortexed for 10 sec. Mixture was transferred to a sealed capillary tube and after 2 min DPPH radical spin resonance was recorded at 1 mW microwave power and 1,000 amplitude.

Cell culture and cytotoxicity determination using MTT assay Human fibrosarcoma HT 1080 cells were grown as monolayers in T-75 tissue culture flasks (Nunc, Roskilde, Denmark) at 5% CO_2 and 37°C humidified atmosphere using Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and 100

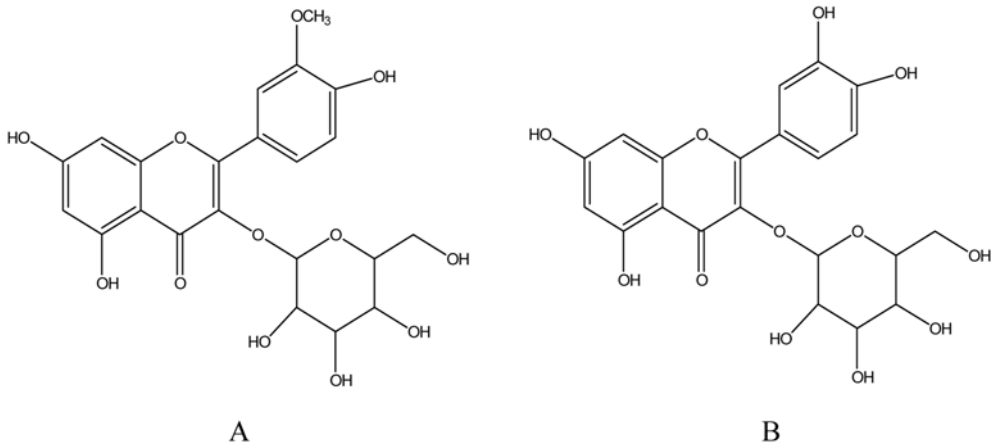

Fig. 1. Chemical structures of compounds isolated from halophyte *S. herbacea*. (A) Compound 1, isorhamnetin 3-*O*-β-D-glucopyranoside; (B) compound 2, quercetin 3-*O*-β-D-glucopyranoside.

μg/mL penicillin-streptomycin (Gibco-BRL). The medium was changed 2 or 3 times each week. Cytotoxic levels of the compounds on cultured cells were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme (21). The cells were grown in 96-well plates at a density of 5×10^3 cells/well. After 24 hr, the cells were washed with fresh medium and were treated with control medium or the medium supplemented with different concentrations of the compounds. After incubation for 48 hr, cells were rewashed and 100 μL of MTT solution (1 mg/mL) was added and incubated for 4 hr. Finally, 150 μL of dimethyl sulfoxide (DMSO) was added to solubilize the formed formazan crystals and the amount of formazan crystal was determined by measuring the absorbance at 540 nm using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instruments Inc., Winooski, VT, USA). Relative cell viability was determined by the amount of MTT converted into formazan crystal. Viability of cells was quantified as a percentage compared to the control and dose response curves were developed.

Determination of intracellular formation of ROS using DCF-DA labeling Intracellular formation of ROS was assessed using oxidation sensitive dye 2,7-dichlorofluorescin diacetate (DCF-DA) as the substrate (22). HT 1080 cells growing in fluorescence microtiter 96-well plates were loaded with 20 μM DCF-DA in Hank's balanced salt solution (HBSS) and incubated for 20 min in the dark. Nonfluorescent DCF-DA dye, that is freely penetrated into cells get hydrolyzed by intracellular esterases to 2,7-dichlorodihydrofluororescein (DCFH), and tarps inside the cells. Cells were then treated with different concentrations of test compounds and incubated for 1 hr. After washing the cells with phosphate buffered saline (PBS) 3 times, 500 μM H_2O_2 dissolved in HBSS was added to the cells. The formation of DCF due to oxidation of DCFH in the presence of various ROS was read after every 30 min at the excitation wavelength (Ex) of 485 nm and the emission wavelength (Em) of 528 nm using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instruments Inc.). Dose-dependant and time-dependant effects of the compound groups were plotted and compared with fluorescence intensity of control and blank groups.

Genomic DNA isolation Genomic high molecular weight DNA was extracted from HT1080 cells using standard phenol/proteinase K procedure with slight modifications (23). Briefly, cells culturing in 10 cm dishes were washed twice with PBS and scraped into 1 mL of PBS containing 10 mM ethylenediaamine tetraacetic acid (EDTA). After centrifugation, the cells were dissolved in RNase (0.03 mg/mL), NaOAC (0.175 M), proteinase K (0.25 mg/mL), and sodium dodecyl sulfate (SDS, 0.6%). The mixture was then incubated for 30 min at 37°C and 1 hr at 55°C. Following incubation, phenol:chloroform:isoamylalcohol (25:24:1) was added at 1:1 ratio and the mixture was centrifuged at 6,000×g for 5 min at 4°C. Following centrifugation, supernatant was mixed with 100% ice cold ethanol at 1:1.5 ratio and kept for 15 min at –20°C. After centrifugation at 16,000×g for 5 min at 4°C, the pellet was dissolved in Tris-EDTA buffer and purity of DNA was spectrophotometrically determined at 260/280 nm. Further, the quality of isolated DNA was evaluated with 1% agarose gel electrophoresis in 0.04 M Tris-acetate-0.001 M EDTA buffer.

Determination of radical-mediated DNA damage H_2O_2 mediated DNA oxidation was determined using the method described by Milne *et al.* (24). DNA reaction mixture (100 μL) was prepared by adding various concentrations of the compounds (or same volume of distilled water as control), final concentrations of 200 μM $FeSO_4$, and 0.1 mM H_2O_2 to 50 μg/mL final concentration of genomic DNA in the same order. Then, the mixture was incubated at room temperature for 30 min and the reaction was terminated by adding 10 mM final concentration of EDTA. Aliquot (20 μL) of reaction mixture containing about 1 μg of DNA was electrophoresed on a 1% agarose gel for 30 min at 100 V. The gels were stained with 1 mg/mL ethidium bromide and visualized by ultraviolet (UV) light using AlphaEase® gel image analysis software (Alpha Innotech, San Leandro, CA, USA).

Measurement of intracellular GSH level Intracellular glutathione (GSH) level in intact cells was determined using monobromobimane as a thiol-staining reagent (25). HT1080 cells were seeded into fluorescence microtiter 96-well plates at a density of 5×10^3 cells/well and allowed to attach completely. Cells were treated with concentrations of the compounds and incubated for 30 min. Monobromobimane in 1% DMSO was added to cells at a final concentration of 40 μM and staining was carried out for 30 min at 37°C in the dark. After staining fluorescence intensity was measured (excitation and emission: 360 and 465 nm) using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instruments Inc.). The averaged fluorescence values of cell populations were plotted and compared with control group in which cells were grown without treatment of the compounds.

Determination of MMP activity by gelatin zymography Activities of MMP-9 in HT1080 cells treated with the compounds were determined by gelatin zymography as described previously (26). HT 1080 cells in serum free DMEM were seeded in 24-well plates with a density of 2×10^5 cells/well and pre-treated with different concentrations of compounds for 1 hr. MMP expression was stimulated by treatment of paramethoxyamphetamine (PMA) (10 ng/mL) and cells were incubated for 36 hr. Total protein content was normalized by Bradford protein determination method. Cell conditioned medium was subjected to substrate gel electrophoresis. Similar amount of protein containing conditioned media was applied under non-reducing conditions on 10% polyacrylamide gels containing 1.5 mg/mL gelatin. After electrophoresis, polyacrylamide gels were washed with 50 mM Tris-HCl (pH 7.5) containing 2.5% Triton X-100 at room temperature to remove SDS. Gels were then incubated overnight at 37°C in a developing buffer containing 10 mM $CaCl_2$, 50 mM Tris-HCl, and 150 mM NaCl to digest gelatin by MMP. Areas of gelatin hydrolyzed by MMP were visualized as clear zones against blue background by Coomassie blue staining

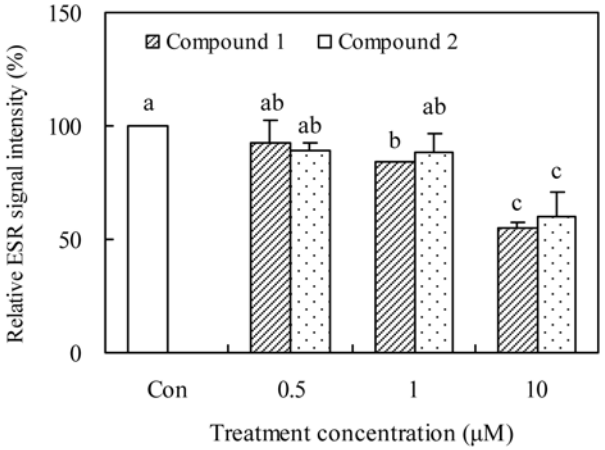

Fig. 2. Scavenging effects of compound 1 and 2 on DPPH radicals by ESR spectroscopy. [a-c]Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

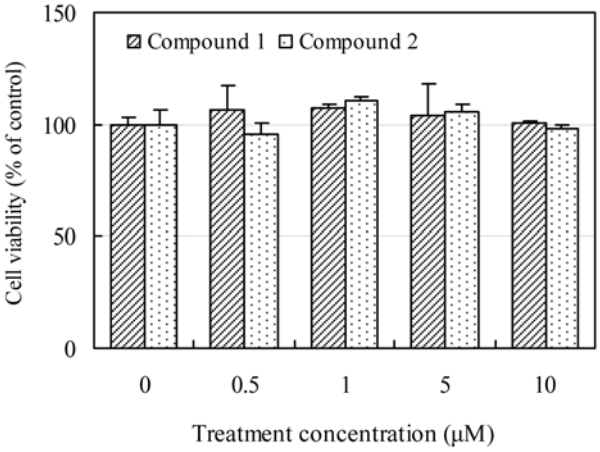

Fig. 3. Cytotoxic effects of compound 1 and 2 in HT1080 cells using MTT assay. Cells were treated with different concentration of the compounds for 24 hr.

and the intensities of the bands were estimated by densitometry (Multi Gauge V3.0 Software, Fujifilm Life Science, Tokyo, Japan).

Statistical analysis Data were analyzed using the analysis of variance (ANOVA) procedure of Statistical Analysis System (SAS Institute, 1999-2001). Significant differences between treatment means were determined by using Duncan's multiple range tests. Significance of differences was defined at the $p<0.05$ level.

Results and Discussion

Scavenging activity on DPPH radicals DPPH radical scavenging abilities of compound **1** and **2** were examined in a cell free system using ESR spectroscopy. Scavenging activities of these compounds were calculated based on relative peak height in ESR spectra of compound treatment groups compared with that of a standard group. As shown in Fig. 2, these compounds suppressed the ESR signal intensity in generation of DPPH radicals in a dose-dependent manner ($p<0.05$). Treatments with compound **1** and **2** led to the increased scavenging activity in generation of DPPH radicals, having 45 and 43% inhibitory activities, respectively, at concentrations of 10 μM compared with control group. No significant differences were found between the 2 compounds at the concentration of 10 μM.

Cell viability The cytotoxic effects of compound **1** and **2** were examined using MTT assay in order to determine non-toxic concentrations for experiment. Any significant toxic effect was not observed on the cells treated with these compounds up to the concentration of 10 μM (Fig. 3).

Cellular radical scavenging effect Intracellular radical scavenging effects of compound **1** and **2** were determined in time and concentration dependent manners using fluorescence sensitive dye, DCF-DA (Fig. 4). This method has been widely used to monitor intracellular oxidative stress and a direct measurement of scavenging of ROS. The cell system was labeled with DCF-DA and followed by adding Fe(II)/H_2O_2. The progressive increments in DCF fluorescence intensity due to H_2O_2 generation were observed with the incubation time up to 2 hr. Treatments with compound **1** and **2** led to the significant reduction of DCF fluorescence intensity, and resulted in the increased scavenging activity against intracellular ROS in a concentration dependent manner ($p<0.05$). These compounds inhibited the radical forming by more than 60% at the concentration of 10 μM. Comparative analysis of ROS scavenging effect in the presence of these compounds revealed that there were significant differences between radical scavenging abilities of these compounds at the concentration lower than 10 μM, but no significant differences at the concentration of 10 μM. DCF-DA is membrane-permeant and diffuses through the cell membrane readily. Within the cell, the acetate groups are enzymatically hydrolyzed by intracellular esterase to nonfluorescent DCFH, which is polar and trapped within the cell and rapidly oxidized to highly DCF in the presence of ROS, particularly H_2O_2 and hydroxyl radial (27). Oxidation of nonfluorescent molecule to fluorescent dichlorofluorescin DCF mainly occurred by action of cellular hydroxyl radical. In the case of natural antioxidants such as flavonoids, their scavenging properties are often associated with their ability to form stable radicals. Antioxidant ability of these compound **1** and **2** might be due to hydrogen-donating ability of OH groups and subsequent removal of ROS initiators. This direct scavenging of cellular hydroxyl radial might contribute to protect DNA damage caused by oxidative stress.

Inhibitory effect on DNA oxidation Inhibitory effects of compound **1** and **2** on DNA oxidative damage were examined using genomic DNA isolated from HT 1080 cells by DNA electrophoresis. DNA oxidation was carried out by Fenton reaction of 200 μM Fe(II) and 0.1 mM H_2O_2 on the integrity of the genomic DNA. After 30 min of reaction, DNA damage was caused in the control group combined only with Fe(II)-H_2O_2, indicating that oxidative stress induced apoptosis (Fig. 5). However, DNA oxidation was inhibited in the presence of these compounds and elevated concentrations of compound mixtures led to the

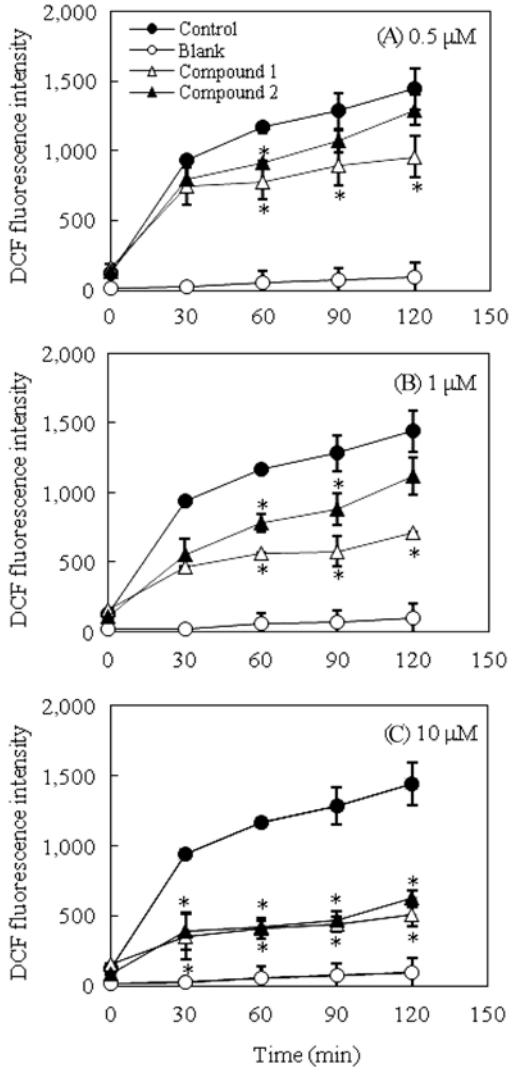

Fig. 4. Inhibitory effects of the compounds on intracellular generation of ROS. Intracellular formation of ROS was assessed using oxidation sensitive dye, DCFH-DA. *Significantly different from control group ($p<0.05$).

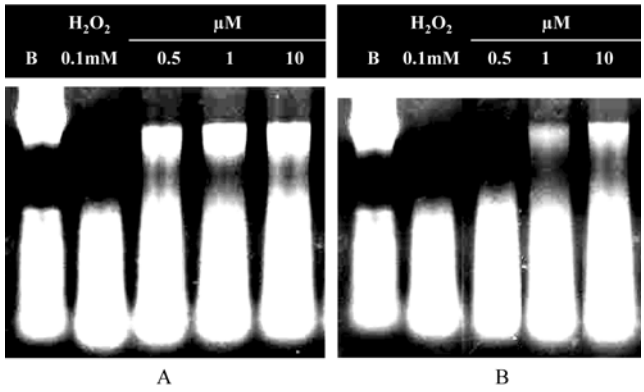

Fig. 5. DNA oxidative protection by isorhamnetin 3-*O*-β-D-glucopyranoside (A) and quercetin 3-*O*-β-D-glucopyranoside (B). Genomic DNA from HT1080 cells was pre-treated with isorhamnetin 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-glucopyranoside and exposed to ·OH using Fenton chemistry.

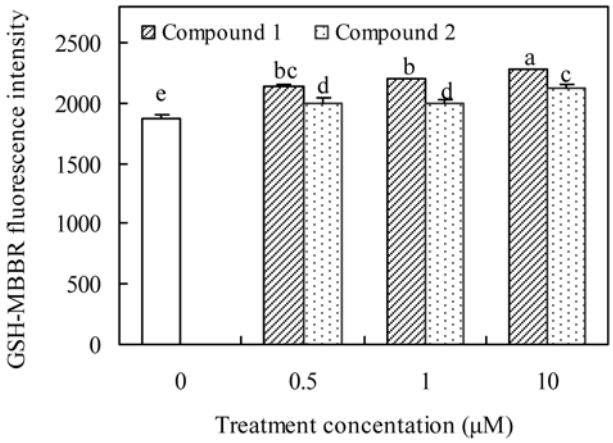

Fig. 6. Effects of compound 1 and 2 on regulation of GSH level in HT 1080 cells. [a-e]Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

extent of DNA band close to that not subjected to the Fenton reaction. At the concentration lower than 10 μM, comparatively compound **1** exhibited higher inhibition effect on DNA damage than compound **2**. On the other hand, at the concentration of 10 μM, there were no significant different in DNA oxidation damages between these compounds. Treatments with these compounds exerted adequate protective effects on radical-mediated DNA damage. Reactive oxygen species are able to attack vital biomolecules and induce all forms of oxidative DNA damage, including base modifications, base-free sites, strand breaks, and DNA-protein cross-link (2,28). These protective effects on DNA damage might be related to radical scavenging ability of these compounds. Cell damage caused by ROS can be protected by intracellular defense system.

Regulation of GSH Level GSH is very important intracellular antioxidant that directly reacts with reactive oxygen species. Increment in GSH regulating protects cells damage either by eliminating free radicals or by making conjugation with toxicants (29). Therefore, in order to investigate the antioxidative ability of these compounds to reduce intracellular radicals according to increasing redox potential in cells, these compounds were treated to the HT 1080 cells in concentration and time dependent manners and followed by measuring intracellular GSH level. Changes in intracellular GSH levels after incubation for 120 min were compared (Fig. 6). Intracellular GSH levels were slightly increased in the presence of compound **1** and **2** compared with those in the absence of compounds with incubation time. Compound **1** showed higher GSH level than that of compound **2**. Even at the concentration lower than 10 μM, intracellular GSH levels were maintained and slightly increased by these compounds. These results suggested that these compounds could be effective against scavenging radicals by increasing cellular GSH level.

Inhibition effect on MMP-9 activity Our results revealed that the compound **1** and **2** had excellent radical scavenging ability. Several different MMPs are known for

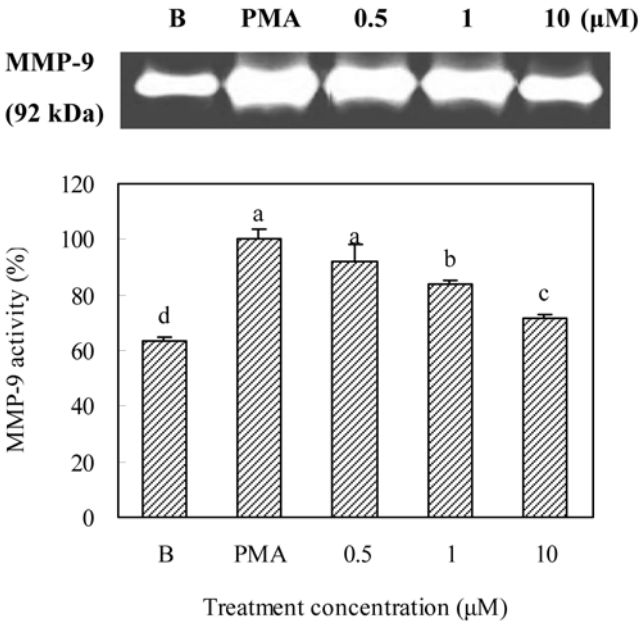

Fig. 7. Effect of isorhamnetin 3-*O*-β-D-glucopyranoside on the gelatinolytic activity of MMP-9 in HT 1080 cell line determined by gelatin zymography. [a-d]Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

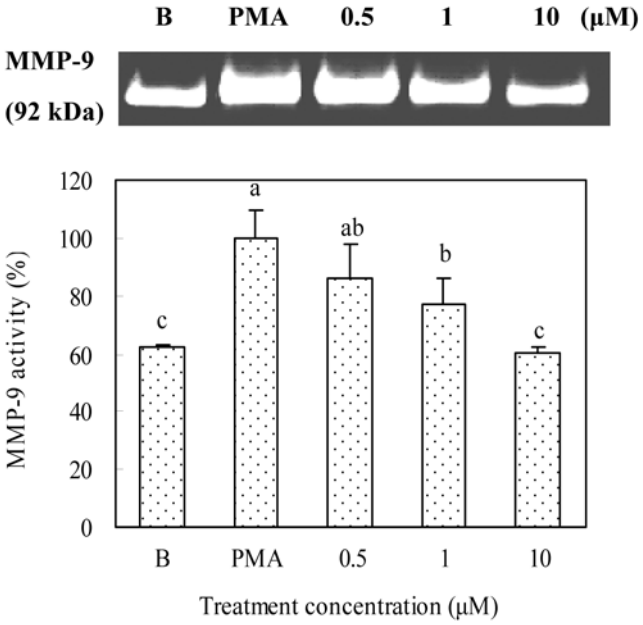

Fig. 8. Effect of quercetin 3-*O*-β-D-glucopyranoside on the gelatinolytic activity of MMP-9 in HT 1080 cell line determined by gelatin zymography. [a-c]Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

having activation by ROS via various intracellular signaling pathways and this activation can be blocked by antioxidants (4,30). Therefore, it can be expected that these compounds contribute to inhibition of MMP activities.

In order to determine whether these compounds affect the gelatinolytic activity of MMP-9 secreted from HT1080 cells, PMA stimulated conditioned medium was treated with various concentrations of these compounds (0.5, 1, and 10 μM) and gelatin zymography was carried out (Fig. 7 and 8). In this study, we selected a fibrosarcoma cell line, HT 1080 cell because this cell has been used widely as a model system to study several MMP-activity and expression (31). HT1080 cells secrete both MMP-2 and MMP-9. However, MMP-9 was selected in order to show clearly the inhibitory effect since MMP-9 expression is much higher than MMP-2 expression. Administration of PMA (10 ng/mL) to HT 1080 cells enhanced MMP-9 expression. The expression levels of MMP-9 were reduced in the presence of these compounds. Compound **1** and **2** exhibited dose-dependent inhibitory effects on MMP-9 activity. At the concentration of 10 μM, these compounds exhibited similar MMP-9 activities to those of non-stimulated cells. From the MTT cell viability test, also, it was revealed that these concentrations were cytocompatible and this MMP-9 inhibition was not due to cytotoxic influence. The position and number of aromatic hydroxyl groups per molecules have been related to the antioxidant activity of flavonoids and the influence of hydroxyl groups is dependent on the position of substitution (32). Therefore, resorcinol phenolic moiety and 3 hydroxyl groups of these compounds might be play an important role in free radicals scavenging and MMP-9 activity mechanisms.

In conclusion, our results revealed that these compound **1** and **2**, from *S. herbacea*, successfully scavenged the intracellular radicals related to radical-mediated cell damage and had inhibitory effect on MMP-9 expression. Therefore, these results suggest that these compounds may be used not only to be developed as a candidate for potential natural antioxidant but also to prevent metastasis involving MMP-9, closely related to ROS.

Acknowledgments

This research was supported by a grant (M2007-03) from the Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Land, Transport, and Maritime Affairs, Korea. Mass spectral data were kindly provided by Korea Basic Science Institute.

References

1. Dawson TM, Snyder SH. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J. Neurosci. 14: 5147-5159 (1994)
2. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J. Functional food science and defence against reactive oxidative species. Brit. J. Nutr. 80: S77-S112 (1998)
3. Ichinose M. Inflammatory mechanisms in bronchial asthma and COPD. Tohuku J. Exp. Med. 200: 1-6 (2003)
4. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases *in vitro*: Implications for atherosclerotic plaque stability. J. Clin. Invest. 98: 2572-2579 (1996)
5. Kohn EC, Jacobs W, Kim YS, Alessandro R, Stetler-Stevenson WG, Liotta LA. Calcium influx modulates expression of matrix metalloproteinase-2 (72 kDa type IV collagenase, gelatinase A). J. Biol. Chem. 269: 21505-21511 (1994)
6. Nagase H, Woessner JF Jr. Matrix metalloproteinases. J. Biol. Chem. 274: 21491-21494 (1999)

7. McCawley LJ, Matrisian LM. Matrix metalloproteinases: Multifunctional contributors to tumor progression. Mol. Med. Today 6: 149-156 (2000)
8. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: Structure, regulation, and biological functions. Eur. J. Cell Biol. 74: 111-122 (1997)
9. Kupferman ME, Fini ME, Muller WJ, Weber R, Cheng Y, Muschel RJ. Matrix Metalloproteinase 9 promoter activity is induced coincident with invasion during tumor progression. Am. J. Pathol. 157: 1777-1783 (2000)
10. Hwang JH, Choi SY, Ko HC, Jang MG, Jin YJ, Kang SI, Park JG, Chung WS, Kim SJ. Anti-inflammatory effect of the hot water extract from *Sasa quelpaetensis* leaves. Food Sci. Biotechnol. 16: 728-733 (2007)
11. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2: 152-159 (1997)
12. Han SK, Kim SM, Pyo BS. Antioxidative effect of glasswort (*Salicornia herbacea* L.) on the lipid oxidation of pork. Korean J. Food Sci. Ani. Resour. 23: 46-49 (2003)
13. Greenway H, Munns R. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31: 149-190 (1980)
14. Han SK, Kim SM. Antioxidative effect of *Salicornia herbacea L.* grown in closed sea beach. J. Korean Soc. Food Sci. Nutr. 32: 207-210 (2003)
15. Oh JH, Kim EO, Lee SK, Woo MH, Choi SW. Antioxidant activities of the ethanol extract of *hamcho (Salicornia herbacea)* cake prepared by enzymatic treatment. Food Sci. Biotechnol. 16: 873-878 (2007)
16. Seo Y, Lee HJ, Kim YA, Park KE. Antioxidative effect of glasswort (*Saliconia herbacea*) extract from Daebudo. Proc. Curr. Biotech. Bioengin. 10: 1-7 (2004)
17. Lee KS, Lee MH, Chang IY, Yoon SP, Lim DY, Jeon YJ. Marcrophage activation by polysaccharide fraction isolated from *Salicornia herbacea*. J. Ethnopharmacol. 103: 372-378 (2006)
18. Park SH, Kim KS. Isolation and identification of antioxidant flavonoids from *Salicornia herbacea* L. J. Korean Soc. Appl. Biol. Chem. 47: 120-123 (2004)
19. ChungYC, Chun HK, Yang JY, Kim JY, Han EH, Kho YH, Jeong HG. Tungtungmadic acid, a novel antioxidant, from *Salicornia herbacea*. Arch. Pharm. Res. 28: 1122-1126 (2005)
20. Nanjo F, Goto K, Seto R, Susuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radical Bio. Med. 21: 895-902 (1996)
21. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods 119: 203-210 (1989)
22. Okimotoa Y, Watanabea A, Nikia E, Yamashitab T, Noguchia N. A novel fluorescent probe diphenyl-1-pyrenylphosphine to follow lipid peroxidation in cell membranes. FEBS Lett. 474: 137-140 (2000)
23. Sambrook J, Russell D. Molecular Clonning a Laboratory Manual. Sambrook J, Russel D (eds). CPHL Press, Cold Spring Harbor, NY, USA (2001)
24. Milne L, Nicotera P, Orrenius S, Burkitt M. Effects of glutathione and chelating agents on copper-mediated DNA oxidation: Pro-oxidant and antioxidant properties of glutathione. Arch. Biochem. Biophys. 304: 102-109 (1993)
25. Poot M, Verkerk A, Koster JF, Jongkind JF. *De novo* synthesis of glutathione in human fibroblasts during *in vitro* ageing and in some metabolic diseases as measured by a flow cytometric method. Biochim. Biophys. Acta 883: 580-584 (1986)
26. Hrabec E, Strek M, Nowak D, Greger J, Suwalski M, Hrabec Z. Activity of type IV collagenases (MMP-2 and MMP-9) in primary pulmonary carcinomas: A quantitative analysis. J. Cancer Res. Clin. 128: 197-204 (2002)
27. Nasr-Esfahani M, Johnson MH. The origin of the reactive oxygen species in mouse embryos cultured *in vitro*. Development 113: 551-560 (1991)
28. Mello LD, Hernandez S, Marrazza G, Mascini M, Kubota LT. Investigations of the antioxidant properties of plant extracts using a DNA-electrochemical biosensor. Biosens. Bioelectron. 21: 1374-1382 (2006)
29. Sanchez-Reus MI, Peinado II, Molina-Jimenez MF, Benedi J. Flaxetin prevents rotenone-induced apoptosis by induction of endogenous glutathione in human neuroblastoma cells. Neurosci. Res. 53: 48-56 (2005)
30. Grimm T, Schäfer A, Högger P. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (pycnogenol). Free Radical Bio. Med. 36: 811-822 (2004)
31. Yoo HG, Shin BA, Park JS, Lee KH, Chay KO, Yang SY, Ahn BW, Jung YD. IL-1beta induces MMP-9 via reactive oxygen species and NF-kappa B in murine macrophage RAW 264.7 cells. Biochem. Bioph. Res. Co. 298: 251-256 (2002)
32. Yesilada E, Tsuchiya K, Takaishi Y, Kawazoe K. Isolation and characterization of free radical scavenging flavonoid glycosides from the flowers of *Spartium junceum* by activity guided fractionation. J. Ethnopharmacol. 73: 471-478 (2000)