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Effect of Microalgal Extracts of *Tetraselmis suecica* against UVB-Induced Photoaging in Human Skin Fibroblasts

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Exposure of cells to ultraviolet B (UVB) radiation can induce production of free radicals and reactive oxygen species (ROS), which damage cellular components. In addition, these agents can stimulate the expression of matrix metalloproteinase (MMP) and decrease collagen synthesis in human skin cells. In this study, we examined the anti-photoaging effects of extracts of *Tetraselmis suecica* (W-TS). W-TS showed the strongest scavenging activity against 2,2-difenyl-1-picrylhydrazyl (DPPH) and peroxyl radicals, followed by superoxide anions from the xanthine/xanthine oxidase system. We observed that the levels of both intracellular ROS and lipid peroxidation significantly increased in UVB-irradiated human skin fibroblast cells. Furthermore, the activities of enzymatic antioxidants (e.g., superoxide dismutase) and the levels of non-enzymatic antioxidants (e.g., glutathione) significantly decreased in cells. However, W-TS pretreatment, at the maximum tested concentration, significantly decreased intracellular ROS and malondialdehyde (MDA) levels, and increased superoxide dismutase and glutathione levels in the cells. At this same concentration, W-TS did not show cytotoxicity. Type 1 procollagen and MMP-1 released were quantified using RT-PCR techniques. The results showed that W-TS protected type 1 procollagen against UVB-induced depletion in fibroblast cells in a dose-dependent manner via inhibition of UVB-induced MMP-1. Taken together, the results of the study suggest that W-TS effectively inhibits UVB-induced photoaging in skin fibroblasts by its strong anti-oxidant ability.

***Key words*:** *Tetraselmis suecica*, Ultraviolet B, Photo aging, Reactive oxygen species, Anti-oxidant

INTRODUCTION

Human skin is constantly exposed to potentially harmful compounds and radiation because it serves as a protective barrier between the environment and internal organs (Ding and Wang, 2003). The skin aging process can be divided into intrinsic aging, a natural course determined by internal factors such as genes, and extrinsic aging, caused by external factors such as sun exposure, gravity, and smoking. In particular, extrinsic aging caused by sunlight is termed photoaging (Varani *et al*., 2000). Ultraviolet (UV) irradiation has deleterious effects on human skin including sunburn, immune suppression, cancer, and photoaging (Offord *et al*., 2002). UVB is especially the most hazardous environmental carcinogen known to human health (Katiyar *et al*., 2001). UVB exposure generally results in increased generation of reactive oxygen species (ROS) within the cell, which can subsequently result in oxidative stress and photodamage to proteins and other macromolecules in the skin (Widmer *et at*., 2006). Oxygen reactive species (superoxide, hydrogen peroxide, hydroxyl radical, alkylperoxyl radical) are naturally occurring and are detoxified by a series of enzymes in the cell. There are three major antioxidant enzymes involved: catalase, SOD and GPx. Superoxide anions are converted

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into H_2O_2 and O_2 by SOD. H_2O_2 is then broken down into H_2O and O_2 by catalase. GPx also catalyzes the reduction of H_2O_2 and lipid hydroperoxides by using glutathione (GSH) as a hydrogen donor. Cell damage from oxygen free radicals occurs when the balance of free radicals and antioxidant enzymes is disturbed. This can occur either by increasing the amount of free radicals or by decreasing the levels of antioxidant enzymes (Sies and de Groot, 1992). In addition, UV-induced ROS also stimulate the abnormal expression of matrix metalloproteinases (MMPs) as a result of intricate signal transduction cascades (Fisher *et al*., 2002). Among the factors responsible for mediating UV-induced skin aging are matrix metalloproteinases (MMPs), which are upregulated in dermal fibroblasts by UV irradiation. MMPs are a family of structurally related matrix degrading enzymes that play important roles in various destructive processes including inflammation, tumor invasion and skin aging (Vincenti and Brinckerhoff, 2002). The most abundant structural protein in skin connective tissue (dermis) is type-1 collagen. Type-1 collagen is synthesized primarily by dermal fibroblasts and is responsible for conferring strength and resiliency to skin.

Botanical antioxidants have been reported to have good potential as photoprotective agents (Berson, 2008). Vitamin E comprises a group of lipid-soluble compounds from which alpha-tocopherol (α-T) is the most abundant and has the highest antioxidant activity *in vivo* (Kamal-Eldin and Appelqvist, 1996). Industrial applications of α-T include its use in the preservation of food, cosmetics and sunscreens, and as an additive in animal feed to ensure the health of animals and to improve meat quality (Dewinne and Dirinck, 1996). The marine species *Dunaliella tertiolecta* and *Tetraselmis suecica* are widely used in aquaculture as feed for fish and mollusk larvae. They produce relatively high concentrations of α-T and other vitamins (Brown *et al*., 1999). *Tetraselmis suecica*, a unicellular flagellated green alga, has been widely used as a live food for bivalves, shrimp larvae, rotifers, and *Artemia* (Guzman *et al*., 2001). However, this species has not yet been shown to exert inhibitory effects on inflammation.

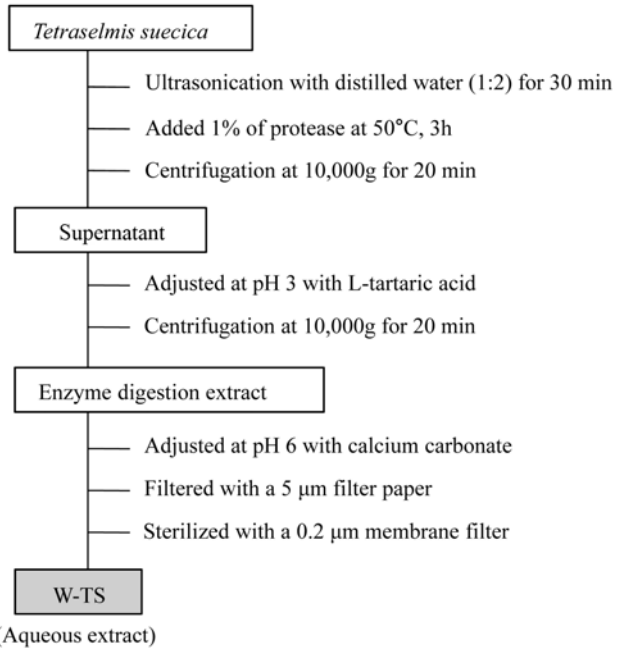

Fig. 1. Procedures for the preparation of crude extracts and fractions from *Tetraselmis suecica*. The extractive procedures described used water and enzyme digestion.

In the present study, we investigated the protective effects of microalgal extracts from *Tetraselmis suecica* against photoaging of human skin fibroblasts irradiated with UVB. Thus, we identified inhibition of ROS generation via antioxidant activity of extracts from *Tetraselmis suecica* (W-TS) and demonstrated that W-TS increased the synthesis of type I procollagen in cultured human fibroblasts and decreased the expression of MMP-1 induced by UV irradiation. Therefore, approaches aimed at reducing ROS production may be useful for the prevention of skin aging and related diseases.

MATERIALS AND METHODS

Preparation of microalgal material. *Tetraselmis suecica* (CCAP-66/22A) used in this study was obtained from NLP. Co. LTD (Busan, Korea). For the preparation of crude extracts with protease (Protamex™, Novo Nordisk Co., Denmark), the powdered *Tetraselmis suecica* (100 g d.w.) was ultrasonicated with distilled water (1 : 2 ratio, w/v) for 30 min, in which 1% of protease (to dried weight of sample) was added. The enzyme reactant was incubated at 50°C for 3 h and centrifuged at 10,000 g for 20 min. The supernatant was adjusted pH with L-tartaric acid and calcium carbonate, filtered with membrane system. This extract was designated as enzyme digestive extract (W-TS).

DPPH radical scavenging activity assay. The principle of the DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay is the reduction of the purple stable free radical DPPH to the yellow diphenylpicrylhydrazine. DPPH radical scavenging activity was according to Brand-Williamsa *et al*. (1995). For DPPH assay, the various concentrations of W-TS were mixed with 0.25 mM DPPH in ethanol, to produce a final DPPH concentration of 0.1 mM, allowed to react for 10 min in the dark, and its absorbance was measured at 517 nm using a microplate reader (Molecular Devices, VersaMax, USA).

Superoxide radical scavenging activity assay. The scavenging potential of W-TS for superoxide radicals was analyzed using a hypoxanthine/xanthine oxidase generating system coupled with nitroblue tetrazolium (NBT) reduc-

tion, with a slight modification involving the use of 96-well plates (Gotoh and Niki, 1992). The reaction mixture contained 136 µl buffer (50 mM KH_2PO_4/KOH, pH 7.4), 10 µl of 20 mM Na_2EDTA, 10 µl of 6 mM hypoxanthine, 2 µl of 10 mM NBT, and 10 µl of various concentrations of W-TS. The microplates were read 2.5 min after adding 32 µl of xanthine oxidase (1 unit per 10 ml buffer) at 550 nm using a microplate reader (Molecular Devices, VersaMax, USA).

Peroxyl radical scavenging activity assay. A hydrophilic radical initiator AAPH (2,2′-azobis-2-amidinopropane) was used to produce peroxyl radicals, and the scavenging activities of antioxidants were measured by monitoring 2,7-dichlorofluorescin-diacetate (DCF) spectrophotometrically (Valkonen and Kuusi, 1997). The activation of DCF was achieved by mixing DCF (3.44 µl of 50 µg/µl solution) and NaOH (1.75 ml of 0.01 N solution) and allowing the mixture to stand for 20 min before adding 18.25 ml of sodium phosphate buffer (25 mM, pH 7.2). Activated DCF was prepared in 96-well plates. The reaction was initiated by adding the AAPH solution to a final concentration of 30 mM and the inhibitory effects of the antioxidants were determined by adding antioxidant to the reaction mixture. Absorbance was determined at room temperature every 10 min at 490 nm using a microplate reader (DU730, Beckman Coulter, USA).

Cell viability assay. Human fibroblasts cells (ATCC, CRL-1634) were grown in monolayer culture using Dulbecco's modified essential medium (DMEM, GIBCO Industries, Inc.) supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 µg/ml of streptomycin, at 37°C in humidified CO_2 incubator (5% CO_2). Fibroblast cells were cultured overnight in 96-well plates, at a density of 1×10^4 cells/200 µl in each well. The next day, cells were co-incubated with different concentrations of W-TS for 24 hr. After incubation, the medium was removed, and 10 µl of 10 mg/ml MTT was then added to each well, followed by incubation for another 4 hr, at 37°C under humidified 5% CO_2 atmosphere. The MTT was removed, and cells were lysed with 150 µl of DMSO. The absorbance was then measured at 550 nm using a microplate reader (Molecular Devices, VersaMax, USA).

UV irradiation and sample treatment. Fibroblasts cells were cultured in serum-free DMEM medium until 90% confluence. They were then treated with different concentrations of W-TS for 24 hr before UVB irradiation. An EB-160C lamp (312 nm, Spectroline, NEW York, NY, U.S.A) was used as a UVB source. The cells were then rinsed twice with phosphate-buffered saline (PBS), and 2 ml of fresh PBS was added before these cells were exposed to UVB (100 mJ/cm^2). After UVB irradiation for 2 min, the cells were washed again with PBS and then incubated with DMEM for 24 hr.

Generation of ROS. The intracellular ROS levels were measured by 2′,7′-diacetyl dichlorofluorescein diacetate (DCFH-DA) method (Hafer *et al*., 2008). Fluorescence was determined after 30 min of incubation with W-TS using a fluorescent microplate reader (485 nm excitation and 538 nm emission, DU730, Beckman Coulter, USA).

Superoxide dismutase (SOD) activity assay. Superoxide dismutase (SOD) activity was determined by an SOD Assay Kit (Dojindo Molecular Technologies, Japan). The colorimetric assay was performed measuring formazan produced by the reaction between a tetrazolium salt (WST-1) and a superoxide anion (O_2^-), produced by the reaction of an exogenous xanthine oxidase. The remaining O_2^- is an indirect hint of the endogenous SOD activity. The absorbance was determined using a spectrometer (Molecular Devices, VersaMax, USA) at 450 nm against 620 nm.

Glutathione levels. Glutathione levels were determined using a BIOXYTECH GSH-420 assay (OxisResearch, Portland, OR). Assay is based on formation of a chromophoric thione. All GSSG is reduced to GSH, thiolethers are then formed by the addition of the chromogen, 4-chloro-1-methyl-7-thrifluoromethylquinolinium, and formation of a chromophoric thione occurs when the pH is then increased to 13. The chromogen thione is then read at 420 nm using a spectrometer (Molecular Devices, VersaMax, USA) and is directly proportional to the amount of GSH that is in the sample. All samples were compared to a GSH standard curve and expressed as GSH nmoles/mg protein.

Malondialdehyde determination. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decomposed to form a complex series of compounds including malondialdehyde (MDA). After irradiation for 24 hr, the cells were centrifuged at 1000 g for 5 min. The cell pellet was homogenized according to the recommendation of the manufacturer and assayed for MDA using the MDA-586 kit (OxisResearch, Portland, USA). MDA levels of W-TS samples were measured using a spectrometer (Molecular Devices, VersaMax, USA) at 586 nm.

Real-time PCR quantification. Total cellular RNA were isolated from fibroblast cells using TRIzol reagent (Invitrogen) and used for reverse transcription. Complementary DNA (cDNA) was synthesized from 1 µg total RNA from each sample using a Maxime RT PreMix (iNtRON), according to the instructions of the manufacturer and used as a template for Taqman™ real-time quantitative PCR. PCR primers and probes specific for human MMP1 (assay ID: Hs 002533958_m1) and COL1A1 (assay ID: Hs 00164004_m1) were obtained as TaqMan® Gene Expression Arrays (Applied Biosystems, Germany). Glyceraldehyde-3-A phosphate-dehydrogenase (GAPDH) (assay ID: Hs 99999905_g1) was

used as an internal control.

Statistical analysis. Results are expressed as means ± standard deviation (SD). Statistical differences between the treatments and the control were evaluated by ANOVA (Dunnett's test). A p value of < 0.05 was defined as significant.

RESULTS

Effect of W-TS from Tetraselmis suecica on free radical scavenging. The DPPH test is a very convenient method for screening small antioxidant molecules because the reaction can be analyzed by simple spectrophotometric assay. The DPPH radical is scavenged by antioxidants through donation of hydrogen to form the stable reduced DPPH molecule. W-TS was found to effectively scavenge free radicals generated by DPPH in dose dependent manner. In the hypoxanthine/xanthine oxidase system, the superoxide anion radical scavenging activity of W-TS was 84.1% at 2000 μg/ml. Peroxyl radical scavenging activity was measured using the DCF/AAPH assay 30 min after treatment of AAPH in the presence of different concentrations of W-TS. W-TS was found to dose-dependently prevent peroxyl radical production (Table 1).

Effect of W-TS from Tetraselmis suecica on generation of ROS in human fibroblast cells. Intracellular generation of ROS following UV exposure was evaluated by the DCF assay. To identify cytotoxicity of W-TS in fibroblast cells, we treated the cells with different concentrations of W-TS for 24 h and then we measured by MTT assay. The MTT test is used to monitor cell growth indirectly, as indicated by the conversion of the tetrazolium salt to the colored product, formazan, the concentration of which can be measured spectrophotometrically. W-TS of *Tetraselmis suecica*

Table 1. Radical scavenging activities of W-TS from *Tetraselmis suecica* extracts determined by the reduction of DPPH radical, superoxide anion radical and peroxyl radical

Extract concentration (μg/ml)		Optical density (OD_{517} nm) OD_{T0}-OD_{TS}	Inhibition percentage (IP) of DPPH radical[1)]
Tetraselmis suecica	0	0.000 ± 0.001	0.0 ± 0.4
	125	0.047 ± 0.004	16.5 ± 1.4 *
	250	0.059 ± 0.009	21.8 ± 1.2 *
	500	0.099 ± 0.004	35.3 ± 1.5 *
	1000	0.121 ± 0.002	43.3 ± 0.3 *
	2000	0.151 ± 0.005	54.7 ± 1.7 *
Ascorbic acid	2000	0.226 ± 0.001	83.3 ± 0.2
Extract concentration (μg/ml)		Optical density (OD_{550} nm) OD_{Ts}-OD_{T0}	Inhibition percentage (IP) of superoxide anion radical[2)]
Tetraselmis suecica	0	0.063 ± 0.001	0.5 ± 0.9
	125	0.046 ± 0.001	27.5 ± 1.8 *
	250	0.034 ± 0.003	46.0 ± 4.2 *
	500	0.021 ± 0.004	66.1 ± 6.0 *
	1000	0.016 ± 0.003	74.6 ± 4.8 *
	2000	0.010 ± 0.002	84.1 ± 2.7 *
Ascorbic acid	2000	0.005 ± 0.006	92.6 ± 8.7
Extract concentration (μg/ml)		Optical density (OD_{490} nm) OD_{T0}-OD_{TS}	Inhibition percentage (IP) of peroxyl radical[3)]
Tetraselmis suecica	0	3 ± 11	0.7 ± 3.3
	125	33 ± 4	11.0 ± 3.8 *
	250	103 ± 3	22.5 ± 0.4 *
	500	151 ± 18	27.1 ± 3.5 *
	1000	301 ± 23	45.1 ± 2.3 *
	2000	367 ± 60	54.0 ± 3.7 *
Ascorbic acid	2000	372 ± 12	70.3 ± 3.1

Three independent assays were performed in triplicate and the data shown are mean ± S.D.
[1),3)]Inhibition percentage (%) = $\{(OD_{T0}-OD_{TS})/OD_{T0}\} \times 100$, where OD_{T0} was the absorbance at time zero and OD_{TS} was the absorbance at the steady state (defined as the state when the absorbance of the sample remained stationary).
[2)]Inhibition percentage (%) = $\{1 - (OD_{sample}/OD_{control})\} \times 100$.
*$p < 0.05$, when compared with the control (0 μg/ml) value.

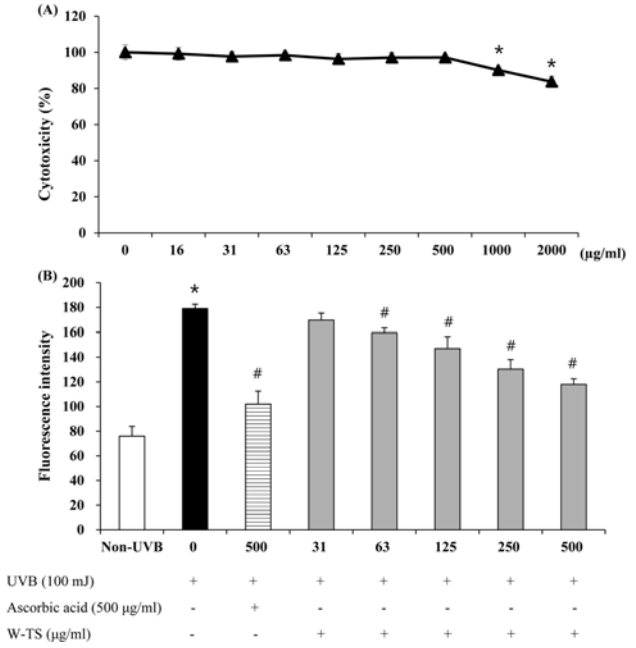

Fig. 2. Cell viability of fibroblast cell with W-TS and generation of ROS in UVB treated human fibroblast cells treated with W-TS from *Tetraselmis suecica*. (A) Fibroblast cells were treated with various concentrations of the crude extracts (aqueous extract, W-TS) from *Tetraselmis suecica* for 24 hr. Cell viability was determined by MTT assay and expressed as a percentage of control growing cells. (B) Effect of W-TS on intracellular ROS in fibroblasts following UVB exposure. Fibroblast cells were pretreated with W-TS (31, 63, 125, 250 and 500 μg/ml) prior to UVB irradiation (100 mJ/cm^2). Three independent assays were performed in triplicate and the data shown are mean ± S.D. *$p < 0.05$ vs. media alone and #$p<0.05$ vs. UVB induced group (control); significance of difference between the treated groups by Students-t tests (*) and ANOVA followed by Dunnett's test (#).

Fig. 3. The effect of W-TS from *Tetraselmis suecica* on the SOD, GSH and MDA levels in UVB irradiated fibroblasts. Fibroblast cells were pretreated with W-TS (31, 63, 125, 250 and 500 μg/ml) prior to UVB irradiation (100 mJ/cm^2) and harvested 24 hr later. Ascorbic acid was used as a positive control. (A) The SOD activity was measured using the SOD assay kit, as described in Materials and Methods. (B) The GSH contents were measured using the DTNB, as described in Materials and Methods. (C) The MDA was estimated with MDA-586 kit and spectrometer at 586 nm. Three independent assays were performed in triplicate and the data shown are mean ± S.D. *$p < 0.05$ vs. media alone and #$p < 0.05$ vs. UVB induced group (control); significance of difference between the treated groups by Students-t tests (*) and ANOVA followed by Dunnett's test (#).

showed 97% cell viability at 500 μg/ml (Fig. 2A). Thus, we selected 31, 63, 125, 250 and 500 μg/ml as the tested concentrations of W-TS in some experiments. We next examined generation of intracellular ROS by W-TS under UVB irradiated fibroblasts. After fibroblasts underwent UVB irradiated condition, considerably more ROS were observed following UVB irradiated condition (2.3-fold), when it compared with the non-irradiated condition. Treatment of ascorbic acid as positive control significantly reduced ROS production compared to the UVB irradiated fibroblasts. W-TS pretreatment significantly inhibited the intracellular ROS production in UVB-irradiated fibroblasts in a dose-dependent manner ($p < 0.05$) (Fig. 2B).

Effect of W-TS from Tetraselmis suecica on antioxidant capacity in human fibroblast cells. Human fibroblasts were treated with W-TS or ascorbic acid for 24 hr and then irradiated with UVB (100 mJ/cm^2). W-TS increased activities of antioxidant enzymes after fibroblasts were irradiated by UVB. Fig. 3A shows the activities of SOD in normal, UVB-irradiated and W-TS pretreated fibroblasts. The activity of SOD was significantly decreased in UV-irradiated fibroblasts (4.3 ± 0.1 U/ml) compared to the non-irradiated cells (6.3 ± 0.1 U/ml). Pretreatment with W-TS significantly increased the activities of SOD in a concentration dependent manner. The levels of non-enzymatic antioxidants such as GSH were found to be decreased in UVB-irradiated fibroblasts. Pretreatment with W-TS at concentrations of 250 and 500 μg/ml elevated the GHS levels by 1.2-

fold and 1.3-fold, respectively ($p < 0.05$) compared to the UVB irradiated fibroblast (Fig. 3B). The MDA level at 100 mJ/cm^2 of UVB irradiation was approximately 1.8-fold greater than that of non-irradiated cells. However, MDA levels were significantly decreased with treatment of W-TS compared to the UVB irradiated fibroblasts ($p < 0.05$) (Fig. 3C).

Effect of W-TS from Tetraselmis suecica on UVB-induced MMP-1 and type-I procollagen expression. We tested the effects of W-TS on MMP-1 and type-1 procollagen expression in UVB-irradiated fibroblasts. Human fibroblasts were treated with W-TS or ascorbic acid for 24 h and then irradiated with UVB (100 mJ/cm^2). In real-time PCR analysis, treatment of fibroblasts with UVB irradiation resulted in 1.8-fold increase in MMP-1 levels compared to the non-irradiated cells. This increase in the MMP-1 content was restored by W-TS treatment at 63~500 μg/ml in the range of 36~79% ($p < 0.05$) (Fig. 4A). Type-I procollagen levels were decreased by 11-fold due to the exposure of fibroblast to UVB irradiation compared to the non-irradiated cells. Treatment of fibroblast with W-TS at 31~500 μg/ml caused an increase in these levels by approximately 15~80% ($p < 0.05$) (Fig. 4B).

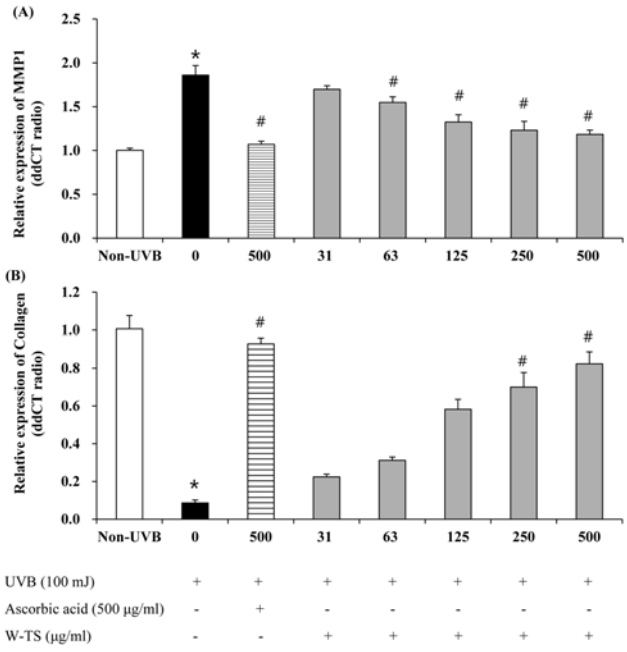

Fig. 4. The effect of W-TS from *Tetraselmis suecica* on the UVB-induced MMP-1 and type I procollagen mRNA production in human skin fibroblasts. Cells were pretreated with W-TS (31, 63, 125, 250 and 500 μg/ml) prior to UVB irradiation (100 mJ/cm^2) and harvested 24 h later. MMP-1 (A) and type 1 procollagen mRNA production (B) was determined by RT-PCR. MMP-1 and type 1 procollagen mRNA was normalized vs. that of the corresponding GAPDH mRNA. Ascorbic acid was used as a positive control. Three independent assays were performed in triplicate and the data shown are mean ± S.D. *$p < 0.05$ vs. media alone and #$p < 0.05$ vs. UVB induced group (control); significance of difference between the treated groups by Students-t tests (*) and ANOVA followed by Dunnett's test (#).

DISCUSSION

Marine organisms are widely used in the life sciences as a source of compounds with diverse structural forms and biological activities. Microalgae are rich in proteins, carbohydrates, minerals, and diverse functional pigments. Their proteins are good health food or animal feed (Fuentes *et al*., 2000), their carbohydrates are useful as stabilizers and emulsifiers in foods, and their bioactive materials have potential medicinal value (Brown, 1991). In addition, their pigments could be used as natural food dyes and they contain many beneficial minerals (Fuentes *et al*., 2000). Therefore, microalgae would be very useful in functional foods. In this study, we demonstrated the anti-aging activities of extracts from *Tetraselmis suecica* (W-TS) in human fibroblasts.

Extrinsic aging is related to environmental effects, mainly UV-induced damage of the dermal connective tissue in the skin (Kang *et al*., 2003). Exposure of skin to ultraviolet UV radiation (200~400 nm), particularly UVA and UVB (290~400 nm), may result in erythema, edema, hyperplasia, hyperpigmentation, sunburn cells, immunosuppression, photoaging, and tumorigenesis in the skin (Afaq and Mukhtar, 2006). UVB interacts with cellular chromotophores and photosensitizers, which results in the generation of ROS, damage to DNA, and activation of cytoplasmic signal transduction pathways, which are related to growth, differentiation, replicative senescence, and connective tissue degradation (Tomaino *et al*., 2006). The free radical theory is one of the major theoretical mechanisms explaining the aging process and has received particular attention in skin aging since skin is constantly exposed to ROS. Our results demonstrate that W-TS showed the strongest scavenging activity against DPPH radicals and peroxyl radicals, followed by superoxide anions from the xanthine/xanthine oxidase system (Table 1). Accumulated ROS as a consequence of repetitive UV exposure has been suggested to play roles in both intrinsic aging and photoaging in the human skin *in vivo* (Kawaguchi *et al*., 1996). Pretreatment with W-TS significantly reduced ROS generation in UVB-irradiated fibroblast cells in a dose-dependent manner (Fig. 2). Taken together, our data suggested that W-TS effectively could eliminate free radical such as ROS under hypoxic condition via anti-oxidant activity.

In this study, we examined the effect of W-TS on cellular antioxidant status in skin fibroblasts under UVB irradiation. Previous reports have shown that enhanced oxidative stress induced by UVB radiation is accompanied with decreased activities of SOD and GSH as well as increased

MDA levels as markers of lipid peroxidation (Merwald *et al*., 2005). GSH is considered to be a free radical-scavengeror and a cofactor for protective enzymes, which plays a pivotal role in the cellular defense against oxidative damage. UVB irradiation leads to decreased levels of GSH due to leakage and oxidation of GSH (Merwald *et al*., 2005). GSH depletion of cultured human skin cells makes them sensitive to UVB induced mutations and cell death (Punnonen *et al*., 1991). The SOD and GSH content was significantly reduced in UVB irradiated cells, while W-TS inhibited UVB-induced decline in SOD and GSH in skin fibroblasts. With the inclusion of W-TS, lipid peroxidation was inhibited during UVB irradiation (Fig. 3).

UVB irradiation of human skin fibroblasts induces the expression of MMPs which degrade the extracellular matrix causing pre-mature aging (photoaging). MMP-mediated collagen damage is a major contributor to the phenotype of photoaged human skin (Fisher *et al*., 2002). Of the various types of MMP, MMP-1 acted more efficiently than the other collagenases (MMP-8 and MMP-13) in inducing collagen damage (Varani *et al*., 2002). Therefore, inhibition of the induction of MMPs has been reported to alleviate UV-induced photoaging in terms of protection from collagen destruction (Fisher *et al*., 1996). We examined the relation of W-TS to MMP-1 expression and found that the UV-induced expression of MMP-1 was significantly reduced by W-TS treatment on human fibroblasts, and W-TS protected type 1 procollagen against UVB-induced fibroblast cell depletion via inhibition of UVB-induced MMP-1 in a dose-dependent manner (Fig. 4).

In conclusion, present results are strongly suggestive of the photo-protective effect of W-TS against UVB irradiation in human skin fibroblasts via its strong free radical scavenging capacity, replenishment of antioxidants stores, MMP-1 inhibitory effect, and protection and enhancement of pro-collagen-1. We conclude that extracts of *Tetraselmis suecica* protect UVB irradiated human skin fibroblasts against photoaging and have the potential to be used in cosmetic products for anti-aging problems.

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