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Anti-Skin Aging Effect of Syriacusins from *Hibiscus Syriacus* on Ultraviolet-Irradiated Human Dermal Fibroblast Cells

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Abstract − Photosensitized peroxidation of membrane lipids has been implicated in skin pathologies such as phototoxicity and premature aging. We have previously reported that syriacusin compounds isolated from *Hibiscus Syriacus* inhibited lipid peroxidation. Here, we investigated whether syriacusins could be effective inhibitor to skin aging using ultraviolet-irradiated human dermal fibroblast cells (HDFCs). Syriacusins A, B, and C inhibit the activity of human neutrophil elastase (HNE), a serine protease to degrade extracellular matrix (ECM) proteins including elastin, with IC_{50}s of 8.0, 5.2, and 6.1 μM, respectively. No changes in cell viability were detected by syriacusins A and B in UV-B (10 mJ/cm^2) irradiated HDFCs. Matrix metalloproteinase (MMP)-1 expression in HDFCs was increased by UV-B irradiation. MMP-1 expression in UV-B irradiated HDFCs was decreased by 10 μM and 20 μM syriacusin A to 50% and 20% of untreated control, respectively. Syriacusin B treated with 20 μM reduced MMP-1 expression in UV-B irradiated HDFCs to 60% of untreated control. Syriacusin A also inhibited MMP-2 expression accompanying the increase of type-I pro-collagen in UV-B irradiated HDFCs. These results demonstrate that syriacusin A could be a more effective compound to inhibit skin aging caused by UV irradiation. It suggests that syriacusins A and B might be developed as possible agents to treat or prevent skin aging.

Keywords: Syriacusin, *Hibiscus syriacus*, MMP-1, Pro-collagen, Human dermal fibroblast

INTRODUCTION

Skin aging is a complex biological process that affects skin function and appearance. Skin connective tissue contains several types of collagen, elastin, fibronectin, proteoglycan and other extracellular matrix (ECM) proteins, among which type-I collagen is the most abundant. (Kligman, 1969; Bernstein *et al*., 1994; Rittie and Fisher, 2002). Human skin aging resulting from ultraviolet (UV) irradiation is a cumulative process that occurs based on the degree of exposure to sunlight (Imokawa, 2008; Imokawa, 2009). Quantitative and qualitative changes in the dermal ECM proteins are associated with photo-damage and eventually skin aging (Imokawa, 2009).

Elastin is an important structural protein of ECM and the major component of elastic fibers that provides resilience and elasticity to many tissues including skin, lungs, ligaments and arterial walls (Wiedow *et al*., 1990; Tsukahara *et al*., 2006). The reduction of elastin in skin plays a role in the formation of wrinkles (Tsuji *et al*., 2001). Human neutrophil elastase (HNE) is a serine protease located primarily in the azurophil granules of polymorphonuclear leukocytes. HNE has a broad substrate specificity being able to degrade ECM proteins such as elastin, collagen, fibronectin, laminin, proteoglycan (Wiedow *et al*., 1990; Tsuji *et al*., 2001; Tsukahara *et al*., 2006) and the other connective tissue proteins such as cartilage tissues (Antonicelli *et al*., 2007). Biologically, elastase activity increases significantly with age and results in reduced skin elasticitic properties (Labat-Robert *et al*., 2000; Tsukahara *et al*., 2001; Tzaphlidou, 2004). Inhibition of elastase activity may protect skin aging (Kim *et al*., 2009; Xu *et al*., 2010).

The matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases that can degrade a wide variety of extracellular matrix compo-

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nents (Kahari and Saarialho-Kere, 1997). These can be classified into the following subgroups: collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs on the basis of their structures and substrate specificities (Rittie and Fisher, 2002). MMP expression is low in unstimulated skin cells and healthy tissues but some MMPs are induced by various extracellular stimuli, such as UV or infrared radiation, growth factors, cytokines and tumor promoters (Rittie and Fisher, 2002; Imokawa, 2009). Gelatinase (*i.e.* MMP-2 and MMP-9) activities were increased by a chronical exposure to UV-B in hairless mice (Inomata *et al*., 2003; Suganuma *et al*., 2010). The inhibition of gelatinase activities suppresses UV-B-induced epidermal thickness enhancement and wrinkle formation (Inomata *et al*., 2003; Suganuma *et al*., 2010). MMP-1 is the major enzyme responsible for collagen 1 digestion (Dong *et al*., 2008). MMP-1 is induced by exposure to sunlight and released from damaged epidermal keratinocytes (Fagot *et al*., 2004) and the fibroblasts (Fagot *et al*., 2002; Dong *et al*., 2008). The level of partially degraded collagen is approximately 3.6-fold greater in photodamaged skin than in sun-protected skin (Varani *et al*., 2001).

The genus *Hibiscus* is widely distributed over Korea, China, India and Siberia. The dried flowers and root bark of *H. syriacus* are used as a folk medicine in the Orient (Hsu *et al*., 1986; Huang, 1993). Active constituents in methanol extract of the root bark of *H. syriacus* are three naphthalene compounds, syriacusins A, B, and C, with the inhibitory effect on lipid peroxidation (Yoo *et al*., 1998). Photosensitized peroxidation of membrane lipids has been implicated in skin pathologies such as phototoxicity, premature aging, and carcinogenesis (Girotti and Kriska, 2004). However, little has been known about the effect of syriacusins on photo-damaged skin.

Here, we investigated whether syriacusins could be effective inhibitor to skin aging by photodamage. We irradiated human dermal fibroblast cells (HDFCs) with UV-B below cytotoxicity energy level. Data showed that syriacusins A, B, and C inhibit HNE activity and the expression of MMP-1 and MMP-2, which led to the increased expression of pro-collagen in HDFCs. It suggests that syriacusins A and B might be developed as possible agents to treat or prevent skin aging by photodamage.

MATERIALS AND METHODS

Plant materials

Hibiscus syriacus was collected at Yuseong, Chungnam Province, Korea, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea. A voucher specimen is deposited in the herbarium of KRIBB.

Extraction and isolation of active constituents from *Hibiscus syriacus*

Active ingredients were extracted and isolated by the described previously method (Yoo *et al.*, 1998). Briefly, the dried root bark and of *H. syriacus* (1.6 kg) were extracted twice with methanol for 2 days at room temperature and then the extracts were partitioned by *n*-hexane, chloroform, ethyl acetate and *n*-butanol in turn. Among them, chloroform layer was subjected to colomn chromatography over a silica gel eluting with *n*-hexane:EtOAc (10:1-1:1, v/v) gradient system. Fractions were collected and combined by monitoring with HNE inhibitory activity in combination with analytical TLC to yieled subfractions 1-1 and 1-2. Subfraction 1-1 was rechromatographed on silica gel column eluting with same solvents as described above, and then purified by Sephadex LH-20 with $CHCl_3$-MeOH (1:1). Syriacusin A (35.5 mg) was finally purified from subfraction 1-1 by HPLC on a Senshu pak ODS column (20× 250 mm) with 70% aq. MeOH at 8 ml min^{-1}. Subfraction 1-2 was further purified by column chromatograpy over silica gel eltuting with $CHCl_3$-MeOH (100:1 to 1:1) followed by

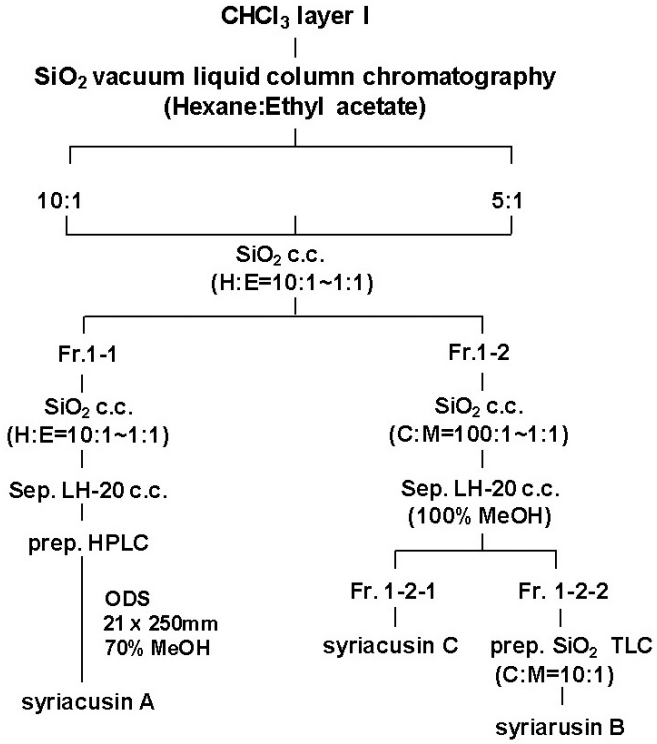

Fig. 1. Isolation procedure of syriacusins A, B, and C from *Hibiscus syriacus.*

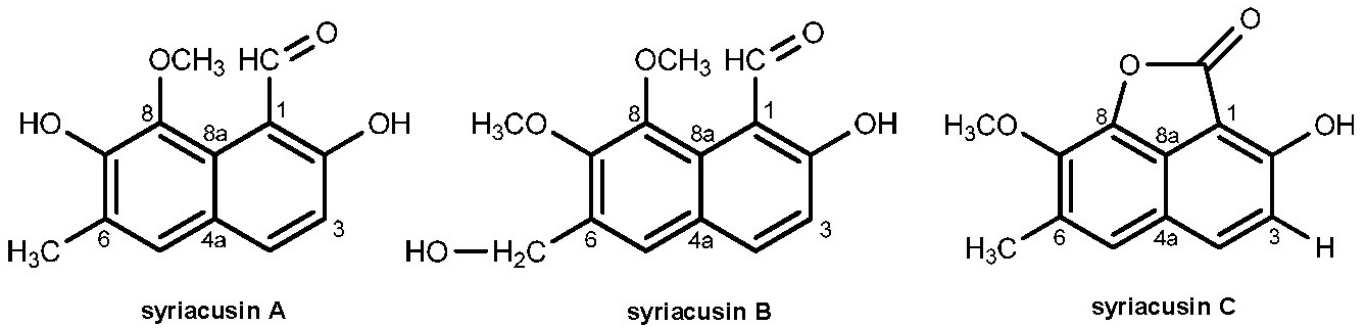

Fig. 2. Chemical structure of syriacusins A, B, and C.

Sephadex LH-20 with methanol, to give syriacusin C (3 mg) and another active subfraction 1-2-2. Syriacusin B (3.5 mg) was purified from subfraction 1-2-2 by silica gel prep TLC developed with $CHCl_3$-MeOH (10:1) (Fig.1, 2). For the bioassy test, compounds were dissolved in DMSO at 100 mM and stored at −20°C. Epigallocatechin gallate (EGCG) was used as a positive control.

Instrumental analysis

NMR spectra were recorded on Varian UNITY 300 spectrometer at 300 and 500 MHz for ^1H, and 75 and 125 MHz for ^{13}C in $CDCl_3 + CD_3OD$ (1:1), $CDCl_3$ and DMSO-d_6 with TMS as an internal standard. Complete proton and carbon assignments were based in 1D (^1H, ^{13}C, DEPT) and 2D (^1H-^1H COSY, ^1H-^{13}C HMQC, ^1H-^{13}C HMBC) NMR experiments. Mass spectra (HREI-MS) were measured using a JMS-SX 102A (JEOL). HPLC was performed on a Senshu pak ODS column (20×250 mm, YMC, RP-18, Japan) using a H_2O-MeOH system and by monitoring with a photodiode-array detector (Waters 515 pump, 2,996 photodiode array detector, USA). HNE (Calbiochem) inhibitory activity was measured according to the ELISA reader VersaMax (Molecular Devices, USA).

Cell culture

Human skin fibrobrast cells (CRL-2076) were purchased from the American Type Culture Collection (ATCC). Cell were cultured in Dulbeccos's Eagle's medium (DMEM: Gibco) supplemented with penicillin A (100 U/ml), streptomycin (100 U/ml), and 10% heat-inactivated fetal bovine serum (Gibco). Cells were maintained in a humidified incubator 5% CO_2 atmosphere at 37°C

MTT assay

The tetrazolium dye colorimetric test was used to determine the viability of fibrobrast cells (Denizot and Lang, 1986). For treatment, cells were maintained on culture media without FBS for 24 h and UV-B irradiation. Then, cells were treated with syriacusins at concentrations range from 0, 3, 10, and 30 μM. MTT solution was added after an incubation period of 48 h. Cells were incubated at 37°C for an additional 3 h. The supernatant was then removed, and 150 μl of dimethyl sulfoxide (DMSO) was added. Absorbance was measured on a microplate reader at 570 nm to obtain the percentage of viable cells as compared to control group.

UV irradiation

Human dermal fibroblast cells (HDFCs) were grown in 6-well culture plate (BD Falcon) and maintained in culture media without FBS overnight. The cells were rinsed twice with phosphate-buffered saline (PBS), and the cells were exposed to UV light under a thin layer of PBS (Gibco). Cells were immediately washed with PBS after irradiation. Then, cells were cultured for 48 h in the serum-free media with or without syriacusins. The same conditions without UV irradiation were used for the control group.

Human neutrophile elastase (HNE) assay

The HNE inhibitory activity of syriacusins A, B, and C were evaluated using a previously described procedure (Kim *et al.*, 2009; Xu *et al.*, 2010). Briefly, each well of 96-well plate containing 40 μl of substrate solution [1.4 mM N-methoxysuccinyl-ala-ala-pro-val-*p*-nitroanilide in 10 mM Tris-HCl buffer (pH 7.5)], 50 μl of test solution (stock solutions of the test compounds were dissolved in DMSO and diluted with Tris-HCl buffer to give the final sample concentrations), adding 10 μl of an enzyme solution (0.18 units HNE) were mixed and incubated for 1 h at 37°C in the dark. After the reaction was quenched by adding 100 μl of soybean trypsin inhibitor at a concentration of 0.2 mg/ml, and the absorbance was immediately measured at 405 nm using ELISA reader. HNE inhibitory activity was expressed as follows:

$$\text{HNE inhibitory activity (\%)} = \left(1 - \frac{Ab\text{-}As}{Ab}\right) \times 100$$

Ab: absorbance of blank
As: absorbance of sample

Western blot analysis

Cells were lysed in ice-cold lysis buffer containing 0.5% Nonidet P-40 (v/v) in 20 mM Tris-HCl (pH 8.3); 150 mM NaCl; protease inhibitors (2 μg/ml aprotinin, pepstatin, and chymostatin; 1 μg/ml leupeptin and pepstatin; 1 mM phenylmethyl sulfonyl fluoride (PMSF); and 1 mM Na_4VO_3. Lysates were incubated for 30 minutes on ice before centrifugation at 12,000×g for 5 minutes at 4°C. Proteins in the supernatant were denatured by boiling for 5 minutes in sodium dodecyl sulfate (SDS) sample buffer. Proteins in equal volume of conditioned culture media or equal number of cells were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Amersham international). To block the non-specific interaction between, membranes were incubated with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) (10 mM Tris-HCl, pH 7.6; 150 mM NaCl; 0.5% Tween 20) for 1 h. To determine the amounts of MMP-1 (42/46 kDa) and type-I pro-collagen (170-190 kDa) secreted into culture media, membranes were incubated with the monocolnal anti-MMP-1 antibody (Calbiochem) and polyclonal anti-pro-collagen type-I antibody (Santa Cruz Biotechnology Inc.) diluted at 1:1,000 and 1:500, respectively. Bound antibodies were incubated with HRP- conjugated anti-mouse IgG and anti-goat IgG antibody as a secondaty antibody diluted at 1:1,000 and 1:500 dilution, respectively. Molecules were detected with the use of enhanced ECL system (Amersham international). Signal strength of each molecule was quantified by using a densitometric program (TINA).

Zymography for MMP-2

MMP-2 was detected by zymography in 10% polyacrylamide gel containing 0.1% gelatin (invitrogen, USA) (Demeule et al., 2000). Insoluble debris was removed by centrifugation at 12,000 g for 5 min at 4°C and 15 μl of supernatant was mixed with Tris-glycine SDS sample buffer (2×) without reducing agent. Without boiling, 25 μl of sample was loaded on to 10% SDS-PAGE. After electrophoresis, SDS gel was incubated with 1× zymogram renaturing buffer (Invitrogen, USA) for 30 min at room temperature. Then, 1× zymogram developing buffer (Invitrogen, USA) was added to the gel. After developing for 30 min, a developing buffer was exchanged with fresh 1× zymogram developing buffer. After the gel was incubated at 37°C overnight, the gel was stained with 0.05% Commassie Brilliant Blue solution and destained with destaining solution containing 10% acetic acid, 40% methanol until protein bands were clearly visible in a blue background.

RESULTS

Inhibitory effect of syriacusins on huma neutrophile elastase (HNE) activity

The chloroform soluble fraction of methanol extract from the root bark of *Hibiscus syriacus* had been bio-assayed for the isolation of active constituents (Fig. 1). Those are three naphthalene compounds, syriacusins A, B, and C (Fig. 2).

Given that lipid peroxidation was inhibited by syriacusins A, B, and C (Yoo *et al.*, 1998) and photosensitized peroxidation of membrane lipids has been implicated in skin aging (Girotti and Kriska, 2004), we examined the effect of syriacusins on HNE activity in human dermal fibroblast cells (HDFCs). Syriacusins were used at 4 different concentrations, 1, 3, 10, and 30 μM. As shown in Table I, syriacusins A, B and C inhibit HNE activity with IC_{50}s of 8.0, 5.2, and 6.1 μM, respectively. Their effect were comparable to the positive control, (-)-epigallocatechin-3gallate (EGCG) with IC_{50} of 1.1 μM. It suggests that syriacusins could be a novel anti-skin aging agent to inhibit the degradation of elastin.

Cell viability had not been changed by syriacusin A and B

To investigate whether the effects of syriacusins A, B and C on the UVB-induced MMP-1 expression and MMP-2

Table I. Inhibitory effect of syriacusins A, B, and C on human neutrophile elastase (HNE) activity[a]

Compounds	Inhibition ratio for HNE (%)[a]				IC_{50} (μM)
	1 μM	3 μM	10 μM	30 μM	
Syriacusin A	19.7 ± 1.6	25.7 ± 1.7	51.9 ± 1.1	76.6 ± 0.7	8.0
Syriacusin B	15.3 ± 1.8	30.1 ± 2.1	86.3 ± 0.6	93.3 ± 1.2	5.2
Syriacusin C	16.5 ± 2.2	22.9 ± 1.0	74.4 ± 1.2	89.6 ± 1.0	6.1
EGCG[b]	45.3 ± 1.6	63.0 ± 0.5	75.8 ± 0.8	83.2 ± 1.2	1.1

[a]Data are expressed as the means ± S.D. (n=3), and IC_{50} indicated the concentration (μM) at which the percentage inhibition of HNE activity was 50%, [b]EGCG, [(−)-epigallocatechin-3-gallate] was used as a positive control.

expression in cultured human dermal fibroblast cells (HDFCs), cells were irradiated with 0, 10, 20, and 30 mJ/cm^2 of UV-B. Cell viability was more than 90% up to 10 mJ/cm^2 as compared to UV-B untreated control (Fig. 3A). When HDFCs were exposed to 10 mJ/cm^2 and immediately further incubated for 48 h in the presence of syriacusins A, B, and C with the concentrations of 3, 10 and 30 μM, no changes were detected in cell viability as compared to syriacusins-untreated control (Fig. 3B). In the meanwhile, cell viability was significantly decreased by the incubation with syriacusin C at 10 μM (data not shown). It demonstrates that syriacusins A and B could be safe compounds to treat UV-exposed skin.

Syriacusin A and B inhibit the expression of MMP-1 and MMP-2

Given that HNE degrade ECM proteins such as elastin and collagen (Wiedow *et al.*, 1990; Tsuji *et al.*, 2001; Tsukahara *et al.*, 2006) and MMP-1 is the major enzyme responsible for collagen 1 digestion (Dong *et al.*, 2008), we investigated the effect of syriacusins A and B on the expression of MMP-1 protein levels in HDFCs. Cells were irradiation with UVB (10 mJ/cm^2) and immediately incubated with 1, 2, 5, 10, and 20 μM syriacusins A or B for 48 h. As shown in Fig. 4A, syriacusin A inhibited UV-B-induced MMP-1 expression in a dose-dependent manner compared to the UV-B-irradiated control. MMP-1 expression in

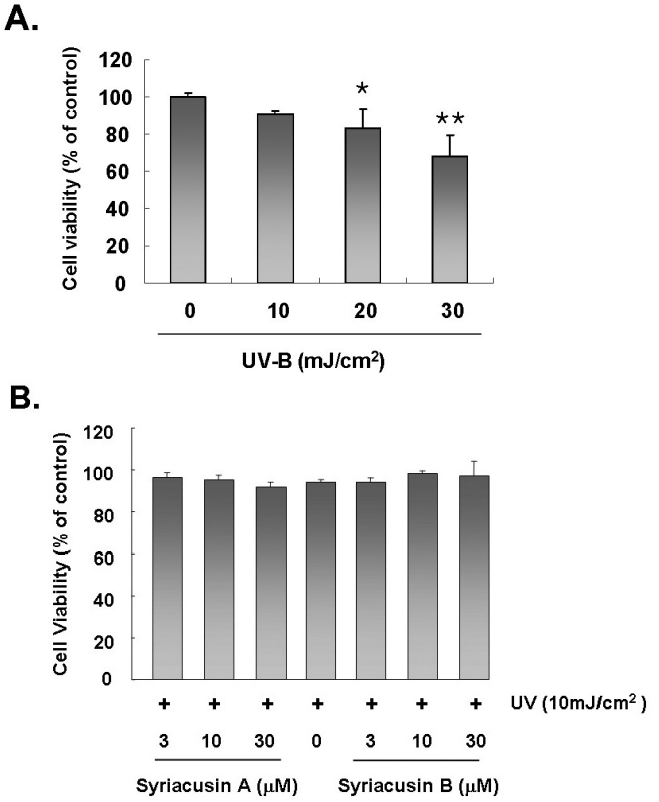

Fig. 3. Syriacusins A and B did not change cell viability exposed to UV-B irradiation. (A) Human dermal fibroblast cells (HDFCs) were irradiated with various energy of UV-B prior to incubation for 48. Cells viability of HDFCs was measured by MTT assay. Data represent mean ± SED. *$p<0.05$, **$p<0.01$, cell viability in UV-B irradiated-treated group was significantly different from control. (B) HDFCs were exposed to UV-B (10 mJ/cm^2) in the presence or absence of various concentrations of syriacusins A and B. Cells viability of HDFCs was measured by MTT assay.

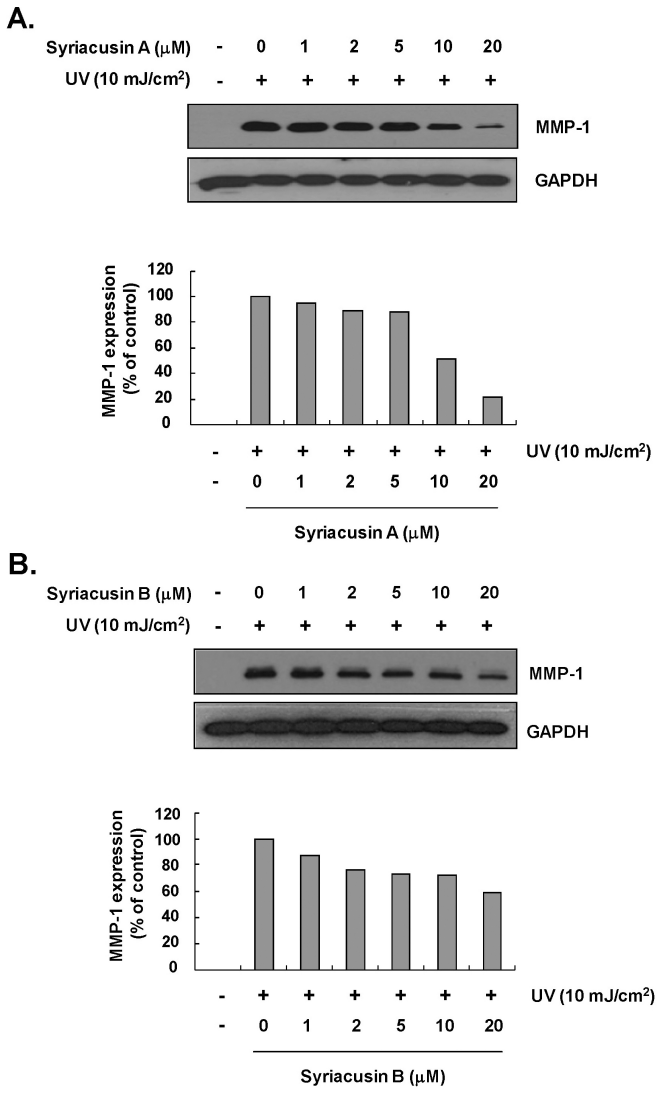

Fig. 4. UV-B-induced matrix metalloproteinase (MMP)-1 expression was inhibited by syriacusins A and B in human dermal fibroblast cells (HDFCs). (A) and (B) HDFCs were exposed to UV-B (10 mJ/cm^2) in the presence or absence of various concentrations of syriacusins A and B, respectively. MMP-1 expression was detected by Western blot analysis as described in materials and methods.

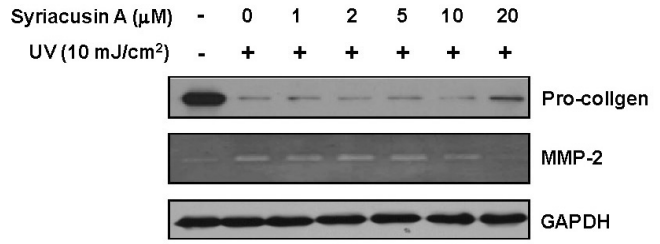

Fig. 5. Syriacusin A enhanced a production of pro-collagen and the enzyme activity of matrix metalloproteinase (MMP)-2 by UV-B irradiated human dermal fibroblast cells (HDFCs). HDFCs were exposed to UV-B (10 mJ/cm^2) in the presence or absence of various concentrations of syriacusin A. Pro-collagen and MMP-2 in UV-B irradiated HDFCs were detected by Western blot analysis and zymography, respectively as described in materials and methods.

UV-B irradiated HDFCs was decreased by 10 μM and 20 μM syriacusin A to 50% and 20% of untreated control, respectively. However, syriacusin B showed slightly decreased expression of MMP-1 (Fig. 4B). Syriacusin B treated with 20 μM reduced MMP-1 expression in UV-B irradiated HDFCs to 60% of untreated control. It demonstrates that syriacusin A could be a more effective compound to inhibit the degradation of collagen caused by UV irradiation.

Pro-collagen expression was increased by syriacusin A

To confirm the inhibitory effect of syriacusin A on MMP-1, we examined the effect of the production of type-I procollagen in UV-B-irradiated HDFCs. As shown in Fig. 5, the exposure of HDFCs to UV-B (10 mJ/cm^2) suppressed the level of type-I pro-collagen in the culture medium. However, the reduced type-I pro-collagen synthesis by UV-B irradiation was significantly attenuated by the incubation with syriacusin A at 20 μM.

Given that MMP-2 activities were increased by a chronical exposure to UV-B in hairless mice (Inomata *et al.*, 2003; Suganuma *et al.*, 2010) and the inhibition of gelatinase activities suppresses UV-B-induced wrinkle formation (Inomata *et al.*, 2003; Suganuma *et al.*, 2010), we measured the changes of MMP-2 activity caused by UV-B irradiation in HDFCs. As shown in Fig. 5, the exposure of HDFCs to UV-B (10 mJ/cm^2) enhanced MMP-2 expression. When HDFCs were incubated with syriacusin A at 20 μM immediately after UV-B irradiation, the increased MMP-2 expression was significantly attenuated to control level. It suggests that syriacusins A and B might be developed as possible agents to treat or prevent skin aging.

DISCUSSION

Regarding the environmental damage to skin, the most common physical injury is caused by UV irradiation. Photoaged skin is biochemically characterized by predominance of abnormal elastic fibers in the dermis and by a dramatic decrease in distinct interstitial collagens. Elastin is an important component of elastic fibers and also involved in inhibiting or repairing wrinkle formation (Labat-Robert *et al*., 2000), although collagen is a major factor in the skin. Elastase is a metalloproteinase which acts on degradation of elastin (Labat-Robert *et al*., 2000; Tsukahara *et al.*, 2001; Tzaphlidou, 2004). It is known that elastase activity is increased by UV-B irradiation (Kim *et al*., 2009; Xu *et al.*, 2010). MMP-mediated collagen damage is a major contributor to the phenotype of photoaged human skin (Varani *et al.*, 2001). Hence, development of MMP inhibitor is considered to be a promising strategy for skin prevention and/or treatment of UV-induced skin damage (Inomata *et al.*, 2003; Suganuma *et al.*, 2010). We have examined the anti-wrinkle effects of various plant extracts including syriacusins A, B and C isolated from *Hibiscus syriacus* by screening the inhibitory effect on human neutophil elastase (HNE). We also investigate whether syriacusins A, B and C are effective on the inhibition of UVB-induced MMP-1 expression and the production of type-I procollagen expression in cultured HDFCs. Data showed that syriacusin A inhibited UV-induced MMP-1 expression and enzymatic activity of MMP-2 and increased type-1 procollagen synthesis in UV-B irradiated HDFCs. However, a little inhibition to MMP-1 expression was shown by syriacusin B and a poor cell viability was detected by syriacusin C. It suggests that syriacusin A could be a more effective compound to inhibit skin aging caused by UV irradiation than syriacusin B or C.

UV irradiation is known to provoke oxidative stress through the generation of reactive oxygen species (ROS) in cells (Lee *et al.*, 1999; Tsukahara *et al.*, 2006; Imokawa, 2008; Imokawa, 2009). ROS generation could results in the subsequent activation of complex signaling pathways, followed by the damage on DNA in skin cell (Dong *et al.*, 2008; Imokawa, 2008). Earlier investigations indicated that the MAP kinase-mediated signal transduction plays an important role in the regulating a variety of cellular functions, including MMP-1 expression (Reunanen *et al.*, 2002; Di Girolamo *et al.*, 2003; Cortez *et al.*, 2007), type-I collagen (Amemiya *et al.*, 1999; Touyz *et al.*, 2001) and elastin (Choi et al., 2009) synthesis. Our result revealed that syriacusin A had no inhibitory effect on the intracellular ROS levels (data not shown). It implicates that the inhibitory ef-

fect of syriacusin A on MMP-1 is not mediated by its antioxidant effect but may be resulted from the modulation of another intracellular signal transduction molecules. It is required to define the effects of syriacusin A on the cell signal pathways in the further study

Collectively, syriacusins A, B and C were isolated from the *Hibiscus syriacus.* Among them, syriacusin A reduced the expression of MMP-1/2 and induced the expression of type-1 procollagen at the protein level in UV-irradiated cultured HDFCs. It suggests that syriacusin A might be developed as a possible agent to treat or prevent skin aging.

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REFERENCES

Amemiya, T., Sasamura, H., Mifune, M., Kitamura, Y., Hirahashi, J., Hayashi, M. and Saruta, T. (1999). Vascular endothelial growth factor activates MAP kinase and enhances collagen synthesis in human mesangial cells. *Kidney Int.* **56**, 2055-2063.

Antonicelli, F., Bellon, G., Debelle, L. and Hornebeck, W. (2007). Elastin-elastases and inflamm-aging. *Curr. Top. Dev. Biol.* **79**, 99-155.

Bernstein, E. F., Chen, Y. Q., Tamai, K., Shepley, K. J., Resnik, K. S., Zhang, H., Tuan, R., Mauviel, A. and Uitto, J. (1994). Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. *J. Invest. Dermatol.* **103**, 182-186.

Choi, W. S., Mitsumoto, A. and Kochevar, I. E. (2009). Involvement of reactive oxygen species in TGF-beta1-induced tropoelastin expression by human dermal fibroblasts. *Photochem. Photobiol.* **85**, 1425-1433.

Cortez, D. M., Feldman, M. D., Mummidi, S., Valente, A. J., Steffensen, B., Vincenti, M., Barnes, J. L. and Chandrasekar, B. (2007). IL-17 stimulates MMP-1 expression in primary human cardiac fibroblasts via p38 MAPK- and ERK1/2-dependent C/EBP-beta, NF-kappaB, and AP-1 activation. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H3356-3365.

Demeule, M., Brossard, M., Page, M., Gingras, D. and Beliveau, R. (2000). Matrix metalloproteinase inhibition by green tea catechins. *Biochim. Biophys. Acta.* **1478**, 51-60.

Denizot, F. and Lang, R. (1986). Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods.* **89**, 271-277.

Di Girolamo, N., Coroneo, M. T. and Wakefield, D. (2003). UVB-elicited induction of MMP-1 expression in human ocular surface epithelial cells is mediated through the ERK1/2 MAPK-dependent pathway. *Invest. Ophthalmol. Vis. Sci.* **44**, 4705-4714.

Dong, K. K., Damaghi, N., Picart, S. D., Markova, N. G., Obayashi, K., Okano, Y., Masaki, H., Grether-Beck, S., Krutmann, J., Smiles, K. A. and Yarosh, D. B. (2008). UV-induced DNA damage initiates release of MMP-1 in human skin. *Exp. Dermatol.* **17**, 1037-1044.

Fagot, D., Asselineau, D. and Bernerd, F. (2002). Direct role of human dermal fibroblasts and indirect participation of epidermal keratinocytes in MMP-1 production after UV-B irradiation. *Arch. Dermatol. Res.* **293**, 576-583.

Fagot, D., Asselineau, D. and Bernerd, F. (2004). Matrix metalloproteinase-1 production observed after solar-simulated radiation exposure is assumed by dermal fibroblasts but involves a paracrine activation through epidermal keratinocytes. *Photochem. Photobiol.* **79**, 499-505.

Girotti, A. W. and Kriska, T. (2004). Role of lipid hydroperoxides in photo-oxidative stress signaling. *Antioxid. Redox. Signal.* **6**, 301-310.

Hsu, H. Y., Chen, Y. P., Shen, S. J., Hsu, C. S., Chen, C. C. and Chang, H. C. (1986). *Oriental Material Medica, a Concise Guide,* pp. 503-504. Healing Arts Institute, Taipei.

Huang, K. C., (1993). *The Pharmacology of Chinese Herbs*, pp. 193-194. CRC Press, Tokyo.

Imokawa, G. (2008). Recent advances in characterizing biological mechanisms underlying UV-induced wrinkles: a pivotal role of fibrobrast-derived elastase. *Arch. Dermatol. Res.* **300(Suppl 1)**, S7-20.

Imokawa, G. (2009). Mechanism of UVB-induced wrinkling of the skin: paracrine cytokine linkage between keratinocytes and fibroblasts leading to the stimulation of elastase. *J. Investig. Dermatol. Symp. Proc.* **14**, 36-43.

Inomata, S., Matsunaga, Y., Amano, S., Takada, K., Kobayashi, K., Tsunenaga, M., Nishiyama, T., Kohno, Y. and Fukuda, M. (2003). Possible involvement of gelatinases in basement membrane damage and wrinkle formation in chronically ultraviolet B-exposed hairless mouse. *J. Invest. Dermatol.* **120**, 128-134.

Kahari, V. M. and Saarialho-Kere, U. (1997). Matrix metalloproteinases in skin. *Exp. Dermatol.* **6**, 199-213.

Kim, Y. H., Ryoo, I. J., Choo, S. J., Xu, G. H., Lee, S. K., Seok, S. J., Bae, K. H. and Yoo, I. D. (2009). Clitocybin D, a novel human neutrophil elastase inhibitor from the culture broth of clitocybe aurantiaca. *J. Micro. Biotech.* **19**, 1139-1141.

Kligman, A. M. (1969). Early destructive effect of sunlight on human skin. *JAMA* **210**, 2377-2380.

Labat-Robert, J., Fourtanier, A., Boyer-Lafargue, B. and Robert, L. (2000). Age dependent increase of elastase type protease activity in mouse skin. Effect of UV-irradiation. *J. Photochem. Photobiol. B* **57**, 113-118.

Lee, S. J., Yun, Y. S., Lee, I. K., Ryoo, I. J., Yun, B. S. and Yoo, I. D. (1999). An antioxidant lignan and other constituents from the root bark of Hibiscus syriacus. *Planta Med.* **65**, 658-660.

Reunanen, N., Li, S. P., Ahonen, M., Foschi, M., Han, J. and Kahari, V. M. (2002). Activation of p38 alpha MAPK enhances collagenase-1 (matrix metalloproteinase (MMP)-1) and stromelysin-1 (MMP-3) expression by mRNA stabilization. *J. Biol. Chem.* **277**, 32360-32368.

Rittie, L. and Fisher, G. J. (2002). UV-light-induced signal cascades and skin aging. *Ageing Res. Rev.* **1**, 705-720.

Suganuma, K., Nakajima, H., Ohtsuki, M. and Imokawa, G. (2010). Astaxanthin attenuates the UVA-induced up-regulation of matrix-metalloproteinase-1 and skin fibroblast elastase in human dermal fibroblasts. *J. Dermatol. Sci.* **58**, 136-142.

Touyz, R. M., He, G., El Mabrouk, M. and Schiffrin, E. L. (2001). p38 Map kinase regulates vascular smooth muscle cell collagen synthesis by angiotensin II in SHR but not in WKY. *Hypertension* **37**, 574-580.

Tsuji, N., Moriwaki, S., Suzuki, Y., Takema, Y. and Imokawa, G. (2001). The role of elastases secreted by fibroblasts in wrinkle formation: implication through selective inhibition of elastase activity. *Photochem Photobiol* **74**, 283-290.

Tsukahara, K., Nakagawa, H., Moriwaki, S., Takema, Y., Fujimura, T. and Imokawa, G. (2006). Inhibition of ultraviolet-B-induced wrinkle formation by an elastase-inhibiting herbal extract: implication for the mechanism underlying elastase-associated wrinkles. *Int. J. Dermatol.* **45**, 460-468.

Tsukahara, K., Takema, Y., Moriwaki, S., Tsuji, N., Suzuki, Y., Fujimura, T. and Imokawa, G. (2001). Selective inhibition of skin fibroblast elastase elicits a concentration-dependent prevention of ultraviolet B-induced wrinkle formation. *J. Invest. Dermatol.* **117**, 671-677.

Tzaphlidou, M. (2004). The role of collagen and elastin in aged skin: an image processing approach. *Micron.* **35**, 173-177.

Varani, J., Spearman, D., Perone, P., Fligiel, S. E., Datta, S. C., Wang, Z. Q., Shao, Y., Kang, S., Fisher, G. J. and Voorhees, J. J. (2001). Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. *Am. J. Pathol.* **158**, 931-942.

Wiedow, O., Schroder, J. M., Gregory, H., Young, J. A. and Christophers, E. (1990). Elafin: an elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. *J. Biol. Chem.* **265**, 14791-14795.

Xu, G. H., Kim, Y. H., Chi, S. W., Choo, S. J., Ryoo, I. J., Ahn, J. S. and Yoo, I. D. (2010). Evaluation of human neutrophil elastase inhibitory effect of iridoid glycosides from *Hedyotis diffusa*. *Bioorg. Med. Chem. Lett.* **20**, 513-515.

Yoo, I. D., Yun, B. S., Lee, I. K., Ryoo, I. J., Choung, D. H. and Han, K. H. (1998). Three naphthalenes from root bark of *Hibiscus syriacus*. *Phytochemistry* **47**, 799-802.