The Protective Effects of Piceatannol from Passion Fruit (*Passiflora edulis*) Seeds in UVB-Irradiated Keratinocytes

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The use of naturally occurring botanicals with substantial antioxidant activity to prevent photoageing is receiving increasing attention. We have previously identified piceatannol and scirpusin B, which is a dimer of piceatannol, as strong antioxidants that are present in passion fruit (*Passiflora edulis*) seeds. In the present study, the effects of passion fruit seed extract, piceatannol, and scirpusin B on human keratinocytes were investigated. The passion fruit seed extract and piceatannol upregulated the glutathione (GSH) levels in keratinocytes in a dose-dependent manner, indicating that piceatannol is an active component of the passion fruit seed extract in keratinocytes. The pretreatment with piceatannol also supressed the UVB-induced generation of reactive oxygen species (ROS) in the keratinocytes. In addition, the transfer of the medium from the UVB-irradiated keratinocytes to non-irradiated fibroblasts enhanced matrix-metalloproteinase (MMP)-1 activity, and this MMP-1 induction was reduced when the keratinocytes were pretreated with piceatannol. These results suggest that piceatannol attenuates the UVB-induced activity of MMP-1 along with a reduction of ROS generation in keratinocytes. Thus, piceatannol and passion fruit seed extract containing high amounts of piceatannol are potential anti-photoageing cosmetic ingredients.

Key words piceatannol; passion fruit seed; keratinocyte; ultraviolet; reactive oxygen species; matrix-metalloproteinase

Solar UV irradiation damages human skin causing it to age prematurely, a process referred to as photoageing, and to develop the characteristics of thickened epidermis and increased melanogenesis.[1–3] In contrast, intrinsic (chronological) ageing is characterised by skin atrophy accompanied by a loss of elasticity and reduced metabolic activity.[4–8]

A hallmark of photoageing is the disorganisation of collagen, the major structural component of the skin. Collagen is produced mainly by dermal fibroblasts and is degraded by the matrix metalloproteinases (MMPs) that are secreted by various cells, including keratinocytes, fibroblasts, and inflammatory cells.[2,8,9] UV irradiation, particularly UVB (290–320 nm) and UVA (315–400 nm), induces the expression of matrix metalloproteinase-1 (MMP-1) in the fibroblasts, which is mainly responsible for the degradation of the dermal collagen in the ageing process of human skin. Therefore, UV-induced MMP-1 is considered to contribute substantially to the connective tissue damage that causes severe collagen deficiency and wrinkling during photoageing.[2,8] Although keratinocytes do not produce MMP-1 in response to UVB irradiation, damaged epidermal keratinocytes indirectly play important roles in the release of MMP-1. It has been reported that the culture medium of UVB-irradiated keratinocytes stimulates MMP-1 release from fibroblasts more efficiently than the direct irradiation of the fibroblasts.[10,11]

The primary mechanism by which UV irradiation initiates molecular responses in human skin is through the photochromic generation of reactive oxygen species (ROS). ROS cause oxidative damage and decrease the levels of non-enzymatic antioxidants, such as glutathione (GSH), resulting in the subsequent activation of complex signalling pathways and MMP induction.[3,12] ROS are also involved in intrinsic ageing, and the main source of excess ROS in intrinsic ageing is mitochondrial oxidative energy generation. During the skin ageing process, the ROS levels increase, and the antioxidant defences decline.[13,14]

Antioxidants are regarded as promising agents that reduce such oxidative stress. In recent years, naturally occurring compounds, such as phenolic acids, flavonoids, and high molecular weight polyphenols, have gained considerable attention as beneficial protective agents.[15] Numerous studies have shown that polyphenols, such as epigallocatechin gallate (EGCG),[16] resveratrol (*trans*-3,5,4′-trihydroxystilbene),[17] and quercetin (3,5,7,3′,4′-pentahydroxyflavon),[18] prevent UV-induced skin ageing.

Passion fruit (*Passiflora edulis* Sims.) is a vine species of the passion flower family (Passifloraceae) indigenous to the tropical regions of America and is known as a medicinal herb. Previous studies have reported that the leaves, vines, and flowers of *P. edulis* contain polyphenolic compounds with many biological effects, including anti-anxiety,[19,20] anti-inflamation,[21,22] and cough-suppressant effects.[23] The fruit (called the passion fruit) is often eaten together with the seeds.

We previously discovered that passion fruit seeds contain large amounts of piceatannol[24] and scirpusin B,[25] natural polyphenolic compounds that have strong antioxidant activities.[25]

In the present study, the effects of a passion fruit seed extract, piceatannol, and scirpusin B on the GSH levels in human keratinocytes were investigated. In addition, the effects of pretreatment with piceatannol on UVB-irradiated keratinocytes were evaluated by examining the ROS generation in keratinocytes and MMP-1 activity in fibroblasts.

The authors declare no conflict of interest.

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MATERIALS AND METHODS

Materials Normal human keratinocytes, normal human fibroblasts, Humedia KG2, and Humedia KB2 were obtained from Kurabo (Osaka, Japan). 2′,7′-Dichlorodihydrofluorescein diacetate (H_2DCFDA) was purchased from Calbiochem (San Diego, CA, U.S.A.). Glutathione reductase, Triton X-100, and trypsin were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB) was purchased from Wako (Osaka, Japan). Nicotinamide-adenine dinucleotide phosphate (NADPH) was purchased from Oriental Yeast Industries (Tokyo, Japan). The bicinchoninic acid (BCA) protein assay reagent was obtained from Pierce (Rockford, IL, U.S.A.). Fluorescein isothiocyanate (FITC)-labelled type I collagen was purchased from the Collagen Research Center (Tokyo, Japan). Collagenase type I was obtained from Worthington Biochemical (Lakewood, NJ, U.S.A.). All of the other chemicals were of analytical reagent grade.

UVB Irradiation For the irradiation, a Philips TL20W/12RS UV-B MEDICAL instrument (Philips, Eindhoven, The Netherlands) equipped with a long-path filter above 300 nm (Asahi Spectra LU0300, Tokyo, Japan) was used. The exposure to UVB irradiation was performed at 40 or 10 mJ/cm^2 for 5 min. In parallel, non-irradiated cells were kept in the dark during the UVB irradiation.

Sample Preparation (Passion Fruit Seeds, Piceatannol, and Scirpusin B) Freeze-dried and milled passion fruit seeds were used. The passion fruit seeds were extracted using 30% 1,3-butylene glycol (BG), which was removed prior to the assay. Briefly, the 30% BG extract was concentrated by evaporation, and the solvent was removed by freeze-drying. The powder contained piceatannol (37.06 μg/mg) and scirpusin B (14.98 μg/mg), which were purified using a previously described protocol[24,25]; 99% pure piceatannol and 91.8% pure scirpusin B were produced. Piceatannol was purchased from Tokyo Chemical Industry (Tokyo, Japan) and used in the GSH and ROS experiments. There was no difference between the extract and the reagent (data not shown).

GSH Level Normal human keratinocytes were seeded at a density of 2.0×10^4 cells per 96-well dish in Humedia KG2 medium. At 24 h after plating, the cells were treated with fresh Humedia KG2 medium containing the indicated treatments for 24 h. The cells were sonicated, and the total GSH level was determined using the glutathione reductase recycling method. A 50 μL aliquot of the suspension was mixed with 125 μL phosphate buffer (0.1 M, pH 7.5) and incubated with 25 μL NADPH and 25 μL glutathione reductase (3.2 units/mL) for 10 min at 37°C. After the incubation, 25 μL of 10 mM DTNB was added. The reaction was followed as the ΔA450, and the total GSH content was calculated using a standard curve. The protein concentration of the suspension was determined using the BCA protein assay reagent.

Intracellular ROS Measurement Normal human keratinocytes were seeded at a density of 2.0×10^4 cells per 96-well dish in Humedia KG2 medium. At 24 h after plating, the cells were treated with fresh Humedia KG2 medium containing piceatannol (0–2 μg/mL) for 24 h. The intracellular ROS levels were determined using the dichlorodihydrofluorescein assay. The polar, non-fluorescent substrate dichlorodihydrofluorescein diacetate (H_2DCFDA) undergoes deacetylation by cytosolic esterases to form dichlorodihydrofluorescein, which reacts with ROS and gives rise to the fluorescent derivative dichlorofluorescein. The fluorescence was monitored at specific excitation/emission wavelengths (488/530 nm).

The cells were incubated with 20 μM H_2DCFDA for 30 min and washed with Hanks' buffered solution without Ca^{2+} and Mg^{2+}. The cells were lysed with 100 μL Triton X-100 (0.5%) after UVB irradiation, and the fluorescence was measured using a fluorescence plate reader. The protein concentration was determined using the BCA protein assay reagent. The level of intracellular ROS was expressed as the relative fluorescence intensity per gram protein; the level of the non-treated cells was set at 100%. The cellular ROS levels were observed using a fluorescence microscope.

Assay of MMP-1 Normal human keratinocytes were seeded at a density of 5.0×10^5 cells per 6-well dish in Humedia KG2. At 24 h after plating, the cells were treated with Humedia KB2 medium containing piceatannol (0–1 μg/mL) for 24 h. The cells were irradiated with UVB in Hanks' balanced salt solution without Ca^{2+} and Mg^{2+}. After irradiation, the cells were cultured in fresh Humedia KB2 without piceatannol for 24 h. This medium was termed the "keratinocyte-conditioned medium."

Normal human fibroblasts were seeded at a density of 2.0×10^4 cells per 96-well dish in Dulbecco's modified Eagle's medium with 5% fetal bovine serum (FBS-DMEM). At 24 h after plating, the cells were treated with the keratinocyte-conditioned medium for 48 h, and the amount of MMP-1 secreted into the culture medium was determined.

An 80 μL sample of the culture supernatant was reacted with 10 μL trypsin (0.05 mg/mL) for 15 min at 37°C, and 10 μL soybean trypsin inhibitor (0.25 mg/mL) was added to terminate the reaction. The MMP-1 activity was estimated at 37°C for 2 h using fluorescein isothiocyanate (FITC)-labelled type I collagen as a substrate. The reacted culture medium was incubated with FITC-labelled type I collagen, and the fluorescence was monitored at specific excitation/emission wavelengths (495/520 nm). For the MMP-1 activity, one unit was defined as the amount of enzyme necessary to degrade 1 μg of type I collagen per min at 37°C. Non-cultured medium and collagenase type I were used as the negative and positive controls, respectively. The protein concentration was determined using the BCA protein assay reagent.

Statistical Analysis The data were expressed as the mean±S.D. A statistical comparison analysis was performed using the Student's *t*-test; $p<0.05$ was considered statistically significant.

RESULTS

The Effect of Passion Fruit Seed Extract on the Glutathione Levels in Human Keratinocytes To investigate the effect of the passion fruit seed extract on human keratinocytes, the level of glutathione (GSH), which is the most important cellular non-enzymatic antioxidant, was measured after the keratinocytes were treated with the passion fruit seed extract, piceatannol, or scirpusin B. The keratinocytes treated with the passion fruit seed extract or piceatannol for 24 h exhibited a dose-dependent GSH induction. The passion fruit seed extract increased the GSH level by 17% ($p<0.0005$) at 6.25 μg/mL, 33% ($p<0.0005$) at 12.5 μg/mL, and 77% ($p<0.005$) at 25 μg/mL. Piceatannol increased the GSH level by 13% ($p<0.05$) at

0.25 μg/mL, 22% ($p<0.05$) at 1 μg/mL, and 63% ($p<0.0005$) at 2 μg/mL. Scirpusin B slightly increased the GSH level by 12% ($p<0.05$) at 2 μg/mL (Fig. 1). The cellular protein levels were not affected by treatment with passion fruit seed extract or scirpusin B, whereas the protein level was lower after treatment with 2 μg/mL piceatannol (data not shown).

The Effect of Piceatannol on UVB-Induced ROS Generation in Human Keratinocytes To investigate the effect of piceatannol on UVB-induced ROS generation, the keratinocytes were treated with piceatannol for 24 h prior to UVB (40 mJ/cm^2) irradiation. Whereas the UVB irradiation induced intracellular ROS 1.8-fold, pretreatment with piceatannol led to a dose-dependent decrease in the ROS level both in non-irradiated and irradiated keratinocytes. The ROS level in the irradiated keratinocytes was decreased by 13% ($p<0.05$) at 0.5 μg/mL, 21% ($p<0.005$) at 1 μg/mL and 58% ($p<0.0005$) at 2 μg/mL compared to the un-treated cells. The ROS level in the non-irradiated keratinocytes was decreased by 8% ($p<0.0005$) at 1 μg/mL and 22% ($p<0.0005$) at 2 μg/mL compared to the un-treated cells. Interestingly, a low concentration (0.0625–0.125 μg/mL) of piceatannol slightly (4–5%, $p<0.05$) increased the ROS level in the non-irradiated keratinocytes (Fig. 2).

Indirect Effect of Piceatannol on MMP-1 Activation in Fibroblasts Treated with UVB-Irradiated Keratinocyte-Conditioned Medium To determine the indirect effect of piceatannol on fibroblasts, the keratinocytes were incubated with piceatannol for 24 h prior to UVB (10 mJ/cm^2) irradiation, and the medium from the UVB-irradiated keratinocytes was applied to non-irradiated fibroblasts. The MMP-1 activity in the non-irradiated fibroblasts increased 2-fold following the addition of the medium from the UVB-irradiated keratinocytes. When fibroblasts were exposed to conditioned medium from cultured keratinocytes treated with piceatan-

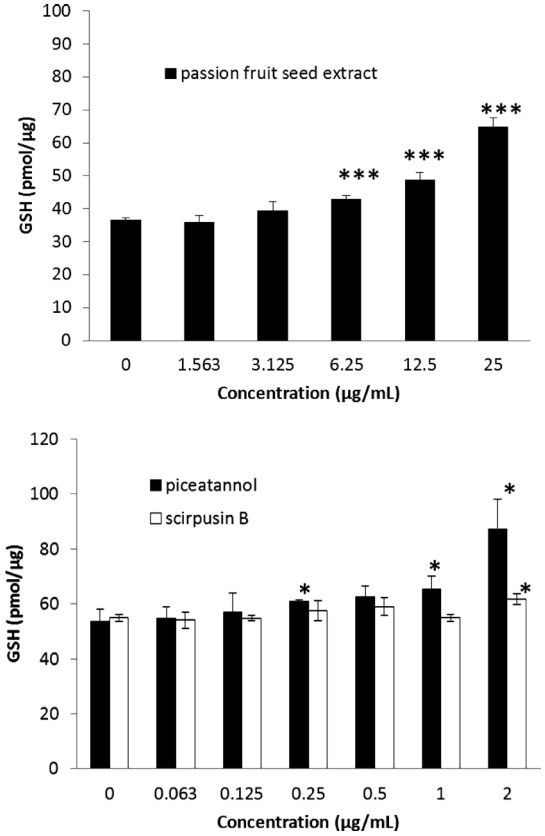

Fig. 1. Intracellular GSH Level in Keratinocytes

Keratinocytes were treated with various concentrations of passion fruit seed extract, piceatannol or scirpusin B for 24 h. The GSH levels were measured using DTNB, as described in Materials and Methods. The values are the mean±S.D. (n=3–5). The statistical analysis was performed using the Student's t-test. *$p<0.05$, ***$p<0.0005$, significantly different from the non-treatment group.

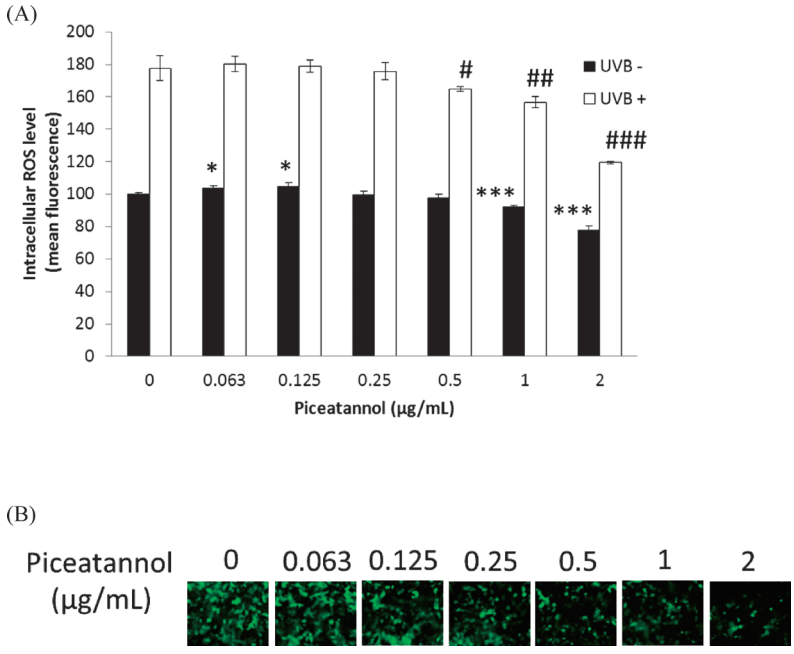

Fig. 2. The Effect of Piceatannol on ROS Generation in Keratinocytes

Keratinocytes were pretreated with various concentrations of piceatannol for 24 h prior to UVB irradiation (40 mJ/cm^2). (A) At 5 min after the UVB irradiation, the intracellular ROS generation was measured using the H_2DCFDA method, as described in Materials and Methods. The values are the mean±S.D. (n=4). The statistical analysis was performed using the Student's t-test. *$^{\#}p<0.05$, $^{\#\#}p<0.005$, ***$^{\#\#\#}p<0.0005$, significantly different from each non-treatment group. (B) Representative fluorescence microscopy images showing the decrease of fluorescence intensity of H_2DCF produced by ROS.

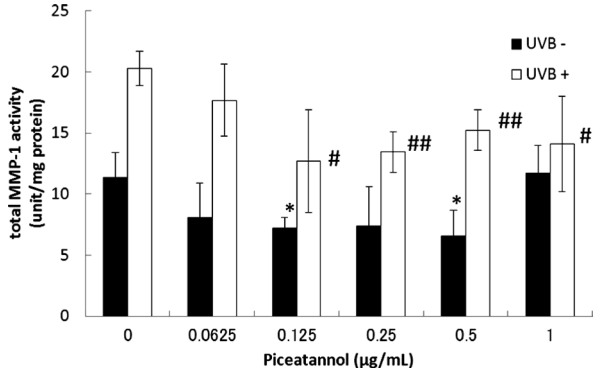

Fig. 3. The Indirect Effect of Piceatannol on the MMP-1 Activity in Fibroblasts

Keratinocytes were pretreated with various concentrations of piceatannol for 24 h prior to UVB irradiation (10 mJ/cm^2). At 5 min after the UVB-irradiation, the keratinocytes were cultured in fresh medium (keratinocyte-conditioned medium) for 24 h. Fibroblasts were then treated with the keratinocyte-conditioned media for 48 h, and the amount of MMP-1 secreted into the cultured medium was determined as described in Materials and Methods. The values are the mean±S.D. (n=4). The statistical analysis was performed using the Student's t-test. $^{*,\#}p<0.05$, $^{\#\#}p<0.005$, significantly different from each non-treatment group.

nol prior to UVB, the MMP-1 activity was suppressed by 37% ($p<0.05$) at 0.125 μg/mL, 34% ($p<0.005$) at 0.25 μg/mL, 25% ($p<0.005$) at 0.5 μg/mL, and 30% ($p<0.05$) at 1 μg/mL compared to the un-treated cells. In addition, the conditioned medium used for cultured keratinocytes with piceatannol without UVB irradiation also suppressed the MMP-1 activity in the fibroblasts by 37% ($p<0.05$) at 0.125 μg/mL and 42% ($p<0.05$) at 0.5 μg/mL (Fig. 3).

DISCUSSION

UV irradiation induces ROS generation in the skin, and it is the primary cause of photoageing.[26] When ROS are not eliminated by the antioxidant defence systems, the results include oxidative stress and increased skin aging. However, botanical antioxidants may be promising reagents for the prevention of photoageing. Cosmetic ingredients are generally applied to epidermis, so we focused on the direct effect on keratinocytes. In this report, we examined the anti-photoageing efficacy of passion fruit seed extract in UVB-irradiated keratinocytes.

Intracellular GSH plays important roles in protecting the skin from the oxidative stress caused by various chemicals and UV irradiation. UV irradiation is known to deplete GSH levels, and UVB-induced GSH depletion is believed to be involved in the pathogenesis of several skin disorders. In this study, we showed that piceatannol and a passion fruit seed extract increased the intracellular GSH levels in a dose-dependent manner in keratinocytes. Piceatannol has previously been reported to increase the GSH level in B16 melanoma cells,[27] while UVB irradiation have no effect on the GSH level in melanocytes.[28] Our finding that GSH levels increased in keratinocytes is important for UV protection in skin.[29] The passion fruit seed extract contains 127.8 μg/mg piceatannol, but the piceatannol concentration was decreased by two thirds during the powdering process (data not shown). The treatment with the passion fruit seed extract showed effects that were similar to those of piceatannol alone (at similar piceatannol concentrations), indicating that piceatannol is the principal active ingredient in the passion fruit seed extract based on the effect on keratinocytes. These data suggest that piceatannol changes the oxidative status in keratinocytes. Scirpusin B, a dimer of piceatannol, has a stronger antioxidant activity than the piceatannol monomer,[25] although the GSH induction of scirpusin B was less than that of piceatannol. This result may be attributed to the cell permeability and distribution and stability of the components.

In addition, we examined the effect of piceatannol on ROS generation in keratinocytes. We found that pretreatment with piceatannol suppressed ROS generation in both non-irradiated and UVB-irradiated keratinocytes. Pretreatment with passion fruit seed extract also suppressed ROS generation similar to piceatannol (data not shown). Thus, piceatannol-induced GSH may contribute to decreasing the levels of ROS: piceatannol acts as an antioxidant, and incorporated piceatannol may directly quench ROS. However, at low concentrations, piceatannol increased the ROS generation in the non-irradiated keratinocytes, which may be an example of the common phenomenon in which antioxidants act as pro-oxidants under certain conditions.

One characteristic feature of photoageing is the breakdown of connective tissue caused by MMP-1. Because it has been reported that the UVB-triggered production of ROS induces MMP-1,[26,30] we focused on MMP-1 induction in fibroblasts to evaluate the anti-ageing efficacy of piceatannol in the UVB-irradiated keratinocytes. First, we confirmed that the medium from the UVB-irradiated keratinocytes stimulated the MMP-1 activity in the fibroblasts. We then found that piceatannol suppressed this MMP-1 induction in the fibroblasts when the keratinocytes were pretreated with piceatannol prior to UVB irradiation. This finding may be linked to the ROS suppression by piceatannol. MMP-1 suppression by piceatannol is not dose-dependent, though ROS suppression by piceatannol is dose-dependent. This result suggests that a little suppression of ROS in keratinocytes is enough to suppress MMP-1 in fibroblasts in this condition. Interestingly, even when the cells were not irradiated, the medium from the keratinocytes treated with piceatannol also suppressed the MMP-1 induction in the fibroblasts. The MMP-1 suppression by the non-irradiated keratinocyte medium could be due to any number of molecular pathways.

Piceatannol is known to display a wide spectrum of biological activities.[31] For instance, piceatannol has been shown to suppress the activation of some transcription factors, including nuclear factor kappa B (NF-κB).[32] Piceatannol also inhibits Janus kinase 1 (JAK1) and spleen tyrosine kinase.[33,34] Piceatannol has positive effects on cultured fibroblasts, including the inhibition of the JAK1/signal transducer and activator of transcription-1 (STAT-1) pathway, which induces the expression of the MMP-1 gene,[35] the inhibition of melanogenesis, and the promotion of collagen synthesis.[24,27] Taken together, these results strongly suggest that piceatannol and passion fruit seed extracts containing high amounts of piceatannol may be used as novel anti-photoageing cosmetic ingredients. Additional *in vivo* studies are needed before this compound can be used by humans.

REFERENCES

1) Kligman LH, Kligman AM. The nature of photoaging: its prevention and repair. *Photodermatology*, **3**, 215–227 (1986).

2) Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N. Engl. J. Med.*, **337**, 1419–1428 (1997).
3) Fisher GJ, Talwar HS, Lin J, Voorhees JJ. Molecular mechanisms of photoaging in human skin *in vivo* and their prevention by all-*trans* retinoic acid. *Photochem. Photobiol.*, **69**, 154–157 (1999).
4) Lavker RM, Zheng PS, Dong G. Aged skin: a study by light, transmission electron, and scanning electron microscopy. *J. Invest. Dermatol.*, **88** (Suppl.), 44s–51s (1987).
5) Gilchrest BA. Skin aging and photoaging: an overview. *J. Am. Acad. Dermatol.*, **21**, 610–613 (1989).
6) Jenkins G. Molecular mechanisms of skin ageing. *Mech. Ageing Dev.*, **123**, 801–810 (2002).
7) Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, Voorhees JJ. Mechanisms of photoaging and chronological skin aging. *Arch. Dermatol.*, **138**, 1462–1470 (2002).
8) Chung JH, Seo JY, Choi HR, Lee MK, Youn CS, Rhie G, Cho KH, Kim KH, Park KC, Eun HC. Modulation of skin collagen metabolism in aged and photoaged human skin *in vivo*. *J. Invest. Dermatol.*, **117**, 1218–1224 (2001).
9) Cutroneo KR. How is Type I procollagen synthesis regulated at the gene level during tissue fibrosis. *J. Cell. Biochem.*, **90**, 1–5 (2003).
10) Fagot D, Asselineau D, Bernerd F. 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 (2002).
11) Dong KK, Damaghi N, Picart SD, Markova NG, Obayashi K, Okano Y, Masaki H, Grether-Beck S, Krutmann J, Smiles KA, Yarosh DB. UV-induced DNA damage initiates release of MMP-1 in human skin. *Exp. Dermatol.*, **17**, 1037–1044 (2008).
12) Kang S, Chung JH, Lee JH, Fisher GJ, Wan YS, Duell EA, Voorhees JJ. Topical *N*-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin *in vivo*. *J. Invest. Dermatol.*, **120**, 835–841 (2003).
13) Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science*, **273**, 59–63 (1996).
14) Ma W, Wlaschek M, Tantcheva-Poór I, Schneider LA, Naderi L, Razi-Wolf Z, Schüller J, Scharffetter-Kochanek K. Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. *Clin. Exp. Dermatol.*, **26**, 592–599 (2001).
15) Svobodová A, Psotová J, Walterová D. Natural phenolics in the prevention of UV-induced skin damage. A review. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, **147**, 137–145 (2003).
16) Bae JY, Choi JS, Choi YJ, Shin SY, Kang SW, Han SJ, Kang YH. (−)Epigallocatechin gallate hampers collagen destruction and collagenase activation in ultraviolet-B-irradiated human dermal fibroblasts: involvement of mitogen-activated protein kinase. *Food Chem. Toxicol.*, **46**, 1298–1307 (2008).
17) Liu Y, Chan F, Sun H, Yan J, Fan D, Zhao D, An J, Zhou D. Resveratrol protects human keratinocytes HaCaT cells from UVA-induced oxidative stress damage by downregulating Keap1 expression. *Eur. J. Pharmacol.*, **650**, 130–137 (2011).
18) Erden Inal M, Kahraman A, Köken T. Beneficial effects of quercetin on oxidative stress induced by ultraviolet A. *Clin. Exp. Dermatol.*, **26**, 536–539 (2001).
19) Barbosa PR, Valvassori SS, Bordignon CL Jr, Kappel VD, Martins MR, Gavioli EC, Quevedo J, Reginatto FH. The aqueous extracts of *Passiflora alata* and *Passiflora edulis* reduce anxiety-related behaviors without affecting memory process in rats. *J. Med. Food*, **11**, 282–288 (2008).
20) Coleta M, Batista MT, Campos MG, Carvalho R, Cotrim MD, Lima TC, Cunha AP. Neuropharmacological evaluation of the putative anxiolytic effects of *Passiflora edulis* Sims., its sub-fractions and flavonoid constituents. *Phytother. Res.*, **20**, 1067–1073 (2006).
21) Montanher AB, Zucolotto SM, Schenkel EP, Fröde TS. Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. *J. Ethnopharmacol.*, **109**, 281–288 (2007).
22) Vargas AJ, Geremias DS, Provensi G, Fornari PE, Reginatto FH, Gosmann G, Schenkel EP, Fröde TS. *Passiflora alata* and *Passiflora edulis* spray-dried aqueous extracts inhibit inflammation in mouse model of pleurisy. *Fitoterapia*, **78**, 112–119 (2007).
23) Dhawan K, Sharma A. Antitussive activity of the methanol extract of *Passiflora incarnata* leaves. *Fitoterapia*, **73**, 397–399 (2002).
24) Matsui Y, Sugiyama K, Kamei M, Takahashi T, Suzuki T, Katagata Y, Ito T. Extract of passion fruit (*Passiflora edulis*) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis. *J. Agric. Food Chem.*, **58**, 11112–11118 (2010).
25) Sano S, Sugiyama K, Ito T, Katano Y, Ishihata A. Identification of the strong vasorelaxing substance scirpusin B, a dimer of piceatannol, from passion fruit (*Passiflora edulis*) seeds. *J. Agric. Food Chem.*, **59**, 6209–6213 (2011).
26) Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, Meewes C, Wlaschek M. Photoaging of the skin from phenotype to mechanisms. *Exp. Gerontol.*, **35**, 307–316 (2000).
27) Yokozawa T, Kim YJ. Piceatannol inhibits melanogenesis by its antioxidative actions. *Biol. Pharm. Bull.*, **30**, 2007–2011 (2007).
28) Larsson P, Andersson E, Johansson U, Ollinger K, Rosdahl I. Ultraviolet A and B affect human melanocytes and keratinocytes differently. A study of oxidative alterations and apoptosis. *Exp. Dermatol.*, **14**, 117–123 (2005).
29) Zhu M, Bowden GT. Molecular mechanism(s) for UV-B irradiation-induced glutathione depletion in cultured human keratinocytes. *Photochem. Photobiol.*, **80**, 191–196 (2004).
30) Brenneisen P, Wenk J, Wlaschek M, Krieg T, Scharffetter-Kochanek K. Activation of p70 ribosomal protein S6 kinase is an essential step in the DNA damage-dependent signaling pathway responsible for the ultraviolet B-mediated increase in interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) protein levels in human dermal fibroblasts. *J. Biol. Chem.*, **275**, 4336–4344 (2000).
31) Piotrowska H, Kucinska M, Murias M. Biological activity of piceatannol: leaving the shadow of resveratrol. *Mutat. Res.*, **750**, 60–82 (2012).
32) Ashikawa K, Majumdar S, Banerjee S, Bharti AC, Shishodia S, Aggarwal BB. Piceatannol inhibits TNF-induced NF-kappaB activation and NF-kappaB-mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. *J. Immunol.*, **169**, 6490–6497 (2002).
33) Su L, David M. Distinct mechanisms of STAT phosphorylation *via* the interferon-alpha/beta receptor. Selective inhibition of STAT3 and STAT5 by piceatannol. *J. Biol. Chem.*, **275**, 12661–12666 (2000).
34) Geahlen RL, McLaughlin JL. Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem. Biophys. Res. Commun.*, **165**, 241–245 (1989).
35) Kim S, Kim Y, Lee Y, Chung JH. Ceramide accelerates ultraviolet-induced MMP-1 expression through JAK1/STAT-1 pathway in cultured human dermal fibroblasts. *J. Lipid Res.*, **49**, 2571–2581 (2008).