

# Protective effects of compounds from *Garcinia mangostana* L. (mangosteen) against UVB damage in HaCaT cells and hairless mice

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**Abstract.** Ultraviolet B (UVB) radiation causes alterations in the skin, such as epidermal thickening, wrinkle formation and inflammation. Therefore, preventing UVB-induced skin damage can promote general health among the human population. *Garcinia mangostana* L. (mangosteen) is a fruit that has become a popular botanical dietary supplement because of its perceived role in promoting overall health. The present study investigated the photoprotective effects of  $\alpha$ -,  $\beta$ -,  $\gamma$ -mangostins and gartanin against UVB radiation using the HaCaT immortalized human keratinocyte cell line as an *in vitro* model and hairless mice as an *in vivo* model. UVB radiation increased the expression of matrix metalloproteinase (MMP)-1 and -9 and decreased the mRNA expression levels of involucrin, filaggrin and loricrin in HaCaT cells; however, these changes were attenuated by pretreating the cells with  $\alpha$ -,  $\beta$ -,  $\gamma$ -mangostins and gartanin. Among these compounds,  $\alpha$ -mangostin exhibited the greatest effects in reducing UVB-induced skin wrinkles, inhibited epidermal thickening in hairless mice *in vivo*. Exposure to UVB radiation increased the expression of MMPs and pro-inflammatory cytokines and activated mitogen-activated protein kinases in hairless mice, but these changes

were attenuated by  $\alpha$ -mangostin. The authors suggested that  $\alpha$ -mangostin exerts anti-wrinkle and anti-aging properties.

## Introduction

Extrinsic aging is caused by environmental oxidative factors primarily resulting from exposure to ultraviolet (UV) radiation (1,2). Skin degeneration by UV radiation is a cumulative process, and the degeneration rate depends on the frequency, duration and intensity of solar exposure, as well as the natural protection provided by skin pigmentation (2). UVB radiation penetrates the superficial layers of the skin down to the basal layer of the epidermis, where it generates harmful reactive oxygen and nitrogen species (ROS and RNS, respectively) and subsequently induces inflammation and sunburn and precipitates skin aging (3).

Oxidative stress plays a synergistic role in UV-induced skin damage. ROS can be induced by UV radiation, and the accumulation of ROS eventually causes inflammation and wrinkle formation in the skin (4). Increased ROS levels induce the production of matrix metalloproteinases (MMPs) and lead to skin damage, subsequently causing skin photoaging (5). Thus, MMPs are used as major markers of UVB-induced wrinkle formation and skin inflammation; additionally, their expression can result in increased skin aging, including the formation of deep wrinkles and thickening of the dermis and epidermis (6).

UVB-induced inflammatory responses involve an increase in pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6, all of which accelerate skin damage and result in MMP activation (7,8). There is considerable evidence that UV-induced oxidative stress mediates the phosphorylation or activation of protein kinases such as mitogen-activated protein kinases (MAPKs) through a series of cascades (9). UVB-induced MAPK phosphorylation has been implicated in various skin diseases, including skin cancer and photoaging (10).

*Garcinia mangostana* (*G. mangostana*) L. (mangosteen) is native to Southeast Asia, and its fruits, which are famous for their remarkably pleasant flavor, have become a popular botanical dietary supplement because of their perceived

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role in promoting overall health (11,12). Various parts of *G. mangostana*, mostly the fruit hull, bark and roots, have been used in Southeast Asia for hundreds of years in herbal medicine to treat a wide variety of medical conditions, including diarrhea, infected wounds and abdominal pain (11,13). Mangosteen fruits are rich in phenolic acids, xanthones, anthocyanins and condensed tannins, including  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin and gartanin (14,15). The most abundant xanthones in mangosteen fruits are  $\alpha$ -mangostin and  $\gamma$ -mangostin, also other xanthones in mangosteen include  $\beta$ -mangostin, gartanin and among others (15).  $\alpha$ -mangostin is the major compound that used as traditional medicine for various activities containing anti-inflammatory, antioxidant and antibacterial effects (16,17). Gartanin is a polyphenolic xanthones was observed to have anticancer activities on human bladder cancer cells (18). To the best of the authors' knowledge, anti-wrinkle activity of *Garcinia mangostana* L. extracts (GME) and its compounds by oral administration in hairless mice was not studied.

In a previous study, mangosteen was reported to contain a variety of bioactive compounds with potential applications as therapeutic agents or functional food additives (19,20). In the current study, the authors isolated  $\alpha$ -mangostin,  $\beta$ -mangostin and  $\gamma$ -mangostin, as well as gartanin from GME and investigated whether these compounds could protect skin from UVB-induced damage.

## Materials and methods

**Preparation of  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin and gartanin.** The compounds  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin and gartanin, which were used in the present *in vitro* study, were obtained from a previous study (21). Large-scale isolation of  $\alpha$ -mangostin for the *in vivo* experiments was conducted as follows: Dried pericarps from *G. mangostana* (7.29 kg) were extracted three times with ethanol at room temperature to obtain 351 g of solid extract. This extract was suspended in water and then aliquoted into hexane, chloroform, ethyl acetate, n-butanol and water, resulting in hexane-soluble (12 g), chloroform-soluble (213 g), ethyl acetate-soluble (15 g), n-butanol-soluble (81 g) and aqueous (126 g) extracts. The chloroform-soluble extract (200 g) was chromatographed on a silica gel column (10x60 cm, 2,000 g) using a gradient mixture of hexane and EtOAc (100:0 to 2:1) followed by a gradient mixture of  $\text{CHCl}_3$  and MeOH (20:1 to 1:1) to produce 21 fractions (GMC-A to GMC-U). The GMC-I fraction yielded 45 g of  $\alpha$ -mangostin. The structures of  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin and gartanin are presented in Fig. 1.

**Cell culture and UVB irradiation.** An immortalized, non-tumorigenic human keratinocyte cell line (HaCaT) was maintained in Dulbecco's modified Eagle's medium (Life Technologies, Gibco-BRL Products, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics at 37°C in a humidified incubator containing 5%  $\text{CO}_2$ . Cells ( $1 \times 10^4$ ) were seeded, allowed to adhere for 24 h, and treated with various concentrations of GME and the purified compounds prior to exposure to UVB radiation at a dose of 20 mJ/cm<sup>2</sup>. Control cells received neither GME nor

purified compounds and were not exposed to UVB radiation. Immediately following UVB irradiation, the cell viability was assessed by incubating the cells with MTS (Promega, Madison, WI, USA) for 1 h, and the MTS reduction to formazan was measured according to the manufacturer's instructions. The absorbance of the samples was measured at 490 nm using a microplate fluorimeter (Molecular Devices, LLC, Sunnyvale, CA, USA).

**Determination of MMP-1 and MMP-9 secretion using enzyme-linked immunosorbent assay (ELISA).** The MMP-1 and MMP-9 levels in the culture media of HaCaT cells ( $5 \times 10^4$ ) exposed to UVB radiation were determined using human total MMP-1 and MMP-9 ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, HaCaT cells were seeded in 96-well plates and treated with GME or the purified compounds. Following exposure to UVB radiation, the cell culture supernatants were collected and centrifuged at 189 x g for 5 min. The levels of MMP-1 and MMP-9 were quantified using colorimetric analysis using a microplate fluorimeter (Molecular Devices, LLC).

**Experimental animals.** HR-1 hairless male mice (weighing 20-23 g, 6 weeks old; Center Laboratory Animal Inc., Seoul, Korea) were stabilized for one week before the study. The animals were maintained at a temperature of 24°C and 50% relative humidity with 12 h light/dark cycles and provided free access to food and water. All animal procedures were performed in accordance to the guidelines for the Care and Use of Laboratory Animals developed by the Institute of Laboratory Animal Resources of the National Research Council, and were approved by the Institutional Animal Care and Use Committee of Daejeon University in Daejeon, Korea. The mice were divided into control (n=5), UVB-irradiated vehicle (n=5) and UVB-irradiated  $\alpha$ -mangostin (n=5) groups. The mice from the  $\alpha$ -mangostin group were orally administered 0.1 ml water containing 100 mg/kg  $\alpha$ -mangostin daily prior to UVB irradiation. Normal drinking water was supplied to the animals in the vehicle group, whereas the unexposed control group was not UV irradiated and received untreated water.

**UVB irradiation.** Mice were subjected to UVB irradiation using an UVM-225D Mineralight UV Display lamp (UVP, Inc., Upland, CA, USA), which emitted radiation at a wavelength of 302 nm. The UV intensity was measured using an HD2102-2 UV meter (Delta Ohm, Padova, Italy). For the *in vivo* experiments, UVB radiation was applied to the backs of the mice three times a week for 12 weeks. The radiation dose was progressively increased from 60 mJ/cm<sup>2</sup> per exposure at week 1 (one minimal erythematous dose = 60 mJ/cm<sup>2</sup>) to 90 mJ/cm<sup>2</sup> per exposure at week 7.

**Histological examination.** Isolated mouse skin tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and sliced into 5  $\mu\text{m}$  sections, which were stained with hematoxylin and eosin. The epidermal thickness was measured under light microscopy with an eyepiece micrometer.

**Antioxidant enzyme activities.** Superoxide dismutase (SOD) and catalase (CAT) activities were measured using a colorimetric

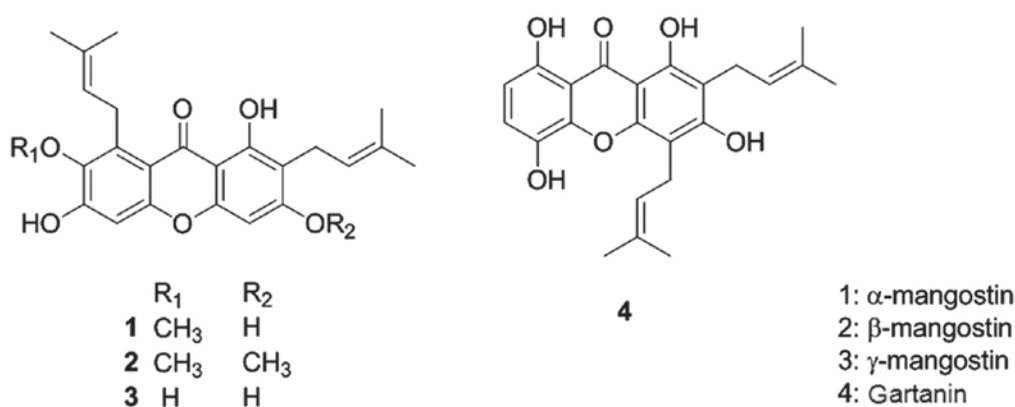


Figure 1. Chemical structures of α-mangostin, β-mangostin, γ-mangostin and gartanin isolated from *Garcinia mangostana* L. (mangosteen).

assay kit (SOD: cat. no. 706002, CAT: cat. no. 707002; Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocol. For protein extraction, skin tissue was homogenized in cold lysis RIPA buffer (Thermo Fisher Scientific, San Jose, CA, USA). The absorbance was measured at 450 and 540 nm to determine the SOD and CAT activity, respectively, using a plate reader (Molecular Devices, LLC).

**RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was extracted from skin tissue from UVB-irradiated mice using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. RT-qPCR was performed using TaqMan assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) specific for involucrin, loricrin, filaggrin, IL-1β, IL-6 and TNF-α on a QuantStudio™ 6 Flex Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Each sample was assayed in triplicate, and the relative mRNA expression levels were calculated using the ΔΔCt method and normalized to the β-actin mRNA levels in each sample (22).

**Determination of MMP-1 and MMP-9 secretion by ELISA.** MMP-1 and MMP-9 levels in the skin tissue after UVB irradiation were determined using total MMP-1 (cat. no. DMP100) and MMP-9 (cat. no. DMP900) ELISA kits according to the manufacturer's instructions. The levels of MMP-1 and MMP-9 were quantified by colorimetric analysis.

**Western blotting.** The protein content in the supernatant was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). The protein lysate was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel, and the proteins were transferred to polyvinylidene difluoride membranes and then blocked with blocking buffer (ATTO, Tokyo, Japan), which were incubated with specific primary antibodies targeting MMP-1 (1:1,000 dilution, ab137332; Abcam, Cambridge, MA, USA), MMP-9 (1:1,000 dilution, cat. no. 3852), p-ERK (1:1,000 dilution, cat. no. 4370), ERK (1:1,000 dilution, cat. no. 9102), p-p38 (1:1,000 dilution, cat. no. 9211), p38 (1:1,000 dilution, cat. no. 9212), p-JNK (1:1,000 dilution, cat. no. 9251), JNK (1:1,000 dilution, cat. no. 9252), and β-actin (1:1,000 dilution,

cat. no. 4970) (all from Cell Signaling Technology, Beverly, MA, USA) at 4°C overnight. The blots were washed for 10 min three times with phosphate-buffered saline (PBS) containing 0.1% Tween-20 and then incubated for 2 h with the appropriate anti-rabbit secondary antibody (1:5,000 dilution, SC-2004; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Protein bands were detected using an enhanced chemiluminescence solution (Bio-Rad) western blot detection system (LAS-4000; Fuji, Tokyo, Japan).

**Statistic analysis.** All measurements were completed in triplicate, and the data are presented as the mean ± standard error of the mean. The results were subjected to analysis of variance using Tukey's multiple comparison test to analyze the differences, and P<0.05 was considered to indicate a statistically significant difference.

## Results

**Protective effects of GME and purified compounds against UVB-induced damage in HaCaT cells.** The authors investigated the effect of GME and the purified compounds on HaCaT cell proliferation after UVB exposure. Cell viability was reduced to 51.7% by UVB irradiation in untreated cells but increased to 82.6% (Fig. 2A) in the presence of α-mangostin.

**Effects of GME and purified compounds on UVB-induced secretion of MMP-1 and MMP-9.** The authors evaluated the effect of GME and the purified compounds on UVB-induced MMP expression in HaCaT cells. UVB radiation resulted in a marked increase in MMP-1 and MMP-9 levels compared with non-irradiated cells (Fig. 2B and C). The ELISA analysis revealed that GME and the individual compound treatments reduced the MMP-1 and MMP-9 protein levels in the culture media of HaCaT cells in a dose-dependent manner.

**Effects of GME and compounds on involucrin, filaggrin, and loricrin levels in UVB-irradiated HaCaT cells.** The results indicated that the expression of involucrin, filaggrin and loricrin was decreased in UVB-irradiated HaCaT cells compared with non-irradiated cells. As presented in Fig. 3, the mRNA expression levels of involucrin, filaggrin and loricrin in UVB-irradiated cells treated with GME or the purified

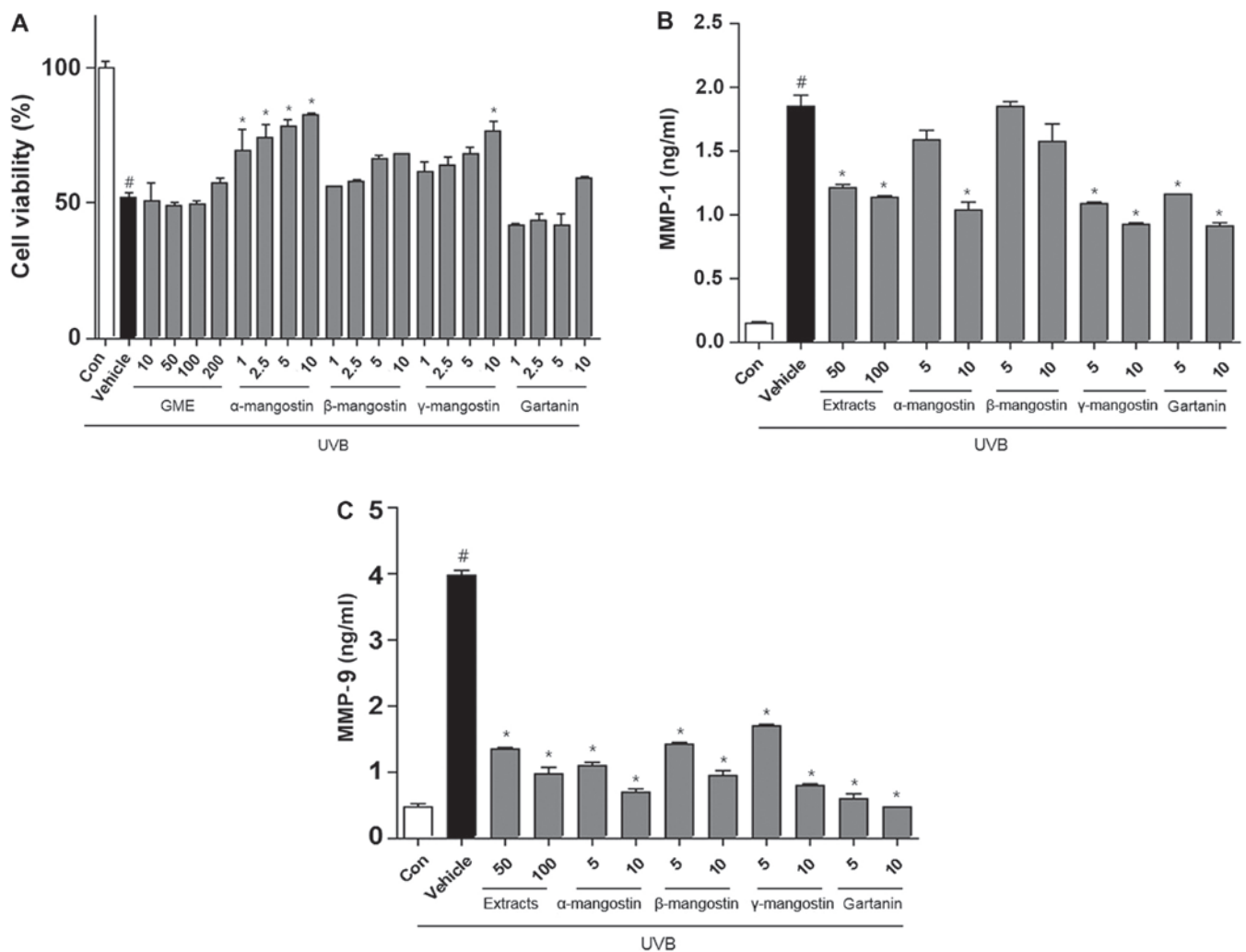


Figure 2. Effects of GME and the purified compounds on cell viability and MMP expression in HaCaT cells subjected to UVB irradiation. (A) HaCaT cells were pretreated with the indicated concentrations for 24 h followed by UVB irradiation of 20 mJ/cm<sup>2</sup>. The expression levels of (B) MMP-1 and (C) MMP-9 in the culture medium of UVB-irradiated HaCaT cells were analyzed using ELISA. Concentration of GME is  $\mu$ g/ml, and other compounds are  $\mu$ M. Data are presented as mean  $\pm$  standard error of the mean. <sup>#</sup>P<0.05 vs. control group; <sup>\*</sup>P<0.05 vs. vehicle group. GME, *Garcinia mangostana* L. extracts; UVB, ultra-violet B; MMP, matrix metalloproteinase; Con, control; ELISA, enzyme-linked immunosorbent assay.

compounds were increased compared to the untreated cells exposed to UVB.

*$\alpha$ -mangostin inhibits UVB-induced wrinkle formation in hairless mice.* Because  $\alpha$ -mangostin was the most effective compound in the *in vitro* studies, the authors investigated the effect of  $\alpha$ -mangostin on UVB-induced wrinkle formation by repeatedly exposed hairless mouse skin to UVB for 12 weeks. Histological evaluation shows that this exposure induced skin wrinkles, which was blocked by  $\alpha$ -mangostin (Fig. 4). To evaluate the effect of  $\alpha$ -mangostin on skin thickening, the epidermal thickness was measured. The epidermal thickness of the UVB-irradiated  $\alpha$ -mangostin-treated group was 118.7 vs. 55.0  $\mu$ m in the UVB-irradiated vehicle-treated group.

*Effects of  $\alpha$ -mangostin on the activity of antioxidant enzymes in UVB-irradiated hairless mice.* To investigate whether the radical-scavenging activity of  $\alpha$ -mangostin was mediated by antioxidant enzymes, the activities of well-known antioxidant enzymes were examined in hairless mice exposed to UVB radiation. The SOD activity was decreased in the

UVB-irradiated hairless mice compared to unexposed control mice (Fig. 5) but was enhanced by  $\alpha$ -mangostin treatment in a dose-dependent manner. Furthermore, compared with the control group, the UVB-irradiated group exhibited decreased CAT activity, but treatment with  $\alpha$ -mangostin ameliorated this loss of CAT activity. These results indicated that  $\alpha$ -mangostin could protect the activities of antioxidant enzymes, which scavenge free radicals, and thereby further inhibit UVB-induced oxidative stress.

*Effects of  $\alpha$ -mangostin on involucrin, filaggrin, and loricrin expression in UVB-irradiated hairless mice.* The authors indicated that the expression of involucrin, filaggrin and loricrin decreased in the UVB-irradiated group compared to the unexposed control group. As shown in Fig. 6, the mRNA expression levels of involucrin, filaggrin and loricrin in the UVB-irradiated hairless mice treated with  $\alpha$ -mangostin were increased compared to the UVB-irradiated vehicle-treated mice.

*Effect of  $\alpha$ -mangostin on the release of inflammatory cytokines in hairless mice.* To assess the regulatory effect of



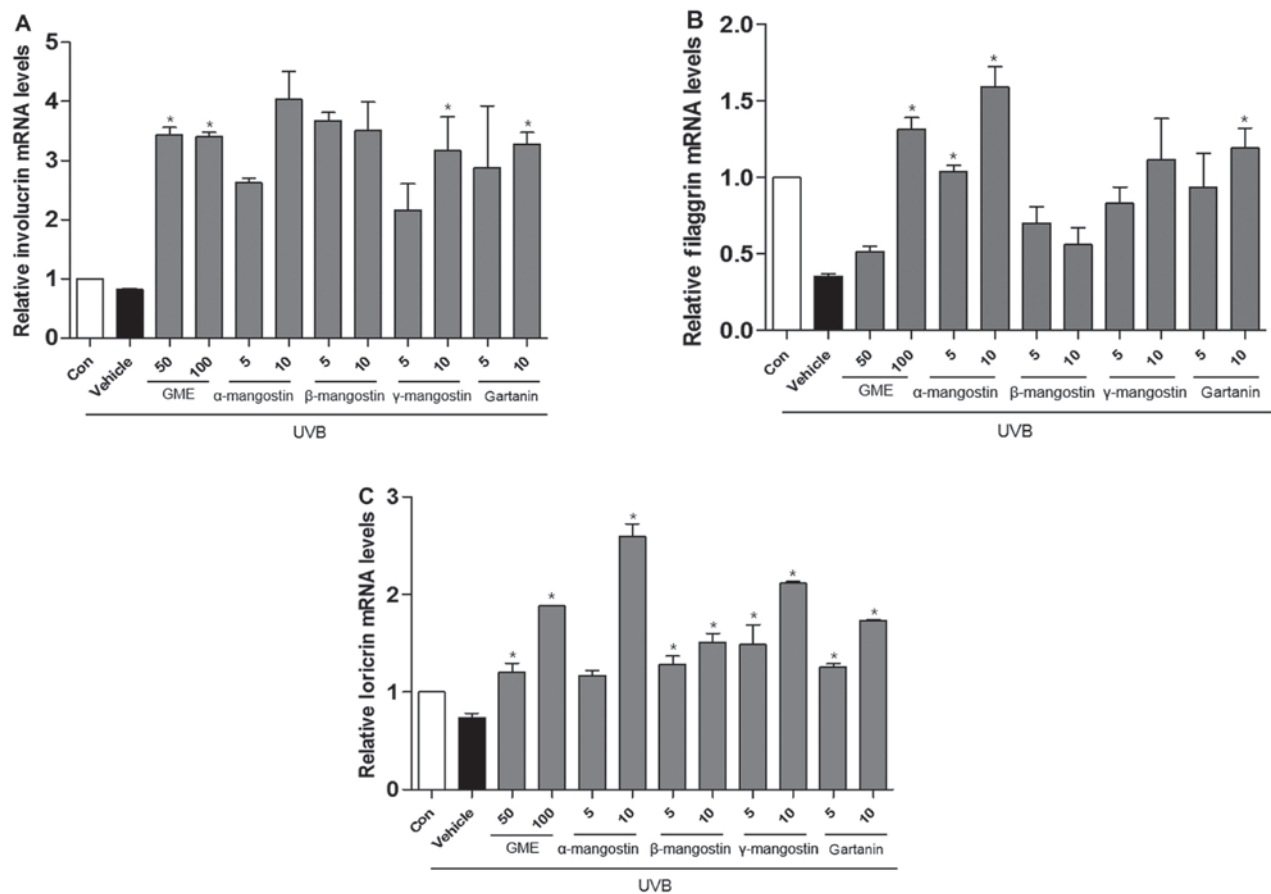


Figure 3. Effects of  $\alpha$ -mangostin on (A) involucrin, (B) filaggrin and (C) lorixin expression in UVB-irradiated HaCaT cells. Concentration of GME is  $\mu\text{g/ml}$ , and other compounds are  $\mu\text{M}$ . Data are presented as mean  $\pm$  standard error of the mean. \* $P<0.05$  vs. vehicle group. GME, *Garcinia mangostana* L. extracts; UVB, ultraviolet B; Con, control.

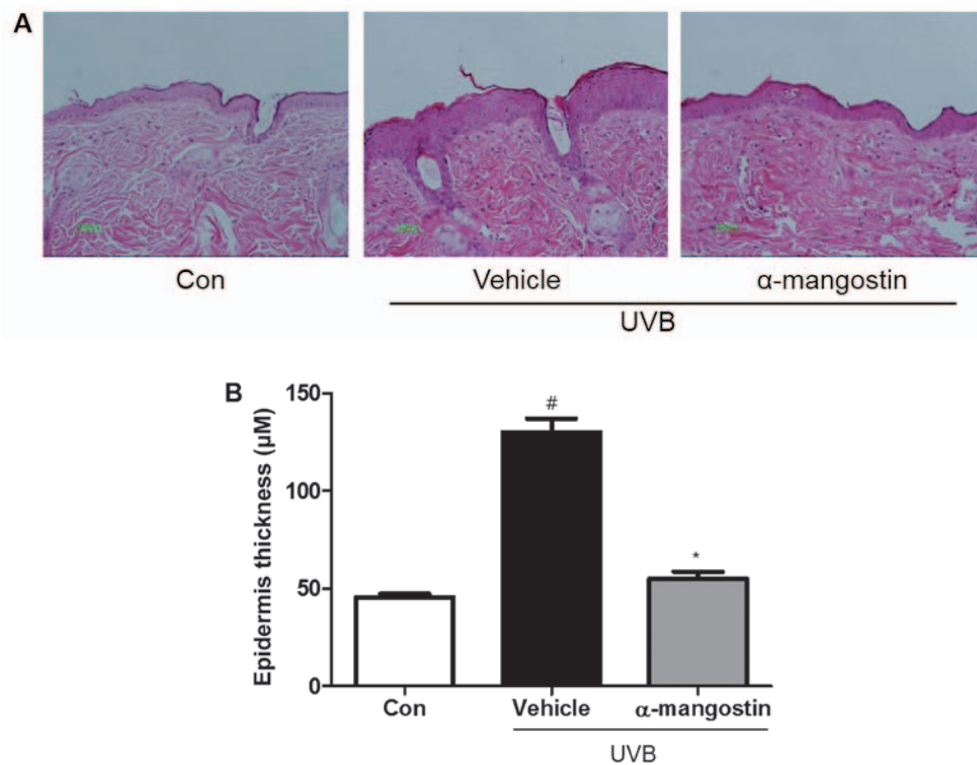


Figure 4. Effect of  $\alpha$ -mangostin treatment on UVB-induced skin thickening in hairless mice. (A) Hematoxylin and eosin staining of UVB-irradiated hairless mouse skin. Original magnification,  $\times 200$ . (B) Dorsal skin epidermal thickness. Data are presented as mean  $\pm$  standard error of the mean. \* $P<0.05$  vs. control group; <sup>#</sup> $P<0.05$  vs. vehicle group. UVB, ultraviolet B; Con, control.

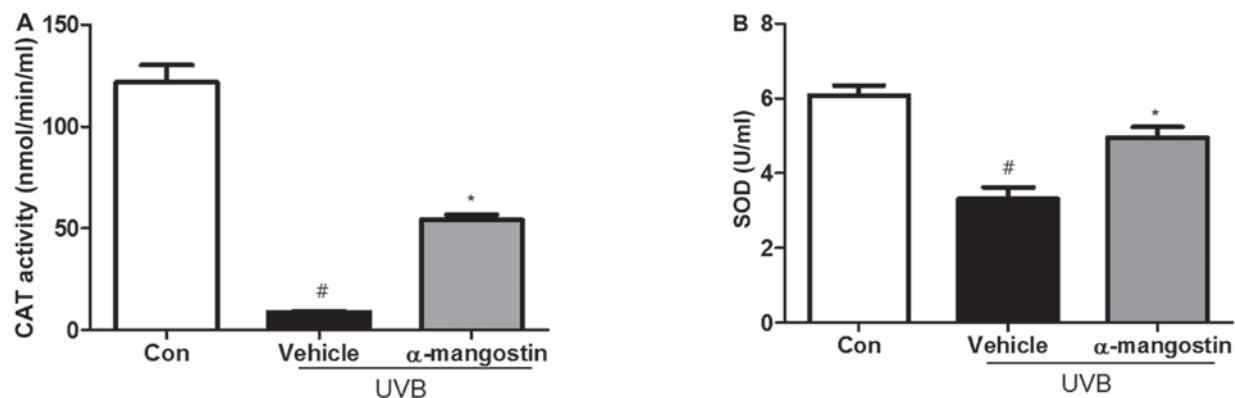


Figure 5. Effects of  $\alpha$ -mangostin on the activity of (A) CAT and (B) SOD in hairless mouse skin exposed to UVB radiation. Data are presented as mean  $\pm$  standard error of the mean. <sup>#</sup>P<0.05 vs. control group; <sup>\*</sup>P<0.05 vs. vehicle group. CAT, catalase; SOD, superoxide dismutase; UVB, ultraviolet B; Con, control.

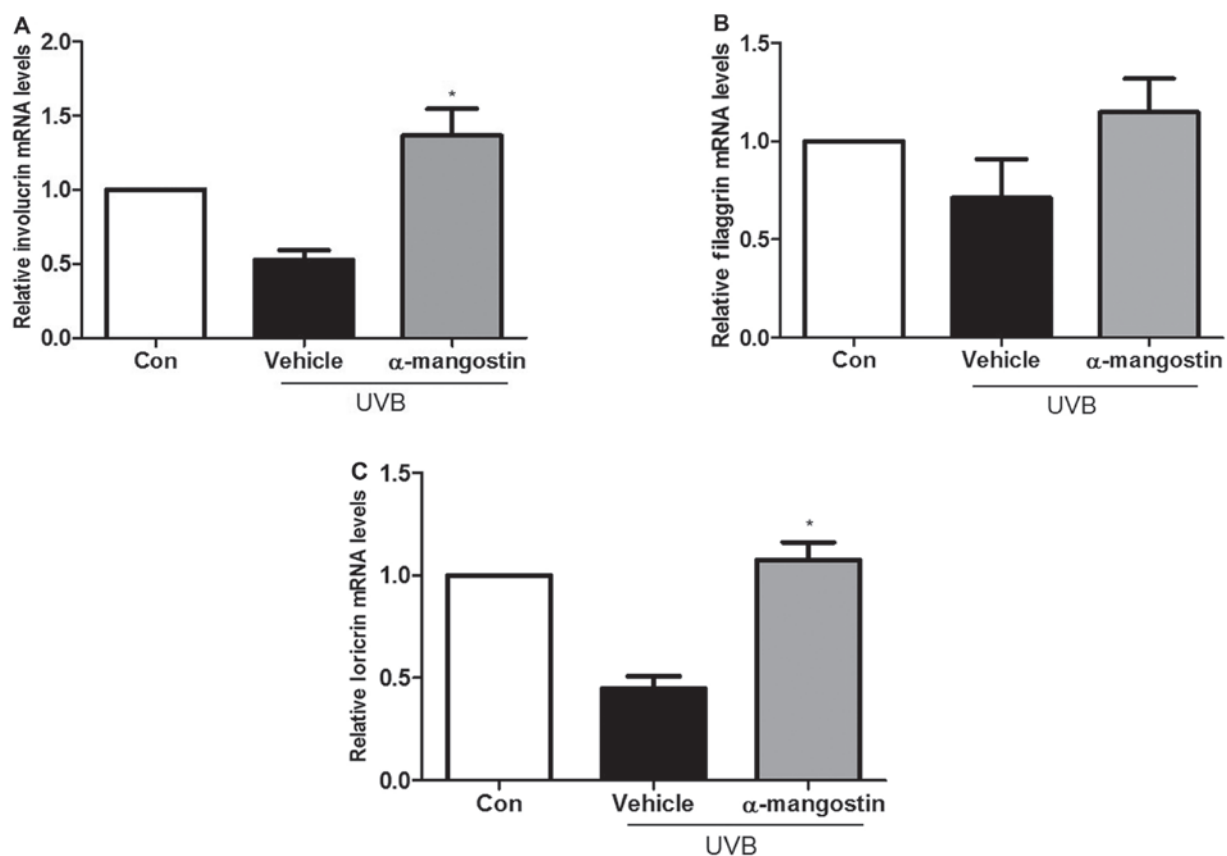


Figure 6. Effects of  $\alpha$ -mangostin on (A) involucrin, (B) filaggrin and (C) loricrin expression in UVB-irradiated hairless mouse skin. Data are presented as mean  $\pm$  standard error of the mean. <sup>\*</sup>P<0.05 vs. vehicle group. UVB, ultraviolet B; Con, control.

$\alpha$ -mangostin on the production of pro-inflammatory cytokines in UVB-irradiated hairless mice, the mRNA expression of pro-inflammatory cytokines was investigated. As presented in Fig. 7, the IL-1 $\beta$ , IL-6 and TNF- $\alpha$  mRNA levels were increased in mice exposed to UVB compared to the unexposed control; however, this increase was suppressed by treatment with  $\alpha$ -mangostin. This suggested that  $\alpha$ -mangostin exerted a photoprotective effect by reducing the inflammatory response to UVB radiation.

*Effects of  $\alpha$ -mangostin on MAPK phosphorylation in UVB-irradiated hairless mice.* Using western blotting,

the authors assessed the effect of oral administration of  $\alpha$ -mangostin on the activation of MAPK family proteins and downstream MMP-1 and MMP-9 proteins in hairless mouse skin exposed to UVB irradiation (Fig. 8A). UVB irradiation increased the skin expression of MMP-1 and MMP-9 levels in hairless mice. Treatment with  $\alpha$ -mangostin reduced the expression of MMP-1 and MMP-9 (Fig. 8B and C). The authors examined whether  $\alpha$ -mangostin could suppress the UVB-induced phosphorylation of MAPKs, including ERK, p38 and JNK. As presented in Fig. 8D, UVB irradiation resulted in the phosphorylation of ERK, p38 and JNK, whereas treatment of mice with  $\alpha$ -mangostin prior to UVB

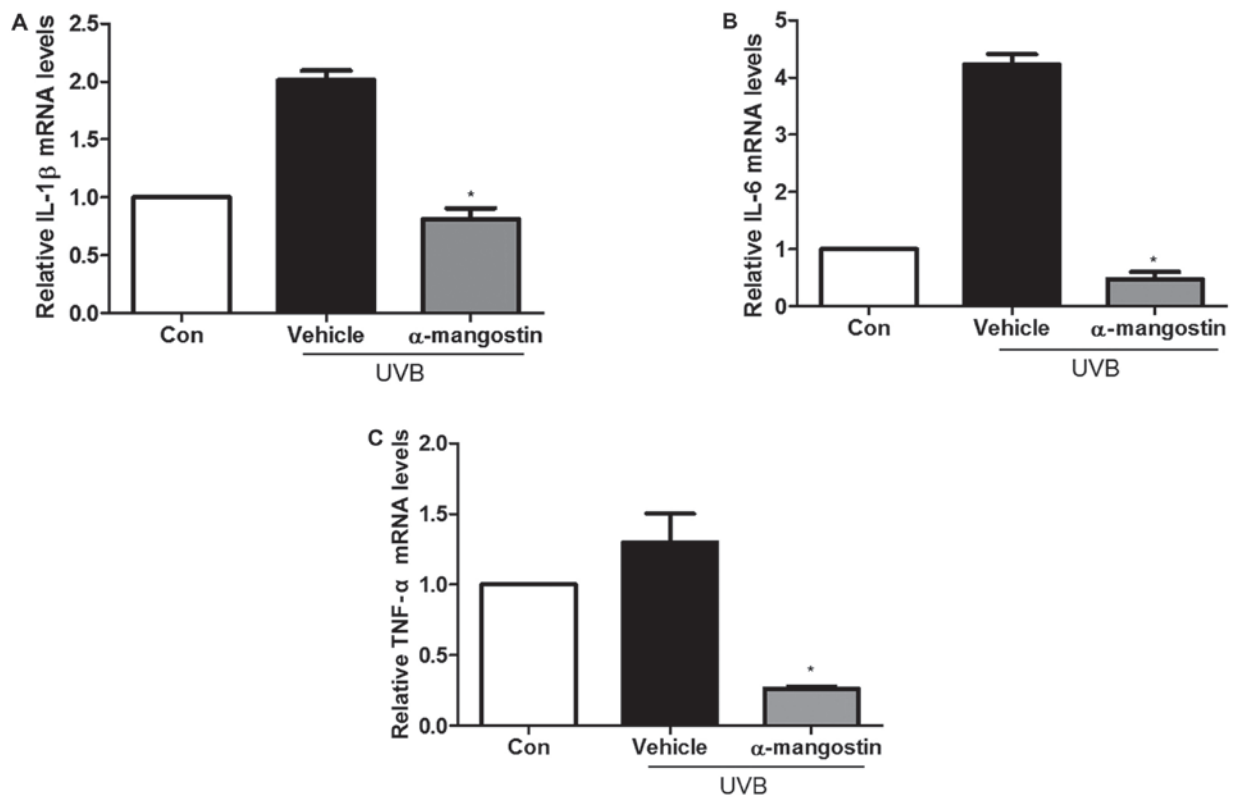


Figure 7. Effects of  $\alpha$ -mangostin on pro-inflammatory cytokines in UVB-irradiated hairless mouse skin. The mRNA expression levels of (A) IL-1 $\beta$ , (B) IL-6 and (C) TNF- $\alpha$  were determined using reverse transcription-quantitative polymerase chain reaction. Data are presented as mean  $\pm$  standard error of the mean. \*P<0.05 vs. vehicle group. UVB, ultraviolet B; Con, control; IL, interleukin; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

irradiation inhibited the UVB-mediated phosphorylation of these MAPKs.

## Discussion

Acute UV exposure on human skin causes sunburn, altered pigmentation, inflammation, immune suppression, and dermal connective tissue damage (3). UV irradiation of the skin enhances collagenase activity and contributes to wrinkle formation via collagen degradation in the dermal extracellular matrix (ECM) (23). The authors investigated the effects of UVB exposure on skin aging in a hairless mouse model, specifically, its effects on the development of skin wrinkles. It was identified that extended UVB radiation induced skin wrinkles in hairless mice after 12 weeks.

Previous pharmacological studies of *G. mangostana* suggested numerous beneficial effects of the mangosteen, such as anti-acne, anti-adipogenic, antibacterial, anticancer, anti-obesity and antioxidative effects (21,24,25). Thus, the effects of  $\alpha$ -mangostin on skin aging were investigated in the same hairless mouse model, especially regarding the development of skin wrinkles.

UVB-induced wrinkle formation is mediated by a complex mechanism involving ECM damage, cell death and the consequent inflammatory responses in both the epidermis and dermis (26). Histological and ultrastructural studies have revealed that photodamaged skin is associated with increased epidermal thickness and alterations in connective tissue organization (27). Epidermal thickness is often used as a parameter to quantitatively evaluate skin photoaging because epidermal

hypertrophy is thought to cause wrinkle formation (28). The thickness of the basal membrane in photodamaged skin is increased, possibly reflecting damage of basal keratinocytes. Furthermore, the distribution of melanocytes along the basal membrane is irregular, and these cells vary widely in size, dendricity and pigmentation (29). Also, a cornified cell envelope, which is composed of involucrin, loricrin and filaggrin is a component of differentiated epidermal keratinocytes and corneocytes, and it is important in the skin barrier and moisture (30). The correct formation of the cornified cell envelope is essential for the barrier function of the skin then barrier inhibits the TEWL and associated loss of solutes (31). In the current study, the mRNA expression levels of involucrin, filaggrin and loricrin in UVB-irradiated cells treated with GME or the purified compounds were increased compared to cells only receiving UVB irradiation. In addition, the epidermal thickness of the dorsal skin of the mice was increased upon UVB exposure but was significantly reduced in mice administered  $\alpha$ -mangostin prior to UVB exposure.

UV irradiation of the skin increases the levels of hydrogen peroxide, other ROS, and antioxidant enzymes (32). Increased ROS production alters the structure and function of multiple genes and protein, leading to skin damage (11). Although ROS are part of normal regulatory circuits and the cellular redox state is tightly controlled by antioxidants, an increased ROS load and the loss of cellular redox homeostasis can promote carcinogenesis and photoaging (33). In the present study, the results revealed that  $\alpha$ -mangostin could protect the activities of antioxidant enzymes, which scavenge free radicals, thereby further inhibiting UVB-induced oxidative stress.

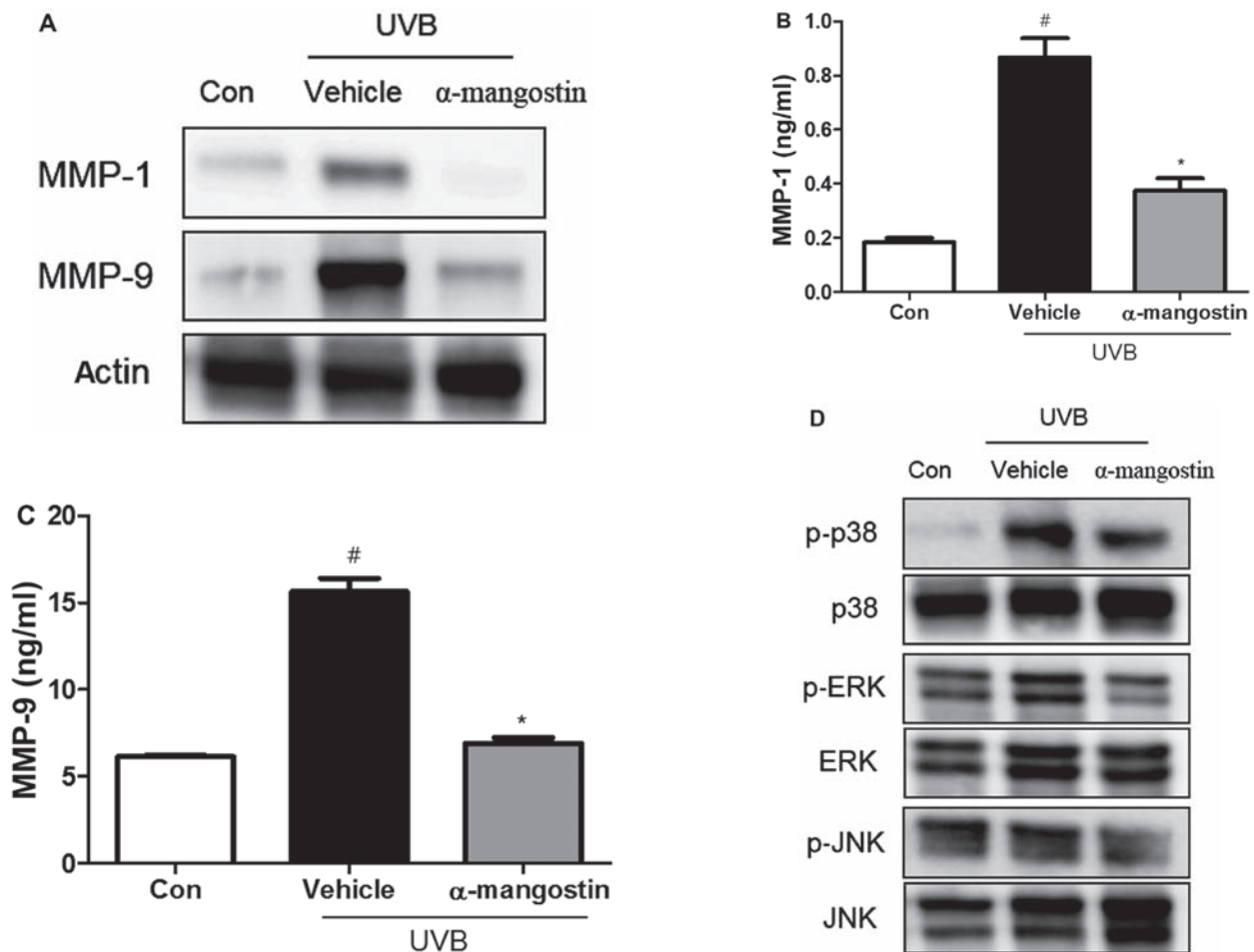


Figure 8. Inhibitory effects of  $\alpha$ -mangostin on UVB-induced (A) MMP-1 and MMP-9 protein levels and (B) inhibition of the UVB-induced MMP-1 and (C) MMP-9 expression by ELISA assay kit. (D) ERK1/2, JNK and p38 phosphorylation in HaCaT cells. The phospho-ERK1/2, phospho-p38, phospho-JNK and  $\beta$ -actin protein levels were determined using western blotting. Data are presented as mean  $\pm$  standard error of the mean. <sup>#</sup> $P < 0.05$  vs. control group; <sup>\*</sup> $P < 0.05$  vs. vehicle group. UVB, ultraviolet B; MMP, matrix metalloproteinase; Con, control; ELISA, enzyme-linked immunosorbent assay.

Upregulated MMP-1 expression following UV irradiation results in collagen degradation, the histopathological hallmark of photoaging (34). A wealth of evidence has indicated that MMP induction plays a major role in the pathogenesis of photoaging (35). MMP-1 initiates the degradation of type I and III collagens and further degrades the collagen fragments generated by collagenases (6). Chronic UV irradiation of human skin results in elevated MMP expression, leading to marked degenerative changes in the upper dermal connective tissue via degradation of elastic and collagen fibers (36). In the current study, treatment with either GME or the purified compounds reduced the MMP-1 and MMP-9 expression levels.

UV radiation stimulates and activates various cells to produce and release cytokines, which likely play a significant role in the process of photoaging (37), and produces free radicals that release pro-inflammatory cytokines and growth factors, which activate proteases that degrade collagen and elastin. This eventually results in structural and functional changes in the ECM (38). UVB radiation triggers cutaneous inflammatory responses by directly inducing epidermal keratinocytes to produce specific pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and cyclooxygenase-2 (39,40). In the current study, the effect of GME was examined and purified compounds on HaCaT cells as well as of  $\alpha$ -mangostin

on UVB-induced pro-inflammatory cytokine production in UVB-irradiated hairless mouse skin; the results indicated that the inhibitory effect of  $\alpha$ -mangostin on the production of inflammatory mediators was accompanied by reduced mRNA expression levels of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ .

UVB irradiation has been shown to stimulate ROS production and activate MAPKs (41), including ERK1/2, JNK and p38 MAPK (42). In the study,  $\alpha$ -mangostin inhibited the UVB-induced activation of JNK, p38 and ERK.

In conclusion, GME and the purified compounds from *G. mangostana* L. could effectively reduce skin damage. Specifically,  $\alpha$ -mangostin ameliorated wrinkling processes induced by UVB irradiation of a hairless mouse model, as indicated by histological examination, increased SOD and CAT activities, and decreased MMP expression; furthermore,  $\alpha$ -mangostin could reduce the protein levels of pro-inflammatory cytokine and multiple MAPKs.

#### Acknowledgements

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