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Coffea arabica extract and its constituents prevent photoaging by suppressing MMPs expression and ~~MAPK~~ pathway

Hsiu-Mei Chiang [a], Tsen-Jung Lin [a], Chen-Yuan Chiu [b], Chiung-Wen Chang [c], Kuo-Chiu Hsu [c], Pei-Ching Fan [a], Kuo-Ching Wen [a,*]

[a] *Department of Cosmeceutics, China Medical University, Taichung 404, Taiwan*
[b] *School of Pharmacy, China Medical University, Taichung, Taiwan*
[c] *Winston Medical Supply Co., Ltd., Tainan, Taiwan*

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ABSTRACT

UV is a potent factor in skin photoaging and photocarcinogenesis. Therefore, investigating the inhibiting mechanisms of photoaging would be useful to enable development of agents to slow down the aging process. UV-irradiation increased metalloproteinase (MMP)-~~1,3 and 9~~ and then causes collagen and elastin degradation, leading to the formation of coarse wrinkles and sagging skin. Polyphenols, a group of compounds, possessing a variety of biological activities including inhibition of MMP-1 and elastase, are widely distributed in plants including *Coffea arabica*. In this study, *Coffea arabica* leaves extract (CAE), its hydrolysates (CAH), chlorogenic acid and caffeic acid, are studied for their anti-photoaging effect. *Coffea arabica* leaves were extracted with methanol, and the extract was hydrolyzed with different concentrations of hydrochloric acid. The various concentrations of CAE, CAH, chlorogenic acid and caffeic acid were subject to MMPs and elastase inhibition tests. The fibroblast was used for collagen synthesis and MMP-1, -3, -9 inhibition tests on herbal extracts. The results showed that CAE stimulated type I procollagen expression, inhibited MMP-1, -3, -9 expression and inhibited the phosphorylation of JNK, ERK and p38. The results suggest that CAE can prevent photo-damage in skin through inhibiting MMP expression and ~~MAPK~~ pathway.

1. Introduction

Skin aging is a progressive process, which can be divided into two basic processes, intrinsic aging and photoaging (Chung, 2003). Intrinsic aging is characterized by smooth, dry, pale and finely wrinkled skin. Environmental factors involving UV irradiation induce photoaging, which is characterized by severe wrinkling and pigmentary changes, such as solar lentigo and mottled pigmentation on exposed areas such as the face, neck and forearm. UV irradiation induces the synthesis of matrix metalloproteinases (MMPs) in human skin *in vivo*. UVB is known to induce the overexpressions of MMP-1, -3, and -9 in the normal human epidermis *in vivo* (Fisher et al., 1996). Some research has proposed that MMP-mediated collagen destruction accounts, in large part, for the connective tissue damage that occurs in photoaging (Rabe et al., 2006).

The most abundant structural protein in skin connective tissue is type I collagen, which is responsible for conferring strength and resiliency (Gelse et al., 2003). Type I collagen is synthesized primarily by fibroblasts residing within skin connective tissue (dermis). It is synthesized as a soluble precursor, type I procollagen, which is secreted from fibroblasts and proteolytically processed to form insoluble collagen fibers. Disorganization, fragmentation, and dispersion of collagen bundles are prominent features of photodamaged human skin.

Polyphenols are abundant in fruits, vegetables, green tea and wine. Tea polyphenol, ~~EGCG~~, showed skin photoprotection through hampering collagen destruction and collagenase activation (Katiyar, 2003; Bae et al., 2008). Rubiaceae is rich in polyphenols, and *Coffea arabica* belongs to Rubiaceae. It has been reported that coffee extract inhibits hepatitis B virus expression (Utsunomiya et al., 2008), enterobacteria (Almeida et al., 2006), super oxide free radicals and lipid peroxidation (Namba and Matsuse, 2002). The components of *Coffea arabica* involve diterpenoid alcohols (such as cafestol and kahweol), alkaloid (caffeine) and organic acids (caffeic acid and chlorogenic acid) (Ranheim and Halvorsen, 2005). Chlorogenic acid and catechin are polyphenols (Kim et al., 2006), which would suggest the potential of *Coffea arabica* as an effective protection against photoaging. In a previous study, caffeic acid applied on abdominal skin suppressed the UVA-induced reactive

Abbreviations: MMP, metalloproteinase; CAE, *Coffea arabica* extracts; CAH, hydrolysates of CAE; MAPK, mitogen-activated protein kinase.

* Corresponding author. Tel.: +886 4 22053366x5302; fax: +886 4 22078083.
E-mail address: kcwen0520@mail.cmu.edu.tw (K.-C. Wen).

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oxygen species (ROS); caffeic acid existed in the skin after oral ingestion (Yamada et al., 2006).

The aim of this study was to investigate the potential and mechanisms of CAE and CAH in counteracting UVB induced MMP-1, -3 and -9 secretion and UVB-induced type I procollagen reduction.

2. Materials and methods

2.1. Materials

The leaves of *Coffea arabica* were harvested in Yunlin County, Taiwan. Human foreskin fibroblasts were obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). Gelatin, agarose, hydrochloric acid, methanol, dimethyl sulfoxide (DMSO), doxycycline hyclate, caffeic acid, chlorogenic acid, calcium chloride ($CaCl_2$), propylene glycol (PG), DL-dithiothreitol, Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazy (DPPH) and 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Fetal bovine serum (FBS), penicillin–streptomycin, trypsin–EDTA, and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco, Invitrogen (Carlsbad, CA, USA). Coomassie blue R-250, dibasic sodium phosphate, Igepal™ CA-630, tris, sodium dodecyl sulfate (SDS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from USB (Cleveland, OH, USA). Collagenase was purchased from Calbiochem, Merck (Darmstadt, Germany). Fluorogenic Peptide Substrate I was purchased from R&D System (Wiesbaden, Germany). Bradford Reagent was supplied by Bio-Rad Laboratories (Hercules, CA, USA). Donkey anti-goat IgG-HRP, ERK 1 (C-16), JNK1 (G-13), MMP-1 (L-20), MMP-3 (1B4), MMP-9 (6-6B), p38 (A-12), p-p38 (Thr 180/Tyr 182)-R, p-JNK (Thr 183/Tyr 185), p-ERK 1/2 (Thr 202/Tyr 204) were purchased from Santa Cruz Biotechnology, Inc. (CA, USA). Elastase substrate IV and porcine elastase were purchased from Calbiochem (San Diego, CA, USA).

2.2. Preparation of Coffee arabica leaves extract (CAE) and its hydrolysates (CAH)

The fresh coffee leaves were dried by oven at 50 °C. The dried leaves were ground and then extracted twice with 30-fold volume of methanol ultrasonically for 1 h. The supernatant was filtered and the filtrate was collected. The filtrate was evaporated to dryness in ~~vaccuo~~. The CAE was dissolved in DMSO, and then 2 mL of 1.2 N and 2.4 N HCl were added to hydrolyze at 80 °C for 30 min and 60 min, respectively. After hydrolysis, the solution was partitioned with ethyl acetate (EA). The EA layer was evaporated to dryness in vacuo. The abbreviation and hydrolytic conditions of Coffee hydrolysates are as follows: CAH1, 1.2 N HCl, 0.5 h; CAH2, 1.2 N HCl, 1 h; CAH3, 2.4 N HCl, 0.5 h; CAH4, 2.4 N HCl, 1 h. The CAE and its hydrolysates were stored at −20 °C before use.

2.3. Total phenolic content of coffee preparation

Total phenolic content was determined by the Folin–Ciocalteu reaction (Ragazzi and Veronese, 1973). A mixture of 100 μL of CAE and 200 μL of 10% Folin–Ciocalteu phenol reagent was prepared and allowed to stand at room temperature for 5 min. Then 800 μL of sodium carbonate (700 mM) was added to the mixture. The resulting blue complex was then measured at 760 nm. Gallic acid was used as a standard for the calibration curve. The phenolic compound contents were calibrated using the linear equation base on the calibration curve. The contents of phenolic compounds were expressed as ~~mg gallic acid equivalent~~/g dry weight. The dry weight indicated was coffee leaves dry weight.

2.4. The antioxidant effects of CAE

2.4.1. DPPH radical scavenging activity

In this assay, ascorbic acid was used as a positive control. Reaction mixtures containing a methanolic solution of 200 μM DPPH (100 μL) and serial dilutions of sample ranging from 25 to 1000 μg/mL were placed in a 96-well microplate at room temperature in the dark for 30 min. After incubation, the absorbance was read at 492 nm by ELISA reader (Tecan, Austria). Scavenging activity was determined by the following equation:

$$\%\ \text{scavenging activity} = \left[1 - \left(A_{\text{sample}}/A_{\text{control}}\right)\right] \times 100$$

2.4.2. Preparation of erythrocyte suspensions and hemolysis assay

Blood was obtained from male SD rats via cardiopuncture, and the whole blood was collected in an EDTA-containing tube. This animal study adhered to The Guidebook for the Care and Use of Laboratory Animals (Published by The Chinese Society for Laboratory Animal Science, Taiwan). The erythrocytes were isolated by centrifugation at 3000*g* for 10 min, washed four times with PBS, and then re-suspended to the desired hematocrit level using the same buffer. In order to induce free radical chain oxidation in the erythrocytes, aqueous peroxyl radicals were generated by thermal decomposition of AAPH in oxygen (Barclay et al., 1984). An erythrocyte suspension at 5% hematocrit was incubated with PBS (control) and preincubated with CAE (10–50 μg/mL) at 37 °C for 30 min, followed by incubation with and without 25 mM AAPH in PBS at pH 7.4. This reaction mixture was shaken gently while being incubated for a fixed interval at 37 °C. Two-hundred microliters of the reaction mixture was removed and centrifuged at 3000*g* for 2 min, with absorbance of the supernatant determined at 540 nm. Reference values were determined using the same volume of erythrocytes in a hypotonic buffer (5 mM phosphate buffer at pH 7.4; 100% hemolysis). The hemolysis percentage was calculated using the formula $[(A_{\text{sample}}/A_{\text{control}})] \times 100$.

2.5. The screening of MMP and elastase inhibition

2.5.1. Gelatin digestion assay (Kim et al., 2006)

Agarose solution (1%) was prepared in a collagenase buffer (50 mM Tris–HCl, 10 mM $CaCl_2$, 0.15 M NaCl, pH 7.8) with 0.15% porcine gelatin (Sigma Aldrich, Cat. G-2500) and allowed to solidify on plates (8 × 6 cm) for 1 h at room temperature. Various concentrations of CAE and CAH (10 μL) dissolved in 50% PG were incubated with 10 μL of bacterial collagenase-1 (0.1 mg/mL) in 80 μL of collagenase buffer for 1 h at room temperature (doxycycline hyclate as a positive control). The samples (40 μL) were loaded onto paper disks placed on gelatin-agarose gel and incubated for 18 h at 37 °C. The degree of gelatin digestion in agarose gel was visualized by Coomassie Blue staining after removal of the paper disks. Following destaining, the area of light translucent zone over blue background was determined by a densitometric program to estimate gelatinase activity.

2.5.2. MMP activity assays by fluorescent gelatin

The assay was followed Kim et al. (2006) with modification. Enzyme activity assays were performed in a 50 mM tris buffer (pH 7.8), 0.15 M NaCl and 10 mM $CaCl_2$. Various concentrations of CAE and CAH were tested for their ability to digest a synthetic fluorogenic substrate (a general MMP substrate). Each concentration of CAE and CAH was incubated with 1 μM substrate at 37 °C for 20 h. Fluorescence intensity was measured at 328 nm (excitation) and 393 nm (emission) with a fluorescence reader (Thermo Electron Corporation, Vantaa, Finland).

2.6. Measurement of elastase activity

The elastase inhibition test on CAE and CAH was investigated using elastase from porcine pancreases. This assay was modified from Kim et al. (2007). Five hundred units of elastase were dissolved in 5 mL of 10 mM tris buffer solution (pH 6.0), and 5 mg of elastase substrate IV was dissolved in 5 mL of 100 mM tris buffer solution (pH 8.0). To measure elastase activity, 100 mM tris buffer solution (pH 8.0) 100 μL, elastase substrate IV solution 25 μL, sample solution 50 μL and elastase solution 25 μL were dispensed into each well of a 96-well plate and then preincubated for 20 min at room temperature. The elastase activity was quantified by measuring light absorbance at 405 nm by ELISA reader (Tecan, Austria). Each assay was carried out in triplicate.

The inhibition rate of elastase was calculated by the following equation:

$$\text{Inhibition}\ (\%) = 1 - \frac{(C-D)}{(A-B)} \times 100$$

where *A* means the absorbance with enzyme but without sample, *B* means the absorbance without enzyme and sample, *C* means the absorbance with enzyme and sample, and *D* means the absorbance without enzyme but with sample.

2.7. Cell culture

Human foreskin fibroblasts (Hs68) were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C in 5% CO_2 humidified air. The cells were subcultured following trypsinization, and cells were used in the 20th to 35th passages.

2.8. UVB irradiation dose

Prior to UV irradiation, cells were washed with PBS twice and covered with PBS. A UV lighter (302 nm, CL-1000 M, UVP, USA) was used. The UVB irradiation doses were 80 mJ/cm^2 (exposure time was 30 s) according to preliminary study. At this UV dose, the cell viability would not significantly reduce and the expression of MMPs and ~~MAPKS~~ were induced (data not shown). After UVB irradiation, PBS was replaced with a serum-free medium and then incubated for 24 h for the thiazolyl blue tetrazolium bromides (MTT) and MMP assay.

2.9. The protection effect and mechanism of coffee preparations on photoaging

2.9.1. Cell viability test

The fibroblasts were plated at a density of 10^4 cells/well in 96 well plates per 100 μL medium. The cells were allowed to attach overnight and were treated with 50 μL of various concentrations of CAE and CAH dissolved in DMEM with few DMSO for 24 h (the final concentration of DMSO was lower than 0.1%). The cytotoxicity of

CAE and CAH was evaluated in the cells cultured for 3 h using the MTT solution, and 75 μL of 10% SDS in 0.01 N HCl was added to the cell culture overnight. Metabolic activity was quantified by measuring light absorbance at 570 nm (Tecan, Austria). Each assay was carried out in triplicate.

2.9.2. The measurement of total collagen

The total collagen synthesis of fibroblast after UVB exposure was measured by Sircol™ soluble collagen assay kit (Biocolor Ltd., UK) following the manufacturer's protocol. Briefly, the sample was mixed with Sircol dye reagent and incubated in room temperature for 30 min. After centrifugation, ice-cold acid-salt wash reagent was added to the precipitate and then centrifuged. The precipitate was dissolved with Alka reagent and the absorption was determined at 555 nm (Tecan, Austria).

2.9.3. Western blot analysis

Cells were harvested and homogenized with lysis buffer (50 mM tris pH 7.4, 150 mM NaCl, 1 mM DL-dithiothreitol, 0.5% sodium deoxycholate, 1 mM EDTA, 1% lgepal CA-630, 0.1% SDS, 0.1 mM Na_3VO_4, 0.02 mg/mL leupeptin and 0.1 mM PMSF (phenylmethanesulfonyl fluoride). The lysates were centrifuged at 12,000*g* for 10 min at 4 °C, and protein content was determined using a Bradford reagent (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (30 μg) were separated on a 10% SDS–PAGE and then transferred to a ~~PVDF~~ membrane (Hybond ECL, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Blots were blocked for 2 h at room temperature with 5% (w/v) non-fat milk in TBS buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20 (TBST). The membrane was incubated overnight at 4 °C with specific antibodies. One is goat polyclonal antibodies against MMP-1 (1:500) and type I procollagen (1:500). Another is mouse polyclonal antibodies against MMP-3 (1:500), MMP-9 (1:500), ERK (1:500), JNK (1:500), p38 (1:500), p-ERK (1:500), p-JNK (1:500) and p-p38 (1:500), respectively (Santa Cruz Biotechnology, Inc.). The membranes were washed with TBST for 40 min. The blot was then incubated with the corresponding conjugated anti-immunoglobulin G-horseradish peroxidase (Santa Cruz Biotechnology Inc.). Immunoreactive proteins were detected with the ECL Western blotting detection system (Fujifilm, LAS-4000). Signal strengths were quantified using a densitometric program (multi Gauge V2.2).

2.10. Statistical analysis

Differences between groups in experiments were analyzed for statistical significance by ANOVA followed by Scheffe's test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The extraction ~~rate~~ quantitaion and total phenolic content of coffee leaves

The extraction yield of coffee leaves was 10.4%. The amount of chlorogenic acid was 48.3 ± 0.4 mg/g but caffeic acid was below lower limit of quantitation, and in CAH1, the chlorogenic acid was 8.1 ± 0.4 mg/g and caffeic acid was 4.2 ± 0.3 mg/g. The amount of total phenols in the extract was determined by the Folin–Ciocalteu method. The total phenolic content expressed as μg gallic acid equivalents per mg of dry weight (coffee leaves) is 26.7 ± 1.6 μg/mg.

3.2. The antioxidant effect of CAE

3.2.1. Scavenging of DPPH radicals

Fig. 1 shows the free radical scavenging activity of CAE (25–1000 μg/mL) and ascorbic acid (12.5 μg/mL). Our results indicated that CAE exhibited the activity of DPPH radial scavenging activity when the dose was higher than 50 μg/mL.

3.2.2. Erythrocyte hemolysis assay

The influence of the CAE on *in vitro* erythrocyte hemolysis was examined by incubating rat erythrocytes in the presence of 25 mM AAPH as an initiator of oxidation. The CAE provided a strong inhibitory effect and in a dose-dependent manner (10–50 μg/mL) against erythrocyte hemolysis when treated time over 1 h (Fig. 2).

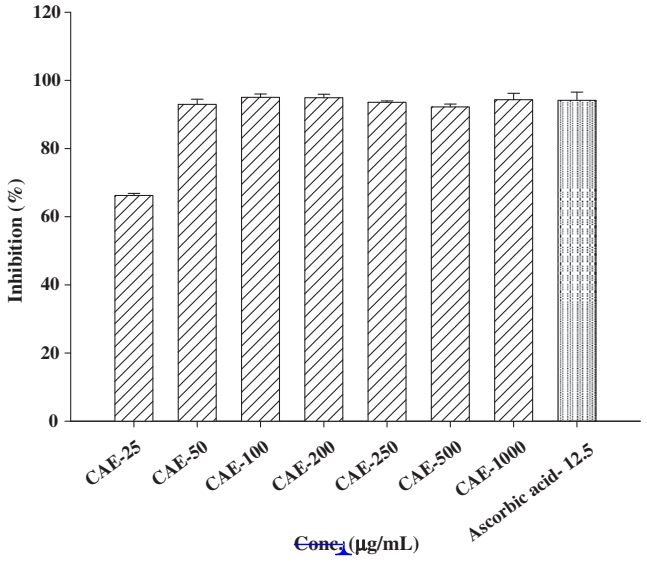

Fig. 1. Antioxidant effect of CAE on DPPH radical-scavenging ($n = 6$). CAE exhibited the activity of DPPH radial scavenging activity when the dose was higher than 50 μg/mL. The IC_{50} of CAE on DPPH radical-scavenging was 9.8 μg/mL.

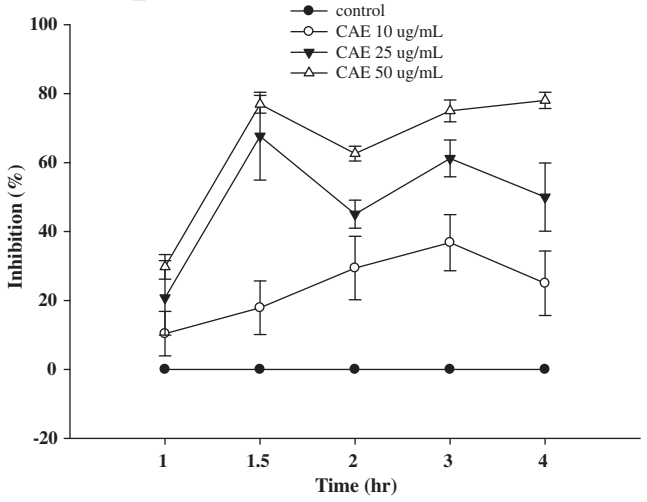

Fig. 2. The time course inhibition of CAE on AAPH-induced lysis of rat erythrocyte. The CAE provided a strong inhibitory effect and in a dose-dependent manner (10–50 μg/mL) against erythrocyte hemolysis. The IC_{50} of CAE on AAPH-induced lysis of rat erythrocyte was 33.5 μg/mL.

3.3. The screening of MMPs and elastase inhibition

3.3.1. Inhibitory effect of CAE and CAH on bacterial collagenase-1 assessed by gelatin digestion assay

For visual investigation of the inhibitory effect of CAE and CAH on MMPs, an indirect assay was developed using bacterial collagenase-1, as described above (Kim et al., 2006). Following incubation of bacterial collagenase-1 with various concentrations of CAE and CAH, the inhibition of enzyme activity was compared with enzyme activity of the control group. As shown in Fig. 3, the control group treated with reaction products of bacterial collagenase-1 and 5% of PG exhibited the highest gelatinolytic activity in the discrete zone, representing no enzyme inhibition. Gelatinolytic activity was decreased following dose-dependent treatment of CAE and CAH.

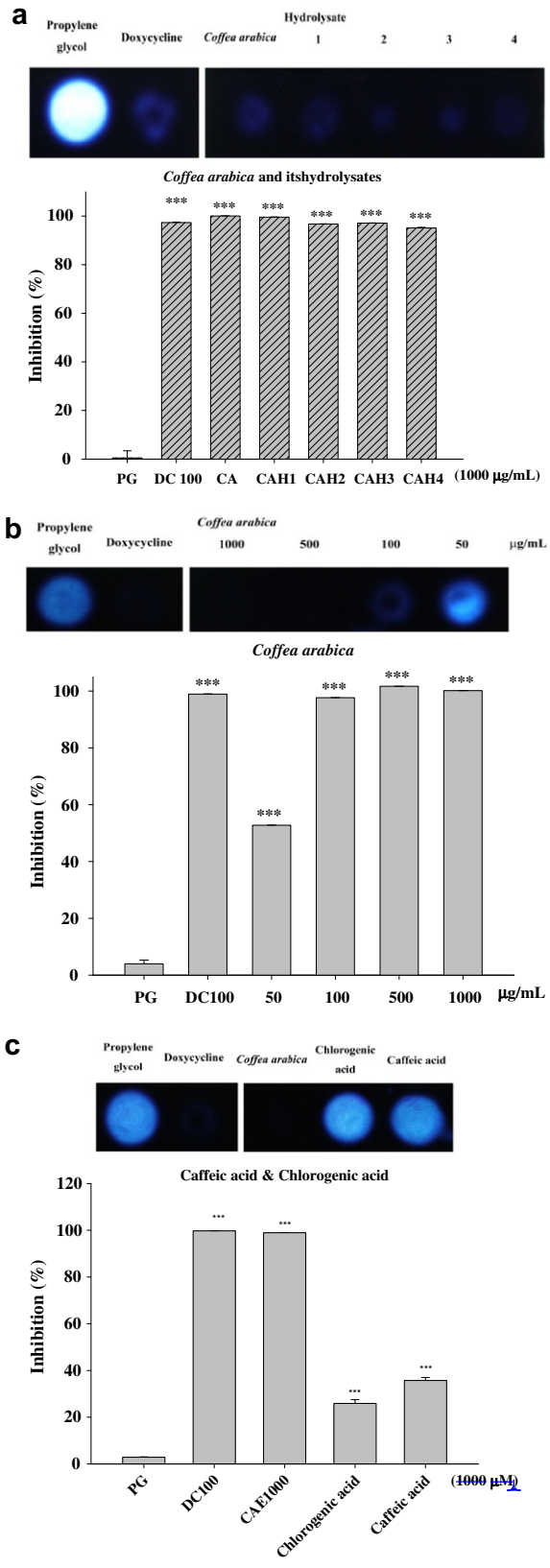

Fig. 3. The inhibition of CAE and CAH, caffeic acid and chlorogenic acid on collagenase activity. (a) CAE and CAH 1000 μg/mL, (b) CAE 50–1000 μg/mL, and (c) CAE (1000 μg/mL), chlorogenic acid and caffeic acid (~~1000 μM~~). CAE significant reduced collagenase activity in gelatin digestion but the collagenase inhibitory ability of CAH was less than which of CAE (hydrolysate 1, hydrolyzed by 1.2 N HCl for 30 min; hydrolysate 2, 1.2 N HCl for 60 min; hydrolysate 3, 2.4 N HCl for 30 min; hydrolysate 4, 2.4 N HCl for 60 min) ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

The inhibition of gelatin digestion by collagenase of CAE, CAH, caffeic acid and cholorogenic acid is shown in Fig. 3a–c. The inhibition of doxycycline (100 μg/mL, as positive control) and 50% PG (as blank) was 97.3 ± 0.02% and 0.4 ± 0.92%, respectively. A significant reduction in gelatin digestion was observed with 100 μg/mL or higher concentrations of CAE, representing inhibition of bacterial collagenase-1 activity more than 50%. However, the collagenase inhibitory ability of CAH was less than that of CAE. The inhibition of CAE (1000 μg/mL), CAH1, CAH2, CAH3 and CAH4 was 100.0 ± 0.05%, 99.5 ± 0.06%, 96.7 ± 0.08%, 97.0 ± 0.07% and 95.1 ± 0.23%, respectively. In addition, the results of 100 μM caffeic acid and chlorogenic acid are shown in Fig. 3c; the inhibition rates were 26.1 ± 0.5% and 35.9 ± 0.9%, respectively.

3.3.2. Fluorometric analysis of inhibitory effect of CAE and CAH on bacterial collagenase-1

In order to elucidate the inhibitory effect of CAE on bacterial collagenase-1, fluorescence-conjugated gelatin was used and compared with the positive control-doxycycline hyclate. In this study, a fluorescence-conjugated substrate was incubated with bacterial collagenase-1 for 20 h in the presence of different concentrations of CAE and CAH and doxycycline hyclate in 37 °C, respectively. CAE exhibited a significant inhibitory effect on bacterial collagenase-1 even at a low concentration (10 μg/mL). The inhibition rate was 80% of that of the control. The hydrolysates in high concentration showed similar inhibition on collagenase but poor inhibitory ability in low concentration. The inhibition of CAE and CAH (10–500 μg/mL) was dose-dependent (Fig. 4). The inhibition of CAE and CAH at high dose (500 μg/mL) was higher than 95%, but the effect was decreased as the concentration was reduced. In addition, the inhibitions of CAH decreased as the concentration of acid and the hydrolysis time increased.

3.4. Measurement of elastase activity

As Fig. 5 shown, the effect of CAE and CAH on elastase activity was not significant, and the inhibition of CAE was similar to the positive control (2 mM elastase inhibitor I) at high concentration (500 μg/mL), but the efficacy of CAH was not obvious.

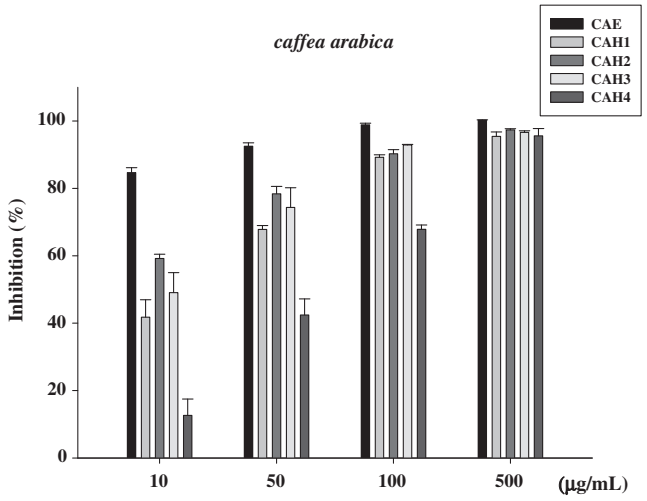

Fig. 4. The inhibition (%) of CAE and CAH on the activity of bacterial collagenase. CAE exhibited a significant inhibitory effect on bacterial collagenase-1 even in low concentration (10 μg/mL). The hydrolysates in high concentration showed similar inhibition on collagenase. The inhibition of CAE and CAH (10–500 μg/mL) were dose-dependent manner. The IC_{50} of CAE, CAH1, CAH2, CAH3 and CAH4 were 3.2, 15.3, 5.6, 10.6 and 32.9 μg/mL, respectively.

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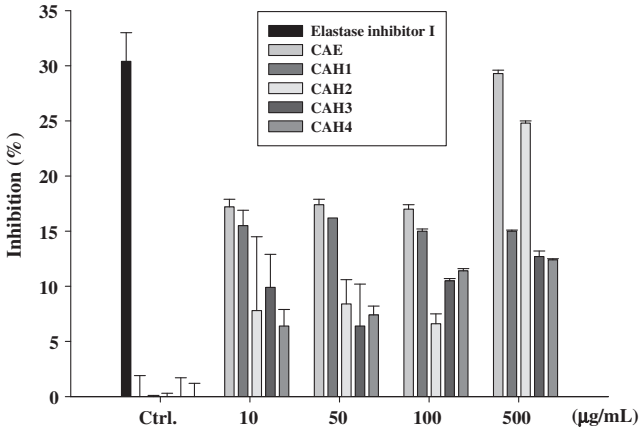

Fig. 5. The inhibition of CAE and its hydrolysates on elastase activity ($n = 4$). Elastase inhibitor I was as positive control.

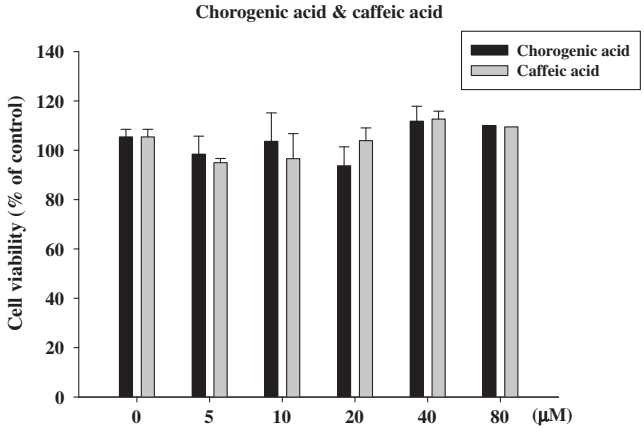

Fig. 7. Cell viability (%) of chlorogenic acid and caffeic acid on human foreskin fibroblasts. Caffeic acid and cholorgenic acid (20–80 μM) did not have cytotoxic effects on the proliferation on fibroblasts.

3.5. Effect of CAE and CAH on the cell viability

Hs68 cells were treated with various concentrations of CAE and CAH, and cell viability was measured using the MTT assay. As shown in Fig. 6, the resulting survival curve indicated that CAE (1–1000 μg/mL) and CAH (1–1000 μg/mL) did not exhibit cytotoxic effects on the proliferation of cells. In addition, the preparations at high concentration (>100 μg/mL) stimulate cell growth (120%). Caffeic acid and chlorogenic acid (5–80 μM) did not have cytotoxic effects on the proliferation on fibroblasts (Fig. 7) and these concentrations were applied for the following experiments.

3.6. Effects of coffee preparation and its constituents on UVB induced photoaging

3.6.1. Effect of CAE, CAH, caffeic acid and chlorogenic acid on MMP expression

According to the preliminary study, UV irradiation induced photodamaged fibroblasts at an exposure dose of 80 mJ/cm^2 has been shown to increase MMP secretion level and to decrease type I procollagen synthesis (data not shown). The cell viability is 98 ± 0.8% of control after 80 mJ/cm^2 UVB exposure (data not shown). This dose equates to about 45 s exposure at the noontime on July in Middle Taiwan measured by a UV meter (UVP, USA). This dose was chosen as the exposure dose in the following experiment. CAE pre-treated cells before UVB (80 mJ/cm^2) irradiation showed significant inhibition of MMP-1, -3, -9 at 5 μg/mL, but CAH did not present the same effect.

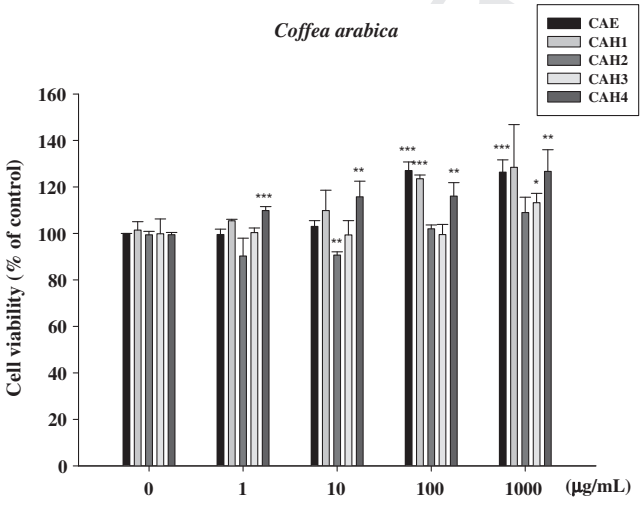

Fig. 6. Cell viability (%) of CAE and CAH on human foreskin fibroblasts. CAE (1–1000 μg/mL) and CAH (1–1000 μg/mL) did not exhibit cytotoxic effects on the proliferation of cells. In addition, the preparations at high concentration (>100 μg/mL) stimulate cell growth (120%) (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

In Fig. 8, MMP-1 expression was increased after UVB irradiation, and CAE (5–25 μg/mL) pretreatment diminished the effect. In addition, the effect of CAE on MMP-1 expression was dose-dependent. The results of MMP-3 and -9 resembled those of MMP-1. Caffeic acid (5–50 μM) inhibited UVB-induced MMP-1 and -9 overexpression, but not MMP-3 (Fig. 9). Chlorogenic acid (5–50 μM) pretreated would inhibit UVB-induced MMP-1 and -3 overexpression but not MMP-9. And chlorogenic acid showed an inhibitory effect on MMP-3 (50 μM) at a high dose.

3.6.2. Effect of CAE, caffeic acid and chlorogenic acid on type I procollagen expression

The fibroblasts were pretreated with CAE (5–25 μg/mL) for 15 min, exposed to UVB, and treated with CAE for 24 h. The expression of type I procollagen is shown in Fig. 8; 10 μg/mL of CAE would restore the type I procollagen to 60% of that of the control group. Caffeic acid and chlorogenic acid would not restore UVB-inhibited type I procollagen expression (Fig. 9).

3.6.3. Effect of CAE on total collagen

The fibroblasts were pretreated with CAE (5–50 μg/mL) for 15 min, exposed to UVB, and treated with CAE for 24 h. The synthesis of collagen is shown in Fig. 10; UVB exposure will significantly suppress the total collagen and CAE will restore that when concentration was higher than 10 μg/mL (Fig. 10).

3.6.4. Effect of CAE, caffeic acid and chlorogenic acid on MAP kinase expression

As Fig. 11 shown, UV (80 mJ/cm^2) will induce the phophorylation of p38, ERK and JNK. The inhibition effect of CAE (5–25 μg/mL) on JNK phosphorylation was dose-dependent (Fig. 11), and the effect was significant when the dose higher than 10 μg/mL. ERK activation was suppressed at a CAE dose of 25 μg/mL. CAE at a low dose (5 and 10 μg/mL) inhibited p38 phosphorylation, but did not do so at 25 μg/mL. Caffeic acid and chlorogenic acid did not inhibit MAP kinase expression (data not shown).

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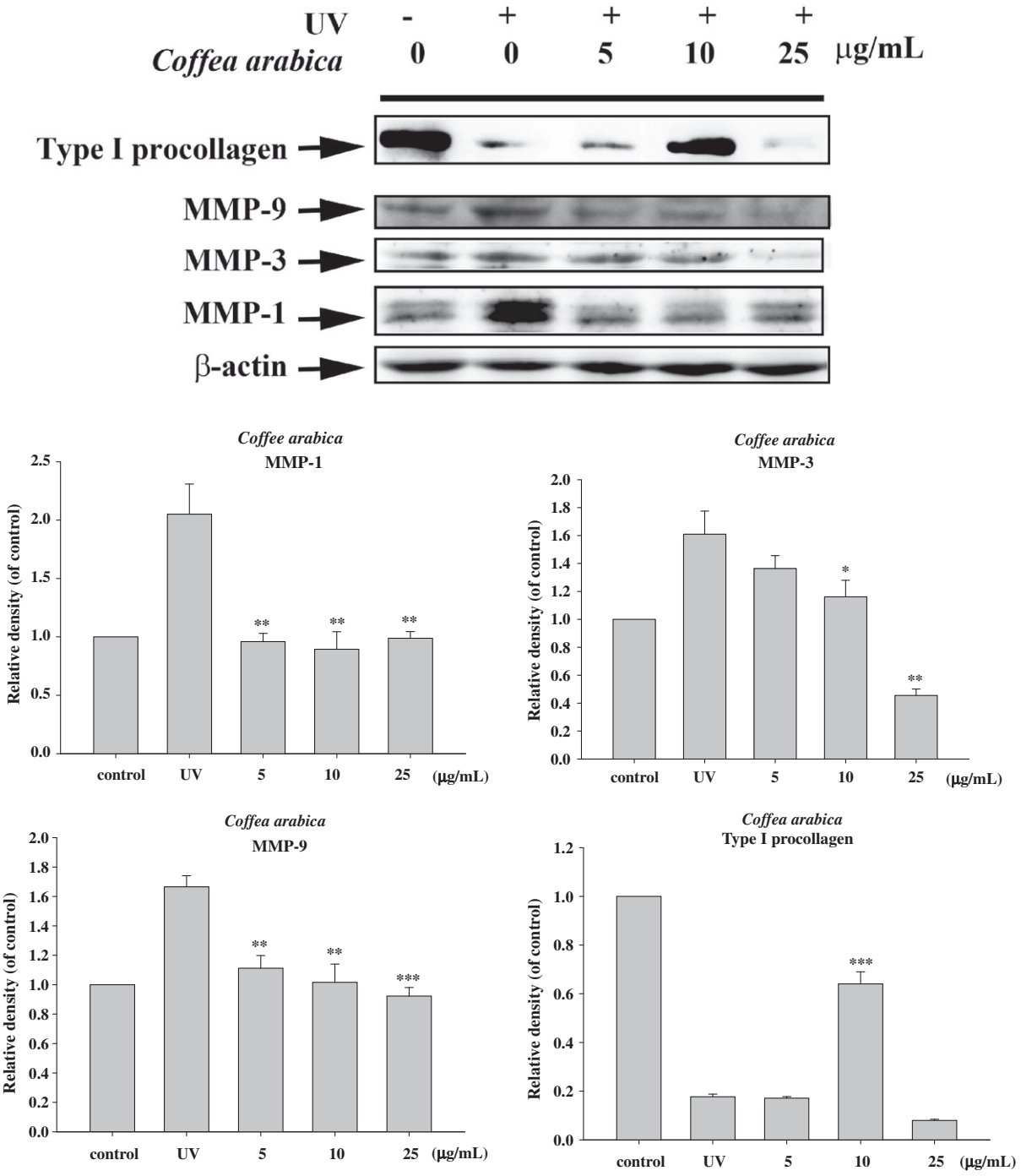

Fig. 8. Effects of CAE on the UVB-induced expression of MMP-~~1, 3 and 9~~ and type I procollagen in human foreskin fibroblasts. MMP-1 expression was increase after UVB irradiation, and CAE (5–25 μg/mL) pretreatment would diminish the effect. The results of MMP-3 and -9 resembled to MMP-1. CAE would restore the type I procollagen to 60% of the control group (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

4. Discussion

In the literature, it has been reported that UV-induced inflammation and the resulting accumulation of ROS play an important role in chronologically aged and photoaged skin *in vivo* (Kawaguchi et al., 1996). Increased ROS production alters gene and protein structure and function, leading to skin damage (Rittié and Fisher, 2002). UV irradiation also enhances collagenase activity and contributes to wrinkle formation through degradation of the collagen in dermal extracellular matrix (Brenneisen et al., 2002; Dong et al., 2008; Uitto, 2008). Collagenase inhibitors have been identified as potential therapeutic candidates for anti-photoaging and prevention of wrinkle formation (Inomata et al., 2003). Polyphenols are predominantly present as glycosides in Chinese herbs, while it has been reported that the aglycone's activity was superior to that of glycones (Kim et al., 2004). Aglycone was hydrophobic and more easily penetrated through the skin and then absorbed. In the literature, it has been reported that numbers of plant-extracts suppressed collagenases (Leu et al., 2006; Lim and Kim, 2007), but studies about the activities of aglycones on collagenase are rare.

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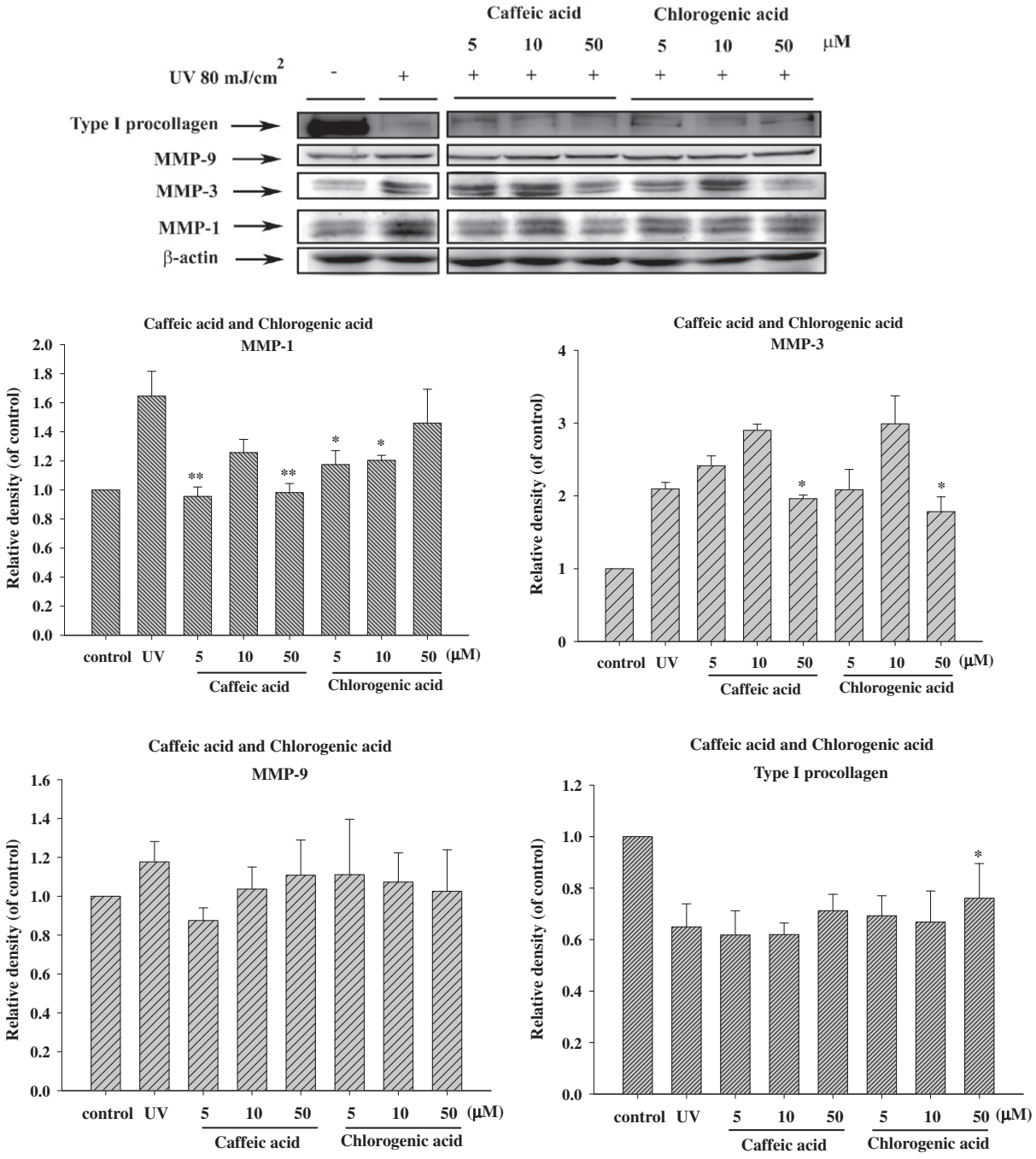

Fig. 9. Effects of caffeic acid and chlorogenic acid on the UVB-induced expression of MMP-~~1, 3, 9~~ and type I procollagen in human foreskin fibroblasts. Caffeic acid (5–50 μM) would inhibit UVB-induced MMP-1 and -9 overexpression, but not MMP-3. Cholorogenic acid (5–50 μM) pretreated would inhibit the UVB-induced MMP-1 and -3 overexpression, but not MMP-9. Caffeic acid and cholorogenic acid would not restore the UVB-inhibited type I procollagen expression (*$P < 0.05$; **$P < 0.01$).

This study attempted to investigate the collagenase inhibition of CAE before and after acid hydrolysis.

The results in Fig. 3 indicate that the collagenase inhibition of CAE and CAH at 1000 μg/mL were higher than 90%, and the inhibition was 84% at 10 μg/mL of CAE. As Fig. 4 shown, the activities of CAH on collagenase inhibition varied with the condition of hydrolysis time, temperature and hydrochloric acid concentration, and the activities were not comparable to extract. While the content of aglycones would elevate after hydrolysis because of the cleavage of glycosides, acid and heat may damage aglycones suppressing the inhibitory effect of CAH on collagenase activity.

Previous studies on natural products have reported that a high content of polyphenols is responsible for some biological activities observed in these plants. The water-soluble extract of *Rosmarinus officinalis* L. suppresses UV-induced MMP-1 through IL-1α and IL-6 modulation, causing MMP-1 translation inhibition in human skin (Martin et al., 2008). Flavonoids like quercetin, kaempferol, apigenin and wogonin inhibit mammalian collagenase, MMP-1, MAP kinase activity, and AP-1 activation (Pillai et al., 2005).

UV irradiation induces MMP-1, MMP-3 and MMP-9 expression (Pillai et al., 2005; Dong et al., 2008). MMP-1 initiates the degradation of types I and III fibrillar collagens, MMP-9 further degrades collagen fragments generated by collagenases, and MMP-3 acti-

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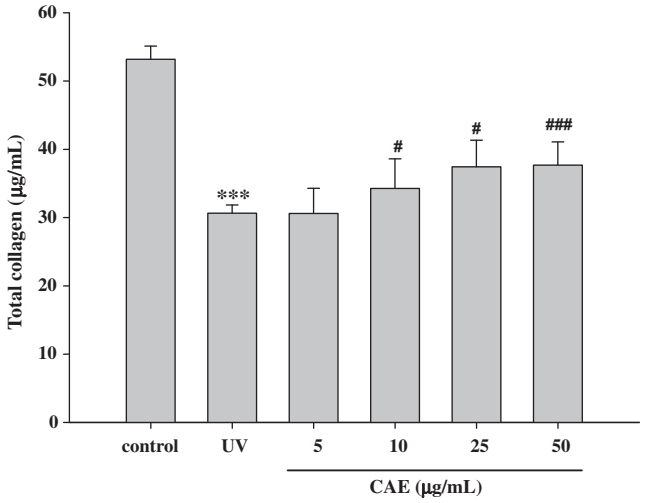

Fig. 10. Effect of CAE on total collagen synthesis in human fibroblasts. Human fibroblasts (Hs68) was treated with/without UV 80 mJ/cm^2 and CAE of 5, 10, 25 and 50 μg/mL ($n = 3$). Significant difference versus control (non-UV-exposed): $^{***}P < 0.001$. Significant inhibition versus UV-exposed group: $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$.

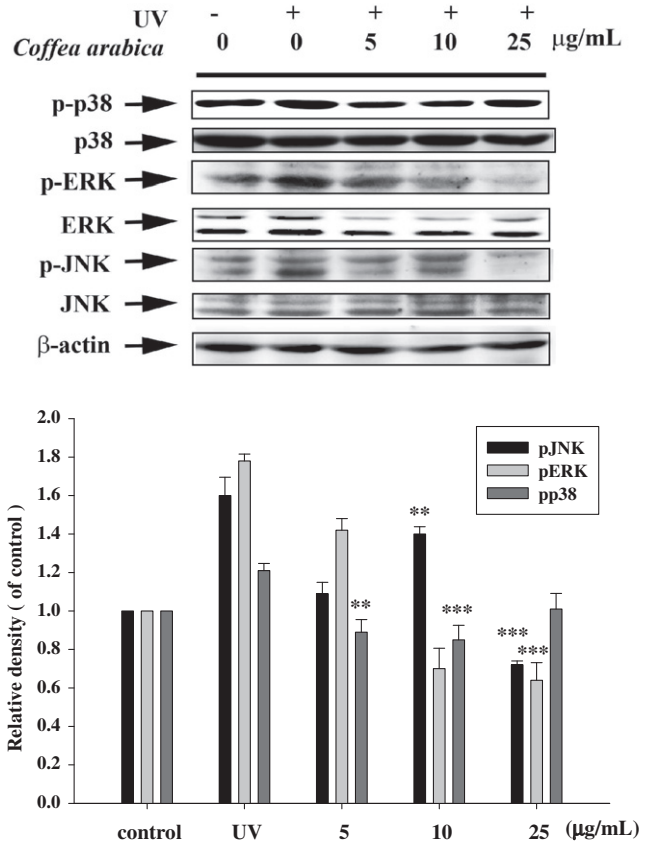

Fig. 11. Effect of CAE on the UVB-induced expression of MAP kinases in human fibroblasts. CAE inhibited JNK phosphorylation as dose-dependent manner. ERK activation would be suppressed as CAE dose at 25 μg/mL. CAE at low dose (5 and 10 μg/mL) would inhibit the p38 phosphorylation, but not at 25 μg/mL ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

vates proMMP-1 (Rittié and Fisher, 2002). *Melothria heterophylla* extract and esculetin isolated from *Fraxinus chinensis* with anti-oxidative activity and inhibiting UVB induced the expression of MMP-1 mRNA and protein. Our results indicated that CAE protects against the photoaging induced by UVB through inhibition of MMP-1 and MMP-9 expression even at a low dose (5 μg/mL) and showed the same effect on MMP-3 (10 μg/mL) (Fig. 8). In the literature, the working dose of natural products on MMP expression inhibition was higher than 10 μg/mL (Kim et al., 2006, 2007; Tanaka et al., 2007). Our study indicated that not only was CAE a potent MMP inhibitor, it also possessed diversity on MMP inhibition, which could inhibit MMP-1 on the degradation of types I and III collagens. In addition, CAE could inhibit MMP-9 to prevent it from degrading collagen fragments generated by MMP-1 and furthermore inhibits MMP-3 to reduce the secretion of proMMP-1.

For the study of the major components of MMP inhibitor, caffeic acid and chlorogenic acid were investigated. The results indicated that caffeic acid inhibits MMP-1 and -9 but not MMP-3 (Fig. 9). However, cholorogenic acid inhibits MMP-3 at 50 μg/mL, but not MMP-1 and MMP-9. It has been reported that caffeic acid selectively suppresses MMP-2 and -9 expression of HepG2 cells induced by PMA (phorbol 12-myristate 13-acetate) by inhibiting the function of NF-κB, but not AP-1 (Chung et al., 2004). However, it has been also reported that chlorogenic acid inhibits the MMP-9 activity of the hepatocellular carcinoma cell line (Hep3B) (Jin et al., 2005). On the other hand, caffeic acid has been reported showing far weaker collagenolytic activity (Kusano et al., 2001). These two components may partly contribute to the anti-photoaging ability of CAE and CAH, and the other constituents in CAE may be involved in MMP inhibition. In animal study, caffeic acid (0.2 mg) significantly inhibited the UVB-induced (4000 J/m^2) activation of c-Jun and p38 mitogen-activated protein kinase in mouse (Staniforth et al., 2006). Besides, caffeic acid could inhibit Fyn kinase activity and UVB-induced COX-2 expression in mouse skin epidermal cells and mouse skin (Kang et al., 2009). Chlorogenic acid could inhibit 12-*O*-tetradecanoylphorbol-13-acetate induced ~~NF-kappaB~~, iNOS and COX-2 activation in mouse epidermis (Cichocki et al., 2010). Q1 Furthermore, chlorogenic acid could protect humans against oxidative damage of macromolecules (Hoelzl et al., 2010). In this study, the MMPs inhibition and of type I procollagen enhancing effect of CAE was superior to caffeic acid and chlorogenic acid, therefore, CAE could be potential in developing of anti-photoaging agents.

Extracellular matrix was the skeleton of skin in dermis. Besides collagen, elastin also responded to skin plasticity (Labat-Robert and Robert, 1988). UV irradiation enhances the secretion of elastase and degrades elastin causing sagging of the skin (Getie et al., 2005; Seite et al., 2006). Some plant extracts have been reported to suppress elastase activity (Kim et al., 2007; Tsukahara et al., 2006), but our results indicated that CAE did not have this effect. UV irradiation would reduce the production of collagen which was the major composition of the dermis and cause an imbalance between MMP synthesis and degradation (Scharffetter-Kochanek et al., 2000; Chung et al., 2003; Ichihashi et al., 2003; Rabe et al., Q2 2006; Bae et al., 2008). The results of this study indicated that CAE prevents UVB-induced type I procollagen damage and enhances the total collagen synthesis even at low dose (10 μg/mL), while caffeic acid and chlorogenic acid do not. It had been reported that the caffeic acid and its derivatives will distributed in the skin after oral administration (Yamada et al., 2006). We supposed that the active components of CAE will be absorbed to exhibit induced collagen synthesis in the skin. The percutaneous absorption of CAE and its active components needed further study.

MAP kinase activation is not only one of the photoaging pathways but also a factor in MMP production in fibroblasts. It has been reported that caffeic acid inhibits UVB-induced IL-10 mRNA expression and MAP kinase activation (Staniforth et al., 2006). If caffeic acid inhibits MAP kinase activation, then it would inhibit MMP expression. The results indicate that CAE inhibited JNK, ERK

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and p38 activation. We speculate that the inhibition of collagen degradation of CAE was related to antioxidant activity, since the direct injury of UVB on skin was due to ROS. Polyphenols would be good ROS scavengers based on their numbers of OH group (Sim et al., 2007). Previous studies have reported that the phenolic content of the plants contributes to the antioxidant activity (Javanmardi et al., 2003; Nazaruk, 2008; Hodzic et al., 2009). UVB irradiation induced ROS production promoted downstream signal transduction in the dermis, causing skin damage and photoaging (Ho et al., 2005). Thus, inhibition ROS production would prevent the skin from photoaging. In this study, CAE containing high total phenolic content (26.7 μg/mg) showed good DPPH radical scavenging activity and protection for AAPH-induced erythrocyte hemolysis (Figs. 2 and 3), indicating that CAE is a potential candidate for the prevention of photoaging.

CAE inhibits MAPKs phosphorylation and causes modulation of c-Fos expression. JNK and p38 modulate c-Fos expression, and c-Fos accompanied by c-Jun would synthesize the translation factor, AP-1. CAE inhibiting ERK, JNK and p38 expression may suppress c-Fos and c-Jun expression and then inhibit AP-1, MMP and type I procollagen expression. We speculate that CAE and its active components may stimulate the proliferation of fibroblasts and TGF-β secretion, activate signal transduction pathway of collagen synthesis and suppress UVB-induced AP-1 activation.

CAE and its constituents, chlorogenic acid and caffeic acid, diminished UVB irritation induced photoaging by inhibiting MMPs and elevating type I procollagen production through ROS scavenging and down-regulation of MAPKs pathway. CAE could be a promising agent for the prevention of cutaneous photodamage.

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References

Almeida, A.A., Farah, A., Silva, D.A., Nunan, E.A., Gloria, M.B., 2006. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. J. Agric. Food Chem. 54, 8738–8743.

Bae, J.Y., Choi, J.S., Choi, Y.J., Shin, S.Y., Kang, S.W., Han, S.J., Kang, Y.H., 2008. (-)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.

Barclay, L.R., Locke, S.J., McNeil, J.M., VanKessel, J., Burton, G.W., Ingold, K.U., 1984. Autoxidation of micelles and model membranes. Quantitative kinetic measurements can be made by using either water-soluble or lipid-soluble initiators with water-soluble or lipid-soluble chain-breaking antioxidants. J. Am. Chem. Soc. 106, 2479–2481.

Brenneisen, P., Sies, H., Scharffetter-Kochanek, K., 2002. Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. Ann. NY Acad. Sci. 973, 31–43.

Chung, J.H., 2003. Photoaging in Asians. Photodermatol. Photoimmunol. Photomed. 19, 109–121.

Chung, T.W., Moon, S.K., Chang, Y.C., Ko, J.H., Lee, Y.C., Cho, G., Kim, S.H., Kim, J.G., Kim, C.H., 2004. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J. 18, 1670–1681.

Cichocki, M., Blumczyńska, J., Baer-Dubowska, W., 2010. Naturally occurring phenolic acids inhibit 12-O-tetradecanoylphorbol-13-acetate induced NF-kappaB, iNOS and COX-2 activation in mouse epidermis. Toxicology 268, 118–124.

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., 2008. UV-induced DNA damage initiates release of MMP-1 in human skin. Exp. Dermatol. 17, 1037–1044.

Fisher, G.J., Datta, S.C., Talwar, H.S., Wang, Z.Q., Varani, J., Kang, S., Voorhees, J.J., 1996. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379, 335–339.

Gelse, K., Poschl, E., Aigner, T., 2003. Collagens–structure, function, and biosynthesis. Adv. Drug Deliv. Rev. 55, 1531–1546.

Getie, M., Schmelzer, C.E., Neubert, R.H., 2005. Characterization of peptides resulting from digestion of human skin elastin with elastase. Proteins 61, 649–657.

Ho, J.N., Lee, Y.H., Lee, Y.D., Jun, W.J., Kim, H.K., Hong, B.S., Shin, D.H., Cho, H.Y., 2005. Inhibitory effect of Aucubin isolated from *Eucommia ulmoides* against UVB-induced matrix metalloproteinase-1 production in human skin fibroblasts. Biosci. Biotechnol. Biochem. 69, 2227–2231.

Hodzic, Z., Pasalic, H., Memisevic, A., Srabovic, M., Saletovic, M., Poljakovic, M., 2009. The influence of total phenols content on antioxidant capacity in the whole grain extracts. Eur. J. Sci. Res. 28, 471–477.

Hoelzl, C., Knasmüller, S., Wagner, K.H., Elbling, L., Huber, W., Kager, N., Ferk, F., Ehrlich, V., Nersesyan, A., Neubauer, O., Desmarchelier, A., Marin-Kuan, M., Delatour, T., Verguet, C., Bezençon, C., Besson, A., Grathwohl, D., Simic, T., Kundi, M., Schilter, B., Cavin, C., 2010. Instant coffee with high chlorogenic acid levels protects humans against oxidative damage of macromolecules. Mol. Nutr. Food Res. 54, 1–12.

Ichihashi, M., Ueda, M., Budiyanto, A., Bito, T., Oka, M., Fukunaga, M., Tsuru, K., Horikawa, T., 2003. UV-induced skin damage. Toxicology 189, 21–39.

Inomata, S., Matsunaga, Y., Amano, S., Takada, K., Kobayashi, K., Tsunenaga, M., Nishiyama, T., Kohno, Y., 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.

Javanmardi, J., Stushnoff, C., Locke, E., Vivanco, J.M., 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem. 83, 547–550.

Jin, U.H., Lee, J.Y., Kang, S.K., Kim, J.K., Park, W.H., Kim, J.G., Moon, S.K., Kim, C.H., 2005. A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metalloproteinase-9 inhibitor: isolation and identification from methanol extract of *Euonymus alatus*. Life Sci. 77, 2760–2769.

Kang, N.J., Lee, K.W., Shin, B.J., Jung, S.K., Hwang, M.K., Bode, A.M., Heo, Y.S., Lee, H.J., Dong, Z., 2009. Caffeic acid, a phenolic phytochemical in coffee, directly inhibits Fyn kinase activity and UVB-induced COX-2 expression. Carcinogenesis 30, 321–330.

Katiyar, S.K., 2003. Skin photoprotection by green tea: antioxidant and immunomodulatory effects. Curr. Drug Targets Immune. Endocr. Metabol. Disord. 3, 234–242.

Kawaguchi, Y., Tanaka, H., Okada, T., Konishi, H., Takahashi, M., Ito, M., Asai, J., 1996. The effects of UV A and reactive oxygen species on the mRNA expression of type IV collagenase and its tissue inhibitor in cultured human dermal fibroblasts. Arch. Dermatol. Res. 288, 39–44.

Kim, D.H., Kim, J.H., Baek, S.H., Seo, J.H., Kho, Y.H., Oh, T.K., Lee, C.H., 2004. Enhancement of tyrosinase inhibition of the extract of *Veratrum patulum* using cellulase. Biotechnol. Bioeng. 87, 849–854.

Kim, M.M., Ta, Q.V., Mendis, E., Rajapakse, N., Jung, W.K., Byun, H.G., Jeon, Y.J., Kim, S.K., 2006. Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. Life Sci. 79, 1436–1443.

Kim, Y.H., Kim, K.S., Han, C.S., Yang, H.C., Park, S.H., Ko, K.I., Lee, S.H., Kim, K.H., Lee, N.H., Kim, J.M., 2007. Inhibitory effects of natural plants of Jeju Island on elastase and MMP-1 expression. Int. J. Cosmet. Sci. 29, 487–488.

Kusano, A.K., Seyama, Y.Y., Nagai, M.H., Shibano, M., Kusano, G.J., 2001. Effects of fukinolic acid and cimicifugic acids from cimicifuga species on collagenolytic activity. Biol. Pharm. Bull. 24, 1198–1201.

Labat-Robert, J., Robert, L., 1988. Aging of the extracellular matrix and its pathology. Exp. Gerontol. 23, 5–18.

Leu, S.J., Lin, Y.P., Lin, R.D., Wen, C.L., Cheng, K.T., Hsu, F.L., Lee, M.H., 2006. Phenolic constituents of *Malus doumeri* var. formosana in the field of skin care. Biol. Pharm. Bull. 29, 740–745.

Lim, H., Kim, H.P., 2007. Inhibition of mammalian collagenase, matrix metalloproteinase-1, by naturally-occurring flavonoids. Planta Med. 73, 1267–1274.

Martin, R., Pierrard, C., Lejeune, F., Hilaire, P., Breton, L., Bernerd, F., 2008. Photoprotective effect of a water-soluble extract of *Rosmarinus officinalis* L. against UV-induced matrix metalloproteinase-1 in human dermal fibroblasts and reconstructed skin. Eur. J. Dermatol. 18, 128–135.

Namba, T., Matsuse, T., 2002. A historical study of coffee in Japanese and Asian countries: focusing the medicinal uses in Asian traditional medicines. Yakushigaku Zasshi 37, 65–75.

Nazaruk, J., 2008. Antioxidant activity and total phenolic content in Cirsium five species from north-east region of Poland. Fitoterapia 79, 194–196.

Pillai, S., Oresajo, C., Hayward, J., 2005. Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation – a review. Int. J. Cosmet. Sci. 27, 17–34.

Rabe, J.H., Mamelak, A.J., McElgunn, P.J., Morison, W.L., Sauder, D.N., 2006. Photoaging: mechanisms and repair. J. Am. Acad. Dermatol. 55, 1–19.

Ragazzi, E., Veronese, G., 1973. Quantitative analysis of phenolics compounds after thin-layer chromatographic separation. J. Chromatogr. 77, 369–375.

Ranheim, T., Halvorsen, B., 2005. Coffee consumption and human health-beneficial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. Mol. Nutr. Food Res. 49, 274–284.

Rittié, G., Fisher, G.J., 2002. UV-light-induced signal cascades and skin aging. Ageing Res. Rev. 1, 705–720.

Scharffetter-Kochanek, K., Brenneisen, P., Wenk, J., Herrmann, G., Ma, W., Kuhr, L., Meewes, C., Wlaschek, M., 2000. Photoaging of the skin from phenotype to mechanisms. Exp. Gerontol. 35, 307–316.

Seite, S., Zucchi, H., Septier, D., Igondjo-Tchen, S., Senni, K., Godeau, G., 2006. Elastin changes during chronological and photo-ageing: the important role of lysozyme. J. Eur. Acad. Dermatol. Venereol. 20, 980–987.

Sim, G.S., Lee, B.C., Cho, H.S., Lee, J.W., Kim, J.H., Lee, D.H., Pyo, H.B., Moon, D.C., Oh, K.W., Yun, Y.P., 2007. Structure activity relationship of antioxidative property of flavonoids and inhibitory effect on matrix metalloproteinase activity in UVA-irradiated human dermal fibroblast. Arch. Pharm. Res. 30, 290–298.

Staniforth, V., Chiu, L.T., Yang, N.S., 2006. Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse. Carcinogenesis 27, 1803–1811.

Tanaka, K., Hasegawa, J., Asamitsu, K., Okamoto, T., 2007. *Magnolia ovovata* extract and its active component magnolol prevent skin photoaging via inhibition of nuclear factor kappaB. Eur. J. Pharmacol. 565, 212–219.

Tsukahara, K., Nakagawa, H., Moriwaki, S., Takema, Y., Fujimura, T., 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.

Uitto, J., 2008. The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. J. Drugs Dermatol. 7, s12–s16.

Utsunomiya, H., Ichinose, M., Uozaki, M., Tsujimoto, K., Yamasaki, H., Koyama, A.H., 2008. Antiviral activities of coffee extracts in vitro. Food Chem. Toxicol. 46, 1919–1924.

Yamada, Y., Yasui, H., Sakurai, H., 2006. Suppressive effect of caffeic acid and its derivatives on the generation of UVA-induced reactive oxygen species in the skin of hairless mice and pharmacokinetic analysis on organ distribution of caffeic acid in ddY mice. Photochem. Photobiol. 82, 1668–1676.

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