

각질세포에서 자외선B가 유도한 세포 사멸, 산화적 스트레스 및 matrix metalloproteinase 1 발현에 대한 죽여추출물의 영향

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Effects of *Bambusae Caulis in Taeniam* Extract on the UVB-induced Cell Death, Oxidative Stress and Matrix Metalloproteinase 1 Expression in Keratinocytes

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요약: 자외선은 피부 광노화의 주요 요인이며, 효과적인 자외선 차단제가 피부의 건강과 아름다움을 위해 필요하다. 본 연구는 세포 실험을 통하여 자외선에 의해 유도된 세포 사멸, 산화적 스트레스, matrix metalloproteinase 1 발현에 미치는 죽여추출물의 영향을 알아보고자 수행하였다. HaCaT 인간 각질세포를 여러 농도의 죽여추출물 유무 조건에서 자외선을 조사하고 세포의 생존율과 생화학적 과정들의 변화를 분석하였다. 죽여추출물은 자외선을 조사한 세포의 생존율을 증진시켰고, procaspase 3가 활성화 형태로 절단되고, Bax/Bcl-2 비율이 증가하는 세포 자살 과정을 완화시켰다. 죽여추출물은 자외선에 노출된 세포에서 활성산소의 발생과 지질 과산화도 감소시켰다. 또한 죽여추출물은 자외선에 의해 자극된 matrix metalloproteinase 1의 발현과 c-Jun N-terminal kinase의 인산화를 억제하였다. 본 연구는 죽여추출물이 자외선에 의한 세포 사멸, 산화적 스트레스, 그리고 matrix metalloproteinase 1 발현을 억제함을 보여 주었으며, 이 추출물이 피부 광노화의 일부 현상을 억제하는 화장품 원료로 유용함을 시사하였다.

Abstract: Ultraviolet radiation (UV) is a major cause of skin photoaging, and effective UV protecting agents are needed for the skin health and beauty. This study was undertaken to examine the effects of *Bambusae caulis in Taeniam* extract (BCTE) on UVB-induced cell death, oxidative stress and matrix metalloproteinase 1 (MMP1) expression in cell-based assays. HaCaT human keratinocytes were exposed to UVB in the presence of BCTE at different concentrations and resulting changes in cell viability and biochemical events were determined. The results showed that BCTE enhanced the viabilities of UVB-exposed cells, and attenuated apoptotic events such as cleavage of procaspase 3 to its active form, and the increase of Bax to Bcl-2 ratios. BCTE also attenuated the reactive oxygen generation and lipid peroxidation in cells exposed to UVB. Additionally, it attenuated the expression of matrix metalloproteinase 1 and the phosphorylation of c-Jun N-terminal kinase stimulated by UVB. Conclusively, the present study demonstrated that BCTE pro

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tected skin cells from the UVB-induced cell death, oxidative stress and MMP1 expression, suggesting its potential use as a cosmetic ingredient mitigating some features of the skin photoaging.

Keywords: UVB, *Bambusae Caulis in Taeniam*, oxidative stress, apoptosis, matrix metalloproteinase 1, keratinocytes

1. Introduction

Intrinsic aging of human skin is dependent on time and genetics, whereas extrinsic skin aging is affected by environmental factors such as solar radiation[1]. Intrinsic aging, also called natural or chronological aging, is inevitable, but extrinsic photoaging can be avoided with good habits minimizing exposure to ultraviolet radiation (UV)[2]. The sun is a primary source of UV inducing skin photoaging which alters the normal phenotypes of the skin such as texture, wrinkles, tone, and color[3]. Of course, UV is a major cause of photo-carcinogenesis[4].

UV-induced apoptotic cell death is involved in skin photoaging[5]. Apoptosis usually involves the changes of pro-apoptotic (Bax, Bak and Bid) and anti-apoptotic (Bcl-2 and Bcl-x) members of the Bcl-2 protein family[6]. Caspases are known to mediate UV-induced apoptosis in keratinocytes[7]. UV induces oxidative stress in cells by stimulating production of reactive oxygen species (ROS) and depleting antioxidants[8,9]. As the results, oxidative damages of biomolecules such as lipids, nucleic acids, and proteins increase in cells exposed to UV[10,11]. Skin photoaging due to UV exposure involves the activation of the matrix metalloproteinases (MMPs), a group of zinc endopeptidases, which regulate the turnover of extracellular matrix macromolecules including type I collagen[12,13]. These MMPs can regulate connective tissue remodeling leading to the formation of wrinkles, and other phenotypes of photoaged skin[14, 15]. MMP1 secreted not only from dermal fibroblasts and but from epidermal keratinocytes is known to participate in collagen destruction in the skin[16,17].

Sunscreen products have become essential products to those who pursue skin health and beauty[18]. Although the weight of current evidence is that sunscreen agents of either inorganic or organic forms remain on the surface

of the skin, there also is a concern regarding the penetration of sunscreen agents into the skin and their harmful effects[19,20]. There is considerable interest in the development of safer, more effective cosmetic ingredients that mitigate the harmful effects of UV. In this regard, plant extracts with UV-shielding and anti-oxidative properties are an attractive prospect. We have previously observed the protective effects of plant extracts derived from *Gardenia jasminoides* and *Sasa quepaertensis* against the UV-induced injury in cells and animal models[21,22].

Bambusae caulis in Taeniam has been used as a health food additive and a traditional medicine for the treatments of atherosclerosis, hyperlipidemia, hypertension, fatigue and so on[23-25]. Its activity is thought to be related partly to potent antioxidant properties[16,26]. In the present study, we examined the effects of BCTE on UVB-induced cell death, oxidative stress and MMP1 expression in HaCaT keratinocytes. This plant material was chosen because it attenuated the UVB-induced HaCaT cell death effectively and showed the lowest cytotoxicity among the numerous plant extracts tested in a preliminary experiment. The present study demonstrated the beneficial properties of BCTE protecting skin cells from the UVB-induced damages, suggesting its potential use in the photoprotection of the skin.

2. Materials and Methods

2.1. Preparation of BCTE

Bambusae caulis in Taeniam (stem) was purchased from a local market (<http://www.jchanbang.com/>), and extracted with 70% aqueous ethanol at room temperature for 7 days, followed by evaporation under a reduced pressure to obtain BCTE at a yield of 4.5%.

2.2. UV spectrophotometry

BCTE was dissolved in phosphate buffered saline (PBS) at 10, 30, and 100 $\mu\text{g}/\text{mL}$, and its absorption spectra were recorded in the 200 ~ 400 nm range using a Shimadzu UV-1650PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.3. HPLC analysis

HPLC analysis of BCTE was done using a Gilson HPLC system (Gilson, Inc. Middleton, WI, USA) equipped with a 321 pump and UV/VIS 151 detector. Aqueous solution of BCTE (1.0 mg/mL) was injected at 20 μL . Separation was done on a 5 mm Hector-M C_{18} column (4.6 mm \times 250 mm)(RStechco, Daejeon, Korea). The mobile phase consisted of 0.5% formic acid (A) and acetonitrile (B). The gradient was programmed as follows: 0 ~ 6 min, 100% A; 6 ~ 10 min, a linear gradient from 0 to 12% B; 10 ~ 35 min, 12 to 21% B; 35 ~ 60 min, 21 to 41%; 60 ~ 70 min, 41 to 100% B; 70 ~ 80 min, 100% B. The flow rate was 0.8 mL/min. The detector was set at 280 nm. p-Coumaric acid (p-CA) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Free radical scavenging effects

The free radical scavenging activities were determined against the radical cation of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)[27], and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)[28]. The ABTS radical was generated by mixing 7.4 mM ABTS (Sigma-Aldrich) and 2.6 mM potassium persulfate (Sigma-Aldrich) in equal quantities, and allowing them to react for 24 h at room temperature in the dark. Each serial dilution of a plant extract in methanol (100 μL) was reacted with 100 μL of 0.2 mM ABTS radical in methanol at room temperature for 3 min, followed by measurement of the absorbance at 734 nm with a BioRad Model 680 microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For DPPH assay, the reaction mixture containing 100 μL of a serially diluted plant extract in methanol and 100 μL of 0.2 mM DPPH (Sigma-Aldrich) in methanol was kept

at room temperature for 3 min, and the absorbance was taken at 517 nm using a microplate reader. Data are presented as SC_{50} ($\mu\text{g}/\text{mL}$) which is the concentration of a sample showing 50% radical scavenging activity.

2.5. HaCaT cells culture

HaCaT cells (a human immortalized keratinocyte cell-line) were grown in DMEM/F-12 medium (GIBCO-BRL, Grand Island, NY, USA) supplemented with a 10% fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 $\mu\text{g}/\text{mL}$ amphotericin B, and 10 $\mu\text{g}/\text{mL}$ hydrocortisone. Cells were cultured at 37 $^{\circ}\text{C}$ in a humidified atmosphere containing 5% CO_2 and 95% air.

2.6. UVB-exposure of HaCaT cells

HaCaT cells were seeded on a six-well plate at a density of 3×10^5 cells per well and grown in culture medium for 24 h. Cells were then exposed to UVB in PBS containing the test material at the specified concentrations. UVB irradiation was conducted using a UVB-18 lamp (ULTRA*LUM. Inc., Claremont, CA, USA) that emitted radiation in the wavelength range 280 to 340 nm with maximum intensity at 300 nm. UV was administered to cells in culture plates at an intensity of 80 $\mu\text{W}/\text{cm}^2$ for different durations to provide different doses of UVB (5, 10, or 15 mJ/cm^2). Following irradiation, cells were fed with a growth medium and incubated for the specified time.

2.7. Cell viability test

Cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Briefly, cells were washed with PBS and incubated in 1 mL of culture media supplemented with 1 mg/mL MTT (AMRESCO, Solon, OH, USA) for 3 h. The medium was aspirated off and cells were extracted with isopropanol to dissolve the formed formazan. The solutions were moved to a 96-well plate and absorbance was determined at 595 nm using a microplate reader.

2.8. Detection of ROS

The production of ROS was determined with the oxidant-sensitive probe dihydrorhodamine 123 (DHR123) (Sigma-Aldrich). Cells were treated with 1.0 μ M DHR123 prior to UVB irradiation. Images of cells fluorescing due to the oxidation of DHR123 to rhodamine 123 were obtained with a Nikon Eclipse TE2000-U microscope (Nikon, Melville, NY, USA). The formed rhodamine123 was extracted from cells using ice-cold 70% ethanol/0.1 N HCl, followed by spinning down at 13,000 rpm for 15 min. The supernatants were neutralized with 1 M NaHCO₃ and spun down again to obtain clear supernatants, of which fluorescence intensity was measured at an excitation wavelength at 485 nm and an emission wavelength of 590 nm, using the Gemini EM fluorescence microplate reader (Molecular devices, Sunnyvale, CA, USA).

2.9. Analysis of lipid peroxidation

As a marker of lipid peroxidation, 2-thiobarbituric acid-reactive substances (TBARS) was quantified[29]. Cell lysates were prepared using a lysis buffer (20 mM Tris-Cl, 2.5 mM EDTA, 1.0% SDS, pH 7.5). Cell lysates (300 mg protein in 100 mL) was mixed with 0.9 mL of 1.0% phosphoric acid and 1.0 mL of 0.9% 2-thiobarbituric acid (Sigma-Aldrich), and then heated on a boiling water bath for 45 min. Standard solutions of 1,1,3,3-tetramethoxypropane (Sigma-Aldrich), a precursor of malondialdehyde, were treated in the same way as cell lysates. After cooling, 1.5 mL of 1-butanol was added and the mixture was centrifuged at 13,000 rpm for 15 min to separate into two layers. The fluorescence intensity of the 1-butanol layer was measured at an emission wavelength of 590 nm (excitation at 540 nm) using a fluorescence microplate reader.

2.10. Western blotting

Whole cell lysates were prepared using a lysis buffer (10 mM Tris-Cl, pH 7.4, 120 mM NaCl, 25 mM KCl, 2 mM EGTA, 1 mM EDTA, 0.5% Triton X-100, and protease inhibitor cocktail). Aliquots of whole cell lysates

were resolved by the electrophoresis on a sodium dodecyl sulfate polyacrylamide under a denaturing condition. Proteins were transferred from the gel to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA), which were incubated with a primary antibody overnight at 4 °C and then with a secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature. Immunoreactive bands were detected using SuperSignal West Femto Western Reagent kit (Thermo Scientific, Waltham, MA, USA) and subjected to densitometric analysis. Rabbit polyclonal antibodies for caspase-3 (#9662), Bax (#2774), Bcl-2 (#2876), c-Jun N-terminal kinase (JNK) (#9252) and phospho-JNK (Thr¹⁸³/Tyr¹⁸²) (#4668) were purchased from Cell Signaling (Danvers, MA, USA). The rabbit polyclonal MMP1 antibody (ab38929) was purchased from Abcam (Life Sciences-Biotech, Cambridge, UK). The mouse monoclonal β -actin antibody (#A5441) was purchased from Sigma-Aldrich.

2.11. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Cellular RNA was extracted with the RNeasy Kit (Qiagen, Valencia, CA, USA) and its 1 mg aliquot was reverse transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) to prepare cDNA. PCR amplification was conducted using the StepOnePlus™ Real-Time PCR System in reaction mixtures (20 μ L) containing SYBR® Green PCR Master Mix, 60 ng cDNA, and 2 pmol gene-specific primer sets (Macrogen, Seoul, Korea). The sequences of primers are as follows: MMP1 (GenBank accession numbers NM_001145938.1) 5' -CAT ATA TGG ACG TTC CCA AAA TCC-3' (forward) and 5' -GTG CGC ATG TAG AAT CTG TCT TTA A-3' (reverse). Reactions were performed under the following conditions: 50 °C for 2 min, 95 °C for 10 min, 40 amplification cycles (95 °C for 15 s and 60 °C for 1 min), followed by a dissociation step. Melting curve analysis showed single peaks, which supported the homogeneity of amplicons. The mRNA expression level relative to the internal control GAPDH

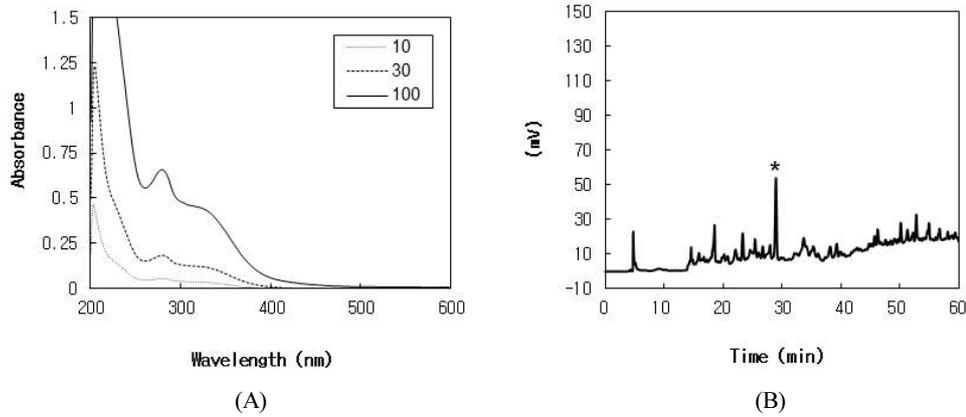


Figure 1. Absorption spectra and fingerprint HPLC of BCTE. In A, the UV absorption spectra of BCTE in PBS were measured at the indicated concentrations ($\mu\text{g/mL}$). In B, a typical HPLC chromatogram of BCTE monitored at 280 nm is shown. *The peak of p-CA was assigned by cochromatography with the authentic standard.

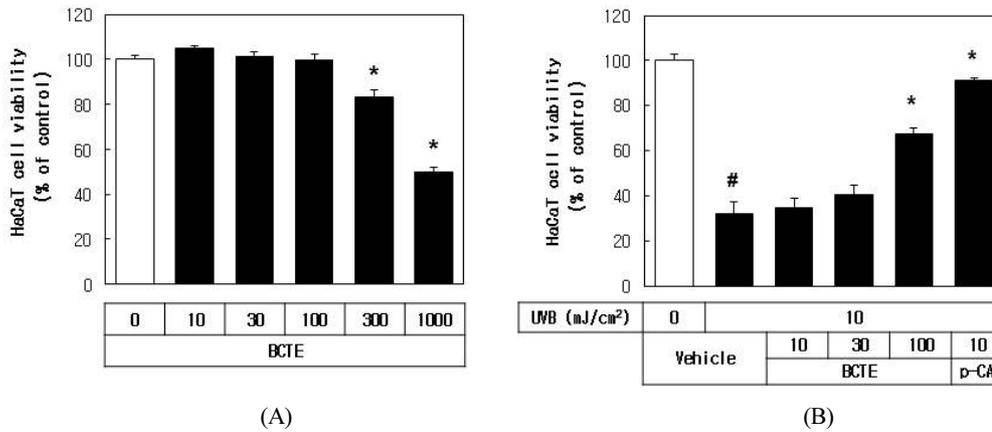


Figure 2. Effects of BCTE on the viability of HaCaT keratinocytes in the absence and presence of UVB irradiation. In A, Cells were treated with BCTE at the indicated concentrations for 24 h and cell viabilities were determined. In B, cells were irradiated with 10 mJ/cm^2 UVB in PBS in the absence and presence of BCTE (10, 30, or 100 $\mu\text{g/mL}$) or p-CA (10 $\mu\text{g/mL}$). Irradiated cells were incubated for 24 h and cell viabilities were determined. Data are presented as percentages of the unirradiated control (means \pm SEs; $n = 3$). # $p < 0.05$ versus the unirradiated control. * $p < 0.05$ versus the irradiated vehicle control.

was calculated using the comparative threshold cycle method.

2.12. Statistical analysis

Data are presented as the means \pm SEs of three or more independent experiments. The significances of differences between groups were determined using the Student's *t*-test, at *p* values < 0.05 .

3. Results

The UV absorption spectra of BCTE at different concentrations are shown in Fig. 1A. Typical HPLC pattern of BCTE is shown in Fig. 1B. Because p-CA has been reported as a major phenolic compound contained in many bamboo species such as *Phyllostachys pubescens* (Pradelle) Mazel ex J. Houz [30] and *Sasa quelpaertensis* Nakai[31], we analyzed the content of p-CA in BCTE using HPLC and it was determined to be 1.7%.

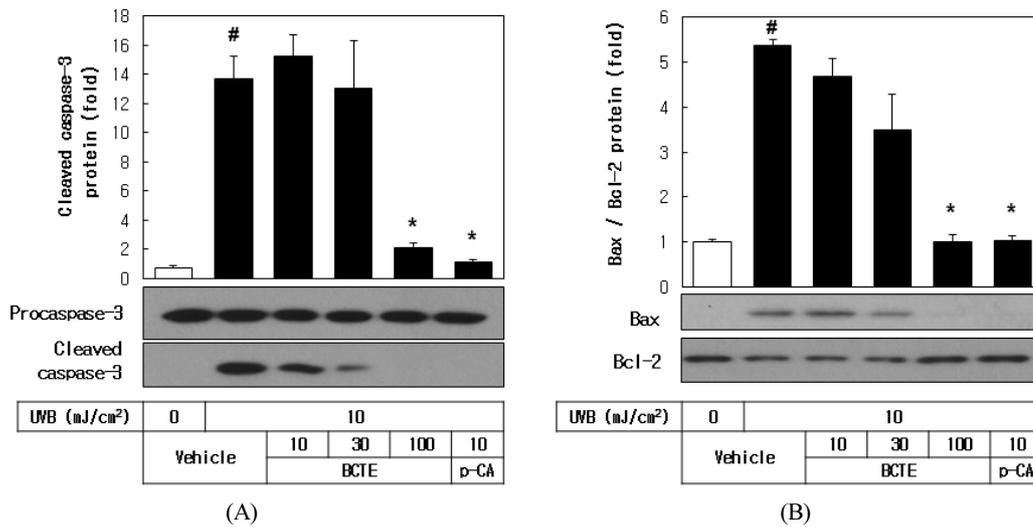


Figure 3. Effects of BCETE on the apoptotic pathway in HaCaT cells. HaCaT cells were irradiated with UVB (10 mJ/cm²) in the absence and presence of BCETE (10, 30, or 100 µg/mL) or p-CA (10 µg/mL), followed by incubation for 24 h. The whole cell lysates were subjected to western blot analysis for procaspase 3 and its cleaved form (A), and Bax and Bcl-2(B). Data are expressed as fold changes (means± SEs; n = 3). [#]*p* < 0.05 versus the unirradiated control. ^{*}*p* < 0.05 versus the irradiated vehicle control.

The cytotoxicity of BCETE was determined in cultured HaCaT human keratinocytes. As shown in Fig. 2A, BCETE was nontoxic up to 100 µg/mL whereas it showed significant cytotoxicity at 300 and 1000 µg/mL. The protective effects of BCETE against UVB-induced cell death were examined at non-toxic concentrations. As shown in Fig. 2B, BCETE enhanced the viabilities of UVB-exposed cells at 100 µg/mL. p-CA was used as a positive control and it exhibited a protective effect at 10 µg/mL.

Occurrence of apoptosis was monitored by the cleavage of procaspase 3 and the increase of pro-apoptotic Bax relative to anti-apoptotic Bcl-2 protein. As shown in Fig. 3A, UVB increased the cleavage of procaspase 3 to its active form, and this change was attenuated by BCETE as well as by p-CA. The relative ratios of Bax to Bcl-2 increased in UVB-irradiated cells and the change was attenuated by either BCETE or p-CA (Fig. 3B).

The effects of BCETE on the UVB-induced ROS generation and lipid peroxidation were determined in cells exposed to UVB. As shown in Fig. 4A, UVB exposure increased ROS generation, and this increase was attenuated by BCETE at 100 µg/mL. p-CA at 10 µg/mL also at-

tenuated the cellular ROS generation due to UVB exposure. We determined the levels of TBARS to monitor lipid peroxidation[29]. The results showed that lipid peroxidation increased in HaCaT cells exposed to UVB and this change was attenuated by BCETE and by p-CA (Fig. 4B).

The free radical scavenging activities of BCETE were determined in vitro against ABTS and DPPH radicals. Ascorbic acid and p-CA were used as positive controls. As shown in Table 1, ascorbic acid showed strongest scavenging activity against DPPH radical, while p-CA was the least active. Crude BCETE appeared to scavenge DPPH radical more effectively than p-CA. In the case of ABTS radical, p-CA showed the strongest free radical scavenging activity, followed by ascorbic acid and BCETE, in the order.

Skin photoaging involves changes in dermal extracellular matrix composition and collagen loss due to overexpression of MMPs via mitogen-activated protein (MAP) kinase-dependent pathways in response to UVB exposure[12-15]. As shown in Fig. 5A and B, UVB exposure increased MMP1 expression at the mRNA and

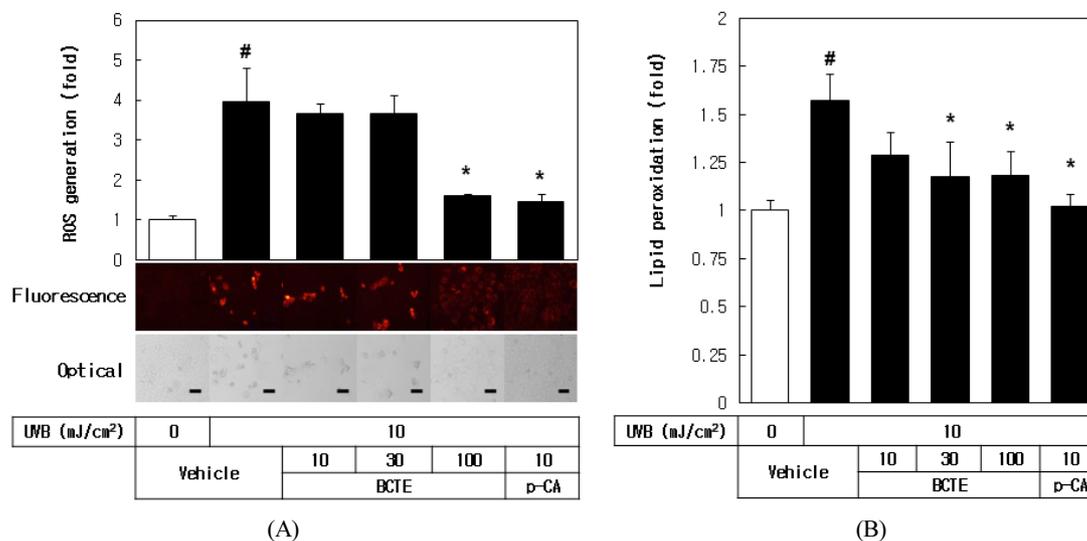


Figure 4. Effects of BCTE on UVB-induced ROS generation and lipid peroxidation in HaCaT cells. HaCaT cells were treated with 1.0 µM DHR123 and irradiated with UVB (10 mJ/cm²) in the absence and presence of BCTE (10, 30, or 100 µg/mL) or p-CA (10 µg/mL). After 24h, fluorescence and optical images of cells were captured and then cells were harvested to extract oxidized rhodamine 123. In B, cells were irradiated with UVB (10 mJ/cm²) in the absence and presence of BCTE (10, 30, or 100 µg/mL) or p-CA (10 µg/mL), followed by incubation for 24 h. Lipid peroxidation was determined by quantifying TBARS. Data are expressed as fold changes versus the vehicle control (means ± SEs; n = 3). #*p* < 0.05 versus the unirradiated control. **p* < 0.05 versus the irradiated vehicle control.

Table 1. Free Radical Scavenging Effects of BCTE, p-CA and Ascorbic acid against ABTS and DPPH Free Radicals

Materials	SC ₅₀ (µg/mL)	
	DPPH	ABTS
Bambusae caulis in Taeniam Extract (BCTE)	293	8.93
Ascorbic acid	3.11	3.04
p-Coumaric acid (p-CA)	10,000	1.21

Data are presented as SC₅₀ which is the concentration of a sample showing 50% free radical scavenging activity.

protein levels in cells and these changes were attenuated by BCTE and by p-CA. As also shown in Fig. 5C, UVB stimulated phosphorylation of JNK in a time-dependent manner and this change were attenuated by BCTE and p-CA.

4. Discussion

Plant extracts enriched with secondary metabolites which can provide UVB-screening and anti-oxidative effects are an attractive prospect as cosmeceuticals. The

present study demonstrated that BCTE attenuated the UVB-induced cytotoxicity and oxidative stress in cultured keratinocytes (Fig. 2 and 4), suggesting its potential use as a cosmeceutical for the photoprotection of the skin.

BCTE was shown to attenuate the UVB-induced apoptotic events such as the cleavage of procaspase 3 to its active form, and the increase of Bax relative to Bcl-2 (Fig. 3). BCTE might have suppressed the apoptotic pathway mainly because of its ability absorbing UV (Fig. 1). Additionally, the extract might have scavenged ROS

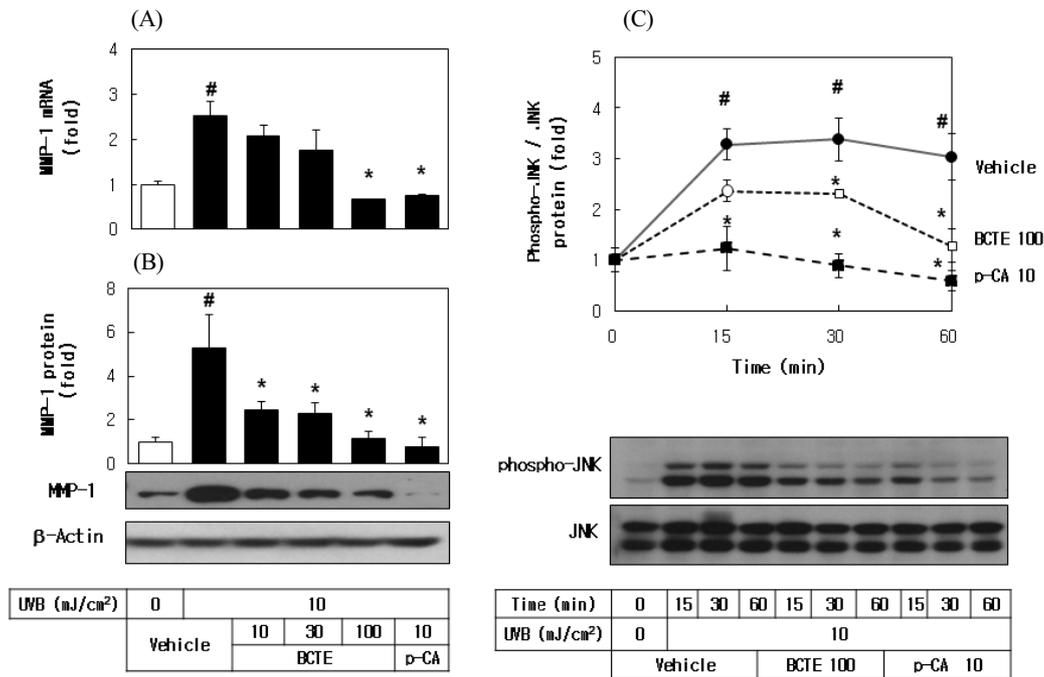


Figure 5. Effects of BCTE on UVB-induced MMP1 expression and JNK phosphorylation in HaCaT cells. (A), HaCaT cells were irradiated with UVB (10 mJ/cm²) in the absence and presence of BCTE (10, 30, or 100 μ g/mL) or p-CA (10 μ g/mL), followed by incubation for 24 h. The MMP1 mRNA levels were determined by qRT-PCR analysis using GAPDH as a control, and MMP1 protein levels were determined by Western blot analysis of whole cell lysates, using β -actin as a control. (B), HaCaT cells were irradiated with 10 mJ/cm² UVB in the absence and presence of BCTE (100 μ g/mL) or p-CA (10 μ g/mL), and then incubated for the specified time. Total and phosphorylated forms of JNK were analyzed by western blot analysis. Data are expressed as fold changes (means \pm SEs; n = 3). #*p* < 0.05 versus the unirradiated control. **p* < 0.05 versus the irradiated vehicle control.

and free radicals implicated in apoptotic signal transduction (Table 1, Fig. 4).

Like the cases of other bamboo species[30,31], p-CA was assumed to contribute to bioactivities of BCTE. The protective effects of p-CA against the UV-induced inflammatory responses or injury has been previously observed in cells and in vivo models[32-34]. We suppose that p-CA is one of the active constituents of BCTE based on the observations in the current study, but other studies reported that BCTE contained various other constituents with antioxidant properties[16,17]. Thus, the activity of BCTE can be attributed to the multiple constituents rather than any single compound. Of interest, BCTE scavenged DPPH free radicals more effectively than p-CA (Table 1), indicating that other constituents al-

so could contribute to the antioxidative effects of BCTE. Both BCTE and p-CA were more reactive to ABTS radical than to DPPH radical whereas ascorbic acid showed similar reactivity to both free radicals (Table 1). Further studies are needed to understand this matter.

MMPs regulate the turnover of extracellular matrix such as type I collagen[12,13]. UV exposure elevates the expression of MMPs in skin cells and triggers skin tissue remodeling, resulting in the formation of wrinkles and other phenotypes of skin aging[14,15,35]. Thus, the suppression of UV-induced MMPs expression is pursued as a strategy to reduce the photoaging process. In the current study, UVB exposure increased MMP1 mRNA and protein levels in cells and these changes were attenuated by BCTE and by p-CA (Fig. 5), suggesting the useful-

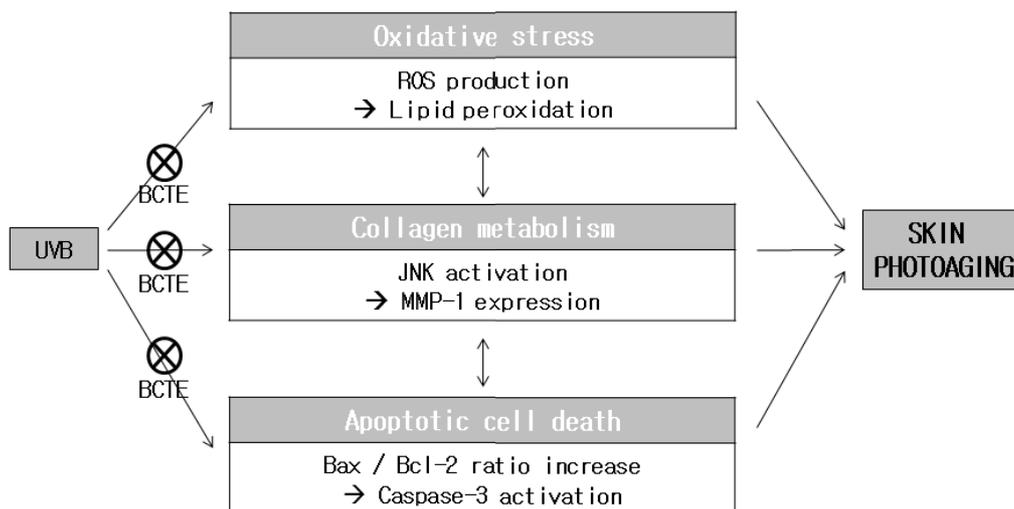


Figure 6. A summary; BCTE attenuates the UVB-induced biochemical events associated with skin photoaging.

ness of these materials as inhibitors of certain features of photoaging processes.

The UVB-induced up-regulation of MMPs is mainly mediated by signal transduction pathways involving the activation of MAP kinases, such as, JNK, extracellular signal-regulated kinase, and p38 kinase[36-38]. We observed that UVB stimulated phosphorylation of JNK and such change was attenuated by BCTE (Fig. 5). BCTE might have attenuated MMP1 expression by blocking the signaling pathway mediated by JNK, and possibly other MAP kinases.

Extracts of various bamboo species have been previously shown to attenuate atherosclerosis, hyperlipidemia, hypertension and fatigue in animal models[23-25]. Bamboo extract has also shown antiapoptotic effects in endothelial cells exposed to a high concentration of glucose[39], and antiinflammatory effects in keratinocytes stimulated by interferon- γ [40]. The present study showed the protective effects of BCTE against the apoptotic cell death in keratinocytes. We suppose the antioxidant activity of BCTE scavenging ROS/free radicals or suppressing redox signaling pathways should be a common mechanism underlying the divergent biological effects.

As summarized in Fig. 6, BCTE attenuated various biochemical events induced by UVB, suggesting its poten-

tial use in preventing skin photoaging. The clinical studies are currently underway to examine its safety and efficacy in human skin, and the results will be published elsewhere.

5. Conclusion

In conclusion, the present study demonstrated, for the first time, that BCTE protects skin cells from the UVB-induced apoptotic cell death, oxidative stress and MMP1 expression involved in skin photoaging process. Thus, BCTE has an attractive prospect as the active cosmetic ingredient.

Acknowledgments

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References

1. J. H. Rabe, A. J. Mamelak, P. J. McElgunn, W. L.

- Morison, and D. N. Sauder, Photoaging: mechanisms and repair, *J. Am. Acad. Dermatol.*, **55**(1), 1 (2006).
2. C. Antoniou, M. G. Kosmadaki, A. J. Stratigos, and A. D. Katsambas, Photoaging: prevention and topical treatments, *Am. J. Clin. Dermatol.*, **11**(2), 95 (2010).
 3. K. Scharffetter-Kochanek, P. Brenneisen, J. Wenk, G. Herrmann, W. Ma, L. Kuhr, C. Meewes, and M. Wlaschek, Photoaging of the skin from phenotype to mechanisms, *Exp. Gerontol.*, **35**(3), 307 (2000).
 4. B. Kozma and M. J. Eide, Photocarcinogenesis: an epidemiologic perspective on ultraviolet light and skin cancer, *Dermatol. Clin.*, **32**(3), 301 (2014).
 5. C. H. Lee, S. B. Wu, C. H. Hong, H. S. Yu, and Y. H. Wei, Molecular Mechanisms of UV-Induced Apoptosis and Its Effects on Skin Residential Cells: The Implication in UV-Based Phototherapy, *Int. J. Mol. Sci.*, **14**(3), 6414 (2013).
 6. N. Pustisek and M. Situm, UV-radiation, apoptosis and skin, *Coll. Antropol.*, **35**, 339 (2011).
 7. L. A. Sitailo, S. S. Tibudan, and M. F. Denning, Activation of caspase-9 is required for UV-induced apoptosis of human keratinocytes, *J. Biol. Chem.*, **277**(22), 19346 (2002).
 8. G. R. Aitken, J. R. Henderson, S. C. Chang, C. J. McNeil, and M. A. Birch-Machin, Direct monitoring of UV-induced free radical generation in HaCaT keratinocytes, *Clin. Exp. Dermatol.*, **32**(6), 722 (2007).
 9. M. Podda, M. G. Traber, C. Weber, L. J. Yan, and L. Packer, UV-irradiation depletes antioxidants and causes oxidative damage in a model of human skin, *Free Radic. Biol. Med.*, **24**(1), 55 (1998).
 10. N. A. Soter, Acute effects of ultraviolet radiation on the skin, *Semin. Dermatol.*, **9**(1), 11 (1990).
 11. F. Afaq and H. Mukhtar, Effects of solar radiation on cutaneous detoxification pathways, *J. Photochem. Photobiol. B*, **63**(1-3), 61 (2001).
 12. V. M. Kahari and U. Saarialho-Kere, Matrix metalloproteinases in skin, *Exp. Dermatol.*, **6**(5), 199 (1997).
 13. W. Bode, C. Fernandez-Catalan, H. Tschesche, F. Grams, H. Nagase, and K. Maskos, Structural properties of matrix metalloproteinases, *Cell Mol. Life Sci.*, **55**(4), 639 (1999).
 14. K. Scharffetter, M. Wlaschek, A. Hogg, K. Bolsen, A. Schothorst, G. Goerz, T. Krieg, and G. Plewig, UVA irradiation induces collagenase in human dermal fibroblasts *in vitro* and *in vivo*, *Arch. Dermatol. Res.*, **283**(8), 506 (1991).
 15. P. Brenneisen, J. Oh, M. Wlaschek, J. Wenk, K. Briviba, C. Hommel, G. Herrmann, H. Sies, and K. Scharffetter-Kochanek, Ultraviolet B wavelength dependence for the regulation of two major matrix-metalloproteinases and their inhibitor TIMP-1 in human dermal fibroblasts, *Photochem. Photobiol.*, **64**(4), 649 (1996).
 16. K. H. Park and M. W. Lee, Anti-oxidative, anti-inflammatory and whitening effects of phenolic compounds from *Bambusae Caulis* in Liquamen, *Nat. Prod. Res.*, **26**(18), 1687 (2012).
 17. Y. M. Lee, Y. K. Kim, K. H. Kim, S. J. Park, S. J. Kim, and J. H. Chung, A novel role for the TRPV1 channel in UV-induced matrix metalloproteinase (MMP)-1 expression in HaCaT cells, *J. Cell Physiol.*, **219**(3), 766 (2009).
 18. M. R. Iannacone, M. C. Hughes, and A. C. Green, Effects of sunscreen on skin cancer and photoaging, *Photodermatol. Photoimmunol. Photomed.*, **30**(2-3), 55 (2014).
 19. J. F. Nash and P. R. Tanner, Relevance of UV filter/sunscreen product photostability to human safety, *Photodermatol. Photoimmunol. Photomed.*, **30**(2-3), 88 (2014).
 20. S. Kim and K. Choi, Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review, *Environ. Int.*, **70**, 143 (2014).
 21. J. Park, J. K. Seok, H. J. Suh, and Y. C. Boo, *Gardenia jasminoides* extract attenuates the UVB-induced expressions of cytokines in keratinocytes and indirectly Inhibits matrix metalloproteinase-1 expression in human dermal fibroblasts, *Evid. Based Complement Alternat. Med.*, **2014**, 429246 (2014).

22. S. M. An, S. J. Lee, J. S. Koh, K. Park, and Y. C. Boo, Effects of plant extract-containing creams on UVB radiation-induced inflammatory responses in mice, *J. Soc. Cosmet. Sci. Korea*, **36**(4), 271 (2010).
23. M. J. Lee, M. J. Kim, Y. S. Song, Y. O. Song, and G. S. Moon, Bamboo culm extract supplementation elevates HDL-cholesterol and ameliorates oxidative stress in C57BL/6 mice fed atherogenic diet, *J. Med. Food*, **11**(1), 69 (2008).
24. J. Jiao, Y. Zhang, D. Lou, and X. Wu, Antihyperlipidemic and antihypertensive effect of a triterpenoid-rich extract from bamboo shavings and vasodilator effect of friedelin on phenylephrine-induced vasoconstriction in thoracic aortas of rats, *Phytother. Res.*, **21**(12), 1135 (2007).
25. Y. Zhang, X. Yao, and B. Bao, Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (*Caulis bambusae in taeniam*), *Phytother. Res.*, **20**(10), 872 (2006).
26. J. Sun, J. Yu, P. C. Zhang, F. Tang, Y. D. Yue, Y. N. Yang, Z. M. Feng, and X. F. Guo, Isolation and identification of lignans from *Caulis Bambusae in Taenia* with antioxidant properties, *J. Agric. Food Chem.*, **61**(19), 4556 (2013).
27. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.*, **26**(9-10), 1231 (1999).
28. J. Xie and K. M. Schaich, Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity, *J. Agric. Food Chem.*, **62**(19), 4251 (2014).
29. M. Mihara and M. Uchiyama, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, *Anal. Biochem.*, **86**(1), 271 (1978).
30. L. Liu, B. Lu, D. Xia, and Y. Zhang, Evaluation of antihypertensive and antihyperlipidemic effects of bamboo shoot angiotensin converting enzyme inhibitory peptide *in vivo*, *J. Agric. Food Chem.*, **60**(45), 11351 (2012).
31. S. M. An, S. I. Lee, S. W. Choi, S. W. Moon, and Y. C. Boo, p-Coumaric acid, a constituent of *Sasa quelpaertensis* Nakai, inhibits cellular melanogenesis stimulated by alpha-melanocyte stimulating hormone, *Br. J. Dermatol.*, **159**(2), 292 (2008).
32. K. Song and Y. C. Boo, UVB shielding effects of para-coumaric acid. *J. Soc. Cosmet. Sci. Korea*, **38**(3), 263 (2012).
33. K. Song, S. M. An, M. Kim, J. S. Koh, and Y. C. Boo, Comparison of the antimelanogenic effects of p-coumaric acid and its methyl ester and their skin permeabilities, *J. Dermatol. Sci.*, **63**(1), 17 (2011).
34. Y. K. Seo, S. J. Kim, Y. C. Boo, J. H. Baek, S. H. Lee, and J. S. Koh, Effects of p-coumaric acid on erythema and pigmentation of human skin exposed to ultraviolet radiation, *Clin. Exp. Dermatol.*, **36**(3), 260 (2011).
35. M. Brennan, H. Bhatti, K. C. Nerusu, N. Bhagavathula, S. Kang, G. J. Fisher, J. Varani, and J. J. Voorhees, Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin, *Photochem. Photobiol.*, **78**(1), 43 (2003).
36. G. J. Fisher, S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, and J. J. Voorhees, Mechanisms of photoaging and chronological skin aging, *Arch. Dermatol.*, **138**(11), 1462 (2002).
37. G. J. Fisher, H. S. Talwar, J. Lin, P. Lin, F. McPhillips, Z. Wang, X. Li, Y. Wan, S. Kang, and J. J. Voorhees, Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin *in vivo*, *J. Clin. Invest.*, **101**(6), 1432 (1998).
38. P. Brenneisen, H. Sies, and K. Scharffetter-Kochanek, Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events, *Ann. N. Y. Acad. Sci.*, **973**, 31 (2002).
39. Y. J. Choi, H. S. Lim, J. S. Choi, S. Y. Shin, J. Y. Bae, S. W. Kang, I. J. Kang, and Y. H. Kang, Blockade of chronic high glucose-induced endothe-

- lial apoptosis by *Sasa borealis* bamboo extract, *Exp. Biol. Med. (Maywood)*, **233**(5), 580 (2008).
40. X. F. Qi, D. H. Kim, Y. S. Yoon, S. B. Song, Y. C. Teng, D. Q. Cai, and K. J. Lee, *Bambusae caulis* in Liguamen Suppresses the Expression of Thymus and Activation-Regulated Chemokine and Macrophage-Derived Chemokine in Human Keratinocytes due to Antioxidant Effect, *Evid. Based Complement Alternat. Med.*, **2012**, 617494 (2012).