Anti-Aging Activity of *Lavandula angustifolia* Extract Fermented with *Pediococcus pentosaceus* DK1 Isolated from *Diospyros kaki* Fruit in UVB-Irradiated Human Skin Fibroblasts and Analysis of Principal Components

Ji Hoon Ha¹, A Rang Kim¹, Keon-Soo Lee¹, Song Hua Xuan¹, Hee Cheol Kang², Dong Hwan Lee², Mi Yeon Cha², Hye Jin Kim², Mi An², and Soo Nam Park^{1*}

¹Department of Fine Chemistry, Cosmetic R&D Center, Cosmetic Industry Coupled Collaboration Center, Seoul National University of Science and Technology, Seoul 01811, Republic of Korea

²Life Science Research Institute, GFC Life Science Co. Ltd., Hwaseong 18471, Republic of Korea

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*Corresponding author
Phone: +82-2-970-6451;
Fax: +82-2-975-9585;
E-mail: snpark@seoultech.ac.kr

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The effects of *Lavandula angustifolia* extract fermented with *Pediococcus pentosaceus* DK1 on UVB-mediated MMP-1 expression and collagen decrease in human skin fibroblasts were determined, and the conversion of its components was also analyzed. Fermentation was performed at varying *L. angustifolia* extract and MRS medium concentrations, and optimal fermentation conditions were selected. *L. angustifolia* extracts showed decreased cytotoxicity after fermentation in the fibroblasts. UVB-irradiated fibroblasts treated with fermented *L. angustifolia* extract showed MMP-1 expression 8.2-14.0% lower than that in UVB-irradiated fibroblasts treated with non-fermented extract. This was observed even at fermented extract concentrations lower than those of non-fermented extracts. Fibroblasts treated with fermented *L. angustifolia* extract showed 20% less reduction in collagen production upon UVB irradiation than those treated with non-fermented extracts. UVB-irradiated fibroblasts treated with fermented *L. angustifolia* extracts showed 50% higher inhibition of ROS generation than those treated with non-fermented extract. Luteolin and apigenin glycosides of *L. angustifolia* were converted during fermentation, and identified using RP-HPLC and LC/ESI-MS. Therefore, the effects of *L. angustifolia* extract on MMP-1 expression and collagen decrease in UVB-irradiated human skin fibroblasts were increased through fermentation by *P. pentosaceus*.

Keywords: *Pediococcus pentosaceus* DK1, fermentation, UVB, matrix metalloproteinase-1, procollagen

Introduction

The skin forms the external surface of the human body, and serves as a barrier protecting the internal organs from ultraviolet radiation, toxins, and bacteria, etc. There are two dependent layers of the skin, the epidermis and dermis, and these consist of many cells such as keratinocytes, melanocytes and fibroblasts [1]. The mechanical strength of the skin is contributed by the dermis, which is composed of the extracellular matrix (ECM) where fibroblasts synthesize ECM components such as collagen and elastin to sustain

the skin's elasticity [2]. However, the structure and function of the dermis can be changed by harmful external factors such as oxidative stress, UV exposure and air pollution. These conditions accelerate aging of the skin by collapsing its dermal structure [3, 4].

Ultraviolet light is a significant cause of exogenous skin damage. The ultraviolet rays that reach the Earth are classified as UVA (320–400 nm) and UVB (280–320 nm) [5]. When exposed to UV on the Earth's surface, the amount of UV radiation that reaches the human skin is known to be 25 J/cm² under natural sunlight in autumn at

38° N for 4–5 h. It has been reported that this corresponds to ten times the minimum erythema dose in skin [6]. In particular, UVB can penetrate the upper layer of the dermis [7]. UVB exposure increases reactive oxygen species (ROS), such as hydroxyl radicals ($\bullet\text{OH}$), superoxide anion radicals ($\text{O}_2^{\bullet-}$), singlet oxygen ($^1\text{O}_2$), and hydrogen peroxide (H_2O_2). To prevent ROS-induced cellular damage, enzymes (superoxide dismutase and catalase) and non-enzymatic antioxidants (L-ascorbic acid and α -tocopherol) are present in skin cells [8, 9]. However, when the balance of this ROS/antioxidant defense system is upset due to excess ROS generation caused by UVB exposure, skin cells become damaged and skin aging is accelerated [10, 11].

ROS induced by UVB exposure increase the expression of matrix metalloproteinase-1 (MMP-1) in fibroblasts, promoting skin photo-aging [12–14]. MMP-1 degrades collagen type 1, which is an ECM component that provides structural support to the skin. This leads to disintegration of the dermis and acceleration of skin aging [15]. Therefore, the development of anti-aging agents to inhibit UVB-induced ROS generation is an essential strategy for suppressing photo-aging [16].

Pediococcus pentosaceus as a lactobacillus is commonly found in fermented foods such as doenjang and kimchi, and is known to produce lactic acid through anaerobic fermentation [17]. Recently, it was reported that *P. pentosaceus* enhanced the immune activity of *Cordyceps militaris* extract, enabling it to boost the phagocytic activity of macrophages in mouse [18]. *P. pentosaceus* increased the anti-aging activity of *Gelidium amansil* extract on MMP-1 expression and decrease of collagen by UVB irradiation [19]. We isolated and identified a new lactobacillus, *P. pentosaceus* DK1, from *Diospyros kaki* fruit. *P. pentosaceus* DK1 donated to KCTC (KCTC12963BP) could degrade the tannins in *Diospyros kaki* fruit and increase the total phenol content by 1.5 fold, and the flavonoid content by 1.4 fold. *Diospyros kaki* fruit fermented by *P. pentosaceus* DK1 also enhanced the inhibitory activity of elastase for improvement of skin aging. Thus, *P. pentosaceus* DK1 was also used to improve the anti-aging activity of *L. angustifolia*.

L. angustifolia, commonly known as lavender, belongs to the Lamiaceae family [20]. Traditionally, *L. angustifolia* extract has been used as a remedy for neurological and rheumatic diseases, due to its antibacterial and relaxing properties, and is mainly obtained by extracting volatile components from oils [21, 22]. Another method involves extracting *L. angustifolia* using water or ethanol, which has been reported to produce extracts that show antioxidant properties, whitening effect, and inhibitory effect on sebum

production [23]. The major components of *L. angustifolia* extract are linalool, linalyl acetate, ladanin, apigenin, apigenin-7-O- β -glucoside, luteolin, luteolin-7-O- β -glucoside, and 5,4'-dihydroxy flavonoid-7-O- β -pyranglycuronate butyl ester [24, 25]. Recently, Ahn, *et al.* [26] reported that the antioxidant, tyrosinase, and elastase inhibitory activities of *L. angustifolia* extract, as well as the ratios of phenolic compounds such as rosmarinic acid, were significantly increased by natural fermentation. However, their fermentation method was very old, and the microorganisms used were also not identified. There have been no studies so far on the anti-aging activity and composition analysis of *L. angustifolia* extracts fermented by *P. pentosaceus* DK1.

In this study, the anti-aging effects of *L. angustifolia* extract fermented by *P. pentosaceus* DK1 were evaluated on MMP-1 expression, collagen production, and antioxidant activity, and analyzed on the converted components during fermentation.

Materials and Methods

Reagents and Chemicals

Human skin fibroblasts (HS68 cells) were purchased from Lonza (Basel, Switzerland). Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), trypsin, and penicillin-streptomycin were obtained from Capricorn Scientific (Ebsdorfergrund, Germany).

Preparation of *L. angustifolia* Extract

Fermented and non-fermented *L. angustifolia* extract were obtained from GFC Life Science (Korea). The dried *L. angustifolia* flowers (2 kg) were homogenized in 70% ethanol (40 L) for 20 days at room temperature. The extract powder (410.93 g) was obtained by filtration and vacuum evaporation drying.

Fermentation of *L. angustifolia* Extract

In order to cultivate the *P. pentosaceus* DK1 strain, lactobacilli MRS broth (BD 288130) purchased from Difco (USA) was selected as the culture medium for the optimal growth conditions of the strain. MRS medium was developed to favor the growth of lactobacilli in 1960 by De Man, Rogosa and Sharpe and is known to support the growth of lactobacilli including *Pediococcus*. MRS broth was dissolved in distilled water, and *L. angustifolia* extract was added (Table 1). *P. pentosaceus* DK1 was pre-cultured in MRS broth and cultured at 37°C for 24 h. Then, 10% of the strains pre-cultured in the broth containing the *L. angustifolia* extract were cultured at 37°C for 14 d. The fermented broth was then centrifuged at 9,010 g for 15 min and the supernatant was filtered using a 0.22 μm cellulose filter. Finally, the fermented broth was fractionated with ethyl acetate. The ethyl acetate fraction was evaporated to obtain fermented *L. angustifolia* extract. All the processes were indicated in Scheme 1.

Table 1. Fermentation conditions of *L. angustifolia* extract.

Sample Group	Fermentation condition	
	<i>L. angustifolia</i> extract Concentration (%)	MRS medium Ratio (%)
A1	0.1%	0.69%
A2	0.1%	1.38%
A3	0.1%	2.75%
A4	0.1%	5.50%
B1	0.5%	0.69%
B2	0.5%	1.38%
B3	0.5%	2.75%
B4	0.5%	5.50%

Cell Culture

Human skin fibroblasts (HS68 cells) were incubated in DMEM medium supplemented with 10% FBS, 100 U/ml, of penicillin and 100 µg/ml of streptomycin. The cells were incubated in medium at 37°C in a humid incubator under 5% CO₂ atmosphere.

UVB Radiation

For supply of UVB radiation, a CL-1000 Ultraviolet Crosslinker (UVP, USA), with an emission spectrum of 280–370 nm and a peak at 312 nm, was used. HS68 cells with 70–80% confluence were treated with fermented and non-fermented *L. angustifolia* extract at various concentrations in a plastic, 60-mm Petri dish in FBS-free medium for 24 h. The medium was then removed, and the cells were rinsed twice with phosphate-buffered saline (PBS). The cells were irradiated through the cover of the dish by UVB after addition of 1 mL PBS in the wells (height; 3 mm). After UV exposure, the medium was replaced with FBS-free medium.

Cell Viability

A 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, USA) assay was used to analyze cell viability. HS68 cells were seeded in 96-well plates at 1×10^4 cells per well and incubated for 24 h at 37°C. After treatment with

various doses of fermented or non-fermented *L. angustifolia* extract for 24 h or exposure to UVB radiation, the medium was replaced with FBS-free medium and the cells were further incubated for up to 72 h. Subsequently, 0.5 mg/ml MTT solution was added and the cells were further incubated for 30 min at 37°C. Then, 100 µl dimethyl sulfoxide (DMSO) was added to the wells respectively, and the absorbance was determined at 570 nm using a microplate reader (TECAN, Austria).

Quantitative Analysis of MMP-1 Expression

HS-68 fibroblasts were incubated up to 70–80% confluence on 60-mm plates in a humid incubator maintained at 37°C. After treatment with different concentrations of the fermented or non-fermented *L. angustifolia* extract, the cells were exposed to 80 mJ/cm² UVB under PBS and then incubated further for 48 h in fresh medium without FBS. Secreted MMP-1 proteins within the culture medium were measured by an ELISA kit (R&D Systems, USA) according to the manufacturer's instruction.

HPLC and LC/ESI-MS Analysis

HPLC analysis of the lavender was carried out using a Shimadzu LC-20A HPLC system (Shimadzu, Japan) equipped with a UVD 170s DIONEX detector and Shim-pack VP-ODS C18 column (L: 250 mm, LD: 4.6 mm, 5 µm). The mobile phase was composed of A (2% acetic acid in H₂O) and B (0.5% acetic acid in 50% acetonitrile aqueous solution). The working conditions were as follows: 0–25 min, 0% (v/v) of B; 25–40 min, 0–25% (v/v) of B; 40–80 min, 25–35% (v/v) of B; 80–110 min, 35–40% (v/v) of B; 110–120 min, 40–20% (v/v) of B; 120–130 min, 20–10% (v/v) of B; 130–140 min, 10–0% (v/v) of B; 140–150 min, 0% (v/v) of B. The flow rate was 1.0 ml/min and the samples were observed at 365 nm. The samples were passed through a 0.2-µm filter and then 20 µl of the samples with 10,000 µg/ml were injected into the HPLC.

The mass spectrometric analysis was performed using an LCQ Ion Trap Mass Spectrometer (Thermo Finnigan, USA) with an ESI interface and detection was done in positive ion mode by the National Instrumentation Center for Environmental Management College of Seoul National University (Seoul, Korea). The operating

Scheme 1. Preparation of fermented and non-fermented *L. angustifolia* extracts.

Fig. 1. Effects of UVB irradiation on human skin fibroblasts. The cells were irradiated with various doses of UVB and incubated 72 h. Then, the levels of MMP-1 proteins (A) and cell viability (B) were measured. Data were presented as mean $\pm$ SD of three independent experiments. * $p < 0.01$ compared with the negative control.

conditions were as follows: capillary voltage, 33 V; capillary temperature, 400°C; nebuliser pressure, 10 psi; and drying gas, N_2 . Compounds within STE were identified by comparing the UV spectra, retention times, and fragment ions of standard materials.

Intracellular ROS Evaluation

The intracellular ROS levels were evaluated using the fluorescence dye 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF -DA, Sigma-Aldrich, USA), which is generated by the conversion of non-fluorescent H_2DCF -DA to highly fluorescent 2',7'-dichlorofluorescein (DCF) by intracellular ROS. HS-68 cells were incubated with 20 μ M H_2DCF -DA for 30 min at 37°C. The cells were then washed twice with PBS and irradiated by 80 mJ/cm² UVB. Fluorescence signals were detected using a fluorescence ELISA reader (Perkin Elmer, USA; excitation, 490 nm; emission, 530 nm).

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 (SPSS, USA) software. The results were presented as mean $\pm$ standard deviation (SD). Statistically significant differences were calculated by one-way ANOVA, where $p < 0.01$ was considered statistically significant.

Results

Effect of Fermented *L. angustifolia* Extract on UVB-Increased MMP-1 Expression in Human Skin Fibroblasts

UVB-treated fibroblasts secrete a greater amount of matrix metalloproteinase-1 (MMP-1) than untreated fibroblasts. MMP-1 is a metalloproteinase with a zinc ion in its center, and promotes skin aging through degradation of collagen type I and III, which act as support fixtures in the dermis.

We studied the effects of fermented and non-fermented *L. angustifolia* extracts on UVB-induced MMP-1 expression in human fibroblasts. To design an optimized system for

measurement of anti-aging activity, the fibroblasts were irradiated by UVB at 20–100 mJ/cm². As the UVB irradiation dose was increased, the amount of MMP-1 expression in the UVB-exposed fibroblasts also increased (Fig. 1A). However, at 100 mJ/cm², there was a slight decrease in cell viability (Fig. 1B). We then set the ultraviolet radiation (UVB) at 80 mJ/cm², which produced a high MMP-1 expression level without cytotoxicity in the fibroblasts.

Fermented and non-fermented *L. angustifolia* extracts (A1-B4) were prepared under various medium conditions, using the concentrations (Table 1) of *L. angustifolia* extract (0.1% or 0.5%) and the concentrations of MRS medium (0.69%, 1.38%, 2.75%, or 5.50%) as described in Materials and Methods.

In order to select the optimal fermentation conditions for the *L. angustifolia* extract in terms of anti-aging activity, 10 μ g/ml of *L. angustifolia* extract fermented product and non-fermented product were used to treat fibroblasts for 24 h and these were then irradiated with 80 mJ/cm² UVB. As shown in Fig. 2A, all fermented and non-fermented *L. angustifolia* extracts reduced the increased MMP-1 protein expression under UVB irradiation. Fermented *L. angustifolia* extract showed a greater inhibitory effect on MMP-1 expression than non-fermented *L. angustifolia* extract. In particular, the inhibitory effect of MMP-1 expression of the *L. angustifolia* extract fermented at B2 condition (0.5% of *L. angustifolia* extract and 1.38% of MRS medium) was the largest of all extracts. All extracts fermented at all conditions showed no cytotoxicity (data not shown). We selected this condition as optimal for fermentation and used these same conditions in subsequent experiments.

As shown in Fig. 2B, non-fermented *L. angustifolia* extracts indicated cytotoxicity at more than 50 μ g/ml but

Fig. 2. Effect of fermented and non-fermented *L. angustifolia* extracts on UVB-induced MMP-1 expression in human skin fibroblasts. (A) MMP-1 expression was determined after pre-treatment with 10 µg/ml of fermented and non-fermented *L. angustifolia* extracts and irradiation of 80 mJ/cm² UVB. The cell viability (B) and MMP-1 expression (C) were determined after pre-treatment of fermented and non-fermented *L. angustifolia* extracts at indicated concentration and irradiation of 80 mJ/cm² UVB. Data are presented as the mean ± SD of three independent experiments. **p* < 0.01 compared with the UVB-treated control. §*p* < 0.01 compared with cells treated with non-fermented *L. angustifolia* extract.

fermented *L. angustifolia* extracts decreased the cytotoxicity at the same concentrations. In particular, fermented *L. angustifolia* extract showed no cytotoxicity up to 50 µg/ml. We selected 25 µg/ml as the maximal concentration for further experiments.

Fermented and non-fermented *L. angustifolia* extracts decreased UVB irradiation-increased MMP-1 expression in a dose-dependent manner (Fig. 2C). The MMP-1 expression levels of fermented *L. angustifolia* extracts were 8.2–14.0% lower than those of non-fermented *L. angustifolia* extracts at all concentrations (3.1–25 µg/ml). These results suggest that *L. angustifolia* extracts fermented by *P. pentosaceus* DK1 are effective in the suppression of MMP-1 protein expression increased by UVB irradiation.

Effect of Fermented *L. angustifolia* Extract on Collagen Production

When the skin is exposed to UVB, the levels of type 1 procollagen decrease. Anti-aging agents can protect against the degradation of collagen in UVB-exposed fibroblasts. The effects of fermented and non-fermented *L. angustifolia* extract were evaluated on UVB irradiation-induced decrease

in collagen production (Fig. 3).

In UVB-irradiated fibroblasts, the level of procollagen type 1 was 42% lower than the levels of collagen in normal cells without UVB irradiation. Fermented and non-fermented *L. angustifolia* extracts increased the levels of collagen reduced by UVB irradiation in a dose-dependent manner. Fermented *L. angustifolia* extracts presented a 20% higher level of collagen production than non-fermented *L. angustifolia* extracts at 12.5 and 25 µg/ml. However, treatment with fermented and non-fermented *L. angustifolia* extracts without UVB irradiation showed no increase in collagen production in fibroblasts (data not shown). These results suggest that the fermentation of *L. angustifolia* extracts recover collagen production after reduction from UVB irradiation.

Effect of Fermented *L. angustifolia* Extract on UVB-Induced ROS Generation

UVB irradiation increases ROS generation in cells and ROS-stimulated fibroblasts increases expression of MMP-1 protein and inhibits collagen production [27]. Therefore, it is important to identify the active compounds that are able

Fig. 3. Effect of fermented and non-fermented *L. angustifolia* extracts on UVB-mediated production of procollagen type I in human skin fibroblasts.

Data are presented as the mean $\pm$ SD of three independent experiments.

* $p < 0.01$ compared with the UVB-treated control. § $p < 0.01$ compared with cells treated with non-fermented *L. angustifolia* extract.

to inhibit ROS generation stimulated by UVB for anti-aging activity [28]. H₂DCF-DA [29] was used to determine the effect of fermented and non-fermented *L. angustifolia* extracts on UVB-induced ROS generation in fibroblasts.

As shown in Fig. 4A, fibroblasts irradiated with UVB showed a 421.2% $\pm$ 20.4% higher ROS generation than cells without UVB irradiation. At all concentrations (3.1–25 µg/ml),

fermented *L. angustifolia* extracts reduced the amount of ROS generation by more than 16% compared to those of non-fermented *L. angustifolia* extracts. Non-fermented and fermented *L. angustifolia* extracts at 6.3 µg/ml indicated 335.4% $\pm$ 11.3% and 283.2% $\pm$ 14.8% of ROS levels on UVB-irradiated fibroblasts.

ROS levels of fermented *L. angustifolia* extract at 25 µg/ml were 116.3% $\pm$ 12.5% lower than that in the non-fermented *L. angustifolia* extract (70.6%) (Figs. 4A and 4B). These results suggest that fermentation using *P. pentosaceus* DK1 enhances the inhibitory effect of *L. angustifolia* extract on UVB-induced ROS generation.

Component Analysis of *L. angustifolia* Extract before and after Fermentation

Changes in the composition of natural products fermented by microorganisms can affect their anti-aging activity [31]. In this study, the anti-aging activity of *L. angustifolia* extract was increased after fermentation, with reduced ROS generation, decreased MMP-1 expression, and increased collagen production.

We observed the changes in the components of the *L. angustifolia* extract after fermentation by *P. pentosaceus* DK1. The components were identified by HPLC, UV-spectrophotometry, and LC/ESI-MS (Table 2), and quantified using standard materials to confirm the change in the amounts of components. As shown in Fig. 5, luteolin-7-O-

Fig. 4. Effect of fermented and non-fermented *L. angustifolia* extracts on UVB-induced ROS generation in human skin fibroblasts.

The cells were treated with different concentrations of fermented and non-fermented *L. angustifolia* extracts for 24 h and then treated with 20 µM H₂DCFH-DA for 30 min. Subsequently, the cells were irradiated with 80 mJ/cm² UVB. ROS generation was measured using a fluorescence reader (A) and fluorescence microscope (B). In Fig. 4B, the cells were treated with (a) non-treatment; (b) 80 mJ/cm² UVB irradiation; treatment of 6.3 (c) and 25 µg/ml (d) non-fermented *L. angustifolia* extract and UVB irradiation; treatment of 6.3 (e) and 25 µg/ml (f) fermented *L. angustifolia* extract and UVB irradiation. Scale bar, 50 µm. Data are presented as the mean $\pm$ SD of three independent experiments. * $p < 0.01$ compared with the UVB-treated control. § $p < 0.01$ compared with cells treated with non-fermented *L. angustifolia* extract.

Table 2. Mass and UV spectrum of identified compounds in *L. angustifolia* extract fermented or non-fermented by *P. pentosaceus* DK1.

HPLC Peak No.	Name of the compound	Molecular formula	Retention time (min)	Measurement		
				$\lambda_{\max}$ (nm)	Negative ions (m/z) [M+H] ⁻	Positive ions (m/z) [M+H] ⁺
1	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	49.754	244, 322	353.4	355.1
2	Luteolin-7-O-glucuronide	C ₂₁ H ₁₈ O ₁₂	69.408	253, 347	-	463.8
3	Luteolin-7-O-glucoside	C ₂₁ H ₂₀ O ₁₁	80.261	254, 346	447.9	449.4
4	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	82.956	290, 331	359.2	-
5	Apigenin-7-O-glucoside	C ₂₁ H ₂₀ O ₁₀	92.844	267, 334	431.8	433.8
6	Luteolin	C ₁₅ H ₁₀ O ₆	98.003	254.349	285.2	287.4
7	Apigenin	C ₁₅ H ₁₀ O ₅	106.566	270, 334	269.1	271.2

glucoside (peak 3) and apigenin-7-O-glucoside (peak 5) in fermented *L. angustifolia* extract were decreased compared with the non-fermented *L. angustifolia* extract and luteolin (peak 6) and apigenin (peak 7), as their aglycone components, were increased, respectively. In addition, chlorogenic acid (peak 1) decreased after fermentation. Interestingly, there was no significant change in luteolin 7-O-glucuronide (peak 2) after fermentation, despite having a similar structure to luteolin-7-O-glucoside.

Fig. 5. HPLC chromatograms of *L. angustifolia* extracts fermented (A) and non-fermented (B).

The peaks were detected at 365 nm of wavelength for 150 min.

Anti-Aging Effect of the Identified Compounds in UVB-Irradiated Human Skin Fibroblasts

Identified components of the *L. angustifolia* extract converted by *P. pentosaceus* DK1 were evaluated on UVB-induced ROS generation, MMP-1 expression and collagen degradation. UVB was irradiated to human fibroblasts after treatment with luteolin, luteolin-7-O-glucuronide, apigenin and apigenin-7-O-glucoside.

Luteolin and apigenin reduced 80% and 85.8% of ROS generation increased by UVB irradiation compared to their glycosides (Fig. 6A). Luteolin and apigenin also reduced 26.5% and 22.4% of UVB-enhanced MMP-1 levels less than their glycosides, respectively (Fig. 6B). In UVB-reduced collagen production, luteolin and apigenin increased 9.5% and 12.5% more than their glycosides (Fig. 6C).

Taken together, these results suggest that aglyconation of the flavonoids in *L. angustifolia* extract through fermentation by *P. pentosaceus* DK1 is a major factor in promoting anti-aging activity through a reduction in UVB-induced ROS production.

Discussion

Recently, fermentation using microorganisms and enzymes has been shown to enhance both the safety and activity of natural products for cosmetics agents [31]. The mechanism of efficacy enhancement of materials by microbial fermentation is as follows: 1) the microorganism breaks down the substance to a small size to increase its absorption into the skin; 2) the microorganism enhances the product's physiological activities by removing glycoside; 3) favorable nutrients including vitamins and organic acids are produced during microbial cultivation [32, 33]. In addition, heavy metals and pesticide residues in the extracts of raw plants

Fig. 6. Effect of identified compounds on ROS generation, MMP-1 expression and collagen production in UVB-irradiated human skin fibroblasts.

Data are presented as mean $\pm$ SD of three independent experiments. * p < 0.01 compared with the negative control. § p < 0.01 compared with cells treated with non-fermented *L. angustifolia* extract.

are harmful to the skin, microorganisms can enhance the safety of the extracts through decomposition and/or adsorption [34].

In this study, *L. angustifolia* extract was fermented using *P. pentosaceus* DK1, a new microorganism isolated from *Diospyros kaki* fruit, commonly known as persimmon, to develop a novel material for the inhibition of skin-aging caused by UVB irradiation. We optimized the fermentation conditions using *P. pentosaceus* DK1 in 1.38% MRS medium supplemented with 0.5% *L. angustifolia* extract. Fermentation of *L. angustifolia* extract by *P. pentosaceus* DK1 increased the anti-aging activities while decreasing MMP-1 expression and ROS generation, and increased collagen production in UVB-irradiated human skin fibroblasts. We identified that the flavonoid glycosides, luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside, contained in *L. angustifolia* extract were decreased and the contents of sugar-removed luteolin and apigenin after fermentation were increased. These results suggest that *P. pentosaceus* DK1 may remove glucosides from flavonoid glucosides as well as degradation of tannins. Luteolin and apigenin indicated higher anti-aging activities than the glucosides of them on MMP-1 expression, collagen production and ROS generation in UVB-irradiated fibroblasts. Sung, *et al.* reported that luteolin had a higher antioxidant activity than the glycoside in intracellular systems [35]. Luteolin and apigenin were also reported to suppress UV-induced MMP-1 expression through anti-oxidative activity on human skin fibroblasts [36] and human keratinocytes (HaCaT cells) [37]. Apigenin increases collagen synthesis in fibroblasts [38].

In conclusion, *P. pentosaceus* DK1 could increase the anti-oxidative and aging activities of the *L. angustifolia* extract through bioconversion which increases the levels of apigenin and luteolin by aglyconation of their glucosides. Thus, *L. angustifolia* extract fermented by *P. pentosaceus* DK1 could have applicability as an anti-aging agent.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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