Antioxidative and Antiaging Activities and Component Analysis of Lespedeza cuneata G. Don Extracts Fermented with Lactobacillus pentosus

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Introduction

There has been a growing interest in beauty in accordance with the economic development in Korea. Korea’s cosmetics industry has thus been growing rapidly with the influence of the Korean Wave, including K-dramas and K-pop. Recently, cosmetic materials developed by Korea’s unique differentiated cosmetics and convergence integration technology have greatly contributed to enhancing the country’s global competitiveness. In particular, the development and application of new biomaterials for cosmetic use have recently been conducted by introducing fermentation and bioconversion technologies to herbal medicines or natural plant extracts based on oriental herbs. Most of the natural plant extracts used as cosmetic materials are present in the form of glycosides, which generally have lower antioxidative and antiaging activities than the aglycone structures in the skin. Recently, studies have focused on converting the glycosides of natural extracts into active materials by using fermentation and bioconversion technologies [1–3]. These processes can reduce the size and polarity of the molecules, thereby improving their skin absorption and efficacy [4–6]. Previous studies have shown that fermented Melissa officinalis and Lavandula angustifolia extracts have higher antioxidative and cytoprotective properties than their non-fermented counterparts [7, 8]. Other studies using these fermentation and bioconversion technologies to improve the functional efficacy of extracted components for antiaging effects and whitening of the skin have recently been actively conducted.

Lespedeza cuneata G. Don is a traditional herb that has been associated with multiple biological activities. In this study, we investigated the antioxidative/antiaging activities and performed an active component analysis of the non-fermented and fermented (using Lactobacillus pentosus) extracts of Lespedeza cuneata G. Don. The antioxidative activities of the fermented extract were higher than those of non-fermented extracts. The elastase inhibitory activity, inhibitory effects on UV-induced MMP-1 expression, and ability to promote type I procollagen synthesis were investigated in Hs68 human fibroblasts cells. These tests also revealed that the fermented extract had increased antiaging activities compared with the non-fermented extract. A component analysis of the ethyl acetate fractions of non-fermented and fermented extracts was performed using TLC, HPLC, and LC/ESI-MS/MS to observe changes in the components before and after fermentation. Six components that were different before and after fermentation were investigated. It was thought that kaempferol and quercetin were converted from kaempferol glucosides and quercetin glucosides, respectively, via bioconversion with the fermentation strain. These results indicate that the fermented extract of L. cuneata G. Don has potential for use as a natural cosmetic material with antioxidative and antiaging effects.

Keywords: Lespedeza cuneata G. Don, Lactobacillus pentosus, fermentation, antioxidative activity, antiaging activity
in the field of cosmetics [9, 10].

When skin is exposed to ultraviolet light, various reactive oxygen species (ROS), such as singlet oxygen (1O2), superoxide anion radical (O2−), hydrogen peroxide (H2O2), hydroxyl radical (•OH), alkoxyl radical (•OR), and hydroperoxyl radical (•OOR), are produced in the skin [11]. These ROS not only oxidize and damage the lipids, proteins, and DNA present in the skin, but also accelerate skin aging, as evidenced by wrinkle formation, through the cleavage and abnormal crosslinking of matrix constituent proteins such as collagen or elastin. In addition, these ROS increase the expression of matrix metalloproteases (MMPs) that degrade collagen and elastin by activating signaling cascades via the phosphorylation of ERK, p38, and JNK kinases and the AP-1 transcription factor, and may also accelerate skin aging by inhibiting the synthesis of procollagen [12]. Therefore, the development of a material that inhibits the expression of MMPs by inhibiting the production of excess ROS generated in the skin, or that promotes the synthesis of procollagen, is important in functional cosmetic research.

Lespedeza cuneata G. Don of the family Leguminosae is a perennial and herbaceous legume widely distributed in Korea, China, Japan, and Australia. As a folk remedy, L. cuneata G. Don extracts have been used to enhance liver and kidney function, remove extravasated blood, and calm swelling [13]. Most of the flavonoid components in L. cuneata G. Don, such as isoorientin, avicularin, and trifolin, were found to exist as glycosides [14]. A study on L. cuneata G. Don extracts has reported that the ethanol extracts possess antioxidative and antimicrobial activities [15]. However, no research on the development of materials from this plant using fermentation or bioconversion technologies has been reported.

Therefore, in this study, we measured the free radical and ROS scavenging activities in a Fe3+-EDTA/H2O2 system, determined the elastase inhibitory effect using human Hs68 fibroblasts, assessed the UV-induced inhibition of MMP-1 expression and the promotion of type I procollagen synthesis, and performed a component analysis of unfermented L. cuneata G. Don extracts and extracts obtained following fermentation by Lb. pentosus. We hypothesized that the extracts of the fermentation product would have higher antioxidative and antiaging activities compared with the unfermented extracts, and would thus have greater potential for cosmetic applications.

Materials and Methods

Materials

The UV-visible spectrophotometer used in this study was Varian Cary 50 (Varian, Australia), and a 6-channel LB9505 LT system (Berthold Technologies, Germany) was used for the chemiluminescence assay. A pH meter (Hanna, Korea) was used in this study. For the component analysis, a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) and a C18 analytical column (5 μm, 250 mm × 4.6 mm (Shimadzu) were used, and LC/ESI-MS/MS (Applied Biosystems, USA) was carried out at the National Instrumentation Center for Environmental Management (Nicem, Korea). The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ethylenediaminetetraacetic acid (EDTA), luminol, Triton X-100, and NP-PEG (natural-products-polyethylene glycol, 2-aminoethyl diphenylborinate) were purchased from Sigma-Aldrich (USA), and H2O2 was purchased from Dae Jung Chemical & Metals (Korea). We purchased the FeCl3·6H2O from Junsei Chemical Co. (Japan). The materials (Na2HPO4·12H2O, NaH2PO4·2H2O, NaCl, trizma base, HCl, and H2SO4) used to prepare buffer solutions, and the solvents ethanol (EtOH), methanol (MeOH), ethyl acetate (EtOAc), and n-hexane, were of extra-pure grade. The reference compounds (+)-α-tocopherol (1,000 IU vitamin E/g), L-ascorbic acid, oleic acid, all-trans retinol, quercetin, kaempferol, isovitexin, quercitrin, avicularin, and juglanin, and the substrate N-succinyl-(Ala)3-p-nitroanilide, were obtained from Sigma-Aldrich. The human Hs68 foreskin fibroblast cell line ATCC CRL-1635 was purchased from the ATCC (USA). The 60 F254 (0.2 mm) thin-layer chromatography (TLC) aluminum sheet gels were purchased from Merck (USA). The whole plant of L. cuneata G. Don was collected from Yangnyeong market, Korea, in November 2015 and was identified by Prof. Soo Nam Park. A voucher specimen was deposited at the department of Fine Chemistry, Seoul National University of Science and Technology.

Extraction and Fractionation

A 100 g portion of the dried whole parts (except the roots) of L. cuneata G. Don was cut finely and dissolved in 4 L of 70% EtOH for 1 week; the resulting powder was obtained by filtration and vacuum evaporation drying. The ethyl acetate fraction from 70% ethanol extracts was obtained by three fractionation cycles and vacuum evaporation drying.

Preparation of the Fermented Extracts

The fermented extract of L. cuneata G. Don was obtained from GFC Co., Ltd. (Korea). Fermented lactic acid strains (Lb. pentosus) were isolated from several microorganisms by selecting acid-producing strains using bromophenol blue [16], isolating strains producing β-glucosidase by the esculin agar method [17], and selecting strains with active glucosidase by the p-nitrophenyl-β-D-glucopyranoside (pNPG) method [18]. The results of β-glucosidase activity in Lb. pentosus are presented in Fig. S1. Lb. pentosus, which is a high β-glucosidase-producing strain, was selected on the basis of these studies, and the homology of Lb. pentosus D79211 was confirmed to be 99% by a 16S rRNA gene sequencing analysis using colony PCR [19, 20]. A liquid fermentation containing 1% (w/v) glucose was used. After adding 70% ethanol extract
powder to distilled water containing 1% (w/v) glucose to a concentration of 1% (w/v), we sterilized the mixture in an autoclave and added *Lb. pentosus* seeded to 3% (w/v) and fermented the reaction mixture for 5 days at 37°C. We called the resulting product the fermented *L. cuneata* extract. To compare the fermented extract with the non-fermented extract, we prepared solutions under the same conditions but did not add the bacterial strain to the unfermented sample. We called the result the non-fermented *L. cuneata* extract.

**Determination of the Free-Radical Scavenging Activity by the DPPH Assay**

The free-radical scavenging activity of the sample was assayed using the DPPH method. One milliliter of ethanol and various concentrations of the sample (in 1 ml) were added to 1 ml of 0.2 mM DPPH solution dissolved in methanol. The samples were then mixed and placed in the dark for 10 min at 25°C. The absorbance was measured at 517 nm using a spectrophotometer. Untreated cells served as the control group, whereas the treated cells were the experimental group. The antioxidative activity of the extract was expressed as the concentration at which the DPPH concentration of the sample was reduced to 50% (free-radical scavenging activity, FSC, μg/ml). The DPPH-radical scavenging rate was calculated using the following formula:

Radical scavenging (%) = \( \left( 1 - \frac{A_{\text{control}} - A_{\text{sample blank}}}{A_{\text{control}}} \right) \times 100 \)

\( A_{\text{experiment}} \): Absorbance of experimental group; \( A_{\text{sample blank}} \): Absorbance of sample; \( A_{\text{control}} \): Absorbance of control.

**ROS Scavenging Activity in a Fe²⁺-EDTA/H₂O₂ System Determined Using the Luminol-Dependent Chemiluminescence Assay**

The Fe²⁺-EDTA/H₂O₂ system generates ROS (O₂⁻, •OH, and H₂O₂) by the Fenton reaction. Therefore, the ROS scavenging activity of a compound or mixture can be measured using this system and involves chelation. The amount of ROS generated was measured using a chemiluminescence method based on the reaction between luminol and ROS. This was done by the same procedures as those described in a previous study [21]. The reactive oxygen scavenging activity was expressed as the concentration of the sample needed for a 50% reduction in the intensity of chemiluminescence (ROS scavenging activity, OSC, μg/ml).

**Measurement of the Elastase Inhibitory Activity Using Hs68 Human Fibroblasts**

The elastase inhibitory activity was measured using human Hs68 fibroblasts. Cultured cells were washed with phosphate-buffered saline (PBS) and then dissolved in a 0.1% Triton X-100/0.2 M Tris solution (pH 8.0 with HCl). The cells were homogenized via ultrasonication and centrifuged to obtain the supernatant with an enzyme solution containing fibroblast elastase. The protein concentration in the prepared elastase solution was determined, and 96 μl of a solution containing 100 μg of protein was added to each well, along with 88 μl of 0.2 M Tris-HCl buffer (pH 8.0). Then, 10 μl of each concentration of the extracts were added to each well, and 2 μl of STANA (N-succinyl-tri-alanyl-p-nitroanilide, 50 mM) was included in each well as an elastase substrate. After 90 min of reaction at 37°C, the absorbance was measured with an ELISA reader at 405 nm. The control contained only the solvent used as the sample solution instead of the protein sample. The blank was made using the same conditions as the experimental wells, but Tris-HCl buffer (pH 8.0) was added instead of the elastase solution. The elastase inhibitory activity was expressed as the concentration required to reduce the activity of the elastase by 50% (IC₅₀) [22, 23].

**Cell Viability Assay**

The 3-(4,5-dimethyl-2-thiazoly)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich) assay was used to determine cell viability. Hs68 cells were seeded in 96-well plates and maintained until 60–80% confluence. Then, the cells were treated with various concentrations of the sample for 24 h. At the end of the incubation, the culture medium was removed and 0.5 mg/ml MTT solution was added for 12 h at 37°C. After removing the medium, the cells were solubilized with dimethyl sulfoxide and the absorbance at 570 nm was measured using an ELISA reader (Tecan, Austria).

**Enzyme-Linked Immunosorbent Assay (ELISA)**

Hs68 human dermal fibroblasts cells were seeded on a 24-well plate at a density of 5 × 10⁵ cells/well in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cultured Hs68 cells were treated with various concentrations of sample in FBS-free medium for 48 h. The immunoreactivity of MMP-1 and type I procollagen within the culture medium was measured via ELISA (R&D Systems, USA) by using a MMP-1 ELISA kit and a type I procollagen ELISA kit according to the manufacturer’s instructions with the supernatant of the culture medium. The concentrations of MMP-1 and type I procollagen were based on the absorbance measured at 450 nm.

**Component Analysis**

Ethyl acetate fractions obtained before and after fermentation of the *L. cuneata* extract were used for the analysis of compositional changes due to fermentation. Ethyl acetate fractions were dissolved in absolute ethanol and the extracts were filtered using a syringe filter (Millipore 0.45 μm) prior to the TLC and HPLC analyses. Among the various bands separated via TLC, the bands showing differences before and after the fermentation were scratched, and the spots were dissolved in absolute ethanol and filtered using a syringe filter (Millipore 0.45 μm, USA). The filtrates were used for
UV-visible absorption spectrometry, HPLC, and LC/ESI-MS/MS analyses. The components were analyzed by comparing the color and Rf values of the bands using the NP-PEG coloring reagent and ultraviolet light (UV-365 nm), with the TLC chromatograms developed using the standard substances and comparisons made by analyzing the peak retention times of the standard substances in the HPLC chromatograms and UV-visible absorption spectra. The HPLC separation conditions are shown in Table S1.

The LC instrument was equipped with a Thermo-Finnigan surveyor (Thermo Scientific, USA) (column spec. U-VDSpher Pur C18-E 1.8 um, 50 × 2.0 mm, Cat. No. N0520E181UVC), and an autosampler, and the mass spectrometric analysis was performed using a Thermo-Finnigan LCQ Deca XP plus ion trap mass spectrometer with ESI interface. An injection volume of 5 μl and a flow rate of 200 μl/min were used. The developing solvent conditions were 0.1% formic acid in H2O (A solvent) and 0.1% formic acid in acetonitrile (B solvent) = 75:25.

The ethyl acetate fractions of non-fermented and fermented extracts of L. cuneata at 10,000 μg/ml were quantitatively analyzed using an HPLC apparatus under the conditions shown in Table S1. Calibration curves were prepared using isovitexin, avicularin, quercitrin, juglanin, quercetin, and kaempferol for the content measurement. Experiments were performed at four concentrations to obtain a sufficient calibration curve. The regression equations were obtained in the form of \(y = ax + b\), and the linearity of the calibration curves was determined based on the R2 value. The calibration curves were used to evaluate the content of the components when the R2 value was 0.99 (Table S2). The chromatograms obtained by HPLC analyses were used to calculate the contents of each component by substituting the area of each component into the regression linear equation.

The areas of peaks 1–6 in the HPLC chromatograms of the ethyl acetate fractions of non-fermented and fermented L. cuneata G. Don extracts were assigned to the linear regression equations for each standard curve, and the amounts of each component in the peaks were calculated. Then, the contents of the respective components in the non-fermented and fermented extracts were calculated in consideration of the yield of each ethyl acetate fraction.

Statistical Analysis
All the results were obtained from three replicate experiments, and the statistical analysis was performed using the Prism 5.0 (GraphPad Software Inc., USA) software program. A one-way ANOVA was used to test for significance. Values of \(p < 0.05\) were considered significant.

Results and Discussion

Yields of L. cuneata G. Don Extracts and Fractions
The yields were calculated on the basis of the weight of dried whole parts (except the roots) of L. cuneata G. Don. The yield of the 70% ethanol extract powder was found to be 8.20 ± 0.24%. The ethyl acetate fraction obtained from non-fermented L. cuneata G. Don extract was 0.55 ± 0.07% and the ethyl acetate fraction obtained from the fermented L. cuneata G. Don extract was 1.16 ± 0.09% (data not shown).

Free-Radical Scavenging Activity
ROS, especially free radicals like O2·− and ·OH, are generated

Fig. 1. Antioxidative activities of the non-fermented and fermented extracts of L. cuneata G. Don and reference compounds. The free-radical scavenging activities (A), and the ROS scavenging activities in the Fe2+-EDTA/H2O2 system as determined by a luminol-dependent chemiluminescence assay (B). Data are presented as the means ± SD. *\(p < 0.05\) compared with reference compounds ((+)−α-tocopherol or L-ascorbic acid)), #\(p < 0.05\) compared with the non-fermented extract.

when the skin is exposed to UV radiation. The reactivity of these radicals is high enough to start autoxidation, leading to lipid peroxidation. The free-radical scavenging activity (free-radical scavenging concentration, FSC<sub>50</sub>) can be measured on the basis of the electron-donating ability of the test agent. The free-radical scavenging activities of non-fermented and fermented <i>L. cuneata</i> G. Don extracts were assayed using DPPH, which is a stable radical. The FSC<sub>50</sub> of the non-fermented extract was 101.83 μg/ml, whereas that of the fermented extract was 76.63 μg/ml (Fig. 1A). Both of these values were lower than that of (+)-α-tocopherol (FSC<sub>50</sub>, 8.98 μg/ml), but after fermentation, the FSC<sub>50</sub> of the extract was approximately 24.7% higher than that of the non-fermented extract. These results indicate that the FSC<sub>50</sub> increases after fermentation.

**ROS Scavenging Activity**

The Fe<sup>3+</sup>-EDTA/H<sub>2</sub>O<sub>2</sub> system generates various ROS through the Fenton reaction. Luminol is oxidized by ROS to form excited aminophthalate. As excited aminophthalate returns to the ground state, light emission occurs at 420–450 nm. Thus, a reduced emission intensity in this system represents the ROS scavenging activity. The ROS scavenging activity (ROS scavenging concentration, OSC<sub>50</sub>) of the non-fermented extract was 7.15 μg/ml, and that of the fermented extract was 3.89 μg/ml (Fig. 1B). These values were both lower than that of L-ascorbic acid (OSC<sub>50</sub>, 1.50 μg/ml), which is a known water-soluble antioxidant, but after fermentation, the OSC<sub>50</sub> was increased by approximately 45.6% compared with the non-fermented extract. These results indicate that the OSC<sub>50</sub> increases after fermentation.

**Elastase Inhibitory Activity**

The MMPs present in the dermal layer of the skin are induced by ultraviolet light and active oxygen, and are closely related to skin aging, especially wrinkle formation. The major components of MMPs are collagenase, gelatinase, and elastase. The reduction in the elasticity of the skin and decrease in the elastase activity are important in wrinkle formation. The elastase inhibitory activity (IC<sub>50</sub>) of the non-fermented extract was 649.40 μg/ml, and that of the fermented extract was 382.80 μg/ml (Fig. 2). This showed that both extracts had lower values than oleanolic acid (IC<sub>50</sub>, 7.24 μg/ml), which was used as a reference compound. However, after fermentation, the OSC<sub>50</sub> of the extract increased by approximately 41.1% compared with the non-fermented extract. These results indicate that elastase inhibitory activity increases after fermentation.

**Cell Viability**

To identify the dose ranges of the non-fermented and fermented extracts of <i>L. cuneata</i> G. Don that were non-toxic to fibroblasts, a MTT assay was performed. Fibroblasts were treated for 48 h with the non-fermented or fermented extract of <i>L. cuneata</i> G. Don (100–400 μg/ml) (Fig. 3A). The viability of the treated fibroblasts was not significantly different from that of untreated cells at concentrations up to 400 μg/ml, and we therefore used doses up to this level in the experiments examining MMP-1 inhibition and type I procollagen production.

**Inhibitory Effect of the Extracts on UV-Induced MMP-1 Expression**

ROS, which are produced on exposure to ultraviolet radiation, activate the expression of MMP-1, which breaks down the extracellular matrix and accelerates collagen degradation, thereby weakening the skin’s elasticity and structural integrity, ultimately creating skin wrinkles [24]. In this study, we evaluated whether the non-fermented and fermented <i>L. cuneata</i> G. Don extracts influenced MMP-1 expression in fibroblasts. As a result, exposure to UV radiation led to a 58.85% increase in MMP-1 expression compared with that in non-irradiated cells. All-trans retinol (10 μg/ml), the positive control, decreased MMP-1 expression by approximately 29.09% compared with the control. Treating cells with 100, 200, and 400 μg/ml of the fermented <i>L. cuneata</i> G. Don extract inhibited the UVB-induced MMP-1 expression.
Effects on Type I Procollagen Production

The extracellular matrix of the skin dermis consists mainly of collagen, elastin, fibrin, and proteoglycans. Collagen is mainly composed of types I–IV. The dermis contains type I collagen mixed with type III collagen, which maintain the elasticity and firmness of skin tissue [11]. There are two distinct mechanisms by which UV irradiation leads to decreased collagen: degradation by enzymes, such as MMPs, and inhibition of collagen synthesis. In this study, we evaluated whether non-fermented and fermented *L. cuneata* G. Don extracts influenced type I procollagen production. The UVB-irradiated group showed a 46.96% reduction in type I procollagen production relative to the untreated group. The positive control group, treated with 10 μg/ml all-trans retinol, showed a 75.00% increase in type I procollagen production, which was reduced by UVB irradiation. Treating cells with 100, 200, and 400 μg/ml of the fermented *L. cuneata* G. Don extract increased the type I procollagen production by 20.00%, 33.58%, and 61.35%, respectively, compared with the UVB-irradiated test group, in a concentration-dependent manner, whereas those in the non-fermented *L. cuneata* G. Don extract group were -5.57%, -0.98%, and 19.00%, respectively. These results suggest that the efficacy of type I procollagen production induced by the fermented extract is higher than that of the non-fermented extract (Fig. 3C).

Component Analysis

In lane ① of Fig. 4, the TLC chromatogram of the ethyl acetate fraction of non-fermented *L. cuneata* G. Don extract was separated into nine bands (LCG1–9). Lane ② shows that the TLC chromatogram of the ethyl acetate fraction of fermented *L. cuneata* G. Don extract was separated into six bands (LCG1–4, LCG8, and 9). When the color intensity of the TLC bands is compared, the amount of LCG1 and LCG2 increased after fermentation, and the amount of LCG5, LCG6, LCG7, and LCG9 decreased after fermentation. The substances obtained by scraping these six bands with different amounts before and after fermentation were analyzed via LC/ESI-MS/MS (Table 1, Figs. S2–S11).

LCG9 (HPLC peak 1) showed [M-H] at m/z 431.3, [M+H] at m/z 433.3, and MS2 at m/z 283.2, 313.2, 337.1, 367.0, 379.1, 397.0, and 415.0 (Figs. S2, S3), which was as confirmed isovitexin (apigenin-6-C-glucoside) from a paper reported by Waridel et al. [25].

LCG6 (HPLC peak 2) showed [M+H]+ at the m/z 435.1, ion peak due to disappearance of the arabinose unit (m/z 435.1 → m/z 303.2), [M-H] at m/z 433.2, and 2[M-H] at m/z

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**Fig. 3.** Cell viability by MTT assay (A), and effects of non-fermented and fermented extracts of *L. cuneata* G. Don and all-trans retinol (10 μg/ml) on UVB-mediated MMP-1 expression (B) and type I procollagen production (C) in Hs68 cells treated with different concentrations of extracts for 48 h after UVB (80 mJ/cm²) irradiation.

All-trans retinol (10 μg/ml) was used as a positive control. Data are presented as the means ± SD. *p < 0.05 compared with the UVB-irradiated control, *p < 0.05 compared with the non-fermented extract. Negative (N) control: untreated control.
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866.9 (Figs. S4, S5), which was confirmed as avicularin (quercetin-3-O-α-arabinofuranoside) in a paper reported by Sánchez-Rabaneda \textit{et al.} [26].

LCG7 (HPLC peak 3) showed [M+H]$^+$ at m/z 449.1, ion
peak due to disappearance of the rhamnose unit (m/z 449.1 \rightarrow m/z 303.2), [M-H] at m/z 447.2, and 2[M-H] at m/z 894.9
(Figs. S6, S7), which was confirmed as quercitrin (quercetin-3-O-rhamnoside) in a paper reported by Ren et al. [27].

LCG5 (HPLC peak 4) showed [M+H]^+ at m/z 419.1, ion peak due to disappearance of the arabinose unit (m/z 419.1
\rightarrow m/z 287.2), and [M-H] at m/z 417.2 (Figs. S8, S9), which was confirmed as juglanin (kaempferol 3-O-arabinoside)
in a paper reported by Kim et al. [28] and De Leo et al. [29].

LCG2 (HPLC peak 5) showed [M+H]^+ at m/z 303.3, [M-H]^− at m/z 301.2, and 2[M-H] at m/z 602.8 (Fig. S10), which
was confirmed as quercitin in a paper reported by Yoo et al. [30].

LCG1 (HPLC peak 6) showed [M+H]^+ at m/z 287.3 and [M-H]^− at m/z 285.3 (Fig. S11), which was confirmed as kaempferol by Yoo et al. [30].

In addition, TLC, HPLC, and UV-Visible absorbance spectra were compared with standard materials to confirm
their composition (Table 1, Figs. 4 and 5).

In the TLC chromatogram, the R_f values and NP-PEG colors of the six bands were LCG1 (0.84, yellow-light green),
LCG2 (0.68, yellow-orange), LCG5 (0.76, yellow-light green), LCG6 (0.66, yellow-orange), LCG7 (0.56, yellow-orange),
and LCG9 (0.32, yellow-light green), which were consistent with the R_f values and NP-PEG colors of kaempferol,
quercitin, juglanin, avicularin, quercitrin, and isovitexin, respectively, and the peak retention times in the HPLC
chromatogram and UV-Visible absorption spectra for the bands were identical to those of the standard materials.
Therefore, LCG1 was identified as kaempferol, LCG2 as quercetin, LCG5 as juglanin, LCG6 as avicularin, LCG7 as
quercitrin, and LCG9 as isovitexin.

The contents of isovitexin, avicularin, quercitrin, and
juglanin decreased by 41.2%, 93.6%, 53.3%, and 60.0%, respectively, after fermentation. The contents of quercetin and kaempferol increased by 242.9% and 266.7%, respectively, after fermentation.

The above results show the bioconverted components formed in the L. cuneata G. Don extract on fermentation via
the \( \beta \)-glucosidase activity of fermentation strain Lb. pentosus, which increases the extract’s antioxidative and antiaging
potential. \( \beta \)-Glucosidases are known to play a role in the hydrolysis of O-glycosidic bonds. Here, it was confirmed
through component and quantitative analyses using TLC, HPLC, and LC/ESI-MS/MS that juglanin (kaempferol-3-O-
\( \alpha \)-L-arabinofuranoside) was bioconverted to kaempferol, which is its aglycone form, and avicularin (quercetin-3-O-
α- L-arabinofuranoside) and quercitrin (quercetin 3-O-α-L-rhamnoside) were bioconverted to quercetin, which is their aglycone form. When flavonoid glycosides are converted into their respective aglycone forms, their O-glycosidic bond is broken to form an OH group; this hydroxyl group can better function as a hydrogen donor as well as a metal-ion chelator-acting site. These molecular changes further result in the reduction of molecular weight [31]. For the same reasons, we also observed that the free-radical (FSC) and ROS scavenging (OSC) activities were higher in the fermented extract than in the non-fermented extract. In addition, there was an increase in the elastase inhibitory activity, inhibition of UV-induced MMP-1 expression, and type I procollagen production efficacy after fermentation due to the increase in the ROS scavenging activity. This increase can also be due to an improvement in the penetration rate of flavonoid glycosides into the cell membrane, when their molecular size decreases and hydrophobicity increases owing to a reduction in polarity, on changing to aglycone forms [21, 32, 33]. These results suggest that the L. cuneata G. Don extract fermented using Lb. pentosus could be used as a natural cosmetic material with increased antioxidative activity and antiaging effects via bioconversion.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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