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Antioxidative and anti-aging activities of *Abeliophyllum distichum* Nakai extracts fermented with *lactobacillus plantarum* and *lactobacillus brevis*

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***Abeliophyllum distichum* Nakai is a deciduous shrub that belongs to Oleaceae Abeliophyllum and grows only in Korea. In this study, the antioxidative activity, cytotoxicity, anti-aging activity, and levels of acteoside and isoacteoside were compared between non-fermented and fermented (using *Lactobacillus plantarum* and *Lactobacillus brevis*) extracts of *A. distichum* Nakai. The cytotoxicity of these extracts, along with their ability to inhibit elastase activity and Matrix metalloproteinase-1 (MMP-1) expression, and their ability to promote type I procollagen synthesis were investigated in human dermal fibroblasts cells. These tests revealed that the fermented extract possessed higher antioxidant and anti-aging activities compared with the non-fermented extract. The levels of acteoside and isoacteoside were about 1.25 and 1.05 times higher in the fermented extract than in the non-fermented extract. It was speculated that they were converted from acteoside glucosides and isoacteoside glucosides via bioconversion by the fermentation strains. Together, these findings indicate that extracts of *A. distichum* Nakai show good potential as antioxidative and anti-aging cosmetic materials.**

Key words: *Abeliophyllum distichum* Nakai, *Lactobacillus plantarum, Lactobacillus brevis,* fermentation, antioxidative activity, anti-aging activity.

INTRODUCTION

Wrinkles naturally occur as skin ages, but the photoaging caused by ultraviolet (UV) rays of sunlight further reduces skin elasticity and increases wrinkles. The active oxygen species generated by UV rays greatly influence this process. For example, active oxygen inhibits the production of collagen, which helps maintain skin elasticity (Kim et al., 2007). Oxidative stress induced by active oxygen increases the production of MMPs, which degrade collagen; leads to decreased skin elasticity and increases wrinkles (Pentland et al., 1995). Therefore, a substance that excels at antioxidation can be a good means for improving skin wrinkles. Various substances are currently being tested with this goal in mind. Many groups are focusing on natural materials, which are expected to be stimulating the skin compared to synthetic materials.

Most of the natural plant extracts used as cosmetic materials are extracted as glycosides. Recent studies

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have focused on converting the glycosides of natural extracts into active materials by using fermentation (Huynh et al., 2016; Wang et al., 2016; Lee and Paik, 2017), which can reduce the size and polarity of the molecules to improve their skin absorption and efficacy (Lee et al., 2014; Hong and Han, 2002; Hirbumi et al., 1998).

Abeliophyllum distichum Nakai is a rare species that is found only in Korea. This member of family Oleaceae is a deciduous broad-leaved shrub that is so rare; it has been designated as a natural monument (Lee et al., 2014; Hong and Han, 2002). Recently, however, it has been mass-produced and grown in the Goesan area of Chungbuk province. Studies on *A. distichum* Nakai extracts have examined their antioxidant and anti-aging efficacy (Hirbumi et al., 1998; Kim and Lee, 2015). The extracts have been found to contain acteoside and isoacteoside, which are phenylpropanoid glycosides that reportedly have antioxidant and anti-aging effects (Yoon et al., 2009).

Most of the previous studies have focused on non-fermented extracts of *A. distichum* Nakai. Here, the cytotoxicity, antioxidative and anti-aging activity, and levels of acteoside and isoacteoside in extracts of *A. distichum* Nakai were compared with and without fermentation using *Lactobacillus plantarum* and *Lactobacillus brevis*.

MATERIALS AND METHODS

Preparation of *A. distichum* Nakai fermented extracts

A. distichum Nakai leaves were collected from a natural habitat in South Korea, Goesan area of Chungbuk province in February 2019. *A. distichum* Nakai leaves were dried at room temperature and subjected to extraction. The distilled water extract of *A. distichum* Nakai (the non-fermented sample) was obtained using 20 volumes of water at 95°C for 18 h. *L. plantarum* and *L. brevis* strains were inoculated to De Man, Rogosa and Sharpe (MRS) broth and grown at 37°C for 24 h. For fermentation, the *A. distichum* Nakai solution (3%) was inoculated with fresh bacterial subculture (4% v/v), incubated at 37°C for 24 h, and subjected to sterilization and filtration. The filtered solution of fermented sample was concentrated and spray-dried.

Measurement of antioxidant activity of the extracts

The antioxidant capacity of extracts was evaluated by measuring free radical scavenging activity using the DPPH assay (Widowati et al., 2003). Samples were prepared at concentrations of 0.1, 0.25, 0.5, and 1.0 mg/ml, with the non-fermented and fermented extracts. After incubation at room temperature for over 30 min, free radical scavenging activity was determined by mixing with 500 μM DPPH solution (1:1) and incubating in the dark, followed by measurement of absorbance at 517 nm using a spectrophotometer.

Measurement of the elastase inhibitory activity

The elastase inhibitory assay was performed as previously described (Thring et al., 2009). Briefly, 20 μl volumes containing various concentrations of non-fermented or fermented extracts, 10 μl elastase from porcine pancreas (2.5 units), and 125 μl Tris buffer were preincubated for 15 min at 25°C, mixed with 0.1 mM N-succinyl-Ala-Ala-Ala-*p*-nitroanilide substrate (20 μl), and incubated for 10 min at 25°C. Absorbance was measured at 410 nm.

Analysis of cytotoxicity of the extracts

Human dermal fibroblasts (Hs68 cells, CRL 1635; American Type Culture Collection, Rockville, MD) were screened for their cytotoxicity following exposure to the various extracts. The MTT assay (Sigma-Aldrich) was used to determine cell viability. Briefly, Hs68 cells were seeded to 96-well plates, grown to 60~80% confluence, treated with 200 μl medium containing various concentrations of non-fermented and fermented extracts at a range of concentrations, and incubated at 37°C for 24 h. Each well was treated with MTT reagent (20 μl), the plate was incubated for an additional 1 h, and absorbance was read at 570 nm using a microplate reader.

Enzyme-linked immunosorbent assay (ELISA)

For analysis of MMP-1 and type I procollagen, Hs68 cells were seeded to a 24-well plate (5×10^4 cells/well) in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were grown for 24 h treated with various concentrations of the non-fermented and fermented extracts in FBS-free medium for 24 h, cultured under 37°C in 5% CO_2 for an additional 24 h, and treated with 10 ng/ml tumor necrosis factor α (TNF-α). The culture media were collected and the immunoreactivities of MMP-1 and type I procollagen were measured by ELISA using commercially available kits, followed by measurement of absorbance at 450 nm.

Component analysis

HPLC was performed using an Agilent 1200 series gradient HPLC system. Briefly, fermented or non-fermented extracts dissolved in methanol (1 mg/ml) were injected (20 μl) onto a reverse-phase column (Agilent-Eclipse Plus C18, 5 μm, 4.9 × 150 mm). The mobile phase was a mixture of water (A) and methanol (B) and progressed from 10 to 90% B over a period of 50 min at a flow rate of 1 ml/min.

Statistical analysis

All data are presented as mean ± standard deviation of three replicates. Differences among treatments were assessed by analysis of variance (ANOVA), followed by Dunnett's test. *p*-value of <0.05 was regarded as significant.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of non-fermented and fermented *A. distichum* Nakai extracts

The scavenging activities of non-fermented and fermented *A. distichum* Nakai extracts increased dose-dependently (Figure 1). The non-fermented extract

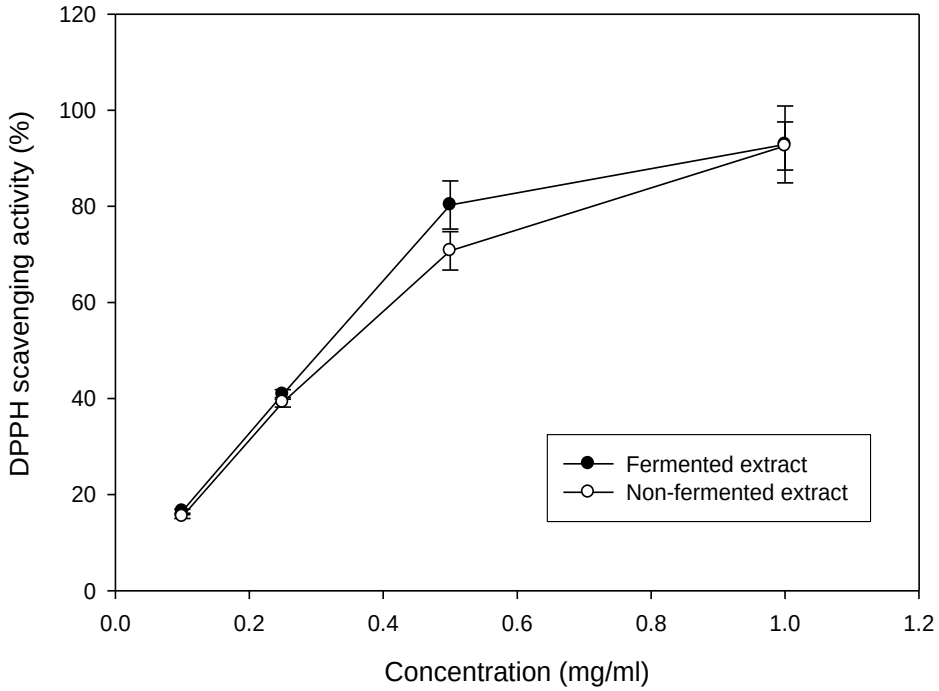

Figure 1. Free radical DPPH scavenging activity of the non-fermented and fermented extracts. Data are presented as the mean ± SD of three independent experiments. Means not sharing a common letter were significantly different at $p<0.05$.

exerted inhibitory effects of 15.43, 39.20, 70.74 and 92.55% at concentrations of 0.1, 0.25, 0.5, and 1.0 mg/ml, respectively, while the same doses of fermented extract exerted inhibitory effects of 16.54, 40.85, 80.27 and 92.87%, respectively. This indicates that fermented *A. distichum* Nakai extract showed higher scavenging activity for DPPH radicals at the concentrations tested. The IC_{50} values were 0.40 and 0.37 mg/ml for non-fermented and fermented *A. distichum* Nakai extracts, respectively.

Cytotoxicity effects

Many lactic acid bacteria-fermented products are known to have various regulatory functions, including anti-diarrheal, antiviral, anti-allergy, and immune effects (Ayivi et al., 2020). Since these fermented products tend to be non-cytotoxic, they have long been ingested as functional foods (Sanders and Huis, 1999). To assess the effect of the non-fermented and fermented extracts on cell viability, fibroblasts cells were herein treated with 0~200 μg/ml of the non-fermented and fermented extracts, and the MTT assay was performed. As shown in Figure 2, significant toxicity was observed within the tested concentration range. This indicates that, consistent with the literature, the lactic acid bacteria-fermented extract did not appear to exert cytotoxicity.

Elastase inhibitory activity

Reduced skin elasticity and decreased elastase activity are important components of wrinkle formation. The elastase inhibitory activity (IC_{50}) of the non-fermented extract was 147.30 μg/ml and that of the fermented extract was 134.57 μg/ml, as shown in Figure 3. Both extracts had lower IC_{50} values than Epigallocatechin gallate (EGCG) (IC_{50} 25.3 μg/ml), which was used as a reference compound. The IC_{50} of the fermented extract was approximately 8.64% higher than that of the non-fermented extract, indicating that the elastase inhibitory activity was increased by fermentation. Studies on the elastase inhibitory activity of many plants have been reported (Thring et al., 2009; Moon et al., 2010). However, this is the first examination of the elastase inhibitory activity of the fermented extract of *A. distichum* Nakai. Additional research will be needed to identify the compound(s) that confer(s) this activity in the fermented product of *A. distichum* Nakai.

MMP-1 inhibitory activity

MMP-1 is a protease that specifically acts on collagen; inhibitors of MMP-1 inhibit collagen degradation and thus help maintain skin elasticity and prevent wrinkle formation (Park et al., 2008). Here, we measured the secretion of

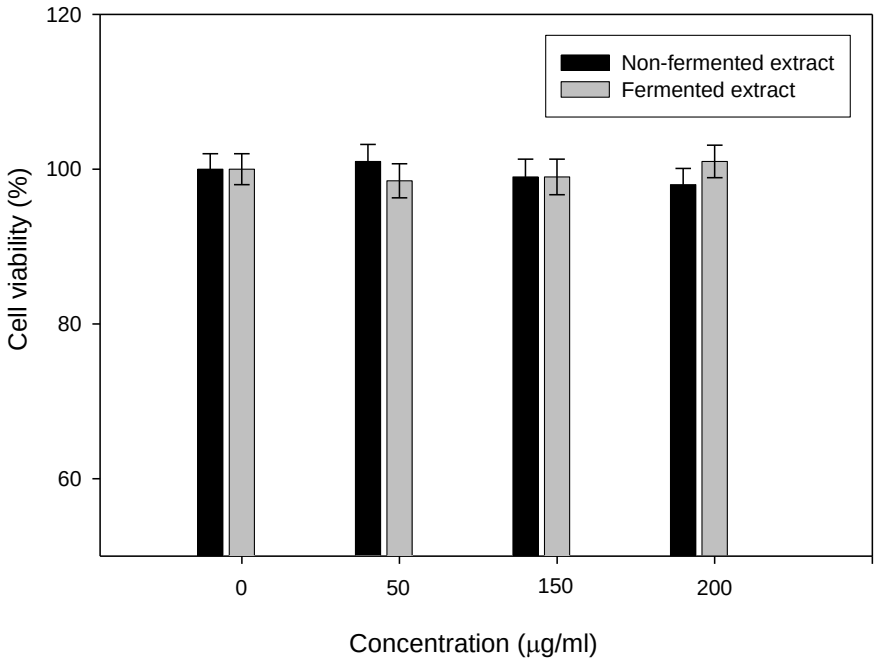

Figure 2. Viability of cells treated with non-fermented and fermented extracts, as assessed by MTT assay. Data are presented as the mean ± SD of three independent experiments. Means not sharing a common letter were significantly different at $p<0.05$.

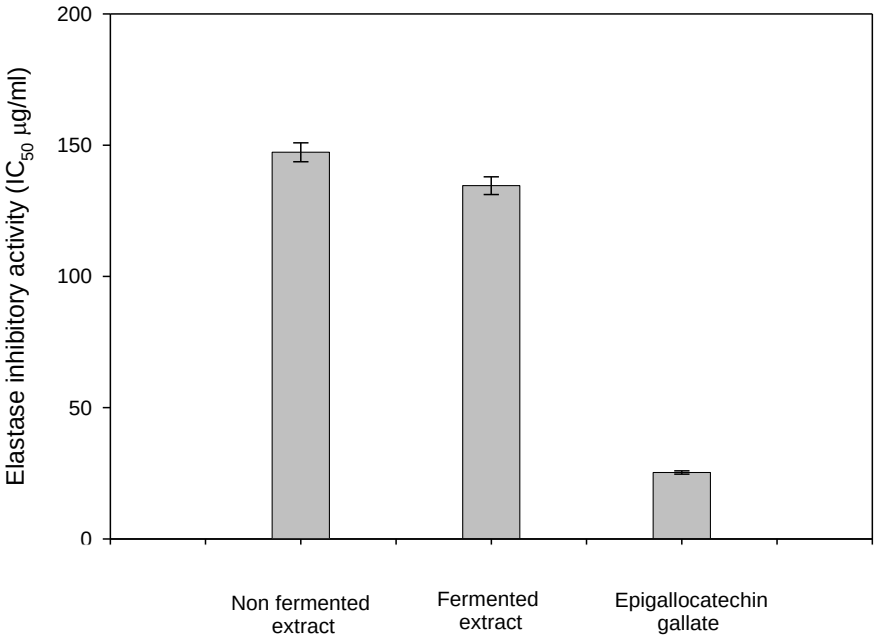

Figure 3. Elastase inhibition by the non-fermented and fermented extracts. Data are presented as the mean ± SD of three independent experiments. Means not sharing a common letter were significantly different at $p<0.05$.

MMP-1 from cells treated with the non-fermented or fermented *A. distichum* Nakai extracts. As shown in Figure 4, the results confirmed the potential wrinkle-improving activity of these extracts, as MMP-1 production

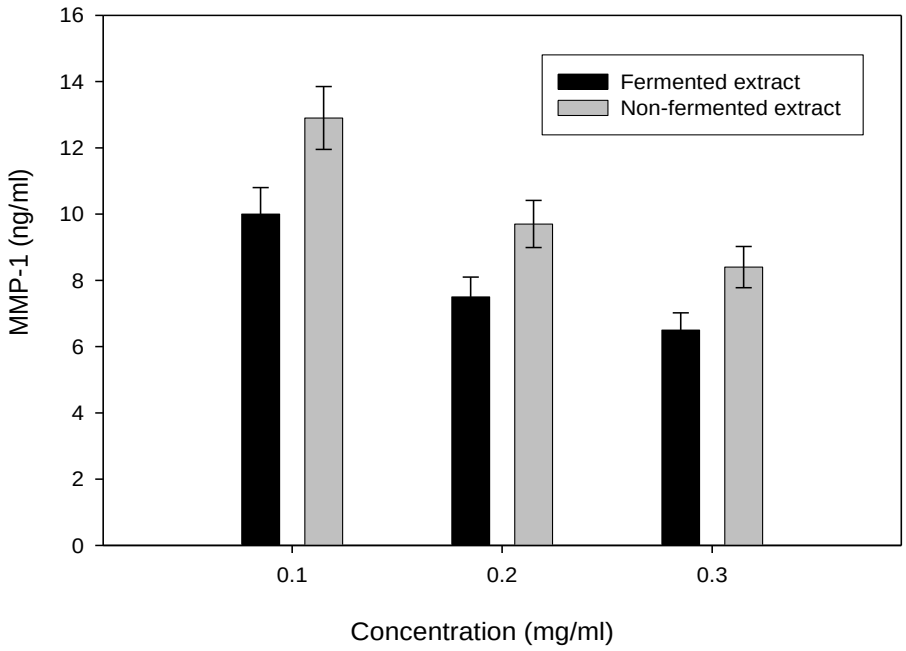

Figure 4. The inhibitory effect of the fermented and non-fermented extracts on MMP-1 production. Data are presented as the mean ± SD of three independent experiments. Means not sharing a common letter were significantly different at $p<0.05$.

was decreased by increasing concentrations of both fermented and non-fermented extracts. Treating cells with 0.1, 0.2, and 0.3 mg/ml of the non-fermented extract inhibited the MMP-1 level by 12.9, 9.7, and 8.4 ng/ml, respectively, whereas treatment of cells with the same concentrations of the fermented extract inhibited the MMP-1 level by 10, 7.5, and 6.5 ng/ml, respectively. These results suggest that the fermented extract has a stronger ability to inhibit MMP-1 production, compared to the non-fermented extract. The mechanism by which the fermented extract additionally suppresses MMP-1 production warrants future study.

Effects on type I procollagen production

It was also evaluated whether non-fermented and fermented *A. distichum* Nakai extracts influenced the production of type I procollagen. Procollagen contains a peptide base sequence called a propeptide at the amino terminus and carboxy terminus. The propeptide is known to facilitate the folding of the procollagen molecule in the endoplasmic reticulum; it is cleaved and separated from the collagen molecule when the collagen-polymerization reaction occurs (Park et al., 2019). Accordingly, the degree of collagen biosynthesis in a cell can be determined by measuring the amount of the separated propeptide (Parfitt et al., 1987). As shown in Figure 5, it was found that treating cells with 0.1, 0.2, and 0.3 mg/ml of the fermented extract increased the type I procollagen production by 22.4, 28.1, and 33.7 ng/ml, respectively, whereas the same doses of non-fermented extract increased this production by 20, 25, and 30 ng/ml, respectively. These results suggest that type I procollagen production is induced more effectively by the fermented extract compared to the non-fermented extract.

Component analysis

HPLC was used to examine the contents of acteoside and isoacteoside in the fermented and non-fermented extracts. Two prominent peaks were observed within the 20-min analysis time (Figure 6). The retention times of peaks 1 and 2 in the HPLC chromatogram were identical to those of the standards for acteoside and isoacteoside, respectively, and thus the peaks were identified as corresponding to these proteins. The contents of acteoside and isoacteoside were 1.25 and 1.05 times higher, respectively, in the fermented extract compared to the non-fermented extract. The amounts of acteoside and isoacteoside were determined to be 372 and 429 mg/100 g for the fermented extract and 298 and 450 mg/100 g for the non-fermented extract. Thus, these components, which were presumably bioconverted via the β-glucosidase activity of *Lactobacillus* strains undergoing fermentation, were increased in the fermented extract. This may explain the apparent improvements in the antioxidative and anti-aging potential of the fermented extract.

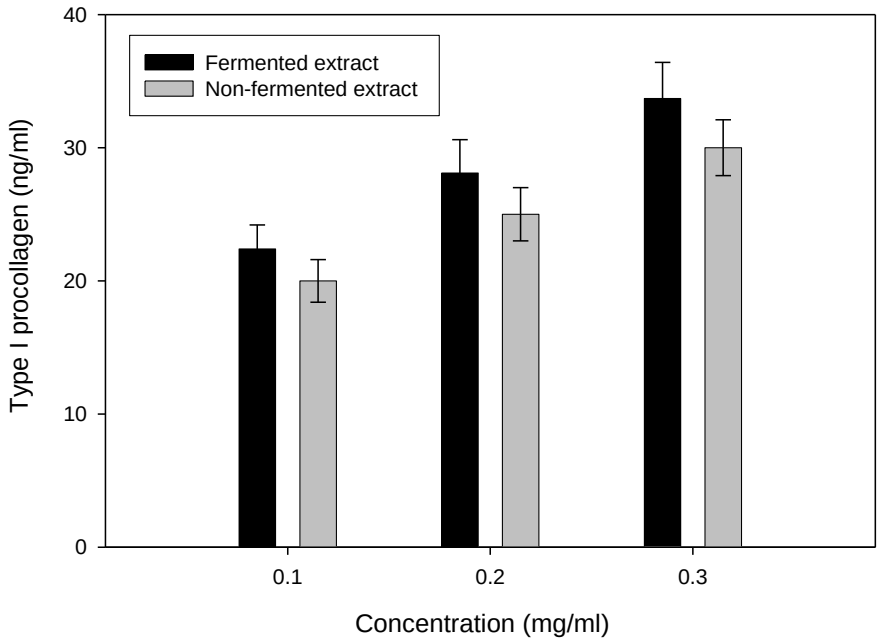

Figure 5. Type 1 procollagen synthesis induced by fermented and non-fermented extracts of *Abeliophyllum distichum* Nakai. Data are presented as the mean ± SD of three independent experiments. Means not sharing a common letter were significantly different at $p<0.05$.

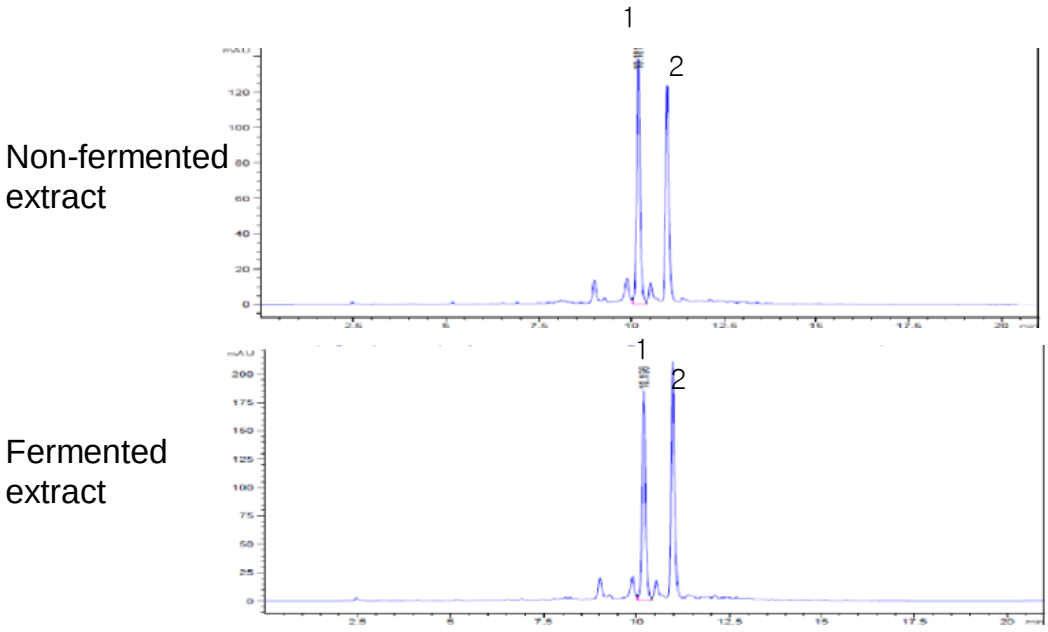

Figure 6. HPLC profiles of non-fermented and fermented extracts of *Abeliopyllum distichum* Nakai (1: acteoside; 2: isoacteoside).

In conclusion, it was observed that the fermented extract from *A. distichum* Nakai more effectively inhibits MMP-1 expression compared to the non-fermented extract. The results suggest that the fermented extract more effectively inhibits intracellular reactive oxygen species (ROS) production and may be useful as an anti- aging

substance for cosmetic applications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ayivi RD, Gyawali R, Krastanov A, Aljaloud SO, Worku M, Tahergorabi R, da Silva RC, Ibrahim SA (2020). Lactic acid bacteria: Food safety and human health applications. Dairy 1(3):2020-232.

Hirbumi Y, Katsuhiko Y, Yoshida Y, Kenichiro IK (1998). Production of cornoside in *Abeliophyllum distichum* cell suspension cultures. Phytochemistry 48(2)273-277.

Hong SP, Han MJ (2002). The floral dimorphism in the rare endemic plant, *Abeliphyllum distichum* Nakai (Oleaceae). Flora-Morphology, Distribution, Functional Ecology **o**f Plants 197(5):317-325.

Huynh TN, Smagghe G, Gonzales GB, Camp JV, Raes K (2016). Extraction and bioconversion of kaempferol metabolites from cauliflower outer leaves through fungal fermentation. Biochemical Engineering Journal 116:27-33.

Kim DW, Hwang I, Kim D (2007). Coenzyme Q10 effectson manganese superoxide dismutase and glutathione peroxidase in the hairless mouse skin induced by ultraviolet b irradiation. Biofactors 30(3):139-147.

Kim NY, Lee HY (2015). Enhancement of anti-wrinkle activities of *Abeliophyllum distichum* Nakai through low temperature extraction process. Korean Journal Medicinal Crop Science 23(3):231-236.

Lee NK, Paik HD (2017). Bioconversion using lactic acid bacteria: ginsenosides, GABA, and phenolic compounds. Journal of Microbiology and Biotechnology 27(5):869-877.

Lee NN, Choi YE, Moon HK (2014). Effect of leds on shoot multiplication and rooting of rare plant *Abeliophyllum distichum* Nakai. Journal of Plant Biotechnology 41(2):94-99.

Moon JY, Yim EY, Song GP, Lee NH, Hyun CG (2010). Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. EurAsian Journal of Biosciences 4:41-53.

Parfitt AM, Simon LS, Villanueva AR, Krane SM (1987). Procollagen type I carboxy-terminal extension peptide in serum as a marker of collagen biosynthesis in bone. Correlation with iliac bone formation rates and comparison with total alkaline phosphatase. Journal of Bone and Mineral Research 2(5):427-436.

Park SM, Lee GW, Cho YH (2008). Effect of Rheum undulatum extract on antioxidant activity and activity of matrix metalloproteinase-1 in human skin fibroblasts. Journal of Life Science 18(12):1700-1704.

Park YS, Nam GH, Jo KJ, Kawk HW, Yoo JG, Jang JD, Kang SM, Kim SY, Kim YM (2019). Adequacy of the anti-aging and anti-wrinkle effects of the *Artemisia vulgaris* fermented solvent fraction. Korean Society for Biotechnology and Bioengineering Journal 34(3):199-206.

Pentland AP, Shapiro SD, Welgus HG (1995). Agonist induced expression of tissue inhibitor of metalloproteinases and metalloproteinases by human macrophages is regulated by endogenous prostaglandin E2 synthesis. Journal of Investigative Dermatology 104(1):52-57.

Sanders ME, Huis J (1999). Bringing a probiotics containing functional food to the market: microbiological, product, regulatory and labeling issues. Antonie van Leeuwenhoek 76:293-315.

Thring TS, Hili P, Naughton DP (2009). Anti-collagnease, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complementary and Alternative Medicine 9(27):1-11.

Wang L, Wei W, Tian X, Shi K, Wu Z (2016). Improving bioactivities of polyphenol extracts from *Psidium guajav*a L. leaves through co-fermentation of *Monascus anka* GIM 3.592 and *Saccharomyces cerevisiae* GIM 2.139. Industrial Crops and Products 94:206-215.

Widowati W, Maesaroh M, Fauziah M, Erawijantari PP, Sandar F (2003). Free radical scavenging and a-/b-glucosidase inhibitory activities of rambutan (*Nephelium lappaceum L*.) peel extract. The Indonesian Biomedical Journal 10(2-3): 165-9.

Yoon MY, Sim SS, Whang WK, Choi BC (2009). Antioxidant activity and whitening effects of acteoside and isoacteoside. Yakhak Hoeji 53(1):1-5.