Anti-Photoaging Effects of Angelica acutiloba Root Ethanol Extract in Human Dermal Fibroblasts

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The effects that ultraviolet rays elicit on collagen synthesis and degradation are the most common causes of wrinkle formation and photo-aging in skin. The objectives of this study were to evaluate the effects of Angelica acutiloba root ethanol extract (AAEE) to promote collagen synthesis and inhibit collagen degradation in human dermal fibroblasts. By examining total polyphenol and flavonoid contents, electron donating ability, radical scavenging activity, and superoxide dismutase-like activity, we found that AAEE exhibited fairly good antioxidant activity. Treatment with AAEE significantly increased type I procollagen production by cultured fibroblasts, as well as reduced ultraviolet-induced matrix metalloproteinase-1 (MMP-1) expression and MMP-2 activity in a dose-dependent manner (p < 0.05). In addition, AAEE significantly increased TIMP-1 mRNA expression (p < 0.05), although without an associated dose-dependent increase in TIMP-1 protein expression. In summary, we suggest that AAEE may be a potentially effective agent for the prevention or alleviation of skin-wrinkle formation induced by ultraviolet rays.

Key words: Angelica acutiloba root, Antioxidant activity, Anti-skin wrinkle, Collagen, Human dermal fibroblasts

INTRODUCTION

Skin aging is a complex biological process that affects both skin function and appearance. The connective tissue of human skin is primarily composed of collagen, elastin, and glucosaminoglycans (1). Collagen and elastin are responsible for the strength and resiliency of skin, and their disarrangement due to photo-aging can lead to an aged appearance.

Collagens are extracellular matrix (ECM) fibrillar molecules found in the dermal connective tissue and make-up approximately 80% of the extracellular materials (2). Collagens are degraded by collagenases, members of the matrix metalloproteinase (MMP) endopeptidase family. MMPs are zinc-dependent endopeptidases that have several pivotal roles in the dynamic remodeling of the ECM. Over time, repeated induction of MMPs by exposure to solar radiation is likely responsible for the increased collagen fragmentation found in sun-damaged skin (3).

With increasing age, collagen synthesis decreases and MMP-1 levels increase naturally in human skin. These alterations can cause changes to the skin, such as wrinkling and loss of elasticity. Exposing cultured human fibroblasts to direct ultraviolet A (UVA) radiation has been shown to increase the abundances of MMP-1 transcript and protein, which in turn leads to the enhancement of MMP-1 activity (4).

MMP activity is carefully regulated by controlling the rate at which proenzymes are converted into catalytic form, and by a family of specific inhibitors, called tissue inhibitors of metalloproteinases (TIMPs) (5). TIMPs directly inhibit the proteolytic activity of MMPs. There are four different types of TIMP (TIMP-1 to -4) and all form physiologically irreversible complexes with MMPs (6). MMP-2 is an important enzyme in the MMP family, and has several essential roles in the skin photo-aging process. MMP-2 has also been shown to degrade type IV collagen, a key component of basement membrane (7).

Elastin, an insoluble fibrous protein, occupies only 2~4% of the dermis matrix but is an important in maintaining the...
elasticity of the skin. Neutrophil elastase and skin fibroblast elastase are two types of elastase enzymes that can degrade elastin in the skin. Neutrophil elastase degrades all types of elastic fibers and is highly sensitive to elaunin and mature elastic fibers, while fibroblast elastase acts on oxytalan and elaunin fibers but has limited effects on mature elastic fibers (8).

Retinol (vitamin A) and its esters, such as retinyl palmitate, are widely used ingredients in cosmetic products. Although retinoids have shown considerable promise for application in dermatologic conditions, they are also associated with local irritation responses that include erythema, burning, itching, scaling, and pruritus. These are collectively referred to as retinoid dermatitis (9). Consequently, plant-derived extracts that can induce anti-wrinkle effects have recently begun to receive much attention (10,11).

Angelica acutiloba (root of Angelica; Danggui) is one of the most popular herbal medicines used in Asian countries, including Korea, China, and Japan. It has traditionally been used in Korean herbal prescriptions for the treatment of gynecological conditions, such as menoxenia and anemia, due to its hemogenic, analgesic, and sedative activities (12,13). The main chemical constituents of Angelica roots are furu lic acid, ligustilide, angelicide, brefeldin A, butylidenephthalide, butyphthalide, succinic acid, nicotinic acid, uracil, and adenine (14). In a previous study, the leaves of Angelica acutiloba were reported to exhibit antioxidant effects (15). However, the inhibitory efficacy of the plant root on skin aging has not been fully investigated.

In this current study, the anti-skin wrinkle effects of A. acutiloba K. (family Apiaceae) root ethanol extract (AAEE) and potential mechanisms were investigated. The effects of AAEE on collagenase activity, elastase activity, collagen synthesis, and the abundances of MMP-1 and TIMP-1 transcript and protein were evaluated using human dermal fibroblasts.

**MATERIALS AND METHODS**

**Plant material.** A. acutiloba roots were purchased from a farm in Bonghwa, Gyeongsangbuk-do, South Korea. Pulverized samples (50 g) were placed into a flask and extracted in 500 mL of 80% ethanol three times for 24 hr each at 25°C. The extract was filtered with filter papers and concentrated using a rotary vacuum evaporator followed by lyophilization (yield 35%).

**Total polyphenol content.** Folin-Denis assays were performed to determine the total polyphenol content of AAEE (16). One milliliter each of the AAEE preparation dissolved in distilled water and Folin reagent were placed in a test tube and allowed to stand for 3 min before adding 1 mL of 10% Na₂CO₃ and shaking vigorously. Tubes were incubated for 1 hr at 25°C before measuring the absorbance at 725 nm. A standard curve was prepared using tannic acid.

**Total flavonoid content.** The total flavonoid content of AAEE was determined by a modified Davis method (17). Each AAEE (100 μL) dissolved in dimethyl sulfoxide (DMSO) was placed in a test tube before adding 1 mL of diethylene glycol reagent and 100 μL of 1 N NaOH. The mixture was shaken vigorously and incubated at 37°C for 1 hr before measuring the absorbance at 420 nm. A standard curve was prepared using rutin.

**Electron donating ability.** The electron donating abilities were evaluated as previously described (18). Briefly, 1 mL of AAEE dissolved in distilled water at 250, 500, or 1,000 μg/mL was placed in a test tube before adding 4 mL of 0.4 mM 1,1-diphenyl-2-picryl hydrazyl (DPPH). The mixture was shaken vigorously and incubated for 10 sec at 60°C before measuring the absorbance at 525 nm. Ascorbic acid was used as a positive control. The DPPH radical scavenging activity of each solution was calculated as a percentage of inhibition using the following equation:

\[
% \text{ donating ability} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control (reagents without test compounds).

**ABTS radical scavenging ability.** The 2,2’-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation decolorization assays were performed as previously described (19), although with slight modifications to compare the antioxidant activity of AAEE with ascorbic acid. ABTS radical cations (ABTS⁺) were produced by reacting 7 mM ABTS solution with 2.4 mM potassium peroxodisulfate at 25°C in the dark for 12 h, and then diluted with ethanol to an absorbance reading of 0.7 ± 0.02 at 734 nm. Samples were then diluted to concentrations of 31.25–1,000 μg/mL and three replicates of each were deposited into a 96-well plate. An equal volume of ABTS⁺ solution was added and the reaction left to proceed for 7 min in the dark at 25°C before absorbance was measured at 734 nm. This was then used to calculate the scavenging ability.

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

**Superoxide dismutase (SOD)-like activity.** The SOD-like activities of AAEE and ascorbic acid were determined as previously described (20) for solutions with concentrations ranging from 31.25 to 1,000 μg/mL. The solution (0.2 mL) was mixed with 2.6 mL of Tris-HCl buffer (50 mM Tris, 10 mM EDTA, pH 8.5) and incubated at 25°C for 10 min to stop the reaction, 0.1 mL of 1 M HCl was added. The absorbance at 420 nm was measured to determine the amount of oxidized pyrogallol with the following equation:

\[
% \text{ SOD-like activity} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]
**Collagenase activity.** Calcium chloride (4 mM) dissolved in 0.1 M Tris-HCl buffer (pH 7.5) was the final buffer assessed. The substrate 4-phenylazo-benzyloxy-carbonyl-Pro-Leu-Gly-Pro-D-Arg (0.15 mg/mL) was dissolved in the buffer and then 250 μL of this solution injected into the test tube with 100 μL of adequate concentration of the sample. *Clostridium histolyticum* collagenase was dissolved in the final buffer to achieve a concentration 0.2 mg/mL, and 150 μL of this solution was added. After incubation at 37°C for 30 min, the reaction was stopped by adding citric acid (6%). The reaction mixture was separated using ethyl acetate. The absorbance of the supernatant was measured at 324 nm. Ascorbic acid was used as the positive control to compare with the test samples. Activity measurement was expressed as the inhibition activity relative to the control.

\[
\text{Inhibition (\%)} = \left[ \frac{(\text{OD}_{410} \text{ of control}) - (\text{OD}_{410} \text{ of sample})}{\text{OD}_{410} \text{ of control}} \right] \times 100
\]

**Elastase activity.** To evaluate the inhibition of elastase activity, the amount of released p-nitroaniline, which is hydrolyzed from the substrate N-succinyl-(L-Ala)_p-p-nitroanilide by elastase, was measured by assessing absorbance at 410 nm. In brief, 2.9 mM N-succinyl-(L-Ala)_p-p-nitroanilide was prepared in a 100 mM Tris-HCl buffer (pH 8.0), and this solution was added to the stock sample. Each sample solution was diluted to final concentrations of 2.5, 5.0, and 10.0 mg/mL. The solutions were mixed thoroughly by tapping before an elastase stock solution (0.2 unit/mL) was added. Solutions were then incubated for 10 min at 37°C, and absorbance measured at 410 nm. The percentage inhibition of the activity of elastase was calculated using the following equation:

\[
\text{Inhibition (\%)} = \left[ \frac{(\text{OD}_{410} \text{ of control}) - (\text{OD}_{410} \text{ of sample})}{\text{OD}_{410} \text{ of control}} \right] \times 100
\]

**Cell culture and cell viability.** Human dermal fibroblasts used in this study were obtained from Amore Pacific Company (Yongin, Korea). Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) in a humidified 5% CO₂ atmosphere at 37°C for 72 hr. Cell respiration, an indicator of cell viability, was assessed by the mitochondrial dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. The reduction of MTT to formazan by viable cells was quantified spectrophotometrically by measuring absorbance at 540 nm.

**Type I procollagen synthesis.** In a 6-well plate, 500 μL of cells were seeded at a density of 0.7 × 10⁵ cells/well and cultured for 24 hr. The media were then removed from the wells, and sample diluted using serum-free media. Cells were diluted to concentrations selected through the previous cell viability tests. After 24 hr, supernatants were obtained by centrifugation for 10 min at 12,000 rpm. Using this supernatant, the degree of collagen synthesis was measured by Procollagen type-1 c-peptide (PIP) enzyme-linked immunosorbent assay (ELISA) kits (TAKARA Biotechnology, Mountain View, CA, USA). The absorbance at 450 nm was determined using a plate reader. The procollagen protein yield was separated from the protein content as previously described (21) and quantified using bovine serum albumin (BSA) standards. Cell lysis buffer (80 μL; CellLytic™ B Cell Lysis Reagent, Sigma, St. Louis, MO, USA) was added to the plate, and samples were freeze (−20°C) thawed three times before removing the lysis mixture from the plate. The protein content of 1/10 dilutions of the freeze-thawed mixtures was quantified using the BSA standard curve.

**Intra-cellular elastase activity.** Cultured human dermal fibroblasts at 80% confluence were washed once with PBS, scraped into PBS, and centrifuged at 4°C, 1,000 rpm for 5 min. Cell pellets were lysed with 0.1 M Tris-HCl (pH 7.6) buffer containing 0.1% Triton-X 100 and 1 mM phenylmethanesulfonyl fluoride, followed by ultrasonication for 5 min on ice. Supernatants that were clear after the removal of cell residues via centrifugation (2,000 rpm, 10 min) were used as the fibroblast enzyme solution. Enzyme solution (100 μL) was dispensed into 96-well plates, which were pre-incubated for 15 min at 37.8°C. After addition of 2 μL 62.5 mM N-succinyl-(L-Ala)_p-p-nitroanilide, the plates were further incubated for 2 hr at 37°C. The release of p-nitroaniline was measured by absorbance at 410 nm.

**Gelatin zymography.** Cultured media were analyzed for gelatin degradation activity through electrophoresis under non-reducing condition in 10% Tris-Glycine gelatin gel (Invitrogen, Carlsbad, CA, USA). Gels were incubated in Novex zymogram buffer containing 2.5% (v/v) Triton® X-100 for 30 min at 25°C with gentle agitation and then equilibrated for 1 hr at 25°C in Novex zymogram developing buffer (Invitrogen) containing 50 mM Tris base, 40 mM HCl, 200 mM NaCl, 5 mM CaCl₂, and 0.02% (w/v) Brij 35. This was followed by an incubation with fresh developing buffer for 24 hr at 37°C. The zymogram gel was washed three times with deionized water and stained with the Simply Blue™ Safe stain (Invitrogen) at 25°C with gentle shaking and washed with deionized water. MMP-2 activity was quantified with an image analyzer.

**Reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA from human dermal fibroblasts was extracted with 1 mL of TRIzol (Invitrogen) per the manufacturer instructions, and 5 μg samples were reverse transcribed in 40-μL of reaction mixture containing 8 μL 5× M-MLV RT (Murine Leukemia Virus Reverse Transcriptase) buffer, 3 μL dNTPs (10 mM), 0.45 μL RNasein (40 U/μL),
0.3 μL M-MLV reverse transcriptase (200 U/μL; Promega, Madison, WI, USA), and 1.5 μL of oligo dT (50 pM/μL; Bioneer, Daejeon, Korea). Single stranded cDNA was amplified by PCR using 4 μL of 5× Green Go-Taq® Flexi reaction buffer, 0.4 μL dNTPs (10 mM), 0.1 μL Taq polymerase (5 U/μL), 1.2 μL MgCl₂ (25 mM; Promega), and 0.4 μL each (20 pM/μL) of specific sense and anti-sense primers for MMP-1, TIMP-1 or β-actin. PCR primer sequences are described in Table 1. PCR conditions were 28 cycles of 94°C for 60 sec, 50°C for 60 sec, and 72°C for 60 sec. PCR products were run on a 1.2% agarose gel with a β-actin internal control to evaluate the relative expression of MMP-1 and TIMP-1.

**Western blotting.** Total cellular protein was extracted from each sample using a RIPA lysis buffer containing protease inhibitors. After electrophoresis, proteins were transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) for immunoblotting using antibodies against MMP-1, TIMP-1, and β-actin.

**Table 1.** Primer sequences of target genes used in the PCR

| Genes    | Primers                                      | Expected size (bp) |
|----------|----------------------------------------------|--------------------|
| MMP-1    | Forward (5’→ 3’): CGACTCTAGAAACACAAGAGCAAGA | 237                |
|          | Reverse (5’→ 3’): AAGGTAGCTTACTGTCACACGCTT   |                    |
| TIMP-1   | Forward (5’→ 3’): AGTCAACCAGACCACCTTATACCA   | 386                |
|          | Reverse (5’→ 3’): TTTCATAGCCTTGGAGGAGCTGGTC  |                    |
| β-Actin  | Forward (5’→ 3’): ACCGTGAAGAATGACCCAG        | 248                |
|          | Reverse (5’→ 3’): TACGGATGTCACACGCA          |                    |

1)MMP-1: matrix metalloproteinase-1, 2)TIMP-1: tissue inhibitor of metalloproteinase-1, 3)bp: basepair.

**Fig. 1.** Antioxidant activity of *Angelica acutiloba* root ethanol extract (AAEE). (A) Total polyphenol and flavonoid content. (B) Electron donating ability. (C) Radical scavenging ability. (D) SOD-like activity. Bars represent mean ± SD (n = 3).
USA) at 100 V for 1 hr. The membrane was blocked for 2 hr in PBST containing 5% dried skimmed milk powder. After three washes in PBST for 10 min, membranes were incubated with primary antibody: MMP-1 (1:1000, ab137332, Abcam, Cambridge, UK), TIMP-1 (1:1000, SC-6832, Santa Cruz Biotechnology, Santa Cruz, CA, USA), or β-actin (1:1000, SC-6832, Santa Cruz Biotechnology) for overnight at 4°C. After three more washes with TBST, membranes were then incubated with a 1:1000 dilution of HRP-conjugated secondary antibody against the appropriate species for 2 hr at 25°C. Protein bands were visualized using a Western Blot Detection Kit (Bio-Rad).

**Statistical analysis.** Differences between the groups were statistically evaluated using one-way analysis of variance (ANOVA) followed by the Duncan multiple range test as the post hoc analysis using SPSS software (v21.0; SPSS Inc., Chicago, IL, USA). The threshold for statistical significance was set at $p < 0.05$.

**RESULTS**

**Antioxidant activity of AAEE.** Total polyphenol and flavonoid contents in AAEE were found to be 28.6 and 12.6 mg/g, respectively (Fig. 1A). The electron donating ability, ABTS radical scavenging ability, and SOD-like activity of AAEE at 1,000 μg/mL were 74.7% (Fig. 1B), 62.5% (Fig. 1C), and 43.7% (Fig. 1D), respectively. These values were lower than those of the positive control ascorbic acid.

**Collagenase and elastase activity inhibition.** Collagenase inhibitory efficacy of AAEE at 10 mg/mL was 50.4%.

**Cell viability and morphological observation.** Ascorbic acid treatment at concentrations of 100 and 200 μg/mL reduced cell viability by 7.5% and 32.5%, respectively, compared to vehicle-treated cells. Thus, the maximum permissible level (MPL) for ascorbic acid application to human fibroblasts was set at 100 μg/mL.
dermal fibroblasts was determined to be 100 μg/mL (Fig. 4A). AAEE treatments at concentrations of 100 and 200 μg/mL reduced cell viability by 0.6% and 7.2% respectively, compared to vehicle-treated cells, therefore exhibiting an MPL of over 200 μg/mL (Fig. 4B). AAEE had lower cytotoxicity than ascorbic acid. Fibroblasts treated with 25–100 μg/mL of ascorbic acid maintained the typical flat, spindle-like shape that was observed in vehicle-treated control cells (Figure not shown).

**Type I procollagen synthesis promotion.** In comparison with the vehicle group, the treatments of AAEE at 25, 50, 100, and 200 μg/mL to human dermal fibroblasts significantly increased collagen production by 31.6%, 47.5%, 55.5%, and 93.7%, respectively, in a dose-dependent manner (Fig. 5) (p < 0.05).

**MMP-1 and TIMP-1 expression.** Treatments of AAEE at 50, 100, and 200 μg/mL to human dermal fibroblasts significantly reduced MMP-1 protein expression by 4.8%, 12.2%, and 14.6% respectively, compared to UV A-irradiated control cells (Fig. 6A) (p < 0.05) as well as MMP-1 mRNA expression by 49.8%, 60.9%, and 85.8%, respectively (Fig. 6C) (p < 0.05). Additionally, at the same concentrations, AAEE significantly increased TIMP-1 protein expression by 14.9%, 15.2%, and 18.3% respectively, compared to UV A-irradiated control cells (Fig. 6B) (p < 0.05) as well as TIMP-1 mRNA expression by 26.4%, 32.5%, and 44.1%, respectively (Fig. 6D) (p < 0.05).

**MMP-2 activity.** AAEE treatment resulted in a dose-dependent decrease in MMP-2 protein expression, as observed by zymography (Fig. 7A). At concentrations of 25, 50, 100, and 200 μg/mL, MMP-2 activity was significantly decreased by 30.8%, 35.5%, 36.7%, and 36.9%, respectively, compared to UVA-irradiated control cells (Fig. 7B) (p < 0.05).

**DISCUSSION**

Danggui is one of the most popular and widely con-
Anti-Photoaging Effects of Angelica acutiloba

Angelica acutiloba is one of the most used medicinal herbs in Asia and is divided into three types; Angelica gigas (Korean Danggui), A. sinensis (Chinese Danggui), and A. acutiloba (Japanese Danggui). In the previous study, A. acutiloba was reported to have anti-allergic (22) and anti-complementary effects (23). However, up to date, it is difficult to contact data reported on the skin anti-ageing effects of A. acutiloba root. Therefore, we investigated the skin anti-aging effects of AAEE using in vitro assays.

UV A rays are absorbed by the skin leading to the generation of reactive oxygen species (ROS) in dermal fibroblasts. ROS subsequently cause oxidative damage to major cellular components that play important roles in different molecular pathways (24). Oxidative damage is the most important contributor to skin aging. One consequence of oxidative damage is up-regulation of elastase and collagenase, leading to degradation of the major dermal components elastin and collagen. This is responsible for the appearance of...
wrinkles and sagging that is characteristic of photo-damaged skin (25).

Plant phenolic compounds have been shown to inhibit collagenase activity, alongside a free-radical scavenging ability that can control aging (26). In this study, total polyphenol and flavonoid contents of AAEE were found to be 28.6 and 12.6 mg/g, respectively. These are higher than those found in A. gigas leaves ethanol extracts (7.87 and 4.23 mg/g, respectively) (27). Additionally, we also found that the electron donating ability, ABTS radical scavenging ability, and SOD-like activity of AAEE were also comparatively high, demonstrating values of 74.7, 62.5, and 43.7% at 1,000 μg/mL, respectively.

The reduction of collagen observed during UV A damage is either due to reduced synthetic activity of dermal fibroblasts or an increased rate of degradation by collagenase. One mechanism that controls type I collagen expression is the collagenase-mediated degradation process (28). In this study, AAEE was found to significantly and dose-dependently inhibit collagenase activity (p < 0.05), exhibiting 50.4% inhibition at 10 mg/mL. Elastin plays a role in maintaining the elasticity of the skin and is a constituent of a network of collagenous fibers under the epidermis. Elastic fibers are interwoven among collagen bundles, where they convey elasticity and resilience (29). In this study, AAEE was found to significantly and dose-dependently inhibit elastase activity both in cell-free assay (30.9% at 10 mg/mL) and in cell assay (27.5% at 100 μg/mL) (p < 0.05).

Skin contains various types of interstitial collagens, including type I, II, III, IV, and V (30). Type I collagen contributes at least 90% to the total collagen content (31) and is synthesized by dermal fibroblasts as procollagen precursor molecules (32). Procollagens are cleaved into collagen molecules, and both carboxy- and amino-terminal propeptides, by specific proteases to form collagen peptides. This is followed by organization into complete collagen fibers (33). In this study, the concentration of carboxy-terminal pro-peptides from type I procollagen in the culture medium was used as a marker of extracellular type I collagen synthesis. AAEE significantly and dose-dependently increased type I collagen production in human dermal fibroblasts (p < 0.05). Type I collagen synthesis at 100 μg/mL AAEE was considerably higher (55.5%) than that of A. gigas extracts at the same concentration (16.9%) (34), indicating that AAEE may be act as a potent collagen synthesis stimulator.

MMP-1 activity is controlled by endogenous inhibitors, such as TIMP-1, that form 1:1 covalent complexes with MMPs in the extracellular or pericellular spaces (35). The balance in expression of MMP-1 to TIMP-1 is therefore considered to regulate degeneration of the ECM (36,37). In this study, AAEE affected the abundance of both protein and mRNA for MMP-1 and TIMP-1. AAEE significantly and dose-dependently decreased MMP-1 protein and mRNA expression (p < 0.05). Additionally, AAEE significantly and dose-dependently increased TIMP-1 mRNA expression (p < 0.05). However, there was no associated dose-dependent increase in TIMP-1 protein abundance even despite significantly increased levels compared to the control (p < 0.05). Similar to our study, some medicinal herb extracts have been reported to have potential activity that prevents collagen degradation by inhibiting MMP-1. For example, A. gigas extracts reduced MMP-1 activity in human dermal fibroblasts (34) and the decursin isolated from A. gigas inhibited UVB-induced MMP-1 expression in human dermal fibroblasts (38).

MMP-2 is thought to play an important role in the final degradation of fibrillar collagens after their initial cleavage by interstitial collagenase MMP-1 (39). It is a secreted endopeptidase and can hydrolyze several components of the ECM, including basement membrane collagen IV (40). In this study, treatment with 100 μg/mL AAEE significantly decreased MMP-2 activity by 36.6% (p < 0.05). This is a larger decrease than that induced by ascorbic acid (27.0%). These results suggest that AAEE could protect dermal fibroblasts against aging by controlling MMP and TIMP expression.

In summary, AAEE was highly effective in increasing collagen synthesis and inhibition of degradation in human
dermal fibroblasts. These findings suggest that AAEE could protect human skin against the effects of photo-aging by suppressing MMP and augmenting TIMP at the transcriptional and protein levels, as well as by stimulating collagen synthesis. Therefore, we suggest that AAEE could serve as a potent anti-wrinkle agent. We analyzed of AAEE used in this study on the ingredients decursin and decursinolangelate, known as skin anti-aging ingredients of A. gigas (41,42), using an ultra-performance liquid chromatograph (UPLC). However, they were below the detection limit (not detected, N.D.). The potential skin anti-aging ingredients in AAEE need to be further investigated.

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