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Anti-aging effect of *Allium pseudojaponicum* in UVA–irradiated human epidermal keratinocytes

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ABSTRACT

This study was conducted to investigate the anti-aging effects of *Allium pseudojaponicum* extract on normal human epidermal keratinocytes (NHEKs). The effects were examined by analyzing gene expressions related to skin hydration using quantitative real-time polymerase chain reaction (qRT-PCR), hyaluronic acid (HA) production using HA-ELISA, cell viability using a cell viability assay, and a phospho-kinase array. *Allium pseudojaponicum* extract increased the gene expression levels of *AQP3*/*HAS2* and HA protein production in NHEKs while decreasing the overexpressed mRNA levels of *KRT1, 10* and *FLG* genes, known as differentiated keratinocyte markers in NHEKs. Additionally, *A. pseudojaponicum* extract reduced the phosphorylation of CHK2 and p53 proteins, which are related to cell cycle or epidermal differentiation. This study demonstrated the anti-aging effects of *A. pseudojaponicum* extract, which could potentially be used as a functional ingredient for skin hydration and anti-aging products.

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Introduction

Human skin is the main barrier that protects the body from environmental factors like ultraviolet (UV) radiation, chemical compounds, fine dust, and microorganisms. When the skin is damaged by various extrinsic factors, living organisms regenerate new skin to restore the damaged area (1,2). UV is beneficial in the medical treatment of certain skin diseases, such as vitiligo and psoriasis, and it also stimulates the biosynthesis of vitamin D3 in the skin. However, exposure to UV can also lead to photoaging of the skin by causing the denaturation of carbohydrates, proteins, lipids, and nucleic acids through oxidative stress (1,3). UV induces the generation of reactive oxygen species (ROS) in skin cells, and ROS not only produces inflammatory cytokines such as TNF-α (tumor necrosis factor-α) but also activates the inflammatory signaling substances, ACE (angiotensin-converting enzyme), COX-2 (cyclooxygenase-2), and cPLA2 (cytosolic phospholipase A2). These signals promote photoaging and induce excessive pigmentation and skin dryness (4,5). When keratinocytes are exposed to UV, the expression of representative biomarkers such as *AQP3* (aquaporin 3) and *HAS2* (hyaluronan synthase 2) decreases and this reduction causes skin dryness (6–9). Aquaporins are transmembrane proteins that facilitate water transportation across cell membranes (10,11). There are various aquaporins in human keratinocytes, and AQP3, in particular, plays a key role in water transportation. Knockout mice with suppressed *AQP3* expression show dry skin and reduced wound healing conditions (10). HAS2 is an enzyme that synthesizes hyaluronic acid (HA), and the HAS2 expression is reduced by UV light (7–9). When UV rays reach the basal layer of the epidermis, keratinocytes of the basal layer are abnormally differentiated, and epidermal turnover is accelerated, thereby causing aging of the skin. UV light reduces the expression of K5/14 (*keratin 5/14)* expressed in keratinocytes of the basal layer and induces the expression of K1/10 (*keratin 1/10)*, FLG (*filaggrin)*, and LOR (*loricrin)*, which are markers for hyperdifferentiation and hyperkeratosis in epidermal keratinocytes (8,9,12).

Allium pseudojaponicum is a perennial plant in the Amaryllidaceae family and is a rare species. The plant was first discovered and reported in the southern part of Japan, near Tsushima. In Korea, its distribution was recently confirmed in the coastal area of Geomundo Island. Compared to *Allium thunbergii*, it has been reported to have characteristics such as evergreen leaves, a chromosome number (2n = 32), and lateral bulbs growing on the peduncle (13). The *A. pseudojaponicum* used in this study was collected from Jeju Island. Studies on *A. pseudojaponicum* are limited to the germination characteristics of seeds based on regional distribution, light conditions, and temperature (13–15). There is no report on the improvement of skin aging or skin moisturizing in human keratinocytes by *A. pseudojaponicum* extract. In this study, I analyzed the effect of *A. pseudojaponicum* extract on the expression of moisturizing-related markers such as *AQP3*/*HAS2* genes and confirmed whether HA production occurs in human keratinocytes. In addition, I confirmed whether *A. pseudojaponicum* extract can reduce hyperdifferentiation and hyperkeratosis caused by UVA. Furthermore, I analyzed the intracellular signal transduction by *A. pseudojaponicum* extract.

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Materials and Methods

Sample preparation and cell culture

Allium pseudojaponicum (NP60210017) was provided by the National Marine Biodiversity Institute of Korea (https://biobank.mbris.kr/biobank/main). The plant specimen used in this study was collected from the Jeju coastal sea in Seogwipo, Jeju, on April 10, 2018. The specimen was lyophilized, extracted by sonication in 70% ethanol as a solvent, and dissolved (10 mg/mL) in ethanol:DMSO (1:1; v/v, Sigma, USA) for DPPH (Sigma) radical assay and cell experiments. NHEKs and KGM™ gold Bullet-Kit™ were purchased from Lonza (Sweden). NHEKs were cultured in an incubator under 5% CO_2 at 37 °C, and they were used within the third passage.

DPPH radical scavenging assay

For the analysis of DPPH scavenging activity, DPPH was dissolved in a mixture of methanol and distilled water (3:2, v/v, Sigma) at a concentration of 0.1 mM. *Allium pseudojaponicum* extract (50 μL) of varying concentrations was added to 500 μL DPPH reagent and allowed to react at room temperature for 20 min. Absorbance was measured using a spectrophotometer (Epoch; Biotek, USA) at 450 nm. The DPPH radical scavenging ability was expressed as a percentage of the difference in absorbance between the blank and sample-treated groups, with L-ascorbic acid (Sigma) used as a positive control group.

Total phenolic compounds (TPC) assay

TPC contents were determined with the Phenolic Compounds Assay Kit (Sigma) according to product instructions. 50 uL of the sample was added with 20 uL of PC Probe and mixed gently. 80 uL of PC assay buffer was added. The mixture was incubated at room temperature for 10 min. The absorbance was measured at 480 nm using a spectrophotometer (Epoch). The concentration of TPC in *Allium pseudojaponicum* extract was calculated from the catechin standard curve with the range of 0.01-0.1 mg/mL and expressed as mg catechin/g dry *A. pseudojaponicum*

UVA irradiation

NEHKs were treated with UVA to induce photoaging. A total of 1.2×10^5 cells were inoculated in a 35-mm tissue culture dish and treated with 1 mL of phenol red-free Dulbecco's Modified Eagle Medium (DMEM; Welgene, Korea). UVA (1.5 J/cm^2) was irradiated using a Bio-Link UVA irradiation system (BLX-365; Vilber Lourmat, France) (8,9).

Cell viability assay

To analyze the survival ratio of NHEKs under *A. pseudojaponicum* treatment, a Cell Counting Kit-8 (CCK-8; DoGenBio, Korea) assay was used. NHEKs were inoculated in a 24-well plate at a concentration of 1.2×10^4 cells and treated with *A. pseudojaponicum* extract at each concentration for 24 h. After removing the culture medium, cells were washed with Dulbecco's phosphate-buffered saline (Welgene) and treated with 30 μL of the CCK-8 solution in 270 μL phenol red-free DMEM. The cells were then incubated for 1 h in a CO_2 incubator. The absorbance was measured at 450 nm using an Epoch spectrophotometer.

RNA isolation and qRT-PCR

To isolate RNA, cells were treated with TRIzol™ reagent (Thermo Fisher, USA), and 2 μg of RNA was reverse transcribed into cDNA using SuperiorScript III Master Mix (Enzynomics, Korea). A qRT-PCR system (LightCycler 96; Roche, Germany) was used to compare the relative expression patterns of genes. The TaqMan™ Gene Expression Assay (Applied Biosystems, USA) used in the experiment is specified in Table 1.

Hyaluronic acid-enzyme-linked immunosorbent assay

To confirm the production of HA by *A. pseudojaponicum* extract, NHEKs (2.5×10^5 cells) were inoculated in a 60-mm tissue culture dish, preincubated for 24 h, and then irradiated with UVA (1.5 J/cm^2). After UVA irradiation, the cells were treated with *A. pseudojaponicum* extract (10 μg/mL) and cultured in a CO_2 incubator for 48 h. HA in the conditioned medium was quantified using a HA-ELISA kit (Corgenix, USA).

Protein kinase array analysis

To investigate the effect of *A. pseudojaponicum* extract (10 μg/mL) on phosphorylated proteins, the cells were preincubated with the extract for 30 min before UVA irradiation. Phosphorylated proteins were analyzed using a human phospho-kinase array following the manufacturer's instructions (R&D Systems). Briefly, cell lysates (300 μg) were collected and incubated with each array overnight on a rocking shaker at 4 °C. The cell lysate was removed, and the array sheets were washed three times with a washing buffer. Next, the arrays were incubated with the primary antibody solution for 2 h at room temperature and washed three times with washing buffer. After incubation with the primary antibody solution, the array was treated with the secondary antibody solution and incubated for 1 h at room temperature on a rocking shaker. The array was then washed three times with washing buffer, and phosphorylated protein spots were visualized using an Amersham Imager 680 (Merck, USA). The density of each phosphorylated protein spot was assessed using the Quick Spots tool

Table 1. Gene symbol, name and assay ID Number in qRT-PCR analysis.

Gene Symbol	Gene Name	Assay ID
AQP3	Aquaporin 3	Hs01105469_g1
HAS2	Hyaluronan synthase 2	Hs00193435_m1
FLG	Filaggrin	Hs00856927_g1
LOR	Loricrin	Hs01894962_s1
KRT1	Keratin 1	Hs01549614_g1
KRT10	Keratin 10	Hs01043114_g1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	43333764F

program (Version 22.0.1b; Western Vision Software), and the density was calculated by subtracting the background and phosphate-buffered saline negative control. The fold change was obtained by comparing UVA-treated samples with the *A. pseudojaponicum* treated samples.

Statistical analysis

The data were statistically analyzed using the Student's T-test. The results are expressed as the mean ± standard deviation of at least three independent experiments, and $p < 0.05$ was considered significant.

Results

Antioxidant effect of Allium pseudojaponicum extract and determination of total phenolic content (TPC)

The antioxidant activity of *A. pseudojaponicum* was evaluated by the 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) free radical scavenging assay. At a concentration of 100 μg/mL, *A. pseudojaponicum* showed an inadequate DPPH scavenging ability of 4.9%. However, at 10 mg/mL, *A. pseudojaponicum* exhibited an antioxidative effect of 51.4%, which was comparable to that of the positive control, L-ascorbic acid (55.4%) (Figure 1). And the TPC of *A. pseudojaponicum* is 1.03 ± 0.06 mg catechin/g dry *A. pseudojaponicum*.

Cell viability analysis of *A. pseudojaponicum* extract

The cytotoxicity of *A. pseudojaponicum* extract in normal human epidermal keratinocytes (NHEKs) was determined by the Cell Counting Kit-8 (CCK-8) assay. *Allium pseudojaponicum* extract was administered at concentrations of 0.1, 1, 10, and 100 ng/mL and 1, 10, and 100 mg/mL. The survival ratio of NHEKs was significantly reduced at *A. pseudojaponicum* concentrations above 100 μg/mL compared with that of the control group (Figure 2). Because the survival ratio of NHEKs was not affected at concentrations of 10 μg/mL or less, *A. pseudojaponicum* extract was used at concentrations of 10, 1, and 0.1 μg/mL for the subsequent experiments.

Gene expression analysis by *A. pseudojaponicum* extract treatment

To investigate the effect of *A. pseudojaponicum* extract on NHEKs, samples were treated for 24 h after UVA irradiation as described in previous studies (8,9). The expressions of *AQP3* and *HAS2*, which are representative markers expressed in NHEKs, were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). *AQP3* and *HAS2* are known to be involved in moisturizing by facilitating the transport of moisture into the cell and the biosynthesis of HA (8,9). In this experiment, the expression of *AQP3* and *HAS2* genes was reduced by 49% and 78% by UVA irradiation, respectively. The expression of *AQP3* increased by 2.12, 1.7, and 1.81 times in the 10, 1, and 0.1 μg/mL treatment groups of *A. pseudojaponicum* extract compared with those of the UVA irradiated group (Figure 3A). And the expression of *HAS2* increased by approximately 4.11 times compared with that of UVA-treated group (Figure 3B).

Effect of *A. pseudojaponicum* extract on hyaluronic acid synthesis

HAS2 is an enzyme responsible for the production of HA in human keratinocytes (16,17). After confirming the increase in HAS2 expression by treating with *A. pseudojaponicum* extract through qRT-PCR (Figure 3B), an enzyme-linked immunosorbent assay for HA (HA-ELISA) was performed to confirm the production of HA protein. HA synthesis, which was reduced by approximately 40% due to UVA irradiation, was increased by approximately 51% by *A. pseudojaponicum* extract compared to that of the UVA irradiation group (Figure 4). This result

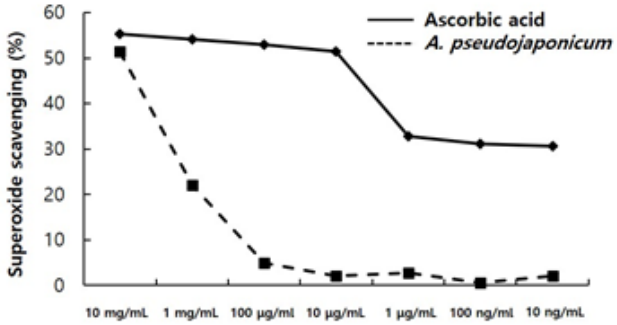

Figure 1. Free radical scavenging assay of *Allium pseudojaponicum* extract by the DPPH assay. The *A. pseudojaponicum* extract has an antioxidant ability similar to that of L-ascorbic acid (positive control) in high concentration (10 mg/mL).

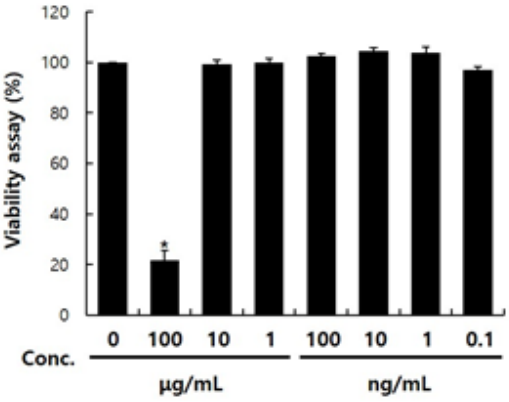

Figure 2. Cytotoxicity of *Allium pseudojaponicum* extract in normal human epidermal keratinocytes (NHEKs). NHEKs (1.2×10^4 cells) were seeded in 24-well plates and treated with *A. pseudojaponicum* extract for 24 h. Cell viability was measured by the CCK-8 assay. The results are presented as the mean ± standard deviation of the percentage of control optical density for triplicate experiments. *, $p < 0.05$ compared to control.

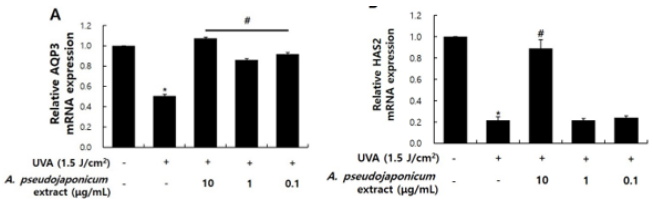

Figure 3. Characterization of *Allium pseudojaponicum* extract treatment on UVA-treated NHEKs. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of hydration markers: *AQP3* (A) and *HAS2* (B). Values represent the mean ± SD of three independent experiments. *: significantly different compared to control, $p < 0.05$. #: significantly different compared to UVA treated condition, $p < 0.05$. AQP3, Aquaporin 3; HAS2, Hyaluronan synthase 2.

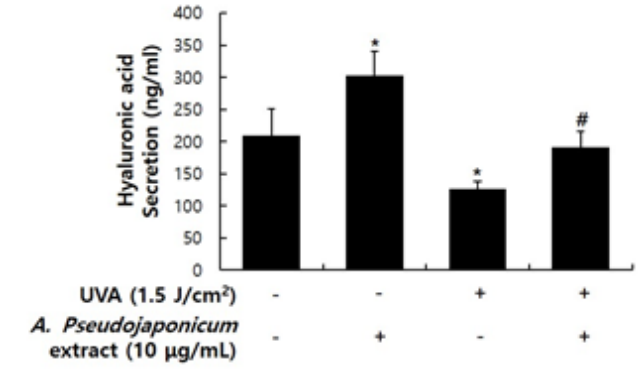

Figure 4. Effects of *Allium pseudojaponicum* extract on hyaluronic acid (HA) synthesis in NHEKs. NHEKs (2.5×10^5 cells) were seeded in a 60-mm tissue culture dish and treated with *A. pseudojaponicum* extract for 48 h. Cell culture medium was collected and analyzed for HA production using HA-ELISA. The data represent the mean ± SD of three independent experiments. *: significantly different compared to control, $p < 0.05$. #: significantly different compared to UVA treated condition, $p < 0.05$.

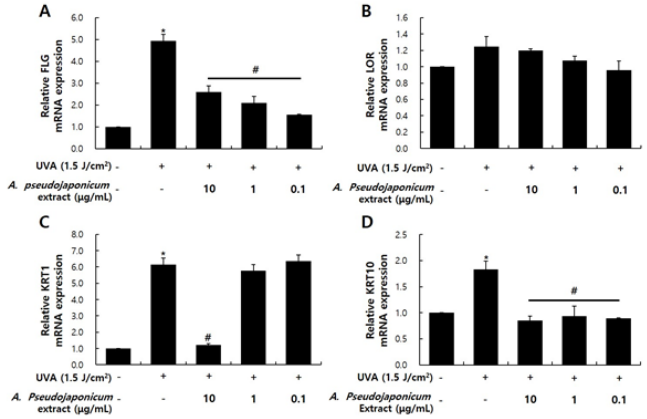

Figure 5. Expressions of differentiated keratinocyte markers upon *Allium pseudojaponicum* extract treatment in UVA-treated NHEKs. qRT-PCR analysis of the representative differentiated markers, *FLG* (A), *LOR* (B), *KRT1* (C), and *KRT10* (D). The graphs are shown as the mean ± SD of three independent experiments. *: significantly different compared to control, $p < 0.05$. #: significantly different compared to UVA treated condition, $p < 0.05$. FLG, Filaggrin; LOR, Loricrin; KRT1, Keratin 1; KRT10, Keratin 10.

is consistent with the *HAS2* gene expression pattern previously confirmed through qRT-PCR (Figure 3B) and shows that *A. pseudojaponicum* extract not only increases the expression of the *HAS2* gene but also increases the HA protein synthesized by *HAS2*.

Effects of *A. pseudojaponicum* extract on epidermal differentiation markers

To analyze the effect on differentiation markers by *A. pseudojaponicum* extracts, the gene expressions of *FLG*, *LOR*, *KRT1*, and *KRT10* genes were analyzed by qRT-PCR. Among the four markers mentioned, UVA significantly increased the expression of *FLG*, *KRT1*, and *KRT10* but did not significantly affect the expression of the *LOR* gene (8,9). Compared to the *FLG* gene expression of the UVA-irradiated group, the gene expression was decreased by 47%, 58%, and 69% in 10, 1, and 0.1 μg/mL *A. pseudojaponicum* treatments, respectively (Figure 5A). Compared to the *KRT1* gene expression of UVA irradiated group, the gene expression was reduced by 80%. Further, the *KRT10* gene was reduced by 54%, 49%, and 51% at 10, 1, and 0.1 μg/mL *A. pseudojaponicum* treatments, respectively (Figure 5C and 5D). In contrast, the *LOR* gene showed no differences between UVA irradiated and *A. pseudojaponicum* extract treatment groups (Figure 5B). These results demonstrated that UVA induces hyperdifferentiation and hyperkeratosis of keratinocytes, but *A. pseudojaponicum* extract suppressed hyperdifferentiation and hyperkeratosis by reducing *FLG*, *KRT1*, and *KRT10* expressions.

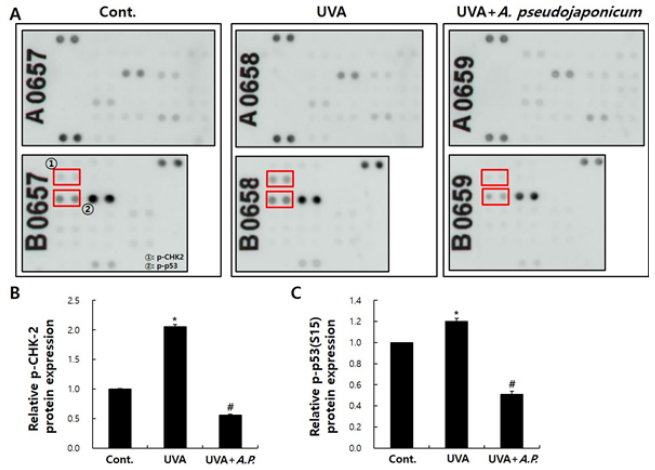

Figure 6. Effect of *Allium pseudojaponicum* extract on phosphorylation of intracellular signaling. Cells were preincubated with *A. pseudojaponicum* extract for 30 min before UVA irradiation. After collecting whole cell lysates, cell lysates were applied to the human phospho-kinase array kit. (A) indicates membranes A and B of the phospho-kinase array kit. The density of each array spot was measured. (B) Relative p-CHK2 protein expression. (C) Relative p-p53 protein expression. The graphs are shown as the mean ± SD of three independent experiments. *: significantly different compared to control, $p < 0.05$. #: significantly different compared to UVA treated condition, $p < 0.05$.

Analysis of intracellular signaling by *A. pseudojaponicum* extract

From the phospho-kinase array experiment, two proteins were identified (Figure 6A, Table 2). These two phosphorylated proteins were increased by UVA irradiation but decreased by *A. pseudojaponicum* extract treatment. In this experiment, the p-CHK2 protein was increased 2.05-fold by UVA but decreased by 73% with *A. pseudojaponicum* extract treatment compared to the protein level of the UVA-treated group (Figure 6B). The phosphorylation of p53 increased by about 20% due to UV irradiation but decreased by about 67% following treatment with *A. pseudojaponicum* extract compared with the UV-treated group (Figure 6C).

Discussion

The *Allium* genus contains the dietary source of carbohydrates, minerals, vitamins, and phenolic compounds. Phenolic compounds are secondary metabolites found abundantly in vegetables, plants, and fruits. Major types of phytochemical phenolic compounds include simple phenolic acids (ex; gallic acid and vanillic acid), stilbenoids, flavonoids (catechin), lignans and polyphenols (proantho-

Table 2. Relative phosphorylated protein analysis of *A. pseudojaponicum* extract treated NHEKs.

Gene Name(phosphorylation site)	Fold change (mean ± S.D) ratio	
	UVA/Cont.	Extract/Cont.
CREB(S133)	1.08±0.05	1.58±0.01
EGF R(Y1086)	0.97±0.01	0.95±0.02
eNOS(S1177)	0.91±0.03	0.76±0.01
ERK1/2(T202/Y204, T185/Y187)	0.96±0.01	0.69±0.01
Fgr(Y412)	1.01±0.15	0.98±0.03
GSK-3α/β(S21/S9)	0.96±0.03	0.87±0.01
GSK-3β(S9)	0.72±0.03	0.66±0.04
HSP27(S78/S82)	0.91±0.25	0.95±0.05
JNK1/2/3(T183/Y185, T221/Y223)	0.95±0.02	1.09±0.04
Lck(Y394)	1.15±0.02	1.14±0.02
Lyn(Y397)	0.92±0.05	0.95±0.04
MSK1/2(S376/S360)	0.79±0.16	0.84±0.08
p38a(T180/Y182)	0.80±0.03	0.74±0.01
PDGF Rβ(Y751)	0.90±0.01	0.94±0.02
PLCγ-1(Y783)	0.85±0.04	0.87±0.05
Src(Y416)	0.72±0.03	0.69±0.05
STAT2(Y689)	0.93±0.02	0.81±0.02
STAT5a/b(Y699)	0.84±0.01	0.79±0.06
WNK1(T60)	0.88±0.06	0.94±0.01
Yes(Y426)	0.86±0.04	0.80±0.04
β-catenin	0.91±0.01	0.76±0.0
Akt1/2/3(S473)	1.03±0.08	0.68±0.02
Akt1/2/3(T308)	1.01±0.15	0.80±0.05
c-Jun(S63)	0.87±0.09	0.91±0.13
p53(S392)	0.92±0.01	0.68±0.09
p53(S46)	0.84±0.01	0.79±0.02
p70 S6 kinase(T389)	0.89±0.03	0.87±0.07
p70 S6 kinase(T421/S424)	0.87±0.02	0.94±0.02
PRAS40(T246)	0.70±0.02	0.53±0.01
Pyk2(Y402)	0.69±0.07	0.72±0.04
RSK1/2(S221/S227)	0.92±0.01	0.85±0.05
RSK1/2/3(S380/S386/S377)	1.01±0.01	0.89±0.01
STAT1(Y701)	0.83±0.02	0.72±0.04
STAT3(S727)	0.95±0.01	0.95±0.01
STAT3(Y705)	0.92±0.10	0.67±0.01
STAT6(Y641)	0.95±0.04	0.84±0.01
HSP60	0.95±0.02	0.54±0.03

cyanidins and tannins). These compounds play an important role in plant defense against UV radiation, serve as a deterrent to herbivores and also act as signaling molecules in plant growth processes. Phenolic compounds have been studied in dietary sources, due to their protective effects against inflammation, allergy, cancer, cardiovascular diseases, and neurodegenerative diseases (18,19). The TPC of *A. pseudojaponicum* is 1.03 ± 0.06 mg catechin/g dry *A. pseudojaponicum*. The TPC of *A. pseudojaponi* seems to confer on the antioxidant properties (Figure 1).

Epidermal keratinocytes present in the basal layer, undergo a differentiation process called the epidermal turnover and migrate toward the stratum corneum. Eventually, they are exfoliated from the epidermis. The expression of undifferentiated markers such as *KRT5*/*14* decreases, and the expression of differentiation markers such as *FLG*, *LOR*, *KRT1*, and *KRT10* increases as keratinocytes in the basal layer differentiate into the spinous layer, the granular layer, and finally the stratum corneum in a sequential manner (12). Therefore, *KRT5/14* are used as markers for keratinocytes present in the basal layer, and *FLG*, *LOR*, and *KRT1/10* are used as markers for differentiated keratinocytes present in the spinous and granular layers. UVA induces hyperdifferentiation of epidermal keratinocytes, and differentiation markers such as *FLR*, *LOR*, and *KRT1*/*10* are overexpressed. The expression of differentiation markers was increased by UVA light, but the *A. pseudojaponi* extract had the effect of reducing the expression of differentiation markers (Figure 5).

A phospho-kinase array was used to screen for intracel-

lular signaling proteins that were increased or decreased by *A. pseudojaponicum* extract treatment in NHEKs compared with that of the UVA-treated group. This technique is commonly used to analyze intracellular signaling proteins in keratinocytes treated with ultraviolet rays as demonstrated in previous studies (20–25). UVA causes DNA damage in organisms (1,3). Damaged DNA can activate ataxia-telangiectasia-mutated (ATM) and ataxia-telangiectasia and Rad3-related (ATR) kinases. The downstream signal of ATM/ATR, p38 MAPK signal, is involved in DNA damage. ATM is known to activate CHK2, which halts the cell cycle (26–29). Figures 1–5 show the anti-aging effects of *A. pseudojaponicum* extract on NHEKs. The extract reduces the damage to keratinocytes, which could lead to a decrease in p-CHK2. This decrease in p-CHK2 is assumed to restore the normal cell cycle from UVA damage. Many studies on CHK2 related to the cell cycle have been reported, but there is no study on the differentiation or moisturizing of keratinocytes. Further studies about the effects of CHK2 on moisturizing effects and epidermal differentiation are needed.

p53 is a protein that has been extensively studied in relation to apoptosis in the past few decades (30–32). Previous studies have shown that p53 expression is increased in human keratinocytes exposed to UVA (33,34). Upon UV exposure, the p53 protein rapidly increases in the epidermis, and serine 9 and serine 15 residues are phosphorylated sequentially. This phosphorylation of p53 induces DNA repair, cell cycle inhibition, and apoptosis of damaged keratinocytes, ultimately leading to tumor suppression (32). Additionally, phosphorylation of p53 has been found to promote the differentiation of the human keratinocyte cell line, HaCaT (35). The phosphorylation of p53 increased by about 20% due to UV irradiation but decreased by about 67% following treatment with *A. pseudojaponicum* extract compared with the UV-treated group (Figure 6C). This suggests that *A. pseudojaponicum* extract has the effect of suppressing the phosphorylation of the p53 protein by pre-emptively preventing the adverse effects of UV rays on NHEKs. The decrease in p-p53 due to *A. pseudojaponicum* extract treatment appears to suppress the excessive differentiation of keratinocytes (Fig. 5 and 6). The phospho-kinase array experiment confirmed that the phosphorylation of CHK2 and p53, which was increased by UV light, was effectively reduced by treatment with *A. pseudojaponicum* extract.

These findings suggest that *A. pseudojaponicum* extract has a potential to improve skin aging and may be utilized in functional cosmetics, functional foods, and pharmaceuticals in the future. The total phenolic contents assay confirms the presence of phenolic compounds in the *A. pseudojaponicum* extract, but further research will be required to determine which bioactive compounds are present in the *A. pseudojaponicum* extract.

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Interest conflict
The author declares that he has no competing interests.

Author contributions
Conceptualization, methodology, experiments, writing-review and editing; JHS.

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