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"Anti-wrinkle Effects of Miquelianin on Human Dermal Fibroblasts"

Minhui Jeon [1,2], Jiyeon Lee[1], Hyeonsun Yang[1], Jun-Hwan Jang[1], Sang-Bae Han[3] ,Jun-Tae Bae[1]

[1] J2KBIO; [2] Cosmetic Industry, Chungbuk National University Graduate school; [3]Pharmacy, Chungbuk National University College of Pharmacy

1. Introduction

Skin aging is a progressive biological process influenced by both intrinsic factors, such as hormonal changes, and extrinsic factors, notably ultraviolet (UV) radiation [1]. Among the external contributors, UVA—characterized by its longer wavelength—penetrates deeply into the dermis and induces complex molecular alterations that result in the breakdown of extracellular matrix (ECM) components. This degradation, primarily mediated by enzymes such as matrix metalloproteinases (MMPs), leads to visible signs of aging , such as the loss of skin elasticity, wrinkles, and sagging [2].

To counteract these signs, the cosmetic and dermatological industries have turned their attention toward natural compounds with potential anti-wrinkle properties. Plant extracts, in particular, are known to be effective in modulating oxidative stress and inflammatory responses induced by UV radiation [3]. Especially, *Nelumbo nucifera* has garnered considerable scientific interest due to its broad spectrum of biological activities, including antioxidant and skin-lightening effects [4, 5].

Within the phytochemical constituents of *Nelumbo nucifera*, miquelianin (Quercetin-3-O-glucuronide) has emerged as a compound of particular interest. Structurally, it is a glucuronide metabolite of quercetin, and has been reported to exhibit not only radical-scavenging activity but also anti-inflammatory and skin-lightening effects. In addition, it has been shown to contribute to moisture retention and enhancement of the skin barrier, thereby suggesting a positive impact on overall skin health. [6, 7].

However, the anti-wrinkle effects of miquelianin in UVA-induced photoaging models have not yet been fully explored. Wrinkle formation is known to result from the overexpression of MMPs

following UV exposure, which leads to the degradation of dermal structural components [2]. Therefore, investigating the molecular mechanisms by which miquelianin regulates MMP-1 expression, promotes collagen synthesis, and inhibits elastase activity holds both scientific and industrial significance.

Given the increasing demand for naturally derived, safe, and multifunctional active ingredients in cosmetic industry, there is a need to investigate miquelianin's specific effects on molecular pathways related to wrinkle formation. In this study, we assess its potential utility as a novel anti-wrinkle agent by evaluating its regulatory impact on ECM-related markers, specifically MMP-1, COL1A1, and elastase activity, in UVA-irradiated human dermal fibroblasts.

2. Materials and Methods

2. 1. Preparation of *Nelumbo nucifera* L. extract

Nelumbo nucifera L. was purchased from Myeongdang Yeonhyang (Republic of Korea). The dried leaves were extracted in 70% ethanol at 55°C for 72 h, filtered through a 150-mesh sieve, and dried in a rotary vacuum evaporator.

2. 2. High-performance liquid chromatography (HPLC) analysis

For high-performance liquid chromatography (HPLC), Alliance Waters e2695 (Waters, USA) was used. The column was Capcellpak C18 column (Osaka soda Co., Japan) and the standard miquelianin used for principal component analysis was purchased from ChemFaces (China) and used (Figure 1).

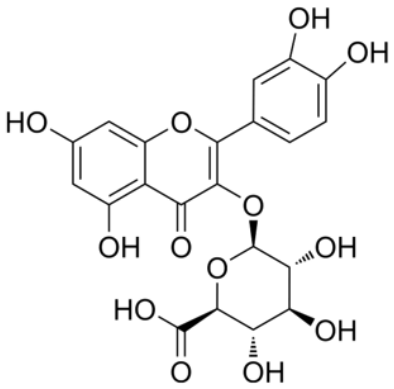

Figure 1. Chemical structure of miquelianin

HPLC analysis was performed to confirm the content of miquelianin, a marker component of *Nelumbo nucifera* L. After accurately weighing miquelianin, *Nelumbo nucifera* L. was dissolved

in 100% (v/v) methanol and filtered through a 0.45 μm PVDF membrane filter before use. Conditions were carried out as in Table 1.

Table 1. Analysis condition of HPLC

HPLC	Waters e2695 / 2998 UVD			
Column	Capcellpak C18 UG 120 5 μm, 4.6 × 250 mm			
Solvent	A : ACN, B : 0.1% Phosphoric acid in D.W.			
Gradient condition	Time	Flow rate	A%	B%
	0	1.0 mL/min	5	95
	5 min	1.0 mL/min	25	75
	15 min	1.0 mL/min	25	75
	25 min	1.0 mL/min	50	50
	28 min	1.0 mL/min	60	40
	33 min	1.0 mL/min	70	30
	40 min	1.0 mL/min	5	95
	50 min	1.0 mL/min	5	95
Detection	UV 334 nm			
Temperature	35 °C			
Injection volume	20 μL			

2. 3. Cell culture

Human dermal fibroblasts (HDF cells) (Cell Engineering For Origin, Republic of Korea) were maintained in Dulbecco's Modified Eagle's Medium / Nutrient Mixture F-12 (DMEM/F-12) 1:1 Mixture (Welgene, Republic of Korea) supplemented with 10% fetal bovine serum (Corning, USA) and 1% penicillin and streptomycin (Hyclone, USA). It was cultured in a 37°C incubator supplied with 5% CO_2, and subculture was performed once every 2 days.

2. 4. Cell viability assay

HDF cells (1.0×10^4/well) were seeded in 96-well plates (Falcon, USA) and incubated overnight at 37°C with 5% CO_2. Serial dilutions of miquelianin were added (20 μL/well), and after 24 h, 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added and incubated for 5 h. The supernatant was removed, and formazan crystals were dissolved in DMSO (SAMCHUN, Republic of Korea). Absorbance was measured at 590 nm using a microplate reader (SpectraMax ABS Plus, Molecular Devices, USA).

2. 5. Quantitative real-time PCR (qRT-PCR)

HDF cells (2.0 × 10⁵/well) were seeded in 6-well plates (Falcon, USA) and incubated for 24 h. After stabilization, the medium was replaced with serum-free DMEM/F12. After 6 h, miquelianin was added and incubated for 2 h, followed by UVA irradiation (10 J/cm²) (Analytik Jena, Germany). Cells were incubated for an additional 48 h, and total RNA was extracted using NucleoZOL (MACHEREY-NAGEL, Germany). cDNA synthesis was performed using the HiSenScript™ RH(-) RT PreMix kit (iNtRON Biotechnology, Republic of Korea) and a thermal cycler (GeneExplorer, BIOER, China). qRT-PCR was conducted on a QuantStudio 3 system (Thermo Fisher Scientific, USA) with Real-Time PCR Master Mix (Biofact, Republic of Korea). Cycling conditions were 95°C for 15 min, followed by 50 cycles of 95°C for 20 s and 60°C for 40 s. Primer sequences are listed in Table 2.

Table 2. Primer sequence

Gene		Primer sequence
MMP-1	Forward	5′- ATGAAGCAGCCCAGATGTGGAG -3′
	Reverse	5′- TGGTCCACATCTGCTCTTGGCA -3′
COL1A1	Forward	5′- GATTCCCTGGACCTAAAGGTGC -3′
	Reverse	5′- AGCCTCTCCATCTTTGCCAGCA -3′
GAPDH	Forward	5′- GGAGCGAGATCCCTCCAAAAT -3′
	Reverse	5′- GGCTGTTGTCATACTTCTCATGG -3′

2. 6. Western blotting

HDF cells (2.0 × 10⁵/well) were seeded in 6-well plates and incubated for 24 h. After stabilization, the medium was replaced with serum-free DMEM/F12. After 6 h, miquelianin was added and incubated for 2 h, followed by UVA irradiation (10 J/cm²) (Analytik Jena, Germany). Cells were then incubated for 48 h, lysed, and proteins were extracted using RIPA buffer (iNtRON Biotechnology, Republic of Korea). Proteins were separated by SDS-PAGE, transferred to PVDF membranes (Bio-Rad, USA), and immunoblotted with primary antibodies against MMP-1, COL1A1 and GAPDH (Cell Signaling Technology, USA). Detection was performed using ChemiDoc™ XRS+ (Bio-Rad, USA), with GAPDH as the internal control.

2. 7. Elastase activity inhibition assay

HDF cell pellets were resuspended in 1% Triton X-100 (Sigma-Aldrich, USA) and subjected to three freeze–thaw cycles. The lysate was centrifuged at 12,000 rpm for 5 min at 4°C, and the supernatant was collected as the fibroblast elastase enzyme solution. Protein

concentration was determined by the Bradford assay (Bio-Rad, USA), and 80 µg of protein per well was transferred to a 96-well plate. Tris-HCl buffer (GoldBio, USA) was added to adjust the final volume to 88 µL, followed by 10 µL of diluted miquelianin and 2 µL of STANA (Sigma-Aldrich, USA). The plate was incubated at 37°C for 90 min, and absorbance was measured at 405 nm. Elastase activity was calculated relative to the control group.

2. 8. Statistical analysis

The data are presented as the mean ± standard deviation. $p < 0.05$ was considered statistically significant compared with the samples from the non-treated groups (control).

3. Result

3. 1. HPLC analysis results

Miquelianin present in *Nelumbo nucifera* L. extract was identified using HPLC analysis. HPLC analysis revealed that the retention time of miquelianin was 9.637 min, matching that of the corresponding standard compound, with a quantified content of 12.093% (Figure 2).

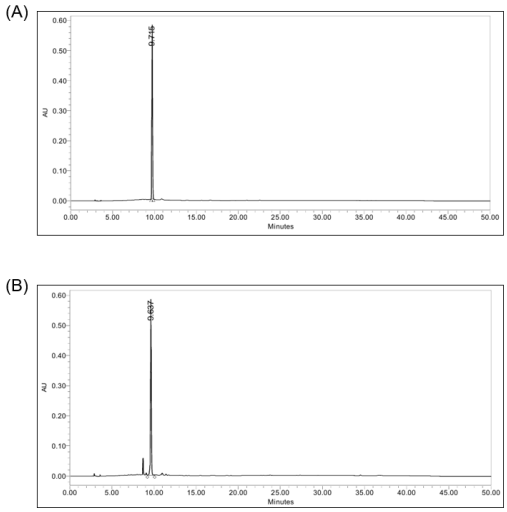

Figure 2. High-performance liquid chromatography (HPLC) chromatograms of standard of miquelianin (A) and sample of *Nelumbo nucifera* L. extract (B). AU indicates the absorbance unit

3. 2. Effect of miquelianin at various concentrations on the viability of HDF cells

Treatment with miquelianin at 25, 50, and 100 µg/mL exhibited no significant cytotoxicity in HDF cells, with cell viability consistently exceeding 95% at all tested concentrations.

These results suggest that miquelianin is biocompatible with human dermal fibroblasts within the tested concentration range (Figure 3).

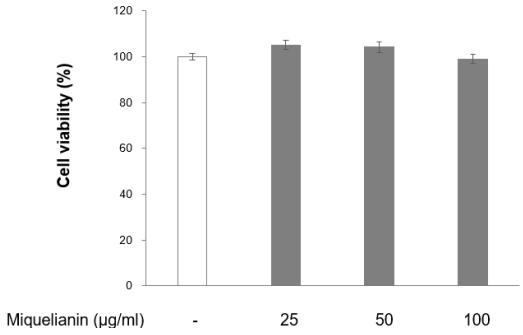

Figure 3. Effect of miquelianin on cell viability in HDF cells. Cell viability was measured by the MTT assay after treatment with various concentrations of miquelianin (25, 50, and 100 μg/mL) for 24 h.

3. 3. Miquelianin suppressed UVA–induced MMP-1 expression and increased COL1A1 expression in HDF cells

To assess the protective role of miquelianin against UVA-induced wrinkle formation, HDF cells, except for the untreated group, were irradiated with 10 J/cm² of UVA. Miquelianin treatment significantly reduced UVA-induced MMP-1 mRNA expression in a concentration-dependent manner, with an inhibition of 88.50% at 100 μg/mL (Figure 4A). COL1A1 mRNA expression was significantly upregulated by 59.16% and 88.98% at 50 and 100 μg/mL, respectively, suggesting enhanced collagen synthesis at higher concentrations (Figure 4B).

These results demonstrate that miquelianin alleviates UVA-induced wrinkle formation by downregulating MMP-1 and promoting COL1A1 expression, thereby contributing to the preservation of dermal structure.

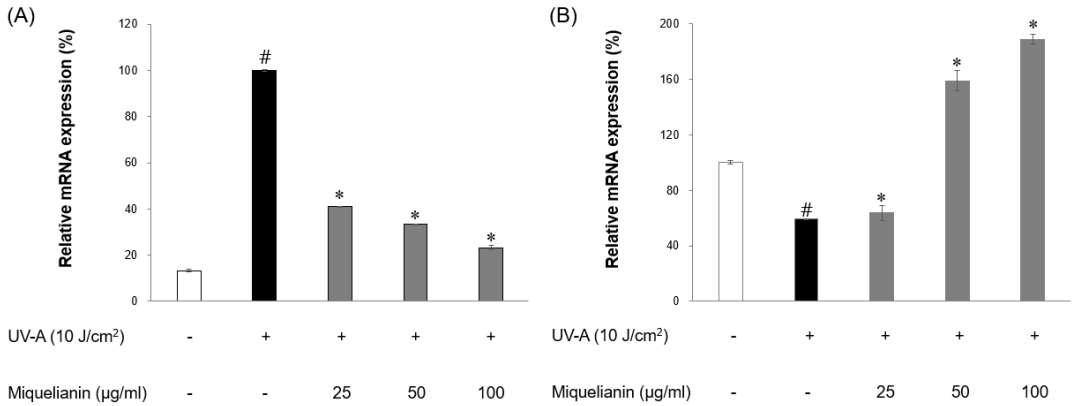

Figure 4. Effect of miquelianin on UVA-induced MMP-1 and COL1A1 expression in HDF cells. Cells were pretreated with miquelianin for 2 h prior to UVA (10 J/cm²) irradiation. Relative mRNA expression levels of MMP-1 (A) and COL1A1 (B) were analyzed by qRT-PCR.

#, Significant difference between the untreated group and the UVA-irradiated control group. ∗, Significant difference ($p < 0.05$) compared to the UVA-irradiated control.

3. 4. Miquelianin reduced UVA–induced MMP-1 protein expression and restored COL1A1 protein expression in HDF cells

To confirm whether the mRNA expression changes were reflected at the protein level, western blot analysis was performed.

After UVA irradiation (10 J/cm²), MMP-1 protein expression was significantly elevated compared to the untreated group. However, treatment with miquelianin at concentrations of 25, 50, and 100 μg/mL led to a concentration-dependent reduction in MMP-1 protein levels, with maximal suppression observed at 100 μg/mL (Figure 5A, B).

In contrast, COL1A1 protein expression was reduced by UVA exposure. Miquelianin treatment resulted in a concentration-dependent restoration in COL1A1 protein levels, with marked upregulation at 100 μg/mL (Figure 5C, D).

These results indicate that miquelianin exerts anti-aging effects at both the transcriptional and protein levels by suppressing MMP-1 and enhancing COL1A1 expression in human dermal fibroblasts.

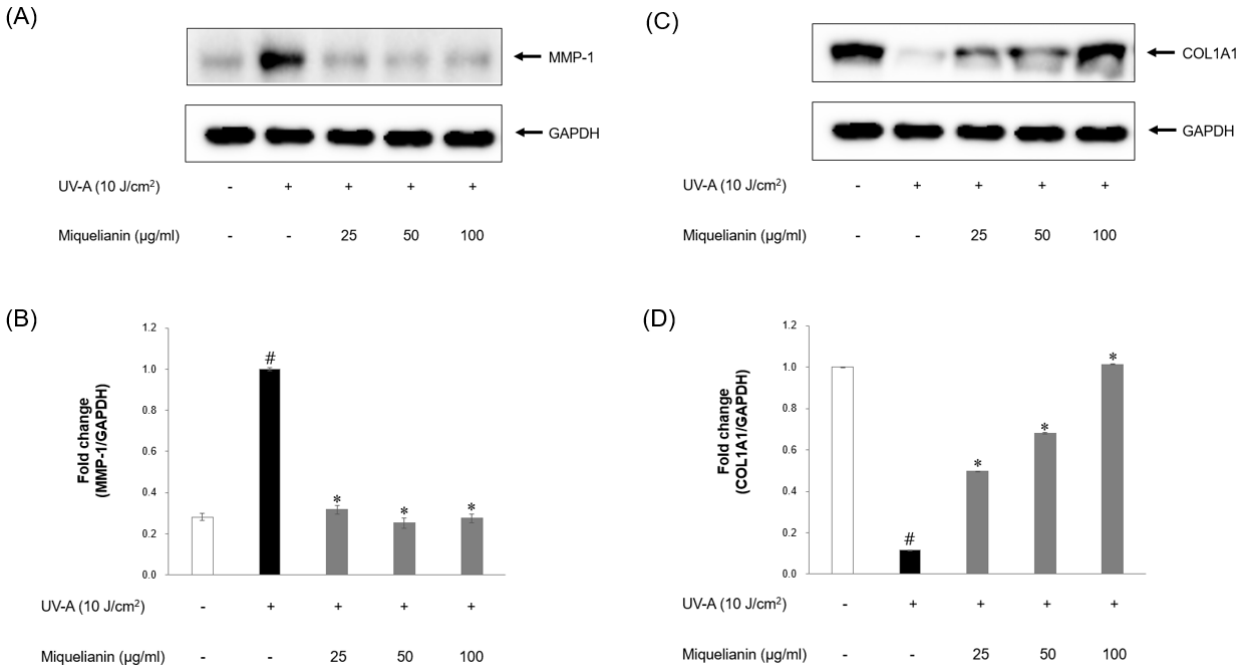

Figure 5. Effect of miquelianin on UVA-induced MMP-1 and COL1A1 protein expression in HDF cells. HDF cells were pretreated with miquelianin for 2 h and then irradiated with UVA (10 J/cm²). Protein expression levels of MMP-1 (A, B) and COL1A1 (C, D) were determined by western blot analysis. GAPDH was used as an internal control. Densitometric analysis is shown in (B) and (D) as fold change relative to GAPDH. #, Significant difference between the

untreated group and the UVA-irradiated control group. ∗, Significant difference ($p < 0.05$) compared to the UVA-irradiated control.

3. 5. Miquelianin enhanced elastase inhibition in HDF cells

Elastin, a major ECM protein, maintains skin elasticity, while increased elastase activity from fibroblasts contributes to wrinkle formation by degrading elastic fibers [8].

In our study, treatment with miquelianin resulted in a significant, concentration-dependent inhibition of elastase activity in HDF cells. Elastase activity was reduced by 10.29%, 15.40%, and 25.51% at 25, 50, and 100 μg/mL, respectively, compared to the control group.

These results indicate that miquelianin effectively suppresses elastase activity, thereby potentially mitigating elastin degradation and contributing to the preservation of skin elasticity (Figure 6).

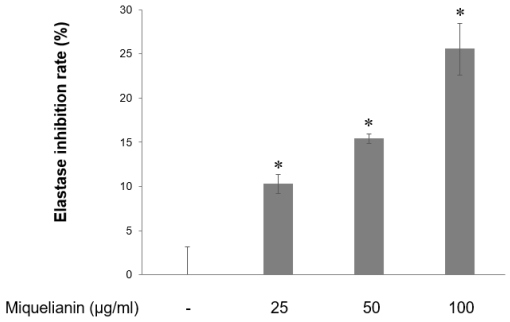

Figure 6. Elastase inhibition rate of miquelianin. HDF cells were treated with miquelianin at various concentrations for 90 min. ∗, Significant difference ($p < 0.05$) compared to the control.

4. Discussion

Wrinkle formation is one of the main consequences of UV-induced skin aging, a process characterized by the degradation of ECM components. UVA, in particular, penetrates deeply into the dermis and induces oxidative stress, activating multiple signaling pathways that elevate the expression of ECM-degrading enzymes and ultimately accelerate wrinkle formation [2].

In this study, we elucidated that the natural flavonoid compound miquelianin exhibits anti-wrinkle effects by inhibiting UVA-induced aging responses. Specifically, miquelianin suppressed the expression of MMP-1, a key enzyme responsible for collagen degradation, and restored COL1A1 expression, thereby supporting collagen synthesis. Additionally, it inhibited elastase activity, helping preserve elastic fibers and maintain skin elasticity. These findings indicate that miquelianin exerts protective effects via multiple mechanisms to prevent UVA-induced wrinkle formation.

MMP-1 expression is known to be regulated by signaling pathways such as MAPK (p38, JNK, ERK), NF-κB, and AP-1 (c-Jun, c-Fos), all of which are activated by UV exposure and associated with wrinkle formation [9]. Although this study did not directly investigate these pathways, additional studies are warranted to determine whether miquelianin modulates critical steps in these signaling cascades, such as the phosphorylation of signaling proteins or the nuclear translocation of transcription factors.

Miquelianin demonstrates strong potential as a functional ingredient that targets UVA-induced molecular changes associated with wrinkle formation. Its ability to regulate multiple factors—MMP-1, COL1A1, and elastase—makes it particularly suitable for use in multi-action anti-aging products.

HPLC analysis confirmed the presence of miquelianin in the 70% ethanol extract of *Nelumbo nucifera* L., as evidenced by matching retention time and UV absorbance spectra (Figure 2). This suggests that miquelianin exists as an active compound in *Nelumbo nucifera* L. extract, highlighting the potential of *Nelumbo nucifera* as a natural source of anti-wrinkle bioactives.

Based on the presence of miquelianin in *Nelumbo nucifera* L. extract, strategies to enhance its concentration such as optimized extraction methods could further increase its anti-wrinkle efficacy. Enhancing the miquelianin content in *Nelumbo nucifera* L. extract may help improve its bioactivity, thereby contributing to the efficacy of the extract as a functional ingredient. This approach supports the potential of *Nelumbo nucifera* as a natural source of bioactive compounds for cosmetic applications.

5. Conclusion

In this study, we investigated the effects of miquelianin, an active compound found in *Nelumbo nucifera* L. extract, on UVA-induced wrinkle formation. Miquelianin suppressed the expression of MMP-1 and restored the reduced expression of COL1A1 in UVA-irradiated human dermal fibroblasts (HDF cells). In addition, it inhibited elastase activity, thereby helping to prevent the degradation of elastic fibers and maintain skin firmness. Taken together, these findings suggest that miquelianin is a promising cosmetic ingredient for alleviating wrinkle formation induced by ultraviolet exposure.

Reference

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