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"The Potential of Thuja orientalis L. *Major Fraction (TOMF) Containing Quercitrin as a Cosmetic Agent for Wrinkle Improvement"*

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1. Introduction

Thuja orientalis L. is an evergreen shrub commonly cultivated in Korea as a hedge or ornamental tree and is also distributed across various regions of China. Its reported pharmacological properties include the treatment of bleeding disorders and chronic bronchitis [1-2].

Previous studies on the phytochemical components of *Thuja orientalis* L. have identified several active compounds, such as rutin, quercetin, quercitrin, and amentoflavone. Among these, quercitrin—a type of flavonoid—is identified as a major active component with demonstrated anti-wrinkle, anti-inflammatory, and reactive oxygen species (ROS) scavenging properties.

Quercitrin is a flavonoid, structurally diverse and widely distributed of natural compounds, and among the most abundant natural phenolics. Flavonoids are well-known for their potent antioxidant activity, enabling them to scavenge ROS, and they also exhibit anticancer, hypolipidemic, anti-aging, and anti-inflammatory properties [3].

Quercitrin has been reported to exert protective effects against bacterial infections, allergic responses, and apoptosis induced by H_2O_2 and ultraviolet(UV) exposure [4]. Furthermore, quercitrin was isolated from the Cosmos caudatus Kunth fraction (CC), which was obtained through extraction and fractionation of Cosmos caudatus Kunth, and research has demonstrated its effectiveness anti-wrinkle formation. Quercitrin has also been shown to inhibit collagenase activity, downregulate MMP-1 and MMP-3 expression at both mRNA and protein levels, and suppress TNF-α-induced activation of the NF-κB signaling pathway [5].

Recent studies have suggested that this inhibitory activity may contribute to anti-aging effects. Of various factors associated with skin aging, photoaging is considered one of the most

prominent. Prolonged exposure to UV induces immune system remodeling and accelerates skin aging, resulting in a phenotype that closely resembles chronological aging [6]. In particular, UVB radiation (280–320 nm) has been reported to cause marked structural alterations in human skin, including epidermal thinning due to atrophy of the basal and spinous layers, a decrease in fibroblast population, and the degradation of extracellular matrix (ECM) components in the dermis.

UVB radiation induces oxidative stress in the skin, leading to sunburn, photoaging, and even skin carcinogenesis. It is widely accepted that UV-induced skin damage is primarily caused by the excessive generation of ROS. In addition, ultraviolet radiation (UVR) can cause structural damage to DNA and proteins in skin cells, particularly in the epidermis. As a result, clinical manifestations of photoaging include increased wrinkle formation, telangiectasia, epidermal atrophy, enhanced melanogenesis, irregular pigmentation, and sunburn [7].

In addition, collagen degradation is a hallmark of photoaging. Matrix metalloproteinase-1 (MMP-1), the primary enzyme responsible for type I collagen degradation, is strongly induced by exposure to sunlight. A significant increase in MMP-1 expression is one of the major contributors to photoaging. In healthy young skin, MMP-1 levels are minimal; however, with age, its mRNA, protein, and enzymatic activity become constitutively elevated. Furthermore, transient upregulation occurs in response to UV exposure or skin injury. MMP-1 is secreted as a catalytically inactive proenzyme that is later activated under stress conditions [8-9]. Therefore, suppression of MMP-1 expression is considered an important mechanism in the prevention of skin aging.

Accordingly, this study provides valuable insights into the mechanisms of skin photoaging and the physiological functions of quercitrin, thereby contributing to the understanding of skin health and anti-aging strategies.

We aimed to focus our research on active compounds isolated from plants. Specifically, we effectively extracted *Thuja orientalis* L. using microbubbles, followed by fractionation, and obtained a high-content of quercitrin-containing *Thuja orientalis L.* Major Fraction (TOMF) using open column chromatography**.**

Microbubble-assisted extraction, a recently developed technique, uses micron-sized gas bubbles to enhance solvent diffusion and mechanically disrupt plant cell walls. Studies have shown that the collapse of these microbubbles generates oxidative species, including hydroxyl radicals, which further facilitate the release of intracellular compounds. This technology has emerged as a promising method for increasing the yield and efficacy of plant-derived compounds, particularly flavonoids, by improving both their extraction and preservation [10].

Additionally, we investigated the efficacy of the TOMF obtained through open column chromatography. Microbubble extracts of Thuja orientalis L. were analyzed using high-

performance liquid chromatography (HPLC) combined with thin-layer chromatography (TLC), followed by the isolation of quercitrin.

In this study, we compared the TOMF obtained by microbubble extraction with the *Thuja orientalis* L. General Fractions (TOGF) extracted without microbubbles to evaluate the differences in efficacy and marker compound content depending on the extraction method.

We showed that the microbubble-extracted fraction exhibited a greater inhibitory effect on MMP-1 expression compared to the non-microbubble-extracted fraction. Based on these findings, we aimed to develop an effective cosmetic composition with potential applications in anti-wrinkle treatment.

2. Materials and Methods

2. 1. Preparation of ethyl acetate fraction from *Thuja orientalis* L. Major Fraction (TOMF) and *Thuja orientalis* L. General Fraction (TOGF)

The *Thuja orientalis* L. used in this experiment was purchased from Dongui herb market (Republic of Korea). As a hybrid microbubble generator for microbubble extraction, O2B-750S (O2Bubble Co., Ltd., Republic of Korea) was used. Rotary vacuum concentrator was purchased (Dooyoung, Republic of Korea).

Thuja orientalis L. (12 kg) was extracted in 70% (w/w) ethanol (120 kg) at 55 °C for 5 h. For TOMF, microbubble-assisted extraction was performed using a hybrid microbubble generator (O2B-750S, O2Bubble Co., Ltd., Republic of Korea), while TOGF was prepared under the same extraction conditions without microbubbles. The extracts were filtered through a 5 μm filter and concentrated under reduced pressure to yield extracts (15.35 kg for TOMF and 15.4 kg for TOGF, respectively). Each extract was subjected to solvent fractionation with dichloromethane (99.5%, SAMCHUN, Republic of Korea) and ethyl acetate (99.5%, SAMCHUN, Republic of Korea). The ethyl acetate fractions were then purified by open column chromatography using a column (CNC Co., Ltd., Republic of Korea) packed with silica gel (SilicaFlash® P60, SiliCycle Inc., Canada). The eluates were concentrated using a rotary vacuum concentrator to obtain TOMF and TOGF, respectively.

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 quercitrin used for principal component analysis was purchased from Sigma-aldrich (USA) and used (Figure 1).

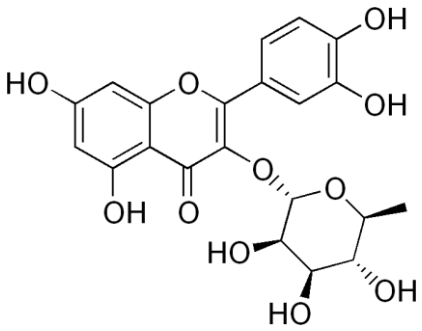

Figure 1. Chemical structure of quercitrin

HPLC analysis was performed to confirm the content of quercitrin, a marker component of TOMF and TOGF. After accurately weighing quercitrin, TOMF and TOGF were 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 : 0.1% Formic acid in D.W. , B : ACN			
Gradient condition	Time	Flow rate	A %	B %
	0	1.0 mL/min	90	10
	5 min	1.0 mL/min	84	16
	10 min	1.0 mL/min	80	20
	15 min	1.0 mL/min	80	20
	20 min	1.0 mL/min	90	10
	30 min	1.0 mL/min	90	10
Detection	UV 360 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 quercitrin, TOMF, TOGF 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 (2.0×10^5 cells/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, the sample was added and incubated for 2 h, followed by UVB irradiation (25 mJ/cm²) (Analytik Jena, Germany). Cells were incubated for an additional 24 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 sequences

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

2. 6. 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

As a result of HPLC analysis to confirm the content of quercitrin in TOMF and TOGF, it was confirmed that quercitrin (RT, 23.447 min and 23.456 min) was an indicator substance, and its content was 31.69% and 17.55% (Figure 2B and 2C).

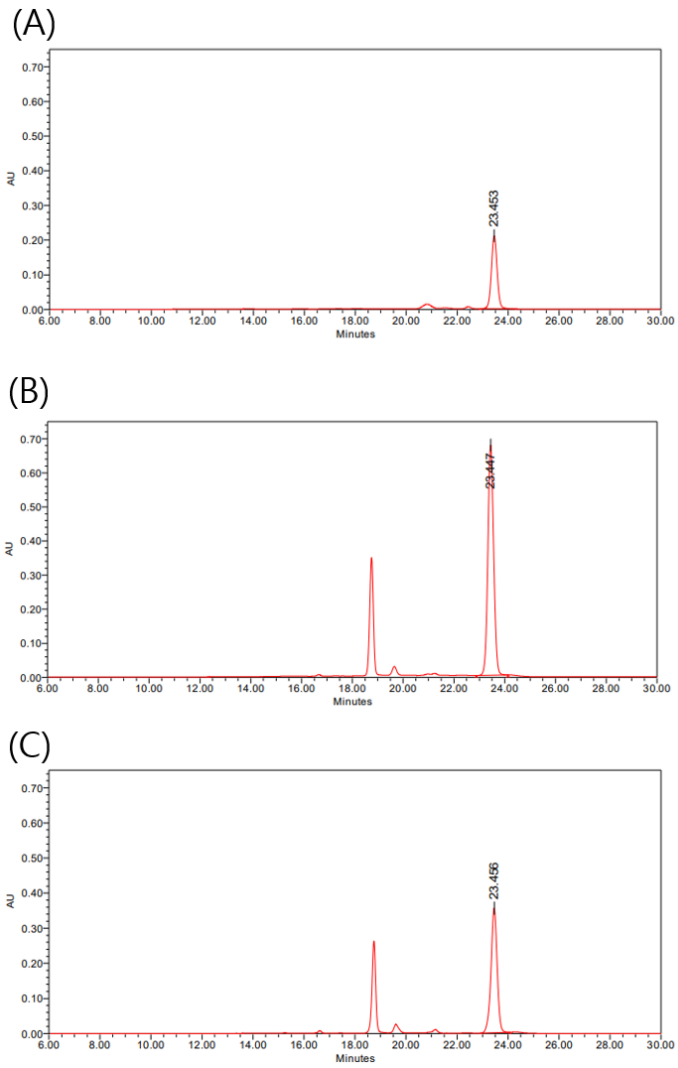

Figure 2. HPLC chromatograms of standard of quercitirn (A) and sample of TOMF (B) and TOGF (C). AU indicates the absorbance unit

3.2. Effect of quercitrin at various concentrations on the viability of HDF cells

Treatment with quercitrin,TOMF,TOGF at tested concentration exhibited no significant cytotoxicity in HDF cells, with cell viability consistently exceeding 95% at all tested concentrations. These results suggest that quercitrin is biocompatible with human dermal fibroblasts within the tested concentration range (Figure. 3).

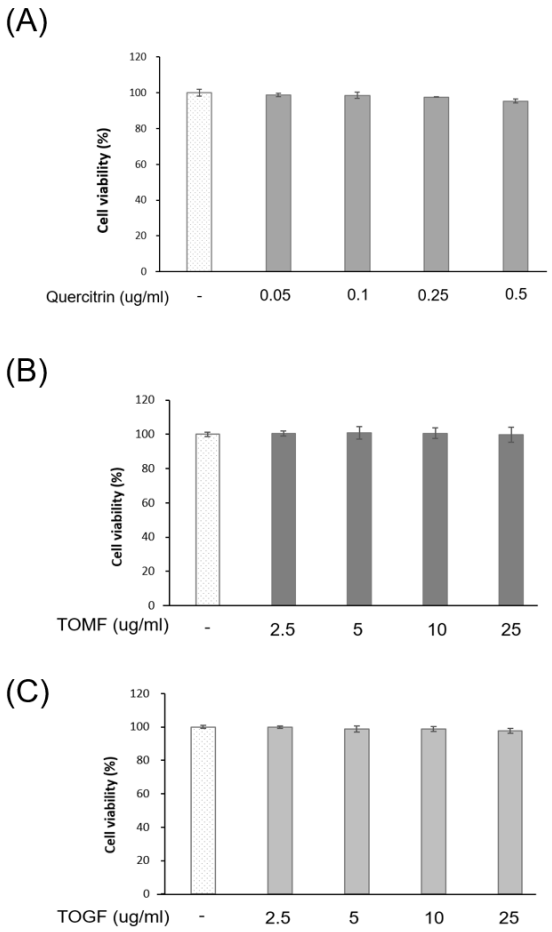

Figure 3. Effect of quercitrin, TOMF, TOGF on cell viability in HDF cells. Cell viability of quercitirn (A) and sample of TOMF (B) and TOGF (C). Cell viability was measured by the MTT assay after treatment with various concentrations of quercitrin, TOMF, TOFG for 24 h.

3. 3. TOMF and TOFG suppressed UVB–induced MMP-1 expression in HDF cells

Real-time qPCR was performed to measure the inhibitory effects on the mRNA expression of MMP-1. The experimental results obtained through real-time qPCR were normalized showed that TOMF and TOGF, which was tested at concentrations of to 25 μg/mL, also showed inhibition of the mRNA expression of the MMP-1, with significant effects observed at all concentrations (Figure 4B). Quercitrin, which was tested at concentrations of 0.05 to 0.5 μg/mL, also showed inhibition of the mRNA expression of the MMP-1, with significant effects observed at all concentrations (Figure 4A).

In conclusion, TOMF inhibited the MMP-1 mRNA expression by 37.81%, and TOGF inhibited it by 20.88%. These results confirm the anti-wrinkle effects of TOMF, TOGF, and quercitrin in UVB-stimulated HDF cells, as all samples significantly suppressed MMP-1 mRNA expression. TOMF, which contained a higher quercitrin content (31.69%) as determined by HPLC, exhibited greater inhibitory activity (37.81%) compared to TOGF (17.55%, 20.88%).

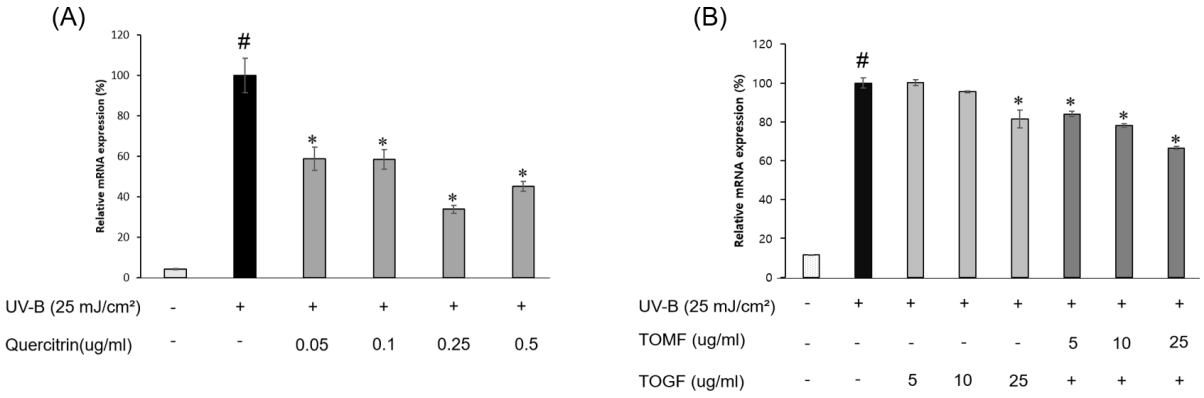

Figure 4. Effect of quercitrin and TOMF, TOGF on UVB-induced MMP-1 in HDF cells. Cells were pretreated with quercitrin for 2 hr prior to UVB (25 mJ/cm²) irradiation. Relative MMP-1 mRNA expression levels of quercitrin (A) and TOMF, TOGF(B) were analyzed by qRT-PCR. #, Significant difference between the untreated group and the UVB-irradiated control group. ∗, Significant difference ($p < 0.05$) compared to the UVB-irradiated control.

4. Discussion

This study demonstrated the efficacy of *Thuja orientalis* L. Major Fraction (TOMF), obtained via microbubble-assisted extraction, in suppressing UVB-induced expression of MMP-1 in human dermal fibroblasts (HDFs). Quercitrin, a bioactive flavonoid and the principal marker compound in *Thuja orientalis* L., was identified as the major constituent of TOMF, comprising 31.69% as determined by HPLC. Compared to the general fraction (TOGF) extracted without microbubble technology and contained 17.55% quercitrin, TOMF exhibited significantly greater inhibition of MMP-1 mRNA expression (37.81% vs. 20.88%, respectively). These findings suggest that microbubble-assisted extraction process not only improves the yield of functional flavonoids but also enhances their biological efficacy. This may be attributed to the enhanced disruption of plant cell walls and improved solubilization of intracellular components, facilitated by microbubble technology. Microbubbles have been reported to generate oxidative radicals such as hydroxyl radicals, which contribute to the breakdown of cellular barriers and promote the efficient release and preservation of bioactive compounds like flavonoids.

These results are consistent with previous studoes that have demonstrated the inhibitory effects of quercitrin in matrix metalloproteinase and its role in mitigating oxidative stress, our data reinforce its relevance in anti-photoaging mechanisms. Quercitrin's ability to suppress MMP-1—an enzyme responsible for collagen degradation—further supports its application as a functional ingredient in anti-wrinkle formulations.

5. Conclusion

In conclusion, our results suggest that quercitrin-enriched *Thuja orientalis* L. Major Fraction (TOMF), is a promising functional cosmetic ingredient for anti-wrinkle applications. The microbubble-assisted extraction not only increased the quercitrin content compared to conventional methods but also significantly enhanced its biological efficacy. TOMF showed marked inhibition of MMP-1 mRNA expression in UVB-stimulated human dermal fibroblasts, suggesting its potential to prevent photoaging by suppressing collagen degradation pathways. These findings provide a scientific rationale for the development of quercitrin-based anti-aging formulations and highlight the value of microbubble-assisted extraction as an effective approach to increasing the yield and efficacy of plant-derived cosmetic actives.

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