

Maple water (*Acer saccharum* sap), a traditional North American beverage: anti-inflammatory activity and potential functional relevance for skin health

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Abstract

Maple water (*Acer saccharum* sap) is a traditional North American sap-derived beverage consumed seasonally; however, its biological functions as a dietary resource remain insufficiently characterized. This study investigates the potential functional properties of maple water extract by assessing its anti-inflammatory activity and its relevance to skin-related cellular responses. In RAW264.7 macrophages, maple water extract significantly inhibited lipopolysaccharide (LPS)-induced nitric oxide production in a dose-dependent manner without inducing cytotoxicity. The treatment downregulated the mRNA expression of key pro-inflammatory mediators, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin (IL)-1 β , and IL-6, and suppressed nuclear factor-kappa B (NF- κ B) signaling. In UV-irradiated HaCaT keratinocytes, maple water extract modulated cellular responses by regulating matrix metalloproteinases (MMP-1 and MMP-2) and their tissue inhibitors (TIMP-1 and TIMP-2), indicating potential relevance to extracellular matrix regulation. Furthermore, maple water enhanced the expression of epidermal differentiation markers, including filaggrin, involucrin, and loricrin. Hydration-related responses were observed, including reduced hyaluronidase expression and increased hyaluronan synthase expression. These findings suggest that maple water, when considered as a traditional dietary beverage, may provide bioactive components that modulate inflammatory responses and support skin-related cellular functions.

Introduction

Maple water, the clear sap harvested from *Acer saccharum* during early spring, has long been traditionally consumed in North America as a natural seasonal beverage [1, 2]. The consumption of plant sap as a beverage is not unique to North America; similar practices have been documented in other regions, including birch sap consumption in Northern Europe and the use of *Acer mono* sap (gorosoe) in Korea, where these seasonal drinks have been traditionally valued for their refreshing properties and perceived health benefits [3]. In particular, historical records indicate that Indigenous peoples of North America consumed maple sap not only as a seasonal beverage but also utilized it in traditional practices associated with recovery and general well-being [4]. Historically appreciated for its refreshing taste and nutritional value, maple water has recently attracted scientific interest as a plant-derived resource with potential health-promoting properties [5, 6]. In recent years, growing consumer interest in natural and culturally rooted dietary resources has led to increased attention toward traditional botanical beverages as potential functional foods [7]. Beyond its mild sweetness, maple water contains a diverse spectrum of nutrients, including essential minerals such as potassium, calcium, magnesium, and zinc, as well as amino acids, organic acids, and polyphenolic compounds [1, 2]. Previous studies have identified numerous antioxidant constituents in maple-derived products [6, 8], and these materials have been associated with biological activities such as antioxidant, antidiabetic, and hepatoprotective effects [9–11]. Despite these findings, research on maple-based resources has largely focused on metabolic health and general antioxidant capacity [2], while other physiologically relevant functions remain insufficiently explored. These traditional uses suggest that plant sap beverages have historically been valued not only

for hydration but also for recovery and skin-related benefits, providing a cultural basis for exploring their biological functions in modern nutritional science.

Among these, the regulation of inflammation represents a key biological function closely linked to overall health and tissue homeostasis. Chronic or excessive inflammatory responses contribute not only to metabolic disorders but also to tissue dysfunction, including processes associated with skin physiology. In particular, inflammatory signaling pathways such as nuclear factor-kappa B (NF- κ B) play central roles in mediating immune responses and are widely recognized as targets for dietary bioactive compounds [12]. From a functional food perspective, evaluating the ability of traditional beverages to modulate inflammatory pathways provides important insight into their potential as dietary resources with broader physiological relevance.

Skin health is increasingly recognized as an important component of overall well-being and is regulated by interconnected biological processes, including inflammatory control, epidermal barrier integrity, extracellular matrix organization, and moisture homeostasis [13, 14]. Environmental stressors, particularly ultraviolet (UV) radiation and air pollutants, disrupt these processes by promoting inflammatory signaling, impairing keratinocyte differentiation, accelerating matrix degradation, and reducing hydration, ultimately contributing to premature skin aging [15, 16]. Accordingly, natural materials capable of modulating these pathways are receiving growing attention as potential functional dietary resources [17, 18]. Despite the phytochemical richness of maple water, its potential role in the regulation of skin physiology remains poorly understood. In particular, whether maple water can modulate key inflammatory signaling pathways such as nuclear factor-kappa B (NF- κ B) has not been systematically investigated, although suppression of inflammatory mediators in macrophages is recognized as a critical strategy for controlling immune activation [12]. Furthermore, evidence regarding its protective capacity against UV-induced skin damage and its influence on extracellular matrix stability and hydration-related mechanisms remains limited. Macrophages and keratinocytes were selected to represent complementary aspects of skin-related responses, as immune cell-derived inflammatory mediators are known to contribute to skin photodamage and barrier dysfunction. Therefore, evaluating both macrophage-mediated inflammatory regulation and keratinocyte responses provides an integrated perspective on its potential biological relevance to skin-related cellular processes. In this context, investigating the effects of maple water on inflammation and skin-related cellular processes may provide scientific support for its traditional use as a natural health-promoting beverage.

Therefore, the present study aimed to evaluate the functional properties of maple water as a traditionally consumed dietary beverage. Using RAW264.7 macrophages and HaCaT keratinocytes, inflammatory mediators, barrier-associated proteins, extracellular matrix regulators, and hydration-related factors were evaluated to establish an integrated understanding of its biological activity. Collectively, this study provides scientific evidence supporting the potential of maple water as a functional dietary resource with relevance to inflammation and skin-related cellular processes.

Materials and methods

Maple water sample

In this study, the term “maple water extract” refers to the ethyl acetate fraction prepared from commercial maple water unless otherwise specified. The maple water used in this study was supplied by Dongkug Industry Co., Ltd. (Seongnam, Republic of Korea). According to the supplier, the maple sap was collected from *Acer saccharum* trees in North America and subsequently imported into Korea as a raw material. Ethyl acetate (EtOAc; Samchun Chemical, Seoul, Republic of Korea) was purchased commercially and used as the extraction solvent. The maple water exhibited a soluble solid content of 2.5–3.0 °Brix and a pH ranging from 6.5 to 7.2, consistent with the typical physicochemical characteristics of fresh maple sap. To ensure batch consistency, these parameters were measured using a digital refractometer (Atago Co., Tokyo, Japan) and a calibrated pH meter (Mettler-Toledo, Columbus, OH, USA), respectively.

Extraction of ethyl acetate fraction from maple water

The filtered maple water (500 mL) was extracted with ethyl acetate (1:1 or 1:2, v/v). After phase separation, the organic layer was collected, and the extraction was repeated to maximize recovery. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure below 40°C. The concentrate was freeze-dried and stored for further analysis.

Determination of total polyphenol and flavonoid contents

Total polyphenol content (TPC) was measured using a modified Folin–Ciocalteu method [19]. After reaction with Folin–Ciocalteu reagent and sodium carbonate, samples were incubated in the dark for 30 min, and absorbance was measured at 750 nm using a microplate reader (INNO-M, LTeK, Republic of Korea). Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoid content (TFC) was determined using a modified method described by Zhishen et al. [20]. Samples were sequentially reacted with sodium nitrite, aluminum chloride, and sodium hydroxide, and absorbance was measured at 510 nm. Catechin served as the reference standard, and results were expressed as mg catechin equivalents (CE)/g extract.

DPPH and ABTS radical scavenging assays

Radical scavenging activities were evaluated using modified methods based on Kim et al. [21] for DPPH and van den Berg et al. [22] for ABTS assays. For the DPPH assay, a 200 µM solution of 2,2-diphenyl-1-picrylhydrazyl was prepared in ethanol. Samples were mixed with the DPPH solution in a 96-well plate and incubated in the dark for 30 min. Absorbance was measured at 520 nm. ABTS radicals were generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate in the dark. After incubation for 60 min with the sample, absorbance was recorded at 414 nm. Ascorbic acid was used as the positive control. Radical scavenging activity was calculated as:

Radical scavenging activity (%) = $(1 - A_{\text{sample}} / A_{\text{control}}) \times 100$

IC₅₀ values were determined from dose–response curves using nonlinear regression analysis.

Cell culture

RAW264.7 murine macrophages (ATCC, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin (PS), and 1.5 g/L sodium bicarbonate. Cells were maintained at 37°C in a humidified incubator with 5% CO₂. For experiments, cells were seeded in 6- or 12-well plates at a density of 1.0×10^5 cells/mL and treated upon reaching approximately 70–80% confluence. Lipopolysaccharide (LPS; Sigma-Aldrich, USA) was applied at a final concentration of 100 ng/mL to induce inflammation. The normal control received distilled water, while the control group was treated with LPS alone. Experimental groups were pretreated with maple water extract for 2 h prior to LPS stimulation. HaCaT human keratinocytes (Amorepacific Co., Republic of Korea) were cultured under identical conditions using DMEM supplemented with 10% FBS and 1% PS.

UV irradiation

For photoaging experiments, HaCaT cells were seeded in 6-well plates at 1.0×10^5 cells/well and incubated for 24 h. Cells were pretreated with maple water extract for 2 h, washed with phosphate-buffered saline (PBS), and exposed to UVB radiation (30 mJ/cm²) using a UVB lamp (312 nm; Vilber Lourmat, France). UVB intensity was calibrated using a UV radiometer prior to each experiment to ensure reproducibility. Following irradiation, PBS was replaced with fresh medium containing the sample, and cells were further incubated for 24 h prior to RNA extraction.

Cell viability assay

Cell viability was assessed using the MTT assay. RAW264.7 cells were seeded at 1.0×10^4 cells/mL in 96-well plates and incubated for 24 h before co-treatment with LPS (100 ng/mL) and maple water extract (0–400 µg/mL). HaCaT cells were seeded at 1.5×10^4 cells/well and treated with maple water extract (3.125–50 µg/mL). After incubation, cells were treated with MTT solution (0.5 mg/mL) for 1 h at 37°C. Formazan crystals were dissolved in DMSO, and absorbance was measured at 550 nm.

Measurement of nitric oxide production

RAW264.7 cells were pretreated with maple water extract (0–100 µg/mL) for 2 h and stimulated with LPS (100 ng/mL) for 24 h. Culture supernatants were mixed with Griess reagent and incubated for 10 min at room temperature. Nitrite concentrations were quantified by measuring absorbance at 550 nm.

Real-time PCR analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). RNA purity was verified using a NanoDrop spectrophotometer, and only samples with an A260/A280 ratio ≥ 1.8 were used. Complementary DNA was synthesized from 1 μ g RNA using a cDNA synthesis kit (iNtRON Biotechnology, Republic of Korea). Real-time PCR was conducted with SYBR Green Master Mix on an AriaMx Real-Time PCR System (Agilent Technologies, USA). Gene expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene. Primer sequences are listed in Table 1.

Table 1
Primers used in this study

Name	Forward (5' to 3')	Reverse (5' to 3')
iNOS	CAGCCAAGCACTCCAATGTA	CCTGAAGGAGCTTTGTCCAC
COX2	TCTCCCCTCTCTACGCATTC	GGCAGAACGACTCGGTTATC
IL-1 β	ACTCATTGTGGCTGTGGAGA	TTGTTTCATCTCGGAGCCTGT
IL-6	CTGCTGCACTTTGGAGTGAT	GCCTCTTTGCTGCTTTCACAC
MMP1	CCCCAGAGACATCCACAGTT	TCCAGGGAAGTCTTCTGCT
MMP2	TGCTACACGGATACCCCAAG	GCAGCATCGATATGCTTCACAG
TIMP1	AGATAACCATGATGGCCCCCT	TCTGGTAGTCCTCAGAGCCC
TIMP2	CTCGGAGCGCAATAAAACGG	TCTTTCCTCCAACGTCCAGC
Collagen-1	GATTCCCTGGACCTAAAGGTGC	AGCCTCTCCATCTTTGCCAGCA
HAS1	CTGCGATACTGGGTAGCCTTCA	CCAGAACTTCTGGTTGTACCAG
HYAL	GGCCAGGGCTTCCTCAAATA	CTGTGACAGTGGCTGAGTGT
Fillagrin	GCTGAAGGAACTTCTGGAAAAGG	GTTGTGGTCTATATCCAAGTGATC
Involucrin	CTGCCTCAGCCTTACTGTGA	GGAGGAACAGTCTTGAGGAG
Loricrin	GCAACCTCGGGTAGCATCA	GCCGTCCAAATAGATCCCC
GAPDH	CTGCGACTTCAACAGCAACT	GAGTTGGGATAGGGCCTCTC
iNOS: inducible nitric oxide synthase, COX-2: cyclooxygenase-2, IL-1 β /6: Interleukin 1 beta/6, MMP1/2: matrix metalloproteinase 1/2, TIMP-1/2: tissue inhibitor of metalloproteinase 1/2, HAS: hyaluronic acid synthase, HYAL1: hyaluronidase.		

Western blot analysis

Cells were lysed in buffer containing protease and phosphatase inhibitors. Protein concentrations were determined using the bicinchoninic acid (BCA) assay. Equal amounts of protein (50 μ g) were separated

on 10% SDS-PAGE gels and transferred onto PVDF membranes. Membranes were blocked with 5% skim milk and incubated overnight at 4°C with primary antibodies (1:1000). After washing, membranes were incubated with HRP-conjugated secondary antibodies for 2 h at room temperature. Protein bands were detected using enhanced chemiluminescence reagents and visualized with the Alliance Q9 imaging system (UVITEC, UK). Relative protein expression levels were normalized to GAPDH using ImageJ software.

Statistical analysis

All experiments were independently performed at least three times. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using IBM SPSS Statistics 27 (IBM Corp., USA). Group differences were evaluated using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Maple water extract attenuates LPS-induced inflammatory responses in macrophages

The cytotoxic effects of maple water extract on RAW264.7 macrophages were first evaluated to determine the appropriate concentration range for subsequent anti-inflammatory assays (Fig. 1). Maple water extract did not exhibit significant cytotoxicity across concentrations ranging from 6.25 to 400 $\mu\text{g/mL}$, indicating good cellular tolerance. Although a slight reduction in cell viability was observed at concentrations $\geq 100 \mu\text{g/mL}$ under LPS-stimulated conditions, the viability remained within an acceptable range for functional assessment (Fig. 1A). Based on these findings, concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were selected for further experiments. To investigate the anti-inflammatory potential of maple water extract, nitric oxide (NO) production was measured in LPS-stimulated macrophages. As expected, LPS treatment markedly increased NO production compared with the normal control. Co-treatment with maple water extract significantly attenuated NO generation, with a marked decrease at 100 $\mu\text{g/mL}$ (Fig. 1B). These findings indicate that maple water extract effectively suppresses LPS-induced inflammatory activation in macrophages without inducing notable cytotoxic effects. Excessive nitric oxide production is a key feature of macrophage-mediated inflammatory responses [23, 24]. These findings suggest that maple water extract may modulate inflammatory activation in macrophages, supporting its potential immunoregulatory role. Further investigation into pro-inflammatory mediators was conducted to better elucidate the underlying mechanisms.

Maple water extract suppresses pro-inflammatory mediators via inhibition of NF- κ B signaling in macrophages

To further elucidate the anti-inflammatory mechanisms of maple water extract, the mRNA expression of key pro-inflammatory mediators was evaluated in LPS-stimulated RAW264.7 macrophages (Fig. 2). LPS markedly upregulated the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), whereas treatment with maple water extract significantly attenuated these increases in a dose-dependent manner (Fig. 2A and B). Consistent with these observations, maple water extract also suppressed the expression of major pro-inflammatory cytokines, including interleukin (IL)-1 β and IL-6, in a dose-dependent manner (Fig. 2C and D). Given that these cytokines play critical roles in amplifying inflammatory cascades and recruiting immune cells to sites of tissue injury, their coordinated downregulation suggests that maple water extract may effectively disrupt the progression of macrophage-driven inflammatory responses. These findings are in agreement with the observed inhibition of nitric oxide production and collectively indicate that maple water extract mitigates inflammatory activation at multiple regulatory levels. The simultaneous suppression of inflammatory enzymes and cytokines further implies that maple water extract does not target a single downstream mediator but rather influences broader transcriptional control mechanisms. Nuclear factor-kappa B (NF- κ B) is widely recognized as a master transcription factor governing the expression of numerous inflammatory genes [25, 26]. Upon activation, NF- κ B translocates to the nucleus and initiates transcriptional programs that promote the production of cytokines, chemokines, and inflammatory enzymes [26]. In the present study, western blot analysis demonstrated that maple water extract markedly suppressed NF- κ B protein expression, with a marked reduction at 100 μ g/mL (Fig. 2E and F). The coordinated downregulation of iNOS, COX-2, IL-1 β , and IL-6 observed in this study is therefore likely attributable to the modulation of NF- κ B-mediated transcriptional activity rather than nonspecific cellular effects. Similar regulatory patterns have been reported following the inhibition of NF- κ B signaling in activated macrophages [27]. Considering the central role of NF- κ B in orchestrating inflammatory responses [26], its substantial inhibition provides strong mechanistic support for the immunomodulatory potential of maple water extract. These regulatory effects were observed at non-cytotoxic concentrations, supporting that the anti-inflammatory activity was not attributable to nonspecific cellular damage. Taken together, the suppression of iNOS, COX-2, IL-1 β , and IL-6 alongside reduced NF- κ B protein expression suggests that maple water extract influences upstream transcriptional control of macrophage activation. This pattern is consistent with an immunomodulatory mode of action relevant to functional food applications.

Maple water extract enhances keratinocyte viability and protects against UV-induced photodamage

The effects of maple water extract on keratinocyte viability were first evaluated to determine its cytotoxic profile and potential proliferative activity in HaCaT cells. Maple water extract promoted cell viability in a concentration-dependent manner up to 50 μ g/mL, resulting in an increase of approximately 43.8% compared with the normal group (NOR) (Fig. 3A). Based on these findings, the photoprotective potential of maple water extract was further investigated under ultraviolet (UV)-induced stress conditions. UV irradiation significantly reduced keratinocyte viability by approximately 30.3%, confirming the induction of cellular photodamage (Fig. 3B). Treatment with maple water extract mitigated this reduction and

restored cell viability in a dose-dependent manner. At 25–50 µg/mL, cell viability increased by approximately 34.0-40.7% relative to the UV-treated control, suggesting a protective effect against UV-mediated cellular injury (Fig. 3B). UV-induced damage is a major contributor to premature skin aging, primarily through the generation of oxidative stress and disruption of cellular homeostasis [28]. The observed recovery of keratinocyte viability suggests that maple water extract may enhance cellular resilience against environmental stressors [29]. This cytoprotective activity is relevant because maintaining keratinocyte viability is a prerequisite for skin barrier maintenance under photostress [30]. Overall, these findings indicate that maple water extract exerts a protective effect against UV-induced photodamage while supporting keratinocyte survival, highlighting its potential as a bioactive candidate for promoting skin homeostasis.

Maple water extract modulates UV-induced extracellular matrix remodeling by regulating MMP and TIMP expression

To evaluate the protective effects of maple water extract against UV-induced extracellular matrix degradation, the expression of matrix metalloproteinases (MMP-1 and MMP-2) and their endogenous inhibitors (TIMP-1 and TIMP-2) was examined in UV-irradiated HaCaT keratinocytes (Fig. 4). MMPs are key enzymes responsible for the breakdown of collagen and other structural proteins within the dermal matrix and are strongly associated with wrinkle formation and the loss of skin elasticity during photoaging [31, 32]. As expected, UV exposure markedly increased the expression of both MMP-1 and MMP-2 compared with the normal control. However, treatment with maple water extract significantly attenuated these elevations (Fig. 4A and B). These results indicate a protective role of maple water extract in preserving dermal structural integrity by limiting UV-driven matrix degradation. Interestingly, TIMP-1 and TIMP-2 expression exhibited a pattern similar to that of MMPs, showing increased levels following UV exposure and subsequent reduction upon maple water extract treatment (Fig. 4C and D). Although TIMPs are traditionally recognized as inhibitors of MMP activity, their upregulation under photostress conditions is often interpreted as a compensatory response to excessive matrix degradation [31, 33]. Therefore, the concurrent normalization of both MMP and TIMP expression observed in this study may reflect the ability of maple water extract to restore extracellular matrix homeostasis rather than simply suppress individual molecular targets. Maintenance of the balance between MMPs and TIMPs is critical for preserving skin architecture, as disruption of this regulatory network accelerates collagen breakdown and contributes to photoaging [31]. In this context, the modulatory effects of maple water extract on these gene families suggest a protective role against UV-induced structural damage. When integrated with the previously observed enhancement of barrier-related gene expression, these findings indicate that maple water extract exerts multi-dimensional protective effects on the skin by supporting both epidermal stability and extracellular matrix regulation. Such coordinated activity highlights the potential of maple water extract as a biofunctional ingredient capable of mitigating photodamage at both the epidermal and matrix levels.

Maple water extract upregulates epidermal barrier-related genes in UV-stressed keratinocytes

To further investigate whether maple water extract contributes to the maintenance of epidermal integrity, the expression of the key skin barrier-related genes, including filaggrin, loricrin, and involucrin, was evaluated in UV-irradiated HaCaT keratinocytes (Fig. 5). UV exposure is well known to disrupt epidermal differentiation and compromise barrier function, thereby accelerating photodamage [34]. Treatment with maple water extract increased the expression of all three barrier markers under UV-stressed conditions, indicating a potential protective role in preserving epidermal structure. Filaggrin expression was increased in a dose-dependent manner (Fig. 5A). Similarly, loricrin and involucrin expression levels were also elevated following treatment (Fig. 5B and C). Filaggrin, loricrin, and involucrin are critical structural proteins involved in keratinocyte differentiation and the formation of the cornified envelope, which collectively determine the mechanical strength and permeability of the epidermal barrier [35]. Therefore, the coordinated upregulation of these genes suggests that maple water extract may support barrier-associated differentiation processes even under photodamaging conditions. Epidermal barrier integrity is essential for preventing excessive transepidermal water loss and protecting the skin from external environmental insults. Disruption of this barrier is a defining characteristic of photoaged skin and is closely associated with increased permeability and reduced tissue resilience [34]. In this context, the observed enhancement of multiple differentiation markers implies that maple water extract does not merely promote isolated gene expression but may facilitate broader epidermal maturation processes, ultimately contributing to improved barrier stability. Notably, these regulatory effects were achieved within non-cytotoxic concentration ranges, reinforcing the physiological relevance of maple water extract as a bioactive candidate for skin protection. The ability to preserve or restore barrier-related protein expression is particularly important because compromised epidermal integrity is a hallmark of UV-induced skin damage [36]. When considered alongside the previously observed cytoprotective effects, these findings indicate that maple water extract not only enhances keratinocyte survival but may also promote functional recovery of the epidermis following photostress. Together with the anti-inflammatory activity observed in macrophages, the present results suggest that maple water extract supports skin homeostasis through multi-level protective mechanisms involving immune regulation, cellular protection, and structural barrier reinforcement. This integrated activity supports the potential of maple water extract as a biofunctional ingredient for functional foods and nutricosmetic applications.

Maple water extract enhances skin hydration-related gene expression in UV-stressed keratinocytes

To further evaluate the moisturizing potential of maple water extract, the expression levels of key hydration-related genes, including collagen type I, hyaluronan synthase (HAS), and hyaluronidase (HYAL), were analyzed (Fig. 6). UV exposure significantly reduced the expression of collagen and HAS while markedly increasing HYAL expression, indicating impaired moisture retention capacity under photostress conditions [37]. Treatment with maple water extract effectively reversed these alterations. Collagen type

I expression was increased following treatment (Fig. 6A). HAS expression exhibited a pronounced increase, suggesting enhanced hyaluronic acid synthesis (Fig. 6B). In contrast, HYAL expression decreased in a dose-dependent manner (Fig. 6C). Hyaluronic acid plays a central role in maintaining skin hydration due to its remarkable water-retaining capacity, while collagen provides structural support that contributes to dermal moisture preservation [38]. Conversely, excessive HYAL activity accelerates hyaluronic acid degradation, leading to reduced skin hydration [37]. Therefore, the simultaneous upregulation of HAS and suppression of HYAL observed in this study suggests that maple water extract may promote moisture homeostasis by favorably regulating hyaluronic acid metabolism. The stronger induction of HAS relative to collagen implies that the hydrating effects of maple water extract may be primarily associated with the modulation of hyaluronic acid synthesis rather than structural reinforcement alone. Such regulation is particularly meaningful under UV-stressed conditions, where disruption of moisture balance is a common feature of photodamaged skin [39]. When interpreted alongside the effects on inflammatory mediators, barrier markers, and matrix-related genes, maple water extract appears to influence multiple pathways relevant to photodamaged skin. This supports its potential as a functional ingredient aimed at skin-related benefits.

Phenolic content and antioxidant characteristics of maple water extract

The phenolic profile and antioxidant capacity of maple water extract were evaluated by measuring total polyphenol and flavonoid contents, along with ferric reducing antioxidant power (FRAP) and radical scavenging activities. Maple water extract contained measurable levels of total polyphenols and flavonoids (Table 2), indicating the presence of phenolic-associated constituents. In the FRAP assay, maple water extract exhibited a reducing capacity of 414.5 mg AAE/g, suggesting its potential electron-donating ability. In contrast, the radical scavenging activities measured by DPPH and ABTS assays showed relatively high IC_{50} values compared with ascorbic acid, indicating comparatively weaker direct free radical scavenging effects (Table 2). These findings suggest that maple water extract may exert antioxidant-related effects primarily through reducing potential rather than strong radical quenching activity. Although the DPPH and ABTS assays revealed relatively weak radical scavenging activity compared with ascorbic acid, chemical antioxidant assays do not necessarily reflect biological efficacy, as cellular systems involve complex oxidative stress regulation and signaling networks. Previous studies have reported that maple-derived products contain diverse phenolic compounds such as gallic acid, caffeic acid, and protocatechuic acid, which are associated with antioxidant properties [40, 41]. While the specific phytochemical composition of the maple water extract used in the present study was not characterized, the detectable polyphenol content suggests that phenolic-associated constituents may contribute to its reducing capacity. Notably, quebecol—a process-derived phenolic compound generated during the thermal concentration of maple sap into syrup—is absent in raw maple water extract. This distinction implies that the biological effects observed in this study are more likely attributable to naturally occurring components rather than heat-induced transformation products. The discrepancy between modest radical scavenging activity and substantial reducing power suggests that maple water extract may support cellular defense mechanisms through redox modulation rather than acting as a

strong direct free radical quencher. Such reducing potential may influence intracellular signaling pathways associated with oxidative stress and inflammation. These chemical properties may partially explain the biological responses observed in this study, suggesting that maple water extract functions as a bioactive modulator rather than a simple radical scavenger.

Table 2
Phenolics content and radical scavenging effect of Maple water

Contents		Sample	
		Maple water	Ascorbic acid
Total polyphenol Content (mg GAE/g)		115.96 ± 1.7	
Total Flavonoid content (mg CE/g)		28.67 ± 0.5	
FRAP (mg AAE/g)		414.5 ± 2.5	
IC ₅₀ (µg/mL)	ABTS	> 30000	204.0
	DPPH	24398.7	57.67
GAE: gallic acid equivalent, CE: catechin equivalent, AAE: Ascorbic acid equivalent, IC ₅₀ : the concentration required to inhibit radical by 50%.			

Overall, this study suggests that maple water extract exerts dermo-protective activity in vitro through concurrent regulation of inflammatory signaling and UV-stressed keratinocyte responses. In macrophages, maple water extract reduced LPS-induced inflammatory activation, as reflected by decreased NO production and reduced expression of NF-κB protein and its downstream mediators. In keratinocytes exposed to UV, maple water extract promoted barrier-associated differentiation marker expression and favorably regulated genes related to extracellular matrix remodeling and hydration, including attenuation of MMP induction and a shift in hyaluronic acid metabolism toward increased synthesis (HAS) with reduced degradation (HYAL). These results are notable because UV-related skin dysfunction is typically driven by multiple interconnected processes—immune activation, impaired differentiation/barrier integrity, matrix remodeling, and moisture imbalance—rather than a single pathway [42]. The combined pattern observed here supports the view that maple water extract may influence more than one biological axis relevant to photoaging and photostress-associated skin changes. Given that maple water is traditionally consumed as a beverage, these findings may have relevance to dietary intake, suggesting that regular consumption of maple water could contribute to systemic regulation of inflammation and support skin health through nutritional mechanisms.

Beyond its nutritional value, maple sap has historically been utilized by Indigenous peoples of North America for various traditional health-related purposes [4]. Ethnobotanical records indicate that sap collected from sugar maple trees (*Acer saccharum*) was consumed not only as a seasonal beverage but

also used as a natural remedy for minor wounds, skin irritation, and inflammatory conditions [4]. The naturally occurring sugars, minerals, and phenolic compounds present in maple sap were believed to support skin soothing and recovery [1, 2]. In this context, the present findings demonstrating anti-inflammatory and skin-protective effects of maple water extract provide scientific evidence that partially supports these traditional observations. Thus, maple water extract may represent an example in which traditional knowledge aligns with modern cellular evidence of dermal protective activity.

Several limitations should be considered. First, the present findings are based on cell models and may not fully represent the complexity of human skin. Second, the active constituents responsible for the observed effects were not identified, and the contributions of individual phytochemicals remain unclear. Future studies should incorporate compositional profiling and mechanistic validation (e.g., pathway-specific inhibition/knockdown approaches), followed by in vivo or clinical evaluation to determine translational relevance.

Conclusions

Maple water extract attenuated LPS-induced inflammatory responses in RAW264.7 macrophages and improved UV-stressed cellular responses in HaCaT keratinocytes, including barrier-related gene expression, extracellular matrix-associated regulators, and hydration-related factors. These findings suggest that maple water extract exerts dermo-protective effects through multi-level regulation of inflammatory signaling and skin-related cellular responses. Importantly, considering that maple water is traditionally consumed as a natural beverage, these results provide scientific support for its potential role as a dietary resource associated with skin-related health benefits. Accordingly, maple water extract may serve as a promising functional dietary resource with relevance to skin-related health benefits, although further studies are required to identify active compounds and validate these effects in in vivo or clinical settings.

Declarations

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Author Contribution

Ye-Lim You: Investigation, Validation, Resources, Methodology, Writing - Review & Editing, Formal analysis. **Ha-Jun Byun:** Investigation, Validation, Resources, Visualization, Methodology. **Kisoo Han and Hyo Chul An:** Investigation, Methodology, Conceptualization. **Hyeon-Son Choi:** Conceptualization, Methodology, Supervision, Writing-Original draft preparation, Project administration, Writing - Review & Editing.

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All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

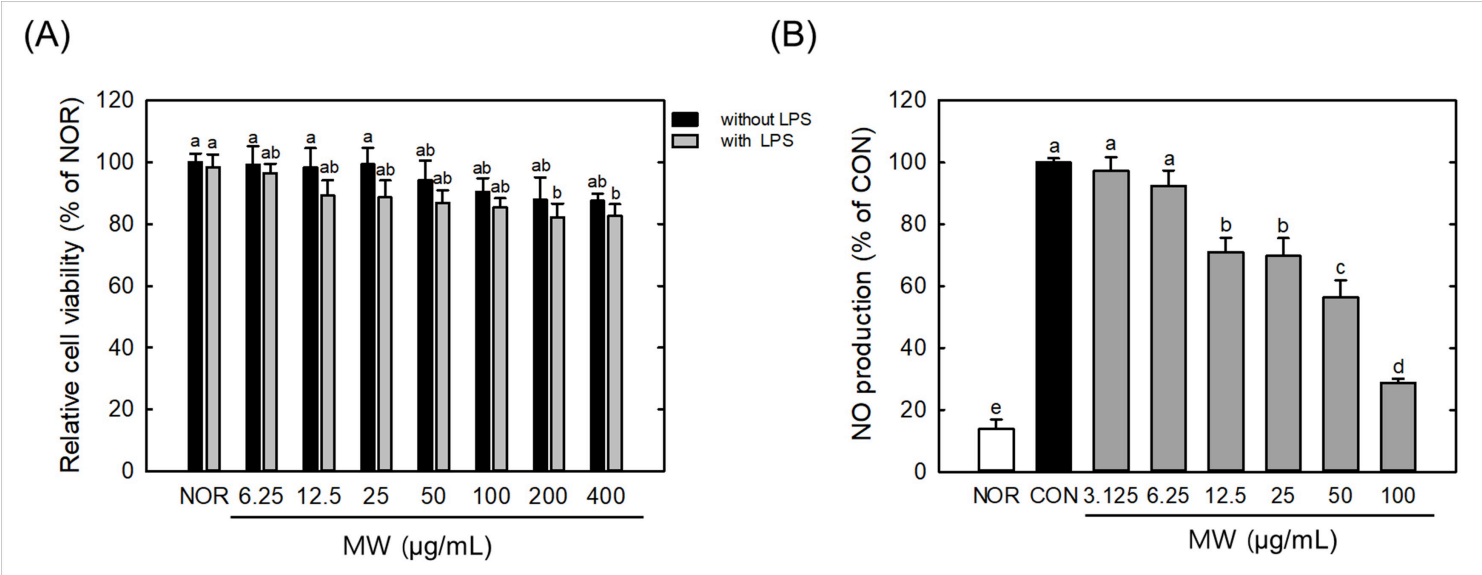


Figure 1

Effects of maple water (MW) on cell viability and nitric oxide (NO) production in RAW264.7 macrophages. (A) Cell viability of RAW264.7 macrophages treated with maple water (MW; 6.25–400 µg/mL) in the absence or presence of lipopolysaccharide (LPS), as assessed by the MTT assay. (B) Nitric oxide (NO) production in RAW264.7 cells treated with MW (3.125–100 µg/mL) under LPS-stimulated conditions. NO levels in the culture supernatants were determined using the Griess reaction. NOR, untreated cells without MW or LPS; CON, LPS-treated cells without MW. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among groups (*p* < 0.05).

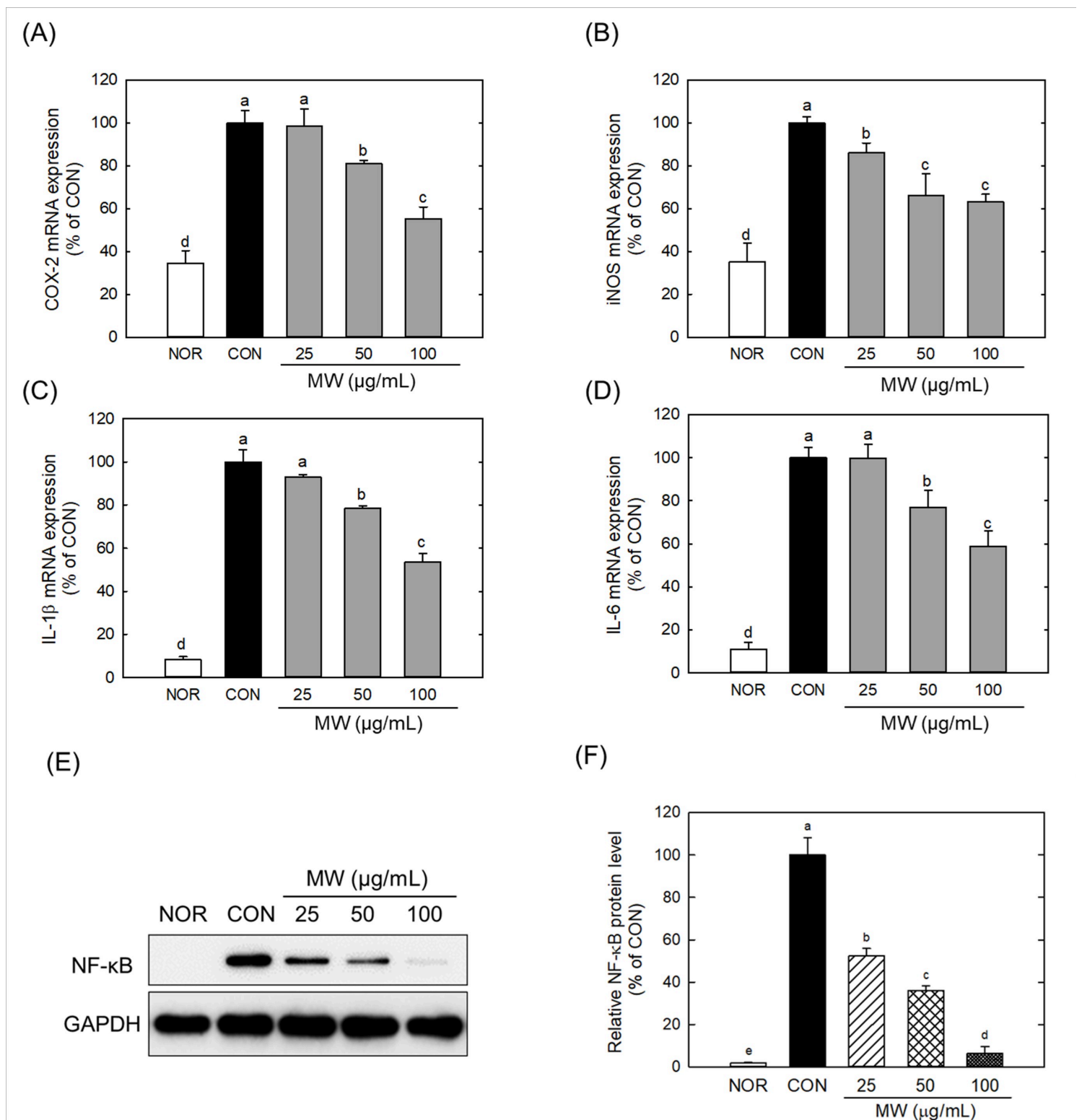


Figure 2

Effects of MW on inflammatory gene expression and NF-κB protein levels in LPS-stimulated RAW264.7 macrophages. (A–D) mRNA expression levels of COX-2 (A), iNOS (B), IL-1β (C), and IL-6 (D) in RAW264.7 cells treated with MW (25–100 μg/mL) under LPS-stimulated conditions. Gene expression was analyzed by quantitative real-time PCR. (E) Representative immunoblot showing NF-κB protein expression in RAW264.7 cells treated with MW under LPS-stimulated conditions, with GAPDH used as a loading

control. (F) Quantitative densitometric analysis of NF- κ B protein bands shown in panel E, performed using ImageJ software (Version 1.53t). NOR, untreated cells without MW or LPS; CON, LPS-treated cells without MW. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($p < 0.05$).

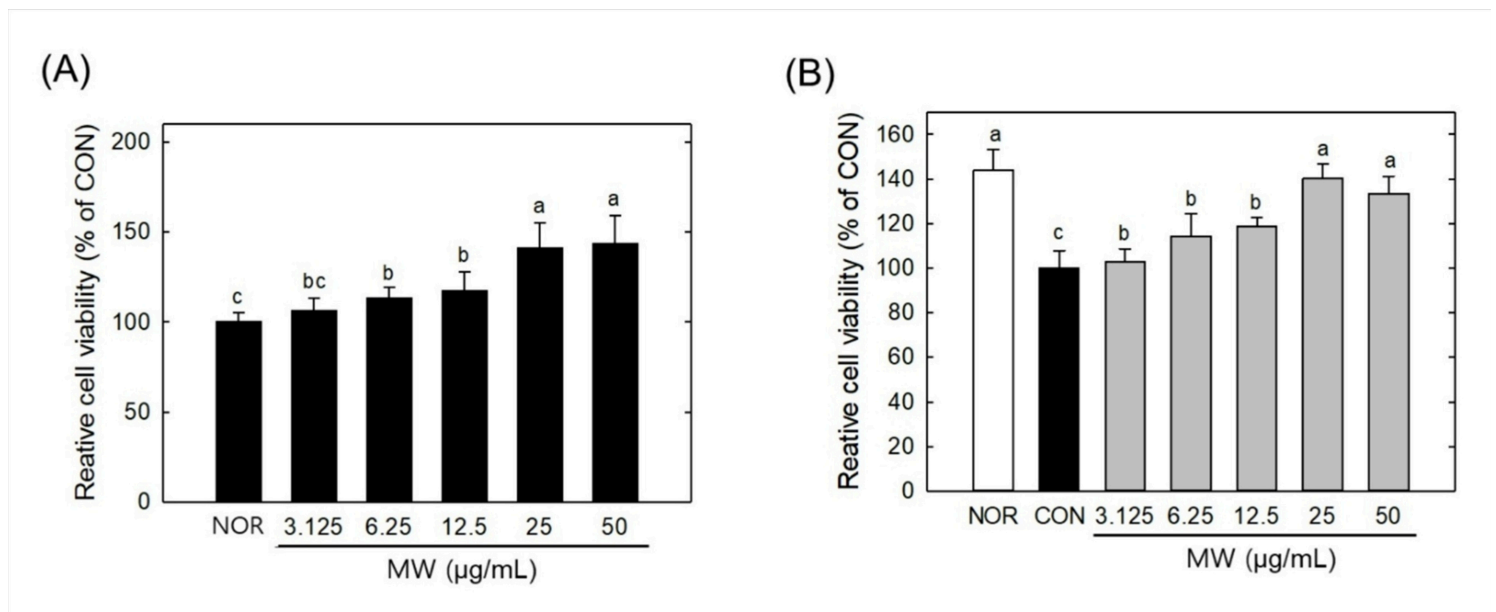


Figure 3

Effects of MW on cell viability and UV-induced cytotoxicity in HaCaT keratinocytes. (A) Cell viability of HaCaT cells treated with maple water (MW; 3.125–50 µg/mL), as assessed by the MTT assay. (B) Cell viability of HaCaT cells treated with MW under UV-irradiated conditions, as assessed by the MTT assay. NOR, untreated cells without MW treatment or UV irradiation; CON, UV-irradiated cells without MW treatment. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($p < 0.05$).

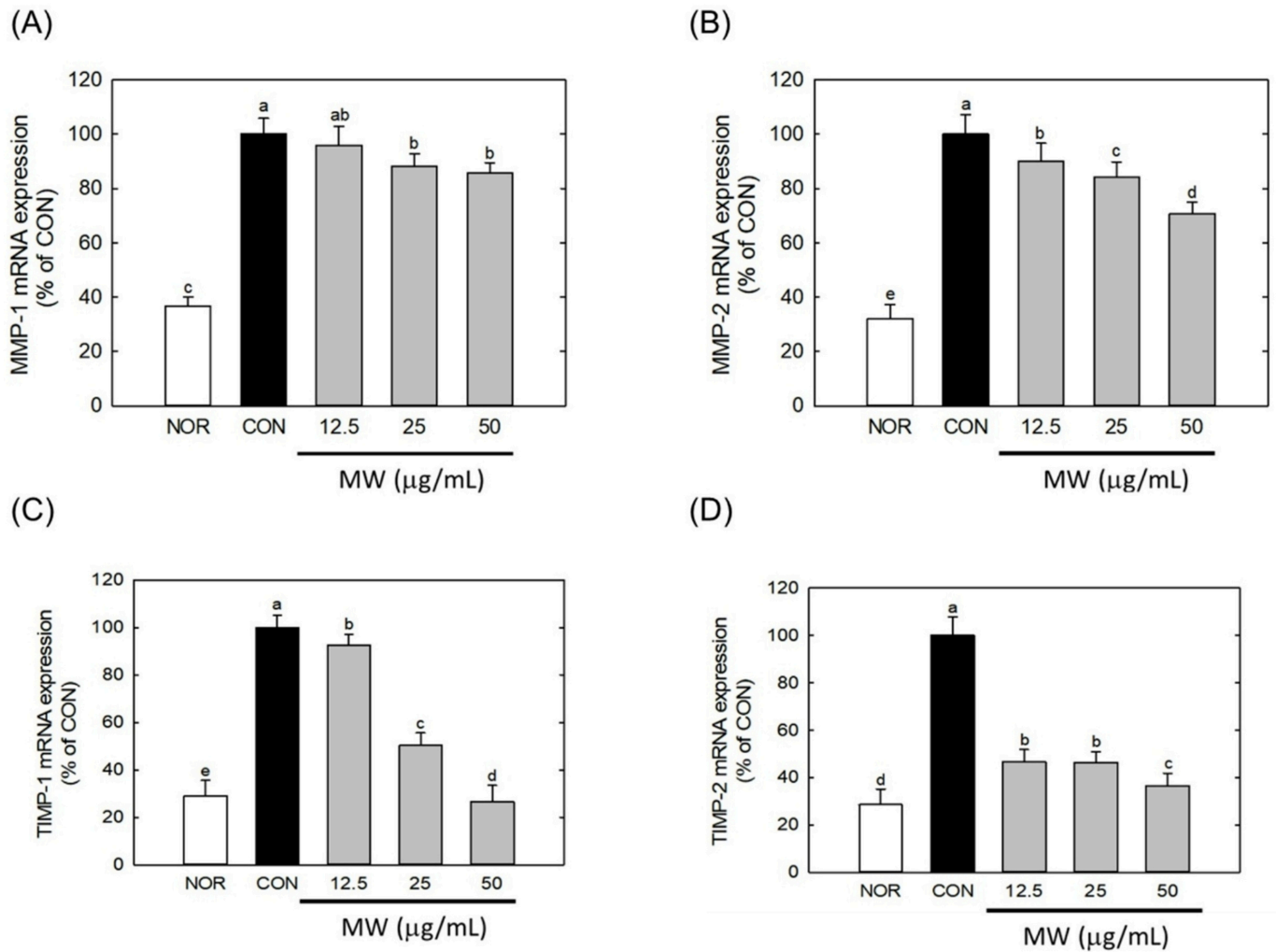


Figure 4

Effects of MW on MMP and TIMP gene expression in UV-stimulated HaCaT keratinocytes. (A, B) mRNA expression levels of matrix metalloproteinase-1 (MMP-1) (A) and matrix metalloproteinase-2 (MMP-2) (B) in HaCaT cells treated with MW (12.5–50 μg/mL) under UV-irradiated conditions. (C, D) mRNA expression levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) (C) and tissue inhibitor of metalloproteinase-2 (TIMP-2) (D) in HaCaT cells treated with MW under UV-irradiated conditions. Gene expression was analyzed by quantitative real-time PCR. NOR, untreated cells without MW treatment or UV irradiation; CON, UV-irradiated cells without MW treatment. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among groups ($p < 0.05$).

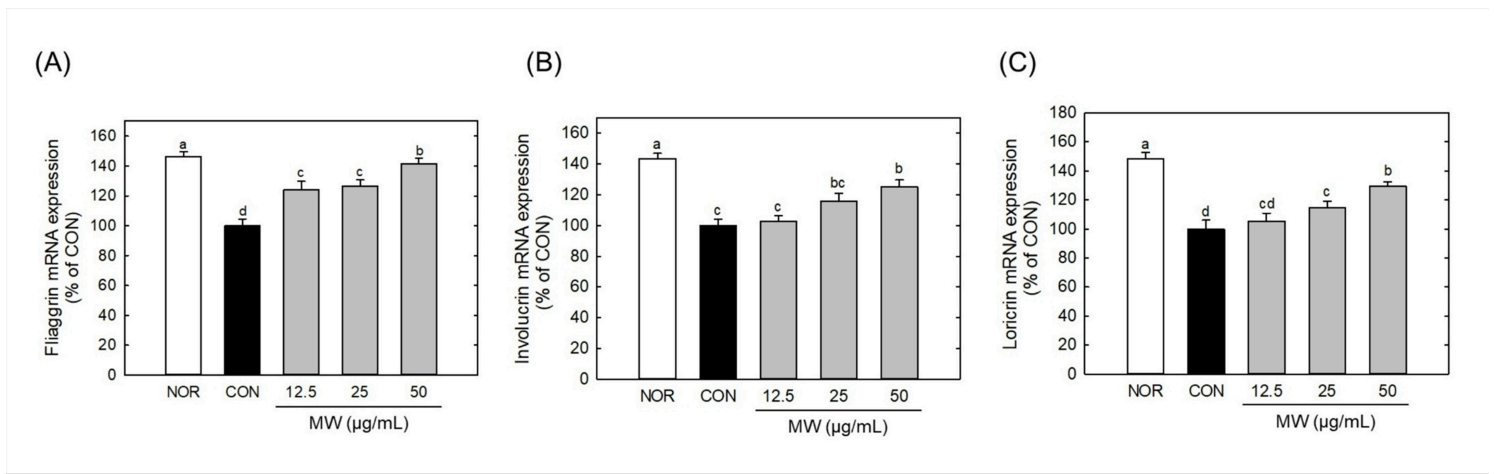


Figure 5

Effects of MW on epidermal differentiation marker gene expression in UV-stimulated HaCaT keratinocytes. (A–C) mRNA expression levels of filaggrin (A), involucrin (B), and loricrin (C) in HaCaT cells treated with MW (12.5–50 µg/mL) under UV-irradiated conditions. Gene expression was analyzed by quantitative real-time PCR. NOR, untreated cells without MW treatment or UV irradiation; CON, UV-irradiated cells without MW treatment. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($p < 0.05$).

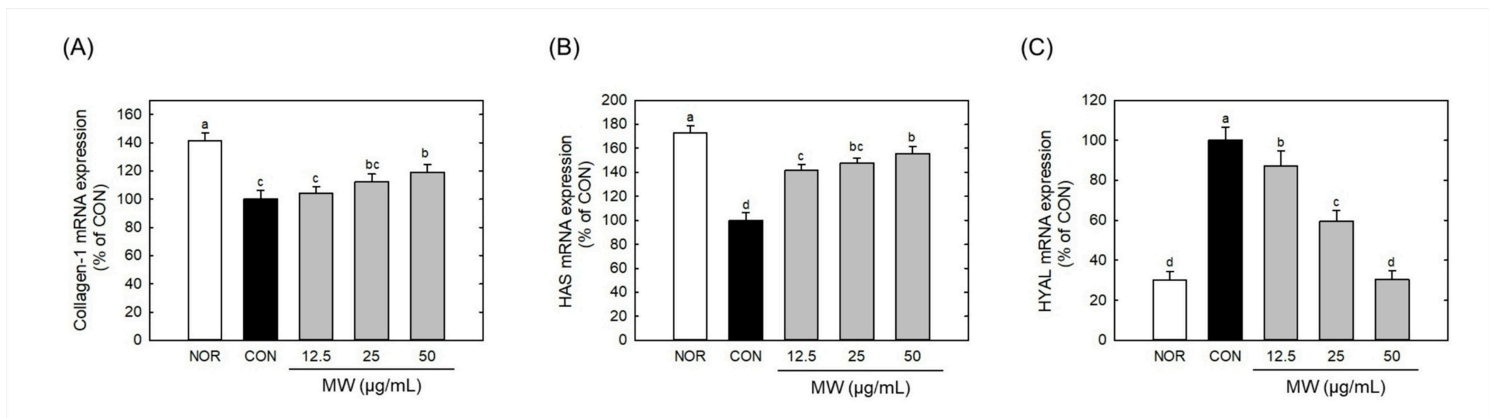


Figure 6

Effects of MW on collagen synthesis- and hydration-related gene expression in UV-stimulated HaCaT keratinocytes. (A–C) mRNA expression levels of collagen-1 (COL1A1) (A), hyaluronan synthase (HAS) (B), and hyaluronidase (HYAL) (C) in HaCaT cells treated with MW (12.5–50 µg/mL) under UV-irradiated conditions. Gene expression was analyzed by quantitative real-time PCR. NOR, untreated cells without MW treatment or UV irradiation; CON, UV-irradiated cells without MW treatment. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($p < 0.05$).