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Liquid Extract from the Bark of *Magnolia officinalis* Rejuvenates Skin Aging Through Mitochondrial ROS Reduction

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Abstract

Reactive oxygen species (ROS) are a major cause of skin aging, leading to oxidation and cleavage of collagen that supports skin structure. Previous studies have demonstrated that *Magnolia officinalis* var. *officinalis* (*M. officinalis*) dry extract reduces mitochondria-enriched ROS production and improves senescence-related phenotypes in vitro. However, its effects on human skin aging have not been investigated. In this study, we conducted both in vitro and clinical trials using an *M. officinalis* liquid extract, which can be directly applied to cosmetic formulations. The *M. officinalis* liquid extract restored mitochondrial function and reduced mitochondria-enriched ROS production. Furthermore, *M. officinalis* liquid extract activated mitophagy, which removes defective mitochondria, a major source of ROS production. In clinical trials, the *M. officinalis* liquid extract reduced the mean depth of neck wrinkles by 12.73% and the maximum depth by 17.44%. It also reduced the mean roughness (Ra), root mean square roughness (Rq), and maximum depth of roughness (Rmax) by 12.73%, 10.16%, and 10.81%, respectively. Furthermore, the key to the skin-improving effects of *M. officinalis* liquid extract lies in its ability to increase skin elasticity by 3.76% and brighten skin tone by 0.76%. In conclusion, this study identified a novel mechanism by which *M. officinalis* liquid extract rejuvenates skin aging. *M. officinalis* can be utilized as a cosmetic ingredient to improve skin aging and therapeutic candidate for the development of anti-aging treatments.

Keywords: ROS; *Magnolia officinalis* liquid extract; skin rejuvenation

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1. Introduction

Cellular senescence is a process characterized by the permanent cessation of cell division and the decline in organelle function. Mitochondria, one of these organelles, undergo structural changes with senescence, increasing in size and volume while decreasing in function [1]. Specifically, mitochondrial dysfunction induces electron leakage from the electron transport chain (ETC), generating reactive oxygen species (ROS) [2]. Excessive ROS production in mitochondria induces a detrimental cycle that further damages mitochondria [2]. Thus, excessive ROS damages the structure and function of organelles other than mitochondria, ultimately leading to senescence [3]. While controlling ROS generation in mitochondria is recognized as crucial for senescence rejuvenation, effective methods to control ROS formation have not yet been identified, and further research in this area is needed.

The skin, the outermost organ of the body, consists of the epidermis, composed of epithelial tissue, and the dermis, composed of connective tissue [4]. Collagen, a protein that makes up about 90% of the dermal layer, is essential for providing a structural framework and contributing to the skin's shape and elasticity [5]. A prominent aspect of skin aging is collagen loss, leading to skin wrinkles [6]. Although several factors are known to cause skin aging, mitochondrial dysfunction is considered the main cause of skin aging. Specifically, mitochondrial dysfunction leads to excessive production of mitochondrial ROS, which in turn activates collagen-degrading enzymes such as matrix metalloproteinases, thereby promoting the breakdown of collagen [7,8]. Excessive levels of ROS also promote oxidative damage to collagen, leading to destabilization and breakdown of collagen–elastin fiber assemblies in the dermal extracellular matrix [8]. For example, vitamin C, an antioxidant that reduces free radical production, protects collagen in skin tissues, thereby maintaining skin elasticity [9]. Niacinamide, a form of vitamin B3, is an antioxidant that protects the skin from free radical damage and improves skin texture [10]. Antioxidant–based approaches have been used as a key strategy to improve skin aging, and further research in this area will help promote skin health.

Magnolia officinalis var. *officinalis* (*M. officinalis*) is distributed in East Asia, with significant populations in China, Japan, and Korea. *M. officinalis* has been widely used in traditional Chinese medicine due to its well-known anti-inflammatory, antibacterial, and antioxidant properties [11]. While many plant extracts are known to possess antioxidant or anti-aging properties, *M. officinalis* stands out from these common free radical scavengers by its ability to reduce mitochondrial ROS in addition to scavenging ROS inside and outside the cell [12]. *M. officinalis*'s ability to reduce mitochondrial ROS plays a key role in restoring mitochondrial function and enhancing its rejuvenating effects [12]. Furthermore, the key active ingredients of *M. officinalis* (e.g., magnolol and honokiol) modulate several senescence-related pathways, including nuclear factor kappa B and nuclear factor erythroid 2-related factor 2, suggesting broad potential for controlling the underlying causes of senescence [13,14]. These properties suggest that *M. officinalis* may offer a mechanism of action advantage over conventional antioxidants, which primarily act as direct free radical scavengers.

Mitophagy offers a unique advantage in that it provides precise and selective quality control for mitochondria [15]. Unlike conventional autophagy, which degrades entire cellular components, mitophagy selectively removes only dysfunctional mitochondria. Mitophagy is a key mechanism for maintaining mitochondrial function and homeostasis. Furthermore, by timely removing dysfunctional mitochondria, it prevents cell death by blocking the release of harmful stress signals and apoptotic factors [15]. Given these cellular roles, the regulation of mitophagy may be an effective strategy to restore mitochondrial function.

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The use of natural products in cosmetics is increasing due to their minimal adverse effects on the human skin [16]. To be used as cosmetic ingredients, natural products are manufactured in dry or liquid form through processes such as cold pressing, carbon dioxide extraction, steeping, infiltration, and solvent extraction. Each extract is diluted to a sufficient amount to provide the intended effects, such as moisturizing, anti-aging, or antioxidant effects, before being added to cosmetic formulations. Among these extracts, liquid extracts are preferred over dry extracts in cosmetic formulations because they can be added directly to cosmetic formulations without prior dissolution [17]. Furthermore, liquid extracts are manufactured using standardized manufacturing protocols, maintaining a consistent concentration of active ingredients, reducing variation in cosmetic production compared to dry extracts [18]. For example, liquid extracts are mixed in various cosmetic bases at concentrations ranging from 0.1% to 3%.

One tool for identifying mitochondrial ROS is dihydrorhodamine 123 (DHR123). Rhodamine 123, a fluorescent dye that selectively stains mitochondria, is reduced to create DHR123 [19]. Cationic rhodamine 123, which is employed to quantify mitochondrial ROS, is produced when DHR123 binds to mitochondrial hydroxyl radicals. However, DHR123 is also susceptible to nonspecific oxidation by various ROS and may respond to ROS other than mitochondrial ROS. Therefore, another mitochondria-specific dye, MitoSOX, was designed to detect mitochondrial ROS. MitoSOX is composed of a superoxide anion-sensitive dihydroethidium compound attached to a triphenylphosphonium moiety found in the mitochondrial matrix [20]. MitoSOX quantifies mitochondrial ROS by binding to superoxide anions in the mitochondrial matrix and oxidizing to a cationic dihydroethidium complex.

This study was conducted to determine whether *M. officinalis* liquid extract is effective in improving skin aging. *M. officinalis* liquid extract reduced the production of ROS in mitochondria, thereby exhibiting anti-aging effects on human skin. This study presents a novel mechanism by which *M. officinalis* liquid extract rejuvenates skin aging, and this could be utilized as a new approach for treating it.

2. Materials and Methods

2.1. Cell Culture

The cell lines presented in Table 1 were used in the experiments. Human dermal fibroblasts were serially passaged in the medium and culture conditions presented in Table 1. At each passage, cell counts were measured using a Cedex HiRes Analyzer (05650216001; Roche, Basel, Switzerland), which was used to calculate the population doubling time (PD). Based on PD measurements, cells exhibiting a PD greater than 14 days were classified as senescent fibroblasts, whereas cells with a PD of less than 2 days were classified as young fibroblasts, as previously reported [21].

Table 1. Information about the cell line.

Cell Line Name	Company Name	Catalogue Number	Medium Condition	Culture Condition
Human dermal fibroblasts	ATCC, Manassas, VA, USA	PCS–201–010	Dulbecco's modified Eagle's medium (10–013–CV; Corning, Corning, NY, USA) 10% fetal bovine serum (30006; SPL Life Sciences, Pocheon, Republic of Korea) 100 U/mL penicillin & 100 µg/mL streptomycin (SV30079.01; Hyclone, Logan, UT, USA)	Cells were cultivated in 5% CO_2 at 37 °C.

2.2. Preparation of M. officinalis Liquid Extract

The bark of *M. officinalis* (Pure Mind, Yeongcheon, Republic of Korea) was extracted by refluxing with 70% ethanol at 60 °C for 3 h at a solid-to-solvent ratio of 1:8 (*w*/*v*). A 5 μm filter was used to filter the extract first, followed by a 0.45 μm filter. Butylene glycol (Amorepacific Japan Co., Ltd., Tokyo, Japan) was used to dissolve the filtrate. The mixture was then concentrated at 60 °C. Polyglyceryl–10 oleate (Taiyo Kagaku Co., Ltd., Tokyo, Japan) was added to the concentrate in a volume ratio of 8:2. After stirring at 80 °C for 30 min, the mixture was diluted fivefold with filtered water. 1,2–Hexanediol (SHD62; SHINSUNG Composites, Jincheon, Republic of Korea) was added to the dilution to a final concentration of 2%. The final mixture was sterilized using a 0.22 μm syringe filter (S25LT022AP1HN–B1H; Hyundai Micro Co., Ltd., Seoul, Republic of Korea). For in vitro testing, the filtrate was diluted in medium to concentrations of 0.03%, 0.05%, and 0.1%.

2.3. Preparation of a Cream Containing M. officinalis Liquid Extract

For the clinical test, the cream base contained purified water, butylene glycol, glycerin, cyclohexasiloxane, cyclopentasiloxane, cetearyl alcohol, cetearyl glucoside, cetyl ethylhexanoate, betaine, 1,2-hexanediol, sodium acrylate/acryloyldimethyltaurate copolymer, isohexadecane, polysorbate 80, β-glucan, sodium hyaluronate, polysorbate 60, glyceryl stearate, PEG–100 stearate, tocopheryl acetate, lavender oil, allantoin, xanthan gum, and disodium EDTA. A cream without *M. officinalis* liquid extract was used as a vehicle group, and a cream containing *M. officinalis* liquid extract (3%) was applied to the test group.

2.4. Flow Cytometric Analysis of ROS, Mitochondrial Membrane Potential (MMP), Autophagy Flux, Mitochondrial Mass and Autofluorescence

Senescent fibroblasts were treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05%, and 0.1%) at 4–day intervals for 12 days. Flow cytometric analysis for ROS, MMP, mitochondrial mass, and autofluorescence was performed according to the conditions in Table 2. Specifically, autofluorescence was measured by incubating cells in dye-free medium for 30 min at 37 °C, followed by detection using the fluorescein isothiocyanate (FITC) channel (excitation at 488 nm, emission at 525 nm). Autophagy flux was measured according to the following procedure. Senescent fibroblasts were cultured overnight at 37 °C in medium containing 20 μM chloroquine (CQ; C6628; Sigma, St. Louis, MO, USA). Then, the senescent fibroblasts were exposed to CYTO–ID®–containing medium (ENZ–51031–0050; Enzo Life Sciences, Farmingdale, NY, USA) at 37 °C for 30 min. [Cyto–ID® FITC mean fluorescence intensity (MFI) (with CQ)—Cyto–ID® unstained FITC MFI (with CQ)]—[Cyto–ID® stained FITC MFI (without CQ)—Cyto–ID® unstained FITC MFI (without CQ)] is autophagic flux.

Table 2. Flow cytometry conditions.

Analysis	Dye	Catalogue Number	Concentration	Staining Condition	Calculation Method
ROS	Dihydrorhodamine (DHR123)	10056–1; Biotium, Fremont, CA, USA	30 μM	30 min at 37 °C	[DHR123 stained FITC MFI] − [DHR123 non–stained FITC MFI]
	MitoSOX	M36008; Life Technologies, Carlsbad, CA, USA	5 μM	30 min at 37 °C	[MitoSOX stained phycoerythrin (PE) MFI] − [MitoSOX non–stained PE MFI]

Table 2. *Cont.*

Analysis	Dye	Catalogue Number	Concentration	Staining Condition	Calculation Method
MMP	JC–10	ENZ–52305; Enzo Life Sciences, Farmingdale, NY, USA	0.6 μg/mL	30 min at 37 °C	[JC–10 stained FITC MFI − JC–10 non–stained FITC MFI]/[JC–10 stained PE MFI − JC–10 non–stained PE MFI]
Mitochondrial mass	MitoTracker™ Deep Red FM Dye (MTDR)	M46753; Invitrogen, Waltham, MA, USA	50 nM	30 min at 37 °C	[MTDR stained APC MFI] − [MTDR non–stained APC MFI].
Autofluorescence	No dye	Not available	Not available	30 min at 37 °C	[FITC MFI]

2.5. Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) Analysis

An XFe96 flux analyzer (Aglient Technology, Santa Clara, CA, USA) was used to examine OCR and ECAR. The Seahorse XF Mito Stress Kit (103015–100; Aglient Technology) and the Seahorse XF Glycolytic Rate Assay Kit (103344–100; Aglient Technology) were used to measure OCR and ECAR, respectively. OCR and ECAR analyses were performed according to the company protocol.

2.6. Fluorescence Analysis of Mitophagy

Immunofluorescence was carried out under the guidelines listed in Table 3.

Table 3. Immunofluorescence conditions.

Analysis	Antibody	Catalogue Number	Dilution in PBS	Staining Condition	Microscope
LC3B staining	anti-LC3B	A19665; Abclonal, Boston, MA, USA	1:200	overnight at 4 °C	A Carl Zeiss LSM 700 (Carl Zeiss, Oberkochen, Germany)
	Alexa Fluor® 488 goat anti-rabbit IgG	A–11008; Invitrogen	1:200	60 min at room temperature	
OXPHOS staining	anti-OXPHOS cocktail	ab110411; Abcam, Cambridge, Cambridgeshire, UK	1:200	overnight at 4 °C	A Carl Zeiss LSM 700 (Carl Zeiss, Oberkochen, Germany)
	Alexa Fluor® 647 goat anti-mouse IgG	A–28181; Invitrogen	1:200	60 min at room temperature	

2.7. Quantitative PCR (qPCR) Analysis

qPCR was carried out as previously mentioned [22]. Table 4 shows the information about primers.

Table 4. Sequence information of primers used in qPCR.

Target	Orientation	Sequence (5′–3′)	Size (bp)
36B4 (Accession number: NM_053275)	forward	CAGCAAGTGGGAAGGTGTAATCC	23
	reverse	CCCATTCTATCATCAACGGGTACAA	25
SLIT2 (Accession number: NM_004787.4)	forward	CAGAGCTTCAGCAACATGACCC	22
	reverse	GAAAGCACCTTCAGGCACAACAG	23

2.8. Western Blot Analysis

The protocol outlined in Table 5 was followed while performing the Western blot analysis. Image J (Version 1.54p, National Institute of Health, Bethesda, MD, USA) was used to evaluate the Western blot quantitatively.

Table 5. Western blot conditions.

Analysis	Antibody	Catalogue Number	Dilution in PBS	Staining Condition
p16 staining	anti-p16	A11337; Abclonal	1:500	overnight at 4 °C
	Horseradish peroxidase–conjugated antibody	sc–2357; Santa Cruz biotechnology; Dallas, TX, USA	1:2000	60 min at room temperature
B-actin staining	anti-β-actin	sc–47778 HRP; Santa Cruz biotechnology	1:5000	overnight at 4 °C

2.9. High-Performance Liquid Chromatography (HPLC) Analysis

The honokiol and magnolol content of the *M. officinalis* liquid extract was analyzed using HPLC (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). The HPLC column utilized was a Capcell Pak C18 4.6 × 250 mm column (a Shiseido, Osaka, Japan). Methanol was used to dilute the *M. officinalis* liquid extract to a concentration of 1 mg/mL. 50 mL methanol was used to dissolve either 4.0 mg honokiol (42612; Sigma) or 3.5 mg magnolol (Y0001289; Sigma) to create a standard solution.

2.10. Clinical Trials

For the clinical experiment, twenty-one Korean women (mean age 56.86 ± 4.94 years) took part. The clinical trial protocol (approval number: DEF-HAAET070(1)-25087) was authorized by Dermapro Co., Ltd.'s Institutional Review Board in Seoul, Republic of Korea. The clinical trial protocol was registered with the Clinical Trial Information Service of the National Institute of Health, Republic of Korea (KCT00100008). The clinical trial protocol was performed in accordance with Dermapro Co., Ltd.'s standard operating procedures and good clinical practice. The period of the clinical trial was from 7 April 2025 to 16 May 2025. The subject selection process excluded participants having skin abnormalities in the test area, such as spots, acne, erythema, or telangiectasia. Written consent for participation was obtained prior to the clinical trial's commencement. Participants were informed of its goal and procedures. To objectively assess efficacy, the clinical trial was designed using randomized, double-blind methods. Specifically, randomization was performed using methods such as block randomization to assign subjects to different treatment groups without bias. Moreover, the double-blind method ensured that neither the investigators nor the participants knew which product was being administered. For 4 weeks, participants applied a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) twice daily, in the morning and the evening. Participants were given 20 min to acclimate in a controlled setting with a temperature of 22 °C ± 2 °C and a relative humidity of 50 ± 5% before the measurements. During the 4-week period, no tolerance and adverse effects were observed in participants who received the vehicle cream or the cream containing *M. officinalis* liquid extract (3%).

2.11. Measurement of Neck Wrinkles

Neck wrinkles were measured using ANTERA® CS (Miravex Limited, Dublin, Ireland). Two-dimensional or three-dimensional image analysis was performed in the same area at the start of treatment and 4 weeks later to determine wrinkle depth. Images of the

left and right neck areas were taken, and wrinkle patterns (depth and maximum depth) were analyzed.

2.12. Measurements of Skin Elasticity

A cutometer (MPA580; Courage and Khazaka Electronic, Cologne, Germany) was used to assess the elasticity of the skin. The measurement's basic concepts are suction and extension. The apparatus pulls the skin in the direction of the probe hole while maintaining a steady negative pressure of 450 mbar for two seconds. After that, the negative pressure is stopped for two seconds to give the skin time to regain its natural contour. The onset/onset cycle is repeated three times for every measurement cycle. The left and right cheeks were used to test the suppleness of the skin.

2.13. Measurements of Skin Texture

Skin texture was measured with an atomic force microscope (Veeco, Plainview, NY, USA). The cheek areas on both sides were measured. Mean roughness (Ra), root mean square roughness (Rq), and maximum depth of roughness (Rmax) were analyzed following the previous study [23].

2.14. Measurements of Skin Complexion

Skin complexion was measured with a spectrophotometer® CM–2500d (Minolta, Tokyo, Japan). The cheek areas on both sides were measured. Skin brightness (L* value) was analyzed following the previous study [24].

2.15. Statistical Analysis

Statistical analysis was performed using an IBM SPSS Statistics version 22 software (IBM, Chicago, IL, USA). Student's *t*-test, two–way ANOVA followed by Bonferroni's post hoc test, RM-ANOVA, and Wilcoxon signed-rank test were used to examine variability between parametric data sets. Specifically, when a significant main effect was detected in RM-ANOVA, post hoc pairwise comparisons were performed using the Bonferroni correction method to correct for multiple comparisons. Cohen's d using pooled standard deviation was used to examine the effect size.

3. Results

3.1. M. officinalis Liquid Extract Ameliorates Mitochondrial Function

In a previous study, we found that the dry extract of *M. officinalis* ameliorated senescence by inhibiting mitochondria-enriched ROS production in senescent fibroblasts [12]. Based on these previous results, we hypothesized that *M. officinalis* liquid extract might have the potential to rejuvenate aged skin in clinical trials. To test this hypothesis, *M. officinalis* liquid extract (0.03%, 0.05%, and 0.1%) suitable for use in cosmetic formulations was prepared. We then examined whether these liquid extracts had antioxidant effects on senescent fibroblasts similar to those of the dry extract used in our previous study. Subsequently, the effects of *M. officinalis* liquid extract on mitochondria-enriched ROS levels were assessed by measuring the amounts of hydroxyl radicals and superoxide using DHR123 and MitoSOX, respectively. Young fibroblasts were used as a positive control. Mitochondria-enriched ROS levels in young fibroblasts were significantly lower than those in DMSO–treated senescent fibroblasts (Figure 1A,B). Moreover, mitochondria-enriched ROS levels in senescent fibroblasts treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%) were significantly reduced compared to those in senescent fibroblasts treated with DMSO (Figure 1A,B). These results suggest that *M. officinalis* liquid extract, as a precursor of cosmetic formulations, reduced mitochondria-enriched ROS production.

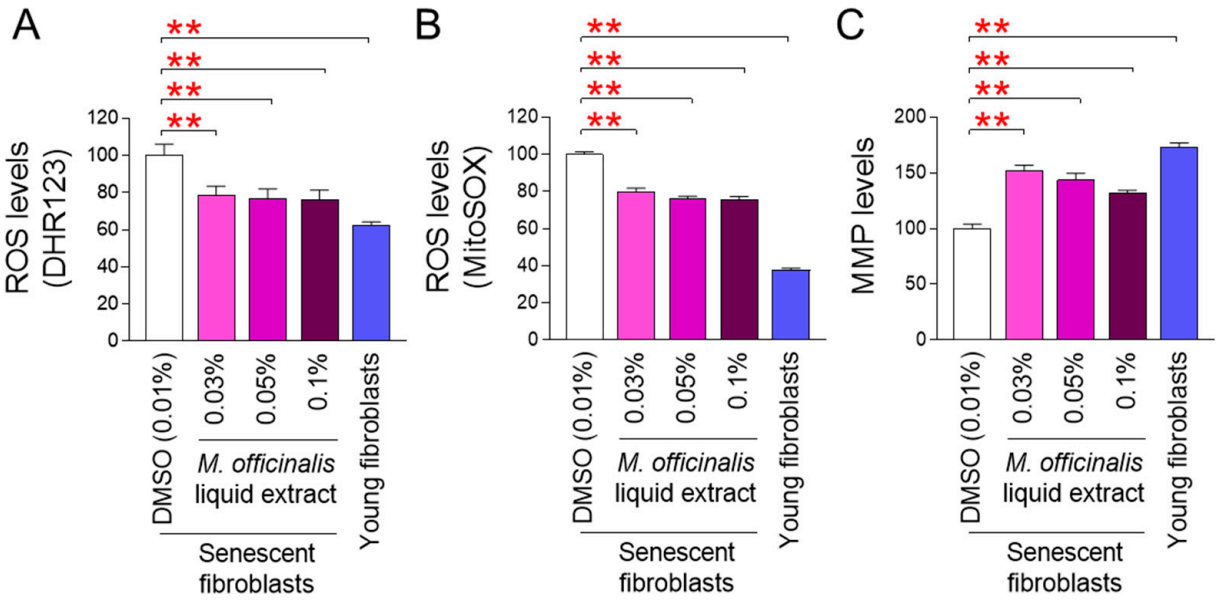

Figure 1. *M. officinalis* liquid extract ameliorates mitochondrial function. (**A**,**B**) Mitochondria-enriched ROS levels were measured in senescent fibroblasts administered with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Use of DHR123 (**A**) and MitoSOX (**B**) for flow cytometry. Young fibroblasts were used as positive control. ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates. (**C**) MMP levels were assessed in senescent fibroblasts administered with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates.

Since the most prominent mitochondrial damage induced by ROS is a reduction in mitochondrial membrane potential (MMP), we evaluated the effect of *M. officinalis* liquid extract on MMP. Young fibroblasts, used as a positive control, showed significantly higher MMP levels than senescent fibroblasts treated with DMSO (Figure 1C). Furthermore, the MMP levels of senescent fibroblasts treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%) were significantly upregulated compared to those of senescent fibroblasts treated with DMSO (Figure 1C). These results suggest that *M. officinalis* liquid extract increases MMP by lowering mitochondria-enriched ROS production.

3.2. M. officinalis Liquid Extract Restores Mitochondrial Metabolic Function

MMP is generated during oxidative phosphorylation (OXPHOS) in mitochondria. After confirming the impact of *M. officinalis* liquid extract on upregulating MMP, the oxygen consumption rate (OCR), a marker of OXPHOS efficiency, was examined [25]. OCR was evaluated sequentially after administration of oligomycin, carbonyl cyanide–p–trifluoromethoxyphenylhydrazone (FCCP), and rotenone/antimycin A. The OCR of young fibroblasts was significantly lower than that of senescent fibroblasts treated with DMSO, indicating that young fibroblasts consume less oxygen due to efficient OXPHOS (Figure 2A). Furthermore, senescent fibroblasts treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%) showed a significantly lower OCR than DMSO–treated senescent fibroblasts, suggesting that *M. officinalis* liquid extract increased OXPHOS efficiency (Figure 2A).

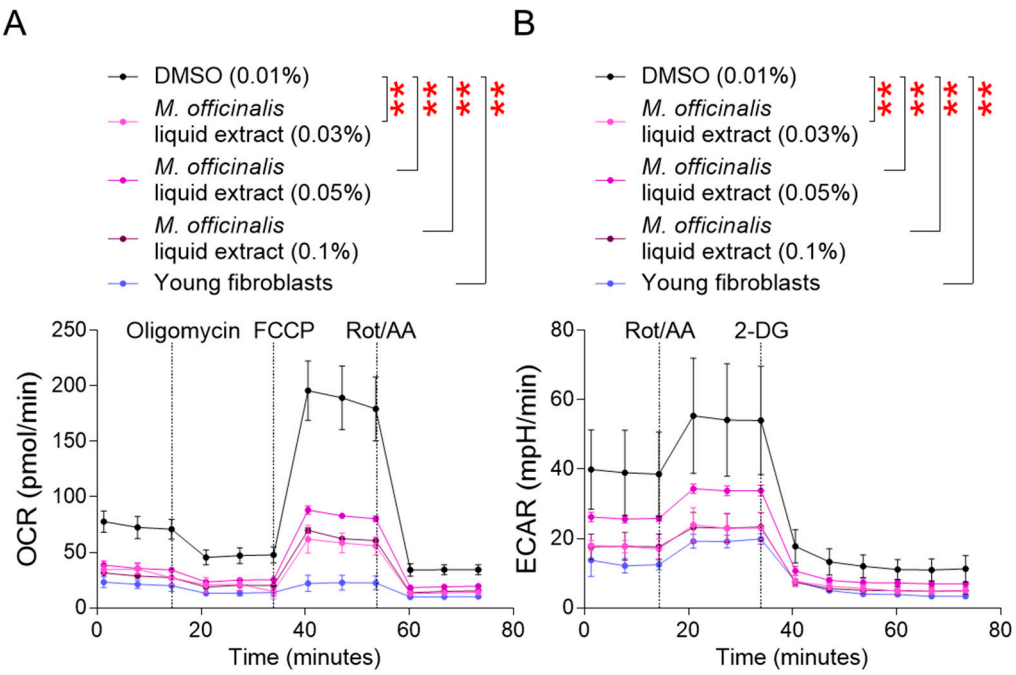

Figure 2. *M. officinalis* liquid extract restores mitochondrial metabolic function. (**A**) Oxygen consumption rate (OCR; pmol/min) was measured in senescent fibroblasts treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. ** $p < 0.01$, two–way ANOVA followed by Bonferroni's post hoc test. Means ± S.D., $n = 3$ as biological replicates. (**B**) Extracellular acidification rate (ECAR; mpH/min) was measured in senescent fibroblasts treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. ** $p < 0.01$, two–way ANOVA followed by Bonferroni's post hoc test. Means ± S.D., $n = 3$ as biological replicates.

Increased OXPHOS efficiency reduces the reliance on glycolysis [25]. The finding that *M. officinalis* liquid extract upregulates OXPHOS efficiency prompted us to study the reliance on glycolysis by measuring the extracellular acidification rate (ECAR) [25]. ECAR was measured before and after rotenone/antimycin A and 2–deoxy–D–glucose (2–DG) treatment [25]. Young fibroblasts' ECAR was significantly lower than that of DMSO–treated senescent fibroblasts, suggesting that young fibroblasts rely less on glycolysis than do senescent fibroblasts (Figure 2B). Additionally, the ECAR of senescent fibroblasts treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%) was significantly reduced compared to that of DMSO–treated senescent fibroblasts, indicating that the *M. officinalis* liquid extract decreased the reliance on glycolysis (Figure 2B).

3.3. M. officinalis Liquid Extract Induces Restoration of Mitophagy and Autophagy Activity

The finding that *M. officinalis* liquid extract restores mitochondrial function led to further investigation into the mechanism by which *M. officinalis* liquid extract exerts this effect. Mitophagy removes damaged mitochondria and restores mitochondrial function [26]. Therefore, we hypothesized that *M. officinalis* liquid extract restores mitochondrial function by activating mitophagy. Because mitophagy specifically removes defective mitochondria using autophagosomes [26], this was assessed by examining the co-localization of the autophagosome membrane protein microtubule-associated protein 1A/1B–light chain 3B (LC3B) with mitochondria. Young fibroblasts exhibited a significant increase in the co-localization compared to DMSO–treated senescent fibroblasts (Figure S1; white arrows). After senescent fibroblasts were treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%), the co-localization significantly increased compared to DMSO–treated senescent fibroblasts

(Figure S1; white arrows). To further confirm the role of *M. officinalis* liquid extract in mitophagy, we included a group treated with chloroquine (CQ). CQ induces autophagosome accumulation by disrupting lysosomal pH, which increases the co-localization of LC3B and mitochondria. CQ treatment increased colocalization in both young and senescent fibroblasts, providing proof of concept for the effect of CQ (Figure 3A,B; white arrows). Furthermore, senescent fibroblasts co-treated with CQ and *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%) showed the significant increase in the co-localization compared to senescent fibroblasts co-treated with CQ and DMSO (Figure 3A,B; white arrows). These results suggest that *M. officinalis* liquid extract promotes mitophagy activation.

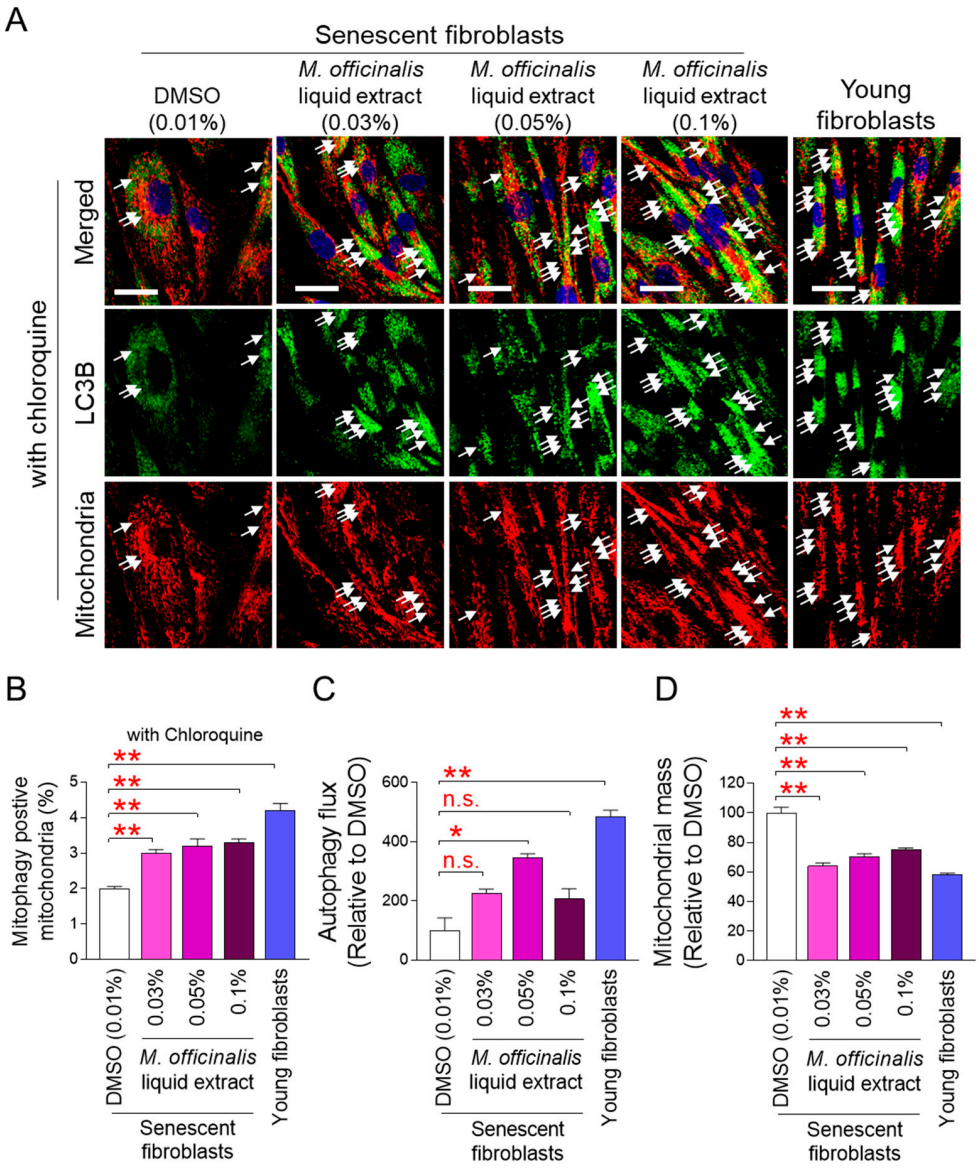

Figure 3. *M. officinalis* liquid extract induces restoration of mitophagy and autophagy activity. (**A**,**B**) Immunostaining for LC3B (green) and mitochondria (red). Senescent fibroblasts were treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. 20 μM chloroquine was added to cells 24 h before immunofluorescence analysis. Scale bar 10 μm. Mitophagy is indicated by a white arrow. ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates. (**C**,**D**) Autophagy flux or mitochondrial mass was assessed in senescent fibroblasts administered with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. n.s. (not significant), * $p < 0.05$, ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates.

Next, we measured autophagic flux to quantify mitophagy activation by the *M. officinalis* liquid extract. Autophagic flux is the rate at which autophagy removes damaged organelles [27]. Young fibroblasts exhibited significantly higher autophagic flux than DMSO–treated senescent fibroblasts, indicating active removal of damaged organelles by autophagy (Figure 3C). Treatment of senescent fibroblasts with the *M. officinalis* liquid extract at concentrations of 0.03% and 0.1% increased autophagic flux compared to DMSO–treated senescent fibroblasts (Figure 3C). Notably, treatment of senescent fibroblasts with the *M. officinalis* liquid extract at a concentration of 0.05% significantly increased autophagic flux compared to DMSO–treated senescent fibroblasts, suggesting that *M. officinalis* liquid extract at 0.05% concentration was more effective in increasing autophagy flux than other concentrations. (Figure 3C).

Because autophagy flux is the rate at which organelles, including damaged mitochondria, are removed, we measured mitochondrial mass to determine whether mitophagy removes damaged mitochondria. Compared to DMSO–treated senescent fibroblasts, young fibroblasts exhibited significantly less mitochondrial mass (Figure 3D). When senescent fibroblasts were administered with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%), mitochondrial mass was significantly decreased compared with DMSO–treated senescent fibroblasts (Figure 3D). These results suggest that mitophagy activation by *M. officinalis* liquid extract plays a key role in reducing mitochondrial mass in senescent fibroblasts.

3.4. M. officinalis Liquid Extract Rejuvenates Senescence-Associated Phenotypes

Restoration of mitochondrial function is essential for rejuvenating senescence [2]. The finding that *M. officinalis* liquid extract restores mitochondrial function prompted us to evaluate the effects of *M. officinalis* liquid extract on senescence. We assessed the impact of *M. officinalis* liquid extract on the quantity of lipofuscin, a cross-linked protein residue produced during senescence by iron-catalyzed oxidation [28]. Lipofuscin was assessed by measuring autofluorescence [28]. Young fibroblasts exhibited significantly lower autofluorescence than DMSO–treated senescent fibroblasts (Figure 4A). Treatment of senescent fibroblasts with *M. officinalis* liquid extract at a concentration of 0.03% did not reduce autofluorescence compared to DMSO–treated senescent fibroblasts (Figure 4A). However, when senescent fibroblasts were administered with the *M. officinalis* liquid extract at concentrations of 0.05% and 0.1%, autofluorescence was significantly reduced compared to senescent fibroblasts treated with DMSO (Figure 4A). These results suggest that autofluorescence was not effectively reduced when senescent fibroblasts were treated with the low concentration (0.03%) of the *M. officinalis* liquid extract, whereas autofluorescence was effectively reduced when senescent fibroblasts were administered with high concentrations (0.05% and 0.1%).

Slit–inducing ligand 2 (*SLIT2*) controls cell–cell connections to aid in the regeneration of skin tissue [29,30]. Since upregulation of *SLIT2* expression increases tissue regeneration capacity and improves skin barrier function, we investigated the impact of *M. officinalis* liquid extract on *SLIT2* expression. Young fibroblasts showed significantly higher *SLIT2* expression than senescent fibroblasts treated with DMSO, suggesting the efficient tissue regeneration capacity of young fibroblasts (Figure 4B). When senescent fibroblasts were administered with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%), *SLIT2* expression increased compared to senescent fibroblasts treated with DMSO (Figure 4B). These results suggest that *M. officinalis* liquid extract promotes regenerative capacity and contributes to the senescence rejuvenation. However, overexpression of *SLIT2* can promote skin tumorigenesis [30]. Therefore, while restoring *SLIT2* levels in senescent fibroblasts may be desirable, increasing *SLIT2* levels in young fibroblasts may be detrimental. Therefore, to rule out these side effects, we examined the expression of *SLIT2* in young fibroblasts treated with DMSO

(0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. When young fibroblasts were treated with *M. officinalis* liquid extract (0.03%, 0.05%, and 0.1%), *SLIT2* expression did not increase compared to young fibroblasts treated with DMSO (Figure S3). These results indicate that *M. officinalis* liquid extract does not promote *SLIT2* expression in young fibroblasts, and thus is unlikely to lead to skin tumorigenesis.

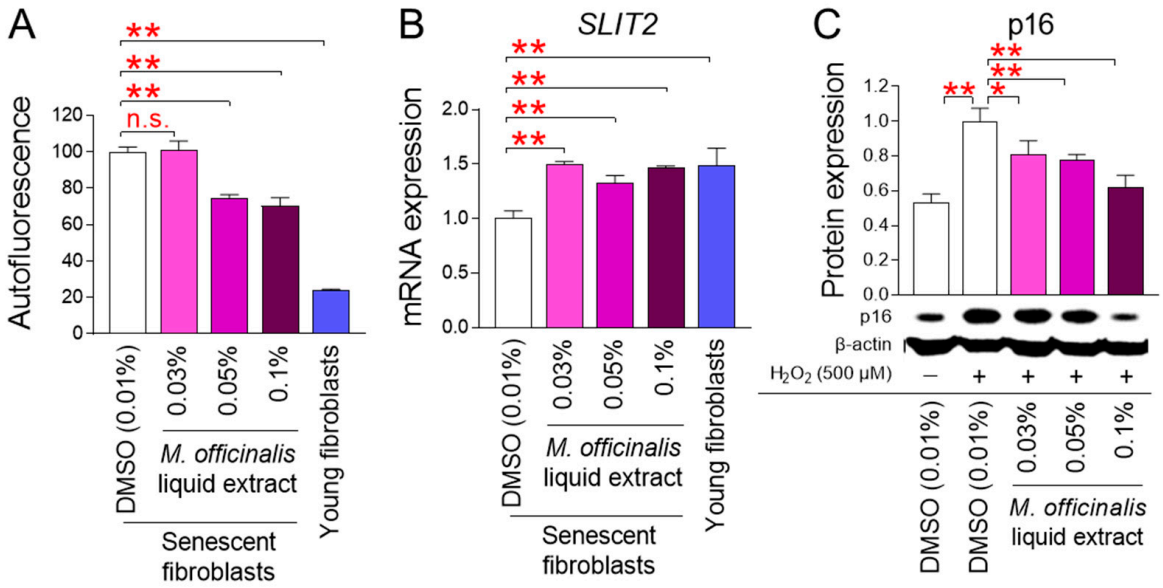

Figure 4. *M. officinalis* liquid extract rejuvenates senescence-associated phenotypes. (**A**,**B**) Autofluorescence (**A**) or *SLIT2* expression (**B**) was assessed in senescent fibroblasts treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. n.s. (not significant), ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates. (**C**) Expression levels of p16 protein after exposure to hydrogen peroxide (H_2O_2). Then, cells were administered with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. Young fibroblasts were used as positive control. * $p < 0.05$, ** $p < 0.01$, Student's *t*-test. Mean ± S.D., $n = 3$ as biological replicates.

Exposure to hydrogen peroxide (H_2O_2), a type of ROS, can induce stress-induced premature senescence (SIPS) [31]. SIPS is a form of senescence that exhibits characteristics similar to replicative senescence [32]. A model that investigates the expression of p16, a cyclin-dependent kinase inhibitor, after induction of SIPS using H_2O_2 has been widely used in senescence research [33]. In this study, young fibroblasts were treated with H_2O_2 to induce senescence. Then, we evaluated whether *M. officinalis* liquid extract could rejuvenate SIPS. Treatment of young fibroblasts with 500 μM H_2O_2 significantly increased p16 expression compared to the untreated group, suggesting that H_2O_2 induces SIPS (Figure 4C). When H_2O_2-induced fibroblasts were treated with *M. officinalis* liquid extract (0.03%, 0.05%, 0.1%), p16 expression was significantly reduced compared with H_2O_2-induced fibroblasts (Figure 4C). These results suggest that *M. officinalis* liquid extract is also effective in restoring SIPS.

The discovery that *M. officinalis* liquid extract is effective in rejuvenating senescence-associated phenotypes led us to provide the basic phytochemical profile of *M. officinalis* liquid extract. *M. officinalis* extract was known to be rich in bioactive phytochemicals, primarily lignans (magnolol & honokiol) along with phenolic compounds, alkaloids, and essential oils [11]. Two primary ingredients, honokiol and magnolol, have been known to have antioxidant effects [34]. Honokiol, a biphenolic lignan, has powerful antioxidant effects that may be beneficial in neuroprotection, anti-inflammatory, and anti-tumorigenic applications [35]. Magnolol is a biphenyl derivative that acts as an oxygen radical scavenger

through redox reactions. Therefore, high-performance liquid chromatography (HPLC) was conducted to evaluate how much honokiol and magnolol were present in the *M. officinalis* liquid extract. The HPLC peak of the *M. officinalis* liquid extract matched the honokiol standard, and the amount of honokiol present in the *M. officinalis* liquid extract was 12.20% (Figure S2). This result was very similar to the standard value of 14.2% from previous tests [36]. The HPLC peak of the *M. officinalis* liquid extract matched the magnolol standard, and its amount was 6.29% of the total extract (Figure S2). This result was similar to values from previous tests (0.78–7.65%) [37].

3.5. The Cream Containing *M. officinalis* Liquid Extract Is Effective in Reducing Neck Wrinkles

The discovery that *M. officinalis* liquid extract is effective in senescence rejuvenation in vitro led to a clinical trial to determine whether it also has anti-aging effects on human skin. The skin of the face and neck is more easily exposed to the external environment than other skin areas [38]. In particular, the skin of the neck is thinner than the facial skin, making it more prone to wrinkles [38]. Therefore, we investigated changes in neck wrinkles after topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) to the neck of participants for 4 weeks. The mean and maximum depth of neck wrinkles in participants who received the vehicle cream did not significantly decrease compared to baseline (Figure 5). Specifically, in participants who received the vehicle cream, the effect sizes for neck wrinkle depth and neck wrinkle maximum depth were 0.32 and 0.22, respectively, indicating a small effect size (Table S1). However, the mean and maximum depths of neck wrinkles in participants who received the cream containing *M. officinalis* liquid extract (3%) were significantly reduced by 12.73% and 17.44%, respectively, compared to baseline (Figure 5). Specifically, in participants who received the cream containing *M. officinalis* liquid extract (3%), the effect sizes for neck wrinkle depth and neck wrinkle maximum depth were 0.65 and 0.78, respectively, indicating a modest magnitude of change (Table S1). These results indicate that the cream containing *M. officinalis* liquid extract is effective in reducing neck wrinkles.

3.6. The Cream Containing *M. officinalis* Liquid Extract Is Effective in Enhancing Skin Elasticity

Loss of skin elasticity is a precursor to wrinkles [39]. Our discovery that a cream containing *M. officinalis* liquid extract was effective in reducing neck wrinkles led us to investigate its effect on skin elasticity, a precursor to wrinkles. Here, changes in skin elasticity were observed after topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) to the participants' skin for 4 weeks. The skin elasticity of participants who received the vehicle cream did not significantly increase compared to the baseline (Figure 6). Specifically, in participants who received the vehicle cream, the effect size for the skin elasticity was −0.08, indicating a negligible effect size (Table S2). However, the skin elasticity of participants who received the cream containing *M. officinalis* liquid extract (3%) significantly increased by 3.76% compared to the baseline (Figure 6). Specifically, in participants who received the cream containing *M. officinalis* liquid extract (3%), the effect size for the skin elasticity was −0.59, indicating a moderate effect size (Table S2). These results suggest that the cream containing *M. officinalis* liquid extract is effective in improving skin elasticity.

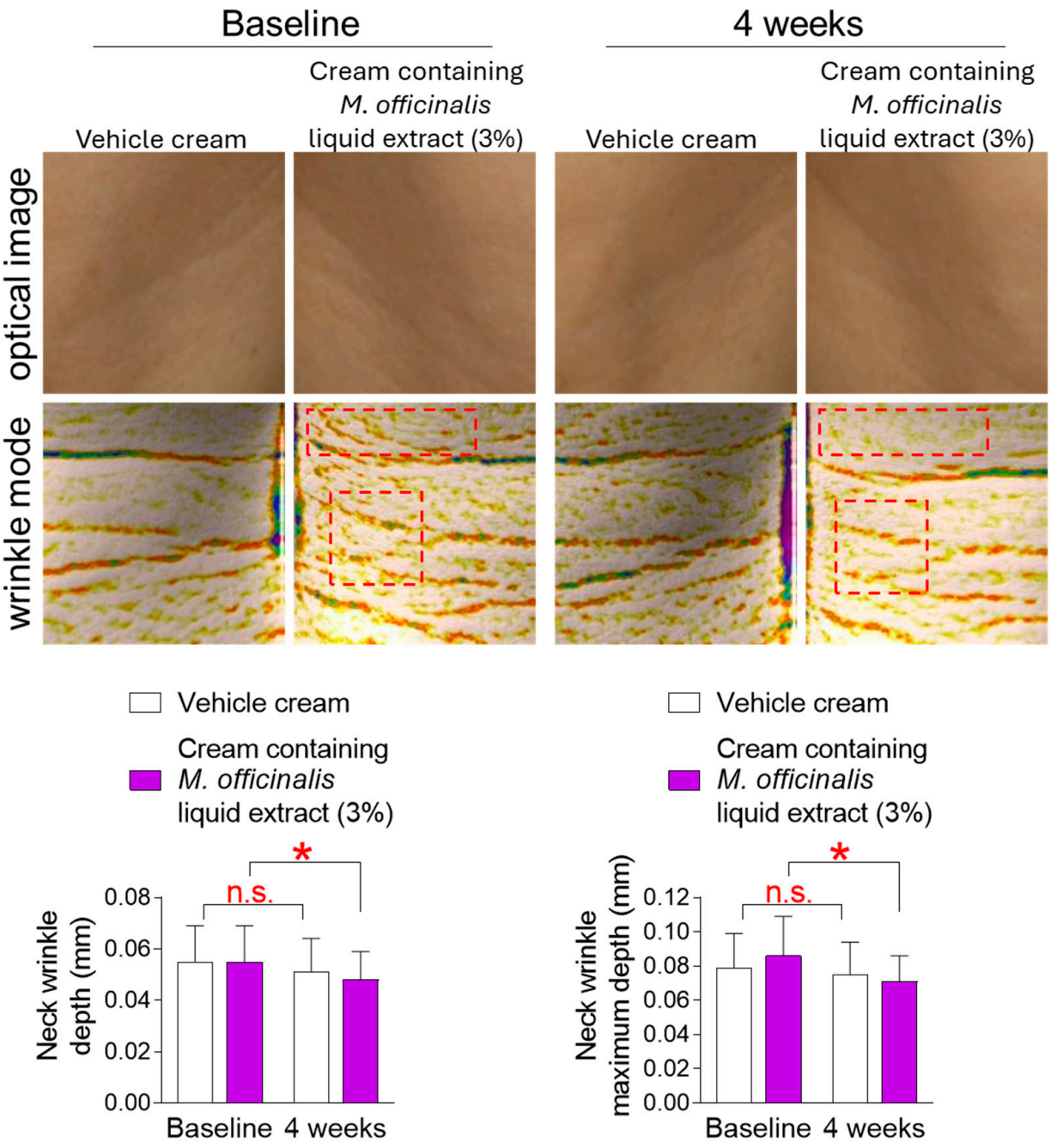

Figure 5. The cream containing *M. officinalis* liquid extract is effective in reducing neck wrinkles. Images of the left and right neck areas were taken after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) to the neck of participants for 4 weeks. The mean and maximum depth of neck wrinkles were measured on the same area at the start of treatment (baseline) and 4 weeks later (4 weeks). Red dotted box: Representative areas showing improvement in neck wrinkles before and after treatment. n.s. (not significant), * $p < 0.05$, RM-ANOVA. Mean ± S.D., $n = 21$. Details for graphs (mean, standard deviation, 95% confidence interval, sample size, and effect size) were shown in Table S1.

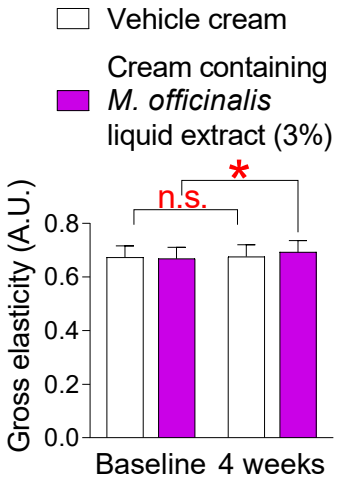

Figure 6. The cream containing *M. officinalis* liquid extract is effective in enhancing skin elasticity. The skin elasticity was measured after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) to the skin of participants for 4 weeks. The elasticity of left and right cheeks was measured on the same area at the start of treatment (baseline) and 4 weeks later (4 weeks). n.s. (not significant), * $p < 0.05$, RM-ANOVA. Mean ± S.D., $n = 21$. Details for graphs (mean, standard deviation, 95% confidence interval, sample size, and effect size) were shown in Table S2.

3.7. The Cream Containing M. officinalis Liquid Extract Is Effective in Improving Skin Texture

An uneven or rough skin surface is caused by age–related changes, such as skin wrinkling, that alter the skin surface [40]. The mean roughness (Ra), root mean square roughness (Rq), and maximum depth of roughness (Rmax) are parameters used to quantify surface texture. Here, changes in skin texture were observed after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) around participants' cheeks for 4 weeks. Ra, Rq, and Rmax in participants who received the vehicle cream did not decrease significantly compared to baseline (Figure 7). Specifically, in participants who received the vehicle cream, effect sizes for Ra, Rq, and Rmax were 0.035, 0.055, and 0.090, respectively, indicating negligible effect sizes (Table S3). However, in participants who received the cream containing *M. officinalis* liquid extract (3%), Ra, Rq, and Rmax were significantly reduced by 12.73%, 10.16%, and 10.81%, respectively, compared to baseline (Figure 7). Specifically, in participants who received the cream containing *M. officinalis* liquid extract (3%), effect sizes for Ra, Rq, and Rmax were 0.48, 0.48, and 0.50, respectively, indicating moderate effect sizes (Table S3). These results suggest that the cream containing *M. officinalis* liquid extract is effective in making the skin surface smoother and more uniform.

3.8. The Cream Containing M. officinalis Liquid Extract Is Effective in Improving Skin Complexion

Skin complexion refers to the overall condition of the skin, including both the skin's natural color and the color beneath the skin's surface [41]. Hyperpigmentation is a darkening of the skin color caused by increased melanin production in melanocytes during the aging process [41]. Skin flushing is another characteristic of skin aging, resulting from the loss of collagen and vascular elasticity in the dermis [6]. The L* value is a parameter used to quantify the degree of skin complexion [42]. Specifically, the L* value represents the skin's brightness, with higher values indicating lighter skin and lower values indicating darker skin. Here, the degree of skin complexion was observed after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) to participants' cheeks for 4 weeks. The L* value of participants receiving the vehicle cream did not significantly increase compared to baseline (Figure 8). Specifically, in participants who received the

vehicle cream, the effect size for the L* value was −0.07, indicating a negligible effect size (Table S4). However, the L* value of participants receiving the cream containing *M. officinalis* liquid extract (3%) significantly increased by 0.76% compared to baseline (Figure 8). Specifically, in participants who received the cream containing *M. officinalis* liquid extract (3%), the effect size for the L* value was −0.25, indicating a moderate amount of change (Table S4). These data indicate that the cream containing *M. officinalis* liquid extract is effective in whitening skin.

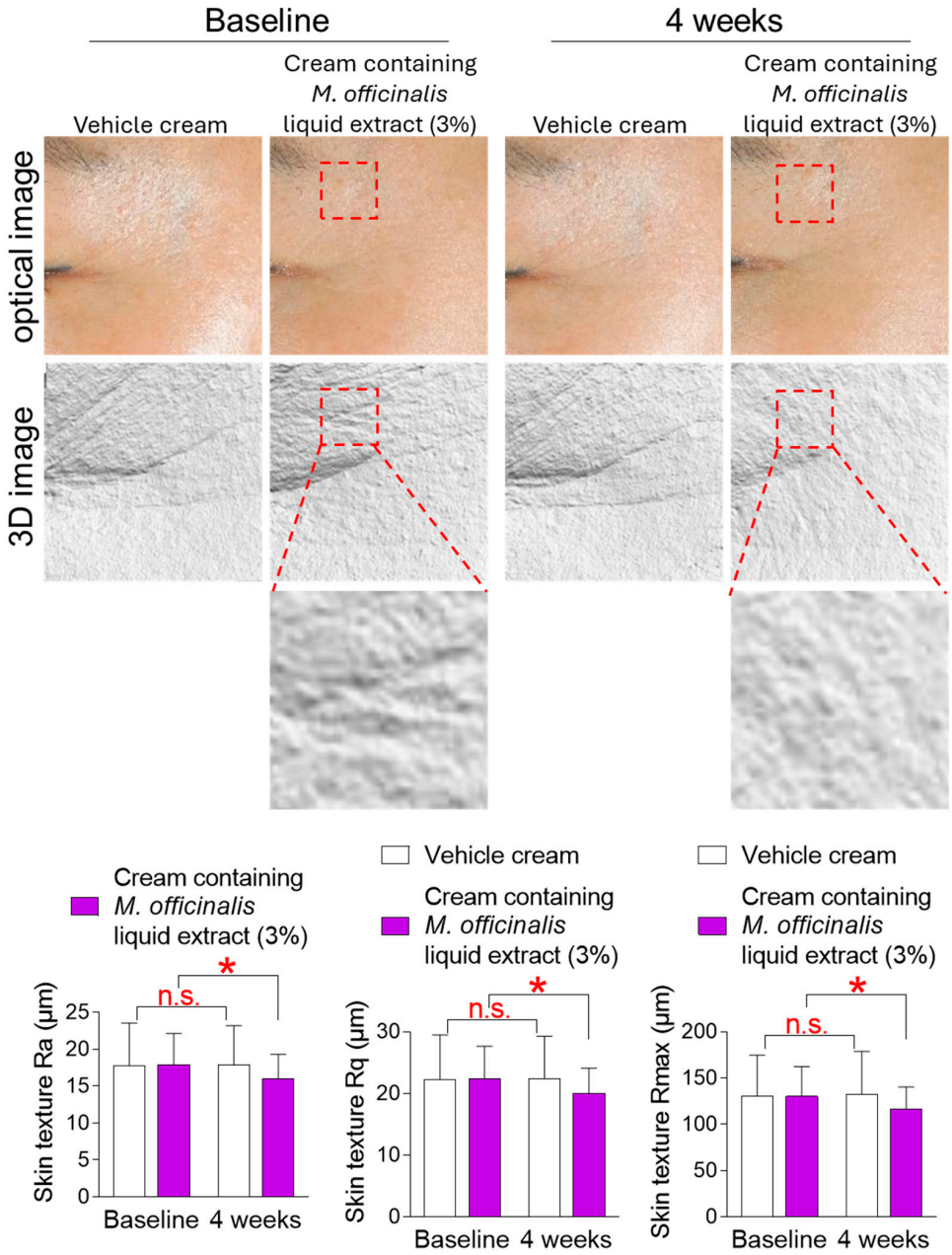

Figure 7. The cream containing *M. officinalis* liquid extract is effective in improving skin texture. Images of the left and right cheek areas were taken after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) around the cheeks of participants for 4 weeks. The mean roughness (Ra), root mean square roughness (Rq), and maximum depth of roughness (Rmax) were measured on the same area at the start of treatment (baseline) and 4 weeks later (4 weeks). Red dotted box: Representative areas showing improvement in skin texture before and after treatment. n.s. (not significant), * $p < 0.05$, RM-ANOVA. Mean ± S.D., $n = 21$. Details for graphs (mean, standard deviation, 95% confidence interval, sample size, and effect size) were shown in Table S3.

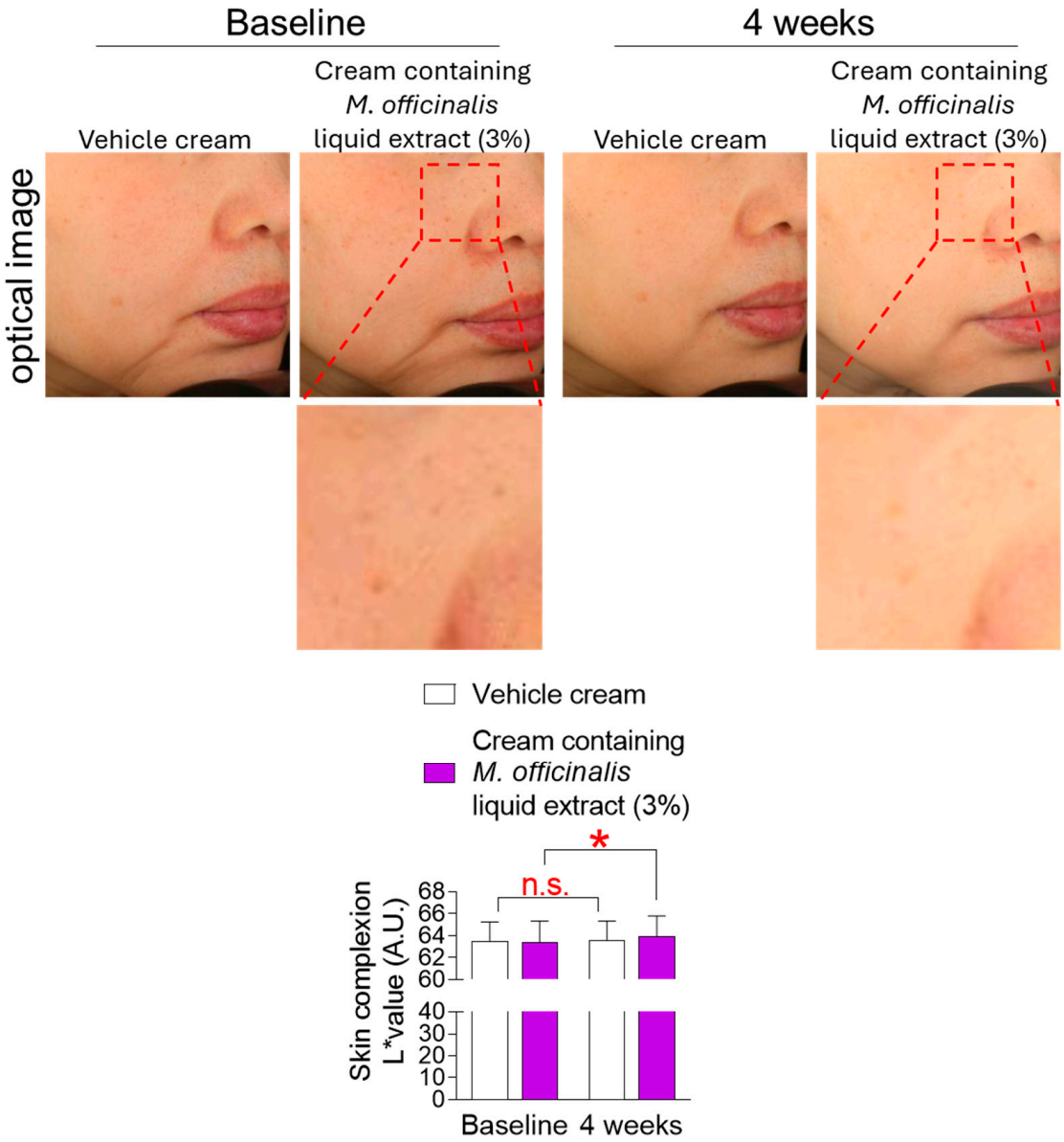

Figure 8. The cream containing *M. officinalis* liquid extract is effective in improving skin complexion. Images of the left and right cheek areas were taken after the topical application of a vehicle cream or a cream containing *M. officinalis* liquid extract (3%) around the cheeks of participants for 4 weeks. The L* value was measured in the same area at the start of treatment (baseline) and 4 weeks later (4 weeks). Red dotted box: Representative areas showing improvement in skin complexion before and after treatment. n.s. (not significant), * $p < 0.05$, RM-ANOVA. Mean ± S.D., $n = 21$. Details for graphs (mean, standard deviation, 95% confidence interval, sample size, and effect size) were shown in Table S4.

4. Discussion

One of the main causes of skin aging is oxidative stress caused by ROS [43]. ROS weakens the collagen that makes up the dermal layer, compromising the structural integrity of subcutaneous tissue [7]. Furthermore, ROS weakens the proteins and phospholipids that make up cell membranes, compromising cell structure. The instability or unpaired electrons of ROS can further exacerbate their detrimental effects on skin aging [44]. This property allows ROS to steal electrons from nearby molecules, triggering an oxidative chain reaction in nearby tissues [44]. Therefore, ROS causes significant damage to the skin, thereby exacerbating skin aging [8]. In this study, we found that *M. officinalis* liquid

extract restored mitochondrial function, thereby reducing mitochondria-enriched ROS levels. Specifically, upregulation of OXPHOS efficiency and downregulation of glycolysis dependence suggest that *M. officinalis* liquid extract restored mitochondrial function. In subsequent clinical trials, *M. officinalis* liquid extract improved skin elasticity loss caused by ROS-induced collagen–elastin chain scission and collagen oxidation. Here, we propose that reducing mitochondria-enriched ROS production using *M. officinalis* liquid extract may be the first step in reducing skin damage, thereby rejuvenating skin aging.

Mitochondria are highly dynamic cellular organelles that constantly undergo cell division and fusion. However, dysfunctional mitochondria fail to reintegrate into the mitochondrial network and are eliminated through autophagy [23]. Maintaining cellular homeostasis relies on the selective removal of damaged mitochondria through mitophagy, as damaged mitochondria have a reduced ability to generate ATP and increase ROS production [24]. In this study, we found that the *M. officinalis* liquid extract stimulates mitophagy. *M. officinalis* liquid extract may contribute to reducing mitochondria-enriched ROS levels by removing dysfunctional mitochondria through this mitophagy activation effect. However, pharmacological or genetic inhibition experiments have not been conducted to confirm the necessity of the mitophagy activation by *M. officinalis* liquid extract. If such experiments were conducted, they would provide evidence that mitophagy induced by *M. officinalis* liquid extract is essential for reducing mitochondria-enriched ROS. Direct evidence of the effect of *M. officinalis* liquid extract on mitochondria-enriched ROS would provide a theoretical basis for its application in the treatment of skin aging.

Skin wrinkles are major visual indicators of skin aging [45]. Wrinkles are caused by damage and degradation of collagen and elastin, which are essential for maintaining skin elasticity [45,46]. In senescent fibroblasts, impaired mitophagy leads to the accumulation of dysfunctional mitochondria, which in turn triggers excessive mitochondrial ROS production, promoting oxidative damage and collagen degradation [47]. Conversely, restoring mitophagy activates a cytoprotective mechanism that reduces wrinkle severity by removing damaged mitochondria and maintaining the structural integrity of the extracellular matrix [48,49]. Previous studies have shown that *M. officinalis* dry extract promotes skin regeneration, demonstrating upregulation of genes associated with skin regeneration and downregulation of genes associated with collagen degradation [12]. However, previous studies were limited to in vitro studies and did not directly investigate the effect of *M. officinalis* in vivo. This study investigated the effects of a cream containing *M. officinalis* liquid extract on skin wrinkles. Topical application of a cream containing *M. officinalis* liquid extract was effective in reducing the average and maximum wrinkle depths on the neck. These beneficial effects are further supported by the result showing that the cream containing *M. officinalis* liquid extract increases skin elasticity, which plays a key role in preventing wrinkles [46]. This study is the first to demonstrate that the cream containing *M. officinalis* liquid extract can improve skin wrinkles by increasing skin elasticity. Although this study suggests that *M. officinalis* liquid extract may reverse signs of skin wrinkles, further studies are needed to confirm the direct effects of *M. officinalis* liquid extract on mitophagy activation, mitochondria-enriched ROS reduction, and subsequent improvement of wrinkles.

Skin quality encompasses the overall condition of the skin, including its texture and complexion [50]. Skin texture refers to the feel of the skin surface (e.g., smooth or rough), whereas skin complexion refers to the overall color and tone of the skin [50]. Previous studies have shown that *M. officinalis* dry extract increases skin turnover, which plays a crucial role in maintaining skin texture [12]. Furthermore, *M. officinalis* dry extract has been shown to reduce skin hyperpigmentation by decreasing the expression of genes that induce skin hyperpigmentation and increasing the expression of genes that reduce skin

hyperpigmentation [12]. Based on these findings, this study investigated the effects of the cream containing *M. officinalis* liquid extract on skin texture and complexion. Topical application of the cream containing *M. officinalis* liquid extract significantly reduced all indices used to quantify skin texture. Furthermore, the cream showed both improved skin color and tone. To our knowledge, this is the first study to demonstrate that the cream containing *M. officinalis* liquid extract improves skin texture and complexion, suggesting its potential as a high-quality cosmetic ingredient.

The clinical results of this study suggest that *M. officinalis* liquid extract can contribute to the recovery of skin aging. The reduction in neck wrinkle depth suggests that the cream containing *M. officinalis* liquid extract significantly improves morphological deterioration of skin caused by aging. Specifically, the results based on objective indicators such as skin texture and complexion suggest that the cream containing *M. officinalis* liquid extract has a positive effect on the overall skin appearance. This study will serve as a touchstone for the development of cosmetics effective in improving skin aging. If this study is validated in a broader clinical setting in the future, the potential of *M. officinalis* liquid extract as a cosmetic ingredient will be further expanded.

The reduction in mitochondrial ROS demonstrated in fibroblast-based experiments is proposed to underlie the clinical improvements observed in this study. However, biomarkers such as mitochondrial ROS have not been evaluated in human skin. Therefore, future clinical studies should directly assess biomarkers linked to the mechanisms identified in fibroblast-based experiments within human skin to determine whether these mechanistic effects are reproducible in a clinical context. Such an approach would help establish the mechanistic findings from fibroblast-based experiments as direct evidence supporting clinical improvement.

In cell-based experiments, *M. officinalis* liquid extract was used at concentrations of 0.03%, 0.05%, and 0.1%, while the clinical study used a 3% concentration. This difference is due to the fact that in cell-based experiments, the active ingredient is directly exposed to cells, allowing it to be effective even at relatively low concentrations. Conversely, topical application to human skin has limited penetration and bioavailability, necessitating higher concentrations [51]. However, further research is needed to determine the optimal concentration of *M. officinalis* liquid extract in a cream that can penetrate the skin barrier and exert physiological effects. Establishing an optimal concentration that satisfies efficacy is expected to strengthen the scientific basis for the use of *M. officinalis* liquid extract in anti-aging skincare.

The 0.76% increase in L* values observed in participants using the cream containing *M. officinalis* liquid extract was statistically significant, suggesting an improvement in skin tone. However, the magnitude of this change is small and may not be noticeable outside of a controlled laboratory setting [52]. Furthermore, the observed increase may not reach the threshold considered clinically significant in cosmetic dermatology [53]. Therefore, the practical significance of this result should be interpreted cautiously. Further studies are needed to elucidate the effect of cream containing *M. officinalis* liquid extract on skin complexion by simultaneously measuring the a* value, which indicates the degree of skin redness, and the b* value, which indicates the degree of skin yellowness, in addition to the L* value.

The in vitro experiments in this study were conducted using three biological replicates, a method commonly used in studies investigating biological effects [54,55]. However, this limited sample size reduces statistical power and increases the risk of false-positive results, especially when measuring multiple comparisons [56]. Therefore, future studies should use larger sample sizes and apply appropriate corrections for multiple comparisons to improve the reproducibility of the results.

There are several considerations when interpreting the results of this clinical study. First, the relatively small sample size (n = 21) may limit the statistical power of the study results. Therefore, larger studies are needed to confirm the reproducibility of the observed skin aging improvement effects. Second, the study subjects consisted of a single ethnic group. Because skin structure and response to topical treatments may vary by ethnicity, it is uncertain whether these results can be applied to other populations. Future studies that include a more diverse ethnic group are needed to assess the broad applicability of the results. Third, the study period was relatively short. While significant improvements in skin aging indicators were observed during the 4-week intervention period, long-term studies are needed to assess the sustained efficacy and safety of a cream containing *M. officinalis* liquid extract (3%).

5. Conclusions

In conclusion, this study found that *M. officinalis* liquid extract restored mitochondrial function and reduced mitochondria-enriched ROS production. Furthermore, activating mitophagy to remove defective mitochondria was one of the strategies by which *M. officinalis* liquid extract reduced mitochondria-enriched ROS production. In a clinical study, the cream containing *M. officinalis* liquid extract improved morphological indicators of skin aging by improving neck wrinkles. Furthermore, the cream containing *M. officinalis* liquid extract improved skin texture and complexion, thereby enhancing overall skin appearance. *M. officinalis* liquid extract has antioxidant properties that help alleviate skin aging and show promise as a next-generation anti-aging cosmetic ingredient.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cosmetics13010022/s1, Figure S1: Immunostaining for LC3B (green) and mitochondria (red); Figure S2: Identification of honokiol and magnolol from *M. officinalis* liquid extracts; Figure S3: *SLIT2* expression was assessed in young fibroblasts treated with DMSO (0.01%) or *M. officinalis* liquid extract (0.03%, 0.05% and 0.1%) every 4 days for 12 days. n.s. (not significant), Student's *t*-test. Mean ± S.D., n = 3; Table S1: Details for graphs shown in Figure 5 (mean, standard deviation, 95% confidence interval, sample size, and effect size); Table S2: Details for graphs shown in Figure 6 (mean, standard deviation, 95% confidence interval, sample size, and effect size); Table S3: Details for graphs shown in Figure 7 (mean, standard deviation, 95% confidence interval, sample size, and effect size); Table S4: Details for graphs shown in Figure 8 (mean, standard deviation, 95% confidence interval, sample size, and effect size).

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