Combating Oxidation, Glycation, and Inflammation in Skin: A Holistic Anti-Aging Complex Containing Ergothioneine

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Abstract

Oxidative stress, inflammatory reactions, and glycosylation reactions interact and influence each other, causing a range of skin issues, including wrinkles, sagging, darkening, and roughness,significantly compromising both skin health and psychological well-being.To address this multifactorial challenge, we developed a novel anti-aging complex comprising ergothioneine, Leontopodium alpinum flower/leaf extract, and Rosa roxburghii fruit extract. The botanical extracts potentiate ergothioneine's anti-glycation and anti-inflammatory properties.Comprehensive in vitro evaluations demonstrated the complex's multifunctional efficacy: it exhibited superior synergistic antioxidant activity and remarkable anti-glycation effects . In UVB-irradiated HaCaT keratinocytes, the formulation significantly suppressed pro-inflammatory cytokine（IL-6 and IL-1β）release. Furthermore, it showed potent anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages.These findings substantiate that our ergothioneine-based composite represents a scientifically validated, multi-targeted therapeutic strategy for age-related skin concerns, particularly offering a promising dermatological solution for individuals with sensitive skin phenotypes.

Keywords: Holistic anti-aging; Synergistic effect; Sensitive skin; Oxidative stress

INTRODUCTION

Under normal physiological conditions, free radicals in the human body maintain a dynamic equilibrium state. Endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), effectively scavenge excess free radicals to maintain homeostasis.

With advancing age and the influence of detrimental lifestyle factors, the activity of these endogenous antioxidant enzymes becomes compromised, disrupting the redox balance and resulting in excessive free radical production. The accumulation of free radicals within cells can induce cellular apoptosis and tissue damage, representing a principal contributor to the aging process.

A close interrelationship exists between cutaneous oxidative stress and glycation. Oxidative stress not only generates substantial reactive oxygen species (ROS), but

also serves as a critical step in the formation of advanced glycation end products (AGEs). Notably, oxidative stress potentiates non-enzymatic glycation, while AGEs reciprocally exacerbate oxidative stress levels.

The AGEs-RAGE interaction activates NADPH oxidase (NOX) activity, thereby amplifying oxidative stress and triggering excessive ROS production. These ROS subsequently activate the NF-κB pathway, a pivotal regulator of inflammatory responses, leading to upregulated expression of various cytokines (including IL-6, TNF-α, and IL-1β), chemokines, and other pro-inflammatory mediators, ultimately resulting in cellular apoptosis and inflammatory cascades.

The interplay among oxidative stress, inflammatory responses, and glycation reactions creates a vicious cycle that manifests clinically as diverse cutaneous manifestations, including wrinkle formation, skin laxity, dull complexion, sallow discoloration, and textural roughness.

During states of intracellular free radical excess, ergothioneine is selectively transported into cellular organelles via the OCTN1 transporter. Upon normalization of cellular redox status, SLC22A4 gene expression is downregulated, OCTN1 transporter activity decreases, and intracellular ROS levels are restored through direct radical scavenging mechanisms. Leontopodium alpinum flower/leaf extract, derived from the alpine edelweiss plant, contains abundant leontopodic acids and flavonoids that exhibit dual functionality: direct free radical neutralization and suppression of AGEs formation pathways. Rosa roxburghii fruit extract, rich in vitamin C derivatives and polyphenolic compounds, demonstrates remarkable anti-inflammatory and antioxidant properties, effectively inhibiting damage-associated molecular patterns (DAMPs) induced by UV irradiation and suppressing IL-8 production.

MATERIALS AND METHODS

Materials

Ergothioneine,Powder, 100% effective content；

Leontopodium alpinum flower/leaf extract,Solution, effective content 7.5%；

Rosa roxburghii fruit extract,Solution, effective content 0.65%；

Novel anti-aging complex,Mix Ergothioneine, Leontopodium alpinum flower/leaf extract and Rosa roxburghii fruit extract in a certain proportion.

Method

Cell culture

HaCaT keratinocytes (Chinese Academy of Sciences, Kunming, China) and RAW264.7 cells (Procell Life Science and Technology Co., LTD,Wu han,China) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco) . Both HaCaT keratinocytes and RAW264.7 cells were incubated at 37 ◦C in a humidified atmosphere containing 5% CO2.

UVB radiation

HaCaT cells were divided into three groups: the control group, the model group, ie UV radiated group, and the test sample group. The control group received no treatment. Prior to UVB irradiation, the culture medium was completely removed from

the culture dishes. To ensure uniform irradiation and eliminate potential interference from medium components, the cells were gently rinsed twice with phosphate-buffered saline (PBS) to remove any residual medium and metabolic byproducts. Then 300 mJ/cm2 doses of UVB was used to irradiate HaCaT keratinocytes, during irradiation, a thin layer of PBS was maintained on the cell surface to prevent dehydration, as PBS exhibits minimal UVB absorption.

Antioxidant Capacity

Principle: ABTS is oxidized by oxidants (e.g., potassium persulfate or manganese dioxide) to form a stable bluish-green cationic radical（ABTS+·）, which exhibits a characteristic absorption peak at 734 nm. Antioxidants scavenge（ABTS+·）, leading to a reduction in absorbance that is proportional to their antioxidant capacity.

Experimental Procedure: The ABTS stock solution was mixed with potassium persulfate solution and incubated in the dark at room temperature for 12–16 h. The resulting （ABTS+·） working solution was diluted with solvent. Using Trolox as a positive control, sample, positive control, blank, and background control groups were established. In a 96-well plate, test samples and blank solvent were added, followed by（ABTS+·） working solution, while the background control received blank solvent. After 6 min of incubation, absorbance was measured, and （ABTS+·） radical scavenging rate (%) was calculated as:

$$\text{Scavenging rate } (\%) = \left[\frac{A_{\text{blank}} - (A_{\text{sample}} - A_{\text{background}})}{A_{\text{blank}}}\right] \times 100\%$$

Experimental Procedure: Using Trolox as the positive control, experimental groups were established including the sample group, positive control group, blank group, and background control group. In a 96-well plate, test samples and blank solvent were added, followed by DPPH solution, while the background control received an equal volume of DPPH solvent. After 30 min of reaction at room temperature, absorbance was measured at 517 nm. The DPPH radical scavenging rate (%) was calculated as follows:

$$\text{Scavenging rate } (\%) = \left[\frac{A_{\text{blank}} - (A_{\text{sample}} - A_{\text{background}})}{A_{\text{blank}}}\right] \times 100\%$$

Anti-glycation Capacity

Advanced glycation end products (AGEs) exhibit intrinsic fluorescence properties. An in vitro glycation model was established by incubating glucose with bovine serum albumin (BSA) to generate AGEs. The anti-glycation efficacy of test compounds was evaluated by quantifying fluorescence intensity, which reflects their inhibitory effects on AGEs formation.

Aminoguanidine (AG) was employed as the reference standard. A dose-response curve was constructed, demonstrating that AGEs inhibition by AG increased proportionally with concentration until reaching a plateau phase at concentrations exceeding 6 mM. In each experiment, AG was included as an internal control to validate system stability.

Anti-inflammatory Capacity：

Total RNA was extracted using HiPure Total RNA Mini Kit (Magen, Guangzhou, China) following the protocol, which was then reverse transcribed to cDNA, then qRT-PCR was performed to assess gene expression level. Target gene PCR reactions were quantitated using Takara's RR820A TB Green Premix Ex Taq II (Tli RNaseH Plus). PCR reaction system (20 μL): TB Green Premix 10 μL, cDNA template 2 μL, forward and reverse primers 0.4 μL each, double distilled water 7.2 μL. PCR reaction conditions: 96 °C 1 min, 95 °C 15s, 60 °C 1 min, 95 °C 15s, total 40 cycles. Using the internal reference gene GADPH, the 2−ΔΔCt technique was used to determine the target gene's relative expression.

Soothing Capacity

RAW264.7 cells were seeded at a density of 1.0x105 cells/ml in 96- well plates for 24h. Then,they were treated with various concentrations of test samples and LPS (1μg/mL) for 24h,respectively,there were four parallel wells in each group; After 24h of treatment, the supernatant wers harvested and subjected to the Griess reaction according to the NO measurement kit protocol,and a 540nm absorbance was used for the measurement by a microplate reader.

Results

Antioxidant Capacity

The anti-aging composite exhibits strong synergistic antioxidant effects against both ABTS and DPPH free radicals.

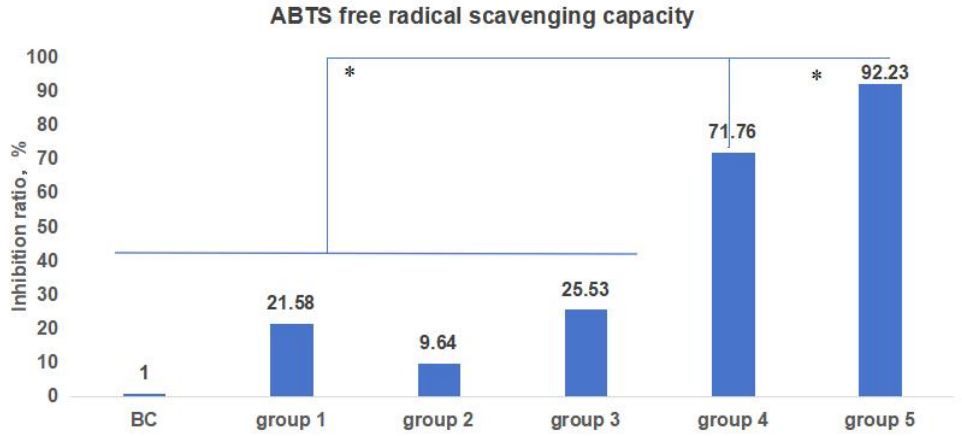

Figure 1.The inhibition ratio of ABTS free radicals（*,P＜0.05）

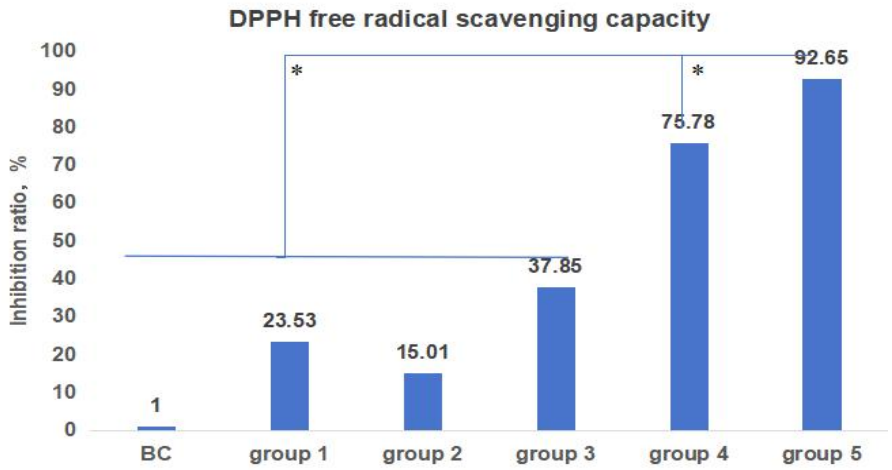

Figure 2.The inhibition ratio of DPPH free radicals（*,P＜0.05）

group 1:Leontopodium alpinum flower/leaf extract; group 2:rosa roxburghii fruit extract; group 3:Leontopodium alpinum flower/leaf extract + rosa roxburghii fruit extract;group 4:ergothioneine;group 5:holistic anti-aging complex

Anti-glycation Capacity

The anti-aging composite demonstrates potent synergistic anti-glycation activity.The Leontopodium alpinum flower/leaf extract and Rosa roxburghii fruit extract potentiate the anti-glycation and anti-inflammatory activities of ergothioneine.

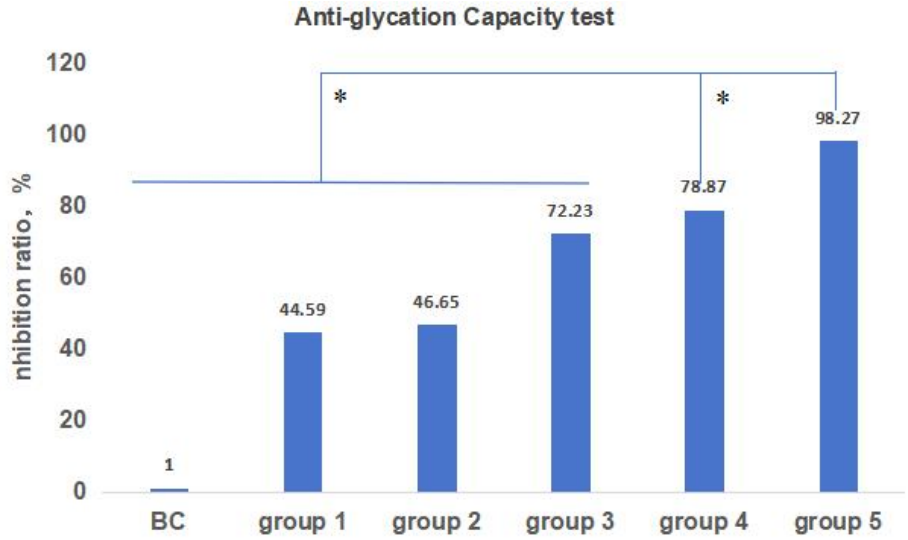

Figure 3.The inhibition ratio of AGEs（*,P＜0.05）

group 1:ergothioneine; group 2:Leontopodium alpinum flower/leaf extract; group 3:rosa roxburghii fruit extract;group 4:ergothioneine+Leontopodium alpinum flower/leaf extract;group 5:holistic anti-aging anti-aging complex

Anti-inflammatory Capacity

The anti-aging composite significantly suppresses the production of pro-inflammatory cytokines, with particularly pronounced inhibitory effects on interleukin-6 (IL-6) and interleukin-1β (IL-1β).

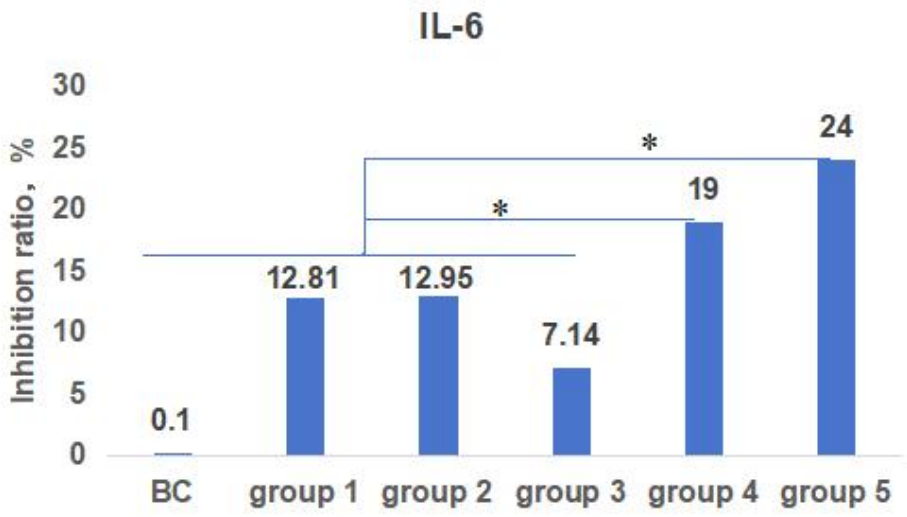

Figure 4. The inhibition ratio of IL-6

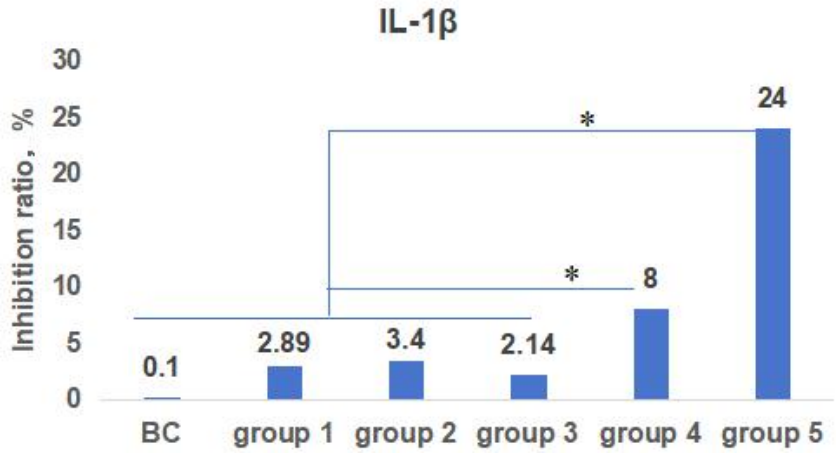

Figure 5. The inhibition ratio of IL-1β（*,P＜0.05）

group 1:ergothioneine; group 2:Leontopodium alpinum flower/leaf extract; group 3:rosa roxburghii fruit extract;group 4:1% holistic anti-aging anti-aging complex;group 5:2% holistic anti-aging anti-aging complex

Soothing Capacity

The anti-aging composite exhibits notable soothing efficacy by effectively suppressing nitric oxide (NO) production.

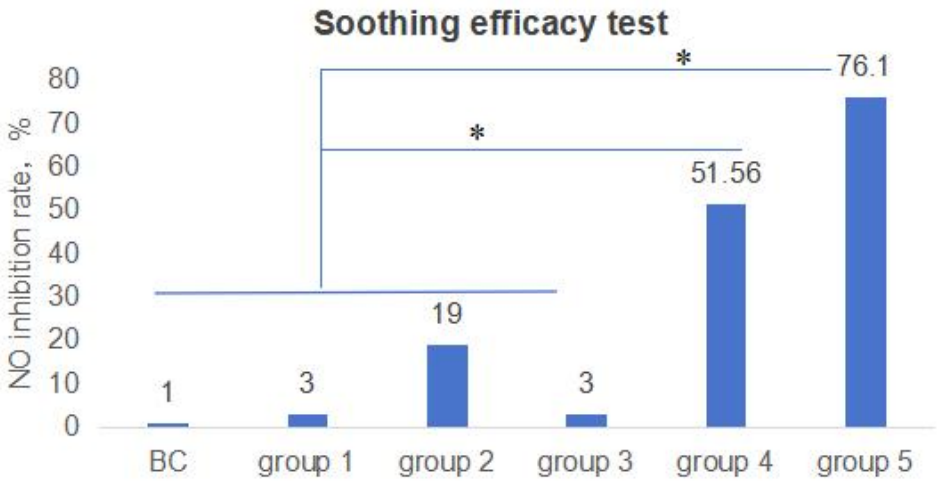

Figure 6. The inhibition ratio of NO（*,P＜0.05）

group 1:ergothioneine; group 2:Leontopodium alpinum flower/leaf extract; group 3:rosa roxburghii fruit extract;group 4:1% holistic anti-aging anti-aging complex;group 5:2% holistic anti-aging anti-aging complex

Discussion

Current therapeutic strategies predominantly employ isolated compounds such as ergothioneine, vitamin C derivatives, and polyphenol-rich botanical extracts (e.g., flavonoids) for antioxidant purposes. However, these approaches often fail to provide a comprehensive solution that simultaneously addresses oxidative stress, glycation, and inflammation in cutaneous tissues.

Notably, ergothioneine demonstrates dual functionality as both a potent antioxidant and an effective anti-glycation agent. The Leontopodium alpinum flower/leaf extract contains unique bioactive constituents, including leontopodic acids and flavonoid glycosides, which exhibit remarkable anti-glycation properties. Specifically, this extract has been shown to reduce type IV collagen glycation at the dermal-epidermal junction (DEJ) and reverse established AGEs formation through carbonyl group sequestration.

Complementarily, Rosa roxburghii fruit extract provides significant anti-inflammatory activity,which can enhanced antioxidant capacity via its high vitamin C content.

This combination of bioactive components represents a multi-target approach to cutaneous aging, addressing the interconnected pathways of oxidative damage, protein glycation, and chronic inflammation that collectively contribute to skin aging pathology.

This study ingeniously combines ergothioneine, Leontopodium alpinum extract, and Rosa roxburghii fruit extract at specific ratios to form an anti-aging composite. The components exhibit synergistic effects: ergothioneine enhances the antioxidant capacity of the two botanical extracts, while Leontopodium alpinum extract improves the anti-glycation activity of ergothioneine, and Rosa roxburghii fruit extract potentiates both the anti-inflammatory and soothing properties of ergothioneine. These in vitro findings suggest the potential application of this composite in skin tone-improving products and anti-aging formulations for sensitive skin.

Conclusion

This study investigates the key factors influencing skin aging, including oxidative stress, glycation, and inflammatory responses, and introduces a novel anti-aging composite composed of ergothioneine, Leontopodium alpinum extract, and Rosa roxburghii fruit extract at optimized ratios. In vitro tests demonstrate that this innovative composite exhibits significant synergistic effects in antioxidant, anti-glycation, anti-inflammatory, and soothing activities, suggesting its considerable potential in addressing skin concerns among individuals with sensitive skin, such as dullness, hyperpigmentation, and post-inflammatory hyperpigmentation. In future studies, we will develop skincare formulations incorporating this composite and conduct clinical efficacy assessments on sensitive skin populations to evaluate its anti-aging benefits, thereby advancing the development of anti-aging solutions for sensitive skin in the cosmetics industry.

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