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Enhancing Skin Transparency Through Epigenetic Modulation: A Novel Complex of Dunaliella Salina Extract and Ascorbic Acid Peptide Derivative

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Abstract

Skin transparency is crucial for maintaining youthful and healthy skin. Environmental stressors, including UV exposure and oxidative damage, compromise the skin's barrier and lead to diminished skin transparency. Recent research suggests that DNA methylation, a key epigenetic regulator, plays an important role in controlling the expression of skin barrier-related genes and can be targeted to restore skin health. This study investigates the synergistic effects of a novel complex composed of *Dunaliella salina* extract and ascorbic acid peptide derivative, designed to modulate DNA methylation and improve skin transparency. The composition was tested using a variety of assays, including in vitro toxicity testing, antioxidant assays, gene expression profiling, high-throughput DNA methylation sequencing, and the evaluation of skin barrier function. Additionally, the composition's whitening and transparency effects were tested using advanced 3D skin models. Results indicate that the complex significantly enhances skin transparency by modulating key barrier-related genes through epigenetic modifications, demonstrating promising potential for future skincare applications.

1. Introduction

Skin transparency, which refers to the smooth, radiant, and clear appearance of skin, plays a significant role in the perception of youth and health [1]. This quality of skin is often impaired due to oxidative stress and UV-induced damage, both of which degrade the skin's structural integrity and barrier function. As a result, the skin becomes more prone to dryness, irritation, and signs of aging, leading to a loss of its natural transparency and clarity.

Epigenetic mechanisms, particularly DNA methylation, are now recognized as important regulators of gene expression in the skin, influencing cellular processes such as differentiation, barrier formation, and response to oxidative stress [2,3]. DNA methylation involves the addition of a methyl group to the cytosine base of DNA, typically repressing gene expression when it occurs at promoter regions [4,5]. The modulation of DNA methylation has emerged as a potential strategy for reversing damage to the skin, particularly for genes involved in skin barrier function.

This study aims to explore a novel synergistic approach to enhancing skin transparency by using a complex formulation consisting of Dunaliella salina extract and an ascorbic acid peptide derivative. This complex is hypothesized to target epigenetic regulators and improve the expression of key skin barrier genes, while also exhibiting antioxidant properties to alleviate oxidative stress. The study includes a comprehensive evaluation of the complex's effects through in vitro assays, high-throughput DNA methylation sequencing, gene expression sequencing, and 3D skin models to test its whitening and transparency effects.

2. Materials and Methods

2.1. Materials

- *Dunaliella salina extract*: Sourced from algae, known for its rich content of carotenoids, particularly beta-carotene, which acts as a potent antioxidant.
- *Ascorbic acid peptide derivative*: A stable form of Vitamin C conjugated to a peptide carnosine to enhance dermal penetration and promote collagen synthesis.
- *HaCaT cells (human keratinocytes)*: A cell line used to study skin biology, including oxidative stress, cell viability, and barrier function.
- *3D Skin Models*: Used to evaluate the composition's effects on skin transparency, pigmentation, and barrier function. The models were sourced from commercial supplier specializing in skin equivalent models.
- *Reagents*: Standard cell culture media, chemicals, and reagents for assays, DNA extraction, and sequencing were sourced from in house established biochemical suppliers.

2.2. Experimental Design

The study was divided into three main phases: in vitro testing (toxicity, antioxidant capacity, gene expression, and skin barrier function), high-throughput DNA methylation and gene expression analysis, and clinical evaluation using 3D skin models for whitening and transparency assessment.

2.3. In Vitro Cellular activity Testing

The CCK-8 assay was used to assess the cellular activity improvement of the complex in HaCaT cells. The cell model induced by 1% SLS, were treated with varying concentrations of the complex and the individual ingredient respectively at the indicated dosage for 24 hours. Cell viability was measured, and the percentage of viable cells was calculated. The results were compared with a vehicle control to assess any cellular activity recovery effects.

2.4. Antioxidant Capacity Testing

To evaluate the antioxidant effects of the complex, HaCaT cells were subjected to 40 mJ/cm² UVB-induced oxidative stress. Cells were pretreated with indicated concentrations of of the ingredients, either individually or in combination. ROS levels were measured using a fluorescent ROS detection assay, and the reduction in ROS levels was quantified relative to the untreated control group.

2.5. Collagen Production Testing

To investigate the synergistic effect of *Dunaliella salina* extract and ascorbic acid peptide derivative on collagen production in human dermal fibroblasts, Cells were treated with various concentrations of the ingredients, either individually or in combination, and the production of collagen type I was assessed using an ELISA. Briefly, the fibroblasts were exposed to UVA for 40 mJ/cm², followed by treatment with the ingredients for 48 hours. After treatment, the cell supernatants were collected and collagen production was quantified using a Collagen Type I ELISA Kit. The absorbance was measured at 450 nm to determine the concentration of collagen I secreted into the extracellular matrix.

2.6. DNA Methylation and Gene Expression Analysis

To explore the epigenetic effects of the complex, high-throughput DNA methylation sequencing was performed on HaCaT cells treated with the complex at 0.2% concentration. DNA was extracted from treated and untreated cells, and DNA methylation profiling was conducted using bisulfite sequencing. This was followed by gene expression profiling using RNA sequencing (RNA-Seq) to evaluate the global effects of the complex on skin barrier-related genes.

2.7. Whitening Efficacy in 3D Melanocyte Model

A 3D melanocyte model (MelaKutis®) was used to assess the whitening effect of the Dunaliella salina extract + Ascorbic acid peptide derivative（complex）and an in-house control group. Both groups were tested at a final concentration of 0.1% + 0.1%, topically applied at 2 mg/cm² on Day 3 and Day 5. The models were exposed daily to UVB radiation (50 mJ/cm²) for 4 days. Brightness (L* value) was measured using a chromameter, and melanin distribution was assessed via silver staining. Statistical differences were analyzed using t-tests ($p < 0.05$ considered significant).

2.8. Anti-Glycation and Anti-Carbonylation Testing in 3D Full-Thickness Skin Model

A full-thickness 3D skin model (FulKutis®) was used to evaluate the anti-glycation and anti-carbonylation efficacy of the complex. After UVA (30 J/cm²) and methylglyoxal (3 mM) stimulation, the test sample was applied topically (0.1% + 0.1%) once daily for 4 consecutive days. Skin yellowness (b* value) was measured by chromameter, while glycation end product (CML) and carbonylated protein levels were assessed via immunofluorescence and DNPH staining, respectively. Suppression rates were calculated relative to the negative control group (NC).

3. Results

3.1. Skin Barrier Activity (SLS-Induced Damage Model)

The complex was tested for its ability to restore skin barrier function in HaCaT cells exposed to 1% SLS. Treatment with 0.2% Dunaliella salina extract or 0.2% Ascorbic acid peptide derivative improved cell viability by 39.79% and 55.87%, respectively, compared to the model group. The combination of 0.1% Dunaliella salina extract + 0.1% Ascorbic acid peptide derivative showed the most significant improvement, with a 97.04% increase in cell viability. A similar trend was observed with 0.5% concentrations, with the combination showing the greatest effect, enhancing cell viability by 38.55%. These results suggest that the combination of both ingredients has a synergistic effect on skin barrier protection following SLS exposure.

3.2. ROS Measurement (UVB-Induced Oxidative Stress Model)

In UVB-exposed HaCaT cells, the complex showed strong antioxidant effects. 0.5% Dunaliella salina extract reduced ROS levels by 99.44%, while 0.5% Ascorbic acid peptide derivative reduced ROS by 30.21%. The combination of 0.25% Dunaliella salina extract + 0.25% Ascorbic acid peptide derivative was the most effective, reducing ROS by 343.71%, demonstrating a synergistic antioxidant effect. Similarly, with 0.2% concentrations, the combination showed the greatest ROS inhibition, reducing levels by 67.75%. These results suggest that the combination of both ingredients has a synergistic effect on skin barrier oxidative stress protection following UVB exposure.

3.3. Collagen Production (UVA-Induced Photo-aging Model)

In UVA-exposed HSF cells, 0.2% Dunaliella salina extract increased collagen production by 79.26%, while 0.2% Ascorbic acid peptide derivative showed a 57.70% increase. The combination of 0.1% Dunaliella salina extract + 0.1% Ascorbic acid peptide derivative showed the highest improvement, increasing collagen by 124.91% compared to the model group. These results suggest that the combination of both ingredients has a synergistic effect on collagen production following UVA exposure.

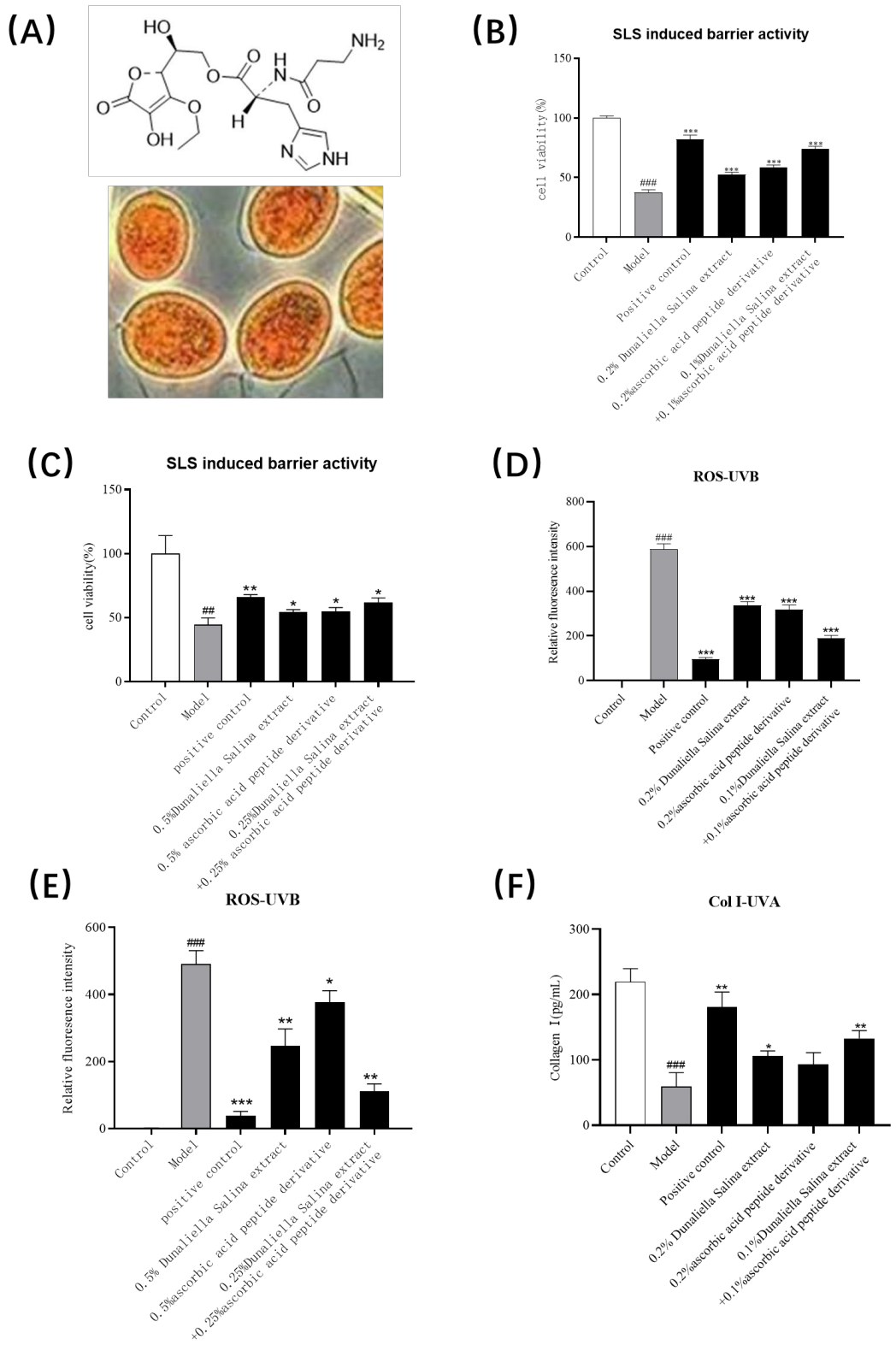

Figure 1. Effect of Dunaliella salina extract and Ascorbic acid peptide derivative on skin barrier activity, ROS inhibition, and collagen production in HaCaT and HSF cells.

(A) Demo snapshoot for *Ascorbic acid peptide derivative* and *Dunaliella salina* for this study. **(B,C)** Skin barrier activity: Both individual ingredients and their combination significantly enhanced cell viability in HaCaT cells subjected to 1% SLS damage, with the combination showing the highest effect. **(D,E)** ROS measurement: The combination treatment reduced ROS levels significantly more than the individual treatments, showing a synergistic antioxidant effect in UVB-exposed HaCaT cells. **(F)** Collagen production: In HSF cells exposed to UVA, the combination of 0.1% *Dunaliella salina* extract and 0.1% Ascorbic acid peptide derivative showed the greatest increase in collagen production, suggesting a synergistic effect on collagen synthesis. Data are presented as mean ± SEM; statistical significance is indicated by $p < 0.05$.

3.4. DNA Methylation and Gene Expression Analysis

High-throughput DNA methylation sequencing revealed that treatment with the complex led to a marked reduction in methylation levels at the promoter regions of multiple genes associated with skin barrier integrity and skin transparency. These epigenetic changes are suggestive of a reprogramming effect toward a healthier skin state, by relieving transcriptional repression at key loci that maintain barrier structure, hydration regulation, and overall epidermal homeostasis.

Complementary RNA sequencing data showed a coordinated upregulation of genes involved in epidermal differentiation, tight junction formation, and antioxidative defense pathways, along with a downregulation of genes associated with oxidative stress responses and chronic inflammation. This parallel between DNA hypomethylation and increased gene expression at critical skin barrier-related sites supports a model wherein the complex exerts dual-layered regulatory effects: first at the epigenetic level through demethylation, and subsequently at the transcriptional level to restore and enhance skin function.

Importantly, several genes that showed epigenetic modulation are also implicated in promoting skin luminosity and evenness, suggesting that the observed improvements in skin transparency may be mechanistically linked to targeted regulation of genes governing epidermal barrier permeability and pigment distribution.

Together, these findings highlight the ability of the complex to beneficially reshape the skin's epigenetic landscape, promoting a more resilient and radiant phenotype. They further underscore the emerging importance of epigenetic strategies in cosmetic science, particularly in developing interventions that strengthen skin barrier function, enhance transparency, and address the root molecular changes induced by environmental aging.

3.5. 3D Skin Model for Whitening and Transparency Effects

For whitening efficacy analysis with 3D skin model. After 4 days of treatment, the complex significantly improved model skin brightness and reduced melanin content. Compared to the negative control, L* value increased by 8.28%, and melanin particle area was reduced by 44.83%. The in-house control group also showed a whitening effect, with a 3.42% increase in L* value and a 35.34% reduction in melanin. These findings suggest that the complex has a superior whitening effect over the in-house reference, though slightly lower than the positive control (Kojic acid).

For anti-glycation and anti-carbonylation effects In the full-thickness skin model, the complex demonstrated marked efficacy in reducing glycation and carbonylation damage. The b* value dropped by 39.38%, and carboxymethyl lysine (CML) levels were suppressed by 65.47%, as shown by decreased fluorescence intensity. In addition, carbonylated protein levels were reduced by 63.60%. All parameters were significantly improved compared to the negative control group ($p < 0.01$), and in some indicators, the effect was comparable to or better than the positive control (aminoguanidine). Collectively, the whitening and transparency effects observed in the 3D skin models support the complex's potential as a cosmetic ingredient for

improving skin clarity.

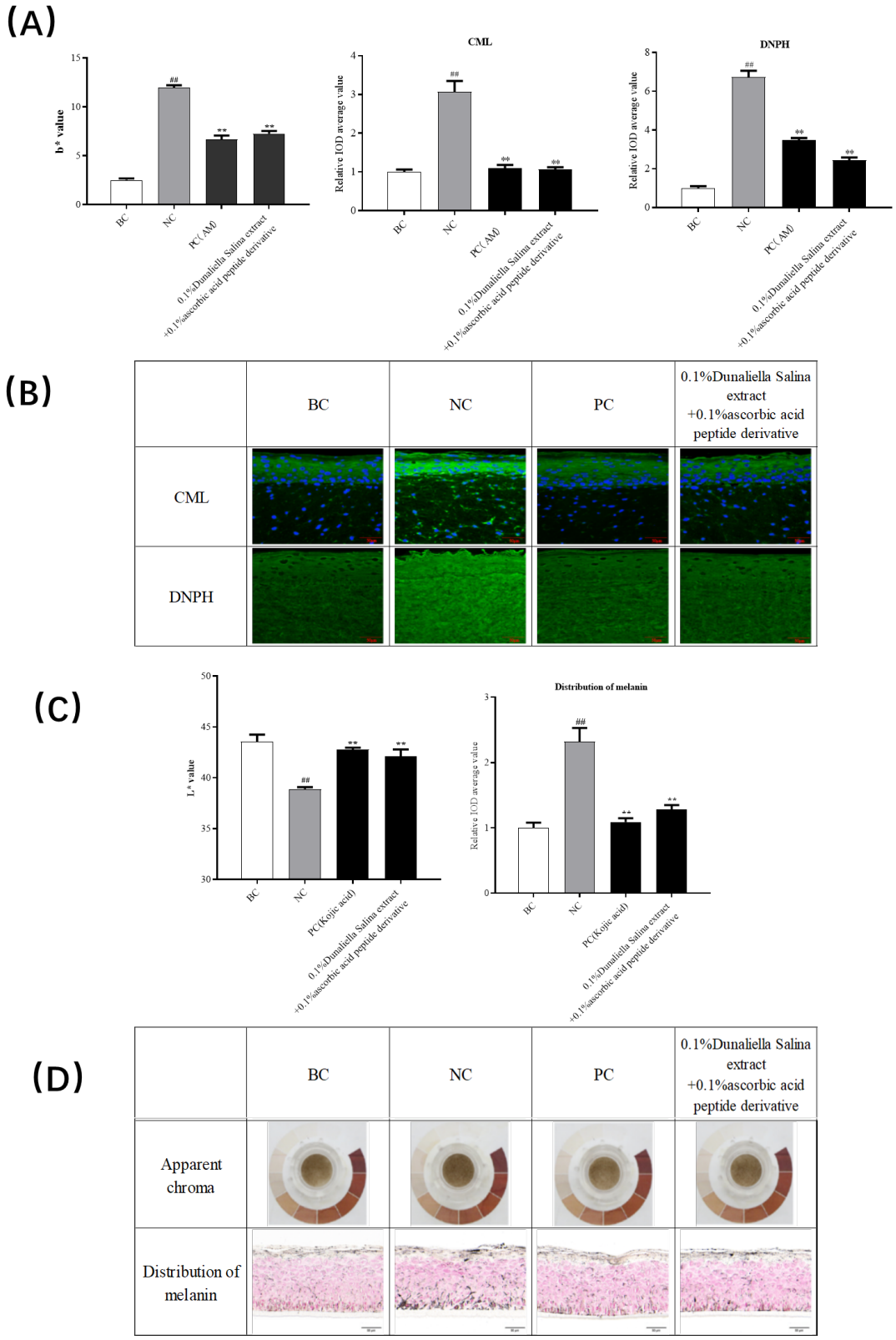

Figure 3: *Effect of Dunaliella Salina Extract + Ascorbic Acid Peptide Derivative on Anti-Glycation , Anti-Carbonylation and Whitening Efficacy in 3D Models*.

(A,B) *Skin yellowness (b* value)*: Compared to the negative control, the tested composition significantly reduced b* value by 39.38%, indicating inhibition of glycation-induced yellowing;

Carboxymethyl lysine (CML) content: Immunofluorescence analysis showed that CML levels were decreased by 65.47% in the tested composition group, comparable to the positive control (aminoguanidine, 64.17%);*Carbonylated protein content*: DNPH-based staining revealed a 63.60% reduction in carbonylation in the tested group, exceeding the 48.14% inhibition observed in the positive control. Data are expressed as mean ± SD; statistical significance determined by t-test ($p < 0.01$). (C,D*) apparent brightness (L* value): After 4 days of UVB irradiation (50 mJ/cm²), the tested compos*ition group increased L* value by 8.28%, indicating enhanced skin brightness, compared to 3.42% by the in-house control group. The positive control group (Kojic acid) showed a 10.00% increase ; *Melanin content analysis*: The tested composition group reduced melanin area by 44.83%, outperforming the in-house control group (35.34%) and approaching the efficacy of the positive control (53.02%). Data are presented as mean ± SD; $p < 0.05$ was considered statistically significant.

4. Discussion

The results of this study demonstrate that the complex of Dunaliella salina extract and ascorbic acid peptide derivative has significant potential for improving skin transparency and barrier function. The cellular activity, ROS and collagen production assay provided compelling evidence that the combination of these two ingredients works synergistically to enhance skin anti-aging effect. The synergistic effect observed in cellular activity, ROS and collagen production assay, where the combined ingredients increased compared to controls, highlights the potential of this complex as a powerful skincare formulation. This finding suggests that the combination of the antioxidants in Dunaliella salina extract and the effects of ascorbic acid can work together to restore and enhance the skin's structural integrity.

Moreover, the epigenetic modulation of key barrier-related genes, as demonstrated by the DNA methylation analysis, offers an innovative approach to improving skin function at the molecular level. By targeting DNA methylation, the complex not only enhances skin barrier integrity but also supports long-term skin health by promoting the expression of essential genes.

The whitening and transparency effects observed in the 3D skin models support the complex's potential as a cosmetic ingredient for improving skin clarity. The significant reduction in melanin production and the increase in skin brightness (L* value) suggest that the complex could be effective in treating skin discoloration and promoting a more youthful, even complexion.

5. Conclusion

This study demonstrates that the complex of *Dunaliella salina* extract and ascorbic acid peptide derivative is a promising skincare formulation that enhances skin transparency, promotes collagen production, and strengthens the skin barrier. The synergistic effects of the two components, combined with epigenetic modulation of key skin barrier genes, make this complex a novel approach to skincare. Future clinical studies and long-term trials will be essential to further evaluate its effectiveness and safety in broader populations, but the current findings indicate significant potential for its application in anti-aging and skin enhancement products.

6. Reference

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