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Synergistic effects of tremella fuciformis polysaccharide and euglena gracilis poplypeptide on skin barrier repair and photoprotection with experimental and bioinformatic approaches

Geng Tian* [1], Junxiang LI[2], Haiwen Zhao[1], Ying Ding[1], Wei Shi [1]

[1] Guofa Haiying Technology, Bejing, China [2] Yangtze delta region institute of Tsinghua University, Agecode R&D Center, Jiaxing, China

1. Introduction

Skin aging is a complex process influenced by a multitude of intrinsic and extrinsic factors, with UV radiation being a key external culprit. As we age, the skin's innate repair capacity wanes, accelerating collagen degradation and compromising the skin barrier. These changes not only mar the skin's appearance, leading to wrinkles, sagging, and hyperpigmentation, but also diminish its defense against environmental aggressors, increasing the risk of skin disorders. Conventional skincare ingredients, while effective to some extent, often come with drawbacks like skin irritation, allergenicity, and environmental burdens.

Amid growing consumer demand for eco-friendly products, the skincare industry is undergoing a green transformation. Plant - and microbe - derived extracts, rich in bioactive compounds, have emerged as promising alternatives. Tremella fuciformis polysaccharide (TFP) and Euglena gracilis extract (EGE) are two such natural ingredients gaining attention for their skin repair and photoprotective potential.

TFP, with its diverse bioactive glycan structures, can activate cell surface receptors and intracellular signaling pathways, stimulating collagen synthesis and enhancing skin elasticity. Its antioxidant and anti - inflammatory properties help neutralize free radicals, reducing UV - induced oxidative damage and alleviating skin inflammation. EGE, abundant in amino acids, peptides, and carotenoids, balances skin metabolism and strengthens natural defenses. The peptides in EGE bind to specific cell surface receptors, mimicking growth factors to promote cell renewal, while carotenoids absorb UV radiation, offering protection against UV - induced damage and modulating the skin's immune response.

This study explores the synergistic mechanisms of TFP and EGE to develop a more effective and safer anti - aging and skin barrier repair product. By merging experimental validation with bioinformatics analysis, we reveal how the TFP - EGE combination enhances collagen synthesis, fortifies the skin barrier, mitigates UV damage, and slows skin aging. These insights could guide the skin care industry to create environmentally friendly formulas to meet

consumer demand for green, pure and efficient skin care products, while guiding the industry towards sustainable development.

2. Materials and Methods

2.1Vitro Cell Models

We utilized in vitro cell models to assess the cytotoxicity and effects of the TFP and EGE composition on UVA-induced Collagen I degradation. Human skin fibroblasts (HSF) were exposed to UVA radiation at a dose of 10J, followed by treatment with varying concentrations of the TFP-EGE composition.Collagen I levels were quantified through ELISA. RNA was collected from the treated HSF cells for subsequent RNA-Seq analysis.

2.2RNA-Seq Analysis

RNA-Seq was employed to identify differentially expressed genes (DEGs) related to skin repair. Cells treated with the TFP-EGE composition were compared to control groups to pinpoint genes crucial for the skin's response to UVA-induced stress. This analysis helped elucidate the molecular mechanisms underlying the composition's effects.

2.33D Epidermal Model

A 3D epidermal model was used to evaluate barrier repair under UVB exposure at a dose of 600mJ/cm². The model was subjected to UVB radiation, followed by treatment with the TFP-EGE composition. Tissue analysis was conducted to assess the number of sunburn cells and the expression levels of key barrier proteins, including filaggrin (FLG), loricrin (LOR), and transglutaminase 1 (TGM1). WY14643 (50μM) was used as a positive control. Additionally, an ex vivo skin model was used to evaluate the effects of UVA + UVB induction (30J/cm² UVA + 50mJ/cm² UVB) on tissue morphology and collagen fibers, including Collagen I, III, IV, and XVII.

2.4Non-Targeted Metabolomics and Network Pharmacology

Non-targeted metabolomics was performed to map metabolic profiles and pathway alterations related to skin aging and barrier function. The composition's impact on metabolic pathways was analyzed using advanced bioinformatics tools. Network pharmacology was employed to identify common targets associated with "skin barrier" and "senescence," highlighting core targets modulated by the composition.

2.5Clinical Testing

Clinical testing was conducted on 31 volunteers to evaluate the practical effects of the TFP-EGE composition on skin hydration, transepidermal water loss (TEWL), complexion brightness, and wrinkle reduction. Volunteers were monitored over a period of eight weeks, with measurements taken at regular intervals to assess improvements in skin condition.

3. Results

3.1Synergistic Effects on Skin Repair and Photoprotection

The TFP and EGE composition demonstrated significant enhancement of Collagen I synthesis following UVA exposure. This superior efficacy was confirmed through ELISA measurements, indicating the composition's ability to counteract UVA-induced collagen degradation. The results showed that the TFP and EGE composition significantly increased

Collagen I levels compared to the control group, highlighting its potential in mitigating UVA-induced skin damage.
In the UVB-induced 3D skin epidermal model, the TFP and EGE composition effectively reduced the number of sunburn cells and increased the expression of key barrier proteins. Specifically, the expression levels of FLG, LOR, and TGM1 were significantly elevated, highlighting the composition's role in skin barrier repair. H&E staining revealed a marked decrease in sunburn cells after treatment with the TFP and EGE composition, further supporting its protective effects against UVB-induced damage.
RNA-Seq analysis revealed a comprehensive set of DEGs crucial for the skin's response to UVA-induced stress. These genes were involved in pathways related to collagen synthesis, inflammation modulation, and barrier function enhancement, providing a molecular basis for the TFP and EGE composition's observed effects. The DEGs identified included several key regulators of skin repair and anti-aging processes, indicating the composition's broad-spectrum activity in promoting skin health.
Notably, at a combined concentration of 0.2 mg/mL TFP and 2% (v/v) EGE, the composition significantly reduced the number of sunburn cells, achieving an inhibition rate of 54.55%. The levels of filaggrin (FLG), loricrin (LOR), and transglutaminase 1 (TGM1) were elevated by 93.33%, 125.64%, and 203.85%, respectively. These results indicate that the composition can improve tissue morphology and enhance the expression of these key proteins, thereby exerting a repair effect. Additionally, the composition increased the content of Collagen IV and Collagen XVII by 126.92% and 138.46%, respectively, suggesting its ability to enhance skin firmness.

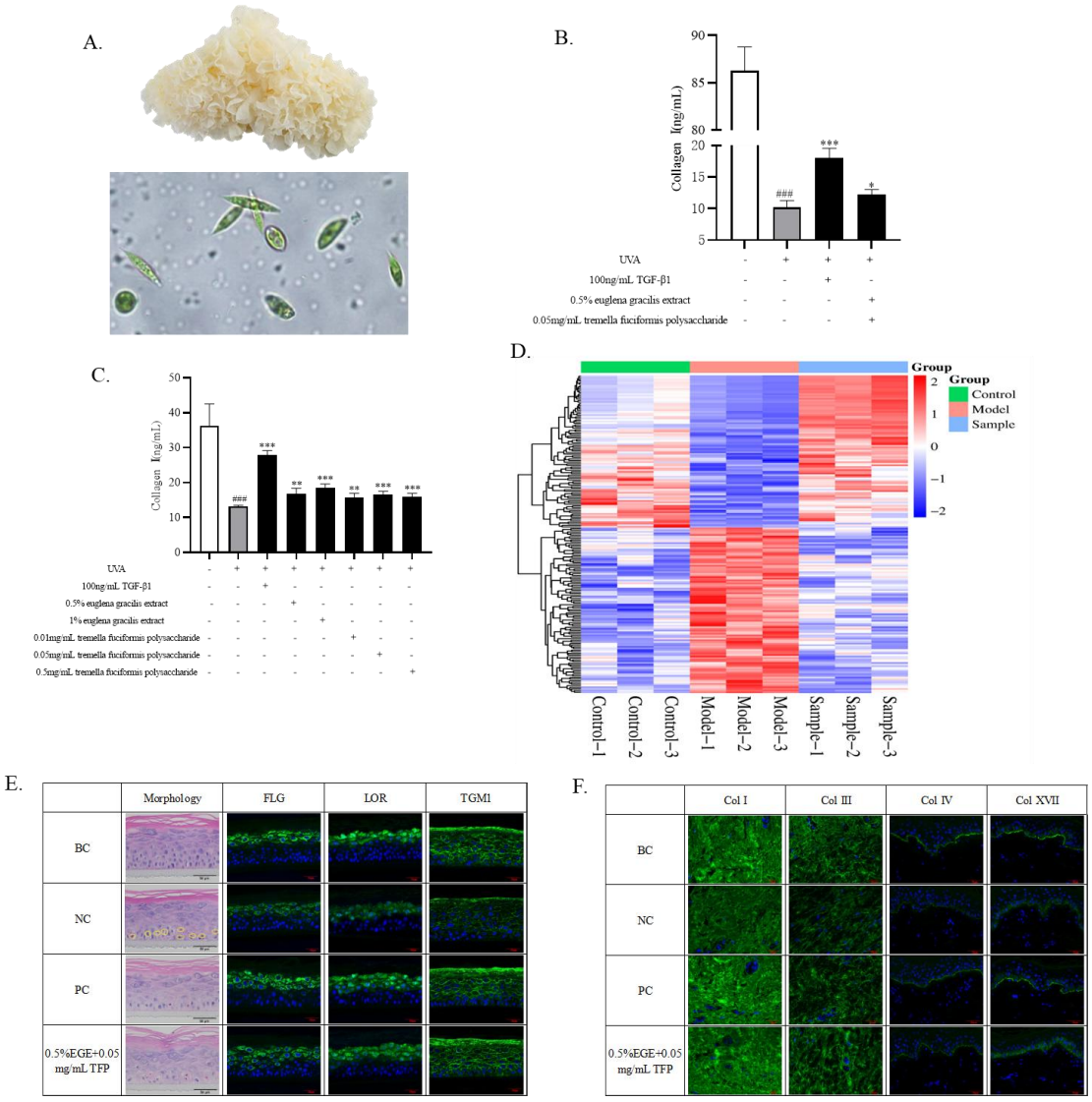

Figure 1. Synergistic Effects of Tremella fuciformis Polysaccharide (TFP) and Euglena gracilis Extract (EGE) on Skin Repair Barrier and Anti-Aging. (A) TFP and EGE; (B-C) Detection of UVA Induced Type I Collagen in HSF Cells; (D) Analysis of differentially expressed genes induced by UVA in HSF cells; (E) 3D epidermal cell barrier repair test; (F) Vitro skin aging test.Values are expressed as mean ± SD (n = 3). Comparisons between groups were performed using the t-test. All statistical analyses involved a two-tailed test. ### p< 0.001 vs. Control group; ** p< 0.01, *** p< 0.001 vs. UVA group.

3.2Metabolomics and Network Pharmacology Findings

The metabolomics analysis demonstrated that the TFP and EGE composition modulates multiple metabolic pathways related to skin health. Network pharmacology identified 181 common targets associated with "skin barrier" and "senescence," 21 of which are core targets modulated by the composition. These targets play critical roles in pathways essential for skin inflammation, barrier repair, and anti-aging effects.

The metabolomics data revealed significant alterations in lipid and amino acid metabolism, which are closely associated with skin barrier function and aging processes. For example, disruptions in lipid metabolism can compromise the skin's protective barrier, leading to

dryness and increased vulnerability to external stressors. Alterations in amino acid metabolism can affect skin cell turnover and repair mechanisms, thereby influencing skin texture and appearance. The TFP-EGE composition's ability to modulate these metabolic pathways suggests its potential to restore and maintain healthy skin function.

PPI analysis showed extensive interactions among the targets regulated by the TFP-EGE combination, forming a complex signaling network that regulates skin function. KEGG analysis identified specific pathways, including the MAPK, PI3K-Akt, and NF-κB signaling pathways, which are key mediators of skin inflammation, repair, and aging processes.

The MAPK signaling pathway is crucial for cellular responses to stress and inflammatory stimuli. Dysregulation of this pathway can result in excessive inflammation and skin tissue damage. By modulating the MAPK pathway, the TFP-EGE composition helps mitigate inflammatory responses, thereby reducing redness and discomfort associated with skin irritation. The PI3K-Akt pathway plays a central role in regulating cell survival, proliferation, and metabolism. Enhancing this pathway can promote skin cell renewal and repair, supporting the restoration of the skin barrier and improving skin texture and appearance. The NF-κB pathway is a key mediator of immune and inflammatory responses. Inhibiting the NF-κB pathway can reduce the production of pro-inflammatory cytokines, alleviating skin inflammation and potentially slowing down the aging process induced by chronic inflammation.

The TFP-EGE combination's regulation of these targets enhances skin repair and photoprotection. By modulating these targets, the combination effectively reduces UV-induced skin damage, promotes regeneration, and strengthens the skin barrier. For instance, modulating the MAPK and NF-κB pathways decreases UV-induced inflammatory responses, minimizing sunburn cell formation. Activating the PI3K-Akt pathway stimulates skin cell proliferation and collagen synthesis, aiding in tissue repair and enhancing skin elasticity and firmness.

These findings provide valuable insights into the mechanistic underpinnings of the TFP-EGE combination's effects in skin care. They also establish a scientific foundation for the development of TFP and EGE-based skin care products. The combination's multi-pathway modulation offers a comprehensive strategy for addressing various skin concerns, including inflammation, damage, repair, and aging. This positions the TFP-EGE combination as a promising candidate for next-generation eco-friendly skincare formulations, potentially offering consumers effective and natural solutions for maintaining healthy skin.

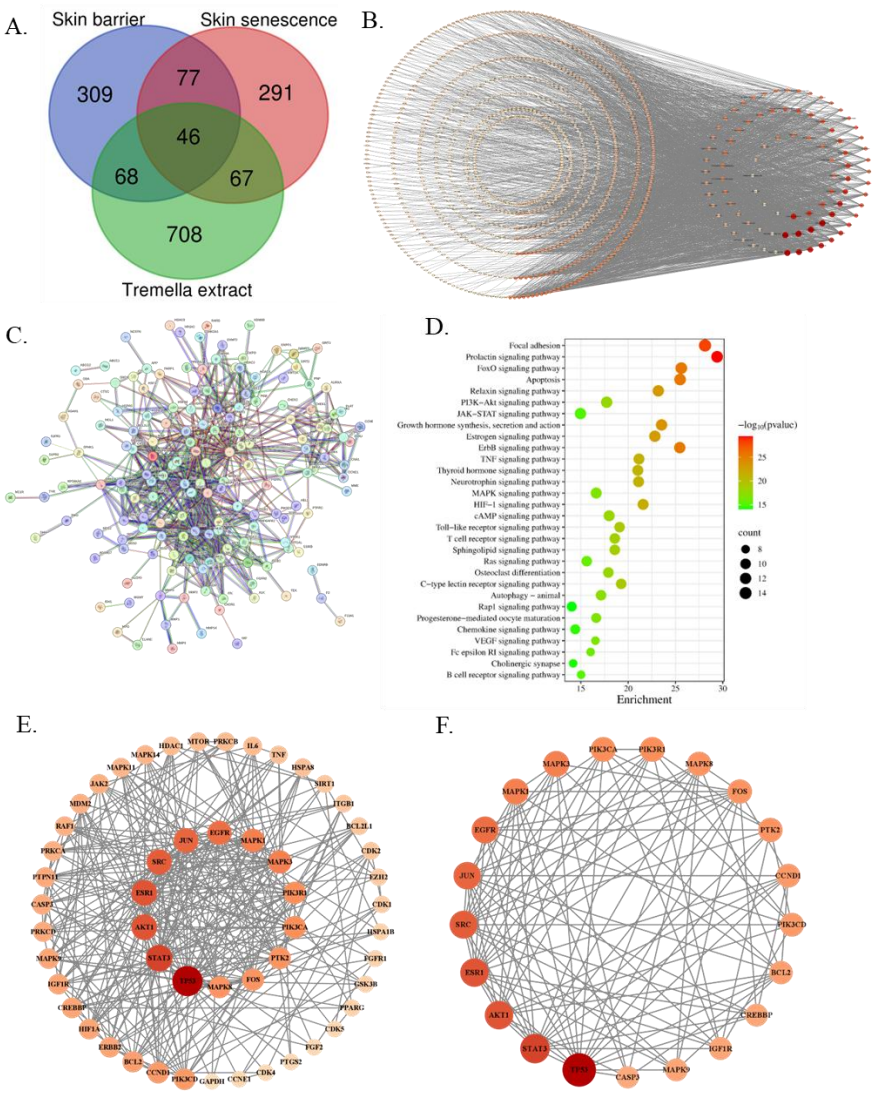

Figure 2. Non-targeted Metabolomics Identification and Network Pharmacology Analysis of Tremella fuciformis Polysaccharide (TFP). (A) Metabolite and genecards database gene wayne map; (B) Metabolite-target network diagram (Circular nodes represent target points, line segments represent relationships between components and target points, and different colors represent degree values).(C)PPI network analysis in String; (D) KEGG analysis (top 30 except disease-related genes); (E-F) Key Target Interaction Diagram (The darker the color, the larger the shape, the higher the degree value).

3.3Clinical Testing Outcomes

Clinical testing on volunteers showed that the combination of TFP and EGE significantly enhanced skin hydration, reduced TEWL, brightened the complexion, and diminished the appearance of wrinkles. These results underscore the practical benefits of the TFP and EGE composition in improving skin condition and appearance. Compared to before use, after 14 and 28 days of using the emulsion containing TFP and EGE, the skin's transepidermal water loss (TEWL) showed a significant decrease (P<0.05), with reduction rates of 9.31% and 20.59%, respectively. Additionally, the reduction in TEWL in the sample area was significantly greater than in the control area (P<0.05).There was a significant increase in the skin color L* value (P<0.05), with enhancement rates of 4.24% and 8.49%, respectively. Moreover, the

increase in the skin color L* value in the sample area was significantly higher than in the control area (P<0.05).The skin color a* value showed a significant decrease (P<0.05), with reduction rates of 4.44% and 4.45%, respectively. Furthermore, the decrease in the skin color a* value in the sample area was significantly greater than in the control area (P<0.05).The skin color b* value exhibited a significant decrease (P<0.05), with reduction rates of 3.86% and 6.09%, respectively. In addition, the decrease in the skin color b* value in the sample area was significantly greater than in the control area (P<0.05).The skin color ITA° value demonstrated a significant increase (P<0.05), with enhancement rates of 5.55% and 10.44%, respectively. Also, the increase in the skin color ITA° value in the sample area was significantly higher than in the control area (P<0.05).The mandibular angle showed a significant increase (P<0.05), with enhancement rates of 1.53% and 3.65%, respectively. Moreover, the increase in the mandibular angle in the sample area was significantly greater than in the control area (P<0.05).The crow's feet area ratio exhibited a significant decrease (P<0.05), with reduction rates of 9.95% and 16.10%, respectively. Additionally, the decrease in the crow's feet area ratio in the sample area was significantly greater than in the control area (P<0.05).Mandible line angle was significantly increased (P<0.05), the rate of elevation was 1.53% and 3.65% respectively;14 days and 28 days later, mandible line angle increment in sample area was significantly higher than that in control area (P<0.05). Volunteers reported noticeable improvements in skin texture and elasticity, with a reduction in fine lines and wrinkles observed after eight weeks of use.

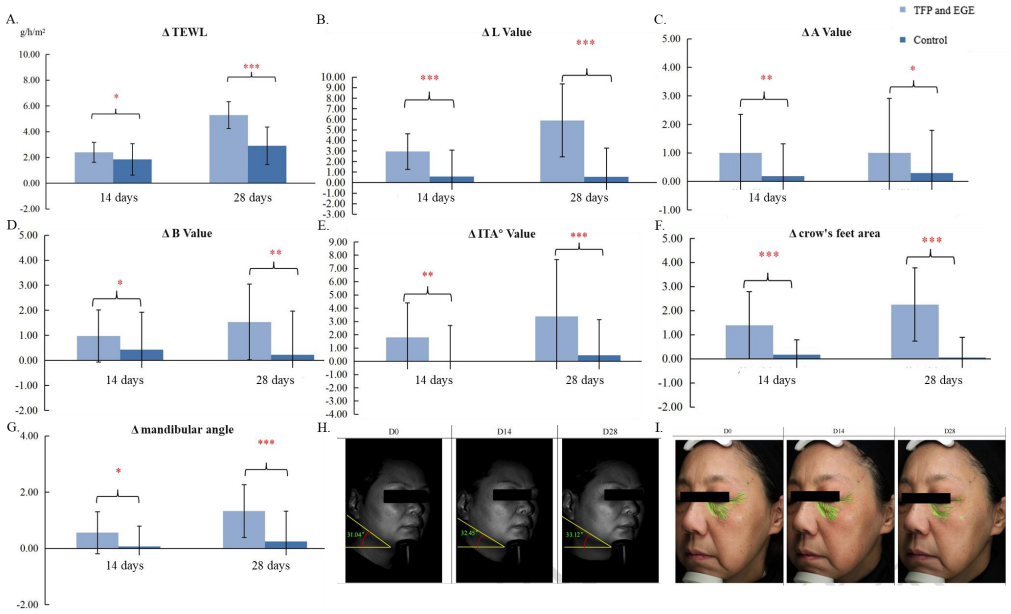

Figure 3.31 subjects continuously used the 28-day efficacy test**.** (A-G); TEWL, skin color, mandibular angle and crow's feet area were measured by the test instrument; (H) Effective case of mandibular line angle improvement (PRIMOS-CR300 image)**;** (**I**) Effective cases of crow's feet improvement (VISIA image).

4. Discussion

Our findings champion the green beauty advantages of TFP and EGE, particularly theirsynergistic effect in skin repair, photoprotection, and anti - aging. The TFP - EGE combination's ability to boost collagen I synthesis, slash sunburn cells, and modulate key metabolic pathways firmly establishes its potential in eco - friendly skincare.

In vitro, the combo significantly ramped up collagen I synthesis in UVA - irradiated fibroblasts, as shown by ELISA, and the RNA - Seq analysis revealed it influenced genes tied to collagen synthesis, inflammation regulation, and barrier function enhancement. These genes are critical for healthy skin, indicating the combo's broad - spectrum activity.

In a 3D skin model, the TFP - EGE combination effectively reduced sunburn cells and increased the expression of key barrier proteins. FLG, LOR, and TGM1 levels rose significantly after treatment, demonstrating the combination's repair capabilities. Metabolomics analysis also revealed that the TFP - EGE combination modulates multiple metabolic pathways related to skin health, with significant changes in lipid and amino acid metabolism, which are closely linked to skin barrier function and aging processes. Network pharmacology identified 181 common targets associated with "skin barrier" and "senescence," with the TFP - EGE combination modulating 21 of these core targets. These targets play crucial roles in pathways critical for skin inflammation, barrier repair, and anti - aging effects.

Overall, the TFP - EGE combination shows great potential as a green, sustainable solution for the skincare industry. Itssynergistic effect in promoting collagen synthesis, enhancing skin barrier function, and providing photoprotection makes it a promising candidate for next - generation eco - friendly skincare products. Future research should further explore its clinical applications, long - term safety, and efficacy across different skin types and environmental conditions to optimize its role in promoting skin health.

5. Conclusion

In summary, our study demonstrates that the combination of Tremella fuciformis polysaccharide (TFP) and Euglena gracilis extract (EGE) enhances collagen synthesis, reduces sunburn cells, strengthens the skin barrier, influences key genes and metabolic pathways related to skin health, and shows significant improvements in skin hydration, TEWL reduction, complexion brightening, and wrinkle diminishment in clinical trials. These findings underscore the potential of TFP and EGE as eco-friendly ingredients that align with the growing consumer demand for sustainable and natural skincare solutions, highlighting their role in promoting skin health through a green beauty approach.

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