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Targeting Skin Barrier Dysfunction: A Green and Synergistic Composition to Address Children and Adolescent Skin Issues

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1. Introduction

Children and adolescent skin are particularly sensitive to both environmental stressors and hormonal fluctuations, leading to compromised skin barrier function[1,2]. The skin barrier plays a critical role in protecting the body from external irritants, preventing excessive water loss, and maintaining skin homeostasis. In children and adolescents, the skin is more prone to disruptions in barrier function due to hormonal changes, which contribute to conditions like acne, eczema, and dryness. Moreover, an imbalance in the skin microbiome can exacerbate these issues, particularly through an overgrowth of Cutibacterium acnes and Staphylococcus aureus, which are commonly associated with acne and inflammation.

In response to these challenges, skincare formulations must offer more than just symptom relief; they must focus on strengthening the skin's natural defense mechanisms and maintaining a healthy balance. This study explores a novel, synergistic composition designed to address both skin barrier dysfunction. The formulation comprises Lactobacillus/Alga Extract Ferment (LAEF), a natural, microbial fermentation product [3], and Glycerol Glucoside (GG), an ingredient derived from microalgaep [4]. Both ingredients are of edible grade, ensuring their safety and suitability for young and sensitive skin.

The objective of this study is to evaluate the efficacy of this formulation in restoring the skin barrier, reducing inflammation, and modulating the skin resistence. To achieve this, we employed a combination of in vitro skin models and network pharmacology analysis The research aims to provide a comprehensive understanding of how this green and synergistic composition can improve skin health in children and adolescent.

2. Materials and Methods

2.1 Sample Preparation and Materials

The formulation used in this study consisted of Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG), both natural ingredients. The Lactobacillus/Alga Extract Ferment (LAEF) was derived from microbial fermentation processes, and Glycerol Glucoside (GG) was sourced from microalgae. These ingredients were prepared in different concentrations (0.6%, 2%) for testing.

2.2 In Vitro Skin Model Testing

2.2.1 3D Epidermal Models

To evaluate the skin barrier repair and integrity, 3D epidermal models (EpiKutis®) were used. The models were exposed to UVB radiation (600 mJ/cm²) to simulate environmental stress. After treatment with the formulation, the models were incubated for 24 hours, followed by H&E staining and immunofluorescence analysis to assess the expression of key skin barrier proteins, including Filaggrin (FLG), Loricrin (LOR), Claudin 1 (CLDN1), and Tight Junction Proteins (TJM1). The relative expression levels of these proteins were compared between treated and control groups.

2.2.2 HaCaT Cell Line Testing

The HaCaT cell line was used to assess cell viability and the formulation's impact on oxidative stress. The cells were exposed to UVB radiation (40 mJ/cm²) or H2O2 (200 μM) to induce oxidative damage, and the formulation was added to evaluate its protective effects. The following assays were performed:

- CCK-8 Cell Viability Test: To assess the cytotoxicity and cell viability after treatment.
- ROS Assay: To measure the production of reactive oxygen species (ROS) induced by UVB or H2O2 exposure.
- Scratch Assay: To evaluate the ability of the formulation to promote cell migration and wound healing by measuring the cell migration distance after creating a scratch in the cell monolayer.

2.2.3 TRPV1 Expression Test

The TRPV1 expression was evaluated in HaCaT cells treated with different concentrations (0.1%, 0.3%, 0.6%) of the formulation. Capsazepine (500 nM) was used as the positive control. The expression of TRPV1 was induced using Capsaicin (50 μM), and the levels of TRPV1 were assessed using fluorescence microscopy.

2.3 Network Pharmacology Analysis

To explore the molecular mechanisms underlying the formulation's effects, network pharmacology was used. This approach involved the following steps:

1. Metabolomics and Target Prediction: Non-targeted metabolomics was used to identify bioactive metabolites from Lactobacillus/Alga Extract Ferment (LAEF). The top 100 metabolites by abundance were used for target prediction using the Swiss Target Prediction database. Glycerol Glucoside (GG) was also included in the target prediction.
2. Gene and Pathway Enrichment: GeneCards was used to identify skin-related targets, focusing on skin barrier and skin repair pathways. From this data, the top 500 genes were selected. The overlap between the targets of the composition and skin-related targets was analyzed.
3. Protein-Protein Interaction (PPI) Network: A PPI network was constructed using the STRING database to understand the relationships between the predicted targets. The analysis was further refined using Cytoscape and CytoNCA plugins to identify key targets with the highest degree, betweenness, and closeness centrality scores. This allowed for the identification of core regulatory pathways involved in skin barrier repair.

3. Results

3.1 Restoration of Skin Barrier Structure and Protein Expression in UVB-Damaged 3D Epidermal Models

To evaluate the efficacy of the formulation in restoring skin barrier function, we employed 3D epidermal skin models (EpiKutis®). These models were exposed to UVB radiation (600 mJ/cm²) to simulate environmental damage, followed by treatment with the Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation at concentrations of 0.6% and 2%. After 24 hours of incubation, the models were analyzed for structural integrity and the expression of key skin barrier proteins.

For sunburn Cells Quantification, UVB radiation caused damage to the epidermal models, resulting in an increased number of sunburn cells. The formulation at both 0.6% and 2% concentrations significantly reduced the number of sunburn cells compared to the negative control group. Specifically, the 2% formulation reduced sunburn cell counts by 85%, while the 0.6% formulation showed a reduction of 65% compared to the untreated group (NC). These results indicate that the formulation significantly alleviates UV-induced cellular damage.

For histological Analysis (H&E Staining), the histological examination revealed that the formulation at both concentrations restored the epidermal structure. The epidermal thickness was notably increased in the treated groups, suggesting enhanced barrier repair. In contrast, the negative control group, which received UVB exposure without treatment, displayed thinner epidermal layers and disrupted tissue architecture. These results suggest that the formulation promotes the recovery of skin structural integrity following UVB-induced damage.

For Immunofluorescence Staining of Skin Barrier Proteins, filaggrin (FLG) is a critical protein involved in maintaining the skin's permeability barrier. The immunofluorescence staining showed a 160.71% increase in FLG expression in the 2% formulation group compared to the negative control (NC). The 0.6% concentration also induced a significant increase in FLG expression (103.57% compared to NC).Loricrin (LOR) is another essential protein that plays a major role in the formation of the skin barrier. Treatment with the formulation led to a 215.38% increase in LOR expression in the 2% group compared to the negative control. The 0.6% formulation also showed a significant increase (142.31% compared to NC).Claudin 1 (CLDN1) is crucial for tight junction formation and maintaining skin cell-to-cell adhesion. Immunofluorescence staining of Claudin 1 revealed a 185.29% increase in expression in the 2% formulation group compared to NC, with a 88.24% increase in the 0.6% formulation group. Finally the formulation significantly enhanced the expression of epidermal barrier proteins Involucrin (IVL) and Transglutaminase 1(TGM1) in this UVB-exposed 3D skin model. At concentrations of 0.6% and 2% (v/v), IVL levels increased by 74.00% and 88.00%, while TGM1 increased by 54.72% and 86.79%, respectively.

These results indicate that the formulation enhances the expression of key proteins involved in skin barrier repair, further supporting its potential as a treatment for skin barrier dysfunction.

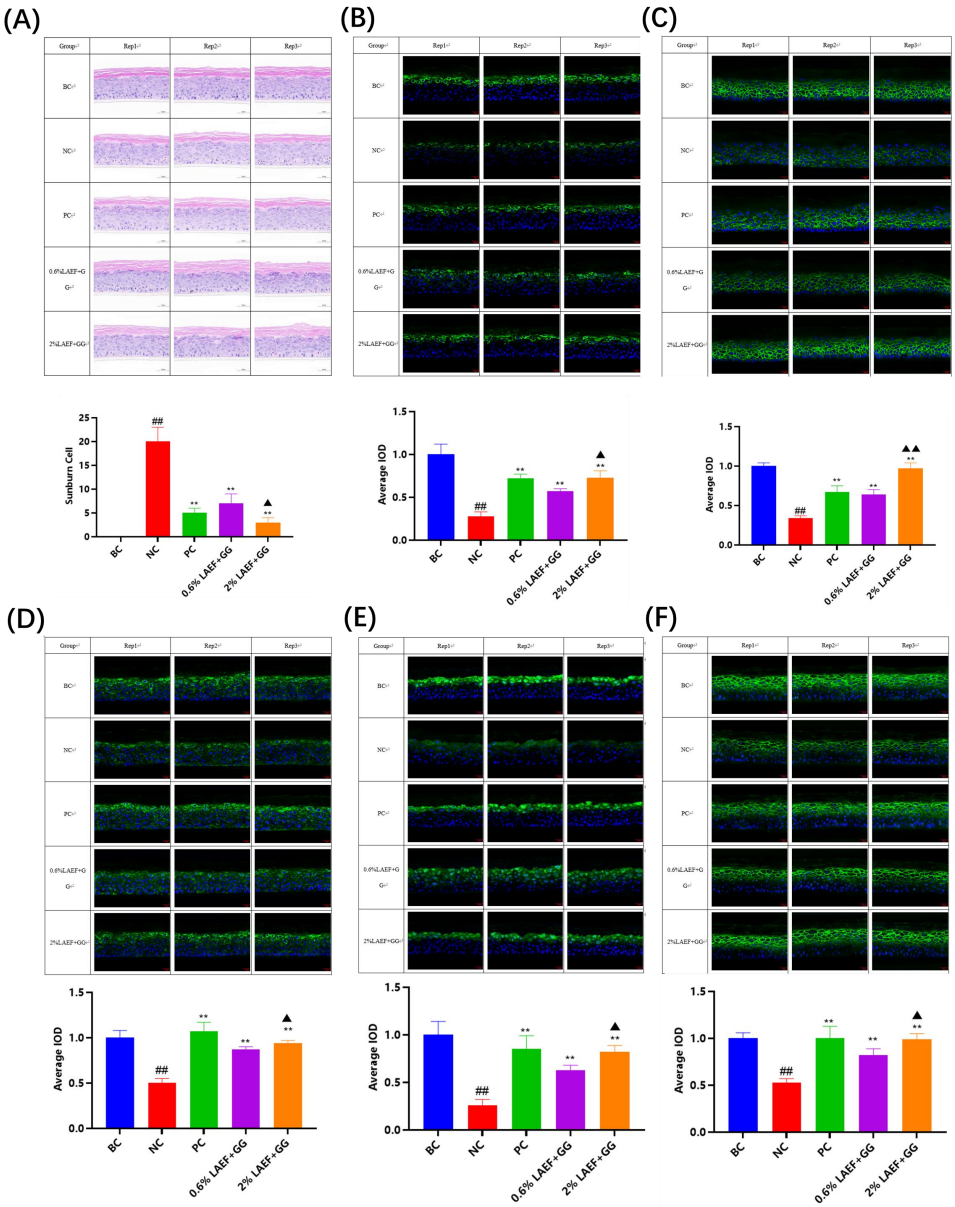

Figure 1:Skin Barrier Repair and Protein Expression in 3D Epidermal Models Post UVB Exposure

(A) H&E staining of 3D epidermal models treated with Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation after UVB exposure (600 mJ/cm²). The 2% formulation significantly restored epidermal thickness and structure compared to the negative control (NC), which showed thinner epidermal layers and disrupted tissue architecture. The 0.6% formulation also demonstrated improved structural integrity.**(B-F)** Immunofluorescence staining of skin barrier proteins Filaggrin (FLG), Claudin 1 (CLDN1), Involucrin (IVL) , Loricrin (LOR) and Transglutaminase 1(TGM1) in 3D epidermal models treated with Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation after UVB exposure. Significant increases were observed in the 2% formulation group compared to the negative control. The 0.6% formulation also enhanced these key proteins involved in maintaining skin integrity.Data are presented as mean ± SD (n=6). Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test. $p < 0.001$ versus negative control.

3.2 Enhanced Cytoprotection, Antioxidant Activity, and Wound Healing in HaCaT Cells Treated with the composition

To comprehensively evaluate the biological activity of the tested formulation in promoting skin barrier repair, a series of in vitro assays were conducted using the human keratinocyte cell line HaCaT. Oxidative stress was induced via either UVB irradiation (40 mJ/cm²) exposure, after which the formulation was applied at various concentrations to assess its protective and restorative effects.

To verify its antioxidative efficacy, intracellular reactive oxygen species (ROS) levels were quantified post-treatment. In the UVB-induced oxidative stress model, ROS levels were reduced significantly with more than 70% in the 0.6% group and 0.3% group, compared to the untreated control. These data highlight the potent antioxidant activity of the formulation, effectively mitigating UV- induced ROS accumulation in keratinocytes.

Additionally, a scratch assay was employed to evaluate the formulation's capacity to enhance cell migration and wound closure, key indicators of regenerative potential. Treated cells were monitored at 12, 24, and 48 hours post-wounding. Notably, after 12,24 and 48 hours, the 0.1%, 0.3% and 2% formulation resulted in an significant increase in wound closure compared to the negative control, indicating robust pro-migratory and wound-healing effects.

Collectively, these in vitro results demonstrate that the Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation significantly exerts potent antioxidant effects, and promotes cell migration. These combined activities support its functional role in strengthening the skin barrier and accelerating epithelial repair, suggesting strong potential for its use in cosmetic or dermatological formulations designed for sensitive or adolescent skin.

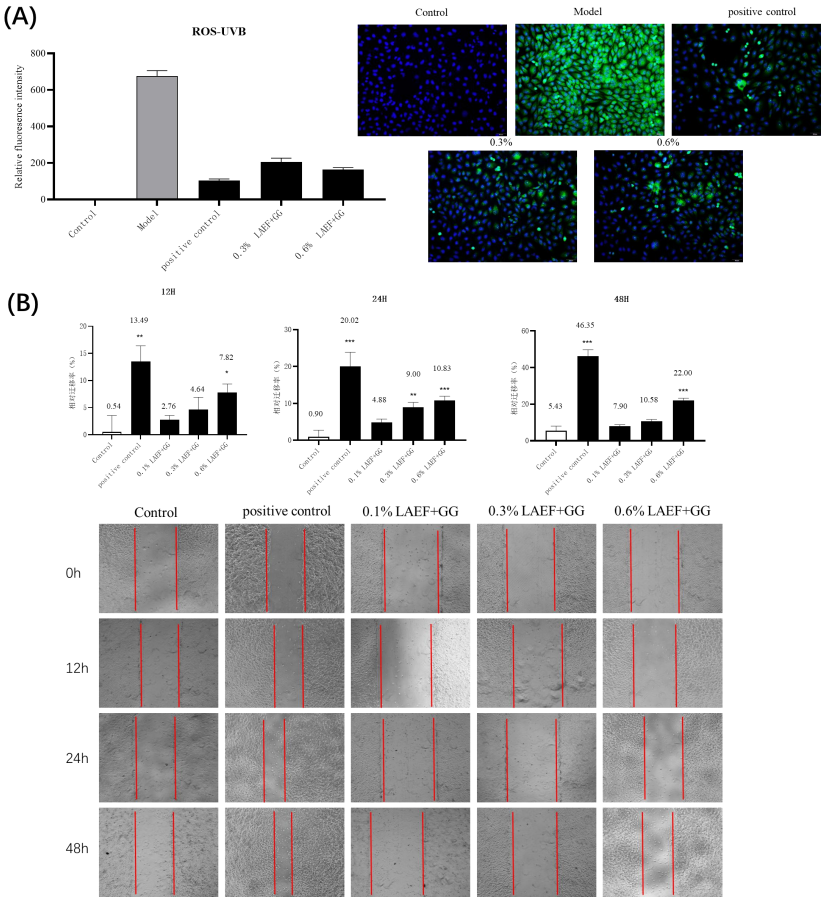

Figure 2: ROS Production, and Wound Healing in HaCaT Cells
(A) Reactive Oxygen Species (ROS) assay in HaCaT cells treated with Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation after UVB (40 mJ/cm²) exposure. The formulation significantly reduced ROS levels, with both 0.6% and 0.3% formulation compared to the negative control.
(B) Scratch assay (wound healing) in HaCaT cells treated with Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation at 0.1%, 0.3% and 0.6% concentrations. All tested formulation concentrations promoted cell migration at12, 24 and 48 hours.
Data are presented as mean ± SD (n=6). Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test. $p < 0.001$ versus negative control for all assays.

3.3 Inhibition of TRPV1 Expression in HaCaT Cells Indicates Anti-Irritation Potential of the Composition

To evaluate the anti-inflammatory and anti-irritant properties of the tested formulation, a TRPV1 expression assay was conducted using the HaCaT human keratinocyte cell line. Transient Receptor Potential Vanilloid 1 (TRPV1) is a nociceptive ion channel activated by physical and chemical stimuli, including thermal stress, UVB radiation, and capsaicin. Its overexpression is associated with cutaneous discomfort, itching, and inflammatory responses, making it a relevant target in the context of sensitive skin care.

In this experiment, TRPV1 expression was induced in HaCaT cells by treatment with Capsaicin (50 μM), a known agonist of the TRPV1 receptor. Following induction, the cells were treated with the Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation at concentrations: 0.1%, 0.3% and 0.6%, to assess its ability to downregulate TRPV1 expression.

Quantitative fluorescence analysis demonstrated that treatment with the 0.3% formulation resulted in a 44.32% reduction in TRPV1 expression relative to the capsaicin-treated control group. The 0.6% formulation exhibited a slightly stronger effect, with a 45.45% inhibition of TRPV1 expression. These results indicate a clear dose-dependent trend and suggest that the formulation effectively attenuates TRPV1 activation in keratinocytes.

Meanwhile, other inflammatory cytokines including IL-6, IL-1β and TNF-α were also effectively reduced by 0.1%, 0.3% and 0.6% formulation.(data not shown)

Collectively, the observed inhibition of TRPV1 as well as reduced inflammatory cytokines implies a potential mechanism by which the formulation may relieve skin irritation and inflammation caused by environmental or chemical stressors. This supports the formulation’s application in products targeting sensitive, inflamed, or reactive skin, particularly for adolescents or individuals prone to discomfort and barrier dysfunction.

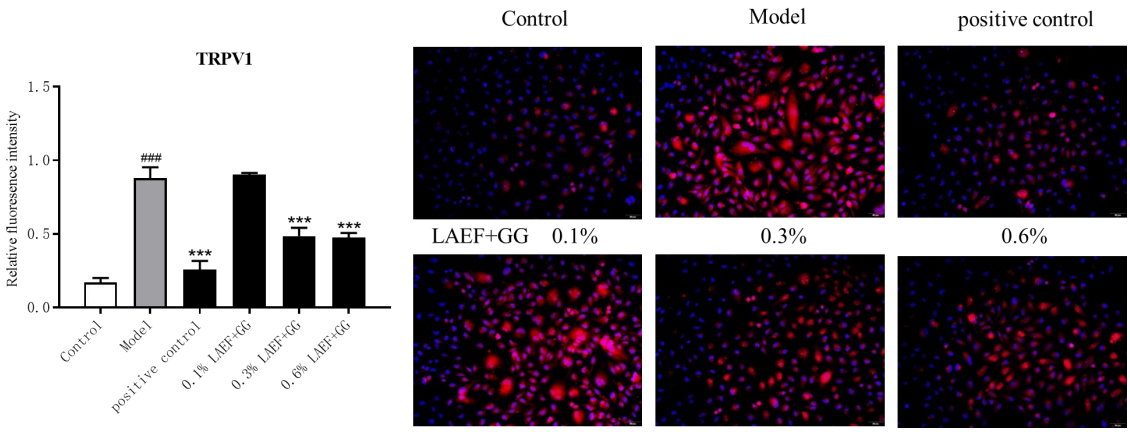

Figure 3: TRPV1 Expression and Inhibition in HaCaT Cells Post Capsaicin Treatment **(A)** TRPV1 expression in HaCaT cells treated with Capsaicin (50 μM) to induce TRPV1 expression and Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation at 0.3% and 0.6% concentrations. The 0.6% formulation inhibited TRPV1 expression by 45.45%, and the 0.3% formulation reduced TRPV1 expression by 44.32% compared to the negative control group.**(B)** Fluorescence imaging of TRPV1 expression in HaCaT cells treated with Capsaicin and Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation. The fluorescence intensity was markedly reduced in the 0.6% formulation group, indicating a significant decrease in TRPV1 expression. Data are presented as mean ± SD (n=4). Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test. $p < 0.001$ versus negative control.

3.4 Network Pharmacology Analysis

To uncover the underlying mechanisms by which the Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation exerts its skin barrier repair and anti-inflammatory effects potentially to meet children skincare demands, a comprehensive network pharmacology approach was implemented. Initially, untargeted metabolomics was used to identify a total of 1,327 metabolites from the Lactobacillus extract. The top 100 metabolites based on abundance were selected, and their corresponding SMILES structures were utilized for target prediction through the Swiss Target Prediction database. In total, 746 targets were predicted for these metabolites and GG. These targets were then cross-referenced with genes associated with skin barrier and skin repair, retrieved from the GeneCards database, resulting in 96 overlapping targets related to skin barrier function and 73 targets linked to skin repair. The combined set of 111 unique targets was subsequently analyzed through protein-protein interaction (PPI) network construction using the STRING database, which allowed for the identification of key target proteins involved in regulating skin physiology. The analysis was visualized with Cytoscape 3.8, where centrality measures such as degree, betweenness, and closeness were calculated, ultimately identifying 12 core targets (including AKT1, PIK3CA, MAPK1, and PIK3R1) that play pivotal roles in regulating skin barrier integrity and repair pathways.

Further analysis of these core targets revealed their significant involvement in a variety of biological processes (BP), molecular functions (MF), and cellular components (CC), as determined by Gene Ontology (GO) enrichment analysis. Notably, the core targets were primarily associated with cellular responses to stress, regulation of protein localization, and key signaling pathways such as insulin receptor signaling and IGF signaling, both of which are essential for skin regeneration and homeostasis. These targets were also involved in

processes such as phosphorylation, kinase activity, and the regulation of cellular chemical homeostasis. In terms of cellular components, the targets enriched cellular structures such as cell-cell junctions, late endosomes, and transcription regulation complexes, which are crucial for maintaining skin integrity and promoting skin regeneration. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified 128 signaling pathways, with 63 related to human diseases. The top 30 pathways were mainly involved in critical processes such as cell proliferation, immune responses, and barrier repair, including well-known pathways like PI3K-Akt, TNF, VEGF, and HIF-1 signaling. Among the most frequently enriched targets in these pathways were AKT1, PIK3CA, PIK3R1, and MAPK1, indicating their central role in modulating cellular responses to environmental stress and regulating skin barrier function. The identification of these key targets provides valuable insights into the molecular basis of the formulation's effects on skin repair.

The network pharmacology analysis thus provides a robust framework for understanding the formulation's multifaceted mechanisms of action, suggesting that it operates through a combination of antioxidant, anti-inflammatory, and skin barrier-enhancing effects via the modulation of key signaling pathways, which is potentially suitable for children skin application.

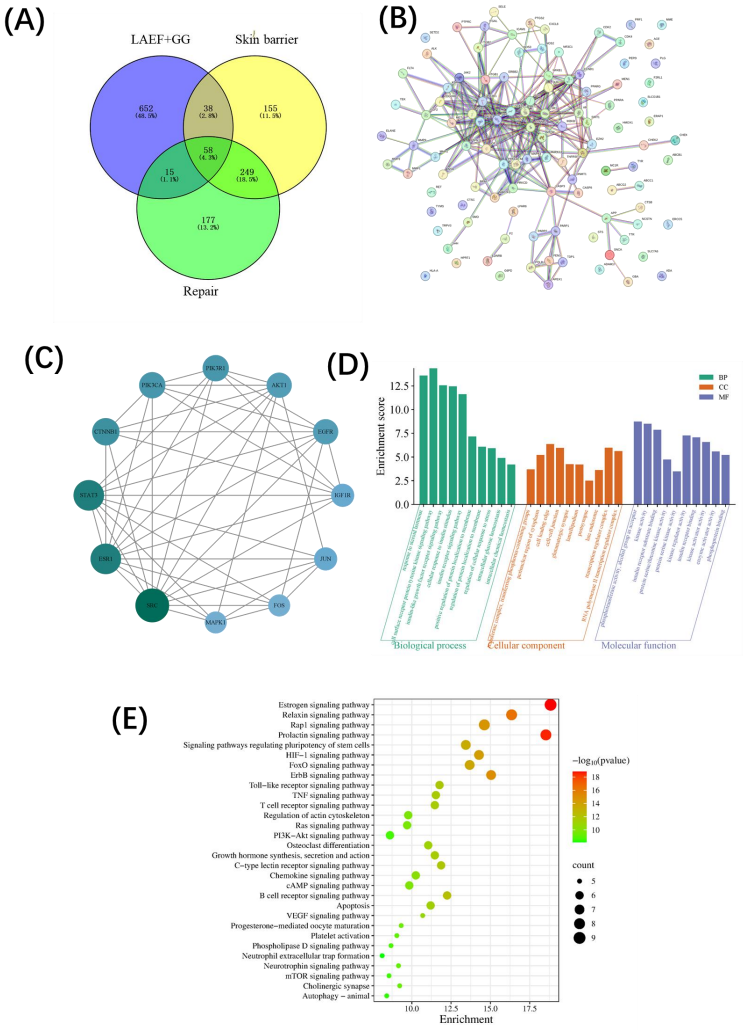

Figure 4: Network Pharmacology Analysis of Key Targets and Pathways Involved in Skin Barrier Repair and Anti-Inflammation for Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) formulation

(A) Venn diagram showing target overlaps between predicted formulation targets and GeneCards-derived “skin barrier” and “skin repair” genes. A total of 111 overlapping targets were identified, with 96 related to skin barrier function and 73 related to skin repair.

(B) Protein-protein interaction (PPI) network of the 111 targets, constructed using STRING (score $\geqslant$ 0.9) and visualized with Cytoscape. Nodes represent proteins, and edge thickness denotes confidence levels.

(C) Core target identification using topological analysis, where 12 high-degree central nodes were identified based on degree, betweenness, and closeness metrics. Node size and color intensity reflect degree value.

(D) GO enrichment analysis (top 10 terms per BP, MF, and CC), showing significant involvement in stress response, protein localization, kinase activity, and insulin/IGF signaling.

(E) KEGG pathway enrichment analysis (top 30 non-disease pathways), highlighting estrogen signaling, PI3K-Akt, VEGF, TNF, HIF-1, and TLR pathways.

Statistical Methods: Target prediction was performed via SwissTargetPrediction; enrichment analyses used DAVID and Metscape with Benjamini-Hochberg correction. P-values < 0.05 were considered significant.

4. Discussion

4.1 Efficacy of Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside

The results of this study strongly support the hypothesis that the Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) composition can enhance skin barrier function, reduce oxidative stress, and modulate inflammatory responses in adolescent skin. In the 3D skin models, the formulation showed significant improvements in key skin barrier proteins, including Filaggrin (FLG), Loricrin (LOR), and Claudin 1 (CLDN1). The increase in these proteins is consistent with enhanced skin barrier function, which is crucial for preventing water loss and protecting the skin from environmental stressors such as UV radiation.

The in vitro tests with HaCaT cells further corroborate these findings, demonstrating that the formulation enhances cell viability, reduces ROS production, and promotes cell migration. These results are especially significant for adolescent skin, which is often more vulnerable to environmental stressors and oxidative damage. By reducing ROS levels and promoting cell migration, the composition helps restore the skin barrier and accelerate wound healing, addressing both the structural and functional needs of the skin.

Moreover, the reduction in TRPV1 expression observed in the TRPV1 test suggests that the formulation can alleviate irritation and inflammation commonly seen in adolescent skin conditions, such as acne and eczema. TRPV1 is known to play a role in skin inflammation and sensory responses to stimuli, and its inhibition could contribute to a reduction in skin sensitivity and irritation.

4.2 Implications for Children and Adolescent Skincare

Children and adolescent face unique challenges in maintaining healthy skin due to hormonal fluctuations, and heightened sensitivity to environmental factors. The findings from this study suggest that the formulation of Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) provides a promising solution for restoring skin health in this age group. By

improving skin barrier function, reducing oxidative stress, and modulating the inflammatory response, the formulation addresses multiple facets of children and adolescent skin health.

This approach is particularly relevant for treating conditions such as acne, dryness, and eczema, which are common during children and adolescence. The ability of the formulation to reduce ROS levels and promote cell regeneration may offer a more effective, long-term solution compared to traditional acne treatments that often focus solely on reducing surface oil production.

5. Conclusion

This study demonstrates that the synergistic composition of Lactobacillus/Alga Extract Ferment (LAEF) and Glycerol Glucoside (GG) offers a promising solution for improving skin barrier function, reducing oxidative stress, and modulating the inflammatory response in children and adolescent skin. The in vitro results, including those from 3D epidermal models, HaCaT cell line testing, and TRPV1 expression assays, show significant improvements in skin barrier proteins, antioxidant activity, cell migration, and inflammation reduction. The formulation's ability to target key signaling pathways involved in skin health further supports its potential for treating common adolescent skin conditions such as acne, dryness, and irritation.

Overall, the findings suggest that this formulation could serve as a safe, effective, and holistic approach to addressing the unique skin concerns of children and adolescents, providing long-term benefits for skin health by improving both its structure and function. Further studies are needed to validate these findings in larger clinical trials and explore the formulation's potential for combination therapy with other skincare treatments.

6. Reference

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