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The mechanism of soothing effect of plant extracts was studied based on network pharmacology and transcriptomics methods

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1. Introduction

With the growing demand for natural, safe, and effective skincare products, plant extracts have garnered significant attention in the cosmetics industry due to their low risk of side effects [1,2]. However, the complex composition of plant extracts, encompassing diverse structural types and numerous isomers, remains a challenge. Their molecular mechanisms of action are still not fully understood, which restricts their in-depth research and broader application.

Network pharmacology, an emerging interdisciplinary field combining bioinformatics, systems biology, medicinal chemistry, and AI algorithms, offers a robust tool for studying intricate drug-target interactions. By constructing drug-target networks and conducting pathway analyses, it can unveil the multi-target and multi-pathway action mechanisms of plant extracts[3-7]. Transcriptomics, which leverages advanced high-throughput sequencing technologies, enables comprehensive and accurate detection of gene expression under specific conditions [8,9]. By comparing transcriptomic data from treated and control groups, it can pinpoint differentially expressed genes following plant extract treatment and delve into their functions, associated signaling pathways, and interactions. This not only helps uncover the direct targets of plant extracts responsible for soothing effects but also explores biological processes indirectly influenced by gene expression regulation [10-12].

This study integrates network pharmacology and transcriptomics, focusing on the soothing mechanisms of plant extracts to deeply analyze their molecular action mechanisms and impacts on intracellular signaling pathways. Through systematic network pharmacology analysis of active components, target networks, signaling pathways, and gene expression changes of plant extracts, this study aims to provide a more scientific basis for the development and application of plant extracts in the health industry. It also seeks to offer new insights and methods for researching the mechanisms of other natural products, thereby driving innovation in biomedicine and cosmetics.

2. MATERIALS & METHODS

Preparation of plant extract formulations

The preparation of the plant extracts formula has been patented. According to the patent, stems of Opuntia, roots of Sophora flavescens, stems of Dendrobium nobile, leaves of Aloe vera from Curacao, fruits of Lycium barbarum from Ningxia, roots of Ophiopogon japonicus, and flowers of Chrysanthemum indicum are weighed in specific proportions, mixed, pulverized, extracted, decolorized, and concentrated. Subsequently, they are combined with pentylene glycol, 1,2-hexanediol, glycerin, and trehalose in specific proportions.

Efficacy verification ideas and methods

Firstly, the components of BSBE were analyzed. The results of the component analysis were then processed using network pharmacology and artificial intelligence (AI). Targets were identified through database analysis. Finally, the efficacy of BSBE was verified using three-dimensional epidermal skin models and transcriptomics, which confirmed its effectiveness.Theoretical experiment and efficacy verification experiment scheme (Figure 1).

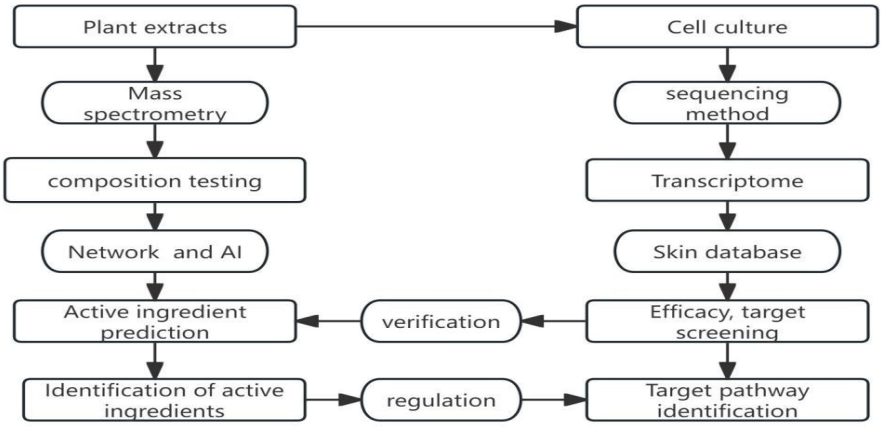

Figure 1. Theoretical experiment and efficacy verification experiment scheme

Chemical composition analysis

To prepare the sample, 1 mL of BSBE extract was vacuum-dried. Subsequently, 300 μL of a 40% methanol aqueous solution was added to reconstitute the dried extract. The resulting mixture was vortexed for 3 min, centrifuged at 16,000 g and 4 ℃ for 20 min, and the supernatant was collected as the sample for analysis. The samples were separated using a Vanquish UHPLC ultra-high performance liquid chromatography system (Thermo Fisher Scientific, Bremen, Germany) equipped with an ACQUITY UPLC HSS T3 chromatographic column (2.1 mm × 100 mm, 1.8μm). The column temperature was maintained at 35℃, and the flow rate was set at 0.3 mL/min. The mobile phase comprised 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) for gradient elution. The separated samples were analyzed using an Orbitrap Exploris 480 mass spectrometer, with electrospray ionization (ESI) in both positive and negative ion modes employed for detection.

Network Pharmacology Analysis

Based on the identified chemical components of BSBE, potential targets of the compounds were searched and summarized using databases such as TCMSP, TCMID, and CTD, along with AI algorithms. Targets related to skin efficacy were then screened out through our internal Skin Molecular Function Knowledge Database. By intersecting the potential targets of the compounds with skin-related targets, we obtained targets relevant to the skin effects of BSBE. AI was used to compare clean data with the reference genome to calculate gene expression levels. Differentially expressed genes were identified by comparing gene expression data between the treatment group and the NC group. These differentially expressed genes were then annotated and enriched through GO (Gene Ontology) and KEGG

enrichment analyses, revealing changes in gene expression in skin cells after BSBE treatment and their potential biological significance.

The potential targets of the compounds in BSBE were screened using the TCMSP [13], TCMID [14], and CTD [15] databases. The targets of all components were identified as official gene names through the Uniprot [16] database. These targets were intersected with skin-related targets from the Skin Molecular Function Knowledge Database (SMD). The intersection targets were imported into the String [17] database to obtain protein interaction relationships. The network diagram was drawn using the Network X [18] software package to identify core targets.

Gene Ontology (GO) analysis [19,20] was performed using the GO database [21] (geneontology.org) to select three modules: biological process (BP), molecular function (MF), and cellular component (CC). KEGG [22] (Kyoto Encyclopedia of Genes and Genomes) analysis was used to select TOP20 data of BP, MF, CC, and KEGG pathways based on P value ($p < 0.05$). The results of the pathway enrichment analysis were visualized using the R language.Through GO function and KEGG function enrichment, we obtained the enrichment results of inflammatory factors, skin barrier, mitochondrial function, autophagy and other efficacy directions. This paper focuses on the selection of inflammatory factors and skin barrier-related pathways for differential analysis and functional analysis.

Transcriptome analysis based on the 3D skin model

Transcriptome analysis helps identify genes activated or inhibited under specific conditions, shedding light on the related biological processes and regulatory mechanisms. The 3D skin model was treated with different concentrations of BSBE. During the culture period, the medium was refreshed regularly, and environmental stability was maintained by controlling temperature, humidity, and CO_2 levels. After a designated culture period, total RNA from each group was extracted using the Trizol method, strictly following kit instructions to ensure RNA integrity and purity. The RNA samples underwent high-throughput sequencing to create a sequencing library. Raw sequencing data were pre-processed by removing low-quality reads, adaptor sequences, and contaminants, yielding clean data ready for further analysis.

Moisturizing test base on the 3D skin model

Preparation of the positive control group solution involves dissolving 400.00 μL of glycerin in 1.60 mL of PBS. This yields a solution with a glycerin concentration of 20.00% (v/v). Subsequently, add 0.90 mL of EpiGrowth culture medium to each well of the 6-well plate, respectively, and transfer the 3D skin model to each well. Ensure the test group numbers are labeled on the 6-well plate. The blank control (BC) group remains untreated, and the positive control (PC) group undergoes treatment with a 20.00% glycerin working solution applied to the model's surface. The sample groups were treated with a 5.00% BSBE solution. Afterward, place the plate in a CO_2 incubator (37℃, 5% CO_2) for 24 hours. The improvement rate of skin water content was calculated by comparing with the NC group to evaluate the moisturizing effect of BSBE.

Detection of anti-inflammatory related factors by ELISA

0.1% sodium lauryl sulfate (SLS), an anionic surfactant, was used as the irritant to establish 3D in vitro skin injury model. The soothing efficacy of the sample formulation was evaluated by assessing changes in the levels of pro-inflammatory cytokines (IL-1α) and mediators (PGE2), following the application of the sample formulation. The experimental design included a blank control (BC) group, a negative control (NC) group, a positive control (PC) group, and a sample group.

Except for the blank control (BC) group, 0.25 μL of 0.10% SLS working solution was added to the surface of each group. The blank control (BC) group received no treatment and was incubated for 30 minutes. After incubation, the positive control (PC) group was treated with 0.01% dexamethasone working solution. The sample group received 12.50 μL of plant extracts solution at the volume concentrations specified in Table 1, which was evenly applied to the surface of the 3D skin model. Subsequently, all groups were placed in a CO_2 incubator (37℃, 5% CO_2) for 24 hours. After 24 hours of incubation, the culture medium from the 3D skin models was collected and transferred to centrifuge tubes. Finally, detection and analysis were conducted according to the instructions provided in the ELISA or IF assay kit.The test scheme refers to Table 1.

Table 1. Detection of anti-inflammatory related factors by ELISA

Groups	Sample names	Dosing concentration	Stimulation condition	Model	Markers	Methods
Blank control (BC)	/	/	/	EpiKutis	IL-1α PGE2	ELISA
Negative control (NC)	/	/	SLS (0.1%)			
Positive control (PC)	Dexamethasone	0.01%				
Samples	BSBE	2% 5%				

3. Results

3.1 Chemical composition analysis

The chemical components in BSBE were analyzed using UHPLC Vanquish ultra-high performance liquid chromatography and Orbitrap Exploris TM 480 high-resolution mass spectrometry. Mass spectrometry was performed in electrospray ionization positive and negative ion modes. After converting the original data files into the appropriate format, the data were processed using XCMS software and searched against a local database for compound identification. The results showed that 772 substances were identified in positive ion mode(Figure 2A), and 425 substances in negative ion mode(Figure 2B), totaling 1,152 substances(Figure 2C). In the positive ion and negative ion mode, if the same compound is identified, there is no repeated counting. These substances covered a variety of plant-related components, and the identification data were stable and reliable.

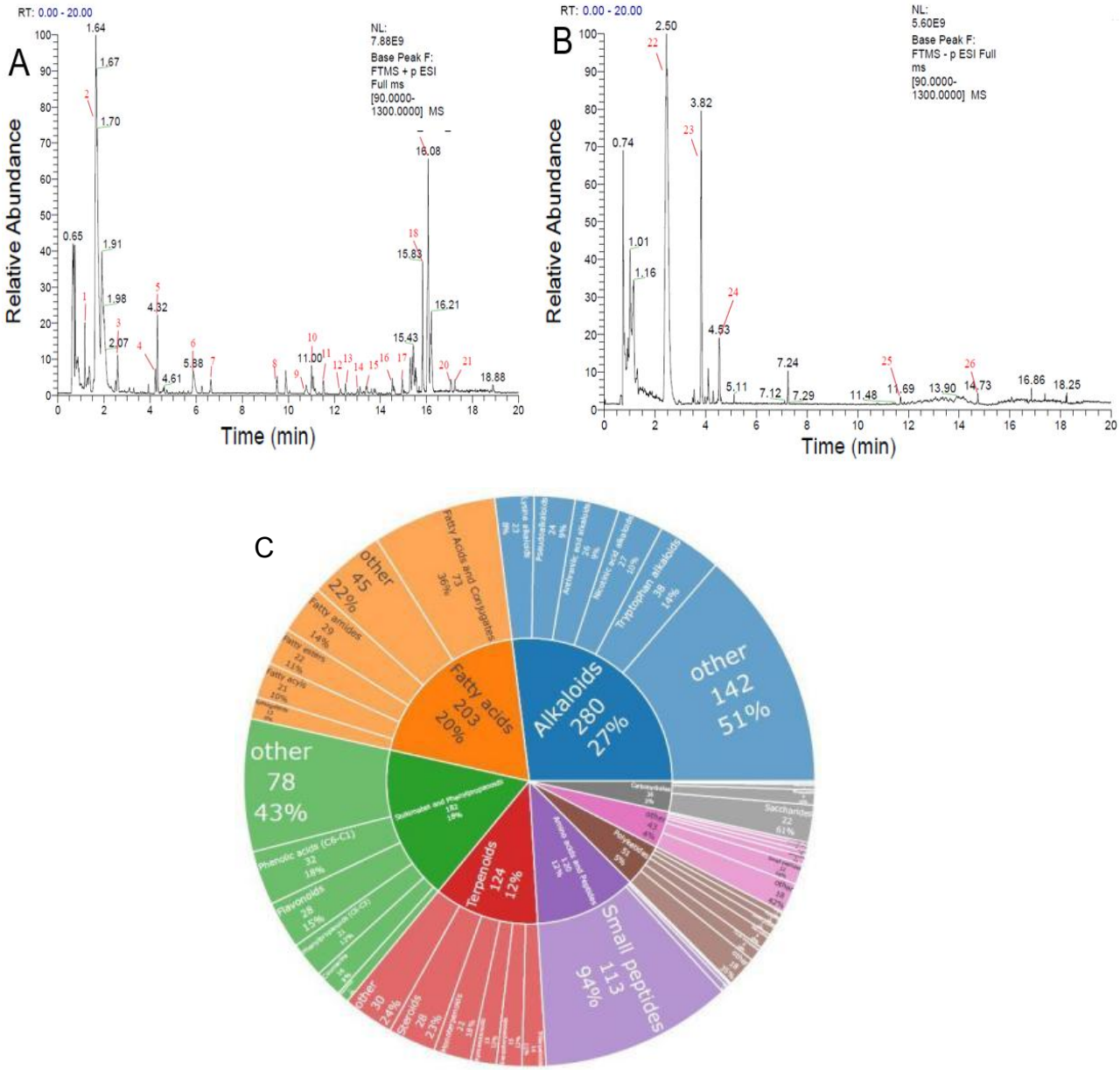

Figure 2. (A) Ion diagram in positive ion mode; (B) ion diagram in negative ion mode; (C) Compound classification diagram.

3.2 Gene expression and functional enrichment results

The gene expression analysis and functional analysis of BSBE in inflammatory factors and repair efficacy were performed by 3D skin model. As shown in Figure 3A and 3B, compared with the control group, BSBE has inhibitory effects on inflammatory factors such as IL1 A and IL1 B (Figure 3A), and has inhibitory effects on repair targets such as TRPV1, DEFB4 A, DEFB103 A and DEFB103 B ; through GO function enrichment, the difference analysis of BSBE in repair effect was carried out(Figure 3B). Compared with the control group, the differentially expressed genes in BSBE were positively regulated in the expression of endopeptidase activity, hyaluronan metabolic process, hyaluronan biosynthetic process and other related signals, indicating that BSBE has soothing and repairing effects.

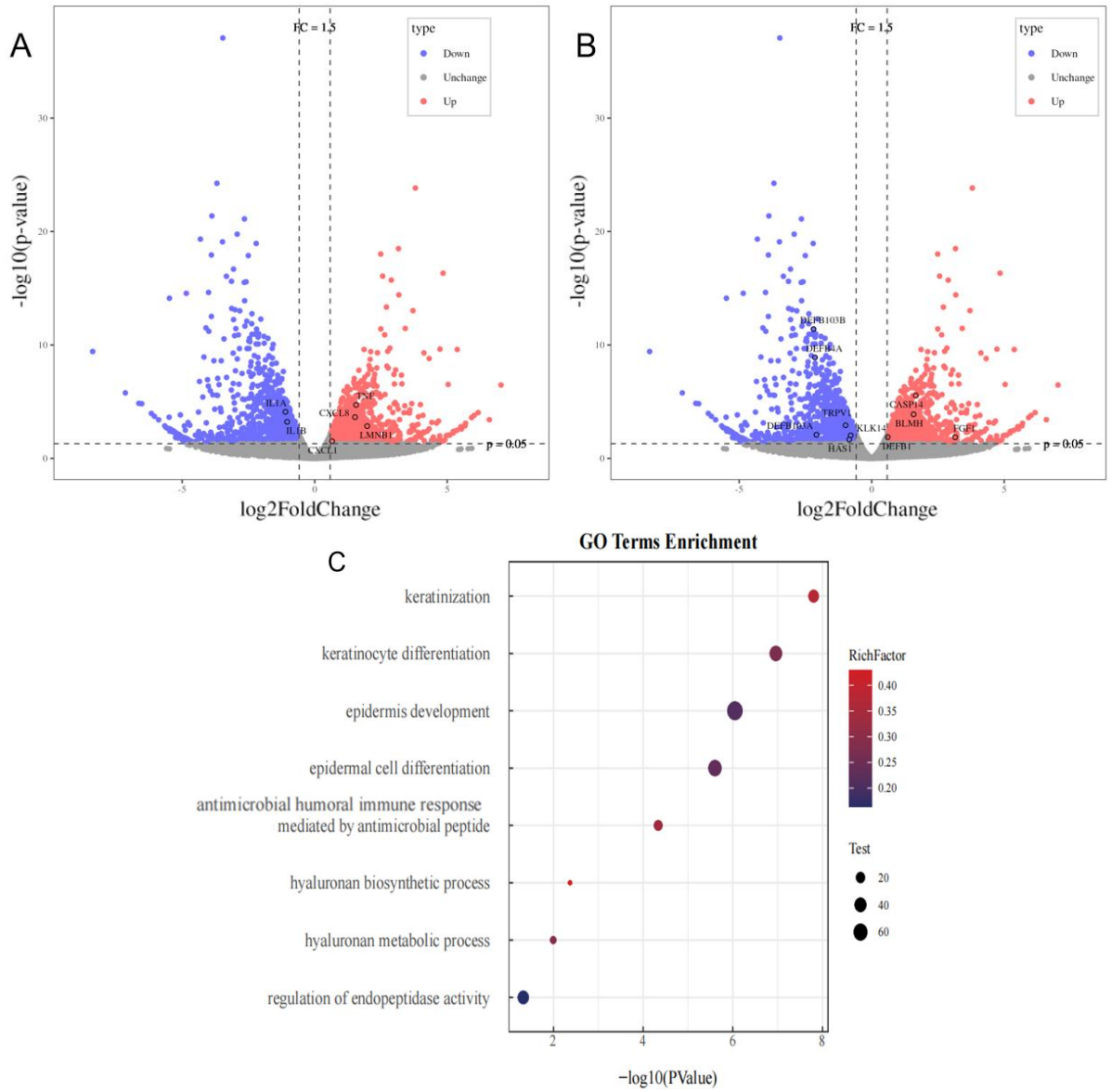

Figure 3. (A) Difference analysis of the direction of inflammatory factors; (B) Difference analysis of skin barrier direction; (C) GO functional bubble diagram in repair direction

3.3 Transcriptome analysis results

In network pharmacology, targets with relatively concentrated anti-inflammatory and soothing effects were selected. GO functional analysis(Figure 4A) and KEGG analysis(Figure 4B) were used to map differentially expressed genes or proteins to metabolic pathways. These analyses revealed the roles of these genes or proteins in various pathways and their interrelationships. Using a 3D skin model, transcriptome sequencing and data analysis were performed. Compared with the control group, the treatment group had a significant inhibitory effect on the expression of IL1 A, IL1 B and other inflammation-related factors, indicating that BSBE has a specific anti-inflammatory pathway and has a soothing effect.

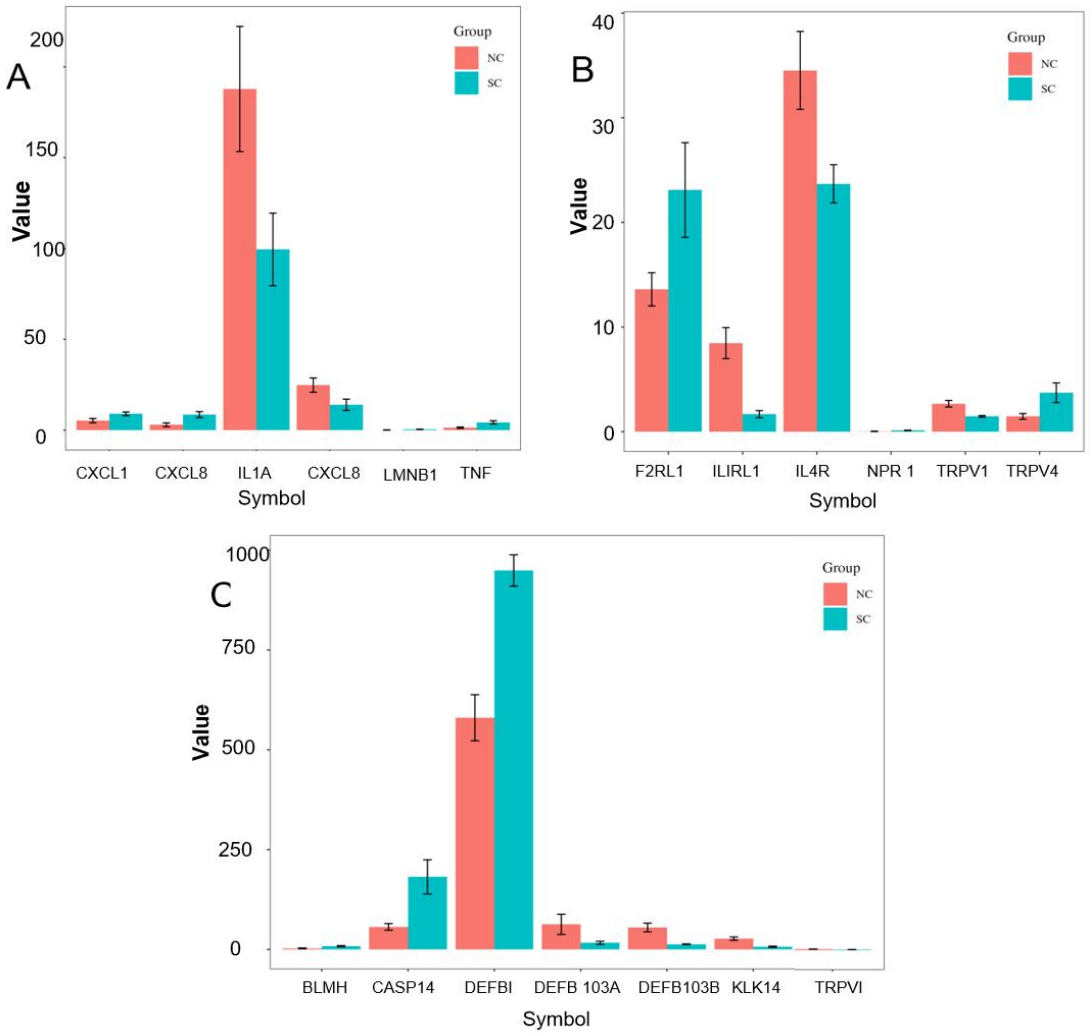

Figure 4. The efficacy of BSBE in the direction of inflammation.
(A) The analysis of KEGG, the changes of skin inflammatory factors were analyzed;
(B) The analysis of KEGG, the changes of inflammatory related nerve receptor pathway;
(C) The analysis of GO, The changes of skin barrier function.

3.4 Detection of soothing and moisturizing effects based on 3D skin model

The soothing effect of BSBE was assessed using an ELISA kit assay. Compared with the control group, the pro-inflammatory factor IL-1α decreased significantly at a concentration of 2.00% (v/v), with an inhibition rate of 15.71%. At 5.00% (v/v), the inhibition rate of IL-1α was 11.11%(Figure 5A). This indicates that BSBE at concentrations of 2.00% and 5.00% (v/v) significantly inhibits IL-1α, demonstrating its soothing effect. Similarly, the PGE2 content decreased significantly at a concentration of 2.00% (v/v), with an inhibition rate of 19.00%. This demonstrates that BSBE at 2.00% (v/v) significantly inhibits PGE2, further confirming its soothing effect(Figure 5B).

The NC group exhibited a skin hydration level of 30.71 (a.u.). In contrast, the BSBE test group at 5.00% (v/v) showed a skin hydration level of 40.51 (a.u.), representing a 31.91% improvement(Figure 5B). This indicates that BSBE at 5.00% (v/v) has a pronounced moisturizing effect.

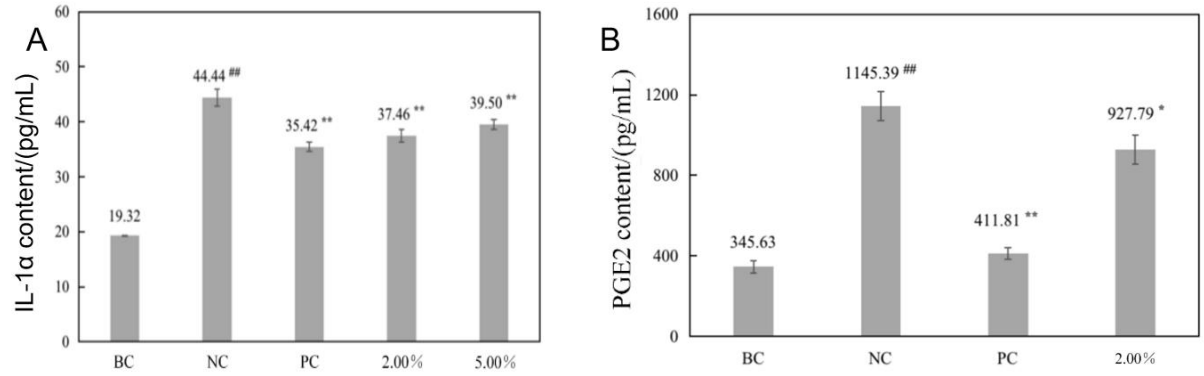

Figure 5. The expression of inflammation-related factors was detected by ELISA. (A) IL-1α Content test results; (B) PGE2 content test results.

4. Discussion

In cosmetics research and development, plant extracts have garnered significant attention for their natural origin and low risk of side effects. However, the complex composition and unclear mechanisms of action have become major bottlenecks in their development. In this study, BSBE was the research focus. A range of techniques was employed to investigate its soothing mechanism, providing a valuable reference for applying plant extracts in cosmetics. Initially, high-resolution mass spectrometry was used to analyze BSBE's chemical composition. A total of 1,152 substances were identified, encompassing various plant-related components and establishing a robust foundation for further mechanistic studies. Network pharmacology, leveraging multiple databases and AI algorithms, was used to predict potential targets and identify those related to skin efficacy.

Subsequent GO and KEGG enrichment analyses revealed that BSBE's multiple components act on various targets, participating in numerous biological processes and signaling pathways, such as inflammatory and cell cycle regulation. This initially delineated its complex yet organized mechanistic network. Transcriptomics verified the targets and pathways predicted by network pharmacology at the gene expression level, revealing substantial changes in skin cell gene expression following BSBE treatment and enhancing the understanding of its mechanism of action.

This study is innovative in several ways. In terms of methodology, it innovatively combines high-resolution mass spectrometry, network pharmacology, and transcriptomics. High-resolution mass spectrometry accurately analyzes complex chemical components. Network pharmacology, using AI algorithms, efficiently mines potential targets and pathways. Transcriptomics verifies and expands the mechanism of action from the genetic level. This multi-technology approach offers a comprehensive and in-depth perspective on plant extract research.

Additionally, the research framework of plant extracts-components-targets-efficacy developed in this study offers a reference model for similar research and advances the field of plant extract research. In vitro experimental results fully confirmed BSBE's efficacy. At various concentrations, BSBE significantly inhibited inflammatory factor expression (IL-1α, PGE2) and effectively increased skin hydration, demonstrating excellent soothing and

moisturizing effects. These findings provide a strong basis for BSBE's cosmetic applications and offer insights for developing other plant extracts.

Nevertheless, this study has limitations. While the soothing mechanism of BSBE is preliminarily revealed, further research is needed on the synergistic effects between components and in vivo efficacy in complex environments. Future in vivo experiments, combined with advanced technologies, could further explore the mechanisms and application potential of plant extracts.

5. Conclusion

This experiment systematically investigated the soothing mechanism of the plant extract BSBE. Firstly, the chemical composition of BSBE was characterized using high-resolution mass spectrometry. This analysis accurately identified numerous plant-related components, thereby clarifying its material basis. Then, network pharmacology was employed, leveraging multiple databases and AI algorithms to predict targets and analyze associated pathways. This step elucidated the complex interactions between the multiple components of BSBE and their multiple targets. Subsequently, the findings from network pharmacology were validated at the gene expression level using transcriptome technology. This further clarified the changes in gene expression within skin cells following BSBE treatment. Finally, a three-dimensional epidermal skin model was utilized to confirm the efficacy. The results demonstrated that BSBE could substantially inhibit IL-1α expression at concentrations of 2% and 5%, markedly reduce PGE2 levels at 2%, and significantly increase skin hydration at 5%. TMoreover, the multi-technology fusion approach employed in this study successfully elucidated its mechanism of action, thereby providing robust support for the application of plant extracts in the cosmetics industry. Nevertheless, the study has certain limitations. Future research could incorporate in vivo experiments to further explore these mechanisms, providing a more comprehensive theoretical foundation for the development and utilization of plant extracts.

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