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# *"Study on the Moisturising and Oil-control Efficacy of Black-red Paeonia Albiflora Ferment Filtrate"*

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## 1. Introduction

Excessive sebum secretion can lead to enlarged pores and acne vulgaris skin problems [1]. Abnormal sebaceous gland function is not only associated with overproduction of sebum but also with alterations in the composition of sebum components. During fatty acid synthesis, a series of enzyme - catalyzed reactions involving acetyl - CoA carboxylase (ACC) and fatty acid synthase (FAS) play a role [2-3]. Research has shown that sebum components with pro - inflammatory properties and involved in inflammatory cascades are related to the development of acne lesions [4]. Among androgens, dihydrotestosterone (DHT) exhibits the strongest androgenic activity and has a higher binding affinity for androgen receptors (AR). The effects of DHT on the skin are primarily mediated through its role in the differentiation and proliferation of sebaceous glands and sebum production [5-6].

Studies have indicated that AR can also regulate the expression of ACC and FAS through different pathways [7]. This implies that AR can bind with DHT to promote abnormal differentiation of sebaceous glands and also play a regulatory role in fatty acid synthesis.

In individuals with oily skin, there is a certain degree of impaired barrier function in the cheek area. This is due to the excessive secretion of sebum, which disrupts the normal arrangement of lipids in the stratum corneum and compromises its integrity, thereby affecting the normal barrier function of the skin.

*Paeonia Albiflora* flowers contains compounds such as gallic acid derivatives, flavonoid polyphenols, and polysaccharides. Among these, paeonia flower polyphenols have shown remarkable effects in free radical scavenging. Research has confirmed that plant - derived extracts obtained through fermentation have enhanced efficacy and reduced toxicological potential [8-10].

In this study, the fermentation of black - red *Paeonia Albiflora* flower using *Galactomyces geotrichum* was conducted to obtain the fermentation filtrate (BPF). The effects of BPF on sebum management - related genetic factors and barrier - related genetic factors were investigated. Additionally, human - based tests were performed to evaluate the effects of BPF on sebum secretion and skin barrier function, in order to explore the efficacy of BPF.

## 2. Materials and Methods

### 2.1 Materials

Candida krusei (KTAPG7 11.67), Peony of the Black Sea Wave variety, 5α - dihydrotestosterone (DHT) standard solution (Aldrich® D - 073), MTT and DMSO, CCK - 8, PBS, Human normal sebaceous gland cells SZ95, Human immortalized keratinocytes SCSP - 5091, Primers (FLG, TGM5, AR, ACC, FAS), Real - time quantitative PCR, using BeyoFast™ SYBR Green qPCR Mix (2X, Low ROX), Salicylic acid.

Carbon dioxide incubator, Skin elasticity tester Corneometer MPA580, Transepidermal water loss and evaporative water loss test probe Tewameter TM HEX, Standardized 3D camera system Antera 3D, Skin oil tester Sebumeter SM815, Facial image analyzer Visia CR (with Image Pro Plus comprehensive skin analysis software).

### 2.2 Methods

### 2.2.1 Preparation of Black Peony Fermentation Liquid (BPF)

Activation and Large-Scale Cultivation of Geotrichum candidum (KTAPG7 11.67). The freeze-dried Geotrichum candidum (KTAPG7 11.67) was retrieved and inoculated onto a solid medium for activation. Subsequent subculturing (two generations) restored its viability, followed by an additional subculture to ensure stable activity. The strain was then expanded in a liquid culture.

Preparation of black - red *Paeonia Albiflora* flower Extrac. Black - red *Paeonia Albiflora* flowers were washed, dried, and milled into a coarse powder, which was sieved through a 50-mesh screen. Two hundred grams of the sieved powder were soaked in 1 L of deionized water for 1 hour, then heated to 60°C. Ultrasonic treatment (30 kHz, 0.5 hours) enhanced extraction efficiency. The mixture was further heated to 75°C with stirring for 1 hour, followed by filtration to obtain the extract.

Sterilization and Inoculation. One liter of the extract was sterilized at 123°C to prepare the culture medium. The activated Geotrichum candidum was inoculated into this medium.

Fermentation and Purification: Fermentation proceeded at 28°C with shaking (200 r/min) for 60 hours. The resulting broth was centrifuged to remove debris, yielding a clear Paeonia suffruticosa fermentation liquid. A preservative system (5% butanediol, 2% 1,2-hexanediol, and 0.05% ethylhexylglycerin) was incorporated to formulate the final product, BPF.

### 2.2.2 Cell culture

Human normal sebaceous gland cells SZ95, Green Flag Bio Shanghai Cell Bank. The cells were inoculated into a 24-well plate and cultured for 24 hours, then replaced with serum-free medium, treated with different concentrations of the test substance, and continued to be cultured for 24 hours. At the end of the incubation, replace the medium with MTT solution and react for 4-6 hours. At the end of the reaction, the supernatant was removed, dimethyl sulfoxide (DMSO) was added, and the methazanate crystals of MTT were completely dissolved by shaking, and then the absorbance was measured at 570 nm using the enzyme marker [11].

Human immortalized keratinocytes SCSP-5091, Chinese Academy of Sciences-Shanghai Cell Bank. The cells were inoculated into 24-well plates, and the supernatant was taken after 24 hours of incubation, and different concentrations of test substances were added for treatment, and the incubation was continued for 24 hours, and the supernatant was collected (for ELISA), and 100 μl of CCK-8 working solution was added to each well, and then the absorbance was measured by using an enzyme labeler at 450 nm after being placed in the incubator for 3 hours [12].

### 2.2.3 Expression of sebum - regulating factors: AR, ACC, FAS

Human normal sebocytes were seeded in 60 mm dishes and cultured for 24 hours. Then, the medium was replaced with serum - free medium containing DHT (Selleckchem) and test substances, and cultured in a microbiological incubator (5% $CO_2$, 37℃). After removing the medium, cells were collected with QIAzol™ reagent and RNA was extracted per the manufacturer's method. The RNA was quantified, reverse - transcribed to cDNA, and subjected to real - time quantitative PCR (using Applied Biosystems reagents and a fast real - time PCR instrument) to assess the expression of target genes (AR, ACC, FAS).

### 2.2.4 Evaluation of the Expression of Barrier - related Genetic Factors: FLG, TGM5

Cells were seeded in a 96 - well plate at a density of $3\times10^5$ cells / mL (100 μL per well) and cultured for 24 hours (5% $CO_2$, 37℃). After 24 hours, 100 μL of test substances and salicylic acid solutions at different concentrations were added to each well, mixed, and incubated for 24 hours ± 1 hour. RNA was extracted using a one - step reverse transcription reagent, and cDNA was synthesized with a cDNA synthesis kit. RT - PCR was performed using BeyoFast™ SYBR Green qPCR Mix (2X, Low ROX) to assess the expression of FLG and TGM5 [13].

### 2.3 Human - efficacy Evaluation

Subject Selection, thirty - four female volunteers aged 18 - 46 were selected, including 16 with dry skin and 16 with oily skin.

Test Sample Preparation, a spray solution of 5% (w/w) BPF in deionized water was prepared.

Test Environment, the tests were conducted in a controlled - environment laboratory maintained at 21 ± 1℃ and a relative humidity of 50 ± 10%. Volunteers were acclimatized in this environment for 15 minutes before each test.

**2.3.1 Evaluation of Facial Moisturizing and Repair Efficacy in Humans**

Before product use, washing their face with cleanser, volunteers rested in the laboratory for 15 minutes. The skin hydration level and transepidermal water loss (TEWL) were measured using a Corneometer MPA580 and Tewameter TM HEX, respectively.

After product use, the same measurements were repeated at 2 - and 4 - week intervals following product application.

**2.3.2 Evaluation of Facial Oil - control Efficacy in Humans**

Before product use, volunteers washed their face with cleanser in the morning and then measured skin sebum levels 8 hours later using a Sebumeter SM815.

After product use, the same procedure was repeated at 2 - and 4 - week intervals following product application.

**2.3.3 Evaluation of Facial Pore - improvement Efficacy in Humans**

Before product use, washing their face with cleanser, volunteers rested in the laboratory for 15 minutes. Baseline images of the volunteers' faces were captured using a Visia CR system.

After product use, Imaging was repeated at 2 - and 4 - week intervals following product application.

**2.3.4 Evaluation of Facial Texture - improvement Efficacy in Humans**

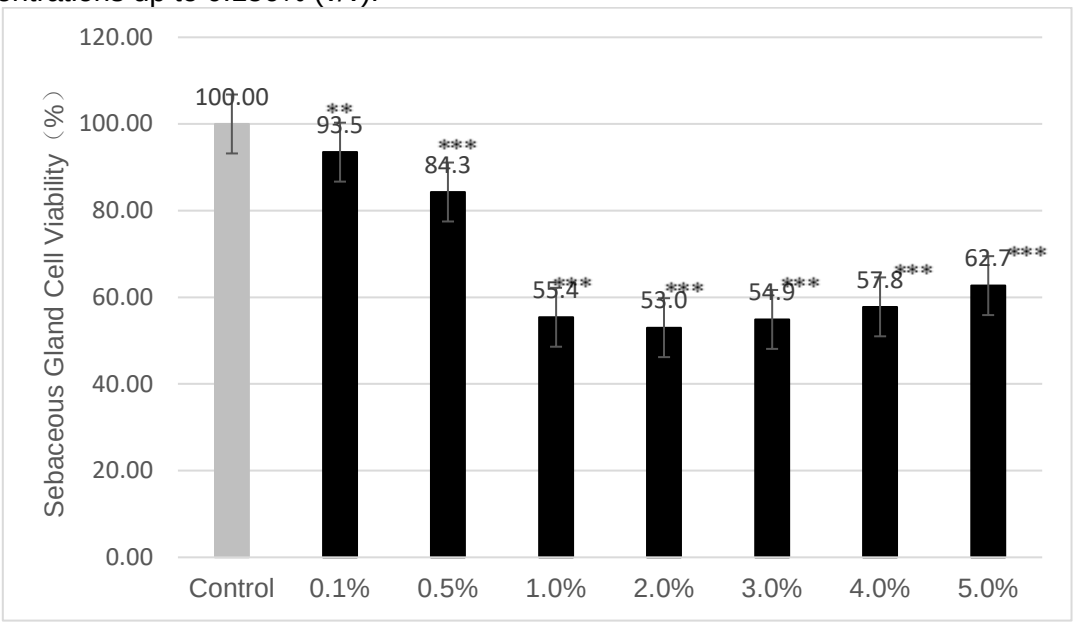
Before product use, washing their face with cleanser, volunteers rested in the laboratory for 15 minutes. Baseline images of the volunteers' faces were captured using an Antera 3D camera.

After product use, imaging was repeated at 2 - and 4 - week intervals following product application.

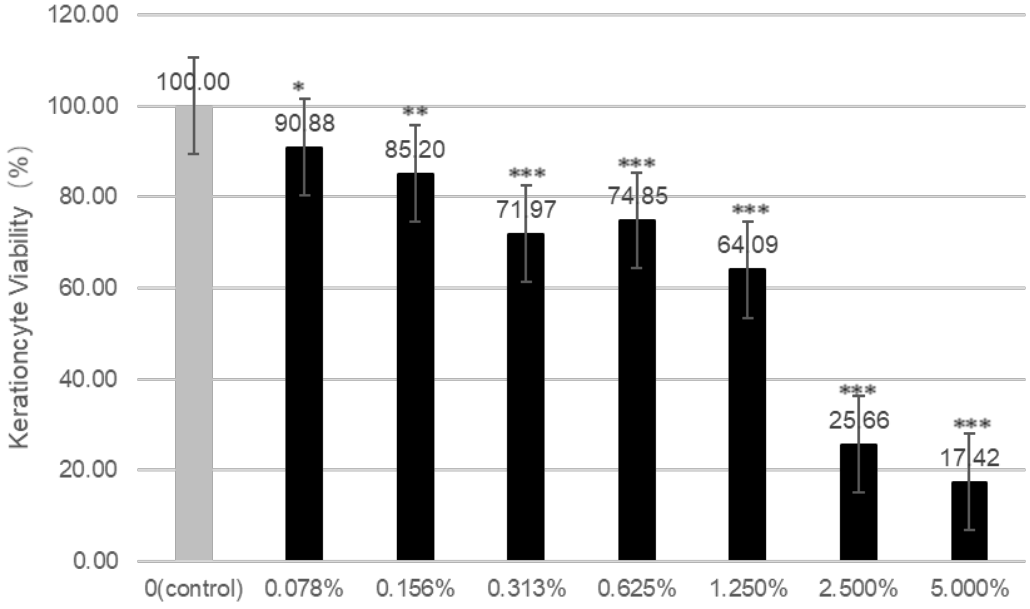
## 3. Results

### 3.1 Cell Viability Assessment

Data are presented in Figure 1. ** denotes $p < 0.01$ and *** denotes $p < 0.001$ vs. the control group. MTT assay results suggest that sample BPF exhibited no significant cytotoxicity to sebaceous gland cells at concentrations up to 0.5% (v/v) or to keratinocytes at concentrations up to 0.156% (v/v).




(a)




（**b**）

**Figure1.** (**a**) MTT-Based Viability Assay of Paeonia Fermentation Broth on Sebaceous Gland Cells (**b**) CCK-8-Based Viability Assay of Paeonia Fermentation Broth on Keratinocytes

### 3.2 BPF Inhibits the Expression of AR, ACC, and FAS in SZ95 Cells

Data are presented in Figure 2. Compared to the blank group, *p* -values less than 0.001 are denoted by ###. When compared to the model group, ** represents $p < 0.01$ and *** represents $p < 0.001$. In vitro test results indicate that BPF at 0.10% (v/v) inhibits AR, ACC, and FAS by 5.57%, 33.20%, and 55.00%, respectively, while BPF at 0.25% (v/v) inhibits these targets by 31.70%, 77.56%, and 81.38%, respectively. Analysis reveals that BPF at 0.25% (v/v) significantly suppresses the expression of sebum - related genetic factors, with a more pronounced downregulation of ACC and FAS than AR.

Previous studies have confirmed the crucial role of androgens in sebaceous gland function [14]. Normal males, with higher androgen secretion, secrete more sebum than normal females. Many oil - control cosmetic ingredients work by inhibiting 5α - reductase to reduce DHT expression. However, literature suggests that reduced 5α - reductase activity and lower DHT levels do not affect sebaceous gland development and function in adults, implying this pathway may not be effective enough in regulating sebum secretion. Yet, as almost every sebaceous gland cell contains androgen receptors (AR), and volunteers with functional AR insensitivity do not produce sebum, it is evident that AR changes underlie sebum secretion differences. This underscores the vital role of AR in sebum secretion mechanisms [15-17].

ACC and FAS, key enzymes in lipid synthesis, can have their expression modulated to regulate lipid production [18-19]. The inhibitory effects of BPF on ACC and FAS may result from both indirect AR - mediated and direct inhibition.

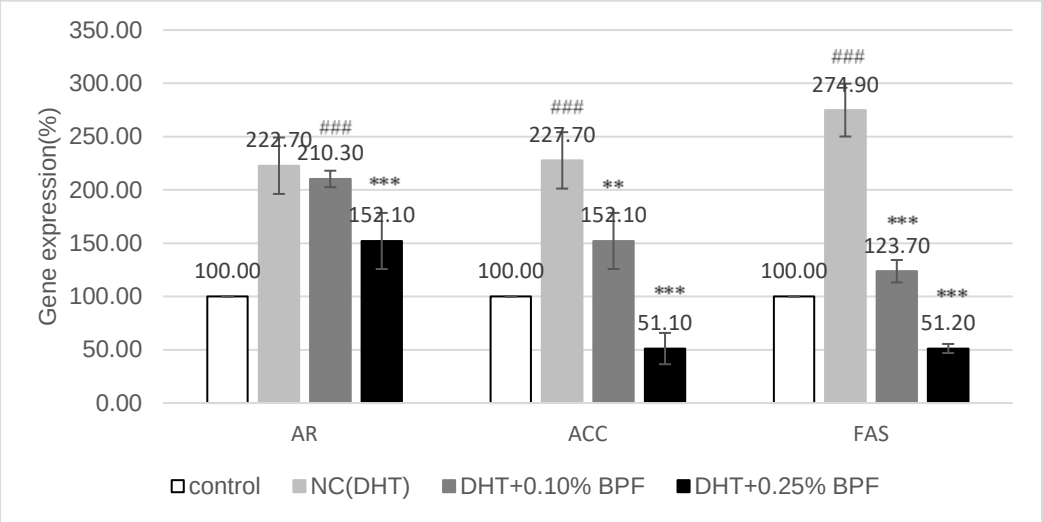



**Figure 2.** Regulatory Efficacy of Paeonia Fermentation Broth on Sebaceous Gland Activity via Suppression of Excessive Sebum Secretion

### 3.3 BPF Upregulates FLG and TGM5 in HaCaT Cells

Data are shown in Figure 3. ** denotes $p < 0.01$ and *** denotes $p < 0.001$ vs. the blank group (###). Compared to the positive control, ** represents $p < 0.01$ and *** represents $p < 0.001$. BPF at 0.078% (v/v) upregulated FLG and TGM5 by 73.253% and 90.187%, respectively, while BPF at 0.156% (v/v) upregulated them by 52.965% and 31.110%. These results indicate significant upregulation effects.

FLG, a structural protein, degrades into products that hydrate the stratum corneum and promote keratinocyte compaction into “building blocks,” forming an impermeable barrier essential for skin barrier function [20-21].

TGM5 cross - links filaggrin monomers with various proteins and is crucial for the epidermal cornified envelope, a proteinaceous layer deposited beneath the keratinocyte membrane during the final differentiation stage, acting as a wear - resistant barrier [22-24].

BPF's upregulation of FLG and TGM5 suggests it can effectively repair the skin barrier.

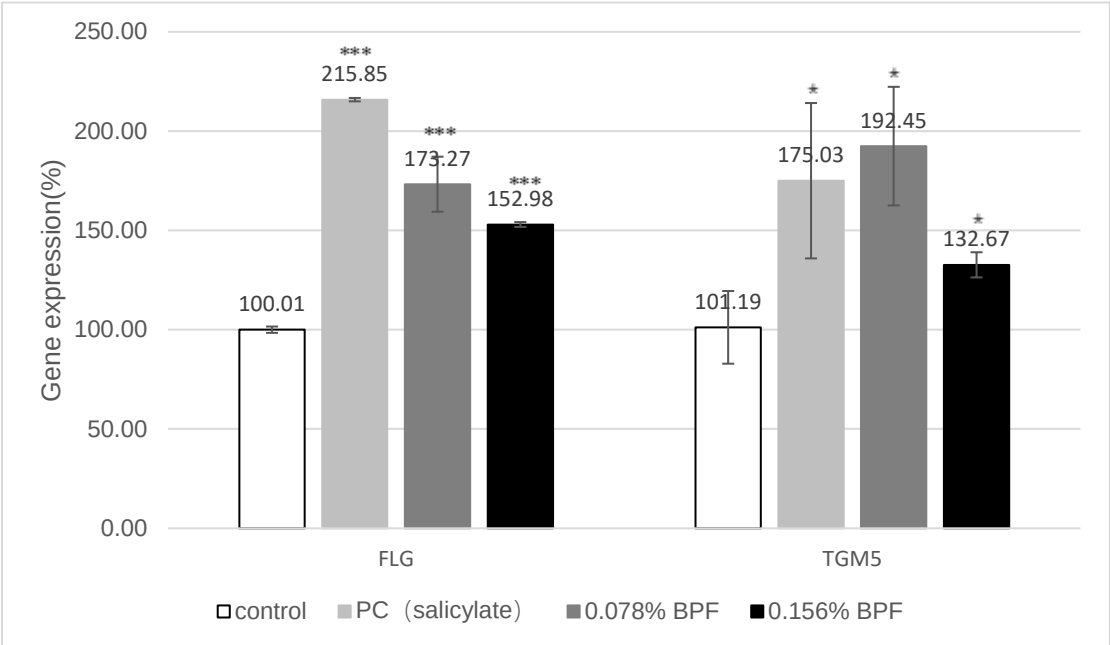


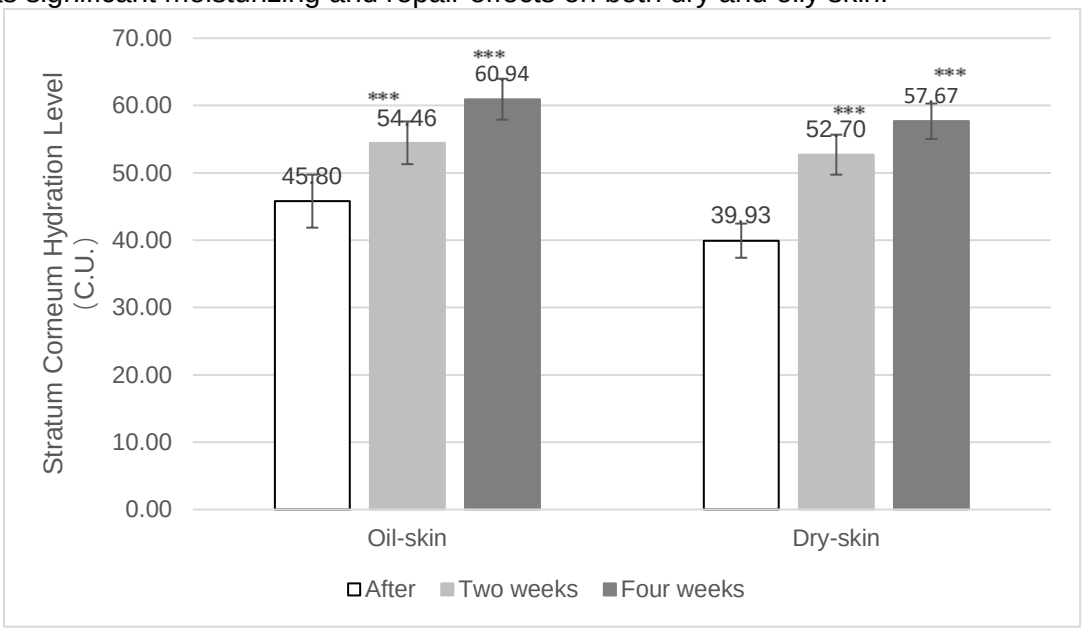

**Figure3.** Modulatory Effects of Paeonia Fermentation Broth on Skin Barrier-Related Factors.

### 3.4 Facial Moisturizing and Repair Efficacy

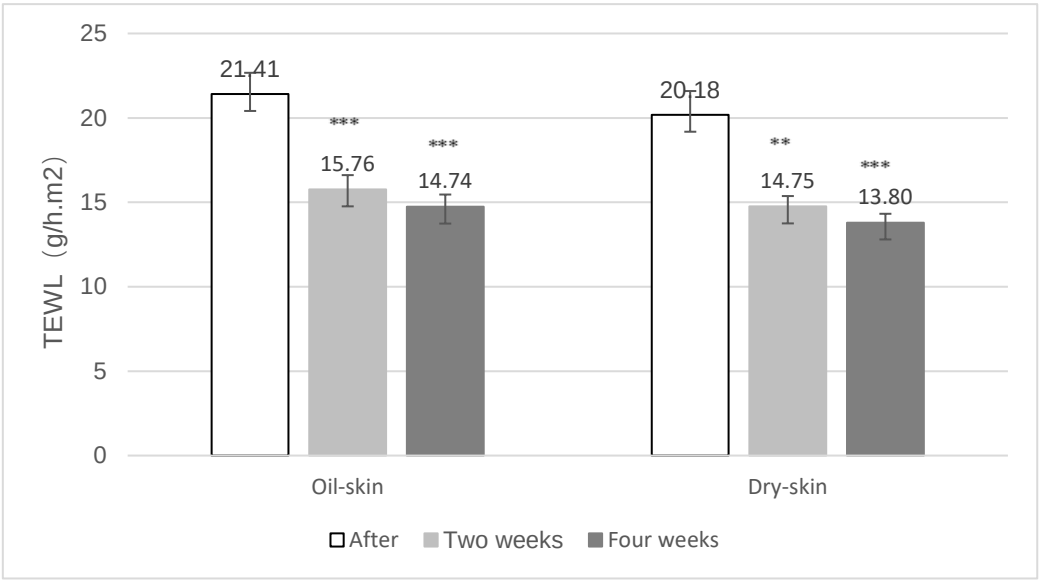
Stratum corneum hydration and TEWL were measured to assess the samples moisturizing and barrier - repair effects. Higher hydration indicates better moisturizing, while lower TEWL signifies better repair .

Data are presented in Figure 2. After 2 weeks of 5% (w/w) BPF spray use, stratum corneum hydration increased by 18.92% (oily skin) and 31.99% (dry skin). After 4 weeks, it rose to 33.07% (oily skin) and 44.43% (dry skin). BPF showed significant moisturizing effects on both skin types, especially dry skin. Initially, oily - skin volunteers had higher hydration, but after 4 weeks of BPF use, hydration levels in both groups were similar.

Initially, TEWL was higher in oily - skin volunteers, indicating more severe barrier damage. After 2 weeks of BPF spray use, TEWL decreased by 26.36% (oily skin) and 26.89% (dry skin). After 4 weeks, it dropped to 31.15% (oily skin) and 31.60% (dry skin). BPF demonstrated significant repair effects on both skin types with no significant difference in efficacy. BPF has significant moisturizing and repair effects on both dry and oily skin.




(**a**)

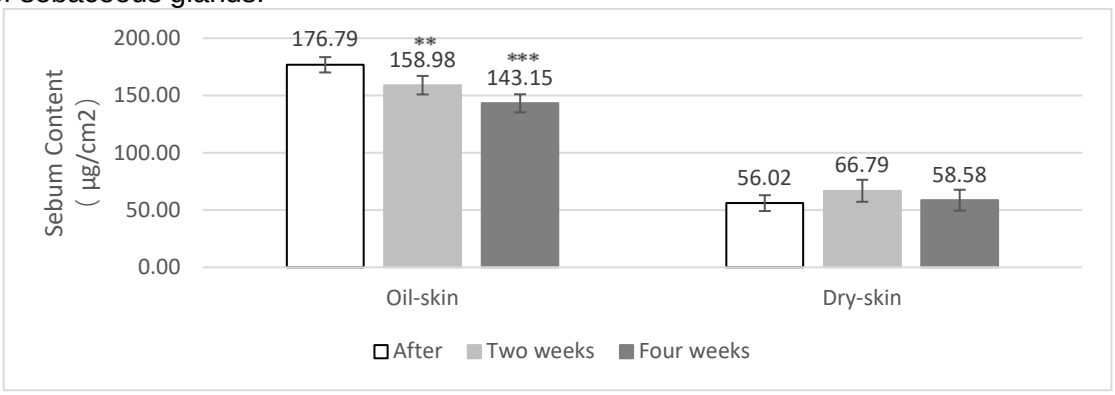



(**b**)

**Figure 4.** (**a**) Moisturizing Effects of Paeonia Fermentation Broth across Different Skin Types (**b**) Repair Effects of Paeonia Fermentation Broth across Different Skin Types

### 3.5 Evaluation of the Oil-control Efficacy of BPF on Human Face

Data are presented in Figure 5. A *p* -value of less than 0.01 is represented by **, and a *p* -value of less than 0.001 is represented by ***.
After the application of a 5% (w/w) BPF solution spray for 2 and 4 weeks, the sebum content of volunteers with oily skin decreased by 10.08% and 19.03%, respectively, while the sebum content of volunteers with dry skin increased by 19.23% and 4.57%, respectively. According to the analysis of the results, BPF significantly reduced sebum content in those with oily skin, but no significant changes were observed in those with dry skin. This indicates that BPF effectively controls oil in individuals with oily skin without affecting sebum levels in those with dry skin.
From the results, it can be inferred that BPF can significantly suppress the overproduction of sebum. However, it does not influence the normal secretion of sebum. This suggests that BPF can resolve the issue of excessive sebum secretion without impairing the normal function of sebaceous glands.

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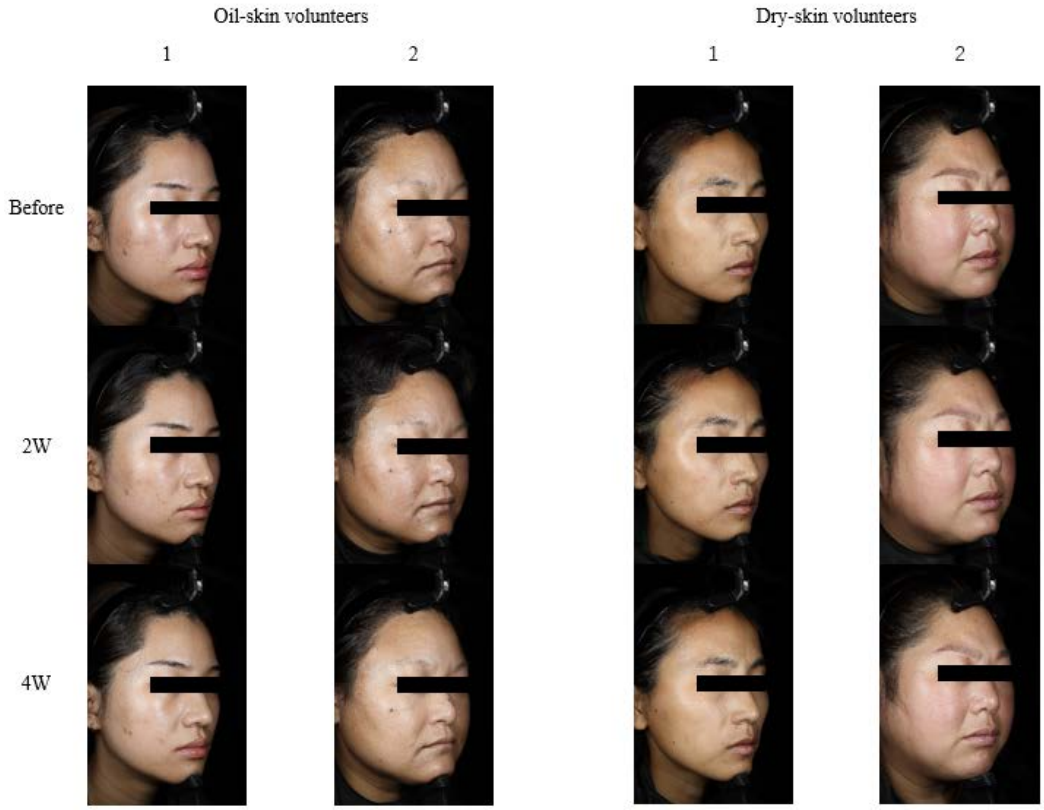
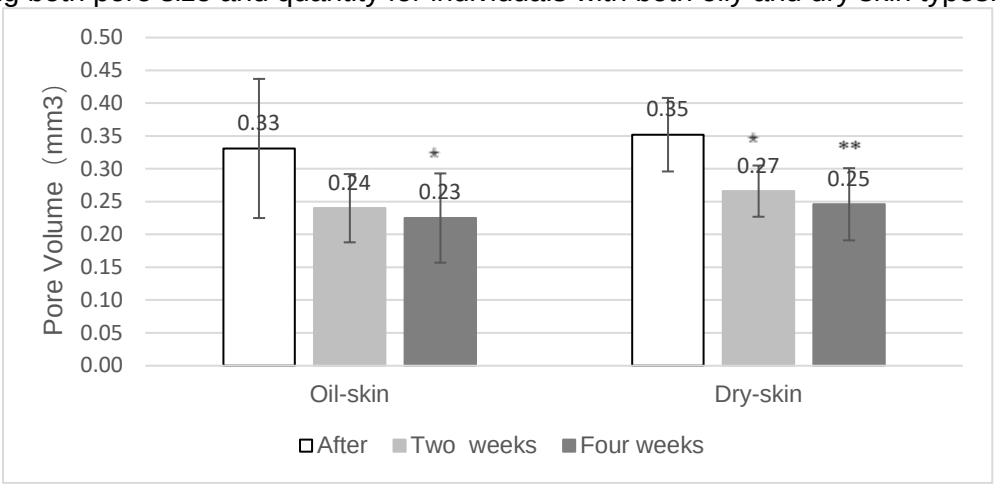



(**b**)

**Figure5.** (**a**) Sebum Regulation Efficacy of Paeonia Fermentation Broth across Different Skin Types (**b**) Demonstration of Facial Shine Improvement Effects

### 3.6 Assessment of the Efficacy of BPF on Improving Facial Pore Size and Quantity

Data are presented in Figure 6. * *p* * values less than 0.05 and 0.01 are indicated by * and **, respectively.
After 2 weeks of use of a 5% (w/w) BPF spray, volunteers with oily and dry skin experienced a reduction in pore volume by 27.49% and 24.64%, respectively, along with a decrease in pore count by 5.13% and 6.11%, respectively. Following 4 weeks of use, pore volume decreased further to 32.00% and 30.24%, while pore count reduced to 7.50% and 9.61% for oily and dry skin volunteers, respectively. These results demonstrate that BPF is effective in reducing both pore size and quantity for individuals with both oily and dry skin types.

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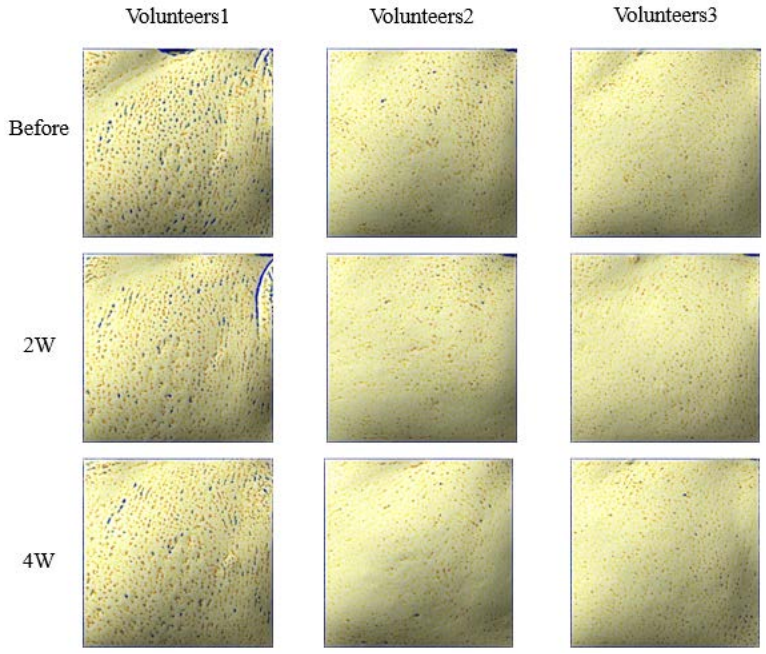


(**b**)

**Figure6.** (**a**) Efficacy of Paeonia Fermentation Broth in Improving Pore Size across Different Skin Types (**b**) Demonstration of Improved Facial Pore Size Effects

## 4. Discussion

Sebaceous glands produce sebum, which is essential for maintaining a healthy skin barrier. Dysfunction of these glands can lead to abnormalities in the lipid profile of the body surface and disrupt the skin barrier function.
The final product of fermentation has a dual value, encompassing both the fermentation strain and the raw material itself. BPF can inhibit the expression of AR to achieve the purpose of inhibiting the abnormal differentiation of sebaceous gland cells. Inhibit the expression of ACC and FAS to achieve the purpose of inhibiting the production of lipids, through these

two directions to solve the problem of excessive sebum secretion, and also has excellent moisturising and repairing effect.

BPF solves the problem of excessive oiliness without affecting the normal function of sebaceous glands， improving skin appearance. BPF can be widely used in various nursing products.

## 5. Conclusion

*In - vitro* and *In - vivo* Testing of BPF for Oil - control, Moisturizing, and Repair Efficacy.

*In - vitro* test results showed significant differences in sebum - related genetic factor expression compared to the model group, demonstrating effective sebum regulation. In moisturizing and repair tests, significant differences in barrier - related genetic factor expression compared to the blank group indicated good skin - barrier repair ability.

*In - vivo* test results demonstrated that after 4 weeks of using a 5% BPF spray solution, volunteers with dry or oily skin showed increased stratum corneum hydration and decreased TEWL (transepidermal water loss). Oiliness was reduced in oily - skin volunteers without affecting sebum levels in dry - skin volunteers. Pore size and texture improved significantly, and the product was well - tolerated.

BPF effectively controls oil, moisturizes, and repairs skin. It suppresses excessive sebum secretion without impairing normal sebaceous gland function. Additionally, it enhances skin appearance by shrinking pores and improving texture, with a gentle and non - irritating formula suitable for facial application.

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