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Study on the Applications of Bifida Ferment Filtrate in Essence and Shampoo

Qiang Li[1], Jianying Zhang[1], Huifang Chen[1], Jingyu He[1], Yuepeng Wan[1], Shengzhao Gong[2,*]

[1,]*Guangzhou Uniasia Cosmetic Technology Co., Ltd., Guangzhou 510663, Guangdong, China;*
[2]*Guangdong Industry Technical College, Guangzhou 510300, Guangdong, China*
Corresponding Author: Shengzhao Gong

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ABSTRACT: The trend of using probiotic-based skincare and scalp care products has been growing steadily, and it is expected to continue growing in the coming years. The main aim of the present researchwork was to examine the benefitsof skin essence and shampoo after adding bifida ferment filtrate. Firstly, TNF-α inhibition rate, wound healing rate and antioxidant test in vitro were used to study the anti-inflammatory, repairing effects and antioxidant capacity of bifida ferment filtrate, then we explored its applications in skin essence and shampoo.The results showed that it could significantly inhibit the production of TNF-α inflammatory factor, promote cell migration and scavenge free radicals. After 28 consecutive days using of the essence with 1.0% bifida ferment filtrate, the average value of facial skin TEWL decreased by 20.62%, the water content of the stratum corneum increased by 17.25%, and the skin erythema contentdecreased by 21.01%. The difference was statistically significant ($p<0.01$), indicating that the product had good moisturizing, repairing and soothing effects.With the increase of bifida ferment filtrate content, it could reduce the irritation of shampoo and TEWL value.Sensory testing evaluation results showed that continuous use of shampoo containing 1% bifida ferment filtrate for 4 weeks has a good effect on improving scalp itching and scalp pain for volunteers.

KEYWORDS: Bifida ferment filtrate, Moisturizing, Repairing, Soothing, Anti-oxidation.

I. INTRODUCTION

As the largest organ of the human body, the skin is exposed to the environment every day, and it endures exogenous oxidative stress from the external environment as well as endogenous oxidative stress. Ultraviolet radiation, environmental pollution, smoking, and cellular metabolism all generate a lot of reactive oxygen species, such as superoxide anions and hydroxyl radicals. Normally, these reactive oxygen species are actively cleared by the antioxidant defense system to maintain a dynamic balance state[1]. When excessive reactive oxygen species are produced, and the body experiences oxidative stress that exceeding the defense capacity of the antioxidant system, or when the antioxidant defense system function decreases, it can cause oxidative damage, promote inflammation, and destroy cell structures such as membranes, lipids, proteins, DNA[2-3]. Oxidative stress and its resulting oxidative damages can exacerbate skin pigmentation and aging, inducing changes in skin complexion homogeneity, wrinkling, sagging, dryness and roughness[4]. Studies have shown that oxidative stress is closely related to dandruff and seborrheic dermatitis, and plays a dominant or accelerating negative role in hair loss[5]. In addition, melanocytes in the hair follicle also produce a large amount of free radicals during melanin synthesis, leading to oxidative stress and accelerating the aging of the follicles, such as graying of the hair[6]. Therefore, reducing oxidative stress and maintaining a healthy skin barrier function are crucial for facial skin care and scalp care.

Bifida ferment filtrate is mostly dominated by bifidobacteria. During the fermentation process, bifidobacteria produce various beneficial metabolites for skin moisturizing and repair, such as polysaccharides and amino acids. It also contains various antioxidants such as glutathione and vitamins,which can neutralize free radicals and reduce oxidative stress on the skin. This study aimed to investigate the inhibitory effect of bifida ferment filtrate on the cell inflammatory factor and its reparative effect on cells. The antioxidant properties of bifida ferment filtrate were also examined

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through in vitro antioxidant model experiments, including DPPH free radical and hydroxyl free radical scavenging rate assays. Additionally, the efficacy of incorporating 1% bifida ferment filtrate into facial essence for moisturizing, repairing, and soothing effects was evaluated. Furthermore, the soothing and anti-itching effects of adding 1% bifida ferment filtrate to shampoo for sensitive scalp were assessed.

II. MATERIALS AND METHODS

Reagents:LPS lipopolysaccharide (catalog number ST1470), high glucose DMEM culture medium, Mouse TNF-α ELISA kit (catalog number PT512), Beyotime Biotechnology; RAW264.7 mouse monocytic leukemia cells, Shanghai Cell Bank of Chinese Academy of Sciences; MTT, Merck KGaA; DPPH (1,1-diphenyl-2-picrylhydrazyl) (purity ≥97%), Shanghai Jinsui Biotechnology Co., Ltd.; Saccharomyces cerevisiae fermentation filtrate, Guangzhou Norvia Biotechnology Co., Ltd.

Instruments:EPOCH2 microplate reader, BioTEK Inc.; UV-1750 UV-visible spectrophotometer, Shimadzu Corporation, Japan; RI-150CN low-temperature incubator, Thermo Fisher Scientific Inc.; inverted microscope, Olympus; LDZF-50KB-II vertical pressure steam sterilizer, Shanghai Shenan Company; INC108MED CO_2 incubator,MEMMERT GmbH, Germany; Delfin VapoMeter transdermal water loss tester, Delfin Ltd., Finland.

Ingredients of Essence: The main components of the essence include water, ethanol, butylene glycol, bifida ferment filtrate, panthenol, dipotassium glycyrrhizinate, and other necessary pH adjusters, moisturizers, thickeners, preservatives, fragrances, etc.

Ingredients of Shampoo: The main components of the shampoo include water, sodium laurethsulfate, cocamidopropyl betaine, cocamide methyl MEA, bifida ferment filtrate, fragrance, glycerin, sodium lauroylsarcosinate, sodium chloride, phenoxyethanol, potassium sorbate, guar hydroxypropyltrimonium chloride,sodium citrate, citric acid, disodium EDTA, etc.

TNF-α Inhibition Rate Test: Mouse macrophages were diluted in cell culture medium to a density of 1×10^5 cells/mL and seeded in a 96-well plate, with a liquid volume of 200 μL in each well, and then cultured for 24 h. After discarding the original culture medium, 200 μL of complete culture medium was added as a control group, and 200 μL of complete culture medium containing 1 μg/mL LPS was added as a negative control group. The complete culture medium containing 1 μg/mL LPS and 0.1%, 0.3%, 0.5%, 0.7%, and 1.0% (w/w) of Saccharomyces cerevisiae fermentation filtrate were added as experimental groups, and the cells were further cultured for 24 h. The cell culture supernatant was collected in a sterile centrifuge tube of 1.5 mL, and the TNF-α content was detected according to the instructions of the ELISA kit. Three duplicate wells were set for each group.

Cell MigrationTest: Horizontal lines were drawn at intervals of 0.5~1 cm on the back of a 6-well plate using a marker pen, with at least 5 lines passing through each well. About 5×10^5 human immortalized epidermal cells (HaCaT cells) were added to each well. The next day, a 200 μL pipette tip was used to make scratches as perpendicular as possible to the horizontal lines on the back, with about 5 scratches per well. The wells were washed twice with sterile PBS to remove the scratched cells and replaced with low serum culture medium, and then placed in a constant temperature incubator at 37 °C with 5% CO_2 for 24 h. During the experiment, photos were taken in the same field of view, and the initial and 24 h areas were compared using Image J image analysis software to calculate the scratch healing rate and evaluate migration function. The scratch healing rate (%) was calculated as (initial scratch area - 24 h scratch area) / initial scratch area × 100%.

DPPH Radical Scavenging Rate Test: According to the reference method[7], 2.5 mg DPPH was dissolved in 95% ethanol and diluted to a 50 mL brown volumetric flask. 1 mL of the DPPH solution was mixed with 2 mL of the test sample solution in an EP tube, and reacted for 30 min at room temperature. The absorbance A_1 was measured at 519 nm wavelength, and the DPPH clearance rate was calculated using the formula: DPPH clearance rate (%)=$[1-(A_1-A_2)/A_3]\times100\%$,where A_2 is the absorbance of the 1 mL test sample solution mixed with 2 mL of 95% ethanol measured at 519 nm wavelength, and A_3 is the absorbance of the 1 mL 95% ethanol mixed with 2 mL of DPPH solution measured at 519 nm wavelength.

Hydroxyl Radical Scavenging Rate Test: The hydroxyl radical scavenging rate was determined using the Fenton system. 0.35 mL of 9 mmol/L

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ferrous sulfate and 0.35 mL of 9 mmol/L hydrogen peroxide were mixed in a test tube, and then 1 mL of the sample was added to the system. Finally, 0.35 mL of an ethanol-salicylic acid solution was added, and the reaction was carried out at 37 °C for 20 min. The absorbance A_x was measured at 510 nm wavelength, and the absorbance value measured with distilled water as a blank control was A_0, and the absorbance value measured with 95% ethanol instead of the ethanol-salicylic acid solution was A_{x0}. Vitamin C was used as a positive control. The hydroxyl radical scavenging rate was calculated using the formula: hydroxyl radical scavenging rate (%) = $[A_0- (A_x-A_{x0}) /A_0]\times100\%$.

Human Test Trial Design: Thirty-three healthy volunteers aged 18 to 60 years, regardless of gender, who met the relevant test requirements were recruited for the study. The test site was the face. The skin essence was used twice a day, once in the morning and once in the evening, for 28 consecutive days. Before each test, the volunteers were reminded to avoid strenuous exercise and rest for 30 minutes in a controlled environment with constant temperature and humidity at the evaluation test center before the evaluation was conducted(temperature 20 °C~22 °C, humidity: 40%~60%). The same evaluator used the same instrument to characterize and measure the relevant indicators.

Recruit 33 healthy volunteers aged 18 to 60 years who meet the relevant test requirements and have scalp sensitivity and itching problems, regardless of gender. The study will be conducted using a single-blind method. The participants will utilize the shampoo containing 1% bifida ferment filtrate a minimum of three times per week for four consecutive weeks. The effectiveness of the product will be assessed via a questionnaire survey.

Skin TransepidermalWater Loss (TEWL): The skin TEWL at the test site was measured using the Finland Delfin company's transepidermal water loss tester at each follow-up visit. The measurement was taken three times, and the mean value was recorded. The reparative efficacy of the product was assessed by comparing the mean difference in skin TEWL values before and after utilization of the product.

Skin Stratum Corneum Water Content: The Skin -pH-Meter of the German CK was used to test the skin's water content. The measurement was taken three times, and the average value was taken. The moisturizing efficacy of the product was assessed by comparing the mean difference in skin stratum corneum water content values before and after utilization of the product.

Skin Erythema Analysis: The Mexameter MX18 tester was used to characterize skin erythema content. The erythema index (EI) was measured at each follow-up visit, and the measurement was taken three times, and the average value was taken. The product's soothing effectiveness was evaluated by comparing the average change in skin erythema content values before and after utilization of the product. The VISCIA-CR of the American CANFIELD company was used to capture images of the subjects' faces at each follow-up visit to assist in evaluating the soothing and repair effects.

Statistical Analysis:SPSS 20.0 software was used for statistical analysis. Measurement values were presented as mean ± standard deviation. The paired t-test was mainly used, and $P < 0.05$ was considered statistically significant. Data visualization was performed using Origin 8.0 software.

III. RESULT AND DISCUSSION

TNF-α Inhibition Rate Test:

The results of testing the inhibition of the inflammatory factor TNF-αby bifida ferment filtrate at different quality fractions (0.1%, 0.3%, 0.5%, 0.7%, 1.0%) are shown in Table 1. As the content of bifida ferment filtrate increased, the inhibition rate of TNF-α gradually increased. When the content of bifida ferment filtrate was 1.0%, the inhibition rate of TNF-α reached 72.13%.Based on the above experiment, we speculate that bifida ferment filtrate may have great potential application value in skin soothing.

Cell MigrationTest: We employed the scratch test method to evaluate cell migration under different mass fractions (0.1%, 0.3%, 0.5%, 0.7%, 1.0%) of bifida ferment filtrate. We found that the addition of bifida ferment filtrate resulted in higher cell scratch healing rates compared to the control group. As the content of bifida ferment filtrate increased, the cell scratch healing rate gradually increased in a dose-dependent manner. When the content of bifida ferment filtrate was 1.0%, the scratch healing rate reached 96.73%. Based on the above experiment, we speculate that bifida ferment filtrate may have great potential application value in skin barrier repair.

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TNF-α Inhibition Table:

Sr.No.	Content(%)	Expression level of TNF-α	TNF-αinhibition(%)
1.	0.1	881.72	40.96
2.	0.3	589.13	60.55
3.	0.5	506.94	66.06
4.	0.7	454.24	69.59
5.	1.0	416.32	72.13
6.	Negative control group (LPS-stimulated group)	1493.52	/
7.	Control	399.80	/

Cell Migration Table:

Sr.No.	Content(%)	Initial area(μm^2)	Area after 24 h(μm^2)	Scratch healing rate (%)
1.	0.1	2709009	611388	77.43
2.	0.3	2719432	557640	79.49
3.	0.5	2813398	411479	85.37
4.	0.7	2794114	192890	93.10
5.	1.0	2831871	92555	96.73
6.	Control	2724353	790210	70.99

DPPH Radical Scavenging Rate Test:

DPPH is a relatively stable free radical that appears purple-red in ethanol solution. With the addition of a free radical scavenger, the single electron of DPPH is paired with a free electron, causing the color to gradually fade. The extent of color fading is quantitatively related to the number of paired electrons, with a greater number of paired electrons resulting in a lighter color and a lower absorbance at the maximum absorption peak. Therefore, the antioxidant capacity of the sample can be evaluated by measuring the clearance of DPPH free radicals. As shown in Figure 1, with the increase of the concentration of bifida ferment filtrate, the ability to clear DPPH free radicals gradually increases. When the mass concentration is 1.0 g/L, the clearance rate of DPPH free radicals is 49.21%.

Hydroxyl Radical Scavenging Rate Test:

Hydroxyl radical (·OH) is one of the most toxic and harmful free radicals produced during human metabolic process. It can cause oxidative damage and destruction of substances such as sugars, amino acids, proteins, nucleic acids, etc. in tissues, leading to cell and tissue organ damage, accelerating aging, and inducing various diseases. In this experiment, the clearance ability of hydroxyl radicals by bifida ferment filtrate at mass concentrations of 0.2~1.0 g/L was studied. The results showed that there was a significant dose-response relationship between the clearance rate of hydroxyl radicals and the mass concentration of bifida ferment filtrate. When the mass concentration was 1.0 g/L, the clearance rate of hydroxyl radicals by bifida ferment filtrate was the highest, reaching

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57.55%.

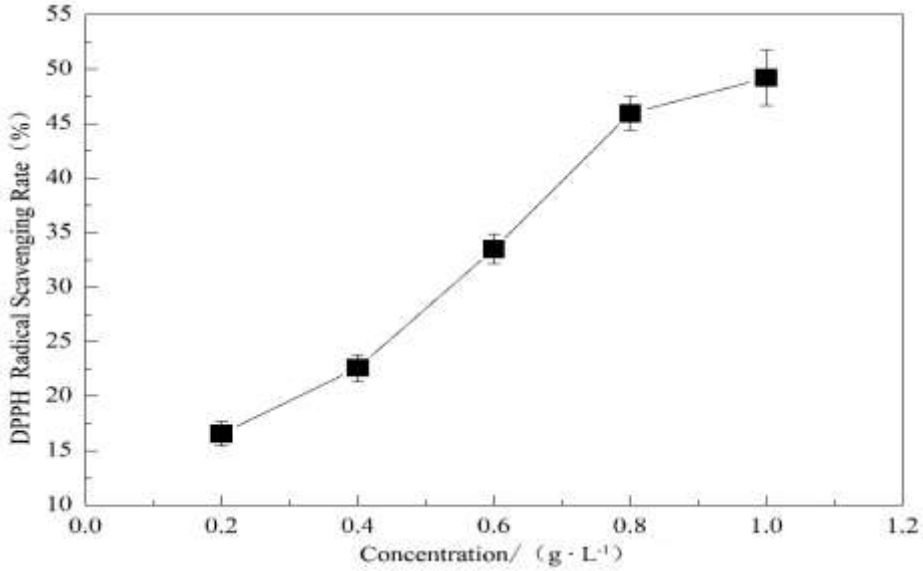

Figure 1: The scavenging rate of DPPH free radicals by bifida ferment filtrate

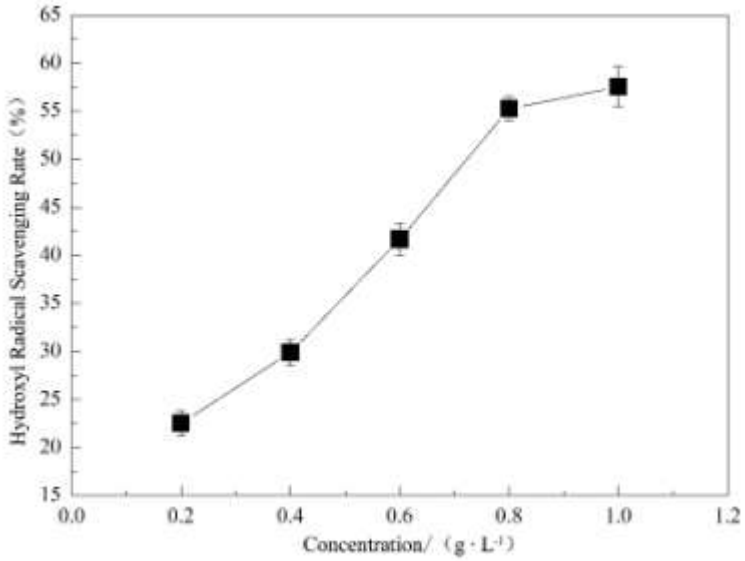

Figure 2: The scavenging rate of Hydroxyl free radicals by bifida ferment filtrate

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Human Test on Facial Skin:

This experiment investigated the alterations in facial skin transepidermal water loss (TEWL), stratum corneum hydration, and skin erythema value before and after utilizing the serum containing 1.0% bifida ferment filtrate. The larger the TEWL value, the more water loss through the epidermis per unit time and unit cross-sectional area. Therefore, the trend of decreasing TEWL values represents the recovery process of barrier-damaged skin. The greater the increase in skin water content compared to the baseline value (D0) before product use, the better the moisturizing effect of the product on the skin.As shown in Table 3, the baseline value D0 was 20.56±4.35 $g/m^2 \cdot h$ before using the skin essence, and the average TEWL value of the skin showed a decreasing trend after using the product, which decreased by 20.62% after continuous use for 28 days, with significant difference compared to the baseline value ($p < 0.01$). Furthermore, the water content of the skin stratum corneum increased by 17.25%, and the skin erythema content decreased by 21.01%, with significant difference compared to the baseline value ($p < 0.01$). Figure 3 also shows that subject 16 had a decrease in facial redness after continuous use of the test product. This indicates that the skin essence containing 1% bifida ferment filtrate is beneficial for skin moisturizing, repairing, and soothing.

Facial Skin Test Table:

Group	Number of testers	TEWL（$g/m^2 \cdot h$）	Stratum corneum water content (C.U.)	Skin erythema value
D0	33	20.56±4.35	61.29±8.78	300.26±60.43
D28	33	16.32±3.59	71.86±9.93	237.19±65.05

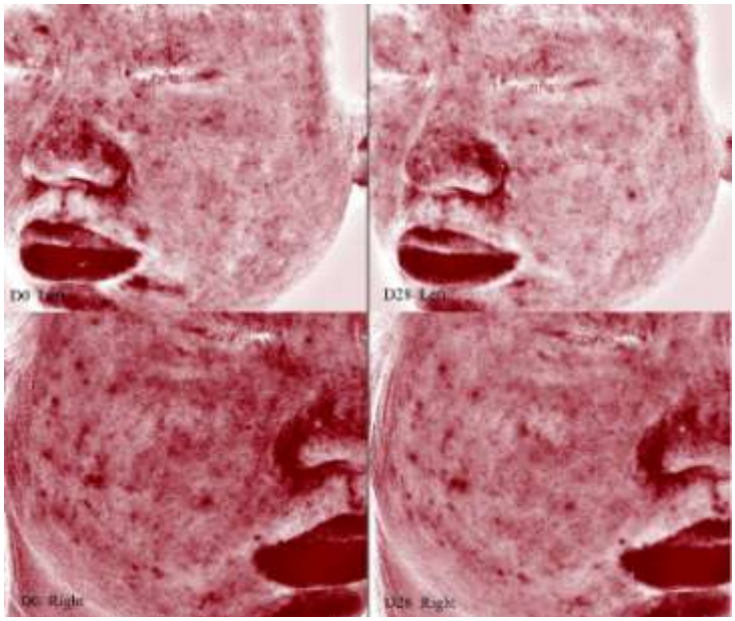

Figure 3: The change in redness of the facial skin of the 16th subject before and after using the skin essence

Human Test on Scalp:

The surfactants in shampoo have amphiphilic properties, which can interact with intercellular lipids and penetrate the skin barrier, leading to skin dryness and tightness. The damage to the skin barrier function further exacerbates the penetration of surfactant molecules or other irritating chemicals through the stratum corneum into the deeper layers

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Volume 8, Issue 2 Mar-Apr 2023, pp: 1318-1325 www.ijprajournal.com ISSN: 2249-7781

of the skin, affecting the living epidermal cells (keratinocytes) and causing local inflammatory reactions.

In our experiment, the transepidermal water loss (TEWL) of the skin treated with shampoo containing 0.3% and 1.0% bifida ferment filtrate was studied and compared with the blank group (shampoo without the addition of bifida ferment filtrate). As shown in the figure 4, the TEWL values before applying any sample were 10.68, 10.75, and 10.85 $g/m^2 \cdot h$. After applying the blank shampoo and the shampoo containing 0.3% and 1.0% bifida ferment filtrate (both the shampoo diluted to 1% solution before application) for 5 minutes, the TEWL values were 14.38, 13.62, and 12.64 $g/m^2 \cdot h$, respectively. A comparison of the results showed that the TEWL value increased by 34.64% after applying the blank shampoo for 5 minutes. However, when the shampoo containing 0.3% and 1.0% bifida ferment filtrate was applied, the TEWL values increased by 26.70% and 16.50%, respectively, showing a significant decreasing trend. In addition, there was a significant difference between the shampoo containing 0.3% and 1.0% bifida ferment filtrate and the blank shampoo solution ($P<0.01$).

We speculate that because of the good anti-inflammatory and repairing effects of bifida ferment filtrate, then it can timely relieve or repair the damage caused by irritants to the scalp. On the other hand, the filtrate may alleviate the penetration of surfactant molecules into the skin and reduce their damage to intercellular lipids. Therefore, the significant decrease in the TEWL value after the addition of the bifida ferment filtrate indicates that it is beneficial for scalp care.

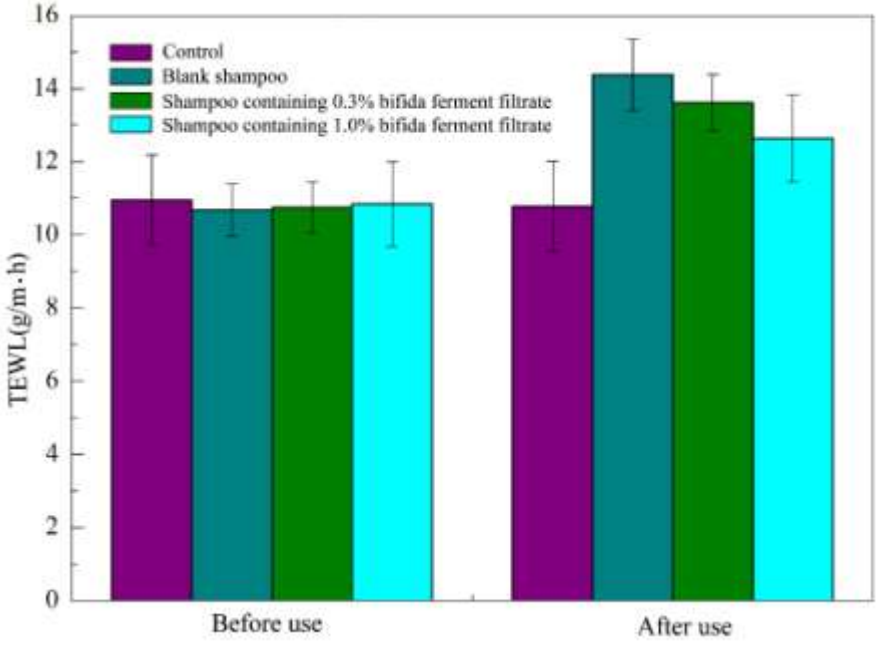

Figure 4: Measurement of transepidermal water loss from the scalp

Questionnaire Results of Shampoo:

In this study, 33 volunteers with sensitive and itchy scalps were recruited. They were required to use a shampoo containing 1% bifida ferment filtrate at least three times a week for four consecutive weeks. The effectiveness of the shampoo in improving scalp itchiness and pain was evaluated through a questionnaire survey. The effectiveness rate in improving scalp itchiness was calculated as (number of obviously improved participants + number of very obviously improved participants) / 33 x 100%, while the effectiveness rate in improving scalp pain was calculated as (number of obviously improved participants + number of very obviously improved participants) / 33 x 100%. The overall effectiveness rate was calculated as (number of obviously improved participants + number of very obviously improved participants) / 33 x 100%. The test results showed that the effectiveness rates in improving scalp itchiness and pain were both 63.64% after using the shampoo for four consecutive weeks, and the overall effectiveness rate was 78.79%. The sensory evaluation test results showed that the shampoo prepared with 1% bifida ferment filtrate had a good

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effect on improving scalp itchiness and pain for volunteers with sensitive and itchy scalps.

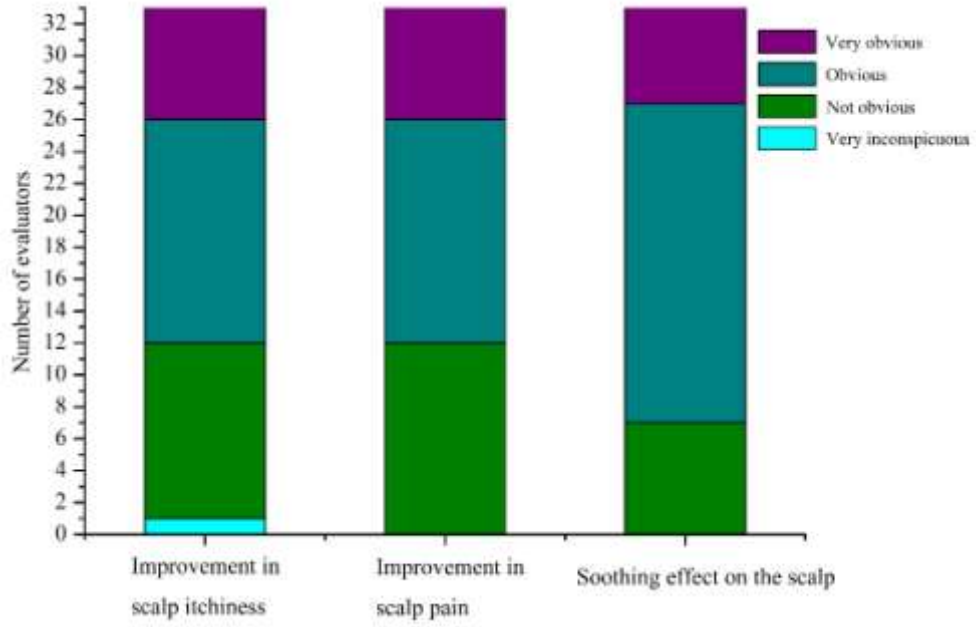

Figure 5: Sensory evaluation test results of shampoo

IV. CONCLUSION

Through measuring skin transepidermal water loss (TEWL), stratum corneum water content, and skin redness value, it was found that the average TEWL value of the skin decreased by 20.62%, the stratum corneum water content increased by 17.25%, and the skin redness content decreased by 21.01% after using the skinessence containing 1.0% bifida ferment filtrate continuously for 28 days, indicating that the product has good moisturizing, repairing, and soothing effects.

This study further explored the effect of shampoo on skin TEWL, and the results showed that after adding 0.3% and 1.0% bifida ferment filtrate to the shampoo, the TEWL values increased by 26.70% and 16.50%, respectively, which was significantly lower than the blank shampoo (without bifida ferment filtrate) of 34.64%, showing a significant downward trend, indicating that the bifida ferment filtratemayhavea good barrier repair effect. Sensory testing evaluation results showed that continuous use of shampoo containing 1% bifida ferment filtrate for 4 weeks has a good effect on improving scalp itching and scalp pain for volunteers with sensitive and itchy scalp.

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