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"Comprehensive evaluation of an essence formulated with a complex enzyme ingredient in the management of closed comedones"

Yina Sheng[1]*, Jianhui Chen[1], Haoyu Wang[1], Peng Han[1] and Jiaming Xu[1]

[1] Shenzhen Yusu Biotechnology Co., Ltd.

1. Introduction

Closed comedones, commonly known as whiteheads, are a prevalent form of non-inflammatory acne characterized by keratin and sebum accumulation within the pilosebaceous canal [1]. These lesions predominantly appear in areas with high sebaceous gland activity, such as the forehead, chin, and cheeks, and are often associated with excessive sebum production [2]. While excessive sebum secretion is widely recognized as a key factor in the formation of closed comedones, excessive keratin accumulation, along with abnormal proliferation and differentiation of keratinocytes at the follicular orifice, also play a crucial role in their pathogenesis [1].

To manage closed comedones, hydroxy acids, such as salicylic acid, lactic acid, and glycolic acid, are commonly incorporated into cosmetic formulations due to their superficial exfoliation effects [3-5]. However, acid-based exfoliation can cause adverse effects, including irritation, redness, increased sensitivity, and allergic reactions, particularly in individuals with compromised skin barriers [6]. It is reported that Asian people have thinner stratum corneum and heightened sensitivity, acid-based exfoliation may not a suitable choice for sensitive Asian skin [7]. As a gentler alternative, enzymatic exfoliation has gained attention for its ability to remove aged keratin while maintaining skin barrier integrity. Proteolytic enzymes, such as keratinases and proteases, selectively degrade keratin, offering a more targeted exfoliation approach than acids [8]. Keratinases, known for their outstanding ability to cleave disulfide bonds, are derived from various microorganisms, such as *Bacillus subtilis*, *Doratomyces microspores*, and *Paecilomyces marquandii* [9-12]. Meanwhile, plant-derived proteases, such as papain, bromelain, and ficin, hydrolyze keratin and desmosomal proteins within the stratum corneum, facilitating gentle yet effective exfoliation [13]. In addition to their keratolytic effects, these enzymes exhibit antioxidative, anti-inflammatory, and potential anti-tumorigenic properties, further benefiting skin health [14-16]. In addition to plant-origin, proteases derived from microorganism also have skin exfoliation activity. *Mucor miehei* proteases, which mimic cathepsin D involved in endogenous keratin renewal, have been shown to significantly improve skin texture and appearance [17]. In addition to enzymatic degradation of excessive keratin, regulating keratinocyte metabolism is essential to prevent further accumulation. Accordingly, certain enzymes, such as superoxide dismutase (SOD), can alleviate oxidative stress in keratinocytes, preventing aberrant keratinocytes proliferation [18].

Our previous research demonstrated that a multi-enzyme complex, containing papain, *Bacillus* keratinases, *Mucor miehei* proteases, and SOD, effectively degraded surface proteins while simultaneously restoring the damaged skin barrier and promoting epidermal cell proliferation in compromised skin [19]. Notably, this enzymatic complex caused no obvious epidermal structure disruption nor inflammatory cytokine expression in 3D epidermal skin model, supporting its safety in short-term and long-term cosmetic application [19]. This study aims to comprehensively evaluate the clinical efficacy and safety of an essence formulated with this enzyme complex as its primary active ingredient in the management of closed comedones, with a particular focus on its anti-closed comedones and anti-acne performance, skin barrier preservation, and irritation potential.

2. Materials and Methods

Subject enrollment

This study enrolled Asian sensitive skin adults aged 18-40 years old with oily or combination skin (baseline sebum level on the forehead exceeding 120 μg/cm²) recruited in Guangzhou, China, in May 2024. All participants provided written informed consent, had no concurrent clinical trial participation, and the study adhered to the Declaration of Helsinki, China's Good Clinical Practice (GCP) guidelines, and relevant national regulations.

Inclusion criteria: This study included subjects who self-identified as having sensitive skin, visible enlarged pores, dullness, and rough skin texture on the face. The acne severity graded of subjects as 1–2 according to the international modified classification system, with visible closed comedones and at least one inflammatory papule. There is no significant scars, excessive facial hair, or other conditions that could interfere with skin assessments in the evaluation area. Subjects were required to comply with the study protocol and maintain a stable lifestyle throughout the evaluation period. Prior to participation, all subjects signed an informed consent form and agreed to refrain from using other skincare products with similar functions during the study.

Exclusion Criteria: Subjects who are unable to read and comprehend the informed consent document will be excluded. Subjects who refuse to sign the informed consent form will not be enrolled. Subjects who are unwilling to comply with the study protocol will be excluded. Subjects who are simultaneously participating in other clinical studies will not be eligible. Subjects who use cosmetics or skincare products on the day of evaluation will be excluded. Subjects who self-report being pregnant, breastfeeding, or planning pregnancy will not be included. Subjects with infectious skin diseases or atopic dermatitis will be excluded. Subjects who have undergone chemical peeling, dermatological treatments, or immunosuppressive therapy within the past three months prior to participation will not be eligible. Subjects who have received systemic steroid therapy or phototherapy within one month before participation will be excluded. Subjects who have used topical medications or specialized skincare products on the test area or experienced excessive sun exposure within one week before the evaluation will not be included. Subjects with severe reactions or allergies to cosmetics, medications, or general light exposure will be excluded. Additionally, subjects deemed unsuitable for participation based on the investigator’s judgment will not be enrolled.

Withdrawal Criteria: Subjects may withdraw from the study due to complications, adverse events, or other reasons occurring during the research period.

Intervention

Participants were given an essence for facial treatment. Initial use of the product was conducted at the research center, where participants were guided on proper application and subsequently asked to complete a questionnaire. The essence was then distributed for home use

with instructions as follows: participants should apply an appropriate amount of the test product evenly to the facial skin and gently massage until fully absorbed every morning after cleaning. On alternate nights, participants should pour a sufficient amount of the test product into a container, and immerse a mask sheet until fully saturated (avoid dripping), then apply it to the facial skin for 10-20 minutes.

Instrumental assessment

In the controlled environment, maintaining uniform temperature and humidity, a series of clinical assessments were performed on participants at baseline, and after 14 days of the intervention. Prior to assessment, participants acclimated to the environment for 30 minutes after washing their faces, excluding the forehead.

The volume of closed comedones was captured using Antera 3D® CS (Miravex, Ireland). The device was applied on the site of closed comedones selected by the dermatologist, providing a reading that represented the volume of closed comedones in certain area.

Skin texture parameters, including roughness (SEr), scaliness (SEsc), and smoothness (SEsm), were quantified using the Visioscan VC20 plus (Courage & Khazaka, Germany). A higher SEr value was associated with less perceived roughness, while lower SEsc and SEsm values indicated reduced scaliness and improved smoothness, respectively.

Frontal images of the face were captured using VISIA-CR (Canifield, USA) and the a* value was analyzed by Image Pro Plus software. A lower a* value correlates with reduced erythema intensity, indicating less redness in the skin.

Skin brightness was quantified using Spectrophotometer CM-700d (Konica Minolta, Japan). Measurements were taken at the cheek region to assess changes in luminance (L* value), the higher L* value indicates greater skin brightness.

Sebum content was assessed using the Sebumeter SM-815 (Courage & Khazaka, Germany). The device was applied once on the forehead, providing a reading that represented the sebum content of the skin in that area.

Transepidermal Water Loss (TEWL) was analyzed using the Tewameter TM300 (Courage & Khazaka, Germany). Three separate measurements were taken and subsequently averaged to obtain a representative value of the skin's waster loss rate.

Clinical assessment

Dermatologists conducted a comprehensive evaluation of acne lesion, counting closed comedones, inflammatory papules, and pustules. Additionally, potential skin irritation and subjective discomfort associated with the test product were closely monitored throughout the study period to ensure participant safety and tolerability.

Self-assessment

Participants' subjective evaluations were obtained using a self-assessment questionnaire designed to assess parameters such as facial smoothness, acne severity, sebum secretion, skin erythema and skin hydration. Each parameter was rated on a 5-point Likert scale (1=very low, 5=very high). The questionnaire was administered at baseline and 14 days thereafter.

Statistical analysis

Statistical analyses were performed to evaluate the differences in skin parameters between the two groups. Normality was assessed using the Shapiro-Wilk test using SPSS software. For normally distributed data, a paired t-test was applied; otherwise, the Wilcoxon signed-rank test was used. A p value < 0.05 was considered statistically significant.

3. Results

Study flowchart

A total of 59 subjects were initially enrolled, with 33 participants (29 females and 4 males, aged 18–38 years) meeting the inclusion criteria and completing the study. Data from these

33 participants were included for subsequent analysis. During the treatment, none of the participants reported irritation or discomfort.

Effects on closed comedones volume and acne lesions count

Significant improvements were observed in both the volume and count of closed comedones following the 14-day treatment. Specifically, the volume of closed comedones decreased by 22.73% (Figure 1B), with a representative case illustrated in Figure 1A. Dermatologist-assessed evaluations (Table 1) revealed a 41.04% reduction in the count of closed comedones. Furthermore, significant improvements were observed in inflammatory lesions: the counts of inflammatory papules, pustules, and total lesions decreased by 51.27%, 63.64% and 42.75%, respectively.

Table 1 Dermatologist-assessed facial acne lesion evaluation results in 14 days clinical trial.

Acne lesion count	Average		Average variation rate (%)	*p* value compared with DAY0
	DAY0	DAY14		
Closed comedones	22.15	13.06	- 41.04	<0.001
Papules	2.36	1.15	-51.27	<0.001
Pustules	0.33	0.12	- 63.64	<0.001
Total lesion count	25.24	14.45	- 42.75	<0.001

Effects on skin texture

After 14 days of product application, significant improvements in skin texture parameters were observed. Figure 2A illustrates the topography of an average case post-treatment, with a more even gray level (GL) indicating enhanced skin texture. The SEr value, which quantifies skin roughness, increased by 17.81%, indicating a reduction in skin roughness and a smoother skin surface. Concurrently, the SEsm value, representing skin smoothness, decreased by 7.31%, further supporting the improvement in skin texture. Additionally, the SEsc value, which reflects skin scaling (scaling index), showed a notable reduction of 33.33%, suggesting a significant decrease in skin flakiness and an improvement in skin barrier integrity.

Effects on skin redness and brightness

The product demonstrated efficacy in reducing facial redness and enhancing skin luminance. VISIA CR images (Figure 3A) revealed a marked reduction in erythema after 14 days of treatment. This improvement was corroborated by a 5.51% decrease in a* values (Figure 3B), which directly reflect skin redness. Additionally, the L* value, representing skin brightness, increased by 2.49% (Figure 3C), indicating a modest but noticeable improvement in skin luminance. These results highlight the product's ability to reduce redness and enhance brightness, contributing to a more even and radiant complexion.

Effects on Sebum and Hydration Regulation

The product effectively regulated sebum production and enhanced skin barrier function. After 14 days of product application, Figure 4A revealed a significant reduction in facial sebum content, with a decrease of 17.11%, indicating the product's efficacy in controlling oil production. Concurrently, Figure 4B showed a 14.2% reduction in TEWL values, suggesting

that the product strengthened the skin barrier and reduced water loss. These findings collectively demonstrate the product's ability to restore sebum-hydration balance, contributing to improved skin health.

Self-assessment

Facial skin condition was assessed via subject self-evaluation before and after the 14-day treatment period. The self-assessment results (Table 2) revealed significant improvements in skin smoothness, overall acne severity, sebum secretion, redness and hydration. Furthermore, 97.0% of participants reported a reduction in closed comedones count, while 93.9% noted a decrease in comedones size. The product achieved an overall satisfaction rate of 97.0%, indicating high efficacy and user acceptability.

Table 2 Self-assessment results in 14 days clinical trial.

Item	Average		Average variation rate(%)	*p* value compared with DAY0
	DAY0	DAY14		
Skin smoothness	1.8	4.2	133.33	<0.001
Facial acne severity	3.9	1.8	- 53.85	<0.001
Sebum secretion level	4.5	2.1	- 53.33	<0.001
Skin erythema severity	4.0	1.7	- 57.50	<0.001
Skin hydration level	2.1	4.3	104.76	<0.001

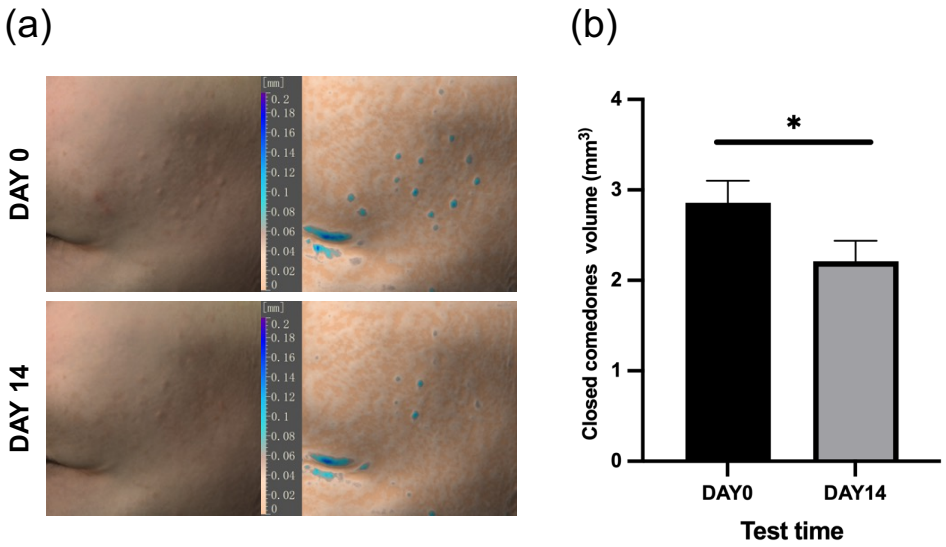

Figure 1 Evaluation of the change of closed comedones volume. (a) The Antera 3D® CS images of an average case before and after 14-day treatment of test product. (b) Statistic data of the closed comedones volume before and after 14-day treatment of test product. *$p < 0.05$ was significantly different compared with the DAY0 group. Error bars represent the standard error of the mean (SEM).

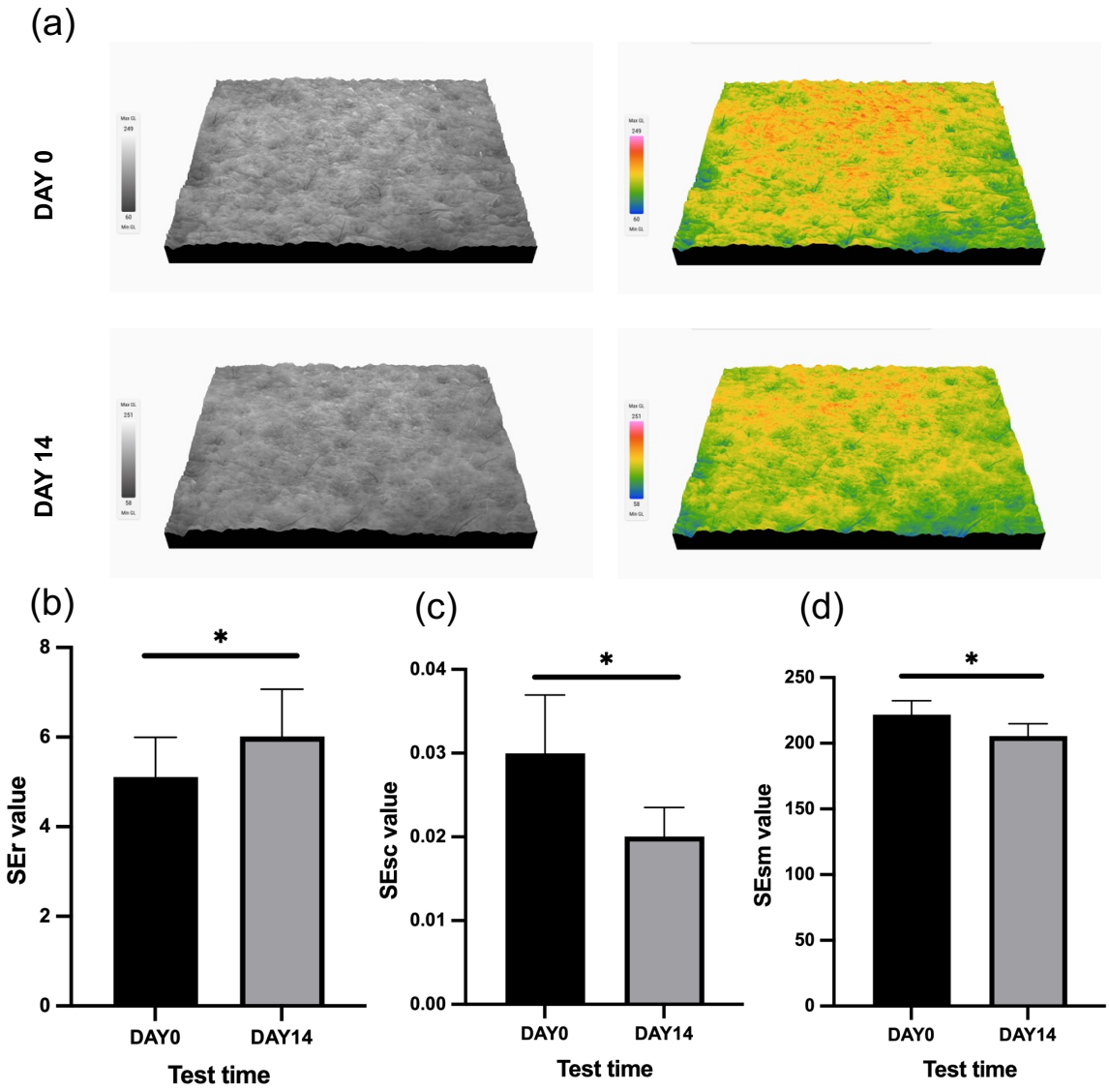

Figure 2 Evaluation of the change of skin texture. (a) The Visioscan VC20 plus topography images of an average case before and after 14-day treatment of test product. (b), (C) and (D) Statistic data of the SEr, SEsc and SEsm value before and after 14-day treatment of test product. *$p < 0.05$ was significantly different compared with the DAY0 group. Error bars represent the standard error of the mean (SEM).

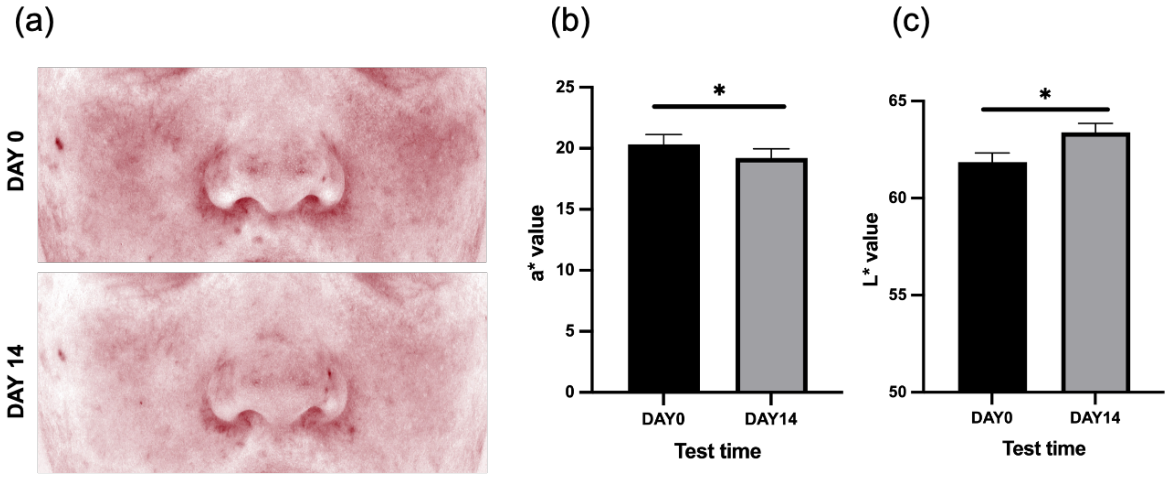

Figure 3 Evaluation of the change of skin color. (a) The VISIA-CR images of an average case before and after 14-day treatment of test product. (b) and (c) Statistic data of the a* and L* value before and after 14-day treatment of test product. *$p < 0.05$ was significantly different compared with the DAY0 group. Error bars represent the standard error of the mean (SEM).

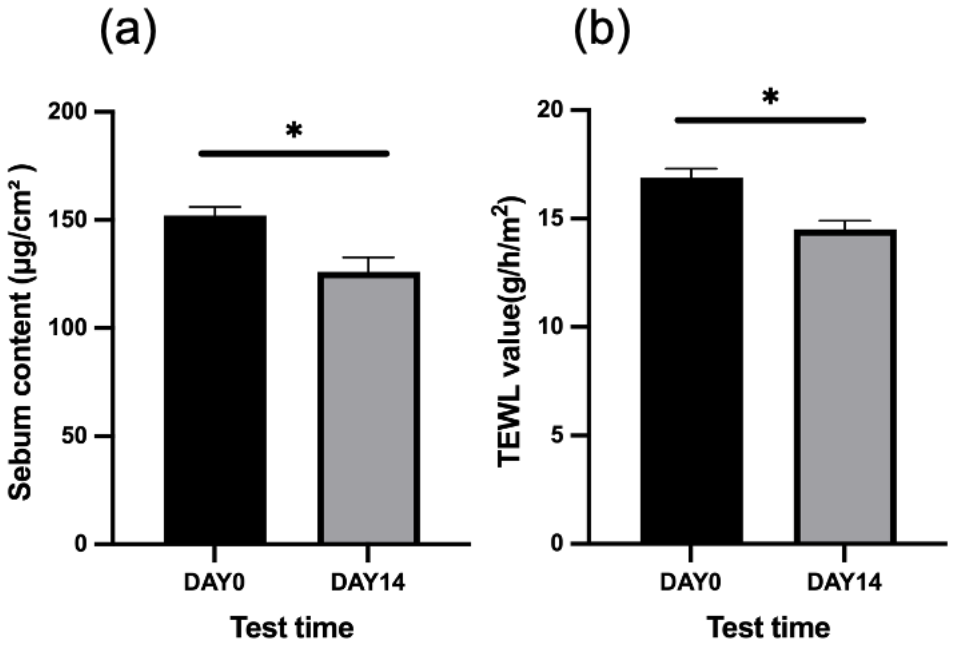

Figure 4 Evaluation of the changes in skin sebum content and TEWL. (a) Changes in skin sebum content over 14 days of product use. (b) Changes in TEWL (Transepidermal Water Loss) over 14 days of product use. *$p < 0.05$ was significantly different compared with the DAY0 group. Error bars represent the standard error of the mean (SEM).

4. Discussion

The present study demonstrates that a novel enzyme-based essence effectively improves closed comedones and overall skin condition in individuals with sensitive Asian skin. Our

findings reveal significant improvements in both objective measurements and subjective assessments after just 14 days of treatment, supporting the hypothesis that enzymatic exfoliation can serve as a gentler alternative to conventional hydroxy acids. The multi-enzyme complex, combined with carefully selected botanical extracts, including *Pueraria lobata*, *Sophora japonica*, *Anemarrhena asphodeloides*, *Silybum marianum*, *Scutellaria baicalensis*, *Sophora flavescens*, and *Glycyrrhiza inflata*, appears to provide comprehensive benefits including keratinolysis, sebum regulation, and anti-inflammatory action without compromising skin barrier function.

Following 14 days of essence application, a significant improvement in closed comedones and overall skin texture was observed through instrumental evaluation, dermatological assessment, and self-assessment. These effects are likely attributed to the keratinolytic activity of the multi-enzyme complex [19]. Previous studies on enzymatic exfoliation have demonstrated the efficacy of various enzymes in promoting stratum corneum turnover, reducing scaling, improving skin smoothness, and evening skin tone; however, their effects on closed comedones have been less frequently explored [20-22]. Our findings provide new evidence supporting the potential of enzymatic exfoliation as an effective strategy for managing closed comedones. Moreover, our previous research demonstrated that the multi-enzyme complex applied in the essence promoted epidermal cell proliferation in a 3D skin model, which may explain the observed decrease in TEWL following treatment [19].

Beyond its efficacy in improving closed comedones, the essence exhibited additional benefits for skin health. Both instrumental measurements and self-assessments confirmed its sebum-regulating effect, which may be attributed to active molecules present in the extracts of *Pueraria lobata*, *Sophora japonica*, and *Anemarrhena asphodeloides* [23-25]. Furthermore, the observed improvement in skin brightness is likely associated with the inhibitory effect of *Silybum marianum* extract on melanin synthesis [26, 27]. The reduction in facial erythema may be attributed to the anti-inflammatory properties of *Scutellaria baicalensis*, *Sophora flavescens*, and *Glycyrrhiza inflata* extracts [28-30].

To evaluate the safety of the essence for individuals with sensitive skin, subjects who self-identified as having sensitive skin were included in the study. Throughout the treatment period, no adverse reactions were reported in clinical observations or self-assessments, suggesting that enzymatic exfoliation represents a well-tolerated and effective approach for managing closed comedones in individuals with sensitive skin.

While this study provides promising evidence for the safety and efficacy of the enzyme-based essence in the treatment of closed comedones, certain limitations should be acknowledged. First, although the multi-enzyme complex is hypothesized to play a crucial role in improving skin condition, direct clinical evidence demonstrating its specific contribution remains limited. Future studies comparing formulations with and without the multi-enzyme complex are necessary to further validate its efficacy. Second, this study was conducted exclusively on East Asian individuals, and thus, the generalizability of these findings to other ethnic groups requires further investigation. Finally, the study duration was relatively short, and long-term effects remain unclear. Future research with larger sample sizes, extended follow-up periods, and diverse populations is warranted to confirm the findings and explore the sustained benefits of enzyme-based exfoliation in acne-prone and sensitive skin.

5. Conclusion

In conclusion, this study provides compelling evidence supporting the efficacy and safety of the enzyme-based essence in improving closed comedones and overall skin condition. The

essence significantly reduced the size and count of closed comedones, enhanced skin texture and tone, regulated sebum production, and contributed to skin barrier repair. Notably, no irritation was reported among participants with sensitive skin, suggesting that enzyme-based essence represents a viable and well-tolerated alternative for individuals with closed comedones and exhibiting poor tolerance to acid-based treatments.

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