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"A Novel Micro-Ecological Lotion for Acne Treatment: Selective Inhibition of Cutibacterium acnes Biofilm Formation"

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1. Introduction

Acne is a multifactorial chronic inflammatory skin disorder, primarily driven by excessive sebum production, follicular hyperkeratinization, colonization of *Cutibacterium acnes* (*C. acnes*), and local inflammatory responses [1, 2]. Among these factors, *C. acnes* is considered a key contributor, exacerbating other pathological processes [3]. Although antibiotics have long been a cornerstone of acne treatment, the emergence of antibiotic-resistant has become a growing concern in clinical dermatology [4, 5].

A major driver of antibiotic resistance in *C. acnes* is its ability to form biofilms—structured microbial communities encased in a self-produced extracellular polymeric substance (EPS) matrix composed of polysaccharides, proteins, lipids, and extracellular DNA [6-8]. These biofilms provide a protective niche, enhancing bacterial survival under antimicrobial stress. Confocal microscopy studies have revealed dense *C. acnes* biofilms spanning the entire hair follicle lumen, with evidence suggesting that acne patients harbor more robust biofilm formations compared to healthy individuals [9, 10]. Furthermore, biofilm-embedded *C. acnes* exhibits increased virulence, secreting virulence factors that exacerbate acne pathogenesis [11].

With growing recognition of the skin microbiome's role in acne, biofilm-disrupting agents are emerging as promising alternatives to conventional antimicrobials in topical acne treatments [12-14]. However, studies evaluating the impact of biofilm-targeting treatments on skin microbiota composition and clinical acne improvement remain limited. Previously, we developed a novel micro-ecological lotion, YS Probalance, containing *Lactobacillus*-fermented *Pinus densiflora* leaf extract and α-glucooligosaccharide, which selectively reduced *C. acnes* biofilm formation without inhibiting bacterial growth [15]. In this study, we further evaluated the lotion's efficacy in disrupting pre-existing biofilms and assessed its impact on skin microbiota composition and acne improvement when incorporated into an essence. These effects were analyzed through in vitro assays and a 28-day controlled clinical study, providing a comprehensive evaluation of the formulation's therapeutic potential.

2. Materials and Methods

C. acnes Biofilm Disruption Assay

C. acnes ATCC 6919 (BeNa Culture collection, China) were cultured anaerobically on glass coverslips in 6-well plates using brain heart infusion (BHI) broth at 37°C for 5 days, with daily medium replacement. Then bacteria with stabilized biofilm was treated with either 2% YS Probalance (diluted in PBS) or PBS control for 12 h under anaerobic conditions. Samples were then fixed with 2.5% glutaraldehyde, dehydrated through an ethanol series (30-95%), critical point dried, gold-coated, and imaged by field-emission scanning electron microscopy (Thermo Fisher, USA).

Skin Microbiome Research

Subject enrollment

This research enrolled healthy Chinese female participants (aged 18-55 years) with dermatologist-confirmed oily skin and mild-to-moderate inflammatory acne lesions (ISGA score 1-3), where non-inflammatory comedones accounted for ≥70% of total acne lesions. Exclusion criteria included pregnancy, allergic conditions, systemic diseases, or recent anti-acne treatments. Participants applied 3-5 drops of the test product to the entire face twice daily for 4 weeks. The study protocol was approved by the Shanghai Ethics Committee for Clinical Research. 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.

Skin microbiome sampling

Standardized skin microbiome sampling (4×4 cm² acne-affected area) was performed at baseline (Day 0) and endpoint (Day 28) under controlled laboratory conditions (20-22°C, 40-60% RH), with morning facial cleansing omitted prior to sampling to ensure microbial biomass preservation. Standardized skin sampling was performed using sterile cotton swabs moistened with 0.9% saline containing 0.1% Tween-20. Each 4×4 cm² cheek area (avoiding active lesions) was swabbed 25 times with continuous rotation, with subsequent follow-ups collected from identical anatomical sites. Swabs were immediately transferred to sterile cryovials and stored at -80°C until processing.

DNA extraction and 16S rRNA sequencing analysis

Microbial genomic DNA was extracted from skin swabs using the FastDNA® Spin Kit (MP Biomedicals, USA) following the manufacturer's protocol. The V3-V4 hypervariable regions of bacterial 16S rRNA genes were amplified using universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which target conserved regions flanking these variable domains. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen, USA), followed by library preparation with the NEXTFLEX Rapid DNA-Seq Kit (Bio Scientific, USA) and paired-end sequencing (2×300 bp) on an Illumina MiSeq platform using the MiSeq Reagent Kit v3, with all procedures performed according to the manufacturers' instructions. Skin microbiome analysis was performed using the BioCloud platform (Shanghai Archgene Biotechnology Co., Ltd., China) for assessing microbial community diversity and composition.

Acne reduction efficacy test

Subject enrollment

This research enrolled healthy Chinese adult participants (aged 18-45 years) with dermatologist-confirmed oily skin and mild-to-moderate inflammatory acne lesions (ISGA score 1-3, where non-inflammatory comedones accounted for ≥70% of total acne lesions), self-reported impaired barrier function, and concomitant symptoms (pruritus/stinging/erythema). Exclusion criteria included pregnancy, allergic conditions, systemic diseases, or recent anti-acne treatments. Participants applied 3-5 drops of the test product to the entire face twice daily for 4 weeks. 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.

Instrumental assessment

In the controlled environment, maintaining uniform temperature and humidity, a series of clinical assessments were performed on participants at baseline, 30 minutes after initial application, and after 28 days of the intervention. 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 hydration levels were measured using a Corneometer® CM825 (Courage+Khazaka, Germany) and 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 hydration level and 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' satisfaction with the product's anti-acne efficacy was assessed via questionnaire, with responses categorized as: "Strongly agree," "Agree," "Neutral,'" "Disagree," or "Strongly disagree." For analysis, respondents selecting "Strongly agree" or "Agree" were grouped as providing positive evaluations. The percentage of participants in this positive cohort was calculated against total respondents at each timepoint (baseline and day 14).

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

Effect on *C. acnes* Biofilm Disruption

Building upon our previous findings that the microecological formulation selectively inhibits *C. acnes* biofilm formation without bactericidal effects [15], we further evaluated its capacity to disrupt established biofilms. Treatment of mature-stage biofilms revealed significant structural disintegration compared to PBS controls (Figure 1), demonstrating YS Probalance's capacity to destabilize pre-existing *C. acnes* biofilms.

Effect on skin microbiome

To investigate whether YS Probalance could modulate facial skin microbiota diversity in acne-prone individuals, we analyzed skin microbiome samples from 32 participants before treatment and after 28 days of application. Alpha diversity indices, commonly used metrics for assessing microbial community diversity, demonstrated significant improvements post-treatment (Figure 2). Specifically, the Ace index, Chao index, and Sbos index all showed marked elevation after 28 days of use, indicating that the lotion effectively regulates facial skin microbiota in subjects with mild-to-moderate acne.

Anti-acne efficacy

To evaluate the anti-acne potential of YS Probalance, 30 subjects applied an essence containing 2% YS Probalance for 28 days. Instrumental assessments revealed significant a* value decrease at acne lesions area within 30 minutes post-application, which indicated the product has the potential in ameliorating acne erythema (Fig. 3A). Dermatologist evaluations by dermatologists demonstrated progressive reduction in acne counts: inflammatory papules decreased by 27.80% and total lesions by 24.87% after 28 days of use (Figure 3B&3C). Subject self-assessments corroborated these findings, with 86.67% of participants reporting perceived efficacy in acne treatment and 86.67% of participants reporting reduced acne frequency following the 28-day intervention.

Skin barrier repair efficacy

The barrier-repairing efficacy of the 2% YS Probalance essence was evaluated through instrumental analysis and self-assessment questionnaire. Instrumental analysis demonstrated rapid hydration enhancement, with significant increases in skin hydration observed within 30 minutes post-application (Figure 4A). Concurrently, TEWL measurement revealed progressive barrier recovery, decreasing by 5.86% at Day 7 and 17.78% at Day 28 compared to baseline (Fig. 4B). These objective findings were supported by participants' self-assessment, with 80% of subjects perceiving barrier repair effects of the essence following the 28-day treatment period.

(a) Negative control (b) 2% YS Probalance

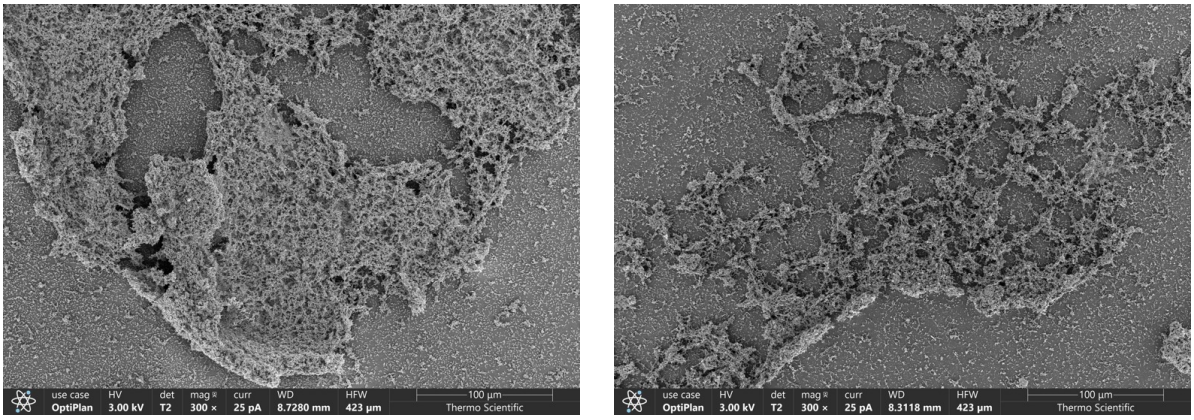

Figure 1 Evaluation of the effect on *C. acnes* biofilm disruption. Scanning electron microscopy (SEM) images showing structural disintegration of mature *C. acnes* biofilms following treatment with the microecological formulation (right panel) compared to intact biofilm architecture in PBS-treated controls (left panel).

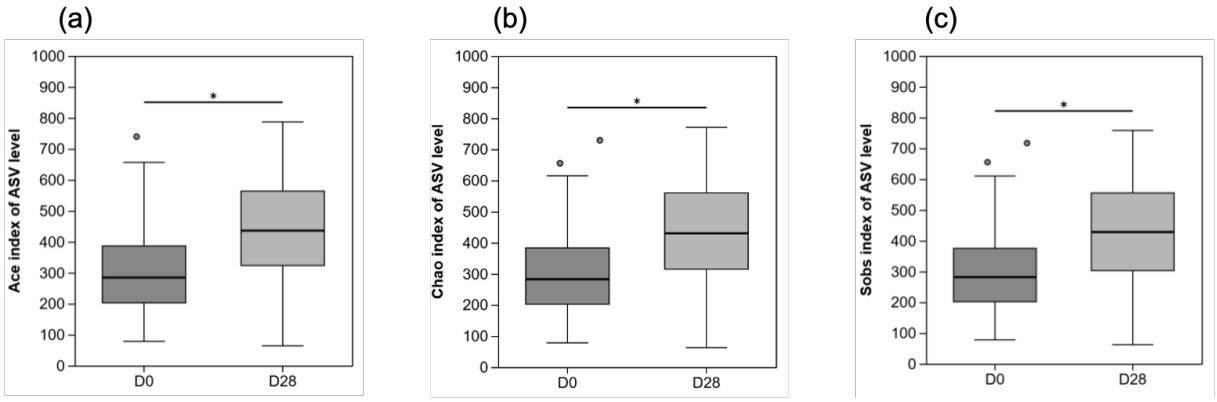

Figure 2 Evaluation of the effect on microbial alpha diversity in acne-prone skin. Box-and-whisker plots comparing pre- and post-treatment alpha diversity indices ((a) Ace, (b) Chao1, and (c) Sbos) of facial skin microbiota from 32 acne patients after 28-day application. *p < 0.05 was significantly different compared with the baseline.

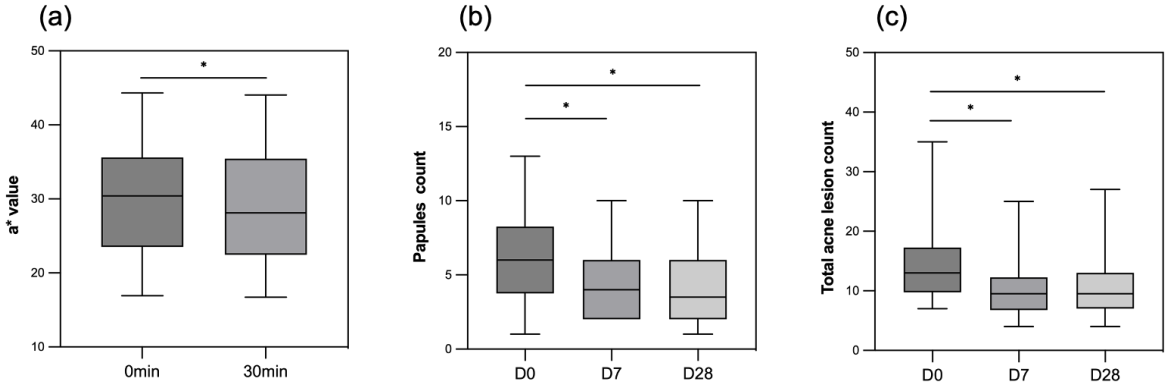

Figure 3 Evaluation of the anti-acne effect. (a) Rapid erythema reduction demonstrated by significant decrease in a* values at acne lesions 30 minutes post-application. Progressive reduction in inflammatory papule counts (b) and total lesion counts (c) over 28-day treatment. *p < 0.05 was significantly different compared with the baseline.

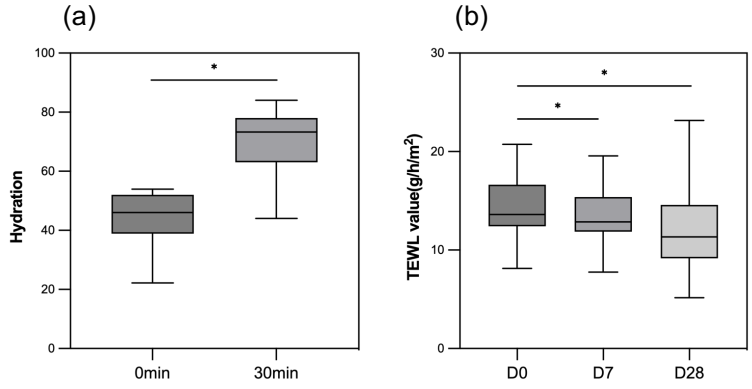

Figure 4 Evaluation of skin barrier repair efficacy. (a) Rapid hydration enhancement demonstrated by significant increase in Corneometer values 30 minutes post-application. (b) Progressive improvement in skin barrier function shown by TEWL reduction at Day 7 and Day 28 compared with baseline. *p < 0.05 was significantly different compared with the baseline.

4. Discussion

The pathogenic role of *C. acnes* in acne vulgaris is multifaceted, involving not only the induction of pro-inflammatory cytokines, but also the promotion of follicular hyperkeratinization and sebaceous gland hyperactivity through various virulence factors [3, 16]. While conventional antibiotic therapies demonstrate efficacy against planktonic *C. acnes* populations, their therapeutic limitations become apparent when confronting biofilm-embedded bacteria [17]. The protective extracellular polymeric matrix of biofilms may confer increased antibiotic resistance compared to their planktonic counterparts, creating a reservoir for bacterial persistence and subsequent relapse following treatment cessation [6]. This phenomenon may explain the frequent recurrence observed in antibiotic-treated acne patients despite initial clinical improvement.

In this study, we evaluated the anti-acne and recurrence-preventing potential of YS Probalance, a novel microecological lotion formulated with a prebiotic (α-glucooligosaccharide) and a postbiotic (*Lactobacillus*-fermented *Pinus densiflora* leaf extract). The therapeutic rationale is based on a dual mechanism: inhibition of *C. acnes* biofilm and enhancement of skin microbiota diversity. α-glucooligosaccharide functions as a selective substrate that promotes the proliferation of commensal and beneficial skin bacteria, thereby supporting microbiome homeostasis [18, 19]. The *Pinus densiflora* extract has previously been reported to exhibit antimicrobial activity against *C. acnes* [20, 21], however, fermentation by *Lactobacillus* may modulate its bioactive components, potentially enhancing or altering its antimicrobial and biofilm-disrupting properties [22, 23]. Our prior in vitro findings demonstrated the ability of YS Probalance to selectively inhibit *C. acnes* biofilm formation [15]. In the current study, SEM further confirmed its capacity to disrupt pre-established biofilms (Figure 1), indicating a dual modulatory role in both the prevention and removal of C. acnes biofilm. Moreover, 16S rRNA sequencing analysis revealed that topical application of YS Probalance led to increased alpha diversity within the skin microbiome (Figure 2), a factor that has been found decreased in acne patients [24, 25]. However, the synergistic interactions between the prebiotic and postbiotic components within YS Probalance remain to be elucidated. Future studies are warranted to clarify whether the observed biofilm-inhibitory and microbiome-modulating effects arise from additive or synergistic mechanisms of the combined ingredients.

Although the use of prebiotics and postbiotics in dermatology has attracted growing interest, robust clinical evidence supporting their efficacy in acne remains limited. Our clinical evaluation provides important validation, showing that a skincare essence containing 2% YS Probalance significantly reduced acne lesion counts, improved skin barrier integrity, and lowered the frequency of acne relapse (Figures 3 and 4). These findings position YS Probalance as a promising topical agent for managing chronic or relapsing acne, especially for individuals seeking alternatives to traditional antibiotics or retinoids.

Despite these encouraging results, several limitations should be acknowledged. First, the exact molecular mechanism by which YS Probalance inhibited *C. acnes* biofilm need further investigation. Second, while changes in microbiota composition were documented, causal links between specific microbial taxa and clinical outcomes were not established. Finally, the long-term durability of the observed effects beyond the intervention period was not assessed. Future research should explore the molecular mechanisms underlying the biofilm-disrupting and microbiota-modulating effects of YS Probalance, including metabolomic profiling of the fermented extract. Additionally, exploring its efficacy in combination with other therapeutic modalities (e.g., light therapy, hormonal treatment) may also broaden its clinical utility.

5. Conclusion

In conclusion, this research demonstrated a novel microecological approach to acne management through actions on *C. acnes* biofilms and the skin microbiome. Its clinical and microbiological effects supported its potential as a non-antibiotic strategy for treating acne and reducing recurrence risk, providing new insights into the development of microbiome-targeted dermatological therapies.

Reference

1. Bharti, S. and H.C. Vadlamudi, *A strategic review on the involvement of receptors, transcription factors and hormones in acne pathogenesis.* Journal of Receptors and Signal Transduction, 2021. **41**(2): p. 105-116.
2. Mias, C., et al., *Recent advances in understanding inflammatory acne: Deciphering the relationship between Cutibacterium acnes and Th17 inflammatory pathway.* Journal of the European Academy of Dermatology and Venereology, 2023. **37**: p. 3-11.
3. Beylot, C., et al., *Propionibacterium acnes: an update on its role in the pathogenesis of acne.* Journal of the European Academy of Dermatology and Venereology, 2014. **28**(3): p. 271-278.
4. Bhate, K., et al., *Is there an association between long-term antibiotics for acne and subsequent infection sequelae and antimicrobial resistance? A systematic review.* BJGP open, 2021. **5**(3).
5. Simonart, T. and M. Dramaix, *Treatment of acne with topical antibiotics: lessons from clinical studies.* British journal of dermatology, 2005. **153**(2): p. 395-403.
6. Aslan Kayiran, M., et al., *Antibiotic resistance in acne: mechanisms, complications and management.* American Journal of Clinical Dermatology, 2020. **21**(6): p. 813-819.
7. Coenye, T., K.-J. Spittaels, and Y. Achermann, *The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of Cutibacterium acnes.* Biofilm, 2022. **4**: p. 100063.
8. Vert, M., et al., *Terminology for biorelated polymers and applications (IUPAC Recommendations 2012).* Pure and Applied Chemistry, 2012. **84**(2): p. 377-410.
9. Jahns, A.C. and O.A. Alexeyev, *Three dimensional distribution of P ropionibacterium acnes biofilms in human skin.* Experimental dermatology, 2014. **23**(9): p. 687-689.
10. Jahns, A.C., et al., *An increased incidence of Propionibacterium acnes biofilms in acne vulgaris: a case–control study.* British journal of dermatology, 2012. **167**(1): p. 50-58.
11. Coenye, T., E. Peeters, and H.J. Nelis, *Biofilm formation by Propionibacterium acnes is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors.* Research in microbiology, 2007. **158**(4): p. 386-392.
12. Attia-Vigneau, J., et al., *Polylysine dendrigraft is able to differentially impact Cutibacterium acnes strains preventing acneic skin.* Experimental Dermatology, 2022. **31**(7): p. 1056-1064.

13. Kim, Y.-G., et al., *Inhibition of Biofilm Formation in Cutibacterium acnes, Staphylococcus aureus, and Candida albicans by the Phytopigment Shikonin.* International Journal of Molecular Sciences, 2024. **25**(4): p. 2426.
14. Lemoine, V., et al., *Nanovectorized microalgal extracts to fight Candida albicans and Cutibacterium acnes Biofilms: Impact of dual-species conditions.* Antibiotics, 2020. **9**(6): p. 279.
15. Hui, Z., et al., *A novel anti-acne method that can directionally inhibit the biofilm of Cutibacterium acnes (in Chinese).* Journal of China Cosmetics Science, 2024(1): p. 43-48.
16. Mayslich, C., P.A. Grange, and N. Dupin, *Cutibacterium acnes as an opportunistic pathogen: an update of its virulence-associated factors.* Microorganisms, 2021. **9**(2): p. 303.
17. Budge, M.D., et al., *The In Vitro Efficacy of Doxycycline over Vancomycin and Penicillin in the Elimination of Cutibacterium Acnes Biofilm.* Antimicrob Comb Devices (2019), 2020. **Stp1630**: p. 53-64.
18. Chung, C. and D. Day, *Glucooligosaccharides from Leuconostoc mesenteroides B-742 (ATCC 13146): a potential prebiotic.* Journal of Industrial Microbiology and Biotechnology, 2002. **29**(4): p. 196-199.
19. Iglesia, S., T. Kononov, and A.S. Zahr, *A multi-functional anti-aging moisturizer maintains a diverse and balanced facial skin microbiome.* Journal of Applied Microbiology, 2022. **133**(3): p. 1791-1799.
20. Oh, Y.J., et al., *Antibacterial and antiviral properties of Pinus densiflora essential oil.* Foods, 2023. **12**(23): p. 4279.
21. Sultan, M.Z., Y.-M. Jeon, and S.-S. Moon, *Labdane-type diterpenes active against acne from pine cones (Pinus densiflora).* Planta medica, 2008. **74**(04): p. 449-452.
22. Chon, H., G. Kim, and S. Kim, *Comparison of aqueous plant extracts before and after fermentation with Lactobacillus paracasei LS-2 on cytokine induction and antioxidant activity.* Natural product communications, 2010. **5**(8): p. 1934578X1000500827.
23. Mousavi, Z.E. and M. Mousavi, *The effect of fermentation by Lactobacillus plantarum on the physicochemical and functional properties of liquorice root extract.* LWT, 2019. **105**: p. 164-168.
24. Maître, M., et al., *Characterization of the forehead skin microbiome in the early phase of acne.* Journal of the European Academy of Dermatology and Venereology, 2024. **38**: p. 3-11.
25. Schneider, A.M., et al., *Evolution of the facial skin microbiome during puberty in normal and acne skin.* Journal of the European Academy of Dermatology and Venereology, 2023. **37**(1): p. 166-175.