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ORIGINAL ARTICLE

Evaluation of a new-formula shampoo containing 6% glycyrrhetinic acid complex for scalp seborrheic dermatitis: A pilot study

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Abstract

Background: Scalp seborrheic dermatitis (SD) is a chronic inflammatory dermatosis associated with sebum imbalance and proliferation of *Malassezia* species. Various antifungal shampoos are commonly used for scalp SD.

Aims: Glycyrrhetinic acid is known to have antioxidative, anti-inflammatory, and anti-allergic effects. This study was designed to evaluate the effectiveness of a new-formula shampoo that contains glycyrrhetinic acid for the treatment of scalp SD.

Patients/Methods: Thirty-four patients were enrolled and treated with the 6% glycyrrhetinic acid complex shampoo. Efficacy was assessed clinically with Dermatology Life Quality Index (DLQI) and Adherent Scalp Flaking Score (ASFS) by the same dermatologist at baseline, week 2, and week 5. Among the 24 subjects with the most significant clinical improvement, four common microorganisms from scalp samples were analyzed by quantitative polymerase chain reaction (qPCR) at baseline, and week 5.

Results: The DLQI and ASFS at week 2 and week 5 improved significantly relative to baseline. The bacteria profiles showed a significant increase of *Cutibacterium acnes* and a decrease of *Staphylococcus epidermidis* at week 5. The fungi profiles showed significant decreases of both *Malassezia restricta* and *Malassezia globosa*. The ratio of *C. acne* to *S. epidermidis* increased significantly from 0.93 at baseline to 1.55 at week 5. The ratio of *M. restricta* to *M. globosa* decreased from 5.02 at baseline to 1.00 at week 5.

Conclusions: The effectiveness of this new regimen was objectively demonstrated at the clinical and microbiological levels. This new formula may alleviate the bacterial and fungal dysbiosis in scalp SD.

KEYWORDS
dysbiosis, glycyrrhetinic acid, scalp, seborrheic dermatitis

Hsiao-Chi Wang and Chii-Shyan Wang should be considered joint first authors.

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

1 | INTRODUCTION

Scalp seborrheic dermatitis (SD) is a chronic type of inflammatory dermatosis associated with the proliferation of *Malassezia* species.[1,2] Several recent scalp microbiome studies on different populations have also revealed the association of dandruff with bacterial and fungal dysbiosis.[3] *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) and *Staphylococcus epidermidis* were noted to be the core bacterial species on the top of the scalp. The former was associated with a healthier scalp, and the latter with a dandruff scalp.[2] Along with the common *Malassezia* species (*M. restricta* and *M. globosa*) on the scalp, a high association of dandruff with other *Malassezia* species was also observed in the mycobiome.[2,4]

Antifungal therapies against *Malassezia* species and anti-inflammation agents are helpful for scalp SD. Traditionally, ketoconazole, ciclopirox olamine, zinc-pyrithione, and selenium sulfide shampoos are common treatments for this condition.[5] However, the treatment response rates varied from 26% to 77%.[6] A new shampoo with different compounds is still needed for patients with a poor response to these conventional shampoos.

Glycyrrhetinic acid, the main metabolite of glycyrrhizin from licorice root, is known to have antioxidative, anti-inflammatory, anti-allergic, and antimicrobial effects. It can be used for various skin conditions including pruritus, atopic dermatitis, acne vulgaris, skin aging, and hyperpigmentation.[7] Its application for scalp SD was rarely found in the literature.[8] This open-labeled pilot study aims to evaluate the clinical efficacy of a new-formula shampoo containing glycyrrhetinic acid. The microorganisms commonly found on the scalp were analyzed to examine a possible dysbiosis restoration in scalp SD patients.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients aged 20–65 years who visited En Chu Kong Hospital for scalp SD from June 2020 to February 2021 were enrolled in this study with inclusion criteria of Dermatology Life Quality Index (DLQI) above 2 and Adherent Scalp Flaking Score (ASFS) above 10.[9–11] The purpose of this non-invasive study was open for all patients, and informed consent forms were signed (IRB No. ECKIRB1090405). Patients were excluded from the study if they were allergic to menthol, had scalp surgery in the previous month, planned to have pregnancy or breastfeeding, had autoimmune, cancer, renal, or liver diseases, or had any other major systemic diseases. In total, 34 patients met the inclusion criteria. The study algorithm was shown in Figure 1.

2.2 | Test products

The ingredients of the new-formula shampoo with 6% glycyrrhetinic acid complex (5α Juniper MediPRO, MacroHi Co. Ltd.) are shown in Table 1. All patients were instructed to rinse and massage their scalps first with the assigned shampoo for 10–20 s and rinse off with water, followed by a second massage for 1–2 min with the

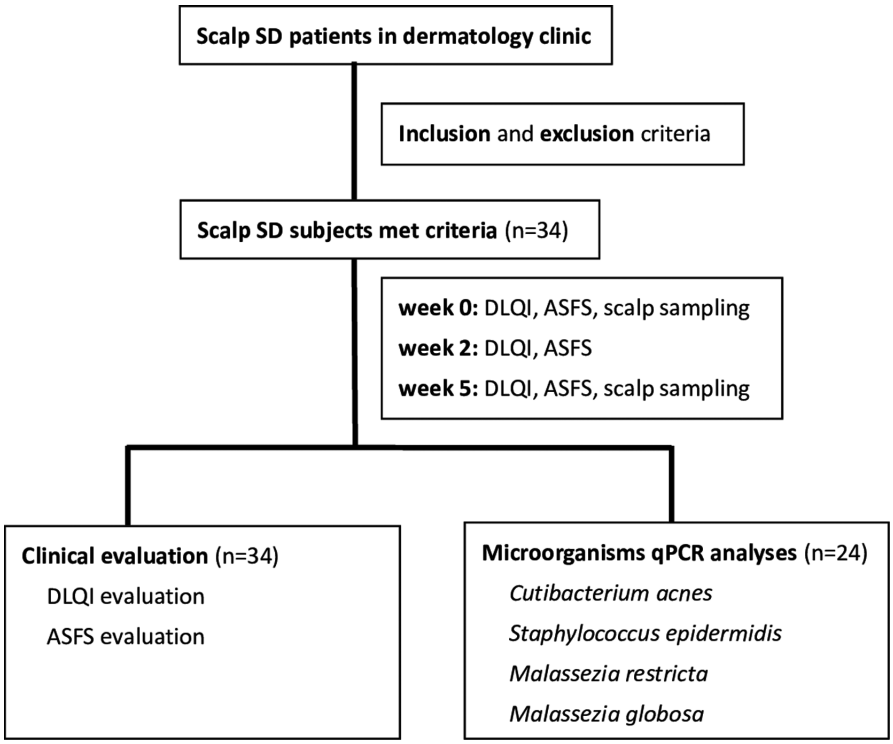

FIGURE 1 Study algorithm. SD, seborrheic dermatitis; DLQI, Dermatology Life Quality Index; ASFS, Adherent Scalp Flaking Score; qPCR, quantitative polymerase chain reaction

TABLE 1 The ingredients of the new-formula shampoo with 6% glycyrrhetinic acid complex

Main ingredient	Glycyrrhetinic acid
Other ingredients	Aqua, Lauramide Propyl Betaine, Sodium Lauroyl Sarcosinate, Citric Acid, Decyl Glucoside, Citrus Grandis Seed Extract, Panthenol, Niacinamide, Glycerin, Serenoa Serrulata Fruit Extract, 1,3-Butylene Glycol, Glycolic Acid, Organic Aloe Vera (Aloe Barbadensis Leaf Powder), Ginger Tincture (Zingiber Cassumunar Root Extract), Oleth-12, Butyl Avocadate, Eucalyptus Globulus (Eucalyptus) Leaf Oil, Rosmarinus Officinalis (Rosemary) Leaf Oil, Laurus Nobilis (Bay leaf) Oil, Citrus Aurantium Bergamia (Bergamot) Fruit Oil, Melaleuca Alternifolia (Tea Tree) Leaf Oil, Mentha Piperita (Peppermint) Oil, Sodium Usnate

TABLE 2 Specific probes used for four microorganism species in real-time quantitative PCR

Species	Primer	Sequence (5′–3′)	Ref
Cutibacterium acnes	PA-F	GCGTGAGTGACGGTAATGGGTA	23
	PA-R	TTCCGACGCGATCAACC	
Staphylococcus epidermidis	SepV58	GCTGTGATGGGGAGAGGAAAT	24
	SepR54bSta59bT	CGGTACGGGCACCTGTTATC	
Malassezia restricta	qMA-F	GTGAATTGCAGAATTCCGTGAAT	25
	qMR-R	GCGAGCCTGTGCTAGGTA	
Malassezia globosa	qMA-F	GTGAATTGCAGAATTCCGTGAAT	25
	qMG-R	GAGCTTTTTCTAGAGAAGAAAAG	

same product and rinse off everyday for 5 weeks. Other drugs or external preparations for scalp diseases were not allowed during the study period.

2.3 | Clinical evaluation

The DLQI questionnaire contains 10 questions and is used to measure the impact of cutaneous disease on patients' quality of life over the past week.[9] Each question is scored from 0 to 3, and the total score ranges from 0 to 30. The higher the score, the higher the impact. The ASFS is evaluated by dermatologists to grade dandruff severity in 8 sections of the scalp on a scale from 0 to 10. The total score ranges from 0 to 80 units, and a higher score indicates a heavier flaking.[10] The scalp was divided into eight sections, and the severity of dandruff was evaluated with the methods proposed by Bacon et al.[10] A comb was used to part the hair in each area to give a clear view of the scalp, and photographs were taken at each evaluation. The clinical severity of DLQI and ASFS was assessed by the same dermatologist at baseline, week 2, and week 5 after using the test product.

2.4 | Sample collection

Four common microorganisms, namely, *C. acnes*, *S. epidermidis*, *M. restricta*, and *M. globosa*, were sampled from scalp and analyzed by quantitative polymerase chain reaction (qPCR) at baseline and week 5. Patients were asked not to wash their hair on the day before the sampling procedure. A controlled environment with a room temperature of 25 ± 2°C and a 50% ± 10% relative humidity was provided during scalp sampling. The sample was collected from the vertex region and temporal region above the ears in triplicate. Sampling was conducted by the swab method as described in previous studies.[12]

Presto™ Mini gDNA Bacteria Kit (GBB100, Geneaid) was used as per the manufacturer's instructions for the preparation of the samples. Sterile swabs (LIBO Medical Products Inc., Cat. No. 30071) were soaked in SCF-1 solution (pH 7.6 Tris buffer 50 mM, pH 8.0 EDTA 1 mM, and 0.5% Tween 20) before sampling. A comb was used to separate hair fibers, and the pre-wetted swab was rubbed back and forth onto the scalp surface for about 30 times. The total covering surface was about 5 cm^2 (10 cm in length and 0.5 cm in width). After sampling, the head of each swab was cut from the handle and placed into a tube containing 1.5 mL SCF-1 solution. All samples were stored at −20°C until DNA extraction.

2.5 | Bacterial and fungal genomic DNA extraction

Among the 24 subjects with the most significant clinical improvement, genomic DNA was extracted from the swab samples using DNeasy® Blood & Tissue Kit (Qiagen, Cat. No. 69504) according to the manufacturer's instructions. The procedure was slightly modified to extract fungal genomic DNA.

The swab samples were vortex mixed at the maximum speed for 30 s, and the swab heads were then removed. Two identical 1.5 mL suspensions were generated from duplicate collection tubes. One was for bacterial DNA, and the other was for fungal DNA extraction.

The suspension from each tube was pelleted by centrifugation at 20 000 × *g* (14 000 rpm) for 3–5 min.

For bacterial DNA extraction, the manufacturer's protocol for genomic DNA isolation from Gram-positive bacteria was followed. For fungal DNA extraction, the protocol with minor modifications was used: The pellet was re-suspended in 150 μL of lysis buffer (200 mM Tris-HCl, pH 8.0; 25 mM EDTA, 250 mM NaCl, and 0.5% SDS) at 100°C for 15 min. Later, 180 μL of buffer ATL was added and incubated with 20 μL of proteinase K at 56°C for 15 min. The remaining steps were performed according to the manufacturer's protocol, and samples were eluted in 150 μL AE buffer. The extracted DNA was stored at −20°C before real-time PCR quantification.

2.6 | Real-time quantitative PCR

The four common species, namely, *C. acnes*, *S. epidermidis*, *M. restricta*, and *M. globosa*, were collected from the scalp and quantified with real-time PCR using TaqMan probes and specific primers listed in Table 2. Real-time quantitative PCR was performed by StepOnePlus™ System (Applied Biosystems) with optical 96-well plates. The reaction mix had a total volume of 15 μL and consisted of 1× KAPA PROBE FAST qPCR Master Mix (Kapa Biosystems), 250 nM forward and reverse primers, 300 nM probe, and 5 μL sample. The reaction conditions were 95°C for 3 min, 40 cycles of 95°C for 3 s, and 60°C for 1 min. Each sample was run in triplicate. The copy numbers of each sample were calculated from the standard curves as follows.

2.7 | Standards curve for quantification

The genomic DNA of *C. acnes* (ATCC 29399), *S. epidermidis* (ATCC 14990), *M. restricta* (ATCC MYA-4611), and *M. globosa* (ATCC 96807) was extracted as mentioned previously. The targeted fragments detected by primers and probes listed in Table 2 were amplified using the new primer sets. The new primer sets designed by using Primer3web (http://primer3.ut.ee/) are listed in Table 3. The amplified products were purified by GenepHlow™ Gel/PCR Kit (Geneaid, Cat. No. DFH100) and cloned into pGEM®-T Easy Vector (Promega, Cat. No. A1360), followed by transformation into DH5α competent *E. coli* cells. Plasmids were extracted using a High-Speed Plasmid Mini Kit (Geneaid, Cat. No. PD300), and the concentrations of the products were determined by Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific Instrument Co. Ltd). The standard curves were generated using a tenfold dilution of the four plasmids separately. Serially diluted plasmids in ddH_2O to a final concentration ranging from 101 to 106 copies/ml were used for real-time PCR. A new standard curve was run for each real-time PCR reaction.

2.8 | Statistical analysis

The therapeutic efficacy analysis of the DLQI and ASFS score during the treatment phase was performed by comparison of mean ± standard deviation (SD) at week 2 and week 5 versus baseline with Scheffé Test, respectively. The log copy numbers of 4 microorganisms at baseline and at week 5 were compared by a paired sample *t*-test. SPSS software 26.0 was used for analysis. All statistical tests were set at a significance level of 0.05.

3 | RESULTS

3.1 | Changes in clinical severity score

A total of 34 patients, 12 males and 22 females, completed the clinical study. The clinical severity score DLQI and ASFS at baseline and after treatment are listed in Tables 4 and 5. The DLQI and ASFS improved significantly relative to baseline at weeks 2 and week 5, respectively ($p < 0.05$). However, the changes in DLQI and ASFS between weeks 2 and week 5 were not significant ($p = 0.652$).

TABLE 3 Sequences of designed primer sets for four microorganism species

Species	Primer	Sequence (5′–3′)	Amplicon Size(bp)
Cutibacterium acnes	CA-F	GCGTGAGTGACGGTAATGGGTA	498
	CA-R	TTCCGACGCGATCAACC	
	CA-TAQ	AGCGTTGTCCGGATTTATTGGGCG	
Staphylococcus epidermidis	SE-F	GCTGTGATGGGGAGAGGAAAT	498
	SE-R	CGGTACGGGCACCTGTTATC	
	SE-TAQ	AGAGGCTTTTCTCGGCAGTGTGAAATCAACGA	
Malassezia restricta	MR-F	GTGAATTGCAGAATTCCGTGAAT	402
	MR-R	GCGAGCCTGTGCTAGGTA	
	MR-TAQ	CTTTGAACGCACCTTGCGCTC	
Malassezia globosa	MG-F	GTGAATTGCAGAATTCCGTGAAT	467
	MG-R	GAGCTTTTTCTAGAGAAGAAAAG	
	MG-TAQ	CTTTGAACGCACCTTGCGCTC	

TABLE 4 The DLQI scores and changes over time after treatment with glycyrrhetinic acid shampoo

Clinical parameter	DLQI (mean ± SD)
Week 0	12.7 ± 5.2
Week 2	6.5 ± 5.7*
Week 5	5.3 ± 5.9**
% change W2 vs. W0	−53.8
% change W5 vs. W0	−54.3

Abbreviations: DLQI, Dermatology Life Quality Index; SD, standard deviation; W0, week 0; W2, week 2; W5, week 5.

*Comparison of mean ± standard deviation (SD) change versus W0 using Scheffé Test, $p < 0.05$.; **Comparison of mean ± standard deviation (SD) change versus W0 using Scheffé Test, $p < 0.05$.

TABLE 5 The ASFS scores and changes over time after treatment with glycyrrhetinic acid shampoo

Clinical parameter	ASFS (mean ± SD)
Week 0	23.1 ± 8.9
Week 2	10.4 ± 10.7*
Week 5	9.1 ± 13.0**
% change W2 vs. W0	−56.9
% change W5 vs. W0	−65.8

Abbreviations: ASFS, Adherent Scalp Flaking Score; SD, standard deviation; W0, week 0; W2, week 2; W5, week 5.

*Comparison of mean ± standard deviation (SD) change versus W0 using Scheffé Test, $p < 0.05$.; **Comparison of mean ± standard deviation (SD) change versus W0 using Scheffé Test, $p < 0.05$.

3.2 | Changes in four common microorganisms

Among the 24 subjects with the most significant clinical improvement, the amount of the four common microorganisms on the scalp showed significant changes after 5 weeks of glycyrrhetinic acid shampoo treatment (Figure 2). The bacteria profiles showed a significant increase of *C. acnes* ($p < 0.05$) and a significant decrease of *S. epidermidis* ($p < 0.001$), respectively (Figure 2A). On the contrary, the fungi profiles showed a significant decrease of both *M. restricta* ($p < 0.001$) and *M. globosa* ($p < 0.001$) (Figure 2B).

We further analyzed the changes in the ratio of *C. acne* to *S. epidermidis* and the ratio of *M. restricta to M. globosa* from week 0 to week 5. The ratio of *C. acne* to *S. epidermidis* increased significantly from 0.93 at baseline to 1.55 at week 5 ($p < 0.001$). In contrast, the ratio of *M. restricta* to *M. globosa* decreased from 5.02 at baseline to 1.00 at week 5, but the difference was not statistically significant ($p = 0.176$) (Figure 3). The 24 individual changes of the bacteria ratio (*C. acne* to *S. epidermidis*) showed increases in 22 subjects after a 5-week intervention of glycyrrhetinic acid shampoo (Figure 4A). The individual changes of the fungi ratio (*M. restricta* to *M. globosa*) showed variable trends from week 0 to week 5 (Figure 4B). Among these 24 subjects, 13 patients had decreasing trends after 5 weeks.

4 | DISCUSSION

The pathogenesis of SD is complex and was hypothesized to result from the interactions among cutaneous microflora, the immune system, and the sebum imbalance in the stratum corneum of the scalp.[13] It is known to be a chronic type of inflammatory dermatosis associated with the proliferation of *Malassezia* species.[1,2] Traditionally, topical steroids have been used to reduce scalp inflammation. However, *Malassezia* species cannot be inhibited by steroids. In addition, the chronic nature of the disease and the need for maintenance therapy should be considered, a long-term treatment is inevitable. It is obvious that steroids are not appropriate for long-term and prophylactic use owing to the relapsing nature of SD.

Various antifungal therapies against *Malassezia* species and anti-inflammation agents are helpful for scalp SD. Ketoconazole, climbazole, ciclopirox olamine, zinc-pyrithione, or selenium sulfide shampoos are also commonly used for scalp SD.[5,14] Ketoconazole and climbazole are both azoles widely used as antifungal agents in cosmetic products. Ciclopirox olamine is a broad-spectrum antifungal agent with an anti-inflammatory activity.[15] Zinc pyrithione exerts an antifungal effect by damaging iron-sulfur proteins and importing copper into cells.[16] Recently, a new shampoo with the combination of zinc-pyrithione, ciclopirox olamine, and glycyrrhetinic acid showed effectiveness in 67 subjects with SD with a decrease in clinical signs, *Malassezia* species, cohesion proteins, and markers for inflammation and pruritus.[8] Most of these agents improved the scalp condition by antifungal or anti-inflammation effect without properly addressing the restoration of scalp dysbiosis.

Previously, glycyrrhetinic acid and its derivatives from licorice root were known to exhibit a broad spectrum of biological and pharmacological activities, including anti-inflammatory and anti-allergic effects by reducing tumor necrosis factor (TNF)-α production and inhibition of nuclear factor kappa-light-chain (NF-κB) activation as well as phosphoinositide 3-kinase (PI3K) activity.[17–19] Glycyrrhetinic acid and its derivatives also showed a remarkably broad spectrum of other biological and pharmacological activities, including antimicrobial effects.[7,20] In the present study, we used glycyrrhetinic acid as the only main ingredient to test its effectiveness in treating scalp SD. The score of DLQI and ASFS both revealed a significant improvement after 2 weeks of therapy. The score remained relatively stable after the following 3 weeks during the treatment period. These results implied the glycyrrhetinic acid shampoo might be suitable as a therapeutic as well as a maintenance regimen for scalp SD.

It has been shown that sebum quantity and water content had a significant impact on the two major reciprocally inhibited bacteria (*Cutibacterium* and *Staphylococcus*) on the scalp.[21] Dandruff was associated with a higher incidence of *S. epidermidis* and a lower incidence of *C. acnes*.[22] The proportion of *Cutibacterium* was also inversely correlated with that of *Staphylococcus* at non-lesional sites of subjects with SD.[2] The ratio of *C. acnes* to *S. epidermidis* was higher in healthy scalp compared to the dandruff scalp, which might be a crucial biomarker for the diagnosis and prevention of the dandruff condition.[4] On the other hand, the *Malassezia* species contributed

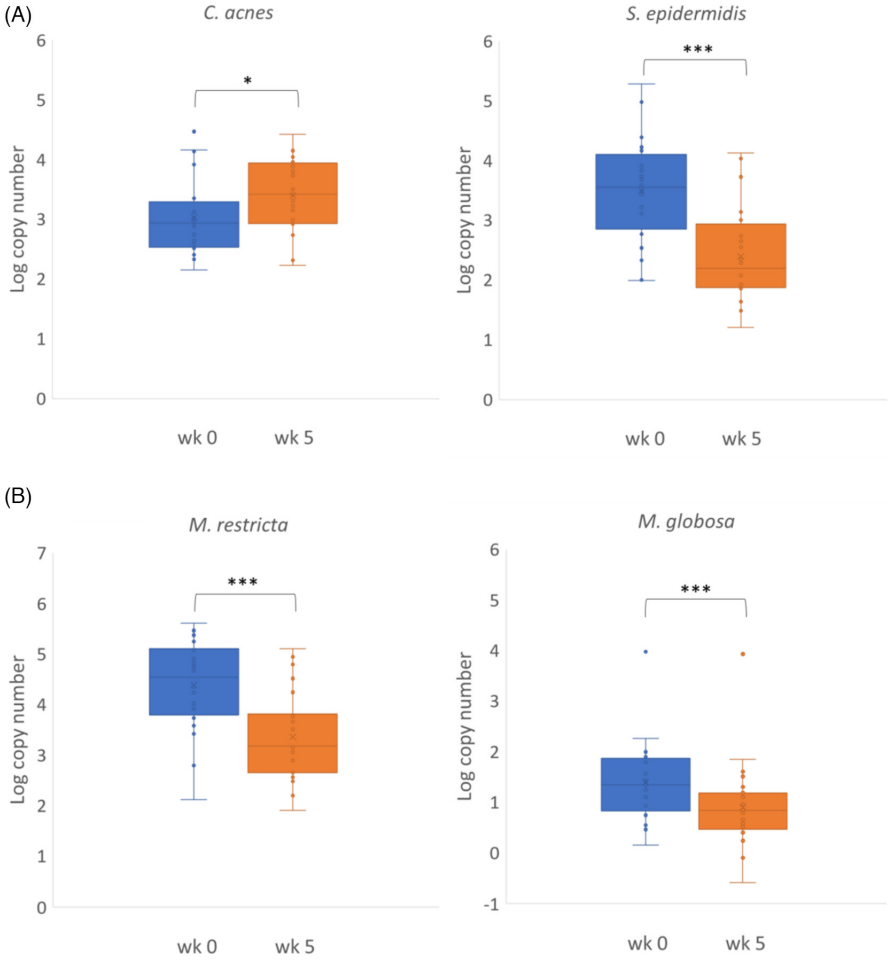

FIGURE 2 (A) Copy number changes of scalp bacteria (*C. acnes* and *S. epidermidis*) between week 0 and week 5 after the use of glycyrrhetinic acid shampoo. (B) Copy number changes of scalp fungi (*M. restricta* and *M. globosa*) between week 0 and week 5 after the intervention of the test product. (Paired sample *t*-test, $^{*}p < 0.05$, $^{***}p < 0.001$)

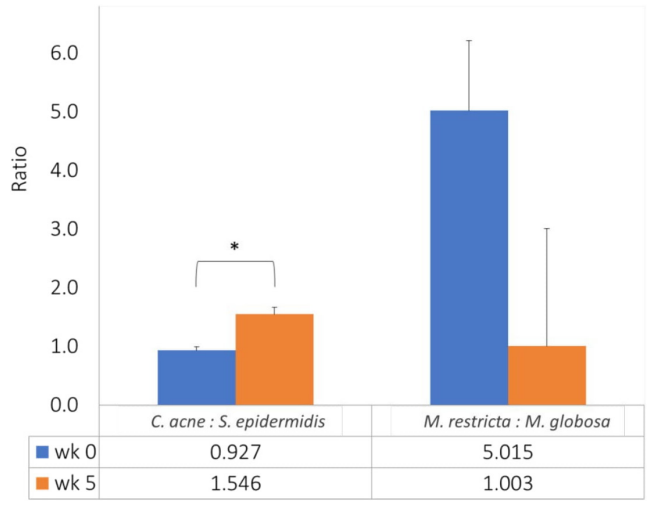

FIGURE 3 Differences in the bacterial ratio of *C. acne* to *S. epidermidis* and fungal ratio of *M. restricta* to *M. globosa* at week 0 and week 5 after the use of glycyrrhetinic acid shampoo. (Paired sample *t*-test, $^{*}p < 0.001$)

contrary roles to the healthy scalp micro-environment. *M. restricta* and *M. globosa* dominated the fungal populations both at lesional and non-lesional sites in the skin of patients with SD.[2] Dandruff was found to be associated with a higher incidence of *M. restricta*.[22] A lower *M. restricta* to *M. globosa* ratio was found to be associated with a healthier scalp.[4] Although the successful treatment of SD is to provide anti-microorganism, antioxidant, and anti-inflammatory therapies, the restoration of dysbiosis found in SD should also be considered, because adjusting the balance of the bacteria on the scalp is a potential solution to lessen dandruff by enhancing *Cutibacterium* and suppressing *Staphylococcus*.[21]

Glycyrrhetinic acid and its derivatives showed a remarkably antimicrobial effect with its minimum inhibitory concentration (MIC) value against *S. epidermidis* at 12.5 ug/ml.[7] Our results showed a significant decrease of scalp *S. epidermidis* after a 5-week use with glycyrrhetinic acid shampoo. This ingredient also helped to significantly increase *C. acnes* accordingly. The overall ratio of *C. acne* to *S. epidermidis* increased significantly from 0.93 at baseline to 1.55 after a 5-week treatment. We also found that 22 of 24 individuals showed similar increasing trends of the bacteria ratio (*C. acne* to *S. epidermidis*). These phenomena proved that the overall increase of *C. acne* may be attributed to the reciprocal inhibition of these two bacteria. Also, the increased ratio of *C. acne* to *S. epidermidis* implies a healthier scalp condition achieved after the glycyrrhetinic acid intervention. This new formula seemed to be an effective way to adjust the balance of these two dominant bacteria on the scalp by the enhancement of *Cutibacterium* and the suppression of *Staphylococcus*.

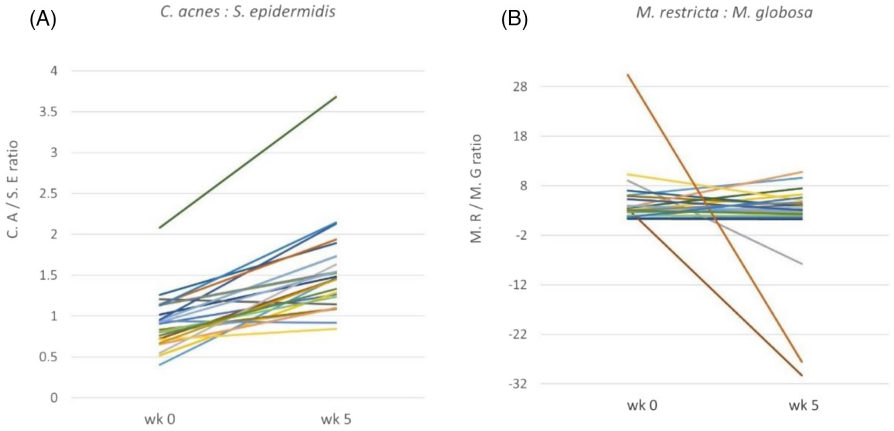

FIGURE 4 (A) The individual bacterial ratio changes (*C. acne* to *S. epidermidis*) of 24 subjects increased mostly from week 0 to week 5 after the use of glycyrrhetinic acid shampoo. (B) The individual fungal ratio changes (*M. restricta* to *M. globosa*) of 24 subjects showed variable trends from week 0 to week 5 after the intervention of the test product

Although glycyrrhetinic acid alone has been found to have a weaker effect against fungi, our results showed a significant reduction ($p < 0.001$) in the number of *M. restricta* and *M. globosa* after 5 weeks of glycyrrhetinic acid intervention. According to Saxena et al., a lower *M. restricta* to *M. globosa* ratio was thought to be associated with a healthier scalp.[4] The overall ratio of *M. restricta* to *M. globosa* decreased from 5.02 at baseline to 1.00 at week 5 in our study, but the result was not statistically significant. The insignificant change may be due to the variable trends among the 24 individual fungi ratios (*M. restricta* to *M. globosa*) and the small sample size of this research. The clinical significance of this ratio and its application in scalp SD severity assessment may need further research to validate this concept. Our result also indicated that bacteria imbalance might have a more substantial impact on the severity of dandruff than fungi. A larger controlled study will be required to give better statistical data and reveal the complex interactions among the bacteria, fungi, sebum, and hydration of the scalp SD.

In conclusion, our research showed a significant treatment response for scalp SD by 6% glycyrrhetinic acid complex shampoo. The most important role of glycyrrhetinic acid is its anti-inflammatory effect and its substantial antimicrobial activity toward some strains of bacteria and fungi. The effectiveness of this new regimen was objectively demonstrated with DLQI and ASFS clinically as early as 2 weeks after treatment. The inhibition of *S. epidermidis* as well as *Malassezia* species alleviated the dysbiosis and restored microbiota. Since these beneficial effects might be attributed to the detergents or other ancillary ingredients in this product, further randomized double-blinded placebo-controlled studies are mandatory in the future.

4.1 | Limitation of Study

The present study did not represent the actual daily scenario because it was designed to use shampoo every day for 5 weeks. The duration of the study was short, and the sample size was small. This non-randomized, open-label research also lack a control group for comparison. The analyses of 4 microorganisms were only performed in 24 out of the 34 subjects due to a limited budget. The long-term results of the product could not be assessed. Finally, the beneficial effects might be attributed to the detergents or other ancillary ingredients in this product. Further randomized double-blinded placebo-controlled studies are mandatory in the future.

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CONFLICT OF INTEREST

This study was supported by MacroHi Co. Ltd., Taiwan. CS Wang and HC Wang received fees for their roles as investigators in this study. HH Chen received fees for manuscript preparation.

AUTHOR CONTRIBUTIONS

HC Wang and CS Wang designed the study and performed clinical assessments. SC Hsieh and YT Hung supervised the sequencing experiments and subsequent analyses of microorganisms. HC Wang, CS Wang, and HH Chen analyzed the data. HH Chen wrote the manuscript. HC Wang, CS Wang, and SC Hsieh reviewed the manuscript. The final manuscript was read and approved by all authors.

ETHICAL APPROVAL

All procedures were in accordance with legal requirements in the country and the ethical principles stated in the Declaration of Helsinki of 1975, as revised in 2000 and 2008. The study was approved by the En Chu Kong Hospital Ethics Committee (IRB No. ECKIRB1090405). All patients signed the informed consent forms before the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the authors. The data are not publicly available due to privacy or ethical restrictions.

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