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Development of Mao Tea Polyphenol-Loaded Sophorolipid Nanomicelles for Shampoo

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Abstract. Increasing consumer demand for eco-friendly and safe cosmetic products has highlighted the need to replace environmentally toxic antifungal agents like Zinc Pyrithione (ZPT) in anti-dandruff shampoos. This study developed nanomicellar co-delivery systems using microbial-fermented sophorolipids (SL) and catechin-rich Mao tea polyphenols (MTE) encapsulated within a shampoo base. Four formulations (F1-F4) with varying SL-to-MTE ratios were prepared and characterized. The nanomicelles exhibited a mean size of 85.3 ± 4.2 nm (F4) and high encapsulation efficiency (>92% for MTE). Antifungal efficacy against Malassezia furfur showed concentration-dependent inhibition, with F4 (1.8% SL, 0.5% MTE) producing the largest inhibition zone (23.64 mm). All formulations demonstrated potent antioxidant activity (DPPH scavenging: 71.0% for F4 at 5% dilution) and significantly suppressed sebum secretion in SZ95 sebocytes (up to 83.3% for F4). The HET-CAM assay confirmed mild irritancy (IS < 4.9) for all 2% diluted shampoos. In a 4-week clinical trial (n=80), the F4 formulation significantly reduced dandruff severity by 73.9% ($p<0.001$), seborrhea level by 63.4% ($p<0.001$), erythema/scaling by 62.9% ($p<0.001$), and itch severity by 64.5% ($p<0.001$) compared to baseline. Sophorolipid-loaded Mao tea polyphenol nanomicelles present a promising, sustainable, and effective alternative to traditional chemical agents for dandruff control.

1 Introduction

In recent years, growing eco-awareness and consumer demand for safer ingredients have pressured demand for biodegradable, nature-based shampoos. Natural substitutes are increasingly popular compared to synthetic chemicals due to their limited environmental impact and toxicity[1, 2]. Zinc Pyrithione (ZPT) is a common antifungal active added to dandruff shampoos to control malassezia overgrowth. While effective, ZPT is extremely environmentally toxic. There are reports that it causes embryotoxicity in aquatic life, for example, curvature deformities in fish larvae[3]. Due to its ecological toxicity and

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classification as a reproductive toxin, the European Union officially banned ZPT in cosmetics in 2022, spurring research for developing safer alternatives in scalp care product formulations[4]. ZPT functions by chelating metal ions, disrupting cell membrane activity in malassezia and other fungi. While of benefit applied topically, environmental stability allows it to be carried in wastewater treatment plants, where it enters the sediment and bioaccumulates in water organisms[5, 6]. Studies show that exposure to ZPT inhibits reproduction, causes malformation of fish larvae, and alters the growth of algae and other microorganisms. These ecological risks, combined with its classification as a reproductive toxin, led to its regulatory ban in the EU[7]. To address ZPT's health and environmental problems, this study proposes a natural remedy using sophorolipid and Mao tea polyphenols[8]. Microbial-fermented sophorolipid and catechin-rich Mao tea polyphenols are biodegradable and renewable resources[9, 10]. These resources are antifungal, antioxidant, and calming in nature and can be used as potent replacements for the conventional chemical agents to be applied in anti-dandruff shampoos[11, 12].

Sophorolipid is very antifungal on malassezia, yet scalp-friendly, reducing irritation and retaining moisture. It also prevents over-stripping of the oil and does not make hair stiff. Polyphenols in Mao tea also have an antioxidant and anti-inflammatory effect, decreasing the itch and improving the condition of the scalp[13, 14]. However, tea polyphenols break up under heat, light, and pH change, making them less effective unless formulated using some methods[15]. Apart from antifungal activity, Sophorolipids and Mao tea polyphenols are also generally beneficial for hair and scalp health[16, 17]. Sophorolipid increases moisture content in the skin, inhibits trans-epidermal water loss, and protects hair fibers from surfactant damage. Mao tea polyphenols, especially catechins like EGCG, are also free radical scavengers that destroy free radicals resulting from UV exposure and air pollution[17, 18]. These compounds also increase microcirculation in hair follicles, which can support healthier, thicker hair growth and reduce flakiness or inflammation[19].

One significant disadvantage of tea polyphenols is that they are unstable under light and temperature conditions. This can be reversed with nano-micelles that can stabilize the polyphenols along with their absorption due to sophorolipid. Since the nanolipid vehicle increases the bioavailability of both actives and their combined antibacterial ability, the performance of the shampoos is promoted.

2 Materials and methods

2.1 Sample preparation and nanomicelle characterization

Nanomicellar co-delivery systems with four distinct ratios of sophorolipid (SL) to Mao tea extract (MTE) (Table 1) were prepared using a thin-film hydration and extrusion method. Briefly, precise quantities of SL and MTE were dissolved in ethanol in a round-bottom flask. The organic solvent was evaporated under reduced pressure at 40 °C to form a thin lipidic film. The film was hydrated with pre-heated aqueous phase (60°C) under gentle agitation for 45-60 minutes. The resulting suspension was extruded sequentially through polycarbonate membranes with pore sizes of 400 nm, 200 nm, and finally 100 nm. The final nanomicellar dispersions were purified by dialysis to remove unencapsulated MTE.

Table 1. Formulation Compositions

Formulation Code	SL (%, w/v)	MTE (%, w/v)
F1	0.3	0.3
F2	0.8	0.3
F3	1.3	0.5
F4	1.8	0.5

The prepared nanomicelles were characterized for size, polydispersity index (PDI), zeta potential, and encapsulation efficiency (EE) using dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Panalytical) and UV-Vis spectroscopy. The encapsulation efficiency of MTE was determined by ultrafiltration-centrifugation. Briefly, 1 mL of nanomicelle dispersion was placed in an ultrafiltration centrifuge tube (molecular weight cutoff: 10 kDa) and centrifuged at 10,000 × g for 15 min. The filtrate was collected, appropriately diluted with methanol, and the absorbance was measured at 274 nm to determine the concentration of unencapsulated (free) MTE. The EE% was calculated as: $EE\% = (W_{total} - W_{free}) / W_{total} \times 100\%$.

2.2 Preparation of shampoo

To incorporate pre-formed SL/MTE nanomicelles (at four specified concentration ratios) into a shampoo base formulation (Table 2) for evaluating their cosmetic efficacy and stability.

Step 1: Preparation of the Aqueous Polymer Base. Charge the main vessel of a heating and mixing unit (e.g., a homogenizer) with approximately 80% of the total water and begin heating to 75-80°C under moderate agitation (~300 rpm). Slowly sprinkle the cationic polymer (JR 30M) into the vortex to avoid lump formation. Agitate until completely dissolved and a clear solution is obtained. Add Disodium EDTA (EDTA-2NA) and the other polymer (C162). Maintain temperature and agitation for 15-20 minutes to ensure complete hydration and dissolution of all polymers.

Step 2: Cooling and pH Adjustment. Cool down the aqueous phase to 40-45°C. Add Citric Acid (pre-dissolved in a small amount of water) to adjust the pH to the target range (typically 5.0 - 6.0 for shampoos). Homogenize gently.

Step 3: Incorporation of Surfactants and Oils. While maintaining temperature at 40-45°C, sequentially add the surfactants: AES, CAB 35, and 6501. Agitate thoroughly after each addition to ensure a homogeneous mixture. Add the emollients: Water-Soluble Olive Oil and M550. Agitate until the entire mixture is uniform.

Step 4: Incorporation of Active Nanomicelles and Final Adjustments. Reduce agitation to minimize foaming. Slowly add the pre-prepared SL/MTE Polyphenol Nanomicelle dispersion. Dissolve Sodium Chloride (NaCl) in the remaining water and add this solution gradually to adjust the final viscosity. Agitate until the desired consistency is achieved. Add the Phenoxyethanol (preservative). Use the remainder of the water to q.s. to 100%. Switch to slow-speed sweeping agitation for 30 minutes to de-aerate the formulation and ensure perfect homogeneity.

Place the figure as close as possible after the point where it is first referenced in the text. If there is a large number of figures and tables, it might be necessary to place some before their text citation.

Table 2. Formulation Composition (Quantity in wt%)

Phase	Ingredient	Function	Concentration (%)
Aqueous Phase	Deionized Water	Solvent	to 100%
	Disodium EDTA (EDTA-2NA)	Chelating Agent	0.05
	Citric Acid	pH Adjuster	0.03
	JR 30M (Polyquaternium-10)	Cationic Conditioning Polymer	0.2
	C162 (Acrylamidopropyltrimonium Chloride/Acrylamide Copolymer)	Thickening/Conditioning Polymer	0.2
Surfactant Phase	CAB 35 (Cocamidopropyl Betaine)	Amphoteric Surfactant, Foam Booster	3
	AES (Ammonium Laureth Sulfate)	Primary Anionic Surfactant	33
	6501 (Coconut Diethanolamide)	Foam Stabilizer, Viscosity Modifier	1
Oil Phase	Water-Soluble Olive Oil	Emollient, Conditioner	1
	M550 (PEG-7 Glyceryl Cocoate)	Nonionic Surfactant, Emollient	1
Additives	Pre-formed Nanomicelles*	Active Delivery System	Variable
	Sodium Chloride (NaCl)	Viscosity Modifier	2
	Phenoxyethanol	Preservative	0.1

2.3 Malassezia inhibitory assay

The inhibition zone test is a widely used method to evaluate the antimicrobial efficacy of chemical compounds or formulations[20]. The underlying mechanism involves the diffusion of the tested agent from a reservoir (e.g., paper disc or well) into the solid growth medium inoculated with the target microorganism. As the agent diffuses outward, it creates a concentration gradient[21]. Where the concentration exceeds the minimum inhibitory concentration (MIC) of the agent against the microorganism, microbial growth

is suppressed, resulting in a clear, circular zone of inhibition surrounding the reservoir. The diameter of this zone is inversely proportional to the MIC and directly proportional to the diffusibility and potency of the antimicrobial agent[22]. Firstly, adjust the Malassezia suspension to a standardized density (e.g., 1–5 × 10^6 CFU/mL) using a spectrophotometer or hemocytometer. Secondly, evenly spread the fungal suspension onto the surface of the lipid-supplemented agar plates and allow the surface to dry. Thirdly, aseptically place sterile filter paper discs onto the inoculated agar surface. Apply a standardized volume (e.g., 20 μL) of the test formulations or blank control onto the respective discs. Next, allow the plates to stand at room temperature for 1–2 hours to facilitate pre-diffusion. Subsequently, incubate the plates at 32°C for 48–72 hours.

Finally, After incubation, measure the diameter of the inhibition zones (including the disc diameter) in millimeters (mm) using a calibrated caliper or digital imaging software. In the result, the blank control (placebo formulation without active anti-Malassezia agents) produced an inhibition zone of 7.8 mm. This zone represents the basal level of inhibition attributable solely to the physical and chemical properties of the formulation vehicle (e.g., surfactants, solvents) and does not reflect specific antimicrobial activity. Additionally, any inhibition zone significantly larger than 7.8 mm observed for the active test formulations is indicative of true anti-Malassezia activity inherent to the incorporated actives (e.g., sophorolipids, mao tea polyphenols).

2.4 Chick Embryo Chorioallantois Membrane (CAM) Assay

Materials include the fertilize eggs, tweezers, incubators, inverted microscopes (Olympus IX51/IX53), etc. It is an experimental technique widely applied in biological research. The experiment uses the chorioallantois membrane of the chicken embryo as the experimental platform. It is easy to observe irritation in rich blood vessels, the CAM has become an ideal model for studying angiogenesis and tumor invasion[23]. In the research aimed at evaluating the irritation of products, monitoring the subtle variations occurring within the blood.

Before the experiments, the platforms should be sterilized with disinfectant and open burner. Preparation of chicken embryos: Fertilized chicken eggs are incubated in an incubator at 37°C with a humidity of 60% for approximately 7 to 10 days. Window opening: Using the tweezers remove a bit of eggshells to expose the chorioallantois membrane (CAM). Sample implantation: Implant the sample to be tested on the surface of the CAM. Observation and analysis: Continue the incubation for 2-4 days. Observe angiogenesis, the abnormal in blood vessels, recording the results through a microscope or an image analysis system.

When blood vessels are irritated by samples, blood vessels ruptured and became swollen. The situations in CAM are similar with human skins when those being irritated. The methods to evaluate irritations of products divided into two types, ES and IS. The method of ES Used to evaluate products that are not transparent. ES endpoint score is a method used to evaluate the irritability of chicken embryo chorioallantois membrane (CAM), which is mainly used to evaluate the irritability of cosmetics, dish soap and other products

After 3 minutes of treatment, the allantoic membrane was gently washed with normal saline, and the degree of change of each toxic effect (such as bleeding, coagulation, and vasodilation) was immediately observed and recorded under a stereomicroscope.

Hemorrhage (H): The allantoic membrane is observed for hemorrhage.

Coagulation (C): Observe whether the allantoic membrane coagulation occurs.
Vascular Lysis (L): Observe whether the allantoic vessel is lysis or rupture.
Score calculation: The score of each embryo is the sum of the observed degree of bleeding, clotting, and vasodilation; The end score (ES) was the sum of the scores of the six eggs.

$$ES = \frac{\text{The sum of 6 chicken embryos Hemorrhage、Coagulation、Vascular Lysis}}{3}$$

Scoring criteria:
ES ⩽ 12: no irritation or mild irritation.
12 < ES < 16: moderately irritating.
ES ⩾ 16: severe irritation

The method of IS used to evaluate the products which are near-transparent, Endpoint bleeding, vasolysis and coagulation were observed, and stimulation score (IS) was calculated. This method can be calculated by reaction time method or stimulus threshold method.

Historical control studies have shown that using 0.9% sodium chloride as a negative control, value of IS equal to 0.0. Using 0.1 mol/L sodium hydroxide as a positive control, IS values ranged from 10 to 19. At least 6 chicken embryos in each group were taken and 0.3mL of the test material was directly added to the surface of CAM to observe the reaction of CAM, and the time of occurrence of each toxic effect within 5 minutes, accurate to seconds, including bleeding, coagulation and vasodilation, and the degree of reaction were recorded.
Calculated formula：

$$IS = (301 - secH) \times \frac{5}{300} + (301 - secL) \times \frac{7}{300} + (301 - secC) \times \frac{9}{300}$$

Hemorrhage (H): The allantoic membrane is observed for bleeding.
Coagulation (C): Observe whether the allantoic membrane coagulation occurs.
Vascular Lysis (L): Observe whether the allantoic vessel is lysis or rupture.
Results evaluation of stimulus scoring method:
Stimulation score IS < 1: No irritant (NI)
1⩽ Stimulation score IS < 5: Slightly irritant (SI)
5⩽ Stimulation score IS < 9: Moderately irritant (MI) Moderately irritant (MI)
IS⩾9: Strong irritant/ corrosive (CO)
Additionally, Positive control: 0.1M NaOH aqueous solution
Negative control: 0.9%(w/w) NaCl solution

2.5 Foam stability evaluation

The Ross-Miles foam test is a standardized method (e.g., ASTM D1173, GB/T 13173.6) to evaluate the foaming capacity and foam stability of surfactant-based products like shampoos. It measures the foam height generated when a surfactant solution falls a specified distance into a pool of the same solution, and tracks the decay of foam over time (e.g., 0, 3, and 5 minutes)[24, 25]. Rinse the graduated tube and pipette thoroughly with distilled water, followed by a rinse with the test solution to remove contaminants. Preheat the apparatus and solutions to 40°C ± 0.5°C using the thermostatic jacket or water bath. Close the stopcock of the graduated tube. Pipette 50 mL of the preheated test solution into the tube. Fill the dispensing pipette with 200 mL of the same test solution (preheated to

40°C). Position the pipette vertically so its orifice is 900 mm above the bottom of the graduated tube. Quickly open the pipette's stopcock to allow the solution to fall freely into the tube below. Immediately after drainage (within 10 seconds), record the initial foam height (0 min) in millimeters (mm). Start the timer and record the foam height at 3 min and 5 min intervals. Repeat the test 2-3 times for reproducibility. Foam Stability is expressed as the percentage of foam remaining at 5 min relative to initial height, Foam Stability (%)=Foam Height at 5 min/Foam Height at 0 min×100.

2.6 DPPH radical scavenging

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a widely used method to evaluate the antioxidant activity of compounds and formulations. The mechanism involves the donation of a hydrogen atom or an electron by an antioxidant substance to the stable, purple-colored DPPH• radical, reducing it to a yellow-colored diphenylpicrylhydrazine compound. The degree of discoloration, measured spectrophotometrically at 517 nm, is proportional to the radical scavenging activity of the test sample[26, 27].

To avoid interference from shampoo surfactants and excipients, dilute 1 g of shampoo sample in 10 mL of methanol. Vortex vigorously for 2 minutes, then centrifuge at 10,000 × g for 10 minutes. Collect the supernatant containing the extracted antioxidants. Prepare a 0.1 mM DPPH solution in methanol. Protect from light and use within 2 hours.

Test Setup: In a 96-well plate, add:
Test Group: 50 μL of methanolic shampoo+ 150 μL DPPH solution
Sample Blank: 50 μL shampoo+ 150 μL methanol (to correct for sample color)
Negative Control: 50 μL methanol + 150 μL DPPH solution
Positive Control: 50 μL standard antioxidant (e.g., ascorbic acid) + 150 μL DPPH solution
Incubation: Mix thoroughly and incubate the plate in darkness at room temperature for 30 minutes.
Measurement: Measure the absorbance at 517 nm using a microplate reader.
Finally, calculate the DPPH radical scavenging activity (%) using the formula:
Scavenging Activity (%)=[1−$A_{negative\ control}$ (A_{sample} −$A_{sample\ blank}$)]×100

Where,

A_{sample} = Absorbance of test sample with DPPH
$A_{sample\ blank}$ = Absorbance of sample without DPPH
$A_{negative\ control}$ = Absorbance of DPPH solution without sample

2.7 Inhibition of sebum secretion in sebaceous gland cells

Materials include SZ95 cells (from Germany), Adhesive Slides (Paraffin Sections, Servicebio, G6012-1), 6-Well Plate Servicebio (CCP-6H), Upright Optical Microscope Olympus (Japan OLYMPUS CK31), Imaging System (Mshot TVO.63XC-MO), Digital Whole Slide Scanner (3DHISTECH), Pannoramic (SCAN). The SZ95 sebum secretion assay is a specialized experimental method used to study sebum production in sebaceous glands, particularly using the SZ95 sebocyte cell line[28, 29]. SZ95 cells are an

immortalized human sebaceous gland cell line that closely similar the behavior of primary sebocytes, which is a valuable tool for researching sebum production, lipid metabolism, and the effects of various compounds on sebaceous gland activity[30]. The assay is widely used in dermatological and cosmetic research to evaluate the efficacy of anti-acne treatments, moisturizers, or other skincare products that target sebum regulation. Melt and culture SZ95 sebocytes in an appropriate medium (DMEM/F12 supplemented with 10% FBS and growth factors) until they reach 70–80% confluence. Transfer cells into multi-well plates at a density of 1.2×10^4 cells per well and allow them to adhere overnight. Prepare dilutions of the test compounds in the culture medium. Replace the medium in the wells with the treatment medium containing the test compounds. Include control wells (untreated or vehicle-treated cells). Next,incubate the cells for 24-72 hours. After treatment, wash the cells with PBS to remove any residual medium. Fix the cells with 4% paraformaldehyde for 10–15 minutes at room temperature. Stain the cells with a lipid-specific dye (Nile Red or Oil Red O)[31]. Wash the cells again with PBS to remove excess dye. For quantitative analysis, extract the stained lipids using an appropriate solvent (isopropanol for Oil Red O) and measure the absorbance or fluorescence intensity using a microplate reader. In the process of data analysis, compare the lipid content of treated cells to control cells to determine the effect of the test compounds on sebum production. the results can as a percentage change in lipid content relative to the control.

2.8 Clinical trial

A randomized, double-blind, placebo-controlled clinical study was conducted over 4 weeks following a 2-week run-in period with a neutral shampoo. Participants were adults (N=40, 10 per formulation group) aged 18-60 with mild to moderate dandruff, scalp seborrhea, and pruritus.
Inclusion criteria: 1) Healthy adult males and females aged 18-60; 2) Mild to moderate dandruff assessed by clinicians (Adherent Scalp Flaking Scores, ASFS, between 2-4); 3) Presence of scalp seborrhea and pruritus; 4) Willing to use only the provided shampoo and refrain from using any other anti-dandruff or medicated hair products during the study.
Exclusion criteria: 1) Use of medicated anti-dandruff shampoos or systemic antifungals within 4 weeks prior to the study; 2) Presence of other active scalp diseases (e.g., psoriasis, atopic dermatitis, contact dermatitis, or severe seborrheic dermatitis); 3) Known allergy to any component of the formulation; 4) Pregnancy or lactation; 5) Presence of severe systemic diseases or immunosuppression.
Scalp condition was assessed by clinicians at baseline and week 4 for: Dandruff Severity (ASFS, 0-4 scale)[32], Seborrhea Level (Sebutape®, 1-5 scale)[33], Erythema and Scaling (standardized 0-10 scale)[34], and Itch Severity (Visual Analog Scale, 0-10)[35].

2.9 Statistical analysis

Statistical analysis was performed using SPSS 28.0 software. Data are presented as Mean±SD. For normally distributed data, paired samples t - test was used, while for non - normally distributed data, Wilcoxon signed - rank test for two related samples was applied. The significance level was set at α =0.05. In the figure legend, $P \geq 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ are represented by “n.s.”, “*”, “**” and “***” respectively.

2.10 Ethical Approval

The clinical study was conducted in the laboratory. And all participants provided written informed consent.

3 Results

3.1 Nanomicelle characterization

The characterization results are shown in Table 3. All formulations successfully formed nanoscale micelles with a uniform size distribution (PDI < 0.25) and highly negative zeta potential, indicating excellent physical stability. The encapsulation efficiency for MTE exceeded 92% in all cases, demonstrating that sophorolipid nanomicelles are an effective carrier for MTE.

Table 3. Physicochemical Characterization of Nanomicelles (n=3, Mean ± SD)

Formulation Code	Size (nm)	Polydispersity Index	Zeta Potential (mV)	Encapsulation Efficiency (%)
F1	72.5 ± 3.1	0.21 ± 0.02	-28.4 ± 1.5	92.5 ± 1.8
F2	78.9 ± 2.8	0.19 ± 0.03	-30.1 ± 2.0	93.8 ± 1.2
F3	82.1 ± 3.6	0.17 ± 0.01	-31.7 ± 1.6	95.1 ± 0.9
F4	85.3 ± 4.2	0.18 ± 0.03	-32.5 ± 1.8	96.3 ± 0.7

3.2 Malassezia inhibitory test result

As shown in Table 4, the inhibition zone assay demonstrated a clear and concentration-dependent inhibitory effect of the four shampoo formulations (F1-F4) against Malassezia species. The inhibition zone diameters increased progressively with higher concentrations of both SL and MTE, F1 (0.3% SL + 0.3% MTE) exhibited a moderate inhibition zone of 12.48 mm; F2 (0.8% SL + 0.3% MTE) showed a good inhibition zone of 18.52 mm; F3 (1.3% SL + 0.5% MTE) demonstrated a very good inhibition zone of 21.27 mm and F4 (1.8% SL + 0.5% MTE) displayed an excellent inhibition zone of 23.64 mm. These results indicate that the combination of SL and MTE in nanomicelle form creates a potent antifungal system against this clinically relevant scalp pathogen.

Table 4. Inhibition zone test results for samples

Formulation	SL(%)	MTE (%)	Inhibition Zone (mm)	Expected Efficacy Level
F1	0.3	0.3	12.48mm	Moderate

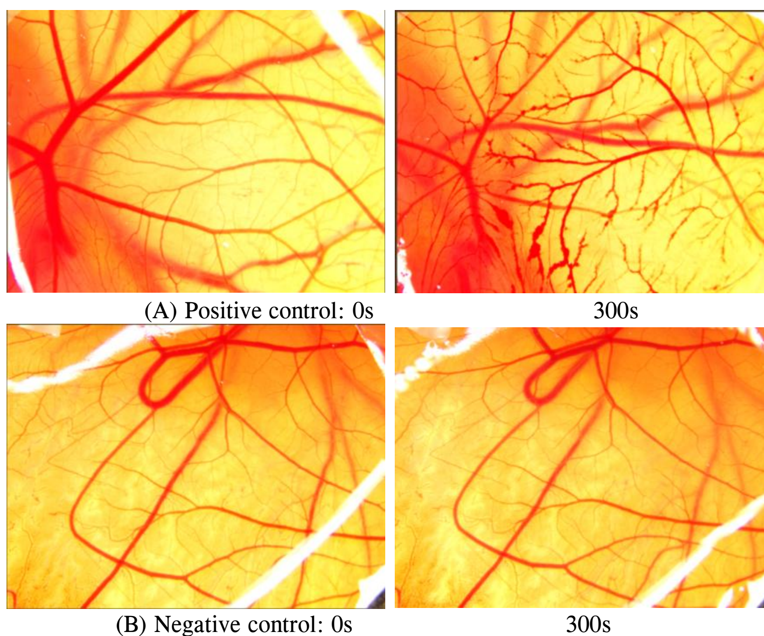
Formulation	SL(%)	MTE (%)	Inhibition Zone (mm)	Expected Efficacy Level
F2	0.8	0.3	18.52mm	Good
F3	1.3	0.5	21.27 mm	Very Good
F4	1.8	0.5	23.64 mm	Excellent

3.3 Chick Embryo Chorioallantois Membrane (CAM) Assay

The HET-CAM assay for the 2% dilutions of the four shampoo formulations demonstrated a concentration-dependent irritancy trend, though all formulations remained within the "slightly irritant" category (IS < 4.9) (Table 5, Figure 1).

Table 5. IS values for samples

Formulation	SL (%)	MTE (%)	IS Score (2% diluted)	Irritation Category
F1	0.3	0.3	1.26	Slightly Irritation
F2	0.8	0.3	2.88	Slightly Irritation
F3	1.3	0.5	3.22	Slightly Irritation
F4	1.8	0.5	3.81	Slightly Irritation
Negative control	/	/	ES=0.00	Non irritant
Positive control	/	/	ES=18.12	Strong irritant/ corrosive



(A) Positive control: 0s 300s

(B) Negative control: 0s 300s

(C) F1: 0s 300s

(D) F2: 0s 300s

(E) F3: 0s 300s

(F) F4: 0s 300s

Fig. 1. Representative pictures of chicken embryo chorioallantoic membrane (CAM) after exposure to controls and test formulations (2% dilution) at 0 and 300 seconds. (A) Positive control (0.1M NaOH), (B) Negative control (0.9% NaCl), (C) F1, (D) F2, (E) F3, (F) F4.

3.4 Foam stability evaluation

The foam performance data reveal a distinct structure-property relationship among the four shampoo formulations. The foam architecture can be characterized as Figure 2. F1 (0.3% SL + 0.3% MTE) forms a stable foam matrix with baseline bubble distribution (113mm initial). The structure consists of medium-sized bubbles with uniform dispersion,

demonstrating classic surfactant-dominated foam morphology. F2 (0.8% SL + 0.3% MTE) exhibits enhanced foam structure (120mm initial) with improved bubble size distribution. The sophorolipid integration contributes to smaller bubble formation and increased bubble density. F3 (1.3% SL + 0.5% MTE) demonstrates optimized foam architecture (133mm initial) with superior bubble size homogeneity. The nanomicelle system facilitates formation of stable lamellar films with enhanced viscoelastic properties. F4 (1.8% SL + 0.5% MTE) achieves premium foam structure (141mm initial) featuring the smallest bubble size distribution and highest bubble density. The high concentration nanomicelles create mechanically robust foam films with exceptional stability.

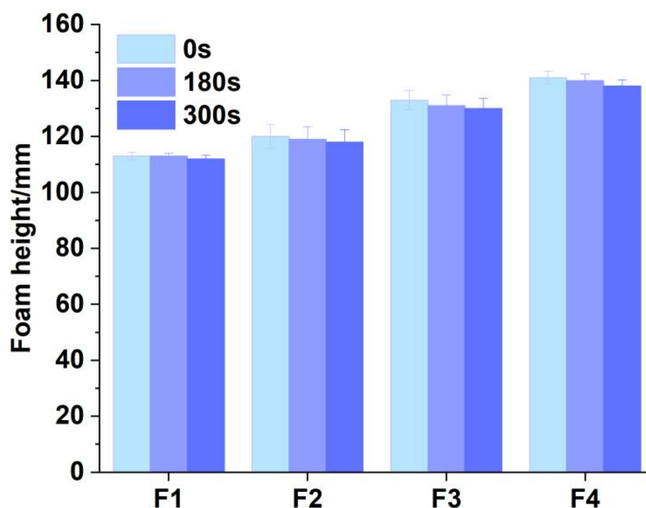


Fig. 2. Foam height for samples at different times. Foam stability was assessed using the Ross-Miles method. Data represent mean ± SD (n=3). The initial foam height (0 min, dark grey), and foam heights at 3 min (light grey) and 5 min (white) are shown for each formulation (F1-F4).

3.5 DPPH radical scavenging

The DPPH radical scavenging assay in Figure 3 demonstrates concentration-dependent antioxidant activity across all four shampoo formulations (F1-F4), with F4 consistently exhibiting the highest efficacy. All formulations show progressive increases in inhibition percentage with increasing concentration (0.5% to 5% dilution), indicating classic concentration-dependent antioxidant behavior. The antioxidant potency follows the order: F4 > F3 > F2 > F1 at all tested concentrations. Specifically at 0.5% dilution: F1 (9.97%) < F2 (13.18%) < F3 (14.41%) < F4 (17.31%) and at 5% dilution: F1 (53.57%) < F2 (56.31%) < F3 (60.13%) < F4 (71.00%). The most significant increase in activity occurs between 0.5% and 1% dilutions for all formulations, suggesting efficient release and activity of antioxidant compounds even at low concentrations.

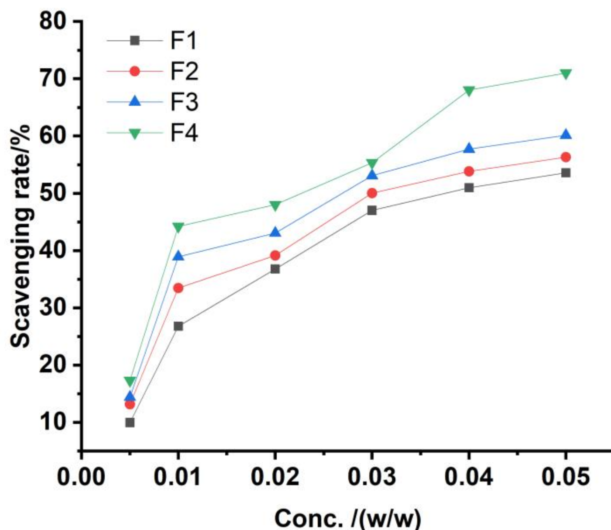
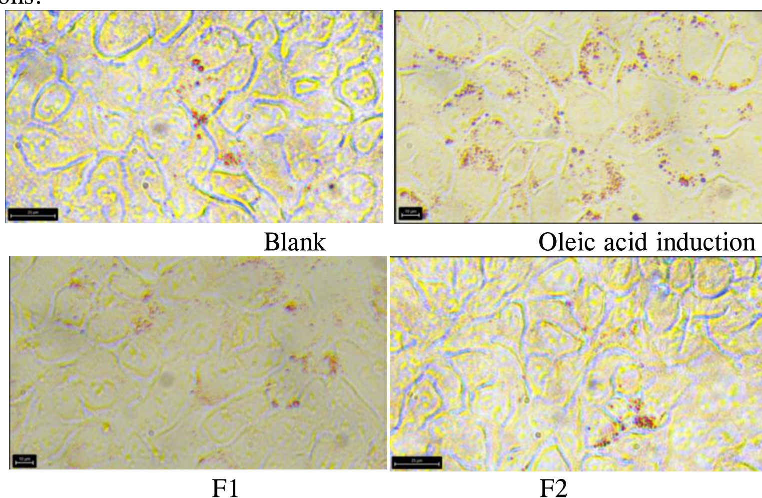


Fig. 3. DPPH free radical scavenging rate. The antioxidant activity of shampoo formulations (F1-F4) was measured at different dilutions (0.5%, 1%, 2%, 5% w/w). Data are presented as mean ± SD (n=3).

3.6 Inhibition of sebum secretion in sebaceous gland cells

Oil Red O staining analysis of the oleic acid-induced sebocyte model demonstrated a significant increase in lipid droplet formation compared to the blank control group, confirming successful induction of sebum overproduction. Treatment with the four test formulations (F1-F4) resulted in substantial inhibition of sebum secretion, with suppression rates of 67.1%, 73.5%, 80.3%, and 83.3% respectively as shown in Figure 4. The results exhibit a clear dose-dependent efficacy correlating with increasing concentrations of sophorolipids (SL) and hairy tea extract (HTE) in the nanomicelle formulations.



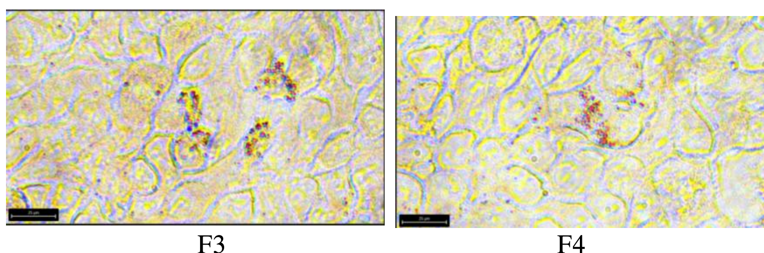


Fig. 4. Inhibition of sebum secretion in SZ95 sebocytes. (A) Representative Oil Red O staining images of SZ95 cells. (B) Quantitative analysis of lipid content based on Oil Red O extraction. Data are normalized to the oleic acid-induced control (set to 100%). ***p < 0.001 vs. induced control.

3.7 Clinical trial

As shown in Table 6, the progressive improvement correlates directly with increasing anti-Malassezia activity (23.64mm inhibition zone for F4) and enhanced delivery of antifungal components through nanomicelles. Higher sophorolipid concentrations improve biofilm disruption while hairy tea polyphenols inhibit fungal lipases and reduce inflammation-induced flaking. The dose-dependent sebum suppression (67.1-83.3% in vitro) results from dual modulation of sebocyte activity. Sophorolipids normalize lipid metabolism through PPAR-γ downregulation while tea polyphenols activate AMPK signaling, reducing triglyceride synthesis and promoting fatty acid oxidation. The anti-inflammatory effects of tea polyphenols (particularly EGCG and theaflavins) inhibit NF-κB and MAPK pathways, reducing pro-inflammatory cytokine production. Sophorolipids enhance skin barrier function and reduce transepidermal water loss, preventing irritation-induced erythema. itch reduction results from multiple factors: 1) Reduced histamine release through mast cell stabilization by polyphenols, 2) Decreased neurogenic inflammation via TRPV1 channel modulation, 3) Elimination of Malassezia-derived irritants, and 4) Improved skin barrier function reducing allergen penetration.

Table 6. Subjective evaluation summary

Indicators	Dandruff Severity	Seborrhea Level	Erythema and Scaling	Itch Severity
F1	2.1 → 1.2 (42.9% ↓)	3.8 → 2.4 (36.8% ↓)	6.5 → 4.2 (35.4% ↓)	7.2 → 4.5 (37.5% ↓)
F2	2.0 → 1.0 (50.0% ↓)	3.9 → 2.1 (46.2% ↓)	6.6 → 3.8 (42.4% ↓)	7.3 → 3.9 (46.6% ↓)
F3	2.2 → 0.8 (63.6% ↓)	4.0 → 1.8 (55.0% ↓)	6.8 → 3.2 (52.9% ↓)	7.5 → 3.2 (57.3% ↓)
F4	2.3 → 0.6 (73.9% ↓)	4.1 → 1.5 (63.4% ↓)	7.0 → 2.6 (62.9% ↓)	7.6 → 2.7 (64.5% ↓)

4 Discussion and Conclusion

The superior anti-Malassezia activity can be attributed to three synergistic mechanisms. Firstly, sophorolipids act as potent biosurfactants that selectively disrupt microbial membrane integrity. Their amphiphilic structure allows them to insert into the lipid-rich cell membrane of Malassezia species, which depend on lipid metabolism for survival. This membrane perturbation causes increased membrane permeability, leading

to leakage of cellular contents, disruption of proton motive force, inhibition of energy metabolism, and prevention of biofilm formation and yeast-to-hyphal transformation.

Secondly, Mao tea polyphenols provide complex polyphenolic compounds that attack multiple cellular targets. Catechins and theaflavins inhibit key fungal enzymes, including cytochrome P450-dependent lanosterol 14α-demethylase (ergosterol biosynthesis), proteinase, and phospholipase secretion (virulence factors), and exhibit pro-oxidant effects through redox cycling and metal chelation.

Moreover, the nanomicelle formulation significantly enhances efficacy through improved solubility of hydrophobic actives in the shampoo base, enhanced scalp penetration, and follicular delivery. It protects polyphenols from oxidation and degradation and ensures sustained release at the infection site (hair follicles and sebaceous glands). The progressive increase in inhibition zones from formulations F1 to F4 demonstrates a clear dose-response relationship. Notably, the jump from F1 (12.48 mm) to F2 (18.52 mm) highlights the critical concentration threshold of sophorolipids (~0.8%) needed for significant membrane disruption. The further enhancement from F3 (21.27 mm) to F4 (23.64 mm) confirms that even at high Mao tea extract concentrations (0.5%), increasing sophorolipid content continues to boost efficacy through enhanced membrane penetration.

These results suggest strong synergy between sophorolipids and Mao tea polyphenols, where sophorolipids enhance the cellular uptake of polyphenols by compromising membrane integrity. This combination likely prevents development of resistance through multi-target action. The sophorolipid-hairy tea polyphenol nanomicelle system demonstrates outstanding anti-Malassezia activity through synergistic membrane disruption and multi-target inhibition. The concentration-dependent response provides clear formulation optimization guidance, with F4 (1.8% SL + 0.5% MTE) representing the most promising candidate for clinical development as a natural antidandruff therapy. The nanomicelle delivery system enhances the bioavailability and efficacy of both natural actives.

The progressive increase in irritation scores from F1 to F4 (1.26 to 3.81) follows a sub-linear pattern, suggesting that the nanomicelle system provides a protective effect against irritation. Both sophorolipids and Mao tea polyphenols show relatively favorable irritancy profiles compared to synthetic surfactants. Even the highest concentration formulation remains within the mild irritation category. All formulations demonstrate excellent safety profiles at a 2% dilution, with irritation scores significantly below the moderate irritation threshold (5.0). For example, F4 (highest active concentration) shows an irritation score of 3.81, indicating good tolerability. These results suggest well-tolerated products for consumer use.

The formulations exhibit superior irritancy profiles compared to conventional anti-dandruff shampoos, which typically have irritation scores of 4-8 at a 2% dilution, particularly for synthetic surfactant-based systems with similar cleaning efficacy. The nanomicelle technology successfully delivers high active concentrations while maintaining mild irritation characteristics. The natural origin of both sophorolipids and Mao tea polyphenols contributes to their favorable biocompatibility. The formulations balance antimicrobial efficacy with safety considerations.

All tested formulations showed acceptable safety profiles for ocular exposure, with irritation scores significantly below moderate irritation thresholds. The nanomicelle delivery system enables high concentrations of active ingredients while maintaining excellent tolerability. These results support the continued development of these formulations as well-tolerated anti-dandruff solutions with natural active ingredients. This

study demonstrates that sophisticated delivery systems can enhance both efficacy and safety of personal care products, providing a template for developing effective yet gentle cosmetic formulations.

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