

Development and Phytochemical Characterization of an Ocimum–Hibiscus Herbal Microemulsion with Antibacterial Activity and In Silico ADME Prediction

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Research Article

Keywords: Ocimum tenuiflorum, Hibiscus rosa-sinensis, herbal microemulsion, chitosan, GC–MS, SwissADME, antimicrobial activity, scalp care

Posted Date: October 6th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9114249/v1>

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Abstract

Herbal bioactives offer promise for sustainable scalp-care products, but poor aqueous solubility and physicochemical instability can limit their formulation. This study developed a chitosan-stabilized combined herbal microemulsion (CHME) containing methanolic leaf extracts of *Ocimum tenuiflorum* and *Hibiscus rosa-sinensis*. Both extracts contained flavonoids, phenolics, tannins, alkaloids, and saponins. *O. tenuiflorum* showed higher total phenolic content (82 ± 1.03 mg GAE g⁻¹), flavonoid content (65 ± 2.14 mg QE g⁻¹), and antioxidant activity (DPPH IC₅₀, 49.60 ± 0.03 µg mL⁻¹) than *H. rosa-sinensis*. GC–MS revealed complementary phytochemical profiles containing phenylpropanoids, terpenoids, fatty acids, phytosterols, and triterpenoid-related compounds. SwissADME predictions were used as preliminary physicochemical screening descriptors only. Phase-diagram-guided CHME exhibited a hydrodynamic diameter of 155 ± 0.21 nm, PDI of 0.29 ± 0.14 , zeta potential of $+32 \pm 0.72$ mV, pH 5.1 ± 0.18 , optical transmittance of $95.33 \pm 0.79\%$, and viscosity of 23.9 mPa·s. FESEM showed predominantly spherical particles with an apparent diameter of 76.08 ± 11.15 nm. CHME remained stable under stress testing and retained > 90% L929 fibroblast viability. It showed concentration-dependent antimicrobial activity against *Staphylococcus aureus* and *Malassezia furfur*, supporting further topical scalp-care evaluation.

Introduction

Plant-derived bioactives have long been used in traditional haircare systems to maintain scalp health, control microbial imbalance, and promote hair growth. Among the widely documented botanicals, *Ocimum* and *Hibiscus* species are valued ingredients in Ayurvedic and traditional medicine [1–4]. *O. tenuiflorum* is recognized for antimicrobial, antioxidant, and anti-inflammatory properties attributed to constituents such as eugenol, β-caryophyllene, and essential fatty acids [2, 5–8], which are reported to support scalp microbiome balance and reduce oxidative follicular damage [2, 4]. *H. rosa-sinensis* has traditionally been used to prevent premature graying and to condition the scalp [9]; its leaves and flowers are rich in amino acids, vitamins, mucilage, flavonoids, and triterpenoids associated with hair and scalp care in traditional and experimental studies [2].

Despite these documented benefits, translating such bioactives into modern haircare formulations is limited by physicochemical challenges: many phytochemicals are hydrophobic and poorly water-soluble, are prone to degradation under light and heat, and show limited penetration through the stratum corneum into hair follicles [10–12], which together reduce topical bioavailability and efficacy. Microemulsion-based delivery systems offer a promising route to overcome these limitations [13]. Microemulsions are thermodynamically stable, optically transparent colloidal dispersions of oil, water, surfactant, and (often) co-surfactant, with droplet sizes typically in the 10–100 nm range [14, 15]; depending on formulation composition, hydration-layer thickness, polymeric stabilizers and measurement conditions, larger hydrodynamic diameters (up to ≈ 200 nm) may also be recorded by dynamic light scattering (DLS) [14–16], so classification should rest on overall physicochemical behavior and formulation architecture rather than droplet size alone. Unlike conventional emulsions, microemulsions form spontaneously and can improve solubilization of lipophilic compounds; the

associated high surfactant content also acts as a penetration enhancer by transiently disrupting the stratum corneum lipid bilayer, which may facilitate transdermal and transfollicular delivery of encapsulated bioactives, subject to confirmation by dedicated release, retention, and permeation studies [14, 17, 18].

Although herbal phytochemistry, topical delivery-system design, and computational pharmacokinetic prediction have each been studied independently, few reports integrate these elements into a unified strategy for botanical haircare formulations [19–21], and the relationships among phytochemical composition, in silico ADME behavior, colloidal formulation characteristics, and biological performance remain largely unexplored for the combined use of *O. tenuiflorum* and *H. rosa-sinensis* [2, 15, 18, 22]. The present study addresses this gap by combining phytochemical profiling, computational prediction, phase-diagram-guided formulation, and biological evaluation within a single workflow. Formulation composition was derived from experimentally observed phase behavior—rather than selected arbitrarily—using pseudo-ternary phase diagrams of the selected oil, surfactant/co-surfactant system, and aqueous phase.

Accordingly, this study comprises (i) GC–MS-based phytochemical profiling of methanolic leaf extracts of *O. tenuiflorum* and *H. rosa-sinensis*; (ii) in silico SwissADME evaluation of the major phytoconstituents to predict drug-likeness, pharmacokinetic behavior, and skin permeability; (iii) phase-diagram-guided formulation and physicochemical characterization of a chitosan-stabilized dual-herbal microemulsion for topical scalp delivery; and (iv) in vitro antibacterial and antifungal evaluation against *Staphylococcus aureus* and *Malassezia furfur*, clinically relevant scalp-associated organisms implicated in folliculitis, dandruff and secondary infection [23–25], together with an assessment of in vitro cytocompatibility. This multidisciplinary approach provides a rational framework for developing multifunctional, nano-enabled botanical haircare formulations with enhanced scalp delivery and potential hair-growth-supporting activity.

Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *O. tenuiflorum* (syn. *O. sanctum*) and *H. rosa-sinensis* were collected from Avadi, Chennai, Tamil Nadu, India, and taxonomically authenticated by a botanist at the Siddha Central Research Institute (SCRI), Ministry of AYUSH, Arumbakkam, Chennai (Figure S1). Leaf specimens were washed, shade-dried, and coarsely powdered prior to extraction.

Preparation of Methanolic Leaf Extracts

Coarsely powdered leaf material (10 g) was subjected to hot Soxhlet extraction with 100% methanol for five continuous cycles until the siphon ran clear. Filtrates were clarified through Whatman No. 1 filter paper and concentrated under reduced pressure at 50°C. Extraction yield was determined gravimetrically

following a standard procedure [26]. Dried extracts were stored in airtight amber glass vials at ambient temperature and redissolved in methanol prior to chemical and biological analysis.

Qualitative and Quantitative Phytochemical Analysis

Preliminary qualitative screening was performed using standard protocols [22] to detect major bioactive classes: flavonoids (ferric chloride test), phenolics (ferric chloride test), tannins (Braymer's test), alkaloids (Wagner's test), proteins and amino acids (xanthoproteic test), saponins (foam test), reducing sugars (Benedict's test), and carbohydrates (Barfoed's test), based on characteristic colour change or precipitate formation. Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (gallic acid standard, 760 nm) and expressed as mg gallic acid equivalents per gram of extract (mg GAE g⁻¹). Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method (quercetin standard, 760 nm) and expressed as mg quercetin equivalents per gram of extract (mg QE g⁻¹), following the reported methods [26, 27].

DPPH Radical-Scavenging Assay

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using a standard method [28]. Freshly prepared 0.1% DPPH in methanol was mixed with test samples (1:3 ratio) and incubated in the dark for 30 min at room temperature; absorbance was read at 517 nm. Methanol served as blank and L-ascorbic acid as positive control. Radical-scavenging activity (%) was calculated and IC₅₀ values were derived from inhibition–concentration plots.

FTIR Spectroscopic Analysis

FTIR spectroscopy was used to identify functional groups in the methanolic leaf extracts using a Cary 630 FTIR spectrometer (4000–400 cm⁻¹). Absorption bands were interpreted by comparison with reference spectra.

GC–MS Phytochemical Profiling

GC–MS analysis was performed following a previously reported method with minor modifications [8]. GC–MS analysis was performed following a reported method with minor modifications¹ at the Sophisticated Analytical Instrument Facility (SAIF), IIT Madras, India, using an Agilent 8890 GC–MS system. Samples (1.0 µL) were injected in split mode and acquired in full-scan mode under electron-ionization (EI) conditions. Separation was achieved on a capillary GC column under a programmed temperature regime with helium as carrier gas at constant flow. Mass spectra were processed using Agilent MassHunter software, and compounds were tentatively identified against the NIST 17 and

Mainlib libraries based on retention time, spectral match, and reverse-match scores, with a minimum match threshold of 500.

SwissADME Analysis (In Silico Prediction)

In silico prediction approach was used evaluate pharmacokinetic predictions [29]. Six representative bioactives were selected from the GC–MS profiles for SwissADME evaluation: eugenol, β -caryophyllene, α -linolenic acid, and β -sitosterol (*O. tenuiflorum*), and lupeol acetate, loliolide, and β -sitosterol (*H. rosa-sinensis*) (Table 4). These molecules were assessed for physicochemical properties, drug-likeness and predicted pharmacokinetic behaviour, with emphasis on parameters relevant to topical and follicular delivery (Table 5, Figure S2).

Preparation and Optimization of Chitosan-Stabilized Microemulsions

A chitosan stock solution (1.5% w/v; low-molecular-weight chitosan, 50–150 kDa) was prepared in 1% (v/v) aqueous acetic acid and adjusted to volume with distilled water. Microemulsion phase space was mapped by water titration across coconut oil (2–10%), Tween 20/glycerol surfactant/co-surfactant mixtures (*Smix*, 1:1 and 2:1 ratios, 10–30%), and distilled water (Tables S1 and S2). The single-phase region obtained at the lowest surfactant level (10% oil, 10% *Smix* [1:1], 80% water; formulation F11) was selected as the phase-diagram-derived reference composition.

For the combined herbal microemulsion (CHME), the oil phase (coconut oil, Tween 20) was added dropwise to the aqueous phase (glycerol, distilled water) under magnetic stirring (1000 rpm), followed by addition of chitosan stock solution and methanolic leaf extracts of *O. tenuiflorum* and *H. rosa-sinensis*. Formulations were bath-sonicated for 20 min and probe-sonicated in an ice bath for 10 min (40% amplitude, 20 kHz, 1-min pulses every 5 min). The final CHME composition (0.3% w/w chitosan, 2% coconut oil, 2% Tween 20, 2% glycerol, 1% w/w each botanical extract) was obtained by proportionally reducing F11 to one-fifth strength and supplementing with chitosan and the two extracts, with purified water added to 100%. Four control formulations; blank microemulsion (BME), chitosan-only microemulsion (CME), *O. tenuiflorum* microemulsion (OTME), and *H. rosa-sinensis* microemulsion (HSME) were prepared under identical conditions.

Physicochemical and Morphological Characterization

Hydrodynamic droplet size, polydispersity index (PDI), and zeta potential were measured by dynamic light scattering (DLS; Malvern Zetasizer Nano ZS) at 25°C after appropriate aqueous dilution. Formulation pH, refractive index, percentage optical transmittance, electrical conductivity and dynamic viscosity were recorded. Morphology and elemental composition were examined by optical microscopy and field-emission scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (FESEM–EDS). Detailed procedures for physicochemical characterization and stability evaluation;

centrifugation, heating–cooling cycling, freeze–thaw cycling, progressive aqueous dilution and pH-dependent zeta-potential profiling are provided in the Supporting Information (Section S1, Figure S3).

Qualitative/Semiquantitative Antibacterial Assessment

Preliminary antibacterial activity of CHME against *S. aureus* was evaluated by well-diffusion and bacterial dip-slide assays (Figure S4). For well-diffusion, CHME and BME were loaded into wells of *S. aureus*-inoculated agar plates (2×10^8 CFU mL⁻¹), incubated, and inhibition zones were assessed visually. For semiquantitative assessment, *S. aureus* cultures were treated with 30, 50, and 70 µL CHME (normalized to 200 µL with sterile water), with an untreated culture as control, and bacterial growth was evaluated using a BACTASLYDE dip-slide system matched against calibrated density indices.

TTC-Based Quantitative Antimicrobial Activity

Antimicrobial activity against *Malassezia furfur* and *Staphylococcus aureus* was quantified using a reported 2,3,5-triphenyltetrazolium chloride (TTC) reduction assay with minor modification [25]. Sterile Sabouraud dextrose broth (for *M. furfur*) or nutrient broth (for *S. aureus*, 1 mL) was dispensed into sterile tubes. CHME and control formulations were added at 20, 40, 60, 80 and 100 µL, followed by 100 µL of standardized inoculum (0.5 McFarland; $\approx 1 \times 10^8$ CFU mL⁻¹ for *S. aureus* and 5×10^5 CFU mL⁻¹ for *M. furfur*) with the working volume adjusted to 1.2 mL. Tubes were incubated at $28 \pm 2^\circ\text{C}$ (*S. aureus*) or $35 \pm 2^\circ\text{C}$ (*M. furfur*) for 12 h, followed by addition of 100 µL of freshly prepared 1% (w/v) TTC and further incubation for 4 h in the dark. Metabolically active cells reduce TTC to red formazan, providing a measure of cellular metabolic activity. Samples were centrifuged (6000 rpm, 30 min), the supernatant discarded and the formazan-containing pellet dissolved in 1 mL acetone; absorbance was measured at 480 nm. All measurements were performed in triplicate (mean $\pm$ SD).

Cytocompatibility Assessment

L929 mouse fibroblasts (National Centre for Cell Science, Pune, India) were used to evaluate cytocompatibility of the optimized CHME by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were maintained in EMEM with 10% FBS (37°C , 5% CO₂), seeded in 96-well plates (1×10^4 cells well⁻¹) and allowed to adhere for 24 h. CHME was serially diluted to five concentrations (12.5–200 µg mL⁻¹) and applied in triplicate, with untreated cells as negative control. After 48 h, MTT solution (15 µL, 5 mg mL⁻¹ in PBS) was added and incubated for 4 h; formazan crystals were dissolved in 100 µL DMSO and absorbance measured at 570 nm. Cell viability was expressed as a percentage of the untreated control.

Results and Discussion

Qualitative and Quantitative Phytochemical Screening

Qualitative screening confirmed flavonoids, phenolics, tannins, alkaloids, and saponins in both extracts (Table 1). Carbohydrates were detected only in *O. tenuiflorum*, while proteins and amino acids were not

detected in either extract. *O. tenuiflorum* contained higher total phenolic content ($82 \pm 1.03 \text{ mg GAE g}^{-1}$) and total flavonoid content ($65 \pm 2.14 \text{ mg QE g}^{-1}$) than *H. rosa-sinensis* ($76 \pm 0.38 \text{ mg GAE g}^{-1}$ and $52 \pm 1.31 \text{ mg QE g}^{-1}$, respectively). Consistent with this, *O. tenuiflorum* showed stronger DPPH radical-scavenging activity, with a significantly lower IC_{50} ($49.60 \pm 0.03 \text{ }\mu\text{g mL}^{-1}$) than *H. rosa-sinensis* ($85.91 \pm 4.10 \text{ }\mu\text{g mL}^{-1}$) (Table 1).

FTIR spectra of the two extracts (Fig. 1) showed broad O–H stretching bands at $\approx 3360\text{--}3380 \text{ cm}^{-1}$ (phenolic/flavonoid O–H), aliphatic C–H stretching ($\approx 2920 \text{ cm}^{-1}$), aromatic C = C stretching ($1617\text{--}1632 \text{ cm}^{-1}$), and C–O stretching ($1036\text{--}1043 \text{ cm}^{-1}$), consistent with previously reported spectral profiles [30, 31].

The complementary phytochemical profiles of the two extracts may be advantageous for combined use in topical haircare formulations. The higher phenolic and flavonoid content of *O. tenuiflorum* is consistent with its greater antioxidant capacity [4, 32, 33]; phenolic hydroxyl groups can contribute to radical stabilization through electron donation, while saponins and other constituents of *H. rosa-sinensis* may contribute surface-active and conditioning properties [2]. Combining the two extracts may contribute to balanced antioxidant and functional attributes relevant to multifunctional haircare formulations.

Table 1
Phytochemical composition and antioxidant activity of the botanical extracts.

Phytochemical test	<i>O. tenuiflorum</i>	<i>H. rosa-sinensis</i>
Flavonoids	Present (+)	Present (+)
Phenolics	Present (+)	Present (+)
Tannins	Present (+)	Present (+)
Alkaloids	Present (+)	Present (+)
Saponins	Present (+)	Present (+)
Carbohydrates	Present (+)	Absent (–)
Proteins and amino acids	Absent (–)	Absent (–)
Total phenolic content (mg GAE g ^{–1})	82 ± 1.03	76 ± 0.38
Total flavonoid content (mg QE g ^{–1})	65 ± 2.14	52 ± 1.31
DPPH IC ₅₀ (µg mL ^{–1})	49.60 ± 0.03	85.91 ± 4.10
Extraction yield (%)	19	22

Phytochemical Profiling by GC–MS

GC–MS chromatograms of the methanolic extracts of *O. tenuiflorum* (Fig. 2) and *H. rosa-sinensis* (Fig. 3) revealed chemically diverse profiles. The extract of *O. tenuiflorum* comprised phenylpropanoid derivatives, sesquiterpenes, fatty acids, oxygenated terpenoids and phytosterols (Table 2), with eugenol as the most abundant constituent (11.14%), followed by hexadecanoic (palmitic) acid (10.01%), α -linolenic acid (9.36%), and β -sitosterol (3.33%). Other notable constituents included caryophyllene (2.49%), caryophyllene oxide (2.78%), phytol acetate (1.65%) and longifolinaldehyde (1.30%), together with several minor sesquiterpenoids (β -acorenol, β -guaiene, γ -himachalene, and oxygenated caryophyllene derivatives), indicating a complex terpenoid fraction.

The GC–MS profile of *H. rosa-sinensis* (Table 3) was dominated by fatty acids, fatty-acid derivatives, terpenoids and sterol-related compounds: methyl α -linolenate (11.42%), palmitic acid TMS derivative (8.50%), betulinaldehyde (8.77%), glyceryl α -linolenate (9.04%), β -sitosterol (3.69%), and lupeol acetate (the acetate derivative of lup-20(29)-en-3-ol, 4.69%), together with methyl hexadecanoate (5.75%), methyl linoleate (6.87%), and α -linolenic acid TMS derivative (5.17%). The co-occurrence of long-chain fatty-acid derivatives with triterpenoid and phytosterol constituents indicates a chemically distinct profile from that of *O. tenuiflorum*.

The occurrence of eugenol, caryophyllene, oxygenated sesquiterpenes, fatty acids and phytosterols in *O. tenuiflorum*, together with the fatty acid-, sterol- and triterpenoid-rich composition of *H. rosa-sinensis*, provides a chemical rationale for evaluating both extracts as multifunctional botanical ingredients for scalp and hair care. Eugenol and caryophyllene have been associated with antimicrobial and anti-inflammatory activity relevant to scalp microbial and inflammatory homeostasis [5, 8, 34, 35]; fatty acids and phytosterols may contribute to skin-barrier support and conditioning [28, 36]; and triterpenoids such as betulinaldehyde and lupeol acetate are consistent with the triterpenoid-rich profile reported for *H. rosa-sinensis* and warrant further evaluation in follicle-relevant models [37–39]. Nevertheless, the biological relevance of individual constituents cannot be inferred from GC–MS relative abundance alone; these identifications provided the chemical basis for the subsequent in silico ADME evaluation and for rationalizing the selection of both extracts for formulation development, while experimental evaluation of the resulting formulation remained necessary to establish its physicochemical stability, compatibility and biological performance.

Table 2
GC–MS identified phytoconstituents in the methanolic leaf extract of *O. tenuiflorum*

Peak	Compound	RT (min)	Area (%)	MF	RMF
1	Glycerin	3.823	2.56	882	943
2	Eugenol	12.535	11.14	918	923
3	β -Acorenol	13.435	0.88	806	838
4	Methyleugenol	13.665	0.48	794	912
5	Caryophyllene	14.141	2.49	870	892
6	β -Guaiene	15.459	0.63	738	775
7	Octahydro-dimethyl-azulene isomer 1	15.743	0.94	849	880
8	Octahydro-dimethyl-azulene isomer 2	15.949	0.85	827	870
9	Caryophyllene oxide	18.004	2.78	890	918
10	Caryophylla-4(12),8(13)-dien-5 α -ol	19.186	0.57	795	892
11	β -Acorenol	19.595	1.57	804	840
12	β -Acorenol	19.771	0.60	737	794
13	γ -Himachalene	19.932	0.44	736	780
14	Isopropenyl-dimethyl-octahydronaphthalenol	20.750	0.70	764	797
15	Longifolenaldehyde	21.043	1.30	799	816
16	Phytyl acetate	23.702	1.65	797	846
17	Methyl hexadecanoate	26.189	1.31	800	849
18	Hexadecanoic acid (palmitic acid)	27.096	10.01	841	869
19	Palmitic acid, TMS derivative	29.305	6.71	887	895
20	Methyl α -linolenate	30.437	1.89	826	842
21	Phytol	30.694	0.71	768	841
22	α -Linolenic acid	31.221	4.08	842	872
23	Glycitin	32.629	5.35	813	929
24	Octadecanoic acid (stearic acid)	31.697	0.49	738	782
25	Linoleic acid, TMS derivative	32.804	0.65	730	873
26	α -Linolenic acid, TMS derivative	32.925	9.36	804	832
27	Stigmasterol	48.423	3.00	759	831

Peak	Compound	RT (min)	Area (%)	MF	RMF
28	β -Sitosterol	49.158	3.33	779	848

MF = match factor; RMF = reverse match factor.

Table 3
GC–MS identified phytoconstituents in the methanolic leaf extract of *H. rosa-sinensis*.

Peak	Compound	RT (min)	Area (%)	MW (g mol ⁻¹)	Formula
1	Glycerin	3.819	6.64	92.09	C ₃ H ₈ O ₃
2	β -Acorenol	14.138	1.74	222.37	C ₁₅ H ₂₆ O
3	6-Hydroxy-trimethyl-tetrahydrobenzofuranone	21.930	1.80	196.24	C ₁₁ H ₁₆ O ₃
4	Methyl hexadecanoate	26.192	5.75	270.50	C ₁₇ H ₃₄ O ₂
5	Ascorbic acid 2,6-dihexadecanoate	27.068	9.31	652.90	C ₃₈ H ₆₈ O ₈
6	Palmitic acid, TMS derivative	29.305	8.50	328.60	C ₁₉ H ₄₀ O ₂ Si
7	Methyl linoleate	30.295	6.87	294.50	C ₁₉ H ₃₄ O ₂
8	Methyl α -linolenate	30.436	11.42	292.50	C ₁₉ H ₃₂ O ₂
9	13-Heptadecyn-1-ol	31.069	3.35	252.40	C ₁₇ H ₃₂ O
10	Glyceryl α -linolenate	31.204	9.04	352.50	C ₂₁ H ₃₆ O ₄
11	Linoleic acid, TMS derivative	32.805	2.20	352.60	C ₂₁ H ₄₀ O ₂ Si
12	α -Linolenic acid, TMS derivative	32.936	5.17	350.60	C ₂₁ H ₃₈ O ₂ Si
13	β -Sitosterol	49.155	3.69	414.70	C ₂₉ H ₅₀ O
14	Lup-20(29)-en-3-ol, acetate (lupeol acetate)	50.829	4.69	468.75	C ₃₂ H ₅₂ O ₂
15	Betulinaldehyde	51.416	8.77	440.70	C ₃₀ H ₄₈ O

In Silico ADME Profiling of GC–MS-Identified Phytoconstituents

Based on relative GC–MS abundance, reported relevance to scalp and hair care, and representation of distinct chemical classes, six constituents were selected for SwissADME evaluation (Table 4): eugenol, β -caryophyllene, α -linolenic acid, and β -sitosterol from *O. tenuiflorum*, and lupeol acetate, loliolide and β -sitosterol from *H. rosa-sinensis*. Because β -sitosterol occurred in both extracts, its inclusion represents a shared phytosterol component rather than an additional independent compound. This selection was intended to provide a representative assessment of the principal chemical classes identified by GC–MS

[29], not to imply that these six compounds alone account for the biological activity of the crude extracts.

SwissADME predictions (Table 5) revealed complementary physicochemical and pharmacokinetic profiles among the selected constituents. Eugenol (MW 164.20 g mol⁻¹) showed a consensus LogP of 2.38, high predicted gastrointestinal (GI) absorption, and a predicted skin-permeation coefficient (log K^p) of - 6.27. β -Caryophyllene was more lipophilic (LogP 4.47; log K^p -5.98). α -Linolenic acid showed LogP 5.73 and log K^p -4.68, while the highly lipophilic β -sitosterol and lupeol acetate (LogP 8.62 and 8.98, respectively) showed log K^p values of - 6.71 and - 6.52. Loliolide was the least lipophilic (LogP 2.12) and had the highest topological polar surface area (TPSA, 43.37 Å²) with log K^p -6.05. None of the six compounds triggered a PAINS alert; the predicted bioavailability score was 0.55 for most compounds and 0.85 for α -linolenic acid.

Lipophilicity (consensus LogP)

Consensus LogP, calculated as the mean of five predictive models (XLogP3, WLogP, MLogP, iLogP, Silicos-IT), was moderate for eugenol and loliolide (LogP $\approx$ 2–2.5) which may favour epidermal diffusion, whereas β -sitosterol and lupeol acetate (LogP > 8) showed strong affinity for lipid matrices and limited aqueous solubility, supporting a microemulsion-based delivery strategy which could be effective in scalp and follicular targeting [17, 18, 40].

Topological polar surface area (TPSA)

All six compounds showed TPSA < 90 Å², theoretically compatible with membrane permeation [3]. Loliolide had the highest TPSA (43.37 Å²), consistent with its predicted high GI absorption, while β -caryophyllene (TPSA = 0) was purely lipophilic.

GI absorption and BBB permeation

Using the BOILED-Egg model, fatty acids, phytosterols and triterpenoids showed predicted GI absorption is an oral-exposure descriptor and should not be interpreted as a measure of systemic exposure following topical application. Whereas eugenol and loliolide showed high predicted GI absorption due to smaller size and moderate polarity. Only eugenol and β -caryophyllene were predicted to be blood–brain-barrier (BBB) permeant; the remaining four compounds were BBB-negative, consistent with a favorable safety profile for topical scalp use.

Skin permeability and PAINS

The predicted log K_p values suggested compound-dependent permeability, with α -linolenic acid showing the least negative predicted value (log K_p = - 4.68 cm s⁻¹), could be effective as a conditioning, follicle-nourishing lipid [29, 41]. The absence of PAINS alerts for all compounds is consistent with a low risk of nonspecific assay interference.

Collectively, these SwissADME outputs indicate that all six compounds possess measurable, compound-dependent potential for dermal partitioning, and that the more lipophilic constituents (β -caryophyllene, α -linolenic acid, β -sitosterol, lupeol acetate) would be expected to partition favorably into the lipid/surfactant domains of a microemulsion, while the less lipophilic constituents (eugenol, loliolide) reflect the chemically heterogeneous character of the extracts. These outputs should, however, be interpreted strictly as preliminary computational indicators of physicochemical behavior; they do not constitute direct evidence of follicular penetration, systemic safety, or therapeutic efficacy, which would require dedicated release, permeation and in vivo studies [12, 29, 41].

The individual biological relevance of the selected constituents further supported their consideration: eugenol has reported antimicrobial and anti-inflammatory activity [5]; β -caryophyllene has reported anti-inflammatory and wound-healing relevance [34]; α -linolenic acid is an essential fatty acid associated with barrier repair and anti-inflammatory function [36]; β -sitosterol has been investigated for anti-androgenic and 5 α -reductase-inhibitory effects relevant to hair loss [42] ; lupeol acetate has reported anti-inflammatory activity [38, 39]; and loliolide has been associated with antioxidant and cytoprotective effects [4]. Together with the GC–MS occurrence of the corresponding constituents, these reported activities provided a rational basis for advancing both extracts toward experimental formulation development.

Table 4
Selected key bioactive compounds and rationale for SwissADME selection.

Source	Compound	Class	GC–MS relevance	Rationale
<i>O. tenuiflorum</i>	Eugenol	Phenylpropanoid	Major constituent (11.14%)	Antimicrobial/anti-inflammatory; dandruff control; moderate lipophilicity
<i>O. tenuiflorum</i>	β -Caryophyllene	Sesquiterpene	Prominent sesquiterpene	CB2 agonist; anti-inflammatory, wound-healing
<i>O. tenuiflorum</i>	α -Linolenic acid	PUFA	High abundance (9.36%)	Barrier repair; anti-inflammatory; follicular nourishment
<i>O. tenuiflorum</i>	β -Sitosterol	Phytosterol	Significant sterol fraction	5 α -reductase inhibition; anti-androgenic relevance
<i>H. rosa-sinensis</i>	α -Linolenic acid	PUFA	Major constituent (11.42%)	Conditioning lipid; anti-inflammatory/antioxidant
<i>H. rosa-sinensis</i>	Lupeol acetate	Triterpenoid	High abundance (4.69%)	Anti-inflammatory; follicular cell protection
<i>H. rosa-sinensis</i>	Loliolide	Norisoprenoid	Characteristic metabolite	Antioxidant/cytoprotective
<i>H. rosa-sinensis</i>	β -Sitosterol	Phytosterol	Consistent sterol presence	Barrier modulation; synergistic with fatty acids

Table 5
SwissADME predicted physicochemical, pharmacokinetic and drug-likeness parameters of selected compounds.

Compound	MW (g mol ⁻¹)	TPSA (Å ²)	LogP	GI absorption	BBB	log Kp (cm s ⁻¹)	Bioavail. score	PAINS
Eugenol	164.20	29.46	2.38	High	Yes	-6.27	0.55	0
β -Caryophyllene	216.36	0.00	4.47	High	Yes	-5.98	0.55	0
α -Linolenic acid	264.40	37.30	5.73	Low	No	-4.68	0.85	0
β -Sitosterol	370.65	20.23	8.62	Low	No	-6.71	0.55	0
Lupeol acetate	316.48	26.30	8.98	Low	No	-6.52	0.55	0
Loliolide	182.22	43.37	2.12	High	No	-6.05	0.55	0

Structures and BOILED-Egg model outputs are provided in Figure S2.

Rationale for Extract Loading in the Hair-Care Microemulsion

Preliminary well-diffusion antimicrobial screening of the individual extracts (Figure S4) showed discernible zones of inhibition for both *O. tenuiflorum* and *H. rosa-sinensis* against the tested scalp-relevant microorganisms. Rather than maximizing extract concentration on the basis of chemical abundance alone, this screening result was used as a practical basis for selecting a conservative loading of 1% w/w for each extract in the combined formulation, providing a balance between measurable botanical activity and the physicochemical requirements of the microemulsion system. The combined 2% botanical loading was incorporated into the phase-diagram-derived microemulsion base; this reflects a formulation-oriented selection rather than a claim of an optimized therapeutic dose and avoids the loss of clarity, phase stability or viscosity control associated with excessive botanical loading.

Pseudo-Ternary Phase Behaviour and Formulation Development

Pseudo-ternary phase diagrams are widely used to identify the composition ranges that form stable, homogeneous microemulsion regions, thereby facilitating the systematic optimization of formulation components [43]. In the present study, pseudo-ternary phase diagrams (Fig. 4) were constructed for coconut oil/Tween 20–glycerol *Smix*/water systems at *Smix* (Tween 20: glycerol) ratios of 1:1 and 2:1, from water-titration data (Tables S1 and S2). Red markers denote compositions that produced clear-to-translucent, homogeneous single-phase systems, while black markers denote turbid or multiphase systems. The 2:1 system exhibited a broader clear/translucent region than the 1:1 system, consistent with the higher Tween 20 content enhancing interfacial coverage and lowering oil–water interfacial tension; however, the associated higher surfactant burden was considered less desirable for the intended topical application.

The 1:1 system produced a clear-to-translucent single-phase formulation at 10% coconut oil, 10% *Smix*, and 80% water (formulation F11, Table S1), which was selected as the reference composition. Proportional reduction of F11 to one-fifth strength yielded a base of 2% coconut oil, 2% *Smix* (1% Tween 20, 1% glycerol), and 16% water. This base was supplemented with 0.3% chitosan and 1% each of the *O. tenuiflorum* and *H. rosa-sinensis* extracts, with purified water added to 100%. This phase-behavior-screening, proportional-reduction, and sequential-supplementation strategy provided an experimentally grounded rationale for the CHME composition.

Physicochemical Characteristics of CHME

Physicochemical properties of the formulations are summarized in Table 6. CHME exhibited a hydrodynamic particle size of 155 ± 0.21 nm and the lowest PDI (0.29 ± 0.14) among the tested formulations, indicating a comparatively narrow size distribution. Incorporation of chitosan reversed the

surface charge from -22 ± 0.23 mV (BME) to $+32 \pm 0.72$ mV (CHME), consistent with the contribution of protonated chitosan to the formulation interface [44]. CHME showed a pH of 5.1 ± 0.18 , high optical transmittance ($95.33 \pm 0.79\%$) and a refractive index of 1.3458 ± 0.011 , supporting its optically clear and homogeneous appearance, with a dynamic viscosity of 23.9 mPa·s ($25 \pm 2^\circ\text{C}$) consistent with a readily spreadable topical formulation.

Table 6

Physicochemical characteristics of blank, chitosan-containing, single-extract and combined herbal microemulsion formulations.

Parameter	BME	CME	OTME	HSME	CHME
Particle size (nm)	151 ± 1.08	259 ± 0.63	200 ± 2.23	176 ± 0.84	155 ± 0.21
PDI	0.441 ± 0.70	0.496 ± 0.86	0.456 ± 0.94	0.947 ± 0.11	0.29 ± 0.14
Zeta potential (mV)	-22 ± 0.23	$+41 \pm 0.03$	$+40 \pm 0.51$	$+39 \pm 0.32$	$+32 \pm 0.72$
Conductivity (mS cm ⁻¹)	0.014 ± 0.28	0.028 ± 0.56	0.041 ± 0.73	0.043 ± 0.27	0.050 ± 0.71
pH	5.1 ± 0.04	5.2 ± 0.01	5.0 ± 0.01	4.8 ± 0.02	5.1 ± 0.18
Refractive index	1.342 ± 0.004	1.3495 ± 0.002	1.3423 ± 0.001	1.3524 ± 0.001	1.3458 ± 0.011
Transmittance (%)	96.33 ± 0.41	97.67 ± 0.22	93.33 ± 0.71	93.01 ± 0.11	95.33 ± 0.79

Physical Stability

CHME and the control formulations showed no visible phase separation, creaming, or sedimentation after centrifugation (5000 rpm, 30 min; Table S3), and retained a clear appearance through six heating–cooling cycles (4–45°C; Table S4) and six freeze–thaw cycles (–20 to 25°C; Table S5). No turbidity, creaming, precipitation, or phase separation was observed after 1:10–1:100 aqueous dilution and 24 h standing (Table S6). Over 60 days of storage (Table S7), CHME retained its nanoscale characteristics: particle size changed from 155 ± 0.21 to 159 ± 0.69 nm at $4 \pm 2^\circ\text{C}$ and to 180 ± 0.32 nm at $25 \pm 2^\circ\text{C}$, while PDI remained low (0.25–0.26) and the positive zeta potential was maintained or increased at both temperatures, indicating good 60-day physical stability with better particle size preservation under refrigerated conditions.

pH-Dependent Interfacial Charge

The zeta potential of CHME decreased progressively with increasing pH, from +41.79 mV at pH 4.5 to +15.25 mV at pH 6.6, followed by charge reversal to –16.78 mV at pH 7.6 and increasingly negative values up to pH 10.6, with an isoelectric point at pH ≈ 7.13 (Figure S3). The formulation pH of ≈ 5.1 lies within the positively charged region, consistent with the measured zeta potential of +32 mV (Table 6) and supporting the contribution of chitosan to the interfacial characteristics of CHME.

Morphological and Elemental Characterization of CHME

FESEM analysis of the optimized CHME (Fig. 5) revealed predominantly quasi-spherical to spherical particles with an apparent mean diameter of 76.08 ± 11.15 nm. This FESEM-derived diameter was lower than the DLS hydrodynamic diameter of 155 ± 0.21 nm (Table 6), as expected because FESEM examines dried particles whereas DLS measures hydrated dispersed entities together with their associated interfacial/solvation layer [16]; the two techniques therefore provide complementary rather than directly equivalent size information. Limited localized aggregation was evident at higher magnification (Fig. 5d), but no extensive morphological agglomeration was observed. EDS analysis showed predominantly carbon (91.56 wt %) and oxygen (5.92 wt %), consistent with the organic composition of the formulation, with a minor silicon signal (2.51 wt %) attributable to the substrate/sample-support material rather than a major CHME constituent.

Comparative FTIR Analysis of the Extracts and Microemulsion Formulations

FTIR spectra of the individual extracts, the combined extract and the microemulsion formulations (Fig. 6) showed characteristic *O. tenuiflorum* (OTE) and *H. rosa-sinensis* (HSE) absorptions associated with hydroxyl, aliphatic C–H, carbonyl/unsaturated, and C–O/C–O–C functionalities. These features were retained, with minor shifts in position and relative intensity, in the combined extract (CPE) and in the BME, CME, OTME, HSME, and CHME formulations: broad O–H stretching at $\approx 3339\text{--}3429$ cm^{-1} , aliphatic C–H stretching at $\approx 2922\text{--}2937$ cm^{-1} , carbonyl/unsaturated bands at $1647\text{--}1744$ cm^{-1} , and C–O/C–O–C absorptions at $1000\text{--}1250$ cm^{-1} . No prominent new absorption band indicative of a major new covalent functional group was evident in CHME; the minor band shifts are consistent with hydrogen bonding or other noncovalent interactions among chitosan, phytoconstituents and formulation components [16, 30, 31]. These results indicate that the principal functional groups of the botanical extracts were retained after formulation, supporting chemical compatibility of the extracts with the formulation matrix without evidence of substantial chemical transformation detectable by FTIR.

Antibacterial Activity against *Staphylococcus aureus*

Antibacterial efficacy was evaluated against *S. aureus*, a common scalp-associated pathogen implicated in dandruff, folliculitis and secondary infection [23, 24]. A clear, concentration-dependent reduction in bacterial count was observed with increasing CHME volume (30, 50, and 70 μL) relative to the untreated control (Figure S4): 30 μL produced a noticeable decrease in colony-forming units, 50 μL (0.5 mg of each extract) produced a further reduction and 70 μL (0.7 mg of each extract) achieved near-complete suppression of bacterial growth. This dose-dependent response likely reflects the combined action of bioactive compounds from *O. tenuiflorum* (e.g., eugenol, β -caryophyllene) and *H. rosa-sinensis* (e.g., fatty acids, lupeol derivatives) and may be assisted by the nanoscale droplet size and positive surface charge of the formulation, which likely facilitate close contact with bacterial cell walls.

Quantitative Assessment of Antimicrobial Activity against *M. furfur* and *S. aureus*

The TTC reduction assay (Fig. 7) showed concentration-dependent antimicrobial responses for the extract-containing formulations against both organisms. BME showed negligible activity throughout, indicating minimal intrinsic contribution from the coconut oil/surfactant–cosurfactant vehicle, whereas CME showed measurable activity consistent with a contribution from chitosan.

Against *M. furfur*, CHME showed the highest activity across the tested range, increasing from $\approx 50\%$ at $20 \mu\text{L mL}^{-1}$ to $> 90\%$ at $100 \mu\text{L mL}^{-1}$, followed by OTME ($\approx 90\%$ at the highest concentration) and HSME ($\approx 75\%$), while CME reached $\approx 50\%$. Against *S. aureus*, CHME increased from $\approx 38\%$ at $20 \mu\text{L mL}^{-1}$ to $\approx 84\%$ at $100 \mu\text{L mL}^{-1}$, followed by OTME ($\approx 72\%$), CME ($\approx 68\%$), and HSME ($\approx 50\%$), with BME again showing minimal activity.

CHME showed the greatest concentration-dependent reduction in TTC metabolic activity against both *M. furfur* and *S. aureus* among the formulations tested. Its response exceeded that of the control formulations. Because BME exhibited negligible activity, the antimicrobial effect of CHME cannot be attributed to the coconut oil/Tween 20/glycerol microemulsion vehicle alone. The measurable activity of CME, OTME and HSME indicates that chitosan and each botanical extract independently contributed to the overall response observed for CHME. Therefore, the enhanced activity of CHME is consistent with the combined contribution of chitosan and the two botanical extracts within the microemulsion system and supports it as a promising multifunctional topical candidate for further evaluation.

In Vitro Cytocompatibility

Phase-contrast microscopy showed that CHME-treated L929 fibroblasts ($12.5\text{--}200 \mu\text{g mL}^{-1}$) retained a largely normal, well-spread, adherent morphology comparable to untreated controls (Fig. 8A), with only minor rounding and particulate deposits at the two highest concentrations tested (100 and $200 \mu\text{g mL}^{-1}$) and no significant cell detachment. Consistent with these observations, the MTT assay showed $> 90\%$ cell viability at all tested concentrations (Fig. 8B), indicating good in vitro cytocompatibility of the optimized CHME over the concentration range studied.

Conclusion

This study demonstrates that *O. tenuiflorum* and *H. rosa-sinensis* extracts possess complementary phytochemical profiles relevant to hair and scalp health, and that phase-diagram-guided, chitosan-stabilized microemulsion encapsulation yields a stable, nanosized, cytocompatible carrier (CHME) with strong antibacterial activity against *S. aureus*. SwissADME provided preliminary compound-level physicochemical and skin-permeability estimates relevant to topical formulation design, while comparative FTIR analysis and multi-stress stability testing (centrifugation, heating–cooling, freeze–thaw, dilution, and 60-day storage) confirmed formulation compatibility and robustness. CHME produced

concentration-dependent antimicrobial activity against both *S. aureus* and *M. furfur* with stronger activity than blank, chitosan-only, and individual-extract formulations under the tested conditions. It also maintained greater than 90% viability in L929 fibroblasts. SwissADME findings should be regarded only as computational indicators. Future work should include quantitative susceptibility testing, interaction analysis, release and skin-permeation studies, irritation testing, and in vivo validation.

Declarations

Supporting Information

The Supporting Information is available and includes plant authentication documentation (Figure S1); BOILED-Egg model and molecular property outputs for the selected SwissADME compounds (Figure S2); pH-dependent zeta potential and conductivity data (Figure S3); qualitative/semiquantitative antibacterial assay results (Figure S4); pseudo-ternary phase-diagram formulation tables (Tables S1, S2); detailed physicochemical and stability-testing procedures (Section S1); and centrifugation, heating–cooling, freeze–thaw, dilution, and 60-day storage stability data (Tables S3–S7).

Authorship contributions

B. Santhoshraman: writing – original draft, formal analysis. K. Jagajjanani Rao: Conceptualization, writing – review and editing, supervision, conceptualization. Tarangini Korumilli: writing – review and editing, supervision, conceptualization.

Compliance with Ethical Standards

Conflict of Interest: All the authors declare that they have no conflict of interest.

Funding

The authors declare that no specific funding was received for this work.

Acknowledgements

The authors thank Research Park, Vel Tech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology, Chennai, India for the research space provided.

Data Availability

The datasets generated during the current study are included in this published article and its Supplementary Information file. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

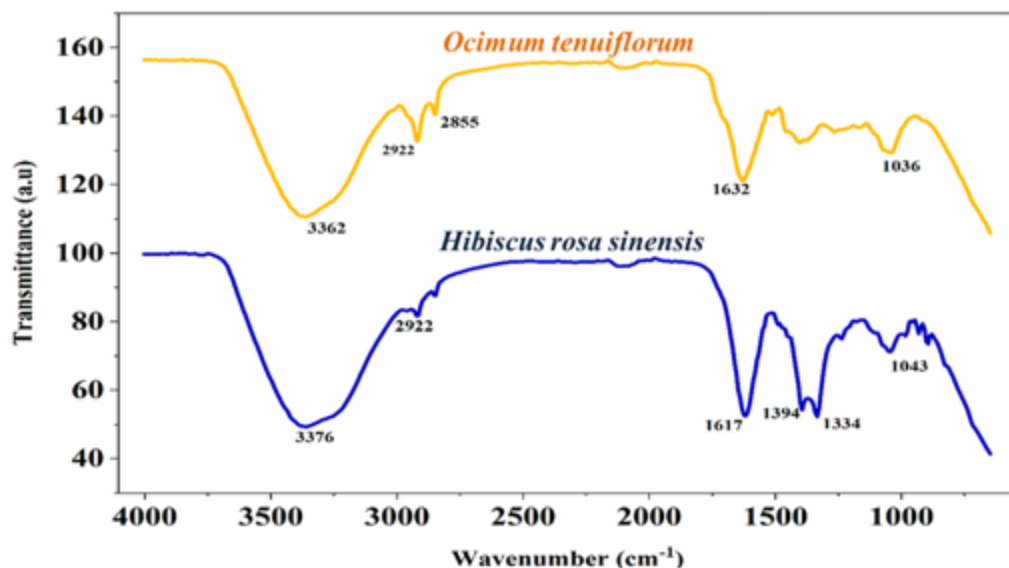


Figure 1

FTIR spectra of the methanolic leaf extracts of *O. tenuiflorum* and *H. rosa-sinensis*.

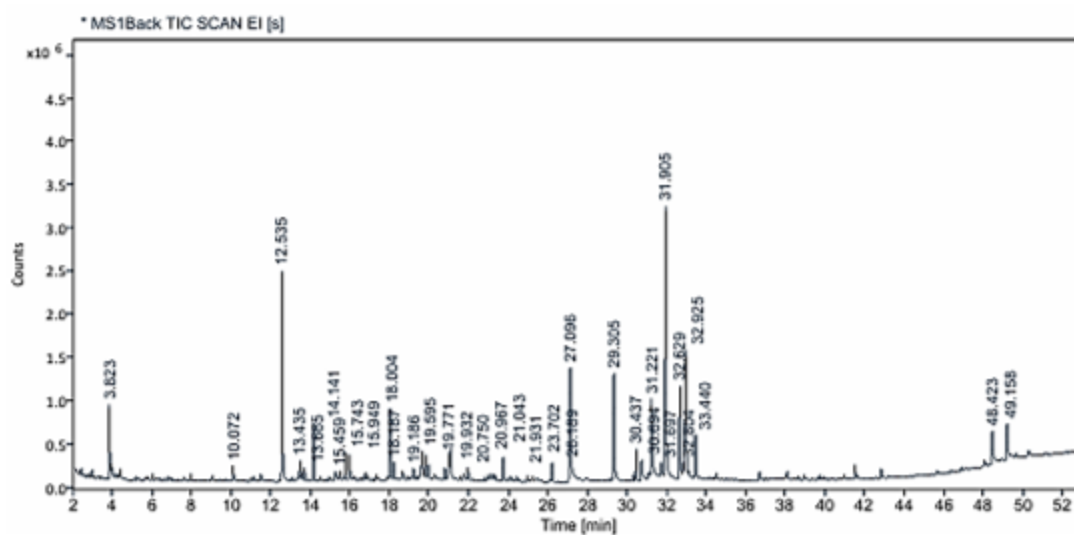


Figure 2

GC-MS chromatogram of the methanolic leaf extract of *O. tenuiflorum*.

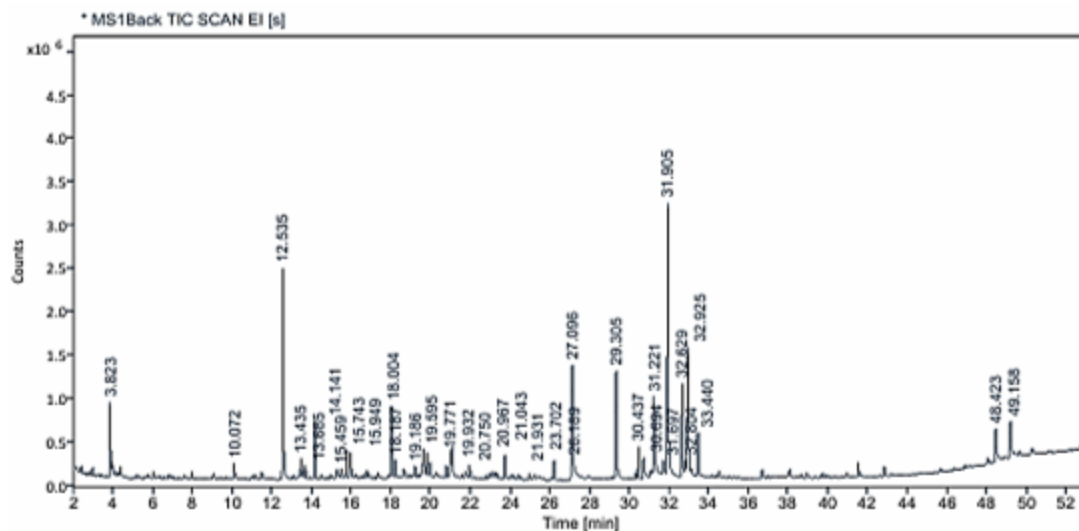


Figure 3

GC–MS chromatogram of the methanolic leaf extract of *H. rosa-sinensis*.

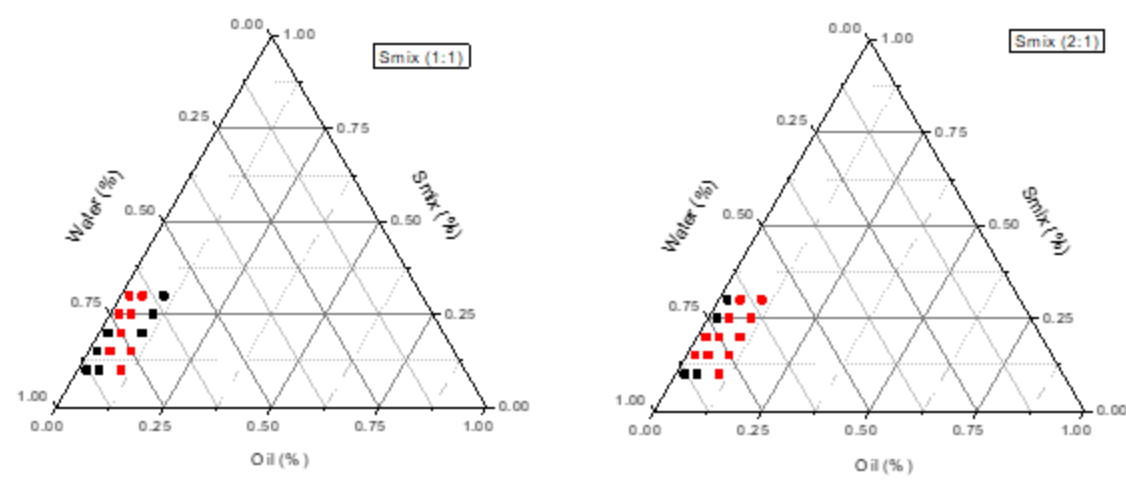


Figure 4

Pseudo-ternary phase diagrams of the coconut oil/Tween 20–glycerol Smix/water system at Smix ratios of (a) 1:1 and (b) 2:1. Red markers denote clear-to-translucent, homogeneous single-phase compositions identified during water-titration screening.

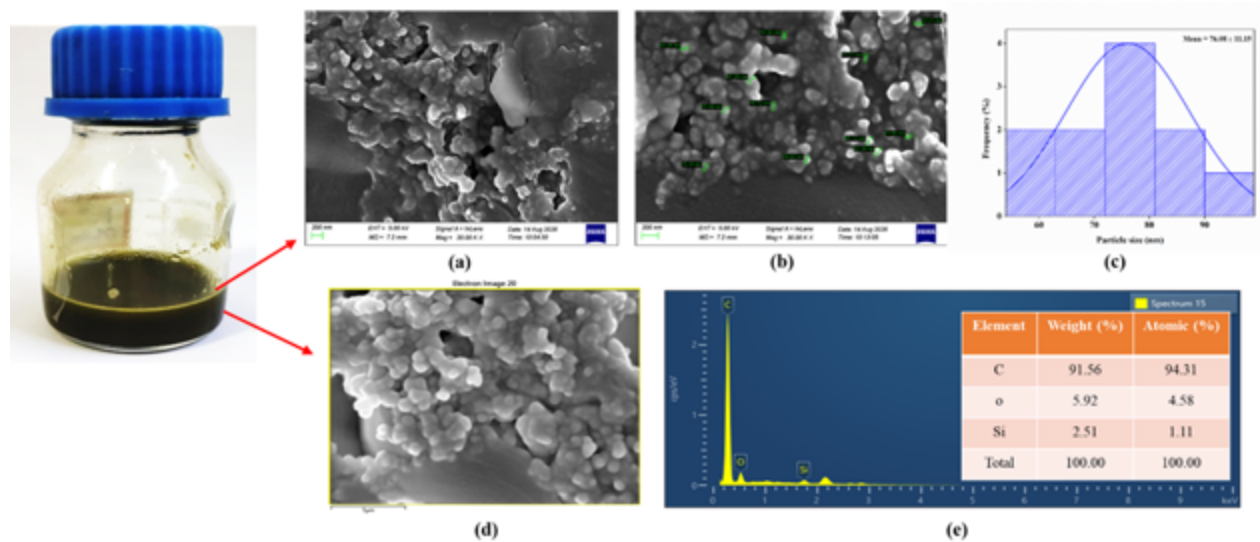


Figure 5

FESEM and EDS characterization of CHME: (a) representative FESEM micrograph; (b) particle-size measurement; (c) particle-size distribution; (d) higher-magnification FESEM image; (e) EDS spectrum with elemental composition. Mean apparent particle diameter: 76.08 ± 11.15 nm.

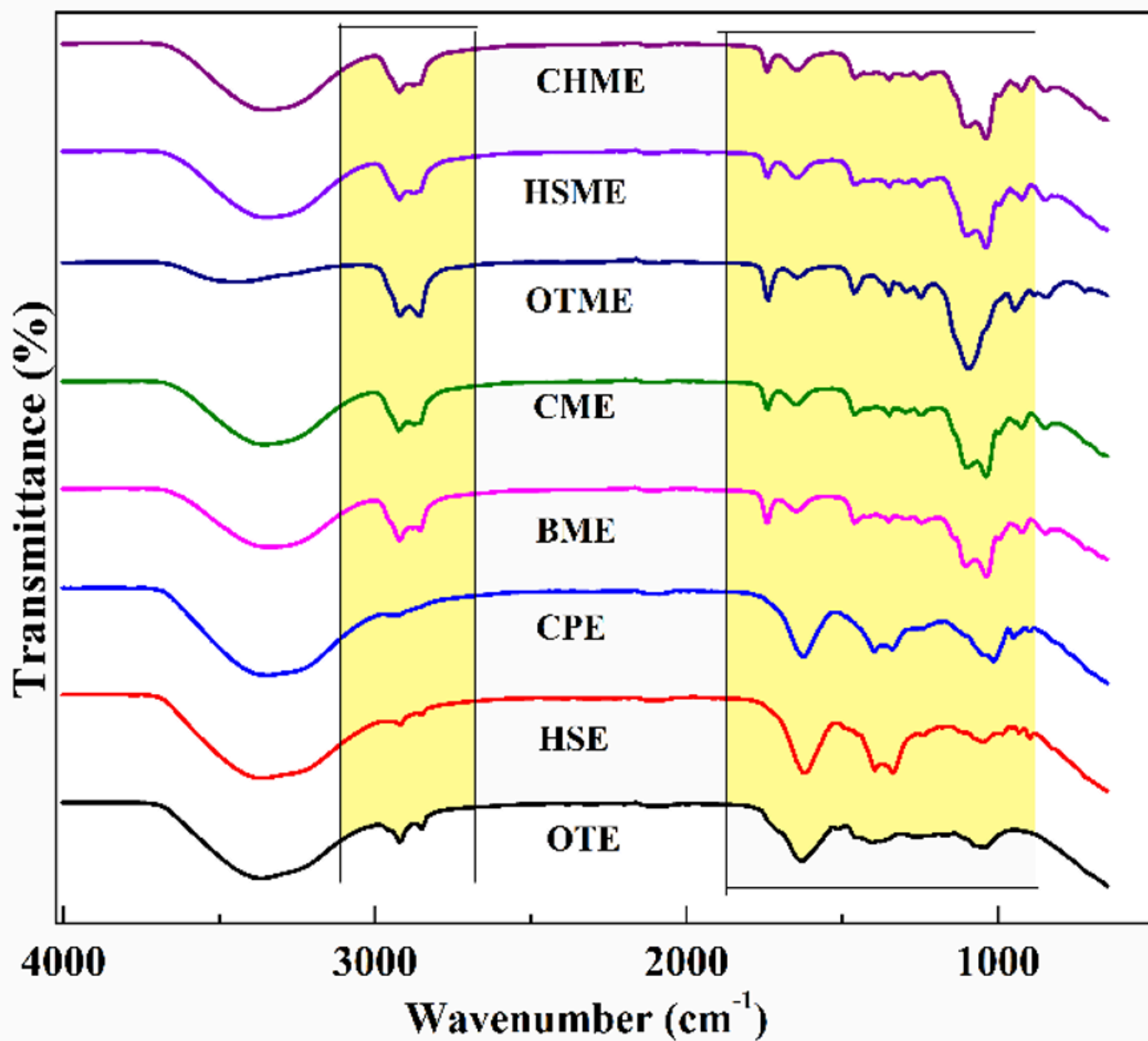


Figure 6

Comparative FTIR spectra of methanolic extracts of *O. tenuiflorum* (OTE) and *H. rosa-sinensis* (HSE), their combined extract (CPE) and blank and herbal microemulsion formulations (BME, CME, OTME, HSME, and CHME).

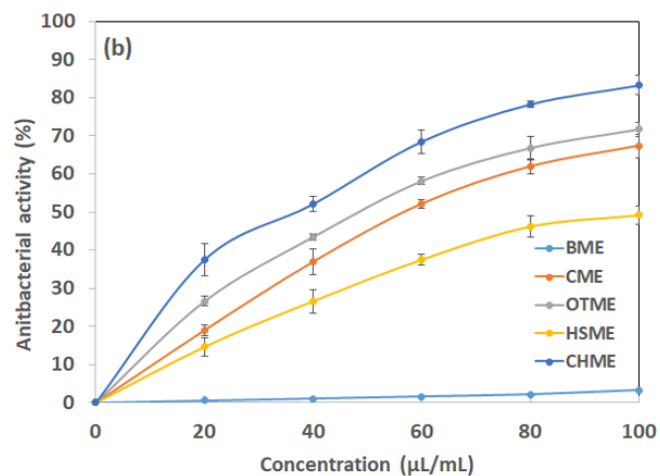
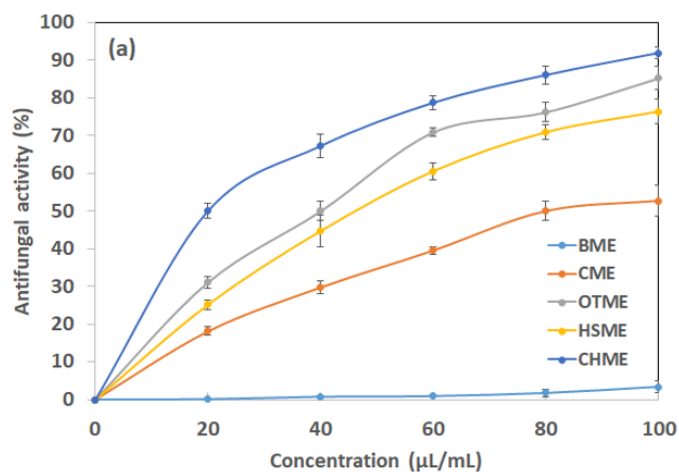
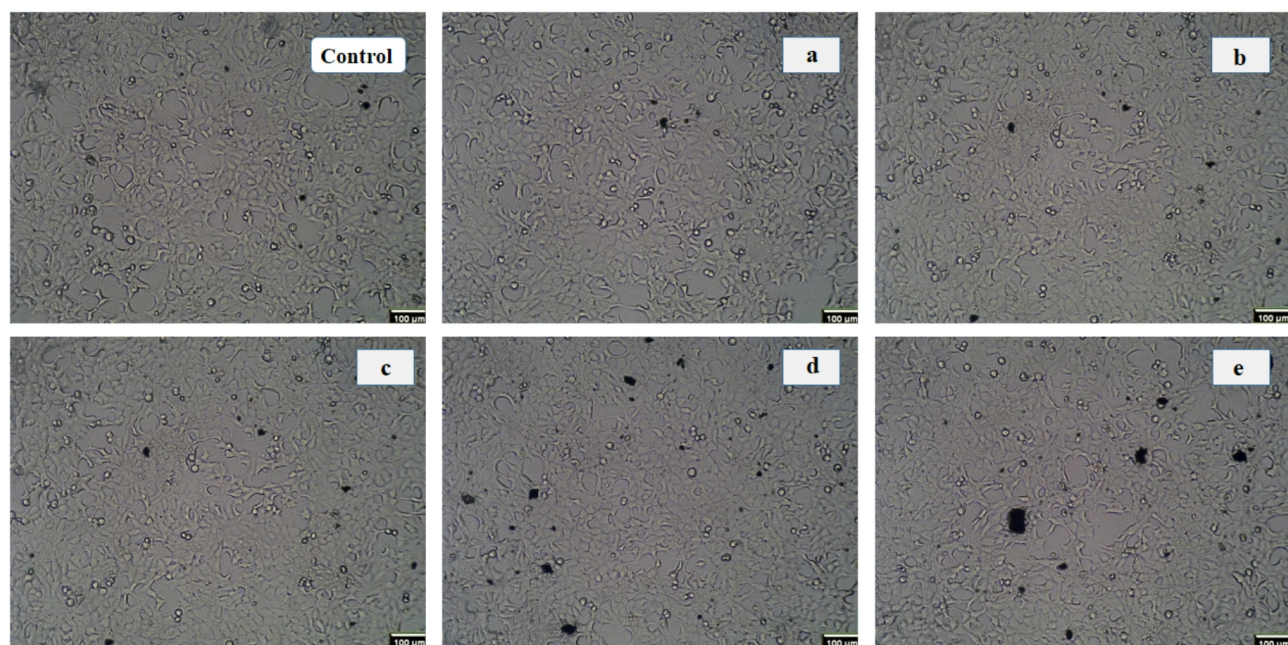
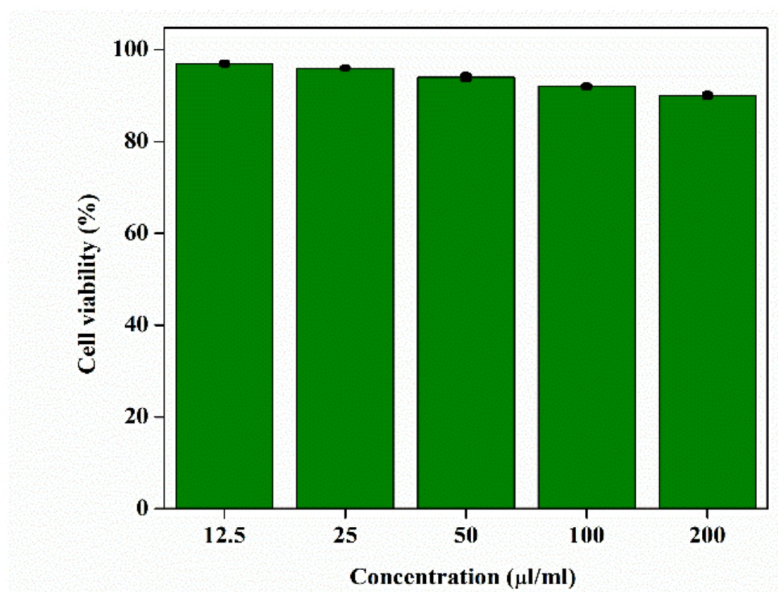


Figure 7

Concentration-dependent antimicrobial activity of blank and herbal microemulsion formulations against (a) *Malassezia furfur* and (b) *Staphylococcus aureus*, determined by the TTC reduction assay at 20, 40, 60, 80, and 100 $\mu\text{L mL}^{-1}$.



(A)



(B)

Figure 8

(A) Phase-contrast morphology of L929 fibroblasts: untreated control and CHME-treated cells (a–e, increasing concentration). (B) MTT cell-viability of L929 fibroblasts treated with CHME (12.5–200 $\mu\text{g mL}^{-1}$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SI4926.docx](#)