

Green Synthesized *Salvia officinalis* Silver Nanoparticles for Topical Anti-Acne Therapy: Formulation, Characterization, and Biological Evaluation

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Abstract

Salvia officinalis-mediated silver nanoparticles were developed and evaluated as a topical anti-acne system with combined antimicrobial and anti-inflammatory activity. Hydroalcoholic and methanolic extracts were prepared and screened for phytochemical content and antioxidant activity. The hydroalcoholic extract showed higher total phenolic and flavonoid contents and stronger antioxidant activity. Silver nanoparticles were synthesized using both extracts and optimized by varying extract proportion and reaction conditions. AgNP-H2 and AgNP-M2 showed the most suitable physicochemical characteristics, with particle sizes of 98.4 ± 4.9 and 112.9 ± 5.2 nm, respectively, along with low PDI and negative zeta potential. Both formulations exhibited enhanced antimicrobial activity against *Cutibacterium acnes* and *Staphylococcus aureus* compared with crude extracts. AgNP-H2 showed an MIC of 31.25 µg/mL against both organisms. Optimized nanoparticles also demonstrated improved protein denaturation inhibition, membrane stabilization, and nitric oxide scavenging. Carbopol-based gels H-G3 and M-G3 showed suitable pH, spreadability, content uniformity, stability, and sustained phytochemical release. In vivo, H-G3 markedly reduced lesion severity and microbial burden and improved histopathological recovery, with performance approaching clindamycin. Both nanoparticle gels showed good dermal compatibility. These findings support *S. officinalis*-derived silver nanoparticles as a promising topical anti-acne platform.

Keywords: *Salvia officinalis*; silver nanoparticles; green synthesis; acne; *Cutibacterium acnes*; topical gel; antimicrobial activity; anti-inflammatory activity.

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Introduction

Acne vulgaris is a common inflammatory disorder of the pilosebaceous unit characterized by follicular obstruction, microbial proliferation, sebum-related changes, and local inflammation. *Cutibacterium acnes* is one of the principal microorganisms associated with acne lesions, while *Staphylococcus aureus* may contribute to secondary infection and worsening of inflammatory lesions. Conventional management commonly includes topical or systemic antimicrobial agents, retinoids, keratolytics, and anti-inflammatory therapies. However, prolonged antimicrobial use may be associated with reduced responsiveness, irritation, and concerns related to antimicrobial resistance. These limitations have encouraged the investigation of plant-derived and nanotechnology-based topical systems with combined antimicrobial and anti-inflammatory properties (Golanka et al., 2021; Hashem et al., 2021; Stein Gold et al., 2022).

Salvia officinalis L. is a medicinal plant rich in phenolic compounds, flavonoids, tannins, terpenoids, and other bioactive constituents. These phytochemicals are associated with antioxidant, antimicrobial, and anti-inflammatory activity. Their

reducing and metal-binding properties also make plant extracts suitable for green synthesis of metallic nanoparticles. Compared with conventional chemical synthesis, plant-mediated nanoparticle preparation can reduce the need for harsh reducing agents because naturally occurring phytochemicals may act simultaneously as reducing and stabilizing components (Gan et al., 2026; Tang et al., 2022; Zhang et al., 2024; Zheng et al., 2025).

Silver nanoparticles have attracted considerable attention because of their broad antimicrobial activity and high surface area. Their nanoscale dimensions can increase contact with microbial cells, while surface-associated phytochemicals may contribute additional biological activity and improve colloidal stabilization. For acne-related applications, this combination is particularly relevant because an effective formulation should ideally control microbial growth while also reducing oxidative and inflammatory damage (Gouda et al., 2024; Morozova, 2021; Xu et al., 2025). Nevertheless, the biological performance of plant-mediated silver nanoparticles depends strongly on synthesis variables such as extract concentration, pH, temperature, and reaction time. Particle size,

polydispersity, surface charge, morphology, and phytochemical capping therefore require careful optimization before biological evaluation. Topical delivery further requires an appropriate vehicle capable of maintaining nanoparticle dispersion while providing suitable pH, spreadability, consistency, and release characteristics. Carbopol-based gels are commonly used for dermatological formulations because they provide a hydrophilic matrix with acceptable application properties and can support localized delivery of dispersed active systems (Devanesan et al., 2021; Enan et al., 2021; Gouda et al., 2024; Li et al., 2026; Morozova, 2021; Rahim et al., 2026).

The present study was therefore designed to develop *Salvia officinalis*-mediated silver nanoparticles for topical anti-acne application. Hydroalcoholic and methanolic extracts were compared for phytochemical content and antioxidant activity and were subsequently used for green synthesis of silver nanoparticles. The nanoparticle formulations were optimized and characterized for particle size, polydispersity, zeta potential, morphology, FTIR profile, and thermal behaviour. Their antimicrobial activity against *C. acnes* and *S. aureus* and their in vitro anti-inflammatory potential was then evaluated. The optimized nanoparticle systems were incorporated into Carbopol gels and assessed for pharmaceutical properties, in vitro phytochemical release, in vivo anti-acne activity, histopathological recovery, and dermal safety. This integrated approach was intended to determine whether phytochemical-capped silver nanoparticles could enhance the anti-acne activity of *S. officinalis* extracts in a topical formulation.

2. Materials and Methods

2.1 Plant material and preparation of extracts

Dried leaves of *Salvia officinalis* were obtained from a certified herbal supplier, Herbal Waves, Himachal Pradesh, India. The plant material was authenticated by a qualified taxonomist, and a voucher specimen (ACP/SO/22/456) was deposited in the departmental herbarium. The leaves were inspected to remove foreign matter and damaged material, powdered using a mechanical grinder, and stored in airtight, light-resistant containers until extraction. Three extraction procedures were initially evaluated. For hydroalcoholic cold maceration, powdered leaves were soaked in ethanol:water (70:30, v/v) at a plant material-to-solvent ratio of 1:10. Maceration was continued for 72 h with intermittent shaking every 6 h. The mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator and dried to obtain the hydroalcoholic cold-macerated extract (HAE-CM). For methanolic cold maceration, the same procedure was followed using 80% methanol to obtain the methanolic cold-macerated extract (ME-CM). A methanolic Soxhlet extract (ME-SE) was also

prepared from approximately 50 g of powdered material by extraction for 6–8 h, followed by rotary evaporation. Percentage extractive yield was calculated from the mass of dried extract relative to the initial mass of plant powder.

2.2 Phytochemical and antioxidant screening

The extracts were subjected to qualitative screening for alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids using standard phytochemical tests. Quantitative evaluation was subsequently carried out for the shortlisted hydroalcoholic and methanolic cold-macerated extracts. Total phenolic content was determined using the Folin–Ciocalteu method. Extract samples were reacted with diluted Folin–Ciocalteu reagent followed by sodium carbonate solution (Wasim et al., 2025; Yolin Angel et al., 2024). After colour development, absorbance was measured spectrophotometrically. Gallic acid was used for calibration, and results were expressed as mg gallic acid equivalents per gram of dried extract. Total flavonoid content was estimated using the aluminium chloride colorimetric method with quercetin as the reference standard and expressed as mg quercetin equivalents per gram of dried extract. Antioxidant activity was assessed using DPPH radical scavenging, ABTS radical cation scavenging, reducing power, superoxide radical scavenging, and metal-chelating assays. Extracts were evaluated at different concentrations, and measurements were performed spectrophotometrically. Results were obtained from triplicate experiments. Extractive yield, phytochemical content, and antioxidant activity were considered collectively for selection of extracts for nanoparticle synthesis. Based on this screening, the hydroalcoholic and methanolic cold-macerated extracts were selected, while the Soxhlet extract was excluded from subsequent nanoparticle development (Harborne, 1973, 1998; Watanabe et al., 2025; Zheng et al., 2024).

2.3 Green synthesis and optimization of silver nanoparticles

Silver nanoparticles were prepared separately using the hydroalcoholic and methanolic extracts as reducing and stabilizing agents. Analytical-grade silver nitrate (AgNO_3) was used as the metal precursor. Fresh aqueous extract solutions were prepared, filtered to remove insoluble material, and used immediately. For each extract, three formulations were prepared by varying the volume of extract while maintaining the AgNO_3 solution at 1 mM. In the hydroalcoholic series, AgNP-H1, AgNP-H2, and AgNP-H3 contained 10 mL of 1 mM AgNO_3 combined with 1, 2, and 3 mL of hydroalcoholic extract solution, respectively. An equivalent design was used for the methanolic series, designated AgNP-M1, AgNP-M2, and AgNP-M3. Nanoparticle formation was monitored visually by development and stabilization of the

characteristic colour of the reaction mixture. Synthesis conditions were further examined using a one-factor-at-a-time approach. The effects of reaction pH, extract concentration, reaction time, and temperature were assessed. Conditions producing rapid nanoparticle formation with minimal visible aggregation or sedimentation were selected. Optimized formulations were prepared independently in triplicate to assess batch reproducibility. The nanoparticle suspensions were purified by centrifugation followed by repeated washing with distilled water to remove unreacted silver ions and excess phytochemicals. Purified nanoparticles were redispersed in distilled water and stored under refrigerated, light-protected conditions until further analysis (Sharma et al., 2025; Wang et al., 2024; Washington et al., 2025).

2.4 Physicochemical characterization of silver nanoparticles

Particle size, polydispersity index (PDI), and zeta potential were determined using a Malvern Zetasizer Nano ZS. Samples were uniformly dispersed before analysis and measured under controlled conditions. Each formulation was analysed in triplicate. Surface morphology was examined by scanning electron microscopy. Nanoparticle suspensions were deposited onto conductive stubs, dried, and coated with a conductive layer when required. Images were recorded at suitable magnifications to examine particle shape, surface characteristics, and aggregation. Fourier-transform infrared spectroscopy was used to evaluate the functional groups involved in reduction and stabilization of the nanoparticles. Spectra of the parent extracts and corresponding nanoparticle samples were compared for changes in characteristic hydroxyl, carbonyl, aromatic, and phenolic bands. Differential scanning calorimetry was used to examine thermal behaviour. Dried extracts and nanoparticle samples were sealed in aluminium pans and heated under controlled conditions to compare thermal transitions and structural changes following nanoparticle formation. Particle size, PDI, zeta potential, morphology, FTIR profile, and thermal characteristics were considered together during selection of structurally acceptable formulations (Sharma et al., 2025; Wang et al., 2024; Washington et al., 2025).

2.5 Antimicrobial evaluation

Antimicrobial activity was evaluated against *Cutibacterium acnes* MTCC 1951 and *Staphylococcus aureus* MTCC 96. *C. acnes* was maintained under anaerobic conditions, while *S. aureus* was cultured aerobically. Fresh bacterial suspensions were adjusted to 0.5 McFarland turbidity before testing. The hydroalcoholic and methanolic extracts and all six nanoparticle formulations were initially screened by agar well diffusion. Standardized bacterial inocula were spread uniformly onto appropriate agar media, wells of equal diameter were prepared, and defined

volumes of test samples were introduced. *C. acnes* plates were incubated anaerobically and *S. aureus* plates aerobically at 37°C. Clindamycin served as the positive control, while sterile solvent was used as the negative control. Zones of inhibition were measured after incubation, and all experiments were performed in triplicate. Formulations showing greater antimicrobial activity were subjected to minimum inhibitory concentration (MIC) determination using a broth dilution method. Serial dilutions were inoculated with standardized bacterial suspensions, and the lowest concentration preventing visible bacterial growth was recorded as the MIC. For minimum bactericidal concentration (MBC), aliquots from clear MIC tubes were subcultured onto fresh agar. The lowest concentration showing no colony growth was recorded as the MBC. One hydroalcoholic AgNP formulation and one methanolic AgNP formulation were selected based on inhibition zone, MIC, MBC, suspension stability, and reproducibility (Luo et al., 2024; M et al., 2023; Tribedi & Cs, 2026).

2.6 In vitro anti-inflammatory evaluation

The selected nanoparticle formulations and their respective crude extracts were evaluated for anti-inflammatory activity using complementary in vitro assays. Protein denaturation inhibition was assessed using bovine serum albumin. Samples were incubated with the protein and exposed to controlled heating. After cooling, absorbance was measured, and inhibition of protein denaturation was calculated relative to the untreated control. Membrane-stabilizing activity was assessed using an erythrocyte haemolysis model. Erythrocytes were exposed to hypotonic stress in the presence of the test samples, and released haemoglobin was measured spectrophotometrically. Lower haemolysis indicated greater membrane stabilization. Nitric oxide scavenging activity was determined using a nitric oxide-generating system followed by quantification of nitrite using Griess reagent. Reduced nitrite formation relative to the control was considered indicative of nitric oxide scavenging activity. Measurements were performed in triplicate (Luo et al., 2024; M et al., 2023; Tribedi & Cs, 2026).

2.7 Preparation and evaluation of nanoparticle-loaded gels

The optimized hydroalcoholic and methanolic nanoparticle formulations were incorporated separately into Carbopol-based topical gels. Five gel batches were prepared for each nanoparticle system by varying the concentration of the gelling polymer while maintaining nanoparticle loading constant. These were designated H-G1 to H-G5 and M-G1 to M-G5. Carbopol was gradually dispersed in purified water under continuous stirring and allowed to hydrate completely. Humectants and the preservative system were incorporated into the hydrated polymer dispersion. The nanoparticle

preparation was initially mixed with a small portion of the gel base and subsequently incorporated into the remaining gel under low-shear mixing. Triethanolamine was used for neutralization and adjustment to a skin-compatible pH. Prepared gels were allowed to stand to remove entrapped air before packaging. Gel formulations were evaluated for visual appearance, colour, homogeneity, pH, spreadability, extrudability, and content uniformity. Spreadability was determined using a sliding plate method. Samples collected from different regions of the container were examined to assess uniformity of nanoparticle distribution. One gel formulation from each series was selected based on overall physical properties, consistency, spreadability, pH, homogeneity, and stability. The optimized gels were monitored for colour change, phase separation, and visible aggregation during storage (Abou-Taleb et al., 2023; Ahmed et al., 2023; Al Homsy et al., 2022; Alioghli Ziaei et al., 2023; Almadani et al., 2025; "Correction: Cell Membrane-Camouflaged Chitosan-Polypyrrole Nanogels Co-Deliver Drug and Gene for Targeted Chemotherapy and Bone Metastasis Inhibition of Prostate Cancer," 2026).

2.8 In vitro release

In vitro release from the optimized hydroalcoholic and methanolic nanoparticle gels was studied using a dialysis membrane diffusion method. Dialysis membranes with a molecular-weight cut-off of approximately 12–14 kDa were conditioned before use. Approximately 1 g of gel was placed in the dialysis bag and immersed in phosphate buffer maintained at $37 \pm 0.5^\circ\text{C}$ with gentle stirring. A skin-relevant receptor pH of 5.5 was used. Samples were withdrawn at predetermined intervals and replaced with an equal volume of fresh receptor medium. Released phytochemicals were quantified using the Folin–Ciocalteu method with gallic acid calibration. Release was expressed as cumulative percentage relative to the theoretical total phenolic content in the applied gel dose. Experiments were performed in triplicate (Abou-Taleb et al., 2023; Ahmed et al., 2023; Al Homsy et al., 2022; Alioghli Ziaei et al., 2023; Almadani et al., 2025; "Correction: Cell Membrane-Camouflaged Chitosan-Polypyrrole Nanogels Co-Deliver Drug and Gene for Targeted Chemotherapy and Bone Metastasis Inhibition of Prostate Cancer," 2026).

2.9 In vivo anti-acne evaluation

The therapeutic activity of the optimized nanoparticle gels was investigated in healthy adult rodents weighing 150–200 g. Animals were acclimatized for at least seven days before experimentation and randomly allocated to seven groups: normal control receiving placebo gel without acne induction; disease control receiving induction and placebo gel; hydroalcoholic extract gel; methanolic extract gel; optimized hydroalcoholic nanoparticle gel; optimized methanolic nanoparticle gel; and clindamycin gel as

the standard treatment. Dorsal hair was removed, and a standardized suspension of acne-associated bacteria was applied to the exposed skin. The area was occluded to facilitate microbial colonization and development of localized inflammation. Successful induction was identified by development of erythema, swelling, and visible lesions. Treatments were applied topically once daily in standardized amounts. Lesions were monitored at predetermined intervals for erythema, edema, and lesion size. Lesion dimensions were measured using digital calipers, and photographic records were obtained during treatment. Microbial burden was assessed by aseptic collection of swabs from lesion sites followed by culture and enumeration of colony-forming units. At completion of the study, skin samples were collected for histopathological examination. Tissues were fixed, processed, embedded in paraffin, sectioned, and stained using standard histological procedures. Epidermal thickness, inflammatory cell infiltration, follicular architecture, and tissue repair were examined microscopically.

2.10 Dermal safety assessment

Dermal compatibility of the optimized gels was assessed separately on healthy shaved skin. Treated areas were examined for erythema, edema, dryness, ulceration, and other visible signs of irritation. Repeated application was also used to evaluate cumulative irritation or hypersensitivity. General behaviour, health status, and body weight were monitored. Skin samples were subsequently examined histologically for epidermal integrity, inflammatory infiltration, and tissue architecture.

2.11 Statistical analysis

In vitro measurements were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Experimental data were tabulated and verified before analysis. Comparisons among treatment groups were performed using one-way analysis of variance followed by an appropriate post hoc multiple-comparison test. Statistical analysis compared crude extracts with nanoparticle formulations, hydroalcoholic with methanolic nanoparticle systems, treatment groups with disease control, and treatment groups with clindamycin. A probability value below the predefined significance threshold was considered statistically significant.

3. Results and Discussion

3.1 Extraction, phytochemical profile and antioxidant activity

Extraction efficiency varied with solvent system and extraction method. Methanolic Soxhlet extraction produced the highest yield ($21.68 \pm 0.72\%$), followed by hydroalcoholic cold maceration ($18.42 \pm 0.56\%$) and methanolic cold maceration ($15.37 \pm 0.44\%$). However, extraction yield alone did not reflect phytochemical quality. The hydroalcoholic cold-macerated extract (HAE-CM) contained 182.4 ± 3.2 mg GAE/g total phenolics and 96.8 ± 2.1 mg

QE/g total flavonoids, compared with 154.6 ± 2.9 mg GAE/g and 81.3 ± 1.8 mg QE/g, respectively, for the methanolic cold-macerated extract (ME-CM). Qualitative screening also showed strong phenolic and flavonoid reactions in HAE-CM. The higher phytochemical content of HAE-CM was reflected consistently in the antioxidant assays. At 100 $\mu\text{g/mL}$, HAE-CM produced $78.9 \pm 1.8\%$ DPPH inhibition, $84.5 \pm 2.0\%$ ABTS inhibition, $72.6 \pm 1.6\%$ superoxide inhibition, and $68.4 \pm 1.5\%$ metal chelation. The corresponding values for ME-CM

Table 1. Phytochemical content and antioxidant activity of the selected extracts

Parameter	HAE-CM	ME-CM
Extraction yield (%)	18.42 ± 0.56	15.37 ± 0.44
Total phenolics (mg GAE/g)	182.4 ± 3.2	154.6 ± 2.9
Total flavonoids (mg QE/g)	96.8 ± 2.1	81.3 ± 1.8
DPPH inhibition at 100 $\mu\text{g/mL}$ (%)	78.9 ± 1.8	70.1 ± 1.6

were $70.1 \pm 1.6\%$, $75.6 \pm 1.9\%$, $65.1 \pm 1.5\%$, and $60.8 \pm 1.3\%$, respectively. HAE-CM also showed greater reducing power, with an absorbance of 1.12 ± 0.04 at 100 $\mu\text{g/mL}$ compared with 0.96 ± 0.03 for ME-CM. The lower IC_{50} values of HAE-CM across the radical-scavenging assays confirmed its stronger antioxidant activity. These findings supported selection of both cold-macerated extracts for comparative nanoparticle synthesis, with HAE-CM showing the stronger reducing potential.

DPPH IC_{50} ($\mu\text{g/mL}$)	47.6	61.2
ABTS inhibition at 100 $\mu\text{g/mL}$ (%)	84.5 ± 2.0	75.6 ± 1.9
ABTS IC_{50} ($\mu\text{g/mL}$)	39.8	54.4
Reducing power at 100 $\mu\text{g/mL}$	1.12 ± 0.04	0.96 ± 0.03
Superoxide inhibition at 100 $\mu\text{g/mL}$ (%)	72.6 ± 1.6	65.1 ± 1.5
Metal chelation at 100 $\mu\text{g/mL}$ (%)	68.4 ± 1.5	60.8 ± 1.3

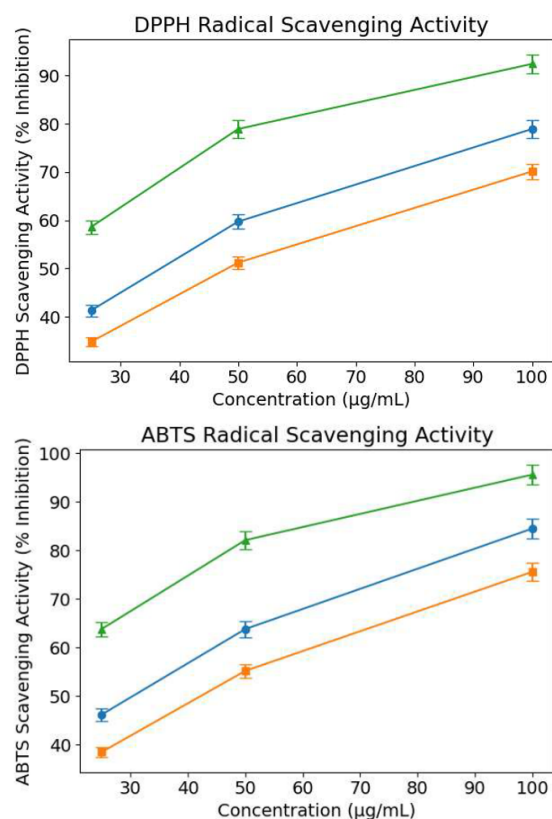


Figure 1. Antioxidant activity of hydroalcoholic and methanolic *Salvia officinalis* extracts showing concentration-dependent DPPH and ABTS radical scavenging.

Table 2. Physicochemical properties of the six silver nanoparticle formulations

The antioxidant profile is relevant to nanoparticle formation because reduction of Ag^+ requires electron-donating constituents. The consistently greater phenolic and flavonoid content of HAE-CM therefore provides a reasonable explanation for the subsequent differences between the hydroalcoholic and methanolic nanoparticle systems.

3.2 Optimization and physicochemical characteristics of silver nanoparticles

Formation of silver nanoparticles was initially indicated by conversion of the reaction mixture from pale yellow to brown. Optimization showed that synthesis was sensitive to pH, temperature, reaction time, and extract proportion. The selected conditions were pH 8.0, 40°C , and a reaction time of 60 min. Intermediate extract proportions, corresponding to H2 and M2, provided the most stable dispersions. Mildly alkaline conditions favoured faster reduction, whereas the highest tested temperature increased heterogeneity. Clear formulation-dependent differences were observed by dynamic light scattering. AgNP-H2 produced the smallest mean particle size in the hydroalcoholic series at 98.4 ± 4.9 nm with a PDI of 0.218 ± 0.012 . AgNP-M2 showed a size of 112.9 ± 5.2 nm and PDI of 0.236 ± 0.013 . The corresponding zeta potentials were -32.8 ± 1.7 and -30.4 ± 1.6 mV. In contrast, H1/M1 showed larger particles, while H3/M3 showed a modest increase in particle size and polydispersity.

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
AgNP-H1	142.6 ± 6.8	0.312 ± 0.018	-24.3 ± 1.4
AgNP-H2	98.4 ± 4.9	0.218 ± 0.012	-32.8 ± 1.7
AgNP-H3	110.7 ± 5.6	0.271 ± 0.015	-28.6 ± 1.5

The intermediate extract concentration therefore appears to have provided an appropriate balance between Ag⁺ reduction and phytochemical stabilization. Insufficient extract may limit nucleation and surface coverage, whereas excessive phytochemical content can increase the hydrodynamic layer or promote secondary association between particles. SEM supported the DLS findings. AgNP-H2 showed predominantly

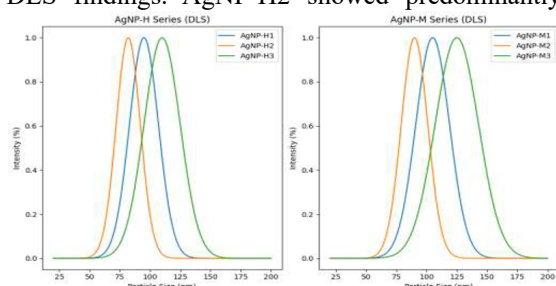


Figure 2. Representative SEM images and DLS particle-size distributions of the optimized formulations: (A, B) AgNP-H2 and (C, D) AgNP-M2.

FTIR spectra showed changes in the 3200–3500 cm⁻¹, 1600–1700 cm⁻¹, and 1000–1300 cm⁻¹ regions after nanoparticle formation. These regions were assigned mainly to O–H, carbonyl/aromatic, and C–O-containing groups. Shifts and changes in intensity supported interaction of plant phytochemicals with the nanoparticle surface. DSC provided complementary evidence, with nanoparticle samples showing attenuated or shifted thermal events relative to the broad endothermic transitions of the crude extracts. Together, these data support formation of phytochemical-capped silver nanoparticles rather than unprotected metallic particles.

AgNP-M1	156.2 ± 7.4	0.338 ± 0.020	-22.7 ± 1.3
AgNP-M2	112.9 ± 5.2	0.236 ± 0.013	-30.4 ± 1.6
AgNP-M3	125.8 ± 6.0	0.289 ± 0.017	-26.1 ± 1.4

near-spherical particles with good dispersion and minimal clustering. AgNP-M2 was also nanosized and predominantly near-spherical, although occasional clustering was observed. Smaller dimensions by SEM than by DLS are expected because DLS measures the hydrated particle together with its associated solvation and surface layer.

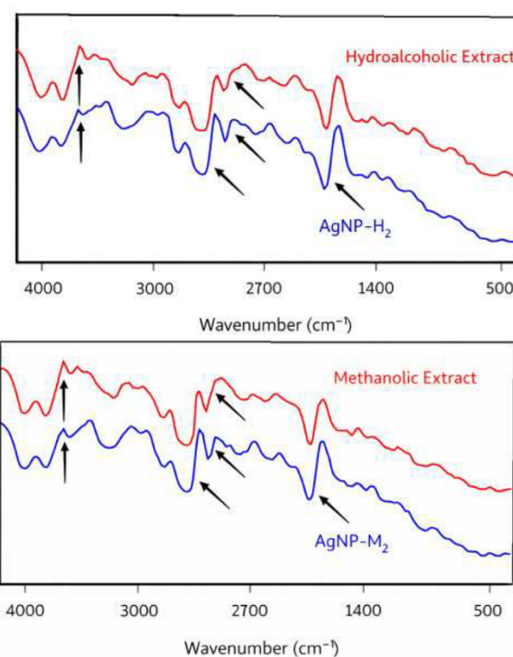


Figure 3. FTIR overlay spectra of the parent extracts and optimized silver nanoparticles showing changes associated with phytochemical-mediated reduction and surface capping.

3.3 Antimicrobial activity

Conversion of the plant extracts into silver nanoparticles substantially increased antimicrobial activity against both *C. acnes* and *S. aureus*. Against *C. acnes*, AgNP-H2 produced a zone of inhibition of 22.6 ± 0.9 mm, compared with 12.4 ± 0.6 mm for HAE-CM. AgNP-M2 produced a 20.3 ± 0.8 mm zone compared with 10.8 ± 0.5 mm for ME-CM. A comparable trend was observed against *S. aureus*. AgNP-H2 produced a zone of 21.2 ± 0.8 mm and AgNP-M2 19.1 ± 0.7 mm, whereas the corresponding crude extracts produced 11.6 ± 0.5 and 9.9 ± 0.4 mm. Clindamycin remained the most active control, with zones of 24.8 ± 1.0 mm against *C. acnes* and 23.5 ± 0.9 mm against *S. aureus*. The MIC data confirmed the zone-of-inhibition results. AgNP-H2 had an MIC of $31.25 \mu\text{g/mL}$ against both organisms, representing an eightfold reduction relative to HAE-CM at $250 \mu\text{g/mL}$. AgNP-M2 showed an MIC of $62.5 \mu\text{g/mL}$ compared with $500 \mu\text{g/mL}$ for ME-CM. MBC values were $62.5 \mu\text{g/mL}$ for AgNP-H2 and $125 \mu\text{g/mL}$ for AgNP-M2, compared with $31.25 \mu\text{g/mL}$ for clindamycin.

Table 3. Antimicrobial activity of selected extracts and optimized nanoparticles

Sample	<i>C. acnes</i> zone (mm)	<i>S. aureus</i> zone (mm)	MIC ($\mu\text{g/mL}$), both strains	MBC ($\mu\text{g/mL}$), both strains
HAE-CM	12.4 ± 0.6	11.6 ± 0.5	250	500
ME-CM	10.8 ± 0.5	9.9 ± 0.4	500	1000
AgNP-H2	22.6 ± 0.9	21.2 ± 0.8	31.25	62.5
AgNP-M2	20.3 ± 0.8	19.1 ± 0.7	62.5	125
Clindamycin	24.8 ± 1.0	23.5 ± 0.9	15.6	31.25

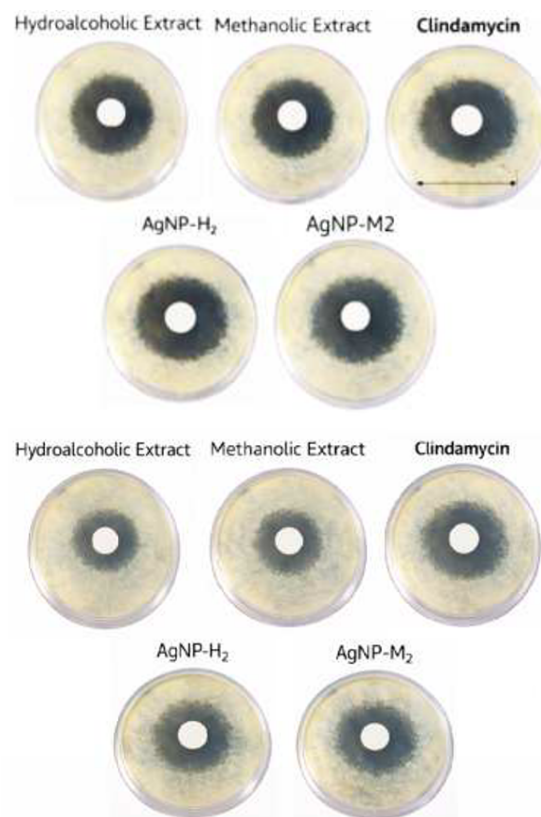


Figure 4. Comparative antimicrobial activity of crude extracts, optimized AgNP formulations, and clindamycin against *C. acnes* and *S. aureus*.

Thus, nanoparticle formation clearly increased antibacterial potency, although the optimized nanoparticles remained less active than clindamycin on an MIC/MBC basis. The stronger activity of AgNP-H2 than AgNP-M2 was consistent with its smaller particle size, lower PDI, greater absolute zeta potential, and the higher phytochemical content of the parent hydroalcoholic extract.

3.4 Anti-inflammatory activity

Both optimized nanoparticle formulations showed concentration-dependent activity in all three anti-inflammatory models. At $200 \mu\text{g/mL}$, AgNP-H2 inhibited protein denaturation by $88.9 \pm 2.4\%$, compared with $71.6 \pm 2.0\%$ for HAE-CM. AgNP-M2 produced $81.5 \pm 2.2\%$ inhibition compared with $63.2 \pm 1.8\%$ for ME-CM. The same ranking was observed for membrane stabilization and nitric oxide scavenging. At $200 \mu\text{g/mL}$, membrane protection reached $85.7 \pm 2.3\%$ for AgNP-H2 and $78.4 \pm 2.1\%$ for AgNP-M2. Nitric oxide scavenging reached $90.1 \pm 2.6\%$ and $83.5 \pm 2.3\%$, respectively. The improvement over the crude extracts demonstrates that nanoparticle formation retained the plant-derived anti-inflammatory activity while increasing the measured response.

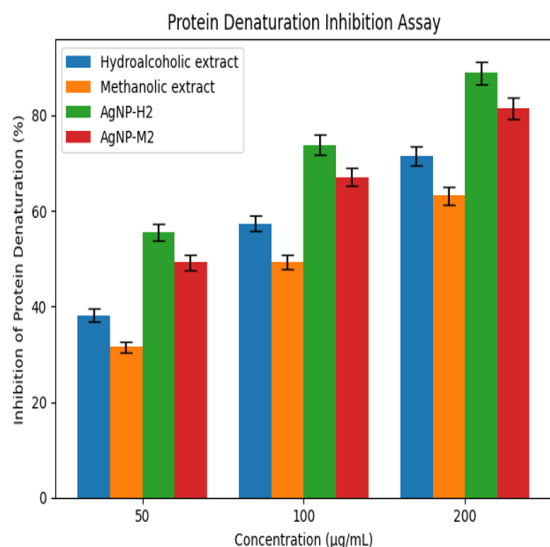


Figure 5. Concentration-dependent protein denaturation inhibition, membrane stabilization, and nitric oxide scavenging by crude extracts and optimized AgNP formulations.

The consistent superiority of AgNP-H2 across the three assays is particularly relevant because acne lesions involve microbial proliferation together with inflammatory and oxidative components. The antimicrobial and anti-inflammatory findings therefore support development of a topical system rather than evaluation of antibacterial activity alone.

3.5 Properties and release behaviour of optimized nanoparticle gels

Increasing Carbopol concentration progressively increased gel firmness and reduced spreadability. H-G3 and M-G3 provided the best balance between structural integrity and ease of application. Their pH values were 6.25 ± 0.04 and 6.24 ± 0.04 , respectively. Spreadability was 12.8 ± 0.5 g·cm/s for H-G3 and 12.5 ± 0.5 g·cm/s for M-G3, while extrusion forces were 338 ± 14 and 332 ± 13 g. Content uniformity was $98.6 \pm 1.2\%$ and $97.9 \pm 1.3\%$, respectively. Both remained physically stable during the short-term observation period, with no phase separation or visible nanoparticle aggregation. In vitro release demonstrated progressive release of phenolic constituents over 24 h. H-G3 released $95.3 \pm 2.9\%$ of its phenolic content by 24 h compared with $90.8 \pm 2.7\%$ from M-G3. At 12 h, the corresponding release values were $88.5 \pm 2.6\%$ and $80.4 \pm 2.4\%$. The profile indicates that incorporation into the Carbopol matrix did not prevent release of the phytochemical-associated fraction and provided gradual release rather than immediate depletion.

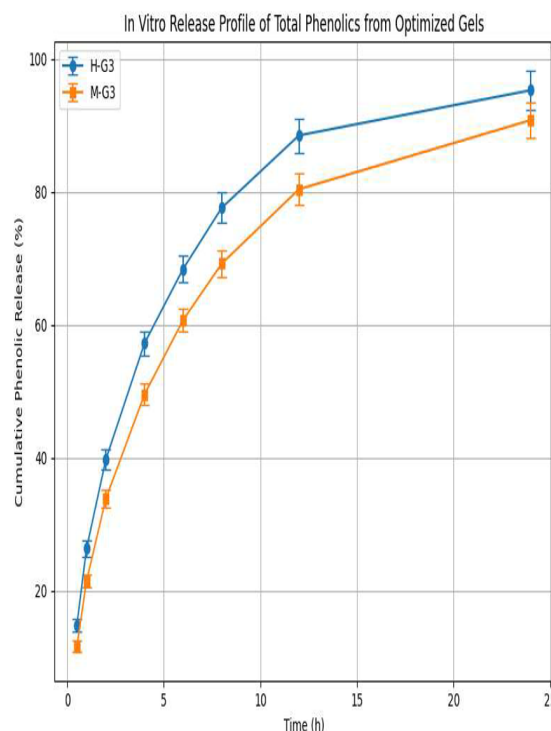


Figure 6. Cumulative in vitro release of total phenolic constituents from optimized H-G3 and M-G3 nanoparticle gels over 24 h.

3.6 In vivo anti-acne efficacy

The in vivo results were consistent with the preceding physicochemical and biological screening. All groups had an initial lesion severity score of 5.0. By day 12, the disease control remained at 3.8 ± 0.5 , whereas HAE and ME gels decreased the scores to 1.4 ± 0.2 and 1.9 ± 0.2 , respectively. Greater improvement occurred with the nanoparticle gels. H-G3 reduced the score to 0.4 ± 0.1 and M-G3 to 0.6 ± 0.1 . Clindamycin produced a final score of 0.3 ± 0.1 . Thus, H-G3 showed a response numerically close to the standard treatment and clearly greater than its parent extract gel. Microbial load measurements provided quantitative support for the lesion response. Disease-control counts changed only from 6.8 ± 0.2 to 6.5 ± 0.3 log CFU by day 12. H-G3 reduced the count from 6.7 ± 0.2 to 2.3 ± 0.2 log CFU, corresponding to a 4.4-log reduction. M-G3 decreased the count from 6.8 ± 0.2 to 2.8 ± 0.2 log CFU, while clindamycin reduced it from 6.7 ± 0.2 to 2.0 ± 0.2 log CFU. The crude HAE and ME gels produced smaller reductions to 4.2 ± 0.2 and 4.8 ± 0.3 log CFU, respectively.

Table 4. In vivo therapeutic response of the topical formulations

Group	Lesi on score Day 0	Lesi on score Day 12	Microb ial load Day 0 (log CFU)	Microb ial load Day 12 (log CFU)
Disease control	5.0 ± 0.0	3.8 ± 0.5	6.8 ± 0.2	6.5 ± 0.3

HAE gel	5.0 ± 0.0	1.4 ± 0.2	6.7 ± 0.2	4.2 ± 0.2
ME gel	5.0 ± 0.0	1.9 ± 0.2	6.8 ± 0.2	4.8 ± 0.3
H-G3	5.0 ± 0.0	0.4 ± 0.1	6.7 ± 0.2	2.3 ± 0.2
M-G3	5.0 ± 0.0	0.6 ± 0.1	6.8 ± 0.2	2.8 ± 0.2
Clindamycin	5.0 ± 0.0	0.3 ± 0.1	6.7 ± 0.2	2.0 ± 0.2

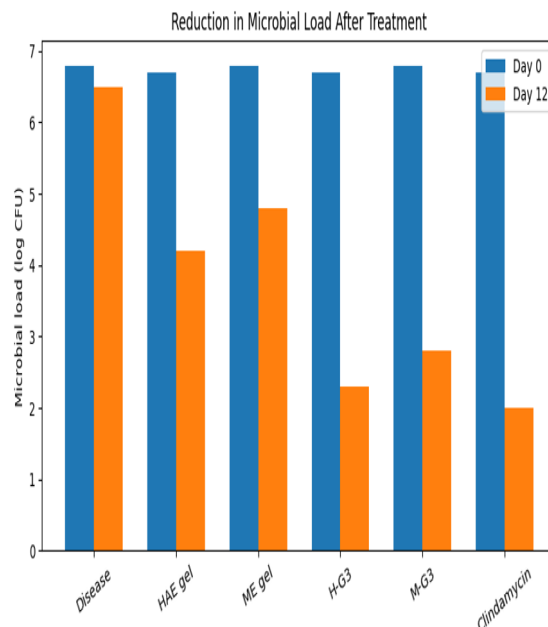
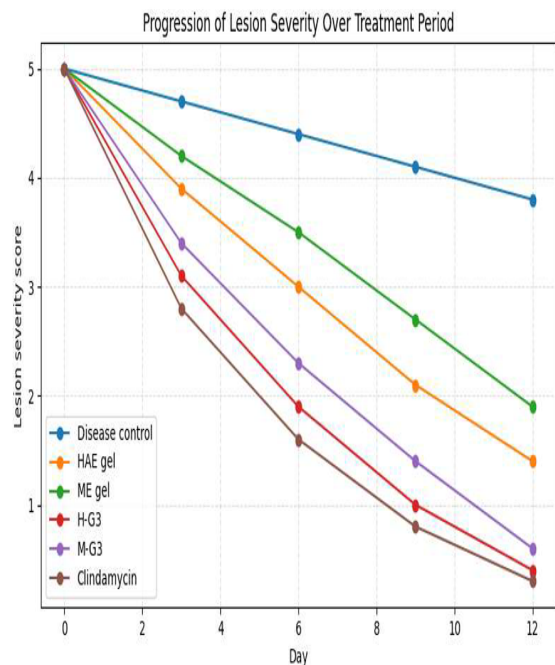


Figure 7. In vivo efficacy of optimized nanoparticle gels: (A) change in lesion severity during 12 days of treatment and (B) microbial load at baseline and Day 12.

Histopathological observations followed the same treatment-dependent pattern. Disease-control skin showed severe inflammatory infiltration, epidermal thickening, and follicular damage. Both extract gels produced moderate improvement. H-G3-treated tissue showed mild inflammatory changes, mild epidermal alteration, and minimal follicular damage, while M-G3 produced mild changes in all three parameters. Clindamycin-treated tissue showed minimal abnormalities. These findings provide tissue-level confirmation that reduction in visible lesion severity was accompanied by recovery of epidermal and follicular architecture.

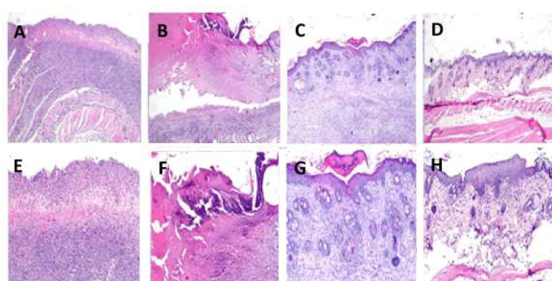


Figure 8A. Representative H&E-stained histological sections of skin across treatment groups. (A) Normal skin. (B) Disease control. (C) Standard drug-treated. (D) Hydroalcoholic nanoparticle gel-treated. (E) Methanolic nanoparticle gel-treated. (F) AgNP-H2-treated group. (G) AgNP-M2-treated group. (H) Best-response/high-dose group showing the greatest histological recovery, characterized by restored epidermal architecture and minimal inflammatory infiltrates

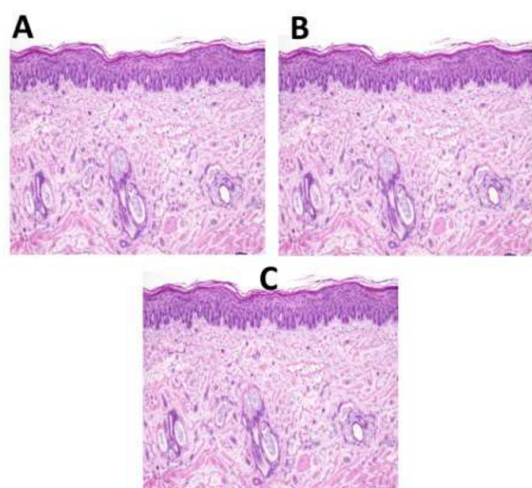


Figure 8B. Representative H&E-stained skin sections showing normal skin, disease control, crude extract gel-treated groups, H-G3, M-G3, and clindamycin-treated groups. Disease control shows marked inflammatory infiltration and epidermal alteration, whereas nanoparticle gel-treated tissue shows substantial restoration of skin architecture. ANOVA confirmed significant treatment effects for lesion severity ($F = 28.6, p < 0.001$), microbial load ($F = 31.2, p < 0.001$), and inflammation score ($F = 24.9, p < 0.001$). Post hoc analysis reported significantly greater responses with H-G3 and M-G3 than with the respective crude extract gels ($p < 0.05$).

3.7 Dermal safety and overall interpretation

Neither H-G3 nor M-G3 produced measurable erythema or edema during the dermal irritation assessment, with irritation scores remaining 0.0 ± 0.0 throughout observation. Repeated topical application for 14 days was not associated with behavioural abnormalities or body-weight loss. Histological examination of healthy treated skin showed intact epidermal layers, preserved tissue architecture, and no inflammatory cell infiltration. These results are relevant because increased antibacterial potency would have limited value if nanoparticle incorporation produced local skin injury. In the present study, the optimized AgNP gels combined antimicrobial activity with acceptable short-term dermal compatibility. Overall, the experimental sequence showed a consistent relationship from extract composition to biological response. HAE-CM contained more phenolic and flavonoid constituents and showed greater antioxidant capacity than ME-CM. Its optimized nanoparticle formulation, AgNP-H2, subsequently exhibited the smaller particle size, lower PDI, greater absolute zeta potential, and stronger antimicrobial and anti-inflammatory activity. Incorporation of AgNP-H2 into H-G3 retained favourable topical properties and produced the greatest response among the test formulations in the animal model. The data therefore support H-G3 as the stronger of the two experimental nanoparticle gels, while clindamycin remained numerically

superior for several antimicrobial and in vivo endpoints. The main advantage demonstrated by the nanoparticle approach was the substantial enhancement of the biological activity of the crude *S. officinalis* extracts after conversion to phytochemical-capped silver nanoparticles, followed by successful delivery through a dermally compatible Carbopol gel.

Conclusion

The present study demonstrated that *Salvia officinalis* extracts can be effectively used for green synthesis of silver nanoparticles with enhanced anti-acne potential. Among the extracts, the hydroalcoholic cold-macerated extract showed higher phenolic and flavonoid contents and stronger antioxidant activity than the methanolic extract. This difference was reflected in the corresponding nanoparticle systems. AgNP-H2 showed a smaller particle size, lower PDI, higher absolute zeta potential, and better antimicrobial performance than AgNP-M2. Both optimized nanoparticle formulations showed stronger activity against *Cutibacterium acnes* and *Staphylococcus aureus* than their parent extracts. They also produced greater protein denaturation inhibition, membrane stabilization, and nitric oxide scavenging. Incorporation into Carbopol gels provided formulations with suitable pH, spreadability, content uniformity, short-term stability, and sustained phytochemical release. In the in vivo acne model, H-G3 and M-G3 reduced lesion severity, microbial burden, and histopathological damage more effectively than crude extract gels. H-G3 produced the stronger response and approached the activity of clindamycin for several evaluated endpoints. Both optimized nanoparticle gels showed good dermal compatibility without visible or histological irritation. Overall, the findings support phytochemical-capped *S. officinalis* silver nanoparticles as a promising topical platform for combined antimicrobial and anti-inflammatory management of acne.

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