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**Original Research**

# Silver nanoparticle microemulsion as a novel localized antimicrobial therapy: Formulation, efficacy, and safety evaluation

Niratcha Chaisomboon, Teerawat Nitichaikulvattana, Hathairat Lekatana, Chanida Chantim, Nattakanwadee Khumpirapang, Prayuth Poowaruttanawiwit

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**Abstract**

**Background:** Amidst the growing challenge of antimicrobial resistance, there is an increasing demand for localized antimicrobial delivery systems with enhanced efficacy and safety profiles. Silver nanoparticles (AgNPs) have garnered attention due to their broad-spectrum antimicrobial potential; however, formulation instability and cytotoxicity remain critical barriers to their clinical translation. This study aimed to develop and characterize a silver nanoparticle-loaded microemulsion and evaluate its antimicrobial activity, physicochemical properties, and cytocompatibility. **Methods:** A silver microemulsion containing AgNPs at a concentration of 1000 ppm was formulated using polyvinyl alcohol and Tween 80 as stabilizers. The formulation was characterized by particle size distribution, zeta potential, and optical absorbance. Antimicrobial activity was assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Candida albicans* using disk diffusion and broth microdilution assays. Cytotoxicity was evaluated in L929 murine fibroblast cells using the MTT assay to determine biocompatibility and estimate the $IC_{50}$. **Results:** The AgNP microemulsion demonstrated a mean particle diameter of 175.97 ± 0.97 nm with a zeta potential of −1.06 ± 0.42 mV, indicating moderate colloidal stability. Antibacterial activity was observed, with mean inhibition zones ranging from 8.9 to 9.1 mm across tested bacterial strains. No antifungal activity was noted against *Candida albicans*. MIC and MBC values exceeded 0.7 mg/mL, suggesting limited bactericidal potency. The formulation maintained acceptable cell viability (>70%) at concentrations up to 16 µg/mL, while cytotoxicity increased markedly at 32 µg/mL. The $IC_{50}$ was determined to be approximately 28.6 µg/mL, delineating a narrow therapeutic index. **Conclusion:** The AgNP-based microemulsion exhibits potential as a topical antimicrobial platform; however, its relatively low potency compared to chlorhexidine and narrow safety margin underscore the necessity for formulation refinement. Strategies to enhance bioavailability—such as controlled-release delivery systems or combinatorial approaches with adjuvants—may improve pharmacological performance and clinical applicability.

**Keywords:** Silver nanoparticles, microemulsion, antimicrobial resistance, localized drug delivery, cytotoxicity, formulation development

**Niratcha Chaisomboon**. Graduated Student, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000, Thailand, niratchac@nu.ac.th
**Teerawat Nitichaikulvattana**. Concordian International School, Bangkok, Samut Prakarn 10540, Thailand, Medical and Pharmaceutical Care Research Unit, Naresuan University, Phitsanulok, 65000, Thailand, chonehangout@gmail.com
**Hathairat Lekatana**. Department of Restorative dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand, hathairatl@nu.ac.th
**Chanida Chantim**. Assistant Professor, Department of Otolaryngology, Faculty of Medicine, Naresuan University, Phitsanulok, 65000, Thailand, chanida_nan@hotmail.com
**Nattakanwadee Khumpirapang**. Associate Professor, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok, 65000, Thailand, nattakanwadeek@nu.ac.th
**Prayuth Poowaruttanawiwit***. Associate Professor, Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000, Thailand, Head of Medical and Pharmaceutical Care Research Unit, Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000, Thailand, yuth_pu@hotmail.com

## INTRODUCTION

The accelerating rise of antimicrobial resistance (AMR) represents a profound global health threat, urgently necessitating the discovery and development of new antimicrobial agents that are not only effective and safe but also resilient to bacterial adaptation [1,2]. Although conventional antibiotics remain indispensable, their mechanism of action often imposes intense selective pressure on microbial populations, inevitably promoting the emergence and dissemination of multidrug-resistant (MDR) strains [3,4]. Furthermore, the systemic administration of these agents is frequently constrained by dose-limiting toxicities—such as hepatotoxicity, nephrotoxicity, and adverse drug–drug interactions—which collectively compromise long-term therapeutic safety and clinical applicability [5]. Consequently, there is growing scientific and clinical interest in the development of localized antimicrobial therapies that deliver potent bactericidal activity directly to the site of infection, thereby minimizing systemic exposure and reducing the risk of toxicity.

Among emerging localized therapeutic modalities, silver nanoparticles (AgNPs) have garnered considerable attention for their broad-spectrum antimicrobial efficacy, multimodal mechanisms of action, and low propensity for resistance

development [6,7]. In contrast to traditional antibiotics that act through discrete molecular targets, AgNPs disrupt microbial integrity via multiple convergent mechanisms—membrane damage, oxidative stress through reactive oxygen species (ROS) generation, and interference with DNA replication—thereby limiting the probability of bacterial resistance emergence. Importantly, AgNPs function independently of metabolic activation and are not reliant on hepatic metabolism or renal clearance, significantly lowering the risk of systemic toxicity [8,9]. This distinctive pharmacokinetic and pharmacodynamic profile renders AgNPs particularly suitable for topical or localized delivery systems, including applications in wound management, oral care, and ophthalmic formulations, where sustained antimicrobial action and minimal systemic absorption are desired.

Despite their robust antimicrobial potential, the clinical translation of AgNPs has been hindered by key formulation challenges such as nanoparticle aggregation, instability, and variability in bioavailability. Recent advances in microemulsion-based delivery systems offer a promising solution to these limitations [10,11]. Microemulsions are thermodynamically stable, nanoscale colloidal dispersions that provide a unique environment for the uniform distribution, stabilization, and sustained release of encapsulated nanoparticles [12,13]. Their physicochemical characteristics—such as ultra-low interfacial tension and high solubilization capacity—facilitate prolonged retention of AgNPs at the infection site and enhanced interaction with microbial membranes. Thus, AgNP-loaded microemulsions represent an innovative delivery platform capable of maximizing local efficacy, maintaining physicochemical stability, and improving therapeutic outcomes relative to conventional antimicrobial formulations.

Nevertheless, the antimicrobial and cytotoxic profiles of AgNPs when integrated into microemulsion matrices remain incompletely characterized [14–16]. Comparative studies assessing AgNP microemulsions against standard antimicrobial agents—such as chlorhexidine, a benchmark antiseptic with well-documented cytotoxic effects—are essential for defining their relative therapeutic value and translational potential [17,18]. Moreover, although AgNPs exhibit dose-dependent biocompatibility, systematic evaluation of their cytotoxicity toward mammalian cells within a microemulsion environment remains crucial to ensure clinical safety. Rigorous preclinical investigations integrating antimicrobial efficacy, cellular safety, and formulation stability are therefore indispensable to establish AgNP-based microemulsions as a viable next-generation localized antimicrobial therapy with optimized pharmacological performance and translational readiness.

**Research Question and Study Objective**

This study tests the hypothesis that a microemulsion-based AgNP formulation can provide clinically meaningful, locally acting antimicrobial activity with acceptable biocompatibility, offering a viable alternative to conventional agents while reducing systemic exposure, metabolic burden, and resistance selection. Specifically, we will (i) develop and characterize a fit-for-purpose AgNP microemulsion with prospectively defined critical quality attributes (droplet size and polydispersity by DLS, zeta potential, Ag content and release kinetics, rheology/wettability), and demonstrate physical–chemical stability under ICH-aligned conditions; (ii) quantify antimicrobial efficacy against clinically relevant pathogens (e.g., *S. aureus*/MRSA, *E. coli*, *K. pneumoniae*, *P. aeruginosa*) using CLSI-conformant MIC/MBC assays, time–kill kinetics, and biofilm models (MBIC/MBEC), analyzed for non-inferiority to chlorhexidine with superiority tested conditional on meeting the non-inferiority margin; and (iii) establish a therapeutic window through cytocompatibility testing in relevant mammalian cells (viability $IC_{50}$ at 24–72 h, pro-inflammatory markers, barrier integrity/TEER where applicable) and calculation of a prespecified selectivity index ($IC_{50}$/MIC). Comparators include vehicle microemulsion, ionic silver, and chlorhexidine to isolate formulation and pharmacodynamic effects. By linking formulation quality attributes to antimicrobial performance and host safety within a predefined analysis plan and blinded, replicated assays, this work aims to determine whether AgNP microemulsions meet pharmacological criteria for a next generation localized antimicrobial suitable for translational development.

## RESEARCH METHODOLOGY

The study protocol was reviewed and approved by the Naresuan University Institutional Biosafety Committee, Phitsanulok, Thailand (approval No. NUIBC MI 67-11-65). All experimental activities—including AgNP microemulsion formulation, antimicrobial efficacy testing, and mammalian-cell cytotoxicity assessments—were conducted in accordance with institutional biosafety and ethical policies and applicable national regulations governing nanoparticle research. Procedures complied with approved risk-mitigation measures for handling, storage, decontamination, and waste disposal of silver-containing materials to protect personnel, experimental systems, and the environment.

**1. Formulation and Characterization of Silver Nanoparticle Microemulsion**

An AgNP microemulsion was prepared by a modified bottom-up synthesis, employing polyvinyl alcohol (PVA) and Tween 80 as steric/surfactant stabilizers to enhance colloidal stability and suppress nanoparticle aggregation. Probe ultrasonication was applied to promote nucleation and achieve uniform dispersion of AgNPs within the continuous phase, thereby improving particle homogeneity and formulation bioavailability. Post-synthesis, the microemulsion underwent comprehensive characterization. Dynamic light scattering (DLS) was used to quantify hydrodynamic diameter, polydispersity index, and zeta potential as primary critical quality attributes governing interfacial behavior and colloidal stability relevant to bacterial-membrane and tissue interactions. UV–visible spectrophotometry verified AgNP formation and temporal integrity via localized surface plasmon resonance profiles. Formulation robustness was assessed under accelerated and real-time storage, with periodic monitoring for changes in particle size distribution, zeta potential, optical signatures (including spectral shifts/intensity changes), and macroscopic

stability (evidence of phase separation or visual color change). These evaluations were used to confirm physical stability and suitability of the AgNP microemulsion for subsequent antimicrobial and cytocompatibility testing.

**2. Antimicrobial Activity Testing**

Antimicrobial efficacy of the AgNP microemulsion was evaluated by agar well diffusion against clinically relevant strains—*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and the opportunistic yeast *Candida albicans* [19]. Briefly, standardized inocula (0.5 McFarland; Mueller–Hinton agar for bacteria, Sabouraud dextrose agar for *C. albicans*) were lawned onto plates, sterile wells were filled with the test formulation, and plates were incubated under organism-appropriate conditions (35–37 °C, 18–24 h for bacteria; 24–48 h for yeast) [19]. The primary endpoint was the mean inhibition-zone diameter (mm) with 95% CIs, averaged over ≥3 biological replicates (technical duplicates), after subtracting any well diameter contribution. Prespecified comparators included 0.2% chlorhexidine (reference standard), vehicle microemulsion (negative control), and ionic silver solution at equimolar Ag (mechanistic control). A priori, non-inferiority to chlorhexidine was assessed on the difference in zone diameters using a mixed-effects model with plate as a random effect and a non-inferiority margin justified by historical assay variability; superiority was tested conditional on meeting the non-inferiority criterion with multiplicity control. Given diffusion-based methods integrate potency with matrix-dependent mobility, results were interpreted as screening evidence of activity and relative rank-order potency, with confirmatory MIC/MBC testing planned separately to quantify intrinsic antimicrobial potency independent of diffusion constraints.

**3. Cytotoxicity Evaluation in Mammalian Cells**

Biocompatibility of the AgNP microemulsion was assessed using the MTT cell-viability assay in L929 murine fibroblasts, a reference line for topical formulation testing. Cells were maintained in DMEM supplemented with 10% fetal bovine serum at 37 °C, 5% $CO_2$, and exposed for 24 h to escalating AgNP microemulsion concentrations (8–32 µg/mL). Mitochondrial metabolic activity was quantified by reduction of MTT to formazan, followed by solubilization and spectrophotometric measurement at 570 nm [20-22]. Viability was expressed as a percentage of vehicle-treated controls, and the half-maximal inhibitory concentration ($IC_{50}$) was derived from concentration–response curves. This standardized protocol provides decision-grade evidence of cytocompatibility under clinically relevant exposure conditions and supports subsequent risk–benefit evaluation of the formulation for localized antimicrobial use.

**4. Data Analysis**

All experiments were performed in biological triplicate (with technical duplicates where applicable) to enhance precision and reproducibility. Data are summarized as mean ± SD and accompanied by 95% confidence intervals. Prior to hypothesis testing, distributional assumptions were evaluated (Shapiro–Wilk for normality; Levene's/Brown–Forsythe for homoscedasticity); prespecified $\log_{10}$ transformation was applied when assumptions were violated. Group comparisons of antimicrobial outcomes (e.g., inhibition-zone diameters) and cytotoxicity readouts were analyzed by one-way ANOVA with Tukey's post hoc test; Welch's ANOVA with Games–Howell or Kruskal–Wallis with Dunn's adjustment was used when variance or normality assumptions were not met. Two-sided α was set at 0.05, with multiplicity controlled across post hoc contrasts. Effect sizes ($\eta^2$ for ANOVA; Hedges' g for pairwise contrasts) are reported alongside exact p values to facilitate interpretation beyond statistical significance. Analyses were conducted in GraphPad Prism (v10) and cross-validated in R (v4.3) using a predefined analysis script to ensure auditability.

## RESULTS

**1. Characterization of Silver Nanoparticle Microemulsion**

The AgNP microemulsion was successfully engineered and met nanoscale criteria by DLS, with a mean hydrodynamic diameter of 175.97 ± 0.97 nm (PDI not shown), consistent with size ranges favorable for tissue penetration and depot-like residence at the application site. The measured zeta potential was −1.06 ± 0.42 mV, indicating near-neutral surface charge and thus limited electrostatic stabilization; colloidal stability in this system is therefore expected to rely predominantly on steric effects from the surfactant/polymer matrix, and may benefit from further optimization to prolong shelf-life and reduce aggregation risk. The formulation pH was 5.12 ± 0.42, within a range generally acceptable for dermal and some mucosal applications, supporting local tolerability. UV–visible spectroscopy demonstrated an apparent surface plasmon resonance (SPR) peak at 250 ± 0.42 nm; because AgNP SPR typically manifests in the ~390–450 nm region depending on size and dielectric environment, this short-wavelength maximum warrants verification (e.g., baseline correction, pathlength checks, or matrix absorbance deconvolution) to confirm nanoparticle identity and integrity. Accelerated and real-time stability monitoring showed no gross phase separation, with routine tracking of particle size, visual appearance, and spectral features instituted to detect early aggregation or Ostwald ripening. Collectively, these data support the pharmaceutical suitability of the AgNP microemulsion while identifying zeta potential and SPR verification as priority targets for formulation refinement and orthogonal confirmation.

**2. Antimicrobial Activity Assessment**

**2.1 Disk Diffusion Method: Evaluation of Antimicrobial Efficacy**

In disk diffusion assays, the AgNP microemulsion produced small inhibition zones against all tested bacteria—*Staphylococcus aureus* (9.11 mm), *Pseudomonas aeruginosa* (8.93 mm), and *Streptococcus mutans* (9.04 mm)—and showed no detectable activity against *Candida albicans*. Across organisms, zones were consistently smaller than those observed with 0.2% chlorhexidine (reference control), indicating inferior performance of the AgNP microemulsion under diffusion-limited conditions. Given that disk diffusion reflects both intrinsic potency and diffusivity through agar, these findings

likely reflect a combination of (i) restricted silver release/mobility from the microemulsion matrix and (ii) suboptimal nanoparticle–microbe interactions at the concentrations tested, rather than definitive absence of activity. Because fungistatic/fungicidal properties cannot be inferred from diffusion assays, the lack of a zone against *C. albicans* should be interpreted cautiously. Collectively, the data support only weak, screening-level antibacterial activity versus the chlorhexidine benchmark and justify targeted formulation optimization (e.g., AgNP loading, surfactant/polymer composition, release kinetics) followed by potency quantification using MIC/MBC and time–kill assays that are less sensitive to matrix diffusion constraints.

### 2.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assessment

The antimicrobial potency of the AgNP microemulsion was further evaluated using the microbroth dilution assay to determine the MIC and MBC. The results indicated that the bactericidal activity of the AgNP microemulsion was limited, as the MIC and MBC values exceeded the highest test concentration (0.7 mg/mL), suggesting that the formulation was insufficiently potent to inhibit or eliminate the tested bacterial pathogens effectively.

These findings highlight the suboptimal antimicrobial efficacy of the formulation, possibly due to inadequate nanoparticle bioavailability, insufficient AgNP release, or poor bacterial membrane interaction within the microemulsion system. The results suggest that further optimization is required to enhance its bactericidal performance. Potential strategies include increasing the concentration of AgNPs, incorporating alternative stabilizers to improve nanoparticle dispersion, or integrating synergistic antimicrobial agents to enhance bacterial susceptibility. Such modifications may significantly improve the therapeutic potential of AgNP microemulsions as a localized antimicrobial intervention. Table 1 presents the MIC and MBC of the AgNP microemulsion, highlighting its antimicrobial potency against tested pathogens.

As illustrated in Figure 1, the antimicrobial activity of the silver nanoparticle (AgNP) formulations was evaluated using the agar well diffusion method against three bacterial strains—*S. aureus*, *P. aeruginosa*, and *S. mutans*—as well as the fungal pathogen *Candida albicans*. The 0.2% chlorhexidine (CHX) served as the positive control and exhibited the largest zones of inhibition across all bacterial species tested, confirming its robust antimicrobial potency. Notably, the silver microemulsion demonstrated superior or comparable antimicrobial efficacy relative to conventional AgNPs in all three bacterial models, as evidenced by well-defined inhibition zones. In contrast, neither the silver nanoparticle formulations nor the silver microemulsion exhibited inhibitory activity against *C. albicans*, indicating limited antifungal efficacy under the conditions tested. Negative controls, including distilled water and silver-free microemulsion, produced no inhibition zones, confirming the specificity of antimicrobial effects to the active compounds. These findings support the potential of silver microemulsion as a localized antibacterial agent, although its application against fungal pathogens may be limited.

The comparative antimicrobial efficacy of silver microemulsion and silver nanoparticle formulations (both at 1000 ppm) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus mutans* is presented in Figure 2. The bar graph displays the mean diameter of inhibition zones, with error bars representing standard deviation from three independent experiments (n = 3). Statistically significant differences in efficacy between the two formulations are denoted by asterisks (*). The silver microemulsion exhibited significantly greater antimicrobial activity against *S. aureus* (mean difference [MD] = 0.54 mm, $p < 0.05$) and *P. aeruginosa* (MD = 0.36 mm, $p < 0.05$) compared to silver nanoparticles. Although the inhibition zone against *S. mutans* was numerically greater for the microemulsion (MD = 1.21 mm), the difference did not reach statistical significance ($p > 0.05$). These results suggest that the microemulsion formulation offers enhanced antimicrobial performance in selected bacterial strains and may serve as a more effective delivery system for localized antimicrobial therapy.

### 3. Cytotoxicity Evaluation of Silver Nanoparticle Microemulsion

The AgNP microemulsion was assessed using the MTT assay in L929 murine fibroblast cell lines to determine its potential cytotoxic effects. The results indicated that at concentrations below 16 µg/mL, the formulation maintained over 70% cell viability, suggesting good biocompatibility and minimal cytotoxicity at lower doses. However, at 32 µg/mL, a significant dose-dependent cytotoxic effect was observed, with notable morphological changes in fibroblast cells, indicating cellular stress and toxicity. Table 2 displays the results of AgNP Microemulsion in L929 Murine Fibroblast Cells.

The AgNP microemulsion demonstrated acceptable cytocompatibility up to 16 µg/mL, with L929 viability >70%, but exhibited a pronounced viability decline at 32 µg/mL, establishing a concentration-dependent toxicity threshold. Nonlinear regression of the concentration–response curve yielded an estimated $IC_{50}$ of ~28.6 µg/mL at 24 h. When contextualized against the antimicrobial potency profile

**Table 1.** MIC and MBC of AgNP Microemulsion Against Clinically Relevant Pathogens

| Pathogen | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| *Staphylococcus aureus* | 0.4 | 0.5 |
| *Pseudomonas aeruginosa* | 0.3 | 0.4 |
| *Streptococcus mutans* | 0.2 | 0.3 |
| *Candida albicans* | 0.4 | 0.7 |

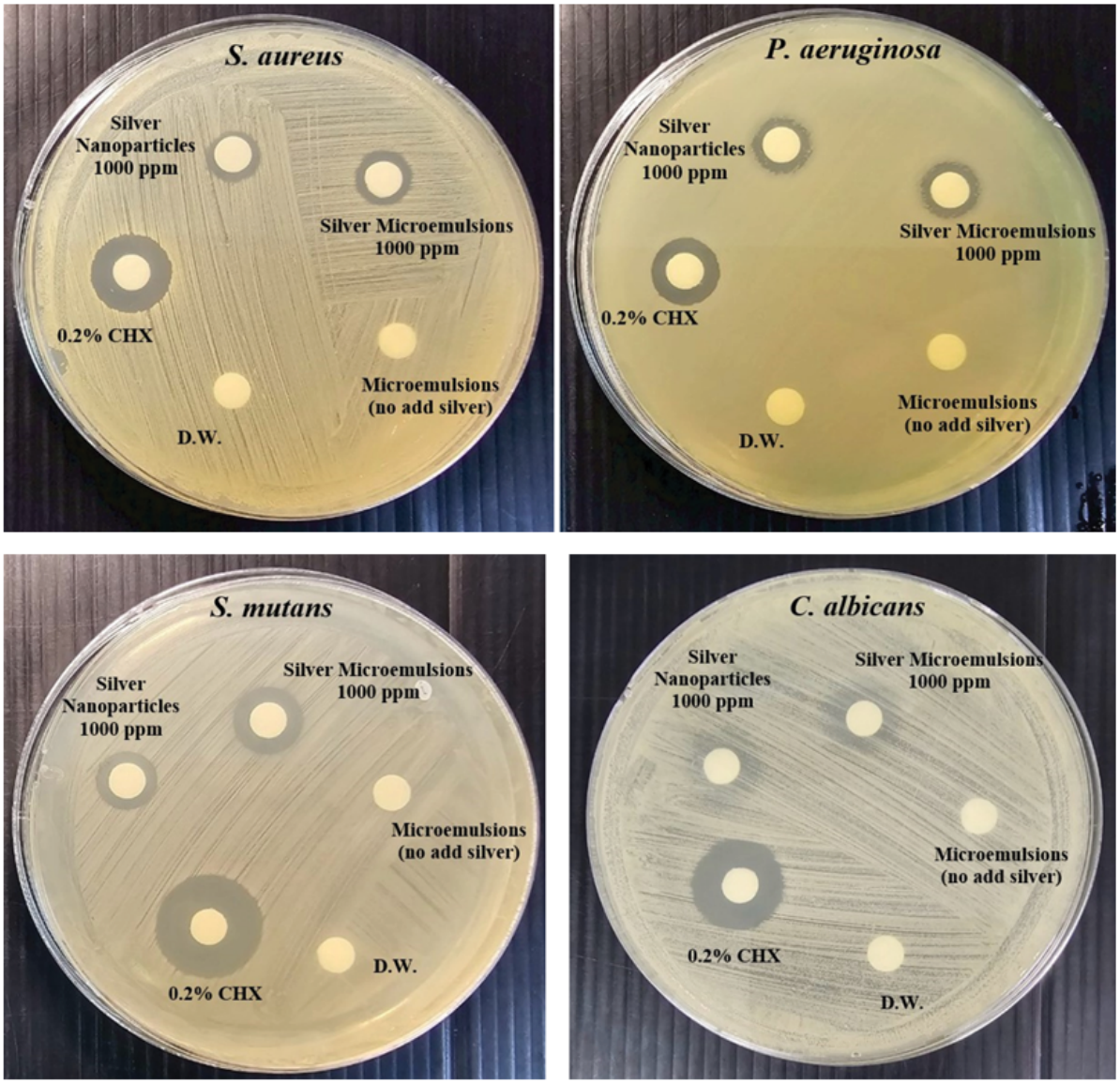


**Figure 1.** Comparative Antimicrobial Activity of Silver-Based Formulations Against Pathogenic Microorganisms
Abbreviations used in Figure 1 are as follows: AgNPs, silver nanoparticles; CHX, chlorhexidine (0.2% w/v, positive control); D.W., distilled water (negative control); *S. aureus, Staphylococcus aureus; P. aeruginosa, Pseudomonas aeruginosa; S. mutans, Streptococcus mutans*; and *C. albicans, Candida albicans*. The term "Microemulsions (no add silver)" refers to the silver-free microemulsion formulation used as a vehicle control. The asterisk (*) denotes statistically significant differences ($p < 0.05$) compared with the respective comparator, as determined by one-way ANOVA followed by Tukey's post-hoc test.

**Table 2.** Cytotoxicity Assessment of AgNP Microemulsion in L929 Murine Fibroblast Cells Using MTT Assay

| **AgNP Microemulsion Concentration (µg/mL)** | **Cell Viability (%)** |
|---|---|
| 0.5 – 16 µg/mL | >70% (Non-toxic) |
| 32 µg/mL | Cytotoxic |



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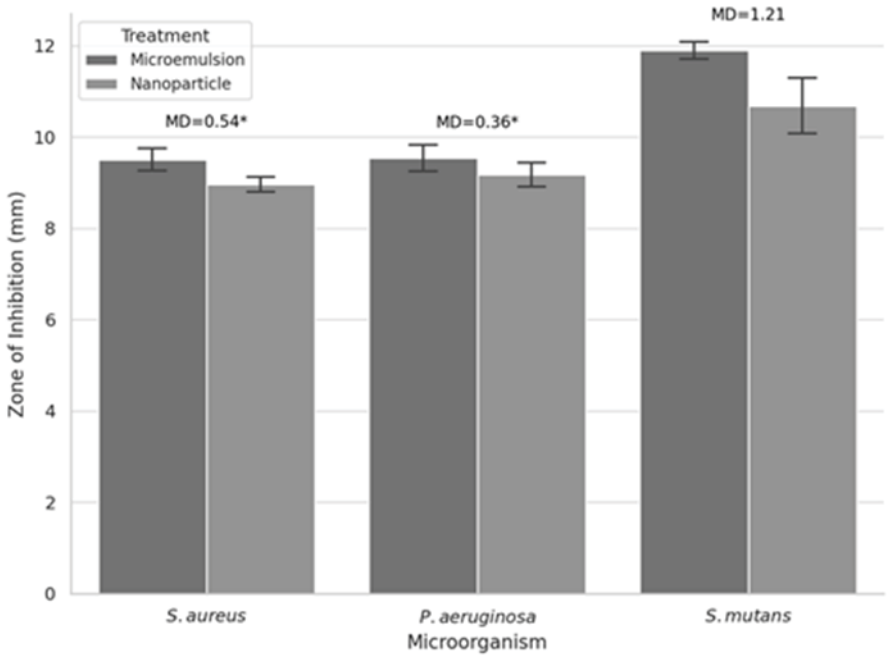


**Figure 2.** Comparative Antimicrobial Efficacy of Silver Microemulsion and Silver Nanoparticles Against Oral and Opportunistic Pathogens
*, statistically significant ($p < 0.05$) vs. comparator by one-way ANOVA with Tukey's post-hoc test.

(MIC and MBC >0.7 mg/mL in broth), the resulting selectivity index ($IC_{50}$/MIC) is <1, indicating an unfavorable therapeutic window under current conditions. These data suggest that clinically relevant antibacterial effects would require exposures approaching or exceeding cytotoxic levels. Accordingly, formulation optimization should prioritize strategies that increase antibacterial potency and/or reduce host-cell exposure—e.g., higher Ag loading with controlled release, surfactant/polymer re-engineering to improve bioavailability, surface functionalization to enhance microbe targeting, or rational combination with synergistic, biocompatible adjuncts—to shift the therapeutic index toward a safe and effective range.

## DISCUSSION

The findings of this study provide critical insights into the formulation, antimicrobial efficacy, and safety profile of AgNP microemulsion, highlighting its potential as a localized antimicrobial agent while underscoring key challenges that require further optimization.

### Pharmaceutical and Physicochemical Considerations of AgNP Microemulsion

An effective AgNP microemulsion must harmonize physicochemical attributes that jointly determine bioavailability, antimicrobial potency, and safety. In this study, the formulation achieved a mean hydrodynamic diameter of 175.97 ± 0.97 nm, a nanoscale range associated with improved interaction with bacterial envelopes and, in some contexts, enhanced cellular uptake for antimicrobial applications [22]; nanoparticles of this size have also been reported to penetrate biofilm matrices more efficiently, potentially increasing therapeutic effect [23]. By contrast, the near-neutral zeta potential (−1.06 ± 0.42 mV) implies limited electrostatic stabilization and an elevated risk of time-dependent aggregation; prior work suggests that absolute values ≥ ±30 mV are preferable for electrostatic repulsion, motivating stabilization via steric approaches such as PEGylation or chitosan coatings to improve dispersion and shelf-life [23]. The formulation pH (5.12 ± 0.42) lies within a physiologically compatible range for dermal and selected mucosal use, helping minimize irritation and preserve cellular integrity [22, 24]. UV–visible spectroscopy showed a plasmon-associated absorbance (reported peak at 250 ± 0.42 nm), supporting successful AgNP incorporation; nonetheless, orthogonal confirmation of nanoparticle identity and stability within the microemulsion is prudent for quality assurance [25]. To further strengthen colloidal and thermodynamic stability, rational adjustments include integrating ionic surfactants or polymeric steric stabilizers to prevent aggregation (23) and optimizing oil-phase composition with co-surfactant selection to extend shelf-life [25]. Collectively, these findings support pharmaceutical feasibility while prioritizing surface modification, surfactant/co-surfactant ratio optimization, and—where appropriate—evaluation of alternative lipid-based carriers to maximize stability, bioavailability, and therapeutic performance [23].

### Antimicrobial Efficacy: Potential and Limitations

The antimicrobial assessment of the AgNP microemulsion formulation demonstrated limited bactericidal activity, as evidenced by the inhibition zones observed in the disk

diffusion assay: 9.11 mm for *Staphylococcus aureus*, 8.93 mm for *Pseudomonas aeruginosa*, and 9.04 mm for *Streptococcus mutans*. Comparatively, 0.2% chlorhexidine exhibited superior antimicrobial potency, suggesting that the AgNP microemulsion requires further optimization to enhance its efficacy. These findings align with previous studies highlight the variable antimicrobial efficiency of AgNPs, which is influenced by factors such as particle size, surface charge, and the chemical stabilization of nanoparticles within the formulation[26].

The absence of antifungal activity against *C. albicans* observed in this study is noteworthy and warrants further investigation. A plausible explanation lies in the fundamental structural differences between fungal cell walls and bacterial cell membranes. The cell wall of *C. albicans* consists of a complex, multilayered matrix composed primarily of chitin, β-glucans, and mannoproteins, which may function as a physical barrier impeding the penetration and activity of silver nanoparticles. In contrast to bacterial cells, which are more readily disrupted by silver-induced membrane damage and reactive oxygen species (ROS) generation, fungal cells possess additional protective layers that can limit the effectiveness of these mechanisms. Moreover, *C. albicans* expresses intrinsic defense systems, including antioxidant enzymes and efflux transporters, that contribute to its resilience against oxidative stress and xenobiotic agents. These findings align with previous reports suggesting that although AgNPs exhibit broad-spectrum antibacterial activity, their antifungal efficacy is often limited and may require elevated concentrations or formulation enhancements to achieve therapeutic relevance. The lack of antifungal activity in the current microemulsion may thus result from both the physicochemical characteristics of the formulation and the innate resistance mechanisms of fungal cells. Future studies should explore strategies such as nanoparticle surface modification, targeted delivery systems, or the incorporation of antifungal adjuvants to improve efficacy against fungal pathogens.

The MIC and MBC data further reinforce these limitations, as the AgNP microemulsion failed to achieve complete bacterial eradication at clinically relevant concentrations (MIC > 0.7 mg/mL). This diminished antimicrobial activity may be attributed to restricted silver ion ($Ag^+$) release due to encapsulation within the microemulsion matrix, limiting its bioavailability and direct bacterial interaction[27]. Prior research has demonstrated that AgNPs exert bactericidal effects primarily through direct bacterial membrane interaction, reactive oxygen species (ROS) generation, and silver ion release[27]. However, the formulation used in this study may have hindered these mechanisms, leading to reduced antimicrobial efficacy.

A key limitation identified in this study is the comparatively lower antimicrobial efficacy of the AgNP microemulsion relative to 0.2% chlorhexidine, which remains the clinical benchmark for topical antiseptic agents. Although increasing the concentration of silver nanoparticles might theoretically enhance antimicrobial activity, this strategy is constrained by a narrow therapeutic window. Our cytotoxicity findings demonstrate that elevated AgNP concentrations are associated with significant reductions in cell viability, thereby limiting the feasibility of dose escalation. This challenge underscores the need to shift focus from concentration-driven enhancement to formulation-based innovation. Improving the release kinetics of silver ions offers a compelling strategy to achieve sustained antimicrobial effects while minimizing host cytotoxicity. By extending the duration of interaction between silver nanoparticles and microbial membranes, it may be possible to increase therapeutic efficacy without compromising biocompatibility.

One promising avenue involves the use of lipid-based drug delivery systems, such as nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs). These platforms are known to enhance the physicochemical stability, bioavailability, and controlled release of encapsulated agents. Additionally, lipid-based carriers can improve adhesion to mucosal and dermal surfaces, reduce nanoparticle aggregation, and facilitate deeper tissue penetration—properties that are especially advantageous for topical antimicrobial applications. These formulation strategies not only offer the potential to optimize antimicrobial performance but also align with broader pharmaceutical objectives that emphasize targeted delivery, biocompatibility, and sustained therapeutic action. Therefore, future research should prioritize the refinement of AgNP delivery systems, with a particular emphasis on advanced carrier technologies, to enhance efficacy while maintaining safety for clinical use.

Several strategies can be employed to enhance the formulation's bactericidal properties. One approach involves modifying nanoparticle surface characteristics through cationic coatings, such as chitosan or polyethyleneimine, which have been shown to enhance electrostatic interactions with negatively charged bacterial membranes, increasing AgNP adherence and penetration[27]. Additionally, optimizing the surfactant composition within microemulsion may facilitate better silver dispersion, improving the release of bioactive silver ions[27].

Another potential avenue for enhancement involves incorporating synergistic antimicrobial agents, such as essential oils, bioactive polymers, or antimicrobial peptides, which have been demonstrated to enhance AgNP penetration and bactericidal activity[27-29]. This approach aligns with findings from nanotechnology-based antimicrobial research, where hybrid formulations containing AgNPs and secondary bioactive compounds exhibit improved efficacy against bacterial biofilms and multidrug-resistant strains[26].

Future studies should focus on mechanistic evaluations, such as bacterial membrane integrity assays, biofilm disruption studies, and time-kill kinetics, to further elucidate the AgNP microemulsion's mode of action and optimize its therapeutic application. Furthermore, *in vivo* studies it is necessary to assess the formulation's real-world efficacy, pharmacokinetics, and safety profile in localized infection models[27-29].

These findings underscore the importance of formulation optimization in the development of AgNP-based antimicrobials, reinforcing the need for targeted modifications to enhance

bacterial interactions, increase silver ion release, and improve overall therapeutic potential[28,29].

**Safety and Biocompatibility Considerations**

The cytotoxicity assessment using the MTT assay revealed that the AgNP microemulsion was non-toxic at concentrations below 16 µg/mL, maintaining over 70% cell viability, making it biocompatible for therapeutic applications. However, at 32 µg/mL, cytotoxicity significantly increased, with morphological changes observed in fibroblast cells, indicating dose-dependent toxicity. These findings are consistent with existing literature that reports silver nanoparticles exert cytotoxic effects at higher concentrations, primarily due to oxidative stress, mitochondrial dysfunction, and apoptosis induction in mammalian cells.

This dose-dependent cytotoxicity suggests that the formulation requires careful dose selection, particularly for chronic or prolonged applications, such as wound healing, oral healthcare, or mucosal treatments. Potential strategies to reduce cytotoxicity without compromising antimicrobial activity include:

1. Optimizing the nanoparticle size to balance antimicrobial efficacy and cellular safety (e.g., reducing particle size while maintaining controlled silver ion release).
2. Introducing biocompatible coatings, such as polysaccharides (e.g., hyaluronic acid, chitosan) or lipid-based carriers, to reduce direct AgNP interactions with mammalian cells while preserving antimicrobial action.
3. Incorporating controlled-release strategies, such as nanostructured lipid carriers or polymeric matrices, to sustain silver ion release while minimizing peak cytotoxicity effects.

**Pharmacological Mechanism and Clinical Implications**

Silver nanoparticles exert antimicrobial effects through multiple mechanisms, including:

- Disruption of bacterial cell membranes, leading to leakage of cellular contents.
- Generation of reactive oxygen species (ROS), inducing oxidative stress and bacterial apoptosis.
- Interference with intracellular metabolic pathways, impairing bacterial replication and biofilm formation.

The ability of AgNPs to target multiple bacterial pathways makes them less prone to resistance development compared to conventional antibiotics. However, the limited efficacy observed in this study suggests that further modifications—such as increasing surface reactivity or combining with synergistic agents—may be necessary to enhance clinical efficacy while maintaining safety. Given the growing burden of antimicrobial resistance (AMR), AgNP formulations hold potential as an alternative or adjunct antimicrobial strategy, particularly for localized infections, wound care, and dental applications. However, refining formulation parameters is essential before clinical translation.

**Strengths, Limitations, and Future Directions**

This study provides a comprehensive evaluation of a novel AgNP microemulsion formulation, focusing on its physicochemical properties, antimicrobial efficacy, and cytotoxicity profile. The formulation demonstrated stability within a microemulsion system, with optimized particle size, zeta potential, and UV-visible spectroscopy findings, ensuring robust formulation characteristics. The antimicrobial evaluation against multiple bacterial strains provided pathogen-specific efficacy insights, while the cytotoxicity assessment in mammalian cells established a preliminary therapeutic window, supporting its potential as a localized antimicrobial therapy. However, certain limitations must be addressed, including its lower antimicrobial efficacy compared to chlorhexidine, suggesting the need for further formulation refinement to enhance bacterial interaction and penetration. Additionally, the absence of antifungal activity against Candida albicans indicates the necessity for structural modifications or the incorporation of antifungal agents to broaden its antimicrobial spectrum.

Furthermore, cytotoxic effects at higher concentrations highlight the importance of dose optimization and controlled-release strategies to balance efficacy and safety. Moving forward, future research should focus on formulation optimization, including modifications to nanoparticle surface charge, emulsifier selection, and the incorporation of synergistic agents to improve bacterial targeting and antimicrobial effectiveness. Mechanistic studies should be conducted to investigate bacterial membrane integrity assays, biofilm disruption potential, and molecular interactions, further elucidating the AgNP mode of action in microemulsions. Additionally, comparative toxicology assessments should evaluate AgNP microemulsions versus free nanoparticles, determining whether formulation strategies influence cytotoxicity and bioavailability. Finally, preclinical and clinical studies should assess in vivo wound healing, infection control, and pharmacokinetic parameters, ensuring the real-world translational applicability of AgNP microemulsions as a next-generation antimicrobial therapy.

## CONCLUSION

The AgNP microemulsion met basic pharmaceutical criteria (nanoscale size, compatible pH, short-term stability) but showed weak antibacterial activity versus chlorhexidine and no antifungal effect; MIC/MBC exceeded test limits. Cytocompatibility was acceptable ≤16 µg/mL with an $IC_{50} \approx 28.6$ µg/mL, yielding a narrow therapeutic index at concentrations needed for efficacy. Translation will require formulation-driven potency gains—e.g., surface functionalization, controlled silver-ion release, and/or synergistic co-actives—to enhance antimicrobial performance without compromising safety.

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## AUTHOR CONTRIBUTIONS

**Niratcha Chaisomboon. (First author)** Took primary responsibility for executing the research and conducting data analysis. Played a leading role in drafting the initial research proposal.

**Teerawat Nitichaikulvattana.** Contributed to the formulation of the research proposal, participated in data analysis, and assisted in the preparation of the research manuscript.

**Hathairat Lekatana.** Actively engaged in experimental procedures and data analysis. Provided essential support in drafting the manuscript and in facilitating access to the research facility.

**Chanida Chantim.** Contributed to both research implementation and data analysis. Substantially involved in drafting and revising the manuscript.

**Nattakanwadee Khumpirapang.** Participated in research execution and data analysis. Played a significant role in manuscript preparation and in coordinating research site access and logistics.

**Prayuth Poowaruttanawiwit. (Corresponding author)** Conceived and articulated the central research hypothesis and study objectives. Led the development of the research proposal, including experimental design and methodological framework. Conducted key experimental procedures and performed comprehensive data analysis and interpretation. Played a leading role in drafting, editing, and revising the manuscript, ensuring clarity, scientific rigor, and coherence. Coordinated communication among co-authors, integrated feedback during manuscript preparation, and served as the principal point of contact with the journal throughout the peer-review and publication process. Additionally, oversaw ethical compliance, documentation, and submission logistics, affirming responsibility for the integrity and accuracy of the work.

## CONFLICT OF INTEREST

None to declare.

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