



Synergistic Antibacterial Effects of Photosensitizing Silver Nanoparticles Synthesized from *Myrmecodia pendans* and Chitosan Nanoparticles against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Introduction: Silver nanoparticles (AgNPs) exhibit potent antimicrobial activity, and their combination with chitosan nanoparticles (CsNPs) may provide enhanced antibacterial effects. Although numerous eco-friendly approaches for AgNP synthesis have been reported, the use of green-synthesized AgNPs in combination with CsNPs for laser-assisted antibacterial applications remains limited. This study aimed to develop a rapid microwave-assisted green synthesis of AgNPs using *Myrmecodia pendans* extract and to evaluate the antibacterial and photodynamic effects of AgNPs–CsNPs formulations.

Methods: AgNPs were synthesized using *M. pendans* extract as a natural reducing and stabilizing agent rich in polyphenolic compounds. Antibacterial activity was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using bacterial growth assessment. Photodynamic inactivation was performed using a 405-nm blue laser with irradiation times of 90–180 s at different nanoparticle concentrations. Experiments were performed in five replicates, and data were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test.

Results: UV-Vis spectroscopy showed characteristic absorption peaks at approximately 430 nm and 290 nm for AgNPs and CsNPs, respectively. Dynamic light scattering revealed particle sizes below 45 nm for AgNPs and approximately 11 nm for CsNPs. The combined AgNPs–CsNPs formulations demonstrated significantly greater antibacterial activity than *M. pendans* extract alone against both *S. aureus* and *P. aeruginosa* ($P < 0.05$). Following 405-nm blue laser irradiation for 90–180 s, the AgNPs–CsNPs formulations produced near-complete bacterial reduction, particularly against *P. aeruginosa*, whereas non-irradiated treatments exhibited lower antibacterial activity.

Conclusion: Green-synthesized AgNPs produced using *M. pendans* extract can be effectively combined with CsNPs to enhance antibacterial activity. The combination of AgNPs–CsNPs with 405-nm blue laser irradiation showed enhanced photodynamic antibacterial performance, indicating its potential as a laser-assisted antimicrobial strategy against bacterial pathogens.

Keywords: Silver nanoparticles, chitosan nanoparticles, microwave-assisted synthesis, *Myrmecodia pendans* extract, photosensitizing antimicrobial materials.



Introduction

The widespread and often indiscriminate use of antibiotics has accelerated the emergence of resistant bacterial strains, contributing to increased rates of illness and death.^{1,2} Treating infections caused by multidrug-resistant pathogens typically involves extended therapeutic regimens and elevated medical expenses, placing a heavy strain on healthcare systems.³ This resistance weakens the effectiveness of conventional antibiotics, leading to both clinical and economic challenges.^{4,5} Bacteria acquire resistance through multiple pathways: genetic mutations, enzymatic modification of drug molecules, and structural changes in their cell membranes.^{6,7}

Certain species also evade treatment by surviving within host cells, where extracellular antibiotics have limited access.^{8–10} Biofilm formation worsens the problem by creating protective barriers that shield bacteria from antibiotics and environmental threats, making biofilm-associated infections particularly difficult to eradicate.^{11–13}

Antimicrobial resistance poses a significant challenge, necessitating the development of novel antimicrobial agents.¹⁴ Conventional antibiotics often fail against resistant and biofilm-associated infections, prompting interest in nanomaterials as alternatives. Due to their unique physicochemical properties, nanomaterials can

effectively target bacterial cells, disrupt cell membranes, and reduce resistance development.¹⁵ They also serve as carriers to protect antimicrobial agents and penetrate biofilm structures, making them promising candidates for next-generation therapies.¹⁶⁻¹⁸

Silver nanoparticles (AgNPs) are notable for their strong antibacterial properties, effective against both Gram-positive and Gram-negative bacteria.¹⁹⁻²¹ Their high surface area-to-volume ratio enables mechanisms such as silver ion release and reactive oxygen species (ROS) generation to disrupt bacterial membranes, contributing to cell death.²² Recent research indicates that AgNPs' antibacterial efficacy improves under light exposure by enhancing ROS production via surface plasmon resonance, offering potential for targeted bacterial inactivation in biomedical applications through photodynamic therapy while preserving surrounding tissues.²³⁻²⁵ This controllability over AgNPs' effects suggests promising future in antimicrobial applications.^{26,27}

Conventional synthesis methods for silver nanoparticles (AgNPs) face challenges such as low yields, high energy consumption, and environmental and safety risks.²⁸ As a solution, green synthesis using plant extracts and microorganisms has emerged, enabling safer, more sustainable production.²⁹ Microwave-assisted synthesis enhances this process by lowering activation energy and accelerating reaction rates, leading to efficient nucleation and nanoparticle growth.^{30,31} Plant extracts containing metabolites such as alkaloids and polyphenols act as natural reducing agents and stabilising ligands, supporting the green chemistry approach.³²⁻³⁶ This integrated method is promising for scalable AgNP production and is applicable in areas such as antimicrobial coatings and water purification.³⁷⁻⁴²

Chitosan nanoparticles (CsNPs) are eco-friendly, biocompatible, biodegradable materials derived from crustacean shells and possess strong antibacterial properties.⁴³ They target both Gram-positive and Gram-negative bacteria by binding to negatively charged components of bacterial cell surfaces, disrupting membrane integrity and causing bacterial death.⁴⁴⁻⁴⁷ While numerous studies have validated their effectiveness, the potential for synergistic combinations with microwave-assisted, plant-derived silver nanoparticles (AgNPs) remains largely unexplored, especially for developing efficient antimicrobial strategies.⁴⁸⁻⁵¹

This work presents a rapid and straightforward synthesis approach utilizing plant extract from *Myrmecodia pendans* as a reducing agent in the microwave-assisted preparation of AgNPs. These nanoparticles are subsequently combined with CsNPs to study the possibility of enhancing antimicrobial efficacy at reduced dosages. The synergistic antibacterial effects of AgNPs and CsNPs were evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* by assessing bacterial growth. Photodynamic inactivation was performed using blue laser irradiation (the wavelength within the 400-500 nm range) at varying exposure times and nanoparticle concentrations.

Materials and Methods

Synthesis of Silver Nanoparticles and Chitosan Nanoparticles

In this study, the bark of the *Myrmecodia pendans* plant, commonly known as 'pokok sarang semut', was ground into a fine powder and sieved prior to extraction.⁵² For the extraction process, 1 g of the powdered material was mixed with 100 mL of distilled water and stirred at room temperature for 10 minutes.⁵³⁻⁵⁷ Subsequently, the mixture was subjected to microwave irradiation at 30 W for 10 minutes.⁵⁸ The resulting plant extract solution was then refrigerated for use in the subsequent reduction process.

Silver nanoparticles (AgNPs) were synthesised using a green synthesis method by combining *Myrmecodia pendans* extract with varying concentrations of silver nitrate (AgNO₃), then irradiating at 30 W for 10 minutes, yielding a deep brown-red colour indicating completion.⁵⁹⁻⁶¹ Chitosan nanoparticles (CsNPs) were produced via ionic gelation by preparing chitosan solutions at 0.05%, 0.08%, and 0.10% in acetic acid. Sodium tripolyphosphate and TWEEN-80 were then added, and the mixture was stirred for 2 hours; the final product was analyzed for particle size to confirm nanoparticle formation.

UV-Vis Spectroscopy and Particle Size Analyses

UV-Vis spectroscopy was conducted in the range of 200 – 800 nm using a Shimadzu 1800 spectrophotometer to evaluate the optical absorption characteristics of the silver nanoparticles (AgNPs) and chitosan nanoparticles (CsNPs). This spectral range enables detection of characteristic absorbance peaks. Particle size distribution of AgNPs and CsNPs was determined using a BIOBASE BK-802N particle size analyser, which employs the Dynamic Light Scattering (DLS) technique.

Bacterial Culture

The initial stage of bacterial cultivation involved the preparation of 10 mL of sterile tryptone soy broth (TSB) medium in a test tube. Gram-positive strain *Staphylococcus aureus* (*S. aureus*) and Gram-negative strain *Pseudomonas aeruginosa* (*P. aeruginosa*) isolates were collected from agar plates using a sterile inoculating loop and subsequently cultured in the TSB medium. The cultures were incubated at 37 °C for 18-24 hours. Following incubation, the bacterial suspension was standardized according to the McFarland turbidity standard prior to plating. The final bacterial concentrations were standardized to 15×10^3 CFU/mL for *S. aureus* and 1.5×10^3 CFU/mL for *P. aeruginosa*.

Bacterial Growth Assessment

The antibacterial activity assay involved adding 50 µL of bacterial culture to solutions of varying concentrations of AgNPs (0.05%, 0.08%, and 0.10%) and CsNPs (1.0 mM, 1.5 mM, and 2.0 mM), mixed in a 1:1:1 ratio. The combinations were denoted as xAgNPs+yCsNPs. After transferring the suspension to Petri dishes and adding sterile tryptone soy agar (TSA), the samples were incubated at 37 °C for 18-24 hours, with five replicates for each group. Following

incubation, bacterial colonies were counted using the total plate count (TPC) method, and the percentage reduction in colonies was calculated⁶²:

$$\text{Colony reduction}(\%) = \frac{N_c - N_t}{N_c} \times 100\%$$

where N_c is the colony count of the untreated control, and N_t is the colony count of the treated sample. The percentage reduction was calculated for each replicate, and the final results are reported as mean $\pm$ standard deviation.⁶³

Photodynamic Laser Treatment of Bacteria Using Laser Treatment

Photodynamic inactivation was carried out using blue laser irradiation within the 400–500 nm range, with a wavelength of 405 nm selected for this study. The peak wavelength was verified using a Jasco CT-10 monochromator.⁶⁴ The laser power output was measured at 2.49 mW with an OMM-6810B-220V power meter, and the beam spot area was 0.13 cm². The irradiation dose was determined by calculating the energy density according to the following equation⁶⁵:

$$\text{Energy density (J / cm}^2\text{)} = \text{Intensity (W / cm}^2\text{)} \times \text{irradiation time (s)}$$

The study involved a laser probe positioned 10 mm from a 96-well microplate containing bacterial cultures. We prepared a treatment suspension by adding bacterial culture to combinations of AgNPs and CsNPs, then diluting with sterile water. Each well received blue laser irradiation for 90s, 120s, 150s, or 180s. After irradiation, we transferred the samples to Petri dishes, mixed them with TSA medium, and incubated them at 37 °C for 18–24 hours. We conducted tests in five replicates and assessed bacterial reduction using the total plate count method.⁶⁶ Five groups were categorised: a control group; groups with *Staphylococcus aureus* and *Pseudomonas aeruginosa* with and without laser treatment; and groups supplemented with either *M. pendans* extract or AgNPs and CsNPs combinations.

Statistical Analysis

All antibacterial experiments were performed in five replicates. Colony-forming units were counted using the total plate count method, and bacterial reduction was calculated for each replicate relative to the untreated control. Results are presented as mean $\pm$ standard deviation. For non-irradiated antibacterial assays, differences among treatment groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. For laser-treated samples, two-way ANOVA was used to evaluate the effects of nanoparticle formulation and irradiation time, followed by Tukey's post hoc test. Statistical significance was accepted at $P < 0.05$. In all figures, error bars represent standard deviation, and different letters indicate significant differences between groups.

Results

Figure 1 illustrates the visual differences between four samples: (a) the *Myrmecodia pendans* extract, which appears light brown; and (b–d) silver nanoparticles (AgNPs) synthesized using precursor concentrations of 1.0 mM, 1.5 mM, and 2.0 mM, respectively. Hereafter, these solutions will be referred to as 1.0 mM AgNPs, 1.5 mM AgNPs, and 2.0 mM AgNPs. A progressive change in colour intensity from pale reddish-brown to deep reddish-brown is observed with increasing AgNPs concentration, indicating possible variations in nanoparticle formation and surface plasmon resonance characteristics. Accordingly, UV-vis spectroscopy and particle size analysis were conducted to evaluate the absorbance characteristics and particle size distribution, respectively.

UV-Vis Spectroscopy

UV-Vis spectroscopy was performed within the visible light range (325–800 nm) to characterize the optical properties of AgNPs and CsNPs. AgNPs synthesized using precursor concentrations of 1.0 mM, 1.5 mM, and 2.0 mM consistently displayed maximum absorbance peaks at 430 nm (Figure 2a). Moreover, the absorbance intensity increases proportionally with the concentration of AgNPs, indicating a direct correlation between nanoparticle amount and optical response.

In contrast, chitosan nanoparticles with concentrations of 0.05%, 0.08%, and 0.10% exhibited absorption peaks at lower wavelengths, approximately 290 nm, as shown in Figure 2b, attributed to the $\pi \rightarrow \pi^*$ electronic transition of the amino groups.^{67–70} This transition involves the excitation of electrons from bonding π orbitals to anti-bonding π^* orbitals upon light absorption. Similarly, the absorbance intensity increases proportionally with the concentration of CsNPs. This size-dependent optical behaviour will be further validated through particle size analysis (PSA) in the following section.

Particle Size Analysis

The particle size of AgNPs synthesized using precursor concentrations of 1.0, 1.5, and 2.0 mM was determined using the dynamic light scattering (DLS) technique, as shown in Figure 3 (a–d). The simple microwave-assisted green synthesis process successfully produced nanoparticles

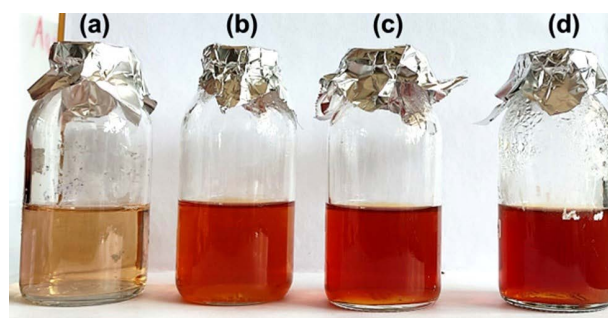


Figure 1. Physical appearance of (a) extract of bark 'pokok sarang semut' (*Myrmecodia pendans*), (b) AgNPs 1.0 mM, (c) AgNPs 1.5 mM, and (d) AgNPs 2.0 mM

with sizes ranging from 41 to 45 nm, with the highest concentration exhibiting the smallest particle diameter. Similarly, chitosan nanoparticles with concentrations of 0.05%, 0.08%, and 0.10% displayed nano-sized particles in a range of 41–45 nm, as illustrated in Figure 4 (a–d). The ion-gelation synthesis method demonstrated effective production of nanoparticles within the size range of 7–11 nm. Notably, the lowest CsNPs concentration (0.05%) resulted in the smallest average diameter, indicating a concentration-dependent effect on particle size reduction.

Synergistic Effect of Silver Nanoparticles and Chitosan Nanoparticles

The bacterial growth of *S. aureus* and *P. aeruginosa* was assessed using two experimental conditions: (a) one without

laser exposure and (b) with laser treatment. In both setups, the bacterial strains were incubated for 18–24 hours at 37 °C in (i) plant extract *M. pendans* and (ii) $x\text{AgNPs}+y\text{CsNPs}$ combinations. Following incubation, the viable bacterial colonies were calculated using the TPC method. The antibacterial effect was evaluated based on the reduction in bacterial colonies compared to the control group.

Synthesis of Silver Nanoparticles and Chitosan Nanoparticles

M. pendans extract is rich in polyphenolic compounds that act as reducing agents, facilitating the reduction of silver ions (Ag^+) to metallic silver (Ag^0).^{71,72} The selected microwave-assisted synthesis technique overcomes the heating inhomogeneity typically encountered in conventional thermal methods by providing rapid volumetric heating

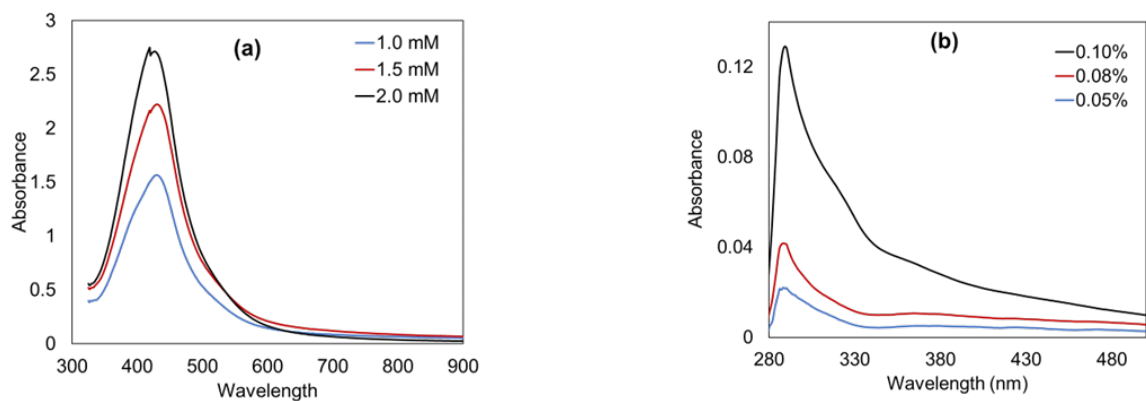


Figure 2. UV-Vis spectra of (a) AgNPs synthesized using precursor concentrations of 1.0 mM, 1.5 mM, and 2.0 mM, (b) CsNPs with different concentrations 0.05%, 0.08%, and 0.10%

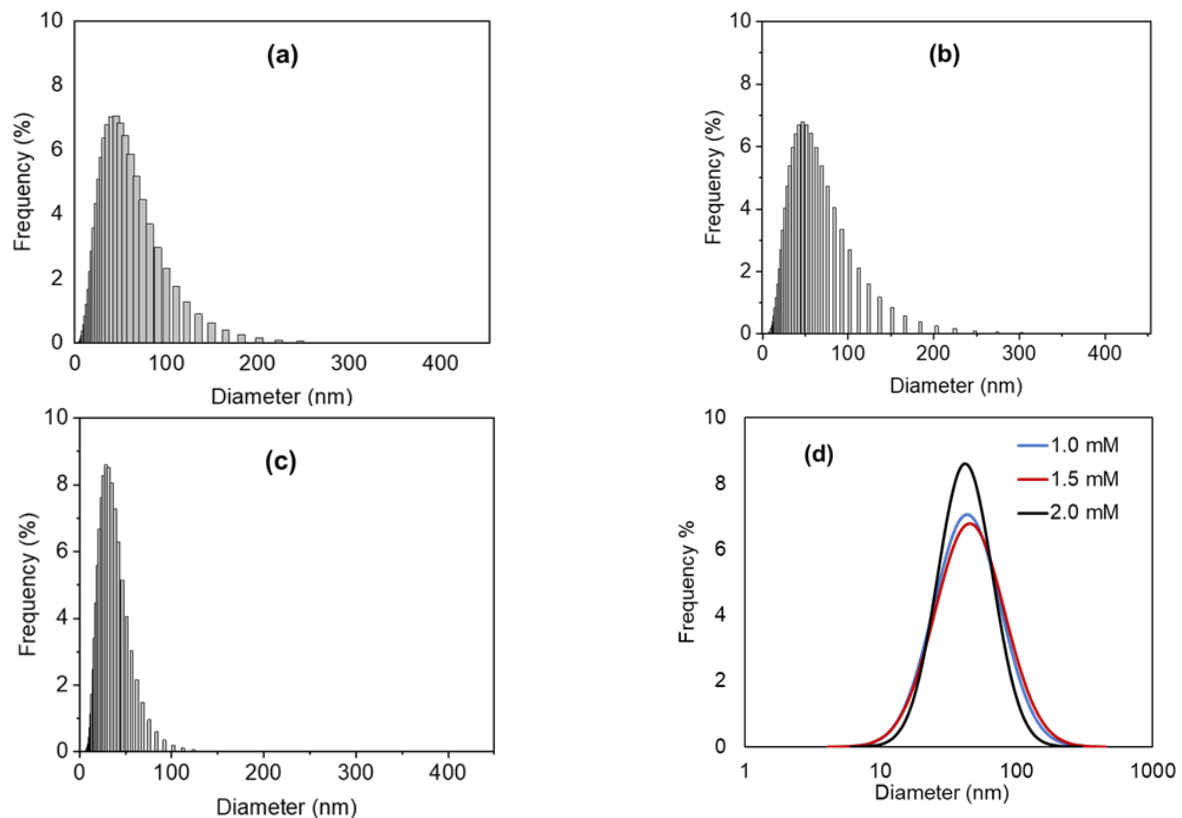


Figure 3. PSA of AgNPs synthesized using precursor concentrations of (a) 1.0 mM, (b) 1.5 mM, and (c) 2.0 mM, and (d) comparison of size at concentration variations

and enhanced reaction kinetics.⁷³ This leads to faster initial heating, accelerated reaction rates, efficient consumption of starting materials, and higher product yields.⁷⁴ The rapid, uniform heating also promotes a high nucleation rate, yielding numerous small nuclei and limiting extensive particle growth.⁷⁵ Simultaneously, polyphenol-rich *M. pendans* extract acts as a capping and stabilizing agent by adsorbing onto emerging nanoparticle surfaces and preventing aggregation. This stabilization influences the observed localized surface plasmon resonance (LSPR) near 430 nm and the DLS-measured size distribution.^{46,76,77} Thus microwave-assisted synthesis combines fast kinetics and particle surface stabilization, explaining the nano-sized AgNPs and their optical signatures reported in this work.

Chitosan nanoparticles were formed via ionic gelation, where protonated amino groups on chitosan electrostatically interact with the polyanionic TPP, forming ionically crosslinked nanoscale domains and generating a stable colloidal system.^{78–80} Rapid complexation upon mixing yields many nucleation sites, and the resulting particle size is governed by the chitosan concentration,

chitosan: TPP ratio, pH, stirring conditions, and presence of surfactants.^{81–83} In our methodology, the addition of Tween-80 improved chitosan solubility and provided steric stabilization, limiting aggregation and contributing to the microspheres.^{74,84}

Without Laser Irradiation on *S. aureus* and *P. aeruginosa*

The synergistic antibacterial properties of $x\text{AgNPs}+y\text{CsNPs}$ formulations against *S. aureus* and *P. aeruginosa* can be observed in Figure 5 (a & b), as bacterial growth was progressively suppressed with increasing nanoparticle concentration. For *S. aureus*, approximately 27% bacterial death was observed in *M. pendans* extract, while mixtures containing increasing concentrations of AgNPs and CsNPs produced progressively greater death (29 – 60%), with the strongest effect at 2 mM AgNPs+0.10% CsNPs resulting in an 86% reduction. In contrast, *P. aeruginosa* exhibited only ~10% reduction in the plant extract alone; however, a marked increase was achieved with the $x\text{AgNPs}+y\text{CsNPs}$ combinations. Complete inhibition (100% bacterial death) was observed at 1 mM AgNPs+0.10% CsNPs and

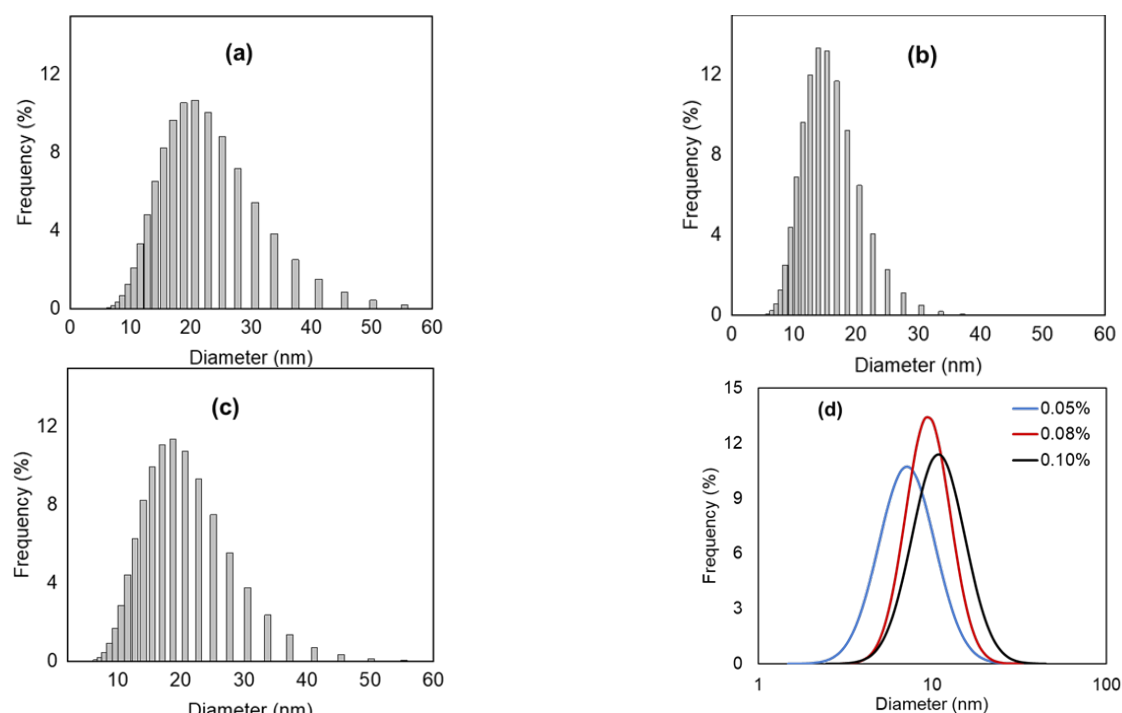


Figure 4. PSA of CsNPs with different concentrations: (a) 0.05 %, (b) 0.08 %, (c) 0.10 %, and (d) comparison of size at concentration variations

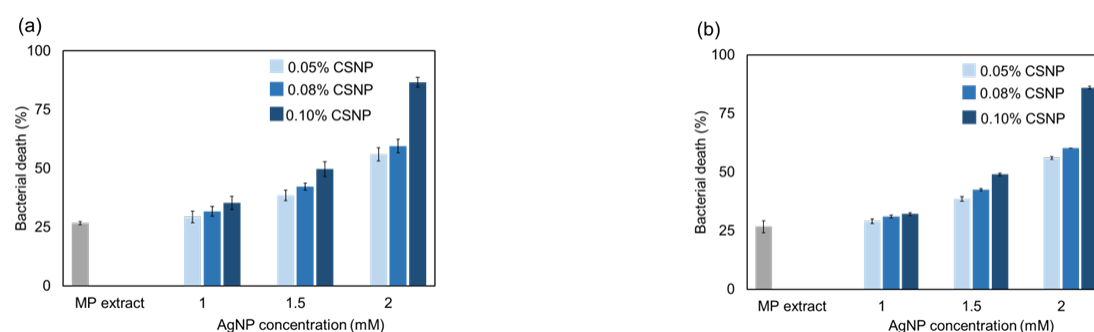


Figure 5. Bacterial reduction of (a) *S. aureus* and (b) *P. aeruginosa* after treatment with *M. pendans* extract and different AgNPs–CsNPs formulations under non-irradiated conditions. Data are presented as mean $\pm$ SD, $n=5$. Different letters indicate statistically significant differences between treatment groups based on one-way ANOVA followed by Tukey's post hoc test, $P<0.05$.

maintained at higher concentrations.

Laser irradiation studies on *S. aureus* and *P. aeruginosa*

The effect of photodynamic treatment against *S. aureus* and *P. aeruginosa* can be observed in Figure 6 and Figure 7 (a - d) respectively, as bacterial growth was completely suppressed with increasing irradiation exposure time. The bacterial suspensions were prepared in two different solutions: (i) *M. pendans* (MP) extract and (ii) various xAgNPs+yCsNPs mixtures prior to blue laser irradiation at varying exposure times of 90, 120, 150, and 180 s, corresponding to different energy densities. The antibacterial effect was evaluated based on the reduction in bacterial colonies compared to the control group, followed by laser irradiation with a blue laser and incubation for 1824 hours.

Figure 7 (a - d) presents the death percentage of *S. aureus* at different exposure times. In the plant extract alone, the bacterial reduction ranged from 32 – 60% after irradiation. In contrast, the xAgNPs+yCsNPs combinations produced nearly complete inhibition (~100% cell death) even at the shortest exposure time. Similarly, Figure 8 (a - d) illustrates the effect on *P. aeruginosa*. In the plant extract alone, bacterial reduction ranged from 35–93%. However, with xAgNPs+yCsNPs combinations, almost complete inhibition (~100% cell death) was achieved across all exposure times. These findings highlight the prominent effect of laser treatment on bacterial colonies in MP extract

and xAgNPs+yCsNPs formulations, even at relatively short exposure times.

Discussion

Green Synthesis and Physicochemical Characteristics of AgNPs and CsNPs

The microwave-assisted green synthesis using *Myrmecodia pendans* extract provided a rapid approach for producing AgNPs. The polyphenolic compounds present in the plant extract can facilitate the reduction of Ag⁺ to metallic

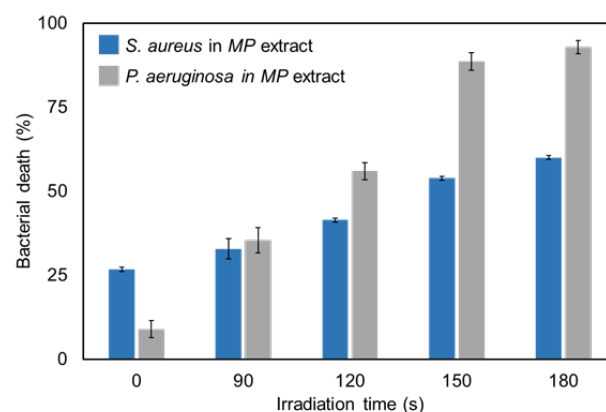


Figure 6: Comparison of bacterial death of *S. aureus* and *P. aeruginosa* in MP extract when exposed under blue laser treatment with time variations of 90 s, 120 s, 150 s and 180 s

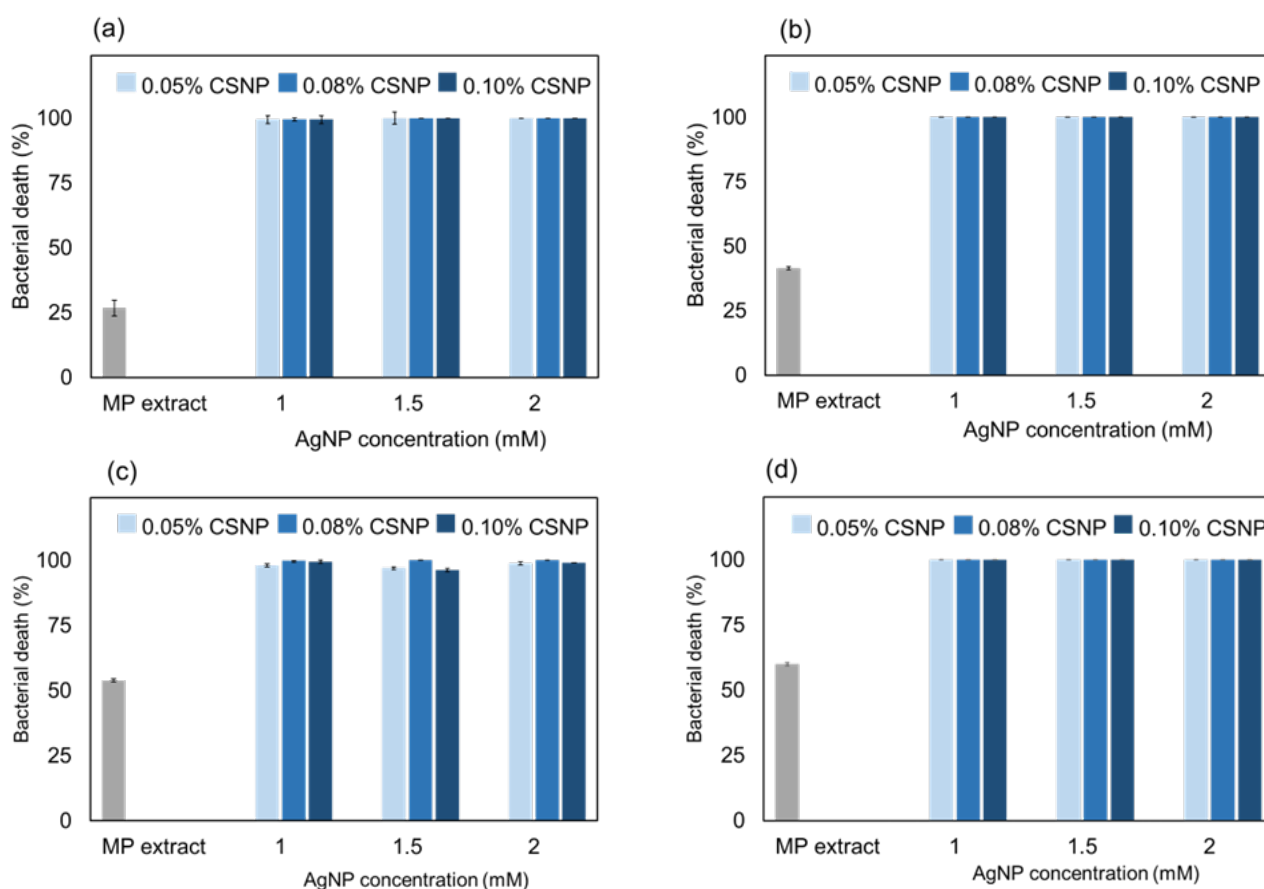


Figure 7. Bacterial reduction of *S. aureus* treated with *M. pendans* extract and different AgNPs–CsNPs formulations under 405-nm blue laser irradiation for 90, 120, 150, and 180 s. Data are presented as mean ± SD, n = 5. Different letters indicate statistically significant differences between treatment groups based on two-way ANOVA followed by Tukey's post hoc test, $P < 0.05$.

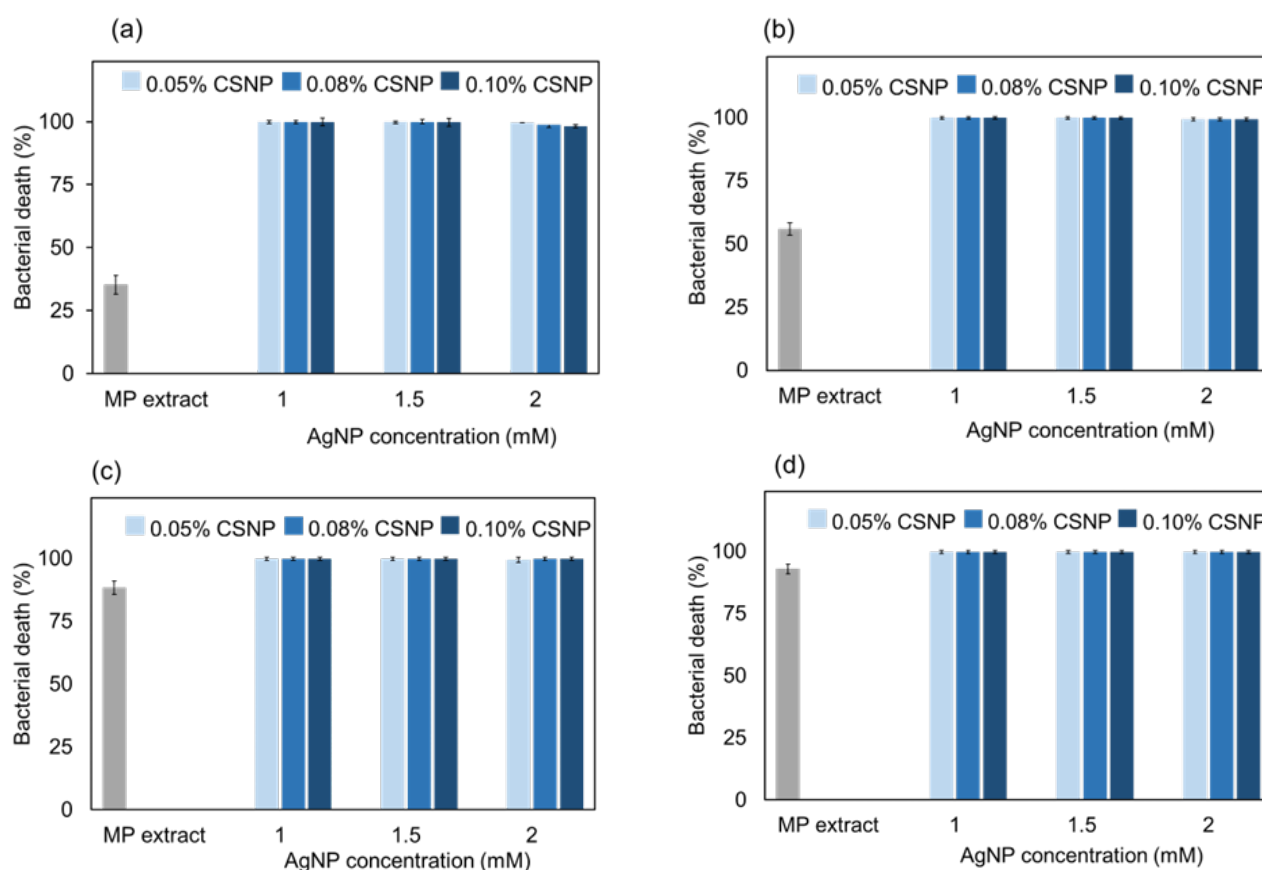


Figure 8. Bacterial reduction of *P. aeruginosa* treated with *M. pendans* extract and different AgNPs–CsNPs formulations under 405-nm blue laser irradiation for 90, 120, 150, and 180 s. Data are presented as mean $\pm$ SD, $n=5$. Different letters indicate statistically significant differences between treatment groups based on two-way ANOVA followed by Tukey's post hoc test, $P<0.05$.

Ag⁰ and may subsequently contribute to nanoparticle stabilization through adsorption onto the particle surface.^{85,86} Microwave irradiation provides rapid and relatively uniform volumetric heating, which can accelerate reaction kinetics and promote efficient nanoparticle formation.^{87,88} The rapid heating process may also favour nucleation over extensive particle growth, contributing to the formation of nanoscale AgNPs.⁸⁹ The observed LSPR peak around 430 nm is consistent with the characteristic optical response of AgNPs and supports the successful formation of the nanoparticles.^{46,90,91}

The CsNPs were prepared through ionic gelation, in which protonated amino groups of chitosan interact electrostatically with the polyanionic TPP to form crosslinked nanoscale structures.⁹² The resulting particle characteristics can be influenced by several formulation parameters, including chitosan concentration, the chitosan-to-TPP ratio, pH, stirring conditions, and the presence of surfactants.⁹³ In the present formulation, Tween 80 may have contributed to improved chitosan solubility and reduced particle aggregation, thereby influencing the observed particle size.^{84,94} The UV–Vis and particle-size results together indicate that the physicochemical characteristics of both AgNPs and CsNPs were dependent on their formulation conditions.

Synergistic Antibacterial Activity of AgNPs–CsNPs

The AgNPs–CsNPs formulations demonstrated greater

antibacterial activity than the *M. pendans* extract alone against both *S. aureus* and *P. aeruginosa*. The concentration-dependent increase in bacterial reduction suggests that increasing the amount of nanoparticles enhanced the overall antibacterial effect. This observation is consistent with previous reports describing enhanced antibacterial activity when silver nanoparticles are combined with chitosan-based materials.^{95,96}

Several mechanisms contribute to the antibacterial activity of silver nanoparticles (AgNPs), including interactions with bacterial cell membranes, increased permeability, and structural damage. AgNPs may affect proteins, nucleic acids, and respiratory enzymes, leading to cell death via reactive oxygen species (ROS). Chitosan enhances antibacterial effects through electrostatic interactions with bacterial surfaces, destabilising membranes and promoting leakage of intracellular components. Its efficacy is pH-dependent, with higher activity in acidic conditions. When combined, chitosan and AgNPs show complementary actions that improve antibacterial effectiveness. Differences in the responses of *S. aureus* and *P. aeruginosa* may stem from variations in their cell-envelope structures, which affect nanoparticle interactions and susceptibility to antimicrobial agents.^{97–99}

Effect of 405-nm Blue-Laser Irradiation

Blue-laser irradiation substantially enhanced the antibacterial activity of the AgNPs–CsNPs formulations.

The combination of nanoparticles with blue-light exposure resulted in almost complete bacterial inhibition at relatively short irradiation times for both bacterial strains. This enhanced effect suggests that photoactivation contributed to the overall antimicrobial activity of the nanoparticle formulations.

The increased antibacterial effect following laser irradiation may be associated with enhanced ROS generation and increased availability of silver ions under illumination.^{100,101} The nanoscale dimensions of the AgNPs–CsNPs system may further facilitate interaction with bacterial surfaces and increase surface reactivity during irradiation.¹⁰² These combined effects provide a possible explanation for the pronounced antibacterial activity observed following blue-laser treatment.

The response to laser irradiation also differed between the two bacterial species. *P. aeruginosa* showed a more pronounced exposure-dependent increase in bacterial reduction, whereas *S. aureus* exhibited a different response pattern. Such differences may arise from variations in cell-envelope architecture and the interaction of the nanoparticles with the respective bacterial surfaces. Nevertheless, the strong reduction observed for both strains following treatment with AgNPs–CsNPs and blue-laser irradiation supports the potential of this combined approach as a laser-assisted antimicrobial strategy.

Limitations and Future Perspectives

Several limitations should be considered when interpreting these findings. First, the study was conducted using planktonic bacteria and did not evaluate established biofilms. Therefore, the observed antibacterial effects may not directly predict activity against mature biofilms or in vivo infections.^{103,104} Second, although increased ROS generation and silver-ion release may contribute to the photoactivated antibacterial effect, these parameters were not directly quantified in the present study. Direct measurements of ROS and Ag⁺ release would therefore be necessary to clarify the relative contributions of these mechanisms.¹⁰⁵ Third, cytotoxicity and biocompatibility toward mammalian cells were not evaluated, limiting conclusions regarding the safety and translational potential of the formulations.¹⁰⁶ Finally, the experiments were performed at laboratory scale using a limited number of bacterial strains and replicates. Further studies involving additional strains, biofilm models, cytotoxicity assessment, mechanistic investigations, and in vivo validation are warranted.

Conclusion

This study demonstrated that green-synthesized AgNPs combined with CsNPs and activated by 405-nm blue laser irradiation produced strong antibacterial activity against both *S. aureus* and *P. aeruginosa*. The antibacterial results were expressed as mean $\pm$ SD from five replicates and statistically analysed using ANOVA followed by Tukey's post hoc test. The AgNPs–CsNPs formulations significantly reduced bacterial colonies compared with *M. pendans* extract alone ($P < 0.05$). Blue laser irradiation

further enhanced bacterial reduction, with near-complete inhibition observed under the tested irradiation times. The improved antibacterial performance may be attributed to AgNP-mediated silver ion release and reactive oxygen species generation, together with the membrane-disruptive and stabilizing effects of CsNPs. However, because AgNP-only and CsNP-only controls were not included, the results should be described as an enhanced combined antibacterial effect rather than definitive synergy. Future studies should include individual nanoparticle controls, formal synergy analysis, ROS measurement, Ag⁺ release quantification, biofilm testing, and cytotoxicity evaluation.

Authors' Contribution

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Validation: Iman Aris Fadzallah, Suryani Dyah Astuti
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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was conducted entirely in vitro and did not involve human participants, animals, or human biological samples. Therefore, ethical approval or clearance was not required.

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References

1. Zhu X, Tang Q, Zhou X, Momeni MR. Antibiotic resistance and nanotechnology: a narrative review. *Microb Pathog* 2024;193:106741. doi:10.1016/j.micpath.2024.106741
2. Albrich WC, Monnet DL, Harbarth S. Antibiotic selection pressure and resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Emerg Infect Dis* 2004;10(3):514-7. doi:10.3201/eid1003.030252
3. Gupta A, Mumtaz S, Li CH, Hussain I, Rotello VM. Combating antibiotic-resistant bacteria using nanomaterials. *Chem Soc Rev* 2019;48(2):415-27. doi:10.1039/c7cs00748e
4. Singh R, Kim K. Environmental occurrence of antibiotic resistance, control measures and challenges in finding therapeutic management. *Emerg Contam* 2025;11(1):100440. doi:10.1016/j.emcon.2024.100440
5. Ahmad M, Khan AU. Global economic impact of antibiotic resistance: a review. *J Glob Antimicrob Resist* 2019;19:313-6. doi:10.1016/j.jgar.2019.05.024
6. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Microbiol Spectr* 2016;4(2):10.1128/microbiolspec.VMBF-

- 0016-2015. doi:[10.1128/microbiolspec.VMBF-0016-2015](https://doi.org/10.1128/microbiolspec.VMBF-0016-2015)
7. Sun S. Emerging antibiotic resistance by various novel proteins/enzymes. *Eur J Clin Microbiol Infect Dis* 2025;44(7):1551-66. doi:[10.1007/s10096-025-05126-4](https://doi.org/10.1007/s10096-025-05126-4)
8. Chen Y, Jiang Y, Xue T, Cheng J. Strategies for the eradication of intracellular bacterial pathogens. *Biomater Sci* 2024;12(5):1115-30. doi:[10.1039/d3bm01498c](https://doi.org/10.1039/d3bm01498c)
9. Hajipour MJ, Fromm KM, Ashkarran AA, Jimenez de Aberasturi D, de Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. *Trends Biotechnol* 2012;30(10):499-511. doi:[10.1016/j.tibtech.2012.06.004](https://doi.org/10.1016/j.tibtech.2012.06.004)
10. Riffaud CM, Rucks EA, Ouellette SP. Persistence of obligate intracellular pathogens: alternative strategies to overcome host-specific stresses. *Front Cell Infect Microbiol* 2023;13:1185571. doi:[10.3389/fcimb.2023.1185571](https://doi.org/10.3389/fcimb.2023.1185571)
11. Almatroudi A. Biofilm resilience: molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology (Basel)* 2025;14(2):165. doi:[10.3390/biology14020165](https://doi.org/10.3390/biology14020165)
12. Liu HY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. *NPJ Antimicrob Resist* 2024;2(1):27. doi:[10.1038/s44259-024-00046-3](https://doi.org/10.1038/s44259-024-00046-3)
13. Wang D, Wang L, Liu Q, Zhao Y. Virulence factors in biofilm formation and therapeutic strategies for *Staphylococcus aureus*: a review. *Animals and Zoonoses* 2025;1(2):188-202. doi:[10.1016/j.azn.2024.11.003](https://doi.org/10.1016/j.azn.2024.11.003)
14. Parvin N, Joo SW, Mandal TK. Nanomaterial-based strategies to combat antibiotic resistance: mechanisms and applications. *Antibiotics (Basel)* 2025;14(2):207. doi:[10.3390/antibiotics14020207](https://doi.org/10.3390/antibiotics14020207)
15. Solanki R, Makwana N, Kumar R, Joshi M, Patel A, Bhatia D, et al. Nanomedicines as a cutting-edge solution to combat antimicrobial resistance. *RSC Adv* 2024;14(45):33568-86. doi:[10.1039/d4ra06117a](https://doi.org/10.1039/d4ra06117a)
16. Makabenta JM, Nabawy A, Li CH, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat Rev Microbiol* 2021;19(1):23-36. doi:[10.1038/s41579-020-0420-1](https://doi.org/10.1038/s41579-020-0420-1)
17. Munir MU, Ahmad MM. Nanomaterials aiming to tackle antibiotic-resistant bacteria. *Pharmaceutics* 2022;14(3):582. doi:[10.3390/pharmaceutics14030582](https://doi.org/10.3390/pharmaceutics14030582)
18. Sedighi O, Bednarke B, Sherriff H, Doiron AL. Nanoparticle-based strategies for managing biofilm infections in wounds: a comprehensive review. *ACS Omega* 2024;9(26):27853-71. doi:[10.1021/acsomega.4c02343](https://doi.org/10.1021/acsomega.4c02343)
19. Pal G, Rai P, Pandey A. Green synthesis of nanoparticles: a greener approach for a cleaner future. In: Shukla AK, Iravani S, eds. *Green Synthesis, Characterization and Applications of Nanoparticles*. Elsevier; 2019. p. 1-26. doi:[10.1016/b978-0-08-102579-6.00001-0](https://doi.org/10.1016/b978-0-08-102579-6.00001-0)
20. Peretyazhko TS, Zhang Q, Colvin VL. Size-controlled dissolution of silver nanoparticles at neutral and acidic pH conditions: kinetics and size changes. *Environ Sci Technol* 2014;48(20):11954-61. doi:[10.1021/es5023202](https://doi.org/10.1021/es5023202)
21. Quinteros MA, Cano Aristizábal V, Dalmasso PR, Paraje MG, Páez PL. Oxidative stress generation of silver nanoparticles in three bacterial genera and its relationship with the antimicrobial activity. *Toxicol In Vitro* 2016;36:216-23. doi:[10.1016/j.tiv.2016.08.007](https://doi.org/10.1016/j.tiv.2016.08.007)
22. Bruna T, Maldonado-Bravo F, Jara P, Caro N. Silver nanoparticles and their antibacterial applications. *Int J Mol Sci* 2021;22(13):7202. doi:[10.3390/ijms22137202](https://doi.org/10.3390/ijms22137202)
23. Qing Y, Cheng L, Li R, Liu G, Zhang Y, Tang X, et al. Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *Int J Nanomedicine* 2018;13:3311-27. doi:[10.2147/ijn.S165125](https://doi.org/10.2147/ijn.S165125)
24. Casals E, Gusta MF, Bastus N, Rello J, Puentes V. Silver nanoparticles and antibiotics: a promising synergistic approach to multidrug-resistant infections. *Microorganisms* 2025;13(4):952. doi:[10.3390/microorganisms13040952](https://doi.org/10.3390/microorganisms13040952)
25. Khalifa HO, Oreiby A, Mohammed T, Abdelhamid MA, Sholkamy EN, Hashem H, et al. Silver nanoparticles as next-generation antimicrobial agents: mechanisms, challenges, and innovations against multidrug-resistant bacteria. *Front Cell Infect Microbiol* 2025;15:1599113. doi:[10.3389/fcimb.2025.1599113](https://doi.org/10.3389/fcimb.2025.1599113)
26. More PR, Pandit S, Filippis A, Franci G, Mijakovic I, Galdiero M. Silver nanoparticles: bactericidal and mechanistic approach against drug resistant pathogens. *Microorganisms* 2023;11(2):369. doi:[10.3390/microorganisms11020369](https://doi.org/10.3390/microorganisms11020369)
27. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Control Release* 2011;156(2):128-45. doi:[10.1016/j.jconrel.2011.07.002](https://doi.org/10.1016/j.jconrel.2011.07.002)
28. Rai M, Kon K, Ingle A, Duran N, Galdiero S, Galdiero M. Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. *Appl Microbiol Biotechnol* 2014;98(5):1951-61. doi:[10.1007/s00253-013-5473-x](https://doi.org/10.1007/s00253-013-5473-x)
29. Loiseau A, Asila V, Boitel-Aullen G, Lam M, Salmain M, Boujday S. Silver-based plasmonic nanoparticles for and their use in biosensing. *Biosensors (Basel)* 2019;9(2):78. doi:[10.3390/bios9020078](https://doi.org/10.3390/bios9020078)
30. Godakhindi V, Kravitz E, Vivero-Escoto JL. Light-activable silver nanoparticles for combatting antibiotic-resistant bacteria and biofilms. *Molecules* 2025;30(3):626. doi:[10.3390/molecules30030626](https://doi.org/10.3390/molecules30030626)
31. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine* 2017;12:1227-49. doi:[10.2147/ijn.S121956](https://doi.org/10.2147/ijn.S121956)
32. Shi J, Fan Y, Zhang Q, Huang Y, Yang M. Harnessing photo-energy conversion in nanomaterials for precision theranostics. *Adv Mater* 2025;37(48): e2501623. doi:[10.1002/adma.202501623](https://doi.org/10.1002/adma.202501623)
33. Sati A, Ranade TN, Mali SN, Ahmad Yasin HK, Pratap A. Silver nanoparticles (AgNPs): comprehensive insights into bio/synthesis, key influencing factors, multifaceted applications, and toxicity-a 2024 update. *ACS Omega* 2025;10(8):7549-82. doi:[10.1021/acsomega.4c11045](https://doi.org/10.1021/acsomega.4c11045)
34. Mahmudin L, Suharyadi E, Bambang Setio Utomo A, Abrahama K. Influence of stabilizing agent and synthesis temperature on the optical properties of silver nanoparticles as active materials in surface plasmon resonance (SPR) biosensor. *AIP Conf Proc* 2016;1725(1):020041. doi:[10.1063/1.4945495](https://doi.org/10.1063/1.4945495)
35. Ovais M, Khalil AT, Ayaz M, Ahmad I, Nethi SK, Mukherjee S. Biosynthesis of metal nanoparticles via microbial enzymes: a mechanistic approach. *Int J Mol Sci* 2018;19(12):4100. doi:[10.3390/ijms19124100](https://doi.org/10.3390/ijms19124100)
36. Sportelli MC, Izzi M, Volpe A, Clemente M, Picca RA, Ancona A, et al. The pros and cons of the use of laser ablation synthesis for the production of silver nano-antimicrobials. *Antibiotics (Basel)* 2018;7(3):67. doi:[10.3390/antibiotics7030067](https://doi.org/10.3390/antibiotics7030067)
37. Vishwanath R, Negi B. Conventional and green methods of synthesis of silver nanoparticles and their antimicrobial properties. *Curr Res Green Sustain Chem* 2021;4:100205. doi:[10.1016/j.crgsc.2021.100205](https://doi.org/10.1016/j.crgsc.2021.100205)
38. Eker F, Akdaşçı E, Duman H, Bechelany M, Karav S. Green synthesis of silver nanoparticles using plant extracts: a comprehensive review of physicochemical properties and multifunctional applications. *Int J Mol Sci* 2025;26(13):6222. doi:[10.3390/ijms26136222](https://doi.org/10.3390/ijms26136222)
39. Rozykulyyeva L, Astuti SD, Zaidan AH, Pradhana AA, Puspita PS. Antibacterial activities of green synthesized silver nanoparticles from *Punica granatum* peel extract. *AIP Conf Proc* 2020;2314(1):060012. doi:[10.1063/5.0034126](https://doi.org/10.1063/5.0034126)
40. Saloga PE, Kästner C, Thünemann AF. High-speed but not magic: microwave-assisted synthesis of ultra-small silver nanoparticles. *Langmuir* 2018;34(1):147-53. doi:[10.1021/acs.langmuir.7b01541](https://doi.org/10.1021/acs.langmuir.7b01541)

41. Bao C, Serrano-Lotina A, Niu M, Portela R, Li Y, Lim KH, et al. Microwave-associated chemistry in environmental catalysis for air pollution remediation: a review. *Chem Eng J* 2023;466:142902. doi:[10.1016/j.cej.2023.142902](https://doi.org/10.1016/j.cej.2023.142902)
42. Restrepo CV, Villa CC. Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: a review. *Environ Nanotechnol Monit Manag* 2021;15:100428. doi:[10.1016/j.enmm.2021.100428](https://doi.org/10.1016/j.enmm.2021.100428)
43. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Talianky ME, et al. "Green" nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae* 2014;6(1):35-44.
44. Zhang Y, Cheng X, Zhang Y, Xue X, Fu Y. Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties. *Colloids Surf A Physicochem Eng Asp* 2013;423:63-8. doi:[10.1016/j.colsurfa.2013.01.059](https://doi.org/10.1016/j.colsurfa.2013.01.059)
45. Anis SN, Liew WC, Mohd Marsin A, Muhamad II, Teh SH, Md Khudzari AZ. Microwave-assisted green synthesis of silver nanoparticles using pineapple leaves waste. *Clean Eng Technol* 2023;15:100660. doi:[10.1016/j.clet.2023.100660](https://doi.org/10.1016/j.clet.2023.100660)
46. Kahrilas GA, Wally LM, Fredrick SJ, Hiskey M, Prieto AL, Owens JE. Microwave-assisted green synthesis of silver nanoparticles using orange peel extract. *ACS Sustain Chem Eng* 2013;2(3):367-76. doi:[10.1021/sc4003664](https://doi.org/10.1021/sc4003664)
47. Rao SS, Saptami K, Venkatesan J, Rekha PD. Microwave-assisted rapid synthesis of silver nanoparticles using fucoidan: characterization with assessment of biocompatibility and antimicrobial activity. *Int J Biol Macromol* 2020;163:745-55. doi:[10.1016/j.ijbiomac.2020.06.230](https://doi.org/10.1016/j.ijbiomac.2020.06.230)
48. Nandhini SN, Sisubalan N, Vijayan A, Karthikeyan C, Gnanaaraj M, Gideon DA, et al. Recent advances in green synthesized nanoparticles for bactericidal and wound healing applications. *Heliyon* 2023;9(2): e13128. doi:[10.1016/j.heliyon.2023.e13128](https://doi.org/10.1016/j.heliyon.2023.e13128)
49. Vigneswari S, Amelia TS, Hazwan MH, Mouriya GK, Bhubalan K, Amirul AA, et al. Transformation of biowaste for medical applications: incorporation of biologically derived silver nanoparticles as antimicrobial coating. *Antibiotics (Basel)* 2021;10(3):229. doi:[10.3390/antibiotics10030229](https://doi.org/10.3390/antibiotics10030229)
50. Habouti S, Solterbeck CH, Es-Souni M. Synthesis of silver nano-fir-twigs and application to single molecules detection. *J Mater Chem* 2010;20(25):5215-9. doi:[10.1039/c0jm00564a](https://doi.org/10.1039/c0jm00564a)
51. Anjana VN, Joseph M, Francis S, Joseph A, Koshy EP, Mathew B. Microwave assisted green synthesis of silver nanoparticles for optical, catalytic, biological and electrochemical applications. *Artif Cells Nanomed Biotechnol* 2021;49(1):438-49. doi:[10.1080/21691401.2021.1925678](https://doi.org/10.1080/21691401.2021.1925678)
52. Liem LN, The NP, Nguyen D. Microwave assisted green synthesis of silver nanoparticles using mulberry leaves extract and silver nitrate solution. *Technologies* 2019;7(1):7. doi:[10.3390/technologies7010007](https://doi.org/10.3390/technologies7010007)
53. Wang M, Wang Y, Chen G, Gao H, Peng Q. Chitosan-based multifunctional biomaterials as active agents or delivery systems for antibacterial therapy. *Bioengineering (Basel)* 2024;11(12):1278. doi:[10.3390/bioengineering11121278](https://doi.org/10.3390/bioengineering11121278)
54. Pathak R, Bhatt S, Punetha VD, Punetha M. Chitosan nanoparticles and based composites as a biocompatible vehicle for drug delivery: a review. *Int J Biol Macromol* 2023;253(Pt 7):127369. doi:[10.1016/j.ijbiomac.2023.127369](https://doi.org/10.1016/j.ijbiomac.2023.127369)
55. Chandrasekaran M, Kim KD, Chun SC. Antibacterial activity of chitosan nanoparticles: a review. *Processes* 2020;8(9):1173. doi:[10.3390/pr8091173](https://doi.org/10.3390/pr8091173)
56. Ke CL, Deng FS, Chuang CY, Lin CH. Antimicrobial actions and applications of chitosan. *Polymers (Basel)* 2021;13(6):904. doi:[10.3390/polym13060904](https://doi.org/10.3390/polym13060904)
57. Rashki S, Asgarpour K, Tarrahimofrad H, Hashemipour M, Ebrahimi MS, Fathizadeh H, et al. Chitosan-based nanoparticles against bacterial infections. *Carbohydr Polym* 2021;251:117108. doi:[10.1016/j.carbpol.2020.117108](https://doi.org/10.1016/j.carbpol.2020.117108)
58. Wei D, Sun W, Qian W, Ye Y, Ma X. The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. *Carbohydr Res* 2009;344(17):2375-82. doi:[10.1016/j.carres.2009.09.001](https://doi.org/10.1016/j.carres.2009.09.001)
59. Guarnieri A, Triunfo M, Scieuzo C, Ianniciello D, Tafi E, Hahn T, et al. Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*. *Sci Rep* 2022;12(1):8084. doi:[10.1038/s41598-022-12150-3](https://doi.org/10.1038/s41598-022-12150-3)
60. Ibrahim HM, El-Bisi MK, Taha GM, El-Alfy EA. Chitosan nanoparticles loaded antibiotics as drug delivery biomaterial. *J Appl Pharm Sci* 2015;5(10):85-90. doi:[10.7324/JAPS.2015.501015](https://doi.org/10.7324/JAPS.2015.501015)
61. Naveedunissa S, Meenalotchan R, Manisha M, Ankul Singh S, Nirenjen S, Anitha K, et al. Advances in chitosan based nanocarriers for targetted wound healing therapies: a review. *Carbohydr Polym Technol Appl* 2025;11:100891. doi:[10.1016/j.carpta.2025.100891](https://doi.org/10.1016/j.carpta.2025.100891)
62. Yaqubi AK, Astuti SD, Zaidan AH, Nurdin DZ. Blue laser-activated silver nanoparticles from grape seed extract for photodynamic antimicrobial therapy against *Escherichia coli* and *Staphylococcus aureus*. *J Lasers Med Sci* 2023;14: e69. doi:[10.34172/jlms.2023.69](https://doi.org/10.34172/jlms.2023.69)
63. Paschoal MA, Tonon CC, Spolidório DM, Bagnato VS, Giusti JS, Santos-Pinto L. Photodynamic potential of curcumin and blue LED against *Streptococcus mutans* in a planktonic culture. *Photodiagnosis Photodyn Ther* 2013;10(3):313-9. doi:[10.1016/j.pdpdt.2013.02.002](https://doi.org/10.1016/j.pdpdt.2013.02.002)
64. McOyi MP, Mpofu KT, Sekhwama M, Mthunzi-Kufa P. Developments in localized surface plasmon resonance. *Plasmonics* 2025;20(7):5481-520. doi:[10.1007/s11468-024-02620-x](https://doi.org/10.1007/s11468-024-02620-x)
65. Alzoubi FY, Ahmad AA, Aljarrah IA, Migdadi AB, Al-Bataineh QM. Localize surface plasmon resonance of silver nanoparticles using Mie theory. *J Mater Sci Mater Electron* 2023;34(32):2128. doi:[10.1007/s10854-023-11304-x](https://doi.org/10.1007/s10854-023-11304-x)
66. Yallappa S, Manjanna J, Peethambar SK, Rajeshwara AN, Satyanarayan ND. Green synthesis of silver nanoparticles using *Acacia farnesiana* (sweet acacia) seed extract under microwave irradiation and their biological assessment. *J Clust Sci* 2013;24(4):1081-92. doi:[10.1007/s10876-013-0599-7](https://doi.org/10.1007/s10876-013-0599-7)
67. Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I. Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques. *Sci Rep* 2016;6:20414. doi:[10.1038/srep20414](https://doi.org/10.1038/srep20414)
68. Aouadi A, Hamada Saud D, Rebiai A, Achouri A, Benabdesselam S, Mohamed Abd El-Mordy F, et al. Introducing the antibacterial and photocatalytic degradation potentials of biosynthesized chitosan, chitosan-ZnO, and chitosan-ZnO/PVP nanoparticles. *Sci Rep* 2024;14(1):14753. doi:[10.1038/s41598-024-65579-z](https://doi.org/10.1038/s41598-024-65579-z)
69. Algethami N, Rajeh A, Ragab HM, Tarabiah AE, Gami F. Characterization, optical, and electrical properties of chitosan/polyacrylamide blend doped silver nanoparticles. *J Mater Sci Mater Electron* 2022;33(13):10645-56. doi:[10.1007/s10854-022-08048-5](https://doi.org/10.1007/s10854-022-08048-5)
70. El-Naggar NE, Dalal SR, Zweil AM, Eltarahony M. Artificial intelligence-based optimization for chitosan nanoparticles biosynthesis, characterization and in-vitro assessment of its anti-biofilm potentiality. *Sci Rep* 2023;13(1):4401. doi:[10.1038/s41598-023-30911-6](https://doi.org/10.1038/s41598-023-30911-6)
71. Negri C, Colombo R, Bracconi M, Atzori C, Donazzi A, Lucotti A, et al. Operando UV-vis spectroscopy for real-time monitoring of nanoparticle size in reaction conditions: a case study on rWGS over Au nanoparticles. *Catal Sci Technol* 2024;14(5):1318-27. doi:[10.1039/d3cy01392h](https://doi.org/10.1039/d3cy01392h)
72. Amaliyah S, Pangesti DP, Masruri M, Sabarudin A, Sumitro SB. Green synthesis and characterization of copper nanoparticles using *Piper retrofractum* Vahl extract as bioreductor and

- capping agent. *Heliyon* 2020;6(8): e04636. doi:[10.1016/j.heliyon.2020.e04636](https://doi.org/10.1016/j.heliyon.2020.e04636)
73. Sukmawati A, Utami W, Yuliani R, Da'i M, Nafarin A. Effect of tween 80 on nanoparticle preparation of modified chitosan for targeted delivery of combination doxorubicin and curcumin analogue. *IOP Conf Ser Mater Sci Eng* 2018;311(1):012024. doi:[10.1088/1757-899x/311/1/012024](https://doi.org/10.1088/1757-899x/311/1/012024)
 74. Hanafy AS, Farid RM, ElGamal SS. Complexation as an approach to entrap cationic drugs into cationic nanoparticles administered intranasally for Alzheimer's disease management: preparation and detection in rat brain. *Drug Dev Ind Pharm* 2015;41(12):2055-68. doi:[10.3109/03639045.2015.1062897](https://doi.org/10.3109/03639045.2015.1062897)
 75. Sanjaya RE, Tedjo YY, Kurniawan A, Ju YH, Ayucitra A, Ismadji S. Investigation on supercritical CO₂ extraction of phenolic-phytochemicals from an epiphytic plant tuber (*Myrmecodia pendans*). *J CO₂ Util* 2014;6:26-33. doi:[10.1016/j.jcou.2014.03.001](https://doi.org/10.1016/j.jcou.2014.03.001)
 76. Zuas O, Hamim N, Sampora Y. Bio-synthesis of silver nanoparticles using water extract of *Myrmecodia pendan* (Sarang Semut plant). *Mater Lett* 2014;123:156-9. doi:[10.1016/j.matlet.2014.03.026](https://doi.org/10.1016/j.matlet.2014.03.026)
 77. Nadagouda MN, Speth TF, Varma RS. Microwave-assisted green synthesis of silver nanostructures. *Acc Chem Res* 2011;44(7):469-78. doi:[10.1021/ar1001457](https://doi.org/10.1021/ar1001457)
 78. Verma R, Khan AK, Amar A, Khan MI, Sah S. Opto-structural characteristics and biomedical applications of microwave irradiated green synthesised AM-AgNP from *Atalantia monophylla* (L.) leaf extract. *ES Energy Environ* 2022;17:44-55. doi:[10.30919/esee8c745](https://doi.org/10.30919/esee8c745)
 79. Hebbalalu D, Lalley J, Nadagouda MN, Varma RS. Greener techniques for the synthesis of silver nanoparticles using plant extracts, enzymes, bacteria, biodegradable polymers, and microwaves. *ACS Sustain Chem Eng* 2013;1(7):703-12. doi:[10.1021/sc4000362](https://doi.org/10.1021/sc4000362)
 80. Balciunaitiene A, Zehra SH, Liaudanskas M, Viskelis J, Trumbeckaite S, Kubiliene L, et al. Microwave-assisted synthesis of antimicrobial silver nanoparticles using propolis extracts. *Processes* 2025;13(9):2861. doi:[10.3390/pr13092861](https://doi.org/10.3390/pr13092861)
 81. Revathy R, Joseph J, Augustine C, Sajini T, Mathew B. Synthesis and catalytic applications of silver nanoparticles: a sustainable chemical approach using indigenous reducing and capping agents from *Hyptis capitata*. *Environ Sci Adv* 2022;1(4):491-505. doi:[10.1039/d2va00044j](https://doi.org/10.1039/d2va00044j)
 82. Sacco P, Paoletti S, Cok M, Asaro F, Abrami M, Grassi M, et al. Insight into the ionotropic gelation of chitosan using tripolyphosphate and pyrophosphate as cross-linkers. *Int J Biol Macromol* 2016;92:476-83. doi:[10.1016/j.ijbiomac.2016.07.056](https://doi.org/10.1016/j.ijbiomac.2016.07.056)
 83. Jardim KV, Siqueira JLN, Bão SN, Parize AL. In vitro cytotoxic and antioxidant evaluation of quercetin loaded in ionic cross-linked chitosan nanoparticles. *J Drug Deliv Sci Technol* 2022;74:103561. doi:[10.1016/j.jddst.2022.103561](https://doi.org/10.1016/j.jddst.2022.103561)
 84. Roacho-Pérez JA, Rodríguez-Aguillón KO, Gallardo-Blanco HL, Velazco-Campos MR, Sosa-Cruz KV, García-Casillas PE, et al. A full set of in vitro assays in chitosan/tween 80 microspheres loaded with magnetite nanoparticles. *Polymers (Basel)* 2021;13(3):400. doi:[10.3390/polym13030400](https://doi.org/10.3390/polym13030400)
 85. Benaissa S, Benmansour K, Zenagui C, Bouchenak Khelladi M, Belyagoubi L. Development of antioxidant and antimicrobial chitosan nanocomposite films incorporating with eco-friendly silver nanoparticles synthesized with Arabic gum. *Polym Bull* 2025;82(14):9275-301. doi:[10.1007/s00289-025-05898-x](https://doi.org/10.1007/s00289-025-05898-x)
 86. Işitan A, Erkan HE, Gök C, Sulak M, Koluman A. Improvement of antibacterial and mechanical properties of chitosan matrix biodegradable polymer coating materials with green synthesized nanosilver obtained from aromatic arugula (*Eruca vesicaria*). *Polym Bull* 2025;82(12):6791-821. doi:[10.1007/s00289-025-05802-7](https://doi.org/10.1007/s00289-025-05802-7)
 87. Arif R, Uddin R. A review on recent developments in the biosynthesis of silver nanoparticles and its biomedical applications. *Med Devices Sens* 2021;4(1): e10158. doi:[10.1002/mds3.10158](https://doi.org/10.1002/mds3.10158)
 88. Das D, Paul P. Environmental impact of silver nanoparticles and its sustainable mitigation by novel approach of green chemistry. *Plant Nano Biol* 2025;14:100210. doi:[10.1016/j.plana.2025.100210](https://doi.org/10.1016/j.plana.2025.100210)
 89. Ammar MM, Ali R, Abd Elaziz NA, Habib H, Abbas FM, Yassin MT, et al. Nanotechnology in oncology: advances in biosynthesis, drug delivery, and theranostics. *Discov Oncol* 2025;16(1):1172. doi:[10.1007/s12672-025-02664-3](https://doi.org/10.1007/s12672-025-02664-3)
 90. Al-Zahrani SS, Bora RS, Al-Garni SM. Antimicrobial activity of chitosan nanoparticles. *Biotechnol Biotechnol Equip* 2021;35(1):1874-80. doi:[10.1080/13102818.2022.2027816](https://doi.org/10.1080/13102818.2022.2027816)
 91. Raafat D, von Barga K, Haas A, Sahl HG. Insights into the mode of action of chitosan as an antibacterial compound. *Appl Environ Microbiol* 2008;74(12):3764-73. doi:[10.1128/aem.00453-08](https://doi.org/10.1128/aem.00453-08)
 92. Qi L, Xu Z, Jiang X, Hu C, Zou X. Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr Res* 2004;339(16):2693-700. doi:[10.1016/j.carres.2004.09.007](https://doi.org/10.1016/j.carres.2004.09.007)
 93. Wu K, Yan Z, Wu Z, Li J, Zhong W, Ding L, et al. Recent advances in the preparation, antibacterial mechanisms, and applications of chitosan. *J Funct Biomater* 2024;15(11):318. doi:[10.3390/jfb15110318](https://doi.org/10.3390/jfb15110318)
 94. Confederat LG, Tuchilus CG, Dragan M, Sha'at M, Dragostin OM. Preparation and antimicrobial activity of chitosan and its derivatives: a concise review. *Molecules* 2021;26(12):3694. doi:[10.3390/molecules26123694](https://doi.org/10.3390/molecules26123694)
 95. Fathil MA, Faris Taufeq FY, Suleman Ismail Abdalla S, Katas H. Roles of chitosan in synthesis, antibacterial and anti-biofilm properties of bionano silver and gold. *RSC Adv* 2022;12(30):19297-312. doi:[10.1039/d2ra01734b](https://doi.org/10.1039/d2ra01734b)
 96. Laanoja J, Sihtmäe M, Vija H, Kurvet I, Otsus M, Šmits K, et al. Particle-driven synergistic antibacterial effect of silver-chitosan nanocomposites against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. *ACS Omega* 2025;10(26):27904-19. doi:[10.1021/acsomega.5c01067](https://doi.org/10.1021/acsomega.5c01067)
 97. Chatterjee T, Chatterjee BK, Majumdar D, Chakrabarti P. Antibacterial effect of silver nanoparticles and the modeling of bacterial growth kinetics using a modified Gompertz model. *Biochim Biophys Acta* 2015;1850(2):299-306. doi:[10.1016/j.bbagen.2014.10.022](https://doi.org/10.1016/j.bbagen.2014.10.022)
 98. Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and gram-negative bacteria differ in their sensitivity to cold plasma. *Sci Rep* 2016;6:38610. doi:[10.1038/srep38610](https://doi.org/10.1038/srep38610)
 99. Yaqubi AK, Astuti SD, Zaidan AH, Syahrom A, Nurdin DZ. Antibacterial effect of red laser-activated silver nanoparticles synthesized with grape seed extract against *Staphylococcus aureus* and *Escherichia coli*. *Lasers Med Sci* 2024;39(1):47. doi:[10.1007/s10103-024-03991-7](https://doi.org/10.1007/s10103-024-03991-7)
 100. Mumtaz S, Ali S, Mumtaz S, Mughal TA, Tahir HM, Shakir HA. Chitosan conjugated silver nanoparticles: the versatile antibacterial agents. *Polym Bull* 2023;80(5):4719-36. doi:[10.1007/s00289-022-04321-z](https://doi.org/10.1007/s00289-022-04321-z)
 101. Mostafa EM, Badr Y, Ramadan MA, Hashem MM, Abo-El-Sooud K, Deif HN, et al. Laser enhanced photothermal effect of silver nanoparticles synthesized by chemical and green method on gram-positive and gram-negative bacteria. *BMC Chem* 2024;18(1):163. doi:[10.1186/s13065-024-01263-7](https://doi.org/10.1186/s13065-024-01263-7)
 102. Liu S, Zhao Y, Xu M, Wen J, Wang H, Yan H, et al. Antibacterial photodynamic properties of silver nanoparticles-loaded curcumin composite material in chitosan-based films. *Int J Biol Macromol* 2024;256(Pt 1):128014. doi:[10.1016/j.ijbiomac.2023.128014](https://doi.org/10.1016/j.ijbiomac.2023.128014)
 103. Roberts AE, Kragh KN, Bjarnsholt T, Diggle SP. The limitations of in vitro experimentation in understanding biofilms and chronic infection. *J Mol Biol* 2015;427(23):3646-61. doi:[10.1016/j.jmb.2015.09.002](https://doi.org/10.1016/j.jmb.2015.09.002)

104. Astuti SD, Hafidiana, Rulaningtyas R, Abdurachman, Putra AP, Samian, et al. The efficacy of photodynamic inactivation with laser diode on *Staphylococcus aureus* biofilm with various ages of biofilm. Infect Dis Rep 2020;12(Suppl 1):8736. doi:[10.4081/idr.2020.8736](https://doi.org/10.4081/idr.2020.8736)
105. Al-Sharqi A, Apun K, Vincent M, Kanakaraju D, Bilung LM. Enhancement of the antibacterial efficiency of silver nanoparticles against gram-positive and gram-negative bacteria using blue laser light. Int J Photoenergy 2019;2019(1):2528490. doi:[10.1155/2019/2528490](https://doi.org/10.1155/2019/2528490)
106. Burduşel AC, Gherasim O, Grumezescu AM, Mogoantă L, Fica A, Andronescu E. Biomedical applications of silver nanoparticles: an up-to-date overview. Nanomaterials (Basel) 2018;8(9):681. doi:[10.3390/nano8090681](https://doi.org/10.3390/nano8090681)