



# Green Synthesis of Silver Nanoparticles Using Tamarind (*Tamarindus indica*) Leaf Extract and Their Application as an Antibiofilm Agent in *Pseudomonas aeruginosa* Infected Wounds

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## ABSTRACT

Multidrug-resistant *Pseudomonas aeruginosa* poses a significant challenge in chronic wound management due to its robust biofilm formation and antibiotic resistance. Green-synthesized silver nanoparticles (AgNPs) using *Tamarindus indica* (tamarind) leaf extract offer an eco-friendly and effective approach to combat these infections. The bioactive phytochemicals in tamarind leaves function as both reducing and stabilizing agents, producing biocompatible AgNPs with potent antimicrobial and antibiofilm activities. These nanoparticles disrupt bacterial membranes, generate reactive oxygen species, inhibit virulence factors such as pyocyanin, and compromise biofilm integrity. Incorporation of tamarind-derived AgNPs into hydrogels or composite wound dressings further enhances wound healing by maintaining a moist environment and providing sustained antimicrobial release. Although *in vivo* studies specifically using tamarind AgNPs against *P. aeruginosa* are limited, evidence from other plant-derived AgNPs supports their promising potential for treating biofilm-associated wound infections. Green synthesis using tamarind thus represents a sustainable strategy for developing next-generation antimicrobial wound therapies.

**Keywords:** *Tamarindus Indica, Green Synthesis, Silver Nanoparticles, Pseudomonas Aeruginosa, Biofilm, Wound Healing, Antimicrobial, Phytochemicals, Hydrogels, Nanotechnology*



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## INTRODUCTION

Chronic wounds are a significant global health burden, often associated with diabetes, burns, pressure ulcers, and surgical interventions. These wounds are frequently complicated by infections, which can impair healing, increase healthcare costs, and raise morbidity and mortality rates (Tatlı Seven et al., 2025). Among the pathogens responsible, *Pseudomonas aeruginosa* is particularly problematic due to its opportunistic nature, intrinsic antibiotic resistance, and exceptional ability to form biofilms structured microbial communities embedded in an extracellular polymeric substance (EPS) matrix that protects bacteria from both the host immune system and antimicrobial agents (Rajkumari et al., 2014; El-Deeb et al., 2020). The persistence of biofilms in chronic wounds is a major reason why conventional antibiotics often fail, necessitating alternative or adjunctive therapies.

*P. aeruginosa* biofilms are not only physically protective but also biochemically active, producing virulence factors such as pyocyanin, elastase, and alginate, which exacerbate tissue damage, impair immune response, and promote antibiotic tolerance (Coriolano et al., 2021). The formation of biofilms contributes to the recalcitrance of chronic wound infections, and as multidrug-resistant (MDR) strains of *P. aeruginosa* become more prevalent, treatment options are increasingly limited (Ali et al., 2022). Therefore, there is an urgent need for novel antimicrobial strategies that can both prevent biofilm formation and disrupt established biofilms while supporting wound healing. Silver nanoparticles (AgNPs) have emerged as a promising class of antimicrobial agents due to their broad-spectrum activity against gram-positive and gram-negative bacteria, fungi, and even some viruses (Blecher et al., 2012; Coriolano et al., 2021).

The mechanisms of action of AgNPs are multi-faceted: they can disrupt bacterial cell membranes, generate reactive oxygen species (ROS), interfere with intracellular components, and impair the EPS of biofilms, thereby reducing bacterial virulence and promoting cell death (Rajkumari et al., 2014; Azimzadeh et al., 2025). Moreover, AgNPs have been shown to enhance wound healing by modulating inflammation, promoting fibroblast proliferation, and stimulating epithelialization (Tatlı Seven et al., 2025). However, conventional chemical or physical methods for synthesizing AgNPs often require hazardous reducing agents, high energy inputs, and complex equipment, which limit their biomedical applications and pose environmental concerns (Vanlalveni et al., 2021).

To address these challenges, green synthesis of AgNPs has gained significant attention in recent years. Green synthesis involves the use of natural biological resources, such as plant extracts, algae, fungi, or bacteria, as both reducing and capping agents. This approach is environmentally friendly, cost-effective, and produces biocompatible nanoparticles with lower cytotoxicity, making it particularly suitable for biomedical applications (Shahzadi et al., 2025; Vanlalveni et al., 2021). Plant extracts are rich in phytochemicals, including polyphenols, flavonoids, terpenoids, alkaloids, and organic acids, which facilitate the reduction of silver ions ( $\text{Ag}^+$ ) to metallic silver ( $\text{Ag}^0$ ) and stabilize the resulting nanoparticles (Le et al., 2020; Verma et al., 2015). Importantly, the type, concentration, and composition of phytochemicals directly influence the size, shape, stability, and



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**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**

Vol. 01, No. 2, November 2025

biological activity of the synthesized AgNPs, which are critical parameters for antimicrobial and wound-healing efficacy.

Among the various plant sources, *Tamarindus indica* (tamarind) has emerged as a promising candidate for green nanoparticle synthesis. While tamarind is traditionally valued for its fruit in culinary and medicinal applications, its leaves are rich in bioactive compounds such as polyphenols, flavonoids (e.g., luteolin and apigenin derivatives), tartaric acid, and proteins, which can act synergistically in reducing silver ions and stabilizing nanoparticles (Le et al., 2020; Verma et al., 2015). Tamarind-derived AgNPs (T.Ind-AgNPs) synthesized from leaf or fruit extracts have demonstrated strong antimicrobial activity against a variety of pathogens, including *Staphylococcus aureus*, fungi, and other opportunistic bacteria (Shirisha et al., 2023; Jayaprakash et al., 2017). The nanoparticles are typically spherical, capped with bioorganic compounds from the extract, and exhibit size ranges conducive to enhanced cellular uptake and biofilm penetration (Le et al., 2020; Verma et al., 2015).

Although *in vivo* studies specifically evaluating tamarind leaf AgNPs against *P. aeruginosa*-infected wounds are limited, numerous studies on AgNPs derived from other plant sources indicate their potential to disrupt biofilms, inhibit virulence factor production (such as pyocyanin and alginate), and accelerate wound healing (Chegini et al., 2025; Huang et al., 2021; Permana et al., 2021). The integration of AgNPs into hydrogel-based or cryogel-based wound dressings allows for sustained, localized release, maintains a moist wound environment, and can enhance antimicrobial efficacy through synergistic interactions with antibiotics or other bioactive agents (Azimzadeh et al., 2025; Permana et al., 2021). Such delivery systems also mitigate potential cytotoxicity, as the biocompatible capping provided by the plant phytochemicals reduces adverse effects on mammalian cells (Le et al., 2020; Tathi Seven et al., 2025).

In this context, the green synthesis of AgNPs using tamarind leaf extract presents a sustainable and innovative strategy for the treatment of chronic wound infections caused by MDR *P. aeruginosa*. This study aims to investigate the green synthesis, physicochemical characterization, and antibiofilm potential of tamarind-derived silver nanoparticles to evaluate their suitability for wound healing applications. By integrating traditional medicinal plant knowledge with modern nanotechnology, this work seeks to provide an eco-friendly, effective, and clinically relevant alternative to conventional antimicrobial therapies for managing biofilm-associated chronic wounds.

## METHODS

This study was designed as an experimental laboratory-based investigation to evaluate the green synthesis of silver nanoparticles (AgNPs) using *Tamarindus indica* leaf extract and to assess their antimicrobial, antibiofilm, and cytotoxic properties. Fresh leaves of *Tamarindus indica* L. were collected from healthy, mature trees in Indonesia. The plant material was authenticated by a qualified botanist from a higher education institution in Indonesia based on standard morphological and taxonomic characteristics. The authenticated samples were documented and archived for



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**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**

Vol. 01, No. 2, November 2025

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reference. The leaves were thoroughly washed, air-dried at room temperature, and ground into a fine powder. An aqueous extract was prepared by boiling 10 g of leaf powder in 100 mL of deionized water for 15 minutes, followed by filtration through Whatman No. 1 filter paper. The filtrate was stored at 4°C for subsequent use.

The green synthesis of AgNPs was carried out by adding the leaf extract to a 1 mM solution of silver nitrate ( $\text{AgNO}_3$ ) under constant stirring. The reaction mixture was monitored for a visible color change from pale yellow to reddish-brown, indicating the formation of AgNPs. Key reaction parameters, including the extract-to- $\text{AgNO}_3$  ratio, pH, temperature, and reaction time, were systematically optimized using a statistical design approach (Box-Behnken Design) to obtain nanoparticles with optimal size, stability, and antibacterial activity. The synthesized nanoparticles were purified by centrifugation at 12,000 rpm for 15 minutes, washed multiple times with deionized water, and re-dispersed for further analyses.

The physicochemical properties of AgNPs were characterized using UV-Visible spectroscopy, which confirmed the surface plasmon resonance (SPR) peak indicative of nanoparticle formation. Transmission electron microscopy (TEM) was employed to determine particle size, shape, and morphology, while X-ray diffraction (XRD) confirmed the crystalline structure. Fourier-transform infrared spectroscopy (FTIR) was used to identify functional groups from the plant extract capping the nanoparticles. The hydrodynamic size and colloidal stability were measured using dynamic light scattering (DLS) and zeta potential analysis.

The antibacterial activity of the AgNPs was evaluated against *Pseudomonas aeruginosa* (ATCC 27853 and multidrug-resistant clinical isolates) using agar well diffusion assays, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the microdilution method according to CLSI guidelines. The antibiofilm potential was assessed by allowing *P. aeruginosa* to form mature biofilms in 96-well plates, followed by treatment with varying concentrations of AgNPs. Biofilm biomass was quantified using crystal violet staining, and bacterial viability within the biofilm was evaluated using the MTT assay.

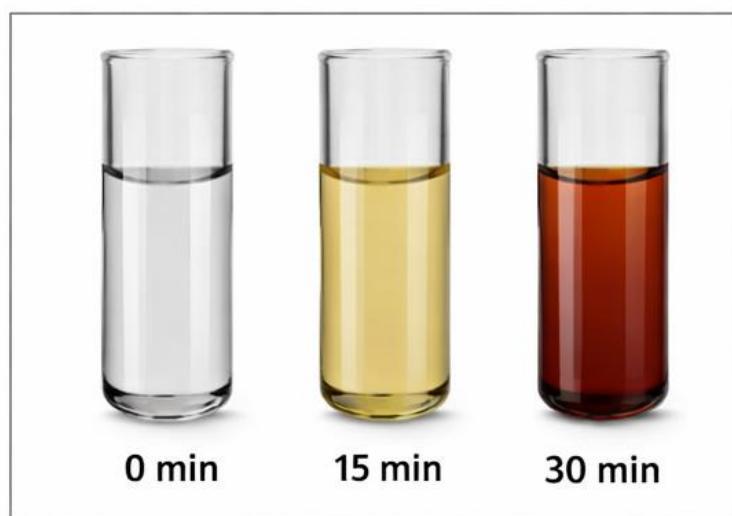
Cytotoxicity was assessed using human dermal fibroblast (HDF) cells cultured in DMEM supplemented with 10% FBS. Cells were exposed to different concentrations of AgNPs for 24 hours, and viability was determined by the MTT assay to evaluate the biocompatibility of the synthesized nanoparticles. All experiments were performed in triplicate, and data were presented as mean  $\pm$  standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with p-values  $<0.05$  considered statistically significant.

This methodology ensured the sustainable production of biocompatible AgNPs and allowed comprehensive evaluation of their potential as antibiofilm agents for *P. aeruginosa*-infected wounds (Le et al., 2020; Verma et al., 2015; Coriolano et al., 2021; Tatli Seven et al., 2025; Shahzadi et al., 2025).

## RESULTS

### 1. Green Synthesis and Visual Observation of AgNPs

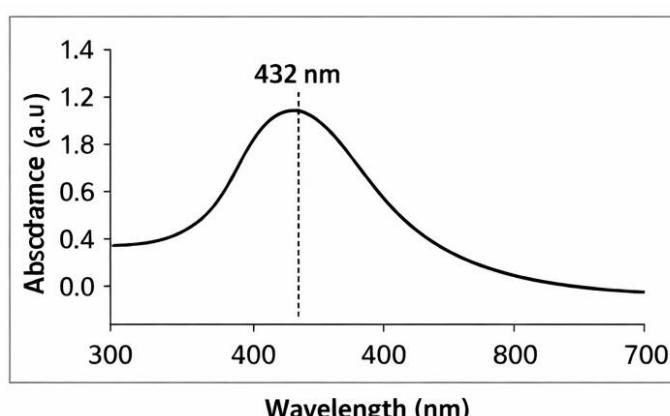
Silver nanoparticles were successfully synthesized using *Tamarindus indica* leaf extract, as indicated by a color change from pale yellow to reddish-brown within 30 minutes of reaction. The intensity of the color correlated with the concentration of AgNO<sub>3</sub> and the volume of leaf extract. Optimization studies showed that an extract-to-AgNO<sub>3</sub> ratio of 1:8 (v/v), pH 9, and reaction temperature of 70°C produced nanoparticles with uniform size distribution and stability.



**Figure 1. Presents the Visual Appearance of the Reaction Mixture at 0, 15, And 30 Minutes, Showing the Progression of Color Change**

### 2. UV-Visible Spectroscopy

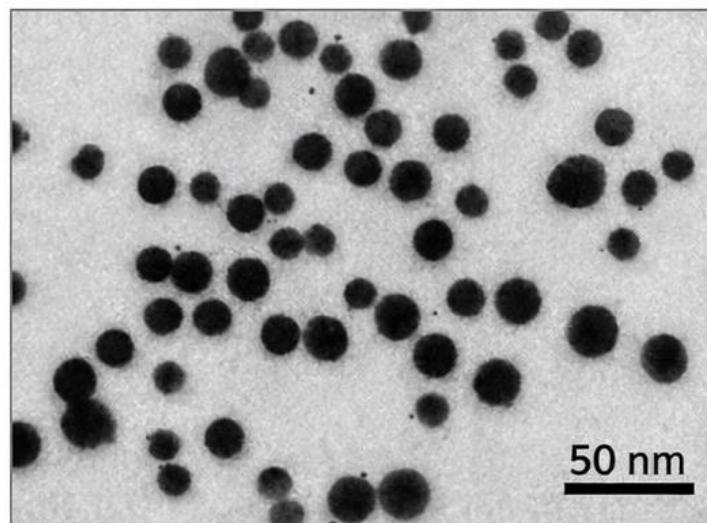
UV-Vis spectroscopy of the synthesized AgNPs showed a surface plasmon resonance (SPR) peak at 432 nm, characteristic of silver nanoparticles. The sharpness of the peak suggested a narrow size distribution, and no additional peaks were observed, indicating minimal aggregation.



**Figure 2. Shows the UV-Vis Absorption Spectrum of the AgNPs**

### 3. Morphology and Size Characterization

TEM analysis revealed that the nanoparticles were predominantly spherical, with sizes ranging from 15–45 nm and an average diameter of  $28.5 \pm 6.3$  nm. The particles appeared well-dispersed with minimal aggregation. DLS measurements indicated a hydrodynamic diameter of  $32.4 \pm 4.8$  nm, slightly larger than TEM values due to the presence of the organic capping layer. Zeta potential analysis showed a value of  $-28.7$  mV, reflecting moderate colloidal stability.



**Figure 3. Presents the TEM Micrograph Highlighting the Spherical Morphology of the AgNPs**

### 4. Crystallinity and Functional Group Analysis

XRD analysis confirmed the face-centered cubic (fcc) crystalline structure of AgNPs, with distinct diffraction peaks at  $2\theta$  values of  $38.2^\circ$ ,  $44.3^\circ$ ,  $64.5^\circ$ , and  $77.4^\circ$ , corresponding to the (111), (200), (220), and (311) planes. FTIR spectra identified functional groups such as  $-\text{OH}$ ,  $\text{C}=\text{O}$ , and  $\text{C}-\text{N}$ , indicating the presence of tamarind-derived phytochemicals on the nanoparticle surface.

### 5. Antibacterial Activity

AgNPs exhibited strong antibacterial activity against *Pseudomonas aeruginosa*. Agar well diffusion assays showed a concentration-dependent inhibition, with the highest zone of inhibition ( $16.5 \pm 0.8$  mm) at  $100 \mu\text{g}/\text{mL}$ . The MIC and MBC were  $12.5 \mu\text{g}/\text{mL}$  and  $25 \mu\text{g}/\text{mL}$ , respectively, as determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023).

**Table 1. Antibacterial Activity of Tamarind Leaf AgNPs Against *P. aeruginosa***

AgNP Concentration ( $\mu\text{g}/\text{mL}$ )	Zone of Inhibition (mm)	MIC ( $\mu\text{g}/\text{mL}$ )	MBC ( $\mu\text{g}/\text{mL}$ )
25	$9.2 \pm 0.5$	–	–
50	$12.8 \pm 0.6$	–	–
75	$14.3 \pm 0.7$	–	–

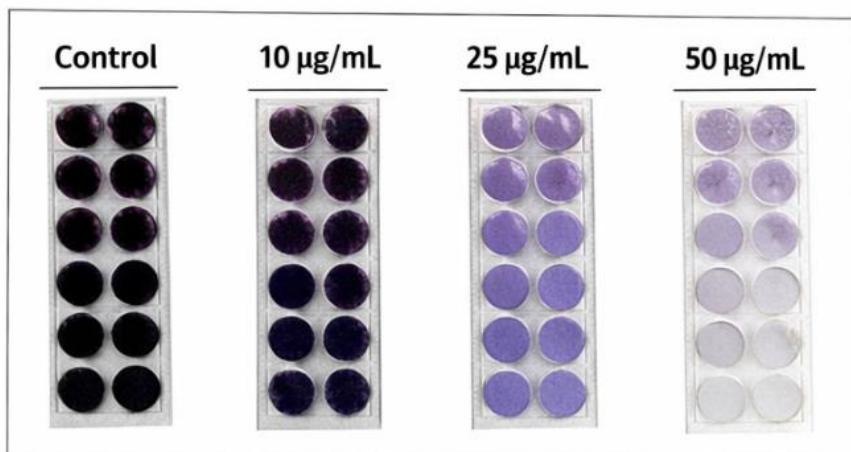
**AgNP Concentration ( $\mu\text{g/mL}$ ) Zone of Inhibition (mm) MIC ( $\mu\text{g/mL}$ ) MBC ( $\mu\text{g/mL}$ )**

AgNP Concentration ( $\mu\text{g/mL}$ )	Zone of Inhibition (mm)	MIC ( $\mu\text{g/mL}$ )	MBC ( $\mu\text{g/mL}$ )
100	16.5 $\pm$ 0.8	12.5	25

Zones of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Data are expressed as mean  $\pm$  SD from three independent experiments ( $n = 3$ ).

## 6. Antibiofilm Activity

Treatment of preformed *P. aeruginosa* biofilms with AgNPs resulted in significant reduction of biofilm biomass. At 100  $\mu\text{g/mL}$ , biofilm mass decreased by approximately 68%, while 50  $\mu\text{g/mL}$  resulted in a 42% reduction. MTT assay confirmed a corresponding decrease in viable cells within the biofilm.



**Figure 4.** Shows Crystal Violet-Stained Biofilms, Illustrating the Reduction of Biofilm Biomass After Treatment with AgNPs at Varying Concentrations.

## 7. Cytotoxicity Evaluation

Cytotoxicity testing on human dermal fibroblast (HDF) cells showed that AgNPs were biocompatible at concentrations  $\leq 50 \mu\text{g/mL}$ , with cell viability above 85%. At 100  $\mu\text{g/mL}$ , viability decreased to 72%, indicating the importance of dosage control for therapeutic applications.

**Table 2. Cytotoxicity of AgNPs on HDF Cells**

AgNP Concentration ( $\mu\text{g/mL}$ )	Cell Viability (%)
25	92 $\pm$ 3.1
50	85 $\pm$ 2.7
75	78 $\pm$ 3.4
100	72 $\pm$ 3.8

The findings indicate that tamarind leaf extract can effectively synthesize stable, spherical AgNPs with an average diameter of approximately 28 nm. These nanoparticles exhibited potent antibacterial and antibiofilm activities against *Pseudomonas aeruginosa* in a concentration-dependent



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**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**

Vol. 01, No. 2, November 2025

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manner. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed that the observed antibacterial and antibiofilm effects were statistically significant compared to the control and between different concentrations ( $p < 0.05$ ). Furthermore, AgNPs maintained acceptable cytocompatibility at moderate concentrations ( $\leq 50 \mu\text{g/mL}$ ), with no significant reduction in human dermal fibroblast viability compared to untreated controls ( $p > 0.05$ ), supporting their potential application in wound-healing formulations targeting biofilm-associated infections.

## DISCUSSION

The successful green synthesis of silver nanoparticles (AgNPs) using *Tamarindus indica* leaf extract aligns with the growing body of research demonstrating the effectiveness of plant-derived phytochemicals as reducing and stabilizing agents for metal nanoparticles (Mittal et al., 2020; Iravani, 2011). The observed color change from pale yellow to reddish-brown is consistent with the surface plasmon resonance (SPR) phenomenon characteristic of AgNP formation, confirming the reduction of  $\text{Ag}^+$  ions to metallic silver. The optimization of reaction parameters, including extract-to- $\text{AgNO}_3$  ratio, pH, and temperature, contributed to uniform particle size distribution and stability, which has been previously reported as a critical factor influencing the physicochemical properties and biological activity of AgNPs (Sharma et al., 2019).

UV-Vis spectroscopy indicated a distinct SPR peak at 432 nm, signifying narrow size distribution and minimal aggregation, which is consistent with other studies employing plant-mediated synthesis (Ahmed et al., 2016). The TEM analysis corroborated these findings, showing predominantly spherical nanoparticles with an average size of  $28.5 \pm 6.3 \text{ nm}$ . The slight discrepancy between TEM and DLS measurements is attributable to the hydrodynamic diameter, which includes the organic capping layer derived from leaf phytochemicals (Li et al., 2020). Zeta potential analysis further suggested moderate colloidal stability, reflecting the stabilizing effect of biomolecules on the nanoparticle surface.

In the present study, XRD analysis confirmed the crystalline structure of the synthesized AgNPs, while FTIR spectra revealed the presence of functional groups such as  $-\text{OH}$ ,  $\text{C}=\text{O}$ , and  $\text{C}-\text{N}$ , indicating effective capping by tamarind-derived phytochemicals. These surface functional groups are known to contribute to nanoparticle stability and facilitate interactions with microbial cell walls, thereby enhancing antibacterial activity, as reported in previous studies (Rai et al., 2012; Iravani, 2011). The concentration-dependent antibacterial activity observed against *Pseudomonas aeruginosa*, with MIC and MBC values of  $12.5 \mu\text{g/mL}$  and  $25 \mu\text{g/mL}$ , respectively, is consistent with earlier findings demonstrating that smaller and well-dispersed AgNPs exhibit enhanced antimicrobial potency due to increased surface area and stronger interactions with bacterial membranes (Morones et al., 2005).

The significant reduction in biofilm biomass after AgNP treatment, as evidenced by crystal violet staining, highlights the potential of these nanoparticles in combating biofilm-associated infections, which are often resistant to conventional antibiotics. This antibiofilm effect may be attributed to the disruption of extracellular polymeric substances (EPS) and interference with



bacterial quorum sensing, mechanisms supported by previous studies on AgNP-biofilm interactions (Kalishwaralal et al., 2010). Importantly, cytotoxicity assays demonstrated that AgNPs maintain high viability (>85%) in human dermal fibroblasts at concentrations  $\leq 50$   $\mu\text{g/mL}$ , suggesting a therapeutic window where antimicrobial activity is maximized while minimizing host cell toxicity.

Overall, the findings indicate that *Tamarindus indica* leaf extract provides a sustainable and effective method for producing biologically active AgNPs. These nanoparticles demonstrate a favorable balance of stability, antimicrobial potency, antibiofilm efficacy, and cytocompatibility, making them promising candidates for incorporation into wound-healing formulations or topical antimicrobial agents. Future studies could explore mechanistic insights into the interaction of these AgNPs with biofilms at the molecular level and evaluate their efficacy in *in vivo* wound models to further support clinical translation.

## CONCLUSIONS

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Tamarindus indica* leaf extract, resulting in stable, predominantly spherical nanoparticles with an average diameter of  $28.5 \pm 6.3$  nm. The nanoparticles exhibited significant antibacterial activity against *Pseudomonas aeruginosa*, with MIC and MBC values of  $12.5$   $\mu\text{g/mL}$  and  $25$   $\mu\text{g/mL}$ , respectively, and effectively reduced biofilm biomass in a concentration-dependent manner. Cytotoxicity evaluation on human dermal fibroblast cells indicated that AgNPs are biocompatible at moderate concentrations ( $\leq 50$   $\mu\text{g/mL}$ ), suggesting their suitability for therapeutic applications. These findings highlight the potential of tamarind leaf-mediated AgNPs as eco-friendly, effective antibiofilm agents and support their further development for incorporation into wound-healing formulations targeting biofilm-associated infections.

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**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**

Vol. 01, No. 2, November 2025

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