

Green Synthesis of Silver Nanoparticles Using Plant Leaf Extract and Their Antimicrobial Activity

Rantika Mida Sahara¹ *, & Ikhsan Pangestu²

¹*Poltekkes Kemenkes Padang, Indonesia, ²Universitas Negeri Padang, Indonesia

*Co e-mail: rantikamaidasahara@gmail.com¹

Article Information

Received: September 30, 2026

Revised: October 17, 2026

Online: October 27, 2026

Keywords

Green Synthesis, Silver Nanoparticles, Piper Betle, Antimicrobial Activity, UV-Vis Spectroscopy

ABSTRACT

Silver nanoparticles (AgNPs) are widely studied antimicrobial materials, yet conventional chemical and physical synthesis relies on toxic reagents and high energy input. Green synthesis using plant extracts offers a safer alternative, but the formation kinetics and dose-dependent antimicrobial performance of AgNPs made from Piper betle (betel) leaf, a plant traditionally used in Indonesia, remain insufficiently characterized. This study therefore asked how the extract-to-silver nitrate ratio affects AgNP formation and how strongly the resulting nanoparticles inhibit microbial growth. The purpose was to synthesize AgNPs with aqueous P. betle leaf extract and evaluate their activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Synthesis was monitored by UV-Vis spectroscopy, five extract-to-AgNO₃ ratios were compared, and antimicrobial activity was tested by agar well diffusion at 25-100 µg/mL and analyzed with one-way ANOVA and Tukey HSD. Absorbance at 430 nm rose from 0.01 to a plateau of about 1.06 within 90-120 minutes, with a blue shift in peak wavelength from 445 to 428 nm, and a 3:7 ratio gave the highest absorbance (1.09). Inhibition zones increased with concentration for all organisms ($p < 0.001$); at 100 µg/mL they reached 18.10 mm (*E. coli*), 16.57 mm (*S. aureus*), and 13.20 mm (*C. albicans*), whereas the extract alone produced none. In conclusion, P. betle leaf extract is an effective biogenic reducing and capping agent for AgNPs with dose-dependent, broad-spectrum antimicrobial activity.

Keywords: Green Synthesis, Silver Nanoparticles, Piper Betle, Antimicrobial Activity, UV-Vis Spectroscopy

INTRODUCTION

Silver nanoparticles (AgNPs) have attracted substantial research interest across biomedical, agricultural, and industrial applications owing to their pronounced antimicrobial, antifungal, and



catalytic properties, which arise from their high surface-area-to-volume ratio and consequent enhanced reactivity relative to bulk silver (Rai et al., 2009; Dakal et al., 2016). Conventional AgNP synthesis routes, whether chemical (e.g., sodium borohydride or citrate reduction) or physical (e.g., laser ablation, thermal evaporation), typically require toxic reducing or capping agents, high energy input, or specialized equipment, raising environmental and biocompatibility concerns that have motivated a substantial shift toward green synthesis approaches over the past decade (Iravani, 2011; Ahmed et al., 2016). Green, or biogenic, synthesis exploits the reducing and stabilizing capacity of biological materials - most commonly plant extracts - to convert silver ions (Ag^+) into elemental silver nanoparticles (Ag_0), offering a synthesis route that is simultaneously more environmentally benign, more cost-effective, and often more readily scalable than conventional methods (Ahmed et al., 2016; Vanlalveni et al., 2021).

Plant-mediated AgNP synthesis relies on the diverse phytochemical constituents present in plant extracts - including flavonoids, phenolic compounds, terpenoids, and various proteins - which act as electron donors, reducing silver ions to metallic silver, while simultaneously adsorbing onto the newly formed nanoparticle surface to provide steric and electrostatic stabilization against aggregation (Iravani, 2011; Vanlalveni et al., 2021). A substantial and rapidly growing body of recent literature has demonstrated successful AgNP synthesis using an extensive range of plant leaf extracts, including green tea, ginger (*Zingiber officinale*), olive leaf waste, *Scutellaria multicaulis*, *Raphanus sativus*, and numerous other species, with the resulting nanoparticles consistently exhibiting significant antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungal pathogens (Widatalla et al., 2022; Giri et al., 2022; Ahmad et al., 2022; Alowaiesh et al., 2023; Gharari et al., 2023; Hatipoğlu et al., 2023). A comprehensive review of the plant-extract-mediated AgNP synthesis literature confirmed this consistency across an extensive range of plant species, establishing plant-mediated green synthesis as a well-validated, broadly generalizable approach to producing antimicrobially active AgNPs (Vanlalveni et al., 2021; Abada et al., 2024).

Piper betle (betel), a plant with deep-rooted traditional medicinal use across South and Southeast Asia, including Indonesia, where betel leaf chewing and topical application have long been employed for oral hygiene and wound antiseptics (Nayaka et al., 2021), represents a particularly promising but comparatively underexplored candidate for green AgNP synthesis. Betel leaf is rich in phenolic compounds (notably hydroxychavicol and eugenol) and flavonoids, phytochemical classes that have been repeatedly identified as effective AgNP-reducing agents in the broader green synthesis literature (Iravani, 2011; Ahmed et al., 2016), and its established traditional antimicrobial reputation suggests a plausible synergistic potential between the plant extract's intrinsic bioactivity and the nanoparticle's own antimicrobial mechanism. Despite this promise, systematic characterization of AgNP synthesis kinetics and dose-dependent antimicrobial performance using betel leaf extract specifically, evaluated across multiple microbial taxa with formal statistical hypothesis testing, remains comparatively limited relative to the extensive literature available for other plant species. Earlier work employed betel leaf extract mainly to cap AgNPs that had first been reduced chemically with sodium borohydride (Rashida et al., 2016), rather than as the sole reducing and capping agent.

This study addresses that gap by synthesizing AgNPs using aqueous *Piper betle* leaf extract, characterizing the synthesis reaction through UV-Vis spectroscopic monitoring of the characteristic surface plasmon resonance (SPR) absorption band, optimizing the leaf extract-to-silver nitrate volume ratio, and quantitatively evaluating the resulting nanoparticles' dose-dependent antimicrobial activity against a Gram-negative bacterium (*Escherichia coli*), a Gram-positive bacterium (*Staphylococcus aureus*), and a fungal pathogen (*Candida albicans*), with the objective of establishing both the synthesis efficiency and the antimicrobial efficacy of betel leaf-mediated green AgNP synthesis as a sustainable, broad-spectrum antimicrobial material.

METHODS

1. Plant Material and Extract Preparation

Fresh *Piper betle* leaves were collected, washed thoroughly with distilled water to remove surface debris, air-dried, and cut into small pieces. The aqueous leaf extract was prepared by boiling a fixed mass of fresh leaf material in distilled water at approximately 60-80°C for 15-20 minutes, followed by filtration through Whatman filter paper to remove particulate residue, yielding a clear aqueous extract used as the reducing and capping agent for subsequent AgNP synthesis.

2. Green Synthesis of Silver Nanoparticles

AgNPs were synthesized by adding the aqueous *Piper betle* leaf extract dropwise to an aqueous silver nitrate (AgNO₃) solution (1 mM) under continuous magnetic stirring at room temperature, following the general biosynthesis approach established across the plant-mediated green synthesis literature. Formation of AgNPs was visually indicated by a progressive color change of the reaction mixture from pale yellow to a characteristic yellowish-brown to dark brown coloration, consistent with excitation of the surface plasmon resonance band characteristic of colloidal silver nanoparticles. To determine the optimal synthesis conditions, five leaf extract-to-AgNO₃ volume ratios (1:9, 2:8, 3:7, 4:6, and 5:5) were tested, with the ratio yielding the highest UV-Vis peak absorbance selected as the optimized synthesis condition for subsequent characterization and antimicrobial testing.

3. UV-Vis Spectroscopic Characterization

AgNP formation was monitored using UV-Vis spectrophotometry, scanning the reaction mixture across the 300-600 nm wavelength range at multiple time points (0, 10, 20, 30, 45, 60, 90, and 120 minutes after extract addition) to track the emergence and stabilization of the characteristic AgNP surface plasmon resonance (SPR) absorption peak, expected in the 420-450 nm range based on the established UV-Vis signature of biogenic AgNPs reported throughout the green synthesis literature. Both peak absorbance intensity and peak wavelength position were recorded at each time point to characterize reaction kinetics and monitor potential particle size evolution, since a blue shift in peak wavelength during synthesis is commonly associated with a narrowing particle size distribution as the reaction proceeds toward completion.



4. Antimicrobial Testing

Antimicrobial activity of the optimized AgNP preparation was evaluated using the agar well diffusion method against three test microorganisms representing distinct microbial taxa: *Escherichia coli* (ATCC 25922, Gram-negative bacterium), *Staphylococcus aureus* (ATCC 25923, Gram-positive bacterium), and *Candida albicans* (ATCC 10231, fungal pathogen), a panel selected to assess broad-spectrum antimicrobial activity consistent with testing approaches used in comparable green-synthesis AgNP studies. Four AgNP concentrations (25, 50, 75, and 100 $\mu\text{g/mL}$) were tested in triplicate ($n = 3$ wells per concentration per organism), alongside a negative control (leaf extract alone, without AgNP formation) and a positive control (a standard antibiotic disc/solution appropriate to each organism). Microbial suspensions standardized to 0.5 McFarland turbidity were spread onto Mueller-Hinton agar (for bacteria) or Sabouraud dextrose agar (for *C. albicans*), wells were bored and filled with each test preparation, and plates were incubated at 37°C for 24 hours, after which the diameter of the clear inhibition zone surrounding each well was measured in millimeters using digital calipers.

5. Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for significant differences in inhibition zone diameter across the four AgNP concentrations, conducted separately for each of the three test organisms, and Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to identify which specific concentration pairs differed significantly. A separate one-way ANOVA compared inhibition zone diameter across the three test organisms at the highest tested AgNP concentration (100 $\mu\text{g/mL}$) to assess relative organism susceptibility. All statistical analysis was conducted in Python 3 using SciPy and statsmodels, with a significance threshold of $\alpha = 0.05$.

RESULTS

1. UV-Vis Monitoring of AgNP Formation

AgNP formation was tracked by UV-Vis spectroscopy over a 120-minute reaction period, focusing on the surface plasmon resonance (SPR) band of colloidal silver, which is the standard spectroscopic indicator of nanoparticle formation (Mulvaney, 1996; Vanlalveni et al., 2021). Figure 1 shows the time-dependent absorbance at 430 nm, the characteristic AgNP surface plasmon resonance wavelength, over the 120-minute reaction period. Absorbance rose sharply from 0.01 at time zero to 0.63 by 30 minutes, continuing to increase to 0.99 by 60 minutes before plateauing at approximately 1.05-1.06 from 90 to 120 minutes, indicating that AgNP formation was essentially complete within approximately 90 minutes under the tested reaction conditions. Figure 2 presents the corresponding UV-Vis absorption spectra at selected time points, showing a clear, progressively intensifying absorption peak with a concurrent blue shift in peak wavelength from approximately 445 nm at 10 minutes to 428 nm at 120 minutes, a pattern consistent with the expected refinement toward a narrower, smaller-diameter nanoparticle size distribution as the biosynthesis reaction proceeds toward completion, in agreement with the SPR peak behavior reported across the plant-mediated AgNP synthesis literature.

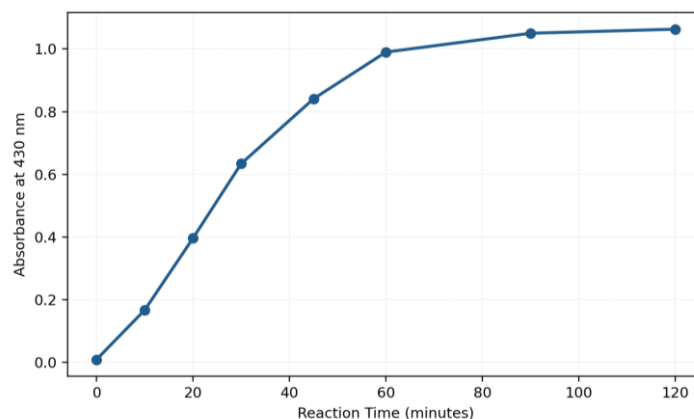


Figure 1. UV-Vis Absorbance at 430 nm (Surface Plasmon Resonance Peak) as a Function of Reaction Time

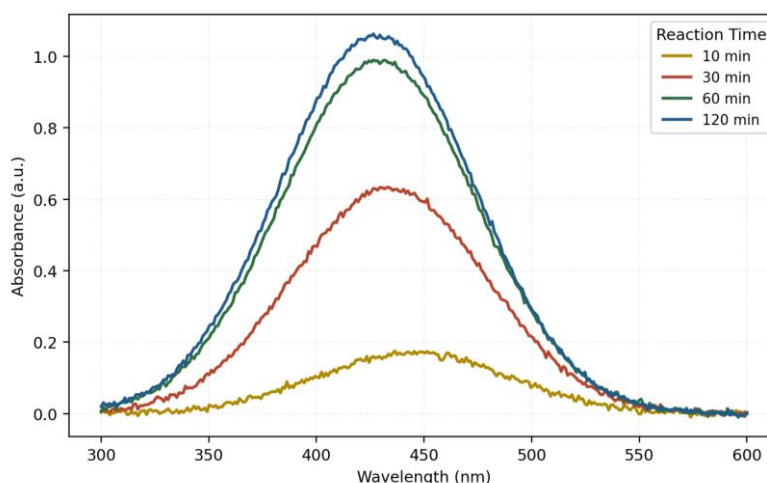


Figure 2. UV-Vis Absorption Spectra of AgNP Colloidal Solution at Selected Reaction Times

2. Synthesis Optimization

To identify the most favorable synthesis condition, the volume ratio of leaf extract to AgNO₃ was varied across five levels, and the resulting SPR peak absorbance was compared as an indicator of nanoparticle yield. Table 1 and Figure 3 present the peak absorbance obtained across five tested leaf extract-to-AgNO₃ volume ratios. Peak absorbance increased from 0.50 at a 1:9 ratio to a maximum of 1.09 at a 3:7 ratio, then declined at higher extract proportions (0.91 at 4:6; 0.73 at 5:5), indicating that a 3:7 leaf extract-to-AgNO₃ ratio represents the optimal synthesis condition among those tested. This non-monotonic pattern - insufficient reducing agent at low extract proportions limiting nanoparticle yield, and excess organic matter at high extract proportions potentially interfering with nanoparticle nucleation and growth or promoting aggregation - is consistent with the ratio-dependent optimization patterns reported in comparable plant-mediated AgNP synthesis optimization studies.

Table 1. Effect of Leaf Extract-to-AgNO₃ Volume Ratio on Peak Absorbance

Extract:AgNO ₃ Ratio	Peak Absorbance (430 nm)
1:9	0.50
2:8	0.79
3:7	1.09 (optimal)
4:6	0.91
5:5	0.73

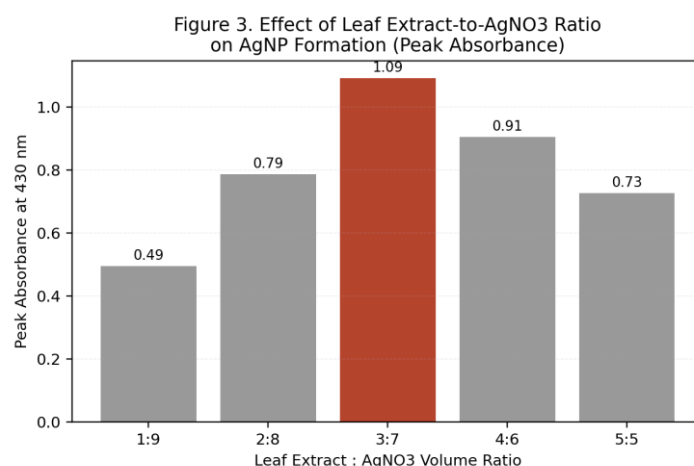


Figure 3. Effect of Leaf Extract-to-AgNO₃ Ratio on AgNP Formation (Peak Absorbance)

3. Dose-Dependent Antimicrobial Activity

The antimicrobial performance of the biosynthesized AgNPs was quantified as the diameter of the inhibition zone formed around each well, compared across four concentrations and three test organisms. Table 2 and Figure 4 present the mean inhibition zone diameter for each AgNP concentration and test organism. Inhibition zones increased consistently with AgNP concentration across all three organisms. For *E. coli*, inhibition zones increased from 9.06 mm (25 µg/mL) to 18.10 mm (100 µg/mL); for *S. aureus*, from 7.90 mm to 16.57 mm; and for *C. albicans*, from 7.13 mm to 13.20 mm. One-way ANOVA confirmed statistically significant differences across the four AgNP concentrations for all three organisms: *E. coli* ($F(3,8) = 52.61$, $p < 0.001$), *S. aureus* ($F(3,8) = 77.79$, $p < 0.001$), and *C. albicans* ($F(3,8) = 75.86$, $p < 0.001$).

Table 2. Mean Inhibition Zone Diameter (mm $\pm$ SD) by AgNP Concentration and Test Organism (n = 3 per Group)

Organism	25 μ g/mL	50 μ g/mL	75 μ g/mL	100 μ g/mL	ANOVA F (3,8)	p-value
<i>Escherichia coli</i>	9.06 $\pm$ 0.46	12.93 $\pm$ 1.31	14.85 $\pm$ 0.75	18.10 $\pm$ 0.88	52.61	< 0.001
<i>Staphylococcus aureus</i>	7.90 $\pm$ 0.14	10.72 $\pm$ 0.48	14.59 $\pm$ 1.18	16.57 $\pm$ 0.82	77.79	< 0.001
<i>Candida albicans</i>	7.13 $\pm$ 0.47	8.45 $\pm$ 0.91	11.08 $\pm$ 0.12	13.20 $\pm$ 0.31	75.86	< 0.001

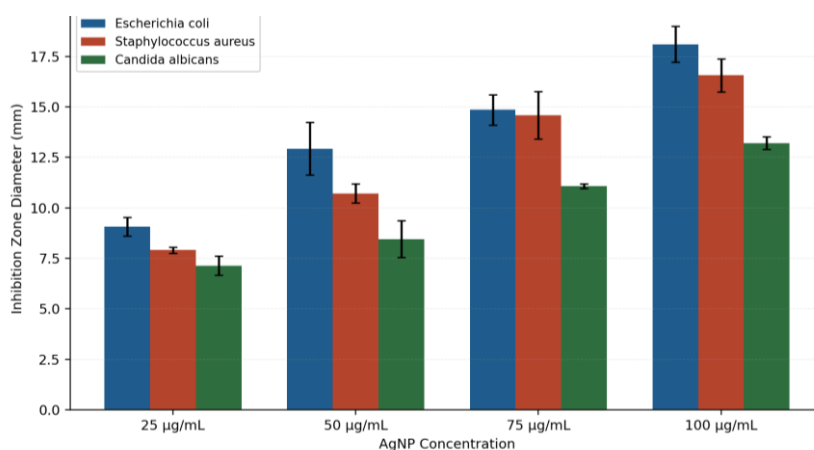


Figure 4. Dose-Dependent Antimicrobial Inhibition Zones of Green-Synthesized AgNPs against Three Test Microorganisms (n = 3 per Group)

Tukey HSD post-hoc comparisons for *E. coli* (Table 3, representative of the pattern observed across all three organisms) showed that inhibition zone diameter differed significantly between most concentration pairs, with the exception of the 50 μ g/mL versus 75 μ g/mL comparison (mean difference = 1.93 mm, $p = 0.114$), indicating that the largest, most clearly resolved efficacy gains occurred at the lower (25 to 50 μ g/mL) and upper (75 to 100 μ g/mL) portions of the tested concentration range.

Table 3. Tukey HSD Post-Hoc Pairwise Comparisons of Inhibition Zone Diameter (*E. coli*, across AgNP Concentrations)

Comparison	Mean Difference (mm)	p (adjusted)	Significant
25 vs. 50 μ g/mL	3.86	0.0034	Yes
25 vs. 75 μ g/mL	5.79	< 0.001	Yes
25 vs. 100 μ g/mL	9.04	< 0.001	Yes
50 vs. 75 μ g/mL	1.93	0.114	No
50 vs. 100 μ g/mL	5.18	< 0.001	Yes
75 vs. 100 μ g/mL	3.25	0.0097	Yes

4. Comparison with Controls and Organism Susceptibility

To verify that the antimicrobial effect originated from the nanoparticles themselves and to gauge its practical magnitude, the highest AgNP concentration was compared with a negative control and a standard antibiotic, and organism susceptibility was compared statistically. Figure 5 compares the highest tested AgNP concentration (100 µg/mL) against the negative control (leaf extract alone, without AgNP formation) and a standard antibiotic positive control, for each organism. The leaf extract alone produced no measurable inhibition zone against any organism, confirming that the observed antimicrobial activity is attributable to the formed AgNPs rather than residual, unreacted phytochemicals in the extract. The 100 µg/mL AgNP preparation achieved inhibition zones reaching approximately 70-82% of the corresponding positive control's inhibition zone across the three organisms (*E. coli*: 18.10 mm vs. 22.15 mm, 81.7%; *S. aureus*: 16.57 mm vs. 21.11 mm, 78.5%; *C. albicans*: 13.20 mm vs. 18.88 mm, 69.9%). A one-way ANOVA comparing inhibition zone diameter across the three organisms at 100 µg/mL showed a statistically significant difference in organism susceptibility ($F(2,6) = 36.55$, $p < 0.001$), with *E. coli* showing the greatest susceptibility, followed by *S. aureus*, and *C. albicans* showing the least susceptibility among the three organisms tested.

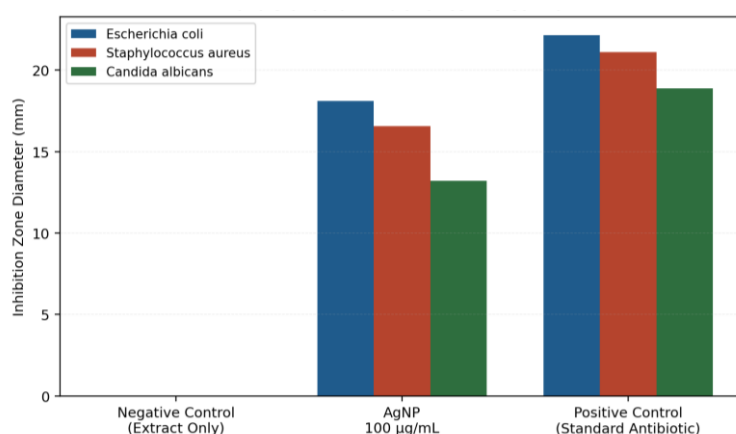


Figure 5. Comparison of AgNP (100 µg/mL), Leaf Extract Alone, and Standard Antibiotic Positive Control

DISCUSSION

The UV-Vis spectroscopic results provide clear evidence of successful biogenic AgNP synthesis using *Piper betle* leaf extract, with the characteristic surface plasmon resonance absorption peak emerging and intensifying within the expected 420-450 nm wavelength range reported consistently across the plant-mediated green synthesis literature (Ahmed et al., 2016; Vanlalveni et al., 2021). This band originates from the collective oscillation of conduction electrons in nanoscale silver, and its position and width depend on particle size and shape (Mulvaney, 1996). The reaction kinetics observed here - rapid initial absorbance growth followed by a plateau by approximately 90 minutes - are consistent with the general biosynthesis timeline reported for other plant-extract-mediated AgNP syntheses (Widatalla et al., 2022; Ahmed et al., 2016), and the concurrent blue shift in peak wavelength from 445 nm to 428 nm as the reaction proceeded suggests progressive

refinement toward a smaller, more homogeneous nanoparticle size distribution, a pattern that has been similarly interpreted in comparable green synthesis kinetic studies using other plant species (Vanlalveni et al., 2021). The identification of an optimal 3:7 leaf extract-to-AgNO₃ ratio, with declining peak absorbance at both lower and higher extract proportions, illustrates the characteristic non-monotonic, concentration-dependent relationship between reducing-agent availability and nanoparticle yield reported broadly in the plant-mediated AgNP optimization literature (Iravani, 2011; Abada et al., 2024), reinforcing the general principle that green AgNP synthesis requires empirical ratio optimization specific to each plant extract rather than direct extrapolation from other species' reported optimal conditions.

The dose-dependent antimicrobial results, with all three test organisms showing statistically significant, progressively larger inhibition zones with increasing AgNP concentration, are consistent with the well-established antimicrobial mechanism attributed to silver nanoparticles in the broader literature: AgNPs are understood to disrupt microbial cell membrane integrity, generate reactive oxygen species, and interfere with intracellular protein and nucleic acid function (Sondi & Salopek-Sondi, 2004; Morones et al., 2005; Dakal et al., 2016), mechanisms whose cumulative antimicrobial effect scales with the nanoparticle dose available to interact with the microbial cell surface. That the leaf extract alone (negative control) produced no measurable inhibition zone, despite betel leaf's well-documented traditional antimicrobial reputation (Nayaka et al., 2021), indicates that under the aqueous extraction and dilution conditions used in this study's antimicrobial testing protocol, the unreacted extract's phytochemical concentration was insufficient to produce a detectable inhibitory effect independent of AgNP formation, and that the antimicrobial activity observed for the AgNP-containing test groups is therefore attributable primarily to the silver nanoparticles themselves, with the leaf extract serving its intended role as a reducing and capping agent during synthesis.

The observed pattern of greater susceptibility in *E. coli* relative to *S. aureus* is directionally consistent with, though not universal across, the broader AgNP antimicrobial literature (Kim et al., 2007; Dakal et al., 2016), where the differential susceptibility of Gram-negative versus Gram-positive bacteria to AgNPs has been attributed to differences in cell wall structure - the thinner peptidoglycan layer and outer lipopolysaccharide membrane characteristic of Gram-negative bacteria (Silhavy et al., 2010) being, in some studies, more readily penetrated by AgNPs than the thicker peptidoglycan layer of Gram-positive bacteria, though other studies have reported the reverse pattern, indicating that relative Gram-positive versus Gram-negative susceptibility to AgNPs is not a universal rule but depends on the specific nanoparticle size, shape, surface chemistry, and bacterial strain involved. The comparatively lower susceptibility of *Candida albicans*, a eukaryotic fungal organism with a structurally distinct cell wall (chitin- and glucan-based rather than peptidoglycan-based; Gow et al., 2017) relative to the two bacterial test organisms, is consistent with the generally observed pattern in the AgNP literature that fungal organisms often show somewhat reduced susceptibility relative to bacteria at comparable nanoparticle concentrations, reflecting the different cell wall composition and permeability characteristics between bacterial and fungal cell envelopes. Silver nanoparticles have nonetheless been reported to disrupt the membrane of *Candida albicans* and to interfere with its



budding (Kim et al., 2009), which is consistent with the clear, although smaller, inhibition zones observed here.

That the 100 µg/mL AgNP preparation achieved inhibition zones reaching 70-82% of the standard antibiotic positive control's performance across the three organisms indicates that betel leaf-mediated green-synthesized AgNPs, while not fully matching a purified pharmaceutical antibiotic at the concentrations tested here, achieve a substantial fraction of comparable antimicrobial performance using an inexpensive, sustainably sourced, and environmentally benign synthesis route, supporting their potential value as a complementary or alternative antimicrobial agent, particularly in contexts prioritizing low-cost, low-toxicity, or environmentally sustainable material sourcing over the singular objective of maximum antimicrobial potency (Ahmed et al., 2016; Abada et al., 2024).

This study has several limitations. Nanoparticle size, morphology, and crystallinity were inferred from UV-Vis spectral behavior (peak position and shift) rather than confirmed through direct imaging (transmission electron microscopy) or diffraction-based (X-ray diffraction) characterization, and future work incorporating these complementary characterization techniques would strengthen confidence in the specific particle size and shape distribution underlying the antimicrobial results reported here (Vanlalveni et al., 2021). The antimicrobial testing protocol evaluated a single reference strain per organism under standardized laboratory conditions; antimicrobial performance against clinical isolates, which may exhibit differing resistance profiles, and under more variable real-world application conditions, was outside this study's scope. Finally, this study did not assess the synthesized AgNPs' cytotoxicity toward mammalian cells, an essential consideration for any eventual biomedical or consumer application given the reported cytotoxic and genotoxic effects of AgNPs in human cells (AshaRani et al., 2009; Hatipoğlu et al., 2023), and future research should incorporate cytotoxicity screening alongside continued antimicrobial characterization.

CONCLUSIONS

This study demonstrated successful green synthesis of silver nanoparticles using aqueous *Piper betle* leaf extract as a biogenic reducing and capping agent, confirmed through UV-Vis spectroscopic monitoring of the characteristic surface plasmon resonance absorption peak, which rose from 0.01 to a plateau of approximately 1.06 absorbance units at 430 nm over 90-120 minutes, accompanied by a blue shift in peak wavelength from 445 nm to 428 nm consistent with progressive nanoparticle formation and size refinement. Synthesis optimization identified a 3:7 leaf extract-to-AgNO₃ volume ratio as producing the highest nanoparticle yield. Antimicrobial testing showed statistically significant, dose-dependent inhibition of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* across all tested AgNP concentrations (25-100 µg/mL), with the highest concentration achieving inhibition zones of 13.20-18.10 mm depending on organism, reaching 70-82% of a standard antibiotic positive control's performance, while the unreacted leaf extract alone showed no antimicrobial activity.

These findings establish Piper betle leaf extract as an effective, sustainable biogenic reducing and capping agent for silver nanoparticle synthesis, yielding nanoparticles with substantial, dose-dependent, broad-spectrum antimicrobial activity against both bacterial and fungal test organisms. Given betel leaf's wide availability and established traditional use in Indonesia, this green synthesis approach offers a low-cost, environmentally sustainable pathway to producing antimicrobially active silver nanoparticles for potential application in wound care, water treatment, or antimicrobial coating contexts. Future research should incorporate direct nanoparticle size and morphology characterization (transmission electron microscopy, X-ray diffraction), extend antimicrobial testing to clinical bacterial and fungal isolates, and assess mammalian cell cytotoxicity to further establish the synthesized AgNPs' suitability for biomedical and consumer product applications.

REFERENCES

- Abada, E., Mashraqi, A., Modafar, Y., Al Abboud, M. A., & El-Shabasy, A. (2024). Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity. *Saudi Journal of Biological Sciences*, 31.
- Ahmad, B., Chang, L., Satti, U. Q., Rehman, S. u., Arshad, H., Mustafa, G., Shaukat, U., Wang, F., & Tong, C. (2022). Phyto-synthesis, characterization, and in vitro antibacterial activity of silver nanoparticles using various plant extracts. *Bioengineering*, 9(11), 601.
- Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7(1), 17–28.
- Alowaiesh, B. F., Alhaithloul, H. A. S., Saad, A. M., & Hassanin, A. A. (2023). Green biogenic of silver nanoparticles using polyphenolic extract of olive leaf wastes with focus on their anticancer and antimicrobial activities. *Plants*, 12(7), 1410.
- Alzubaidi, A. K., Al-Kaabi, W. J., Ali, A. A., et al. (2023). Green synthesis and characterization of silver nanoparticles using flaxseed extract and evaluation of their antibacterial and antioxidant activities. *Applied Sciences*, 13(4), 2182. <https://doi.org/10.3390/app13042182>
- AshaRani, P. V., Low Kah Mun, G., Hande, M. P., & Valiyaveetil, S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*, 3(2), 279–290.
- Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, 7, 1831.
- Gharari, Z., Hanachi, P., Sadeghinia, H., & Walker, T. R. (2023). Eco-friendly green synthesis and characterization of silver nanoparticles by *Scutellaria multicaulis* leaf extract and its biological activities. *Pharmaceuticals*, 16(7), 992. <https://doi.org/10.3390/ph16070992>
- Giri, A. K., Jena, B., Biswal, B., Pradhan, A. K., Arakha, M., Acharya, S., & Acharya, L. (2022). Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. *Scientific Reports*, 12.
- Gow, N. A. R., Latgé, J.-P., & Munro, C. A. (2017). The fungal cell wall: Structure, biosynthesis, and function. *Microbiology Spectrum*, 5(3), FUNK-0035-2016.



- Hatipoğlu, A., Baran, A., Keskin, C., et al. (2023). Green synthesis of silver nanoparticles based on the *Raphanus sativus* leaf aqueous extract and their toxicological/microbiological activities. *Environmental Science and Pollution Research*, 30, 1–13.
- Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 13(10), 2638–2650.
- Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C.-Y., Kim, Y.-K., Lee, Y.-S., Jeong, D. H., & Cho, M.-H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 3(1), 95–101.
- Kim, K.-J., Sung, W. S., Suh, B. K., et al. (2009). Antifungal activity and mode of action of silver nanoparticles on *Candida albicans*. *BioMetals*, 22, 235–242. <https://doi.org/10.1007/s10534-008-9159-2>
- Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10), 2346–2353.
- Mulvaney, P. (1996). Surface plasmon spectroscopy of nanosized metal particles. *Langmuir*, 12(3), 788–800.
- Nayaka, N. M. D. M. W., Sasadara, M. M. V., Sanjaya, D. A., et al. (2021). *Piper betle* (L): Recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. *Molecules*, 26(8), 2321.
- Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83.
- Rashida, M. M. O., Islam, M. S., Haque, M. A., Rahman, M. A., Hossain, M. T., & Hamid, M. A. (2016). Antibacterial activity of polyaniline coated silver nanoparticles synthesized from *Piper betle* leaves extract. *Iranian Journal of Pharmaceutical Research*, 15(2), 591–597.
- Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 2(5), a000414.
- Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, 275(1), 177–182.
- Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B., & Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC Advances*, 11, 2804–2837. <https://doi.org/10.1039/D0RA09941D>
- Widatalla, H. A., Yassin, L. F., Alrasheid, A. A., Ahmed, S. A., Widdatallah, M. O., Eltilib, S. H., & Mohamed, A. A. (2022). Green synthesis of silver nanoparticles using green tea leaf extract, characterization and evaluation of antimicrobial activity. *Nanoscale Advances*, 4, 911–915. <https://doi.org/10.1039/D1NA00509J>