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Green Synthesis of Silver Nanoparticles Using *Curcuma xanthorrhiza* as an Antibacterial Agent Against *Staphylococcus aureus*

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ABSTRACT

This study synthesized silver nanoparticles (Cur-AgNPs) using *Curcuma xanthorrhiza* rhizome extract via a green synthesis method to address the growing concern of nosocomial infections. Nosocomial infections, particularly those caused by *Staphylococcus aureus*, remain a significant healthcare challenge due to increasing antimicrobial resistance. The aim of this study was to evaluate the antibacterial activity of Cur-AgNPs against *Staphylococcus aureus*. Silver nanoparticles were synthesized using 1 mM $AgNO_3$ at 60°C for 24 h, followed by characterization using UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Antibacterial activity was assessed using the disk diffusion method at concentrations of 10, 20, 40, 80, and 160 µg/mL, with Clindamycin as a positive control. The results showed that Cur-AgNPs exhibited concentration-dependent antibacterial activity, with inhibition zones ranging from 5 ± 0.25 mm to 15 ± 0.89 mm, while the positive control (Clindamycin) produced an inhibition zone of 14 ± 0.2 mm. These findings suggest that Cur-AgNPs possess promising in vitro antibacterial activity against Staphylococcus aureus. However, this study is limited to a single bacterial species and in vitro evaluation. Further studies are required, including minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, cytotoxicity testing, nanoparticle stability analysis, and evaluation in in vivo models to confirm their potential biomedical applications.

Keywords: *Curcuma xanthorrhiza*; Silver nanoparticle; Green synthesis; *Staphylococcus aureus*; Antibacterial activity

INTRODUCTION

Health is a fundamental and vital need for every individual and society. One of the major global challenges in healthcare is the increasing incidence of nosocomial infections, also known as Healthcare-Associated Infections (HAIs), which are acquired during a patient's stay in healthcare facilities. HAIs are generally defined as infections that occur after 48 hours of hospitalization, with no evidence of infection at the time of admission. These infections significantly worsen patient outcomes and place a substantial burden on healthcare systems. The impact of HAIs includes prolonged hospital stays, increased mortality rates, and higher healthcare costs.[1] Previous reports have shown that certain pathogens, such as *Acinetobacter baumannii*, are associated with mortality rates of up to 40–60% in critically ill patients.[1] The World Health Organization (WHO) estimates that

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the prevalence of HAIs ranges from 5.7% to 19.1% in developing countries, with a global average of approximately 10.1%.[2] In Indonesia, the prevalence is reported at 7.1%, indicating the need for more effective interventions.

Among the causative agents of HAIs, *Staphylococcus aureus* is one of the most significant pathogens. This Gram-positive bacterium is known for its ability to form biofilms on medical devices, which enhances its persistence and resistance to treatmen.[3] The increasing resistance of S. aureus to antibiotics further complicates treatment outcomes and highlights the urgent need for alternative antimicrobial strategies.[4] Antimicrobial resistance is a growing global concern, with hundreds of thousands of deaths annually attributed to resistant infections.[5]

In response to this challenge, nanotechnology has emerged as a promising approach, particularly through the development of silver nanoparticles (AgNPs). AgNPs exhibit broad-spectrum antibacterial activity and have been reported to be effective against various bacterial pathogens, including *Staphylococcus aureus*.[6] Their antibacterial mechanisms include disruption of cell membranes, inhibition of DNA replication, and the induction of reactive oxygen species (ROS), leading to bacterial cell death.[78]

Despite their effectiveness, conventional chemical and physical methods for synthesizing AgNPs often involve toxic reagents and high energy consumption, which may pose environmental and health risks[9]. Therefore, green synthesis approaches using plant extracts have gained increasing attention as safer and more sustainable alternatives. These methods utilize natural phytochemicals as reducing and stabilizing agents, eliminating the need for hazardous substances.[1011]

Curcuma xanthorrhiza (temulawak), a medicinal plant widely found in Indonesia, is a promising candidate for green synthesis of AgNPs. It contains bioactive compounds such as curcumin and xanthorrhizol, which have demonstrated antibacterial, antioxidant, and anti-inflammatory activities.[1213] These phytochemicals can act as both reducing and stabilizing agents in nanoparticle synthesis.[1415] In addition, temulawak extract itself has shown antibacterial activity against *Staphylococcus aureus*.[1617]

However, studies specifically investigating the biosynthesis of AgNPs using Curcuma *xanthorrhiza* extract and evaluating their antibacterial activity against Staphylococcus aureus relevant to nosocomial infections—particularly in the Indonesian context—remain limited. This indicates a clear research gap in the development of locally sourced, plant-based nanomaterials for antimicrobial applications.

Therefore, this study aims to synthesize silver nanoparticles using *Curcuma xanthorrhiza* extract and evaluate their antibacterial activity against *Staphylococcus aureus*. We hypothesize that *Curcuma xanthorrhiza*-mediated AgNPs will exhibit concentration-dependent antibacterial effects against *Staphylococcus aureus*, supporting their potential as an alternative antimicrobial agent.

METHODS

Materials

Fresh rhizomes of temulawak were obtained from Blambangan Market, Banyuwangi, Indonesia. The reagents used included silver nitrate ($AgNO_3$, Sigma-Aldrich, ≥99.0%, analytical grade), sterile deionized water (18.2 MΩ·cm), and Plate Count Agar (PCA, Merck). Filtration was carried out using Whatman No.1 filter paper (GE Healthcare), and the bacterial strain used was *Staphylococcus aureus* (nosocomial isolate). A Clindamycin disk (Oxoid, 160 µg/mL) was used as a positive control in antibacterial testing. The instruments utilized were a drying oven (Memmert UFE500), ultrasonic water bath (Sibata), centrifuge (Kokusan H-103N), UV-Vis spectrophotometer (Thermo Scientific Genesys 10 UV), FTIR spectrophotometer (Shimadzu 8400), and a Scanning Electron Microscope (SEM, JEOL JSM-6510LA, resolution 200, 11 kV, 3000x

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magnification). Freeze-drying was performed using a Labconco FreeZone 2.5 Liter Benchtop Freeze Dryer.

Methods

This experimental research involved six treatments and three replications. Treatments were based on the concentration of silver nanoparticles synthesized using *C. xanthorrhiza* extract (Cur-AgNPs): 10, 20, 40, 80, and 160 µg/mL, along with a positive control of chlorhexidine at 160 µg/mL. The response variable was the diameter of the inhibition zone formed against S. aureus. Statistical analysis was conducted using One Way ANOVA with IBM SPSS Statistics 26.

Preparation of *C. xanthorrhiza* Extract

The rhizomes were washed, sliced, and dried in a Memmert UFE500 oven at 50°C. Once dried, the rhizomes were ground into powder and sieved using a 50-mesh filter. Five grams of the powder were mixed with 50 mL of sterile deionized water at a 1:10 ratio. The mixture was sonicated using a Sibata ultrasonic water bath at 60°C for 4 hours. It was then filtered through Whatman No.1 filter paper and stored at 4°C. Plant residues were removed by centrifugation at 4,000 rpm for 10 minutes using a Kokusan H-103N centrifuge. The supernatant was collected as extract and used for nanoparticle synthesis.

1. Synthesis of Silver Nanoparticles (Cur-AgNPs).

Ten milliliters of *C. xanthorrhiza* extract were added to 90 mL of 1 mM silver nitrate (Sigma-Aldrich, ≥99.0%). The mixture was incubated in the dark at 60°C for 24 hours. A gradual color change from colorless to reddish-brown indicated nanoparticle formation. The mixture was then centrifuged at 12,000 rpm for 30 minutes to collect the nanoparticles, followed by two washes with sterile deionized water using the same centrifugation settings. The resulting pellet was freeze-dried using the Labconco FreeZone Freeze Dryer to obtain stable Cur-AgNPs.

2. Characterization of Cur-AgNPs

Surface plasmon resonance of the Cur-AgNPs was confirmed using a Thermo Scientific Genesys 10 UV spectrophotometer, with absorption peaks observed in the 400–430 nm range. FTIR analysis was performed using a Shimadzu 8400 spectrophotometer in the 500–4000 cm^{-1} range to detect phytochemical involvement in nanoparticle synthesis. Particle shape and size were analyzed using SEM (JEOL JSM-6510LA, 11 kV, 3000x, resolution 200).

3. Antibacterial Activity Assay

The antibacterial activity of Cur-AgNPs was tested against *Staphylococcus aureus* using an agar well diffusion method. PCA (Merck) was poured into sterilized Petri dishes and left overnight. A bacterial suspension equivalent to 0.5 McFarland standard (~1.5×10^8 CFU/mL) was prepared and spread onto the agar surface. Paper disks were impregnated with Cur-AgNPs at concentrations of 10, 20, 40, 80, and 160 µg/mL. A positive control disk containing Clindamycin (Oxoid, 160 µg/mL) was also included. Plates were incubated at 37°C for 24 hours. Inhibition zones were measured horizontally and vertically, then averaged.

RESULTS AND DISCUSSION

1. Synthesis and Characterization of Silver Nanoparticles from *Curcuma xanthorrhiza* Rhizome

The synthesis process of silver nanoparticles from *Curcuma xanthorrhiza* rhizome was successfully carried out using a green synthesis approach, utilizing the phytochemical compounds from temulawak extract as reducing and stabilizing agents. The initial weight of the fresh rhizome was 1.384 grams, which significantly decreased to 138 grams after the drying process, indicating a highwater content in the temulawak rhizome. From the dried rhizome, 5 grams were extracted and used in the nanoparticle synthesis. Five grams of dried rhizome powder were

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extracted using 50 mL of sterile deionized water at a 1:10 (w/v) ratio. The extraction yield was not determined in this study due to the absence of dried extract weight measurement.

Initial indications of successful synthesis were visually observed through the color change of the solution from yellow to dark brown, which is characteristic of silver nanoparticle formation due to surface plasmon resonance (Figure 1). In contrast, the control mixture without $AgNO_3$ did not show any color change, confirming that the reduction reaction occurred only in the solution containing silver ions.

Further confirmation of nanoparticle formation was carried out through UV-Vis spectrophotometric analysis (Figure 2). The absorption spectrum showed a peak at a wavelength of 410 nm, which is a typical range for small and stable silver nanoparticles. This result suggests the formation of nanoscale particles; however, UV-Vis analysis alone cannot fully determine particle size distribution or aggregation state. The synthesis process produced a nanoparticle suspension that yielded approximately 0.013 g of dried nanoparticles after freeze-drying. This corresponds to an approximate nanoparticle concentration of 0.13 mg/mL based on the total reaction volume. However, the overall synthesis yield was not calculated due to the absence of recovery efficiency data.

Further characterization using Fourier Transform Infrared Spectroscopy (FTIR) revealed absorption peaks at 3511, 1628, 1510, and 1282 cm^{-1} (Figure 3). The peak at 3511 cm^{-1} corresponds to O–H stretching vibrations of phenolic compounds, while the peak at 1628 cm^{-1} is attributed to C=O stretching or aromatic C=C vibrations associated with flavonoids and curcuminoids. The peak at 1510 cm^{-1} represents aromatic ring vibrations, and the peak at 1282 cm^{-1} corresponds to C–O stretching of alcohol or ether groups[18] [7,11]. Comparison between the extract and Cur-AgNPs spectra revealed slight shifts and changes in peak intensity, particularly in the O–H and C=O regions. These changes indicate the involvement of these functional groups in the reduction of Ag^+ ions and stabilization of the nanoparticles.

The presence of these functional groups on the nanoparticle surface suggests that phytochemicals from *Curcuma xanthorrhiza* act as both reducing and capping agents during nanoparticle synthesis.

These findings are consistent with previous studies reporting the role of plant-derived biomolecules in nanoparticle formation and stabilization.[1920]

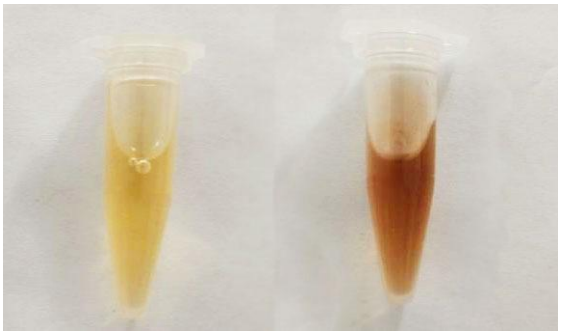

Figure 1. Catechin A noticeable color change appears in the mixture of rhizome extract and AgNO3, which differs from the control. On the left is the mixture of rhizome extract and AgNO3, while the right shows the control mixture of rhizome extract and sterile deionized water.

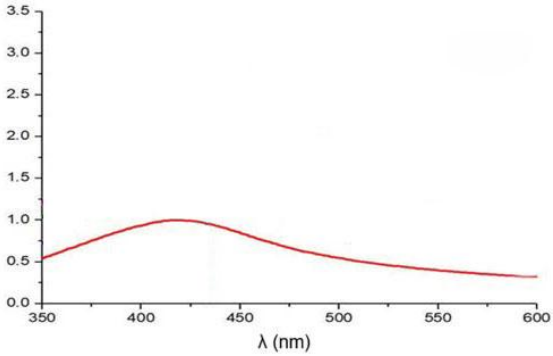

Figure 2. UV-Vis Spectrophotometric Analysis of Silver Nanoparticles

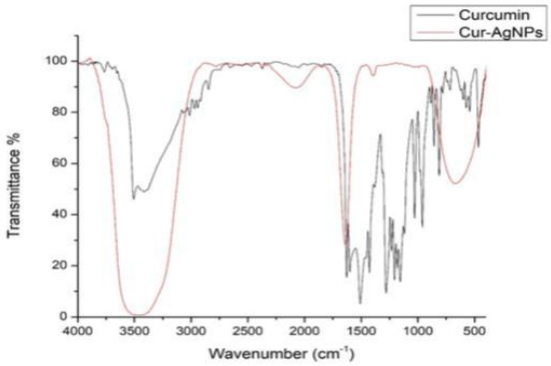

Figure 3. FTIR Analysis of Silver Nanoparticles-Temulawak Rhizome

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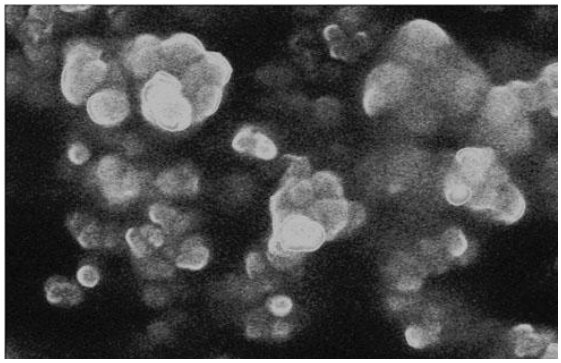

Figure 4. Morphology of silver nanoparticles from temulawak rhizome observed using SEM analysis at 3,000x magnification

2. Scanning Electron Microscope (SEM) analysis

SEM analysis was performed to evaluate the surface morphology and particle size of the synthesized silver nanoparticles (Cur-AgNPs). The sample was pretreated at 80°C for 2 hours to remove residual moisture prior to imaging. The SEM micrograph (Figure 4) shows that the nanoparticles are predominantly spherical with slight aggregation observed in some regions. The particle size ranged from approximately 5 to 90 nm.

To obtain quantitative data, particle size distribution analysis was performed by measuring approximately 50 individual particles using ImageJ software. The average particle size was calculated to be 38.6 ± 18.4 nm (mean ± SD, n = 50). The relatively broad size distribution indicates heterogeneous nucleation and growth during the biosynthesis process, which is commonly reported in plant-mediated nanoparticle synthesis.[18,21] Furthermore, the nanoparticle surfaces appear to be coated with a thin organic layer, which is attributed to phytochemical compounds from the *Curcuma xanthorrhiza* extract. These compounds likely act as stabilizing (capping) agents that prevent aggregation between particles.[19] These findings are consistent with FTIR analysis, which revealed functional groups such as O–H, C=O, and C–O associated with phenolic and flavonoid compounds.[18,19] The observed morphology is also consistent with previous studies reporting spherical silver nanoparticles synthesized using plant extracts, including *Curcuma longa*.[20]

However, SEM analysis alone has limitations in accurately determining particle size distribution due to possible aggregation and limited sampling area. Therefore, further characterization using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and zeta potential analysis is recommended to provide more precise information on particle size distribution, dispersion, and stability.

Table 1. Inhibition zone diameter of Cur-AgNPs against *Staphylococcus aureus*

Treatment	Concentration (µg/mL)	Inhibition zone (mm, mean ± SD)
Cur-AgNPs	10	5.00 ± 0.25[a]
Cur-AgNPs	20	7.00 ± 0.85[b]
Cur-AgNPs	40	8.00 ± 0.47[b]
Cur-AgNPs	80	9.00 ± 0.86[c]
Cur-AgNPs	160	15.00 ± 0.89[d]
Positive Control (Clindamycin)	160	14.00 ± 0.20[d]

Values are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD test, with significance set at $p < 0.05$.

3. Antibacterial Activity Test of Cur-AgNPs

The antibacterial activity of Cur-AgNPs against *Staphylococcus aureus* was evaluated using the disk diffusion method, and the results are presented in Table 1. Cur-AgNPs exhibited concentration-dependent antibacterial activity, as indicated by the increase in inhibition zone diameter with increasing nanoparticle concentration. The inhibition zones ranged from 5.00 ± 0.25 mm at 10 µg/mL to 15.00 ± 0.89 mm at 160 µg/mL.

Statistical analysis using one-way ANOVA followed by Tukey's HSD test showed that there were significant differences between treatment groups ($p < 0.05$). The inhibition zone at 10 µg/mL (5.00 ± 0.25 mm) was significantly lower than those at higher concentrations, while concentrations of 20 and 40 µg/mL did not

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differ significantly from each other ($p > 0.05$).

A significant increase in antibacterial activity was observed at higher concentrations, with 80 µg/mL (9.00 ± 0.86 mm) showing greater inhibition than lower concentrations. The highest inhibition was observed at 160 µg/mL (15.00 ± 0.89 mm), which was not significantly different from the positive control, Clindamycin (14.00 ± 0.20 mm) ($p > 0.05$).

These results indicate that Cur-AgNPs exhibit a dose-dependent antibacterial effect against Staphylococcus aureus under the experimental conditions. The antibacterial activity of Cur-AgNPs is likely associated with multiple mechanisms, including disruption of bacterial cell membranes, increased membrane permeability, and the generation of reactive oxygen species (ROS), which lead to cellular damage.[1819]

These findings are consistent with previous studies reporting inhibition zones of approximately 12–14 mm for plant-mediated silver nanoparticles against *Staphylococcus aureus*.[18] The slightly higher inhibition observed in this study may be influenced by the presence of bioactive compounds in *Curcuma xanthorrhiza*, which could contribute to antibacterial activity.

However, the comparison with Clindamycin should be interpreted cautiously, as the concentration used in this study (160 µg/mL) differs from standardized antibiotic disk concentrations. This study has several limitations. The antibacterial activity was evaluated only against a single bacterial species using the disk diffusion method. In addition, vehicle control and plant extract-only control were not included, limiting the ability to distinguish nanoparticle-specific effects. Furthermore, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were not determined.

Therefore, further studies are required to determine MIC and MBC values, evaluate cytotoxicity, assess nanoparticle stability, and investigate antibacterial activity against a broader range of microorganisms.

CONCLUSION

This study successfully synthesized silver nanoparticles (Cur-AgNPs) using *Curcuma xanthorrhiza* rhizome extract through a green synthesis method. The synthesized nanoparticles were confirmed by UV-Vis, FTIR, and SEM analyses, demonstrating the formation of predominantly spherical nanoparticles with nanoscale dimensions. Cur-AgNPs exhibited concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones ranging from 5 ± 0.25 mm to 15 ± 0.89 mm. These findings indicate that Cur-AgNPs demonstrate promising in vitro antibacterial activity. However, further studies are required to evaluate their efficacy and safety before practical application.

Future work should include determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), cytotoxicity assessment, nanoparticle stability evaluation, and testing against a broader spectrum of microorganisms, as well as in vivo studies. Overall, this study provides preliminary evidence supporting the potential of Curcuma xanthorrhiza-mediated silver nanoparticles as a plant-based antimicrobial agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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