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Green Synthesis and Antibacterial Activity of Silver Nanoparticles Using Ethanolic Extract from *Clerodendrum fragrans* Leaves

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ABSTRACT

Green synthesized silver nanoparticles using plant extract (AgNPs) have emerged as promising biomaterials for biomedical applications, particularly as antimicrobial agents. This study aimed to synthesize AgNPs using ethanolic leaf extract of sarang banua (Clerodendrum fragrans) and to evaluate their physicochemical characteristics and antibacterial activity for skin-related applications. C. fragrans leaf contains secondary metabolite bioactive compounds that can be involved in nanoparticle synthesis. In this approach plant-derived phytochemicals functioned as reducing and stabilizing agents. Antibacterial activity was tested using the disc diffusion method followed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results of the study showed an average diameter of 114 nm with a polydispersity index (PDI) of 0.5, indicating moderate uniformity.The AgNPs showed strong inhibitory activity against Staphylococcus aureus as a representative skin-associated pathogenic bacteria with an inhibition zone of 11.06 mm. The minimum inhibitory concentration value was 3.375 µg/mL, while the minimum bactericidal concentration value exceeded 27 µg/mL, indicating bactericidal effects at higher concentrations. These findings highlight the potential of C. fragrans-mediated AgNPs as biomaterials for skin-related biomedical applications.

Keywords: Antibacterial Activity, *Clerodendrum Fragrans*, Silver Nanoparticles

1. INTRODUCTION

Skin disorders associated with bacterial infection remain a recurring clinical concern, particularly when disruption of the skin barrier facilitates microbial colonization and prolongs inflammatory processes [1]. In conditions such as dermatitis, eczema, and wound infections, the presence of pathogenic bacteria can stimulate the release of pro-inflammatory mediators and interfere with tissue repair mechanisms [2]. Among the microorganisms associated with skin-related infections, Staphylococcus aureus is recognized as one of the most common pathogens involved in inflammation and delayed wound healing [3]. Therefore the development of biomaterials with antibacterial properties has become an important focus in biomedical research especially for skin-related applications.

As a country with extensive biodiversity, Indonesia possesses various medicinal plants that have not been fully explored for scientific and pharmaceutical applications [4]. One example is *Clerodendrum fragrans*, locally

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known as sarang banua, which is traditionally used in North Sumatra for minor health conditions. The results of the "Herbarium Bogoriense" Botany Division of the Biology Research Center - LIPI Bogor, determined that the sarang banua plant is *Clerodendrum fragrans*, belonging to the Verbanaccae family. Previous phytochemical studies have reported that the leaves of *C. fragrans* contain several secondary metabolites including flavonoids, phenolic compounds, and alkaloids[5] . These bioactive constituents are considered to play important roles in various biological activities and may contribute to nanoparticle formation through reduction and stabilization mechanisms.

However the direct use of plant extracts in biomedical applications still faces several limitations, particularly related to stability and effectiveness[6]. To overcome these limitations, nanotechnology-based approaches have increasingly been explored. Among various nanomaterials silver nanoparticles have attracted considerable attention because of their favorable physicochemical characteristics and antibacterial potential [7,8]. Their nanoscale size provides a larger surface area that may enhance interaction with bacterial cells and improve biological performance in biomedical applications [9].

The synthesis of silver nanoparticles using plant extracts, commonly referred to as green synthesis, has emerged as an environmentally friendly approach. In this method, phytochemical compounds present in the extract act as reducing and stabilizing agents minimizing the use of synthetic chemicals during nanoparticle production[10]. In addition, plant-mediated synthesized silver nanoparticles have been reported to exhibit enhanced antibacterial activity compared to crude plant extracts alone[11].

Based on these considerations, this study aimed to synthesize silver nanoparticles using ethanolic leaf extract of *Clerodendrum fragrans*, perform phytochemical screening and particle size analysis, and evaluate their antibacterial activity against *Staphylococcus aureus* as a representative skin-associated pathogen. The findings of this study are expected to support the development of plant-mediated silver nanoparticles as potential biomaterials for skin-related biomedical applications.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The main chemicals used in this study included silver nitrate ($AgNO_3$, ≥99%, analytical grade, Merck KGaA), ethanol, dimethyl sulfoxide (DMSO), sodium chloride (NaCl), and distilled water. Mueller–Hinton agar and Mueller–Hinton broth used for antibacterial assays were obtained from Oxoid. Chloramphenicol discs (30 µg) were used as positive controls in the antibacterial test. Fresh leaves of *Clerodendrum fragrans* were collected from North Sumatra, Indonesia.

2.2. Research Procedure

Plant Preparation and Extraction

Fresh leaves of *Clerodendrum fragrans* (4,0 kg) were washed, drained, and air-dried under shade to avoid direct sunlight exposure. Dried material was subjected to sequential maceration using solvents of increasing polarity (n-hexane, ethyl acetate, and ethanol). Extraction was carried out at room temperature with a solvent-to-material ratio of 4:1 (v/w) for 72 h per solvent. The residue was re-extracted with the subsequent solvents under identical conditions. The ethanolic filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a viscous extract [12].

Synthesis of Silver Nanoparticles Clerodendrum fragrans Leaf Extract (AgNPs)

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Silver nanoparticles were synthesized using a a silver nitrate precursor, 10 mM $AgNO_3$ stock solution was prepared by dissolving 0.169 g of $AgNO_3$ in 100 mL of distilled water and subsequently diluted to 1 mM. The plant extract solution was prepared by dissolving 1 g of extract in 100 mL of distilled water. Synthesis was carried out by mixing $AgNO_3$ (1 mM) and extract solution at a 1:1 (v/v) ratio, followed by incubation at room temperature for 24 h under low-light conditions. The formation of AgNPs was indicated by a visible color change of the reaction mixture[13]. Particle size distribution and average diameter were determined using a Particle Size Analyzer (PSA).

Antibacterial Activity Assay

Preparation of Media and Test Solutions

All glassware and materials were sterilized by autoclaving at 121 °C for 15 min. Mueller–Hinton agar (15.2 g) was dissolved in 400 mL of distilled water and heated under continuous stirring until completely dissolved. Test solutions were prepared using AgNPs, *C. fragrans* extract, and $AgNO_3$ in DMSO (1 mL) as the solvent, followed by vortexing to ensure homogeneity[14]

Rejuvenation and preparation of Inoculun Suspension

Bacterial strains were pre-cultured on Mueller–Hinton agar plates and incubated at 37 °C for 24 h. Colonies of *Staphylococcus aureus* were suspended in 2 mL of 0.9% NaCl and vortexed to achieve turbidity equivalent to the 0.5 McFarland standard [14].

Disc Diffusion Method

A 100 µL aliquot of bacterial suspension was uniformly spread onto solidified Mueller–Hinton agar plates and allowed to dry for 5 min. Sterile discs were then placed on the agar surface for each treatment and control. Test discs were impregnated with 20 µL of AgNPs, *C. fragrans* extract, or $AgNO_3$ solutions, while DMSO served as the negative control and a 30 µg chloramphenicol disc as the positive control. The plates were incubated at 37 °C for 24 h, after which the diameters of inhibition zones were measured to evaluate antibacterial activity [15,16].

Minimum inhibitory concentration

Minimum inhibitory concentration was determined using a broth microdilution method. Bacterial suspension (100 µL) was added to 4.9 mL of Mueller–Hinton broth. Aliquots (100 µL) of the inoculated medium were dispensed into wells 2–12, while well 1 served as the negative control containing only broth. The test solution (100 µg/mL) was added to well 12 and serially two-fold diluted across the plate to well 3, maintaining a final volume of 100 µL per well. The microplate was incubated at 37 °C for 24 h, and bacterial growth was assessed visually based on turbidity [17].

Minimum bactericidal concentration

Minimum bactericidal concentration was determined by subculturing 10 µL aliquots from each well of the microdilution assay onto Mueller–Hinton agar plates, followed by incubation at 37 °C for 24 h. Bacterial growth was then assessed to identify the lowest concentration showing no visible colony formation [18].

3. RESULTS AND DISCUSSION

3.1 Formation of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized using the ethanolic extract of *Clerodendrum fragrans* selected for its enrichment in polar phytochemicals particularly phenolics and flavonoids. These compounds are known to actively participate in redox processes where hydroxyl groups facilitate electron transfer enabling the reduction of Ag^+ to Ag^0 [19] according to the reaction mechanism shown in Figure 1. In this system the extract appears to

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play a dual role influencing both reduction kinetics and nanoparticle stabilization rather than acting solely as a passive reagent [20,21].

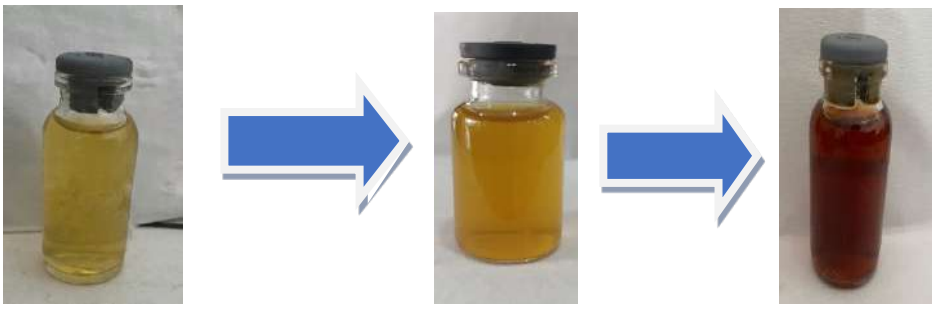

Figure 1. Color change of the $AgNO_3$–extract solution after 24 h

The use of a 1% extract concentration likely provided a sufficient density of reducing agents without introducing excessive organic content that could promote uncontrolled aggregation[22] . The relatively low concentration of $AgNO_3$ (1 mM) and the 1:1 mixing ratio suggest a system in which nucleation proceeds in a moderated manner [13]. Such conditions are generally associated with more controlled particle formation as opposed to rapid nucleation that often results in broad size distributions. This balance between metal ion availability and phytochemical reducing capacity is critical in determining nanoparticle characteristics .

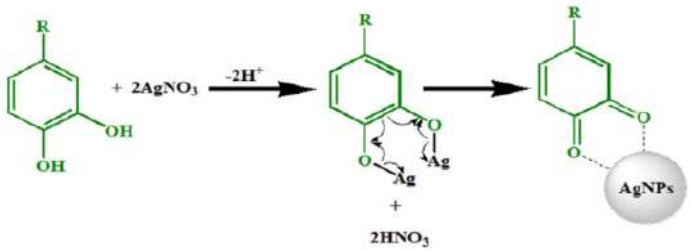

Figure 2. Schematic of AgNPs formation via phytochemical reduction of Ag^+ [23]

A visible color shift from green to reddish brown after 24 h indicated nanoparticle formation consistent with the emergence of surface plasmon resonance [24] as shown in Figure 1. Beyond this qualitative observation the concurrent processes of reduction and growth likely involved continuous adsorption of biomolecules onto the nanoparticle surface. Functional groups such as hydroxyl and carbonyl are expected to contribute to capping thereby limiting excessive growth and minimizing aggregation. Collectively these interactions highlight the importance of extract composition in governing both nanoparticle formation and colloidal stability[25].

3.2 Particle Size and Distribution (PSA/DLS)

The analysis revealed an average particle size of 114 ± 5.4 nm with a polydispersity index of 0.5 indicating a moderately polydisperse system. This value is consistent with numerous plant mediated synthesis studies where particle sizes often fall below 200 nm. The particle size distribution obtained from PSA–DLS analysis is presented in Figure 4. The relatively larger size is commonly attributed to the presence of phytochemical capping layers derived from the plant extract, which contribute to an increased hydrodynamic diameter and enhanced colloidal stability [26,27].

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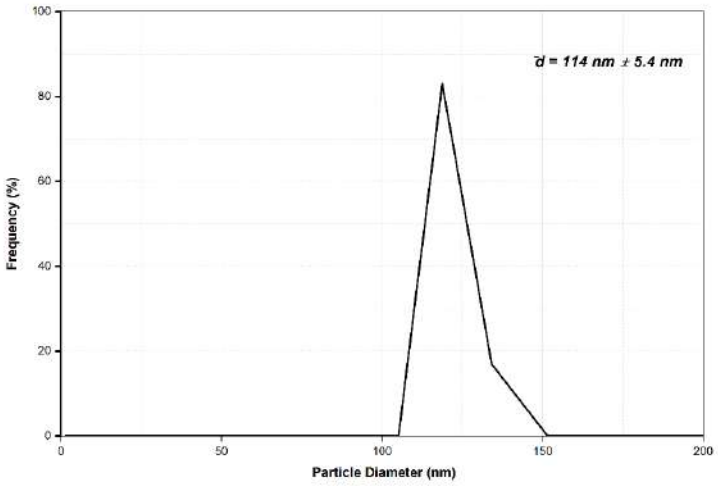

Figure 3. Particle size distribution of biosynthesized AgNPs determined by dynamic light scattering (DLS).

In plant mediated synthesis various constituents such as phenolics, flavonoids, and other organic compounds are known to interact with the nanoparticle surface. These molecules form a stabilizing layer which contributes to a larger measured size compared to the actual metallic core [28]. As a result DLS measurements typically report higher values due to the inclusion of this surface bound layer. The obtained size is consistent with commonly reported ranges for silver nanoparticles synthesized via green methods which often span from tens to several hundred nanometers depending on the extract composition and reaction conditions. Variations in size are largely influenced by the availability of reducing agents and the dynamics of nucleation and growth during synthesis [29]. The relatively low standard deviation (± 5.4 nm) suggests a fairly narrow size distribution indicating that particle formation occurred in a controlled manner. This uniformity may reflect the dual role of phytochemicals in both reducing silver ions and stabilizing the resulting nanoparticles [30 31]. Overall the results indicate that the synthesis approach produced well dispersed nanoparticles with a reasonably consistent size profile.

3.3 Antibacterial activity of AgNPs C. fragrans

Media preparation is an important stage in antibacterial testing as it greatly determines the validity and reproducibility of the results. The medium internationally recommended for bacterial sensitivity testing is Mueller-Hinton Agar (MHA) as it has a composition that supports bacterial growth and allows optimal diffusion of antibacterial agents [32].

3.3.1 Disk Diffusion method

In the disk diffusion method, antibacterial activity can be observed through the diameter of the clear inhibition zone. The disk diffusion test was conducted in triplicate. Figure 7 and Table 1 present the results of the test on *S. aureus*.

Figure 4. Results of the Growth Inhibition Test Against *Staphylococcus aureus*

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Table 1. Results of Growth Inhibition Measurement Against *Staphylococcus aureus*

NO.	Test Substance	Status	Inhibition Zone Diameter(mm)			Average (mm)
			Test1 (mm)	Test2 (mm)	Test3 (mm)	
1	Chloramphenicol	Positive Control	26,6	25,8	25,4	25,93
2	DMSO	Negative Control	0	0	0	0
3	Extract *C. fragrans*	Comparator	9,4	8,6	9,8	9,26
4	AgNO3	Comparator	6,8	7,5	6,4	6,9
5	AgNPs	Test Sample	10,4	11,3	11,5	11,06

The synthesized nanoparticles from the ethanolic leaf extract of *C. fragrans* exhibited notable antibacterial activity against *Staphylococcus aureus*, as indicated by the formation of clear inhibition zones. The AgNPs showed a mean inhibition zone of 11.06 mm, classified as strong which was higher than that of the extract (9.26 mm) and $AgNO_3$ solution (6.9 mm), both categorized as moderate. Inhibition strength was classified as strong (10–20 mm), moderate (5–10 mm), and weak (<5 mm) [33].

3.3.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is defined as the lowest concentration of an agent that inhibits visible bacterial growth, assessed by turbidity in liquid medium; clear broth indicates inhibition, whereas turbidity reflects growth. This method provides a quantitative measure of antibacterial efficacy [17].

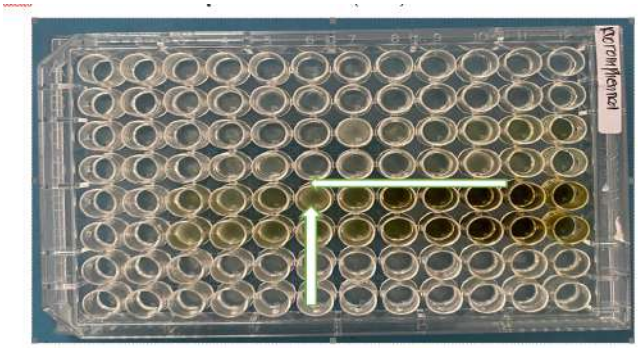

Figure 5. Results of the MIC Test on *Staphylococcus aureus*

Based on Figure 5 it can be seen that in the synthesized nanoparticles the area showing clarity was in row E, columns 12–8. In chloramphenicol, the clarity was in row A columns 12–3.

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Table 2. Results of the MIC Test on *Staphylococcus aureus*

Test Solution	Concentration (µg/mL)
Chloramphenicol (5000 ppm)	4.88
AgNPs (2500 ppm)	1.6875

The activity of an extract is categorized as strong when its MIC value is < 625 µg/mL [34].
Based on Table 3 it can be seen that the AgNPs had a minimum inhibitory concentration value of 1.6875 µg/mL. It can be stated that the MIC test of the *C. fragrans* extract against *S. aureus* was classified as strong.

3.3.3 Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of an antibacterial agent that is capable of killing ≥99.99% of the bacterial population which is indicated by the absence of colony growth after incubation. This parameter is important for distinguishing whether a substance is bacteriostatic or bactericidal. The determination of MBC is carried out by subculturing the suspension from the MIC test onto solid media and observing colony growth after incubation at 37°C [35]. The minimum bactericidal concentration (MBC) of AgNPs against *Staphylococcus aureus* are presented in Figure 6 and Table 3.

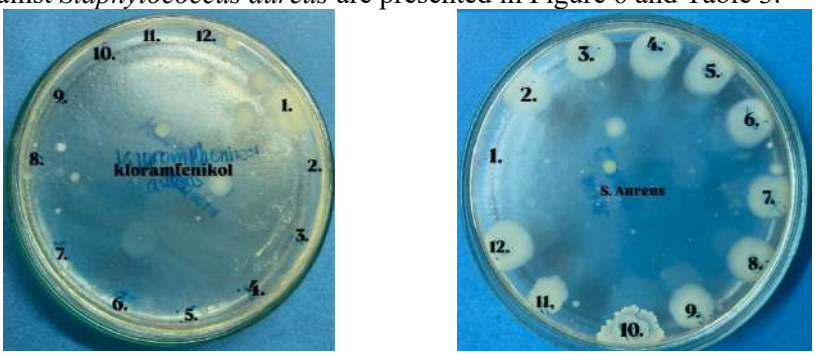

Figure 6. Results of the AgNPs MBC Test Against *Staphylococcus aureus*

In this study, the MBC test was carried out by inoculating the solution from each microplate well onto Mueller-Hinton Agar (MHA) media, followed by incubation for 24 hours. The absence of colony growth indicated that the concentration was bactericidal, whereas the appearance of colonies indicated that the test substance only inhibited bacterial growth. This procedure is widely used because it can provide direct confirmation of the ability of an agent to kill bacteria [36].

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Table 3. Results of the AgNPs MBC Test Against *Staphylococcus aureus*

Test Solution	Concentration (µg/mL)
Chloramphenicol (5000 ppm)	9.76
AgNPs (54 ppm)	>54

Based on Table 3. the MBC value of chloramphenicol was 9.76 µg/mL against *Staphylococcus aureus*. This indicates that chloramphenicol was bactericidal whereas the AgNPs from *C. fragrans* required a concentration higher than 54 µg/mL in other words the *C. fragrans* extract was not bactericidal.

The antibacterial activity of flavonoid mediated silver nanoparticles against *Staphylococcus aureus* is enhanced through the synergistic action of flavonoids and AgNPs. Flavonoids disrupt the bacterial cell membrane facilitating the penetration of silver nanoparticles and the release of Ag^+ ions into bacterial cells. Subsequently AgNPs further damage the membrane induce reactive oxygen species (ROS) production, and interfere with DNA replication and protein synthesis. In addition flavonoids stabilize the nanoparticles by preventing aggregation thereby increasing their interaction with bacterial cells. This synergistic mechanism results in greater cellular damage and more effective inhibition of bacterial growth than flavonoids or silver nanoparticles alone [35].

4. CONCLUSION

The results showed the physicochemical characteristics and antibacterial activity of silver nanoparticles (AgNPs) synthesized using *Clerodendrum fragrans* extract. Particle size analysis showed an average particle size of 114 nm with a polydispersity index of 0.5, indicating moderate particle distribution. Antibacterial testing showed that the AgNPs were able to inhibit the growth of *Staphylococcus aureus* with an inhibition zone of 11.06 mm (strong activity). The minimum inhibitory concentration was 3.375 µg/mL while the minimum bactericidal concentration was above 27 µg/mL indicating bactericidal activity at higher concentrations. These findings suggest that *C. fragrans*-mediated AgNPs have potential as environmentally friendly nanomaterials for biomedical applications.

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