

Green Synthesis Reviews: Synthesis of Silver Nanoparticles from Galangal Rhizome Extract (*Alpinia galanga*) and *Centella asiatica* Leaves as Bacterial Inhibitors

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Abstract: This review will discuss the green synthesis process of silver nanoparticles using galangal rhizome extract and centella leaf extract as capping agents and discuss their application as antibacterials. Both of these capping agents were obtained by the extraction process of the maceration method in which the macerated water is used as a reducing agent to precipitate silver nanoparticles. The characterization was carried out by looking at the absorbance at a wavelength of 350-450 nm because at that wavelength the silver nanoparticle solution is able to experience maximum absorption. The particle size obtained ranges from 50-90 nm depending on the concentration of silver used. The antibacterial results obtained on silver nanoparticles of galangal rhizome extract against *Pseudomonas aeruginosa* were able to obtain a clear zone with a diameter of 6.2 mm while the *Staphylococcus epidermidis* did not show any inhibition. Meanwhile, the silver nanoparticles of *Centella asiatica* leaf extract showed an inhibition in the form of a clear zone on the two test bacteria, namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively of 22.5 and 25 mm.

Keywords: green synthesis, nanoparticles, *Alpinia galanga*, *Centella asiatica* L.

Introduction

Nanoparticles are a technology that is currently developing. The advantage of nanoparticles is their very small particle size, which is <100 nm. Metal nanoparticles attract a lot of attention because of their broad applications and can be applied to detectors, electronics, catalysts, cosmetics and medicines (Wahyudi & Rismayani, 2008). Silver nanoparticles have been shown to have the ability to both as antimicrobials, namely against bacteria, viruses and eukaryotic microorganisms (Gong et al., 2007). Silver nanoparticles are synthesized by several methods and conditions such as chemical reduction methods, photochemistry, sonochemistry, solvothermal synthesis and others (Wahyudi & Rismayani, 2008). Currently, the utilization of living things such as microorganisms (Shankar,

2004), plant extracts (Chandran et al., 2006) is developing.) for the synthesis of nanoparticles, which is known as nanoparticle biosynthesis. Galangal impregnation has been studied to contain flavonoids, saponins, tannins, polyphenols and essential oils (Mishra, 2009). Concentration, shape, size, and contact time of bacteria with silver nanoparticles are factors that can affect the antibacterial activity of nanoparticles. Silver nanoparticles (AgNP) are substances that have antimicrobial properties that are effective for various organisms including pathogenic bacteria. Therefore, silver nanoparticles are used in the disinfection process in water and wastewater treatment. Synthesis of silver nanoparticles can be done in various ways. Synthesis of colloidal silver nanoparticles by chemical reduction and silver nitrate solution (AgNO₃) is a method that is often carried out

because of the simple process, using the reducing compound trisodium citrate (Haryono et al., 2008). Nanotechnology is an important field in the field of modern research. The nanotechnology approach is related to the design, synthesis, and manipulation carried out to obtain particle structures with sizes ranging from 1-1000 nm (Kim et al., 2016) which are then called nano-sized particles or nanoparticles. Nanotechnology is an important field in the field of modern research. The nanoparticle synthesis method is based on three process approaches namely chemistry, physics, and biology (Iravani et al., 2014). A similar study was also conducted by Jun Tian et al regarding the use of silver nanoparticles in wound healing therapy, where the observations made showed an accelerated healing of wounds treated with silver nanoparticles (Tian et al., 2007). In the last decades the biosynthesis of silver nanoparticles has developed. Silver nanoparticle biosynthesis is the synthesis of silver nanoparticles using reduction from natural materials. Several natural materials are capable of being reducing agents in the biosynthesis of silver nanoparticles such as *Capsicum Annuum* L (Li et al., 2007), *Azadirachta* (Ahmed et al., 2016), and *Brucea Javanica* L. Merr (Yudha et al., 2013)

Materials and Methods

The method used in this article review is to conduct a review of several journals related to extraction of natural materials to be used as capping agents in the synthesis of nanoparticles, characterization, and application of silver nanoparticles to the activity of bacterial inhibitors.

Tools and Material

The tools used in this study were UV-Vis spectrophotometer (Thermoscientific genesis 10), autoclave (All American Mode 25X-2), vortex (H-VM-300), microcentrifuge (HITACHI), oven (Fisher Scientific Model 655F), incubator (Heraeus Instrument D6450), incubator shaker (Barnstead, Lab-Line), micropipette, analytical balance (Mettler AE 200), vacuum rotary evaporator (Rotavapor Buchr-114), Buchner vacuum and

other standard laboratory glassware according to the procedure. autoclave, stir bar, suction ball, petri dish, erlenmeyer, hot plate, ncubator, wire netting, tripod, portable stove, cuvette, magnetic stirrer, round loop, volume pipette, dropper pipette, tube rack, spiritus, test tube, and analytical scales. The materials used in this study were sterile distilled water, gotu kola leaves, galangal rhizome, metal salts AgNO_3 , FeCl_3 .

Preparation of galangal rhizome extract and gotu kola leaves

Galangal rhizomes that have been cut and cleaned, weighed as much as 50 grams. Boiled with 120 ml of distilled water, boiled for 25 minutes and ultrasonicated for 30 minutes. Filtered using filter paper with the help of a Buchner vacuum, the filtered results were concentrated using a rotary evaporator to obtain a thick extract. (Chalish, et al, 2016)

Fresh centella leaves were cleaned, then cut into small pieces, then weighed as much as 10 grams. Macerated with 50 ml of distilled water, boiled for 5 minutes, then allowed to stand until it follows room temperature and then filtered using Whatmann No.42 filter paper. The filtered solution is ready to be used as a reducing agent for silver nanoparticles. (Indah ,et al, 2022)

Green Synthesis of Silver Nanoparticles Extract of Galangal Rhizomes and Centella Asiatica Leaves

Synthesis of silver nanoparticles was carried out by reacting galangal extract and 10-3 M AgNO_3 solution with a ratio of 1:1, 1:5, 1:10, and 1:20. Then stirring was carried out using an incubator shaker at room temperature with a speed of 150 rpm. The color change was observed every 60th minute. The solution was centrifuged at 10,000-12,000rpm and the residue was stored in the refrigerator. (Chalish, et al, 2016)

Synthesis of silver nanoparticles of *Centella asiatica* leaves was carried out by mixing a solution of AgNO_3 with boiled water from *Centella asiatica* leaves. 40 mL of 1.5 M, 1 M and 0.5 M AgNO_3 solutions were pipetted and each solution was put into a beaker, then 1 mL of boiled water from fresh gotu kola leaves was added.

After that, stirring was carried out with a magnetic stirrer at a speed of 1500 rpm at room temperature until a color change occurred in the solution. Then stored to see a significant difference on day 1 to day 7. (Indah ,et al, 2022).

Green Characterization of Silver Nanoparticle Synthesis of Galangal Rhizome and Centella Leaf.

Characterization of the synthesized silver nanoparticles of galangal extract and gotu kola leaf extract. The characters observed during the biosynthesis process included changes in the color of the solution which were observed visually during the synthesis process, microscopic characters using a UV-Vis spectrophotometer where the absorbance of the synthesized products was measured with a UV-Vis spectrophotometer. Absorption spectra were observed at a wavelength of 300-700 nm for Centella asiatica leaves and at a wavelength of 414-471 nm at a ratio of 1:5 for galangal rhizome extract. The stability of silver nanoparticles was observed based on changes in λ_{max} at certain times with a UV-Vis spectrophotometer. (Chalish, et al, 2016) (Indah, et al, 2022).

Bacterial Test of Silver Nanoparticles of Galangal Rhizome and Centella Leaf

Testing galangal rhizome extract on bacteria. Bacteria that have been inoculated into the Nutrient Broth growth medium, each is streaked on the surface of the Nutrient Agar medium using a sterile cotton bud. A total of 10 μ L of each synthesized silver nanoparticle and the extract of each rhizome was pipetted onto a paper disc (6 mm in diameter) that had been placed on a drip plate using a micro pipette. Amoxsan® was used as a positive control for *Pseudomonas aeruginosa* while chloramphenicol® was used as a positive control for *Staphylococcus epidermidis*. Positive control was made with a concentration of 30 μ g/mL.(Chalish, et al, 2016)

DM aqua was used as a negative control. Paper discs were placed on 4 Nutrient Agar media then incubated in an incubator at 37°C. The diameter of the clear zone around the discs was measured after 24 hours of incubation using a caliper. Qualitative

testing of anti-bacterial activity was carried out using the disc diffusion method where disc paper was moistened with silver nanoparticles (AgNPs) and affixed to nutrient agar which had been added to the test bacteria *pseudomonas aeruginosa* and *staphylococcus aureus*. Incubated for 24 hours at 37 oC. In the boiled water of fresh centella leaves, AgNO₃, positive control and negative control were carried out. The positive control was used to test *pseudomonas aeruginosa* bacteria using gentamicin and for *staphylococcus aureus* bacteria using chloramphenicol as a positive control. In both the sterile distilled water test bacteria were used as a negative control. The clear zone around the disc paper on the surface of the media was observed to determine the inhibition power. (Indah, et al, 2022).

Results and Discussion

Characterization of silver nanoparticles of galangal rhizome using a UV-Vis spectrophotometer aims to determine and detect the formation of silver nanoparticles. Based on research conducted by Sileikate et al (2006), silver nanoparticle solutions have a maximum wavelength in the range of 350-450 nm. Characterization of silver nanoparticles from galangal extract using a UV-Vis spectrophotometer was carried out at a ratio of 1:1, 1:5, 1:10 and 1:20. The spectrum of the solution characterization results in a ratio of 1:5. Data from the characterization of galangal nanoparticle solutions are presented in Table 1.

Table 1. Galangal nanoparticle solution characterization data at various ratios. using a UV-Vis spectrophotometer.(Chalish, et al, 2016)

Ratio	Wavelength (nm)	absorbance
1:1	414-463	<0.5
1:5	435-471	>1
1:10	432-458	>0.8
1:20	426-456	>0.7

Based on Table 1, the 1:5 ratio has a higher absorbance value than the other ratios.

The ratio of 1:5 is the optimum ratio used in the antibacterial activity test.

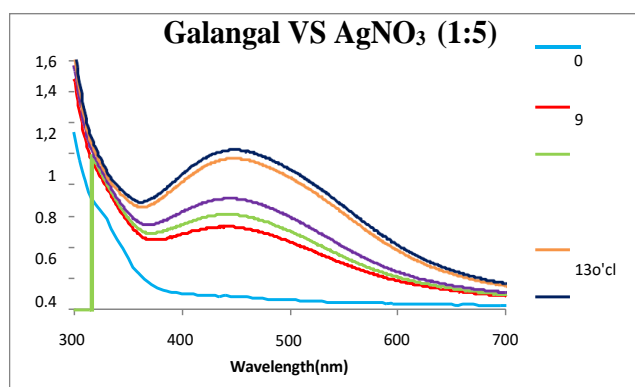


Figure 1. UV-Vis spectrum of silver nanoparticles from galangal in a ratio of 1:5 (Chalish, *et al*, 2016)

Following the bacterial test on galangal rhizome extract, the bacteria that had been inoculated into the NB growth medium were streaked on the surface of the sterile NA medium using a cotton bud. As much as 10 μ L of each synthesized silver nanoparticle and the extract of each rhizome was pipetted onto paper discs (6 mm in diameter) which had been placed on the drip plate using a micro pipette. Amoxsan® was used as a positive control for *Pseudomonas aeruginosa* while chloramphenicol® was used as a positive control for *Staphylococcus epidermidis*. The positive control was made at a concentration of 30 μ g/mL. DM aqua was used as a negative control. 4 paper discs were placed on NA medium and then incubated in an incubator at 37°C. The diameter of the clear zone around the disc was measured after 24 hours of incubation using a caliper. The silver nanoparticles used for the test were silver nanoparticles in a 1:5 ratio contact time 19 hours. The results of the antibacterial activity test of galangal silver extract and nanoparticles are presented in Table 2.

Table 2. Antibacterial test of galangal rhizome(Chalish, *et al*, 2016)

Pathogenic Bacteria	Average inhibition zone diameter (mm)		
	(+)	Galangal water extract	Silver nanoparticles
<i>S. epidermidis</i>	29	-	-
<i>P. aeruginosa</i>	24	-	6,5

Based on the results of the antibacterial activity test between silver nanoparticles and extracts listed in Table 2, silver nanoparticles have activity in inhibiting the growth of *P. aeruginosa* bacteria

The formation of silver nanoparticles was reviewed based on the color change of the solution. The color change of the solution is shown in Figure 2. As the contact time increases, the color of the solution will become darker. The initial color change was formed at the 9th hour of stirring. The color change is an indication of the silver ion reduction process, resulting in the formation of silver nanoparticles (Bakir, 2011). In this research, the synthesis of silver nanoparticles was carried out using a biological method that utilized secondary metabolites from *Centella asiatica* to reduce silver. Biosynthesis of nanoparticles was carried out using AgNO_3 with three different concentrations, namely 0.5 M, 1 M, and 1.5 M, as well as different synthesis times, namely 1 day, 2 days, 3 days, 5 days, 6 days, and 7 days. This was done to see the effect of precursor concentration and synthesis time on the synthesis of silver nanoparticles. The formation of silver nanoparticles can be observed visually by looking at the darker color changes that occur with the passage of synthesis time in (figure 2).

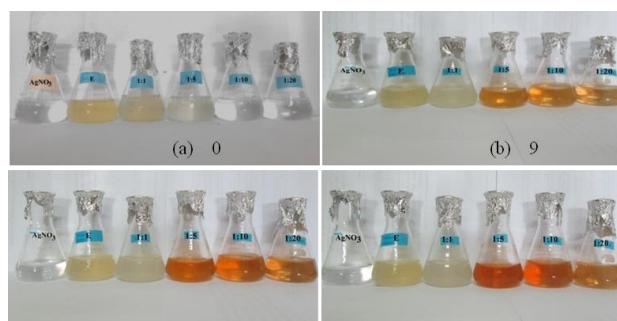


Figure 2. Characterization of the color of the solution in the synthesis of nanoparticles using water extract of galangal rhizome. (Chalish, *et al*, 2016)

Silver nanoparticles (AgNPs) have been widely synthesized, especially by the greensynthesis method and are also widely used in various fields, one of which is as an antimicrobial. Greensynthesis of silver nanoparticles is carried out by utilizing plant bioactive compounds to reduce Ag^+ ions to Ag^0 . This is done so that the process of forming silver nanoparticles becomes more environmentally friendly. Based on the literature, the process of reducing Ag^+ ions to Ag^0 cannot be separated from the role of certain bioreductor compounds such as phenolic compounds, terpenoids, flavonoids and other

compounds in plants that contain aldehyde, carboxylic acid and amide functional groups. From the results of phytochemical screening, the boiled water of fresh gotu kola leaves positively contained saponins and flavonoids. The results can be seen in table 3. The presence of these two compounds allows *Centella asiatica* leaves to be used as a bioreductor in the synthesis of silver nanoparticles. From some literature it is stated that these compounds can reduce Ag⁺ ions from silver nitrate to Ag⁰. The general mechanism of the reaction for the formation of silver nanoparticles by flavonoids is illustrated in table 3.

Table 3. Phytochemical screening results for secondary metabolite compounds in boiled water of fresh *Centella asiatica* leaves. (Indah ,et al, 2022)

Compound	Solvent	Description
Alkolooid	Aquades	Negative (-)
Flavonoids	Aquades	Positive (+)
Saponins	Aquades	Positive (+)
Tannins	Aquades	Negative (-)
Triterpenoids	Aquades	Negative (-)

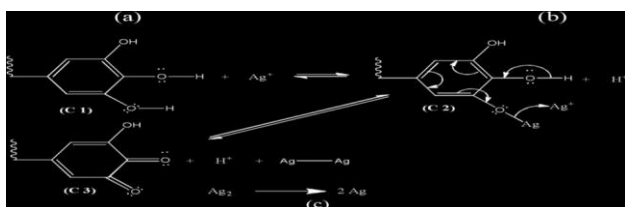


Figure 3. The reaction for the formation of silver nanoparticles (AgNPs) (Indah,et al, 2022)

The formation of silver nanoparticles can be further confirmed by observing the absorbance spectrum of the solution with a UV-Vis spectrophotometer at a wavelength of 400-500 nm which is a typical wavelength for silver nanoparticles. For reference the range of particle sizes based on wavelength can be seen in table 5.

Table 4. Differences in Absorbance and Maximum Wavelength obtained from variants of AgNO₃ concentrations and different synthesis times. (Indah ,et al, 2022)

Concentration AgNO ₃ (M)	Boiled water of fresh gotu kola leaves (mL)	Time Synthesis (days)	Maximum wavelength (Amax) nm	absorbance (AU)	nanoparticle size Silver (n.m.)
0.5	1	1	476.80	4.1693	70-90
		2	475.60	4.1559	
		3	466.90	5.0247	
		5	481.75	4.1566	
		6	456.75	3.6175	
		7	459.30	3.6468	
		1	1	1	
2	476.80			3.8693	
3	480.55			3.8654	
5	486.65			4.1350	
6	454.20			3.5921	
7	471.90			4.1230	
1.5	1			1	439.40
		2	445.55	3.8411	
		3	432.05	3.0630	
		5	470.65	4.1058	
		6	464.35	4.0076	
		7	455.45	3.0904	

Table 5. Range of particle sizes with particle wavelengths for silver nanoparticles. (Indah ,et al, 2022)

Particle size(nm)	Lambda range(nm)
20	405
30	410
40	416
50	423
60	441
70	451
80	467
90	493
100	501
110	523

Antibacterial testing was carried out by the diffusion method using a paper disc on *Pseudomonas aeruginosa* for gram-negative bacteria and *Staphylococcus aureus* for gram-positive bacteria. The silver nanoparticle samples tested were AgNPs with a concentration of 1.5

M. Antibacterial testing for gram-negative bacteria used Gentamicin (100 ppm) as a positive control, while for gram-positive bacteria, Chloramphenicol (250 ppm) was used. As well as sterile aqua destillata as a negative control. In this test, an antibacterial test was also carried out on 1.5 M AgNO₃ and boiled water from fresh gotu kola leaves as a comparison, which is the starting material or composition of silver nanoparticles, so it must be analyzed to see if there is an increase in antibacterial inhibition after the two This material forms silver nanoparticles. The incubation process was carried out at 37°C for 24 hours. After 24 hours of incubation, the diameter of the inhibition zone was measured. Table 6 and Table 7 show the results of the inhibition zone measurements on the results of the antibacterial activity test.

Table 6. Interpretation of inhibition. (Indah, et al, 2022)

Bright Zone Diameter (nm)	Inhibitory Strength
<5	Weak
6-10	Currently
11-20	Strong
>21	Very strong

Table 7. Results of inhibition zone measurements on antibacterial activity testing. Inhibition zone (mm). (Indah, et al, 2022)

Bacteria	3rd replication	Silver nanoparticles	AgNO ₃	gotu kola	Positive control	Negative control
Pseudomonas aeruginosa	1	26	12	12	28	0
	2	25	13	11	33	0
	3	24	11	9	32	0
	Average	25	12	10	31	0
Staphylococcus aureus	1	22	10	10	26	0
	2	23	9	8	28	0
	3	23	13	12	27	0
	Average	22,6	11	10	27	0

In the test, it was found that the AgNO₃ solution and the boiled water of *Centella asiatica* leaves showed the presence of an inhibition zone on the two test bacteria. However, the resulting inhibition zone was smaller than the inhibition zone generated by AgNPs. In testing the gram-positive bacteria, *Staphylococcus aureus*, the average inhibition zone produced by AgNO₃ solution was 11 mm, and gotu kola was 10 mm, while the average AgNPs inhibition zone was 22.6 mm. This means that there is an increase in the value of the inhibition zone in the test using silver nanoparticles compared to the AgNO₃ solution and gotu kola itself. Similar results were also shown in testing the antibacterial activity against gram-negative bacteria, *Pseudomonas aeruginosa* where the average inhibition zone produced by AgNO₃ solution was 12 mm, and *Centella asiatica* was 10 mm while the average AgNPs inhibition zone was 25 mm. The difference in the inhibition zone between AgNPs (silver nanoparticles) and their precursors is due to the fact that the smaller the particle size, the greater the surface area which increases contact with bacteria. Then there will be interactions between silver ions and sulfhydryl thiol groups in proteins. This silver ion will replace the hydrogen cation (H⁺) in the sulfhydryl thiol group (SH) which will produce a stable S-Ag group on the surface of the bacterial cell which causes the protein to become deactivated, which has an impact on decreasing membrane permeability, and ultimately causing cellular death. Then there will be interactions between

silver ions and sulfhydryl thiol groups in proteins. This silver ion will replace the hydrogen cation (H⁺) in the sulfhydryl thiol group (SH) which will produce a stable S-Ag group on the surface of the bacterial cell which causes the protein to become deactivated, which has an impact on decreasing membrane permeability, and ultimately causing cellular death. Then there will be interactions between silver ions and sulfhydryl thiol groups in proteins. This silver ion will replace the hydrogen cation (H⁺) in the sulfhydryl thiol group (SH) which will produce a stable S-Ag group on the surface of the bacterial cell which causes the protein to become deactivated, which has an impact on decreasing membrane permeability, and ultimately causing cellular death.

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Conclusions

Silver nanoparticles can be synthesized using the bioreductor method of galangal rhizome water extract with stirring using a shaker. The best silver nanoparticles synthesized from galangal are the ratio of 1:5 at a contact time of 19 hours. Silver nanoparticles have high antibacterial activity compared to extract antibacterial activity.

(Chalish, et al, 2016)

Based on the research that has been done, it can be concluded that boiled water from fresh *Centella asiatica* leaves can be used as a bioreductor in synthesizing silver nanoparticles for 1 week which is indicated by the presence of absorption peaks at a wavelength of 400-500 nm. The size of the silver nanoparticles produced ranges from 50 to 90 nm. Biosynthesized silver nanoparticles had better antibacterial activity than silver nitrate and boiled water from *Centella asiatica* leaves, judging from the inhibition zone for *Pseudomonas aeruginosa* of 25 mm for AgNPs, 12 mm for AgNO₃ and 10 mm for *Centella asiatica*. Likewise with *Staphylococcus aureus* 22.6 mm for AgNPs, 11 mm for AgNO₃ and 10 mm for gotu kola. (Indah, et al, 2022)

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