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Green Synthesis of Antimicrobial Silver Nanoparticles using Green Tea Extract: The Role of Concentration and pH

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ABSTRACT: Silver nanoparticles (AgNP) have been proven effective against many microbial strains with their superior antibacterial properties, due to their nano size and large surface area that can interact directly with the bacterial structure. Green synthesis for silver nanoparticles is the process of reducing Ag^{+} ions to Ag^{0} using plant bioactive compounds. Green synthesis is safer, environmentally friendly, and cost-effective. *Camellia sinensis* L. (green tea) containing polyphenols that can be used as a bioreductant in the formation of silver nanoparticles. This study aims to determine the effect of various concentration of $AgNO_3$, green tea extract, and pH on the physical characteristics and antibacterial activity of silver nanoparticles. The AgNPs were evaluated by UV-Vis Spectrophotometer, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The result demonstrated that the optimal AgNP was obtained from the $AgNO_3$ concentration of 1.5 mM with 0.003% green tea extract at pH 10 which showed the highest absorbance value of 4.420 at λ 435.3 nm, with a particle size of 47.1 nm and a PDI of 0.243. The AgNPs showed growth inhibition on *Staphylococcus aureus* and *Escherichia coli* and the antibacterial activity enhance compare to $AgNO_3$.

Keywords: **silver nanoparticles; green synthesis; green tea extract; pH; antibacterial activity.**

Introduction

Nanoparticles (NPs) are organic and inorganic structures with sizes ranging from 1 to 100 nm, which can be obtained by physical, chemical, and recently environmentally friendly synthesis methods [1]. Recent findings show that when particles of a certain material are reduced to the nanometer scale, they will have different properties compared to the original particles which include larger surface areas (enhancing physical, chemical, and biological activity), increased solubility, and a higher level of sensitivity [2]. In the medical field, silver is a non-toxic and safe inorganic antibacterial agent that can kill about 650 types of pathogenic microorganisms, but the increasing number of bacterial strains resistant to some antibiotics has led to the development of new antibacterials [3]. Silver is frequently used as an antibacterial agent in open wound infections [4]. Silver nanoparticles (AgNP) offer the benefit of a large surface area due to their small size, enabling direct interaction with bacterial cells [5]. AgNP was also formed based on the Surface Plasmon Resonance (SPR), as the higher concentration of $AgNO_3$, green tea extract, and pH. The absorbance value increases which indicates the amount of silver nanoparticles increases [6]. The manufacture of silver nanoparticles is often carried out using chemical method that is expensive and also uses toxic and hazardous chemicals that may have potential environmental and biological hazards. Since the physical methods provide low yields and require high energy [7]. Meanwhile, biological method is an alternative that provides an environmentally friendly way to synthesize nanoparticles. In addition, this method is characterized as economical, easy to synthesize, does not require expensive costs, harmless, and non-toxic [8]. The nanoparticle synthesis involving reducing of ions and the formation of AgNP from plant metabolite compounds, such as terpenoves, flavones, ketones, aldehydes, amides, and carboxylic acid, is known as biosynthesis or green synthesis [7]. Most NPs synthesized using plant extracts exhibit sphere sizes ranging from 5 to 50 nm and superior antibacterial activity [9].

Green tea extract from the leaves of *Camelia sinensis. L* has antibacterial activity due to the presence of naturally reducing polyphenolic components [10]. Green tea extract can be used as a bioreductor and stabilizer,

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in the process of of AgNP formation [11]. Therefore, green tea extract replaces organic solvents and chemical compounds in aqueous media, eliminating several steps and thus reducing costs [12]. The size and shape of NPs synthesized using plant extracts can be controlled and modified by changing parameters such as pH, temperature, reaction time [13], and concentration $AgNO_3$ [14].

Antibacterial activity on silver nanoparticles is generally influenced by several factors including shape, size, unique chemical and physical properties, and high spesific surface [15]. I n t his s tudy, w e f abricated s ilver nanoparticles using an environmentally friendly method to investigate the effect of $AgNO_3$, green tea extract, and pH, then evaluate the antibacterial potential of silver nanoparticles.

Methods

Materials

Silver nitrate ($AgNO_3$) (Merck KgaA, Germany), green tea extract (IMAHERB BIOTECH Co., LTD), nutrient broth (Merck KgaA, Germany), sodium hydroxide (NaOH) (Merck KgaA, Germany), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) from the Assessment Service Unit at Universitas Airlangga, Indonesia.

Green synthesis of silver nanoparticles

The synthesis of nanoparticles used $AgNO_3$ with concentrations of 0.5 mM, 1.0 mM, and 1.5 mM and green tea extract with concentrations of 0.001%, 0.002%, and 0.003%.

25 mL of green tea extract solution was added to 25 mL of $AgNO_3$ solution and stirred at 250 rpm. The pH was adjusted using 1N NaOH to obtain pH 8,9 and 10. The reaction was allowed for 1.5 h at room temperature.

Characterization of AgNPs

Spectrophotometry UV-Vis

The UV-Vis spectrophotometer (Hitachi UH5300) was used to measure the Surface Plasmon Resonance (SPR) of the AgNP. The SPR measurement was carried out with a wavelength of 300 - 550. From the measurement, the maximum wavelength and absorbance of the AgNP solution will be obtained.

Dynamic Light Scattering (DLS)

Particle size and polydispersity index of AgNP particles were analyzed using Delsa Nano C particle analyzer (Beckman Coulter) with Dynamic Light Scattering (DLS). The average diameter and polydispersity index (PDI) were calculated by the software from the measured particle distribution.

Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The analysis was carried out using (Brucker Eco-ATR) with a sample of silver nanoparticles placed in a holder to be observed in the wave number range 4000-500 cm^{-1} with a spectrum resolution of 4 cm^{-1} to determine the functional groups contained in the AgNP sample.

Scanning Electron Microscopy (SEM)

The morphology of the particle was determined using SEM (Hitachi FlexSEM). The AgNP was mounted on a carbon-coated holder, which was then inserted into a sputter coater for gold palladium plating that lasted for approximately 120 seconds. Examination was carried out using an inspect S50 type FP 2017/12 at various magnifications, with measurements taken on multiple particles.

Antibacterial activity Assay

Bacterial suspension preparation

The bacteria used in this evaluation were *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739. Suspensions of each bacterium were prepared by adding 0.9% NaCl solution. The turbidity of bacterial suspension was measured using UV-Vis spectrophotometer at λ 580 nm. The bacteria suspension can be used if the % transmittance is 25 ± 1%

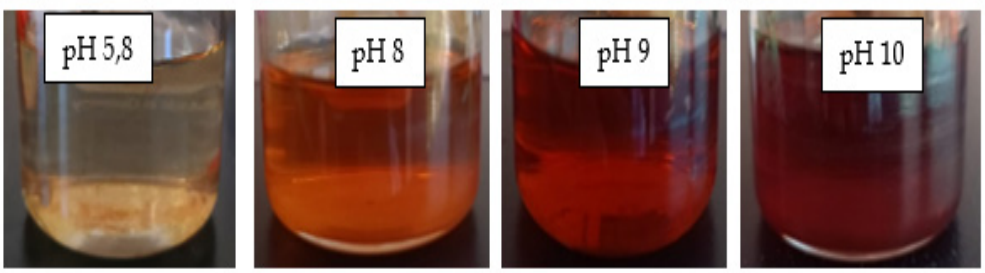

Figure 1. The color difference of AgNP at different pH

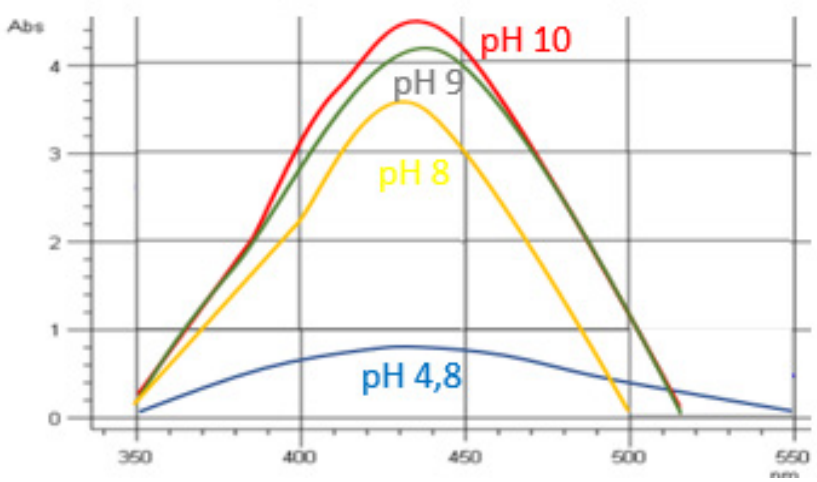

Figure 2. Absorbance spectra of AgNP with various pH at λ 350-550 nm

Antibacterial activity of AgNP

200 µl of cultured bacterial suspension in 0.9% NaCl was added into an Erlenmeyer containing 19.8 mL of NB liquid media. Further AgNP solutions with the volume of 5 mL, 10 mL, 15 mL and 20 mL equivalent to 0.187 mM, 0.375 mM, 0.562 mM and 0.75 mM respectively were added into the erlenmeyer. The mixtures were shaken for 30 minutes to maximize contact between bacteria and AgNPs. Then 1 ml of each mixture was added to 9 mL of NB, then incubated at 37°C for 24 h. The turbidity was observed by measuring the %transmittance using a UV-Vis spectrophotometer at λ 580 nm. The evaluation also performed for $AgNO_3$. Kanamycin at 50 ppm was used as a positive control.

Results and Discussion

Synthesis of (AgNP) and Characterization

In this study, silver nanoparticles were synthesized with green tea extract. The formation of AgNP can be determined by a UV-Vis spectrophotometer to observe the maximum wavelength that corresponds to the wavelength absorption of AgNP in the 400-450 nm range [16]. In green synthesis methods the formation of silver particles were observed in nanoscale with the presence of a large absorption band in the visible region. It is known that AgNPs had different colors, depending on the pH and size of AgNP [2]. The AgNPs formed were characterized by a change in the color of the solution from clear to bright yellow. Adjusting the pH of the solution causes a change in color to blackish brown (Figure 1), The color changed as the pH solution increased which indicated that AgNP had been formed and the color was the characteristics of the AgNP' Surface Plasmon Resonance (SPR) [13].

SPR depend on several factors that can affect the electron charge density on the surface of a particle, such as the type of metal, particle size, particle shape, particle structure, and dielectric constant of a medium [17]. The absorbance at a wavelength of about 400 nm indicates

Table 1. Absorbance value and particle size of AgNP synthesized with various concentrations of $AgNO_3$, green tea extract, and pH

Observation	Variable	Green Tea Extract								
	$AgNO_3$	pH 8			pH 9			pH 10		
		0.001%	0.002%	0.003%	0.001%	0.002%	0.003%	0.001%	0.002%	0.003%
Particle Size (nm)	0.5 mM	50.4	43	58	36.9	42.5	56.8	65	60.1	42.8
Absorbance		2.512	2.474	2.505	2.480	2.739	2.817	2.093	2.104	2.328
Particle Size (nm)	1 mM	80.4	69.7	66.2	46.2	51.7	77.6	38.4	37.8	35.6
Absorbance		2.798	2.921	3.006	2.969	3.393	3.388	3.561	3.485	3.917
Particle Size (nm)	1.5 mM	47	54.8	48.5	37.8	40.3	46.3	60.1	52.8	47.1
Absorbance		3.432	3.794	3.895	3.907	4.084	4.270	4.266	4.385	4.420

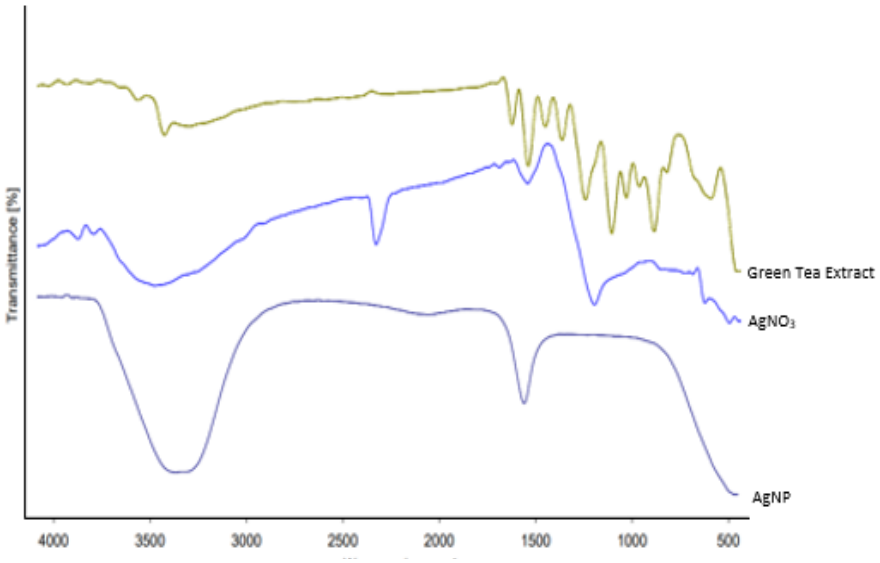

Figure 3. FTIR spectra of AgNP pH 10

the presence of silver nanoparticles formed. This can be seen in Figure 2, the synthesis of AgNPs with increasing pH solution caused the absorption spectra shift towards narrower wavelength range, and the absorbance value increased, which indicated the amount of AgNP formed became higher [18]. Table 1 shows that absorbance values increased from 2.093 to 4.420 as the pH elevated from 8 to 10.

Table 1 also presents the results of the evaluation using DLS to determine the particle size and particle distribution. It was known that the particle size was in the range of 35.6 nm – 80.4 nm with PDI value was 0.095 – 0.271 indicated a homogeneous particle size [13]. PDI values close to 0 or < 0.3 indicate homogeneous particle size, while PDI value > 0.3 indicate heteregeneous particle size[19]. From the results, the AgNP synthesis with 1.5 mM $AgNO_3$, 0.003% green tea extract at pH 10 had highest absorbance value indicated higher NPAg formed compare to other condition.

The ATR-FTIR was used to determine the functional groups and chemical interactions that occur in the formation of AgNP. The results of ART-FTIR (Figure 3) showed the peak at the wavenumber 3321.30 cm^{-1} and the hydroxyl group (-OH) presence of hydrogen bonds in AgNP [20]. The wavenumber 2104.96 cm^{-1} indicated that the formation of AgNP also involved stretching of symmetric and asymmetric C-H and CH aliphatic from CH_2 group. When compared with the green tea extract spectrum, there was a slight shift from 3223.38 cm^{-1} to 3415.42 cm^{-1}. This showed the role of the phenolic group

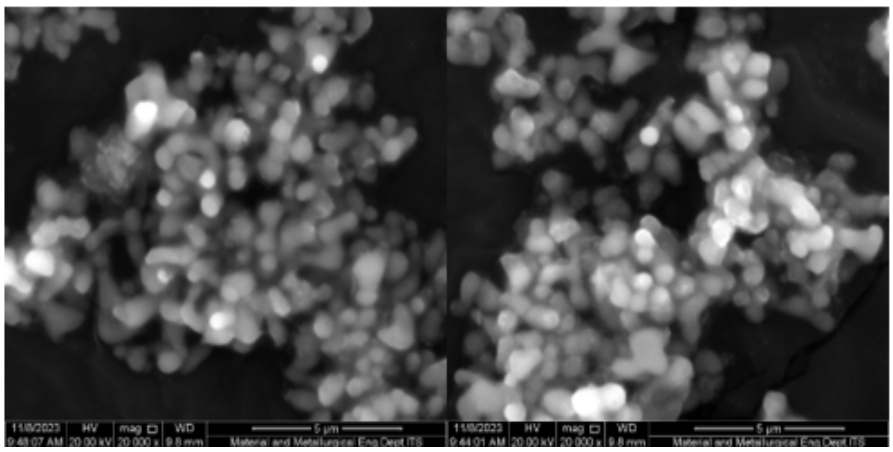

Figure 4. SEM results of AgNP with magnification 20.000x formed from a concentration of 1.5 mM $AgNO_3$ and 0.003% green tea extract at pH 10

Table2. Antibacterial activity of $AgNO_3$ and AgNP againts *Staphylococcus aureus* and *Escherichia coli*

Bacteria	Transmittance (%)									
	Control (+)	Control (-)	$AgNO_3$ concentration (mM)				AgNP concentration (%)			
			0.187	0.375	0.562	0.75	0.187	0.375	0.562	0.75
S.aureus	96.17 ± 0.26	28.4 ± 0.29	34.63 ± 0.12	50.97 ± 0.25	57.30 ± 0.08	66.69 ± 0.09	75.3 ± 0.4	80.5 ± 0.031	82.2 ± 0.24	83.7 ± 0.17
E.coli	90.47 ± 0.09	30.5 ± 0.25	30.53 ± 0.12	38.37 ± 0.12	46.57 ± 0.34	57.80 ± 0.34	48.3 ± 0.08	79.6 ± 0.12	81.1 ± 0.12	82.8 ± 0.17

of the green tea extract compound in the formation of AgNP which act as reducing agent [2].

The SEM was used to determine the morphology of the particle surface. Figure 4 showed that AgNP with concentration 1.5 mM $AgNO_3$ and 0.003% green tea extract at pH 10 had spherical shape, irregular surface and tended to aggregate. Nanoparticles were known to be easily aggregated due to the high surface area, especially when the sample was kept in liquid form [21].

Antibacterial Activity of AgNP

The antibacterial activity was analyzed by comparing the % transmittance of AgNP with the % transmittance of $AgNO_3$, the positive control (Kanamycin), and the negative control. AgNPs <100 nm in size can effectively attack the bacterial cell membrane, the smaller the size of AgNPs, the easier it is to penetrate and causing toxicity the bacterial cell wall [22]. Silver ions attach to the cell wall or cytoplasmic membrane, it enhances the permeability of the cell and ultimately leads to cell distruption [23].

The results of the antibacterial activity test can be seen in Table 2 and Figure 5. The % transmittance value of AgNP was greater compared to $AgNO_3$ on both bacteria tests, *Staphylococcus aureus* and *Escherichia coli*. Otherwise, the activity of AgNP against *Escherichia coli* was more concentration-dependent. The antibacterial activity of AgNPs depends on several parameters, namely particle size, particle shape, and particle surface charge [24]. Positively charged AgNP were the most effective particles and the negatively charged ones were the least effective particles againts the microorganisms tested. This is due to the repulsion between bacteria and negatively charged NPAg, which can form an electrostatic barrier, thus limiting the interaction between AgNPs and bacteria [24]. This study revealed that AgNPs were more effective against gram-positive bacteria (*Staphylococcus aureus*) than to gram-negative (*Escherichia coli)*. This is due to the difference of membrane and cell wall structure between gram-positive and gram-negative bacteria [25]. Gram-negative cell walls have an outer membrane, a lipopolysaccharide layer that contains polysaccharides and protein, a thicker periplasm, a gel-like matrix between the inner cytoplasmic membrane [26].

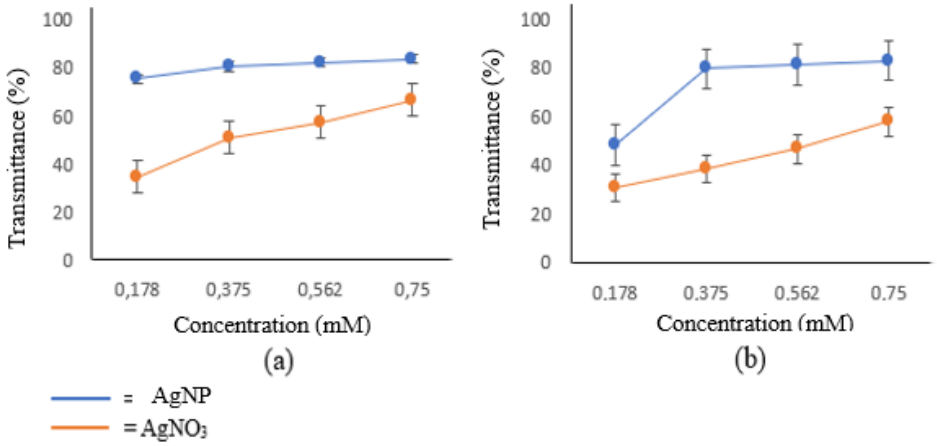

Figure 5. Antibacterial activity of AgNP and $AgNO_3$ against (a) *Staphylococcus aureus* (b) *Escherichia coli.*

Conclusion

The formation of AgNPs is affected by $AgNO_3$, green tea extract concentration, and pH. AgNP obtained from 1.5 mM $AgNO_3$, 0.003% green tea extract and pH 10 has the smallest particles size and highest absorbance. The antibacterial activity of AgNP is higher compared to the $AgNO_3$ and concentration-dependent against *Escherichia coli*.

Conflict of Interest

The authors declared no conflict of interest.

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