The Potency of Biologically Synthesized Silver Nanoparticles Using *Andrographis Paniculata* Extract (S-AgNp) as an Antibacterial Agent

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**Abstract.** This study reported the potency of ‘biologically synthesized silver nanoparticles using *Andrographis paniculata* extract’ (S-AgNp) as an antibacterial agent and its minimum inhibitory concentration (MIC). The antibacterial test was conducted to pathogenic bacteria of *S. aureus* and *E. coli* by using a diffusion-well method. The media used were Nutrient Agar (NA) and Nutrient Broth (NB). The tests were carried out in two steps. The first step was determining the potency of S-AgNp as an antibacterial agent. This step used S-AgNp with the concentration of 100%, *Andrographis paniculata* extract and AgNO₃ solution as a positive control, and distilled water as negative control. It was found that S-AgNp were able to inhibit the growth of both bacteria. The second step was finding out the MIC of S-AgNp to both bacteria. In this step, the concentration of S-AgNp tested from 70% to 10% and amoxicillin of 5% was used as the positive control. It was found that the antibacterial activity on both bacteria increased as increasing the concentration of S-AgNp tested. The growth inhibition of *S. aureus* was greater than that of *E. coli* (p < 0.05). The MIC value of both bacteria was 0.0005 g/mL. Based on this result, it is concluded that S-AgNp has a potency to be used as an antibacterial agent, especially for *S. aureus*.

**Keywords:** Silver nanoparticle, *Andrographis paniculata*, biosynthesis, antibacterial agent

1. Introduction

In general, antibacterial agents can be classified as bactericidal, bacteriostatic, and germicidal [1]. There is a critical requirement to produce new antimicrobial agents from numerous sources to fight microbial resistance. Based on the previous works, it has been well known that silver nanoparticles (AgNp) can prevent bacterial development and even destroy microorganisms. Interestingly, it can be used in some common public places in daily activities [2]. Silver has been used for years in the medical field for antimicrobial applications such as removal of microorganisms on textile fabrics, burn treatment, disinfection in water treatment and prevention of bacterial colonization on catheters [3,4].

A previous work reported that AgNp has an important antibacterial activity to *E. coli* and *S. aureus*, which are multi recessive to various drugs [5]. Furthermore, Pal et al. found that the antibacterial activity of AgNp to *E. coli* was influenced by the structure of the nanoparticles [6]. Meanwhile, Kim et al.,
reported that AgNp damaged the fungal cells by attacking their membrane cells [7]. This has been also strengthened by the results of the analysis using transmission electron microscopy (TEM) where the interaction between AgNp and the membrane structure of C. albicans cells occurred when the cell membrane was exposed to AgNp. This caused the formation of holes in the cell membrane then eventually the membrane became hollow and finally, the cells were dead.

In this study, we have examined the antibacterial activity of “AgNp made by a biosynthesis method using Andrographis paniculata extract” (S-AgNp). Biosynthesis is a method to prepare nanoparticles by using biological materials like plant extract, bacteria, and fungi [8,9]. Silver nanoparticle is one of the metal nanoparticles which have become the focus of research because of its unique properties that can be adjusted in its application e.g. by controlling the size, morphology, and shape of the nanoparticles [8,10]. Physical and chemical methods that are commonly used for the synthesis of metal nanoparticles produce the low levels of production, high spending and produce additional toxic substances [8]. Likewise, the production of large-scale nanomaterials experiences definite losses such as polydispersity and steadiness. Therefore, the synthesis of silver nanoparticles by using biological method provides promising and beneficial alternatives such as environmentally friendly, easy to increase for large-scale synthesis and does not need to use high pressure, temperature and toxic chemicals.

Biosynthetic nanoparticles by utilizing microorganisms such as bacteria, viruses, and fungi are able to collect metals and can be used in the reducing reaction and controlling structural topography of metal ions in the nanoparticle synthesis [9]. Endophytic fungi can produce the big amounts of biomass and it resists agitation and pressure compared to plant material and bacteria, so it is good to synthesize nanoparticles. Fungus is easy to be cultivated, produce big amounts of enzymes and the extraction process is easy so that it is easy to synthesize AgNp and can be used in numerous uses straightly. In the medicinal manufacturing, AgNp is the most expectant because it shows high activity as antibacterial to various pathogenic bacteria. AgNp synthesized by the biological method has been widely applied to prevent infections in food protection, biomedical devices, water purification, cosmetics, fashion, and various other medicinal products. Therefore, the use of biologically kind materials to synthesize AgNp is more acceptable as compared with chemical methods due to many advantages like the conditions of high temperature, pressure, and toxic chemicals that are not essential in the synthesis procedure [8,9]. Consequently, the preparation of AgNp by green synthesis method has compatibility for biomedical and pharmaceutical applications.

*Andrographis paniculata* is one of the traditional medicinal plants. *Andrographis paniculata* is rich in polyphenol compounds such as flavonoids, phenols, and tannins. It also contains the other chemical compounds such as andrographolide, panulide, farnesol, arabinogalactant proteins, and saponins [11]. Polyphenol compounds are one of the chemical compounds that are thought to act as reducing agents. Therefore, *Andrographis paniculata* plants can be used to synthesize nanoparticles, especially silver nanoparticles.

In this study by using S-AgNp, the antibacterial activity test was carried out by a diffusion-well method. The study included the finding the antibacterial activity of S-AgNp, the minimum inhibitory concentration (MIC) and grouping the antibacterial activity of S-AgNp according to previous work [12]. The grouping purpose is to determine whether the substance belongs to a very strong, strong, moderate or weak antibacterial agent.

2. Methods

2.1. Materials

The bacterial isolates used in this study were *S. aureus* and *E. coli*. The other materials used were nutrient agar (NA), 5% amoxicillin, nutrient broth (NB), distilled water, and biologically synthesized silver nanoparticles using *Andrographis paniculata* leaf extract (S-AgNp). The synthesized method of S-AgNp has been explained in previous work [11]. The characteristics of S-AgNp are the Surface Plasmon Resonance (SPR) wavelength of 423 nm, the particle size distribution of 10-30 nm, and the crystal structure of face center cubic (FCC) with lattice parameter “a” of 4.03 Å [11].
2.2. Antibacterial test method

The method used to evaluate the antibacterial activity was a diffusion-well method. This method is commonly used to assess the antimicrobial activity of plants or microbial extracts. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 5 mm was punched with a sterile tip, and the volume of 20 µL of the antimicrobial substance or extract solution at wanted concentration was introduced into the well. The Agar plates were incubated under a temperature of 37 °C for 24 h. Then, the clear zone (inhibition zone) formed was observed which indicated inhibits of the growth of the microbial strain.

2.3. Testing procedure

The tests have been performed in two steps. The first step, the concentration of S-AgNp tested was 100%, *Andrographis paniculata* leaf extract and AgNO₃ solution became the positive control, and distilled water became the negative control. The first step was finding out whether S-AgNp has ability as an antibacterial agent especially on *S. aureus* and *E. coli*. The second step, the concentration of S-AgNp tested were 70%, 40%, 30%, 20% and 10%; the distilled water became the negative control and 5% amoxicillin became the positive control. This step performed to determine the relationship between the concentration of S-AgNp and their antibacterial activity, also to find out the minimum inhibitory concentration (MIC).

2.4. Data analysis

All the measurements were replicated three times and the results are presented as average ± SD (standard deviation). The data were analyzed using windows Statistical Product Services Solution program (SPSS 17) using One way ANOVA method (analysis of one-way variance) with 95% confidence level or α = 0.05.

3. Results and Discussion

3.1. Antibacterial test

The inhibition zone diameters obtained in the first and second step tests are shown in Figures 1 and 2 respectively. The analysis results are listed in Table 1 and Table 2. Figure 1 shows that *Andrographis paniculata* leaf extract and S-AgNp have the ability to inhibit *S. aureus* more strongly than *E. coli*. The inhibit effect by *Andrographis paniculata* leaf extract was more strong than by S-AgNp. The wider the diameter of the inhibition zone formed showed the greater the resistance of the nanoparticles to the growth of bacteria. The AgNO₃ solution (as the positive control) and the distilled water (as the negative control) had no ability to inhibit because no inhibition zones formed around the diffusion-wells.

For better descriptions, the data in Table 1 and Table 2 are plotted as shown in Figure 3. The graph shows that S-AgNp inhibited the growth of *S. aureus* and *E. coli*, which decreased as decreasing the concentration of S-AgNp used. The positive control e.g. 5% amoxicillin has inhibited the ability to both bacteria with the average inhibition zone diameters of 1.25 and 5.025 cm respectively, whereas the negative control e.g. distilled water had not inhibited the ability to both bacteria. Figure 3 also shows that, at the same concentration, the inhibition zone diameters produced on both bacteria were different (p < 0.05). The growth inhibition of *S. aureus* was greater than that of *E. coli* (p < 0.05). These can be due to the differences on the cellular wall of each bacterium; the cellular wall of gram-positive is wider than the cellular wall of gram-negative [13,14]. Also, it depends on the difference in their resistance to antibiotics, in this case to the positive controls i.e. 5% amoxicillin as well as S-AgNp. *E. coli* are the gram-negative which have higher resistance to 5% amoxicillin than S-AgNp. This is also seen on the more turbid color of media at *E. coli* (Figure 1b and Figure 2b) compared with Figure 1a and Figure 2a for *S. aureus*. 
Figure 1. The inhibition zones obtained on the first step test: (a) *S. aureus* and (b) *E. coli*. Where A, B, C, and D are Andrographis paniculata leaf extract, S-AgNp 100%, distilled water, and AgNO₃ solution, respectively.

Figure 2. The inhibition zones obtained on the second step test: (a) *S. aureus* and (b) *E. coli*. Where 1, 2, 3, 4, and 5 are the concentrations of S-AgNp applied such as 10%, 20%, 30%, 40%, and 70% respectively. C+ is the positive control and C- is the negative control.

3.2. Antibacterial activity

Based on the average inhibition zone diameters in Table 1 and Table 2, the antibacterial activity of *S. aureus* and *E. coli* could be calculated by using Equation (1) [15]. The values obtained are shown in Table 3.

\[
\text{Antibacterial activity (\%)} = \frac{d}{C^{+}} \times 100\%
\] (1)

Where \(d\) is the average inhibition zone diameter of a sample and \(C^{+}\) is the average inhibition zone diameter of the positive control (5% amoxicillin).
Table 1. The inhibition zone diameter obtained on the first step.

| Bacteria | The concentration of S-AgNp | The average inhibition zone diameter ± SD (cm) |
|----------|-----------------------------|-----------------------------------------------|
| S. aureus | 100%                        | 1.250 ± 0.008                                 |
|          | C+ (1)                      | 3.725 ± 0.085                                 |
|          | C+ (2)                      | 0                                             |
|          | C-                          | 0                                             |
| E. coli  | 100%                        | 0.890 ± 0.008                                 |
|          | C+ (1)                      | 3.300 ± 0.041                                 |
|          | C+ (2)                      | 0                                             |
|          | C-                          | 0                                             |

- C+ (1) is the positive control 1 = 100% Andrographis paniculata leaf extract,
- C+ (2) is the positive control 2 = 100% AgNO3 solution (the concentration of 0.005 g/mL),
- C- is the negative control = distilled water
- SD is standard deviation.

Table 2. The inhibition zone diameter obtained on the second step.

| Bacteria | The concentration of S-AgNp | The average inhibition zone diameters ± SD (cm) |
|----------|-----------------------------|-----------------------------------------------|
| S. aureus | 70%                         | 1.200 ± 0.071                                 |
|          | 40%                         | 1.025 ± 0.025                                 |
|          | 30%                         | 1.175 ± 0.025                                 |
|          | 20%                         | 1.075 ± 0.048                                 |
|          | 10%                         | 0.900 ± 0.000                                 |
|          | C+                          | 5.025 ± 0.342                                 |
|          | C-                          | 0                                             |
| E. coli  | 70%                         | 0.900 ± 0.058                                 |
|          | 40%                         | 0.875 ± 0.048                                 |
|          | 30%                         | 0.875 ± 0.025                                 |
|          | 20%                         | 0.625 ± 0.063                                 |
|          | 10%                         | 0.625 ± 0.075                                 |
|          | C+                          | 1.250 ± 0.096                                 |

- C+ is the positive control = 5% amoxicillin,
- C- is the negative control = distilled water,
- SD is standard deviation.

For explanation, the data in Table 3 have been plotted in a graph as shown in Figure 4. The graph shows that greater the concentration of nanoparticles used, the greater the antibacterial activity produced. This was observed on both bacteria. In addition, it has been observed that S-AgNp has a greater antibacterial activity on E. coli than on S. aureus (p < 0.05). The difference in the antibacterial activity obtained is affected by the resistance of the bacteria to the antibiotic e.g. 5% amoxicillin, where the more resistant a bacterium to an antibiotic the greater the antibacterial activity produced. The lowest antibacterial activity was 50% on E. coli and 17.91% on S. aureus. This means that the diameter of the inhibition zone against 5% amoxicillin in E. coli is greater than S. aureus. This explains that E. coli more resistant to 5% amoxicillin compared with S. aureus. This is because S. aureus has a cell wall composed of thicker peptidoglycan layers, while E. coli has a thinner peptidoglycan layer and has a
thick lipopolysaccharide layer. The mechanism of action of amoxicillin is by inhibiting the biosynthesis of cell walls, especially peptidoglycan [16], while the lipopolysaccharide layer is a substance that in certain circumstances is toxic which can be trigger the activation of the cell's immune system [17].

The mechanism by which silver nanoparticles can cause antimicrobial effects is not well known. Numerous theories about this mechanism have been reported. Silver nanoparticles have the capability to be on the cell wall of bacteria, and with the increasing time these particles collect and are able to press more deeply into the cell surface [1]. Therefore, in a certain period, they can enter the cell wall; in this way, nanoparticles can cause structural changes in cell membranes such as penetration of cell layers and even kill the cells. In general, Morones et al. reported that there were four stages of the antibacterial mechanism of silver nanoparticles [18], including silver nanoparticles connected to the cell surface and increasing their capacity, infiltration of microorganisms, and the release of silver ions. This mechanism occurs in both gram-negative microbes such as *E. coli*, Vibrio cholerae, P. aeruginosa, and Salmonella typhi, as well as in the gram-positive bacteria, such as B. subtilis, *S. aureus*, and Enterococcus faecalis [1,19]. It has also been reported that the mechanism of archaea growth inhibition by silver nanoparticles is related to the disruption and destabilization of the haloarchaeal membrane which is highly resistant and the releases of respiratory dehydrogenase along with lipid peroxidation [2].

![Figure 3. Inhibition zone diameter of S-AgNp on *S. aureus* and *E. coli*](image)

The biologically synthesized silver nanoparticles using *Andrographis paniculata* leaf extract (S-AgNp) have the antibacterial resistance to the growth of *S. aureus* and *E. coli* bacteria. This could be seen from the diameter of the clear zone produced when the antibacterial test was carried out on each of these bacteria. The interaction or mechanism that occurred so that S-AgNp could inhibit the growth of both bacteria is explained as follows. When S-AgNp was injected into NA media that already contained the bacterial culture, the diffusion process occurred between S-AgNp and bacteria. The nano-sized silver nanoparticles were ten times smaller than micro-sized bacteria, approaching the bacterial cell membrane and even entering into the bacterial cells. The bacterial cell membrane contains protein with sulfur compounds as its main component. Silver nanoparticles interact with this protein, and then interact again with phosphorus which is a compound contained in DNA. When the nanoparticles enter the bacterial cell, the bacteria will increase their resistivity to the nanoparticles by forming the clots to protect DNA [10]. Then the nanoparticles attack the bacterial respiratory chain, until eventually the cell becomes dead. The antibacterial mechanism of silver nanoparticles is preceded by the silver nanoparticles releasing Ag⁺ ions and then the interaction between Ag⁺ silver ion and thiol group (-S-H) occurs in bacterial cell membrane proteins. The silver ion will replace the hydrogen (H⁺) cation from the sulfidryl thiol group to produce a more stable S-Ag group on the surface of the bacterial cell. This can deactivate
enzymes and reduce membrane permeability. Furthermore, silver ions enter bacterial cells and alter the structure of DNA, which in turn causes the cell death.

Numerous principal mechanisms to explain how AgNp work as antimicrobes have been recognized. First, AgNp was embed to the surface of cells with negatively charged, thus changing the chemical and physical properties of walls and membranes cells. This act knew to disrupt essential functions such as osmoregulation, electron transport, cell respiration, and permeability [10]. Second, AgNp can enter cells efficiently due to their nano size, thus they interact with proteins, DNA and components comprising proteins [13]. Third, AgNp discharge the silver ions to generate imbalances in cells and produce reinforced biocidal effects [10].

Table 3. The antibacterial activity of S-AgNp obtained on *S. aureus* and *E. coli*.

| Bacteria | The concentration of S-AgNp | The antibacterial activity of S-AgNp (%) |
|----------|-----------------------------|----------------------------------------|
| *S. aureus* | 100% | 24.87 |
| | 70% | 23.88 |
| | 40% | 20.40 |
| | 30% | 23.38 |
| | 20% | 21.39 |
| | 10% | 17.91 |
| *E. coli* | 100% | 71.20 |
| | 70% | 72.00 |
| | 40% | 70.00 |
| | 30% | 70.00 |
| | 20% | 50.00 |
| | 10% | 50.00 |

Figure 4. Antibacterial activity of S-AgNp on *S. aureus* and *E. coli*. 
3.3. Classification of antibacterial activity of S-AgNp.

According to Davis and Stout (1971), the antibacterial strength of a substance can be grouped into a very strong, strong, moderate, and weak correlated to the diameter of the inhibition zone formed; those were ≥ 20 mm, 10-20 mm, 5 - 10 mm, and < 5 mm, respectively [10]. Therefore, the antibacterial activity of S-AgNp was grouped as. Silver nanoparticles with the concentrations of 100% - 20% classified as strong antibacterial to S. aureus, whereas S-AgNp with the concentration of 10% classified as a moderate antibacterial. S-AgNp with the concentration of 100% - 10% classified as moderate antibacterial to E. coli. Based on those results, it can be recommended that S-AgNp synthesized by biologically method using Andrographis paniculata leaf extract can be used more effectively as antibacterial agent for S. aureus compared to E. coli.

4. Conclusion

This study has described the antibacterial potential application of “silver nanoparticles synthesized by biosynthesis method using Andrographis paniculata leaf extract” (S-AgNp). The results showed that S-AgNp has the ability to inhibit the growth of pathogenic bacteria S. aureus and E. coli. The growth inhibition of S. aureus was greater than that of E. coli (p < 0.05). The antibacterial activity increased as increasing the concentration of S-AgNp applied. The MIC value of both bacteria was 0.0005 g/mL. The results of this study indicated that silver and plants in nano size have definite components or elements that have antibacterial characteristics that can be used as antibacterial agents in the manufacture of new antibacterial pathogenic drugs.

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