UTILIZATION OF KETAPANG LEAF ETIL ASETIC FRACTION (*Terminalia catappa*) AS A BIOREDUCTOR IN SYNTHESIS OF SILVER NANOPARTICLES AND ANALYSIS OF THE ANTIBACTERIAL PROPERTIES

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Abstrak. Nanopartikel perak telah disintesis dengan metode reduksi menggunakan fraksi etil asetat daun ketapang (*Terminalia catappa*) sebagai bioreduktor. Nanopartikel yang terbentuk dimonitoring dengan spektrofotometer UV-Vis menunjukkan bahwa nilai absorbansi meningkat dengan meningkatnya konsentrasi umpan $AgNO_3$, serapan maksimum nanopartikel perak 1 mM dan 2 mM antara 418-436,5 nm dan 421-434 nm. Analisis gugus fungsi yang berperan dalam sintesis menggunakan *Fourier Transform Infrared Spectroscopy* (FTIR). Ukuran partikel ditentukan menggunakan (*Particle Size Analyzer*) menunjukkan ukuran rata-rata nanopartikel perak 1 mM dan 2 mM adalah 53 nm dan 158 nm. Pengujian antibakteri dilakukan dengan metode difusi agar terhadap bakteri *Staphylococcus aureus*, *Basillus subtilis* dan *Escherichia coli.* Hasil pengujian menunjukkan nanopartikel perak dapat menghambat aktivitas bakteri *Staphylococcus aureus*, *Eschericia coli*, dan *Bacillus Subtilis* dengan diameter zona hambat masing-masing adalah 7,2 mm, 6,9 mm dan 6,6 mm dengan masa inkubasi selama 48 jam.

Kata Kunci: Antibakteri, Bioreduktor, Nanopartikel Perak, *Terminalia catappa*

Abstract. Silver nanoparticles had been synthesis conducted in reduction method by using ethyl acetate fraction of catappa leaf (*Terminalia catappa*) as bioreductor. Silver nanoparticles were characterized by UV-Vis, FTIR, and PSA measurement. The results showed that absorbance values increased with the increase of $AgNO_3$ concentration. Maximum absorption of silver nanoparticles one mM dan two mM silver nanoparticles between 418-436,5 nm and 421-434 nm respectively. Functional groups that contribute to the synthesis was analyzed using *Fourier Transform Infrared Spectroscopy* (FTIR). Particle size was determined by using PSA. The result showed that one mM and two mM of silver nanoparticles were 53 nm dan 158 nm respectively. Antibacterial testing conducted by using agar diffusion method against *Staphylococcus aureus*, *Bacillus subtilis* dan *Escherichia coli* activity inhibition diameter zone were 7,2 mm, 6,9 mm, and 6,6 mm with incubation period was 48 hours.

Keywords: Antibacteria, Bioreductor, Silver Nanoparticles, *Terminalia catappa*

INTRODUCTION

Technology that is currently developing is nano-based technology or often called nanotechnology. Nanotechnology is the science and engineering in the creation of materials, functional structures, and devices on a nanometer scale. Materials or structures that have nano size will have different properties from the original material. The specific characteristics of these nanoparticles depend on size, distribution, morphology, and phase (Willems and Wildenberg, 2005).

Silver nanoparticle preparation has been carried out through a chemical synthesis process using bottom-up techniques. The synthesis of silver nanoparticles can be done by several methods such as electrochemical methods, chemical reduction, ultrasonic irradiation, photochemistry, and sonochemistry. The synthesis of silver nanoparticles is the most commonly used method of chemical reduction using silver salts with plants as reducing agents. For reasons of convenience, relatively low costs, and the possibility to be produced on a large scale (Lu and Chou, 2008).

Ketapang contains compounds such as flavonoids (Lin et al., 2000), triterpenoids (Gao et al., 2004), tannins (Ankamwar, 2010), alkaloids (Mandasari, 2006), steroids (Babayi et al., 2004), and fatty acids (Jaziroh , 2008). Various extracts from the leaves of Ketapang have also been investigated (Pauly, 2001). The secondary metabolite content of ketapang leaf extract has antioxidant activity so it is used as a reducing agent in the synthesis of silver nanoparticles (Lembang, 2013).

The extract used in the synthesis of nanoparticles is ethyl acetate fraction from ketapang leaves. Ethyl acetate is a good solvent used for extraction because it can be easily evaporated, not hygroscopic, and has low toxicity (Wardhani and Sulistyani, 2012). Ethyl acetate is a solvent that can attract alkaloid compounds, flavonoids, saponins, tannins, polyphenols and triterpenoids (Packirisamy and Krishnamorthi, 2014)

Nanoparticles tend to experience aggregation (large size). The stability of silver nanoparticles plays a very important role when it will be characterized and applied to a product. Efforts to prevent the occurrence of aggregates between nanoparticles can be done by adding particle coating material or molecules (Haryono et al., 2008).

Silver has long been known to have antimicrobial properties. Silver's antimicrobial ability can kill all pathogenic microorganisms and there have been no reports of silver-resistant microbes (Ariyanta et al., 2014).

The antibacterial properties of silver nanoparticles are influenced by particle size. If the particle size gets smaller, the surface area of silver nanoparticles gets bigger so that it increases contact with bacteria or fungi and is able to increase the effectiveness of bactericides and fungicides. When contact occurs between silver nanoparticles and bacteria and fungi, silver nanoparticles affect cell metabolism and inhibit cell growth. Silver nanoparticles penetrate into cell

membranes and then prevent protein synthesis and then decrease membrane permeability, and ultimately cause cell death (Montazer et al., 2012).

Based on the description, this research is directed at synthesizing silver nanoparticles by utilizing the ethyl acetate fraction of the leaves of Ketapang (Terminalia catappa) as a conductor. The silver nanoparticles produced will be tested for antibacterial activity against Staphylococcus aureus, Escherichia coli and Bacillus subtilis

MATERIAL AND METHOD

Instruments

The instruments used in this study are glassware commonly used in chemistry laboratories, paper discs, slide ruler, oceans, incubators, autoclaves, methylated lamps, Elmasonic S40H devices, hotplates, rotary evaporators Heidolph Hei-Vap Value, vacuum filters, balance sheets analytic, blender, magnetic stirrer, Shimadzu UV-2600 UV-Vis spectrophotometer, spray dryer Labplant, Shimadzu 820 IPC FTIR, and DLS VASCO PSA.

Materials

The materials used in this study were silver nitrate with Merck grade pro analyst, ketapang leaf (Terminalia catappa), poly acrylic acid (PAA) obtained from Sigma-Aldrich, nutrient broth (NB), nutrient agar (NA), medium MHA (Muller Hinton Agar), physiological NaCl 0.9%, chloramphenicol, methanol pa, Bovine Serum Albumin (BSA), ethyl acetate pa, culture of Staphylococcus aureus bacteria, culture of Bacillus subtilis bacteria, culture of Escherichia coli bacteria and aquabidest.

Methods

1. Extraction of Ketapang Leaves

Ketapang leaves are picked and washed thoroughly with distilled water. The leaves are dried in room temperature and then mashed. The dried powder of Ketapang leaves was weighed as much as 25 grams then added 25 mL of methanol and 25 mL of aquabidest. Furthermore, it is sonicated for 1 hour. After that the suspension was filtered, then methanol was evaporated from the filtrate using a rotary evaporator to obtain thick extracts of ketapang leaves (Mittal et al., 2014)

2. Solvent Partition of Ketapang Leaf Extract

Ketapang leaf extract was partitioned with ethyl acetate p.a (3x50 mL). Furthermore, the organic phase was isolated using a rotary evaporator. The extract can then be used as a bioreductor (Mittal et al., 2014).

3. Synthesis of Silver Nanoparticles

Silver nanoparticle synthesis was carried out by mixing 100 mL of 1 mM and 2 mM $AgNO_3$ solution with 2.5 mL ethyl acetate extract from ketapang leaves as a bioreductor and then heating for 1 hour and leaving it for 1 day until the yellow color formed. Then added 10 mL of 1% PAA and the distirer for 2 hours. Characterization of the solution in the form of color, UV-Vis spectrum, and pH at 1 day; 3 days; 5 days and 7 days. The product is then characterized using

FTIR, and PSA to determine the functional groups that play a role and particle size. Characterization of the solution in the form of color, UV-Vis spectrum, and pH at one day; 3 days; 5 days and seven days. The product is then characterized using FTIR, and PSA to determine the functional groups that play a role and particle size

4. Coating of Silver Nanoparticles at Paper Disc

Paper discs are washed, sterilized, and dried. Clean and sterile paper discs were soaked in silver nanoparticles for 12 hours then left for 5 minutes. The paper disc that has been coated with silver nanoparticles is dried again
with the oven at 70oC for 5 minutes (Ariyanta, 2014).

5. Antibacterial Activity Testing

Testing the inhibition of silver nanoparticles on the growth of Staphylococcus aureus, Escherichia coli, and Bacillus subtilis bacteria was carried out by the diffusion method to use paper discs with a diameter of 5 mm. The sterile MHA medium (Muller Hilton) is cooled at a temperature of 40-45 ºC. Then poured aseptically into the petri dish as much as 15 mL and put the test suspension as much as 0.2 mL. After that, four paper discs were placed aseptically using sterile tweezers on the surface of the medium. Label petri dishes to distinguish samples tested. Then incubated for 24 and 48 hours at 37 °C and then observed and measured the resistance zone with a sliding ruler (Ariyanta, 2014)

RESULTS AND DISCUSSION

Color characterization and pH

The color characterization of solutions and pH was carried out to determine the effect of contact time in the formation of silver nanoparticles by the addition of PAA (Poly acrylic acid) from the time of manufacture to 7 days. The synthesis of silver nanoparticles was carried out by mixing AgNO3 solution and bioreductors of the ethyl acetate fraction of Ketapang leaves (Terminalia catappa) then heated for 1 hour and left for 1 day to form a yellow solution with pH 4. Then PAA was added to the pH 5.

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The synthesized silver form nanoparticles are colloidal. Figure 1 shows silver nanoparticles with a reaction time of 1 day which is a yellow solution and changes to a greenish yellow solution with the length of reaction time. The color change of the solution from colorless to yellow indicates that silver ions have been reduced (Bakir, 2011).

The formation of these colors is due to the absorption of light and the emission of light in visible areas due to plasmon resonance or commonly called localized surface plasmon resonance (LSPR). LSPR is a combination of charged electron oscillations excited by light on nanoparticles (Megasari and Abraha, 2012).

Beginning 1 day 7 days

Figure 1. The character of silver nanoparticle color from the beginning of the making to 7 days

Characterization of UV-Vis Spectrophotometers

The UV-Vis spectrum was used to see the formation of nanoparticles by looking at the absorbance and maximum wavelength. The ethyl acetate fraction of Ketapang leaf (*Terminalia catappa*) absorbs energy at a maximum wavelength of 195-556 nm, $AgNO_3$ solution at a maximum wavelength of 216 nm, PAA at a maximum wavelength of 273 nm and silver nanoparticles at a maximum wavelength of 418 nm and 421 nm with time one day reaction.

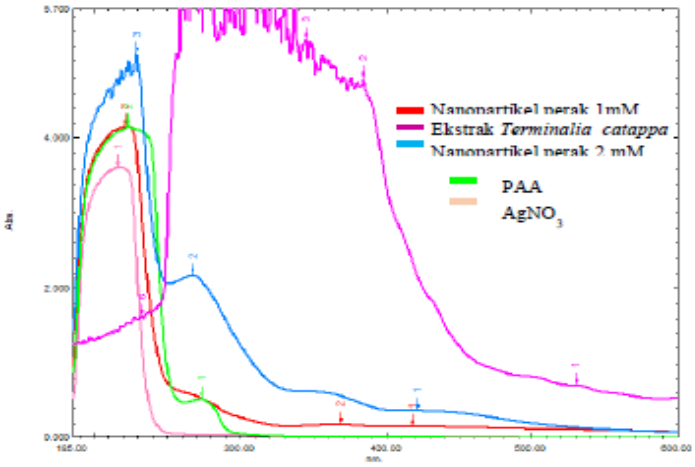

Figure 2. UV-Vis absorption of formation of silver nanoparticles at wavelengths of 185-600 nm.

Measurement of the UV-Vis spectrum was also used to determine the stability of silver nanoparticles synthesized based on the time function. If there is a shift in absorption peak to a larger wavelength, the agglomeration has occurred in silver nanoparticles. If agglomeration occurs, the color of the solution changes so that the absorption peak will shift (Ristian, 2014).

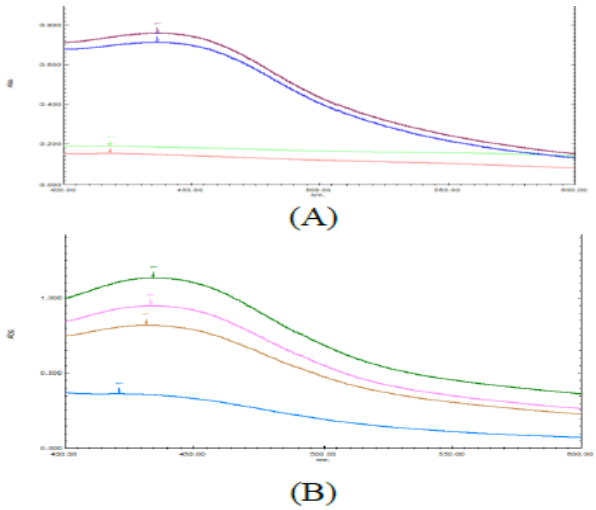

Figure 3. The spectrum of UV-Vis stability of silver nanoparticles (A) silver nanoparticles 1 mM (B) silver nanoparticles 2 mM

Silver nanoparticles with a concentration of 1 mM $AgNO_3$ have a maximum wavelength of 418-436.5 nm and silver nanoparticles with a concentration of 2 mM AgNO3 having a maximum wavelength of 421-434.5. Changes in the peak of the wavelength that occurs are not significant until the 7th day. This situation shows that the silver nanoparticles synthesized are relatively stable. The shift of absorption peak to a larger wavelength is directly proportional to the increase in absorbance value, which means that the silver nanoparticles formed are increasing with the length of contact time (Handayani et al., 2010)

FTIR Characterization

Characterization using FTIR aims to identify biomolecules in bioreductors, in this study using ethyl acetate fraction of Ketapang leaf (Terminalia catappa) which is responsible for reducing Ag + ions to Ag.

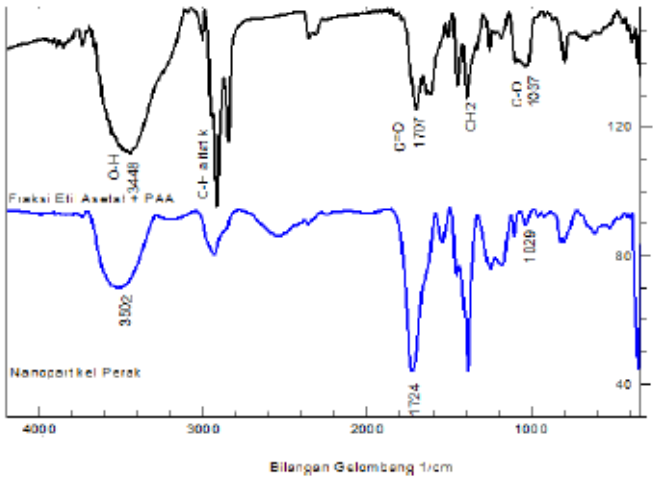

Figure 4. Results of IR Nanoparticle Silver Spectrum

Figure 4 (a) shows the results of FTIR analysis of ethyl acetate fraction of ketapang leaves (Terminalia catappa) showing the absorption of prominent bands at 1707 cm^{-1}, 1037 $cm^{-1,}$ and 3448 cm^{-1}. The absorption is at 1741 cm^{-1} 1mM silver nanoparticles Terminalia catappa extract 2 mM silver nanoparticles PAA $AgNO_3$ is a characteristic of the carbonyl group of carboxylic acid and phenol, this is confirmed by the absorption at 1047 which is the characterization of the C-O group. The presence of absorption bands with a wide and strong intensity at 3448 cm-1 is due to the presence of O-H groups from phenol and O-H from the poly acrylic acid group.

Figure 4 (b) shows the FTIR spectrum of silver nanoparticles synthesized. There was a shift in the spectrum wavelength from ketapang leaf extract before and after reducing. The shift in wave number occurs in the -OH group of 3448 cm-1- 3502 cm^{-1} with moderate absorption intensity, this indicates that there is an interaction between the -OH group and Ag due to the reduction oxidation process. This is also proven by the increasing intensity of C = O which is increasingly sharp at wave number 1724 cm^{-1}.

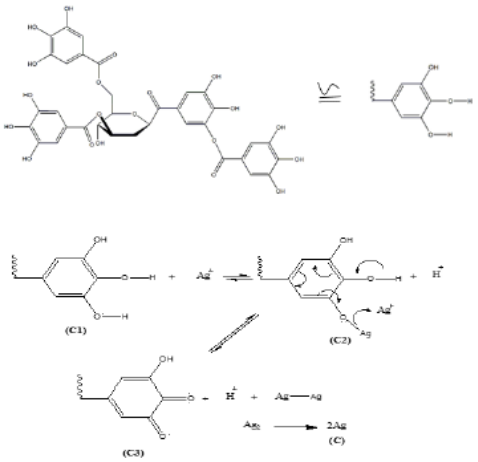

Figure 5. Estimated Mechanism of Synthesis of Silver Nanoparticles Using Ketapang Leaf Extract (Terminalia catappa).

Ethyl acetate extract of ketapang leaves contains tannins, phenolics, flavonoids, and alkaloids (Packirisamy, 2012). The functional groups of tannins which play an active role in reducing Ag^{+} to Ag are estimated in Figure 5.

The process that might occur in the formation of silver nanoparticles is the formation of an Ag polymer and then hydrolyzed to form the Ag nucleus as in the following scheme.

$$Ag\text{-}Ag \longrightarrow Ag\ Core \longrightarrow AgNP \longrightarrow Colloid$$

The formation is associated with the emergence of nuclei in saturated conditions. After that, Ag nanoparticle is formed, which will grow into colloids (Zakir, 2005).

Characterization of Particle Size Distribution

From the results of particle size analysis, samples of silver nanoparticles with a concentration of 1 mM $AgNO_3$ had an average particle size of 53.06 nm, and silver nanoparticles with a concentration of 2 mM $AgNO_3$ were 158.64 nm. The greater the concentration of AgNO3 used in the synthesis causes the number of Ag^+ to be reduced. This causes a reduction in the PAA function as a stabilizer so that the likelihood of agglomeration is greater, and consequently, the size distribution of silver nanoparticles becomes even greater (Ristian, 2013).

Table 1. Particle Size of Silver Nanoparticles

Sample	Average Particle Size (nm)	Polidispersity Index
Silver Nanoparticles 1mM	53,06	0,2660
Silver Nanoparticles 2mM	158,64	0,1600

Based on Table 1. it can be seen that nanoparticles with variations in the concentration of $AgNO_3$ have an index value small polydispersity, which means that the level of confidence in particle size dispersed on silver nanoparticle colloids is still good.

Antibacterial Activity Testing

Antibacterial testing of silver nanoparticles was carried out to show that silver nanoparticles have good antibacterial abilities. This study used test bacteria consisting of Staphylococcus aureus, Escherichia coli, and Bacillus subtilis.

Table 2. The diameter of silver nanoparticle barriers to testing bacteria

Sample	Barriers Diameter (mm)					
	S.aureus		E. coli		B. subtilis	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Silver mop nanoparticles 1 mM	6,5	7,2	6,6	6,9	7,4	6,6
2 mM Silver Nanoparticles	6,2	7	6,2	6,4	7,2	6,2
Positive Control	9,4	10,4	24,1	26	23,8	24
Negative Control	0	0	0	0	0	0

Table 2 and Figure 6 appears that silver nanoparticles can inhibit bacterial growth. This can be seen from the width of the clear zone around the Paper disc on bacteria-grown media. The greater the diameter of the clear zone indicates the stronger the inhibition of silver nanoparticles on bacterial growth. In the antibacterial test showed the diameter of the inhibitory zone for Staphylococcus aureus bacteria was 6.5 mm for silver nanoparticles with a concentration of 1 mM $AgNO_3$, and 6.2 mm for silver nanoparticles with $AgNO_3$ 2 mM concentration with a 24-hour incubation period. In the antibacterial test showed the inhibition zone diameter for Bacillus subtilis bacteria was 7.4 mm for silver nanoparticles with $AgNO_3$ concentration of 1 mM, and 7.2 mm for silver nanoparticles with $AgNO_3$ 2 mM concentration with a 24-hour incubation period. The diameter of the inhibitory zone for Escherichia coli bacteria was 7.0 mm for silver nanoparticles with a concentration of 1 mM $AgNO_3$ and 6.5 for silver nanoparticles with $AgNO_3$ 2 mM concentration with a 24-hour incubation period. Figure 18 shows that the width of the clear zone is the largest and most clearly shown by the Paper disc soaked in nanoparticles with a concentration of 1 mM $AgNO_3$ for the three test bacteria.

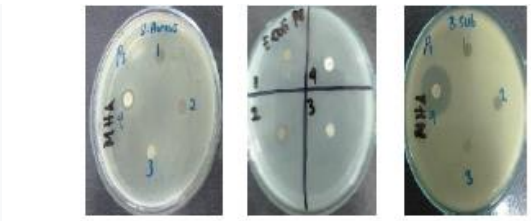

Figure 6. Visualization of antibacterial nanoparticle tests against bacteria with a 24-hours incubation period, (1) Silver nanoparticles with 1 mM $AgNO_3$ concentration, (2) Silver nanoparticles with $AgNO_3$ 2 mM concentration, (3) BSA (negative control) 4: Chloramphenicol (positive control).

In the antibacterial test Figure 7 and Table 2 show that the diameter of the inhibitory zone for Staphylococcus aureus bacteria is 7.2 mm for silver nanoparticles with a concentration of 1 mM $AgNO_3$ and 7.0 mm for silver nanoparticles with $AgNO_3$ concentration of 2 mM. The diameter of the inhibitory zone for Bacillus subtilis bacteria was 6.6 mm for silver nanoparticles with a concentration of 1 mM $AgNO_3$, and 6.2 mm for silver nanoparticles with a concentration of 2 mM $AgNO_3$. The diameter of the inhibition zone for Escherichia coli bacteria was 6.9 mm for silver nanoparticles with a concentration of 1 mM $AgNO_3$, and 6.4 mm for silver nanoparticles with a concentration of 2 mM $AgNO_3$. With an incubation period of 48 hours for all three test bacteria.

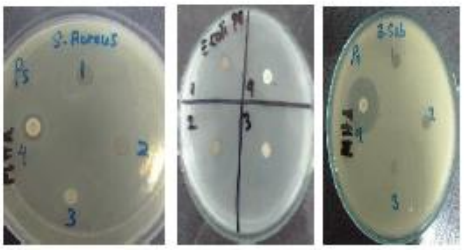

Figure 7. Visualization of antibacterial nanoparticle tests against bacteria with a 48-hours incubation period, (1) Silver nanoparticles with 1 mM $AgNO_3$ concentration, (2) Silver nanoparticles with $AgNO_3$ 2 mM concentration, (3) BSA (negative control), (4) Chloramphenicol (positive control)

Figures 6 and 7 show that the width of the clear zone is the largest and most clearly shown by Paper discs soaked in nanoparticles with a concentration of 1 mM $AgNO_3$ for 24 and 48 hour incubation periods. This proves that small size nanoparticles have greater bacterial inhibitory ability with particle distribution sizes of 53.06 nm. The smaller the size of silver nanoparticles, the greater the antimicrobial effect (Guzman et al., 2009).

According to Feng et al. (2000), the antibacterial mechanism of silver nanoparticles is preceded by silver nanoparticles releasing Ag^+ ions and further interactions between silver Ag^+ ions and thiol groups (-SH) on surface proteins. Like proteins on bacterial cell membranes. Silver ion will replace the hydrogen cation (H^+) from the sulfhydryl thiol group to produce a more stable S-Ag group on the surface of the bacterial cell. Furthermore, silver ions will enter the cell and change the structure of DNA and ultimately cause cell death.

CONCLUSION

Based on the research that has been done, it can be concluded that silver nanoparticles can be synthesized by the reduction method using ethyl acetate fraction of Ketapang leaves (*Terminalia catappa*). Silver nanoparticles can inhibit the activity of *Staphylococcus aureus, Escherichia coli*, and *Bacillus Subtilis* bacteria with inhibition zone diameters of 7.2 mm, 6.9 mm and 6.6 mm respectively, with an incubation period of 48 hours. Silver nanoparticles with a concentration of 1 mM $AgNO_3$ with a particle size of 53.06 mm have a larger inhibition zone diameter compared to silver nanoparticles with a concentration of 2 mM $AgNO_3$ to *Staphylococcus aureus, Escherichia coli*, and *Bacillus Subtilis* test bacteria.

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