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The Synthesize of Silver Nanoparticle (Ag-Np) Made from Waste of Kotagede Silver Handicraft Industry as an Antimicrobial Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: *The activity of human life can never be separated from the presence of microorganisms found in the surrounding environment. Pathogenic microorganisms can cause various diseases, it can even cause death. Nano-sized silver ions (Ag nanoparticles) has been widely used as an antimicrobial material. This study aims i) to determine the silver content in waste water of silver handicraft industry in Kotagede, ii) to determine the size of the silver nanoparticles (Ag-Np) which was formed, iii) to determine the diameter of bacteria inhibition zone of S. aureus and E. coli after exposure to Ag-Np silver waste water, This type of research was an experimental study of a single factor with three variables, namely, the independent variables such as the concentration of Ag-Np with a level of 100%, 75%, 50%, and 25%, the control variable which is the microbes without Ag-Np treatment, and the dependent variable was the diameter of bacteria inhibition zone using The Kirby-Bauer disk diffusion method. The chemical and physical properties of Ag-Np was characterized using AAS, XRD, and UV-Visible. The data analysis was done by One Way Annova. The result showed that the amount of silver in the wastewater was 2.3 g / L, the size of silver nanoparticles (Ag-Np) was 423.6 nm, the resulting inhibition zone diameter of E. coli was 4,39 mm and S. aureus was 4,97 mm which has significant value with P (0,00)<0.05. The most effective dose was the concentration of Ag-Np 100% (1 mM).*

Keywords: *antimicrobial, Ag-Np, Staphylococcus aureus, Escherichia coli*

1. Introduction

Activity of human life can never be separated from the presence of microorganisms these found in the surrounding environment. Microorganisms can be bacteria, fungi, and viruses. The existence of pathogenic microorganisms can cause various diseases, even may cause death. Scientists have developed an antimicrobial that was able to inhibit the growth of microorganisms to resolve the issue.

Silver nanoparticles have been widely used as an antimicrobial material. This was related to its ability to inhibit cell division and damaging the sheath cells and cellular components contained in the bodies of living microorganisms, so that microorganisms experiencing abnormal state and then die. Ag elements in nano size of inorganic particles appear to be more effective by removing the cation Ag^+ as antimicrobial rather than the size of the micro elements that show only a low ability as an antimicrobial [1].

Manufacture of nanoparticles used in previous research still came from the finished product (main) silver industry. On the other hand, silver liquid waste was a waste material or an adverse outcomes, which was harmful

if it left in the environment and exceeded the normal threshold. This waste normally produced by the silversmith industry by way of gilding.

Ag ion content in the silver liquid waste can be recovered (recovery) and separated from the waste liquid through using electrodeposition method (precipitation) using formaldehyde at a concentration of 0.2 M to obtain the Ag and Cu ions separately [2].

One tourist destination in Indonesia, especially in the Special Region of Yogyakarta was Kotagede silver handicraft industry. There, many merchandise were sold, for example jewelry, ring, and ribbon. The production of this merchandise may always followed with the liquid waste as its residue. The amount of the liquid waste more than 10 L of each home industry every month.

2. Materials and Methods

2.1. Materials and Reagens

The materials used in this study were glassware, platinum electrode, filter paper, analytical balance, atomic absorption spectrophotometer (AAS), X-ray Defraction (XRD), UV-Visible spectroscopy (UV-vis), potentiometer, petri dish, test tubes, ose, erlenmeyer, micropipette, bunsen lamps, counting chamber, laminar air flow (LAF), autoclave, incubator, Pasteur pipette, pH meter, spectrophotometry, microscopy, silver liquid waste, $AgNO_3$ standard solution, a solution of HNO_3, 37% formaldehyde, H_2SO_4 solution, acetone, distilled water, *Staphylococcus aureus*, *Escherichia coli*, nutrient agar, nutrient broth, potato dextrose agar, potato dextrose broth.

2.2. Sampling the silver waste

The silver wastewater was taken from the Kotagede silver handicraft industry and put into plastic bottle with an amount of 1 L.

2.3. Separation of the Ag elements from waste liquid

Samples of 500 ml silver waste liquid put in a 1000 ml baker glass. 37% formaldehyde solution was added as much as 0.4 ml to produce formaldehyde of 0.2 M. The two electrodes immersed in the sample solution with a certain distance of the two electrodes. Electrodeposition was carried out for 45 minutes at a potential of 4 Volts. After electrodeposition, drying and weighing were done with heavy cathode electrode as the cathode end. To determine the Ag^+ ions remaining in the waste liquid of electroplating, the AAS test was done. To determine the crystal structure of the deposit, the XRD test was done. Data collection was performed three times and the results were averaged. Ag^+ ion concentration was obtained from the data of AAS absorbance measurement results.

2.4. Synthesis Of Silver Nanoparticle (Ag-Np)

The synthesis of colloidal silver nanoparticles (Ag-NPs) involved a simple aqueous phase mixing of $AgNO_3$ with varying concentration of $C_6H_5Na_3O_7 \cdot 2H_2O$ (sodium citrate) solution. For the preparation of Ag-NPs, a solution of $AgNO_3$ 1mM in deionized water was heated until it began to boil. Sodium citrate solution was added dropwise to the $AgNO_3$ solution as soon as the boiling commenced. The color of the solution slowly turned into grayish yellow, indicating the reduction of the Ag^+ ions [3]. The formation of silver nanoparticles were detected by using UV-Vis [4]. Then determine the concentration of Ag nanoparticles were determined by dilution with aquadest.

2.5. Preparation of Medium

14 grams of nutrient agar powder were weighed using a clean electronic weighing balance and then 500 ml of distilled water was poured into conical flask containing 14 g of nutrient agar. The mixture was stirred with a sterilized glass rod and covered with a cotton wool, over which an aluminum foil was tightly wrapped and then autoclaved for 15 min at 121°C. The agar was then allowed to cool [5].

2.6. Growing The Microbes In This Research

Microorganism used was a strain of *Staphylococcus aureus* and *Escherichia coli*. Microbial suspension obtained from a pure culture in the form of slant from LPPT UGM. Bacterial strains were grown on OXOID nutrien agar plates at 37 °C for one night. The bacteria strains have grown, the microbial cells were incubated on Nutrien Broth at 37 °C for one night untill they become a suspension of white color.

2.7. Testing The Silver Nanoparticles (Ag-Nps) To The Microbes

Antibacterial activity was shown by measuring the diameter of inhibition zone according to *the Kirby Bouer* method. Relative antibacterial potency was measured by comparing the growth inhibition zone on the plates. The resultant color clear zone was measured in units of mm [6].

3. Results & Discussion

3.1. AAS Analysis

The **AAS analysis** results showed the levels of **silver in the** industry waste was equal to 2,030 ppm. Silver content data that have been obtained from the waste liquid **Kotagede silver industry** used as a reference for the addition of HCL and **form**aldehydes in electroplating methods**.** Electroplating experiment was conducted at a voltage of 4 volts for 45 minutes**.** Results obtained **from** electroplating silver deposits with a shiny white color as much as 0.2 grams per electroplating**.** After getting the silver deposit**,** XRD analysis was conducted to characterize and determine the crystal structure**.**

3.2. XRD Analysis

XRD results showed that a crystalline form of silver ions shown with one the highest peak, while the other two peaks were an amorphous form of silver ions. After silver deposit was characterized, followed on the stage was the manufacture of Ag nanoparticles. Silver deposits that have been obtained through electroplating method were dissolved by adding HNO_3 as much as 100 mL at 0.014 grams silver deposit. This solution was used as a main liquid to make $AgNO_3$. Then, the $AgNO_3$ obtained from the waste liquid was mixed with standard $AgNO_3$ with ratio of 1:1.

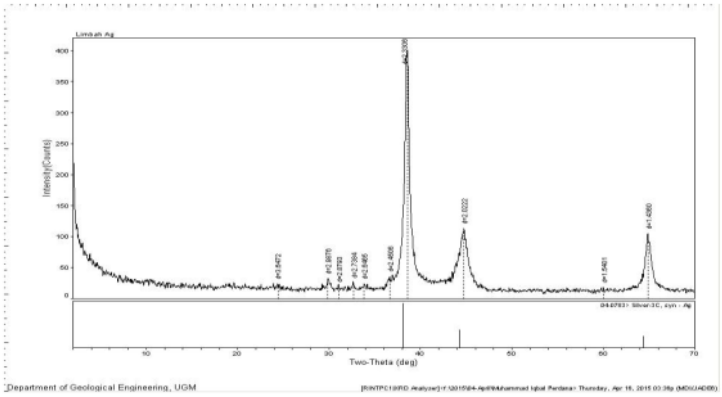

Fig. 1: The XRD analysis of Ag deposite from waste after electrodeposition

3.3. The UV-Vis Analysis

UV-Vis analysis result was obtained for Ag-Np obtained from the combination above. The silver nanoparticles had the wavelength of 423.6 nm. For the reference of [7] it was obtained that the size of the silver nanoparticles according to the wavelength which has mentioned had a particle size 68.82 nm.

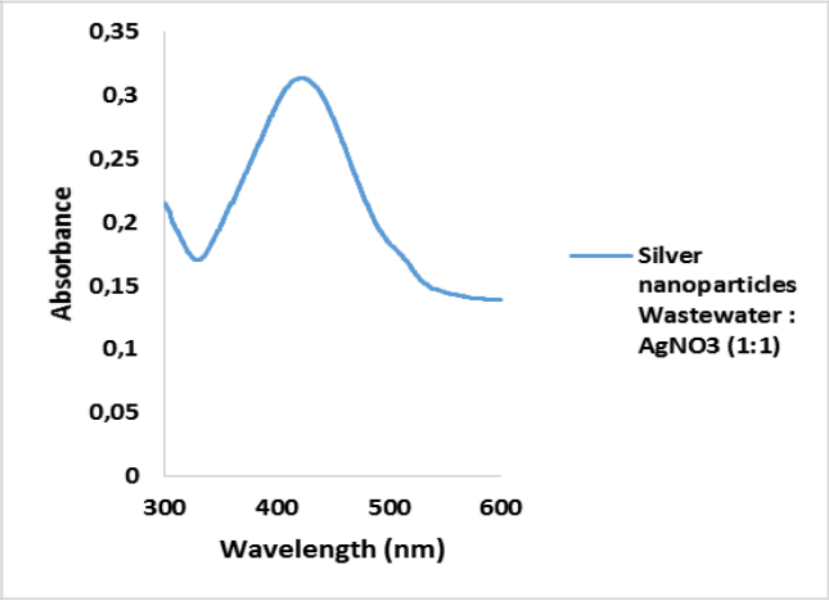

Fig. 2: The graph of absorbances vs wave length for silver nanoparticles combination from AgNO-wasteliquid and standard AgNO.

3.4. The Inhibition Zone Of Staphylococcus Aureus And Escherichia Coli

Results of analysis of variance (ANOVA) test 4 variations of silver nanoparticles concentration which were treated to the bacteria Staphylococcus aureus, Escherichia coli showed both has a P value (0.00) <0.05. This means that there was a significant difference in the 4 series of silver nanoparticle concentration. Then the data were tested with Duncan test which indicated that the effect was most noticeable on the silver nanoparticles with a concentration of 100%. Figure 3 showed a graph nanoparticle concentration variation with a diameter of inhibition zone formed by the time the growth of E. coli bacteria. The graph showed a polygonal shape of the curve at 24th hour and the curve was linear in the 48th hour. Figures 4 showed the relationship nanoparticle concentration variation and the diameter of inhibition zone with a bacterial growth of S. aureus. The second graph showed the polygonal shape of the curve at 24th hour and 48th hour.

This clearly demonstrated that the antimicrobial activity was only due to nanosilver shapes impregnated inside the bacterial and not due to individual bacterial. The Ag^{+} ions form insoluble compounds with sulphydryl group in the cellular wall of the microorganism that were responsible for the inhibition zone in the seeded culture media. The surface of the cell walls of *S. aureus* was covered with substance resulted from the cell disruption after the nanosilver shapes treatmemt. The surface of the cell walls of *E. coli* treated with nanosilver shapes was severely distrupted compared to the non-treated *E. coli* [8]. It indicated that the AgNPs have an antimicrobial activity against *E. coli* and *S. aureus* by disrupting cell and require a the maximum concentration (100%) to inhibit development of the *S. aureus* and *E. coli*.

TABLE I: THE AVERAGE INHIBITION ZONE DIAMETER OF *ESCHERICHIA COLI*

Concentration of Silver nanoparticle (Ag-Np)	100 %	70%	50%	25%
24 hours	3,88 mm	3,07 mm	3,08 mm	3,45 mm
48 hours	3,39 mm	3,15 mm	2,59 mm	2,41 mm

TABLE II: The Average Inhibition zone diameter of *Staphylococcus aureus*

Concentration of Silver nanoparticle (Ag-Np)	100 %	70%	50%	25%
24 hours	4,70 mm	3,75 mm	3,08 mm	3,45 mm
48 hours	4,97 mm	3,56 mm	3,24 mm	3,16 mm

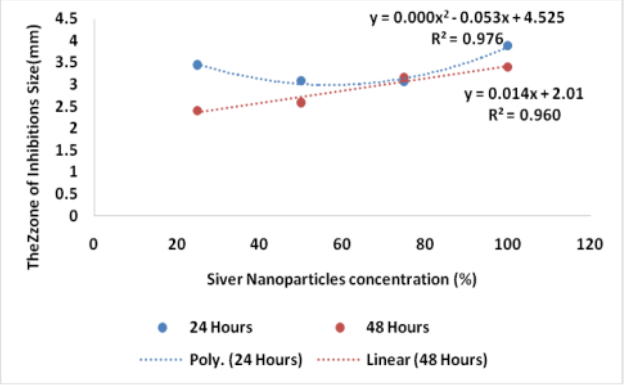

Fig. 3: The graph of relationship between silver nanoparticle concentration vs the zone of inhibition diameter for *Escherichia coli*

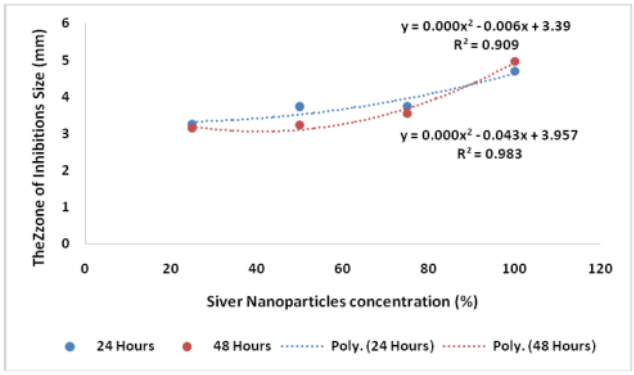

Fig. 4: The graph of relationship between silver nanoparticle concentration vs the zone of inhibition diameter for *Staphylococcus aureus*

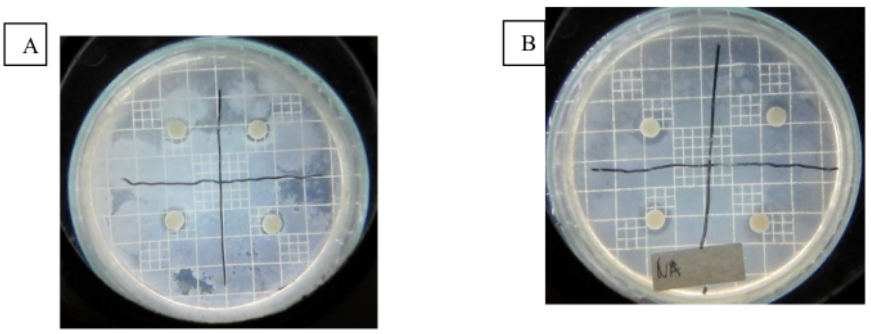

Fig. 5: The zone of inhibition diameter of A) *E. coli* and B) *S. aureus*, after being exposured with silver nanopartices

4. Acknowledgements

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