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The effect of temperature solubility on particle size and antibacterial activity of nanosilver of gembili's inulin

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ABSTRACT

A local Indonesian tuber such gembili (*Dioscorea esculenta* L.) is reported as a source of inulin potentialy. Inulin is one of the fiber types and carbohydrate sources derived from plants and has prebiotic and antibacterial activity. However, inulin long chain has low solubility in water, so it is necessary to modify the temperature solubility in the biosynthetic process to enhance its solubility. In this study, the biosynthetic process used inulin as a reducer of silver ions. And also to help increase the absorption of inulin. Gembili inulin was mixed with silver nitrate solution at variation temperatures of 25 ℃, 60 ℃, and 90 ℃ of a biosynthetic process to produce a nano particle size and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacterias. This study showed that nanosilver biosynthetic has a a particle size of 12-49 nm with a round shape. The temperature solubility of inulin affect to particle size, but did not show any significant difference toward the antibacterial activity against *S.aureus* and *E.coli* bacterias. FTIR spectra showed an interaction between silver nitrate and inulin of gembili functional groups. Inulin acts as a capping agent of nanosilver but does not induce the antibacterial activity.

Keywords: gembili, inulin, nanosilver, antibacterial, biosynthesis

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INTRODUCTION

Inulin commercially was produced by the chicory plant tuber (*Cichorium intybus*) from the United States and England. However, chicory was not the endemic plant in Indonesia, so the fulfillment needs for inulin for industry and research are still imported from England. Therefore, research was conducted investigating local plants containing inulin to overcome the problem. Gembili was one of the local tubers reported having potential as a source of inulin (Pratiwi et al., 2016). Gembili's inulin has low water solubility, so inulin solubility into water temperature modification is needed. Gembili was a local Indonesian plant with high inulin (14.77%) (Winarti et al., 2013; Yuniastuti & Iswari, 2018), so it can be used as a source of inulin and was expected to increase the Phyto-pharmacy repertoire in Indonesia and reduce imported materials. Inulin, as crude fiber, cannot be absorbed by the intestinal wall and will remain in the colon. Inulin was a Fructooligosaccharide (FOS) general crude fiber that can enter the plasma through the process of monolayer cell particulate in a small amount (Chi et al., 2018). In this study, the biosynthesis process was conducted to form a nanosilver using inulin of gembili to increase the absorption of inulin (Ermawati et al., 2021).

The application of nanotechnology in the pharmacy has various advantages, including increasing the solubility of compounds, reducing the dosage of medication, increasing absorption, and decreasing the frequency of medication so that patients were more compliant. Nanoparticle was material at the nanoscale in dimensions range of <100 nanometers. The nanoparticle size was used to design and compile or manipulate the material to produce a material with new properties and functions (Albrecht et al., 2006). However, silver nanoparticle preparation was formed by mixing silver metal with stabilizing and reducing agents from toxic and dangerous chemicals to the environment. Therefore, a simple, safe and environmentally friendly process is developed using plant extracts (Kowshik et al., 2003; Shahverdi et al., 2007).

Silver nanoparticle formed by biosynthesis process using plant extracts has been widely used (Dubey et al., 2010; Narayanan & Sakthivel, 2008), most recently with *Camellia sinensis* (Vilchis-Nestor et al., 2008), *Aloe vera*, Mangosteen leaf (Veerasamy et al., 2011). Silver nanoparticles were anti-fungal, antimicrobial, anti-inflammatory, anti-platelete, anti-angiogenetic, and anti-viral. Biosynthesis process of silver nanoparticles was carried out at 60 ^{0}C because that temperature showed the optimum size (Kaviya et al., 2011). This research aims to determine the effectiveness of nanosilver biosynthetic reactions using gembili inulin at variations in inulin solubility temperature (25 ^{0}C, 60 ^{0}C, and 90 ^{0}C) to produce the aimed particle size, and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* bacterias.

MATERIALS AND METHOD

Materials

Gembili's Inulin from Independent UKM (Plalangan Village, Semarang, Indonesia), Silver nitrate ($AgNO_3$) (Merck, German); aquadest repacking from CV. Agung Jaya Kimia, Surakarta, Indonesia; Whatman paper No.1; *S. aureus* ATCC 25923, and *E. coli* ATCC 25922 (Microbiology Laboratory of Medical Faculty, UNS, Surakarta, Indonesia). *Instrumenents*: UV-Vis spectrophotometer (GenesysTM, Japan), FTIR (Shimadzu, Japan), TEM instrument (JEOL/EO JEM-1400 Japan), digital calipers (Krisbow, USA), climatic chamber (Thermo Scientific Series 8000 WJ), thermometer (Zeal), glassware (Pyrex), and hotplate (thermo scientificis).

Methods

Biosynthesis Process

Silver nitrate (AgNO3) powder weighed 85.0 mg and dissolved into 500 mL of water at 40 ºC until homogenous. Inulin of Gembili from the species of gembili tuber (*Dioscorea esculenta* L.) was isolated according to (Glibowski, 2010) method. Gembili tuber powders of 10 grams were weighed, then dissolved into 250.0 mL of water at temperatures of 25 ^{0}C, 60 ^{0}C, and 90 ^{0}C. The mixture was stirred until the inulin powder dissolved homogeneously. The mixture was then filtered using

Whatman paper no.1 to obtain a clear inulin filtrate. Added inulin solution of 7.0 mL to 36 mL of silver nitrate solution and measured the mixing temperature at 60 ^{0}C. The inulin of gembili and silver nitrate solution were incubated for 24-72 hours to maximize the biosynthesis process (Kaviya et al., 2011).

Nanosilver of gembili's inulin characterization

The chosen variations solution of inulin's solubility temperature (25 ^{0}C and 60 ^{0}C) were scanned in the wavelength range of 190-500 nm by a UV-Vis Spectrophotometer, where distilled water is used as blank. The samples were tested using a Transmission Electron Microscope performed at a magnification of 200.0; 100.0; 50.0; and 20.0 nm (Sathishkumar et al., 2010). The powder sample was made by centrifuge of solution for 10 minutes with a speed of 10,000 rpm to obtain powder from the solution. The powder was dried using an oven at 50 ºC for 24 hours to remove any remaining water in the sample (Waidha et al., 2015). Added the KBr powder pellets for analysis using Fourier Transform Infra-Red (FTIR).

The antibacterial activity test

S. aureus and *E. coli* bacterias were spreaded in nutrient agar media, then made in a well with a diameter of 10 mm. Poured an amount of 50 μL nanosilver biosynthesis solution, silver nitrate solution, inulin of gembili solution, and water into of agar medium's well. The agar medium was incubated for 24 hours at 37 °C, and the clear zone was measured as a diameter of the inhibition zone, indicating the ability of nanosilver biosynthesis to inhibit bacteria. Added the nanosilver biosynthesis solution to Nutrient agar media bland with *Lactobacillus* bacteria. The petri dish was then incubated at 37 °C for 24 hours to observe the growth of *Lactobacillus* bacteria. Calculate ALT of Lactobacillus bacteria.

Data Analysis

Statistical data analysis was used to compare the effect of temperature of inulin on particle size and diameter of inhibition zone with the One Way ANOVA analysis and the Paired Sample T-Test using the IBM SPSS Statistic 21 software.

RESULT AND DISCUSSION

Results of Transmittance Electron Microscopy (TEM) analysis were performed to determine the sample's particle size also surface morphology. Based on Figure.1, the sample results of nanosilver of gembili's inulin at a inulin solubility temperature of 25 °C indicate particle sizes in the range of 12.0−49.0 nm with a spherical shape. The results of the sample results of nanosilver of gembili's inulin at a inulin solubility temperature of 60 °C showed particle sizes in the range of 27.0-48.0 nm with a round shape without any aggregation. The solubility temperature of 90 °C was not recommended because the inulin becomes gel at that temperature. In the pharmaceutical, nanotechnology is used as a technology designed to produce materials with unique properties and functions compared to the original material. One application is in the biosynthesis process using gembili inulin with silver nitrate. Size control during the biosynthesis process is an important criterion. PSA ensures that the particle size is still in the nanometer range. Nanoparticles definision was a particle that has a scale size of 1–100 nm (Tripathi et al., 2017). TEM results were found in the study by (Charoenwongpaiboon et al., 2019), which states that the synthesis of inulin nanoparticles (INNP) shows the morphology of INNP, which has a spherical shape with a diameter range of 25−60 nm, and also found that the biosynthesis of silver nanoparticles using *Dioscorea bulbifera* showed a generally spherical structure.

Fourier Transform Infrared (FTIR) is an instrument to detect functional groups of a sample's compound. The working principle of FTIR is to collect infrared light spread on the sample's surface to determine the frequency of the absorbed waves. Furthermore, it will interpret this readable frequency to find out the specific group of the sample. The previous study reported that wave numbers of 900 cm^1 are characteristic of inulin carbohydrates. The chemical interaction between inulin and AgNO3 was evaluated using FTIR spectroscopy at a wave of 500-4000 cm^{-1}. FTIR analysis was carried out on samples of AgNO3 powder, inulin powder, and inulin-AgNO3 biosynthetic solutions at different inulin solubility temperatures at 25°C and 60°C. The presence of a wave shift in the samples produced by inulin-AgNO3 biosynthesis, whether the inulin solubility temperature of 25 °C or 60 °C (Table 1), indicates that there has been an interaction between functional groups and nanosilver due to the oxidation process. Temperature variations in inulin solubility may cause the oxidation process. Her research also reported that derived of inulin from dahlia tubers has the same FTIR spectra as inulin arthicoke and inulin chicory, characterized by the appearance of the O-H stretching absorption peak at a wave number of 3300 cm-1, which is characteristic of inulin. The results of FTIR analysis on inulin powder with NO2- functional groups were not found. FTIR result indicates that AgNO3 has a role in biosynthesis as an antimicrobial agent. Meanwhile, the inhibitory activity of the biosynthetic results showed that the specific biosynthetic samples inhibited specific bacteria.

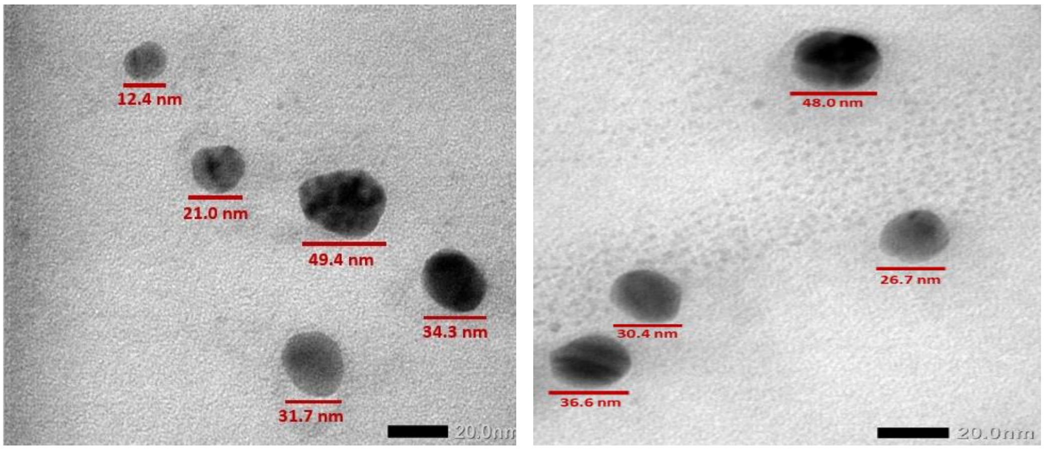

Figure 1. **The particle size results using TEM of nanosilver using gembili's inulin as bioreducer at an inulin solubility temperature of 25 °C (left) and 60 °C (right) with magnification until 250.000 times**

Table1. Results of FTIR analysis on functional groups of AgNO3 powder; inulin powder; nanosilver of gembili's inulin in inulin solubility at 25 °C and 60 °C

Functional group	Wave Number (cm^{-1})			
	AgNO3 Powder	**Inulin Powder**	**Nanosilver 25 ºC**	**Nanosilver 60 ºC**
Ag-N	408, 439	-	-	-
Pyranose ring	-	574	528; 577; 605	578
C-C vibration	-	709	-	-
C-H bending	-	763	-	-
NO3	823	-	-	-
Fructofluranose	-	858; 928	855; 931	-
Alcohol	-	1018; 1082	1022; 1079	1021; 1079
C-O-C	-	1154	1153	1152
Fenol	-	1366	-	-
NO2-	1382	-	1338; 1367	1318; 1421
C=C	-	1647	1637	1639
O-H stratching	2735	3379	3379	3246

Table2. Results of antibacterial activity test of inulin solution, AgNO3 solution, aquadest and nanosilver of gembili's inulin

Samples	Diameter of Inhibition zone (mm)	
Inulin solution	0.00±0.00	0.00±0.00
AgNO3 solution	11.59±1.66	2.32±0.05
Nanosilver of gembili's inulin at 25ºC	10.47±2.02	2.12±0.20
Nanosilver of gembili's inulin at 60ºC	10.25±1.62	2.18±0.25
aquadest	0.00±0.00	0.00±0.00

*X±SD

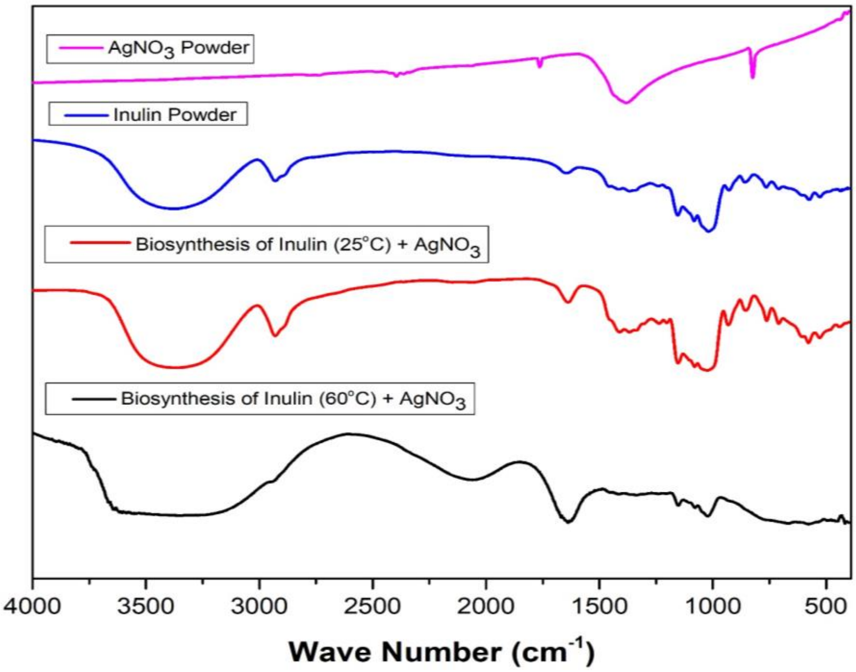

Figure 2. FTIR analysis results that showed shifting of several functional groups, indicate some interactions of initial materials

The antimicrobial activity in this study was conducted using the well diffusion method. The test measures the diameter of the inhibition zone, marked by the clear zone around the sample. Based on Table 2, the solubility of inulin at temperatures of 25 °C and 60 °C after being biosynthesized with AgNO3 shows that the diameter of inhibition zone of *S. aureus* and *E. coli* bacteria was not significantly different from the AgNO3 control. Meanwhile, the results of inulin-AgNO3 biosynthetic at both inulin solubility temperatures of 25 °C and 60 °C showed that the diameter of the inhibition zone in *S. aureus* and *E. coli* was significantly different from inulin control. Antibacterial activity of gram-positive bacteria (*S. aureus*) on AgNO3 solution and nanosilver-gembili inulin solubility at both 25 °C and 60 °C is classified as a strong antimicrobial activity because the diameter of inhibition zone value obtained between 10-19 mm. Meanwhile, the inhibitory activity of gram-negative bacteria on AgNO3 solution and nanosilver inulin solubility at both temperatures of 25 °C and 60 °C was classified as a weak antimicrobial activity because the diameter of inhibition zone value obtained is ≤ 5 mm, inulin solution in this test has no antibacterial activity. The strain of bacteria determines the antimicrobial activity of the nanosilver (Ruparelia et al., 2008). Gram-positive bacteria consist of a thick cell wall structure with multilayer peptidoglycan, which will facilitate the penetration of nanoparticles into cells and cause leakage of intracellular components, which leads to cell death. Meanwhile, gram-negative bacteria consist of a complex cell wall structure with a thin peptidoglycan layer surrounded by an outer membrane which can reduce the adhesion function of nanoparticles (Glibowski, 2010).

A test was carried out on *Lactobacillus sp* to find out whether nanosilver also affects the growth of lactic acid bacteria.The test was carried out by inoculating 1.0 µL of 24-hour-old culture into 5 mL of Na Agar media using a spread plate technique and adding a solution of nanosilver biosynthetic at a concentration of 10µL; 20µL; 40µL, and 50µL, then incubated at 37 ℃ for 24 hours. During the incubation process, the amounts of bacteria were calculated using the total plate count method on NA agar. The total colonies counted must meet the standard (ICMF), where the colonies are between 30 and 300 for each petri dish. Growth is one of the characteristics or properties of microorganisms that are important in fermentation. The increase in *Lactobacillus* can be defined as the growth of lactic acid bacteria. Previous studies stated that the greater the concentration of nanosilver added, the lower the growth of S.aureus and Lactobacillus bacteria (Azarsina et al., 2013). The results showed no difference in the growth of *Lactobacillus* bacteria in either the biosynthetic agent of gembili's inulin or control bacteria. Based on the results showed that nanosilver of gembili’s inulin have no significant increase in the growth of *Lactobacillus sp*, and it is may not harmful for *Lactobacillus* growth. Different results may be that the nanosilver formed in the Azarsina et al. (2013) study used a chemical method, while the nanosilver in this study used a biological method using inulin from tubers. We will carry out our further research testing of in vivo immunomodulator and antidiabetic test.

CONCLUSION

This research results in variations in solubility temperature may play a role in determining targets for inhibition of certain types of microbes. Inulin’s solubility at a temperature of 25 °C has a smaller particle size of nanosilver than solubility of inulin at a temperature of 60 °C. Inulin functions as a capping agent in the biosynthesis process and does not provide antibacterial activity of nanosilver. Nanosilver of gembili’s inulin have no significant increase in the growth of *Lactobacillus*, also its may not harmful for *Lactobacillus* growth.

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