Indonesian Journal of Pharmacy

VOL 32 (2) 2022: 234–243 | RESEARCH ARTICLE

The Effect of Turmeric Juice Volume on the Characteristics and Antibacterial Activity of Nanosilver Biosynthetic and Hydrogel Formulation

Dian Eka Ermawati*, Safira Rahma Novachiria, Berliana Rizka Ramadhan and Saptono Hadi

Department of Pharmacy, Mathematics and natural Science Faculty, Sebelas Maret University, Surakarta, Central Java, Indonesia

Info Article

Submitted: 01-01-2021
Revised: 22-01-2022
Accepted: 13-04-2022

*Corresponding author
Dian Eka Ermawati

Email:
dianekae@staff.uns.ac.id

ABSTRACT

This study aimed to determine the effect of turmeric juice volume on the characteristics and antibacterial activity of nanosilver (AgNPs) and its concentration on gel's physicochemical properties.The biosynthetic process involved mixing turmeric juice and silver nitrate ($AgNO_3$) solution for 24 h at 25°C and analyzed using Spectrophotometer UV VIS, TEM, SEM, and FTIR. The gel made in varying concentrations of AgNPs was evaluated based on pH, viscosity, and dispersibility test during storage for 12 days at 25°C. The formula of AgNPs biosynthetic with a 5:38 mL ratio has absorption peak at 407 nm is selected. The preferred AgNPs has round shape with a particle size of 17.96 nm and broad-spectrum antibacterial activity. Based on the results, FTIR spectra showed interaction with specific peaks, while the AgNPs concentration affected the gel's physicochemical properties. The formula with 30% AgNPs has a pH of 5.7±0.4, a viscosity of 20.3±0.6, and a dispersibility of 6.6±0.1. Therefore, it meets the requirements of gel based on SNI 16-4380-1996 and it did not significantly change during 12 days at 25°C.
Keywords: nanosilver, biosynthesis, turmeric juice, antibacterial, hydrogel

INTRODUCTION

Silver is a metal applied for medical purposes, and its ion has antibacterial activity that could be induced as nanoparticles. Subsequently, nanosilver was prepared by mixing silver with a reducing agent to remove a toxic and environmentally harmful chemical. Nanoparticles contain colloidal active ingredients with nano range dimensions between 10-100 nm. This small particle size increased the surface area and effectiveness of inhibiting bacteria (Khezri *et al*., 2018). Meanwhile, the bioreducer method was used since it does not cause problems such as toxic solvents usage, releasing hazardous waste, and high energy consumption (Rasyid *et al*., 2015). The plant extract was then preferred to be developed as an AgNPs biosynthetic agent considering it was safe, simple, and environmentally friendly. Moreover, the plant extracts as nanosilver bioreduction agents were favored based on the presence of metabolite compounds that convert silver ion (Ag^+) into Ag^0 (silver). They include terpenoids, phenolics, flavonoids, and other components with carboxylic acid, amide, and aldehyde functional groups (Shankar *et al*., 2004).

Turmeric, known scientifically as *Curcuma longa* L., is potentially useful as a nanosilver biosynthetic agent. The aldehyde groups in flavonoid compounds contained potential reducing agents (Shameli *et al*., 2012). According to Kurian *et al*. (2016), using turmeric rhizome to infuse nanosilver biosynthetic produces a nanosilver particle measuring 20-50 nm, with a round shape and 14 mm as a diameter of inhibition zone against the *Staphylococcus aureus* bacteria. Moreover, a previous study stated that the particle size of nanosilver produced through biosynthesis using water extract of turmeric rhizome powder ranged between 5-35 nm, with an average dimension of 18±0.56 nm (Alsammarraie *et al*., 2018). In contrast, this study used turmeric juice as a reducing agent with five variations of the volume ratio of $AgNO_3$ solution. The volume variation aimed to establish the optimum mixture that fulfills the absorption peak of the Surface Plasmon Resonance (SPR) range of nanosilver at 400-500 nm. This would help identify the antibacterial activity based on the diameter of the inhibition zone against the *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. The

Copyright © 2022 by Indonesian Journal of Pharmacy (IJP). The open access articles are distributed under the terms and conditions of Creative Commons Attribution 2.0 Generic License (https://creativecommons.org/licenses/by/2.0/).

nanosilver biosynthetic from the best mixture's ratio that has strong category of antibacterial activity was carried by characterizing using Transmission Electron Microscope (TEM), Scanning Microscope Electron (SEM), and Fourier Transform Infra-Red (FT-IR). Also, it involved examining the antibacterial activity against *Pseudomonas Aeruginoisa* and *Escherichia coli* bacteria. This study used turmeric juice as a bioreducer by modifying its volume to assess its effect on particle size and antibacterial activity, and its concentration on gel's physicochemical properties for topical dosage form. AgNPs have been extensively adopted for medical purposes, including topical preparations, in which Nanosilver inhibits bacteria with a minimum concentration of 10 mg/Kg BW (Pulit-Prociak *et al*., 2019). In line with this, the Science Committee on Consumer Safety (SCCS) recommended less than 10.000 ppm of nanosilver concentration in cosmetics preparation (Pulit-Prociak *et al*., 2019).

Formulating the nanosilver biosynthesis solution into a hydrogel preparation involved choosing Carbopol 940 as a gelling agent with an optimum concentration of 0.5-2%. This is because Carbopol 940 produces a stable viscosity than Carbopol 934 (Paul *et al.,* 2020). The experiment used five formulas with active substances in various concentrations of nanosilver biusynthetic that dispersed into the hydrogel. This helped know the effect of nanosilver biosynthetic concentration on the physicochemical properties of hydrogel preparation during storage for 12 days at room temperature. The physicochemical properties include organoleptic, pH, viscosity, and dispersibility-test of AgNPs biosynthetic hydrogel according to Indonesian Standard Product of gel (SNI 16-4380-1996).

MATERİALS AND METHODS

Turmeric rhizome-identified in Taxonomy of Plant Laboratory, Faculty of Biology, Universitas Gadjah Mada-from Pasar Gede, Surakarta, Central Java, Indonesia; $AgNO_3$ (Merck, German), Triethanolamine (repacked by PT. Luxchem, Indonesia), glycerine (repacked by PT. Brataco, Indonesia), Carbopol 940 (repacked by PT. Luxhem, Indonesia), aqua dest (repacked by PT. Brataco, Indonesia), phenoxyethanol, Whatmann Paper No.1; *S. aureus* (ATCC 25923); *S. epidermidis* (ATCC 12228); *P. aeruginosa* (ATCC 27853); *E. coli* (ATCC 25922) Microbiology Laboratory of Medical Faculty, UNS, Indonesia. Clindamycine 1% and Gentamycine 1% (prerared by Laboratory of Medical Faculty, UNS, Indonesia). Spectrophotometer UV – Vis (Genesys™), Fourier Transform-IR (Shimadzu, Japan), Transmission Electron Microscope (JEOL/EO 1400, Japan), Scanning Electron Microscope (FEI Quanta 200), oven (Memmert), incubator (Thermo Scientific Series 8000, WJ Heratherm IGS60, USA), digital calipers (Krisbow), hot plate (IKA C-MAG), magnetic stirrer (Labtech ST6), analytic neraca (Precisa), pH meter (Pen Type PH-009, China), analytical weight (Ohaus PA413, USA), vortex mixer (Maxi Mix II Thermolyne, USA), centrifugation (Mini Spin Plus, USA).

Samples preparation

Turmeric juice was prepared by washing the turmeric rhizome before blending, squeezing, and filtering the extract with Whatman paper no 1. The filtered juice was then centrifuged at 10,000 rpm for 10 min at 25°C. The clear liquid was separated using spuit injection and stored in a flacon disk (Kurian *et al*., 2016). Additionally, a Silver nitrate 1.0 mM solution. Silver nitrate 1.0 mM solution was made by weigh $AgNO_3$ powder of 85.0 mg then dissolved in 500 mL distilled water at 40°C.

Biosynthetic process

Turmeric juice was mixed with 1.0 mM $AgNO_3$ solution in 5 ratios of 1: 42; 3:40; 5: 38; 7: 36 and 9: 34 mL. The biosynthesis process was conducted at room temperature and protected from light. The solution was then stirred for 24 h using a magnetic stirrer at room temperature. This resulted in a color change to yellowish-brown, indicating the formation of nanosilver (Sathishkumar *et al*., 2010). The result was observed using spectrophotometer UV-VIS, with a 300 - 700 nm wavelength range to determine the success of the biosynthesis process, where aquadest as the blank solution.

Antibacterial activity against *S.aureus* and *S. epidermidis*

Muller Hinton media was poured into sterile petry dishes then incubated at 37 ºC for 24 hours. The bacterias were taken from culture using ose, then suspended into 0.9 N NaCl sterile solution. The turbidity was measured using the McFarland Equivalens Turbidity Standard. The positive control, formula control, and negative control used are Clindamycin 2.0 µg, $AgNO_3$ 1.0 mM solution, and aqua dest respectively. Bacterial culture was prepared in media with a diameter of 10 mm, where 50 µL of the AgNPs biosynthetic solution

was poured into the well. The medium was incubated for 24 h at 37 °C, and the clear zone measured indicated the nanosilver biosynthetic's ability to inhibit bacteria.

Characteristics of the Selected AgNPs Biosynthetic Volume Ratio

Transmission electron microscope analysis of AgNPs biosynthetic

The biosynthetic solution was analyzed using a TEM for the particle size and shape. The sample tested was the selected formula of the AgNPs biosynthetic solutions that met the AgNPs SPR range and antiacterial activity to Gram Positif Bacterias. The TEM test was performed at a magnification of 200.0, 100.0, 50.0, and 20.0 nm (Sathishkumar *et al*., 2010).

Fourier transform infra-red analysis

The powder sample was prepared by centrifuge of solution for 10 minutes at 10,000 rpm to obtain powder from the solution. It was dried in an oven at 50°C overnight to remove the remaining water (Waidha *et al*., 2014) before adding KBr pellets for analysis using FT-IR.

Scanning electron microscope analysis

The powder sample was made by centrifuge of solution for 10 minutes at 10,000 rpm to obtain powder from the solution. It was dried in an oven at 50°C overnight to remove the remaining water (Waidha *et al*., 2014). The powder was analyzed using SEM equipped with carbon coating to establish the surface morphology of nanosilver biosynthetic.

Antibacterial activity against Pseudomonas aeruginosa and Escherichia coli

Bacterial culture was prepared in media with a diameter of 10 mm, where 50 µL of the AgNPs biosynthetic solution was poured into the well. The medium was incubated for 24 h at 37 °C, and the clear zone measured indicated the nanosilver biosynthetic's ability to inhibit bacteria. The positive and negative controls used are Gentamicin 1% for gram-negative bacteria and aquadest, respectively.

Hydrogel formulation of AgNPs biosynthetic nanosilver

This study used the nanosilver biosynthetic with five different concentrations dispersed into the hydrogel, using the gel preparation formula by Jadhav *et al*. (2016) (Table I). The formula uses a minimal nanosilver in cosmetics of 10 mg/Kg BW (Pulit-Prociak *et al*., 2019). Also, the Nanosilver powder of 1.0 mg is equivalent to a 9.27 g nanosilver liquid.

Carbopol of 0.5 g was dissolved into distilled water at 70 °C, stirred to homogeneity before adding Triethanolamine to form a gel mass. Glycerin was added as a humectant and stirred to homogeneity before adding the nanosilver biosynthetic. Finally, phenoxyethanol was added as a preservative, dissolved first into the remaining glycerin, and stirred. The physical and chemical properties test of nanosilver biosynthesis hydrogel during 12 days are as follows :

pH test

The pH of hydrogel preparations was measured using a pH meter that has calibrated using a buffer solution of pH 4.01 and 6.86 (Adnan, 2017). Based on the Indonesian Standard Product of Gel [SNI No. 06-2588], the pH value of the gel preparation ranged between 4.5-6.5.

Viscosity test

Viscosity was measured using a Rion viscometer. The number 2 spindle was dipped into the hydrogel until the spindle immersed. The tool was turned on and waited for 1.0 min to record the viscosity of the hydrogel preparation (Sinko *et al*., 2011). According to the Indonesian Standard Product of Gel [SNI 16-4380-1996], the viscosity value of gel preparations ranged between 3.000-50.000 cps.

Dispersibility test

The hydrogel was weighed 0.5 g and placed in the middle of a round glass scale. Another round glass weighing 200 g was placed on top of the hydrogel and waited for 1.0 min before recording the spread diameter (Sinko *et al*., 2011). The value of the dispersibility test that meets SNI No. 06-2588 ranges between 5-7 cm.

Data Analaysis

Statistical data analysis was conducted to compare the effect of nanosilver biosynthetic concentrations between formulas, and analyze changes in the test results of the five formulas before and after storage. The data from the evaluation test of the physical and chemical properties of the hydrogel preparations to see the difference using One Way ANOVA analysis and the Paired Sample T-Test using the IBM SPSS Statistic 21 software.

Table I. The hydrogel formula of nanosilver biosynthetic with five different concentrations

Ingredients	Function	Weight (grams)				
		F1	F2	F3	F4	F5
Nanosilver biosynthesis	Active substance	5	10	15	20	25
Carbopol 940	Gelling agent	0.5	0.5	0.5	0.5	0.5
Triethanolamine	Alkalizing agent	1.0	1.0	1.0	1.0	1.0
Glycerine	humectant	10	10	10	10	10
Phenoxyethanol	preservative	0.5	0.5	0.5	0.5	0.5
Aquadest	solvent	83	78	73	68	63

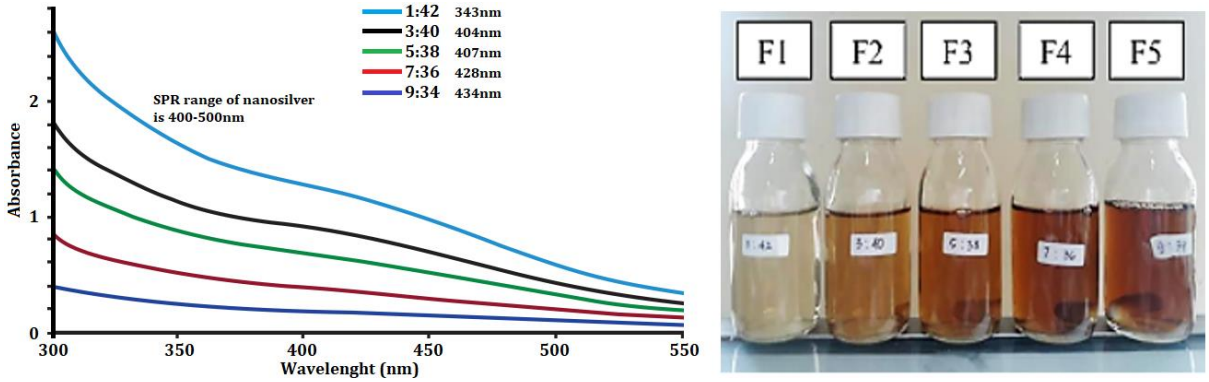

Figure 1. The results of Spectrophotometer analysis spectra produced from the nanosilver biosynthetic with volume variation of turmeric juice-$AgNO_3$ solution with 1: 42; 3: 40; 5: 38; 7: 36 and 9: 34 mL ratios had absorption peaks at 343, 404, 407, 428, 434 nm

RESULTS AND DISCUSSION

The spectra produced from the nanosilver biosynthetic with volume variation of turmeric juice-$AgNO_3$ solution with 1: 42; 3: 40; 5: 38; 7: 36 and 9: 34 mL ratios had absorption peaks at 343, 404, 407, 428, 434 nm, respectively (Figure 1). Nanosilver biosynthetic spectra with 3: 40; 5: 38; 7: 36 and 9:34 mL ratios showed nanosilver formation characterized by peaks in the wavelength range of 400 - 500 nm (Figure 1). In this case, the peak around 350 - 385 nm is the absorption range of turmeric flavonoids (Rahayu *et al*., 2015). Based on the wavelength scanning spectra, the amount of turmeric juice added increased the absorption peak and the resulting AgNPs (Haryani *et al*., 2016). The ratios of turmeric juice-$AgNO_3$ of 5:38, 7:36, and 9:34 mL were selected for antibacterial activity tests against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Furthermore, the pH value test of the mixture was used to establish stability during storage at room temperature. Turmeric juice and $AgNO_3$ solution have pH values of 5.6 and 7.2, respectively, while Curcumin is stable at low pH (El Khoury *et al*., 2015). The results of the mixture's pH value during the nanosilver biosynthetic process from the 1:42; 3:40; 5:38; 7:36, and 9:34 ratios were 6.8; 6.6; 6.4; 6.2 and 6.0 respectively. After the biosynthetic process for 24 hours, the pH of the AgNPs decreased from 6.8 to 6.7. In contrast, the pH values for nanosilver biosynthetic of the 3: 40; 5:38 and 9:34 mL ratios increased to 6.7; 6.5 and 6.1 respectively. Nanosilver biosynthetic of 7:36 mL was stable before and after 24 h.

Antibacterial Activity Test

The antibacterial activity of nanosilver biosynthetic using turmeric juice was tested using the green method. It is easy, simple, practical, and useful in establishing the sensitivity of various microbes to antimicrobial agents (Falugah *et al*., 2019). *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria were selected because they are pathogenic bacteria that cause skin infections such as redness, pus, and acne. In the experiment, the nanosilver biosynthetic was formulated into topical products. The antibacterial activity test was run on the nanosilver biosynthetic formula with 5:38; 7:36 and 9:34 mL ratios.

Table II. the results of an average diameter of inhibition zone against *S. aureus* and *S. epidermidis* bacteria

Sample	Diameter of inhibition zone (mm)		Activity description
	S. aureus	***S.epidermidis***	
Aquadest	0.00[a]±0.00	0.00[a]±0.00	no activity
Silver nitrate	14.44±0.29	13.45±0.69	strong
Clindamycine 2µg	23.91±0.22	23.21±0.88	very strong
Nanosilver biosynthesis [5:38]	14.86±0.39	13.97±0.76	strong
Nanosilver biosynthesis [7:36]	13.80±0.69	13.40±0.48	strong
Nanosilver biosynthesis [9:34]	13.28±1.19	12.84±1.08	strong

[a] significant difference $p<0.05$, all values are means±SE; n=3

Subsequently, Clindamycin was selected as a positive control because its narrow-spectrum antibiotic is effective against gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. Also, the Muller Hinton recommended by the World Health Organization for antibacterial tests against aerobic and facultative anaerobes was the preferred media that gave best results (Putra, 2015). Results of an average diameter of inhibition zone against *S. aureus* and *S. epidermidis* bacteria (Table II). According to Davis and Stout (1971), the diameter of more than 20 nm has an effective inhibition, 10 - 20 mm has a potent inhibition, 5 - 10 mm has moderate inhibition, while less than 5 mm has weak inhibition or resistance. The nanosilver biosynthetic formula with 5: 38; 7: 36 and 9: 34 mL ratios fell under a potent category, with the 5: 38 mL ratio having the highest inhibition diameter value. This indicates that the formula of 5: 38 mL inhibits the growth of *S. aureus* and *S. epidermidis* bacteria more efficiently than the AgNO3 solution. Therefore, biosynthesis using turmeric juice with a 5:38 mL turmeric juice-AgNO3 ratio has antibacterial activity against S. aureus as potent. This is in line with a previous study that used turmeric infuse with an inhibition zone diameter of 14 mm.

In the nanosilver biosynthetic process, turmeric juice acts as a capping agent that envelops the silver ion. The turmeric juice added affects the thickness of the capping agent layer to encapsulate silver ions and increases the size of the nanosilver (Table II). The nanosilver size affects its antibacterial activity because an optimum particle size enters the bacterial cells. Therefore, adding too many bioactive compounds reduces antibacterial activity. The formula ratio of 5:38 mL shows the higher diameter inhibition zone against *S.aureus* and *S.epidermidis* bacterias than a ratio of 7: 36 and 9: 34 mL. Additionally, a ratio of 5: 38 mL estimated has a smaller particle size.

Characteristic of Nanosilver Biosynthetic Formula with a ratio of Turmeric Juice-$AgNO_3$ of 5: 38 mL

FTIR Analysis

FTIR spectra identify biomolecules reducing silver ion (Ag+) and capping agents in the biosynthesis process. The FTIR spectra of AgNO3 powder, turmeric juice, and AgNPs biosynthetic ratio of 5: 38 mL (Figure 2). AgNPs biosynthetic absorption bands around 816, 1030, 1237, 1323, and 1383 cm^{-1} show the absorption bands –C– N vibrational amine strain and –C – O – C–, or –C – O groups (Table III). Functional groups such as –C – O – C– and –C – O originate from heterocyclic components such as alkaloids or flavonoids. Moreover, the Amide (I) bonds found in proteins present in turmeric juice act as nanosilver capping agents. The broad bands at 3411 and 2926 cm^{-1} are aliphatic hydroxyl (–OH) and C-H bonds from turmeric juice. In this study, the absorption band at 1635 cm^{-1} was associated with the vibrational strain of the carboxyl group (–C = O). The absorption band between 1323 - 1385 cm^{-1} indicates the presence of –NO3 residue. In contrast, the presence of a broad absorption band at about 500 cm^{-1} is associated with nanosilver bands (AgNPs) with oxygen from the hydroxyl groups of turmeric juice (Shameli *et al*., 2012). Based on FTIR analysis, the –C – O – C–, and –C – O groups in the flavonoid compounds and the –C – N groups in turmeric juice potentially bind silver ions. Furthermore, these biomolecules coat the nanosilver, acting as a capping agent to prevent particle agglomeration. They stabilize the nanosilver particles in aqueous media (Kurian *et al*., 2016; Ermawati *et al*., 2021).

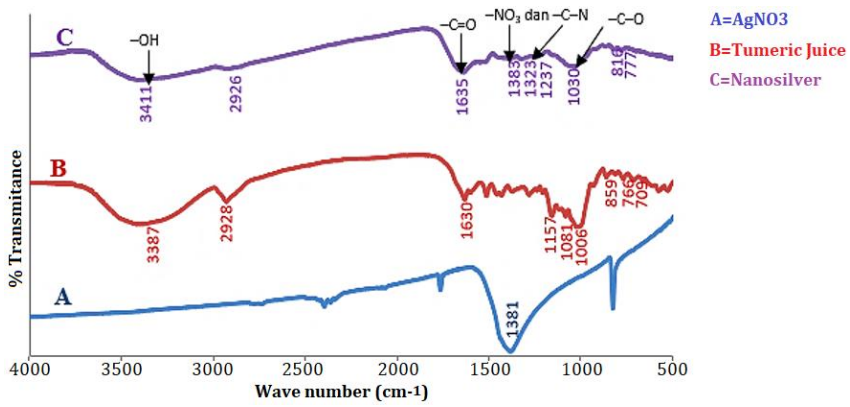

Figure 2. Result of FTIR Analysis of $AgNO_3$ powder [A], turmeric juice [b], and nanosilver biosynthetic of 5:38 mL. It was that there are similarities in the absorption peak of turmeric juice and nanosilver biosynthetic, also $AgNO_3$

Table III. Results of the wavenumber of $AgNO_3$ powder, turmeric juice, and nanosilver biosynthetic ratio of 5: 38 mL with their functional groups

Wave Number (cm^{-1})			Fungtional Groups
Turmeric Juice powder	**NPs Biosynthesis 5:38 mL powder**	**$AgNO_3$ powder**	
3387	3411	-	–OH
2928	2926	-	C–H aliphatic/ alkane
1630	1635	-	–C=O
-	1383, 1323	1381	residue $–NO_3$
1280, 1205	1383, 1323	-	–C–N
1157, 1081, 1006	1237, 1030	-	–C–O
859, 766, 709	816, 777	-	C–H alkene

FTIR analysis showed biosynthesis nanosilver process in existing –C–N–, –C–O–C–, or –C–O, and –OH groups with peaks at 816, 1030, 1237, 1323, 1383, and 3411 cm^{-1}.

TEM and SEM analysis

TEM analysis determines the sample's surface morphology and particle size. Based on Figure 3, the sample results of nanosilver biosynthetic with the ratio of 5:38 mL has an average particle size of 17.97 nm, spherical in shape, and without aggregation at a magnification of 250,000 times. All particles in the samples were evenly distributed in the mixed matrix of turmeric juice and $AgNO_3$. Furthermore, the nanosilver biosynthetic using turmeric juice in average particle size produced a smaller size compared with turmeric infuse used by Kurian *et al.* (2016) and water extract of dry rhizome used by Alsammarraie *et al.* (2018). However, it was a bit bigger than the particle size of nanosilver biosynthesis using water extract of dry rhizomes (Shameli *et al.*, 2012). Therefore, biosynthesis using turmeric juice produces smaller particle sizes than turmeric infuse or water extract of dry turmeric rhizome.

Characterization using SEM aimed to determine the surface morphology of nanosilver biosynthetic, whose 5: 38 mL ratio showed various round shapes, such as the oval morphology of $AgNO_3$ powder. The SEM analysis implied that the size of the nanosilver biosynthetic is smaller than the $AgNO_3$ powder at the same magnification (Figure 4). Several studies on silver biosynthesis showed that the dominant shape of nanosilver particles is spherical, though it does not disregard the formation of nanosilver with other forms. Factors such as temperature, AgNO3 concentration, pH of the solution, and duration of synthesis process affected the shape of the nanosilver produced (Soleimani *et al.*, 2018).

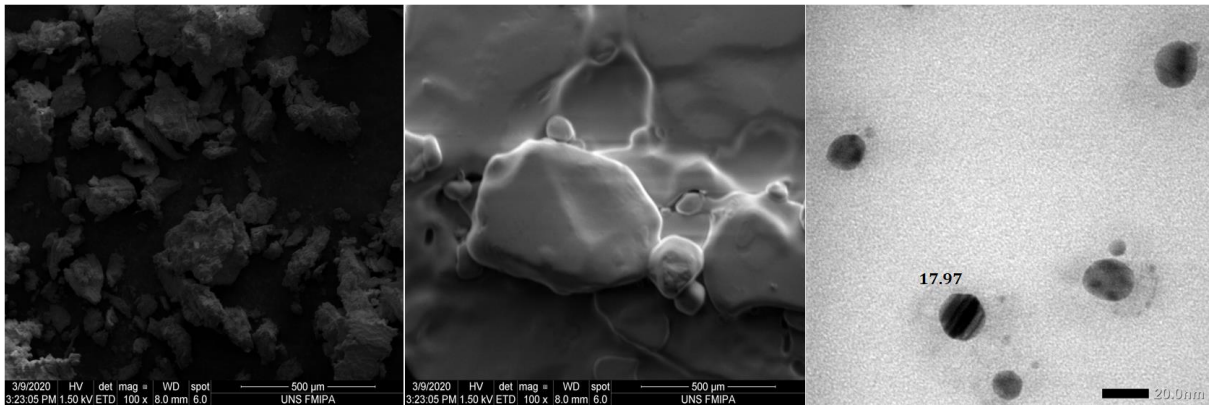

Figure 3 **.** Result of SEM analysis of $AgNO_3$ powder [a] and nanosilver biosynthetic [b]. The morphology surface of nanosilver covered by capping agent compare with $AgNO_3$ powder with the magnification of 200,000 times. Results of TEM analysis of nanosilver biosynthetic shows an average particle size of 17.97 nm with a spherical shape without any aggregation in magnification 250,000 times [c]

Table IV. Results of physicochemical properties of nanosilver biosynthetic hydrogel during storage for 12 days at room temperature

Formula	Physical and Chemical Properties of Nanosilver Biosynthesis Gel					
	Viscosity (dPas)		pH value		Dispersibility (cm)	
	D-0	D-12	D-0	D-12	D-0	D-12
1	30±0	28±0.3	6.3±0.3	6.1±0.1	4.80±0.2	5.20±0.4
2	30±0	30±0	6.2±0.2	6.0±0.1	4.98±0.4	5.31±0.4
3	25±0	25±0	5.7±0.6	5.63±0.1	5.50±0.2	6.15±0.1
4	21±0	21±0	5.7±0.0	5.6±0.1	5.7±0.3	6.1±0.4
5	21±0	20.3±0.6	5.7±0.0	5.7±0.1	5.7±0.4	6.6±0.1

all values are means±SE; n=3

Antibacterial activity against gram-negative bacteria

The average diameters of the inhibition zone of the nanosilver biosynthetic formula with a ratio of 5: 38 mL against *P. aeruginosa* and *E.coli* were 13.55±0.01 and 10.92±0.04 mm, respectively, implying a strong category. Antibacterial activity against *P.aeruginosa* showed that nanosilver biosynthetic has an inhibition zone diameter higher than 13.10 mm $AgNO_3$ solution and 9.83 mm Gentamycin 1%. This means that 5:38 mL of the nanosilver biosynthetic is more potent than the $AgNO_3$ solution and Gentamycin 1% in inhibiting the growth of *P. aeruginosa*. Moreover, the diameter of the inhibition zone of the nanosilver is relatively higher than the silver solution. It indicates that turmeric has antibacterial activity and improves the nanosilver's potential antibacterial activity. The nanosilver biosynthetic on gram-negative produced the inhibition zone diameter not as large as in gram-positive bacteria. This is because gram-positive bacteria have a single-layer cell wall structure, while gram-negative bacteria have a double-layer cell wall structure (Ahluwalia *et al*., 2018). Therefore, the nanosilver biosynthetic of turmeric juice provides broad antibacterial activity against gram-positive and gram-negative bacteria.

Formulation of nanosilver biosynthetic hydrogel

In this study, nanosilver biosynthetic with a ratio volume of 5:38 mL was dispersed into hydrogel polymer to treat and prevent acne topically. Carbopol 940 was the hydrogel polymer used in five formulas with different concentrations of nanosilver biosynthetic. The minimum concentration of nanosilver to inhibit bacteria in topical preparations was 10 mg/Kg B.W (Pulit-Prociak *et al*., 2019). Moreover, the calculation was performed to deduce the nanosilver solutions needed to inhibit bacteria. The minimum weight of

the nanosilver solution calculated was 9.27 mL, which dispersed into 100 grams of hydrogel, resulting in 9.27 grams. The spherical nanosilver biosynthetic measuring 12 nm is nontoxic to human lung epithelial A549 cells (Ahamed, AlSalhi, *et al*., 2010). In line with this, a previous study found that AgNPs measuring 10 nm may be toxic. This is because 50 and 100 µg/mL concentrations could up-regulate the expression of heat shock protein 70 and induce oxidative stress in *D. melanogaster (Ahamed, Posgai, et al., 2010).*

Based on these data, this study used variations in nanosilver biosynthetic concentrations of 5, 10, 15, 20, and 25 g into 100 g of hydrogel to observe their effect on the physical and chemical properties hydrogel preparation during 12 days of storage (Table IV). Carbopol 940 was applied as a polymer because it is hydrophilic, stable, and produces a preparation with high viscosity. Also, it is non-irritative and provides a gel with good physical properties (Allen, 2001) and a more distinct appearance than carbopol 934 (Paul *et al.,* 2020). Carbopol is useful as a gelling agent at a concentration of 0.5% -2%, where a 0.5% solution has an acidic pH of 2.7-3.5. The pH of carbopol is neutralized by adding 1.0% of Triethanolamine, into which glycerin is added as a humectant. The physical and chemical properties of nanosilver biosynthesis hydrogel results are as follows:

pH Test

The pH test ensures that the gel preparation does not irritate the skin due to pH measurements on five nanosilver biosynthetic gel with various active substances. Formulas 4 and 5 have a constant pH value when replicated three times. The higher nanosilver biosynthetic decreased pH value, whose recommended range is 4.5-6.5. However, five formulas have a pH value that fulfills of pH requirement of SNI No. 06-2588 for gel preparation. The statistical analysis showed no significant difference in pH values between formulas. Therefore, nanosilver biosynthesis concentration variation did not affect pH value after storage for 12 days at room temperature.

Viscosity Test

The viscosity test determines the thickness of the gel, where formula 1 with the minimum concentration of nanosilver biosynthetic shows a high viscosity value. In this study, the viscosity values with the slightest results among the five formulas are produced by formula 5 with the highest concentration of an active substance. The increased concentration of active substances reduced viscosity. This is because a smaller pH value reduced the number of ionization groups and intermolecular repulsive force, decreasing the viscosity (Gutowski, 2010). Furthermore, the statistical analysis of the five formulas shows no significant differences between prescriptions. Although the viscosity decreased in formulas 1 and 5 after storage for 12 days at room temperature, it fulfills the range requirement of SNI No. 06-2588 for gel preparation.

Dipersibility Test

The dispersibility test determines the ability of the gel preparation to spread when applied to the skin. The dispersibility test value that meets the Indonesian Standard Product [SNI No. 06-2588] is 5-7 cm. In this case, formulas 1 and 5 have the smallest and highest diameter values of the dispersibility test, respectively. This could be because the increasing concentration of the active substance affected the gel's watery consistency, widening the dispersibility. The statistical analysis implied that the existing substance concentration difference in the gel preparation did not differ in the dispersibility value. The five formulas have a dispersibility that fulfills the requirement range after 12 days of storage. Therefore, the next step was to check nanosilver biosynthetic hydrogel's skin irritation and antibacterial activity.

CONCLUSION

The volume of turmeric juice affects the SPR and antibacterial activity of nanosilver. In this study showed that turmeric juice is a bioreducer that potentially induces the nanosilver's antibacterial activity. The 5:38 mL ratio is the choosen formula that has absorption peak at 407 nm, round shape with a particle size of 17.96 nm and broad-spectrum antibacterial activity. Based on the results, FTIR spectra showed interaction with specific peaks, while the AgNPs concentration affected the gel's physicochemical properties. The formula with 30% AgNPs has a pH of 5.7±0.4, a viscosity of 20.3±0.6, and a dispersibility of 6.6±0.1. Therefore, it meets the requirements of gel based on SNI 16-4380-1996 and it did not significantly change during 12 days at 25°C.

ACKNOWLEDGMENT

Research "Unggulan Terapan" Grant, Dana Non APBN Tahun Anggaran 2021 LPPM Universitas Sebelas Maret, Surakarta, Indonesia

REFERENCES

Adnan, J. (2017). Formulası gel ekstrak daun beluntas (Pluceaindicaless) dengan na-cmc

sebagaı basıs gel. *Journal of Pharmaceutical Science and Herbal Tec*hnology, 1(1), 41-44.

Ahamed, M., AlSalhi, M. S., & Siddiqui, M. K. J. (2010). Silver nanoparticle applications and human health. *Clinica Chimica Acta*, *411*(23), 1841–1848. https://doi.org/https://doi.org/10.1016/j.cca.2010.08.016.

Ahamed, M., Posgai, R., Gorey, T. J., Nielsen, M., Hussain, S. M., & Rowe, J. J. (2010). Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. *Toxicology and Applied Pharmacology*, *242*(3), 263–269. https://doi.org/https://doi.org/10.1016/j.taap.2009.10.016.

Ahluwalia, V., Elumalai, S., Kumar, V., Kumar, S., & Sangwan, R. S. (2018). Nano silver particle synthesis using Swertia paniculata herbal extract and its antimicrobial activity. *Microbial Pathogenesis*, *114*, 402–408. https://doi.org/https://doi.org/10.1016/j.micpath.2017.11.052.

Allen, L. V. (2020). The Art, Science, and Technology of Pharmaceutical Compounding. sixth edition. APhA Publications; 6th edition (October, 2020). https://doi.org/10.21019/9781582123578

Alsammarraie, F. K., Wang, W., Zhou, P., Mustapha, A., & Lin, M. (2018). Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. *Colloids and Surfaces B: Biointerfaces*, *171*, 398–405. https://doi.org/10.1016/j.colsurfb.2018.07.059.

El Khoury, E., Abiad, M., Kassaify, Z. G., & Patra, D. (2015). Green synthesis of curcumin conjugated nanosilver for the applications in nucleic acid sensing and anti-bacterial activity. *Colloids and Surfaces B: Biointerfaces*, *127*, 274–280. https://doi.org/10.1016/j.colsurfb.2015.01.050.

Ermawati DE., Yuniastuti A., Fajrin HI. (2021). Effectiveness of Nanosilver Biosynthesis using Inulin Gembili Tuber (Dioscorea esculenta L.) on Variation of Inulin Solution Towards Particle Sizes and Antibacterial Activities. *Journal of Physics: Conference Series 1912 (2021)*. doi:10.1088/1742-6596/1912/1/012042.

Falugah, F., Posangi, J., & Yamlean, P. (2019). ujı efek antıbakterı jamur endofit pada tumbuhan sereh (*Cymbopogon citratus*) pada bakterı ujı Staphylococcus aureus dan Escherichia coli. Pharmacon, 8(3), 292–302. https://doi.org/10.35799/pha.8.2019.29395.

Gutowski, The effects of pH and concentration on the rheology of carbopol gels. *Thesis*, 1–115. Fraser University: Canada.

Haryani, Y., Kartika, G. F., yuharmen, y., putri, e. m., alchalish, d. t., & melanie, y. (2016). pemanfaatan ekstrak aır rımpang jahe merah (zingiber officinale linn. var. rubrum) pada bıosıntesıs sederhana nanopartıkel perak. *Chimica et natura acta*, *4*(3), 151. https://doi.org/10.24198/cna.v4.n3.10989.

Jadhav, K., Dhamecha, D., Bhattacharya, D., & Patil, M. (2016). Green and ecofriendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns. *Journal of Photochemistry and Photobiology*, *155*, 109–115. https://doi.org/10.1016/j.jphotobiol.2016.01.002.

Khezri, K., Saeedi, M., & Maleki Dizaj, S. (2018). Application of nanoparticles in percutaneous delivery of active ingredients in cosmetic preparations. *Biomedicine & Pharmacotherapy*, *106*, 1499–1505. https://doi.org/https://doi.org/10.1016/j.biopha.2018.07.084.

Kurian, M., Varghese, B., Athira, T. S., & Krishna, S. (2016). Novel and Efficient Synthesis of Silver Nanoparticles Using Curcuma Longa and Zingiber Officinale Rhizome Extracts. *International Journal of Nanoscience and Nanotechnology*, *12*(3), 175–181.

Pulit-Prociak, J., Grabowska, A., Chwastowski, J., Majka, T. M., & Banach, M. (2019). Safety of the application of nanosilver and nanogold in topical cosmetic preparations. *Colloids and Surfaces B: Biointerfaces*, *183*, 110416. https://doi.org/https://doi.org/10.1016/j.colsurfb.2019.110416.

Putra, I. M. A. S. (2015). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (Annonae muricata L.) Dengan Metode Difusi Agar Cakram Terhadap Escherichia coli. *Jurnal Ilmiah Medicamento*, *1*(1), 15–19. https://doi.org/10.36733/medicamento.v1i1.721.

Rahayu, S., Kurniasih, N., & Amalia, V. (2015). Ekstraksi Dan Identifikasi Senyawa Flavonoid Dari Limbah Kulit Bawang Merah

Sebagai Antioksidan Alami. *Al-Kimiya*, *2*(1), 1–8. https://doi.org/10.15575/ak.v2i1.345.

Rasyid, M., Raya, T., & Trak, A. (2015). Biosintesis Partikel-nano Perak Menggunakan Ekstrak Metanol Daun Manggis (Garcinia mangostana L.) Biosynthesis of Silver Nanoparticles using Methanol Extract of Mangosteen Leaves (Garcinia mangostana L.). Jurnal Sainsmat, 4(1), 28-41.

Sathishkumar, M., Sneha, K., & Yun, Y.-S. (2010). Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bactericidal activity. *Bioresource Technology*, *101*(20), 7958–7965. https://doi.org/https://doi.org/10.1016/j.biortech.2010.05.051.

Shameli, K., Ahmad, M. Bin, Zamanian, A., Sangpour, P., Shabanzadeh, P., Abdollahi, Y., & Zargar, M. (2012). Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder. *International Journal of Nanomedicine*, *7*, 5603–5610. https://doi.org/10.2147/IJN.S36786.

Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. *Journal of Colloid and Interface Science*, *275*(2), 496–502. https://doi.org/10.1016/j.jcis.2004.03.003.

Sheskey, P.J., Hancock, B.C., Moss, G.P. (2020). Handbook of Pharmaceutical Excipients Ninth edition. Pharmaceutical Press: Washington: American Pharmacists Association, 110-114

Sinko, P. J., Manurung, J., & Djajadisastra, J. (2011). Martin: Farmasi Fisika dan Ilmu Farmasetika. ISBN: 978-979-044-081-4. Penerbit Buku EGC, Jakarta, Indonesia, 16-17.

Soleimani, F. F., Saleh, T., Shojaosadati, S. A., & Poursalehi, R. (2018). Green Synthesis of Different Shapes of Silver Nanostructures and Evaluation of Their Antibacterial and Cytotoxic Activity. *BioNanoScience*, *8*(1), 72–80. https://doi.org/10.1007/s12668-017-0423-1.

Waidha, A. I., Pandiyarasan, V., Anusya, T., Waidha, K. M., & Shah, A. H. (2014). Synthesis and characterization of silver nano rod like structures by green synthesis method using Curcumin longa. *International Journal of ChemTech Research*, *7*(3), 1504–1508.