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Synthesis of Silver Nanoparticle Using Bio-Reductant from Wuluh Star Fruit (*Averrhoa Bilimbi* L.) Extract and Their Activity as Sunscreen

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Abstract

In recent times, silver nanoparticles can be applied in many fields, including food, energy, biomaterial, nanomedicine, hospital liquid waste purifier, and other applications in similar fields. Silver nanoparticles can also be applied as cosmetics, including sunscreen or sunblock, due to their antioxidant and biocidal properties, making them effective in neutralizing radical agents and also serving as non-toxic antibacterial agent. This study introduces an eco-friendly and innovative synthesis method by utilizing wuluh star fruit as a natural bio-reductant agent, offering a promising solution for environment-friendly sunscreen. Furthermore, researches also leverage the Surface Plasmon Resonance (SPR) properties from silver nanoparticles to perform a development towards sunscreen agent applications. Synthesis of silver nanoparticles has been carried out using $AgNO_3$ solution as precursor with wuluh star fruit extract as bio-reductant and reacted under the sunlight. The synthesized silver nanoparticles were tested for its sunscreen activity. The synthesized has been done with optimization of $AgNO_3$ precursor and bio-reductant. Based on the absorbances value using UV-Vis Spectrophotometer, it is shown that silver nanoparticles from 1.5 mM $AgNO_3$ and 25% ($^v/_v$) extract are more stable than the other variation for 4 days. The characterization of silver nanoparticles using Transmission Electron Microscope (TEM) and Particle Size Analyzer (PSA) instrument result shows the morphology of rounded silver nanoparticles with diverse sizes at range 6.9 – 27.7 nm, and with average particle size of 14.8 nm. Particle Size Analyzer (PSA) instrument shows the distribution of silver nanoparticles at range under 100 nm. Sunscreen activity of silver nanoparticles shows the SPF value of 44.14 with ultra protection type and on high level. The SPF value obtained indicates that silver nanoparticles can be applied as sunscreen.

INTRODUCTION

Silver nanoparticles are nano-sized materials in the range of 1-100 nm. Nanoparticles are quite widely highlighted because they have many advantages, such as biocidal properties as a non-toxic antibacterial agent and radical-stress antidotes applied in biomaterial, nanomedicine, sunscreen agent, *etc* (Zulaicha et al., 2021).

The effectiveness of sunscreen agent can be measured by the SPF value or the result of the amount of UV light to reach the MED value on the unprotected skin (Pratama & Zulkarnain, 2015). The table of SPF values by the Food and Drug Administration (FDA) can be seen in Table 1 and the table of SPF values by Indonesian Food and Drug Authority can be seen in Table 2.

Silver nanoparticles have strong antibacterial properties and provide absorbance

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values in the UV B range so that it can be utilized as a sunscreen. Silver nanoparticles have good UV-shielding properties and can be utilized to protect the skin from UV B damage (Ho et al., 2020)

Table 1. SPF values by the Food and Drug Administration (FDA)

Protection Type	SPF Values
Minimal	1 – 4
Medium	4 – 6
Extra	6 – 8
Maximal	8 – 15
Ultra	> 15

(Nabila, 2021).

Table 2. SPF values by Indonesian Food and Drug Authority.

Level	SPF Values
Low	≥ 6 – < 15
Medium	≥ 15 – < 30
High	≥ 30 – < 50
Very High	≥ 50

(BPOM, 2020)

Silver nanoparticles have the SPR (Surface Plasmon Resonance) properties in the UV-Visible range due to the presence of free electrons in the conduction band due to the small particle size. Silver nanoparticles can counteract photoreaction to the UV light because it can provide a physical barrier and prevent direct contact or reduce the intensity of UV radiation. It is due to the SPR properties that make silver nanoparticles very effective in light absorption and scattering properties (Ghzawani et al., 2023).

In general, silver nanoparticles are synthesized using the bottom-up method as a practical and inexpensive synthesis method. This method utilizes the use of reductants and stabilizers to reduce Ag^+ ions into colloidal silver nanoparticles Ag^0 (Kosimaningrum et al., 2020). Reductants can be replaced using bio-reductants as a more environmentally friendly alternative because it reduces the use of chemical compounds (Fabiani et al., 2019).

In the synthesis process, various synthesis methods are used, one of which is synthesis with the assist of sunlight irradiation. Sunlight can accelerate redox reactions in the formation of silver nanoparticles because it can function as external energy (Firdaus, 2019). Based on the previous statement, it can be concluded that sunlight provides the eco-friendly external energy source, eliminating the requirement of additional instruments. This method also demonstrates the efficiency of using sunlight irradiation compared to a conventional method (e.g., ultrasonic irradiation, thermal stirring, etc.).

The bio-reductant candidate is wuluh star fruit. This fruit has potential as a bio-reductant in the synthesis of silver nanoparticles. Based on the research from Azhar et al. (2019), wuluh star fruit has high enough vitamin C content, amounting to 25 mg/100 grams of fruit. Therefore, the notably high vitamin C content in wuluh star fruit renders it as an ideal bio-reducing agent in the manufacture of silver nanoparticles.

Thus, the synthesis of silver nanoparticles using the bio-reductant of star fruit extract aims to determine the stability of silver nanoparticles that have been formed based on UV-Vis Spectrophotometer data, characterization of silver nanoparticles based on TEM and PSA data, and determine the potential sunscreen activity of silver nanoparticles.

MATERIALS AND METHOD

Materials

The materials used in this research were Whatman No.1 filter paper, aluminum foil, plastic wrap, tissue, wuluh star fruit (*Averrhoa bilimbi* L.), distilled water, silver nitrate (Merck. 99.8%), and polyvinyl alcohol (PVA) (Sigma-Aldrich).

Method

Preparation of Wuluh Star Fruit (Averrhoa bilimbi L.) Extract

200 grams of wuluh star fruit that has been washed clean were weighed, and then then it was cut into small pieces with a size of 1 cm × 1 cm. the fruit was then pureed and filtered. The filtrate was then centrifuged at 4000 rpm for 15 minutes. Finally the extract was then separated from the precipitate (Isaac et al., 2013).

Silver Nanoparticles Synthesis and Stability Test with UV-Vis Spectrophotometer

Silver nanoparticles were synthesized by mixing 25; 50; 75; and 100% wuluh star fruit extract drop by drop into 1.0 mM $AgNO_3$ solution (1:1 ratio) until a color change appeared from clear to brownish yellow with the assist of sunlight irradiation for 0; 3; 6; and 9 minutes (Azhar et al.,

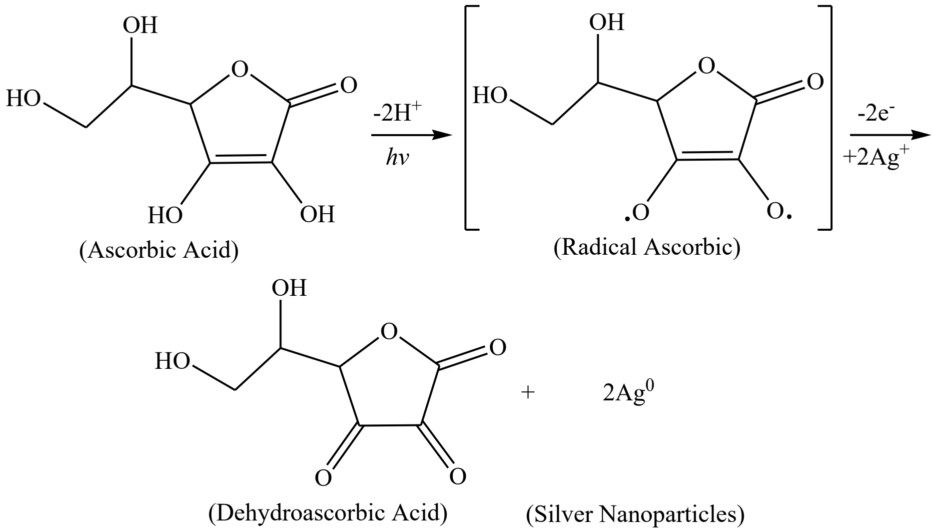


Figure 1. Synthesis reaction mechanism of silver nanoparticles using bio-reductant of ascorbic acid (Sari et al., 2017).

2019). The synthesis process was carried out with the same steps with variations of 25% wuluh star fruit extract and 0.5; 1.0; and 1.5 mM $AgNO_3$. Then, 1% PVA solution were added as a stabilizer for silver nanoparticles. The absorbances of the mixture were then measured with a UV-Vis Spectrophotometer at a wavelength of 300-650 nm. Silver nanoparticles with selected variations will be continued with characterization tests using Transmission Electron Microscope (TEM) and Particle Size Analyzer (PSA) instruments.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles obtained using Transmission Electron Microscope (TEM) instrument aims to determine the morphology of silver nanoparticles and displayed them in the form of images. Colloidal silver nanoparticles were dripped on to the TEM grid, then filtered with filter paper under the TEM to remove residue. After that, the imaging results were processed using ImageJ software to determine the average diameter of the particles formed.

The characterization of silver nanoparticles obtained using Particle Size Analyzer (PSA) instrument aims to determine the size of silver nanoparticles. The optimum concentration of $AgNO_3$ to produce silver nanoparticles with the smallest size can be determined using PSA instrument.

Sunscreen Activity Test

Sunscreen activity testing refers to the research from Payapo et al. (2017), which tests sunscreen activity by determining the SPF value of the silver nanoparticles solution using a UV-Vis Spectrophotometer. The the absorbance value was measured at a wavelength of 290 nm to 400 nm with an interval of 5 nm with three times repetition (triplo). The absorbances value obtained was used to calculate the SPF value with the Equation (1-2).

$$[AUC] = \frac{A_a + A_b}{2} \times (dP_{a-b}) \quad (1)$$

Where, AUC is the Average absorbances value, A_a is the absorbances at a nm wavelength, A_b is the absorbances at b nm wavelength and dP_{a-b} is a and b wavelength difference which is 5 nm.

$$\log SPF = \frac{\Pi AUC}{\lambda_1 - \lambda_0} \quad (2)$$

Where, SPF is *Sun Protection Factor*, ΠAUC is the total AUC value, λ_1 is the largest wavelength (400 nm) and λ_0 is the smallest wavelength (290 nm).

RESULTS AND DISCUSSION

In this study, silver nanoparticles have been successfully synthesized using wuluh star fruit extract. Wuluh star fruit extract used as the bio-reductant contains vitamin C or ascorbic acid which can reduce Ag^+ ions to form Ag^0 in the form of colloidal silver nanoparticles with the following reaction in Figure 1.

Based on the mechanism provided in Figure 1, vitamin C or ascorbic acid has a –OH group that functions as a proton donor, with the reduction potential of ascorbic acid (+0.35 V) being lower than the reduction potential value of Ag^+ ions (+0.80 V), so that in the synthesis process, Ag^+ ions will be reduced by ascorbic acid to form Ag^0 colloidal silver nanoparticles shown by the formation of a brownish yellow solution color appeared as in Figure 2.

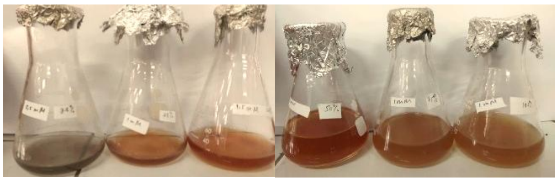


Figure 2. Silver nanoparticles' synthesis using bio-reductant of wuluh star fruit extract.

The obtained nanoparticles were then tested for stability by measuring their absorbances value using a UV-Vis Spectrophotometer at a wavelength of 300-650 nm to observe and obtain the graph of the shift in the absorbances value of silver nanoparticles, which was marked by the appearance of the SPR peak at 400-450 nm as a specific peak for silver nanoparticles. The measurement results can be seen in Figures 3 and 4.

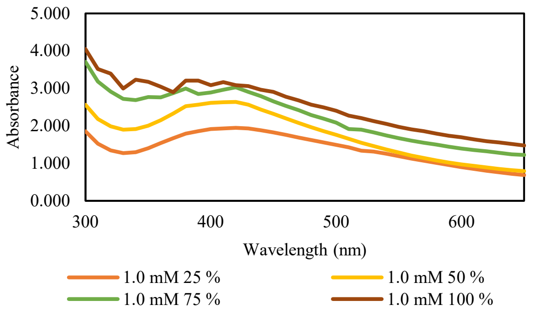


Figure 3. Absorbance of Silver nanoparticles with the variation of wuluh star fruit extract's concentration.

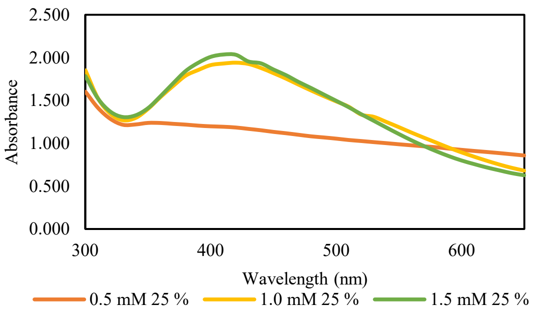


Figure 4. Silver nanoparticles with the variation of $AgNO_3$ concentration.

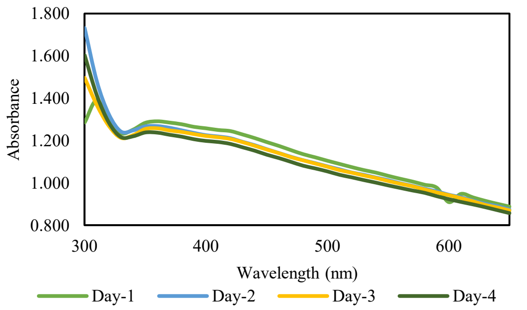
Based on Figure 3, for silver nanoparticles with 1.0 mM $AgNO_3$ and 25% $(^{v}/_{v})$ star fruit, a maximum wavelength value of 420 nm was obtained with an absorbance of 1.941; on silver nanoparticles with 50% $(^{v}/_{v})$ star fruit, a maximum wavelength value of 420 nm was obtained with an absorbance value of 2.639; on silver nanoparticles with 75% $(^{v}/_{v})$ star fruit, a maximum wavelength value of 300 nm was obtained with an absorbance value of 3.708; and for silver nanoparticles with 75% $(^{v}/_{v})$ star fruit, a maximum wavelength value of 300 nm was obtained with an absorbance value of 4,050. In the synthesis of silver nanoparticles with varying bio-reductant concentrations of 75 and 100%, no nanoparticles were formed because according to Solomon et al. (2007) silver nanoparticles are formed in the wavelength range of 400-450 nm.

Furthermore, silver nanoparticles with 1.0 mM $AgNO_3$ and 25% $(^{v}/_{v})$ star fruit extract were chosen as the bio-reductant composition for the next optimization steps. This choice was made because they exhibited a maximum wavelength of 420 nm and a color that was not too intense. A more intense color was suspected to indicate the formation of a greater quantity of silver nanoparticles. In accordance with statement from (rasetiowati et al. (2018), a higher absorbance value implies that a greater number of nanoparticles were formed. This led the researchers to avoid selecting silver nanoparticles with a 50% bio-reductant composition. The formation of a larger quantity of nanoparticles could potentially lead to faster agglomeration, as particles in a liquid medium tend to come into close proximity and agglomerate. This raised concerns about the long-term stability of the silver nanoparticles, as increased agglomeration could result in larger particle sizes, rendering them less effective in their performance.

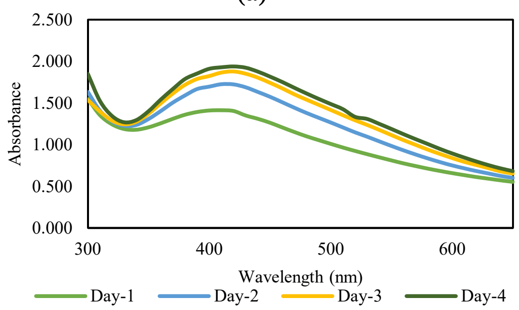
Based on Figure 4, silver nanoparticles with a concentration of $AgNO_3$ at 0.5 mM and 25% wuluh star fruit extract exhibited a maximum wavelength value of 300 nm with an absorbance of 1.603. This wavelength does not indicate the presence of silver nanoparticles, suggesting that at the $AgNO_3$ concentration of 0.5 mM, silver nanoparticles have not yet formed. For silver nanoparticles with a concentration of $AgNO_3$ at 1.0 mM, the maximum wavelength observed was 420 nm with an absorbance of 1.941. Furthermore, for silver nanoparticles with a concentration of $AgNO_3$ at 1.5 mM, the maximum wavelength recorded was

410 nm with an absorbance of 2.037. Silver nanoparticles with a concentration of $AgNO_3$ at 1.5 mM exhibit a stable maximum wavelength profile compared to other concentration variations. Therefore, the concentration of $AgNO_3$ at 1.5 mM is considered the optimum concentration among the different concentration variations. According to Prasetiowati et al. (2018), quantitatively, a higher absorbance value suggests a greater quantity of silver nanoparticles formed.

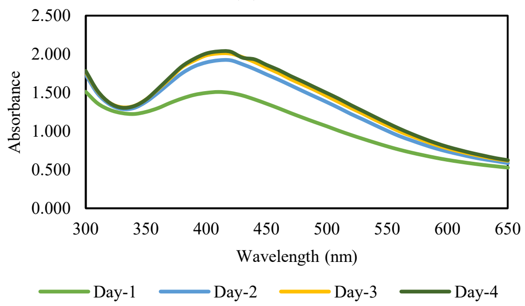
According to Sari et al. (2017), a high absorbance value indicates the formation of a significant number of silver nanoparticles, which can potentially lead to the production of high-quality silver nanoparticles suitable for various applications. The stability test results for the silver nanoparticles obtained over a period of 4 days can be seen in Figure 5.



(a)



(b)



(c)

Figure 5. Characterization results of silver nanoparticles from 25% wuluh star fruit extract by using $AgNO_3$ with a concentration of (a) 0.5 mM (b) 1.0 mM and (c) 1.5 mM.

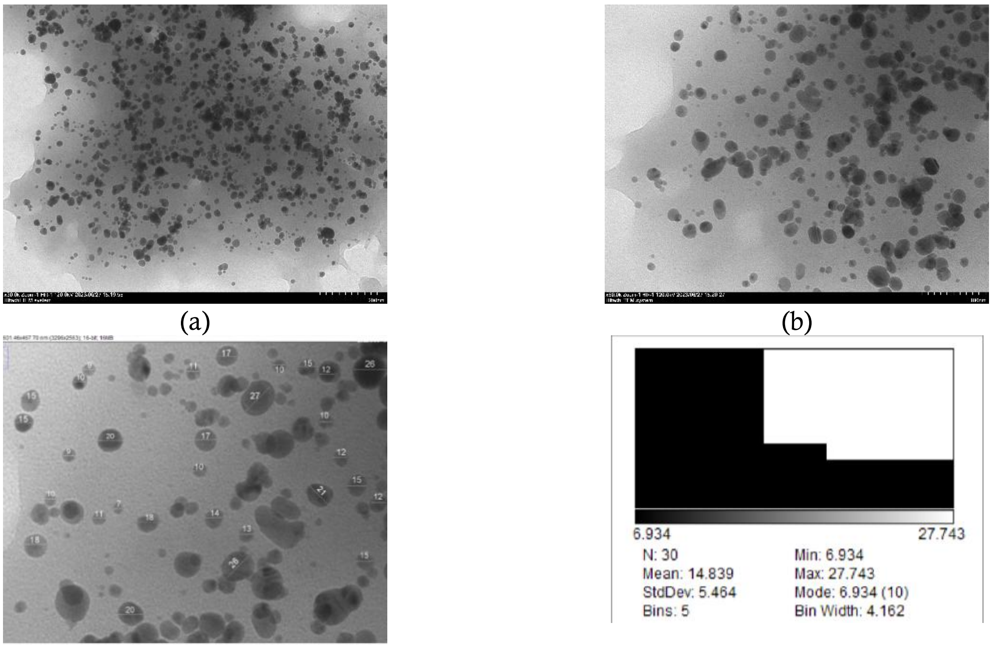
Based on the measurements of silver nanoparticles over 4 days using a UV-Vis spectrophotometer, it is evident that each concentration exhibits a stable wavelength pattern. The measurement results indicate a shift in the wavelength for silver nanoparticles with concentrations of 0.5; 1.0; and 1.5 mM in combination with 25% star fruit extract, ranging approximately from 330-360 nm, 400-420 nm, and 400-410 nm, respectively (Figure 5).

The synthesis of silver nanoparticles with $AgNO_3$ concentration at 0.5 mM and 25% star fruit extract from day 1 to day 4 does not show any absorption peaks in the nanoparticle range. Therefore, it can be concluded that no silver nanoparticles were formed at this concentration. In contrast, for $AgNO_3$ concentrations of 1.0 and 1.5 mM, it is evident that there is an absorption peak at 400-450 nm from day 1 to 4. Silver nanoparticles with a concentration of 1.5 mM show greater stability compared to those with a concentration of 1.0 mM. This is because silver nanoparticles with a concentration of 1.5 mM exhibit a smaller shift in wavelength compared to the 1.0 mM concentration.

The best-performing variation of silver nanoparticles will undergo further characterization tests using Transmission Electron Microscope (TEM) and Particle Size Analyzer (PSA). The characterization results of silver nanoparticles using Transmission Electron Microscope (TEM) can be seen in Figure 6.

The morphology of silver nanoparticle showed from Figure 6. TEM analysis at scales of 100 nm and 200 nm, showing the spherical silver nanoparticles with varying dominant particle sizes were obtained. Through analysis using ImageJ software, particle sizes ranging from 6.93 nm to 27.74 nm were determined, with an average particle size of 14.84 nm. The formed silver nanoparticles did not aggregate or form larger particle clusters.

The characterization results of silver nanoparticles using a Particle Size Analyzer (PSA) instrument can be observed in Figure 7. From the PSA instrument, it shows that the particle size distribution of silver nanoparticles were found to have an average of 110 nm. However, the size range varied from approximately 80-280 nm. The variation in nanoparticle sizes is suspected to be due to agglomeration during the testing process, which may have occurred because of extended shipping and waiting times in the laboratory. This discrepancy between the TEM and PSA



(a) (b) (c) (d)

Figure 6. Morphology of silver nanoparticle using Transmission Electron Microscope (TEM). Silver nano particles on a scale of (a) 200 nm (b) 100 nm (c) Particle size analysis by using ImageJ software (d) Particle size distribution measurement by using ImageJ Software.

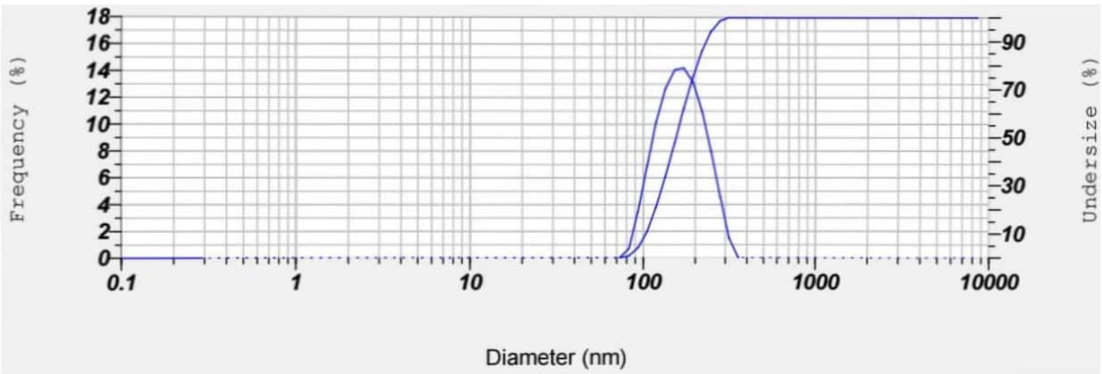


Figure 7. Characterization results using the Particle Size Analyzer (PSA) instrument for silver nanoparticles.

characterization results suggests that the sizes of the silver nanoparticles obtained generally fall within the range of 40-100 nm. This aligns with research from Prasetiowati et al. (2018) that reported an average particle size of 112.8 nm.

The selected variation of silver nanoparticles was further subjected to a sun protection factor (SPF) testing to determine their sunscreen activity. According to Widyawati et al. (2019), UVB radiation is not entirely filtered by the ozone layer and can reach the Earth's surface, causing sunburn. Meanwhile, UVA radiation can penetrate the ozone layer, reach the Earth, and human skin. UVA can penetrate the epidermis and dermis layers deeper into the skin's surface, potentially causing premature aging. The effects of sunlight exposure or UV radiation are divided into two categories: acute effects such as erythema or sunburn, phototoxic reactions, photoallergic reactions, and photosensitivity, as well as chronic effects including photoaging and skin cancer. Based on these impacts, sunscreen activity testing was conducted at wavelengths of 290-320 nm (UVB) and 320-400 nm (UVA). The SPF measurement results can be seen in Table 3.

The sunscreen activity testing in Table 3, it can be observed that the SPF values increase when higher concentrations of $AgNO_3$ are used. Sunscreen activity of silver nanoparticles with a concentration of $AgNO_3$ at 1.0 mM and 25% star

Table 3. The SPF value of silver nanoparticles.

Sample	SPF Value	Protection Type*	Level**
Silver Nanoparticles 1.0 mM 25%	38.25	Ultra	High
Silver Nanoparticles 1.5 mM 25%	44.14	Ultra	High

*Protection Type based on Table 1 SPF Values by the Food and Drug Administration
**Level based on Table 2 SPF Values by the Indonesian Food and Drug Authority

fruit extract has an SPF value of 38.25, which falls into the "ultra" protection type with a high level of protection. The sunscreen activity of silver nanoparticles with a concentration of $AgNO_3$ at 1.5 mM has an SPF value of 44.14, also belonging to the "ultra" protection type with a high level of protection. Silver nanoparticles with a concentration of $AgNO_3$ at 1.5 mM exhibit a higher SPF value compared to those with a concentration of AgNO3 at 1.0 mM. This is due to the quantitative aspect, where a greater quantity of silver nanoparticles forms at the $AgNO_3$concentration of 1.5 mM.

The sun protection ability of silver nanoparticles in this research, with an SPF value of 38.25 for the 1.0 mM concentration and 44.14 for the 1.5 mM concentration, is higher compared to the study of Payapo et al. (2017) (SPF value of 3.39) that also used a precursor concentration of 1.0 mM $AgNO_3$. Based on the study of Payapo et al. (2017), silver nanoparticles were combined with hydroxycinnamic acid. This suggests that silver nanoparticles without the combination of cinnamic acid have better sunscreen activity, and the use of natural substances as bio-reducing agents in the production of silver nanoparticles is considered superior to the use of chemicals as reducing agents.

The SPF values in Table 3, silver nanoparticles with star fruit extract as the bio-reductant have an 'ultra' protection type, indicating their sunscreen capability. This is because, according to Ghzawani et al. (2023), UVB sunlight can cause skin reddening, sunburn effects, DNA damage, cell death, and increased production of reactive oxygen species (ROS). Silver nanoparticles possess strong antioxidant properties because they can reduce the increase in ROS. Silver nanoparticles can counteract photoreactions to UV radiation because they provide a physical barrier, limiting the possibility of photoreactions by preventing direct contact or reducing the intensity of UV radiation. Silver nanoparticles can also block UV radiation due to their Surface Plasmon Resonance (SPR) properties, making them highly effective in light absorption and scattering.

CONCLUSION

Silver nanoparticles synthesized from a solution of 1.5 mM $AgNO_3$ with 25% of star fruit (*Averrhoa bilimbi* L.) extract demonstrated stability over a period of 4 days. Characterization of the silver nanoparticles using TEM and PSA revealed a spherical morphology with an average particle size of 14.8 nm, and the particle size distribution fell within the range of less than 100 nm. Additionally, the Sun Protection Factor (SPF) value for the sunscreen activity of silver nanoparticles with star fruit extract as the bio-reductant at a concentration of 1.5 mM $AgNO_3$ and 25% of star fruit extract was determined to be 44.14, categorizing it as ultra protection with a high level of effectiveness.

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