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OPTIMIZATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *ARECA CATECHU* L. SEED EXTRACT WITH VARIATIONS OF SILVER NITRATE AND EXTRACT CONCENTRATIONS USING SIMPLEX LATTICE DESIGN METHOD

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Abstract

Silver nanoparticles have been widely used because of their antibacterial properties. Green synthesis of silver nanoparticles was carried out using the ethanolic extract of *Areca catechu* L. seed because it can act as a bioreductor and stabilizer. This study aims to optimize the formula for silver nanoparticles using the simplex lattice design method with various concentrations of silver nitrate ($AgNO_3$) and extract. The study used 9 formulas to determine the surface plasmon resonance (SPR) of silver nanoparticles. The ethanolic extract of *Areca catechu* L. seed contains flavonoid compounds, alkaloids, polyphenols, tannins, and saponins which can reduce and stabilize silver nanoparticles. The flavonoid content was 110.849 mg CE/g extract. Optimum formula obtained from the concentration of silver nitrate ($AgNO_3$) 3 mM and 3 mL of extract concentration of 10% w/v. The maximum wavelength of SPR was 420 nm and the absorbance was 1.427, particle size was 122.53 nm, PDI was 0.35, and zeta potential was -21.17 mV. The results of the thermodynamic stability test showed the formation of an irreversible precipitate, a bathochromic and hypochromic shift in the SPR spectrum, and a decrease in the pH value. The optimum formula has quite good particle characteristics but is still not physically stable.

Rezumat

Nanoparticulele de argint au fost utilizate pe scară largă datorită proprietăților lor antibacteriene. Sinteza “verde” a nanoparticulelor de argint a fost realizată folosind extractul etanolic de semințe de *Areca catechu* L., deoarece acesta poate acționa ca bioreductor și stabilizator. Prezentul studiu are ca scop optimizarea formulei nanoparticulelor de argint folosind metoda de proiectare a rețelei simplex cu diferite concentrații de nitrat de argint ($AgNO_3$) și extract etanolic. Studiul a utilizat 9 formule pentru a determina rezonanța plasmonică de suprafață (RPS) a nanoparticulelor de argint. Extractul etanolic de semințe de *Areca catechu* L. conține flavonoide, alcaloizi, polifenoli, taninuri și saponine care pot reduce și stabiliza nanoparticulele de argint. Conținutul de flavonoide determinat a fost de 110,849 mg CE/g extract. Formula optimă a fost obținută din concentrația de nitrat de argint ($AgNO_3$) 3 mM și 3 ml de extract cu concentrație de 10% p/v. Lungimea de undă maximă a RPS a fost de 420 nm, iar absorbția a fost de 1,427, dimensiunea particulelor a fost de 122,53 nm, indicele de polidispersitate (IPD) a fost de 0,35, iar potențialul zeta a fost de -21,17 mV. Rezultatele testului de stabilitate termodinamică au arătat formarea unui precipitat ireversibil, o schimbare batocromică și hipocromică în spectrul RPS și o scădere a valorii pH-ului. Formula optimă are caracteristici destul de bune ale particulelor, dar nu este încă stabilă din punct de vedere fizic.

Keywords: *Areca catechu* L. seed, silver nanoparticles, simplex lattice design, surface plasmon resonance

Introduction

Nanoparticles are particles that have a size of 10 - 1000 nm [1]. Silver nanoparticles are currently widely used in the health sector, one of which is as an antibacterial. Silver nanoparticles are known to be able to fight 650 types of bacteria [2]. The presence of these antibacterial properties causes silver nanoparticles to become a substance that has the potential to be further developed in the health sector.

In general, the synthesis of nanoparticles can be carried out using a top-down Method and a bottom-up Method using synthetic compounds. However, the use of synthetic compounds is not environmentally friendly. Therefore, a green synthesis nanoparticle method was developed which is safer, economical, and environmentally friendly [3]. The green synthesis of nanoparticles uses plant extracts that act as reducing agents and stabilizers of nanoparticles [4, 5]. Phenolic and flavonoid compounds in plant extracts have antioxidant properties that can to reduce metals [6].

Areca catechu L. is one of the plants that can be used for the synthesis of silver nanoparticles. *Areca catechu* L. seed contains major alkaloid compounds such as arecaidine and arecoline as well as major polyphenolic compounds such as tannic acid, catechin, epigallocatechin, epicatechin, epigallocatechin gallate (EGCG) and gallic acid [7]. Amudhan's research [8] stated that *Areca catechu* L. seed contains higher polyphenol compounds and lower alkaloid compounds. Based on Zhang *et al.*'s research [9], the ethanol extract of areca nut has strong antioxidant activity with an EC50 value of 0.409 mg/mL.

The preparation of silver nanoparticles in this study was carried out by varying the concentration of silver nitrate ($AgNO_3$) and ethanolic extract of *Areca catechu* L. seed. Both of these parameters have an important role to produce good nanoparticles. The higher the concentration of silver nitrate ($AgNO_3$) and the concentration of the extract used, the more silver nanoparticles formed [10]. However, research by Sunita and Palaniswamy [11] stated that lower extract concentrations could provide more stable particles.

Based on the description above, it is necessary to optimize the process and characterization of silver nanoparticles with varying concentrations of silver nitrate and ethanol extract of *Areca catechu* L. seed using the simplex lattice design method. The concentration range of silver nitrate used is 1 - 5 mM. Meanwhile, the ethanolic extract of *Areca catechu* L. seed used was 10% w/v with varying volumes was 1 - 5 mL. The characterization of silver nano-particles was carried out using a UV-Vis spectro-photometer to observe the maximum wavelength and absorbance values of surface plasmon resonance (SPR). The optimum silver nanoparticle formula was further characterized using a particle size analyser (PSA) to observe the size, distribution, and zeta potential of the nanoparticles. In addition, the optimum formula of silver nanoparticles was also tested for stability using the heating cooling method to determine the thermodynamic stability.

Materials and Methods

Materials

The materials used in this study were *Areca catechu* L. seeds, 70% ethanol (Bratachem®, Indonesia), absolute ethanol (Emsure®, Indonesia), $AgNO_3$ (Emsure®, Indonesia), Mg powder, HCl p.a (Merck®, Singapore), 0.1 N NaOH (Emsure®, Indonesia), aquadest, Dragendorff reagent (Sigma-Aldrich®, Singapore), Mayer reagent (Sigma-Aldrich®, Singapore), Wagner reagent (Sigma-Aldrich®, Singapore), 2% $FeCl_3$ (Sigma-Aldrich®, Singapore), glacial acetic acid (Emsure®, Indonesia), NaCl (Merck®, Singapore), gelatin, methanol (Bratachem®, Indonesia), chloroform (Merck®, Singapore), H_2SO_4 p (Merck®, Singapore), catechins (Sigma-Aldrich®, Singapore), aluminum foil and Whatmann filter paper no. 42.

Preparation of ethanolic extract of Areca catechu L. seed

Areca catechu L. seeds are separated from the skin of the fruit and the seeds are washed with running water. After washing, the *Areca catechu* L. seeds are then chopped and dried. The dried seeds are then mashed to obtain Simplicia powder. A total of 300 g of Simplicia powder was put into a maceration container then added 70% ethanol solvent and stored in a dark place. The extract solution was soaked for 48 hours. The filtrate is filtered and the residue is re-maceration for 24 hours [12]. The filtrate was collected and evaporated using a rotary evaporator at a temperature of 65°C to obtain a thick extract. The % extract yield value can be calculated by the formula in Equation 1 as follows:

$$\%\ Yield = \frac{\text{Weight of extract obtained in grams}}{\text{Weight of simpisia in grams}}\ x\ 100\% \quad (1)$$

Phytochemical Screening

Phytochemical screening carried out included examination of flavonoids, alkaloids, polyphenols, tannins, steroids and triterpenoids, and saponins. The test was carried out qualitatively using colour reagents and visual observation [13].

Determination of Total Flavonoid Content in Extract

The measurement of total flavonoid content in the extract was carried out based on the research of Abdeltaif *et al.* [14] with minor modifications. Catechins are used as standards to make calibration curve equations. The ethanol extract of *Areca catechu* L. seeds were made to a concentration of 50 g/mL. The sample solution was then measured its absorbance using a UV-Vis spectrophotometer (Biobase®) using the maximum wavelength obtained on the calibration curve. The total flavonoid value contained in the extract can be calculated by the formula in Equation 2 as follows:

$$TFC = \frac{Volume\ (mL) x\ Concentration\ \left(\frac{mg}{mL}\right) x\ dilute\ factor}{g\ Extract} \quad (2)$$

Design Formula of Silver Nanoparticles

The optimization of the silver nanoparticle formula was carried out by determining the composition of the mixture of silver nitrate ($AgNO_3$) and ethanolic extract of *Areca catechu* L. seeds using the Simplex Lattice Design method from the Design-Expert 12®. The range of silver nitrate ($AgNO_3$) concentration and extract concentration refers to the research of Ahmed *et al.* [15] and Manosalva *et al.* [16]. The concentration range for the silver nitrate ($AgNO_3$) was 1 - 5 mM, and for the extract 1 - 5 mL, respectively. The design formula for silver nanoparticles reduced by ethanolic extract of *Areca catechu* L. seeds using the Simplex Lattice Design method from the Design-Expert 12® device can be seen in Table I.

Table I

Formula design of silver nanoparticles

Formula	**Concentration**	
	Silver Nitrate (mM)	**Extract (mL)**
1	5	1
2	3	3
3	2	4
4	1	5
5	1	5
6	5	1
7	5	1

Formula	Concentration	
	Silver Nitrate (mM)	Extract (mL)
8	3	3
9	4	2

Preparation of Silver Nanoparticles

The extract solution was pipetted according to the volume in Table I. The silver nitrate ($AgNO_3$) solution was heated at a temperature of 60°C and the extract solution was added drop by drop with a volume ratio of 9:1 silver nitrate solution and extract solution [17]. The solution was added with 0.1 N NaOH until a solution pH of 9. The solution was stirred with a stirring speed of 750 rpm for 15 minutes. The process of reducing silver ions (Ag^+) to silver nanoparticles (Ag^0) is characterized by a change in colour to reddish-brown or blackish-brown [18].

Characterization of Silver Nanoparticle

Determination of Surface Plasmon Resonance

Absorption band measurements of surface plasmon resonance silver nanoparticles were performed using a UV-Vis spectrophotometer (Biobase®). A sample was pipetted into a cuvette and analysed for the maximum wavelength and absorbance value in the wavelength range of 370 - 600 nm.

Determination of size, polydispersity index, and Zeta potential

Determination of particle size, polydispersity index, and zeta potential was carried out on the optimum formula of silver nanoparticles using a particle size analyser (Malvern®) using the dynamic light scattering (DLS) method. The diluted suspension was put into a zeta sizer cell/cuvette. Then size was measured, the polydispersity index (PDI) value with a scattering angle of 90°, and the zeta potential with a scattering angle of 173° [19].

Thermodynamic Stability Test

A stability test was carried out on the optimum formula for silver nanoparticles using the heating-cooling cycle test method. The heating-cooling cycle test was carried out for 6 cycles between 4°C and 40°C with storage at each temperature for 24 hours [20]. Organoleptic observations, namely colour changes and the formation or absence of precipitates were carried out in each cycle. Meanwhile, the value of max, absorbance and pH value of silver nanoparticles were characterized.

Statistical analysis

Statistical analysis was performed with the program Design-Expert 12®. Observational data on the physical characterization of silver nanoparticles or the response from the experimental results were carried out by ANOVA test to determine the significance of the response analysis between variables and to find out the model suggested by Design-Expert 12®. Optimum formula analysis is done by determining the desired response criteria with a range that is possible to achieve. The most optimum formula is the formula with the maximum desirability value.

Results and Discussion

The Ethanolic Extract of Areca catechu L. Seeds

The ethanolic extract of *Areca catechu* L. seeds obtained was brown in colour, had a characteristic odour, and was thick. The yield percentage produced was 59.21%. The higher yield is produced because catechin compounds can be extracted well in 70% ethanol solvent. 70% ethanol solvent is a polar solvent that can extract polyphenolic compounds in *Areca catechu L.* seeds which is also polar. The polarity of catechins and other polyphenolic compounds results from the large number of hydroxyl groups (OH).

The results of phytochemical screening showed that the ethanolic extract of *Areca catechu* L. seeds contains secondary metabolites such as flavonoids, polyphenols, tannins, saponins, and alkaloids which have electron donor groups so it can reduce Ag^+ metal ions to Ag^0. Flavonoid compounds and polyphenols are the main compounds in the extract that most act as bioreductors because they have several functional groups such as carbonyl and hydroxy groups, as well as phi electrons that can reduce metals. In addition, the ability of tautomeric transformation of flavonoid compounds from enol to keto form can release reactive hydrogen atoms which can also reduce metal ions into nanoparticles [5]. The total flavonoid content obtained in the ethanolic extract of *Areca catechu* L. seeds was 110.849 ± 0.548 mg CE/g extract.

Silver Nanoparticles

Silver nanoparticles were synthesized using a bioreductor of ethanolic extract of *Areca catechu L.* seeds which produced reddish-brown or blackish-brown in colour.

Silver nanoparticles have unique optical properties due to the surface plasmon resonance (SPR) phenomenon. SPR is a resonant phenomenon of oscillation or vibration of metal valence electrons on the surface of nanoparticles [21]. The SPR phenomenon can explain the colour change during the synthesis of silver nanoparticles. The reddish-brown or blackish-brown colour indicates the formation of silver nanoparticles. This colour results from the wavelength-dependent absorption and scattering of silver nanoparticles from the SPR. In silver nanoparticles, the maximum wavelength of SPR is in visible light, which is in the range of 400 - 450 nm [22]. In this study, the maximum wavelength and absorbance of the 9 formulas were found in the range of 419 - 430 nm and 0.600 - 1.600.

The maximum wavelength and absorbance of the SPR are strongly influenced by the concentration of

$AgNO_3$ and the extract used, so in this study optimization of the concentration of $AgNO_3$ and the ethanolic extract of *Areca catechu* L. seeds were carried out. The spectrum of the SPR of silver nanoparticles showed the differences in maximum wavelength and absorbance of each formula. The smaller the maximum wavelength of the SPR indicates the smaller the nanoparticle size and the increase in the absorbance of the SPR can be associated with an increase in the number of nanoparticles formed [10, 23]. An increase in the concentration of silver nitrate will increase the absorbance of silver nanoparticles. Formulas 1, 6, and 7 with a concentration of 5 mM silver nitrate and 1 mL extract gave a fairly high absorbance. However, the large concentration of silver ions (Ag^+) also requires a high enough concentration of extract to produce silver nanoparticles. Formulas 2 and 8 with a concentration of 3 mM silver nitrate and 3 mL extract gave the highest absorbance.

Model Analysis of Silver Nanoparticle Formula

Analysis of the silver nanoparticle formula model was carried out using the Design-Expert 12®. In this study, a formula modelling was made to obtain the optimum formula of silver nanoparticles from the ethanolic extract of *Areca catechu* L. seeds. The results of the modelling analysis obtained can be seen in Table II.

Table II

Model Analysis

Response	Parameter						
	Mean	SD	CV (%)	R^2	Adjusted R^2	Predicted R^2	Adequate precision
Max. Wavelength	425.56	1.44	0.3393	0.9161	0.8658	0.6980	11.4290
Absorbance	1.11	0.16	14.59	0.8769	0.8030	0.4687	7.9970

The model of the SPR silver nanoparticles from the ANOVA analysis was significant ($p < 0.05$) with an insignificant lack of fit ($p > 0.05$) so the obtained model can be used for the formula optimization process. Based on Table V, this model has a difference in the value of Adjusted R^2 and Predicted R^2 which is less than 0.2 and the value of an adequate precision model is greater than 4 so there is no significant difference between the predicted value of the system and the actual value obtained from the research data. The maximum wavelength and absorbance data of the SPR of silver nanoparticles produced in this study were normally distributed.

Data Analysis of Maximum Wavelength of Silver Nanoparticles

Data analysis of the maximum wavelength of silver nanoparticles was carried out using the simplex lattice design method using the Design-Expert 12®. This data analysis was carried out to see the effect of each factor ($AgNO_3$ and extract concentration) and the interaction of the two factors on the maximum wavelength response. The relationship between factors and responses can be seen in Table III and Figure 1.

Table III

The coefficient of factor to maximum wavelength response

	A	B	AB	AB(A-B)
Coefficient	427.744	427.616	-21.9279	-59.0077
p-values	0.4037	0.4037	0.0041*	0.0036*

* factors that have a significant effect ($p < 0.05$)

Based on Table III, the max SPR equation for silver nanoparticles is obtained, namely:

$$Y = -21.9279AB - 59.0077AB(A-B) \quad (3)$$

Where: Y = The maximum wavelength of silver nanoparticles; A = Factor of silver nitrate ($AgNO_3$); B = Factor of the ethanolic extract of *Areca catechu* L. seeds; AB = Interaction factor of silver nitrate and the ethanolic extract of *Areca catechu* L. seeds.

Based on the Table III, silver nitrate concentration and the extract concentration separately have no significant effect (p > 0 0.05) but the combination of concentration of silver nitrate and extract had a significant effect ($p < 0.05$) on the maximum wavelength SPR of silver nanoparticles. The combination of concentration of silver nitrate and extract has a negative correlation with the maximum wavelength SPR of silver nanoparticles. This indicates that the higher the combination of silver nitrate and extract used, the smaller the maximum wavelength SPR value of silver nanoparticles obtained.

However, the use of the same high concentration of silver nitrate and extract can produce a large maximum wavelength SPR value of silver nanoparticles. This is similar to the research of Edison and Sethuraman [24] which states that a lower concentration of extract will result in a smaller maximum wavelength SPR value. Figure 1 showed the relationship between variations in the concentration of silver nitrate and extract on the maximum wavelength SPR value of the resulting silver nanoparticles. The use of high concentrations of silver and extracts can cause the maximum wavelength value of the SPR of silver nanoparticles to increase or undergo a bathochromic shift. This is because the higher the concentration of silver nitrate and extracts used, the more nanoparticles formed so that the tendency to interact and agglomerate is greater. Meanwhile, the use of low concentrations of silver or extracts will cause lower silver nanoparticles to form but smaller in size as a result of the unavailability of silver ion concentration and the low reduction ability of the extract. Figure 1 shows formulas 2 and 8 with 3 mM silver concentration and 3 mL extract, and formula 9 with 4 mM silver nitrate concentration and 2 mL extract giving a low maximum wavelength SPR value of silver nanoparticles.

Data Analysis of Absorbance of Silver Nanoparticles

Data analysis of the absorbance of silver nanoparticles was carried out using the simplex lattice design method using the Design-Expert 12®. This data analysis was carried out to see the effect of each factor ($AgNO_3$ and extract concentration) and the interaction of the two factors on the absorbance response. The relationship between factors and responses can be seen in Table IV and Figure 2.

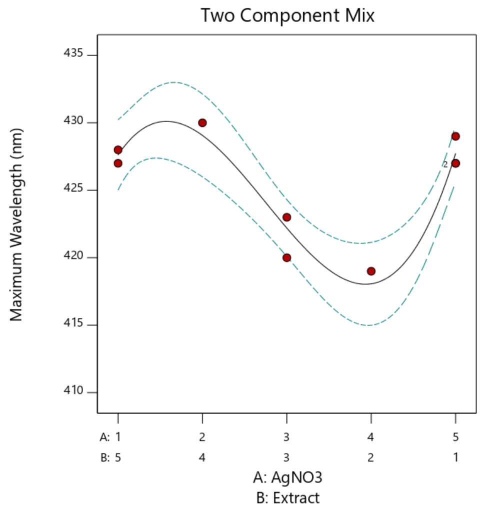

Figure 1.
The correlation curve of the concentration of silver nitrate and extract on the maximum wavelength

Table IV
The coefficient of factor to absorbance response

	A	B	AB	AB(A-B)^2
Coefficient	1.3125	0.7607	1.80751	-13.0247
p-values	0.0119*	0.0119*	0.0209*	0.0082*

* factors that have a significant effect (p <0.05)

Based on Table IV the absorbance equation for silver nanoparticles is obtained, namely:

$$Y = 1.3125A + 0.7607B + 1.8075AB – 13.0247AB(A-B)^2 \quad (4)$$

Where: Y = The absorbance of silver nanoparticles; A = Factor of silver nitrate ($AgNO_3$); B = Factor of the ethanolic extract of *Areca catechu* L. seeds; AB = Interaction factor of silver nitrate and the ethanolic extract of *Areca catechu* L. seeds.

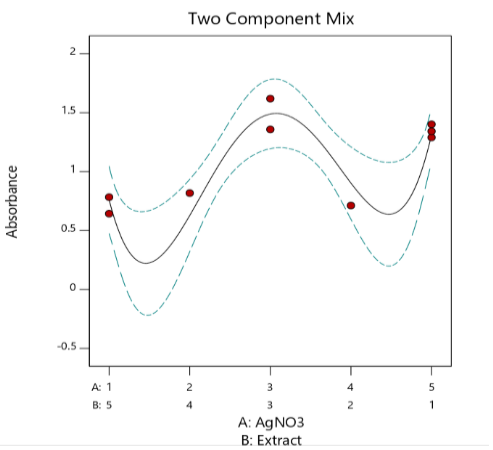

Figure 2.
The correlation curve of the concentration of silver nitrate and extract on the absorbance

In the absorbance response in Table IV, the concentration of silver nitrate and the concentration of the extract or the combination of both had a significant effect ($p < 0.05$). Silver nitrate and extract concentration have a positive correlation to the absorbance of nanoparticles. This indicates that an increase in the concentration of silver nitrate or extract will increase the absorbance of nanoparticles. The combination of silver nitrate and extract concentration also had a positive correlation to the absorbance of silver nanoparticles. This indicates that an increase in the combined concentration of silver nitrate and extract will increase the absorbance of silver nanoparticles. This is similar to the research of Ahmed *et al*. [15] which states that increasing the concentration of silver nitrate and the concentration of the extract will increase the absorbance of silver nanoparticles. The relationship between variations in the concentration of silver nitrate and extract on the absorbance value of the resulting silver nanoparticles is shown in Figure 5. The concentration of silver nitrate is related to the availability of Ag^+ ions which will be reduced to Ag^0 atoms. The higher the concentration of Ag^+ ions, the more reduction, and nucleation processes occur so that more nanoparticles are formed. Meanwhile, the extract concentration was related to the extract's ability to reduce Ag^+ ions to Ag^0 atoms. The higher the concentration of the extract, the greater the ability of the extract to reduce Ag^+ ions to Ag^0 atoms. Figure 2 shows formula 2 with 3 mM silver concentration and 3 mL extract, giving the highest absorbance of silver nanoparticles.

Silver Nanoparticle Optimum Formula

The desirability value is an important indicator in determining an optimum formula. The desirability value that is getting closer to 1 indicates the program's ability to produce formulas with the desired criteria is getting more perfect. The formula criteria were designed to obtain a nanoparticle formula with a minimum max SPR value in the range of 415 - 420 nm and a maximum absorbance in the range of 1.4 - 1.6. The concentration of silver nitrate and extract is a significant factor to obtain the optimum formula with a small wavelength and large absorbance, so the importance level is set to 5. The optimum formula suggested by the system is a formula with a silver nitrate concentration of 3 mM and an extract of 3 mL with a desirability value of 1. The predicted value and the verification range of the optimum formula are shown in Table V.

Table V

Prediction value and verification range of the optimum formula

Response	Predicted Mean	Observed	95%Cl		95% PI	
			Low	High	Low	High
Maximum Wavelength	422.198	420	420.089	424.307	412.776	431.621
Absorbance	1.488	1.427	1.1948	1.7822	0.38871	2.5883

Verification of the optimum formula is done by looking at the predictive value consisting of 95% CI (Confidence Interval) and 95% PI (Predicted Interval). The CI value is the value interval for the average value of the observations at the 95% confidence level and the PI value is the value interval for the individual's predicted value at the 95% confidence level [25]. In the optimum formula, the maximum wavelength of the SPR of silver nanoparticles is 420 nm and the absorbance of silver nanoparticles is 1.427. This shows that the value of max and the absorbance of the SPR of silver nanoparticles are theoretically still within the 95% CI and 95% PI values.

Silver nanoparticle optimum formula characterization

Characterization includes determining particle size, Poly Dispersity Index (PDI), and zeta potential value of particles. The results of the characterization can be seen in Table VI.

Table VI

Characterization results

Parameter	Mean ± SD
Particle size (nm)	122.53 ± 14.85
PDI	0.35 ± 0.09
Zeta Potential (mV)	-21.17 ± 3.18

Silver nanoparticles in the optimum formula have an average particle size that is still classified as having good antibacterial activity, which is 122.53 ± 14.85 nm. This is supported by the research of Pei *et al*. [26] which tested the antibacterial activity of silver nanoparticles with a size of 135.8 nm on several types of bacteria. The results of Pei *et al*. [26] obtained silver nanoparticles capable of inhibiting the growth of *Staphylococcus aureus* bacteria at a minimum inhibitory concentration of 50 µg/mL, and *Pseudomonas aeruginosa* bacteria at a minimum inhibitory concentration of 100 µg/mL. In addition, the results of research by Raza *et al*. [27] also showed that silver-chitosan nanoparticles with a size of 941 nm still had good antibacterial activity. Silver nanoparticles from the optimum formula also have a fairly homogeneous particle size distribution and uniformity. This can be seen from the resulting Poly Dispersity Index (PDI) which is less than 0.5, which is 0.35 ± 0.09. Particle sizes with a PDI value of 0.1-0.4 belong to the moderate dispersion distribution [28]. The results of the measurement of the zeta potential value showed the average zeta potential value of the optimum formula was -21.17 ± 3.18 mV. Based on these results, the silver nanoparticle suspension still tends to experience aggregation because of the weak repulsion between particles. Nanoparticles are said to be stable if they have a zeta potential value of more than ± 30 mV [19]. The negative zeta potential value in this study was due to the nanoparticles being synthesized in an alkaline environment containing a lot of hydroxyl ions (OH^-) creating a dispersion medium with a negative charge. The zeta potential value of silver nanoparticles is related to the antibacterial properties produced. Silver nanoparticles with negative zeta potential have stronger antibacterial properties against gram-positive bacteria. This is because the negative charge on the cell wall of gram-positive bacteria is weaker than that of gram-negative bacteria, so it tends to be easier to interact with nanoparticles that have a negative zeta potential [29]. Electrostatic interactions between nanoparticles and bacterial cell walls can release energy to form ROS (Reactive Oxygen Species) which can damage cells and cause bacterial death [30]. The research of Solviani *et al*. [31] showed that nanoparticles with a zeta potential value of -18.3 ± 1.9 mV had stronger antibacterial activity on gram-positive bacteria (*Staphylococcus aureus*) compared to gram-negative bacteria (*Escherichia coli*).

Thermodynamic stability of silver nanoparticle optimum formula

The optimum formula for silver nanoparticles was tested for stability by heating-cooling cycle test for 6 cycles. Silver nanoparticles in the optimum formula had poor stability which was indicated by a bathochromic shift in the SPR spectrum and a decrease in pH (Figure 3). In the 1st cycle, the maximum wavelength value of silver nanoparticles was 426 nm with an absorbance of 1.385. Meanwhile, in the 6th cycle, the maximum wavelength SPR value of silver nanoparticles was 429 nm with an absorbance of 1.246. The bathochromic shift indicates an increase in particle size and a decrease in absorbance indicates the nanoparticles have changed in size to silver ions [32]. The existence of a widening peak in the 6th cycle nanoparticle spectrum also indicates that aggregation has occurred which is also characterized by the formation of sediment on organoleptic observations [33].

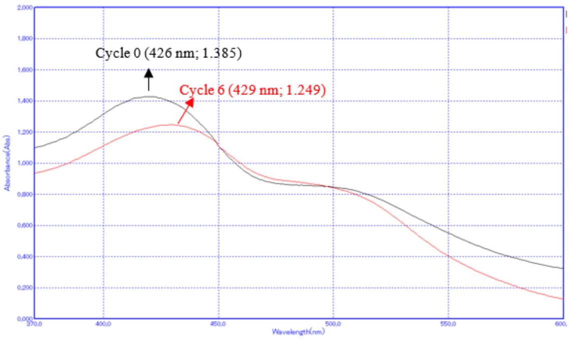

Figure 3.

UV-Vis Spectrum of Silver Nanoparticles Cycle 0 and Cycle 6

The instability of the silver nanoparticle suspension was also characterized by a decrease in the pH value of the silver nanoparticle suspension. The decrease in the pH value of the silver nanoparticle suspension was due to the instability of flavonoid compounds due to the influence of extreme temperatures resulting in the release of acidic hydrogen ions (H^+). So it is necessary to study further the stability of silver nanoparticles from the ethanol extract of *Areca catechu L.* seeds.

Conclusions

The combination of using silver nitrate and extract has a negative correlation to the maximum wavelength SPR of silver nanoparticles. Meanwhile, the concentration of silver nitrate, the concentration of the extract, and their combination had a positive correlation with the absorbance produced. Optimum formula optimization results were obtained at a concentration of 3 mM silver nitrate and extract concentration of 10% w/v as much as 3 mL with a maximum wavelength SPR value of 420 nm and an absorbance of 1.427. The value of particle diameter size, polydispersity index (PDI), and zeta potential of the optimum formula were 122.53 ± 14.85 nm, respectively; 0.35 ± 0.09; and -21.17 ± 3.18 mV. The optimum formula for silver nanoparticles was less stable which was indicated by the formation of brown sediment, a bathochromic and hypochromic shift from the UV-Vis spectrum, and a decrease in pH.

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Conflict of interest

The authors declare no conflict of interest.

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