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THE ROLE OF TEMPERATURE AND pH IN THE SYNTHESIS OF SILVER NANOPARTICLES USING ARECA CATECHU L. SEED EXTRACT AS BIOREDUCTOR

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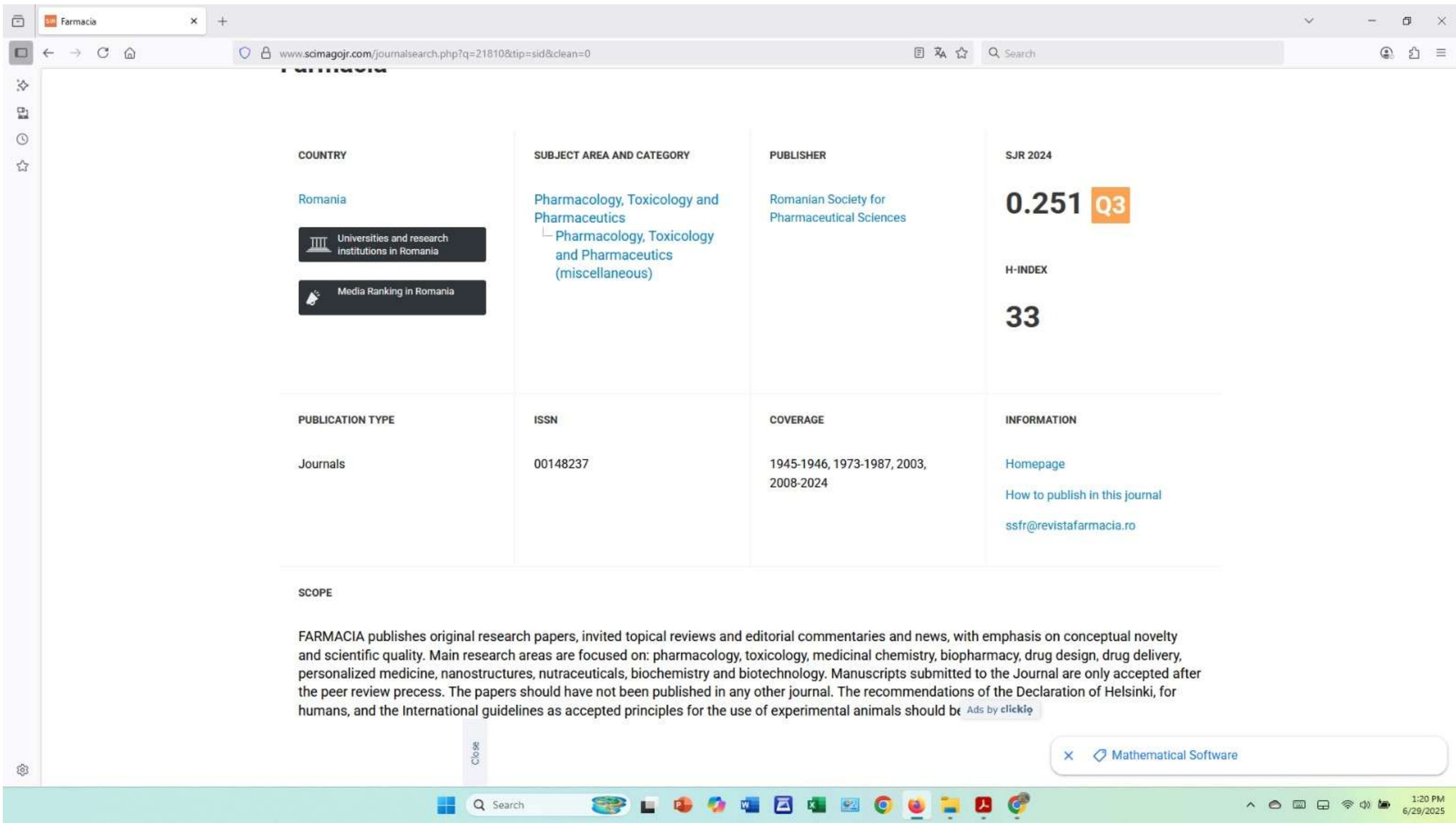

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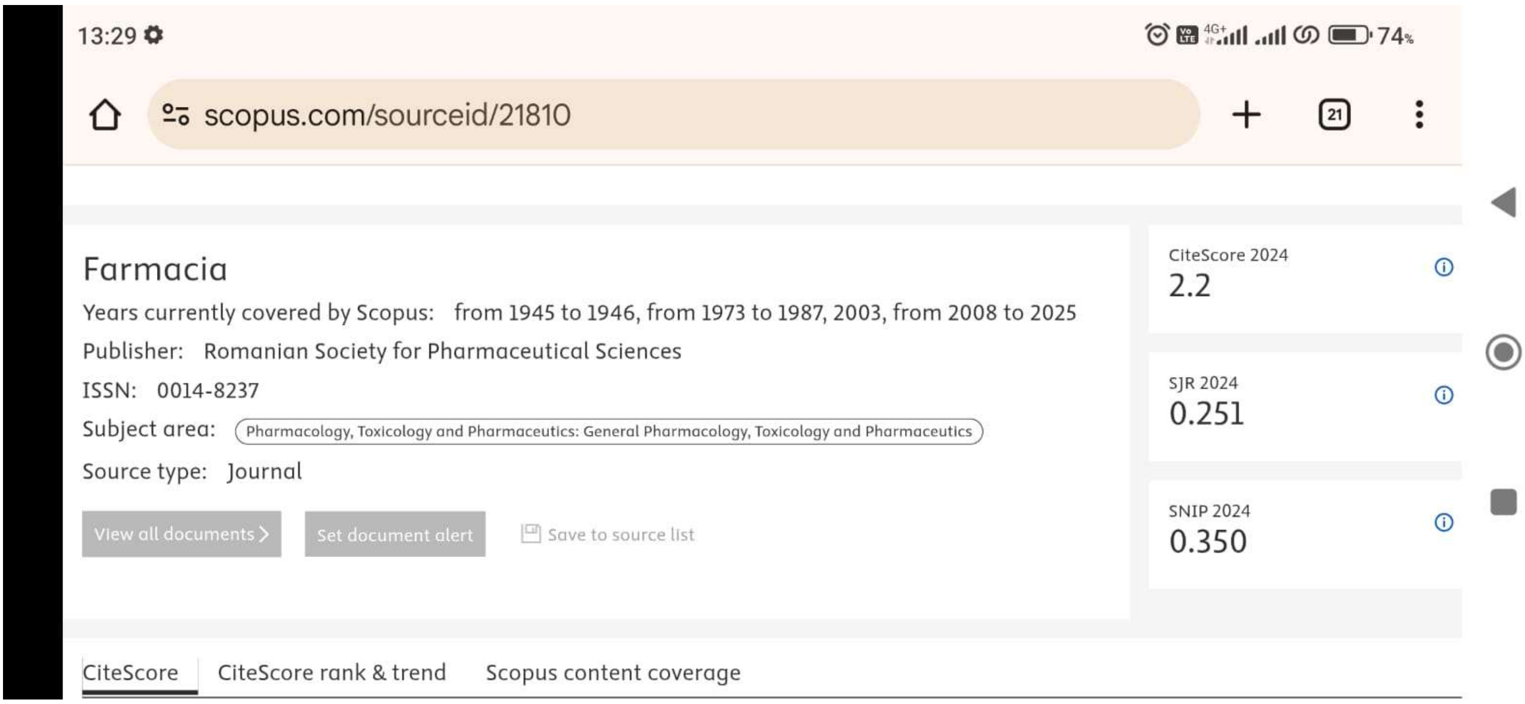

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THE ROLE OF TEMPERATURE AND pH IN THE SYNTHESIS OF SILVER NANOPARTICLES USING *ARECA CATECHU* L. SEED EXTRACT AS BIOREDUCTOR

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Abstract

Silver nanoparticles are prepared using natural bioreductors such as *Areca catechu* ethanol extract, which is rich in phenolic content. This study aims to optimize the temperature and pH conditions for synthesizing silver nanoparticles using the response surface methodology (RSM) - central composite design (CCD) method. The phenolic content of the *Areca catechu* ethanol extract was 111.14 ± 3.41 mg CE/g extract. Temperature and pH conditions significantly affect maximum wavelength and absorbance values. The optimum condition was obtained at a temperature of 30oC and a pH of 10.5. Silver nanoparticles at optimum conditions had a wavelength of 423 nm, absorbance was 1.148, particle size was 161.7 ± 46.1 nm, PDI was 0.286 ± 0.035, and zeta potential was - 16.1 ± 3.7 mV. The stability of silver nanoparticles at the optimum conditions produced is relatively stable, characterized by no significant changes in organoleptic, pH, and wavelength, but the absorbance value has increased. The resulting silver nanoparticles have good characteristics and good stability.

Rezumat

Nanoparticulele de argint pot fi preparate folosind bioreducători naturali, cum este extractul etanolic de *Areca catechu*, bogat în fenoli. În cadrul acestui studiu ne-am propus optimizarea condițiilor de temperatură și pH pentru sintetizarea nanoparticulelor de argint folosind metoda suprafeței de răspuns (MSR). Conținutul fenolic al extractului etanolic de *Areca catechu* a fost de 111,14 ± 3,41 mg/g extract. Temperatura și pH-ul afectează semnificativ reacția, condițiile optime de formare a nanoparticulelor fiind la 30°C și un pH de 10,5. Stabilitatea nanoparticulelor de argint obținute este relativ optimală, caracterizată prin faptul că nu există modificări semnificative în ceea ce privește proprietățile organoleptice și pH-ul.

Keywords: *Areca catechu* L. seed, pH, response surface methodology, silver nanoparticles, temperature

Introduction

Nanotechnology in the pharmaceutical field is currently developing very rapidly. Silver nanoparticles are among the most researched and set in the medical world because they have been shown to have anti-bacterial, anti-inflammatory, anti-angiogenesis, anti-fungal, antiviral, and antiplatelet activities [1-4].

The production of silver nanoparticles has shifted towards green synthesis methods that are more environmentally friendly. The green synthesis method requires the help of secondary metabolites in plants as a source of bioreductors [5]. Secondary metabolites that can act as bioreductors usually act as antioxidants, such as flavonoids, phenolics, and alkaloids [6]. *Areca catechu* L. is a plant rich in alkaloid compounds such as guvacine, guvacoline, arecaidine, and arecoline and also rich in polyphenolic compounds such as catechin and quercetin [7, 8].

The formation of silver nanoparticles through the green synthesis method can be influenced by several factors, such as temperature and pH [9]. Temperature is another critical factor affecting the formation of nanoparticles in plant extracts [10]. In general, an increase in temperature will increase nanoparticle synthesis's reaction rate and efficiency [11]. This statement is proven by research conducted by Jiang *et al* [12], where a temperature of 17 to 55°C will accelerate the reaction and cause the particle size to increase from 90 nm to 180 nm. Besides temperature, changes in pH will also affect the process of forming silver nanoparticles. Changes in pH will result in changes in the natural phytochemical charge contained in the extract. This effect will affect its ability to bind and reduce metal cations while synthesizing silver nanoparticles. According to research by Marciniak *et al*. [13], the reaction for forming silver nanoparticles runs at a pH of 6.0 to 11.0 for citric acid and 7.0 to 11.0 for malic acid. Increasing the pH can also affect the particle size of silver nanoparticles, where the higher the pH value, the smaller the particle size [14].

Based on the description above, researchers are interested in conducting research in the form of optimizing the effect of temperature and pH on λmax surface plasmon resonance (SPR) and absorbance in the manufacture of silver nanoparticles using ethanol extract of young areca nut seeds as a bioreductor agent.

Optimization was carried out using the response surface methodology (RSM) - central composite design (CCD) method for temperature and pH. The temperature and pH used in this study were 30 - 70°C, respectively, and the pH range was 7.5 - 10.5, referring to the research by Prodjosantoso *et al.* [15] and Seifipour *et al.* [16] with slight modifications. The formula of silver nanoparticles with optimum temperature and pH will be further characterized in particle size, polydispersity index (PDI), zeta potential and thermodynamic stability tests using the cycling test method.

Materials and Methods

Materials

The materials used in this study were *Areca catechu L.* seed s, AgNO3 (Emsure®, Indonesia), 70% ethanol (Bratachem®, Indonesia), absolute ethanol (Emsure®, Indonesia), NaOH 0.1 M (Bratachem®, Indonesia), catechins (Sigma-Aldrich®, Singapore), and aqua deionized (Bratachem®, Indonesia).

Preparation of Ethanolic Extract of Areca catechu L. Seed

Areca catechu L. seeds were washed, dried and crushed to obtain Simplicia powder. The simplicia powder was macerated using 70% ethanol solvent and stored in a dark place for 48 hours. The filtrate was then evaporated using a rotary evaporator to obtain a thick extract.

Determination of Total Phenolic Content in Extract

The measurement of total phenolic content in the extract was carried out based on the research of Indarti *et al.* [17] and Sudirman *et al.* [18] with modifications. The standard used in this procedure is catechin. The extract is put at 10 mg into a 100 mL measuring flask, then the volume is made up to obtain a 100 µg/mL concentration. The absorbance of the sample solution was measured using a UV-Vis Spectrophotometer at 281 nm. The formula can calculate the total phenolic content in the extract in Equation 1.

$$\text{TPC} = \frac{Volume\ (mL) x\ Concentration\ \left(\frac{mg}{mL}\right) x\ dilute\ factor}{g\ Extract}. \quad (1)$$

Formula of Silver Nanoparticles

The formula for making silver nanoparticles using the ethanol extract of *Areca catechu* L. refers to research by Apriani *et al.* [19]. The concentration of silver nitrate ($AgNO_3$) used was 3 mM and 3 mL of extract at a concentration of 10% w/v with a ratio of 1:9.

Temperature and pH Optimization

Optimization of temperature and pH in the manufacture of silver nanoparticles was carried out using the response surface methodology (RSM) - central composite design (CCD) method referring to the research of Prodjosantoso *et al.* [15] and Seifipour *et al.* [16] with slight modifications. The levels used consist of low, middle, and high levels. The design of the levels for each factor, namely temperature and pH can be seen in Table I. Based on the conditions in Table I, nine formulas were produced for manufacturing silver nanoparticles which can be seen in Table II.

Table I

Level design for temperature and pH

Factor	Level		
	Low	Medium	High
Temperature	30	50	70
pH	7.5	9	10.5

Table II

Formula and condition of silver nanoparticles

Formula	$AgNO_3$ 3 mM (mL)	Extract 10% b/v (mL)	Temp (°C)	pH
1	27	3	70	7.5
2	27	3	30	9
3	27	3	70	10.5
4	27	3	50	9
5	27	3	50	7.5
6	27	3	70	9
7	27	3	30	10.5
8	27	3	30	7.5
9	27	3	50	10.5

Preparation of Silver Nanoparticles

As much as 3 mL of 10% w/v young areca seed ethanol extract was added drop by drop in 27 mL of 3 mM silver nitrate solution at 750 rpm for 15 minutes [19]. The solution was heated and maintained at the temperature and pH according to Table II using 0.1 M NaOH. The formation of yellow-brown, reddish-brown, or dark brown colour indicated the formation of silver nanoparticles [20, 21].

Determination of Surface Plasmon Resonance

Surface plasmon resonance was determined by observing the wavelength and absorbance of the sample solution in the range of 370 - 600 nm using a UV-Vis Spectrophotometer. The blank used in this procedure is distilled water.

Determination of Optimum Conditions for Silver Nanoparticles

Optimum conditions (temperature and pH) were determined to manufacture silver nanoparticles by analyzing surface plasmon resonance data using the Design Expert 13® program. The program will examine the effect of temperature and pH factors on surface plasmon resonance's wavelength response and absorbance. The program suggests optimum conditions when these conditions have a desirability value close to 1.

Characterization of Silver Nanoparticles at Optimum Condition

Silver nanoparticles under optimum conditions were subjected to further characterization, such as the determination of surface plasmon resonance, particle size, polydispersity index, and zeta potential. Particle size, polydispersity index, and zeta potential were measured using a Particle Size Analyzer. The sample solution is diluted using aqua deionized. Particle size and polydispersity index were measured at a scattering angle of 90°, while measured the zeta potential at a scattering angle of 173° [22].

Stability Test of Silver Nanoparticles at Optimum Condition

A stability test was carried out using the cycling test method. The cycling test was carried out at temperatures 4°C and 40°C with storage at each temperature for 24 hours. The procedure was repeated for six cycles [23, 24]. Organoleptic observations, pH, and surface plasmon resonances were observed in cycles 0 and 6.

Data Analysis

Data analysis for optimization was performed using program Design-Expert 13®.

Results and Discussion

Areca catechu L. Seeds Extract

The *Areca catechu* L. extract obtained in this study had a distinctive odour, brown colour and thick. The total phenolic content obtained was 111.4 ± 0.411 mg CE/g extract. The results were not much different from those of Zhang *et al*. [25], who got a total phenolic content of 111.14 ± 3.41 mg CE/g extract.

Silver Nanoparticles

Areca catechu L. seed extract is a bioreductor in forming silver nanoparticles. The silver nanoparticle preparations obtained in this study were blackish-brown in colour. *Areca catechu* L. seed extract can act as a bioreductor due to the presence of phenolic compounds. Phenolics can actively chelate and reduce metal ions into nanoparticles due to the presence of carbonyl groups or phi (π) electrons. The transformation of the phenolic tautomer from the enol to the keto form by releasing reactive hydrogen atoms can reduce metal ions to form nanoparticles [11]. An overview of the mechanism for creating silver nanoparticles from catechin compounds can be seen in Figure 1.

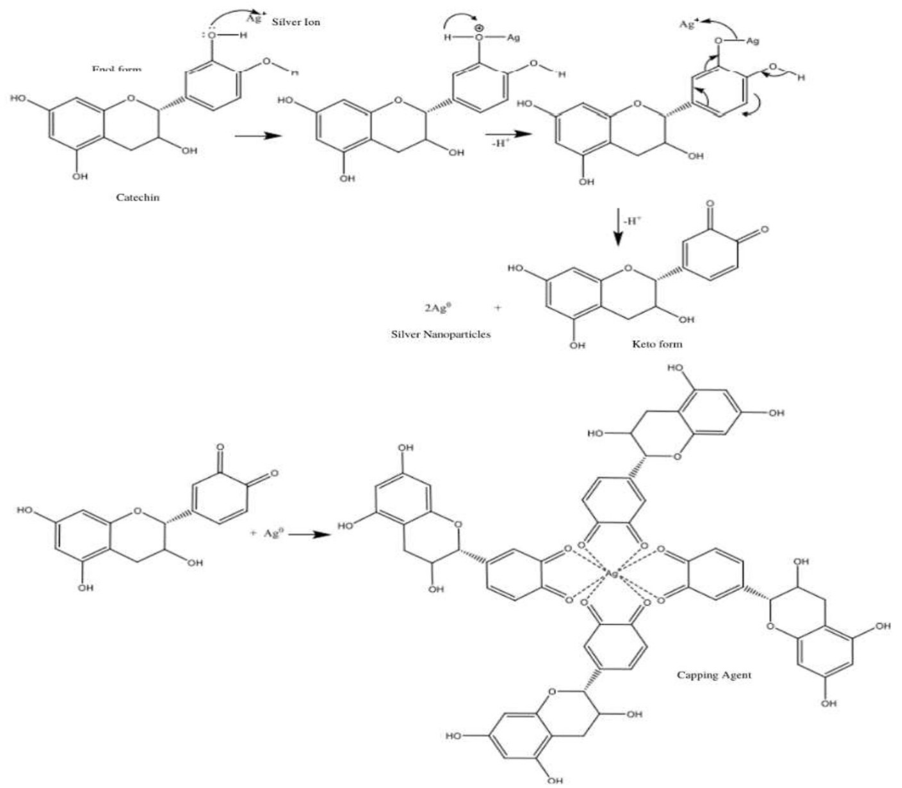

Figure 1.
Silver nanoparticle formation mechanism

The formation of silver nanoparticles can be observed from surface plasmon resonance events. Surface plasmon resonance (SPR) is a free electron resonance oscillation on a metal surface layer that is excited by an incident light source [26]. The oscillation is determined by the absorption of the wavelength obtained [27]. Surface plasmon resonance (SPR) wavelengths are 400 - 450 nm due to the interaction between light and surface electrons moving from silver nanoparticles [28]. In addition, the absorbance produced at surface plasmon resonance wavelengths can describe the colour intensity and the number of silver nanoparticles formed during the synthesis process. The higher the absorbance, the more silver nanoparticles are formed. The surface plasmon resonance results obtained in this study were in the wavelength range of 424 - 431 nm and absorbance of 0.431 - 1.265 (Table III).

Table III

Data maximum wavelength and absorbance of silver nanoparticles

Formula	Temperature (°C)	pH	Max. Wavelength (nm)	Absorbance
1	70	7.5	429	0.446
2	30	9	424	0.769
3	70	10.5	427	1.265
4	50	9	425	0.962
5	50	7.5	431	0.431
6	70	9	430	1.059
7	30	10.5	424	1.004
8	30	7.5	427	0.435
9	50	10.5	423	1.121

The maximum wavelength and absorbance data from the SPR obtained are closely related to each formula's different temperature and temperature conditions. Based on Figure 2, F3 has the highest absorbance of 1.265 while F9 has the lowest wavelength of 423 nm.

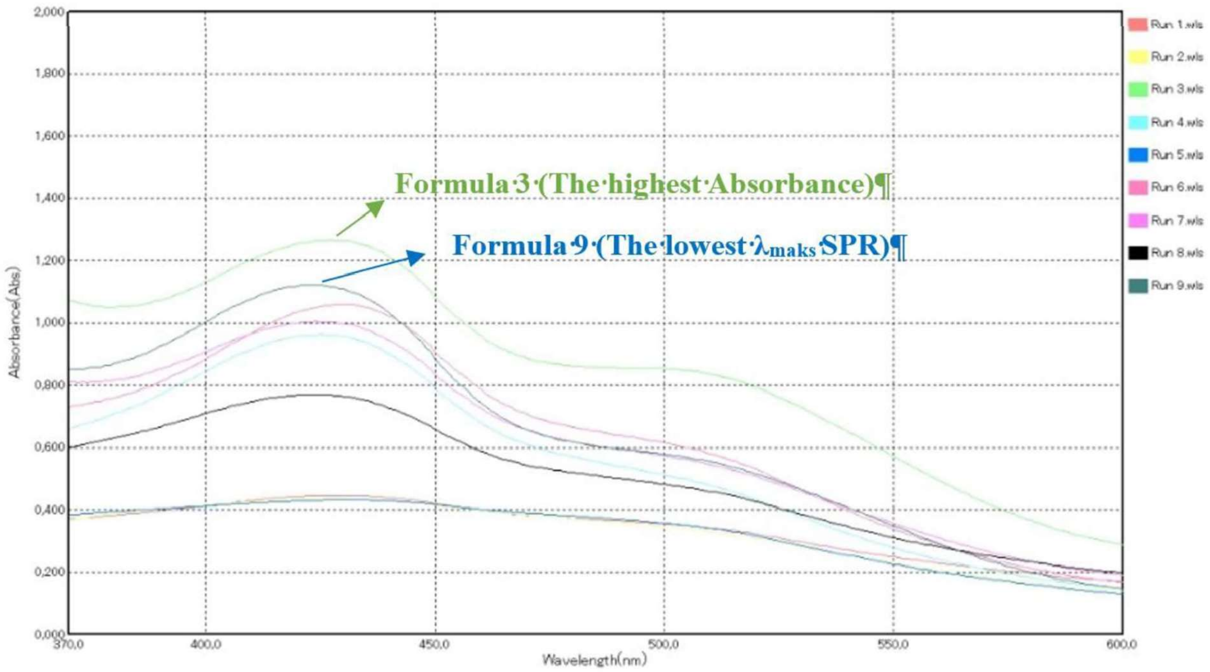

Figure 2.

UV-Vis Spectrum of Silver Nanoparticles

The Role of Temperature and pH to λmax of Silver Nanoparticle

The role of temperature and pH on the maximum wavelength of silver nanoparticles was analyzed using the Design Expert 13® program. The initial step is to analyze the model and then analyze the response. The model analysis obtained at the maximum wavelength response can be seen in Table IV.

Table IV
Model Analysis of λmax Responses

Response	Parameter				
	R^2	Adjusted R^2	Predicted R^2	Adequate precision	p-value
Max. Wavelength	0.9161	0.8658	0.6980	11.4290	0.0192

Model analysis on λmax has an R^2 value of more than 0.7, namely 0.9161, which shows that 91.61% of the data on the λmax response is influenced by temperature and pH factors. In comparison, the remaining 8.39% is an estimation error. This result is supported by the normal curve plot of residuals (Figure 3A) which shows that the data points are close to a straight line so that the data can be said to be normally distributed. Furthermore, the adjusted R^2 and predicted R^2 values obtained in the λmax model analysis have a difference of less than 0.2 namely 0.1678. The difference between adjusted R2 and predicted R2 values illustrates the similarity of the model obtained from the relationship between temperature and pH to the λmax response. This result is supported by the predicted vs. actual curve in Figure 3B. The predicted *vs*. actual curve illustrates the results of the adjusted R^2 and predicted R^2 values with points to the right and left of the line. The closer the distance between the dots to the right and left of the line indicates that the data values from the research are more precise than those predicted by the system, where 86.58% of the existing data already represents the population and can explain a good linear relationship. The adequate value obtained in the model analysis is more than 4, which is 11.4290. The greater the adequate precision value, the more resistant the model will be to noise (disturbance). In addition, the p-value of less than 0.05 in the model indicates that the model used significantly affects the λmax response. Based on the model analysis, it is said that the model is good to be continued in the response analysis process.

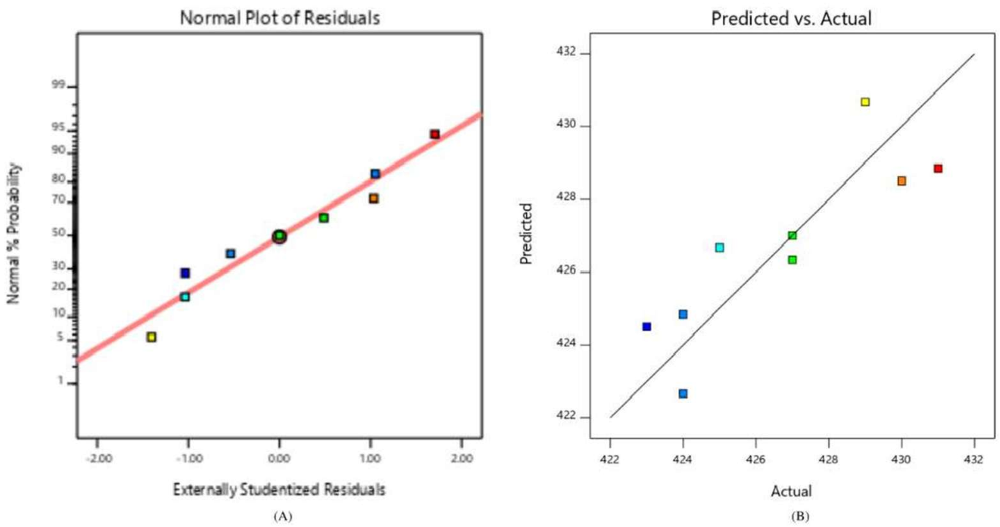

Figure 3.
Normal Plot of residual (A) and predicted *vs*. actual (B) curves of λmax

Analysis of the λmax response was carried out to observe the effect of each factor and the interaction between the two on the response. Response analysis was carried out by following the results of the ANOVA in the form of coefficient values and p-values of the temperature (A) and pH (B) factors. The results of the ANOVA analysis on the pH response can be seen in Table V.

Table V
Response analysis of λmax

Respon	*Intercept* (nm)	A	B
λmaks SPR	426.667	1.833	-2.167
p-values		0.0397*	0.0213*

A: Temperature; B: pH; *factors that have a significant effect (p-value < 0.05)

Based on the results of the ANOVA analysis in Table V, temperature and pH significantly affect the λmax response where the p-value obtained is less than 0.05. Factors that have a significant effect are then entered into the response equation so that the response equation λmax is accepted, namely y = 426.667 + 1.833A - 2.167B. The notation for factors A and B is (+) and (-), respectively. This notation shows that the A-temperature factor has a positive correlation, meaning that the greater the A value, the higher the resulting λmax value. In contrast, the B-pH factor has a negative correlation, meaning that the greater the pH, the smaller the resulting λmax value. This result is supported by the research of Yeshchenko *et al*. [29], which showed that an increase in temperature causes a redshift (a shift in wavelength to a more significant value). This condition is due to the thermal volume expansion of the silver nanoparticles with increasing temperature. Thermal volume expansion is when a substance experiences a change in temperature so that the substance can expand (expand) or shrink (shrink) depending on the increase or decrease in temperature. Research conducted by Alqadi *et al*. [30] also supported the effect of pH in this study. The study showed that increasing the pH resulted in a smaller λmax and was followed by the formation of smaller silver nanoparticle sizes. According to Sathishkumar *et al*. [31], when the pH is acidic, the aggregation of silver nanoparticles to form larger nanoparticles is believed to be preferable to the stages of creating new nanoparticles (nucleation). However, at alkaline pH, it will show a large number of functional groups available for silver binding, thereby facilitating a higher number of Ag^+ ions to bind and subsequently form a large number of smaller diameter nanoparticles.

The Role of Temperature and pH to Absorbance of Silver Nanoparticle

The model analysis obtained at the maximum wavelength response can be seen in Table VI. The model analysis results indicate that the model is robust and significant enough to proceed to the response analysis process.

Table VI

Model analysis of absorbance responses

Response	Parameter				
	R^2	Adjusted R^2	Predicted R^2	Adequate precision	p-value
Absorbance	0.9700	0.9519	0.8822	18.5757	0.0003

Model analysis on the absorbance response has an R^2 value of 0.9700, which indicates that 97% of the data on the absorbance response is influenced by temperature and pH factors. In comparison, the remaining 3% is an estimation error. This result is supported by the normal plot of the residual curve (Figure 4A), where the data points are close to a straight line so that the data can be said to be normally distributed.

Furthermore, the adjusted R^2 and predicted R^2 values obtained in the absorbance model analysis have a difference of less than 0.2 namely 0.0697, so there is a similarity in the model obtained from the relationship between temperature and pH on the absorbance response. This result is supported by the predicted vs. actual curve in Figure 4B, which shows the distance between the points to the right and left of the near line where 95.19% of the existing data already represents the population and can explain a good linear relationship. The adeq precision value obtained in the model analysis is more than 4 namely 18.5757 so the model is robust against noise (disturbance). In addition, the p-value of less than 0.05 in the model indicates that the model used significantly affects the absorbance response.

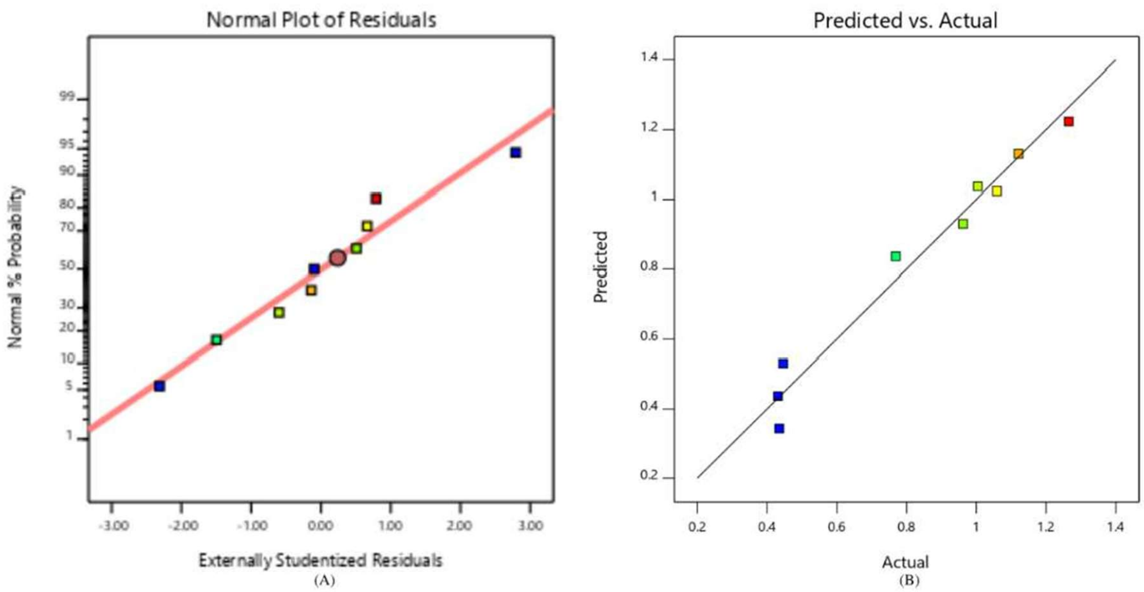

Figure 4.

Normal plot of residual (A) and predicted *vs*. actual (B) curves of absorbance

The absorbance response analysis was continued, and the results obtained can be seen in Table VII. Based on the results of the ANOVA analysis in Table VII, temperature and pH have a significant effect on the absorbance response.

Table VII

Response analysis of absorbance

Response	*Intercept* (nm)	A	B	B2
Absorbance	0.930	0.094	0.346	-0.146
p-values		0.0232*	0.0001*	0.0333*

A: Temperature; B: pH; *factors that have a significant effect (p-value < 0.05)

The absorbance response equation obtained is y = 0.930 + 0.094A + 0.346B - 0.146B2. The notation on an equation for factors A and B is (+), but there is a new factor, namely B2, which has a value of (-). Factor B2, marked (-), means that when the pH used is too high, the absorbance produced will be smaller. When viewed from the p-value, factor B has the smallest p-value, so that factor B has the greatest influence on the absorbance response.

The temperature parameter is directly proportional to the absorbance value. This shows that when there is an increase in temperature, the absorbance value of the silver nanoparticles produced will increase. Based on research by Prodjosantoso *et al.* [15], when the temperature increases, the formation rate of silver nanoparticles will be faster so that many silver nanoparticles are formed. The more silver nanoparticles formed, the higher the absorbance [32].

In addition, the pH parameter is also directly proportional and has the most significant effect on the absorbance value. This shows that when there is an increase in pH, the absorbance value of the silver nanoparticles will be higher. These results are supported by research by Alqadi *et al.* [30], which showed that an increase in pH resulted in a higher absorbance of silver nanoparticles. In addition, Roopan *et al.* [33] research showed that at pH 2 no reaction to form silver nanoparticles occurred, while at pH 11, the silver nanoparticles formed were highly monodispersed. Research by Tagad *et al.* [34] also showed that at acidic pH (pH 4 and 6), there is no surface plasmon resonance absorption which is characteristic of silver nanoparticles. The formation of silver nanoparticles is more suitable to occur in alkaline pH conditions so that many small silver nanoparticles are formed, and the resulting absorbance value will be higher [35]. However, the synthesis of silver nanoparticles at extreme alkaline pH (> 12) can produce low stability and cause aggregation [36].

Optimum Condition for Silver Nanoparticles

Determination of optimum conditions is done by looking at the system's desirability value closest to 1. The desirability value close to 1 indicates that the situation meets the target desired by the researcher. Based on the analysis results, the optimum conditions for making silver nanoparticles are recommended for the system to be at a temperature of 30°C and pH of 10.5 with a desirability value of 0.932.

Characterization of Silver Nanoparticles at Optimum Condition

Silver nanoparticles at optimum conditions were tested for characterization, such as surface plasmon resonance, particle size, polydispersity index, and zeta potential. The results of the characterization can be seen in Table VIII.

Table VIII

Characterization results

Parameter	Mean ± SD
λmax (nm)	423 ± 2.156
Absorbance	1.148 ± 0.076
Particle size (nm)	161.7 ± 46.1
PDI	0.286 ± 0.035
Zeta Potential (mV)	-16.1 ± 3.7

Silver nanoparticles in the optimum condition have an excellent λmax value and absorbance for silver nanoparticles. The size of the silver nanoparticles obtained was 161.7 ± 46.1 nm. The particle size results obtained were smaller than previous studies conducted by Choi *et al.* [37] at 233.4 - 238 nm and Bhat *et al.* [38] at 553 - 610 nm. The polydispersity index value was 0.286 ± 0.035, indicating that the particle size obtained was uniform. The zeta potential value obtained from the optimum formula for silver nanoparticles is - 16.1 ± 3.7 mV. These results indicate that the zeta potential of the silver nanoparticle optimum formula is still relatively stable. The negative zeta potential value is due to the alkaline pH (pH 10.5) during the biosynthesis of silver nanoparticles. With an increase in pH (alkaline pH), the organic functional groups on the surface of silver nanoparticles reach a higher level of deprotonation (release of H^+ protons) so that the amount of negative surface charge increases simultaneously [39]. The characterization results obtained have met the requirements for nanoparticle preparations.

Stability of Silver Nanoparticles at Optimum Condition

The silver nanoparticles in optimum condition were tested for stability by cycling for six cycles. The stability results of silver nanoparticles can be seen in Table IX.

Table IX

The stability results

Cycle	Sedimentation	pH	λmax (nm)	Absorbance
1	None	10.50 ± 0.008	423	1.148
6	Slight	10.10 ± 0.008	425	1.396

Organoleptic results showed the formation of a slightly precipitate in the 6th cycle. The results obtained are better than the previous reference research conducted by Apriani *et al*. [19], which showed many deposits formed on colloidal silver nanoparticles in the 6th cycle. The difference in stability results obtained can be due to differences in the conditions for synthesizing silver nanoparticles. This study used a temperature of 30°C and pH 10.5 as the optimum conditions, while the study of Apriani *et al*. [19] used a temperature of 60°C and pH 9. Research by Pinto *et al*. [40] showed that high temperatures can produce unstable silver nanoparticles and can cause damage or decomposition of plant secondary metabolites, which function as stabilizing agents (capping agents).

Changes in the pH value in this study were also said to be relatively stable, where the pH changed from 10.50 ± 0.008 to 10.10 ± 0.008. In contrast to previous research from the same researcher, Apriani *et al*. [19], with the conditions of nanoparticle synthesis at a temperature of 60°C and pH 9, the decrease in pH was significant, namely pH 9.00 ± 0.10 to 7.97 ± 0.03. This stable pH also affects changes in λmax values and absorbance. The shift in λmax in the 6th cycle was not too far and was still in the range of silver nanoparticles. In contrast, the absorbance in the 6th cycle increased, indicating that more and more silver nanoparticles were formed, as shown in Figure 5. This is because phenolic stability depends on pH. Phenolic compounds are in the enol form at acidic pH and are in the keto form at an alkaline pH. The keto form of phenolic is helpful as a stabilizing agent (capping agent). In this study, the reaction to form silver nanoparticles continued because the environmental pH was relatively stable at around 10.

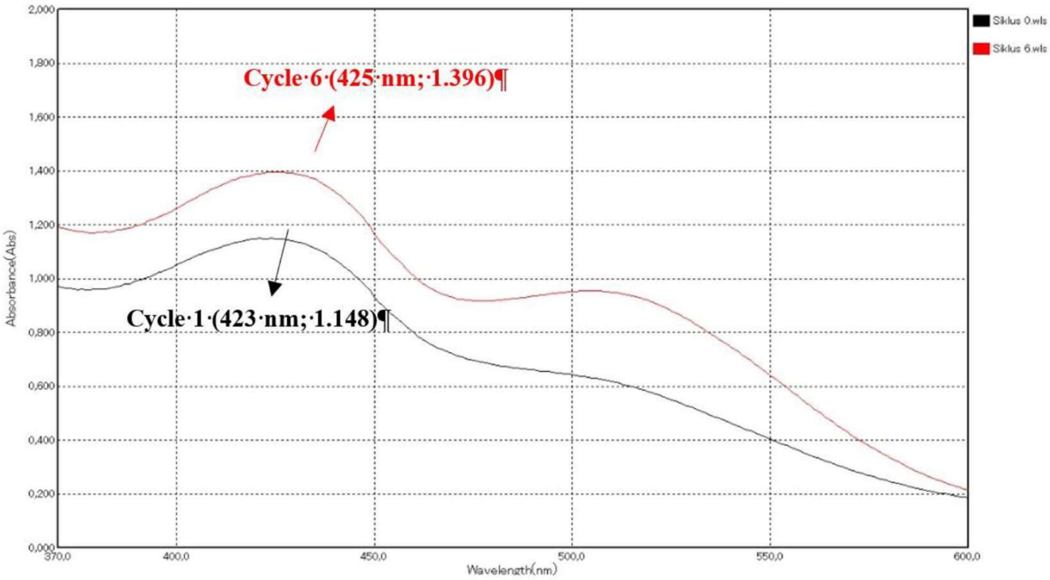

Figure 5.
Comparison of the UV-Vis spectra of the 1st and 6th cycles

Conclusions

Temperature and pH conditions in manufacturing silver nanoparticles have been shown to affect the surface plasmon resonance characteristics. The temperature has a positive effect on λmax and the absorbance of SPR, while pH harms λmax and has a positive impact on the absorbance of SPR. The optimum temperature and pH conditions for the synthesis of silver nano-particles using ethanol extract of young areca nut seeds were found at 30°C and pH 10.5 with a surface plasmon resonance silver nanoparticle λmax value of 423 nm and an absorbance of 1.148. Particle size, polydispersity index (PDI), and zeta potential of silver nanoparticles at optimum conditions were 161.7 ± 46.1 nm, 0.286 ± 0.035, and -16.1 ± 3.7 mV, respectively. The stability of the silver nanoparticles at the optimum conditions produced was relatively stable, characterized by not too many organoleptic changes, pH, which was still stable in the range of 10 small wavelength shifts, and increased absorbance values.

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Conflict of interest

The authors declare no conflict of interest.

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