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Optimization of Silver Nanoparticle Biosynthesis with Papaya *(Carica Papaya L)* Seed Ethanol Extract and its Evaluation on Antibacterial Effects

Alifa Noora Rahma [1*], ***Suratno*** [2]

[1]Department of Pharmacy, the Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Central Java, Indonesia
[2]Department of Pharmacognosy, Semmelweis University, Budapest, Hungary

*Corresponding author: alifanoora2@gmail.com

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Abstract

The utilization of papaya plants (Carica papaya L) heretofore is focusing on their aril/mesocarp, leaves, and flowers, while the seeds are still neglected. Thus, this recent study attempts to make use of papaya seeds as a green-synthesis bioreductor of silver nanoparticles. It aims at finding out the characteristics of silver nanoparticles of ethanol extract of papaya seeds (AgNPs-EEPS), investigating the optimization as well as the characteristics of the AgNPs-EEPS anti-acne cream formula, and examining the antibacterial activity of Propionibacterium acnes on the AgNPs-EEPS anti-acne cream. The software design expert with factorial design method was applied to optimize the AgNPs-EEPS biosynthesis and cream formula. The analysis of the formation of AgNPs-EEPS characteristics, employing various levels of temperature and extract concentration, and applying UV- VIS spectrophotometry, resulted in a wavelength of 415 nm. Meanwhile, the implementation of particle size distribution testing, using PSD on the optimum concentration by 6% with temperature 65°C, generated a wavelength of 54 nm, pI 0.820, and zeta potential -7,7 mV. The optimization of the AgNPs-EEPS cream formula obtained optimum results at a stearic acid level 7 and cetyl alcohol 6. The characteristics of the cream produced a cream that was physically stable and met the requirements of good topical preparation. The anti-acne cream from silver nanoparticles ethanol extract of papaya seeds has the antibacterial activity of Propionibacterium acnes which belongs to the moderate category.

Keywords: *Optimization; silver nanoparticles; Carica papaya L.; Propionibacterium acnes*

Introduction

Acne vulgaris is a disease of the pilosebaceous unit characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (pustules, papules, and blemishes) (Aparajita *et al.*, 2014). Acne pathology is generated by the overproduction of sebum, changes in skin keratinization, *propionibacterium acnes,* and inflammatory processes. In pediatric patients, the development of acne can occur due to the endocrine changes caused by the onset of puberty, the increased androgenic hormones, and the proliferated sebum production (Chim, 2016). The response to some of these factors is different for each individual.

Papaya (*Carica papaya L.*) is one of the plants that can be utilized as an alternative solution for the issue. Based on several previous studies, the papaya plant can be utilized as an antibacterial, one of which is acne-causing bacteria. Rinita (2017) and Humaira (2018) revealed that papaya seed extract inhibited the activity of *propionibacterium acnes* bacterial cells. However, the utilization of papaya is still limited to its aril, leaves, and flowers, while the seeds are not used. People often perceive papaya seeds as waste generated by fruit traders and households, thus most of them are still not utilized properly.

Many studies found that the content of terpenoids, karpain, and flavonoids in papaya seeds possess antibacterial activity that can kill bacteria by damaging the integrity of the bacterial cell membrane (Mulyono, 2013). Further, Kale *et al.* (2018) stated that the development of green-synthesis-based nanoparticle technology showed that AgNPs was synthesized using papaya seeds as a reducing agent. Papaya seeds have a double function in the formation and stabilization process of AgNPs due to the natural presence of polyols, such as terpenoids and flavones within the reaction. The size of the synthesized nanoparticles is in the range of 12 nm to 70 nm

The implementation of this research deals with the investigation of parameters affecting AgNPs biosynthesis, i.e., the temperature level and the amount of ethanol extract of papaya seeds. In optimizing the AgNP biosynthesis of papaya seed ethanol extract, this study applied a *software design expert* with a factorial design method.

Methods

Tools and Materials

The list of tools used in this research were stir bar, measuring cup (Pyrex), beaker (pyrex), hotplate (Thermo Scientific), cuvette, magnetic stirrer, Particle Size Analyzer SZ-100 (Horiba), UV-Vis Spectrophotometry (Shimadzu-UV 1280), dropper pipette, spatula, measuring flask, analytical balance, centrifuge tube, centrifuge, pH meter Petri dish, ose, object glass, and stamper mortar. Meanwhile, the materials applied in the study were $AgNO_3$ (Sigma Aldrich), aqua destillata, 96% ethanol, California variety papaya seeds, acetone, aquades, NaOH, and *Propionibacterium acnes.*

Work Procedures

Material Collection

The test material was California variety *papaya* (*Carica papaya L.*) seeds obtained from a local market in Semarang. Papaya seeds were taken from ripe papaya fruit. Wet papaya seeds were subjected to wet sorting, washing, and drying by wind or under sunlight indirectly. Then the dried seeds were pulverized to reduce the particle size of the simplicia. Papaya seeds were washed with distilled water and dried at 50°C in an oven for 24 hours until they became brittle and crushed into powder (Kale *et al.*, 2018).

Simplicia Preparation

Dry powder of *papaya* fruit (*Carica papaya L.*) seeds as much as 300 g was added with 96% ethanol in 1500 ml, and then the extraction process was carried out by the maceration method (1 hour stirring, 24 hours standing) every day for 3 days. The result was filtered, and the filtrate was obtained. The filtrate was concentrated with a rotary evaporator (70°C).

Biosynthesis of Silver Nanoparticles with Ethanol Extract from Papaya Seeds

The experiment was conducted by using a magnetic stirrer in the laboratory of Universitas Muhammadiyah Surakarta. A silver nitrate solution with a concentration of 1 mM was prepared using aqua destillata water in a 100-ml volumetric flask. The amount of papaya seed ethanol extract was then added to the silver nitrate solution. The solution was heated and stirred continuously at 70 rpm for 30 minutes. The solution turned brown which indicates the formation of AgNPs. The solution was centrifuged for 15 minutes at 12,000 rpm, and the residue was further washed with distilled water with 3 times repetitions and *freeze-dried* for further testing (Kale *et al.*, 2018).

Preparation of a 1 mM $AgNO_3$ Concentration

A total of 42.25 mg of $AgNO_3$ (p.a.) powder was put into a beaker and dissolved with 250 ml of aqua distillate. After stirring the $AgNO_3$ solution until homogeneous, a concentration of $AgNO_3$ 1 mM was obtained.

Optimization of AgNPs - EEPS

In this study, different parameters were optimized to synthesize AgNPs, including the amount of papaya seed ethanol extract and temperature (°C). The observed object was the absorbance response of the UV-Vis spectrum.

Factorial design with two factors and two levels, namely the concentration of papaya seed ethanol extract is 6%, 12%, and the temperature is 50°C, 65°C. The biosynthesis optimization design of AgNPs - EEPS can be seen in Table 1.

Table 1. Optimization Design of AgNPs - EEPS

Level	Experimental factors	
	EEPS (%) / $AgNO_3$ (1:9)	temperature (°C)
Low level	6	50
High level	12	65

Characterization of AgNPs Biosynthesis

The UV-VIS spectrophotometer aims to test the characteristics in determining the maximum wavelength that identifies the formation of silver nanoparticles, ranging from 400 up to 500 nm.

Particle Size Analyzer (PSA)

Characterization using the PSA aims to determine the size and distribution of particles that have been formed.

Zeta Potential

Zeta potential is used to characterize the surface charge properties of nanoparticles and it relates with their electrostatic interactions. Electrostatic interactions will determine the aggregation and repulsion tendencies of the particles. Zeta potential is a measure of the surface charge of particles dispersed in a dispersing medium. Ideally, it should be higher than the dispersing medium to prevent aggregation.

FT-IR Spectrum Analysis

FTIR analysis aims to characterize the prospective biomolecules responsible for the formation of AgNPs by reducing silver ions to Ag° and its stabilization as a capping agent (Jassim *et al.*, 2019).

SEM Analysis

Scanning Electron Microscopy (SEM) analysis was employed for morphological observation of the surface and shape of silver nanoparticles. The determination of size and shape has a greater impact on their optical behavior and surface plasmon effects. Nanoparticles can be triangular, spherical, or hexagonal shapes (Halima & Archna, 2016).

Antibacterial Activity Test

Tested bacteria that have been cultured and made in suspension form with turbidity based on the *McFarland* standards. Prepare nutrient agar that solidified, then planted with a suspension of test bacteria which was divided into six wells. Three wells contained silver nanoparticles of papaya seed ethanol extract, a negative control (Aqua bidest), and a positive control (medi-klin® gel (clindamycin)). The media was incubated for 24 hours at 35-37°C. After 24 hours, the diameter of the inhibition zone (clear zone) produced around the wells was measured.

Data Analysis

The data of optimization of papaya seed ethanol extract biosynthesis was analyzed using Design Expert *13* trial software with the factorial design method. The inhibition zone data obtained were compared with the literature on antibacterial activity.

Results and Discussion

The synthesis of AgNPs is influenced by several parameters, including reaction temperature, metal ion concentration, and extract content. This greatly affects the size, shape, and morphology of AgNPs (Srikar *et al.*, 2016). Elevated temperatures can increase the reaction rate and synthesis efficiency.

The result of ethanol extraction of papaya seeds using 95% ethanol solvent is a thick yellow-brown extract with a distinctive smell. Figure 1 shows the powder and filtrate forms of papaya seed ethanol extract.

Figure 1. Dried papaya seed powder and filtration result of papaya seed ethanol extract

Optimization of Biosynthesis AgNPs - EEPS

Biosynthesis employing the mother of papaya seed ethanol extract was added $AgNO_3$ reagent 1 mM in a ratio of 1:9. Determination of mixing ratio based on research of Abd Karim *et al.*, (2021). The solution was added with a 0.05 M NaOH catalyst until Ph 8 when the color of the solution changed to a clear pseudo-yellow, heated at 65°C.

The formation of silver nanoparticles in this study is evidenced by the process of color change from a colorless solution to brownish yellow when the solution is heated. The color change process was measured at a wavelength of 350-650 nm using a spectrophotometer UV-Vis Shimadzu-UV 1280, with time variations of 15 minutes, 30 minutes, 45 minutes, 60 minutes, 120 minutes, and 180 minutes. UV-Vis spectrophotometer readings with temperature variations showed 415 nm with different absorbance values.

According to Solomon *et al.* (2007), the wavelength of maximum plasmon absorption can be used to indicate particle size. As the particle size increases, the plasmon peak shifts to longer wavelengths and widens.

The optimization of AgNPs - EEPS was inputted into the *Design Expert* 13 *trial software*. The display of 2^2 factorial run design can be seen in Table 2.

Table 2. The 2^2 factorial run design using *software design expert* 13 *trial* on AgNPs - EEPS

Run	Factor 1 Extract concentration	Factor 2 Temperature	Response Absorbance
1	6	65	2.16
2	12	50	1.907
3	6	50	1.58
4	12	50	1.785
5	12	65	2.02
6	6	65	2.156
7	12	65	2.03
8	6	65	2.169
9	6	50	1.657
10	6	50	1.569
11	12	65	2.02

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The effect of each factor in the form of temperature and extract concentration, as well as the interaction of the two factors, were observed to see which factor had a more dominant effect on the response. Factors that can significantly affect the response will have a *p-value* <0,05 (Ernes *et al.*, 2014).

The results of data processing for absorbance is shown at the table 3.

Table 3. Effect analysis data and *Anova* test of absorbance response

Factors	Effect	p-value	p-value Model
Extract concentration	0.0528333	0.0776	<0.0001
Temperature	0.3685	<0.0001	(significant)

In the ANOVA test, the tested model has a significant criterion with a p-value of 0.0001, which is <0.05 (95% confidence level). These results indicate that the test model can be used to predict the absorbance response. The positive value of the ANOVA data shows that the factor can increase the absorbance produced. The interaction between the two factors is shown in Figure 2.

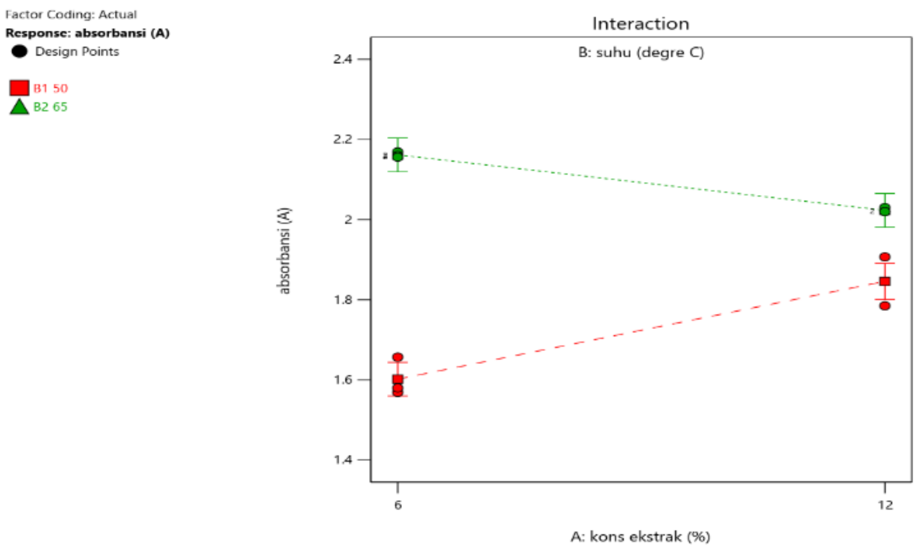

Figure 2: Diagram of the Interaction of Extract Concentration with Temperature

Table 4. ANOVA data

Std. Dev.	0.0417	**R^2**	0.9771
Mean	1.91	**Adjusted R^2**	0.9673
C.V. %	2.18	**Predicted R^2**	0.9241
		Adeq Precision	22.2356

Analysis of the interaction diagram of extract concentration with temperature shows that they do not intersect each other, and are proportional to the increase in temperature and extract concentration. It means that the absorbance will also increase.

In the ANOVA analysis, the R^2 value in this research model is 0.9771, which shows that the extract concentration and temperature have a large influence of 97.71%. ANOVA data can be seen in Table 4.

The results of the factorial design optimization data showed that the extract concentration of 6% with an optimum temperature of 65°C gave a high absorbance. The optimization data can be seen in Table 5.

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Table 5. Factorial design optimization data

Number	extract	temperature	absorbance	Desirability	
1	**6**	**65**	**2.162**	**0.988**	**Selected**
2	12	65	2.023	0.757	
3	12	50	1.846	0.462	
4	6	50	1.602	0.055	

Based on the optimization data, the result of silver nanoparticles in 6% papaya seed ethanol extract with 65°C heating was employed for the nanosilver characteristic test, further formulation, and antibacterial activity test.

PSA *(*Particle Size Analyzer*)* testing showed a silver nanoparticle size of 54 nm with a PI value of 0.820, as shown in Table 6. The particle size meets the range of 1-100 nm. The polydispersion index value of 1 means that it has a very wide particle size distribution and contains large particles or aggregates that can undergo sedimentation (Abdassah, 2017). PSA test results show a PI value below 1, which indicates that the particle size distribution is good.

Table 6. PSA (*Particle Size Analyzer*) Testing

Sample	Size (nm)	PI
AgNPs of papaya seed ethanol extract at 65°C	54	0.820

The zeta potential value of AgNPs sample of papaya seed ethanol extract at 65°C of -7.7 mV can be seen in Table 7. Nanoparticles that have zeta potential values smaller than -30 mV and greater than +30 mV are having higher stability. Therefore, it can be said that the nanoparticles produced have good stability.

Table 7. Zeta Potential Testing

Sampel	Zeta Potensial (mV)
AgNPs of papaya seed ethanol extract at 65°C	-7,7

FTIR Test

A silver nanoparticle solution of papaya seed ethanol extract was analyzed by FTIR spectroscopy. In Figure 3A of the FTIR analysis, there are two peaks, 3326 cm^{-1} and 1636 cm^{-1} are carboxyl and amine groups. These peaks indicate the presence of secondary amines, a sign of protein. It confirmed the biofabrication of silver nanoparticles by the action of proteins or phytochemicals. This suggests that proteins are involved in the capping and stabilization of the synthesized silver nanoparticles.

Figure 3B, A broad peak at wavenumber 3338 cm^{-1} with a fair intensity indicates the presence of stretching and bending vibrations of carboxylic acids and stretching of free -OH alcohols. At 3100-3500 cm^{-1}, the frequency with fair intensity is characteristic of amide groups. There are several weak peaks at 2974 cm^{-1} to 2889 cm^{-1}, indicating the stretching of the -CH group of the methylene group. The sharp peak at the wavenumber 1647cm^{-1} indicates primary amide vibrations. Several bands around the frequency of 1452 cm^{-1} indicate the C-H bending vibrations of the cyclohexane ring of the methylene group. The frequency bands 1326 cm^{-1}-1381 cm^{-1} indicate the stretching of the amine group. Peaks at 1085 cm^{-1} and 1044 cm^{-1} indicate the presence of ether (C-O) groups. The peak at 878 cm^{-1} indicates the presence of the CH_2 group.

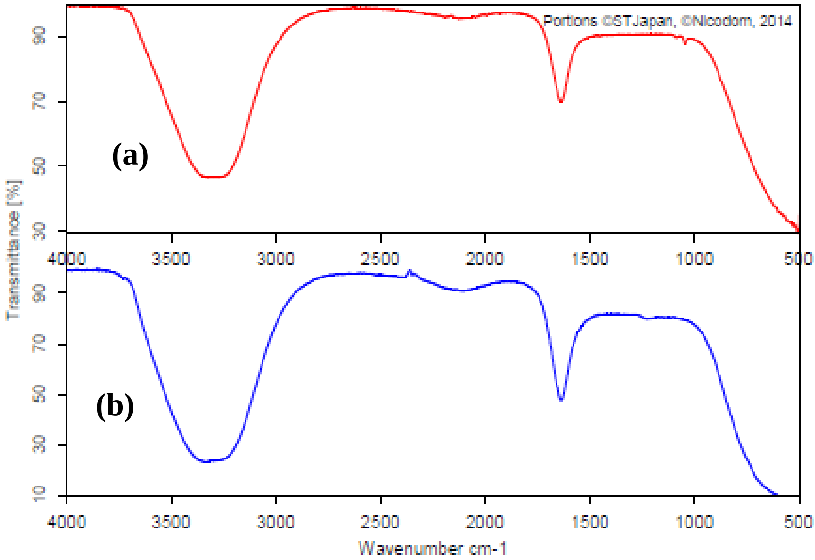

Figure 3. FTIR Spectrum of (a) Silver Nanoparticle Solution of Ethanol Extract of Papaya Seeds, and (b) Papaya Seed Ethanol Extract Solution

SEM Test

Silver nanoparticles with papaya seed ethanol extract were subjected to SEM testing. A SEM view of silver nanoparticles of papaya seed ethanol extract is shown in Figure 4.

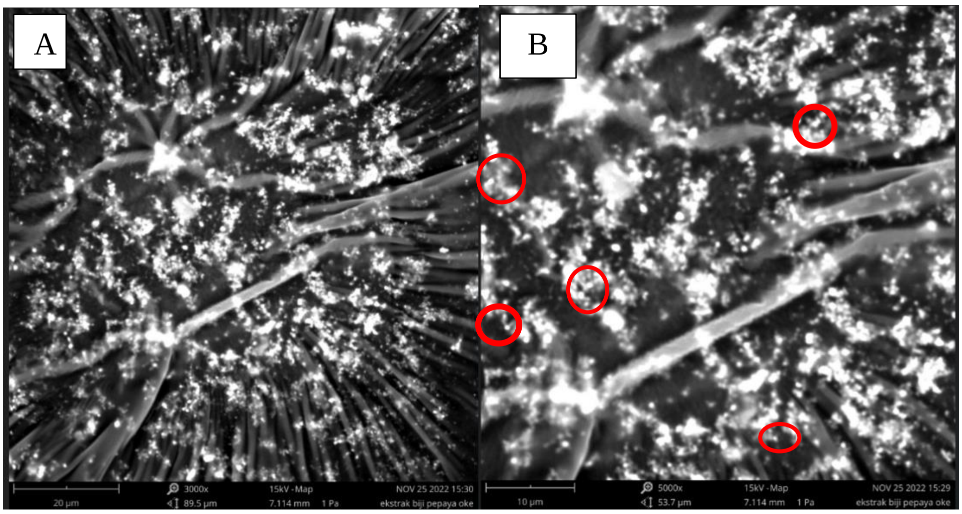

Figure 4. (A) 3000x Magnification particle (B) 5000x Magnification Particles

The results of SEM analysis show that the morphology of silver nanoparticles has a smooth surface structure with non-uniform particles and a round, asymmetric shape. Previous research with papaya samples gave the same results of spherical particle shape with different particle sizes, namely Banala *et al.* (2015) particle sizes of 5-200 nm, Mohammed *et al.* (2019) 40-150 nm, Kale *et al.* (2018) 12-69 nm, and Balavijayalakshmi *et al.* (2017) 5-20 nm. The difference in particle size obtained is due to differences in the biosynthesis process.

Antibacterial Activity Test against Propionibacterium Acnes

Testing of antibacterial activity against *Propionibacterium acnes* employing the well diffusion method. The working principle of this method is the diffusion of antibacterial compounds into the solid medium where the tested microbes have been inoculated. The wells diffusion method is done by making holes perpendicular to the solid agar that has been inoculated with tested bacteria (Balouiri *et al.*, 2016).

The antibacterial test treatment was carried out in two replicates with three wells in each replicate. The negative control used aqua destillata. The positive control used Medi-klin gel (clindamycin 1%).

Table 8. Antibacterial Test Results

Test	diameter replication 1	diameter replication 2	Description
Formulation 1	9 mm	10 mm	concentration 30mg/ml
Formulation 2	8 mm	7 mm	concentration 30mg/ml
Mediclin cr 1%	15 mm	17 mm	positive control
AgNPsEEBP	12 mm	11 mm	Positive control optimum formula (AgNPsEEBP 6%, temperature 65°C, concentration 2 mg/10 ml
F1 Cream Base	0	0	negative control
F2 Cream Base	0	0	concentration 30mg/ml

A silver nanoparticle solution of papaya seed ethanol extract has antibacterial activity against *Propionibacterium acnes,* as indicated by the inhibition zone. The silver nanoparticles from papaya seed ethanol extract belong to the strong category.

Table 9. Categories of Antibacterial Activity According to Davis and Stout (Khairani *et al.*, 2017)

Antibacterial Activity	Inhibition Zone Diameter (mm)
Weak	< 5
Medium	5 – 10
Strong	10 – 20
Very strong	>20

Conclusion

Characteristics of silver nanoparticles biosinthesys with ethanol extract of papaya seed (AgNPs-EEPS) were formed with variations in temperature and extract concentration by employing UV-Vis spectrophotometry to obtain a wavelength of 415 nm, particle size tests employing PSA at 6% extract concentration with a temperature of 65°C to obtain a size of 54 nm, and a PI value of 0.820. A nanoparticle cream of papaya seed ethanol extract has *Propionibacterium acnes* antibacterial activity.

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