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The Effect of Extraction Time of Raja Nangka Banana Peel as Capping Agent on the Characteristic and Antibacterial Activity of ZnO Nanoparticles against *Staphylococcus epidermidis*

Adinnia Apriandini*, Fauziatul Fajaroh, Adilah Aliyatulmuna, Endang Ciptawati

Departement of Chemistry, Faculty of Mathematics and Natural Science,
Universitas Negeri Malang, Jalan Semarang, Kota Malang, Indonesia

**Corresponding Author: adinniaa@gmail.com*

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Abstract: *A green chemistry-based ZnO nanoparticle synthesis method based on plant extracts has been developed, and raja nangka banana peel is one of them. The extraction time is one element that influences the amounts of secondary metabolites. The longer the extraction time, the more secondary metabolites are obtained. As the optimal time approaches, the secondary metabolite compounds will decrease. This research aimed to determine the optimal time to extract secondary metabolites from the raja nangka banana peel and to know the effect of extraction time on the characteristics of ZnO nanoparticles, which include morphology, size, and antibacterial activity against Staphylococcus epidermidis. The steps of this research: maceration, phytochemical tests and total levels tests, synthesis of ZnO nanoparticles, characterization, and antibacterial activity test against Staphylococcus epidermidis. Maceration for 24 hours is the best time for extracting secondary metabolites from raja nangka banana peels. The SEM test results reveals that the morphology of the three samples had agglomeration. The ZnO nanoparticles with 24-hour raja nangka banana peel extract had a smaller size of 295.2 nm and were spherical. The inhibition zone diameter from ZnO nanoparticles with 24-hour raja nangka banana peel extract has a larger area of 5.65 mm.*

Keywords: *capping agent, maceration time, raja nangka banana peel, Staphylococcus epidermidis, ZnO nanoparticles*

INTRODUCTION

Nanoparticles are currently being developed as antibacterial agents because they can overcome bacterial resistance and inhibit biofilm growth. ZnO nanoparticles are one of them. The advantages of ZnO nanoparticles are that they are non-toxic, easy to use, and biocompatible (Vishnupriya et al., 2020). ZnO nanoparticles can be synthesized using precipitation, sol-gel, thermal decomposition, hydrothermal synthesis, and electrochemical methods (Nurlina & Syahbanu, 2020; Vanathi et al., 2014). However, this method applies a capping agent that is harmful to the environment. Thus, the alternative method uses a green chemistry-based method to produce environmentally friendly ZnO nanoparticles.

Green chemistry-based synthesis utilizes ingredients from plants as capping agents, one of which uses raja nangka banana peels. Raja nangka banana is commonly used as banana chips (Mukhoyyaroh & Hakim, 2020). Therefore, there is plenty of banana peel accumulation. A study by Nursanti et al. (2018) dicsloses that the raja nangka banana peel contains flavonoid compounds, alkaloids, saponins, and polyphenols that can be applied as capping agents.

Raja nangka peel provides secondary metabolite compounds that can be extracted. High levels of secondary metabolites can be obtained if the extraction is carried out for a long time (Febrina et al., 2015). Nonetheless, if the extraction time exceeds the optimal time, it can cause secondary metabolites to decrease and damage (Chintya & Utami, 2017; Widodo et al., 2021). Using secondary metabolites compounds from banana peel extract as a capping agent can reduce the toxicity of the synthesized ZnO nanoparticles, making it safer to use as an antibacterial compound to inhibit the growth of *Staphylococcus epidermidis*.

Thus, the researcher conducted this study to determine the optimum extraction time of secondary metabolites compounds from the raja nangka banana peel and to discover the effect of extraction time on the characteristics of ZnO nanoparticles, which include morphology, size, as well as antibacterial activity against *S. epidermidis*.

RESEARCH METHODS

Materials and Tools

The tools used in this research included laboratory glassware, analytical balance, knife, hotplate stirrer, universal indicator, oven, watch glass, Whatman No. 42 paper, disc paper, aluminum foil, plastic wrap, and blender. Meanwhile, the instrumentations used are XRD (PanAlytical Type Expert Pro), SEM (FEI Type INSPECT-S50), and PSA (Horiba-SZ 100z).

The materials used in this study included raja nangka banana peel, technical ethanol (96%), demineralized water, Dragendorff reagent (Merck), Mayer reagent (Merck), magnesium powder (Merck), technical H_2SO_4 (95%), $FeCl_3$ powder (Merck), quercetin p.a, technical methanol (70%), $AlCl_3$ 10%, CH_3COONa p.a, gallic acid p.a, Folin-ciocalteu reagent (Merck), Na_2CO_3 p.a, $ZnSO_4.7H_2O$ (Merck), NaOH (Merck), *S. epidermidis* bacteria, amoxicillin, and DMSO.

Methods

Banana Peel Extraction

To prepare the banana peel extraction, the researcher used the following procedures. It began with washing the banana peel waste and drying it in the sun for ± 8 hours. After that, the dried banana peel was mashed. In addition, 15 grams of banana peel powder was extracted by maceration with an ethanol-water ratio of 2:1 for 12, 24, and 72 hours. The extract of banana peel was separated using a Buchner funnel (Adhayanti et al., 2018; Pardede et al., 2019; Yulianti et al., 2021).

Phytochemical Test of Banana Peel Extract

Alkaloid Test

An alkaloid test was conducted by preparing two tubes containing 1 mL of banana peel extract, 3 drops of Dragendorff's reagent were added to the first tube, and 3 drops of Mayer's reagent were added to the second tube. A positive test with Dragendorff's reagent was indicated by the appearance of an orange precipitate, while the presence of a white precipitate for Mayer's reagent (Lumowa & Bardin, 2018; Priyardarshini et al., 2021).

Flavonoids Test

The preparation of the flavonoids test began with 1 mL of banana peel extract mixed with 0.5 grams of magnesium powder and 1 mL of H_2SO_4 2 M solution. Then, allow it to stand for 1 minute. Next, 5 drops of concentrated H_2SO_4 solution were added to the sample. A change in the color of the solution to orange-red indicated a positive result for this test -(Lumowa & Bardin, 2018).

Polyphenol Test

Polyphenol test was prepared with 1 mL of banana peel extract added with 3 drops of $FeCl_3$ 10%. A positive result of this test was indicated by the color change of the solution to blackish green (Priyardarshini et al., 2021).

Saponins Test

The saponin test was assisted by combining 10 mL of boiling water and 1 mL of raja nangka banana peel extract and mixing the combination shaken violently. The production of steady bubbles for 10 minutes indicated a successful test outcome. (Lumowa & Bardin, 2018).

Determination of Total Flavonoid Level

Preparation of Quercetin Standard Solution

To determine the total flavonoid level, the researcher prepared the quercetin standard solution by dissolving 100 mg of quercetin in 100 mL of distilled water. Later, the standard quercetin solution was diluted to obtain solutions with 1, 2, 3, 4, and 5 ppm concentrations. In the quercetin standard solution, 3 mL of methanol, 0.2 mL of $AlCl_3$ 10%, and 0.2 mL of CH_3COONa 1 M were added. Then, distilled water was added to the mark in a 10 mL volumetric flask and incubated for 30 minutes. A spectrophotometer measured the absorbance value of each standard quercetin solution at a wavelength of 432 nm (Ratulangi et al., 2016).

Determination of Total Flavonoid Levels of Raja Nangka Banana Peel Extract

The determination of total flavonoid levels of banana peel extract was then continued by adding 3 mL of methanol, 0.2 mL of $AlCl_3$ 10%, and 0.2 mL of CH_3COONa 1 M to 1 mL of each raja nangka banana peel extract. After that, distilled water was added to the mark of the 10 mL volumetric flask and incubated for 30 minutes. The absorbance value of each raja nangka banana peel extract was measured using a spectrophotometer at a wavelength of 432 nm (Ratulangi et al., 2016).

Determination of Total Polyphenol Level

Preparation of Gallic Acid Standard Solution

The researcher dissolved 100 mg of gallic acid in 100 mL of distilled water to determine the total polyphenol level. After that, the gallic acid solution was diluted to obtain solutions with concentrations of 2, 4, 6, 8, and 10 ppm. Then, 2.5 mL of Folin-Ciocalteu reagent was mixed into 0.25 mL gallic acid solution. The mixture was shaken and left for 4–8 minutes. Then, 2 mL of 7.5% Na_2CO_3 solution and distilled water were added to a 10 mL volumetric flask. The solution was incubated for 2 hours, and its absorbance was measured at a wavelength of 765 nm. (Mukhriani et al., 2019).

Determination of Total Polyphenol Levels of Raja Nangka Banana Peel Extract

The determining total polyphenol levels of banana peel extract continued by mixing 2.5 mL of Folin-Ciocalteu reagent into 0.25 mL of raja nangka banana peel extract. Then, the mixture was homogenized and left for 4–8 minutes. After that, 2 mL of Na_2CO_3 7.5% solution and distilled water were added to the 10 mL volumetric flask. The solution was incubated for 2 hours, and the absorbance was measured later at a wavelength of 765 nm (Mukhriani et al., 2019).

ZnO Nanoparticle Synthesis

In this research, ZnO nanoparticle synthesizing was conducted by reacting 7,07 grams of $ZnSO_4.7H_2O$ with 86,4 mL of banana peel extract and stirring it using a hotplate stirrer at a speed of 450 rpm and a temperature of 70°C for 2 hours. After that, NaOH 2 M was added slowly until pH 12 was obtained. The solution in the beaker glass was covered then with aluminum foil and allowed to stand for 24 hours. After the precipitate formed, filtered the solution was using a Buchner funnel and washed with demineralized water until the filtrate became neutral. Later, the precipitate was dried in an oven at 70°C for 2 hours. Calcination of the precipitate was carried out at 500°C for 3 hours. The synthesized ZnO nanoparticles were then characterized using XRD, SEM, and PSA instruments (Gnanasangeetha & Saralathambavani, 2013).

Antibacterial Activity Test

The last procedure was the antibacterial activity test, and this test was conducted with dissolving 1 mg of ZnO nanoparticles in 10 mL of DMSO using ultrasonic for 15 minutes. 3 discs were inserted into that solution and left for 20 minutes later. On the other hand, *S. epidermidis* bacteria were taken on NB media from incubation for 24 hours using a cotton swab. Then, streaked the bacteria on solidified NA media in a sterile petri dish. On top of the layer was placed a paper disc containing ZnO nanoparticles, and it was incubated for 24 hours at 37°C. after that, Amoxicillin 100 ppm was used as a positive control, while distilled water was used as a negative control. As a result, a clear zone around the paper disc indicated that ZnO nanoparticles could inhibit the growth of *S. epidermidis*. While the area of the clear zone formed was measured to determine the strength of its inhibitory power.

RESULTS AND DISCUSSION

Phytochemical Test of Banana Peel Extract

Based on the results of phytochemical tests, as shown in Table 1, the three raja nangka banana peel extracts with different maceration times contained polyphenols and saponins. The highest levels of these two secondary metabolites were found in the extract resulting from 24-hour maceration.

Table 1. Phytochemical Test Results of Secondary Metabolic Compounds from Raja Nangka Banana Peel Extract

Maceration Time (Hour)	Alkaloid	Flavonoids	Polyphenol	Saponins
12	-	-	+	+
24	-	-	++	++
72	-	-	+	+

Determination of Total Flavonoids and Polyphenol Level

Raja nangka banana peel extract with a maceration time of 24 hours had higher levels of flavonoids and polyphenols compared to a maceration time of 12 and 72 hours, as shown in Table 2.

Table 2. Concentration and Total Flavonoids and also Polyphenol Level of Raja Nangka Banana Peel Extract

Maceration Time (Hour)	Flavonoids		Polyphenol	
	Concentration (ppm)	Level (%)	Concentration (ppm)	Level (%)
12	1.788	0.018	223.188	0.223
24	2.228	0.022	359.434	0.359
72	2.091	0.021	250.951	0.251

There was different time variation in the maceration process to determine the optimum time for extracting secondary metabolites from the raja nangka banana peel to obtain the maximum results. An escalation interaction between the solvent and the simplicia by a longer maceration time resulted in high levels of secondary metabolite production. However, if it exceeds the optimum maceration time, the number of secondary metabolite compounds extracted would reduce because it was damaged. This result was in line with the research conducted by Wahyuni & Widjanarko (2015) that the length of extraction time increases the interaction between the solvent and the extracted substance, affecting the increased extraction.

The qualitative test in this study revealed no color change, indicating a lack of flavonoids. However, the quantitative tests revealed flavonoids in all three extracts. It was due to the three extracts' relatively low flavonoid content, which ranges from 0.018 to 0.022%. These levels were significantly different from those discovered by Adhayanti et al. (2018). After 24 hours of extraction, banana peel extract had flavonoid levels of 3.501%, and the color changed from orange to red.

Based on the results of the secondary metabolite compound test both qualitatively and quantitatively, it revealed that the extract with 24 hours maceration time was able to extract secondary metabolite compounds at highest levels compare to other extracts.

ZnO Nanoparticle Characterization

The synthesized result of the ZnO nanoparticle diffractogram was analyzed using Match! 3. The result found that ZnO nanoparticles with raja nangka banana peel extract have been successfully carried out and crystallized in hexagonal wurtzite. The occurance of synthesis was indicated by the appearance of typical peaks for ZnO nanoparticles similar to the standard diffractogram (ICSD No. 57450), as seen in Figure 1.

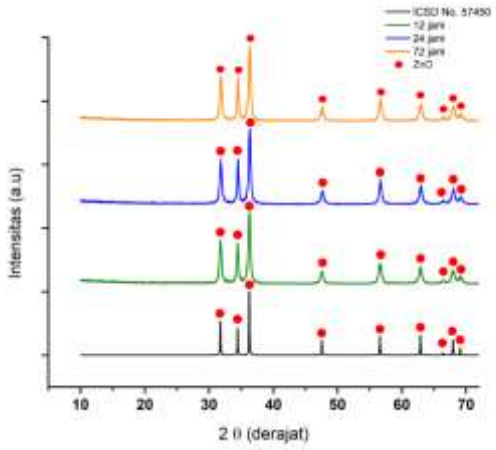

Figure 1. ZnO Nanoparticle Diffractogram with Time Variation of Raja Nangka Banana Peel Extract

The results of the SEM analysis revealed that ZnO nanoparticles with 12-hour raja nangka banana peel extract had a needle-like shape, as in the study of Wahab et al. (2011), while the other two extracts had a spherical shape. All three had a non-uniform size, as shown in Figure 2. It happened because the sample undergoes agglomeration, indicated by the presence of lumps. Agglomeration occurred due to low levels of flavonoids and polyphenols because they could not cover the maximum binding of the Zn^{2+} ion surface. As a result, the Zn^{2+} ions were interrelated, which caused the molecule to become macro-sized.

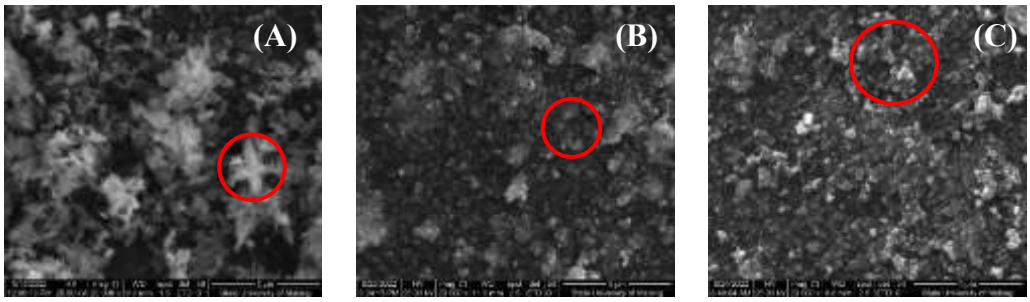

Figure 2. Morphology Shape of ZnO Nanoparticles with Raja Nangka Banana Peel Extract with Maceration Time (A) 12, (B) 24, dan (C) 72 hours

The levels of secondary metabolites as capping agents also affected particle size. Based on the test results, the size of ZnO nanoparticles with variations in the extraction time of the raja nangka banana peel is shown in Table 3.

Table 3. Size and *Polydispersity Index* of Synthesized ZnO Nanoparticles

Maceration Time (Hour)	Particle Size (nm)	*Polydispersity Index* (PI)
12	551.2	0.441
24	295.2	0.298
72	337.6	0.337

The levels of flavonoids and polyphenols caused the difference in particle size as capping agents in synthesizing ZnO nanoparticles. The more secondary metabolite compounds, the more the Zn^{2+} ion surface was covered by these secondary metabolites. It reduced the possibility of interaction between Zn^{2+} ions, which can trigger agglomeration. Based on the PSA test results, the three synthesized ZnO nanoparticles were included in the nanoparticle material. The size of the synthesized nanoparticles was generally in the range of 1–1000 nm (Sabdoningrum et al., 2021). In this study, the size of the ZnO nanoparticles was 295.2 to 551.2 nm. This size was smaller than the maximum size of the synthesized nanoparticles, so it can be concluded that the extraction time affected the size of the nanoparticles.

The polydispersity index (PI) was a value that determine the particle size distribution to determine the uniformity of the particle (Taurina et al., 2017). The PI value < 0.05 was the standard for particles with a very good or homogeneous size distribution, while > 0.7 indicated a widened particle size distribution (Danaei et al., 2018). The PI value of the synthesized ZnO nanoparticles was between 0.05 and 0.7, which indicated that the particle size distribution had moderate uniformity. It could indicate the occurrence of agglomeration in ZnO nanoparticles such that the particle size was not uniform, as shown in Figure 2.

Antibacterial Activity Test

The antibacterial activity of ZnO nanoparticles in inhibiting the growth of *Staphylococcus epidermidis* was carried out using the paper disc diffusion method, characterized by the appearance of a clear zone around the paper disc. The results of the antibacterial activity of ZnO nanoparticles against *Staphylococcus epidermidis* can be seen in Table 4.

Table 4. ZnO Nanoparticle Antibacterial Test Results *Staphylococcus epidermidis*

Maceration Time (Hour)	Clear Zone Diameter (mm)			Average (mm)
	1	2	3	
12	5.03	5.05	5.03	5.04
24	5.60	5.65	5.55	5.60
72	5.15	5.18	5.25	5.19
Negative control (distilled water)	0	0	0	0
Positive control (*amoxicillin*)	6.33	6.10	6.24	6.22

Their particle size influenced the antibacterial activity of ZnO nanoparticles. The smaller the particle size, the greater the antibacterial activity of ZnO nanoparticles (Romadhan et al., 2016). The diameter of the clear zone of nanoparticles synthesized using the raja nangka banana peel extract with an extraction time of 24 hours was greater than that of 12 and 72 hours. It was inversely proportional to the particle size of the synthesized ZnO nanoparticles. The smaller the particle size, the easier it was to penetrate the bacterial wall and inhibit the metabolism and growth of these bacteria.

ZnO compounds could inhibit bacterial growth because these compounds disrupt bacterial DNA's structure. As a result, bacteria could not replicate and metabolize proteins, which caused inhibition of the growth process and cell multiplication of the bacteria (Romadhan & Pujilestari, 2018). The formation of hydrogen peroxide was the key to antibacterial agents. In addition, the accumulation of ZnO nanoparticles on the surface of bacterial cells was also one of the reasons why these nanomaterials can inhibit bacterial growth. ZnO nanoparticles produced zinc ions, triggering reactive oxygen species (ROS) formation. The presence of reactive oxygen species around the bacterial cell wall caused bacterial cells to be damaged and made it easier for them to enter bacterial cells. Reactive oxygen species would inhibit the DNA replication process and denature proteins in bacterial cells (Hoseinzadeh et al., 2017). If the DNA replication process is disrupted, it caused the growth of bacteria is inhibited, which could cause death.

CONCLUSIONS

The optimum time for maceration of secondary metabolites from raja nangka banana peels using ethanol-water (2:1) was 24 hours. The SEM test results revealed that the three samples' morphology had agglomeration. The ZnO nanoparticles with 24-hour raja nangka banana peel extract had a smaller size of 295.2 nm and were spherical. An antibacterial test against *S. epidermidis* revealed that ZnO nanoparticles with 24-hour raja nangka banana peel extract could act as an antibacterial agent with an inhibition zone diameter of 5.65 mm.

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