Green Synthesis of ZnO Nanoparticles by Using Banana Peel Extract as Capping agent and Its Bacterial Activity

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Abstract. One of the nanoparticles used in the medical world is ZnO nanoparticles. The purpose of this study was to synthesize ZnO nanoparticles by using peel’s extract of kepok, ambon, and tanduk bananas (Musa paradisiaca L., Musa acuminata Cavendish Subgroup, and Musa x paradisiaca) as a capping agent. This research was conducted in four main steps, namely: extraction of three kind of bananas peel, phytochemical test on the bananas peel extract, synthesis of ZnO nanoparticles by using the extracts, characterization of ZnO nanoparticles by X-Ray Diffraction (XRD), and antibacterial activity test of ZnO nanoparticles. Based on the phytochemical test it was proven that bananas peel extract contained secondary metabolite such as flavonoids, polyphenols, alkaloids, and saponins in various concentration. The XRD analysis concluded that ZnO nanoparticles were successfully synthesized. Through the agar diffusion test, it can be showed that ZnO nanoparticles are a promising material as antibacterial agents. This statement is based on evidence that the diameter of the clear zone produced ranges from 8 to 10 nm.

1. Introduction

One of the metal oxide nanoparticles which has many benefits for humans is zinc oxide nanoparticles (ZnO NPs). ZnO NPs are used in a variety of applications including antibacterial agents, solar cell manufacturing materials, cosmetic ingredients, and gas adsorbents such as NOx, CO2, CO, and NH3 [1]. Currently, the development of ZnO NPs is not only limited to how it is applied, but also how to synthesize the nanoparticles which produces the desired properties. Many ZnO NPs synthesis methods have been developed, one of them is a chemical method. Unfortunately, this method has disadvantages consider with the environmental impact due to waste from the synthesis process using hazardous chemicals as capping agents (PEG polymers, PVC, and anionic surfactants and others). Moreover, the application of chemical methods is also expensive, requires a long process, and high temperatures [2].

One of environmental-friendly method for synthesis of ZnO NPs nanoparticles is green method. In this method, the use of chemicals as reducing or capping agent is replaced by natural ingredients like plant extracts or mushrooms or algae. Green synthesis of ZnO NPs has been carried out by many researchers including using Bauhinia tomentosa leaf extract [3], Citrus sinensis extract [4], cavendish banana peel extract [5] Sargassum sp [6] and garlic extract [7].

To the best of our knowledge, there is no researchers uses peel extract of Kepok, Ambon, and Tanduk bananas (Musa paradisiaca L., Musa acuminata Cavendish Subgroup, and Musa x paradisiaca) as capping agents in ZnO NPs synthesis. The peels of these three types of bananas are accumulated as
waste. Data from Ministry of Agriculture showed that the prospect of banana production until 2020 is about 8000 tons/year [8]. While the waste of banana peels production is about 40% of total production [9]. The use of banana peel extract as a capping agent is possible because the banana peel extract contains secondary metabolite compounds such as flavonoids, polyphenols, alkaloids and saponins with various amounts [3]. Flavonoids are natural compounds that contain 15 carbon atoms arranged in a C6-C3-C6 configuration. Flavonoids are predicted to be the compounds that produce the most nanoparticles in the Green Synthesis process both as capping agents and reducing agents [10]. Polyphenols are secondary metabolites that have many phenol groups in their structure. Alkaloids are secondary metabolites that have one or more nitrogen atoms in their heterocyclic rings, whereas saponins are compounds composed of glycons (sugars) and aglycones (non-sugars) that are rich in hydroxyl groups. Functional group in the secondary metabolites will act as Lewis bases in water. Metal cations that are in the same solution with the compounds will be stabilized and subsequently undergo conversion to hydroxide after passing through the saturation point. Different parts of plant material such as fruit extracts, bark, fruit bark, and roots have been studied for the synthesis of silver, gold, platinum and titanium nanoparticles in various sizes and shapes [2]. According to Ibrahim synthesis of nanoparticles mediated by plant extracts is preferred because it is cheaper, environmental-friendly, and the products are safe for medical purposes [2].

This study aims to carry out phytochemical tests on bananas peel extract, synthesis and characterization of ZnO NPs by using bananas peel extract as a capping agent, followed with antibacterial test. This last goal relates to the ability of ZnO NPs to interfere the permeability of bacterial cell walls which in turn makes bacterial cells undergo a lysis or damage so that the cytoplasmic fluid in cells will come out and cause bacterial death [11]. The results of Manyasree's research [12] show that ZnO NPs is highly reactive to S. aureus gram-positive and gram-negative E. coli bacteria.

2. Materials and Method

The equipments used in this study were some glass wares, an analytical balance (SHIMADZU) with accuracy of 0.0001 g, hot plate with magnetic stirrer, mortar and pestle, centrifuge, vortex mixer, and XRD (Panalytical Xpert Pro).

The materials used in this study were kepok, ambon, and tanduk bananas (Musa paradisiaca L., Musa acuminata Cavendish Subgroup, and Musa x paradisiaca) peel, ZnSO4.7H2O p.a. Merck, NaOH p.a. Merck, ethanol p.a. Merck, universal pH paper, HgCl2, KI, demineralized water, DMSO p.a, nutrient agar, Merck, E. coli and S. aureus bacteria. Filter paper (Whatman-42) and universal indicator.

Bananas peels were first cleaned using demineralized water and cut into pieces, then weighed in the amount of 150 grams. The banana peels that have been placed in a beaker glass were added with 150 mL of demineralized water and heated at 80 °C for 10 minutes. The banana peels then mashed and macerated, and then filtered off.

Phytochemical tests were carried out by referring to the procedure in the study of Rao et al (2016). Phytochemical tests consist of flavonoid, polyphenol, alkaloid, and saponin tests. The tests are described below.

i. Flavonoid Test
   The flavonoid test was carried out by adding concentrated of HCl solution dropwise to the banana peel extract. If the color changes to red, it can be said that the extract contains of flavonoids.

ii. Polyphenol Test
   Banana peel extract was added with solution of FeCl3 1%. A positive reaction gives a strong green, red, purple, blue or black color.

iii. Alkaloid Test
   Banana peel extract was added with 0.2 mL of HCl solution and then was added with 1 mL Mayer reagent. A positive test is given by the formation of white deposits to yellowish.
iv. Saponin Test

A 1 mL of banana peel extract was added with 20 mL of hot water then shaken vigorously. Positive result is indicated by the formation of bubbles that are stable for not less than 15 minutes.

Synthesis of ZnO nanoparticles was carried out by mixing 500 mL of 0.0369 M of zinc sulfate solution with 129 mL of banana peel extract and stirred for 10 minutes, then added 2.0 M of NaOH solution dropwise (8 grams of NaOH dissolved into 100 mL of demineralized water) to reach pH 12. The mixture was stirred for 2 hours, then filtered using a Buchner funnel. After that, the solid was rinsed with ethanol until the filtrate had the same color as the ethanol, then the solid was rinsed with demineralized water until the filtrate reached pH 7. The solid was dried in an oven at 60 °C until it reached a constant mass and dried for 48 hours.

ZnO nanoparticles were characterized using X-Ray Diffraction (XRD) to determine crystallinity, phase identification and particle size. This characterization was carried out in the range of 2θ angles at 10°-90° at room temperature, with an X-ray source produced by copper anode material.

The antibacterial test was carried out in 2 stages, namely the stage of making agar medium and the stage of antibacterial testing. The bacteria used were S. aureus as representative of gram-positive bacteria and E. coli as representative of gram-negative bacteria.

i. Agar Medium Preparation

The agar medium was prepared in 24 medium plates made from solid agar nutrient powder. Each plate containing nutrients was wrapped in a cover paper and tied. Next, the plate was sterilized in an autoclave at 121 °C for 15 minutes, then cooled down in a refrigerator.

ii. Antibacterial Testing

0.5 g of ZnO NPs powder was dispersed in 5 mL of DMSO solvents, then vortexed until completely mixed. Furthermore, wells are made in the medium so that previously contaminated with the bacteria used, namely E. coli and S. aureus. Two drops of ZnO NPs solution was put into a well and the cup was tightly closed then allowed to stand in an incubator at 37° C for 24 hours. The same procedure is performed for each bacterium and each sample. Antibacterial activity was measured based on the diameter of the clear zone produced.

3. Result and Discussion

3.1. The results of phytochemical tests

The results of phytochemical tests on Musa paradisiaca L., Musa acuminata Cavendish Subgroup, and Musa x paradisiaca peel extracts are presented in Table 1.

| No | Banana                               | Flavonoid | Polyphenol | Alkaloid | Saponin |
|----|--------------------------------------|-----------|------------|----------|---------|
| 1  | Musa paradisiaca L.                  | +         | +          | +        | +       |
| 2  | Musa acuminata Cavendish Subgroup    | +         | ++         | +        | +       |
| 3  | Musa x paradisiaca                   | +++       | +++        | ++       | ++      |

As shown in Table 1, all three types of bananas contain secondary metabolites. The levels of these secondary metabolites are only stated qualitatively, based on the intensity of changes shown by each type of banana in the phytochemical test. Musa x paradisiaca peel extracts have the highest levels of secondary metabolites than the other two bananas whether it's the levels of flavonoids, polyphenols, alkaloids, or saponins. This levels difference is predicted to affect the properties of ZnO NPs produced and discussed in the following analysis results.
3.2. X-Ray Diffraction

Characterization using powder XRD aims to determine the synthesis of ZnO nanoparticles by using banana peel extract as a capping agent. The characterization was based on the standard ZnO nanoparticle Diffractogram JCPDS Card No: 36-1451. The XRD profile of the product is presented in Figure 1. Based on the diffractogram, ZnO NPs have been successfully synthesized using extracts of all 3 types of banana peel varieties, which obtained a value of 2θ angles at 31.78°, 34.42°, 36.25°, 47.53°, 56.57°, 62.87°, 66.42°, 67.93°, and 69.09°. These peaks correspond to JCPDS card No: 36-1451. The peaks marked with the sign (*) on the diffractogram in Figure 1 (b) is the diffraction peak of the compound Zn(OH)₂ according to the JCPDS Card No 89-0138 as checked by the application of Match software. This means that Zn(OH)₂ also crystallized because of the possibility of temperature process used is not too high and not long enough so that the Zn(OH)₂ does not undergo the H₂O molecules releasing into ZnO and remains stable in the form of Zn(OH)₂.

![Figure 1](image-url)

**Figure 1.** XRD diffractograms of ZnO NPs synthesized from banana peel extract of (a) Musa paradisiaca L.; (b) Musa acuminata Cavendish Subgroup; (c) Musa x paradisiaca peel extracts.

As shown in Figure 1, ZnO NPs synthesized with Tanduk (Musa x paradisiaca) banana showed the lowest peak intensity compared to others due to the levels of secondary metabolites in the Tanduk banana that prevent further crystallization.

3.3. The Crystallite Size

The XRD results data are then processed using the RIETICA application. The crystalite size of each ZnO NPs sample obtained are presented in Table 2. XRD analysis for each type of bananas is only represented by one sample each. Calculation of the crystalite size of each sample is based on the results of the XRD analysis.
3.4. Antibacterial Activity
Antibacterial test results expressed as clear zone diameters around the wells in each plate is presented in Table 3 and Table 4. Based on the results of ZnO NPs antibacterial test against \textit{E. coli} and \textit{S. aureus} bacteria, it can be said that all of the three samples have antibacterial activity as shown from the clear zone that produced which is greater than 5 mm. DMSO solution that is used as a negative control does not form a clear zone so that the DMSO (dimethylsulfoxide) has no effect on the antibacterial test. ZnO NPs which were synthesized using capping agent extract of Tanduk banana peel has antibacterial activity > that of Ambon banana peel extract > that of red kepok banana peel extract. The antibacterial test results are directly proportional to the particle size of each ZnO NPs. The smallest particle size of ZnO NPs will be more effective to be used as an antibacterial because the nanoparticles will be easier to penetrate the cell walls of the bacteria and easier to damage the bacterial cells so that cells will undergo lysis quicker and resulting in more damage. All the three ZnO NPs have better antibacterial activity against \textit{E. coli} gram-negative bacteria compared to that of \textit{S. aureus} gram-positive bacteria, because gram-negative bacteria have a thinner layer of peptidoglycan which making it easier to be penetrated by the ZnO nanoparticles. These results are in accordance with those obtained by Diez-Pascual [13].

Table 2. The Crystallite Size

| Banana                                      | Crystallite Size (nm) |
|---------------------------------------------|-----------------------|
| Red Kepok (\textit{Musa paradisiaca L.})    | 33.3                  |
| Ambon (\textit{Musa acuminata Cavendish Subgroup}) | 26.5                  |
| Tanduk (\textit{Musa x paradisiaca})        | 24.5                  |

Table 3. Antibacterial test against \textit{S. aureus}

| No | Banana                                      | Average Clear Zone Diameter against \textit{S. aureus} (mm) | Conclusion     |
|----|---------------------------------------------|-------------------------------------------------------------|----------------|
| 1  | \textit{Musa paradisiaca L.}                | 8.16                                                        | Antibacterial  |
| 2  | \textit{Musa acuminata Cavendish Subgroup}  | 8.20                                                        | Antibacterial  |
| 3  | \textit{Musa x paradisiaca}                 | 9.36                                                        | Antibacterial  |
| 4  | DMSO                                        | 0                                                           | -              |

Table 4. Antibacterial test against \textit{E. coli}

| No | Banana                                      | Average Clear Zone Diameter against \textit{S. aureus} (mm) | Conclusion     |
|----|---------------------------------------------|-------------------------------------------------------------|----------------|
| 1  | \textit{Musa paradisiaca L.}                | 8.28                                                        | Antibacterial  |
| 2  | \textit{Musa acuminata Cavendish Subgroup}  | 8.38                                                        | Antibacterial  |
| 3  | \textit{Musa x paradisiaca}                 | 10.73                                                       | Antibacterial  |
| 4  | DMSO                                        | 0                                                           | -              |

4. Conclusion
ZnO nanoparticles were successfully synthesized using banana peel extract as a capping agent. The extract from the Tanduk banana peel gives the best results. This is evidenced by the similarity between the diffractogram and the standard diffractogram. In addition, antibacterial test results showed that all three types of bananas have antibacterial activity, where the clear zone produced is more than 5 mm.

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