



Biosynthesis of Zinc Oxide Nanoparticles with Horned Banana Peel Waste Extract (*Musa paradisiaca* fa. *corniculata*)

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Abstract. The high amount of banana consumption in Indonesia has caused banana peel waste to increase, which can cause environmental pollution. One of the utilizations of banana peel waste is as a metal nanoparticle synthesis. This study aims to synthesize ZnO nanoparticles with banana peel extract as a capping agent and determine the effect of solvent volume variation on the characterization of ZnO particles using variable volumes of ethanol-water solvent with a ratio of 2:1 and 1:1 (v/v). This research uses the maceration extraction method for 24 hours. ZnO particles were characterized, including Fourier Transform Infrared Spectroscopy (FTIR) and Particle Size Analyzer (PSA). In this study, the total polyphenol and flavonoid content in ethanol-water 1:1 (v/v) horn banana peel extract was higher at 4.944 % and 5.940 % than in ethanol-water 2:1 (v/v) at 4.114% and 4.131%. Based on the results of FTIR testing, both samples have ZnO peaks where in ethanol-water 1:1 (v/v), 441 cm⁻¹, and 619 cm⁻¹, while ethanol-water 2:1 (v/v) is 428 cm⁻¹. Then, from the PSA test results, the ethanol-water 1:1 (v/v) sample has a smaller average nanoparticle diameter of 135.6 nm than the ethanol-water 2:1 (v/v) sample, which is 153.6 nm. ZnO nanoparticles were successfully synthesized using the natural capping agent banana peel extract. Different levels of secondary metabolites in each extract influence the diameter of the synthesized ZnO nanoparticles.

Keywords: *capping agent, banana horn skin, ZnO nanoparticles*

1. Introduction

Banana plants are one of the tropical fruit commodities that are widely found in Indonesia. Indonesia is one of the world's banana-producing countries, with banana tons in 2020 of 8,182,756 tons, an increase of around 12.39% compared to 2019. As the population in Indonesia increases and the public's nutritional awareness increases, the demand for bananas also increases [1]. This is proven by the increasing amount of banana production in Indonesia in 2022 of 9,245,427 tons [2]. Banana peels are often overlooked and even considered waste, accounting for 35-50% of the total mass of banana fruit [3]. The high production and

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consumption of bananas in Indonesia has led to an increase in banana peel waste. If banana waste is not processed correctly, it will cause environmental pollution. One study showed that plant waste such as orange peel, lemon peel, mango peel, rice husk, and banana peel can be utilized as a metal nanoparticle synthesis. So, banana peel waste can be used as a nanoparticle synthesis agent [4].

Nanoparticles are one of the nanotechnologies that are experiencing rapid development. This is due to the high utilization of nanoparticles in human life, such as biomedicine, energy, industry, environment, electronics, and textiles [5]. Metal oxide nanoparticles are one of the promising types of nanoparticles due to their unique biological, chemical, and physical properties. Based on the research that has been done, some metals such as ZnO, TiO₂, CaO, and MgO have anti-microbial abilities, and these compounds are considered harmless to humans [6]. Economically, ZnO has a production cost of 75% cheaper than TiO₂ in industrial-scale production. Therefore, ZnO nanoparticles are widely used in various interests, one is an anti-microbial agent because ZnO nanoparticles have a longer life than organic-based disinfectants [4].

Zinc oxide (ZnO) is a nanometer-sized material with photocatalytic ability and has been tested to slow the development of gram-negative or gram-positive bacteria [7]. In addition, ZnO is classified as a non-toxic, biocompatible, and bio-safe material [8]. The antibacterial performance of ZnO is highly dependent on size and morphology. The smaller the diameter of ZnO particles, the better the antibacterial performance [9].

The synthesis of ZnO nanoparticles faces serious challenges in the form of agglomeration that can reduce the effectiveness and unique properties of the particles. Using capping agents is a potential solution to prevent aggregation, with natural capping agents from secondary metabolite compounds (such as tannins, alkaloids, polyphenols, and flavonoids) offering a more environmentally friendly and sustainable alternative [10], [11]. Agglomeration is the clumping of particles that causes non-uniform size and reduces the concentration of ZnO in the nanoparticles. Capping agents coat ZnO particles during synthesis to prevent accumulation [10]. The synthesis of ZnO nanoparticles also provides the potential to provide new insights into the interactions between organic compounds and inorganic nanoparticles. Based on the research, secondary metabolite compounds can be found in banana peel extracts, such as saponins, alkaloids, flavonoids, and polyphenols [12]. In addition, the determination of solvents in the extraction process influences the yield of secondary metabolites. The solvent

used in the extraction must have low toxicity, be non-volatile, and not damage the extract components [13], the content of saponins, flavonoids, glycosides, and phenols. [14] Obtained reducing sugars from research on banana peel extraction with ethanol and water solvents. Ethanol and water solvents were used to determine the quality of ZnO nanoparticles produced with volume variations. A mixture of ethanol and water solvents can increase the extraction of secondary metabolite compounds soluble in solvents [15].

Research on nanoparticles using various natural materials, such as star apple leaf, water hyacinth leaves, tin leaf, and pectin, has been explored through nanoparticle synthesis. For star apple leaf, extract volumes (2-10 mL) and pH variations (7-9) were tested, with an FTIR result showing a ZnO peak at 405-768 cm^{-1} [16]. Water hyacinth leaves had volume variations (20-40 mL), and PSA tests revealed particle sizes between 15.6 and 76.9 nm [17]. Tin leaf showed volume variations (30-40 mL), and PSA tests recorded an average ZnO nanoparticle size of 49.62 nm [18]. Pectin synthesis with temperature variations (60-100°C) resulted in an FTIR wavelength peak of around 435-566 cm^{-1} [19].

Based on the description above, this study aims to synthesize ZnO nanoparticles with the essential ingredients of horned banana peel extract as a capping agent and determine the effect of variation in the volume of ethanol-water solvent on the characterization of ZnO particles produced from horned banana peel on ZnO particle synthesis. ZnO particles were characterized, including Fourier Transform Infrared Spectroscopy (FTIR) and Particle Size Analyzer (PSA). Through this research, it is expected to reduce banana peel waste and become literature for further studies related to this research.

2. Research Method

2.1 Materials

Banana horn peel waste, 96% ethanol ($\text{C}_2\text{H}_5\text{OH}$) (Sigma Aldrich, USA), distilled water (H_2O), zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (The Emsure, Germany), and sodium hydroxide (NaOH) (The Emsure, Germany).

2.2 Equipment

The tools used in this research include 100 mL Pyrex beaker, 50 mL Pyrex measuring cup, Hot plate stirrer Thermo Scientific, Thermolyne 30420C-33-80 Muffle Furnace, OHAUS CP214 analytical balance, Panasonic oven, blender, Pyrex measuring pipette, dropper pipette,

Digital Photo Tachometer pH meter, 250 mL Pyrex Erlenmeyer, 50 mL Pyrex glass funnel, and 8 cm watch glass.

2.3 Methods

2.3.1 Horn Banana Peel Extraction

The banana peels were washed, cut into small pieces, and dried in the sun for \pm 15 hours. The banana peels were pulverized with the help of a blender. The powder of the horned banana peel was weighed in the amount of 15 grams, and the weighing was repeated 2 times. The extraction process began using the maceration method. Divided the sample into two, namely ethanol-water 2:1 (v/v) with a ratio of 96% ethanol totaling 60 mL and water totaling 30 mL and ethanol-water 1:1 (v/v) with a ratio of 96% ethanol totaling 30 mL and water totaling 30 mL. The two samples were allowed to stand for 1x24 hours, then filtered using filter paper to obtain a solution of ethanol-water extract of banana horn skin [20].

2.3.2 Assay of Total Flavonoid and Polyphenol Content of Horn Banana Peel Extract

2.3.2.1 Total Flavonoid Content

The procedure that needs to be done in determining the total flavonoid content is to prepare 1 mL of horn banana peel extract and then put it in a 10 mL measuring flask. Added methanol in the amount of 3 mL, AlCl_3 10% in the amount of 0.2 mL, CH_3COOK 1M in the amount of 0.2 mL, and included distilled water until the limit mark of 10 mL. Incubated the solution for 30 minutes and measured the absorbance with the help of a UV-Vis spectrophotometer at a wavelength of 432 nm [21]. Determination of total flavonoid content using the help of a 1-5 ppm quercetin standard curve [22].

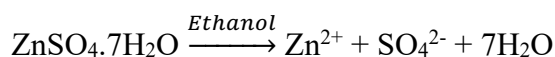
2.3.2.2 Total Polyphenol Content

In testing the total polyphenol content using the help of a standard curve of 1-5 ppm gallic acid solution. Prepared 0.25 mL of horned banana peel extract, then Folin-Ciocalteu 10% reagent was added as much as 500 μL and 4 mL of 7.5% Na_2CO_3 and distilled water until the limit mark of 10 mL. Shaken until homogeneous, then incubated the solution for 60 minutes at room temperature. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm [21], [23].

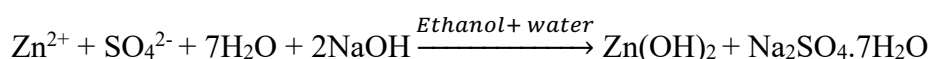
2.3.3 Synthesis of ZnO Particles with Horn Banana Peel Extract Material

Synthesized ZnO particles through the reaction process between $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and banana horn skin extract solution [24]. In the ethanol-water 2:1 (v/v) sample, 3.28 grams of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was reacted with 40 mL of banana horn skin extract solution. In the ethanol-water 1:1 (v/v) sample, 1.64 grams of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was reacted with 20 mL of banana peel horn extract solution and stirred each sample with the help of a hotplate stirrer for 2 hours at 70 °C with a speed of 450 rpm. After stirring, 2 M NaOH was added slowly until pH 12. Closed the glass beaker using plastic wrap and aluminum foil, then allowed to stand for 24 hours at room temperature. Filtered the resulting precipitate using filter paper. Washed with demineralization until pH 7. Dried the washed precipitate using an oven for 4 hours at 80 °C. Calcined the precipitate using a furnace for 3 hours at 500 °C. The calcination process is an endothermic decomposition reaction that aims to remove gases from hydroxides or carbonates to obtain high-purity powders in the form of oxides [25]. Below is the reaction mechanism in the formation of ZnO particles [26]:

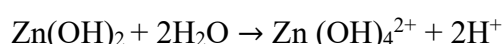
- First stage (Solvation stage):



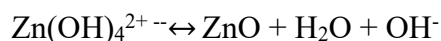
- The second stage (Hydrolysis stage):



- Third stage (Polymerization stage):



- Fourth stage (Transformation stage):



2.3.4 Characterization of ZnO Nanoparticles

Fourier Transform Infrared (FTIR) is useful as a determinant of the presence of functional groups based on wave numbers when vibrations occur. FTIR analysis is performed in wave numbers around 600 to 4000 cm^{-1} [27]. The study observed several peaks and functional groups where the Zn-O functional group occurred at wave numbers 400-600 cm^{-1} [28].

Particle Size Analysis (PSA) is one of the nanoparticle analyses that aims to determine the size of nanoparticles dispersed in colloidal nanoparticles. Based on the results of previous research, the particle size that meets the requirements to be classified as nanoparticles is 10-

1000 nm [29]. The principal PSA uses is Dynamic Light Scattering (DLS), where light is scattered during measurement. When particles are irradiated with light, light scattering occurs, fluctuating with a speed that depends on the particle size. The smaller the particle size, the faster the particle distribution fluctuates. Particle distribution and size are key characteristics in nanoparticle systems because they affect the targeting ability of the nanoparticle [30].

3. Result and Discussion

3.1 Extraction of Secondary Metabolites of Horn Banana Peels

Figure 1 shows the results of the extraction of horned banana peel using various ethanol-water ratios. The extraction process produced a brownish-yellow solution with different levels of color concentration. As shown in **Figure 1**, (a) 1:1 (v/v) ethanol-water extract has a more intense color compared to (b) 2:1 (v/v) ethanol-water extract.



Figure 1. Horn Banana Peel Extract with Ethanol-Water Variation (a) (1:1) and (b) (2:1)

This study uses the maceration method because it has an easy process and limits the degradation of secondary metabolite compounds due to high heat exposure. The choice of solvent used must be appropriate to attract metabolite compounds. Solvents attract extracts more quickly if they have the same polarity properties [31]. Ethanol has a high polarity, extracting polar compounds such as phenolic compounds [32]. This study uses a combination of ethanol and water as solvents to extract secondary metabolite compounds from samples. Ethanol and water were chosen because they can dissolve secondary metabolite compounds soluble in organic solvents and water. The ethanol-water solvent volume ratio aims to find the ideal solvent volume ratio that can extract more secondary metabolite compounds, especially

flavonoids, and polyphenols, which serve as capping agents for forming nanometer-sized materials.

3.2 Analysis of Total Polyphenol and Flavonoid Content of Horn Banana Peel Extract

The results showed that the total polyphenol content in ethanol-water 1:1 (v/v) was more significant than with ethanol-water 2:1 (v/v), namely 4.944% and 4.114%, as shown in **Table 2**. Furthermore, the total flavonoid content test showed that ethanol-water 1:1 (v/v) was more significant than ethanol-water 2:1 (v/v), namely 5.940% and 4.131%, as in **Table 2**. These two tests show that the content of secondary metabolite compounds in ethanol-water 1:1 (v/v) is more significant than in ethanol-water 2:1 (v/v). This is because, in the variation (1:1), the ethanol used is less, namely 30 mL, compared to the variation (2:1), which is 60 mL, so that in the variation (1:1), the hydrogenation bond that occurs is lower. Based on previous research, hydrogenation bonds can break some bonds in the structure of polyphenols and flavonoids, thereby reducing their levels [38].

Table 1. Total Polyphenol and Flavonoid Content of Horn Banana Peel Extract

Sample Extract	Total Polyphenol Content	Total Flavonoid Content
(1:1)	4.944 %	5.940 %
(2:1)	4.114 %	4.131 %

Based on previous research explains that the more concentrated the color of the extract, the more secondary metabolite content [24]. According to the research that has been done, the total polyphenol and flavonoid content in the ethanol-water variation 1:1 (v/v) is more significant than ethanol-water 2:1 (v/v) as in **Table 2**, where the variation (1:1) has a more concentrated according to extract according to color according than according to the variation (2:1).

Polyphenols are compounds found in plants with more than one phenol group, divided into several parts, such as flavonoids, tannins, stilbenes, and phenolic acids. Flavonoids are one part of polyphenols that have the basic structure of the flavon ring and are divided into several parts, such as flavones, flavonols, flavones, isoflavones, and anthocyanins. Polyphenols and flavonoids have several similarities, namely having a hydroxyl group (-OH) that can bind to the surface of nanoparticles, where this bond can prevent nanoparticle agglomeration. In addition, polyphenols and flavonoids have antioxidant properties that can protect nanoparticles from free radical damage, thus extending the life of nanoparticles and increasing their efficiency [33]. Free radicals are unstable molecules that can damage the surface of nanoparticles, resulting in

decreased stability and function [34]. In synthesizing ZnO nanoparticles using natural capping agents, testing the total polyphenol and flavonoid content is needed because it determines the effectiveness of the capping agent and understands the interaction between nanoparticles and the resulting capping agent.

In determining the total polyphenol content, Folin-Ciocalteu reagent was added to the sample, which aims to oxidize phenolic compounds into blue, as shown in **Figure 2**. The density of the blue color formed is proportional to the amount of phenolic compounds in the sample [35]. However, the reaction process between the Folin-Ciocalteu reagent and phenolic compounds runs slowly in an acidic atmosphere, so adding sodium carbonate is needed to create an alkaline atmosphere, and the reaction can run faster [36].

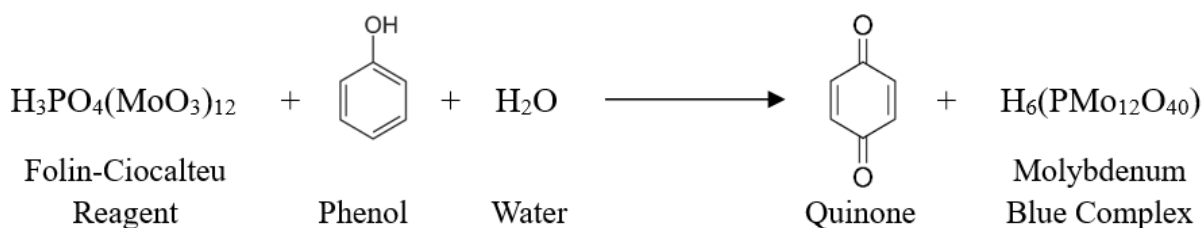


Figure 2. Reaction of phenol with Folin-Ciocalteu reagent

The total flavonoid content of horned banana peel extract was determined using colorimetry. AlCl_3 solution is used to form colored complex compounds with flavonoids so that there is a shift in wavelength towards visible, marked by the solution changing color to yellow [22]. The purpose of adding CH_3COOK solution is to stabilize and maintain the wavelength in the visible part.

The principle of determining the total flavonoid content using AlCl_3 is the formation of complexes between AlCl_3 with keto groups at C-4 atoms and with hydroxyl groups at adjacent C-3 or C-5 atoms of flavones and flavonols, as in **Figure 3**. Quercetin is a standard solution because quercetin is a flavonoid of the flavonol group with a keto and a hydroxyl group [37].

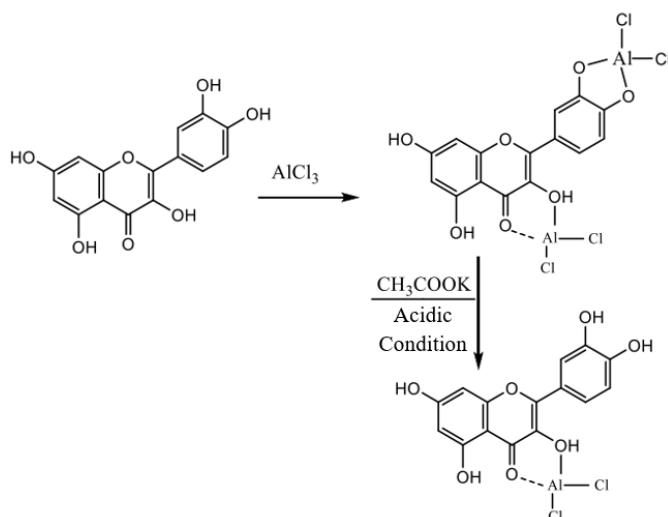


Figure 3. Reaction of Flavonoids with AlCl₃

3.3 Synthesis of ZnO Particles Using Horned Banana Peel Extract

The formation of ZnO particles from zinc sulfate heptahydrate typically involves four stages: solvation, hydrolysis, polymerization, and transformation. Zinc sulfate heptahydrate is initially dissolved in a banana peel extract and ethanol mixture. The ethanol slows hydrolysis, removing sulfate ions to form Zn(OH)₂ [26]. This Zn(OH)₂ then undergoes polymerization, forming Zn-O-Zn bonds that eventually transform into ZnO compounds. The final step is calcination, where the mixture is heated at high temperatures. This process results in smaller, more crystalline ZnO nanoparticles, which appear white, as shown in **Figure 4**.

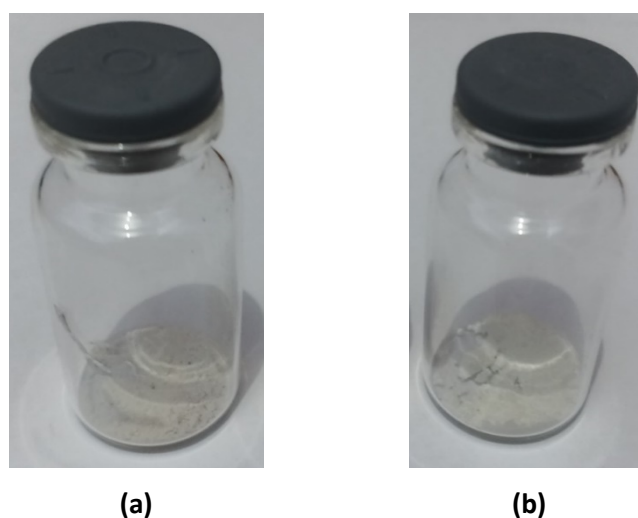


Figure 4. ZnO Synthesis Results Using Horned Banana Peel Extract with Ethanol-Water Variation (a) (1:1) and (b) (2:1)

Secondary metabolite compounds of banana peel extract, especially flavonoids and polyphenols, function as capping agents that interact with the surface of Zn^{2+} metal ions and bind Zn^{2+} metal ions to form a stable structure [24]. This interaction occurs at the polar head of secondary metabolite compounds, namely functional groups such as hydroxyl. The interaction between metabolite compounds and Zn^{2+} metal ions is to prevent aggregation. Agglomeration can cause the particle size to become non-uniform, with some particles being more significant than others. Therefore, increasing the amount of secondary metabolite compounds in ZnO synthesis produces particles with better morphology and size. The reaction equation occurs when synthesizing ZnO particles using a capping agent made from banana horn skin extract.

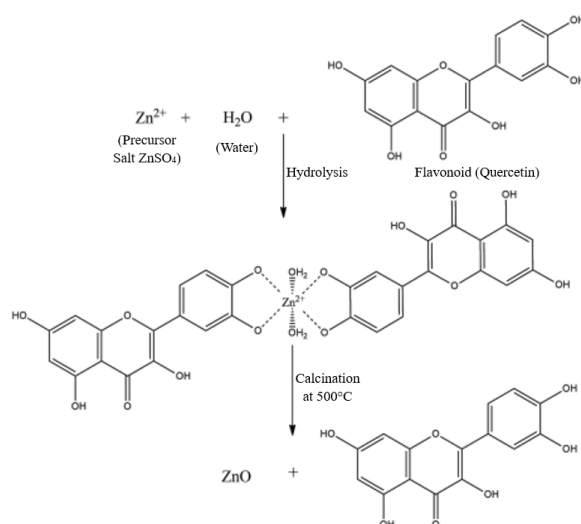


Figure 5. Mechanism of Interaction between ZnO Particles and Flavonoid Capping Agent [39]

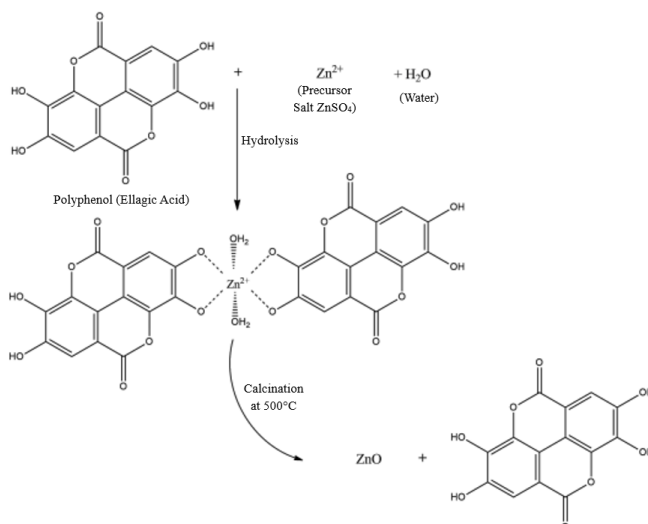


Figure 6. Mechanism of Interaction between ZnO Particles and Polyphenol Capping Agent [40]

3.4 Characterization of ZnO Particles

3.4.1 *Fourier Transform Infrared (FTIR)*

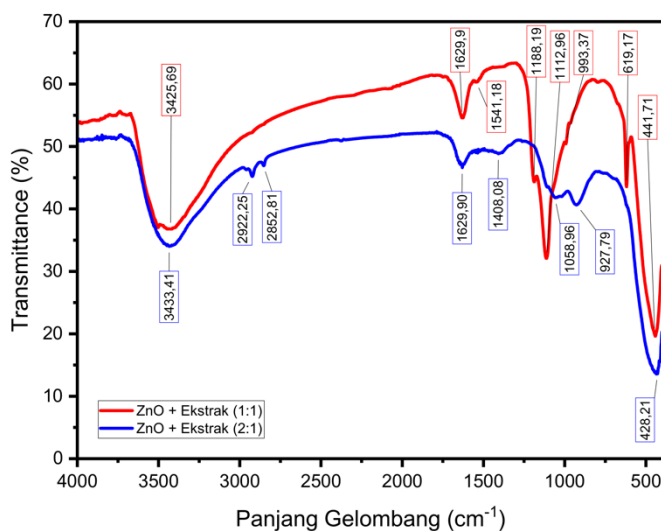


Figure 7. FTIR Spectrum of Horn Banana Peel Extract at Ethanol-Water Variation (1:1) and (2:1)

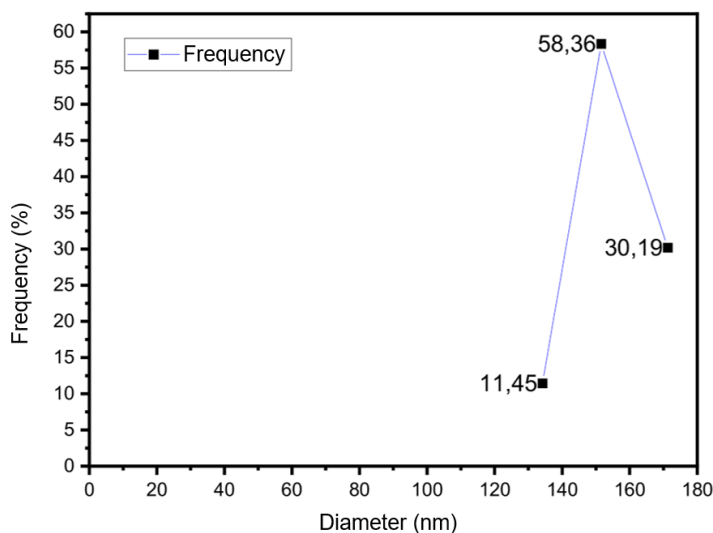
The FTIR analysis identified that the ZnO 1:1 and 2:1 sample extract shows a distinctive broad peak at wave numbers 3425cm⁻¹ and 3433cm⁻¹, respectively. In the variation (1:1), several peaks appear, including 3425 cm⁻¹, which is the O-H group on polyphenols, 1629 cm⁻¹, which is the C=C group of aromatic rings, 1541 cm⁻¹, which is the C-C stretching group, 1188 cm⁻¹, and 1112 cm⁻¹ shows the C-O-C or C-O polysaccharide group, 993 cm⁻¹ shows the C-O-C stretching group, and 441 cm⁻¹ and 619 cm⁻¹ which are ZnO groups [28], [41]–[44].

Meanwhile, the variation (2:1) has several peak points, namely 3433 cm^{-1} , the O-H group on polyphenols. 2922 cm^{-1} and 2852 cm^{-1} are C-H stretching, 1629 cm^{-1} is an aromatic ring C=C group, 1408 cm^{-1} is a deformed CH_2 group, 1058 cm^{-1} is C-N stretching, 927 cm^{-1} is a C-O-C stretching group and 428 cm^{-1} is a ZnO peak [28], [41], [42], [45].

Functional groups such as CO-C, C-O, and C=C are derivatives of proteins contained in banana horn peel extract and serve as capping agents in the synthesis of nanoparticles [41]. From the curve seen in **Figure 7**, there is a prominent difference in the variation (1:1) at a wavelength of 1112 cm^{-1} , namely the C-O-C or C-O group, the difference seen is at a wavelength of 1112 cm^{-1} diving sharply down, which means the concentration of the C-O-C or C-O group is lower. This is because, in the variation (1:1), the amount of ethanol used is less than the variation (2:1), so the concentration of C-O-C or C-O groups is lower, and hydrogenation bonds as well [46].

3.4.2 Particle Size Analyzer (PSA)

ZnO synthesis results through the PSA characterization process, which aims to determine the average particle diameter and PI (Polydispersity Index) value, which indicates the uniformity of particle size. The results of PSA test data analysis of ZnO samples are shown in **Figure 8** and **Table 3**.



(a)

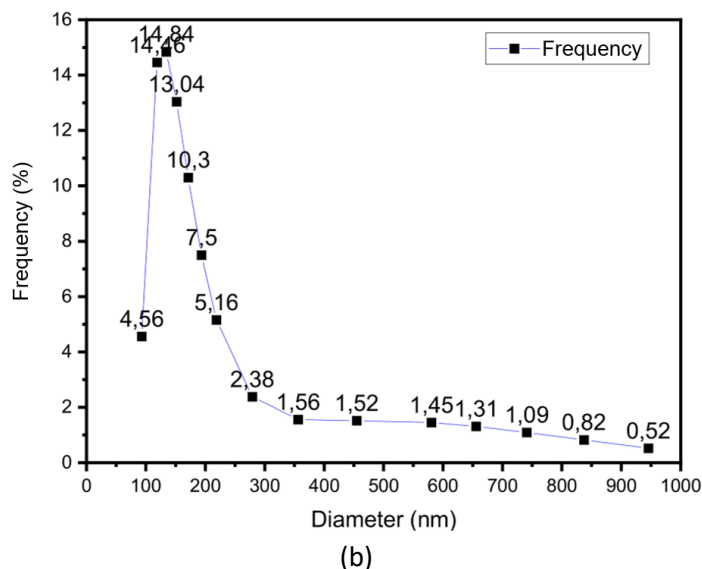


Figure 8. Diameter Distribution Chart of Synthesized ZnO Using Horn Banana Peel Extract (a) Ethanol (96%)-water 2:1 (v/v) (b) Ethanol (96%)-water 1:1 (v/v)

Table 2. PSA Test Result Data of Synthesized ZnO

PSA Analysis Result Data	ZnO + Extract 1:1	ZnO + Extract 2:1
Average Diameter (nm)	135.6	153.6
Polydispersity Index (PI)	0.33	0.221
Dispersity	Polydisperse	Monodisperse

The PSA test showed that the particles obtained were 135.6 nm for the 1:1 sample and 153.6 nm for the 2:1 sample. The particle size obtained met the requirements of a nanoparticle size of 10-1000 nm [29]. The polydispersity index (PI) value is used to estimate a sample's particle size distribution range and determine the aggregation's presence or absence. A low PI value indicates higher particle size homogeneity. The polydispersity index value is divided into three, namely monodisperse (less than 0.3), polydisperse (0.3 to 0.7), and superdispersed (more than 0.7). A polydispersity index value below 0.3 indicates that the particle size has a narrow distribution, while a polydispersity index above 0.3 indicates that the particle size has a broader distribution [47]. The study's results on the sample of ZnO nanoparticles ethanol-water 1:1 classified as polydisperse with a PI value of 0.33, while the sample of ZnO nanoparticles ethanol-water 2:1 classified as monodisperse with a PI value of 0.221. The difference in PI value is due to the amount of ethanol used in the ethanol-water variation (1:1) being less than the ethanol-water variation (2:1), so particle aggregation is more inhibited, and solvent-polymer interactions are weaker, so the particle size distribution is more expansive. However, the average diameter of the particles is smaller in the ethanol-water variation (1:1) [47].

4. Conclusions

The ratio of ethanol and water in the extraction solvent affects the number of secondary metabolites extracted from the horned banana peel in synthesizing ZnO nanoparticles. At a 1:1 ratio, the color of the solution was more intense than the 2:1 ratio, indicating higher metabolite levels. FTIR results showed ZnO peaks at 428 cm^{-1} , 441 cm^{-1} , and 619 cm^{-1} . PSA test showed the diameter of nanoparticles at a 1:1 ratio was smaller (135.6 nm) than at 2:1 (153.6 nm) due to higher flavonoid and polyphenol content at a 1:1 ratio, reducing agglomeration. Both meet the classification of nanoparticles (10-1000 nm).

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