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Characterization and Antibacterial Activity Test of Green Synthetic ZnO Nanoparticles Using Avocado (*Persea americana*) Seed Extract

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Abstract

The ability of cotton fabrics to absorb water creates several problems, such as providing an environment for bacterial growth. Antibacterial properties of textiles can be conducted by coating with nanoparticles with antibacterial activity. This study aimed to synthesize ZnO via green process nanoparticles using avocado seed extract (*Persea americana*), then characterize and evaluate its antibacterial activity on cotton fabrics. This research began with extracting avocado seed powder with distilled water. Then the avocado seed extract was mixed with $Zn(CH_3COO)_2.2H_2O$ and heated in a water bath at 70°C. The mixture was stirred while NaOH was added until the mixture reached pH 7, 8, and 9. The FTIR measurement of the avocado seed extract showed the presence of free hydroxyl and amino groups that act as reducing agents, capping agents, and stabilizers in the synthesis of ZnO nanoparticles. The XRD pattern of synthesized ZnO nanoparticles was hexagonal. The SEM results showed that the morphology of ZnO nanoparticles was spherical, with a particle size of 19.965 nm. Antibacterial activity was carried out on the cotton cloth coated with ZnO nanoparticles, resulting in an inhibition zone of 1.8 cm against *E. coli* and 1.97 cm against *S. aureus* bacteria. This study result indicated that ZnO nanoparticles have antibacterial activity by producing inhibition against *E. coli* and *S. aureus*.

1. Introduction

Cotton fabric is one of the most popular materials in apparel manufacture. The characteristics of cotton that make this material popular are that it is comfortable to use, degradable, renewable, and environmentally friendly. The surface of cotton fabric has a hydroxyl group (-OH) which makes this material hydrophilic. The ability of cotton fabrics to absorb water creates several problems, such as fabrics being easily stained and providing an environment for bacterial growth [1]. Therefore, the required material has an antibacterial activity to be applied to textiles. Semiconductor nanoparticles such as zinc oxide (ZnO) have broad applications in electronics, optics, optoelectronics, and biomedicine [2]. ZnO is a semiconductor material II and VI with a wide bandgap of 3.37 eV and an energy of 60 MeV. The wide bandgap in ZnO can be utilized in the semiconductor field [3]. ZnO possesses particular activity, heat resistance, resistance to UV rays, and the ability to absorb UV rays [4]. With the help of light, photocatalytic materials produce radical compounds that will bind to bacterial cells, resulting in bacterial damage and death [5]. The photocatalytic activity of ZnO can be utilized more optimally by fabricating ZnO into nanoparticles.

Nanoparticles can be synthesized using the sol-gel method. Sol-gel is a method of synthesizing nanoparticles in which the sample must go through the sol and gel stages [6]. The sol-gel method was chosen because it has several advantages: low temperatures, adjustable composition, and a homogeneous and pure layer [7]. The sol-gel method is carried out by adding

surfactants such as Polyvinyl Pyrrolidone (PVP) [8], Cetyl trimethylammonium bromide (CTAB), and Sodium Dodecyl Sulfate (SDS) [9], which are not environmentally friendly and offering a toxic effect on living organisms.

Research on the green synthesis of nanoparticles continues to develop because it is more environmentally friendly. The green synthesis process does not use toxic solvents, a time-consuming reflux process, high pressure, and high temperature [10]. Green synthesis of nanoparticles can be performed using yeast, fungi, algae, plant extracts, and microorganisms that have a role as reducing agents and capping agents [11]. The avocado seed is one of the materials that contribute to organic waste. It can be reduced by using it as reducing agents, capping agents, and stabilizers to synthesize nanoparticles. Avocado seeds contain fat, protein, fiber, minerals, and carbohydrates. Free amino groups present in proteins also have an essential role in the synthesis of nanoparticles [12].

Rajeshkumar and Rinitha (2018) have synthesized Cu nanoparticles using avocado seed extract and produced spherical nanoparticles 42–90 nm in size [13]. Girón-Vázquez *et al*. (2019) have successfully synthesized Ag nanoparticles using avocado seed extract and had antibacterial activity on *E. coli* bacteria [14]. Saridewi *et al*. (2021) have successfully synthesized ZnO nanoparticles using pumpkin seed extract with zinc acetate as a precursor with an optimum concentration of 1.5 M and pH of 8 [15]. Cakir *et al*. (2012) have researched fabrics that have been coated with ZnO nanoparticles that have antibacterial activity on *E. coli* and *S. aureus* bacteria [16]. Therefore, this study used the sol-gel method to synthesize ZnO nanoparticles using avocado (*Persea americana*) seed extract. Furthermore, its activity as antibacterial was evaluated against *E. coli* and *S.aureus* bacteria. This research aimed to produce ZnO nanoparticles with antibacterial activity.

2. Methods

2.1. Materials

The main ingredient used in this research was avocado seeds (*Persea americana*). Other ingredients were $Zn(CH_3COO)_2.2H_2O$ (Merck), NaOH (Merck), distilled water, NA media (Nutrient Agar), cotton combed 30s. *E. coli* test bacteria, *S. aureus* test bacteria (obtained from the Integrated Laboratory Center/ PLT UIN Syarif Hidayatullah Jakarta), and Amoxicillin 2%.

2.2. Equipment

The equipment used in this research were X-ray Diffraction (XRD) Shimadzu 7000, Fourier Transform Infrared (FTIR) Alpha II, and Scanning Electron Microscopy (SEM) Jeol, hot plate, magnetic stirrer, freeze dryer, oven, pH indicator, furnace, thermometer, centrifuge, blender. 500 mL Erlenmeyer, 250 mL beaker glass, 25 mL measuring cylinder, 100 mL measuring cylinder, 100 mL volumetric flask, 250 mL volumetric flask, drop pipette, stirring rod, petri dish, spatula, separating funnel, evaporating dish porcelain.

2.3. Synthesis of ZnO Nanoparticles

A 10 grams mashed avocado seeds (*Persea americana*) were put into a beaker, then added 100 mL of distilled water and a magnetic stirrer, then heated in a water bath at 100°C for 25 minutes, constantly stirring at 400 rpm. The extract was filtered using Whatman Grade 42 filter paper [13]. The avocado seed extract was then used to synthesize ZnO nanoparticles.

Ten mL avocado seed extract was added with 90 mL of $Zn(CH_3COO)_2.2H_2O$ 0.15M solution. After that, the solution was heated at 70°C for 1 hour in a water bath and constantly stirred. A 0.1 M NaOH was added to the mixture until pH 7, 8, 9 reached, then stirred for one hour to form colloidal ZnO nanoparticles. The resulting pale white solid ZnO was centrifuged at 25°C at 4000 rpm for 10 minutes. The solid was carefully washed with distilled water and dried in an oven at 100°C for 18 hours. After that, the solid ZnO was calcined using a furnace at 450°C for 4 hours [12].

2.4. Characterization

The avocado seed extract was characterized by Fourier Transform Infrared (FTIR) spectrophotometry to identify the functional groups contained therein. The crystallinity of synthesized ZnO nanoparticles was measured by X-ray Diffraction (XRD). Particle size and distribution were determined by Scanning Electron Microscopy (SEM). Then it was applied to Combed 30s cotton fabric. Cotton cloth was soaked in a solution of ZnO nanoparticles with various solution concentrations (2.5%, 5%, 7.5%, 10%), then placed on Nutrient Agar media which had been overgrown with *E. coli* and *S. aureus* bacteria. The antibacterial activity of the fabric was evaluated by the diameter of the zone of inhibition of bacterial growth.

3. Results and Discussion

3.1. Functional Group Analysis of Avocado Seed Extract (*Persea americana*)

The analysis of avocado seed extract using FTIR aimed to identify the functional groups of avocado seed extract. Analysis of avocado seed extract using FTIR was performed in the wavenumber range of 400–4000 cm^{-1}. Figure 1 shows the FTIR spectrum of the avocado seed extract.

Table 1 shows the FTIR spectrum of avocado seed extract generating the main peak positions, such as 3164–3308 cm^{-1}, 2831–2962 cm^{-1}, 1598 cm^{-1}, 1400 cm^{-1}, 1033 cm^{-1}, and 509 cm^{-1}. A wide peak at 3164–3308 cm^{-1} indicates the presence of the O-H functional group from the alcohol compound [17, 18, 19]. The bands at 2831–2962 cm^{-1} are associated with C-H stretching in the alkane chain. The existence of the N-H group is evidenced by the absorption of the wavenumber 1598 cm^{-1} contained in the amine compound. A peak of 1400 cm^{-1} corresponds to the bending O-H group found in alcohol compounds [12]. The vibrations of the C-O group in alcohol compounds and C-N stretching in aliphatic amine compounds appear at 1033 cm^{-1} [20]. The hydroxy group is important in synthesizing ZnO nanoparticles as a

reducing agent and stabilizer [12]. The free amine group in avocado seed extract acts as a stabilizer in synthesizing nanoparticles [21].

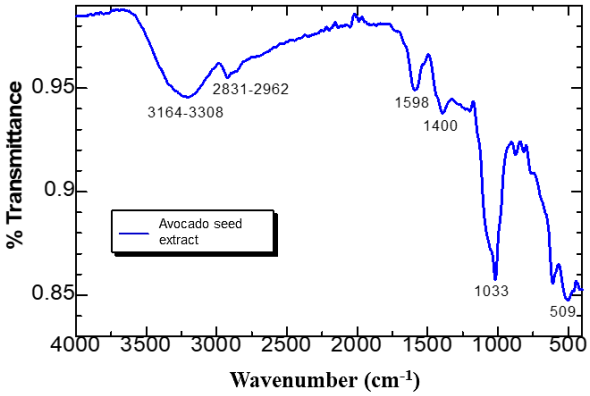

Figure 1. FTIR spectrum of avocado seed extract

Table 1. Results of the FTIR spectrum of avocado seed extract

Number	Wavenumber (cm^{-1})	Functional group vibration
1	3164–3308	O-H *stretching*
2	2831–2962	C-H *stretching*
3	1598	N-H *bending*
4	1400	O-H *bending*
5	1033	C-N *stretching* C-O *stretching*

Reactions that can occur in the process of synthesizing ZnO nanoparticles:

$$Zn(CH_3COO)_2.2H_2O + 2NaOH \rightleftarrows Zn(OH)_2 + 2CH_3COONa + 2H_2O \quad (1)$$

$$Zn^{2+} + 2OH^- \rightleftarrows Zn(OH)_2(l) \quad (2)$$

$$Zn^{2+} + 4OH^- \rightleftarrows Zn(OH)_4^{2-} \quad (3)$$

$$Zn^{2+} + 2OH^- \rightleftarrows ZnO + 2H_2O \quad (4)$$

In ZnO nanoparticles synthesis, H_2O and constant stirring aimed to re-dissociate $Zn(OH)_4^{2-}$ into Zn^{2+} and OH^- ions [12, 22]. The formation mechanism of ZnO nanoparticles is illustrated in Figure 2.

The hydroxy groups in avocado seed extract will reduce Zn^{2+} ions to Zn by adding NaOH. After that, Zn combines and forms clusters that will cause particle growth, where the growth rate can affect particle size. The functional groups in the avocado seed extract will interact with the Zn surface and envelop the formed Zn cluster, commonly known as capping. Capping agents are organic ligands used to bind selectively specific nanocrystals through an oriented chelating process [23], resulting in no aggregation between Zn clusters and the formation of stable ZnO nanoparticles. The hydroxy group plays a role in binding the Zn cluster, allowing the negatively charged ions to cover the particle's surface. This negative ion causes repulsion between similar charges, preventing aggregation between nanoparticles [24].

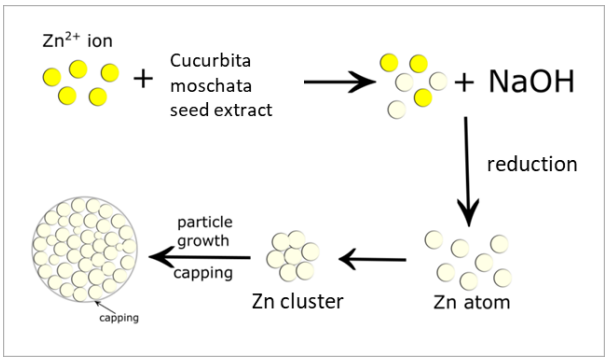

Figure 2. Formation mechanism of ZnO nanoparticles [11]

3.2. ZnO Nanoparticle Crystal Analysis Results

Analysis of ZnO nanoparticles using XRD aimed to measure the crystal size and level of crystallinity of the nanoparticles. XRD analysis was characterized the synthesized ZnO nanoparticles with variations in pH 7, 8, and 9. The pH of the sample was varied because the amount of OH^- and H^+ greatly affected the crystal formation process. The XRD results of several pH variations are as follows.

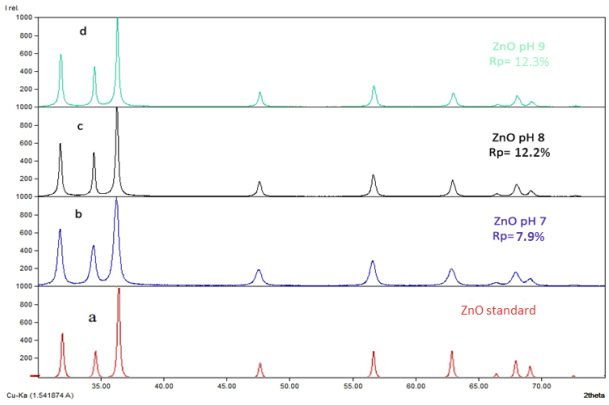

Figure 3. Diffraction pattern of standard ZnO (a) ZnO nanoparticles, (b) ZnO pH 7, (c) ZnO pH 8, (d) ZnO pH 9

From the XRD analysis, pH 7, 8, and 9 have the same diffraction pattern as the standard ZnO based on the Joint Committee on Powder Diffraction Standards (JCPDS) No. 36–1451, namely ZnO wurtzite with a hexagonal crystal system. The diffraction peaks in all ZnO nanoparticle samples produced narrow and strong peaks, indicating good crystallinity of ZnO nanoparticles [25].

Crystal size can be determined by calculating quantitative data from XRD analysis with Debye Scherrer equation. After being calculated by the Debye Scherrer equation, the crystal size can be seen in Table 2.

Table 2. Crystal size of ZnO nanoparticles

Number	Sample	Crystal Size (nm)
1	ZnO pH 7	19.532
2	ZnO pH 8	34.273
3	ZnO pH 9	36.832

Table 2 reveals that variations in pH affected the crystal size of the synthesized ZnO nanoparticles. ZnO nanoparticles that have the smallest crystal size were ZnO nanoparticles synthesized with pH 7 of 19.532 nm.

Meanwhile, the largest crystal size was found in ZnO nanoparticles synthesized with pH 9 of 36.832 nm. From the crystal size results, the optimum pH for synthesizing ZnO nanoparticles using avocado seed extract was pH 7.

The number of H^+ and OH^- ions in solution significantly affects the synthesis of ZnO nanoparticles. When the pH is increased, which increases the concentration of OH^-, the concentration of H^+ ions decreases compared to when the solution was acidic. The presence of OH^- ions will affect the formed crystal structure. A highly concentration of OH^- ions will cause the hydrolysis and condensation processes during the synthesis of ZnO nanoparticles to be increasingly uncontrolled, resulting in nanoparticles with irregular shapes and sizes [26].

3.3. Morphological Analysis Results of ZnO Nanoparticles

SEM analysis of ZnO nanoparticles aimed to determine the surface morphology and particle size. ZnO nanoparticles synthesized at pH 7 were characterized by SEM because they have the smallest crystal size. The SEM image was obtained at 5,000x magnification (Figure 4). The data obtained were processed using Image J software to determine the particle size of ZnO nanoparticles.

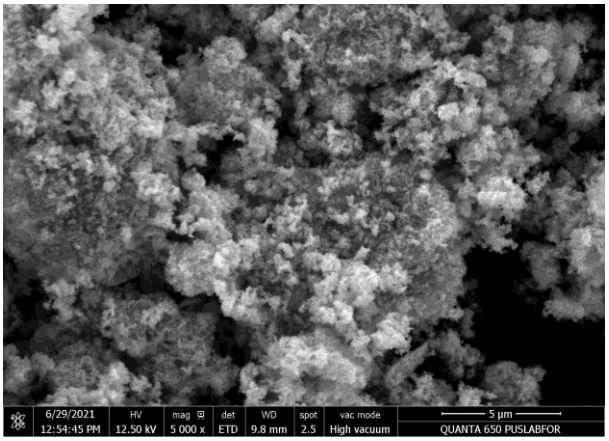

Figure 4. SEM image of ZnO nanoparticles

SEM image shows ZnO nanoparticles that are spherical and clustered. The particle size of the synthesized ZnO at pH 7 was 19.965 nm. The particle size distribution can be seen in Figure 5 after processing the data using Origin.

Figure 5 shows a graph of the particle size distribution of ZnO nanoparticles. The graph shows that ZnO nanoparticles with a 10–15 nm particle size have the most significant frequency, and this indicates that the particles with the most frequencies are in the range of 10–15 nm. The particle size distribution graph shows that the synthesized ZnO nanoparticles were evenly distributed even though particles indicated more than 10–15 nm agglomeration. Agglomeration can be caused by ZnO nanoparticles' polarity and electrostatic attraction [27]. The particle size in this study was smaller than the ZnO nanoparticles synthesized by Saridewi *et al.* (2021) using pumpkin seed extract (*Cucurbita moschata*), which was 28.07 nm [15]. In addition, the particle size in this study is smaller than the ZnO nanoparticles synthesized by Nurbayasari *et al.* (2017) using green seaweed extract (*Caulerpa sp*), which is 370.72 nm [12]. In conclusion, the synthesis of ZnO nanoparticles was successfully performed using avocado seed extract as a reducing agent, capping agent, and stabilizer.

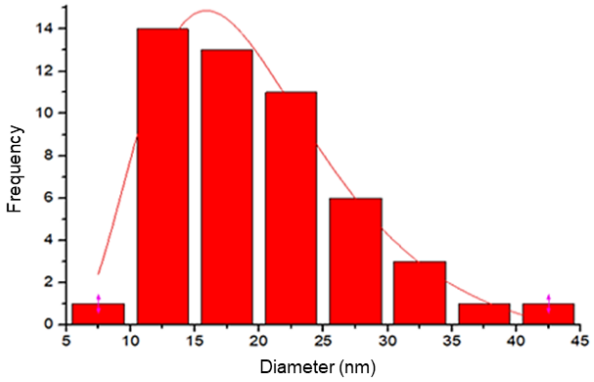

Figure 5. Particle size distribution graph of ZnO nanoparticles synthesized at pH 7

3.4. Isolation and Partial Purification of Compounds

In this study, antibacterial testing was conducted using the agar diffusion method. The medium used was Nutrient Agar (NA). The agar diffusion method generally utilizes disc paper, but this study directly used Combed 30s cotton cloth. The ZnO nanoparticles were synthesized at pH 7 because they had the smallest crystal size and the largest surface area. The nanoparticle solution has concentration variation of 2.5%, 5%, 7.5%, and 10% with 95% ethanol as a solvent. Amoxicillin 2% was used as the positive control, while the negative control used distilled water. The bacterial growth inhibition zone was calculated in this antibacterial test, which is presented in Table 3.

Table 3. Antibacterial inhibition of ZnO nanoparticles

Number	Bacteria	Repetition	Zone of Inhibition (cm)					
			ZnO 2.5%	ZnO 5%	ZnO 7.5%	ZnO 10%	Distilled water	Amoxicillin
1	*S. aureus*	1	1.6	1.7	1.9	1.8	0	4.3
		2	1.6	1.6	2	1.8	0	4.5
		3	1.4	1.7	2	1.5	0	4.1
	Average		1.53	1.67	1.97	1.7	0	4.3
2	*E. coli*	1	1.4	1.3	1.8	1.3	0	3
		2	1.4	1.6	1.7	1.6	0	2.9
		3	1.2	1.5	1.9	1.5	0	2.9
	Average		1.33	1.47	1.8	1.47	0	2.93

The antibacterial test on ZnO nanoparticles generated data on the diameter of the clear zone (zone of inhibition), indicating bacterial growth inhibition in all variations in the concentration of ZnO nanoparticles (Figure 6). The diameter of the largest inhibition zone was found at 7.5% ZnO nanoparticles in *S. aureus* and *E. coli* with an average inhibition of 1.97 cm and 1.8 cm, respectively. Meanwhile, the smallest inhibition zone diameter was seen in *S. aureus* and *E. coli* at a 2.5% ZnO nanoparticles concentration with an average inhibition zone diameter of 1.53 cm and 1.33 cm, respectively. This study resulted in a larger inhibition zone diameter than the research conducted by Nithya and Kalyanasundharam (2019) using ZnO nanoparticles synthesized by a chemical

method with inhibition zones of 10 mm (*E. coli*) and 13 mm (*S. aureus*) [28].

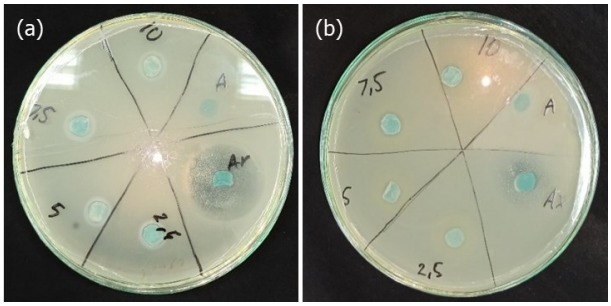

Figure 6. Antibacterial test results (a) *S. aureus* (b) *E. coli*

From the data in Table 3, it is known that the high concentration of dissolved ZnO nanoparticles leads to more significant inhibition of bacterial growth [29], as seen from the concentration of ZnO nanoparticles 2.5%–7.5%, while the inhibition of bacterial growth decreased at 10% ZnO nanoparticle concentration. At a concentration of 10% ZnO nanoparticles, the inhibition of bacterial growth decreased because the solution was too saturated, resulting in a decreased inhibition of bacterial growth. Increasing the concentration can also affect the interaction or adsorption process of nanoparticles on the surface of cotton fabrics. Another study conducted by Musdalifa *et al.* (2019) also reported that increasing the concentration of ZnO nanoparticles to 10% in cotton fabrics decreased the inhibition zone for *S. aureus* bacteria [30].

From the observation of the inhibition zone diameter, it was found that the inhibition zone diameter for *S. aureus* bacteria was more significant than for *E. coli* bacteria. *E. coli* (gram-negative) bacteria have a more complex cell wall than *S. aureus* (gram-positive) bacteria. The cell wall of gram-negative bacteria consists of three polymers, namely lipoprotein (outer layer), lipopolysaccharide (middle layer), and peptidoglycan (inner layer). The outer membrane of gram-negative bacteria is in the form of a bilayer; thus, gram-negative bacteria have good resistance to compounds that enter their cells and toxic properties [31]. The cell wall of *S. aureus* (gram-positive) bacteria is composed of polysaccharides, so it is easier to denature than *E. coli* bacteria (gram-negative) cell wall, which is composed of phospholipids [32].

The antibacterial mechanism by ZnO nanoparticles involves the formation of ROS (Reactive Oxygen Species) and the release of Zn^{2+} ions. The ROS produced can cause mitochondrial weakness, intracellular outflow, and the release of oxidative stress gene expression that causes inhibition of bacterial growth and even bacterial cell death [5]. Metal protein is suspected of assisting the release of Zn^{2+} and penetrating that ion into bacterial cytoplasm. Furthermore, Zn^{2+} ions penetrate the cytoplasm, causing bacterial cell death. ZnO nanoparticles have good antibacterial activity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria.

4. Conclusion

Avocado seed extract contains free hydroxyl and amino groups that can act as reducing agents, capping agents, and stabilizers in the synthesis of ZnO nanoparticles. XRD results showed ZnO nanoparticles in the form of hexagonal wurtzite with different crystal sizes, depending on the pH used for preparation. Experiments with pH 7, 8, and 9 resulted in particle sizes of 19.532, 34.273, and 36.832 nm, respectively. Based on the SEM results, the particle size was 19.965 nm. The zone of inhibition of bacterial growth by ZnO nanoparticles was 1.8 cm for *E. coli* and 1.97 cm for *S. aureus*.

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