Original Research Article

Biosynthesis of ZnO Nanoparticles Using *Spirulina platensis* Based on Calcination Temperature Changes and Its Antioxidant Activity

ABSTRACT

The effect of different calcination temperatures on molecular structure, morphology, and antioxidant activity was investigated for Zinc Oxide nanoparticles synthesized using the sol-gel method and the capping agent *Spirulina platensis*. The prepared nanoparticle ZnO was calcined at 160°C, 300°C, and 600°C according to the results of the DTA-TGA analysis. The effect of different calcination temperatures on the characterization of the prepared samples was studied using Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), and Field Emission-Scanning Electron Microscope (FE-SEM). In addition, the potential antioxidant activity of ZnO nanoparticles was investigated using the DPPH method. The results showed that FT-IR and XRD confirmed the presence of ZnO nanoparticles with good purity and small crystal size found in calcified ZnO nanoparticles at 600°C. FE-SEM confirmed the morphology ZnO nanoparticle produced at 600°C calcination are spherics, cubes, and nanorods with different particle sizes with range 50 – 150 nm. ZnO nanoparticles calcined at 600°C also showed higher antioxidant activity when compared to other calcination temperatures.

Keywords: biosynthesis, zinc oxide, capping agent, Spirulina platensis, calcination, antioxidant

1. INTRODUCTION

Nanotechnology is widely used in research to synthesize nanomaterials whose size and shape can be controlled so that they can function as desired [1]. Nanotechnology using environmentally friendly materials is increasingly being developed because it can affect the size and quality of the resulting nanomaterial products and can reduce or eliminate pollution from hazardous materials [2]. Nanomaterials/ nanoparticles are nano-sized materials that can be synthesized using a simple method, and the resulting product can be adjusted to the desired morphology [3]. Nanoparticles have unique characteristics, such as large surface

area, high reactivity, and high catalytic efficiency. Because they have a very small size (high surface area), nanoparticles have high absorption and efficiency compared to their bulk materials [4].

Nanomaterials with environmentally friendly methods (green method) can be synthesized by various methods such as sol-gel [5], hydrothermal [6], solvothermal [7], and precipitation [8]. The synthesis of nanoparticles using the sol-gel method is a cheap and simple method with many advantages, including producing high-quality nanoparticles, relatively low processing temperatures, and the resulting nanoparticles being more homogeneous [9]. Biosynthesis with the green method usually involves natural ingredients as capping agents. Capping agents are very important as stabilizers that prevent their aggregation/coagulation and steric hindrance in the synthesis of nanoparticles, change the biological activity and surface chemistry, and stabilize the interactions of nanoparticles in the prepared media [10], [11]. The capping agent must be biodegradable, well dispersed and soluble, biocompatible, and non-toxic so that the waste material does not damage the environment [10]. Capping agents commonly used are carbohydrates (glucose, lactose, sucrose, fructose, cellulose, starch, and chitosan), proteins (collagen, enzymes, and albumin), amino acids (protein and non-protein), lipids, honey, nucleic acids (DNA), and biological extracts (plants, algae, bacteria, viruses, and fungi) [11]. Recently, algae have often been used as a capping agent in the synthesis of nanoparticles. Algae can be used in nanoparticle biosynthesis in intercellular or extracellular ways, depending on their cellular reactions within the cell, their primary and secondary metabolites, or the extraction of other algal contents. Algae are autotrophic unicellular or multicellular organisms categorized between prokaryotes and eukaryotes. Its size varies between microalgae (microscopic) and macroalgae (large size), and it has been classified into Bacillariophyta, Chlorophyta Chrysophyta, Cyanophyta, Dinophyta, Phaeophyta, and Rhodophyta [12]. *Spirulina platensis* is a cyanobacterium known as blue-green microalgae, which has important appeal as a natural source of bioactive compounds with a range of biological activities such as antimicrobial, antiviral, anticancer, antioxidant, and anti-inflammatory [13]. *Spirulina platensis* lives naturally in tropical and subtropical lakes with high pH and high carbonate and bicarbonate concentrations [14]. Mostafa M. El-Sheekh et.al. (2022), reported that nanoparticles synthesized with microalgae can be applied as drug delivery and biosensors which can be used in tissue engineering, tumor destruction, anticancer treatment, antimicrobial, biosensors, food preservation and packaging, water treatment, and many other applications that can improve human life [12].

ZnO nanoparticles have received extraordinary attention because of their potential use as chemotherapeutic agents (anticancer) and antimicrobials, and antioxidants because ZnO nanoparticles are capable of absorbing UV rays and have high photostability [15-16]. ZnO nanoparticles are included in the category of metal oxides which are generally recognized as safe metal oxides (generally recognized as safe-GRAS) [15]. A.M. Ismail et al., (2019) reported that the effect of different calcination temperatures will affect the purity of the resulting ZnO nanoparticles, crystal size, and morphology of the product produced [4]. In this research, the synthesis of ZnO nanoparticles was carried out using the sol-gel method and *Spirulina platensis* as a capping agent, with different calcination temperatures of 160°C, 300°C, and 600°C. The resulting ZnO nanoparticles will be studied for their antioxidant activity.

2. METHODOLOGY

2.1. Preparation of *Spirulina platensis*

One liter of *Spirulina platensis* was added to bottles containing 10 liters of water. Then, fertilizer, KCL, NPK, ZA, urea, baking soda, and table salt were added and stirred until

completely dissolved. The bottle was installed with an air bubble and kept for ± 10 days. After 10 days, *Spirulina platensis* was harvested by filtering water in nursery bottles, and the biomass was collected to the Petri dish, then dried at room temperature. The dried biomass was milled to obtain *Spirulina platensis* powder

2.2. Biosynthesis of ZnO Nanoparticles

ZnO was synthesized with 0.126 M $Zn(NO_3)_2.6H_2O$ and 0.25 M citric acids ($C_6H_8O_7$) in 100 mL of distilled water and stirred at 70°C for 2 hours to form a solution. Subsequently, the solution was placed in the oven at 100- 110°C for 5 hours for gel formation. The 1M NaOH solution was dripped directly into the gel with constant stirring, and the pH of the suspension was adjusted to 9, and stirred for 2 hours. Afterward, ZnO seeds, 1.089 grams of *Spirulina platensis*, and 5% PEG were added and stirred for 2 hours. Then, the resulting suspension (±100 mL) was transferred into a Teflon-coated stainless-steel autoclave and heated at 160°C for 12 hours. The product was dried and calcinated at 300 and 600°C for 4 hours [17]. ZnO nanorods were characterized using DTA-TGA, FTIR, XRD, and FE-SEM.

2.3. Antioxidant Activity

A total of 0.1 g of ZnO-NPs was added to 1 mL of a methanol solution containing the DPPH radical (2,2-diphenyl-1-picryl hydroxyl) (0.012 g/100 mL). The solutions were mixed until homogeneous and then incubated for 30 minutes at room temperature in a dark room. The absorbance was then read at 517 nm against a blank. DPPH solution and ascorbic acid were used as a control and positive control, respectively. The scavenging ability was calculated using the following equations [18]:

$$\text{Scavenging activity} = \frac{A\,517\ control - A\,517\ sample}{A\,517\ control}\, x\ 100\%$$

3. RESULTS AND DISCUSSION

3.1. DTA-TGA (*Thermogravimetric Analysis* - Differential Thermal Analyzer)

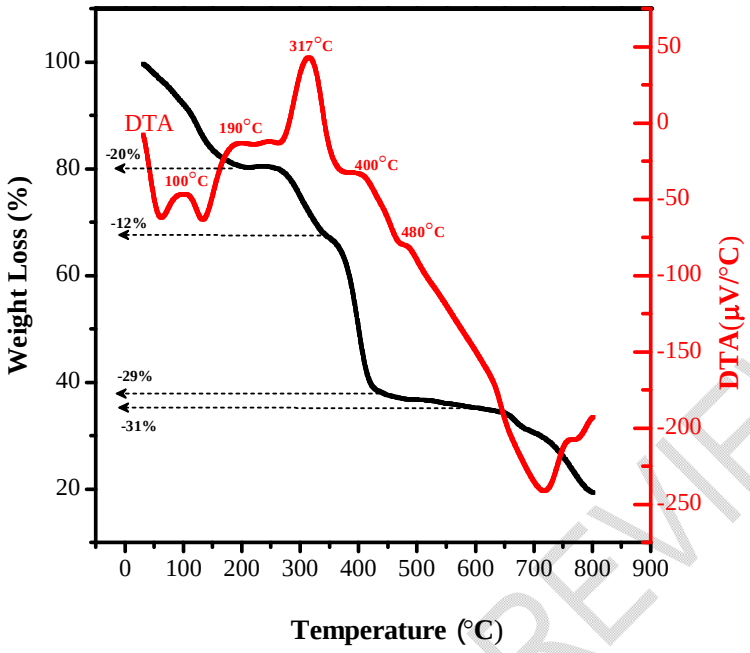

Figure 1. TGA-DTA pattern of ZnO nanoparticles synthesized with *Spirulina platensis* as a capping agent.

The DTA-TGA data were taken at a temperature range of 30°C to 800°C and the ZnO nanoparticle samples had been dried at 100°C. The TGA pattern in Figure 1 above illustrates an overall weight/mass reduction of 63% which results in excellent thermal stability of the synthesized ZnO nanoparticles. This proves that mass reduction 33 consists of four distinct stages, which are shown together with their respective endothermic peaks in the DTA spectrum. In the temperature range of 30-200°C there was a mass decrease of 20% and the endothermic peaks at 100°C and 190°C indicated the loss of water content and volatile components on the surface of the ZnO nanoparticles. The mass is reduced by 12% in the next step over a temperature range of 270-340°C with an endothermic peak at 312°C. In this range, the weight loss is caused by the loss of amine compounds, hydroxyl, and some carboxyl compounds according to the FTIR analysis in Figure 1. The next weight loss occurs in the temperature range of 340-430°C by 29% and the endothermic peak is at 400°C, which is a decrease in the mixture of organic residues. The reduction of organic residues was continued at a temperature range of 430-500°C by 2% and there was no further reduction in the mass of ZnO nanoparticles up to a temperature of 700 °C which indicated that the thermal stability of the formation of ZnO nanoparticles had been achieved. Based on the pattern of TGA and DTA, the calcination temperature was chosen at 600°C. The stages of mass reduction are in accordance with those reported by R. Vinayagam et al. (2022) who synthesized ZnO anoparticles using Muntingia calabura leaf extract. [19]

3.2. FTIR (Fourier Transform Infra-red)

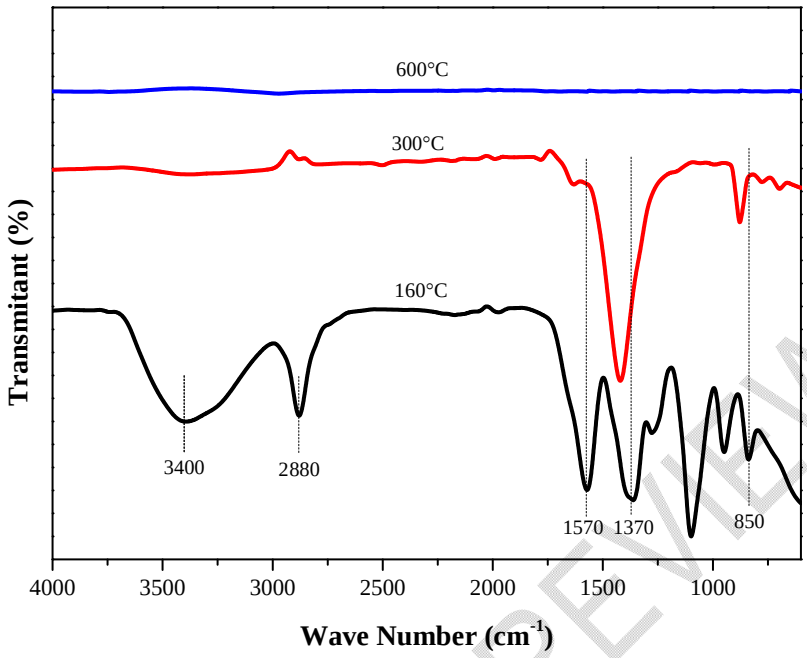

Figure 2. FTIR spectrum of ZnO synthesized at pH 9 at temperatures of 160°C, 300°C and 600°C.

The FTIR characterization of the ZnO NPs product aims to identify the functional groups present in the *Spirulina platensis* microalgae and the biosynthetic products. In Figure 2 above, it can be seen how the stability of the capping agent of the microalgae *Spirulina platensis* when used for ZnO biosynthesis at pH 9 is affected by temperature. At a temperature of 160°C (hydrothermal yield), there are still organic compounds marked by an absorption peak at wave numbers 3402.18 cm^{-1}; 2876.47 cm^{-1}; 1577.93cm^{-1}; 1368, 62 cm^{-1}; 1100.51 cm^{-1} and 837.31 cm^{-1}. This absorption peak is the same as the peak of *Spirulina platensis* as shown in Figure 2. Whereas ZnO nanoparticles at a temperature of 300°C show that many organic compounds have been lost during heating, and there are still carboxyl groups (-CO) at wave number 1422.52 cm^{-1} and stretching of the amide group C=O, vibration of the C=C carbonyl stretch, and symmetrical or asymmetric stretching of the carboxylate and C-O groups at wave number 869.50 cm-1. Calcination at 600°C has no visible absorption peaks, which indicates that the organic compounds and other impurities are gone and pure ZnO nanoparticles have been formed.

3.3. XRD (X-ray Diffraction)

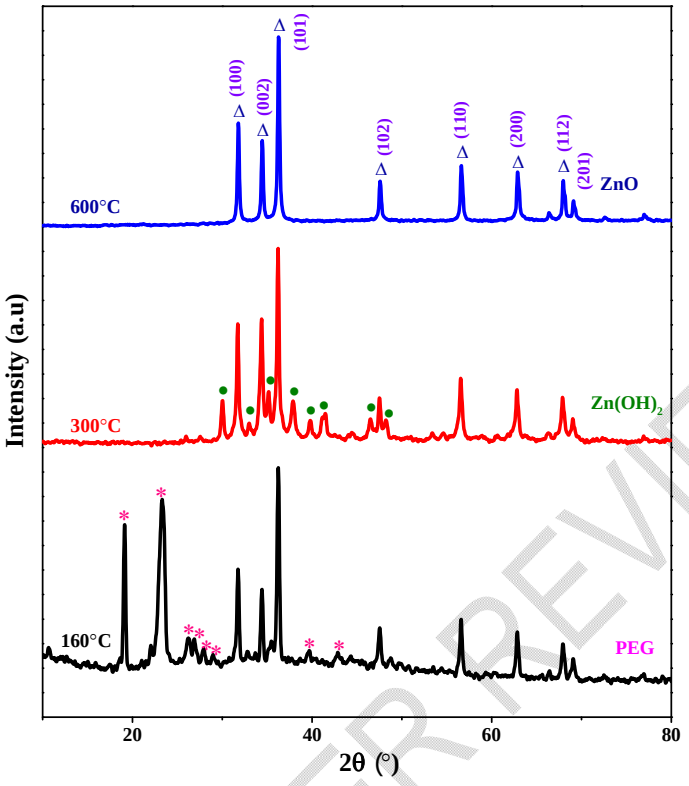

Figure 3. XRD diffraction pattern of ZnO nanoparticles at a temperature calcination of 160°C-600°C (Rev: hkl on picture added)

In Figure 3, it can be seen that after the hydrothermal process, ZnO crystals have formed at angles of 2θ 31.74° ; 34.40° ; 36.23° ; 47.53° ; 56.59° ; 62.86° ; 66.38° ; 67.96° and 69.10°, but there are still other peaks at angles of 2θ 19.11° and 23.53° which are the peaks of polyethylene glycol (PEG), and peaks at 2θ 32.93° and 39.46° which are the peaks of Zn(OH)2. After calcining at 300°C, the PEG peak has disappeared and the $Zn(OH)_2$ peak is still present at an angle of 2θ 30.01°; 33.00°; 37.87°; 39.78° and 41.45°. Whereas after calcining at 600°C, no other peaks associated with impurities appeared, indicating that high-purity ZnO nanoparticles had been obtained. In addition, the peak of ZnO nanoparticles at 600°C is also higher, sharper, and has a small FWHM intensity. Akbarian (2018) reported that the smaller the FWHM and the higher the peak intensity, the higher the crystallinity. [20]. The sharp and narrow peaks on the ZnO nanoparticle samples which were calcined at 600ºC prove that the nanoparticles have high crystallinity when compared to the ZnO nanoparticle samples which yielded hydrothermal at 160ºC and calcination at 300ºC. The XRD pattern of the synthesized metal oxide ZnO at a calcination temperature of 600°C can be observed, where the intensity is at 2θ = 31.74° ; 34.40° ; 36.23° ; 47.53° ; 56.59° ; 62.86° ; 66.38° ; 67.96° and 69.10° show the crystal structure of ZnO is wurtzite and have crystal planes with hkl values (100), (002), (101), (102), (110), (200), (112), and (201) with the hexagonal unit cell conforms to the ICSD standard -15772476.

3.4. FE-SEM (Field Emission-Scanning Electron Microscope)

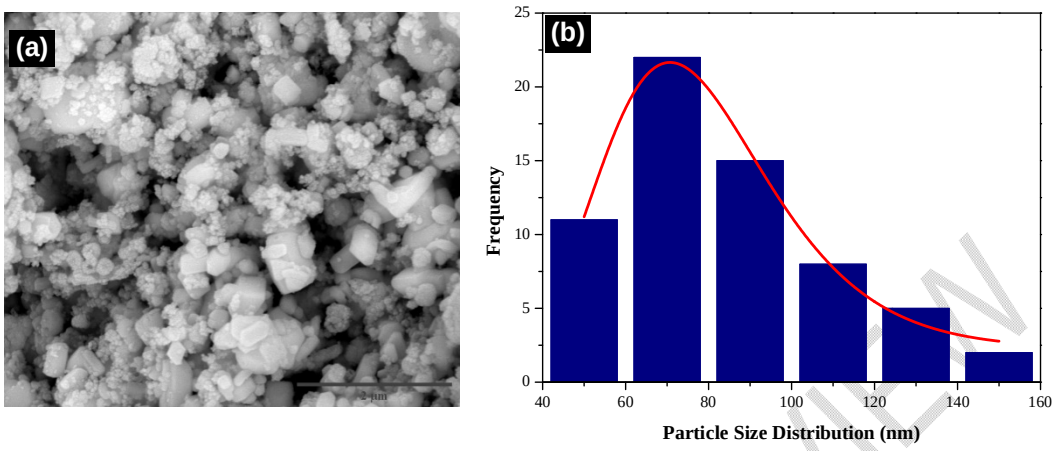

Figure 4. FE-SEM pattern of ZnO nanoparticles (a) and particle size distribution (b) from calcination at 600°C.

FE-SEM is an electron microscope that images the surface of a sample by scanning it with high-emission electrons. Field Emission Scanning Electron Microscopy (FE-SEM) analysis is used to determine the surface morphology with a certain magnification and particle size distribution. Figure 4 (a) below shows the FE-SEM pattern of ZnO nanoparticles after being calcined at 600°C. This figure shows that the ZnO nanoparticles produced have various morphologies, namely nanocubes, nanospheres, and nanorods. There is a lot of agglomeration, hence, the morphology formed is not clearly visible. Agglomeration usually occurs during the synthesis of NPs in aqueous media. It also occurs due to polarity, electrostatic attraction, and high surface energy of ZnO-NPs Figure 4 (b) shows the particle sizes of ZnO nanoparticles are in range of 50 - 150 nm.

3.5. Antioxidant Activity

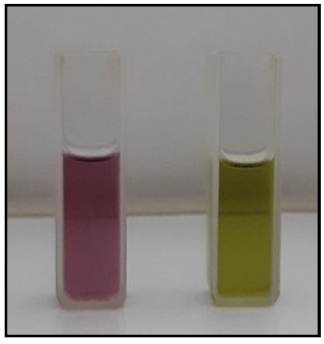

Figure 5. DPPH with dark purple color changed to yellow color after added by ZnO nanoparticle.

Determination of antioxidant levels aims to see the ability of ZnO to scavenge free radicals from DPPH (2,2-diphenyl-1-pikrilhidrazyl). DPPH is a stable radical whose color is dark purple and will turn yellow when reduced by other substances or other radicals as show at Figure 5. In table 1 above, the antioxidant activity of ZnO calcined at 600C shows a higher number, namely 84.0%. Ascorbic acid was used as a control, with an antioxidant level of 98.3%.

Table 1. Antioxidant activity of ZnO nanoparticles

Calcination Temperature	Antioxidant Activity (%)
160°C	63,9
300°C	72,1
600°C	84,0
Ascorbic acid	98,3
Spirulina platensis	54,4

4. CONCLUSION

ZnO nanoparticles have been successfully synthesized using the sol-gel method with the addition of *Spirulina platensis* as a capping agent. The difference in calcination temperature affects the purity and crystal size of the resulting ZnO nanoparticles. Calcination at 600°C showed high purity of ZnO nanoparticles, high crystallinity, and smaller crystal size than other calcination temperatures. ZnO nanoparticles resulting from calcination at 600°C also showed high antioxidant activity of 84.0%.

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