EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 1
265-276
Year: 2024

BIOSYNTHESIS OF ZNO NANOPARTICLES USES BINAHONG (ANREDERA CORDIFOLIA (TEN) STEENIS) LEAF EXTRACT

Delvina Lumban Gaol, Anak Agung Bawa Putra*, I. Wayan Suarsa, I. Made Siaka, Ni Luh Rustini and Sri Wahjuni

Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia.

***Corresponding Author: Anak Agung Bawa Putra**

Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia.

Article Received on 21/11/2023 **Article Revised on 11/12/2023** **Article Accepted on 01/01/2024**

ABSTRACT

Nanoparticles have superior properties compared to similar particles which have a larger size. Nanoparticle biosynthesis was developed as an alternative method by utilizing reductants from plants so they are more environmentally friendly. This research aims to carry out biosynthesis and characterization of ZnO nanoparticles using binahong (*Anredera cordifolia* (Ten) Steenis) leaf extractas a bioreductor and stabilizer. ZnO nanoparticles were synthesized from the precursor material $Zn(CH_3COO)_2.2H_2O$ 0.15 M at pH 8 and calcination was carried out at 400°C for 2 hours. Biosynthesisof ZnO nanoparticles with a bioreductant from binahong leaf extract (*Anredera cordifolia* (Ten) Steenis) produces ZnO particles with an average diameter of 46.07 nm. Secondary metabolites which are thought to act as bioreductors and stabilizing agents from binahong leaf extract are polyphenolic compounds. Synthesis of ZnO nanoparticles produces an elemental composition of 67.8% Zn and 28.2% O with a ratio of 2:1.

KEYWORDS: Bioreductor, Binahong leaves, ZnO nanoparticles.

INTRODUCTION

Nanotechnology research continues to develop, both for the purposes of developing nanoscience and the application of technology. Therefore, researchers continue to compete to realize new discoveries in the field of nanotechnology. One nanotechnology that has attracted a lot of interest from researchers is the development of nanoparticle synthesis methods.

Nanoparticles can be formed either naturally or through a synthetic process. Existing nanoparticles can be metals, semiconductors, metal oxides, polymers, and organic compounds (Nurbayasari *et al*., 2017). Nanoparticle synthesis aims to create particles with a size of less than 100nm and have specific properties and functions (Fazrin *et al*., 2020).

The metal oxide is one of the nanoparticles that are interesting to synthesize because the change in particle size of the metal oxide shows physical properties such as dimensions and uniform size distribution, morphology, crystallinity, and better chemical properties compared to the metal oxide when it is large or bulk material (Saravanadevi *et al*., 2020). ZnO (Zinc Oxide) is a metal oxide compound that has great potential to be used as metal oxide nanoparticles because it is non-toxic and able to provide high mobility and good thermal stability (Preethi *et al*., 2020).

ZnO nanoparticles can be synthesized conventionally on a large scale using physical (top- down) and chemical (bottom-up) methods such as sol-gel, hydrothermal, and mechanochemical methods (Chan *et al*., 2021). These methods show inefficient results because the process is still complex, low product yields, long reaction times, the use of high temperatures, the use of large amounts of energy, and the use of toxic chemicals as reducing and stabilizing agents such as polyetherimides (PEI), polyethylene glycol (PEG), and polyacrylic acid (PAA) which have the potential to be pollutants for the environment (Nasrollahzadeh *et al*., 2019).

To control the adverse consequences of the ZnO nanoparticle synthesis process using conventional methods, it is necessary to develop a method for synthesizing ZnO nanoparticles so that the effects of environmental pollution can be controlled. One of the methods developed is a biosynthesis method (green synthesis) which is environmentally friendly, but still produces ZnO nanoparticles with good characteristics (Nurbayasari *et al*., 2017).

Green synthesis is a modified chemical method that utilizes extracts of animal organisms, plants/plants, and microorganisms as metal bioreductors. The synthesis of nanoparticles using the green synthesis method is supported by the availability of natural resources in Indonesia which are very abundant and of various types, making it possible to obtain natural reducing agents and particle stabilizers in the synthesis process. Plants containing secondary metabolites have the potential to be used as bioreductors in the synthesis process of metal nanoparticles (Sugiyarti *et al*., 2021). Agarwal (2017) states that the use of phytochemical compounds contained in plants such as alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids can be used as reducing and stabilizing compoundsso that they can be used to reduce metals into metal nanoparticles. The ability of plants containing antioxidant compounds and polyols has the potential to reduce zinc oxide to nanometer size (Saravanadevi *et al*., 2020).

ZnO nanoparticles synthesized using plant extracts are more stable than those using extracts from other organisms (Rajakumar *et al*., 2018). Various kinds of plant extracts have been used as bioreductors in the green synthesis nanoparticle process, including the biosynthesis of ZnO with *caulera* sp extract by Nurbayasari *et al*., (2017) produces Spherical shaped nanoparticles, biosynthesisof ZnO with *Carica papaya* L (papaya leaves) by Ramadanti *et al*., (2022) produces an average size of 39.60 nm which is hexagonal, and the biosynthesis of ZnO nanoparticles with muicle plant extract(*Justicia spicigera*) by Soto-Robles *et al*., (2020) produces nanoparticles with an average size of 55 nm in the shape of rods.

One plant extract that can also be used as a bioreductor in the green synthesis process of ZnO is the Binahong plant (*Anredera cordifolia* (Ten) Steenis) (Tjiang *et al*., 2020). In his research, Tjiang*et al*., (2020) succeeded in synthesizing Ag/CoFe2O4 nanoparticles using binahong leaf extract with an average size of 47 nm. Binahong is a type of medicinal plant that has many benefits and is well- known as a medicinal plant that has been used by the community to treat diseases (Wattimena and Patty, 2017). This plant is known to have a high antioxidant content (IC50 of 40.27 ppm(Veronita *et al*., 2017).

Based on the background above, in this research, ZnO nanoparticles were synthesized using binahong (*Anredera cordifolia* (Ten) Steenis) leaf extract which can function as a reducing agent or capping agent to provide a strong coating on metal nanoparticles. Therefore, this experiment was carried out to determine the results of the synthesis of nanoparticles formed using a bioreductant frombinahong leaf extract.

MATERIALS AND METHODS

Research Materials and Equipment

Binahong leaves (*Anredera cordifolia* (Ten) Steenis) taken in the South Kuta area, Badung Regency, Bali Province, Indonesia, $Zn(CH_3COO)_2.2H_2O$ (pa), standard ZnO (pa), NaOH (pa), distilled water, KBr (pa), Dragendorff reagent (pa), anhydrous acetic acid (pa), concentrated sulfuricacid (pa), and $FeCl_3$ reagent (pa).

The equipment used is a measuring flask, beaker glass, Whatman filter papernumber 1,stir bar, measuring cup, drop pipette, drop plate, watch glass, mortar, funnel, test tube, pH meter, analytical balance, hotplate stirrer, magnetic stirrer, oven, centrifuge, furnace, UV-Vis Double Beam spectrophotometer (Shimadzu/ UV - 18000), Shimadzu FTIR-21 spectrophotometer, Particle Size Analyzer (PSA) pro blue Malvern, Scanning Electron Microscopy (SEM) SEM JSM IT-200, and Transmission Electron Microscopy(TEM) TEM JEOL JEM-1400).

Method

Extraction of binahong leaves (Anredera cordifolia (Ten) Steenis)

Leaf binahong (*Anredera cordifolia* (Ten) Steenis) used for the green synthesis process was taken in the South Kuta area, Badung Regency, Bali Province, Indonesia. A total of 100 g of binahong leaf powder was dissolved in 500 mL of distilled water. The solution was stirred using a stirrer at a temperature of 50^{o}C with a speed of 300 rpm for 3 hours. Next filtered using Whatman paper No.42 (Daphedar and Taranath, 2018).

The extract obtained was then subjected to phytochemical testing to test the content of secondary metabolite compounds contained in the binahong leaf extract. Phytochemical tests were carried out by reacting several reagents with binahong leaf extract. Binahong leaf extract was characterized using FTIR to determine the functional groups contained in binahong leaf extract (Puspitasari *et al*., 2021).

Biosynthesis of ZnO nanoparticles

The source of Zn was a zinc acetate dihydrate solution ($Zn(CH_3COO)_2.2H_2O$) by weighing 1,650 g of zinc acetate dihydrate then dissolving it in distilled water and then setting the volume to 50mL (Iwan *et al*., 2020).

A total of 25 mL of thick binahong leaf extract was mixed with 50 mL of $Zn(CH_3COO)_2.2H_2O$ solution. Then the mixture was stirred using a magnetic stirrer at a speed of 400–600 rpm at room temperature. During the stirring process, the NaOH solution was added drop by drop until the solution reached pH 8. Stirring was continued for 3 hours until the solution turned pale yellow. The change in color of the solution to pale yellow indicates that ZnO nanoparticles have been formed (Santhoshkumar *et al*., 2017). The next stage was centrifuged for 20 minutes at a speed of 4000 rpm. The resulting sediment is washed using distilled water to remove organic residue or dirt that is still present in the sediment. After that, the resulting precipitate was dried using an oven at 150^{o}C for 3 hours (Fastaqibul and Dina, 2022). ZnO nanoparticles were obtained through a calcination process using a furnace at a temperature of 400^{o}C for 2 hours.

The ZnO nanoparticles formed were analyzed using a UV-Vis spectrophotometer, FTIR spectrophotometer, SEM, and PSA to determine the characteristics of the ZnO nanoparticles.

Characterization of ZnO nanoparticles

The ZnO nanoparticles produced from the synthesis process are then characterized to determine the characteristics of the synthesized ZnO nanoparticles. In this research, measurements were also carried out on standard ZnO as a comparison to show that there had been a shift/difference in the characteristics of the synthesized ZnO nanoparticles with standard ZnO.

Characterization of ZnO nanoparticles aims to determine the particle size and morphology of the ZnO nanoparticles formed. Therefore, ZnO was characterized using UV-Vis spectrophotometer, FTIR spectrophotometer, Particle Size Analyzer (PSA), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).

Characterization using UV-Vis spectrophotometry

A total of 0.01 g of ZnO nanoparticles was dissolved in 10 mL of distilled water. Then the dissolved sample was then measured using a UV-Vis spectrophotometer to determine the absorbance peak produced. UV-Vis spectrophotometric measurements were carried out with a wavelength range of 250-700 nm to obtain peak absorbance and transmittance results (Yedurkar *et al.*, 2017).

Characterization using Fourier-Transform Infrared Spectroscopy *(FTIR)*

Functional group analysis using FTIR was carried out on ZnO nanoparticle colloids that had been dried using an oven. A sample of 1-2 mg was weighed and 100-200 mg of KBr was added, then the mixture was crushed until smooth. Characterization using FTIR aims to determine and analyze thefunctional groups on ZnO nanoparticles in the wave number range 650–4000 cm^{-1}. FTIR results show the appearance of vibration peaks which indicate the presence of functional groups in a compound. Based on the observed functional groups, we can then determine the bonds between atoms in the sample being measured (Yedurkar *et al.*, 2017).

Characterization using Particle Size Analyzer *(PSA)*

A total of 0.25 mg of ZnO nanoparticles was dissolved in ethanol until an emulsion was formed. The emulsion is then homogenized and put into a cuvette. The cuvette containing the emulsion was analyzed with a PSA instrument. Measured sample size distribution via the resulting graph (Nurbayasari *et al.*, 2017).

Characterization using Scanning Electron Microscopy *(SEM)*

The sample is placed in a measuring chamber 80 100 35 mm^3 with a diameter of 200 mm which has been coated with carbon tape and inserted into the smart couter for coating. The chamber is then inserted into the specimen holder on the SEM. The sample was observed at a voltage of 20 kV. Magnification was carried out 15,000 times for surface morphology and determination of chemical composition by EDX (Jayakar *et al*., 2021)

Characterization using Transmission Electron Microscopy *(TEM)*

The sample was sonicated for 5 minutes and analyzed at a voltage of 200 kV. The sample is then placed on a copper grid and allowed to dry. Images were captured at different magnetic strengthsand diffractogram areas were selected to analyze the structure of the ZnO nanoparticles (Jayakar *et al*., 2021)

RESULTS AND DISCUSSION

Binahong leaf extract

Phytochemical testing is a qualitative analysis of secondary metabolite compounds that can be identified using reagents that can provide characteristic characteristics of each secondary metabolite group (Kumalasari *et al*., 2020). The results of the phytochemical test of binahong leaf extract are presented in Table 1.

Table 1: Phytochemical test results of binahong leaf extract.

PhytochemicalTest	Reactor	Results	Information
Polyphenols	$FeCl_3$	Color changes from yellow to blackishgreen	Positive Polyphenols
Tannin	$FeCl_3$	Color changes from yellow to blackishgreen	PositiveTannin
Flavonoids	Mg powderand HCl	The color changes from yellow to a redprecipitate	Positive Flavonoids
Alkaloids	PlusWagner reagent	A red precipitate is formed	Positive Alkaloids
Steroids/ Terpenoids	Anhydrous acetic acid and concentratedsulfuric acid	Color changes from yellow to darkbrown	Positive for terpenoids NegativeSteroids

Phytochemical test results with several reagents showed that binahong leaf extract positively contained polyphenols, tannins, flavonoids, alkaloids and terpenoids. The characterization of binahong leaf extract aims to determine the presence of phenolic compounds that function as reducing agents and capping agents which inhibit the formation of agglomeration (Dwiastuti *et al*., 2022). Based on the data from the phytochemical test results, binahong leaf extract was then analyzed using an FTIR spectrophotometer and the results are shown in Figure 1.

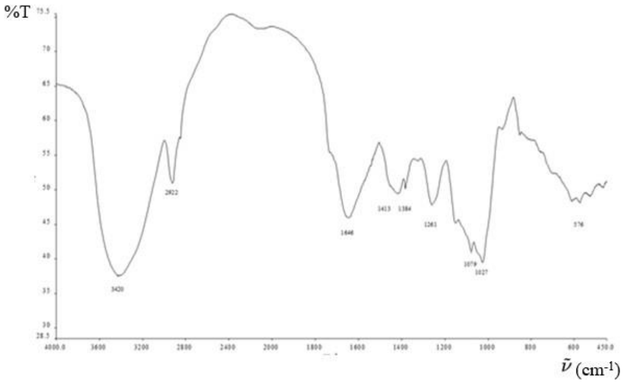


Figure 1: FTIR spectrum of binahong leaf extract.

Binahong leaf extract shows the absorption of OH stretching, C=O stretching, CH stretching, N=O stretching, and CN stretching in the areas 3420, 1646, 2922, 1384, and 1079 cm^{-1}. The wide absorption peak in the 3420 cm^{-1} region indicates the vibration of the OH group, which typically comes from polyphenols (Ismail *et al*., 2019). The absorption peak that occurs in the 2922 cm^{-1} area shows the absorption band for the CH stretching polyol functional group (Edison *et al*., 2016). The sharp band observed in the 1646 cm^{-1} region indicates the stretching of the symmetric C=O group (Rajendran *et al*., 2017). The absorption that occurs in the 1384 cm^{-1} area indicates the presence of an alkane group (C=H) and also the presence of NO stretching (Sari & Hawari., 2022). The positions of the absorption peaks in the 1079 and 1027 cm^{-1} areas are the CN stretching vibrations and flexible OH groups in the protein (Vijayakumar *et al*., 2018). These absorption peaks indicate the presence of polyol compounds (phenolic acids and flavonoids), terpenoids, and tannins in binahong leaf extract. This secondary metabolite compound has a hydroxy functional group.

Biosynthesis of ZnO Nanoparticles

The precursor solution plays an important role in the chemical process of ZnO nanoparticle fabrication. The formulation of the number of moles of NaOH added to the zinc acetate solution provides optimization in the fabrication of ZnO nanoparticles using the precipitation method followedby pre-hydrothermal treatment (Hossary *et al*., 2018).

The reaction that occurs in the process of mixing zinc acetate and NaOH is shown in the equation $(CH_3COO)_2Zn$ + 2NaOH → $2CH_3COOHNa$ + $Zn(OH)_4^{2-}$. $Zn(OH)^2$ and $Zn(OH)_4^{2-}$ precipitates are formed according to the stoichiometric ratio of Zn^{2+} and OH^- used. Zinc ions come from zinc acetate solution, while hydroxide ions come from sodium hydroxide solution, according to the chemical reaction in the equations $Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2$ and $Zn^{2+} + 4OH^- \rightarrow Zn(OH)^{2-}$ (Rhamdiyah *et al*., 2022).

The NaOH formulation used in this research is 10 mL NaOH to be reacted with 50 mL Zinc acetate dihydrate, where this condition is the optimum condition for the reaction between zinc and hydroxide ions to occur in conditions where the ions are still dissolved. These ions can then become precipitates as a precursor to the formation of ZnO nanoparticles when given hydrothermal treatment(Prakorso *et al*., 2019)

The formation of ZnO nanoparticles occurs through a reaction mechanism between zinc acetate dihydrate precursor solution ($Zn(CH_3COO)_2.2H_2O$), binahong leaf extract, and NaOH. Zinc acetate dihydrate is used as a precursor because it has more stable properties, produces good typography and has stable and uniform crystallinity (Nasser *et al*., 2020).

The precursor concentration used for the biosynthesis of ZnO nanoparticles is 0.15 M. Nurbayasari *et al* (2017) and Tamtowi (2020) states that a concentration of 0.15 M and pH 8 are goodconditions for the biosynthesis of ZnO nanoparticles. The reaction between the precursor solution andNaOH produces $Zn(OH)_2$, CH_3COONa, and H_2O. The $Zn(OH)_2$ compound is formed starting with a cloudy solution. The cloudy solution turned into a milky yellow colloid after adding binahong leaf extract. Colloidal solutions are formed after Zn^{2+} and OH^- are at the critical point of solubility. Meanwhile, excess OH^- ions will react with $Zn(OH)_2$ and then form a complex $Zn(OH)_4^{2-}$ (Nurbayasari *et al*., 2017). The stirring process and the avet availability of H_2O cause the $Zn(OH)_4^{2-}$ compound to dissociate into Zn^{2+} and OH^- again and then change to ZnO due to the reduction reaction of Zn^{2+} ions to Zn by functional groups originating from binahong leaf extract (Alamdari *et al*., 2020).

In the biosynthesis of ZnO nanoparticles, polyphenolic compounds in binahong leaf extract act as a stabilizing agent as well as a reducing agent (Nurbayasari *et al*., 2017). The functional groups from binahong leaf extract and NaOH will reduce Zn^{2+} ions into Zn atoms, allowing the Zn atoms to approach each other and interact with each other through intermetallic bonds to form a nano-sized Zn cluster (Marslin *et al*., 2018). Next, particle growth occurs where the growth rate will affect the size of the resulting particles (Rhamdiyah *et al*., 2022).

The functional groups from binahong leaf extract interact with the Zn compound interface and envelop the Zn cluster, this event is called capping so that in the formation of ZnO nanoparticles there is no aggregation between the nanoparticles and it forms stable ZnO nanoparticles. This is due to the repulsive force between similar charges. Hydroxy (OH^-) plays a role in binding Zn clusters so that the particles are surrounded by negatively charged ions (Marslin *et al*., 2018).

Functional groups such as hydroxy (OH^-) act as stabilizing agents in the biosynthesis of ZnO nanoparticles. According to Yusof *et al*., (2018), the OH^- functional group acts as a ligand that donateslone pairs of electrons to the Zn^{2+} orbital, then Zn^{2+} and the OH^- group form a complex compound in a nano-sized template. Complex compounds are formed through coordinating covalent bonds between ligands and metals. The ligand will donate a lone pair of electrons to the metal ion providing an emptyorbital. The metal ion acts as a Lewis acid while the ligand acts as a Lewis base. The complex compound formed has a more stable chelating effect (Sari *et al*., 2017).

The tendency of particles to aggregate is caused by the effect of Brownian motion or the continuous movement of particles that occurs in solution. This tendency causes the particle diameter to be non-uniform. Nanoparticle aggregation occurs in two stages. In the first stage, the particles approach each other and collide with each other and in the second stage the colliding particles stick to each other (Astuti *et al.*, 2020).

The functional groups in binahong leaf extract, such as hydroxy, act as stabilizing agents or surfactants. Surfactants or surface active agents are molecules that contain hydrophilic (like water) andlipophilic (like oil/fat) groups in the same molecule (Anwar *et al*., 2017). The polar (hydrophilic) groupcan have a positive, negative or neutral charge. Generally, the polar part contains a hydroxy group, while the non-polar (lipophilic) part is a long alkyl chain. The presence of these two parts encouragesthe formation of homogeneous nanoparticles (Marslin *et al*., 2018).

The hydroxy groups contained in binahong leaf extract undergo a complexation reaction with Zn^{2+}. Binahong leaf extract as a ligand and Zn^{2+} as the central atom form a coordinating covalent bond. The ligand donates a lone pair of electrons to the metal ion providing an empty orbital. The hydroxy groups in binahong leaf extract act as ligands that donate free electron pairs to Zn^{2+} orbitals to form nano-sized complex compounds so that ZnO nanoparticles are formed after the calcination process (Sari *et al*., 2017).

The process of drying precipitates from ZnO nanoparticles using an oven is not carried out at a temperature of 100°C because it can cause a dehydration reaction, namely the release of hydrates into the environment in the form of water vapor. Meanwhile, $Zn(OH)2$ has not completely undergone decomposition. In research Gill *et al.*, (2018) state that $Zn(OH)2$ undergoes decomposition at temperatures above 125°C, so in this study, a temperature of 150°C was used.

The calcination treatment in the process of forming ZnO nanoparticles is very important because it can affect the properties of the nanoparticle material, one of which is reducing the size of the ZnO crystals. Calcination carried out at a temperature of 4000°C as carried out by Kumaresan *et al*. (2017) and Septiani *et al*. (2017), that a calcination temperature of 400°C can form good ZnO particles. A calcination temperature that is too high will allow an increase in the nucleation and growth of ZnO nanoparticles. This is because, at higher calcination temperatures, the release of OH^- ions occurs excessively so the reaction rate for the formation of ZnO increases (Mediouni *et al*., 2022). In addition, at higher calcination temperatures, more energy is given to atoms to diffuse so that particleswith smaller surface energy tend to expand (Gill *et al.*, 2018).

Calicination at a temperature of 400°C will generate driving energy which can break the Zn and OH (HO○●Zn●○OH) bonds found on the surface or interface of ZnO to form Zn^{2+}● and OH^-○ radicals which then rearrange and formation of nanoparticles (Gill *et al.*, 2018) as in the equations $2Zn(OH)2(s) \rightarrow Zn^{2+} + 2OH^-$ and $Zn^{2+} + 2OH^- \rightarrow ZnO(s) + H2O$. These results are also confirmed by research by Mornani *et al*. (2016) which produces ZnO nanoparticles with increasingly smaller sizes, namely from 66 nm to 46 nm with calcination temperatures from 400°C to 650°C. Apart from being a driving energy, calcination also aims to remove other compounds such as sodium hydroxide, sodium acetate and zinc acetate to increase the crystallinity of ZnO nanoparticles. The mechanism that occurs in the calcination process is heating the synthesized zinc oxide powder at a temperature of 400°C for 3 hours to remove compounds other than zinc oxide, such as sodium acetate, sodium hydroxide and zinc acetate by evaporating the dirty pounds. As a result of the calcination process, zinc oxide powder has a denser and more uniform crystal structure compared to the normal drying process.

(a) ZnO Micro **(b) ZnO nano**

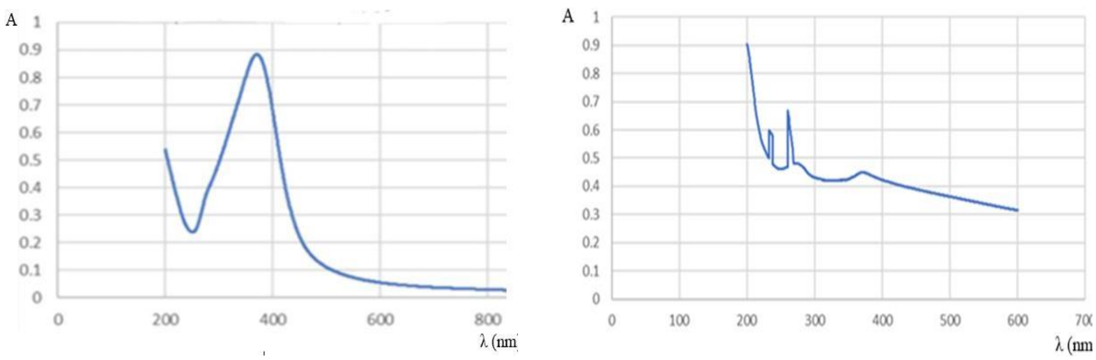
Figure 2: ZnO crystals.

The crystal form of the ZnO nanoparticles that were successfully synthesized has different shapes and colors compared to micro ZnO. Nano ZnO is in the form of crystals which are denser and harder while micro ZnO is in the form of fine powder.

ZnO nanoparticle characterization results

Characterization using a UV-Vis spectrophotometer

UV-Vis spectrophotometer is used to characterize and validate the formation and stability of metal nanoparticles in aqueous solutions. Figure 4 shows the absorption peak at a maximum wavelength of 260 nm and an absorbance value of 0.6 indicates that ZnO nanoparticles have been formed. This is in accordance with research conducted by Jayakar *et al.*, (2021) obtained data that the absorption peak of ZnO nano appears at 260 nm which is related to the intrinsic band gap absorption, where the electron transition occurs from the valence band to the conduction band. The appearance ofan absorption peak at a wavelength of 260 nm predicts that ZnO nanoparticles have an SPR (surface plasmon resonance) phenomenon.



(a) UV-Vis Absorption Spectrum of ZnO micro **(b) UV-Vis Absorption Spectrum of ZnO Nano**

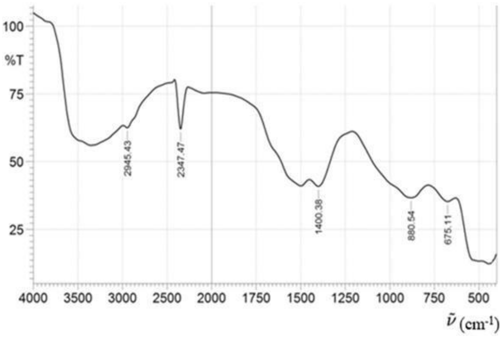
Figure 3: UV-Vis Absorption Spectrum.

Wavelength is closely related to the UV-Vis absorption spectrum. Spectrum peaks that appear at large wavelengths have small wave frequencies, so the waves will also have small energy (Yudono, 2017). Based on the results of the characterization of the spectral properties using a UV-Vis spectrophotometer, it was found that the relationship between the absorbance spectrum was in the wavelength range between 200 nm to 800 nm and the greatest absorption occurred at wavelengths in the Ultraviolet region (200 nm-380 nm).

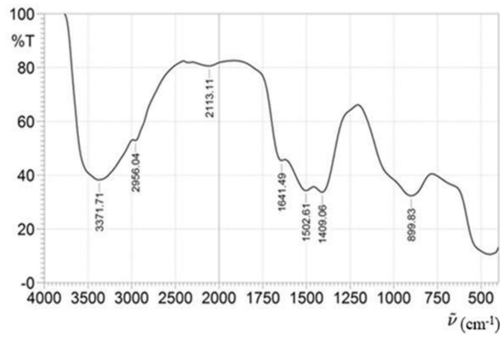
From the results of the UV absorption spectrum, it can be observed that there is a change in theabsorption spectrum at wavelengths between ZnO micro and ZnO nano. The highest absorption value for micro ZnO occurs at a wavelength of 378 nm, while for nano ZnO the highest absorption occurs at a wavelength of 260 nm. The maximum wavelength of ZnO nano is smaller than that of micro ZnO compounds. Therefore, it can be concluded that the energy possessed by ZnO nano in absorbing UV light is greater than that of micro ZnO. Apart from that, there are more absorption peaks produced by ZnO nano in the absorption spectrum compared to ZnO micro. This indicates that nano ZnO has a much greater ability to absorb energy compared to micro ZnO.

Characterization using an FTIR Spectrophotometer

Information regarding the interactions that occur between the biological components of binahong leaf extract and the IR spectra solution in the ZnO nanoparticle synthesis process is observed through peak shifts and intensity changes in the data.8pectra FTIR.



(a) FTIR spectra of ZnO Micro



(b) FTIR spectra of ZnO Nano

Figure 4: FTIR spectra.

Table 2: FTIR spectra of ZnO Micro and ZnO Nano.

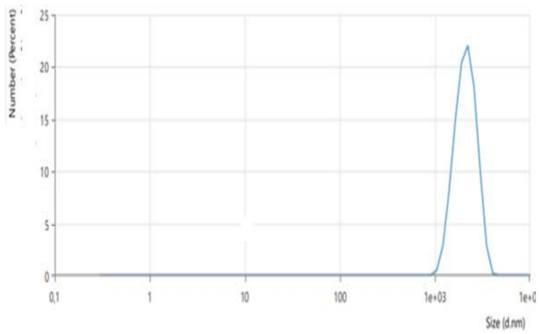
ZnO	Wave Number(cm-1)	Functional group vibration	Literature
Micro	2945 675	CH stretchingZnO	Edison *et al*., 2016 Abaoelfetoh *et al*., 2017Nur *et al*., 2022
Nano	3371 1502 1641	OH stretching NH stretching ZnO stretching	Edison *et al*., 2016 Abaoelfetoh *et al*., 2017Amanda *et al*., 2022

The spectra resulting from FTIR characterization can be seen in Table 2. Several peaks can beseen forming in the wave number range of 500 cm^{-1} to 4000 cm^{-1}. The peak at wave number 1,641 cm^{-1} for ZnO nano indicates stretching vibrations in the Zn-O functional group. This peak also proves that the Zn-O compound has been formed (Amanda *et al*., 2022). At a wave number of around 889 cm^{-1} for nano ZnO a peak forms due to the tetrahedral bonds of Zn while in micro ZnO absorption occurs at a wave number of 675 cm^{-1} (Amanda *et al*., 2022). Another significant difference produced by these two compounds is that in micro ZnO, OH groups were found, whereas in nano ZnO an absorption peak was found which indicates the presence of OH functional groups. This indicates that the residue from binahong leaf extract in ZnO nano has not completely disappeared so bonds are still found in carboxyl. This is because the heating temperature given to the ZnO nanoparticles is still insufficient to be able to release the bonds of the residual compounds (Amanda *et al*., 2022).

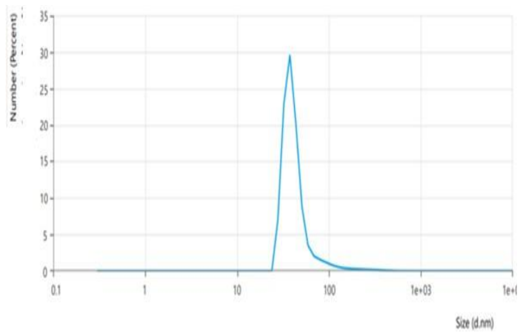
The decrease in intensity that occurs in the 3400 cm^{-1} area indicates the suspected involvement of the OH group in the Zn^{2+} reduction process. In the FTIR spectrum of ZnO nanoparticles, an absorption band appears in the 1500 cm^{-1} area which is assumed to be the NH group (Abaoelfetoh *et al*., 2017). The peptide bonds in the amide compounds contained in the binahong leaf extract are thought to play a role during the reduction process which gives rise to the NH group, which is the cause of the increased sharpness in the 1500 cm^{-1} absorption area. Based on the FTIR results, it is suspected that the main groups involved in the Zn^{2+} bioreduction process are the OH and NH functional groups which are derivatives of heterocomplex compounds, namely derivatives of proteins contained in binahong leaf extract (Nur *et al*., 2022).

Characterization using PSA

The results of analysis using PSA obtained comparative data for ZnO micro and ZnO nano, as presented in Figure 5.



(a) ZnO microparticle size curve



(b) ZnO nanoparticle size curve

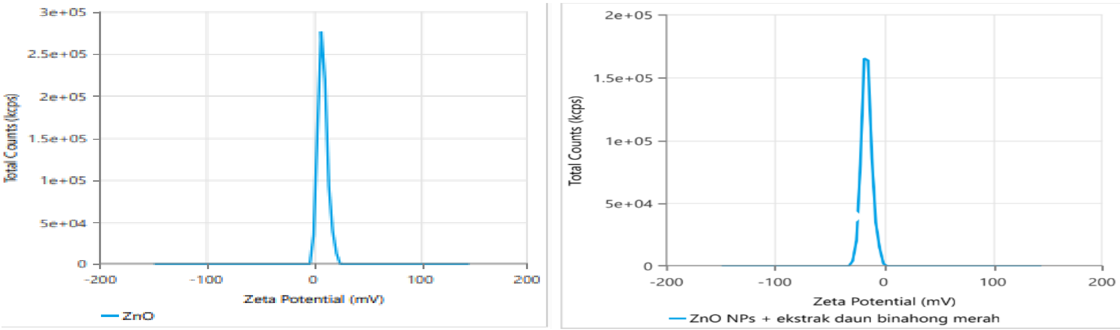
Figure 5: Particle size curve.

Based on Figure 5, it is found that the difference in diameter size between ZnO micro and ZnO nano is very large, where the diameter of ZnO nano is 46.07 nm while the diameter of ZnO micro is 2147 nm. This proves that the synthesized ZnO is nano-sized (below 100 nm).

Nanoparticles show distinctive properties when they have a diameter below 100 nm. This is in accordance with the research results that the diameter of ZnO resulting from synthesis using a reductant from binahong leaf extract is 46.07 nm, causing the ZnO nano colloid system to tend to be stable as a result of the effectiveness of binahong leaf extract which has acted as an excellent stabilizing agent inthe formation of ZnO nanoparticles.

The polydispersion index value is used to estimate the range of nanoparticle size distribution and determine the presence or absence of aggregation (Amyliana and Agustini, 2021). The smaller the polydispersity index value, the more homogeneous the particle size. The polydispersion index value has three ranges, namely monodispersion (less than 0.3), polydispersion (0.3-0.7), and superdispersion(more than 0.7). Polydispersion index values below 0.3 indicate a narrow distribution (Liana, 2016). Based on research data, shows that the polydispersity index value of ZnO nano is less than 0.3, namely 0.2913, which describes very homogeneous particle size uniformity, where the tendency of particles to agglomerate to form large particle aggregates is very small (Ningsih *et al*., 2017).

Zeta potential was determined to determine the surface charge on ZnO nanoparticles. The determination was carried out at a temperature of 250C with a refractive index of 1.330; viscosity of 0.887 cP; and scattering intensity of 78.5 kcps. Data from zeta potential measurements are shown in Figure 6.



(a) ZnO micro zeta potential curve **(b) ZnO nano zeta potential curve**

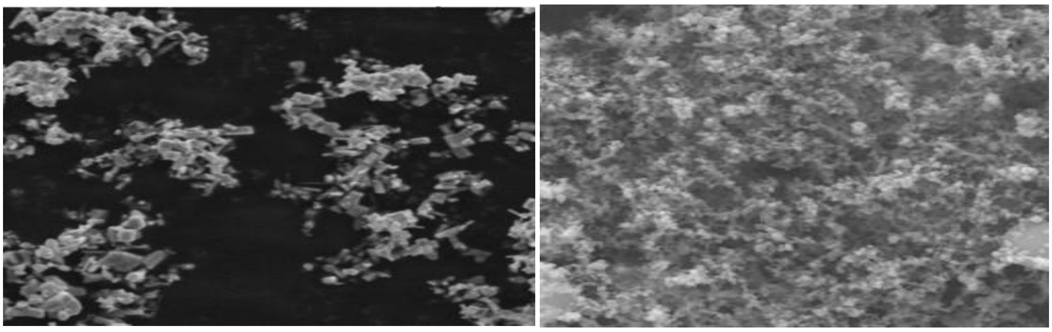
Figure 6: Zeta potential curve.

The zeta potential value is expected to be less than -30 mV and greater than +30 mV because the particles will have a repulsive force between particles that have the same charge so that it can produce nanoparticles that tend to be stable (Nugroho and Sari., 2018). If the zeta potential value is low it will tend to agglomerate or experience flocculation which then causes poor physical stability (Haidar *et al*., 2017). However, zeta potential is not the main parameter in determining the stability of a nanoparticle, other factors that also influence include the size, distribution and morphology of the particle (Menichetti *et al*., 2023)

The results of measuring the zeta potential of ZnO nanoparticles are -15.89 mV and can be said to be stable because they have a zeta potential value below -30 mV. A negative zeta potential indicates that ZnO nanoparticles have a negative surface charge. Meanwhile, the zeta potential of micro ZnO can be said to be unstable because it is below 30 mV or 8.772.

Characterization using SEM

Sample testing uses a test SEM-EDX produces images of the surface morphology structure and elemental content of the samples tested. SEM results can be images of the morphological structure for each sample at a certain magnification. The SEM observations carried out had detection limitations because the ZnO sample imaging tool could only be carried out at a magnification of 15,000 times to produce a clear image.



(a) SEM micrograph of ZnO Micro **(b) SEM micrograph of ZnO Nano**

Figure 7: SEM micrograph.

SEM micrographs of ZnO nano show that the particles are distributed unevenly but have a uniform shape. The SEM micrograph of ZnO micro shows that there are many gaps between the particles produced and the structure is not dense, but the micrograph of ZnO nano shows that the particle distribution is even, the gaps between particles are not clearly visible, and small round shapesare the main clusters.

Image of surface morphology of ZnO nanoparticles derived from Zn^{2+} ions. The resulting particles are generally round in shape and have gaps between the particles that are not clearly visible. However, gaps between the particles remain, this is due to agglomeration between the ZnO particles (Saridewi *et al.,* 2023). This agglomeration is due to the influence of polarity, electrostatic power of ZnO, and large energy on the surface of the sample which usually occurs when the synthesis process takes place (Kamli *et al.,* 2021). Agglomeration can also occur because there are still many secondary metabolite compounds from binahong leaf extract that act as templates for zinc acetate dihydrate precursors. Another factor that also influences the morphology of the ZnO nanoparticles formed is the reaction temperature when biosynthesis takes place (Fawcett *et al*., 2017)

After knowing the size distribution of ZnO particles produced from the sample, the SEM- Maping and SEM-EDX tests were also used to determine the elemental content in the sample. The results of mapping the elemental content of ZnO nanoparticles are shown in Figure 8.

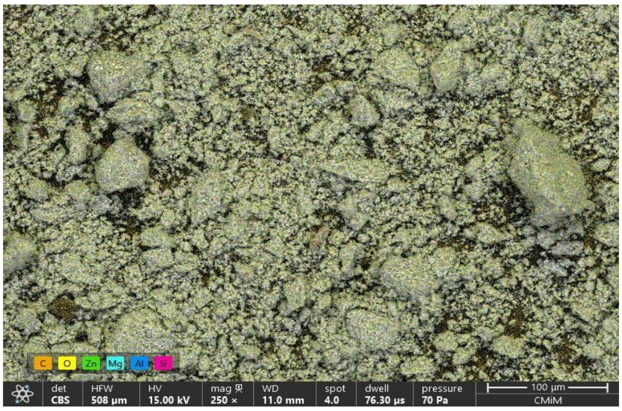


Figure 8: SEM-Mapping micrograph of ZnO nano.

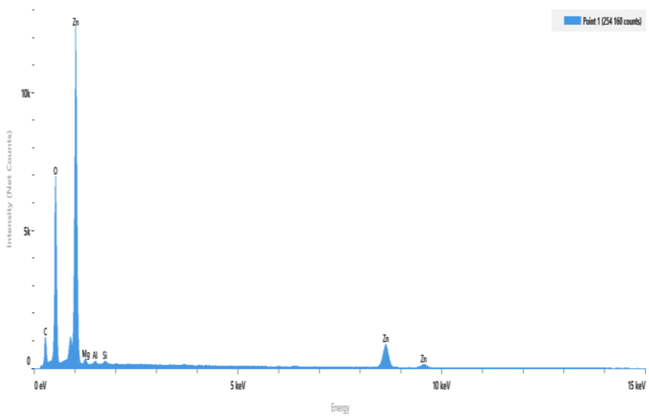


Figure 9: Graph of SEM-EDX test results on ZnO nano samples.

Table 3: SEM-EDX test results on ZnO nano samples.

Elements	Atomic %	Atomic %Error	Weight %	Weight %Error	Net Counts
S	0.1	0.0	0.1	0.0	254
C	0.9	0.1	0.7	0.1	3 975
O	23.0	0.3	28.2	0.2	21 758
Zn	73.5	0.3	67.8	0.8	65 566
Mg	0.5	0.0	0.5	0.1	1 295
Al	0.6	0.0	0.6	0.0	1 926
Si	0.6	0.0	0.6	0.0	2 439
Ca	0.2	0.0	0.3	0.1	924
Fe	0.6	0.1	1.2	0.2	1 451

The SEM-EDX results in Figure 9 and Table 3 for ZnO nanoparticles show that the element Zinc (Zn) has the largest mass percentage, namely 67.8% with an atomic percentage of 73.5%. Followed by a mass percentage of oxygen (O) of 28.2% with an atomic percentage of 23.0% and a mass percentage of carbon (C) of 0.7% with an atomic percentage of 0.9%. There are several other constituent elements with a mass percentage and atomic percentage of less than 1%. Based on the percentage of the majority atomic arrangement, it can be observed that this is in accordance with the atomic arrangement of ZnO nanoparticles, where the Zn atom indicates the main element that makes up the material and oxygen indicates the material in its oxide form. The zinc and oxygen elements indicate that the material is ZnO. Meanwhile, the carbon element (C) which has a fairly large mass percentage below the oxygen element is due to the secondary metabolite content of binahong leaf extract which predominantly contains carbon elements from polyphenolic compounds.

To confirm the results of the characterization of ZnO nanoparticles from the SEM results, we continued testing the ZnO nanoparticles using TEM to see a clearer morphology at different magnifications.

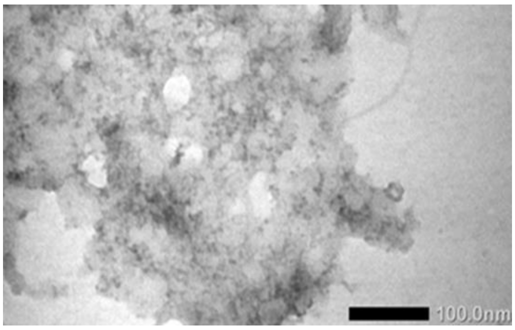


Figure 10: TEM analysis results of ZnO nano at 5000x magnification.

Figure 10 shows that ZnO nanoparticles are round or said to be spherical (Putri and Atun, 2017). According to Yusuf *et al*., (2023), the morphological shape of the nanoparticles is very important because if the shape is not spherical it will very easily come into contact with other nanoparticles so that the preparation will easily form aggregates.

CONCLUSION

Based on the results of research on the biosynthesis and characterization of ZnO nanoparticles, it can be concluded as follows:

1. Binahong leaf extract contains polyphenol secondary metabolite compounds and is confirmed by identification results using an FTIR spectrophotometer which shows that it contains the functionalgroups C=O, OH, and NH.
2. Nanoparticle biosynthesis using the bioreduction binahong leaf extract has succeeded in producingZnO nanoparticles with a particle size of 46.07 nm, spherical morphology, and a ratio of Zn to O element composition of 2:1.

ACKNOWLEDGMENTS

The author would like to thank the Chancellor of Udayana University, the dean of the Faculty of Mathematics and Natural Sciences, Udayana University, and the coordinator of the chemistry study program, Faculty of Mathematics and Natural Sciences, Udayana University for permission to use the laboratory and instruments for this research.

REFERENCES

1. Agarwal, H. A review on green synthesis of zinc oxide nanoparticles -an ECP-friendly approach. *Res. Eff. Technol*, 2017; 3(4): 406-413.
2. Alamdari, S., Ghamsari, M.S., Lee, C., Han, W., Park, H.H., Tafreshi, M.J., Afarideh, H. and Ara, M.H.M. Preparation and Characterization of Zinc Oxide Nanoparticles Using Leaf Extract of Sambucus ebulus. *Applied Sciences (Switzerland*), 2020; 10(10): 1-19.
3. Amanda, A.N., Safriani, L., Aprillia, A. and Ayi, B. The Influence of Precursor Type on the Characteristics of Zno Particles and Testing of Their Photocatalytic Properties. *Indonesian Journal of Materials and Energy*, 2022; 12(1): 7-17.
4. Amyliana, N.A. and Agustini, R. Formulation and Characterization of Nanoencapsulation Yeast Black Rice by Sonication Method with Poloxamer. *UNESA Journal of Chemistry*, 2021; 10(2): 184- 191.
5. Anwar, HM. and Karim, M. Phenolic Analysis and Inhibitory Power of Binahong (*Anredera cordifolia* (Ten) Steenis) Leaves Against *Escherichia coli* and *Staphylococcus aureus Bacteria*. *Indonesian Chemistry and Application Journal*, 2017; 1(1): 1-9.
6. Astusti, A., Sari. S.P. and Geraldine, A. Effects of Brown Motion and Nanofluid Stability On Convection Coefficients In Double Pipe Heat Exchangers. *Proceedings of National Seminar on Research and Community Service*, 2020; 2698(1): 2987-2480.
7. Chan, Y.Y., Pang, Y.L., Lim, S. and Chong, W.C. Facile Green Synthesis of ZnO Nanoparticles Using Natural Based Materials: Properties, mechanisms, surface modification and application. *Journal of Environmental Chemical Engineering*, 2021; 9(4): 110-128.

8. Chikkana, M.M., Neeglagund, S.E. and Rajashekarappa, K. Green Synthesis of Zinc Oxide Nanaoparticles (ZnO NPs): Antibacterial and Their Biological Activity. *Springer Nature Journal*, 2018; 1(7): 117-128.
9. Dwiastuti, R., Irnandari, E., Gani, M.R., Yuliani, S.H. and Nastiti, M.R.R. Optimization of Nanosilver Synthesis Proscess with Bioreductor of Binahong Leaf Extract. *J Pharmacy*, 2022; 19(2).
10. Fastaqibul, K.R. and Dina, K.M. Biosynthesis of ZnO Nanoparticles from Aqueous Extract of Moringa Oleifera L: Its Application as Antibacterial and Photocatalyst. *Indonesian Journal of Chemical Science*, 2022; 11(2): 1-20.
11. Fawcett, D., Verduin, J.J., Shah, M., Sharma, S.B. and Poinem, G. Review of Current Researchinto the Biogenic Synthesis of Metal and Metal Oxide Nanoparticles via Marine Algae and Seagrasses. *Journal of Nanoscience*. Article1-15 ID 8013850, 2017; 20(17): 1-15.
12. Goswami, M., Adhikary, N.C. and Bhattacharjee, S. Effect of Annealing Temperatures on the Structural and Optical Properties of Zinc Oxide Nanoparticles Prepared by Chemical Precipitation Method. *Optics*, 2018; 158(25): 1006-1015.
13. Haidar, H., H., C., B., DS, E. and WN, C. The Role of Lecithin Degradation On The pH- Dependent Stability of Halofantrine Encapsulated Fat Nano-Emulsion. *International Journal of Pharmaceutics*, 2017; 528(12): 524-535.
14. Ismail, A.M., Manaeza, A.A., Kabary, H.A., El-Serbiny, A.E. and Samy, A. The Influence of Calcination Temperature of Zinc Oxide Nanoparticles Synthesized by Solgel Method. *Journal of molecular structure*, 2019; 1196(2): 332-337.
15. Iwan, S.S., Siti, S., Yoki, Y. and Sudirman. Green Synthetic ZnO Nanoparticles Using Fig LeafExtract Media (Ficus carica Linn). *Journal of Chemistry and Packaging*, 2020; 42(1): 1–6.
16. Jayakar, V., Lokapur, V., Nityasree, B.R., Raju, K.C., Lyned, D. and Manjula, S. Optimization and green synthesis of zinc oxide nanoparticles using *Garcinia cambogia* leaves and evaluation of their antioxidant and anticancer properties in kidney cancer (A498) cell lines. *Biomedicine*, 2021; 41(2): 206-222.
17. Kamli, M.R., Malik, M.A., Vartika, S., Sabil, J.S.M., Ehab, H.M. and Ahmad, A. Biogenic ZnO nanoparticles synthesized from *Origanum vulgare* abrogate qorum sensing and biofilm formation in opportunistic pathogen *Chromobacterium violaceum*. *Pharmaceutics*, 20121; 13(11): 1743-1756.
18. Kumalasari, M.L.F. and Andiarna, F. Phytochemical Screening of Basil Leaf Extract (*Ocimum basilicum L*). *Indonesian Journal for Health Science*, 2020; 4(1): 39-44.
19. Marslin, G., Siram, K., Maqbool, Q., Selvakesan, R.K., Kruszka, P. and Franklin, G. Secondary Matabolites in The Green Synthesis of Metallic Nanoparticles. *Materials*, 2018; 11(6): 1-25.
20. Mediouni, N., Guillard, C., Dappozze, F., Khoruz, L., Parola, S., Justin, C., Amara, A.B.H., Rhaiem, H.B., Renault, N.J. and Phillippe, N. Impact of structural defects on the photocatalytic properties of ZnO. *Journal of Hazardous Materials Advances*, 2022; 6(10): 1-31.
21. Menichectti, A., Printezi, A.M., Mordini, D. and Montalti, M. Effect of Size, Shape, and Surface Functionalization on The Antibacterial Activity of Silver Nanoparticles. *J. Funct. Biomater*, 2023; 14(5): 244-289.
22. Mornani, E.G., Mosyebian, P., Dorranian, Dand Behzae, K. Effect of calcination temperature on the size and optical properties of synthesized ZnO nanoparticles. *Jornal od nanobiotechnology, 2016;* 11(2): 39-50.
23. Nasrollahzadeh, M., Atarod, M., Sajjadi, M., Sajadi, S.M. and Issaabadi, Z. *Plant-Mediated Green Synthesis of Nanostructures: Mechanisms, Characterization, and Applications. In Interface Science and Technology* Elsevier Ltd, 2019; 1: 28.
24. Nasser, M., Aslam, U., Khalid, B. and Chen, B. Green route to synthesized Zinc Oxide Nanoparticles using leaf extract of *Cassia fistula* and *Melia azadarach* and their antibacterial potential. *Scientific Reports*, 202; 10(1): 55-90.
25. Nurbayasari, R., Saridewi, N.and Shofwatunnisa. Biosynthesis and Characterization of ZnO Nanoparticles with Extract of Green Seaweed *Caulerpa* sp. *Gadjah Mada University Fisheries Journal*, 2017; 19(1): 17-28.
26. Prakorso, A.B., Agustini, R.D., Aprillia, A., Suryaningsih, S., Lusi, S. and Ayi, B. Effect of NaOH Concentration on the Structure, Morphology and Properties of Zinc Oxide Photocatalyst Doped with Aluminum Atoms. *Indonesian Journal of Materials and Energy*, 2019; 9(2): 86-97.
27. Preethi, S., Abama, K., Nithyasari, M., Kishore, P., Deepika, K., Ranjithkumar, R., Bhuvaneshwari,V. and Bharathi, D. Synthesis and Characterization of Chitosan/Zinc Oxide Nanocomposite for Antibacterial Activity onto Cotton Fabrics and Dye Degradation Applications. *International Journal of Biological Macromolecules*, 2020; 164(1): 2779-2787.
28. Puspitasari, L., Suci, M.and Amilus, T. Characterization of Mint Leaf Chemical Compounds (Mentha sp.) using FTIR and Chemometric Methods. *Journal of Pharmaceutical Sciences*, 2021; 14(1): 5-11.
29. Rajakumar, G., Thiruvengadam, M., Mydhili, G., Gomathi, T. and Chung, I.M. Green Approach for Synthesis of Zinc Oxide Nanoparticles from Andrographis paniculate Leaf Extract and Evaluation of Their Antioxidant, Anti-Diabetic, and Anti-Inflammatory Activities. *Bioprocess and Biosystems Engineering*, 2018; 41(1): 21-30.
30. Rajendran, P. and Sengodan, K. Synthesized and

Characterization of Zinc Oxide and Iron Oxide Nanoparticles Using *Sesbania grandiflora* Leaf Extract as Reducing Agent. *Journal of Nanoscience*, 2017; 19(8): 1-7.
31. Rofi, S.N. and Maharani, D.K. Synthesis and Characterization of ZnO for the Application of Hydrophobic Properties in Glass. *UNESA Journal of Chemistry*, 2020; 9(1): 111-115.
32. Santhoshkumar, J., Kumar, S.V. and Rajeshkumar, S. Synthesis of Zinc Oxide Nanoparticles Using Plant Leaf Extract Against Urinary Tract Infection Pathogen. *Resource-Efficient Technologies*, 2017; 3(4): 459-465.
33. Saravanadevi, K., Kavitha, M., Karpagavinayagam, P., Saminathan, K. and Vedhi, C. Biosynthesis of ZnO and Ag-doped ZnO Nanoparticles from Vitis vinifera Leaf for Antibacterial, Photocatalytic Application. *Materials Today*: Proceedings, 2020.
34. Sari, S.A. and Hawari, D. Biosynthesis of ZnO Nanoparticles using Lime Leaf Extract (Citrus auraantifolia) for identification of Latent Fingerprints. *JKPK*, 2022; 7(2): 171-180.
35. Saridewi, S., Riyanti, A., Aziz, I., Kumila, B.N. and Adinda, A.L. Synthesis of ZnO-TiO_2 nanoparticles by sol-gel process and its application for solar cell semiconductor. *Jurnal Kimia Valensi*, 2023; 9(2): 261-270.
36. Tjiang, D., Aritonang, H.F. and Koleangan, H.S.J. Synthesis of Ag/$CoFe_2O_4$ Nanoparticles Using Binahong (*Anredera cordifolia* (Ten) Steenis) Leaf Extract and Its Application as a Photocatalyst for Degrading Methylene Blue Dyes. *Chemistry Program*, 2020; 12(2): 59-66.
37. Veronita, F. Isolation and Testing of the Antibacterial Activity of Binahong Leaves and Their Application as a Hand Sanitizer. *Indonesian Journal of Chemical Science*, 2017; 6(2): 1-7.
38. Wattimena, S.G. and Patty, P.J. Synthesis of Silver Nanoparticles Mediated by stem extract of *Anredera cordifolia* and study of their antimicrobial properties. *The Pharmaceutical and Chemical Journal*, 2017; 4(5): 129-136.
39. Yedurkar, S., Maurya, C. and Mahanwar, P. Biosynthesis of Zinc Oxide Nanoparticles Using Ixora Coccinea Leaf Extract- A Green Approach. Open *Journal of Synthesis Theory and Applications*, 2017; 5(1): 1-4.
40. Yudono, B. and Akhmad, A.B. *Spectrometry*. Palembang: Simetri, 2017.
41. Yusuf, A., Almatoairy, A.R., Henidi, H., Alshehri, O.Y. and Aldughaim, M.S. Nanoparticles as Drug Delivery Systems. *Polymers, 2023;* 15(7): 15-25.